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(54) Title: KIDNEY DISEASE BIOMARKER

(57) Abstract: The present invention provides methods and solid state devices for detecting and staging chronic kidney disease in a patient, where the levels of biomarkers in a sample obtained from a patient are elevated or reduced compared to the levels in a sample obtained from healthy subject. The invention also relates to the use of methods and solid state devices for measurement of specific biological markers for determining the efficacy of a treatment for chronic kidney disease and for determining a drug treatment protocol for a subject suffering from chronic kidney disease.

## KIDNEY DISEASE BIOMARKER

### Field of the Invention

The present invention relates to kidney disease and methods for its diagnosis.

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### Background of the Invention

Kidney disease is a general term, which describes a class of conditions in which the kidneys fail to filter and remove waste products from the blood. There are two forms of kidney disease; acute kidney injury (AKI) and chronic kidney 10 disease (CKD). CKD is usually asymptomatic, except in its most advanced state. Consequently, blood and/or urine tests generally are required to make a diagnosis.

15 The definition of CKD developed by the Kidney Disease Outcomes Quality Initiative (KDOQI) was:

1. Kidney damage present for at least 3 months, as defined by structural or functional abnormalities (most often based on increased albuminuria e.g. urinary albumin / creatinine ratio [UACR]  $\geq 30$  mg/g) and/or
2. Glomerular filtration rate (GFR)  $< 60$  mL/min/1.73 m<sup>2</sup> present for at least 3 months.

Within this framework, KDOQI then classified CKD into five stages, as follows:

- 25 • Stage 1: Kidney damage with GFR  $\geq 90$  mL/min/1.73 m<sup>2</sup>.
- Stage 2: Kidney damage with GFR 60-89 mL/min/1.73 m<sup>2</sup>.
- Stage 3: GFR 30-59 mL/min/1.73 m<sup>2</sup>.
- Stage 4: GFR 15-29 mL/min/1.73 m<sup>2</sup>.
- Stage 5: GFR  $< 15$  mL/min/1.73 m<sup>2</sup> or kidney failure treated by dialysis or

30 transplantation.

In the United States, based on data from the 1999-2006 National Health and Nutrition Examination Survey (NHANES) study, an estimated 11.1 percent (22.4

million) of adults aged 20 or older have CKD stages 1–3. An additional 0.8 million U.S. adults aged 20 or older have CKD stage 4, and more than 0.3 million have stage 5 CKD and receive hemodialysis.

- 5 Analyses of NHANES data between 1988-1994 and 1999-2004 suggest that the prevalence of CKD is rising for every CKD stage, but with a particular increase in the prevalence of individuals classified with CKD stage 3. The number of patients with stage 5 CKD requiring dialysis also has increased. It has been estimated that more than 700,000 individuals will have End Stage Renal
- 10 Disease (ESRD) by 2015.

Although CKD can be caused by primary kidney disease (e.g. glomerular diseases, tubulointerstitial diseases, obstruction, and polycystic kidney disease), in the vast majority of patients with CKD, the kidney damage is associated with

- 15 other medical conditions such as diabetes and hypertension. In 2008, excluding those with ESRD, 48 percent of Medicare patients with CKD had diabetes, 91 percent had hypertension, and 46 percent had atherosclerotic heart disease. Other risk factors for CKD include age, obesity, family history, and ethnicity.

- 20 CKD has been associated with numerous adverse health outcomes. Many studies have reported that a GFR of 30-59 mL/min/1.73 m<sup>2</sup> is associated with an increased risk of mortality, cardiovascular disease, fractures, bone loss, infections, cognitive impairment, and frailty. Similarly, there appears to be a graded relationship between the severity of proteinuria or albuminuria and
- 25 adverse health outcomes, including mortality, ESRD, and cardiovascular disease. Further, the risk for adverse outcomes conferred by reduced GFR and increased albuminuria (or proteinuria) appears to be independent and multiplicative.
- 30 The rationale for considering screening for early-stage CKD includes the high and rising prevalence of CKD, its known risk factors, its numerous adverse health consequences, its long asymptomatic phase, the availability of potential screening tests for CKD, and the availability of treatments that may alter the

course of early-stage CKD and reduce complications of early-stage CKD or its associated health conditions.

Some organizations already recommend CKD screening in selected 5 populations. Kidney Disease: Improving Global Outcomes (KDIGO) recommends screening of all patients with hypertension, diabetes, or cardiovascular disease. The American Diabetes Association recommends annual screening of all adults with diabetes, based on “expert consensus or clinical experience.” The Joint National Committee on Prevention, Detection, 10 Evaluation, and Treatment of High Blood Pressure (JNC7) recommends annual screening of all patients with combined hypertension and diabetes. Also advocating selected screening, the National Kidney Foundation sponsors free CKD screening for all adults with hypertension, diabetes, or a primary relative with a history of kidney disease, hypertension, or diabetes.

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In most patients with CKD stages 1 to 3 GFR declines slowly. However, the rate of decline varies among individuals, and many factors appear to impact progression. Because CKD stages 1 and 2 usually progress asymptotically, detection of early-stage CKD requires laboratory testing.

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Some organizations recommend monitoring for changes in kidney function or damage in patients with CKD. For example, the Kidney Disease Outcomes Quality Initiative (KDOQI) recommends at least annual estimated GFR measurement in adults with CKD in order to predict onset of ESRD and evaluate 25 the effect of CKD treatments. JNC7 recommends annual quantitative measurement of albuminuria in all patients with “kidney disease.” KDOQI also recommends more frequent monitoring of CKD patients with worsening kidney function.

30 Despite the importance of measuring clinical parameters for CKD in serum or urine, there are few diagnostic tests to detect early stage CKD and monitor the progression of this disease. Measurement of GFR is not sufficiently sensitive for early detection of kidney disease, while the measurement of urinary protein is not specific for kidney disease, nor is it suitable for monitoring the progression of

the disease. Therefore, there is a requirement for a specific and sensitive clinical marker or combination of markers for the diagnosis of early CKD and staging of kidney disease.

5 **Summary of the Invention**

The present invention provides methods for the diagnosis of kidney disease. More specifically the invention provides biomarkers which provide a means for an early diagnosis and staging of CKD.

10 The present invention is based on the surprising finding by the inventors that altered levels of individual biomarkers can be detected in serum from patients with stage 1, stage 2 and stage 3 CKD compared with serum from control individuals. Biomarkers which can be detected at altered levels in serum from patients with stage 1, stage 2 and stage 3 CKD compared with serum from control individuals include complement C3a(des)Arg (C3a desArg), interleukin 8 (IL-8), macrophage inflammatory protein 1 alpha (MIP 1 $\alpha$ ), adepnectin (ADPN), dipeptidyl peptidase 4 (CD26), Creatinine, c-reactive protein (CRP), cystatin c (CYSC), D-dimer, endothelial growth factor (EGF), e selectin (ESEL), fatty acid-binding protein 1 (liver fatty acid-binding protein; FABP1 or LFABP), granulocyte 15 and macrophage colony stimulating factor (GMCSF), intercellular adhesion molecule 1 (ICAM1), interferon gamma (IFN $\gamma$ ), interleukin 10 (IL10), interleukin 20 15 (IL15), interleukin 1 alpha (IL1 $\alpha$ ), interleukin 1 beta (IL1 $\beta$ ), interleukin 2 (IL2), interleukin 4 (IL4), interleukin 5 (IL5), interleukin 6 (IL6), l selectin (LSEL), monocyte chemotactic protein 1 (MCP1), matrix metalloproteinase 9 (MMP9), 25 neutrophil gelatinase-associated lipocalin (NGAL), neuron specific enolase (NSE), p selectin (PSEL), soluble interleukin 2 alpha (sIL2 $\alpha$ ), soluble interleukin 6 receptor (sIL6R), soluble tumour necrosis factor receptor 1 (STNFR1), soluble tumour necrosis factor receptor 2 (STNFR2), tumour necrosis factor alpha (TNF $\alpha$ ), vascular endothelial growth factor (VEGF) and vascular cell adhesion 30 molecule 1 (VCAM1).

In a first aspect, the invention provides a method for detecting kidney disease in a subject, comprising measuring the amount of a biomarker in a sample obtained from

the subject, and determining whether the amount of the biomarker is altered compared to a normal control, wherein the biomarker is selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, 5 IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.

In a second aspect, the invention provides a solid state device comprising a substrate comprising an antibody that binds to a biomarker selected from the group 10 consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.

15 In a third aspect, the invention provides a method of determining the efficacy of a treatment for chronic kidney disease, comprising measuring the amount of a biomarker in a sample obtained from a subject receiving such treatment, wherein the biomarker is selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, 20 GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1, and comparing the level of the biomarker to that of a sample from an untreated control to determine whether the treatment has had the effect of altering the biomarker level.

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In a fourth aspect, the invention provides a method of determining the efficacy of a treatment for chronic kidney disease, comprising measuring the amount of a biomarker in a sample obtained from a subject receiving such treatment, wherein the biomarker is selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , 30 ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1, and comparing the level of the biomarker to that of a sample obtained from the subject

before treatment, wherein a reduction or maintenance in the stage of chronic kidney disease or a slowing of progression through stages of chronic kidney disease following treatment indicates that the treatment has been successful.

- 5 In a fifth aspect, the invention provides a method of determining a drug treatment protocol for a subject suffering from chronic kidney disease, comprising, in a sample taken from a subject treated with the drug, comparing the level of a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1 to the level in sample obtained from an untreated control to determine whether the drug has had the effect of altering the biomarker level, and selecting a drug treatment protocol based on whether the subject has stage 1, stage 2 or stage 3 chronic kidney disease.

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- In a sixth aspect, the invention provides the use of a kit for screening for kidney disease in a method according to any of the proceeding aspects of the invention, the kit comprising a probe that binds specifically to a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1, or reagents for an immunoassay or 1D or 2D gel electrophoresis for detecting the level of a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, FABP1, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.

30 **Description of the Drawings**

The invention is described with reference to the following Figures in which:

Figure 1 shows boxplots that summarise the levels of C3a desArg measured in serum samples taken from Healthy controls, patients suffering from Stage 1 or Stage 2 CKD and patients suffering from Stage 3 CKD;

5 Figure 2 shows boxplots that summarise the levels of MIP 1 $\alpha$ , measured in serum samples taken from Healthy controls, patients suffering from Stage 1 or Stage 2 CKD and patients suffering from Stage 3 CKD;

10 Figure 3 shows boxplots that summarise the levels of STNFR1 measured in serum samples taken from Healthy controls, patients suffering from Stage 1 or Stage 2 CKD and patients suffering from Stage 3 CKD;

15 Figure 4 shows boxplots that summarise the levels of STNFR2, measured in serum samples taken from Healthy controls, patients suffering from Stage 1 or Stage 2 CKD and patients suffering from Stage 3 CKD;

Figure 5 shows boxplots that summarise the levels of IL-8 measured in serum samples taken from Healthy controls, patients suffering from Stage 1 or Stage 2 CKD and patients suffering from Stage 3 CKD;

20 Figure 6 shows boxplots that summarise the levels of EGF measured in serum samples taken from Healthy controls, patients suffering from Stage 1 or Stage 2 CKD and patients suffering from Stage 3 CKD;

25 Figure 7 shows boxplots that summarise the levels of Creatinine measured in serum samples taken from Healthy controls, patients suffering from Stage 1 or Stage 2 CKD and patients suffering from Stage 3 CKD;

30 Figure 8 shows the aggregated AUC values achieved for discriminating control subjects from stage 1, stage 2 and stage 3 chronic kidney disease patients for all combinations that include each biomarker;

Figure 9 shows the aggregated AUC values achieved for discriminating stage 1 or stage 2 chronic kidney disease patients from control subjects for all combinations that include each biomarker;

- 5 Figure 10 shows the aggregated AUC values achieved for discriminating stage 3 chronic kidney disease patients from control subjects for all combinations that include each biomarker; and

- 10 Figure 11 shows the aggregated AUC values achieved for overall discrimination between all three groups of patients (Control, Stage 1/2, Stage 3) for all combinations that include each biomarker.

