

biopsy. These techniques are invasive and cause significant discomfort to the subject under examination.

A general need exists for convenient, reliable and accurate tests for diagnosing prostate disease. Preferably, the tests may have the capacity to discern between prostate cancer and non-cancerous forms of prostate disease (e.g. benign prostatic hyperplasia (BPH)).

Summary

The present inventors have identified combinations of biomarkers effective for the detection of prostate disease. In particular, the biomarker combinations may be capable of discerning between cancerous and non-cancerous forms of prostate disease. Accordingly, the present disclosure relates to these biomarker combinations and assays that utilize them for the detection of prostate disease.

The present disclosure thus relates at least to the following series of numbered examples below, wherein reference to Example 1 includes each of Examples 1a, 1b and 1c as numbered below:

Example 1a: A method for determining whether a subject has benign prostatic hyperplasia (BPH) from prostate cancer (CaP), comprising:

(a) generating a threshold value for discerning between BPH and prostate cancer by:

detecting at least two analytes in a series of biological samples obtained from a population of subjects having BPH and a population of subjects having CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series;

combining the analyte levels in a manner that allows discrimination between the BPH and CaP samples; and

selecting a threshold value from the combined analyte levels and using said threshold value as a basis to discriminate between BPH and CaP in the sample; and

(b) detecting said at least two analytes in a biological sample from a subject to thereby obtain an analyte level for each said analyte in the subject's biological sample; and

(c) applying a suitable algorithm and/or transformation to the individual or combined analyte levels obtained from the subject's biological sample to thereby generate a patient analyte value for comparison to the threshold value; and

(d) determining whether the subject has BPH or CaP by comparison of the patient analyte value to the threshold value,

wherein the at least two analytes comprise glypican-1 (GPC-1) and are selected from any one of the following analyte combinations:

GPC-1 and hepatocyte growth factor (HGF);
 GPC-1 and epidermal growth factor (EGF);
 GPC-1 and plasminogen activator inhibitor 1 (PAI-1);
 GPC-1 and granulocyte colony stimulating factor (G-CSF);
 5 GPC-1 and human Interleukin 18 (HuIL-18);
 GPC-1 and platelet derived growth factor AB.BB (PDGFAB.BB);
 GPC-1 and platelet derived growth factor BB (PDGFBB);
 GPC-1 and soluble CD40 Ligand (sCD40L);
 GPC-1 and human granulocyte macrophage colony stimulating factor
 10 (HuGM-CSF);
 GPC-1 and interferon gamma (IFN γ); and
 GPC-1 and follistatin.

Example 1b: A method for determining whether a subject has benign prostatic hyperplasia (BPH) or prostate cancer (CaP), comprising:

- 15 (a) generating a threshold value for discerning between BPH and prostate cancer by:
 detecting at least two analytes in a series of biological samples obtained from a population of subjects having BPH and a population of subjects having CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series;
 combining the analyte levels in a manner that allows discrimination between the BPH
 20 and CaP samples; and
 selecting a threshold value from the combined analyte levels and using said threshold value as a basis to discriminate between BPH and CaP in the sample; and
- (b) detecting said at least two analytes in a biological sample from a subject to thereby obtain an analyte level for each said analyte in the subject's biological sample; and
- 25 (c) applying a suitable algorithm and/or transformation to the individual or combined analyte levels obtained from the subject's biological sample to thereby generate a patient analyte value for comparison to the threshold value; and
- (d) determining whether the subject has BPH or CaP by comparison of the patient analyte value to the threshold value.

30 wherein the at least two analytes comprise glypican-1 (GPC-1) and are selected from any one of the following analyte combinations:

GPC-1 and hepatocyte growth factor (HGF);
 GPC-1 and epidermal growth factor (EGF);
 GPC-1 and plasminogen activator inhibitor 1 (PAI-1);

GPC-1 and granulocyte colony stimulating factor (G-CSF);
GPC-1 and human Interleukin 18 (HuIL-18);
GPC-1 and platelet derived growth factor AB.BB (PDGFAB.BB);
GPC-1 and platelet derived growth factor BB (PDGFBB);
5 GPC-1 and soluble CD40 Ligand (sCD40L);
GPC-1 and human granulocyte macrophage colony stimulating factor
(HuGM-CSF);
GPC-1 and interferon gamma (IFN γ); and
GPC-1 and follistatin.

