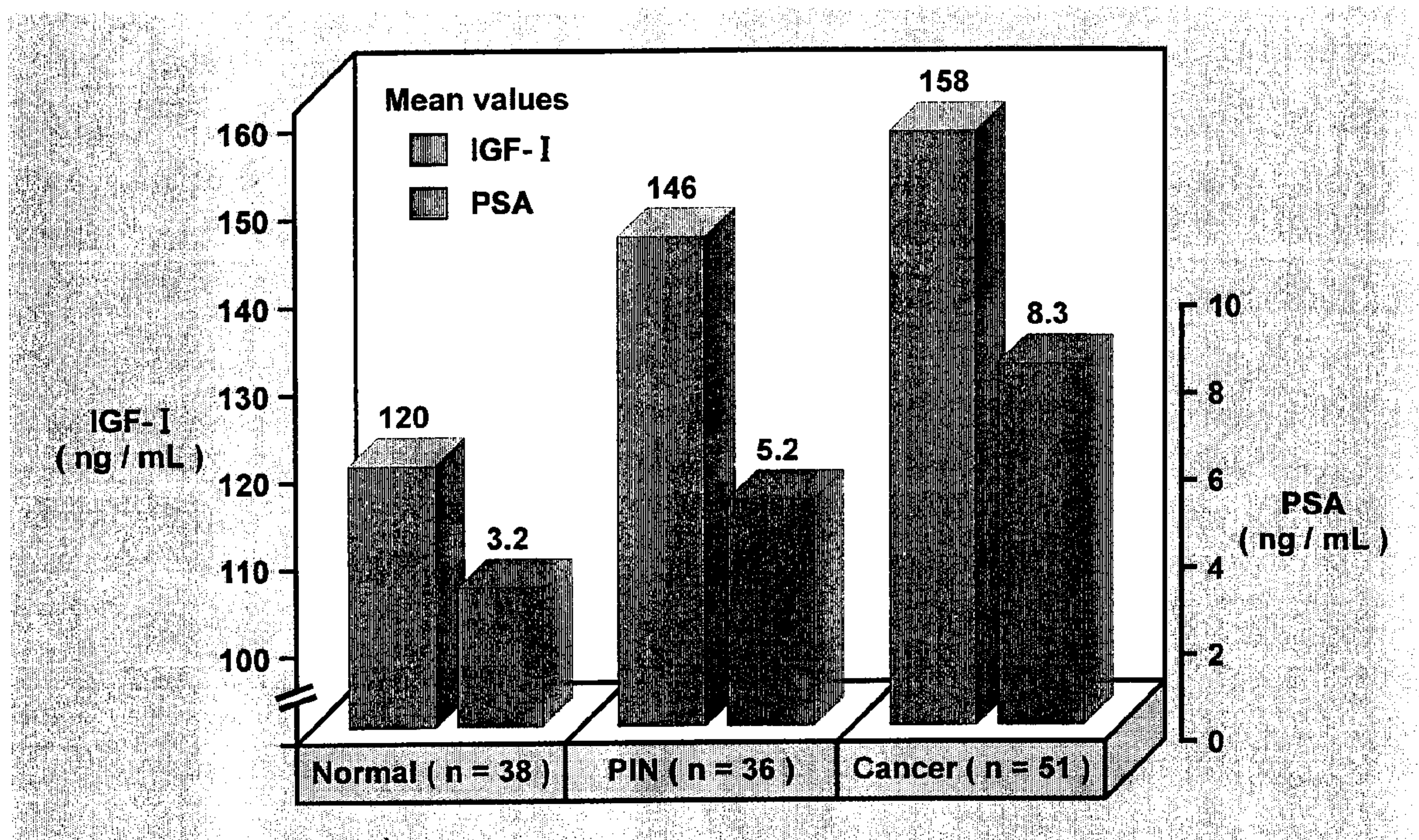




(86) Date de dépôt PCT/PCT Filing Date: 2000/10/09  
(87) Date publication PCT/PCT Publication Date: 2001/04/12  
(85) Entrée phase nationale/National Entry: 2002/04/05  
(86) N° demande PCT/PCT Application No.: GB 2000/003854  
(87) N° publication PCT/PCT Publication No.: 2001/025790  
(30) Priorités/Priorities: 1999/10/07 (9923599.6) GB;  
2000/05/27 (0012869.4) GB