#### **Detailed Description of the Invention**

The present invention provides a method for the detection of kidney disease by 15 determining the level of a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1 in a sample obtained from the patient and comparing 20 the level with a control. An altered level of the biomarker in the sample compared to the control indicates that the patient suffers from, or is at risk of developing, kidney disease.

The term "kidney disease" in the context of the present invention is understood 25 to mean conditions or diseases characterised by a decrease in renal function compared to healthy patients. Kidney disease may include chronic kidney disease (CKD), acute kidney injury (AKI), diabetic nephropathy, glomerulonephritis, focal glomerulosclerosis, immune complex nephropathy or lupus nephritis. Kidney disease may be caused by drug-induced renal injury or 30 kidney graft rejection. Kidney disease may be characterised as nephrotic syndrome or renal insufficiency.

The “amount” of a biomarker refers to the quantity, expression level or concentration of the biomarker within the sample. The amount of a biomarker may also refer to the biomarker measurement expressed as a ratio or percentage of the amount of one or more other analytes. The amount of one or 5 more such other analytes may remain consistent in the majority of samples or conditions. By way of example, the other analytes could be albumin,  $\beta$ -actin or total matrix protein.

10 The amount of a biomarker may also refer to the biomarker measurement expressed as a ratio or percentage of the amount of one or more other analytes, where the amount of the one or more other analytes is proposed to hold some biochemical significance to the clinical condition of interest.

15 The term “probe” refers to a molecule that is capable of specifically binding to a target molecule such that the target molecule can be detected as a consequence of said specific binding. Probes that can be used in the present invention include, for example, antibodies, aptamers and oligonucleotides.

20 The term “antibody” refers to an immunoglobulin which specifically recognises an epitope on a target as determined by the binding characteristics of the immunoglobulin variable domains of the heavy and light chains ( $V_{H}S$  and  $V_{L}S$ ), more specifically the complementarity-determining regions (CDRs). Many potential antibody forms are known in the art, which may include, but are not limited to, a plurality of intact monoclonal antibodies or polyclonal mixtures 25 comprising intact monoclonal antibodies, antibody fragments (for example  $F_{ab}$ ,  $F_{ab}'$ , and  $F_v$  fragments, linear antibodies single chain antibodies and multi-specific antibodies comprising antibody fragments), single-chain variable fragments (scF<sub>v</sub>S), multi-specific antibodies, chimeric antibodies, humanised antibodies and fusion proteins comprising the domains necessary for the 30 recognition of a given epitope on a target. Preferably, references to antibodies in the context of the present invention refer to monoclonal antibodies. Antibodies may also be conjugated to various detectable labels to enable detection, including but not limited to radionuclides, fluorophores, dyes or enzymes including, for example, horse-radish peroxidase and alkaline phosphatase.

The term “aptamer” refers to an oligonucleotide molecule or a polypeptide molecule that specifically binds to a target molecule. Oligonucleotide aptamers may be ribonucleotides (RNA) or deoxyribonucleotides (DNA) and typically 5 consist of short strands of oligonucleotides. Polypeptide aptamers typically consist of short peptide domains that may be attached at one end or at both ends to a protein scaffold.

The term “binds specifically”, in the context of antibody-epitope interactions, 10 refers to an interaction wherein the antibody and epitope associate more frequently or rapidly, or with greater duration or affinity, or with any combination of the above, than when either antibody or epitope is substituted for an alternative substance, for example an unrelated protein. Generally, but not necessarily, reference to binding means specific recognition. Furthermore, it is 15 appreciated that an antibody may recognise more than one antigen specifically. Techniques known in the art for determining the specific binding of a target by a monoclonal antibody or lack thereof include but are not limited to, FACS analysis, immunocytochemical staining, immunohistochemistry, western blotting/dot blotting, ELISA, affinity chromatography. By way of example and not 20 limitation, specific binding, or lack thereof, may be determined by comparative analysis with a control comprising the use of an antibody which is known in the art to specifically recognise said target and/or a control comprising the absence of, or minimal, specific recognition of said target (for example wherein the control comprises the use of a non-specific antibody). Said comparative 25 analysis may be either qualitative or quantitative. It is understood, however, that an antibody or binding moiety which demonstrates exclusive specific recognition of a given target is said to have higher specificity for said target when compared with an antibody which, for example, specifically recognises both the target and a homologous protein.

30

In the context of the present invention, a “control value” is understood to be the level of a particular biomarker, such as C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ ,

IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 typically found in healthy individuals. The control level of a biomarker may be determined by analysis of a sample isolated from a healthy individual or may be the level of the

5 biomarker understood by the skilled person to be typical for a healthy individual. The “control value” may be a range of values considered by the skilled person to be a normal level for the biomarker in a healthy individual. The skilled person would appreciate that control values for a biomarker may be calculated by the user analysing the level of the biomarker in a sample from a healthy individual or

10 by reference to typical values provided by the manufacturer of the assay used to determine the level of the biomarker in the sample.

Preferably the sample isolated from the patient is a serum sample, but may also be blood, plasma, urine or saliva (particularly with respect to salivary creatinine).

15 The determination of the level of a biomarker may be done on one or more samples from the patient. The sample may be obtained from the patient by methods routinely used in the art.

The present inventors have found that detection of altered levels of a biomarker

20 selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1 in samples can be used to identify patients suffering from CKD or to stage the progression

25 of the disease. Thus, the present invention may be used to diagnose early stage CKD. In the context of the present invention, “early stage” is understood to mean any of the first or second stages of CKD as defined by the KDOQI classification.

30 In a first aspect, the present invention provides a method of identifying patients suffering from CKD or staging the progression of CKD in a patient comprising measuring the amount of a biomarker in a sample obtained from the subject, and determining whether the amount of the biomarker is altered compared to a normal

control, wherein the biomarker is selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , 5 VEGF and VCAM1. The control range of values of a biomarker may vary dependent on demographic of population and the sample being tested. For example, the control range of values from a healthy individual may vary from a blood sample compared to a urine sample. The upper and lower threshold for a given sample and patient demographic may be determined by the skilled person 10 by analyzing samples from a patient cohort to find average values. The levels of each biomarker measured in control patients and in patients suffering from CKD are presented in Table 1 below.

In a further aspect, the present invention provides a method of identifying 15 patients at various stages of CKD or staging the progression of CKD comprising measuring the amount of two or more biomarkers in one or more samples obtained from the subject, and determining whether the amount of the biomarkers are altered compared to a normal control, wherein the two or more biomarkers are selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, 20 IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.

Accuracy of a diagnostic method is best described by its receiver-operating 25 characteristics (ROC) (Zweig, M. H., and Campbell, G., Clin. Chem. 39 (1993) 561-577). The ROC graph is a plot of all of the sensitivity/specificity pairs resulting from continuously varying the decision threshold over the entire range of data observed. A ROC plot depicts the overlap between the two distributions by plotting the sensitivity versus 1 - specificity for the complete range of decision 30 thresholds. On the y-axis is sensitivity, or the true-positive fraction defined as [(number of true-positive test results)/ (number of true-positive + number of false-negative test results)]. This has also been referred to as positivity in the presence of a disease or condition. It is calculated solely from the affected

subgroup. On the x-axis is the false-positive fraction, or 1 - specificity [defined as (number of false-positive results)/(number of true-negative + number of false-positive results)]. It is an index of specificity and is calculated entirely from the unaffected subgroup. Because the true- and false-positive fractions are 5 calculated entirely separately, by using the test results from two different subgroups, the ROC plot is independent of the prevalence of disease in the sample. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions of results) has an ROC plot 10 that passes through the upper left corner, where the true-positive fraction is 1.0 or 100% (perfect sensitivity), and the false-positive fraction is 0 (perfect specificity). The theoretical plot for a test with no discrimination (identical distributions of results for the two groups) is a 45° diagonal line from the lower left corner to the upper right corner. Most plots fall in between these two 15 extremes. Qualitatively, the closer the plot is to the upper left corner, the higher the overall accuracy of the test.

One convenient goal to quantify the diagnostic accuracy of a laboratory test is to express its performance by a single number. The most common global measure 20 is the area under the curve (AUC) of the ROC plot. The area under the ROC curve is a measure of the probability that the perceived measurement will allow correct identification of a condition. By convention, this area is always  $\geq 0.5$ . Values range between 1.0 (perfect separation of the test values of the two 25 groups) and 0.5 (no apparent distributional difference between the two groups of test values). The area does not depend only on a particular portion of the plot such as the point closest to the diagonal or the sensitivity at 90% specificity, but on the entire plot. This is a quantitative, descriptive expression of how close the ROC plot is to the perfect one (area = 1.0). In the context of the present invention, Table 2 presents AUC values for individual biomarkers where the 30 altered levels of the biomarkers are used to identify patients suffering from CKD or to stage the progression of CKD.

Where two or more biomarkers are used in the invention, a suitable mathematical or machine learning classification model, such as logistic

regression equation, can be derived. The skilled statistician will understand how such a suitable model is derived, which can include other variables such as age and gender of the patient. The ROC curve can be used to assess the accuracy of the model, and the model can be used independently or in an algorithm to aid 5 clinical decision making. Although a logistic regression equation is a common mathematical/statistical procedure used in such cases and an option in the context of the present invention, other mathematical/statistical, decision trees or machine learning procedures can also be used. The skilled person will appreciate that the model generated for a given population may need to be 10 adjusted for application to datasets obtained from different populations or patient cohorts.

The present inventors have further found that detection of altered levels of combinations of two or more biomarkers selected from the group consisting of 15 C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1 can be used to identify patients suffering from CKD or to stage the progression of the disease. In the context of the present invention, 20 Table 3 presents AUC values for combinations of two or more biomarkers where altered levels of the biomarkers are used to identify patients suffering from CKD or to stage the progression of CKD, and where the AUC values for the combination of biomarkers is greater than any single one of the individual biomarkers alone.

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In yet a further aspect, the present invention provides a method to support the decision to effect therapeutic intervention in a patient suspected of having renal disease comprising measuring the amount of a biomarker in a sample obtained from the subject, and determining whether the amount of the biomarker is 30 altered compared to a normal control, wherein the biomarker is selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ ,

sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1, and based on the amount of biomarker measured declining or effecting a therapeutic intervention.

The decision to effect a therapeutic intervention will be made by a physician, 5 and said intervention will be designed to combat renal disease. Such therapeutic intervention may include one or more of adult stem cell therapy, transplant therapy, dialysis, diet management, exercise or drug therapy. Drug therapy may be selected from the group consisting of: a diuretic, an angiotensin-converting enzyme inhibitor, angiotensin II receptor antagonist, a 10 beta-adrenergic antagonist, an alpha-adrenergic antagonist, a calcium channel antagonist, a statin, erythropoietin, vitamin D, vitamin C, vitamin B, folic acid, a hypouricaemic, a phosphate binder, a potassium binding resin, an immunosuppressant and a calcium supplement.