10 Example 1c: A method of generating a threshold value for use in discriminating
between benign prostatic hyperplasia (BPH) and prostate cancer (CaP), comprising:

(a) detecting at least two analytes in a series of biological samples obtained from a
population of subjects known to have BPH and a population of subjects known to have CaP,
to thereby obtain an analyte level for each said analyte in each said biological sample of the
15 series;

(b) combining the analyte levels in a manner that allows discrimination between the BPH
and CaP samples; and

(c) selecting the threshold value from the combined analyte levels to serve as a basis to
discriminate between BPH and CaP;

20 wherein the at least two analytes comprise glypican-1 (GPC-1) and are selected from
any one of the following analyte combinations:

GPC-1 and hepatocyte growth factor (HGF);
GPC-1 and epidermal growth factor (EGF);
GPC-1 and plasminogen activator inhibitor 1 (PAI-1);
25 GPC-1 and granulocyte colony stimulating factor (G-CSF);
GPC-1 and human Interleukin 18 (HuIL-18);
GPC-1 and platelet derived growth factor AB.BB (PDGFAB.BB);
GPC-1 and platelet derived growth factor BB (PDGFBB);
GPC-1 and soluble CD40 Ligand (sCD40L);
30 GPC-1 and human granulocyte macrophage colony stimulating factor
(HuGM-CSF);
GPC-1 and interferon gamma (IFN γ); and
GPC-1 and follistatin.

Example 2. The method according to example 1, wherein the at least two analytes are selected from any one of the following analyte combinations:

GPC-1, HGF and PAI-1;

GPC-1, HGF and human fibroblast growth factor beta (HuFGFb);

5 GPC-1, HGF and EGF;

GPC-1, HGF and PDGFBB;

GPC-1, HGF and follistatin;

GPC-1, HGF and G-CSF;

GPC-1, HGF and human interleukin 8 (HuIL-8);

10 GPC-1, HGF and soluble CD40 ligand (sCD40L);

GPC-1, HGF and human cutaneous T-cell attracting cytokine (CTACK – also known as C-C motif ligand 27 or CCL27);

GPC-1, HGF and PDGFAB.BB;

GPC-1, HGF and IFN γ ;

15 GPC-1, HGF and human monocyte chemotactic and activating factor (HuMCP1/MCAF);

GPC-1, HGF, and HuIL-18;

GPC-1, HGF, HuGM-CSF; GPC-1, HGF, and urokinase plasminogen activator (uPA);

20 GPC-1, HGF, and human melanoma growth stimulating activity alpha (HuGROa, also known as CXC motif ligand 1, CXCL1);

GPC-1, HGF and soluble vascular endothelial growth factor receptor (sVEGFR);

GPC-1, EGF and follistatin;

GPC-1, EGF and tumour necrosis factor alpha (TNF α); and

25 GPC-1, G-CSF and soluble tyrosine kinase with immunoglobulin like and EGF-like domains 2 (sTIE2).

Example 3. The method of example 1 or example 2, wherein said selecting the threshold value from the combined analyte levels comprises selecting a subset of the combined analyte levels.

30 Example 4. The method according to any one of examples 1 to 3, wherein said combining of the analyte levels in a manner that allows discrimination between the BPH and CaP samples comprises combining a weighted linear sum of these analyte levels in a manner that maximizes the discrimination between the BPH and CaP samples in accordance with the formula:

$$S = w_1A_1 + w_2A_2 \dots w_nA_n$$

wherein S is the weighted sum,

w is the final analyte weighting,

and A is the level of a given analyte from n different analytes, or a transformation of

5 the level of a given analyte either numerically or as a ratio of analyte values.

Example 5. The method of example 4, wherein said transformation of the level of a given analyte numerically comprises use of an exponential function, a power function and/or a root function.