(51) Cl.Int.<sup>7</sup>/Int.Cl.<sup>7</sup> G01N 33/574  
(71) Demandeur/Applicant:  
JOKEN LIMITED, GB  
(72) Inventeurs/Inventors:  
GRIFFITHS, KEITH, GB;  
TURKES, ATILLA T., GB  
(74) Agent: OGILVY RENAULT

(54) Titre : SYSTEME DE DETECTION DU CANCER DE LA PROSTATE PAR MESURE OU RAPPORT PSA/IGF-1  
(54) Title: DETECTION OF PROSTATE CANCER MEASURING PSA/IGF-1 RATIO



(57) **Abrégé/Abstract:**

An assay to assist in the detection of prostate cancer in a patient, said assay comprising the steps of: collecting a sample of biological fluid from said patient, and measuring the amount of free IGF-1 and free PSA, or the ratio between these amounts, in said sample. Another aspect of the invention is a device to be used in the assay. The device comprises a solid phase matrix having a surface and first antibodies which can specifically form immunocomplexes with free PSA and second antibodies which can form immunocomplexes with free IGF-1. The invention further relates to a kit which comprises such device.



## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number  
**WO 01/25790 A1**(51) International Patent Classification<sup>7</sup>: G01N 33/574

(74) Agent: MURGITROYD &amp; COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).

(21) International Application Number: PCT/GB00/03854

(22) International Filing Date: 9 October 2000 (09.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

|           |                             |    |
|-----------|-----------------------------|----|
| 9923599.6 | 7 October 1999 (07.10.1999) | GB |
| 0012869.4 | 27 May 2000 (27.05.2000)    | GB |

(71) Applicant (for all designated States except US): JOKEN LIMITED [GB/GB]; 1 Des Roches Square, Witan Way, Witney, Oxon OX8 6BE (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): GRIFFITHS, Keith [GB/GB]; Laurel Cottage, Castleton, Cardiff (GB). TURKES, Atilla, T. [GB/GB]; 23 Heol Aradlir, Cardiff CF5 2RE (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