15 The biomarker measuring step may be replaced by measurement of an immune complex formed on addition to the patient sample of an antibody capable of specifically binding to the biomarker. Binding of the antibody to the biomarker forms an immune complex that can then be detected and measured using standard techniques known in the art.

20 Table 1 presents the levels of each biomarker measured in serum. For the avoidance of doubt, an increased expression level (when compared to a healthy non-diseased control) of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, ICAM1, IL10, IL1 $\beta$ , IL5, IL6, MCP1, MMP9, 25 NGAL, PSEL, sIL2 $\alpha$ , STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 indicates the presence of or risk of stage 1 or stage 2 chronic kidney disease. An increased expression level (when compared to a healthy non-diseased control or a stage 1 or stage 2 chronic kidney disease patient) of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, 30 IL10, IL15, IL1 $\beta$ , IL5, IL6, MCP1, MMP9, NGAL, PSEL, sIL2 $\alpha$ , STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 indicates the presence of or risk of stage 3 chronic kidney disease. A reduced expression level (when compared to a healthy non-diseased control) of Creatinine, IL4, LSEL or NSE indicates the presence of or

risk of stage 1 or stage 2 chronic kidney disease. A reduced expression level (when compared to a healthy non-diseased control or a stage 1 or stage 2 chronic kidney disease patient) of IL1 $\alpha$ , IL4 or NSE indicates the presence of or risk of stage 3 chronic kidney disease.

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Table 2 presents AUC values for individual biomarkers where altered levels of the biomarkers are used to identify patients suffering from CKD or to stage the progression of CKD. The data indicate AUC values for individual biomarker's use to discriminate between healthy control subjects and chronic kidney disease patients (Control v CKD), between healthy control subjects and stage 1 or stage 2 chronic kidney disease patients (Control v Stage 1/2), between healthy control subjects and stage 3 chronic kidney disease patients (Control v Stage 3) and between healthy control subjects, stage 1 or stage 2 chronic kidney disease patients and stage 3 chronic kidney disease patients (Overall).

Table 3 presents AUC values for combinations of two or more biomarkers where altered levels of the biomarkers are used to identify patients suffering from CKD or to stage the progression of CKD. The data indicate AUC values for combinations of biomarkers used to discriminate between healthy control subjects and chronic kidney disease patients (Control v CKD), between healthy control subjects and stage 1 or stage 2 chronic kidney disease patients (Control v Stage 1/2), between healthy control subjects and stage 3 chronic kidney disease patients (Control v Stage 3) and between healthy control subjects, stage 1 or stage 2 chronic kidney disease patients and stage 3 chronic kidney disease patients (Overall).

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**Table 1**  
**Summary of biomarker levels measured in serum**

Marker	Units	Control			Stage 1/2			Stage 3					
		N	Median	5-95 %	N	Median	5-95 %	N	Median	5-95 %	N	Median	5-95 %
C3a desArg	ng/ml	51	4458.6	1308.8 - 9540	89	16525.61	6376.3 - 33913.2	<0.0001	44	19279.24	4539.5 - 43390	<0.0001	1.0000
CD26	ng/ml	51	821.84	466.12 - 1349.6	89	996.31	495.7 - 2064.2	0.0517	44	895.60	323.05 - 1765.9	1.0000	0.5324
Creatinine	umol/l	64	87.47	64.38 - 115.26	89	82.66	59 - 108.29	0.1219	44	137.87	80.5 - 254.91	<0.0001	<0.0001
CRP	mg/L	163	2.75	0.51 - 13.69	294	4.61	0.59 - 15.5	<0.0001	104	4.88	0.67 - 14.01	<0.0001	0.9459
DDIMER	ng/ml	163	42.03	9.56 - 97.46	295	97.03	16.42 - 277.11	<0.0001	105	141.95	22.52 - 498.59	<0.0001	0.0021
NSE	ng/ml	163	4.94	1.89 - 10.65	295	2.95	0.8 - 6.27	<0.0001	105	2.94	0.56 - 9.43	<0.0001	0.2150
NGAL	ng/ml	163	650.34	295.13 - 1089.4	295	721.52	284.09 - 1373.14	0.4302	105	1093.78	396.41 - 1843.17	<0.0001	<0.0001
VCAM1	ng/ml	211	561.35	355.34 - 851.02	273	689.46	416.89 - 1050.88	<0.0001	105	825.37	456.68 - 1507.6	<0.0001	0.0002
ICAM1	ng/ml	211	264.35	171.23 - 381.46	273	316.55	189.78 - 497.49	<0.0001	105	341.22	175.73 - 645.56	<0.0001	0.3170
ESEL	ng/ml	211	17.02	7.61 - 31.26	273	21.72	8.14 - 46.83	<0.0001	105	19.73	8.05 - 37.33	0.0174	0.7830
PSEL	ng/ml	211	165.89	96.74 - 239.44	273	213.30	113.09 - 322.73	<0.0001	105	207.42	117.7 - 330.97	<0.0001	1.0000
LSEL	ng/ml	211	1696.5	1032.23 - 2677.06	273	1660.49	978.47 - 2562.48	0.6135	105	1695.86	1023.12 - 2810.2	0.0798	0.6309
IL5	pg/ml	50	1.48	0.48 - 4.54	98	1.84	0.47 - 4.85	1.0000	52	1.88	0.62 - 8.35	1.0000	1.0000
IL15	pg/ml	50	1.09	0.43 - 1.87	102	1.02	0.53 - 1.67	0.9433	52	1.30	0.52 - 3.91	1.0000	0.3000
GMC SF	pg/ml	50	1.01	0 - 1.89	102	1.01	0 - 2.87	0.2554	52	1.24	0 - 6.12	0.9645	1.0000
MIP1A	pg/ml	50	5.41	3.02 - 8.23	102	11.60	2.89 - 29.54	<0.0001	52	15.59	4.01 - 34.34	<0.0001	0.1575
sIL2a	ng/ml	163	0.17	0.08 - 0.26	295	0.20	0.08 - 0.38	0.0743	105	0.26	0.07 - 0.5	<0.0001	0.0002
sIL6R	ng/ml	163	1.78	0.68 - 3.15	295	1.72	0.54 - 3.28	0.8181	105	2.07	0.89 - 3.93	0.0854	0.0015
STNFR1	ng/ml	163	0.44	0.23 - 0.65	295	0.68	0.36 - 1.16	<0.0001	105	1.27	0.59 - 2.69	<0.0001	<0.0001
STNFR2	ng/ml	163	0.34	0.11 - 0.67	295	0.76	0.24 - 1.57	<0.0001	105	1.58	0.28 - 3.71	<0.0001	<0.0001

Marker	Units	Control			Stage 1/2			Stage 3			p vs Control	p vs Stage 1/2	
		N	Median	5-95 %	N	Median	5-95 %	N	Median	5-95 %			
MMP9	ng/ml	163	185.88	39.34 - 392.04	295	341.22	76.77 - 725.82	<0.0001	105	320.99	54.05 - 734.84	<0.0001	1.0000
IL2	pg/ml	164	3.03	0 - 6.3	295	3.00	0 - 9.05	1.0000	105	3.06	0 - 6.02	0.3807	1.0000
IL4	pg/ml	164	3.03	1.48 - 5.36	295	2.64	1.79 - 3.99	1.0000	105	2.53	1.66 - 3.95	0.3568	0.0210
IL6	pg/ml	164	2.30	0.64 - 5.21	295	8.08	0.87 - 11.08	<0.0001	105	8.93	1.3 - 21.38	<0.0001	<0.0001
IL8	pg/ml	164	10.63	4.4 - 20.83	295	111.42	9.04 - 510.1	<0.0001	105	108.26	11.96 - 650.72	<0.0001	1.0000
IL10	pg/ml	164	1.12	0 - 2.54	295	3.30	0 - 3.33	0.2737	105	1.51	0 - 3.62	0.2761	1.0000
VEGF	pg/ml	164	113.55	15.82 - 266.05	295	136.23	31.04 - 319.48	0.0005	105	122.89	37.53 - 271.83	0.0245	1.0000
IFNG	pg/ml	164	0.57	0.26 - 1.32	295	0.58	0 - 1.25	0.4417	105	0.57	0 - 1.9	1.0000	1.0000
TNFA	pg/ml	164	2.42	1.43 - 3.41	295	2.64	1.43 - 4.26	0.2495	105	3.47	1.98 - 5.28	<0.0001	<0.0001
IL1A	pg/ml	164	0.45	0.04 - 0.56	295	0.46	0.13 - 0.95	1.0000	105	0.31	0 - 0.73	1.0000	0.8398
IL1B	pg/ml	164	1.54	0 - 4.37	295	2.27	0 - 4.89	0.0004	105	1.87	0 - 3.9	0.2326	0.3362
MCP1	pg/ml	164	234.97	80.93 - 440.1	295	259.55	101.42 - 533.3	0.3432	105	276.85	112.64 - 528.43	0.0178	0.2368
EGF	pg/ml	164	53.39	2.84 - 115.55	295	147.39	27.7 - 327.5	<0.0001	105	139.33	27.92 - 281.65	<0.0001	1.0000
ADPN	ng/ml	89	4372.6	812 - 11022	134	4949.61	955 - 13746	1.0000	24	9472.29	1603.67 - 43539.5	0.0037	0.0070
CYSC	ng/ml	89	335.02	54 - 707	134	574.74	151.43 - 1337.3	<0.0001	24	1244.60	467.25 - 4081.62	<0.0001	<0.0001
FABP1	ng/ml	140	11.55	2.20-27.67	112	33.20	2.07-116.7	0.0005	44	80.53	4.53-322.65	0.0001	0.0061

**Table 2**

AUC values calculated for altered levels of individual biomarkers in diagnosis and staging of CKD

Biomarker	Control vs CKD	Control Vs Stage 1/2	Control Vs Stage 3	Overall
C3aDA	0.96	0.976	0.932	0.817
CD26	0.583	0.634	0.496	0.585
Creatinine	0.613	0.57	0.926	0.81
CRP	0.643	0.622	0.701	0.636
DDMER	0.763	0.738	0.828	0.734
NSE	0.725	0.715	0.752	0.682
NGAL	0.636	0.56	0.839	0.726
VCAM1	0.697	0.659	0.788	0.697
ICAM1	0.664	0.645	0.711	0.65
ESEL	0.595	0.602	0.578	0.568
PSEL	0.694	0.697	0.687	0.628
LSEL	0.583	0.58	0.588	0.556
IL5	0.497	0.496	0.499	0.497
IL15	0.508	0.553	0.633	0.631
GMCSF	0.532	0.578	0.562	0.588
MIP1A	0.86	0.836	0.91	0.802
sIL2a	0.59	0.53	0.749	0.657
sIL6R	0.521	0.544	0.541	0.559
STNFR1	0.863	0.815	0.989	0.886
STNFR2	0.866	0.841	0.931	0.843
MMP9	0.747	0.745	0.752	0.674
IL2	0.495	0.498	0.486	0.491
IL4	0.488	0.474	0.526	0.52
IL6	0.765	0.731	0.853	0.747
IL8	0.929	0.925	0.939	0.79
IL10	0.589	0.57	0.637	0.586
VEGF	0.596	0.594	0.602	0.565
IFNG	0.533	0.539	0.515	0.526
TNFA	0.605	0.534	0.793	0.685
IL1A	0.479	0.487	0.458	0.488
IL1B	0.631	0.626	0.645	0.587
MCP1	0.571	0.563	0.593	0.564
EGF	0.868	0.87	0.865	0.742
ADPN	0.52	0.547	0.647	0.621
CYSC	0.727	0.691	0.947	0.827
FABP1	0.655	0.624	0.734	/

**Table 3**

AUC values calculated for altered levels of combinations of two or more biomarkers in diagnosis and staging of CKD.