Example 6. The method according to example 4 or example 5, wherein obtaining
10 the final analyte weightings w comprises generating initial analyte weightings (W) that are set to 1, or generated according to the formula:

$$W = \max(M)/M, \text{ where } W = \{w_1, \dots, w_n\} \text{ and } M = \{\text{median}(A_1), \dots, \text{median}(A_n)\};$$

wherein max (M) is a maximum median level of the analyte levels obtained, and M is a vector containing the median analyte level obtained for each said analyte.

15 Example 7. The method according to example 6, wherein each said final weight is determined using a discriminating function for optimization in discerning between BPH and CaP in the series of biological samples.

Example 8. The method according to example 7, wherein the discriminating function is any one or more of:

- 20 (i) the area under a curve generated by a ROC analysis;
- (ii) a combination of True Positive Rates (TPR) and True Negative Rates (TNR);
- (iii) the area under a curve generated by a ROC analysis within a restricted specificity range;
- (iv) the area under a curve generated by a ROC analysis within a restricted
25 sensitivity range.

Example 9. The method according to example 7 or example 8, wherein the discriminating function for optimization is any one or more of the Nelder-Mead (simplex method), a stochastic method, a gradient descent method, a stochastic gradient descent method, a genetic algorithm, a particle-swarm method, and/or a brute force method.

30 Example 10. The method according to any one of examples 1 to 9, wherein the suitable algorithm and/or transformation applied to the individual or combined analyte levels obtained from the subject's biological sample is in accordance with the formula:

$$S = w_1A_1 + w_2A_2 \dots w_nA_n$$

wherein S is the weighted sum,

w is the final analyte weighting,

and A is the level of a given analyte from n different analytes, or a transformation of the level of a given analyte either numerically or as a ratio of analyte values.

5 Example 11. The method according to any one of examples 1 to 10, wherein said combining the analyte levels maximizes discrimination between the BPH and CaP samples.

Example 12. The method of any one of examples 1 to 11, comprising selecting the threshold value from the combined analyte levels in a manner that:

- (i) reduces the misclassification rate between BPH and CaP; and/or
- 10 (ii) increases sensitivity in discriminating between BPH and CaP; and/or
- (iii) increases specificity in discriminating between BPH and CaP.

Example 13. The method of example 12, wherein said selecting the threshold value from the combined analyte levels in a manner that reduces the misclassification rate between BPH and CaP comprises selecting a suitable true positive and/or true negative rate.

15 Example 14. The method example 12 or example 13, wherein said selecting the threshold value from the combined analyte levels in a manner that reduces the misclassification rate between BPH and CaP minimizes the misclassification rate.

Example 15. The method according to any one of examples 12 to 14, wherein selecting the threshold value from the combined analyte levels in a manner that reduces the misclassification rate between BPH and CaP comprises minimizing the misclassification rate between BPH and CaP by identifying a point where the true positive rate intersects the true negative rate.

25 Example 16. The method according to example 12, wherein selecting the threshold value from the combined analyte levels in a manner that increases sensitivity in discriminating between BPH and CaP comprises using a weighted sum Nelder-Mead optimization procedure to optimize for a partial area under a curve generated by a ROC analysis (pAUC), wherein the pAUC represents the area under the ROC within a restricted (1- sensitivity) range that is between value 0 and a specified value e (e.g. 0.5).

30 Example 17. The method example 12 or example 16 wherein said selecting the threshold value from the combined analyte levels in a manner that increases sensitivity in discriminating between BPH and CaP maximizes said sensitivity.

Example 18. The method example 12 or example 16 wherein said selecting the threshold value from the combined analyte levels in a manner that increases specificity in discriminating between BPH and CaP maximizes said specificity.

Example 19. The method according to example 12 or example 18, wherein selecting the threshold value from the combined analyte levels in a manner that increases specificity in discriminating between BPH and CaP comprises using a weighted sum Nelder-Mead optimization procedure to optimize for a partial area under a curve generated by a ROC analysis (pAUC), wherein the pAUC represents the area under the ROC within a restricted (1- specificity) range that is between values 0 and a specified value e (e.g. 0.5).

Example 20. The method according to any one of examples 12, 16, 17, 18 or 19, wherein the at least two analytes are selected from any one of the following analyte combinations:

GPC-1/EGF/follistatin; and

GPC-1/EGF/TNF α .