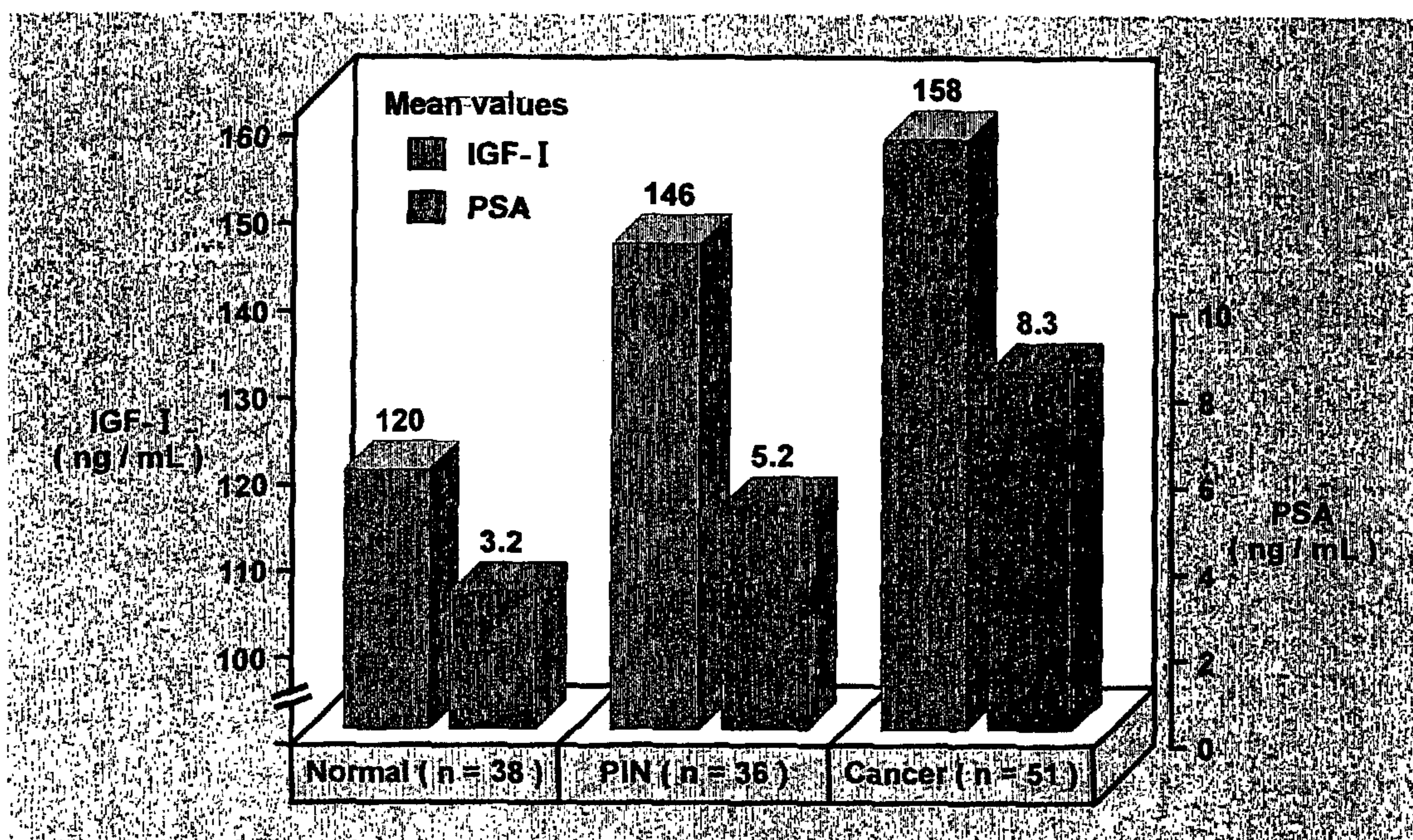
(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

[Continued on next page]

(54) Title: DETECTION OF PROSTATE CANCER MEASURING PSA/IGF-1 RATIO



(57) Abstract: An assay to assist in the detection of prostate cancer in a patient, said assay comprising the steps of: collecting a sample of biological fluid from said patient, and measuring the amount of free IGF-1 and free PSA, or the ratio between these amounts, in said sample. Another aspect of the invention is a device to be used in the assay. The device comprises a solid phase matrix having a surface and first antibodies which can specifically form immunocomplexes with free PSA and second antibodies which can form immunocomplexes with free IGF-1. The invention further relates to a kit which comprises such device.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## 1 DETECTION OF PROSTATE CANCER MEASURING PSA/IGF-1 RATIO

2

3

4 The present invention relates to a method of analysis  
5 of free prostate specific antigen (PSA) and free  
6 insulin-like growth factor-1 (IGF-1) levels to provide  
7 a more accurate differential diagnosis of prostate  
8 cancer. Advantageously the analysis of free PSA and  
9 free IGF-1 is made in a simultaneous manner, time-  
10 related changes are also monitored and a modified dual-  
11 antibody immunoassay (sandwich assay) is used.

12

13 Until recently, total prostate specific antigen (PSA)  
14 was recognised as the most sensitive and reliable index  
15 of prostatic carcinoma (Oesterling, 1993; J Urol 145:  
16 907-923). Indeed over the last decade, particularly in  
17 the USA, it has become the major focus of prostate  
18 cancer screening programmes. However, there have been  
19 increasing concerns with respect to the fidelity and

1 reproducibility of the measurement of and hence the  
2 value of the traditional assay procedures.

