



(51) International Patent Classification:
G01N 33/50 (2006.01)

(21) International Application Number:
PCT/US2015/042976

(22) International Filing Date:
30 July 2015 (30.07.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/031,132 30 July 2014 (30.07.2014) US
62/031,834 31 July 2014 (31.07.2014) US
62/115,077 11 February 2015 (11.02.2015) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: METHODS AND COMPOSITIONS FOR DIAGNOSING, PROGNOSING, AND CONFIRMING PREECLAMPSIA

(57) Abstract: Methods, compositions, kits, algorithms, systems, specialized computer, software, business methods and reagents are provided for diagnosing, prognosing, monitoring, characterizing, determining the severity of preeclampsia, confirming the presence of preeclampsia or confirming the absence of preeclampsia in a female subject. The methods and compositions find use in a number of applications, including, for example, predicting if an individual will develop preeclampsia, diagnosing whether the individual has preeclampsia, characterizing preeclampsia, monitoring an individual with preeclampsia, determining the severity of preeclampsia, confirming the presence of preeclampsia or confirming the absence of preeclampsia in a subject.



METHODS AND COMPOSITIONS FOR DIAGNOSING, PROGNOSING, AND CONFIRMING PREECLAMPSIA

[0001] This application claims the benefit of and priority from United States provisional patent application 62/031,132, filed July 30, 2014, United States provisional patent application 62/031,834, filed July 31, 2014, and United States provisional patent application 62/115,077, filed February 11, 2015. The contents and disclosures of each of the foregoing patent applications are incorporated herein by reference in their entirety.

RELEVANT FIELD

[0002] This disclosure pertains to providing a preeclampsia diagnosis and prognosis.

BACKGROUND

[0003] Preeclampsia (PE) is a serious multisystem complication of pregnancy with adverse effects for mothers and babies. The incidence of the disorder is around 5-8% of all pregnancies in the U.S. and worldwide, and the disorder is responsible for 18% of all maternal deaths in the U.S. The causes and pathogenesis of preeclampsia remain uncertain, and current laboratory signs and clinical symptoms of PE occur late in the disease process, sometimes making the determination of PE and clinical management decisions difficult. Specifically, it is crucial to distinguish preeclampsia from complication of pregnancy symptoms, such as gestational hypertension, chronic hypertension, and gestational diabetes, each of which require different treatment options. Earlier and more reliable diagnosis, prognosis, confirmation and monitoring of the disease will lead to more timely and personalized preeclampsia treatments and as such, will significantly advance the understanding of preeclampsia pathogenesis.

SUMMARY

[0004] The disclosure provides a method for confirming preeclampsia in any subject, preferably a pregnant subject, comprising: evaluating a plurality of biomarkers in a sample derived from the subject to calculate an index or to confirm if the subject has preeclampsia wherein the confirmation has a sensitivity of greater than 90%, a specificity of greater than 90%, or greater than 0.9 area under the receiver operating characteristic curve (ROC and/or AUC).

[0005] The disclosure provides a test for confirming preeclampsia in a subject, preferably a pregnant subject, wherein the test is able to discern subjects not having PE but having one or more symptoms associated with PE from subjects having by PE, with a ROC value of at least 0.80, 0.85, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 0.995 or more. The one or more symptoms associated with PE can be diabetes (e.g. gestational, type I or type II), higher than normal glucose level, hypertension (e.g. chronic or non-chronic), excessive or sudden weight gain, higher than normal weight, obesity, higher than normal body mass index (BMI), abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results (Pap smear), prior preeclampsia episodes (e.g., personal history of PE), familial history of PE, PE in prior pregnancies, renal disease, thrombophilia, or any combination thereof.

[0006] The disclosure provides a test for confirming preeclampsia in a subject, preferably a pregnant subject, wherein the test is able to discern subjects not having PE but having one or more symptoms associated with PE from subjects having PE, with a sensitivity, specificity and/or negative predictive value (NPV) of at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or more. The one or more symptoms associated with PE can be diabetes (e.g. gestational, type I or type II), higher than normal glucose level, hypertension (e.g. chronic or non-chronic), excessive or sudden weight gain, higher than normal weight, obesity, higher than normal body mass index (BMI), abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results (Pap smear), prior preeclampsia episodes (i.e. personal history of PE), familial history of PE, PE in prior pregnancies, renal disease, thrombophilia, or any combination thereof.

[0007] The disclosure provides a method for confirming preeclampsia in a subject, preferably a pregnant subject, comprising performing a test on a sample derived from the subject, wherein the test comprises measuring the levels of a plurality of markers and using the levels to confirm PE with a sensitivity, specificity and/or negative predictive value (NPV) of at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or more, or a ROC value of at

least 0.80, 0.85, 0.90, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 0.995 or more. The one or more symptoms associated with PE can be diabetes (e.g. gestational, type I or type II), higher than normal glucose level, hypertension (e.g. chronic or non-chronic), excessive or sudden weight gain, higher than normal weight, obesity, higher than normal body mass index (BMI), abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results (Pap smear), prior preeclampsia episodes (i.e. personal history of PE), familial history of PE, PE in prior pregnancies, renal disease, thrombophilia, or any combination thereof.

[0008] The disclosure further provides a method for confirming if a subject, preferably a pregnant subject, does not have preeclampsia, the method comprising: evaluating a sample derived from the subject to determine levels of a plurality of biomarkers in the sample, using the levels of the plurality of biomarkers to calculate an index representative of a likelihood that the subject does not have preeclampsia; and based upon the index, confirming if the subject does not have preeclampsia.

[0009] A method for confirming if a subject, preferably a pregnant subject, does have preeclampsia, the method comprising: (a) evaluating a sample derived from the subject to determine levels of a plurality of biomarkers in the sample, (b) using the levels of the plurality of biomarkers to calculate an index representative of a likelihood that the subject does have preeclampsia; and (c) based upon the index, confirming if the subject does have preeclampsia.

[0010] The disclosure further provides method for confirming if a subject, preferably a pregnant subject, does not have preeclampsia, the method comprising: (a) evaluating a sample derived from the subject to determine a level of a biomarker in the sample; and (b) using the level of the biomarker to calculate an index representative of the likelihood that the subject does not have preeclampsia, wherein the biomarker is not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) or heme.

[0011] The disclosure provides a method for confirming if a subject, preferably a pregnant subject, does have preeclampsia, the method comprising: (a) evaluating a sample derived from the subject to determine a level of a biomarker in the sample; and (b) using the level of the biomarker to calculate an index representative of the likelihood that the subject does have preeclampsia, wherein the biomarker is not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) or heme.

[0012] The disclosure further provides a method for confirming if a subject, preferably a pregnant subject, having at least one symptom associated with PE has PE, the method comprising: (a) evaluating a sample derived from the subject to determine a level of one or more biomarkers in the sample; and (b) calculating an index representative of a likelihood that the woman does have PE using the levels of the one or more biomarkers to calculate an index, wherein the one or more biomarkers are not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) or heme.

[0013] The disclosure further provides a method for confirming if a subject, preferably a pregnant subject, not having at least one symptom associated with PE does not have PE, the method comprising: (a) evaluating a sample derived from the subject to determine a level of one or more biomarkers in the sample; and (b) calculating an index representative of a likelihood that the subject does not have PE using the levels of the one or more biomarkers to calculate an index, wherein the one or more biomarkers are not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) or heme.

[0014] The disclosure further provides a method for diagnosing or confirming a presence of preeclampsia in a subject, preferably a pregnant subject, the method comprising: (a) performing a plurality of different assays that determine a level of fibronectin in a sample

derived from the subject; and (b) evaluating the sample and using the levels from the plurality of different assays to diagnose or confirm the existence of preeclampsia and calculate an index.

[0015] The disclosure further provides a method for confirming that a subject, preferably a pregnant subject, does not have preeclampsia, the method comprising: performing a plurality of different assays that determine a level of fibronectin in a sample derived from the subject; and evaluating the sample and using the levels from the plurality of assays to confirm the subject does not have preeclampsia and calculate an index.

[0016] The disclosure further provides a method for diagnosing or confirming a presence of preeclampsia in a subject, preferably a pregnant subject, the method comprising: (a) performing at least one assay which utilizes an antibody which binds fibronectin or an antibody that selectively binds a same antigen of fibronectin as the antibody, wherein the binding of the antibody determines a level of fibronectin in a sample derived from the subject; and (b) evaluating the sample and using the level of fibronectin from the at least one assay to diagnose or confirm the existence of preeclampsia and calculate an index.

[0017] The disclosure further provides a method for diagnosing or confirming a presence of preeclampsia in a subject, preferably a pregnant subject, the method comprising: (a) measuring a level of a ratio of sFlt-1 and PlGF (PLGF) and a level a plurality of different biomarkers in a sample derived from the subject, wherein none of the different biomarkers is ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) or heme; and (b) evaluating the sample and using the level from step (a) to determine an index to diagnose or confirm the presence of preeclampsia and calculate an index.

[0018] The disclosure further provides a method for diagnosing, monitoring, characterizing or confirming preeclampsia by evaluating a sample derived from a subject, preferably a pregnant subject, by using the levels of at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 markers (e.g., biomarkers) selected from Figures 5A-5F (or Table 2) to calculate an index value, wherein

the index value is used to determine a diagnosis, relative level, or characterization preeclampsia in the subject.

[0019] The disclosure further provides a method for confirming a subject, preferably a pregnant subject, does not have preeclampsia consisting of: measuring a level of a ratio of sFlt-1 and PlGF and a level of a plurality of different biomarkers in a sample derived from the subject, wherein none of the different biomarkers is ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) or heme; and evaluating the sample and using the level from step (a) to determine an index to confirm the absence of preeclampsia and calculate an index.

[0020] The disclosure further provides a method for distinguishing a subject having preeclampsia from a subject not having preeclampsia but having symptoms associated with preeclampsia. Such symptoms associated with preeclampsia include, e.g., chronic hypertension, gestational hypertension, autoimmune disorders and/or gestational diabetes. The methods and tests herein have a specificity, sensitivity and/or negative predictive value (NPV) of at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%. The methods and tests herein preferably distinguish between a subject having preeclampsia from a subject not having preeclampsia but having symptoms associated with preeclampsia with a ROC value or area under the curve value of at least 0.8, 0.85, 0.9, or 0.95. The methods herein comprise measuring the level of a plurality of different biomarkers (e.g., such as those selected from the list in Figures 5A-5F (or Table 2)) in a sample derived from a subject, generating an index using the levels of the different index, and using the index as a means to confirm the presence of preeclampsia, absence of preeclampsia, and/or severity of preeclampsia.

[0021] The disclosure further provides a method for confirming the presence, absence or severity of preeclampsia in a subject, preferably a pregnant subject, the method comprising: utilizing a monoclonal antibody that selectively binds fibronectin to determine the levels of fibronectin in a sample derived from the subject, generating a report indicating the presence, absence or severity of preeclampsia based on the levels and containing an index; evaluating

the sample; and based upon the index, suggesting a treatment for preeclampsia, , wherein the treatment involves aspirin, preterm labor, treatment with anti-hypertensive or anti-preeclampsia drugs, or bedrest.

[0022] The disclosure further provides a method for confirming the presence, absence or severity of preeclampsia in a sample derived from a subject, preferably a pregnant subject, the method comprising: utilizing an antibody directed to the antigen of the fibronectin antibody in at least one fibronectin ELISA kit to analyze and evaluate a sample derived from the subject.

[0023] The disclosure further provides a method for confirming the presence, absence or severity of preeclampsia in a subject, preferably a pregnant subject, the method comprising: performing at least one assay which utilizes an antibody that selectively binds fibronectin, a portion of fibronectin, a part of fibronectin, or a fragment of fibronectin, wherein the binding of the antibody determines the level of fibronectin in a sample derived from the subject; evaluating the sample; and using the level of fibronectin from the at least one assay to confirm the presence, absence, or severity of preeclampsia and calculate an index.

[0024] The disclosure further provides a test for confirming an absence of preeclampsia in a subject, preferably a female subject, wherein the test measures one or more biomarkers from a sample derived from the subject, wherein the test has an overall ROC value of at least 0.8. In some examples, the test measures one or more biomarkers from a sample derived from a subject, wherein the test has an overall ROC value of at least 0.7, 0.8, 0.85, 0.9, 0.95, 0.96, 0.97, 0.98, 0.99 or more. In some examples, the test measures one or more biomarkers from a sample derived from a subject and has an overall ROC value of at 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.9, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, 0.995 or more.

[0025] The disclosure further provides a kit for confirming, diagnosing, prognosing, monitoring or characterizing preeclampsia in a subject, preferably a pregnant subject, said kit comprising at least two reagents that are specific for determining level of fibronectin in a sample derived from the subject.

[0026] The disclosure further provides a business method comprising the step of determining presence, absence, forecast, severity or character of preeclampsia in a subject, preferably a pregnant subject,, said method comprising the steps of: (a) evaluating levels of

sFLT-1, PlGF and a plurality of different biomarkers in a sample derived from the subject, wherein the none of the different biomarkers is ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) or heme, (b) determining a biomarker index value, said index comprising sFLT-1/PlGF and the addition of a plurality of different biomarkers, (c) employing said biomarker index to provide a preeclampsia determination, confirmation of preeclampsia diagnosis, confirmation of preeclampsia absence, prognosis of preeclampsia, or characteristics of preeclampsia, and (d) providing a report in exchange for a fee, wherein the report indicates the index value based on the analysis of said biomarkers, and specifies whether said subject is at low risk of preeclampsia, high risk of preeclampsia, or has preeclampsia.

[0027] The disclosure further provides a system for confirming , diagnosing, prognosing, monitoring or characterizing preeclampsia in a subject, preferably a pregnant subject, comprising: (a) an input module for receiving as an input levels of sFLT-1, PlGF and a plurality of different biomarkers, (b) a processor optionally configured to perform a log transformation of said levels to obtain log transformed levels, normalize each of said log transformed levels to normalized levels, adjust each of said normalized levels to a weighted normalized level, total each of the adjusted levels, average each of the adjusted levels; and provide a preeclampsia index based on said score wherein the index score comprises sFLT-1/PlGF and an addition of the plurality of different biomarkers. In some instances, the log transformation is a natural, common, binary, rational or irrational log transformation. In some examples, the log transformation is $\log_2$, $\log_{10}$ or $\log_e$ transformation. In some instances, the log transformation is $\log_b$, where the logarithmic base is any real number (including natural numbers, rational number or irrational number).

[0028] This disclosure further provides a method for confirming that a subject, preferably a pregnant subject, does not have preeclampsia comprising: evaluating a plurality of biomarkers in a sample derived from the subject to confirm the subject does not have preeclampsia wherein the confirmation has a specificity of greater than 90% or has an 0.9 AUC and is used to calculate an index.

[0029] Some embodiments of this disclosure are:

1. A method for determining the severity of preeclampsia or for confirming the presence or absence of preeclampsia in a female subject comprising:

a) measuring levels of one or more biomarkers in a sample derived from the pregnant female;

b) calculating an index based on the levels of the one or more biomarkers; and

c) confirming whether the pregnant female is experiencing preeclampsia or whether the pregnant female is not experiencing preeclampsia, based on the index.

2. A method for diagnosing, pronging, monitoring, characterizing, determining the severity of preeclampsia or confirming the presence or absence of preeclampsia in a female subject the presence or absence of preeclampsia in a female subject comprising:

a) measuring levels of one or more biomarkers in a sample derived from the pregnant female;

b) comparing the levels of one or more biomarkers to a respective recombinant protein level; and

c) confirming whether the pregnant female is experiencing preeclampsia or whether the pregnant female is not experiencing preeclampsia, based on the comparing.

3. The method of claim 2, further comprising calculating an index based on the levels of the one or more biomarkers.

4. A method for diagnosing, pronging, monitoring, characterizing, determining the severity of preeclampsia or confirming the presence or absence of preeclampsia in a female subject comprising:

a) measuring levels of fibronectin (FN) and two or more biomarkers in a sample derived from the pregnant female, wherein at least two of the two or more biomarkers are different from fibronectin,

b) calculating an index based on the levels of fibronectin and the two or more biomarkers; and

c) confirming whether the female subject is experiencing preeclampsia or whether the female subject is not experiencing preeclampsia, based on the comparing.

5. The method of claim 4, wherein the two or more biomarkers are selected from the group consisting of sFLT-1, P1GF, ADAM-12, HPX and PAPP-A.

5 6. The method of claim 4, wherein the one or more biomarkers are sFLT-1, P1GF and PAPP-A.

7. The method of claim 4, wherein the one or more biomarkers are sFLT-1, P1GF, PAPP-A and ADAM-12.

8. The method of claim 4, wherein the one or more biomarkers are sFLT-1, P1GF, PAPP-A and HPX.

9. The method of claim 4, wherein the one or more biomarkers are P1GF, PAPP-A and ADAM-12.

10. The method of claim 4, wherein the one or more biomarkers are sFLT-1 and P1GF.

11. The method of claim 4, wherein the one or more biomarkers are P1GF and PAPP-A.

12. The method of claim 4, wherein the one or more biomarkers are sFLT-1, P1GF and ADAM-12.

13. The method of claim 4, wherein the one or more biomarkers are sFLT-1 and ADAM-12.

14. The method of claim 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 further comprising comparing the index to a threshold value.