Example 21. The method according to any one of examples 12, 16, 17, 18 or 19, wherein the at least two analytes are selected from any one of the following analyte combinations:

GPC-1/HGF;

GPC-1/HGF/PAI-1;

GPC-1/HGF/HuFGFb;

GPC-1/HGF/EGF;

GPC-1/HGF/HuPDFGBB;

GPC-1/EGF;

GPC-1/PAI-1;

GPC-1/G-CSF; and

GPC-1/PDGFAB.BB.

Example 22. The method according to any one of examples 1 to 19, wherein the at least two analytes are selected from any one of the following analyte combinations:

GPC-1/HGF;

GPC-1/PAI-1;

GPC-1/G-CSF;

GPC-1/EGF;

GPC-1/PDGFAB.BB;
GPC-1/HGF/FGFb;
GPC-1/HGF/PAI-1;
GPC-1/HGF/EGF;
5 GPC-1/HGF/Follistatin; and
GPC-1/HGF/G-CSF.

Example 23. The method according to any one of examples 1 to 22, wherein the subject has previously received a positive indication of prostate disease.

10 Example 24. The method according to any one of examples 1 to 23, wherein the subject has previously received a positive indication of prostate disease by digital rectal exam (DRE) and/or by PSA testing.

Example 25. The method according to any one of examples 1 to 24, wherein said detecting at least two analytes in the subject's biological sample comprises:

- 15 (i) measuring one or more fluorescent signals indicative of each said analyte level;
- (ii) obtaining a measurement of weight/volume of said analytes in the samples;
- (iii) measuring an absorbance signal indicative of each said analyte level; or
- (iv) using a technique selected from the group consisting of: mass spectrometry, a protein array technique, high performance liquid chromatography (HPLC), gel
- 20 electrophoresis, radiolabeling, and any combination thereof.

Example 26. The method according to any one of examples 1 to 25, wherein said obtaining an analyte level in the biological samples of the series and the subject's biological sample comprises contacting each said sample with first and second antibody populations, wherein each said antibody population has binding specificity for one of said analytes, and

25 the first and second antibody populations have different binding specificities.

Example 27. The method according to example 26, wherein the first and/or second antibody populations are labelled.

Example 28. The method according to example 27, wherein said label is selected from the group consisting of a radiolabel, a fluorescent label, a biotin-avidin amplification

30 system, a chemiluminescence system, microspheres, and colloidal gold.

Example 29. The method according to any one of examples 26 to 28, wherein binding of each said antibody population to the analyte is detected by a technique selected from the group consisting of: immunofluorescence, radiolabeling, immunoblotting,

Western blotting, enzyme-linked immunosorbent assay (ELISA), flow cytometry, immunoprecipitation, immunohistochemistry, biofilm test, affinity ring test, antibody array optical density test, and chemiluminescence.

Example 30. The method according to any one of examples 1 to 29, wherein the biological samples of the series and the subject's biological sample are each whole blood, serum, plasma, saliva, tear/s, urine, or tissue.

Example 31. The method according to any one of examples 1 to 30, wherein said subject, said population of subjects having BPH, and said population of subjects having CaP, are human.

Example 32. The method of any one of examples 1 to 31, wherein said detecting of the at least two analytes in the biological samples of the series and/or the subject's biological sample comprises detecting the analytes directly.

Example 33. The method of any one of examples 1 to 32, wherein said detecting of the at least two analytes in the biological samples of the series and/or the subject's biological sample comprises detecting a nucleic acid encoding the analytes.

Example 34. The method of any one of examples 1 to 33, wherein said detecting of the at least two analytes in the biological sample from the subject is performed *in vitro* or *ex vivo*.

Definitions

As used in this application, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the phrase "an antibody" also includes multiple antibodies.

As used herein, the term "comprising" means "including". Variations of the word "comprising", such as "comprise" and "comprises," have correspondingly varied meanings. Thus, for example, a biomarker combination "comprising" analyte A and analyte B may consist exclusively of analyte A and analyte B or may include one or more additional components (e.g. analyte C).