3

4 It is well documented that different commercial assays  
5 for total PSA give quantitatively different results on  
6 the same patient samples (Nakamura et al 1997. In 4<sup>th</sup>  
7 International Consultation of BPH; Committee on The  
8 Detection of Prostate cancer in a patient with BPH: Ed  
9 Denis et al; SCI, Plymouth: 381-387). This creates a  
10 considerable degree of uncertainty and unreliability in  
11 the precision of the diagnosis of prostate cancer. On  
12 this basis, the committee recommended that free rather  
13 than total PSA would be a more reliable diagnostic  
14 tool. Only recently has the methodology become  
15 available for the reliable quantification of free PSA.  
16 The diagnostic value using PSA alone can be further  
17 improved by analysing the change in free PSA levels  
18 over a period of time (PSA velocity) (Oesterling et al  
19 1993; JAMA 270: 860-864).

20

21 There is now considerable evidence linking the peptide  
22 growth factor, insulin-like growth factor-1 (IGF-1) to  
23 the development of prostatic hyperplasia and carcinoma.  
24 Recent work has indicated that IGF-1 levels are higher  
25 in men with prostate cancer, even when PSA is in the  
26 "normal" range. In a recent prostate cancer study in  
27 the county of Gwent, the levels of PSA have been  
28 measured in more than 3000 men between the ages of 55  
29 and 75, and prostate cancer identified in approximately  
30 3% of the screened group. A retrospective  
31 determination of IGF-1 in samples of serum from  
32 screened subject patients with low total PSA levels

1 (less than 4 ng/l), those with identified pre-  
2 neoplastic lesions (PIN) and those diagnosed with  
3 cancer of the prostate, revealed a close relationship  
4 between levels of PSA and IGF-1 (see Figure 1).

5  
6 Apart from the inter-assay variability, the major  
7 problem with the use of PSA as a predictive marker and  
8 test for prostate cancer is that certain cancer (about  
9 30%) will not secrete PSA and other subjects with high  
10 PSA levels have benign prostatic hyperplasia (BPH) and  
11 not cancer. Thus, existing technology creates the  
12 opportunity for both false negative and false positive  
13 results thereby reducing both the fidelity and value of  
14 the diagnosis.

15  
16 When PSA levels are greater than 20ng/ml, the  
17 probability of prostate cancer is about 90%. Levels of  
18 PSA of between 10 and 20 ng/ml could indicate cancer or  
19 benign prostatic hyperplasia (BPH). A difficulty  
20 however, lies in the assessment of patients having PSA  
21 levels, in the so-called "diagnostic grey zone", namely  
22 between 4-10 ng/ml. The levels could be normal because  
23 of old age, or could indicate a disease state and be  
24 elevated due to cancer, BPH or prostatitis. In the  
25 dual assay herein described we can differentially  
26 diagnose prostate cancer and eliminate unnecessary  
27 biopsies, particularly for those patients who exhibit  
28 PSA levels in the "diagnostic grey zone".

29  
30 The analytical difficulties and the imprecision of the  
31 diagnosis are well recognised and, until recently,

1 physician, patient and healthcare provider, alike  
2 accepted the limitations.

3

4 More recently, the use of dual markers, including PSA  
5 and IGF-1, has been advocated (see Djavan et al, 1999;  
6 Urology 54: 603-606; WO-A-99/38011). However, the  
7 differential diagnosis of prostate cancer described is  
8 based on single time point measurements of both PSA and  
9 IGF-1; concerns measurement of total PSA, (rather than  
10 free PSA); and involves different assay conditions.

11

12 The invention provides a method for a more accurate and  
13 less ambiguous differential diagnosis of prostate  
14 cancer.

15

16 It is therefore one object of the invention to provide  
17 an assay to assist in the detection of prostate cancer  
18 in a patient, which comprises the steps of :

- 19                   - collecting a sample of biological fluid  
20                   from the patient; and  
21                   - measuring, preferably simultaneously, the  
22                   amount of free IGF-1 and free PSA or the  
23                   ratio between these amounts.