	Control vs CKD	Control Vs Stage 1/2	Control Vs Stage 3	Overall	Number
C3aDA, CYSC, CRP	0.985	0.979	1	0.859	106
C3aDA, GMCSF	0.992	0.992	0.992	0.84	108
C3aDA, IL5	0.992	0.991	0.995	0.84	104
C3aDA, MIP1A	0.998	1	0.995	0.833	108
C3aDA, GMCSF, CYSC	1	1	1	0.884	97
C3aDA, PSEL, GMCSF	1	1	1	0.878	108
C3aDA, PSEL, IL5	0.988	0.985	0.995	0.906	104
C3aDA, MIP1A, CYSC	1	1	1	0.873	97
C3aDA, GMCSF, IL6	0.994	0.995	0.992	0.89	108
C3aDA, ESEL, MIP1A	1	1	1	0.865	108
C3aDA, GMCSF, IL4	0.995	0.995	0.995	0.879	108
C3aDA, Creatinine, IL2	0.994	0.991	1	0.877	159
C3aDA, NGAL, PSEL, IL5	0.999	1	0.997	0.912	104
C3aDA, NGAL, PSEL, GMCSF	1	1	1	0.899	108
C3aDA, GMCSF, MIP1A, CYSC	1	1	1	0.897	97
C3aDA, IL5, IFNG, CYSC	1	1	1	0.892	93
C3aDA, DDMER, IL5, CYSC	1	1	1	0.891	93
C3aDA, NGAL, PSEL, IL15	0.993	0.992	0.995	0.911	108
C3aDA, MIP1A, TNFA, CYSC	1	1	1	0.889	97
C3aDA, ICAM1, IL5, IL1A	0.987	0.981	1	0.921	104
MIP1A, IL8, IL1B, CYSC	0.995	0.994	1	0.89	121
VCAM1, IL15, IL8, CYSC	1	1	1	0.862	121
VCAM1, MIP1A, IL8, CYSC	1	1	1	0.861	121
NSE, MIP1A, IL8, CYSC	1	1	1	0.807	121
NSE, VCAM1, MIP1A, IL8	0.999	1	0.999	0.87	191
FABP1, IL8, MIP1A, DDMER	0.985	0.978	1	0.831	114
FABP1, IL8, MIP1A, NGAL	0.991	0.986	1	0.822	114
FABP1, IL8, MIP1A, EGF	0.977	0.969	0.996	0.818	114

The stratification of patients into one of the stages of CKD is useful to assess whether a patient would benefit from one treatment type compared to another and also to monitor whether a treatment is successful. Stratification of patients also assists in determining the prognosis of the patient thereby enabling future care requirements.

The methods of the present invention may use methods for determining the level of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, 5 IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 known in the art, such as enzymatic and/or chemical protein determination or immunological assay based methods. Immunological assays for determining the level of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, 10 ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 can be performed in a variety of assay formats, including sandwich assays e.g. (ELISA), competition assays (competitive RIA), bridge immunoassays, immunohistochemistry (IHC) and immunocytochemistry (ICC). 15 Methods for determining the level of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 include contacting a patient sample with antibodies that bind to C3a desArg, IL-8, MIP 20 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 and detecting binding. Antibodies having specificity for C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, 25 ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 can be immobilised on a support material using conventional methods. Binding of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, 30 IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 to the antibodies on the support can be detected using further antibodies having specificity or reactivity (which may be for the biomarker or other discriminating

analyte) for C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 or by using physical methods such 5 as surface plasmon resonance (Biacore Int, Sweden).

Preferably, a solid state device may be used to determine the level of one or more biomarkers selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, 10 GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 in the sample isolated from the patient. The solid state device comprises a substrate having an activated surface on to which is applied an antibody to C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D- 15 dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 to discreet areas of the activated surface. Preferably the solid state device may perform multi-analyte assays such 20 that the level of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 in a sample isolated from the patient may be determined simultaneously with the level of any others of C3a 25 desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 in the sample. In this embodiment, the solid 30 state device has a multiplicity of discrete reaction sites each bearing a desired antibody covalently bound to the substrate, and in which the surface of the substrate between the reaction sites is inert with respect to the target biomarker. The solid state, multi-analyte device used in the present invention may therefore exhibit little or no non-specific binding.

A device that may be used in the invention may be prepared by activating the surface of a suitable substrate, and applying an array of antibodies on to discrete sites on the surface. Suitable substrates are produced from any inert materials such as plastics, ceramics or glass. If desired, the other active areas

5 may be blocked. The ligands may be bound to the substrate via a linker, e.g. by covalent attachment. In particular, it is preferred that the activated surface is reacted successively with an organosilane, a bifunctional linker and the antibody. The solid state device used in the methods of the present invention may be manufactured according to the method disclosed in, for example, GB-A-

10 2324866 the contents of which is incorporated herein in its entirety. Preferably, the solid state device used in the methods of the present invention is a biochip which forms part of the Biochip Array Technology system (BAT) (available from Randox Laboratories Limited).

15 The solid state device used in the method of the present invention comprises an antibody to C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1. Preferably the antibody is specific

20 for C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 and exhibits a cross-reactivity of less than 0.5%, preferably less than 0.1%, to other antigens. Preferably, the antibody is a

25 monoclonal antibody. The solid state device used in the method of the present invention can be processed employing BAT analysers from Randox.

The present invention further provides assays to measure the amount of two or more biomarkers in a sample obtained from the subject, wherein the biomarkers

30 are selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL,

NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1. The assays of the invention can be used to measure the levels of combinations of three, four, five, six, seven or more of these biomarkers in a sample. Such assays can be carried out using the solid state devices of the invention, and they 5 may be used in the methods of the invention.

The methods of the present invention may be used to monitor a patient post-operatively to assess the risk of them developing renal complications. Specifically, a sample may be obtained from a patient at given intervals post 10 operatively and the level of any one or more biomarkers selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 in the sample determined. An 15 assessment of the level of any one or more biomarkers selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF or VCAM1 over a particular time frame provides 20 the clinician with an indication of whether the patient is suffering early stage CKD and medical intervention can be taken accordingly.

### **Example**

Serum samples from 376 patients suffering from chronic kidney disease and 211 25 healthy controls were taken by means of venepuncture in a method known in the art and stored at -80°C until ready for use. Patients' CKD was staged using the KDOQI guidelines, and patients suffering from Stage 1 and Stage 2 CKD were combined for the purpose of this analysis as being representative of early kidney disease that is not easily determined through conventional biomarker-based 30 methods.

The sample cohort was composed of:

- 587 serum samples in total

25

- 271 CKD stage 1/2 patients
- 105 CKD stage 3 patients
- 211 donor controls

5 In order to determine the levels of biomarkers in the sample, immunoassays using the biochip array technology (BAT) were employed, with the exception of creatinine measurement. Creatinine was measured using a commercially available clinical chemistry assay (Product number CR3814; Randox Laboratories), and samples were analysed on the RX Daytona (available from 10 Randox Laboratories).

#### Protocol description

15 Data were reported in the respective units of the assay and no normalisation techniques were used prior to analysis. Data were first analysed through conventional univariate non-parametric testing. Table 1 reports the median and 5 to 95 percentile of each analyte studied. Significance was determined using 20 mann-whitney with bonferroni correction for multiple comparisons (Control vs Stage 1/2, Control vs Stage 3, Stage 1/2 vs Stage 3). Receiver-operator characteristics (ROC) analysis was also performed on this data and the area under the curve determined for the identification of; all CKD patients (Stage 1/2/3) versus healthy controls, Stage 1/2 versus healthy controls, Stage 3 versus healthy controls and for differentiating between healthy controls, Stage 1/2 and Stage 3 (Overall). These AUC values have been summarised in table 2.

25

In order to determine whether potential combinations of markers may have improved diagnostic and stratification performance, two methods were employed. The first was the use of logistic regression, where a subset of the cohort was used to develop a model for distinguishing CKD patients from healthy 30 controls. Backward entry was used to select the optimal variables required. This model was then validated on a validation subset, the predicted probability of CKD was calculated using the logistic regression equation and then ROC analysis was performed.

The second method was carried out to fully examine the value in combining these biomarkers to improve diagnostic performance and disease stratification, random forests were grown using a randomly selected subset of the data for all possible single, double, triple and quadruple biomarker combinations. In total 5 66,675 models were generated and the AUC was calculated from a separate validation subset. The AUCs for all the combinations that contain each value were aggregated and those markers whose average aggregated AUC were highest were deemed to be the most important markers. In this C3a desArg was shown to perform best in almost any combination and was present in 78% of the 10 top performing models. Table 3 represent the AUCs of some combinations in discriminating CKD and differentiating the stage of disease.

**Claims**

1. A method for detecting kidney disease in a subject, comprising measuring the amount of a biomarker in a sample obtained from the subject, and determining whether the amount of the biomarker is altered compared to a normal control, wherein the biomarker is selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.
2. A method for determining the efficacy of a treatment for chronic kidney disease, comprising measuring the amount of a biomarker in a sample obtained from a subject receiving such treatment, wherein the biomarker is selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1, and comparing the level of the biomarker to that of a sample from an untreated control to determine whether the treatment has had the effect of altering the biomarker level.
3. A method for determining the efficacy of a treatment for chronic kidney disease, comprising measuring the amount of a biomarker in a sample obtained from a subject receiving such treatment, wherein the biomarker is selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1, and comparing the level of the biomarker to that of a sample obtained from the subject before treatment, wherein a reduction or maintenance in the stage of chronic kidney disease or a slowing of progression through stages of chronic kidney disease following treatment indicates that the treatment has been successful.
4. A method of determining a drug treatment protocol for a subject suffering from chronic kidney disease, comprising, in a sample taken from a subject treated

with the drug, comparing the level of a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, FABP1, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ ,  
5 VEGF and VCAM1 to the level in sample obtained from an untreated control to determine whether the drug has had the effect of altering the biomarker level, and selecting a drug treatment protocol based on whether the subject has stage 1, stage 2 or stage 3 chronic kidney disease.

10 5. A method according to claim 4, wherein the drug treatment protocol is selected based on whether the subject has stage 1 or stage 2 chronic kidney disease or whether the subject has stage 3 chronic kidney disease.

15 6. A method according to any one of claims 1 to 5, wherein the level of C3a desArg is measured.

7. A method according to any one of claims 1 to 5, wherein the level of MIP 1 $\alpha$  is measured.

20 8. A method according to any one of claims 1 to 5, wherein the kidney disease is chronic kidney disease.