As used herein, the term "prostate disease" refers to a disease of the prostate including, but not limited to, benign prostatic hyperplasia (BPH), prostatic intraepithelial neoplasia, prostatitis, prostate cancer, prostatic adenocarcinoma, prostatic leiomyosarcoma and prostatic rhabdomyosarcoma.

As used herein, the terms "biological sample" and "sample" encompass any body fluid or tissue taken from a subject including, but not limited to, a saliva sample, a tear

CLAIMS

1. A method for determining whether a subject has **benign prostatic hyperplasia** (BPH) from prostate cancer (CaP), comprising:

- (a) generating a threshold value for discerning between BPH and prostate cancer by:
 - detecting at least two analytes in a series of biological samples obtained from a population of subjects having BPH and a population of subjects having CaP, to thereby obtain an analyte level for each said analyte in each said biological sample of the series;
 - combining the analyte levels in a manner that allows discrimination between the BPH and CaP samples; and
 - selecting a threshold value from the combined analyte levels and using said threshold value as a basis to discriminate between BPH and CaP in the sample; and
- (b) detecting said at least two analytes in a biological sample from a subject to thereby obtain an analyte level for each said analyte in the subject's biological sample; and
- (c) applying a suitable algorithm and/or transformation to the individual or combined analyte levels obtained from the subject's biological sample to thereby generate a patient analyte value for comparison to the threshold value; and
- (d) determining whether the subject has BPH or CaP by comparison of the patient analyte value to the threshold value,
 - wherein the at least two analytes comprise glypican-1 (GPC-1) and are selected from any one of the following analyte combinations:
 - GPC-1 and hepatocyte growth factor (HGF)
 - GPC-1 and epidermal growth factor (EGF);
 - GPC-1 and plasminogen activator inhibitor 1 (PAI-1);
 - GPC-1 and granulocyte colony stimulating factor (G-CSF);
 - GPC-1 and human Interleukin 18 (HuIL-18);
 - GPC-1 and platelet derived growth factor AB.BB (PDGFAB.BB);
 - GPC-1 and platelet derived growth factor BB (PDGFBB);
 - GPC-1 and soluble CD40 Ligand (sCD40L);

GPC-1 and human granulocyte macrophage colony stimulating factor (HuGM-CSF);
GPC-1 and interferon gamma (IFN γ); and
GPC-1 and follistatin.

2. The method according to claim 1, wherein the at least two analytes comprise glypican-1 (GPC-1) and are selected from any one of the following analyte combinations:

GPC-1, HGF and PAI-1;
GPC-1, HGF and human fibroblast growth factor beta (HuFGFb);
GPC-1, HGF and EGF;
GPC-1, HGF and PDGFBB;
GPC-1, HGF and follistatin;
GPC-1, HGF and G-CSF;
GPC-1, HGF and human interleukin 8 (HuIL-8);
GPC-1, HGF and soluble CD40 ligand (sCD40L);
GPC-1, HGF and human cutaneous T-cell attracting cytokine (CTACK – also known as C-C motif ligand 27 or CCL27);
GPC-1, HGF and PDGFAB.BB;
GPC-1, HGF and IFN γ ;
GPC-1, HGF and human monocyte chemotactic and activating factor (HuMCP1/MCAF);
GPC-1, HGF, and HuIL-18;
GPC-1, HGF, HuGM-CSF; GPC-1, HGF, and urokinase plasminogen activator (uPA);
GPC-1, HGF, and human melanoma growth stimulating activity alpha (HuGROa, also known as CXC motif ligand 1, CXCL1);
GPC-1, HGF and soluble vascular endothelial growth factor receptor (sVEGFR);
GPC-1, EGF and follistatin;
GPC-1, EGF and tumour necrosis factor alpha (TNF α); and
GPC-1, G-CSF and soluble tyrosine kinase with immunoglobulin like and EGF-like domains 2 (sTIE2).

3. The method of claim 1 or claim 2, wherein said selecting the threshold value from the combined analyte levels comprises selecting a subset of the combined analyte levels.