24

25 Preferably several samples are collected at separate  
26 pre-determined time periods to be assayed as described  
27 above. Advantageously dual antibody techniques are used  
28 to simultaneously measure free IGF-1 and free PSA in a  
29 single sample of biological fluid. Such an assay  
30 represents a substantial qualitative and quantitative  
31 improvement on existing methodology and thereby offers

1 the opportunity for precise differential diagnosis of  
2 prostate cancer. As both peptide hormones PSA and IGF-  
3 1 will be measured in one sample and therefore under  
4 identical assay conditions accuracy of the measurement  
5 will be provided. Fidelity of the approach is likewise  
6 considerably enriched by basing the diagnosis on free  
7 levels and on the ratio of concentration of PSA/IGF-1.

8  
9 It is preferred to use a sandwich immunoassay to  
10 measure the amount of free IGF-1 and free PSA or their  
11 ratio. Such assay may further comprise the steps of :  
12 - providing first antibodies which can  
13 specifically form immunocomplexes with free PSA  
14 and second antibodies which can form  
15 immunocomplexes with free IGF-1, these first and  
16 second antibodies being attached to a solid phase  
17 matrix; and  
18 - contacting the solid phase matrix to the sample  
19 under conditions suitable to allow the formation  
20 of immunocomplexes;  
21 - subsequently contacting the solid phase matrix  
22 with third antibodies which can specifically form  
23 immunocomplexes with free PSA or said first  
24 antibodies and fourth antibodies which can form  
25 immunocomplexes with free IGF-1 or second  
26 antibodies, these third and fourth antibodies  
27 being labelled and the contacting step being made  
28 under suitable conditions to allow immunocomplexes  
29 formation; and  
30 - measuring, preferably simultaneously, the amount  
31 of labels of the third antibodies and of the  
32 fourth antibodies and/or the ratio between them.

1 It is also preferred that these third and fourth  
2 antibodies be labelled with distinct first and second  
3 labels.

4

5 Another aspect of the invention is a device to assist  
6 in the detection or diagnostic of prostate cancer.

7 Such device comprises a solid phase matrix having a  
8 surface and first antibodies which can specifically  
9 form immunocomplexes with free PSA and second  
10 antibodies which can form immunocomplexes with free  
11 IGF-1 located thereon.

12

13 For example the antibodies may be chemically bound to  
14 or physically entrapped in the surface. Alternatively,  
15 the antibodies may be localised by means of  
16 electrostatic or ionic attraction. Desirably the ratio  
17 of anti-PSA antibodies: anti-IGF-1 antibodies located  
18 on said surface is in the range 1:2 to 2:1, preferably  
19 is approximately 1:1.

20

21 In a further aspect, the present invention provides a  
22 kit to assist in the detection or diagnosis of prostate  
23 cancer. The kit comprises a device as defined above,  
24 and packaged separately thereto, third antibodies which  
25 can specifically form immunocomplexes with free PSA or  
26 said first antibodies and fourth antibodies which can  
27 specifically form immunocomplexes with free IGF-1 or  
28 second antibodies, said third and fourth antibodies  
29 being labelled with a first and second labels,  
30 respectively. Advantageously, the kit may further  
31 comprises means to detect and measure the amount of

1 label(s) retained on said apparatus following its  
2 exposure to said labelled antibodies.

3

4 The above generally describes the present invention  
5 which can be better understood with reference to the  
6 following example.

7

8 **Brief Description of the Figure.**

9

10 Figure 1 shows mean levels of PSA and IGF-1 for  
11 patients with low levels of PSA and the correlation  
12 with prostate cancer.

13

14 **Example**

15

16 The principle of the conventional two-site (sandwich)  
17 immunoassay which is preferred requires two antibodies,  
18 one of which is linked to a solid-phase matrix in order  
19 to provide a means of separation, and the other is  
20 "labelled" to allow the end point determination. Both  
21 antibodies recognise the analyte to be measured and  
22 form an antibody-analyte-antibody immunocomplex. The  
23 amount of complex formed is directly related to the  
24 concentration of analyte present in the serum sample.

25

26 Two different antibodies raised against PSA and IGF-1  
27 are linked to the solid phase matrix and two  
28 corresponding labelled antibodies will be used to  
29 measure both analytes, PSA and IGF-1, simultaneously in  
30 a single sample of biological fluid. Assay conditions  
31 can be optimised to provide the analytical sensitivity  
32 required for the detection of free PSA and free IGF-1.