15. A method for confirming preeclampsia or the absence of preeclampsia in a female subject comprising:

a) measuring levels of fibronectin (FN) or FN fragment in a sample derived from the female subject using a monoclonal antibody that selectively binds FN or FN fragment; and

b) confirming whether the female subject is experiencing preeclampsia or whether the female subject is not experiencing preeclampsia, wherein the confirming is based on the comparing.

16. The method of claim 15, further comprising measuring levels of two or more biomarkers in a sample derived from the female subject.

17. The method of claim 16, wherein the two or more biomarkers are selected from the group consisting of sFLT-1, P1GF, ADAM-12, HPX and PAPP-A.

5 18. The method of claim 16, wherein the one or more biomarkers are sFLT-1, P1GF and PAPP-A.

19. The method of claim 16, wherein the one or more biomarkers are sFLT-1, P1GF, PAPP-A and ADAM-12.

10 20. The method of claim 16, wherein the one or more biomarkers are sFLT-1, P1GF, PAPP-A and HPX.

21. The method of claim 16, wherein the one or more biomarkers are P1GF, PAPP-A and ADAM-12.

22. The method of claim 16, wherein the one or more biomarkers are sFLT-1 and P1GF.

15 23. The method of claim 16, wherein the one or more biomarkers are P1GF and PAPP-A.

24. The method of claim 16, wherein the one or more biomarkers are sFLT-1, P1GF and ADAM-12.

20 25. The method of claim 16, wherein the one or more biomarkers are sFLT-1 and ADAM-12.

26. The method of claim 15, further comprising calculating an index based on the levels of the bound monoclonal antibodies.

25 27. The method of claim 16, 18, 19, 20, 21, 22, 23, 24 or 25 further comprising calculating an index based on the levels of the bound monoclonal antibodies and the two or more biomarkers.

28. The method of claims 26 or 27 further comprising comparing the index to a threshold value, wherein the index is indicative of the presence or absence of preeclampsia in a female subject.

29. A method for diagnosing, pronging, characterizing, monitoring, determining the severity of preeclampsia or confirming preeclampsia or the absence of preeclampsia in a female subject comprising:

- 5 a) measuring levels of sFLT, PlGF and one or more biomarkers in a sample derived from the pregnant female, wherein the one or more biomarker is different from only VEGF, wherein VEGF excludes VEGF R-1.
- b) calculating an index based on the levels of sFLT, PlGF and the one or more biomarkers; and
- 10 c) confirming whether the female subject is experiencing preeclampsia or whether the female subject is not experiencing preeclampsia, based on the index.

30. The method of claim 29, wherein the one or more biomarkers are selected from the group consisting of fibronectin (FN), ADAM-12, HPX and PAPP-A.

31. The method of claim 29, wherein the one or more biomarkers are ADAM-12.

32. The method of claim 29, wherein the one or more biomarkers are PAPP-A.

15 33. The method of claim 29, wherein the one or more biomarkers are fibronectin (FN).

34. The method of claim 29, wherein the one or more biomarkers are fibronectin (FN) and PAPP-A.

20 35. The method of claim 29, wherein the one or more biomarkers are fibronectin (FN) and ADAM-12.

36. The method of claim 29, wherein the one or more biomarkers are fibronectin (FN), ADAM-12 and PAPP-A.

37. The method of claim 29, wherein the one or more biomarkers are fibronectin (FN), HPX and PAPP-A.

25 38. The method of claims 29, 30, 32, 33, 34, 35, 36 or 37 further comprising comparing the index to a threshold value.

39. A method for diagnosing, pronging, characterizing, monitoring, determining the severity of preeclampsia or confirming preeclampsia or the absence of preeclampsia in a female subject comprising:

- a) measuring levels of least one fibronectin (FN) fragment in two different assays, wherein the assays determine the level of FN in a sample derived from the pregnant female; and
- b) diagnosing, pronging, characterizing, monitoring, determining the severity of preeclampsia or confirming preeclampsia or the absence of preeclampsia in a female subject, based on the FN levels measured in the two different assays.

40. The method of claim 39, wherein each of the different assays utilizes a different monoclonal antibody.

41. The method of claim 40, further comprising measuring levels of one or more biomarkers in the sample derived from the pregnant female, wherein the one or more biomarkers are different from fibronectin (FN).

42. The method of claim 41, wherein the one or more biomarkers are selected from the group consisting of sFLT-1, P1GF, ADAM-12, HPX and PAPP-A.

43. The method of claim 40, wherein the one or more biomarkers are sFLT-1 and P1GF.

44. The method of claim 40, wherein the one or more biomarkers are sFLT-1, P1GF and PAPP-A.

45. The method of claim 40, wherein the one or more biomarkers are sFLT-1, P1GF and ADAM-12.

46. The method of claims 41, 43, 44 or 45, further comprising, calculating an index based on the levels of the bound monoclonal antibodies and the two or more biomarkers.

47. The method of claim 1, 3, or 46 further comprising comparing the index to a threshold value.

48. The method of claim 14, 28, 38, 47, wherein the index is calculated by a real function algorithm for totaling the one or more biomarker levels comprising one or more variables multiplied by one or more corresponding weight factors, wherein the level of each of the one or more biomarker levels is input into a specific variable of the one or more variables,

wherein the corresponding weight factor is unique for each specific variable,
wherein at least one of the corresponding weight factor is different from one.

49. The method of claim 48, wherein the algorithm comprises at least one binary operation.

5 50. The method of claim 49, wherein at least one binary operation is division.

51. The method of claim 49, wherein at least one binary operation is addition or subtraction.

52. The method of claim 1, 3, 4, 15, 29 or 39, further comprising generating a report indicative of the presence or absence of preeclampsia.

10 53. The method of claim 1, 3, 4, 15, 29 or 39, wherein the method excludes measuring blood pressure, sugar blood level, urine protein level, familial preeclampsia history, or weight gain.

54. The method of claim 1, 3, 4, 15, 29 or 39, wherein the female subject was diagnosed as having least one of the symptoms of the group consisting of: blood
15 pressure above 140/90 mm Hg, sugar blood level above 100 mg/dL while fasting, urine protein level more than 5 gram in a 24 hour collection or more than 3+ on two random urine samples collected at least four hours apart, weight gain of more than two pounds in a week, platelets level below 155,000(per microliter) in the second trimester or below 145,000 (per microliter) during the third trimester , oliguria of less
20 than 400 mililiters in 24 hours, high body-mass index above 25, familial preeclampsia history or preeclampsia, maternal history of preeclampsia, pulmonary edema, cyanosis and change in vision.

55. The method of claim 1, or 2, wherein the one or more biomarkers are selected from the group consisting of sFLT-1, PlGF, fibronectin (FN), ADAM-12, HPX and
25 PAPP-A.

56. The method of claim 1, 3, 4, 15, 29 or 39, wherein the biomarkers exclude ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB),

fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) and heme.

57. The method of claim 14, 38 or 47, wherein the comparing is comparing of the one or more biomarkers to a single pregnant female or to a group of pregnant females experience PE and a group of pregnant females not experiencing PE.

58. The method of claim 57, wherein the single pregnant female is the female being tested.

59. The method of claim 57, wherein the comparing comprises comparing of the one or more biomarkers to a respective recombinant protein index value.

60. The method of claim 1, 3, 4, 15, 29 or 39, wherein the biomarkers comprise one or more proteins or protein fragments.

61. The method of claim 1, 3, 4, 15, 29 or 39, wherein the biomarkers comprise polynucleotides.

62. The method of claim 1, 3, 4, 15, 29 or 39, wherein the measuring is measuring by a method selected from the group consisting of an immunological assay, mass spectrometry, chromatography, nephelometry, radial immunodiffusion and single radial immunodiffusion assay.

63. The method of claim 1, 3, 4, 15, 29 or 39, wherein the measuring is measuring by an immunological assay.

64. The method of claim 63, wherein the immunological assay is selected from the group consisting of ELISA, sandwich ELISA, competitive ELISA and IgM antibody capture ELISA.

65. A kit for diagnosing, prognosing, monitoring, characterizing, determining the severity of preeclampsia or for confirming the presence or absence of preeclampsia in a pregnant female, the kit comprising: at least two different reagents that are specific for determining a level of fibronectin (FN) in a sample from the pregnant female.

66. The kit of claim 65, further comprising two or more reagents measuring levels of two or more biomarkers in a sample derived from the female subject.

67. The kit of claim 66, wherein the two or more biomarkers are sFLT-1, P1GF and PAPP-A.

68. The kit of claim 66, wherein the two or more biomarkers are sFLT-1, P1GF, PAPP-A and ADAM-12.

69. The kit of claim 66, wherein the two or more biomarkers are sFLT-1, P1GF, PAPP-A and HPX.

5 70. The kit of claim 66, wherein the two or more biomarkers are P1GF, PAPP-A and ADAM-12.

71. The kit of claim 66, wherein the two or more biomarkers are sFLT-1 and P1GF.

10 72. The kit of claim 66, wherein the two or more biomarkers are P1GF and PAPP-A.

73. The kit of claim 66, wherein the two or more biomarkers are sFLT-1, P1GF and ADAM-12.

74. The kit of claim 66, wherein the two or more biomarkers are sFLT-1 and ADAM-12.

15 75. The kit of claim 65 or 66, wherein the kit does not include a reagent measure the levels of biomarkers selected from the group consisting of ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha
20 (FGA), pikachurin (EGFLAM), free beta hPC and heme.

76. A kit for confirming the presence or absence of preeclampsia in a pregnant female, the kit comprises

a) a first reagent specific for determining level of PAPP-A; and

b) a second reagent specific for determining ADAM12.

25 77. The kit of claim 76, further comprising one or more reagents measuring levels of one or more biomarkers in a sample derived from the female subject.

78. The kit of claim 77, wherein the one or more biomarkers are sFLT-1, P1GF and fibronectin (FN).

30 79. The kit of claim 77, wherein the one or more biomarkers are P1GF and fibronectin (FN).

80. The kit of claim 76 or 77, wherein the kit does not include a reagent measuring the levels of biomarkers selected from the group consisting of ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free beta hPC and heme.

81. A kit for diagnosing, prognosing, monitoring, characterizing, determining the severity of preeclampsia, or for confirming the presence or absence of preeclampsia in a pregnant female, the kit comprises:

- a) a first reagent specific for determining level of one of sFLT-1 or P1GF;
- b) a second reagent specific for determining fibronectin (FN); and
- c) a third reagent specific for determining a level of a biomarker that is different from the biomarker determined by the first and second reagent.

82. The kit of claim 81, wherein the first reagent determines the levels of sFLT-1, the third reagent determines the levels of P1GF, and the kit further comprises a fourth reagent determining the level of a biomarker different from sFLT-1, P1GF and FN.

83. The kit of claim 82, wherein the fourth reagent determines the levels of PAPP-A, HPX or ADAM12.

84. The kit of claim 81, 82 or 83 wherein the kit does not include reagents which determine the levels of the biomarkers selected from the group consisting of ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free beta hPC and heme.

85. A test for confirming the presence or the absence of preeclampsia in a pregnant female, wherein the test measures one or more biomarkers from a sample derived from the pregnant female and has an overall ROC value of at least 0.8 or more.

86. The test of claim 85, wherein the overall ROC value is at least 0.9 or more.

87. The test of claim 85, wherein the overall ROC value is at least 0.95 or more.

88. The test of claim 85, wherein the overall ROC value is at least 0.98 or more.

89. The test of claim 85, wherein the overall ROC value is at least 0.984 or more.

90. A computer readable medium having an executable logic for diagnosing,
pronging, characterizing, monitoring, determining the severity of preeclampsia or
confirming the presence or absence of preeclampsia in a female subject comprising:

(a) input fields for providing levels of one or more biomarkers,
(b) algorithm for adjusting the levels of one or more biomarkers to a training set,
thereby providing one or more adjusted biomarker levels;

(c) algorithm comprising at least one binary operation performed using the
adjusted biomarker levels, wherein the algorithm is a real function that results in an
index value; and

(d) output field presenting the index value, wherein the index value indicates
diagnosis, prognosis, characterization, monitor , determining the severity of
preeclampsia or confirmation of the presence or absence of preeclampsia in a female
subject.

91. A computer readable medium having an executable logic for confirming the
presence or absence of preeclampsia in a female subject comprising:

(e) input fields for providing levels of one or more biomarkers,

(f) algorithm for adjusting the levels of one or more biomarkers to a control
value, thereby providing one or more adjusted biomarker levels;

(g) algorithm comprising adding or subtracting the one or more adjusted
biomarker levels, wherein the algorithm is a real function that results in an index
value; and

(h) output field presenting the index value, wherein the index value indicates the
absence or presence of preeclampsia in the female subject.

92. A computer readable medium having an executable logic for confirming the
presence or absence of preeclampsia in a female subject comprising:

(i) input fields for providing levels of one or more biomarkers,

(j) algorithm for adjusting the levels of one or more biomarkers to a control value, thereby providing one or more adjusted biomarker levels;

(k) algorithm comprising a ratio between two of the one or more adjusted biomarker levels, wherein the algorithm is a real function that results in an index value; and

(l) output field presenting the index value, wherein the index value indicates the absence or presence of preeclampsia in the female subject.

93. The computer readable medium of claim 90, 91 or 92 wherein the real function is a complex statistical algorithm.

94. The computer readable medium of claim 90, 91 or 92, wherein the real function comprises at least one additional binary operation.

95. The computer readable medium of claim 94, wherein the real function comprises a variable multiplied by a corresponding weight factor.

96. The computer readable medium of claim 95, wherein the level of each biomarker of the one or more biomarkers is input into a specific variable corresponding to the specific biomarker, and wherein the corresponding weight factor is unique for each specific variable or unique for each specific ratio of two variables.

97. The computer readable medium of claim 90, further comprising an algorithm for averaging each of the one or more adjusted biomarker levels.

The computer readable medium of claim 90, wherein the computer readable medium further comprises an algorithm for performing a logarithmic transformation of the levels to obtain log transformed levels; algorithm for normalizing each of the log transformed levels to normalized levels; algorithm for adjusting each of the normalized levels to a weighted normalized level.

98. A computer readable medium having an executable logic for diagnosing, prognosing, monitoring, characterizing, determining the severity of preeclampsia or for confirming the presence or absence of preeclampsia in a pregnant female comprising:

(a) input fields for providing levels of one or more biomarkers,

(b) adjusting algorithm for adjusting the levels of each of the one or more biomarkers to a corresponding control value, wherein the adjusting algorithm provides one or more adjusted biomarker levels;

(c) real function algorithm for manipulating the one or more adjusted biomarker levels comprising one or more variables multiplied by one or more corresponding weight factors,

wherein the level of each of the one or more adjusted biomarker levels is input into a specific variable of the one or more variables,

wherein the corresponding weight factor is unique for each specific variable,

wherein at least one of the corresponding weight factor is different from one; and

(d) output field presenting the index value, wherein the index value indicates the absence or presence of preeclampsia in the female subject.

99. The computer readable medium of claims 90, 91, 92 or 98 wherein the control value is established using a training set.

100. The computer readable medium of claim 98, wherein the training set is based on a model.

101. The computer readable medium of claim 98, wherein the training set is based on real values obtained from subjects.

102. The computer readable medium of claim 98, wherein the subjects are at least 150 subjects or more.

103. The computer readable medium of claim 98, wherein the actual subjects comprise complex subjects.

104. The computer readable medium of claim 98, wherein the complex subjects comprise at least 10% of the total subjects used for the training set.