4. The method according to any one of claims 1 to 3, wherein said combining of the analyte levels in a manner that allows discrimination between the BPH and CaP samples comprises combining a weighted linear sum of these analyte levels in a manner that maximizes the discrimination between the BPH and CaP samples in accordance with the formula:

$$S = w_1A_1 + w_2A_2 \dots w_nA_n$$

wherein S is the weighted sum,

w is the final analyte weighting,

and A is the level of a given analyte from n different analytes, or a transformation of the level of a given analyte either numerically or as a ratio of analyte values.

5. The method of claim 4, wherein said transformation of the level of a given analyte numerically comprises use of an exponential function, a power function and/or a root function.

6. The method according to claim 4 or claim 5, wherein obtaining the final analyte weightings w comprises generating initial analyte weightings (W) that are set to 1, or generated according to the formula:

$$W = \max(M)/M, \text{ where } W = \{w_1, \dots, w_n\} \text{ and } M = \{\text{median}(A_1), \dots, \text{median}(A_n)\};$$

wherein max (M) is a maximum median level of the analyte levels obtained, and M is a vector containing the median analyte level obtained for each said analyte.

7. The method according to claim 6, wherein each said final weight is determined using a discriminating function for optimization in discerning between BPH and CaP in the series of biological samples.

8. The method according to claim 7, wherein the discriminating function is any one or more of:

- (i) the area under a curve generated by a ROC analysis;
- (ii) a combination of True Positive Rates (TPR) and True Negative Rates (TNR);
- (iii) the area under a curve generated by a ROC analysis within a restricted specificity range;
- (iv) the area under a curve generated by a ROC analysis within a restricted sensitivity range.

9. The method according to claim 7 or claim 8, wherein the discriminating function for optimization is any one or more of the Nelder-Mead (simplex method), a stochastic method, a gradient descent method, a stochastic gradient descent method, a genetic algorithm, a particle-swarm method, and/or a brute force method.

10. The method according to any one of claims 1 to 9, wherein the suitable algorithm and/or transformation applied to the individual or combined analyte levels obtained from the subject's biological sample is in accordance with the formula:

$$S = w_1A_1 + w_2A_2 \dots w_nA_n$$

wherein S is the weighted sum,

w is the final analyte weighting,

and A is the level of a given analyte from n different analytes, or a transformation of the level of a given analyte either numerically or as a ratio of analyte values.

11. The method according to any one of claims 1 to 10, wherein said combining the analyte levels maximizes discrimination between the BPH and CaP samples.

12. The method of any one of claims 1 to 11, comprising selecting the threshold value from the combined analyte levels in a manner that:

- (i) reduces the misclassification rate between BPH and CaP; and/or
- (ii) increases sensitivity in discriminating between BPH and CaP; and/or
- (iii) increases specificity in discriminating between BPH and CaP.

13. The method of claim 12, wherein said selecting the threshold value from the combined analyte levels in a manner that reduces the misclassification rate between BPH and CaP comprises selecting a suitable true positive and/or true negative rate.

14. The method claim 12 or claim 13, wherein said selecting the threshold value from the combined analyte levels in a manner that reduces the misclassification rate between BPH and CaP minimizes the misclassification rate.

15. The method according to any one of claims 12 to 14, wherein selecting the threshold value from the combined analyte levels in a manner that reduces the misclassification rate

between BPH and CaP comprises minimizing the misclassification rate between BPH and CaP by identifying a point where the true positive rate intersects the true negative rate.

16. The method according to claim 12, wherein selecting the threshold value from the combined analyte levels in a manner that increases sensitivity in discriminating between BPH and CaP comprises using a weighted sum Nelder-Mead optimization procedure to optimize for a partial area under a curve generated by a ROC analysis (pAUC), wherein the pAUC represents the area under the ROC within a restricted (1- sensitivity) range that is between value 0 and a specified value e (e.g. 0.5).

17. The method claim 12 or claim 16 wherein said selecting the threshold value from the combined analyte levels in a manner that increases sensitivity in discriminating between BPH and CaP maximizes said sensitivity.

18. The method claim 12 or claim 16 wherein said selecting the threshold value from the combined analyte levels in a manner that increases specificity in discriminating between BPH and CaP maximizes said specificity.