1 The sample may be diluted with suitable like BSA  
2 (Bovine serum albumine), PBS (Phosphate Buffer Saline),  
3 or BSA (Bovine gamma globuline) before being contacted  
4 with the solid phase matrix. The sample is then  
5 allowed to incubate for a period of time, like a few  
6 hours, to allow for the formation of immunocomplexes  
7 and the solid phase matrix is then washed, for example  
8 with PBS, to remove non-immunocomplexed material. Then  
9 the solid matrix can be contacted with labelled  
10 antibodies and then incubated and washed before the  
11 amount of labels being measured.

12

13 The mono- and/or poly-clonal antibodies raised against  
14 PSA and IGF-1, can be purified either using salt  
15 fractionation, or Protein-A or affinity purification  
16 procedures and together will be attached to a solid  
17 phase matrix. The solid phase matrix can be prepared  
18 by linking antibodies to plastic test tubes, beads,  
19 microtitre plates/strips, microcrystalline cellulose,  
20 glass of magnetic particles, or membranes used in semi-  
21 quantitative assays. Particularly preferred support is  
22 tubes or microtitre plates.

23

24 Suitable commercially available anti-PSA antibodies  
25 include Nos. 7820-0004 and 7820-0350 of Biogenesis Ltd,  
26 Poole, Dorset, UK; MAB4082 of Chemicon International  
27 Inc, Temecula, CA, USA; SC-7638 and SC-7816 of Santa  
28 Cruz Biotechnology Inc, Santa Cruz, CA, USA; and MAS  
29 343cf of Harlan Sera-Lab Ltd, Loughborough,  
30 Leicestershire, UK.

31

1    Suitable commercially available anti-IGF antibodies  
2    include Nos. 5345-0329 and 5345-0209 of Biogenesis Ltd,  
3    Poole, Dorset, UK; GF006 of Chemicon International Inc,  
4    Temecula, CA, USA; SC-7144 and SC-1422 of Santa Cruz  
5    Biotechnology Inc, Santa Cruz, CA, USA; and MAS 974p of  
6    Harlan Sera-Lab Ltd, Loughborough, Leicestershire, UK.

7  
8    The second pair of antibodies recognising the different  
9    antigenic determinants of free PSA and free IGF-1 can  
10    be used with labels. Labelled antibodies can be  
11    prepared by conjugating with enzymes (horseradish  
12    peroxidase or alkaline phosphate), or a fluogenic agent  
13    (fluorocein), or a chemiluminescent agent (luminol or  
14    an acridinium derivative). It is proposed to use rare  
15    earth chelates, like europium and samarium, in the  
16    preparation of the labelled antibodies. On the basis  
17    of their different absorption/emission characteristics,  
18    they can be used at the same time in an assay system:  
19    europium for labelled IGF-1 antibody and samarium for  
20    PSA, or vice versa. However, other pairs of labels  
21    like erythrin/iron can also be effective in giving  
22    different emissions easy to differentiate.

23  
24    The principle of underlying the invention, therefore,  
25    is that antibodies against free PSA and IGF-1 on the  
26    same solid matrix, will form antibody-analyte complexes  
27    with free PSA and free IGF-1 in the same sample of  
28    serum and labelled PSA and IGF-1 will bind to their  
29    respective analytes. This completes the antibody-  
30    analyte-antibody sandwich. The end point analysis will  
31    be undertaken on an instrument capable of determining

1 time-lapse, or time-resolve fluorescence, like a  
2 spectrophotometer.

3

4 It should be understood that this invention is not  
5 limited to the particular embodiments shown and  
6 described above, but various changes and modifications  
7 can be made without departing from the spirit and scope  
8 of the novel invention.