105. The computer readable medium of claim 98, wherein the algorithm comprises at least one binary operation.

106. The computer readable medium of claim 105, wherein at least one binary operation is division.

107. The computer readable medium of claim 105, wherein at least one binary operation is addition or subtraction.

[0030] Some embodiments of this disclosure are:

1. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
 - a) measuring levels of one or more biomarkers in a sample derived from the female subject;
 - b) calculating an index based on the levels of the one or more biomarkers; and
 - c) confirming the presence or absence of preeclampsia in the female subject, based on the index.
2. The method of claim 1, wherein the measuring levels of one or more biomarkers comprise measuring levels of three or more biomarkers.
3. The method of claim 1, wherein the measuring levels of one or more biomarkers comprise measuring levels of four or more biomarkers.
4. The method of claim 1, wherein the measuring levels of one or more biomarkers comprise measuring levels of five or more biomarkers.
5. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
 - a) measuring levels of one or more biomarkers in a sample derived from the female subject;
 - b) comparing the levels of the one or more biomarkers to a respective recombinant protein level or to a standard value; and
 - c) confirming the presence or absence of preeclampsia in the female subject, based on the comparing.
6. The method of claim 5, further comprising calculating an index based on the levels of the one or more biomarkers.
7. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
 - a) measuring levels of fibronectin (FN) and two or more biomarkers in a sample derived from the female subject, wherein at least two of the two or more biomarkers are different from fibronectin,

- b) calculating an index based on the levels of FN and the two or more biomarkers; and
 - c) confirming the presence or absence of preeclampsia in the female subject, based on the index.
- 5 8. The method of claim 7, wherein the two or more biomarkers are selected from the group consisting of sFLT-1, P1GF, ADAM-12, HPX and PAPP-A.
9. The method of claim 7, wherein the biomarkers are sFLT-1, P1GF and PAPP-A.
- 10 10. The method of claim 7, wherein the biomarkers are sFLT-1, P1GF, PAPP-A and ADAM-12.
11. The method of claim 7, wherein the biomarkers are sFLT-1, P1GF, PAPP-A and HPX.
12. The method of claim 7, wherein the biomarkers are P1GF, PAPP-A and ADAM-12.
- 15 13. The method of claim 7, wherein the biomarkers are sFLT-1 and P1GF.
14. The method of claim 7, wherein the biomarkers are P1GF and PAPP-A.
15. The method of claim 7, wherein the biomarkers are sFLT-1, P1GF and ADAM-12.
16. The method of claim 7, wherein the biomarkers are sFLT-1 and ADAM-12.
- 20 17. The method of claim 7, wherein the biomarkers are P1GF, ADAM-12, sFLT1, PAPP-A2, and HPX.
18. The method of claim 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17, further comprising comparing the index to a threshold value.
19. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
- 25 a) measuring levels of fibronectin (FN) or FN fragment in a sample derived from the female subject using a monoclonal antibody that selectively binds the FN or the FN fragment;
- 30 b) comparing the levels of fibronectin (FN) or FN fragment to a respective recombinant protein level or to a standard value; and

- c) confirming the presence or the absence of preeclampsia, based on the comparing.
20. The method of claim 19, further comprising measuring levels of two or more biomarkers in a sample derived from the female subject.
- 5 21. The method of claim 20, wherein the two or more biomarkers are selected from the group consisting of sFLT-1, P1GF, ADAM-12, HPX and PAPP-A.
22. The method of claim 20, wherein the biomarkers are sFLT-1, P1GF and PAPP-A.
23. The method of claim 20, wherein the biomarkers are sFLT-1, P1GF, PAPP-A and ADAM-12.
- 10 24. The method of claim 20, wherein the biomarkers are sFLT-1, P1GF, PAPP-A and HPX.
25. The method of claim 20, wherein the biomarkers are P1GF, PAPP-A and ADAM-12.
- 15 26. The method of claim 20, wherein the biomarkers are sFLT-1 and P1GF.
27. The method of claim 20, wherein the biomarkers are P1GF and PAPP-A.
28. The method of claim 20, wherein the biomarkers are sFLT-1, P1GF and ADAM-12.
29. The method of claim 20, wherein the biomarkers are sFLT-1 and ADAM-12.
- 20 30. The method of claim 20, wherein the biomarkers are P1GF, FN, ADAM-12, sFLT1, PAPP-A2, and HPX.
31. The method of claim 19, further comprising calculating an index based on levels of bound monoclonal antibodies.
32. The method of claim 20, 22, 23, 24, 25, 26, 27, 28 or 29 further comprising calculating an index based on levels of (1) bound monoclonal antibodies and (2) the two or more biomarkers.
- 25 33. The method of claims 31 or 32 further comprising comparing the index to a threshold value, wherein the index is indicative of the presence or absence of preeclampsia in the female subject.

34. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
- 5 a) measuring levels of sFLT, PlGF and one or more biomarkers in a sample derived from the female subject, wherein the one or more biomarker is different from VEGF, wherein VEGF excludes VEGF R-1;
- b) calculating an index based on the levels of sFLT, PlGF and the one or more biomarkers; and
- c) confirming the presence or absence of preeclampsia in the female subject, based on the index.
- 10 35. The method of claim 34, wherein the one or more biomarkers are selected from the group consisting of fibronectin (FN), ADAM-12, HPX and PAPP-A.
36. The method of claim 34, wherein the biomarkers are ADAM-12.
37. The method of claim 34, wherein the biomarkers are PAPP-A.
38. The method of claim 34, wherein the biomarkers are fibronectin (FN).
- 15 39. The method of claim 34, wherein the biomarkers are fibronectin (FN) and PAPP-A.
40. The method of claim 34, wherein the biomarkers are fibronectin (FN) and ADAM-12.
41. The method of claim 34, wherein the biomarkers are fibronectin (FN),
- 20 ADAM-12 and PAPP-A.
42. The method of claim 34, wherein the biomarkers are fibronectin (FN), HPX and PAPP-A.
43. The method of claim 34, wherein the biomarkers are FN, ADAM-12, PAPP-A2, and HPX.
- 25 44. The method of claims 34, 35, 36, 37, 38, 39, 40, 41 or 42 further comprising comparing the index to a threshold value.
45. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
- 30 a. measuring levels of biomarkers consisting of: sFLT and PlGF;
- b. calculating an index based on the levels of sFLT and PlGF; and

- c. confirming the presence or absence of preeclampsia in the female subject, based on the index.
46. The method of claim 45, wherein the calculating comprises multiplying each of the measured levels of sFLT and P1GF by a unique weight factor, and applying one or more binary functions to weighted measured levels of sFLT and P1GF.
47. A method for diagnosing, prognosing, characterizing, monitoring, determining a severity of, confirming a presence of, or confirming an absence of preeclampsia in a female subject, the method comprising:
- a) measuring levels of least one fibronectin (FN) fragment in two different assays, wherein the assays determine the level of FN in a sample derived from the female subject; and
- b) diagnosing, prognosing, characterizing, monitoring, determining the severity of, confirming the presence of, or confirming the absence of preeclampsia in the female subject, based on the levels of at least one FN fragment measured in the two different assays.
48. The method of claim 47, wherein each of the two different assays utilizes a different monoclonal antibody.
49. The method of claim 48, further comprising measuring levels of one or more biomarkers in the sample derived from the female subject, wherein the one or more biomarkers are different from fibronectin (FN).
50. The method of claim 49, wherein the biomarkers are selected from the group consisting of sFLT-1, P1GF, ADAM-12, HPX and PAPP-A.
51. The method of claim 49, wherein the biomarkers are sFLT-1 or P1GF.
52. The method of claim 49, wherein the biomarkers are sFLT-1, P1GF or PAPP-A.
53. The method of claim 49, wherein the biomarkers are sFLT-1, P1GF or ADAM-12.
54. The method of claim 49, wherein the biomarkers are P1GF, ADAM-12, sFLT1, PAPP-A2, and HPX.

55. The method of claims 49, 50, 51, 52, 53, or 54, further comprising calculating an index based on the levels of (1) bound monoclonal antibodies and (2) the one or more biomarkers.
56. The method of claims 1, 5, 7, 34, or 47, wherein the measuring comprises:
5 coating an immunoassay plate with antibodies exhibiting an affinity for a biomarker to be measured; and
coating the immunoassay plate with a non-specific blocking protein.
57. The method of claim 56, further comprising:
mixing labeled biomarkers with the sample resulting in a mixture; and
10 adding the mixture to an immunoassay plate.
58. The method of claim 56, further comprising:
introducing the sample to the immunoassay plate; and
introducing secondary conjugated antibodies to the immunoassay plate.
59. The method of claim 1, 6 or 55 further comprising comparing the index to a
15 threshold value.
60. The method of claim 7, 18, 31, 32, 33, 34, 44 , or 59, wherein the index is calculated by a real function algorithm for totaling measured levels of biomarker levels, wherein the algorithm comprises multiplying one or more variables by one or more corresponding weight factors,
20 wherein the level of each of the biomarker levels is input into a specific variable of the one or more variables,
wherein a corresponding weight factor is unique for each specific variable,
wherein at least one of the one or more corresponding weight factors is different from one.
61. The method of claim 60, wherein the algorithm comprises at least one binary operation.
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62. The method of claim 61, wherein the at least one binary operation is division.
63. The method of claim 61, wherein the at least one binary operation is addition or subtraction.

64. The method of claim 60, wherein the one or more weight factors is a ratio of measured levels of two biomarkers.
65. The method of claim 1, 5, 7, 19, 34 or 47, further comprising generating a report indicating the presence or absence of preeclampsia in the female subject.
66. The method of claim 1, 5, 7, 19, 34 or 47, wherein the method excludes consideration of blood pressure, sugar blood level, urine protein level, familial preeclampsia history, or weight gain.
67. The method of claim 1, 5, 7, 19, 34 or 47, wherein the female subject has at least one symptom in the group consisting of: blood pressure above 140/90 mm Hg, sugar blood level above 100 mg/dL while fasting, urine protein level more than 5 grams in a 24 hour collection or more than 3+ on two random urine samples collected at least four hours apart, weight gain of more than two pounds in a week, platelets level below 155,000 (per microliter) in a second trimester or below 145,000 (per microliter) during a third trimester, oliguria of less than 400 milliliters in 24 hours, high body-mass index above 25, familial history of preeclampsia, pulmonary edema, cyanosis and change in vision.
68. The method of claim 1 or 5, wherein the one or more biomarkers are selected from the group consisting of sFLT-1, P1GF, fibronectin (FN), ADAM-12, HPX and PAPP-A.
69. The method of claim 1, 5, 7, 19, 34 or 47, wherein the biomarkers exclude ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) and heme.
70. The method of claim 18, 44 or 59, wherein the comparing comprises comparing the biomarkers to (1) that of a single pregnant female or a group of

pregnant females having preeclampsia and (2) that of a group of pregnant females not having preeclampsia.

71. The method of claim 70, wherein the single pregnant female is the female subject.

5 72. The method of claim 70, wherein the comparing comprises comparing the biomarkers to a respective recombinant protein index value.

73. The method of claim 1, 5, 7, 19, 34 or 47, wherein biomarkers comprise one or more proteins or protein fragments.

10 74. The method of claim 1, 5, 7, 19, 34 or 47, wherein the biomarkers comprise polynucleotides.

75. The method of claim 1, 5, 7, 19, 34 or 47, wherein the measuring comprises utilizing an immunological assay, mass spectrometry, chromatography, nephelometry, radial immunodiffusion or single radial immunodiffusion assay.

15 76. The method of claim 1, 5, 7, 19, 34 or 47, wherein the measuring comprises measuring by an immunological assay.

77. The method of claim 76, wherein the immunological assay is selected from the group consisting of ELISA, sandwich ELISA, competitive ELISA and IgM antibody capture ELISA.

20 78. A kit for diagnosing, prognosing, monitoring, characterizing, determining a severity of, confirming a presence of, or confirming an absence of preeclampsia in a female subject, the kit comprising: at least two different reagents that are specific for determining a level of fibronectin (FN) in a sample derived from the female subject.

25 79. The kit of claim 78, further comprising two or more reagents for measuring levels of two or more biomarkers in the sample derived from the female subject.

80. The kit of claim 79, wherein the biomarkers are sFLT-1, PlGF and PAPP-A.

81. The kit of claim 79, wherein the biomarkers are sFLT-1, PlGF, PAPP-A and ADAM-12.

82. The kit of claim 79, wherein the biomarkers are sFLT-1, P1GF, PAPP-A and HPX.
83. The kit of claim 79, wherein the biomarkers are P1GF, PAPP-A and ADAM-12.
- 5 84. The kit of claim 79, wherein the biomarkers are sFLT-1 and P1GF.
85. The kit of claim 79, wherein the biomarkers are P1GF and PAPP-A.
86. The kit of claim 79, wherein the biomarkers are sFLT-1, P1GF and ADAM-12.
- 10 87. The kit of claim 79, wherein the biomarkers are sFLT-1 and ADAM-12.
88. The kit of claim 79, wherein the biomarkers are P1GF, FN, ADAM-12, sFLT1, PAPP-A2, and HPX.
89. The kit of claim 78 or 79, wherein the kit does not include a reagent for measuring the levels of biomarkers selected from the group consisting of
- 15 ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free beta hPC and heme.
- 20 90. A kit for confirming the presence or absence of preeclampsia in a female subject, the kit comprising:
- a) a first reagent specific for determining level of PAPP-A; and
- b) a second reagent specific for determining level of ADAM12.
91. The kit of claim 90, further comprising one or more reagents for measuring
- 25 levels of one or more biomarkers in a sample derived from the female subject.
92. The kit of claim 91, wherein the biomarkers are sFLT-1, P1GF and fibronectin (FN).
93. The kit of claim 91 wherein the biomarkers are P1GF and fibronectin (FN).
94. The kit of claim 90 or 91, wherein the kit does not include a reagent for
- 30 measuring levels of biomarkers selected from the group consisting of ferritin

(FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free beta hPC and heme.

95. A kit for diagnosing, prognosing, monitoring, characterizing, determining a severity of, confirming a presence of, or confirming an absence of preeclampsia, the kit comprising:
 - a) a first reagent specific for determining a level of one of sFLT-1 or PlGF;
 - b) a second reagent specific for determining fibronectin (FN); and
 - c) a third reagent specific for determining a level of a biomarker that is different from biomarker determined by the first and second reagent.
96. The kit of claim 95, wherein the first reagent is specific for determining the level of sFLT-1, the third reagent is specific for determining the level of PlGF, and the kit further comprises a fourth reagent specific for determining a level of a biomarker different from sFLT-1, PlGF and FN.
97. The kit of claim 96, wherein the fourth reagent is specific for determining the levels of PAPP-A, HPX or ADAM12.
98. The kit of claim 95, 96 or 97 wherein the kit does not include reagents specific for determining levels of biomarkers selected from the group consisting of ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free beta hPC and heme.
99. A test for confirming a presence or absence of preeclampsia in a female subject, wherein the test measures one or more biomarkers from a sample derived from the female subject, wherein a receiver operating characteristic (ROC) value associated with the biomarkers is at least 0.8.
100. The test of claim 99, wherein the ROC value is at least 0.9.

101. The test of claim 99, wherein the ROC value is at least 0.95.
102. The test of claim 99, wherein the ROC value is at least 0.98.
103. The test of claim 99, wherein the ROC value is at least 0.984.
- 5 104. A test for confirming a presence or absence of preeclampsia in a subject, wherein the test measures one or more biomarkers from a sample derived from the subject, wherein a receiver operating characteristic (ROC) value associated with the biomarkers is greater than a ROC value associated with sFLT/P1GF.
105. The test of claim 104, wherein the female subject exhibits clinical symptoms of preeclampsia.
- 10 106. The test of claim 104, wherein the test comprises measuring a ratio of measured levels of sFLT/P1GF.
107. The test of claim 104, wherein the ratio of measured levels of sFLT/P1GF is normalized, raw, adjusted, or a combination thereof.
108. A system for diagnosing, prognosing, characterizing, monitoring, determining
15 a severity of, confirming a presence of, or confirming an absence of preeclampsia in a female subject, the system comprising:
- (a) an input module for receiving as an input levels of one or more biomarkers;
- (b) a processor configured to:
perform a first algorithm adjusting the levels of the one or more biomarkers to a
20 training set, thereby providing one or more adjusted biomarker levels; and
perform a second algorithm that applies at least one binary operation using the
adjusted biomarker levels, wherein the second algorithm is a real function that
results in an index value; and
- (c) an output module for outputting the index value, wherein the index value
25 indicates diagnosis, prognosis, characterization, a monitored aspect, determination of the severity, confirmation of the presence, or confirmation of the absence, of preeclampsia in the female subject.
109. A system for confirming a presence or absence of preeclampsia in a female subject, the system comprising:
- 30 (a) an input module for receiving as an input levels of one or more biomarkers;

(b) a processor configured to:
perform a first algorithm adjusting the levels of the one or more biomarkers to a control value; and
perform a second algorithm adding or subtracting the one or more adjusted
5 biomarker levels, wherein the second algorithm is a real function that results in an index value; and

(c) an output module for outputting the index value, wherein the index value indicates the absence or the presence of preeclampsia in the female subject.

110. A system for confirming a presence or absence of preeclampsia in a female
10 subject, the system comprising:

(a) an input module for receiving as an input levels of two or more biomarkers;
(b) a processor configured to:

perform a first algorithm adjusting the levels of the two or more biomarkers to a control value, thereby providing two or more adjusted biomarker levels; and

15 perform a second algorithm calculating a ratio between two of the two or more adjusted biomarker levels, wherein the second algorithm is a real function that results in an index value; and

(c) an output module for outputting the index value, wherein the index value indicates the absence or presence of preeclampsia in the female subject.

20 111. The system of claim 108, 109 or 110 wherein the real function comprises a complex statistical algorithm.

112. The system of claim 108, 109 or 110, wherein the real function comprises at least one binary operation.

113. The system of claim 112, wherein the real function comprises a multiplying a
25 variable by a corresponding weight factor.