9. A method according to claim 8, wherein the chronic kidney disease is stage 1 or stage 2 chronic kidney disease.

25 10. A method according to claim 8, wherein the chronic kidney disease is stage 3 chronic kidney disease.

11. A method according to any one of claims 1 to 10, wherein the sample is  
30 urine, blood, plasma, serum or saliva.

12. A method according to claim 11, wherein the sample is plasma or serum.

13. A method according to any one of claims 1 to 12, wherein the amount of  
35 the biomarker in the sample is measured by binding of an antibody that binds specifically to the biomarker.

14. A method according to claim 13, wherein the antibody that binds specifically to the biomarker is detectably-labelled.
15. A method according to claim 14, wherein the label is horse-radish peroxidase.
16. A method according to any one of claims 1 to 15, wherein the amount of a biomarker in the sample is measured using biochip array technology.
- 10 17. A method according to any one of claims 1 to 16, wherein the amount of a biomarker in the sample is measured using a sandwich ELISA assay.
18. A method according to any one of claims 1 to 17, wherein the level of two or more biomarkers are measured in a sample obtained from the subject.
- 15 19. A method according to claim 18, wherein the levels of C3a desArg and GMCSF are measured.
20. A method according to claim 18, wherein the levels of C3a desArg and IL5 are measured.
21. A method according to claim 18, wherein the levels of C3a desArg and MIP 1 $\alpha$  are measured.
- 25 22. A method according to claim 18, wherein the levels of C3a desArg, CRP and CYSC are measured.
23. A method according to claim 18, wherein the levels of C3a desArg, GMCSF and CYSC are measured.
- 30 24. A method according to claim 18, wherein the levels of C3a desArg, GMCSF and PSEL are measured.
- 25 25. A method according to claim 18, wherein the levels of C3a desArg, IL5 and PSEL are measured.
- 35 26. A method according to claim 18, wherein the levels of C3a desArg, CYSC and MIP 1 $\alpha$  are measured.

27. A method according to claim 18, wherein the levels of C3a desArg, GMCSF and IL6 are measured.

5 28. A method according to claim 18, wherein the levels of C3a desArg, ESEL and MIP 1 $\alpha$  are measured.

29. A method according to claim 18, wherein the levels of C3a desArg, GMCSF and IL4 are measured.

10 30. A method according to claim 18, wherein the levels of C3a desArg, creatinine and IL2 are measured.

31. A method according to claim 18, wherein the levels of C3a desArg, 15 NGAL, PSEL and IL5 are measured.

32. A method according to claim 18, wherein the levels of C3a desArg, NGAL, PSEL and GMCSF are measured.

20 33. A method according to claim 18, wherein the levels of C3a desArg, GMCSF, CYSC and MIP 1 $\alpha$  are measured.

34. A method according to claim 18, wherein the levels of C3a desArg, IL5, IFNG and CYSC are measured.

25 35. A method according to claim 18, wherein the levels of C3a desArg, D-dimer, IL5 and CYSC are measured.

36. A method according to claim 18, wherein the levels of C3a desArg, 30 NGAL, PSEL and IL15 are measured.

37. A method according to claim 18, wherein the levels of C3a desArg, TNFA, CYSC and MIP 1 $\alpha$  are measured.

35 38. A method according to claim 18, wherein the levels of C3a desArg, ICAM1, IL5 and IL1 $\alpha$  are measured.

39. A method according to claim 18, wherein the levels of MIP 1 $\alpha$ , IL8, IL1 $\beta$  and CYSC are measured.

40. A method according to claim 18, wherein the levels of VCAM1, IL15, IL8 and CYSC are measured.

41. A method according to claim 18, wherein the levels of VCAM1, MIP 1 $\alpha$ , IL8 and CYSC are measured.

10 42. A method according to claim 18, wherein the levels of NSE, MIP 1 $\alpha$ , IL8 and CYSC are measured.

43. A method according to claim 18, wherein the levels of NSE, VCAM1, MIP 1 $\alpha$  and IL8 are measured.

15 44. A method according to claim 18, wherein the levels of EGF, FABP1, IL8, sTNFR1, sTNFR2, D-dimer, NGAL and MIP 1 $\alpha$  are measured.

20 45. A method according to claim 18, wherein the levels of C3a desArg, CRP, CYSC, EGF, FABP1, IL8, sTNFR1, sTNFR2, D-dimer, NGAL and MIP 1 $\alpha$  are measured.

25 46. Use of a kit for screening for kidney disease in a method according to any one of claims 1 to 45, the kit comprising a probe that binds specifically to a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1, or reagents for an immunoassay or 1D or 2D gel electrophoresis for 30 detecting the level of a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.

47. The use according to claim 46, wherein the kidney disease is chronic kidney disease.

48. The use according to claim 46, wherein the probe binds specifically to a biomarker selected from the group consisting of C3a desArg, C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.

49. The use according to claim 46, wherein the probe is an antibody or an aptamer that binds specifically to a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP, LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.

50. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg.

51. The use according to claim 46, wherein the kit comprises a probe that binds specifically to MIP 1 $\alpha$ .

52. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg and a probe that binds specifically to GMCSF.

53. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg and a probe that binds specifically to IL5.

54. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg and a probe that binds specifically to MIP 1 $\alpha$ .

55. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to CRP and a probe that binds specifically to CYSC.

56. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to GMCSF and a probe that binds specifically to CYSC.

5 57. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to GMCSF and a probe that binds specifically to PSEL.

10 58. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to IL5 and a probe that binds specifically to PSEL.

15 59. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to CYSC and a probe that binds specifically to MIP 1 $\alpha$ .

20 60. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to GMCSF and a probe that binds specifically to IL6.

25 61. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to ESEL and a probe that binds specifically to MIP 1 $\alpha$ .

30 62. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to GMCSF and a probe that binds specifically to IL4.

35 63. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to creatinine and a probe that binds specifically to IL2.

64. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to NGAL, a probe that binds specifically to PSEL and a probe that binds specifically to IL5.

65. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to NGAL, a probe that binds specifically to PSEL and a probe that binds specifically to GMCSF.

5

66. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to GMCSF, a probe that binds specifically to CYSC and a probe that binds specifically to MIP 1 $\alpha$ .

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67. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to IL5, a probe that binds specifically to IFNG and a probe that binds specifically to CYSC.

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68. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to D-dimer, a probe that binds specifically to IL5 and a probe that binds specifically to CYSC.

20

69. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to NGAL, a probe that binds specifically to PSEL and a probe that binds specifically to IL15.

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70. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to TNFA, a probe that binds specifically to CYSC and a probe that binds specifically to MIP 1 $\alpha$ .

30

71. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to ICAM1, a probe that binds specifically to IL5 and a probe that binds specifically to IL1 $\alpha$ .

72. The use according to claim 46, wherein the kit comprises a probe that binds specifically to MIP 1 $\alpha$ , a probe that binds specifically to IL8, a probe that binds specifically to IL1 $\beta$  and a probe that binds specifically to CYSC.

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73. The use according to claim 46, wherein the kit comprises a probe that binds specifically to VCAM1, a probe that binds specifically to IL15, a probe that binds specifically to IL8 and a probe that binds specifically to CYSC.

5 74. The use according to claim 46, wherein the kit comprises a probe that binds specifically to VCAM1, a probe that binds specifically to MIP 1 $\alpha$ , a probe that binds specifically to IL8 and a probe that binds specifically to CYSC.

10 75. The use according to claim 46, wherein the kit comprises a probe that binds specifically to NSE, a probe that binds specifically to MIP 1 $\alpha$ , a probe that binds specifically to IL8 and a probe that binds specifically to CYSC.

15 76. The use according to claim 46, wherein the kit comprises a probe that binds specifically to NSE, a probe that binds specifically to VCAM1, a probe that binds specifically to MIP 1 $\alpha$  and a probe that binds specifically to IL8.

20 77. The use according to claim 46, wherein the kit comprises a probe that binds specifically to EGF, a probe that binds specifically to FABP1, a probe that binds specifically to IL8, a probe that binds specifically to sTNFR1, a probe that binds specifically to sTNFR2, a probe that binds specifically to D-dimer, a probe that binds specifically to NGAL and a probe that binds specifically to MIP 1 $\alpha$ .

25 78. The use according to claim 46, wherein the kit comprises a probe that binds specifically to C3a desArg, a probe that binds specifically to CRP, a probe that binds specifically to CYSC, a probe that binds specifically to EGF, a probe that binds specifically to FABP1, a probe that binds specifically to IL8, a probe that binds specifically to sTNFR1, a probe that binds specifically to sTNFR2, a probe that binds specifically to D-dimer, a probe that binds specifically to NGAL and a probe that binds specifically to MIP 1 $\alpha$ .

30 79. A solid state device comprising a substrate that comprises an antibody that binds specifically to a biomarker selected from the group consisting of C3a desArg, IL-8, MIP 1 $\alpha$ , ADPN, CD26, Creatinine, CRP, CYSC, D-dimer, EGF, ESEL, GMCSF, ICAM1, IFN $\gamma$ , IL10, IL15, IL1 $\alpha$ , IL1 $\beta$ , IL2, IL4, IL5, IL6, LFABP,

LSEL, MCP1, MMP9, NGAL, NSE, PSEL, sIL2 $\alpha$ , sIL6R, STNFR1, STNFR2, TNF $\alpha$ , VEGF and VCAM1.

80. The solid state device according to claim 79, wherein the substrate  
5 comprises an antibody that binds specifically to C3a desArg.

81. The solid state device according to claim 79, wherein the substrate  
comprises an antibody that binds specifically to MIP 1 $\alpha$ .

10 82. The solid state device according to claim 79, wherein the substrate  
comprises an antibody that binds specifically to C3a desArg and an antibody that  
binds specifically to GMCSF.

15 83. The solid state device according to claim 79, wherein the substrate  
comprises an antibody that binds specifically to C3a desArg and an antibody that  
binds specifically to IL5.

20 84. The solid state device according to claim 79, wherein the substrate  
comprises an antibody that binds specifically to C3a desArg and an antibody that  
binds specifically to MIP 1 $\alpha$ .

85. The solid state device according to claim 79, wherein the substrate  
comprises an antibody that binds specifically to C3a desArg, an antibody that  
binds specifically to CRP and an antibody that binds specifically to CYSC.

25 86. The solid state device according to claim 79, wherein the substrate  
comprises an antibody that binds specifically to C3a desArg, an antibody that  
binds specifically to GMCSF and an antibody that binds specifically to CYSC.

30 87. The solid state device according to claim 79, wherein the substrate  
comprises an antibody that binds specifically to C3a desArg, an antibody that  
binds specifically to GMCSF and an antibody that binds specifically to PSEL.

35 88. The solid state device according to claim 79, wherein the substrate  
comprises an antibody that binds specifically to C3a desArg, an antibody that  
binds specifically to IL5 and an antibody that binds specifically to PSEL.

89. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to CYSC and an antibody that binds specifically to MIP 1 $\alpha$ .

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90. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to GMCSF and an antibody that binds specifically to IL6.

10

91. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to ESEL and an antibody that binds specifically to MIP 1 $\alpha$ .

15

92. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to GMCSF and an antibody that binds specifically to IL4.

20

93. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to creatinine and an antibody that binds specifically to IL2.

25

94. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to NGAL, an antibody that binds specifically to PSEL and an antibody that binds specifically to IL5.

30

95. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to NGAL, an antibody that binds specifically to PSEL and an antibody that binds specifically to GMCSF.