1       **CLAIMS**

2

3       1.   An assay to assist in the detection of prostate  
4           cancer in a patient, said assay comprising the  
5           steps of :

- 6               -   collecting a sample of biological fluid
- 7               -   from said patient; and
- 8               -   simultaneously measuring by a sandwich
- 9               -   immunoassay the amount of free IGF-1
- 10              -   and free PSA in said sample, or the
- 11              -   ratio between these amounts in said
- 12              -   sample.

13

14       2.   The assay claimed in Claim 1 or 2, wherein more  
15           than one sample from the same patient taken at  
16           separate and pre-determined time period is  
17           submitted to the assay.

18

19       3.   The assay of Claim 2, wherein said assay further  
20           comprises the steps of :

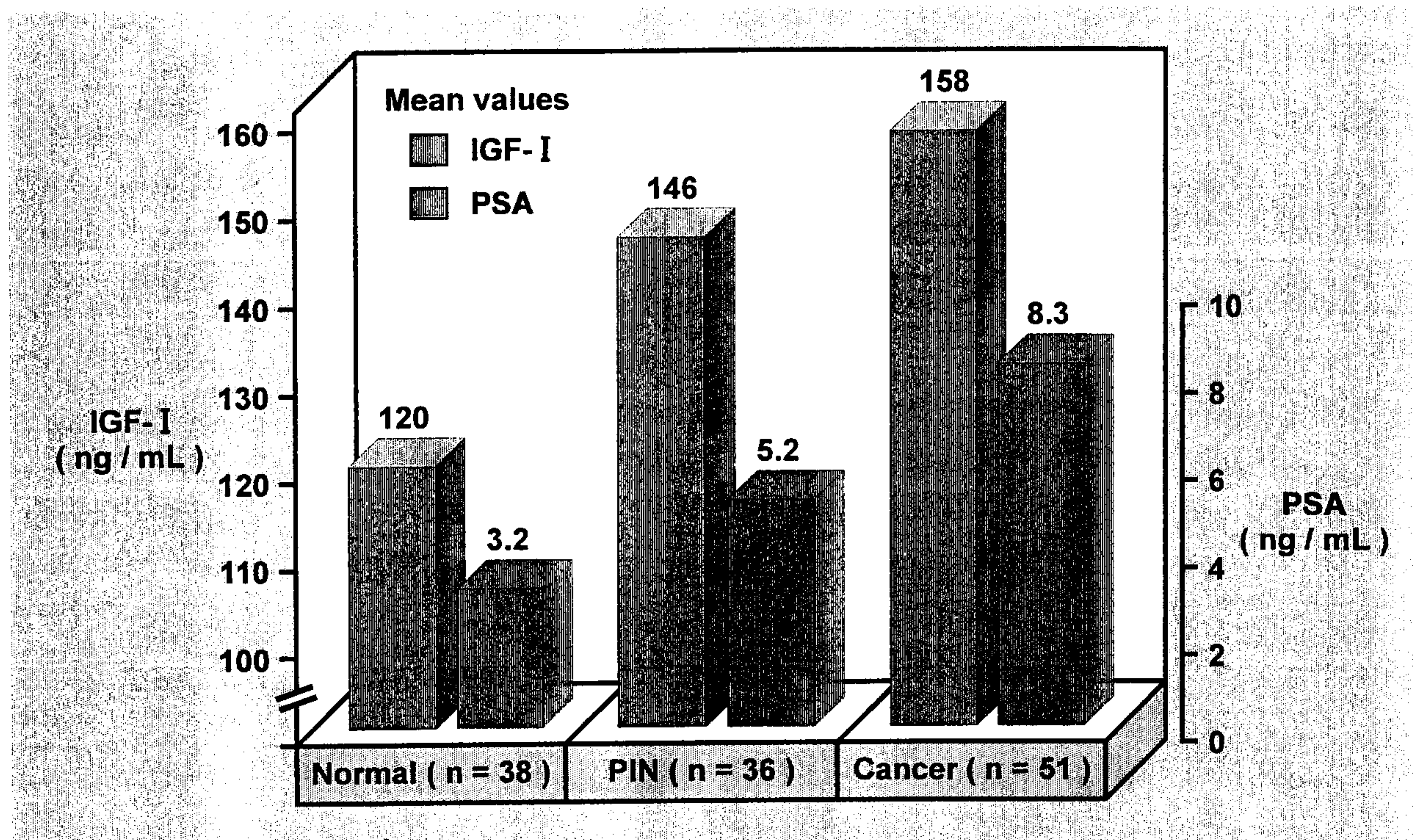
- 21               -   providing first antibodies which can
- 22               -   specifically form immunocomplexes with
- 23               -   free PSA and second antibodies which
- 24               -   can form immunocomplexes with free IGF-
- 25               -   1, said first and second antibodies
- 26               -   being attached to a solid phase matrix;
- 27               -   and
- 28               -   contacting the solid phase matrix to
- 29               -   said sample under conditions suitable
- 30               -   to allow the formation of
- 31               -   immunocomplexes;