114. The system of claim 113, wherein a level of each biomarker is input into a specific variable corresponding to the biomarkers, and wherein the corresponding weight factor is unique for each variable or unique for each ratio of two variables.

115. The system of claim 108, wherein the processor is further configured to perform a third algorithm averaging each of the one or more adjusted biomarker levels.

116. The system of claim 108, wherein the processor is further configured to perform a third algorithm applying a logarithmic transformation of the levels to obtain log transformed levels; a fourth algorithm normalizing each of the log transformed levels to normalized levels; and a fifth algorithm adjusting each of the normalized levels to a weighted normalized level.

117. A system for diagnosing, prognosing, monitoring, characterizing, determining a severity of, confirming a presence of, or confirming an absence of preeclampsia in a female subject, the system comprising:

- (a) an input module for receiving as an input levels of one or more biomarkers;
- (b) a processor configured to:

perform an algorithm adjusting the levels of each of the one or more biomarkers to a corresponding control value, thereby providing one or more adjusted biomarker levels;

perform a real function algorithm manipulating the one or more adjusted biomarker levels by multiplying one or more variables by one or more corresponding weight factors, wherein a level of each of the one or more adjusted biomarker levels is input into a specific variable of the one or more variables, wherein the corresponding weight factor is unique for each specific variable, wherein at least one of the corresponding weight factors is different from one; and

- (c) an output module for outputting an index value, wherein the index value indicates diagnosis, prognosis, characterization, a monitored aspect, determination of the severity, confirmation of the presence, or confirmation of the absence, of preeclampsia in the female subject.

118. The system of claims 109, 110 or 117 wherein the control value is established using a training set.

119. The system of claim 117, wherein the training set is based on a model.

120. The system of claim 117, wherein the training set is based on real values obtained from subjects.
121. The system of claim 120, wherein the subjects comprise at least 150 subjects.
122. The system of claim 120, wherein subjects comprise complex subjects.
- 5 123. The system of claim 122, wherein the complex subjects comprise at least 10% of all subjects used for the training set.
124. The system of claim 117, wherein the algorithm applies at least one binary operation.
125. The system of claim 124, wherein the at least one binary operation is division.
- 10 126. The system of claim 124, wherein the at least one binary operation is addition or subtraction.
127. A test for confirming preeclampsia in a subject, wherein the test is able to discern subjects that do not have preeclampsia but have one or more symptoms associated with preeclampsia, from subjects having preeclampsia, with a receiving operating characteristic (ROC) value of at least 0.8.
- 15 128. The test of claim 127, wherein the ROC value is of at least 0.9.
129. The test of claim 127, wherein the one or more symptoms associated with preeclampsia are selected from the group consisting of diabetes, higher than normal glucose level, hypertension, excess or sudden weight gain, overweight, obesity, higher than normal body mass index, abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results, prior preeclampsia episodes, familial history of PE, renal disease and thrombophilia.
- 20 25 130. The test of claim 129, wherein the diabetes is gestational, type I or type II.
131. The test of claim 129, wherein the hypertension is chronic hypertension.
132. A test for confirming preeclampsia in a subject, wherein the test is able to discern subjects not having preeclampsia but having one or more symptoms

associated with preeclampsia, from subjects having preeclampsia, with a sensitivity of at least 80% .

133. A test for confirming preeclampsia in a subject, wherein the test is able to discern subjects not having preeclampsia but having one or more symptoms associated with preeclampsia, from subjects having preeclampsia, with specificity of at least 80%.

134. A test for confirming preeclampsia in a subject, wherein the test is able to discern subjects not having preeclampsia but having one or more symptoms associated with preeclampsia, from subjects having preeclampsia, with a negative predictive value (NPV) of at least 80%.

135. The test of claim 132, 133 or 134, wherein the one or more symptoms associated with preeclampsia are selected from the group consisting of diabetes, higher than normal glucose level, hypertension, excess or sudden weight gain, overweight, obesity, higher than normal body mass index, abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results, prior preeclampsia episodes, familial history of PE, renal disease and thrombophilia.

136. The test of claim 135, wherein the diabetes is gestational, type I or type II.

137. The test of claim 135, wherein the hypertension is chronic hypertension.

138. The test of claim 135, wherein the sensitivity is of at least 90%.

139. The test of claim 133, wherein the specificity is of at least 90%.

140. The test of claim 134, wherein the NPV is of at least 90%.

141. A method for confirming preeclampsia, the method comprising performing a test on a sample derived from a female subject wherein the test comprises measuring levels of a plurality of markers and using the levels of the plurality of markers to confirm preeclampsia with a receiving operating characteristic (ROC) value of at least 0.90.

142. The method of claim 141, wherein the ROC value is at least 0.95.

143. A method for confirming preeclampsia, the method comprising performing a test on a sample derived from a female subject wherein the test comprises measuring levels of a plurality of markers and using the levels of the plurality of markers to confirm preeclampsia with a specificity of at least 80%.
- 5 144. The method of claim 143, wherein the specificity is at least 90%.
145. A method for confirming preeclampsia, the method comprising performing a test on a sample derived from a female subject wherein the test comprises measuring levels of a plurality of markers and using the levels of the plurality of markers to confirm preeclampsia with a sensitivity of at least 80%.
- 10 146. The method of claim 145, wherein the sensitivity is at least 90%.
147. A method for confirming preeclampsia, the method comprising performing a test on a sample derived from a female subject wherein the test comprises measuring levels of a plurality of markers and using said levels to confirm preeclampsia with a negative predictive value of at least 80%.
- 15 148. The method of claim 147, wherein the negative predictive value is at least 90%.
149. The test as in any of claims 127-140, wherein the sample is selected from the group consisting of whole blood, urine, serum and plasma.
150. The method as in one of claims 141-148, wherein the sample is selected from
- 20 the group consisting of whole blood, urine, serum and plasma.
151. The method as in any of claims 1-7, 31, 34, 60, 141, 143 or 147, wherein the biomarker comprises a biomarker of Group-1.
152. The test as in any of claims 30, 126 or 131-134, wherein the biomarker comprises a biomarker of Group-1.
- 25 153. The system as in any of claims 108-110 or 116, wherein the biomarker comprises a biomarker of Group-1.
154. The kit as in any of claims 78, 90 or 95, wherein the biomarker comprises a biomarker of Group-1.
- 30 155. A computer readable medium containing instructions which, when executed by a computer system, cause the computer system to:

receive a first data set pertaining to first levels of a plurality of preeclampsia
biomarkers in a first biological sample derived from a subject at a first point-
in-time;
perform a first analysis on the first levels to obtain a first assessment of preeclampsia
5 in the subject;
receive a second set of data pertaining to second levels of the plurality of
preeclampsia biomarkers in a second biological sample derived from the
subject at a second point-in-time;
perform a second analysis on the second levels to obtain an assessment of
10 preeclampsia in the subject;
compare the first assessment with the second assessment; and
confirm preeclampsia or lack thereof based on the comparison.

156. A method for diagnosing or confirming preeclampsia in a subject, the method
comprising:

15 detecting protein levels of sFLT, PIG, and a protein or protein fragment binding to
pikachurin antibody in a biological sample derived from the subject; and
calculating a preeclampsia index score using the detected protein levels, wherein the
preeclampsia score is indicative of the presence or absence of preeclampsia in
the subject.

20 157. The method of claim 156, further comprising diagnosing or confirming
preeclampsia in the subject.

158. The method of claim 156, wherein the calculating comprises multiplying the
detected protein levels by a unique weight factor, and applying one or more
binary functions to weighted detected protein levels.

25 159. The method of claim 3, wherein each of the four or more biomarkers is
selected from the group consisting of sFLT-1, PIGF, abFN, PAPP-A, TakFN,
ADAM-12, and HPX, wherein each of the four or more biomarkers is different
from each other.

30 160. The method of claim 5 or 49, wherein the one or more biomarkers comprise
four or more biomarkers, wherein each of the four or more biomarkers is

selected from the group consisting of sFLT-1, PIGF, abFN, PAPP-A, TakFN, ADAM-12, and HPX, wherein each of the four or more biomarkers is different from each other.

5 161. The method of claim 7 or 20, wherein the two or more biomarkers comprise four or more biomarkers, wherein each of the four or more biomarkers is selected from the group consisting of sFLT-1, PIGF, abFN, PAPP-A, TakFN, ADAM-12, and HPX, wherein each of the four or more biomarkers is different from each other.

10 162. The kit of claim 79, wherein the two or more biomarkers comprise four or more biomarkers, wherein each of the four or more biomarkers is selected from the group consisting of sFLT-1, PIGF, abFN, PAPP-A, TakFN, ADAM-12, and HPX, wherein each of the four or more biomarkers is different from each other.

INCORPORATION BY REFERENCE

15 [0031] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

20 [0032] The disclosure is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

25 [0033] **Figure 1** depicts the performance of a series of penalized models with increasing number of markers. The figure shows the mean cross-validated performance and corresponding standard error. The number on the top represents the size of the model while the arrows identify the models with the highest area under the curve (AUC) and the one with a mean AUC within 1-se ("1-se" rule for model selection) of the maximum.

[0034] **Figure 2** depicts the performance of a series of penalized models with increasing number of markers excluding sFlt-1/PlGF. The figure shows the mean cross-validated performance and corresponding standard error. The number on the top represents the size of the model while the arrows identify the models with the highest AUC and the one with mean AUC within 1-se (“1-se” rule for model selection) of the maximum.

[0035] **Figure 3** is a diagram of a duplicate template depicting 32 samples with 2X duplicate Hi and Lo quality control.

[0036] **Figure 4** is a diagram of a triplicate template depicting 20 samples with 3X triplicate Hi and Lo quality control.

[0037] **Figures 5A-5F** provide a listing of various PE biomarkers (see also Table 2).

[0038] **Figure 6** is a diagram of an example 20 compound Master Block.

DETAILED DESCRIPTION

[0039] Provided herein are methods, compositions, systems and software for confirming preeclampsia diagnosis, confirming the presence and/or absence of “pre-eclampsia” or “preeclampsia” or “PE”, predicting the likelihood that a subject will develop PE, determining and/or confirming the severity of PE, determining the susceptibility of a subject not pregnant developing PE if the subject becomes pregnant, and monitoring PE progression in a subject already diagnosed with PE, all with greater sensitivity, specificity, confidence, accuracy, or area under the curve values than traditional PE tests.

[0040] The methods involve analyzing a sample or samples derived from a subject to confirm presence, absence, quantity and/or conformation of one or more PE biomarkers. A sample derived from the subject can be whole blood, urine, serum, plasma and other liquid samples of biological origin or cells derived therefrom and the progeny thereof. Samples can be manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components. A “marker” or “biomarker” is any biological entity that is represented differently in a sample from an individual that will get or has preeclampsia as compared to an individual that will not get preeclampsia. A biomarker is differently represented if, e.g., it is found in a different level (e.g., amount of protein, RNA or DNA), different three dimensional state (native form, mis-folded, alternative conformation), or different arrangements (e.g., complex, aggregates, mis-folded assemblies).

[0041] A PE biomarker can be a protein, a protein fragment, a peptide, a polynucleotide, a gene (DNA) or a gene fragment, an RNA transcript, or other forms of RNA such as snRNA, siRNA and micro-RNA. The terms “protein”, “peptide” and “polypeptide” as used in this application are interchangeable. “Polypeptide” refers to a polymer of amino acids and includes post-translationally modified polypeptides, glycosylated polypeptide, acetylated polypeptide, phosphorylated polypeptide and the like.

[0042] Examples of PE biomarkers contemplated herein include, but are not limited to the markers listed in Figures 5A-5F (or Table 2).

[0043] The presence, absence, monitoring or prediction of PE can be performed by obtaining a “preeclampsia profile”, “PE profile” or a “profile”. A PE profile is the level of one or more preeclampsia biomarkers in a patient sample. PE biomarkers can be determined by measuring protein levels or expression levels. A PE profile can include any one or more of the following sets (panels) of PE biomarkers shown in Table 1.

Table 1.

ADAM12
FN
FN, ADAM12
HPX
HPX, ADAM12, PAPPa
HPX, FN, ADAM12
HPX, PAPPa
PAPPa
PlGF
PlGF, ADAM12
PlGF, ADAM12, PAPPa, FN
PlGF, ADAM12, PAPPa, TakFN
PlGF, abFN, PAPPa, TakFN
PlGF, FN, PAPPa
PlGF, FN, ADAM12, sFLT1,

PAPP-A2, HPX
P1GF, PAPPa, TakFN
sFLT, PAPPa, FN
sFLT, P1GF
sFLT, P1GF, ADAM12, PAPPa, FN
sFLT, P1GF, FN
sFLT, P1GF, FN, ADAM12
sFLT, P1GF, FN, PAPPa
sFLT, P1GF, HPX, PAPPa, FN
sFlt1
sFlt1, FN, ADAM12
sFlt1, abFN, ADAM12, TakFN
sFLT1/P1GF, abFN, PAPPa, TakFN
sFLT1/P1GF, ADAM12, PAPPa, TakFN
sFLT1/P1GF, sFlt1, PAPPa, TakFN
sFLT1/P1GF, PAPPa, TakFN
sFLT1/P1GF, P1GF, PAPPa, TakFN
sFLT1/P1GF, sFlt1, abFN, PAPPa
sFLT1/P1GF, HPX, PAPPa, TakFN
sFLT1/P1GF, abFN, TakFN
sFLT1/P1GF, abFN, ADAM12, TakFN

sFLT1/P1GF, P1GF, abFN, TakFN
sFLT1/P1GF, sFlt1, abFN, TakFN

[0044] Other examples of PE profiles include any one or more of the following:

- the ratio sFLT1/P1GF and at least one biomarker selected from the group consisting of FN, PAPP-A, HPX, and ADAM12,
- at least two biomarkers selected from the group consisting of P1GF, ADAM12, FN, PAPP-A and HPX.
- FN, FG and at least one or two biomarkers selected from the group consisting of HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), P1GF and ADAM12.
- HPX in combination with FN, FG, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), P1GF and/or ADAM12
- sFlt-1 in combination with FN, FG, HPX, PAPP-A, VEGF (excluding VEGF-R1), P1GF and/or ADAM12.
- PAPP-A in combination with FN, FG, HPX, sFlt-1, VEGF (excluding VEGF-R1), P1GF and/or ADAM12.
- VEGF (excluding VEGF-R1) may be measured in combination with FN, FG, HPX, PAPP-A, sFlt-1, P1GF and/or ADAM12.
- P1GF in combination with FN, FG, HPX, PAPP-A, sFlt-1, VEGF (excluding VEGF-R1) and/or ADAM12.
- ADAM12 in combination with FN, FG, HPX, PAPP-A, sFlt-1, P1GF and/or VEGF (excluding VEGF-R1).
- sFlt-1, P1GF, FN, FG and PAPP-A;
- sFlt-1, P1GF, ADAM12 and FN, FG,;
- sFlt-1, P1GF, PAPP-A and FN, FG;
- sFlt-1, P1GF, HPX, PAPP-A and FN, FG;
- P1GF, ADAM12, PAPP-A, FN, FG;

- sFlt-1, PlGF and FN, FG, PlGF, FN, FG, PAPP-A
- FN, FG, sFlt-1, PlGF and FN, FG;
- sFlt-1, FN, FG, and ADAM12;
- PlGF, FN, FG, and PAPP-A;
- 5 • PlGF and ADAM12;
- FN, FG, and ADAM12, HPX, FN, FG, and ADAM12, HPX, ADAM12 and PAPP-A, HPX and PAPP-A, sFlt-1 and PlGF.

Any of the PE biomarker profiles or panels herein can optionally exclude one or more of the following biomarkers: ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin
 10 (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM) and heme.

In some cases, the panel of biomarkers includes at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or 15 biomarkers. Preferably, methods of the disclosure comprise determining protein levels of
 15 the one or more panels described herein. When a panel includes a ratio of sFlt1 and PlGF, the ratio can be of raw levels of sFlt-1 and PlGF, normalized or adjusted levels of sFlt-1 and PlGF (e.g., relative to housekeeping genes, e.g., ABL1, GAPDH, PGK1, or relative to signal across a whole panel), averaged levels, or levels as compared to a control (e.g., purified, recombinant proteins).

20 When a plurality of PE biomarkers is analyzed, the results can be subjected to an algorithm, or PE score function, that provides a single score, e.g., a PE score. A preeclampsia score is a single metric value that represents the one or more preeclampsia biomarkers in a patient sample. The PE score can be reported in a report to the subject or a healthcare service provider of the subject.

25 **[0045]** The PE score can be reported as a PE index. A “PE index” or an “index” is a metric system that indicates the likelihood PE is confirmed, severity of PE or the degree of likelihood of developing PE. The PE index can be calculated from the PE score, using a classification algorithm. Examples of supervised and unsupervised classification processes are described in Jain, “Statistical Pattern Recognition: A Review,” IEEE Transactions on
 30 Pattern Analysis and Machine Intelligence, Vol. 22, No. 1, January 2000.