35

96. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to GMCSF, an antibody that binds specifically to CYSC and an antibody that binds specifically to MIP 1 $\alpha$ .

97. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to IL5, an antibody that binds specifically to IFNG and an antibody that binds specifically to CYSC.

5

98. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to D-dimer, an antibody that binds specifically to IL5 and an antibody that binds specifically to CYSC.

10

99. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to NGAL, an antibody that binds specifically to PSEL and an antibody that binds specifically to IL15.

15

100. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to TNFA, an antibody that binds specifically to CYSC and an antibody that binds specifically to MIP 1 $\alpha$ .

20

101. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to ICAM1, an antibody that binds specifically to IL5 and an antibody that binds specifically to IL1 $\alpha$ .

25

102. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to MIP 1 $\alpha$ , an antibody that binds specifically to IL8, an antibody that binds specifically to IL1 $\beta$  and an antibody that binds specifically to CYSC.

30

103. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to VCAM1, an antibody that binds specifically to IL15, an antibody that binds specifically to IL8 and an antibody that binds specifically to CYSC.

35

104. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to VCAM1, an antibody that binds specifically to MIP 1 $\alpha$ , an antibody that binds specifically to IL8 and an antibody that binds specifically to CYSC.

5

105. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to NSE, an antibody that binds specifically to MIP 1 $\alpha$ , an antibody that binds specifically to IL8 and an antibody that binds specifically to CYSC.

10

106. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to NSE, an antibody that binds specifically to VCAM1, an antibody that binds specifically to MIP 1 $\alpha$  and an antibody that binds specifically to IL8.

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107. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to EGF, an antibody that binds specifically to FABP1, an antibody that binds specifically to IL8, an antibody that binds specifically to sTNFR1, an antibody that binds specifically to sTNFR2, an antibody that binds specifically to D-dimer, an antibody that binds specifically to NGAL and an antibody that binds specifically to MIP 1 $\alpha$ .

20

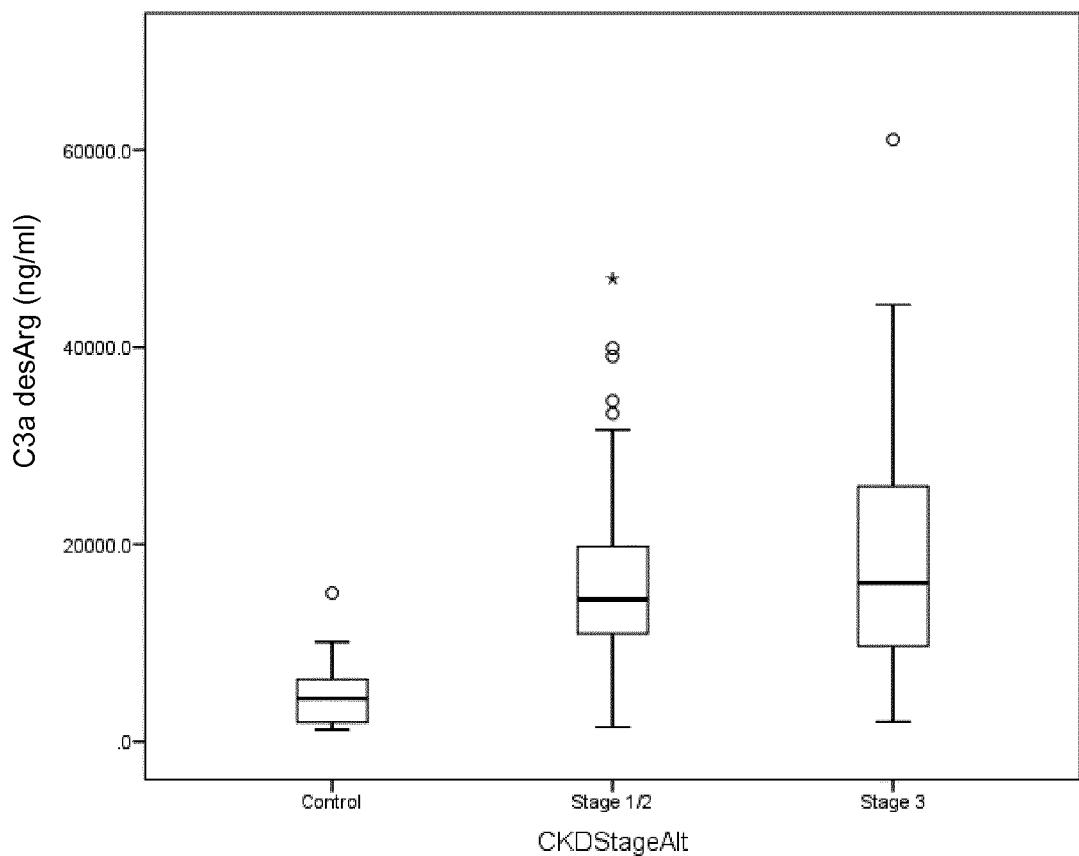
108. The solid state device according to claim 79, wherein the substrate comprises an antibody that binds specifically to C3a desArg, an antibody that binds specifically to CRP, an antibody that binds specifically to CYSC, an antibody that binds specifically to EGF, an antibody that binds specifically to FABP1, an antibody that binds specifically to IL8, an antibody that binds specifically to sTNFR1, an antibody that binds specifically to sTNFR2, an antibody that binds specifically to D-dimer, an antibody that binds specifically to NGAL and an antibody that binds specifically to MIP 1 $\alpha$ .

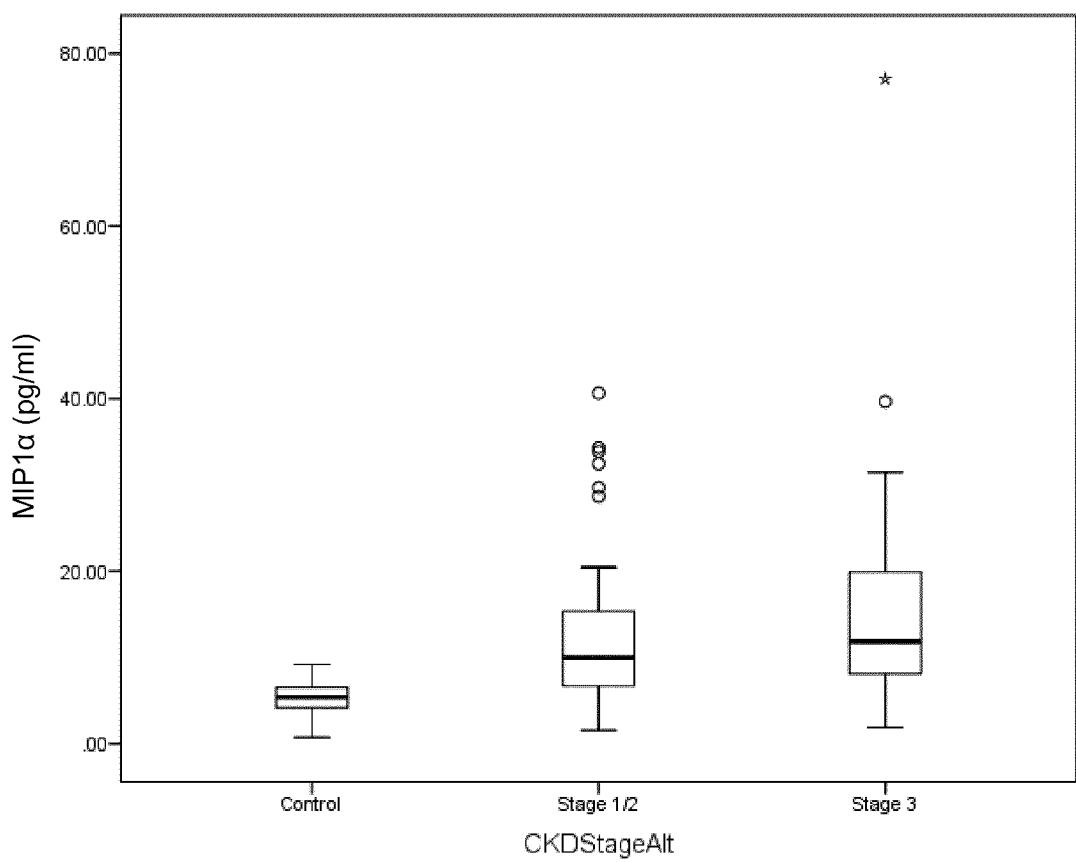
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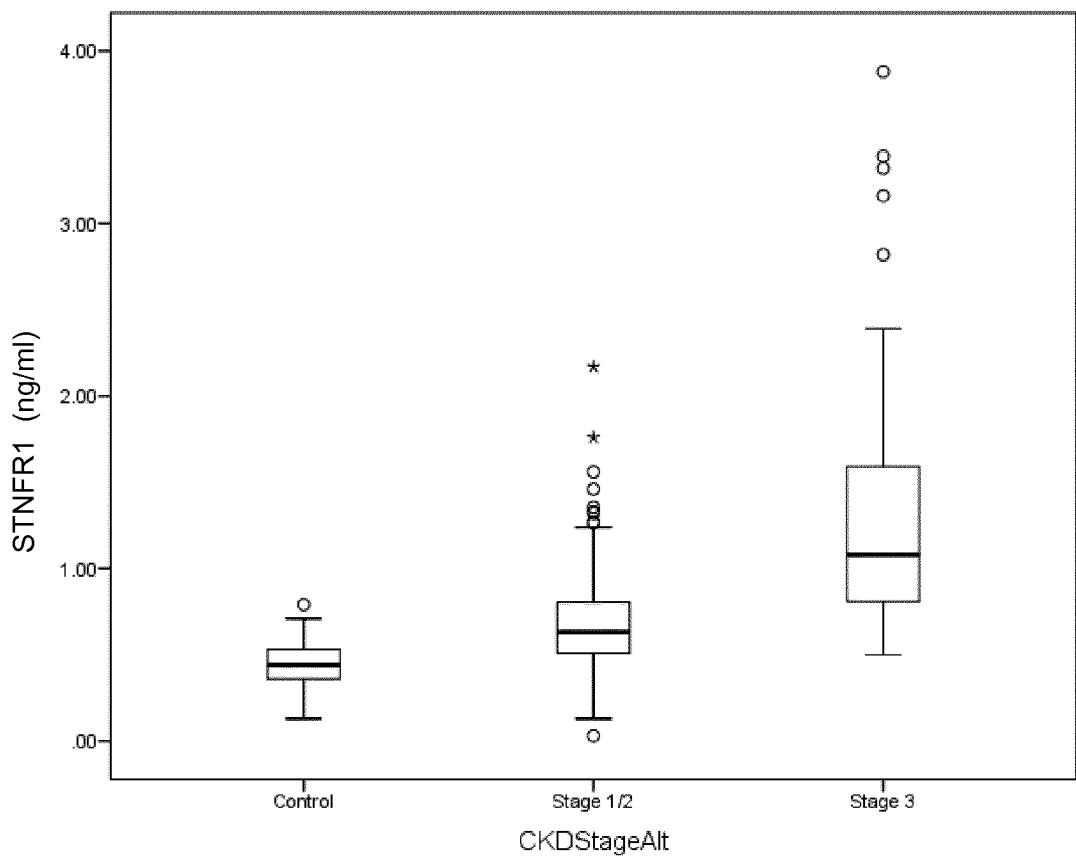
109. A solid state device according to any one of claims 79 to 108, wherein the antibody is a monoclonal antibody.