- 1                   - subsequently contacting said solid  
2                   phase matrix with third antibodies  
3                   which can specifically form  
4                   immunocomplexes with free PSA or said  
5                   first antibodies and fourth antibodies  
6                   which can form immunocomplexes with  
7                   free IGF-1 or second antibodies, said  
8                   third and fourth antibodies being  
9                   labelled, said contacting step being  
10                  made under suitable conditions to allow  
11                  immunocomplexes formation; and  
12                  - measuring the amount of labels of the  
13                  third antibodies and of the fourth  
14                  antibodies and/or the ratio between  
15                  them.  
16
- 17       4.   The assay of Claim 3, wherein said third and  
18           fourth antibodies are labelled with distinct  
19           first and second labels.  
20
- 21       5.   A device to assist in the detection of prostate  
22           cancer, said device comprising a solid phase  
23           matrix having a surface and first antibodies  
24           which can specifically form immunocomplexes with  
25           free PSA and second antibodies which can form  
26           immunocomplexes with free IGF-1.  
27
- 28       6.   The device as claimed in Claim 5, wherein the  
29           ratio of first and second antibodies provided on  
30           the surface is ranging from 1:2 to 2:1.  
31

- 1       7. The device of Claim 6, wherein said ratio is  
2           substantially 1:1.  
3
- 4       8. The device of any one of Claims 5 to 7, wherein  
5           said solid phase matrix is a plastic test tube,  
6           beads, microtitre plates/strips,  
7           microcrystalline cellulose, glass of magnetic  
8           particles or a membrane used in semi-  
9           quantitative assay.  
10
- 11      9. A kit to assist in the detection of prostate  
12           cancer, said kit comprising a device as  
13           described in any one of Claims 5 to 7 and,  
14           packaged separately, third antibodies which can  
15           specifically form immunocomplexes with free PSA  
16           or said first antibodies and fourth antibodies  
17           which can form immunocomplexes with free IGF-1  
18           or second antibodies, said third and fourth  
19           antibodies being labelled with a first and  
20           second labels, respectively.  
21
- 22      10. The kit of Claim 9, wherein said first and  
23           second labels are distinct from each other and  
24           are colour labels which under suitable  
25           conditions generate distinct absorption/emission  
26           characteristics so that the amount of first and  
27           second label can be measured simultaneously.  
28
- 29      11. The kit of Claims 9 or 10, wherein said first  
30           and second labels are distinct from each other  
31           and are chosen in the group consisting of

- 1 enzymes, fluogenic agents and chemiluminescent  
2 agents.  
3
- 4 12. The kit of Claim 9 or 10, wherein said first and  
5 second labels are distinct from each other and  
6 comprise rare earth chelates.  
7
- 8 13. The kit of Claim 12, wherein said first and  
9 second labels are distinct from each other and  
10 comprise either europium or samarium chelates.  
11
- 12 14. The kit of Claim 9 or 10, wherein said first and  
13 second labels are distinct from each other and  
14 comprise either erythrin or iron.  
15
- 16 15. The kit of any of Claims 9 to 12, wherein said  
17 kit further comprises means to detect the amount  
18 of labels retained on the device following its  
19 exposure to said labelled antibodies.

1/1

*Figure. 1*