[0046] The PE score or index can be indicative of the diagnosis, prognosis, or confirmation of PE diagnosis. The report can provide, in addition to the PE score or PE index, a set of suggested treatments or assessment of effectiveness of current treatment. The term “diagnosing” or “diagnosis” as used herein refers to a determination of whether a subject has or does not have PE. The term “confirming” or “confirmation” as used herein generally includes a determination of whether a subject suspected of having PE or previously diagnosed with PE has or does not have PE. The term “prognosing” or “prognosis” as used herein generally includes a prediction of the likely course of PE in a subject, such as the likelihood of increasing severity of PE or a subject’s responsiveness to treatment. The term “treating” or “treatment” as used herein generally means obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof (e.g., reducing the likelihood of incidence), reducing the incidence or severity of the disease, and/or therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. In determining a PE score, raw levels of the biomarkers are obtained by obtaining e.g., an optical density (OD) value. The raw data may be used to determine the concentration of a biomarker in a sample using the methods described herein which may include a comparison against a standard curve. The standard curve may have a coefficient of determination. In some cases, the coefficient of determination may be an R^2 value, for example, an R^2 value of $\geq 0.5, 0.6, 0.7, 0.75, 0.8, 0.85, 0.9$ or 0.95 may be used with the methods described herein. In an exemplary case, the R^2 of a standard curve using the methods described herein is ≥ 0.95 . Additional cases of the data may be evaluated using statistical methods known to those of ordinary skill in the art. Software such as SoftMax Pro may be used to perform at least some of the calculations and analysis described herein. Acquired data which falls outside of the range of the standard curve will not be analyzed or calculated further.

[0047] Raw levels of a biomarker can be optionally normalized, e.g., to a blank, a control or to another sample described herein. In some cases, normalization may include subtracting the OD value of a blank, a control or to another sample from the OD value of the sample. Normalization may also include first taking an average, mean or median of the OD of the

sample and second, taking an average, mean or median OD of the blank, control or another sample before subtracting the two OD values.

[0048] In some cases, the raw OD values of individual samples, average, mean or median of a set of samples, a blank, a control, another sample, a set of blanks, a set of controls or a set of another samples are log transformed. Often, the log transformation may include a comparison with a standard curve.

[0049] Biomarker levels can be adjusted relative to one or more of the following: a control derived from a training set as discussed in more detail below, a subject being tested prior to pregnancy, a subject being tested prior to onset of PE, a mean value from pregnant subjects not having PE, a value derived from specified laboratory subjects, a calculated value, a corresponding purified biomarker or a corresponding recombinant biomarker, a control level derived from a sample of a pregnant subject that does not have PE or does not have symptoms of PE, control level derived from a sample of a pregnant subject that is not diagnosed as having PE or does not have symptoms of PE, a control level derived from a sample of a pregnant subject that has (or is diagnosed as having) symptoms of PE, such as complications of pregnancy symptoms, but does not have (or is not diagnosed as having) PE, a control level derived from a sample of a pregnant subject that does not have PE but has one or more preeclampsia symptoms (e.g., a pregnant subject having complicated pregnancy) such as diabetes (e.g., gestational, type I or type II), higher than normal glucose level, hypertension (e.g., chronic or non-chronic), higher than normal weight, obesity, higher than normal body mass index (BMI), abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinurea, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results (Pap smear), prior preeclampsia episodes (e.g., personal history of PE), familial history of PE, PE in prior pregnancies, renal disease, thrombophilia, or any combination thereof; a control level derived from a sample of a pregnant subject that is not diagnosed with PE but has one or more preeclampsia symptoms (e.g., a pregnant subject having complicated pregnancy) such as those mentioned above.

[0050] When a control value is derived from a training set, the training set can be based on a theoretical model, real (measured) values obtained from subjects, or a combination of both.

Preferably, a training set is based on real values obtained from at least 10, 50, 100, 150, 200, 250, 300, 400, or 500 subjects. More preferably, at least 5, 10, 20, 30, 40, 50, or 60% of the subjects in the training set have PE while the remaining subjects are pregnant and do not have PE.

5 [0051] In one instance, PE biomarker levels are adjusted by multiplying each biomarker level (or normalized level) by a weighting factor, or “weight”, to arrive at weighted levels.

[0052] A biomarker score or biomarker index is then calculated using an algorithm, a function, a real function, a polynomial or the like. Such algorithm is referred to herein as PE score function. The weight for each biomarker in the PE score function can be unique. The weight for each biomarker can be a positive or a negative real number. The weight can be a ratio of two or more biomarkers. The PE score function can be a real function algorithm comprising binary operations such as addition, subtraction, multiplication and division. In some instances, the PE score function comprises at most one, two, three, four, five, six or seven binary operations. In some instances all binary operations are additions or subtractions of variables (the variables being biomarker values whether adjusted or non-adjusted). In some instances the binary operations include at least one division of variables. In some instances the binary operations include only one division of variables. In some examples, the binary operations exclude division of variables. In some instances, the binary operations exclude multiplication of variables.

20 [0053] PE score functions can be linear, exponential, logarithmic, quadratic, or any combination thereof.

[0054] A PE score function for determining a PE score can be represented according to the following formula:

[0055]
$$\text{PE Score} = a_0 + a_1(\text{ratio}) + a_2(\text{sFLT-1}) + a_3(\text{PlGF}) + a_4(\text{FN1}) + a_5(\text{FN2}) + a_6(\text{PAPPA}) + a_7(\text{HPX}) + a_8(\text{ADAM12}) + a_9(\text{FG}) + a_n(\text{biomarker or ratio of biomarkers selected from Table 1}),$$
 wherein, (i) a_0 is zero or at most -0.5, -1, -5, -10, -20, -30, -40, -50, -60, -70, -80, -90, -100, -110, -120, -130, -140, or -150 or a_0 is zero, (ii) a_1 is at least 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.10, (iii) a_2 is zero or at least -5.0, -4.0, -3.5, -3.0, -2.5, -2.0, -1.5, -1.0, 0.001, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, or 5.0, (iv) a_3 is zero or at least -5.0, -4.0, -3.5, -3.0, -2.5, -2.0, -1.5, -1.0, 0.001, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0,

3.5, 4.0, or 5.0, (iv) a_4 is zero or at least 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, or 15.5, (v) a_5 is zero or at least -13.0, -12.0, -11.0, -10.0, -9.0, -8.0, -7.0, -6.0, -5.0, -4.0, -3.5, -3.0, -2.5, -2.0, -1.5, -1.0, 0.001, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, or 5.0, (vi) a_6 is zero or at least -5.0, -4.0, -3.5, -3.0, -2.5, -2.0, -1.5, -1.0, -0.5, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0, (vii) a_7 is zero or at least 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 2.0, or 2.5, (viii) a_8 is zero or at least -13.0, -12.0, -11.0, -10.0, -9.0, -8.0, -7.0, -6.0, -5.0, -4.0, -3.5, -3.0, -2.5, -2.0, -1.5, -1.0, 0.001, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, or 15.5, (ix) a_9 is zero or at least -13.0, -12.0, -11.0, -10.0, -9.0, -8.0, -7.0, -6.0, -5.0, -4.0, -3.5, -3.0, -2.5, -2.0, -1.5, -1.0, 0.001, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, or 15.5, (x) a_n is zero or at least -13.0, -12.0, -11.0, -10.0, -9.0, -8.0, -7.0, -6.0, -5.0, -4.0, -3.5, -3.0, -2.5, -2.0, -1.5, -1.0, 0.001, 0.01, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, or 15.5, and (xi) a ratio can be any ratio or two or more biomarkers, including, e.g., sFLT-1/P1GF, P1GF/sFLT-1, (sFLT-1)/(VEGF excluding VEGF-R1), (VEGF excluding VEGF-R1)/P1GF, (VEGF excluding VEGF-R1)/sFLT-1, P1GF/(VEGF excluding VEGF-R1); (xii) FN1 designates an isoform of fibronectin detected by a first antibody and FN2 designates a fibronectin isoform detected by a second antibody, wherein the two isoforms can be the same or different, and wherein the two antibodies are different.

[0056] Non-limiting examples of linear models for determining a PE score are provided as following: $L = -52.651 + 0.214 \cdot \text{ratio} - 0.877 \cdot \text{PlGF} - 0.715 \cdot \text{HPX} - 0.884 \cdot \text{ADAM12} + \text{PAPPA} \cdot 3.85 + 4.473 \cdot \text{FN}$; $L = -46.789 + 0.018 \cdot \text{ratio} - 0.879 \cdot \text{PlGF} - 0.587 \cdot \text{HPX} - 0.973 \cdot \text{ADAM12} + \text{PAPPA} \cdot 3.121 + 4.007 \cdot \text{FN}$; $L = -49.789 + 0.012 \cdot \text{ratio} - 0.984 \cdot \text{PlGF} - 0.652 \cdot \text{HPX} - 0.968 \cdot \text{ADAM12} + \text{PAPPA} \cdot 3.22 + 4.105 \cdot \text{FN}$; $L = -50.789 + 0.019 \cdot \text{ratio} - 0.977 \cdot \text{PlGF} - 0.695 \cdot \text{HPX} - 0.910 \cdot \text{ADAM12} + \text{PAPPA} \cdot 3.62 + 4.351 \cdot \text{FN}$; $L = -48.989 + 0.022 \cdot \text{ratio} - 0.998 \cdot \text{PlGF} - 0.731 \cdot \text{HPX} - 0.971 \cdot \text{ADAM12} + \text{PAPPA} \cdot 3.41 + 4.317 \cdot \text{FN}$; $L = -58.899 + 0.014 \cdot \text{ratio} - 0.912 \cdot \text{PlGF} - 0.486 \cdot \text{HPX} - 0.853 \cdot \text{ADAM12} + \text{PAPPA} \cdot 2.191 + 4.097 \cdot \text{FN}$; $L = -$

49.211 + 0.017*ratio -0.974 PlGF -0.785 * HPX-0.957*ADAM12+PAPPA*3.68+ 4.324*FN;
 L = -48.789 + 0.011*ratio -0.899*PlGF -0.487 * HPX-0.873*ADAM12+PAPPA*2.121 +
 4.007*FN; L = -52.838 + 0.009*ratio -0.942*PlGF -0.533 * HPX-
 0.899*ADAM12+PAPPA*2.460 + 4.212*FN; L = -51.828 + 0.119*ratio -0.762*PlGF -
 5 0.618* HPX-0.711*ADAM12+PAPPA*2.243 + 5.921*FN; L = -47.298 + 0.122*ratio -
 1.298*PlGF -0.723 * HPX-0.932*ADAM12+PAPPA*1.920 + 3.929*FN; L = -47.562 +
 1.292*ratio -0.298*PlGF -0.722* HPX-0.921*ADAM12+PAPPA*3.291+ 4.118*FN.

[0057] Additional non-limiting examples of linear models for determining a PE score

include the following: L = -20.0484 + 0.1478(ratio) + 3.2970(PAPPA); L = -20.1484 +
 10 0.0478(ratio) + 3.0970(PAPPA); L = -21.0484 + 2.1478(ratio) + 4.2970(PAPPA); L = -
 20.3484 + 0.4478(ratio) + 3.5970(PAPPA); L = -77.6525 + 0.1405(ratio) + 5.1705(FN1); L = -
 79.6525 + 1.0405(ratio) + 7.1705(FN1); L = -78.7525 + 0.2405(ratio) + 6.2705(FN1); and L =
 -78.6525 + 0.0405(ratio) + 6.1705(FN1).

[0058] Additional non-limiting examples of linear models for determining a PE score

15 include the following: L = -77.0827 + 9.0491(ratio) + 8.5207(PlGF) + 7.1632(FN1); L = -
 79.0827 + 0.1491(ratio) + 0.2207(PlGF) + 6.3632(FN1); L = -78.0827 + 0.0491(ratio) +
 0.5207(PlGF) + 6.1632(FN1); L = -76.0827 + 0.4491(ratio) + 0.6207(PlGF) + 6.7632(FN1);

[0059] L = -87.8431 + 0.0422(ratio) + 0.9659(HPX) + 6.3886(FN1). L = -86.8431 +
 0.1422(ratio) + 0.8659(HPX) + 6.2886(FN1); L = -88.8431 + 0.1422(ratio) + 1.9659(HPX) +
 20 7.3886(FN1); and = -85.8431 + 0.2422(ratio) + 0.3659(HPX) + 6.4886(FN1);

[0060] Additional non-limiting examples of linear models for determining a PE score

include the following: L = -49.0767 + 0.0462(ratio) + 3.2997(FN2) + 3.8873(PAPPA); L = -
 48.0767 + 0.0462(ratio) + 2.2997(FN2) + 2.8873(PAPPA); L = -48.1767 + 0.2462(ratio) +
 2.3997(FN2) + 2.4873(PAPPA) and L = -48.5767 + 0.6462(ratio) + 2.7997(FN2) +

25 2.9873(PAPPA).

[0061] Additional non-limiting examples of linear models for determining a PE score

include the following: L = -85.7092 + 0.0492(ratio) -5.8358(FN2) + 12.7388(FN1); L = -
 85.1092 + 0.2492(ratio) -5.3358(FN2) + 12.4388(FN1); L = -86.7092 + 0.1492(ratio) -
 6.8358(FN2) + 13.7388(FN1); and L = -85.5092 + 0.6492(ratio) -5.7358(FN2) +

30 12.8388(FN1).

[0062] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -68.2829 + 1.0405(\text{ratio}) + 2.0848(\text{ADAM12}) + 3.1148(\text{FN1})$; $L = -88.2829 + 2.0405(\text{ratio}) + 3.0848(\text{ADAM12}) + 4.1148(\text{FN1})$; $L = -79.2829 + 0.1405(\text{ratio}) + 0.2848(\text{ADAM12}) + 7.1148(\text{FN1})$; and $L = -78.2829 + 0.0405(\text{ratio}) + 0.0848(\text{ADAM12}) + 6.1148(\text{FN1})$.

[0063] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -12.2190 + 2.0500(\text{ratio}) + 3.7240(\text{PAPPA}) + 4.2174(\text{FN1})$; $L = -82.2190 + 0.0500(\text{ratio}) + 2.7240(\text{PAPPA}) + 5.2174(\text{FN1})$; $L = -22.2190 + 3.0500(\text{ratio}) + 4.7240(\text{PAPPA}) + 6.2174(\text{FN1})$; and $L = -32.2190 + 4.0500(\text{ratio}) + 5.7240(\text{PAPPA}) + 7.2174(\text{FN1})$.

[0064] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -29.9815 + 4.0348(\text{sFLT1}) - 5.5288(\text{FN2}) + 16.2160(\text{FN1})$; $L = -39.9815 + 4.0348(\text{sFLT1}) - 5.5288(\text{FN2}) + 17.2160(\text{FN1})$; $L = -19.9815 + 2.0348(\text{sFLT1}) - 3.5288(\text{FN2}) + 14.2160(\text{FN1})$; and $L = -59.9815 + 3.0348(\text{sFLT1}) - 7.5288(\text{FN2}) + 12.2160(\text{FN1})$.

[0065] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -12.4750 + -3.8232(\text{PIGF}) + 4.8961(\text{PAPPA}) + 6.7670(\text{FN1})$; $L = -92.4750 + -2.8232(\text{PIGF}) + 2.8961(\text{PAPPA}) + 5.7670(\text{FN1})$; $L = -22.4750 + -3.8232(\text{PIGF}) + 4.8961(\text{PAPPA}) + 7.7670(\text{FN1})$; and $L = -32.4750 + -4.8232(\text{PIGF}) + 5.8961(\text{PAPPA}) + 9.7670(\text{FN1})$.

[0066] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -14.0940 + 0.0441(\text{ratio}) + 2.4773(\text{sFLT1}) + 3.5748(\text{PIGF}) + 4.8088(\text{FN1})$; $L = -24.0940 + 3.0441(\text{ratio}) + 4.4773(\text{sFLT1}) + 5.5748(\text{PIGF}) + 6.8088(\text{FN1})$; $L = -74.0940 + 0.0441(\text{ratio}) + 0.4773(\text{sFLT1}) + 0.5748(\text{PIGF}) + 5.8088(\text{FN1})$; and $L = -34.0940 + 4.0441(\text{ratio}) + 5.4773(\text{sFLT1}) + 6.5748(\text{PIGF}) + 7.8088(\text{FN1})$.

[0067] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -70.3822 + 1.0796(\text{ratio}) - 3.7798(\text{sFLT1}) + 4.8134(\text{FN2}) + 6.2025(\text{PAPPA})$; $L = -77.3822 + 0.0796(\text{ratio}) - 2.7798(\text{sFLT1}) + 3.8134(\text{FN2}) + 5.2025(\text{PAPPA})$; $L = -71.3822 + 2.0796(\text{ratio}) - 3.7798(\text{sFLT1}) + 4.8134(\text{FN2}) +$

6.2025(PAPPA); and $L = -72.3822 + 3.0796(\text{ratio}) - 4.7798(\text{sFLT1}) + 5.8134(\text{FN2}) + 7.2025(\text{PAPPA})$.