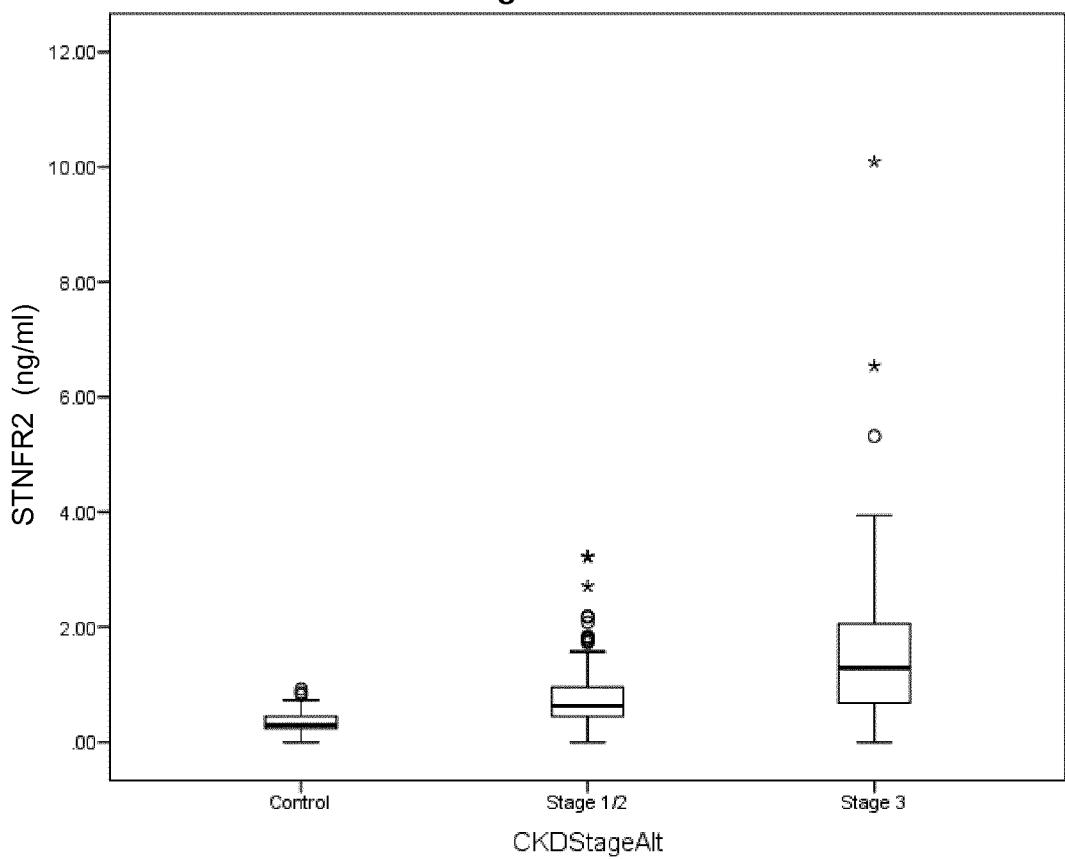
40

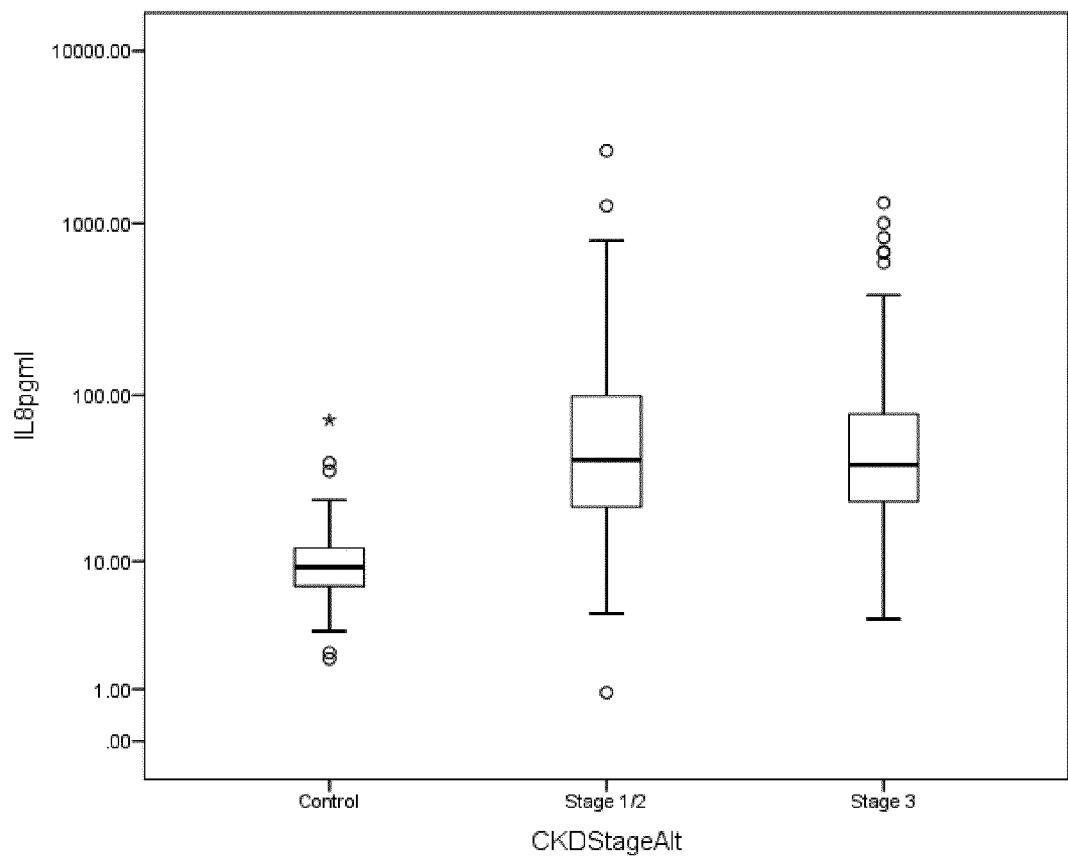
110. Use of a solid state device according to any one of claims 79 to 109 to screen for chronic kidney disease in a subject.
111. Use of a solid state device according to any one of claims 79 to 109 to 5 screen for stage 1 or stage 2 chronic kidney disease.