19. The method according to claim 12 or claim 18, wherein selecting the threshold value from the combined analyte levels in a manner that increases specificity in discriminating between BPH and CaP comprises using a weighted sum Nelder-Mead optimization procedure to optimize for a partial area under a curve generated by a ROC analysis (pAUC), wherein the pAUC represents the area under the ROC within a restricted (1- specificity) range that is between values 0 and a specified value e (e.g. 0.5).

20. The method according to any one of claims 12, 16, 17, 18 or 19, wherein the at least two analytes comprise glypican-1 (GPC-1) and are selected from any one of the following analyte combinations:

GPC-1/EGF/follistatin; and
GPC-1/EGF/TNF α .

21. The method according to any one of claims 12, 16, 17, 18 or 19, wherein the at least two analytes comprise glypican-1 (GPC-1) and are selected from any one of the following analyte combinations:

GPC-1/HGF;
GPC-1/HGF/PAI-1;
GPC-1/HGF/HuFGFb;
GPC-1/HGF/EGF;
GPC-1/HGF/HuPDFGBB;
GPC-1/EGF;
GPC-1/PAI-1;
GPC-1/G-CSF; and
GPC-1/PDGFAB.BB.

22. The method according to any one of claims 1 to 19, wherein the at least two analytes comprise glypican-1 (GPC-1) and are selected from any one of the following analyte combinations:

GPC-1/HGF;
GPC-1/PAI-1;
GPC-1/G-CSF;
GPC-1/EGF;
GPC-1/PDGFAB.BB;
GPC-1/HGF/FGFb;
GPC-1/HGF/PAI-1;
GPC-1/HGF/EGF;
GPC-1/HGF/Follistatin; and
GPC-1/HGF/G-CSF.

23. The method according to any one of claims 1 to 22, wherein the subject has previously received a positive indication of prostate disease.

24. The method according to any one of claims 1 to 23, wherein the subject has previously received a positive indication of prostate disease by digital rectal exam (DRE) and/or by PSA testing.

25. The method according to any one of claims 1 to 24, wherein said detecting at least two analytes in the subject's biological sample comprises:

- (i) measuring one or more fluorescent signals indicative of each said analyte level;
- (ii) obtaining a measurement of weight/volume of said analytes in the samples;
- (iii) measuring an absorbance signal indicative of each said analyte level; or
- (iv) using a technique selected from the group consisting of: mass spectrometry, a protein array technique, high performance liquid chromatography (HPLC), gel electrophoresis, radiolabeling, and any combination thereof.

26. The method according to any one of claims 1 to 25, wherein said obtaining an analyte level in the biological samples of the series and the subject's biological sample comprises contacting each said sample with first and second antibody populations, wherein each said antibody population has binding specificity for one of said analytes, and the first and second antibody populations have different binding specificities.

27. The method according to claim 26, wherein the first and/or second antibody populations are labelled.

28. The method according to claim 27, wherein said label is selected from the group consisting of a radiolabel, a fluorescent label, a biotin-avidin amplification system, a chemiluminescence system, microspheres, and colloidal gold.

29. The method according to any one of claims 26 to 28, wherein binding of each said antibody population to the analyte is detected by a technique selected from the group consisting of: immunofluorescence, radiolabeling, immunoblotting, Western blotting, enzyme-linked immunosorbent assay (ELISA), flow cytometry, immunoprecipitation, immunohistochemistry, biofilm test, affinity ring test, antibody array optical density test, and chemiluminescence.

30. The method according to any one of claims 1 to 29, wherein the biological samples of the series and the subject's biological sample are each whole blood, serum, plasma, saliva, tear/s, urine, or tissue.

31. The method according to any one of claims 1 to 30, wherein said subject, said population of subjects having BPH, and said population of subjects having CaP, are human.

32. The method of any one of claims 1 to 31, wherein said detecting of the at least two analytes in the biological samples of the series and/or the subject's biological sample comprises detecting the analytes directly.

33. The method of any one of claims 1 to 32, wherein said detecting of the at least two analytes in the biological samples of the series and/or the subject's biological sample comprises detecting a nucleic acid encoding the analytes.