[0068] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -70.3510 + 1.0348(\text{ratio}) + 2.4503(\text{sFLT1}) - 3.5868(\text{FN2}) +$

5 $14.8250(\text{FN1}); L = -78.3510 + 0.0348(\text{ratio}) + 1.4503(\text{sFLT1}) - 7.5868(\text{FN2}) + 13.8250(\text{FN1}); L = -71.3510 + 2.0348(\text{ratio}) + 3.4503(\text{sFLT1}) - 4.5868(\text{FN2}) + 15.8250(\text{FN1});$ and $L = -72.3510 + 3.0348(\text{ratio}) + 4.4503(\text{sFLT1}) - 5.5868(\text{FN2}) + 16.8250(\text{FN1})$.

[0069] Additional non-limiting examples of linear models for determining a PE score

10 include the following: $L = -74.5657 + 0.0300(\text{ratio}) + 0.8356(\text{sFLT1}) - 0.5594(\text{ADAM12}) + 5.9467(\text{FN1}); L = -72.5657 + 0.0370(\text{ratio}) + 0.2756(\text{sFLT1}) - 0.3894(\text{ADAM12}) + 4.6467(\text{FN1}); L = -79.5657 + 0.0350(\text{ratio}) + 0.8756(\text{sFLT1}) - 0.5894(\text{ADAM12}) + 5.6467(\text{FN1});$ and $L = -67.5657 + 0.0230(\text{ratio}) + 0.4756(\text{sFLT1}) - 0.1494(\text{ADAM12}) + 4.7867(\text{FN1})$.

15 **[0070]** Additional non-limiting examples of linear models for determining a PE score include the following: $L = -100.6298 + 1.0845(\text{ratio}) + -3.8124(\text{sFLT1}) + 4.0643(\text{PAPPA}) + 6.5308(\text{FN1}); L = -108.6298 + 0.0845(\text{ratio}) + -2.8124(\text{sFLT1}) + 5.0643(\text{PAPPA}) + 6.5308(\text{FN1}); L = -101.6298 + 2.0845(\text{ratio}) + -3.8124(\text{sFLT1}) + 4.0643(\text{PAPPA}) + 5.5308(\text{FN1});$ and $L = -102.6298 + 3.0845(\text{ratio}) + -4.8124(\text{sFLT1}) + 6.0643(\text{PAPPA}) + 7.5308(\text{FN1})$.

[0071] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -41.5704 + 2.0489(\text{ratio}) + 3.1839(\text{PIGF}) + 4.2834(\text{FN2})$

25 $+ 5.8683(\text{PAPPA}); L = -42.5704 + 3.0489(\text{ratio}) + 4.1839(\text{PIGF}) + 5.2834(\text{FN2}) + 6.8683(\text{PAPPA}); L = -40.5704 + 1.0489(\text{ratio}) + 2.1839(\text{PIGF}) + 3.2834(\text{FN2}) + 4.8683(\text{PAPPA}); L = -47.5704 + 0.0489(\text{ratio}) + 0.1839(\text{PIGF}) + 2.2834(\text{FN2}) + 2.8683(\text{PAPPA}); L = -80.6889 + 1.0607(\text{ratio}) + 2.6545(\text{PIGF}) - 3.9105(\text{FN2}) + 14.8608(\text{FN1}); L = -85.6889 + 0.0607(\text{ratio}) + 0.6545(\text{PIGF}) - 5.9105(\text{FN2}) + 12.8608(\text{FN1}); L = -81.6889 + 2.0607(\text{ratio}) + 3.6545(\text{PIGF}) - 4.9105(\text{FN2}) + 15.8608(\text{FN1});$ and $L = -82.6889 + 3.0607(\text{ratio}) + 4.6545(\text{PIGF}) - 6.9105(\text{FN2}) + 17.8608(\text{FN1})$.

[0072] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -77.8703 + 0.0491(\text{ratio}) + 0.5191(\text{PIGF}) + 0.0523(\text{ADAM12}) + 6.1300(\text{FN1})$; $L = -71.8703 + 2.0491(\text{ratio}) + 3.5191(\text{PIGF}) + 4.0523(\text{ADAM12}) + 5.1300(\text{FN1})$; $L = -72.8703 + 3.0491(\text{ratio}) + 4.5191(\text{PIGF}) + 5.0523(\text{ADAM12}) + 7.1300(\text{FN1})$; and $L = -70.8703 + 1.0491(\text{ratio}) + 2.5191(\text{PIGF}) + 3.0523(\text{ADAM12}) + 4.1300(\text{FN1})$.

[0073] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -81.9135 + 0.0433(\text{ratio}) + 0.3154(\text{PIGF}) + 1.7019(\text{PAPPA}) + 5.2189(\text{FN1})$; $L = -81.9125 + 0.0533(\text{ratio}) + 0.2154(\text{PIGF}) + 2.7019(\text{PAPPA}) + 5.2199(\text{FN1})$; $L = -82.9125 + 0.0553(\text{ratio}) + 0.3154(\text{PIGF}) + 2.8019(\text{PAPPA}) + 4.2199(\text{FN1})$; and $L = -71.9125 + 0.0633(\text{ratio}) + 0.2254(\text{PIGF}) + 2.7519(\text{PAPPA}) + 5.2399(\text{FN1})$.

[0074] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -49.5169 + 0.0464(\text{ratio}) + 0.1807(\text{HPX}) + 2.3236(\text{FN2}) + 2.8744(\text{PAPPA})$; $L = -47.5169 + 0.1464(\text{ratio}) + 0.1857(\text{HPX}) + 2.4236(\text{FN2}) + 2.6744(\text{PAPPA})$; $L = -45.2169 + 0.0344(\text{ratio}) + 0.2707(\text{HPX}) + 1.2236(\text{FN2}) + 2.4544(\text{PAPPA})$; and $L = -48.5169 + 0.0564(\text{ratio}) + 0.2807(\text{HPX}) + 2.1236(\text{FN2}) + 2.7744(\text{PAPPA})$.

[0075] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -68.9243 + 0.0583(\text{ratio}) - 0.7927(\text{HPX}) - 6.2143(\text{FN2}) + 10.7478(\text{FN1})$; $L = -78.9243 + 0.0483(\text{ratio}) - 0.6927(\text{HPX}) - 6.0143(\text{FN2}) + 12.7478(\text{FN1})$; $L = -77.9243 + 0.0583(\text{ratio}) - 0.7727(\text{HPX}) - 6.1243(\text{FN2}) + 11.8478(\text{FN1})$; and $L = -79.9243 + 0.1483(\text{ratio}) - 0.6227(\text{HPX}) - 6.2143(\text{FN2}) + 12.9478(\text{FN1})$.

[0076] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -88.0679 + 0.0309(\text{ratio}) + 0.5208(\text{HPX}) + 2.6887(\text{PAPPA}) + 5.2737(\text{FN1})$; $L = -97.0679 + 0.0589(\text{ratio}) + 0.6708(\text{HPX}) + 2.7787(\text{PAPPA}) + 5.2937(\text{FN1})$; $L = -77.0679 + 0.0609(\text{ratio}) + 0.6308(\text{HPX}) + 2.8787(\text{PAPPA}) + 5.3937(\text{FN1})$; and $L = -87.0679 + 0.0509(\text{ratio}) + 0.6208(\text{HPX}) + 2.6787(\text{PAPPA}) + 5.2937(\text{FN1})$.

[0077] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -53.7575 + 0.0542(\text{ratio}) + 3.2134(\text{FN2}) - 1.5150(\text{ADAM12}) + 3.6894(\text{PAPPA})$; $L = -56.7575 + 0.0342(\text{ratio}) + 4.0134(\text{FN2}) - 1.4160(\text{ADAM12}) + 3.7094(\text{PAPPA})$; $L = -55.7575 + 0.0442(\text{ratio}) + 3.0134(\text{FN2}) - 1.4150(\text{ADAM12}) + 3.6094(\text{PAPPA})$; and $L = -45.7575 + 0.0443(\text{ratio}) + 3.2134(\text{FN2}) - 1.6150(\text{ADAM12}) + 3.6084(\text{PAPPA})$.

[0078] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -85.1599 + 0.0529(\text{ratio}) - 6.5276(\text{FN2}) + 0.8455(\text{ADAM12}) + 13.1454(\text{FN1})$; $L = -85.1499 + 0.0509(\text{ratio}) - 6.5286(\text{FN2}) + 0.8435(\text{ADAM12}) + 13.1434(\text{FN1})$; $L = -85.1399 + 0.0409(\text{ratio}) - 6.2286(\text{FN2}) + 0.7435(\text{ADAM12}) + 13.0434(\text{FN1})$; and $L = -87.1499 + 1.0509(\text{ratio}) - 8.5286(\text{FN2}) + 1.8435(\text{ADAM12}) + 11.1434(\text{FN1})$.

[0079] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -94.7950 + 0.0632(\text{ratio}) - 7.5691(\text{FN2}) + 3.0857(\text{PAPPA}) + 13.8372(\text{FN1})$; $L = -95.7950 + 0.1632(\text{ratio}) - 7.5491(\text{FN2}) + 3.2857(\text{PAPPA}) + 15.8372(\text{FN1})$; $L = -94.8950 + 0.0642(\text{ratio}) - 7.5591(\text{FN2}) + 3.0957(\text{PAPPA}) + 13.2372(\text{FN1})$; and $L = -94.7960 + 0.0631(\text{ratio}) - 7.5791(\text{FN2}) + 4.0857(\text{PAPPA}) + 11.8372(\text{FN1})$.

[0080] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -91.5034 + 0.0566(\text{ratio}) - 1.8810(\text{ADAM12}) + 3.5597(\text{PAPPA}) + 6.1198(\text{FN1})$; $L = -92.5034 + 0.0466(\text{ratio}) - 1.8910(\text{ADAM12}) + 3.6597(\text{PAPPA}) + 6.2198(\text{FN1})$; $L = -93.5034 + 0.0467(\text{ratio}) - 1.7910(\text{ADAM12}) + 3.6797(\text{PAPPA}) + 7.2198(\text{FN1})$; and $L = -82.5034 + 0.0476(\text{ratio}) - 1.9910(\text{ADAM12}) + 4.6597(\text{PAPPA}) + 6.5198(\text{FN1})$.

[0081] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -73.6418 + 2.3065(\text{sFLT1}) - 1.3341(\text{PIGF}) - 7.4383(\text{FN2}) + 13.0395(\text{FN1})$; $L = -72.3418 + 2.2365(\text{sFLT1}) - 1.3131(\text{PIGF}) - 7.4332(\text{FN2}) + 13.3595(\text{FN1})$; $L = -71.6418 + 2.1065(\text{sFLT1}) - 1.1141(\text{PIGF}) - 7.1382(\text{FN2}) + 13.1595(\text{FN1})$; and $L = -72.6418 + 2.2065(\text{sFLT1}) - 1.3141(\text{PIGF}) - 7.4382(\text{FN2}) + 13.0595(\text{FN1})$.

[0082] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -103.5305 + -0.3764(sFLT1) - 3.3328(PIGF) + 3.4350(PAPPA) + 6.3890(FN1)$; $L = -105.5305 + -0.6564(sFLT1) - 3.5928(PIGF) + 3.4550(PAPPA) + 6.5890(FN1)$; $L = -104.5305 + -0.6764(sFLT1) - 3.3928(PIGF) + 3.4850(PAPPA) + 6.4890(FN1)$; and $L = -104.4305 + -0.6464(sFLT1) - 3.4928(PIGF) + 3.4450(PAPPA) + 6.4490(FN1)$.

[0083] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -74.1756 + 3.9467(sFLT1) - 8.4664(FN2) - 2.4423(ADAM12) + 15.3474(FN1)$; $L = -75.1756 + 3.9167(sFLT1) - 8.7664(FN2) - 2.4923(ADAM12) + 15.3674(FN1)$; $L = -76.1756 + 3.9667(sFLT1) - 8.6664(FN2) - 2.4623(ADAM12) + 15.6674(FN1)$; and $L = -73.1756 + 3.9367(sFLT1) - 8.7364(FN2) - 2.4323(ADAM12) + 13.3674(FN1)$.

[0084] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -76.5467 + 2.6634(sFLT1) - 3.2682(ADAM12) + 1.9649(PAPPA) + 6.6178(FN1)$; $L = -68.5467 + 2.6634(sFLT1) - 3.2682(ADAM12) + 1.6349(PAPPA) + 6.6178(FN1)$; $L = -74.5467 + 2.4634(sFLT1) - 3.4282(ADAM12) + 1.9449(PAPPA) + 6.4178(FN1)$; $L = -78.5467 + 2.5634(sFLT1) - 3.2282(ADAM12) + 1.9349(PAPPA) + 6.1178(FN1)$; $L = -78.8718 - 2.6772(PIGF) - 1.7373(HPX) + 2.9707(PAPPA) + 5.5077(FN1)$; $L = -75.8618 - 2.5762(PIGF) - 1.5573(HPX) + 2.9507(PAPPA) + 5.5557(FN1)$; $L = -77.8618 - 2.6772(PIGF) - 1.5673(HPX) + 2.9106(PAPPA) + 5.5056(FN1)$; and $L = -78.8618 - 2.6762(PIGF) - 1.5373(HPX) + 2.9107(PAPPA) + 5.5057(FN1)$.

[0085] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -109.2319 - 3.7617(PIGF) - 6.7308(FN2) + 3.5194(PAPPA) + 13.6500(FN1)$; $L = -107.2319 - 3.5617(PIGF) - 6.3308(FN2) + 3.4194(PAPPA) + 13.6700(FN1)$; $L = -108.2319 - 3.6617(PIGF) - 6.6308(FN2) + 3.2194(PAPPA) + 11.6500(FN1)$; and $L = -119.2319 - 3.7117(PIGF) - 6.1308(FN2) + 3.5114(PAPPA) + 13.7500(FN1)$.

[0086] Additional non-limiting examples of linear models for determining a PE score include the following: $L = -112.4589 - 2.7282(PIGF) - 2.7122(ADAM12) + 4.4379(PAPPA)$

+ 7.6259(FN1); L= -113.4589 -2.7281(PIGF) -2.7121(ADAM12) + 4.4279(PAPPA) + 7.6159(FN1); L= -114.4589 -2.7381(PIGF) -2.7221(ADAM12) + 4.5379(PAPPA) + 7.7159(FN1); and L= -133.4589 -2.8281(PIGF) -2.7131(ADAM12) + 4.5279(PAPPA) + 7.6169(FN1).

- 5 **[0087]** In some cases, a linear and an exponential model is utilized to determine the probability using the following algorithm: $PE = 1/(1+\exp(-L))$.
- [0088]** Weighted levels of all biomarkers in a panel can then be totaled and in some cases, such as in the levels of sFlt and PlGF, the weighted levels can be formed into a ratio. The sum of the weighted levels and optionally a ratio results in a single weighted level or “PE score”. Each biomarker can have a unique weighting factor, or a combination of biomarkers
- 10 can have a unique weighting factor. In some instances, a preeclampsia score may be determined by methods similar to those described for a preeclampsia signature, e.g. the levels of each of the one or more preeclampsia markers in a patient sample may be $\log_2$, $\log_e$ or $\log_{10}$ transformed and normalized as described above for generating a preeclampsia profile.
- 15 **[0089]** The weighted levels for calculating the score can be defined by a reference dataset, “training dataset,” or “training set.” The training set can be based on a model, actual values obtained from subjects, or a combination thereof. A training set can comprise subjects diagnosed as having symptoms of PE. A training set can comprise subjects having symptoms of PE, but not PE. A training set can comprise subjects having symptoms of PE, but not PE,
- 20 and having one or more other disorders (e.g., subjects having pregnancies with complications) such as diabetes (e.g., gestational, type I or type II), higher than normal glucose level, hypertension (e.g., chronic or non-chronic), higher than normal weight, obesity, higher than normal body mass index (BMI), abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema,
- 25 abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results (Pap smear), prior preeclampsia episodes (e.g., personal history of PE), familial history of PE, PE in prior pregnancies, renal disease, thrombophilia, or any combination thereof. A training set can comprise subjects diagnosed as having PE with one or more disorders (e.g., subjects having pregnancies with complications) such as diabetes
- 30 (e.g., gestational, type I or type II), higher than normal glucose level, hypertension (e.g.,

chronic or non-chronic), higher than normal, obesity, higher than normal body mass index (BMI), abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results (Pap smear), prior preeclampsia episodes (e.g., personal history of PE), familial history of PE, PE in prior pregnancies, renal disease, thrombophilia, or any combination thereof. The training set can include at least 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, or 400 subjects. The training set can include data from at least 15, 50, 100, 150, 200 or 250 subjects having a normal pregnancy, and at least 15, 50, 100, 150, 200 or 250 pregnant subjects with PE. In some instances, more than 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50% of the preeclamptic subjects have at least one additional condition (e.g., hypertension, diabetes, overweight, etc.).