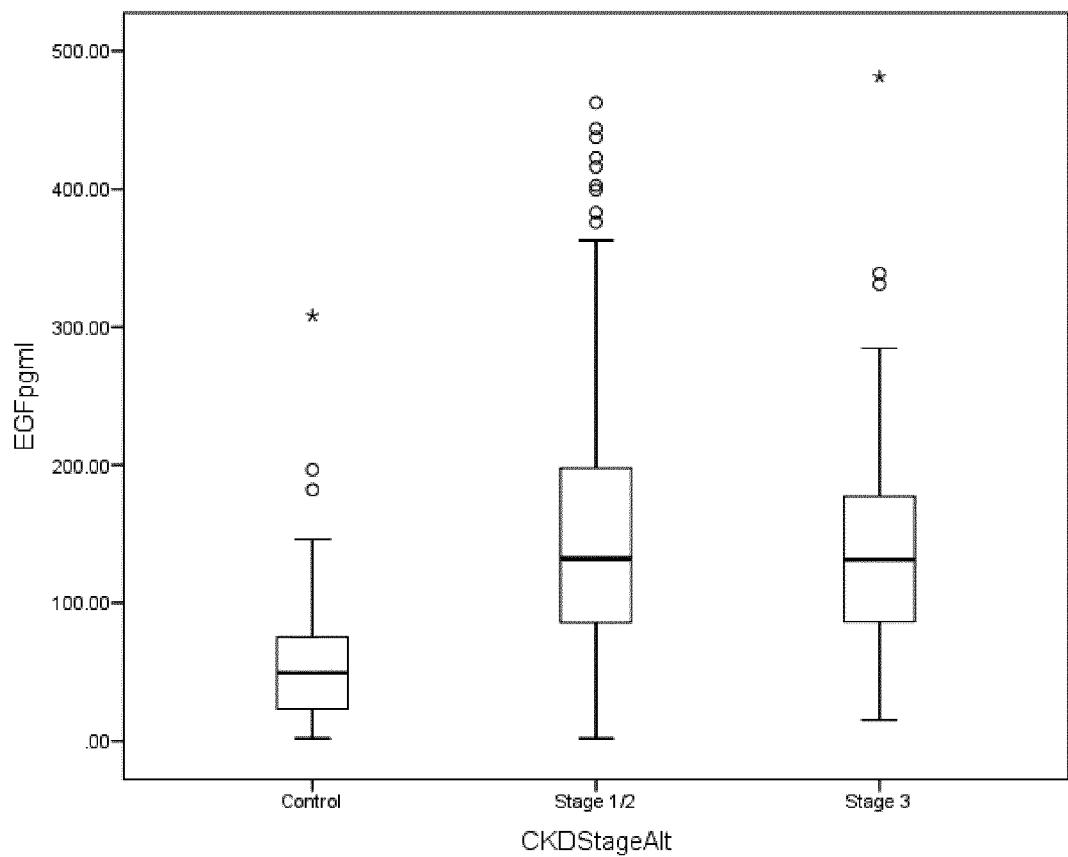
**Figure 1**

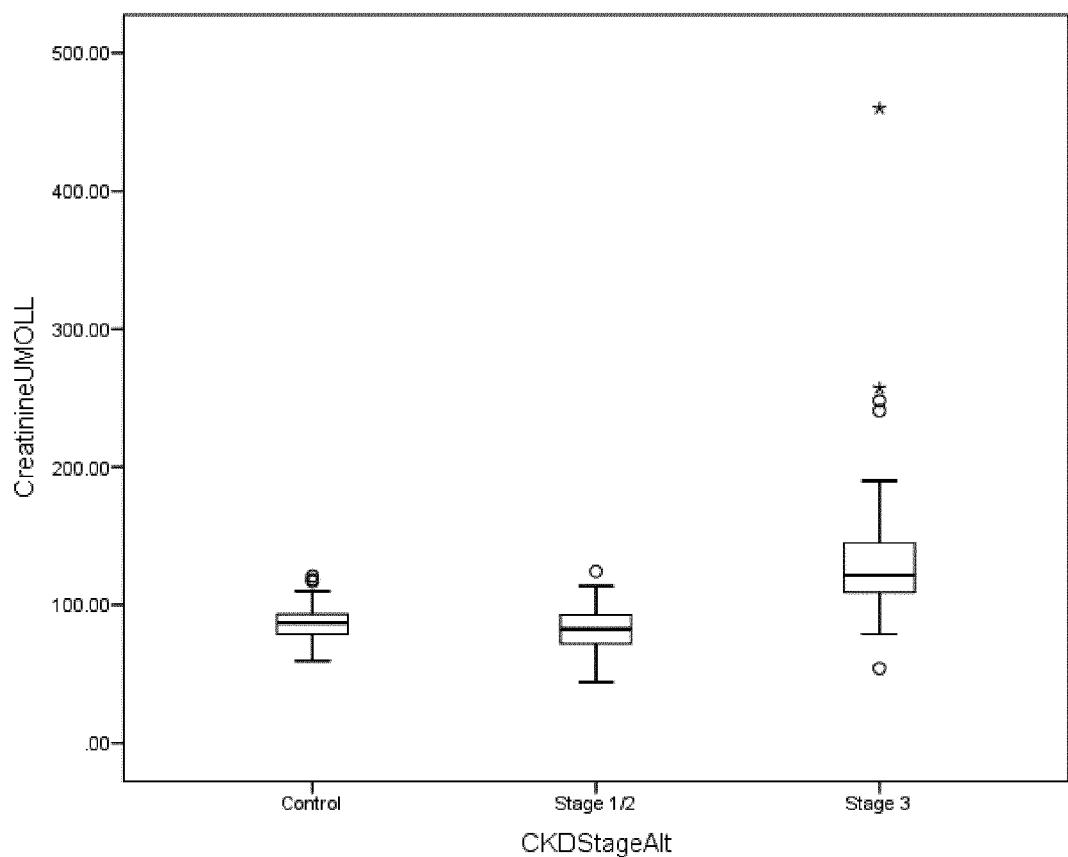
**Figure 2**

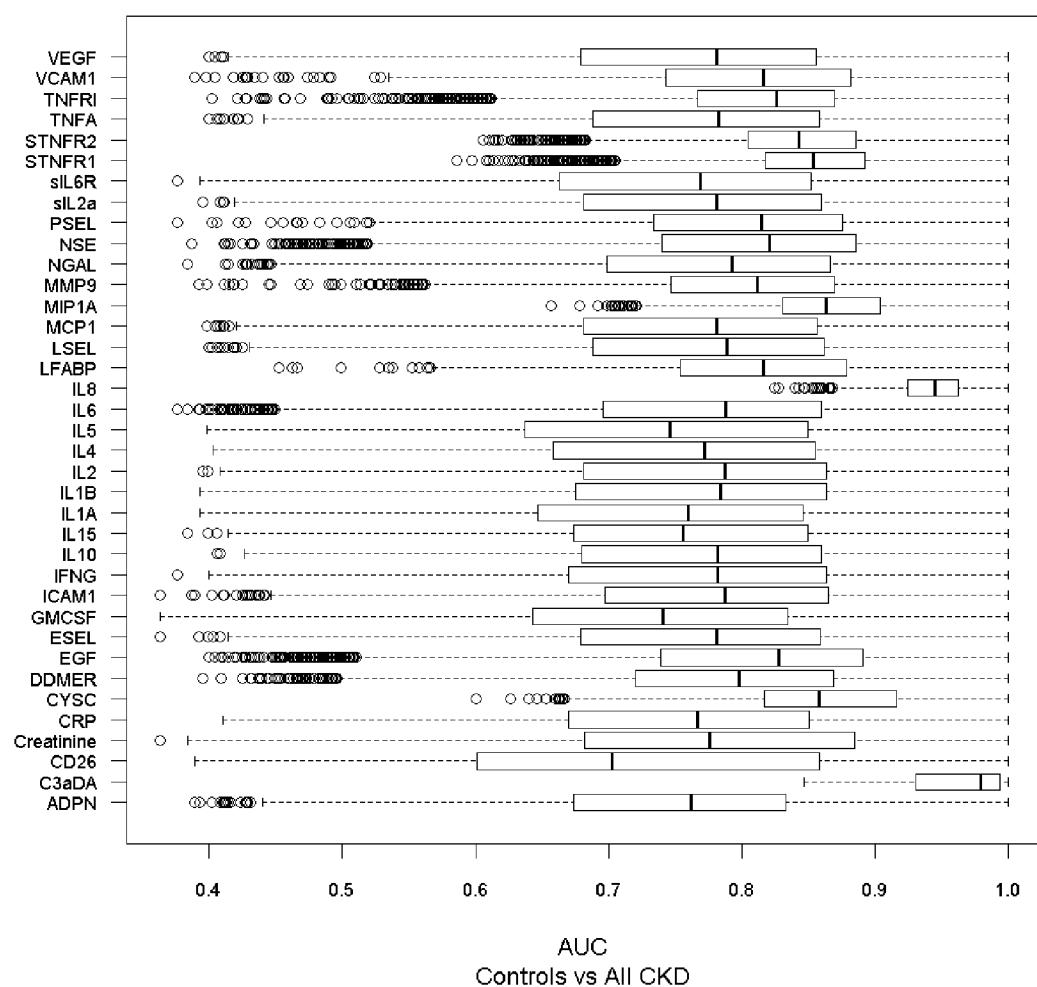
**Figure 3**

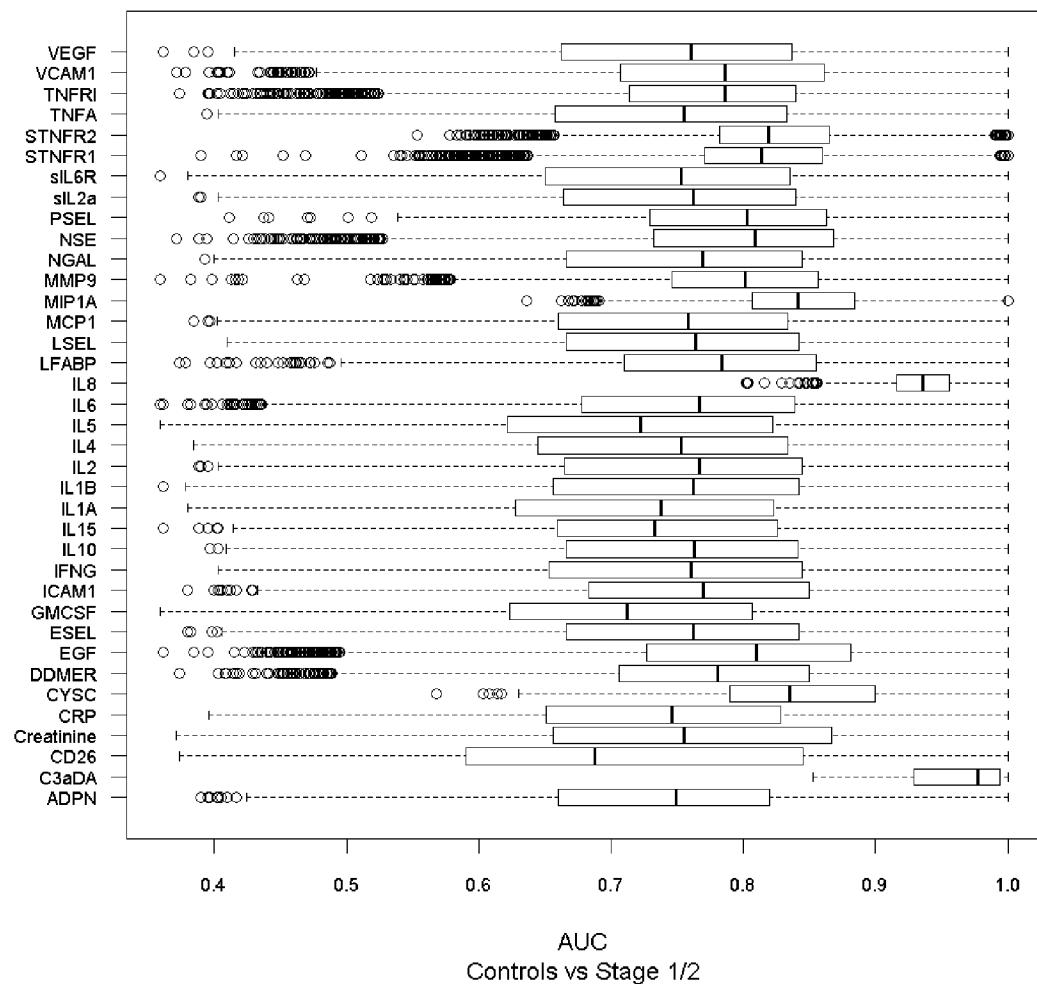
**Figure 4**

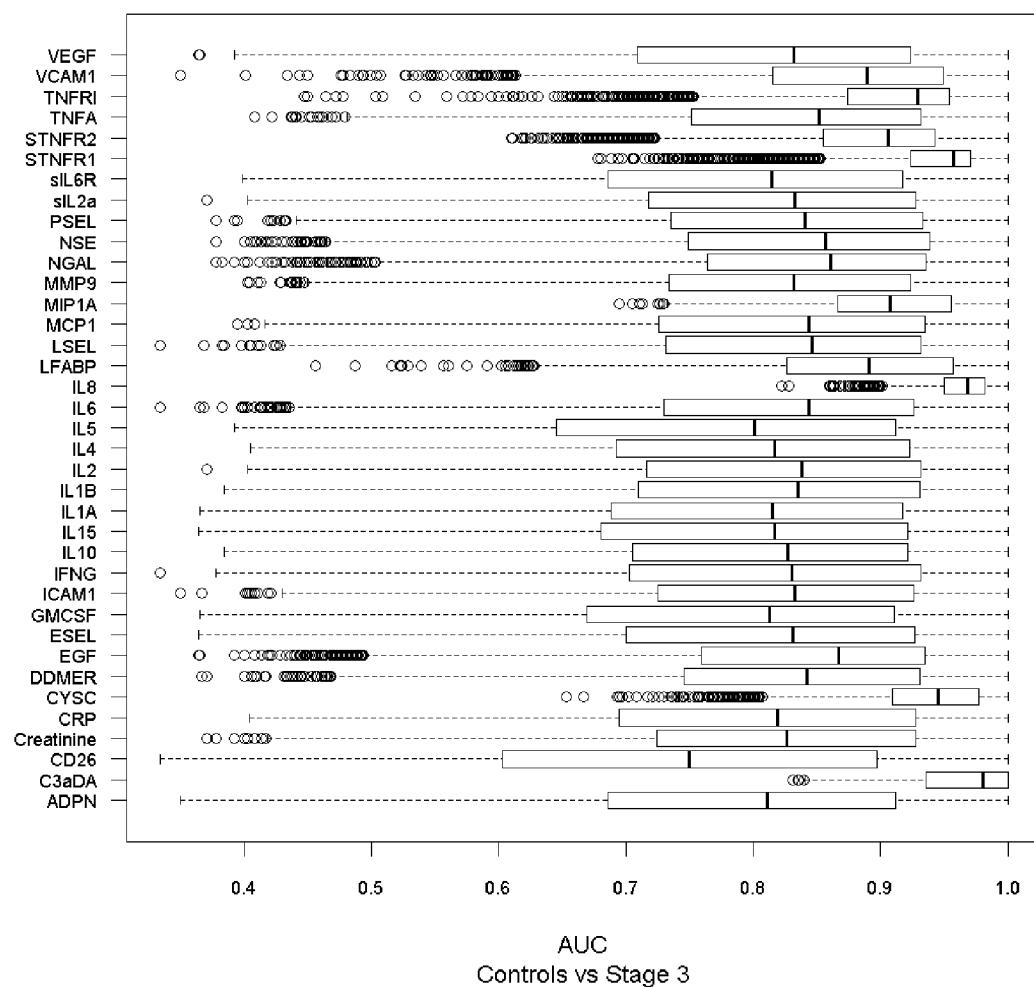
**Figure 5**

**Figure 6**

**Figure 7**

**Figure 8****AUCs for combinations containing each factor**

**Figure 9****AUCs for combinations containing each factor**

**Figure 10****AUCs for combinations containing each factor**

**Figure 11****AUCs for combinations containing each factor**