[0090] In some instances, the classification of PE that is used to provide the “preeclampsia index” described herein is not based on for example blood pressure, weight gain, water retention, hereditary factors, proteinuria, headache, edema, protein/creatinine ratio, platelet count, stress, PE in prior pregnancies, nulliparity, age, age less than 20 years, age greater than 35, race, African-American and Filipino decent, serotype, Papanicolaou test results (Pap smear), prior preeclampsia episodes (e.g., personal history of PE), familial history of PE, number of pregnancies, number of miscarriages, body mass index (BMI), gestational diabetes, type I diabetes, obesity, glucose level, current and past medications, stress, PE in prior pregnancies (of the subject or her family members), chronic hypertension, renal disease and thrombophilia. In some examples, the classification of PE is not based on any of the characteristics of pregnancy just mentioned. In some examples, the classification of PE is based on at least one of the characteristics of pregnancy just mentioned. In some cases, the PE index may be based on the female subject’s gestational period.

[0091] A “report,” as described herein, is an electronic or tangible document which includes report elements that provide information relating to a subject. A subject report optionally includes one or more of the following: information about the subject, a PE profile, a PE score, a PE index, PE confirmation, PE diagnosis, PE prognosis, PE monitoring status, and/or suggested treatments. A subject report can further include one or more of: 1) information regarding the testing facility; 2) service provider information; 3) patient data; 4) sample data;

5) an assessment report, which can include various information including: a) reference values employed, and b) test data, where test data can include, e.g., a protein level determination; and 6) other features. The report may be for positive confirmation of PE, negative confirmation of PE, diagnosis of PE, characteristics of PE, progress of PE, severity of PE, or prognosis of PE. Positive confirmation of PE refers to a situation where a subject having PE symptoms is confirmed as having PE. Negative confirmation of PE refers to a situation where a subject not having symptoms of PE is confirmed as not having PE. Such report may include relative weight or signature values of biomarkers, PE score or PE index score. The report may include recommendation as to treatment recommendations (e.g., bed-rest, aspirin, drinking extra water, a low salt diet, medicines to control blood pressure, corticosteroids, or recommendation for early delivery).

Subjects and Samples

[0092] The term “biological sample” and “sample” encompasses blood, urine, serum, plasma, and other liquid samples of biological origin or cells derived therefrom. Once a sample is derived from a subject, it can be used directly, frozen, or maintained in appropriate culture medium for short periods of time. A sample that is derived from blood may be allowed to clot, and the serum separated and collected to be used in the assay.

[0093] A sample volume of blood, serum, or urine between 2 μ l to 2,000 μ l, may be sufficient for determining the PE score. In some examples, the sample volume ranges from 10 μ l to 1,750 μ l, from 20 μ l to 1,500 μ l, from 40 μ l to 1,250 μ l, from 60 μ l to 1,000 μ l, from 100 μ l to 900 μ l, from 200 μ l to 800 μ l, from 400 μ l to 600 μ l. In some instances a sample volume is 2-10mL or 0.5-5 mL or up to 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 mL.

[0094] A subject analyzed may have zero, or at least one, two, three, four, or five factors which confound a diagnosis of preeclampsia. In some cases, a confounding factor may be selected from the group consisting of: high blood pressure, age over 35 years, higher than normal weight, quick weight gain, gestational period greater than 20 weeks, ethnicity, diabetes (Type I or II), high proteinurea, kidney disease, autoimmune disease, prior PE by the subject in an earlier pregnancy, and a family or maternal history of PE.

[0095] In practicing the subject methods, a sample from a subject is evaluated to obtain a representation of the level(s) of one or more PE biomarkers. The levels of one or more PE

biomarkers can be used to provide, for example, a PE profile, PE signature, PE score, or PE index as described in greater detail below.

[0096] A subject sample may be treated in a variety of ways so as to enhance detection of the preeclampsia marker. For example, where the sample is blood, the red blood cells may be removed from the sample (e.g., by centrifugation) prior to assaying. Such a treatment may serve to reduce the non-specific background levels of detecting the level of a preeclampsia marker using an affinity reagent. Detection of a preeclampsia marker may also be enhanced by concentrating the sample using procedures well known in the art (e.g., acid precipitation, alcohol precipitation, salt precipitation, hydrophobic precipitation, filtration (using a filter which is capable of retaining molecules greater than 30 kD, e.g., Centrion 30™) or affinity purification. In some cases, the pH of the test and control samples will be adjusted to, and maintained at, a pH which approximates neutrality (e.g., pH 6.5-8.0). Such a pH adjustment will prevent complex formation, thereby providing a more accurate quantitation of the level of marker in the sample. In cases where the sample is urine, the pH of the sample is adjusted and the sample is concentrated in order to enhance the detection of the marker. pH may be adjusted using methods known to those of ordinary skill in the art, for example, adding an acid to a basic or neutral pH sample or adding a base to an acidic or neutral pH sample.

[0097] Buffers and/or other reagents may be added to the sample to facilitate preparation of the sample prior to determining a level of at least one biomarker in the sample. In some cases, a buffer and/or other reagent may include at least, but is not limited to, one of the following: ethylenediaminetetraacetic acid (EDTA), phosphate buffered saline, Hanks balanced salt solution, Ficoll, sodium chloride, sodium citrate, silica, thrombin, teophylline, adenosine, dipyridamole, aprotinine, heparin, lithium heparin, fluoride, potassium oxalate, tri-sodium citrate, citric acid, and/or dextrose. The buffer and/or other reagent may be compounded in an inert base, for example, a gel, water, saline or the like.

[0098] In a particular case, a sample of blood may be collected using a serum separator tube (SST). The SST may contain a buffer and/or other reagent. In another particular case, a sample of blood may be collected using a clot-2 serum separator tube which may contain a buffer and/or reagent. The SST and/or clot-2 tube may be obtained from a manufacturer such as Becton Dickinson although any comparable tube may be used. In some cases, the sample

may be treated using a method, reagent or chemical known to one of ordinary skill in the art such that components of the sample become separated from one another. Sometimes, the sample of blood is separated such that the serum is in a layer comprising the top of the sample.

5 [0099] A subject sample is typically obtained from the individual during the second or third trimester of gestation. By “gestation” it is meant the duration of pregnancy in a mammal, e.g., the period of development in the uterus from conception until birth. A subject sample may be derived early in gestation, for example, on or before 34 weeks of gestation, e.g., at weeks 20-34 of gestation, at 24-34 weeks of gestation, at weeks 30-34 weeks of gestation. A
10 subject sample may be derived late in gestation, for example, after 34, 35, 36, 37, or 38 weeks of gestation.

[00100] A PE profile, signature, score, or index may be determined soon after or at least 2, 3, or 4, weeks from the time a sample is derived from a subject. In some cases, a PE profile, signature, score, or index is determined at most 1, 2, 3, or 4 days from the time a sample is
15 derived from a subject.

[00101] Once a sample is derived from a subject, the sample can be processed (e.g., plasma or serum isolated). The sample, or portion thereof, can further be diluted. A sample, or portion of a sample, can be diluted by a factor of at least 5, 10, 50, 100, 500, 1,000, 5,000, 10,000, 15,000, 20,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000,
20 70,000, 75,000 or 80,00 fold.

[00102] Techniques for Detecting Biomarkers.

[00103] When a biomarker is a differentially expressed protein level, the biomarker can be detected by measuring the levels or the amounts of one or more proteins, protein fragments, peptides, nucleic acid transcripts (e.g. mRNA), genes, or gene fragments.

25 [00104] Each biomarker assayed using the methods and protocols described herein may have a threshold. Often the threshold of a biomarker is the performance of a biomarker in an assay, method or protocol. In some cases, the methods, protocols and assays described herein may be accurate, sensitive, and specific and may be used as a positive or a negative predictive value. The methods, protocols and assays described herein may be at least 70%, 75%, 80%,
30 85%, 90%, 95% or 99% accurate. The methods, protocols and assays described herein may

be at most 70%, 75%, 80%, 85%, 90%, 95% or at most 99% accurate. The methods, protocols and assays described herein may be at least 70%, 75%, 80%, 85%, 90%, 95% or 99% sensitive. The methods, protocols and assays described herein may be at most 70%, 75%, 80%, 85%, 90%, 95% or about 99% sensitive. The methods, protocols and assays described herein may be at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or more specific. The methods, protocols and assays described herein may be at most 70%, 75%, 80%, 85%, 90%, 95%, 99% or less specific. The methods, protocols and assays described herein may have a positive predictive value of at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or more. The methods, protocols and assays described herein may have a positive predictive value of at most 70%, 75%, 80%, 85%, 90%, 95%, 99% or less. The methods, protocols and assays described herein may have a negative predictive value of at least 70%, 75%, 80%, 85%, 90%, 95%, 99% or more. The methods, protocols and assays described herein may have a negative predictive value of at most 70%, 75%, 80%, 85%, 90%, 95%, 99% or less.

[00105] In some cases, a biomarker assayed using the methods and protocols described herein

may be present in a sample above the lowest concentration of a standard curve sample. In other cases, a biomarker assayed using the methods and protocols described herein may be present in a sample below the lowest concentration of a standard curve sample. In some cases, a biomarker assayed using the methods and protocols described herein may be present in a sample above the highest concentration of a standard curve sample. In other cases, a biomarker assayed using the methods and protocols described herein may be present in a sample below the highest concentration of a standard curve sample. In some cases, a biomarker assayed using the methods and protocols described herein may be present in a sample above the lowest concentration of a high quality control sample. In other cases, a biomarker assayed using the methods and protocols described herein may be present in a sample below the lowest concentration of a high quality control sample. In some cases, a biomarker assayed using the methods and protocols described herein may be present in a sample above the highest concentration of a high quality control sample. In other cases, a biomarker assayed using the methods and protocols described herein may be present in a sample below the highest concentration of a high quality control sample. In some cases, a biomarker assayed using the methods and protocols described herein may be present in a

sample above the lowest concentration of a low quality control sample. In other cases, a biomarker assayed using the methods and protocols described herein may be present in a sample below the lowest concentration of a low quality control sample. In some cases, a biomarker assayed using the methods and protocols described herein may be present in a sample above the highest concentration of a low quality control sample. In other cases, a biomarker assayed using the methods and protocols described herein may be present in a sample below the highest concentration of a low quality control sample.

[00106] Often, a level of a biomarker assayed using the methods and protocols described herein may increase between a first assay and a second assay. In some cases, a level of a

biomarker assayed using the methods and protocols described herein may increase by about two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or 10 000 fold between the first assay and the second assay. In some cases, a level of a biomarker assayed using the methods and protocols described herein may increase by at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500, 10,000 fold or more between the first assay and the second assay.

In some cases, a level of a biomarker assayed using the methods and protocols described herein may increase by at most two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or less than 10 000 fold between the first assay and the second assay.

[00107] Often, a level of a biomarker assayed using the methods and protocols described herein may decrease between a first assay and a second assay. In some cases, a level of a

biomarker assayed using the methods and protocols described herein may decrease by about two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30,

35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or 10 000 fold between the first assay and the second assay. In some cases, a level of a biomarker assayed using the methods and protocols described herein may

5 decrease at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or 10 000 fold or more between the first assay and the second assay. In some cases, a level of a biomarker

10 assayed using the methods and protocols described herein may decrease at most two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500, 10 000 fold or less, between the first assay
15 and the second assay.

[00108] Often, a level of a biomarker assayed using the methods and protocols described herein may increase between a first assay and a second assay. In some cases, a level of a biomarker assayed using the methods and protocols described herein may increase by about two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30,
20 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or 10 000 percent between the first assay and the second assay. In some cases, a level of a biomarker assayed using the methods and protocols described herein may increase at least by two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14,
25 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or 10 000 percent or more between the first assay and the second assay. In some cases, a level of a biomarker assayed using the methods and protocols described herein may increase by at most
30 two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19,

20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or 10 000 percent or less, between the first assay and the second assay.

5 [00109] Often, a level of a biomarker assayed using the methods and protocols described herein may decrease between a first assay and a second assay. In some cases, a level of a biomarker assayed using the methods and protocols described herein may decrease by about two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 10 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or 10 000 percent between the first assay and the second assay. In some cases, a level of a biomarker assayed using the methods and protocols described herein may decrease by at least two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 15 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500, 10 000 percent or more between the first assay and the second assay. In some cases, a level of a biomarker assayed using the methods and protocols described herein may decrease by at most two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1250, 1500, 1750, 2000, 2500, 5000, 7500 or 10 000 percent or less, between the first assay and the second assay.

[00110] The methods and protocols described herein may be considered in combination with 25 clinical measures. Clinical measures may be associated with, but are not limited to, total blood pressure, diastolic blood pressure, systolic blood pressure, mean arterial blood pressure, proteinuria detected by, for example, dipstick method or 24-hour collection method, body mass index, swelling, abdominal pressure, uterine pulsatility index, uterine Doppler measurements, circulating free DNA, circulating free fetal DNA, fetal DNA and/or 30 thrombocytopenia, fetal abnormalities, gestational period, age of mother, previous case of

preeclampsia during pregnancy, race or ethnicity, history of preeclampsia with mother or farther, multiple births, first birth, and subject's smoking history.

[00111] The disclosure provides a method for diagnosing or confirming an existence of preeclampsia in a subject comprising: performing at least two different assays that determine a level of fibronectin in a sample from the subject; and evaluating the sample and using the levels from the plurality of assays to diagnose or confirm the existence of preeclampsia and calculate an index. In some cases, the method further comprises, based upon the index, suggesting a treatment for preeclampsia, the treatment selected from the group consisting of aspirin, preterm labor or bedrest.

[00112] The disclosure provides a method for diagnosing or confirming an existence of preeclampsia in a subject comprising: evaluating a level of a ratio of sFlt-1 and PlGF and a level of a plurality of biomarkers in a sample derived from the subject, wherein the different biomarker is not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) or heme; and evaluating the sample and using the levels from the previous step to determine an index to diagnose or confirm the existence of preeclampsia and calculating an index. In some cases, the method further comprises, based upon the index, suggesting a treatment for preeclampsia, the treatment involving aspirin, preterm labor or bedrest. In some cases, the method further comprises performing replicates of identical assays for each biomarker using the sample. In some cases, the method further comprises determining a mean level for each of the biomarkers using levels derived from each of the multiple identical assays. In some cases, the method further comprises performing a $\log_2$, $\log_e$ or $\log_{10}$ transformation of the mean levels. In some cases, the method further comprises comparing the levels of each biomarker to a standard curve for that biomarker. In some cases, the method further comprises weighting each of the biomarkers, wherein the weighting includes providing numbers into a polynomial such that each marker has a distinct weight. In some cases, the calculating comprises determining a ratio of adjusted levels of sFlt-1 and PlGF. In some cases, the calculating comprises determining a ratio of normalized levels of sFlt-1 and PlGF. In some

cases, the calculating comprises determining a ratio of raw levels of sFlt-1 and PlGF. In some cases, the calculating comprises determining a ratio of adjusted levels of sFlt-1 and PlGF compared to a control. In some cases, the calculating comprises determining a ratio of normalized levels of sFlt-1 and PlGF compared to a control. In some cases, the calculating comprises determining a ratio of raw levels of sFlt-1 and PlGF compared to a control.

[00113] The disclosure provides a method for diagnosing or confirming an existence of preeclampsia in a subject comprising: evaluating a level of a ratio of sFlt-1 and PlGF and a level of a plurality of biomarkers in a sample derived from the subject, wherein the different biomarker is not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) or heme; and evaluating the sample and using the levels from step (a) to determine an index to diagnose or confirm the presence of preeclampsia. In some cases, the plurality of biomarkers is selected from the group consisting of sFlt-1, PlGF, FN and PAPP-A; sFlt-1, PlGF, FN, ADAM12 and PAPP-A; sFlt-1, PlGF, PAPP-A and FN; sFlt-1, PlGF, HPX, FN and PAPP-A; PlGF, ADAM12, FN and PAPP-A; PlGF, FN and PAPP-A; sFlt-1, PlGF and FN; PlGF, FN and PAPP-A; sFlt-1, PlGF, FN and ADAM12; sFlt-1, PlGF and FN; PlGF, sFlt-1 and FN; sFlt-1, FN and ADAM12; PlGF, FN and PAPP-A. In some cases, the method further comprises, based upon the index, suggesting a treatment for preeclampsia, the treatment involving aspirin, preterm labor or bedrest. In some cases, the method further comprises performing replicates of identical assays for each biomarker using the sample. In some cases, the method further comprises determining a mean level for each of the biomarkers using levels derived from each of the multiple identical assays. In some cases, the method further comprises performing a $\log_2$, $\log_e$ or $\log_{10}$ transformation of the mean levels. In some cases, the method further comprises comparing the levels of each biomarker to a standard curve for that biomarker. In some cases, the method further comprises weighting each of the biomarkers, wherein the weighting includes providing numbers into a polynomial such that each marker has a distinct weight. In some cases, the calculating comprises determining a ratio of adjusted levels of sFlt-1 and PlGF. In some cases, the calculating comprises

determining a ratio of normalized levels of sFlt-1 and PlGF. In some cases, the calculating comprises determining a ratio of raw levels of sFlt-1 and PlGF. In some cases, the calculating comprises determining a ratio of adjusted levels of sFlt-1 and PlGF compared to a control. In some cases, the calculating comprises determining a ratio of normalized levels of sFlt-1 and PlGF compared to a control. In some cases, the calculating comprises determining a ratio of raw levels of sFlt-1 and PlGF compared to a control.

Detection Reagents and Antibodies

[00114] The disclosure includes detection reagents and antibodies that may be used to determine levels of a plurality of biomarkers disclosed herein. Often, the detection reagents may include reagents useful for performing an ELISA.

[00115] Examples of commercially available antibody kits that can be used in an ELISA protocol include, but are not limited to, anti-PlGF (distributed by USCN Life Science Inc., Roche and R&D Systems), anti-sFlt-1 (distributed by Boster Bio., R&D systems, MyBioSource.com, antibodies-online, Biotrend Chemikalien GmbH, and Enzo Life Sciences), anti-PAPP-A (distributed by R&D systems, RayBioTech, IBL Japan, DRG International, Abnova, USCN Life Science, Novus Bio, Rapid Test, MyBioSource, antibodies-online.com, Fisher Scientific, elabscience, Sigma Aldrich, C USA Bio, ANSH Labs, Demeditec, Alpco, AMS Bio, NovaTeinBio, Creative Biomart, Biorbyt, Biomartik Corporation), anti-VEGF (distributed by AMS Bio, Mybiosource, Abnova, antibodies-online.com, United States Biological, Biomartik Corporation, Cloud-Clone Corp, Biovendor, Boster Immunoleader, Enzo Life Sciences, Fitzgerald, Abnova, Aviva Systems Biology and Creative Biomart), anti-fibronectin (distributed by Biovendor, Boster Immunoleader, QED Bioscience, eBioscience, Biorbyt, Fitzgerald, Amsbio, MyBioSource, Nova TeinBio, Abnova, Aviva Systems Biology, Creative Biomart, antibodies-online.com, Abcam, Novus Biologicals, United States Biological, EIAAB (Hong Kong) Company Limited, Biomartik Corporation, Cloud-Clone Corp), anti-fibrinogen (distributed by Molecular Innovations, Fitzgerald, Ams Bio, Biorbyt, MyBioSource, NovaTein Bio, Abnova, Creative BioMart, Aviva Systems Biology, antibodies-online.com, Abcam, Novus Biologicals, United States Biological, EIAAB (Hong Kong) Company Limited, Biomartik Corporation, Cloud-Clone Corp) and anti-ADAM12 (distributed by Boster Immunoleader, AMS Bio, MyBioSource,

Abnova, Creative Biomart, antibodies-online.com, GeneTex, Biorbyt, United States Biological, EIAAB (Hong Kong) Company Limited, R&D Systems and Cloud-Clone Corp).

[00116] The methods include detecting the levels of one biomarker described herein using

one ELISA kit and/or antibody described herein. In some cases, the methods include

detecting the levels of one biomarker described herein using two ELISA kits and/or
antibodies described herein. In other cases, the methods include detecting the levels of one

biomarker described herein using three ELISA kits and/or antibodies described herein. In

other cases, the methods include detecting the levels of one biomarker described herein using
four ELISA kits and/or antibodies described herein. In other cases, the methods include

detecting the levels of one biomarker described herein using five ELISA kits and/or
antibodies described herein. In other cases, the methods include detecting the levels of one

biomarker described herein using more than five ELISA kits and/or antibodies described

herein. Often the one biomarker is independently measured using one, two, three, four, five

or more than five ELISA kits. In some cases, the one, two, three, four, five or more than five

ELISA kits may be the same or different ELISA kits.

[00117] The methods include detecting the levels of two biomarkers described herein using

two ELISA kits and/or antibodies described herein. In other cases, the methods include

detecting the levels of two biomarkers described herein using three ELISA kits and/or

antibodies described herein. In other cases, the methods include detecting the levels of two

biomarkers described herein using four ELISA kits and/or antibodies described herein. In

other cases, the methods include detecting the levels of two biomarkers described herein

using five ELISA kits and/or antibodies described herein. In other cases, the methods include

detecting the levels of two biomarkers described herein using more than five ELISA kits

and/or antibodies described herein. Often the two biomarkers are independently measured

using two, three, four, five or more than five ELISA kits. In some cases, the two, three, four,

five or more than five ELISA kits may be the same or different ELISA kits.

[00118] In other cases, the methods include detecting the levels of three biomarkers described

herein using three ELISA kits and/or antibodies described herein. In other cases, the methods

include detecting the levels of three biomarkers described herein using four ELISA kits

and/or antibodies described herein. In other cases, the methods include detecting the levels of

three biomarkers described herein using five ELISA kits and/or antibodies described herein. In other cases, the methods include detecting the levels of three biomarkers described herein using more than five ELISA kits and/or antibodies described herein. Often the three biomarkers are independently measured using three, four, five or more than five ELISA kits.

5 In some cases, the three, four, five or more than five ELISA kits may be the same or different ELISA kits.

[00119] In other cases, the methods include detecting the levels of four biomarkers described herein using four ELISA kits and/or antibodies described herein. In other cases, the methods include detecting the levels of four biomarkers described herein using five ELISA kits and/or
10 antibodies described herein. In other cases, the methods include detecting the levels of four biomarkers described herein using more than five ELISA kits and/or antibodies described herein. Often the four biomarkers are independently measured using four, five or more than five ELISA kits. In some cases, the four, five or more than five ELISA kits may be the same or different ELISA kits.

15 [00120] In certain cases, the antibodies against the selected biomarkers may be monoclonal antibodies. In some cases the antibodies against the selected biomarkers may be polyclonal antibodies. Of particular interest are antibodies against a plurality biomarkers selected from a group consisting of PlGF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12. Such antibodies may be monoclonal or polyclonal antibodies. Such antibodies
20 may be commercially available or generated by the user using methods known to those of ordinary skill in the art.

[00121] Any commercially available antibody known to one of ordinary skill in the art may be used to detect a biomarker listed herein and may be used in combination with the subject methods. For example, commercially available antibodies can include, but are not limited to,
25 anti-PlGF (distributed by Amb, Novus Biologicals, Nordic Biosite or Tebu Biologicals), anti-HPX (distributed by Sino Biological, Pierce, Sigma Aldrich, Origene, Lifespan, Proteintech Group, AbD Sertotec, BioRad, ThermoFisher, Agrisera, Angio-Proteomie, Enzo Life Sciences, Aviva Systems Biology, Everest Biotech, R&D systems, St. John's Laboratory, Abbotec, Biorbyt, Acris Antibodies, MyBioSource, AmsBio, Abgent, Santa
30 Cruz Biotechnology, Creative Biomart, Nova TeinBio, Raybiotech, Gene Tex, United States

Biological, Abcam, Cedarlane Labs, Gallus Immunotech, Abnova and Cloud-Clone Corp), anti-sFlt-1 (distributed by Cell Signaling Technology, Lifespan Biosciences, Antibodies-online.com, Sino Biological, AbD Serotec, Proteintech Group, Boster Immunoleader, Thermo Fischer Scientific, Merck Millipore, Agrisera, Atlas Antibodies, Fitzgerald, Aviva Systems Biology, Angio-Proteomie, eBioscience, Genway, Biorbyt, R&D systems, Life Technologies, St. John's Laboratory, Abbiotec, Acris Antibodies, MyBioSource, Amsbio, Santa Cruz Biotechnology, Creative Biomart, Origene, Nova TeinBio, Raybiotech, Novus Biologicals, ProSci, Gene Tex, United States Biological, Abbexa, Abcam, Bioworld Technology Inc., Abnova, Spring Bioscience and Cloud-Clone Corp), anti- PAPP-A (distributed by Lifespan Biosciences, Thermo Fisher Scientific, AbD Serotec, Bio-Rad, Merck Millipore, antibodies-online.com, R&D systems, Genway, Atlas Antibodies, Abbiotec, Amsbio, MyBioSource, SantaCruz Biotechnology, Aviva Systems Biology, Biorbyt, Creative Biomart, Nova TeinBio, Raybiotech, GeneTex, United States Biological, Fitzgerald, Novus Biologicals, Abcam, BBI Solutions, Abnova and Cloud-Clone Corp), anti-VEGF (distributed by Cell Signaling Technology, Lifespan Biosciences, antibodies-online.com, Epigentek, Angio-Proteomie, Aviva Systems Biology, R&D Systems, St. John's Laboratory, Thermo Fisher Scientific Inc., Biorbyt, Acris Antibodies, MyBioSource, SantaCruz Biotechnology, Gene Tex, United States Biological, Abbexa, Fitzgerald, Novus Biologicals, Abcam, Bioworld Technology Inc., Abnova, Sino Biological, Merck Millipore, Aviva Systems Biology, Origene, Life Technologies, Amsbio, Abgent, Creative Biomart, Raybiotech, IBL America, Boster Immunoleader, Atlas Antibodies, Bioworld Technology Inc., and Cloud-Clone Corp), anti-fibronectin (distributed by Proteintech Group, Fitzgerald, Lifespan Biosciences, Boster Immunoleader, Abcam, QED Bioscience, Sino Biological, AbD Serotec, Bio-Rad, Proten Biotechnik GmbH, Beckman Coulter, antibodies-online.com, Takara, Merck Millipore, Atlas Antibodies, Agrisera, Thermo Fisher Scientific, Inc., Rockland, Immquest, Enzo Life Sciences, Aviva Systems Biology, Genway, eBioscience, Biorbyt, R&D Systems, Amsbio, St. John's Laboratory, Abbiotec, Acris Antibodies, MyBioSource, Santa Cruz Biotechnology, Novus Biologicals, Creative Biomart, Nova TeinBio, Raybiotech, GeneTex, ProSci, United States Biological, Abbexa, Abcam, Cedarlane Labs, Molecular Innovations, Southern Biotech, Bioworld Technology Inc., Gallus

Immunotech, Abnova, Alfa Aesar and Cloud-Clone Corp), anti-fibrinogen (distributed by Lifespan Biosciences, antibodies-online, AbD Serotec, Bio-Rad, Absea Biotechnology, Thermo Fischer Scientific Inc., Merck Millipore, Agrisera, Atlas Antibodies, Aviva Systems Biology, Enzo Life Sciences, Rockland, Genway, R&D Systems, St. John's Laboratory, 5 Abbiotec, Amsbio, Acris Antibodies, MyBioSource, Abgent, Biorbyt, Santa Cruz Biotechnology, Creative Biomart, Origene, Nova TeinBio, Raybiotech, Gene Tex, Pro Sci, United States Biological, Fitzgerald, Cedarlane Labs, Novus Biologicals, Molecular Innovations, Haematologic Technologies Inc., Dako, Oxford Biomedical Research, Gallus Immunotech, Bioworld Technology Inc., Abnova, Nordic Immunological Laboratories, and 10 Cloud-Clone Corp) and anti-ADAM12 (distributed by Lifespan Biosciences, Sino Biological, AbD Serotec, Bio-Rad, Thermo Fischer Scientific Inc., Merck Millipore, antibodies-online.com, Atlas Antibodies, Enzo Life Sciences, Everest Biotech, Angio-Proteomie, Aviva Systems Biology, Proteintech Group, Genway, R&D Systems, St. John's Laboratory, Acris Antibodies, MyBio Source, Amsbio, Santa Cruz Biotechnology, Biorbyt, Creative Biomart, 15 Origene, Nova Tein Bio, Raybiotech, GeneTex, ProSci, United States Biological, Fitzgerald, Novus Biologicals, Abcam, Abnova and Cloud-Clone Corp).

[00122] Monoclonal antibodies that specifically bind to any of the biomarkers listed herein may be produced using methods known to those of ordinary skill in the art. These methods include the methods of Kohler and Milstein (Nature, 256: 495-497, 1975) and Campbell 20 ("Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam, 1985), as well as methods described by Huse et al. (Science, 246, 1275-1281, 1989).

[00123] Monoclonal antibodies may be prepared from supernatants of cultured hybridoma 25 cells or from ascites induced by intra-peritoneal inoculation of hybridoma cells into mice. These methods are described in Kohler and Milstein (Eur. J. Immunol, 6, 511-519, 1976). The route and schedule of immunization of the host animal or cultured antibody-producing cells may follow with route and schedules known to those of ordinary skill in the art for antibody stimulation and production. Typically, mice are used as the test model, however,

any mammalian subject or antibody producing cells therefrom can be used for production of mammalian, including human, hybrid cell lines.

[00124] Following immunization, immune lymphoid cells can be fused with myeloma cells to generate a hybrid cell line that can be cultured indefinitely, to produce monoclonal antibodies. For example, lymphocytes may be selected for fusion and may be isolated either from lymph node tissue or the spleens of immunized animals. Murine myeloma cell lines can be obtained, for example, from the American Type Culture Collection (ATCC; Manassas, VA). Human myeloma and mouse-human heteromyeloma cell lines have also been described (Kozbor et al., J. Immunol., 133:3001-3005, 1984; Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, pp. 51-63, 1987).

[00125] The hybrid cell lines can be maintained in vitro and stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely. The secreted antibody can be recovered from tissue culture supernatant by conventional methods such as precipitation, ion exchange chromatography, affinity chromatography, or the like. The antibody may be from any of one of the following immunoglobulin classes: IgG, IgM, IgA, IgD, or IgE, and the subclasses thereof, and preferably is an IgG antibody.

[00126] In certain cases, a first antibody set may be included, that is specifically designed to interact with selected biomarkers. For example, the first antibody set may be designed to interact with proteins selected from the group consisting of PlGF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12.

[00127] In some cases, detection reagents other than antibodies may be used to practice the methods described herein. A detection reagent specifically binds to a biomarker as described herein. In addition to antibodies, detection reagents may further comprise aptamers, Fc fragments, Fab fragments, Fab2 fragments, ScFv domains, diabodies, non-antibody ligands, small molecules, peptides, polypeptides, proteins, nanoparticles, affibodies or the like.

[00128] The disclosure provides a method for diagnosing or confirming a presence of preeclampsia in a subject comprising: performing at least two different assays that determine a level of fibronectin in a sample derived from the subject; and evaluating the sample and using the levels from the plurality of assays to diagnose or confirm the presence of

preeclampsia and calculate an index. In some cases, the method further comprises, based upon the index, suggesting a treatment for preeclampsia, the treatment involving aspirin, preterm labor or bedrest.

[00129] The disclosure provides a method for diagnosing or confirming an existence of

5 preeclampsia in a subject comprising: measuring a level of a ratio of sFlt-1 and PlGF and a level of a plurality of biomarkers in a sample derived from the subject, wherein none of the biomarkers is ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3),
10 apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) or heme; and evaluating the sample and using the levels from the first step to determine an index to diagnose or confirm the presence of preeclampsia and calculate an index. In some cases, the method further comprises step, based upon the index, suggesting a treatment for preeclampsia, the treatment involving aspirin, preterm labor or bedrest. In some cases, the
15 method further comprises performing replicates of identical assays for each biomarker using the sample. In some cases, the method further comprises determining a mean level for each of the biomarkers using levels derived from each of the multiple identical assays. In some cases, the method further comprises performing a $\log_2$, $\log_e$ or $\log_{10}$ transformation of the mean levels. In some cases, the method further comprises comparing the levels of each biomarker
20 to a standard curve for that biomarker. In some cases, the method further comprises weighting each of the biomarkers, wherein the weighting includes providing numbers into a polynomial such that each marker has a distinct weight. In some cases, the calculating comprises determining a ratio of adjusted levels of sFlt-1 and PlGF. In some cases, the calculating comprises determining a ratio of normalized levels of sFlt-1 and PlGF. In some
25 cases, the calculating comprises determining a ratio of raw levels of sFlt-1 and PlGF. In some cases, the calculating comprises determining a ratio of adjusted levels of sFlt-1 and PlGF compared to a control. In some cases, the calculating comprises determining a ratio of normalized levels of sFlt-1 and PlGF compared to a control. In some cases, the calculating comprises determining a ratio of raw levels of sFlt-1 and PlGF compared to a control.

[00130] The disclosure provides a method for diagnosing or confirming a presence of preeclampsia in a subject comprising: measuring a level of a ratio of sFlt-1 and PlGF and a level of a plurality of biomarkers in a sample derived from the subject, wherein none of the biomarkers is ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) or heme; and evaluating the sample and using the levels from the first step to determine an index to diagnose or confirm the existence of preeclampsia and calculate an index. In some cases, the plurality of biomarkers is selected from the group consisting of sFlt-1, PlGF, FN and PAPP-A; sFlt-1, PlGF, FN, ADAM12 and PAPP-A; sFlt-1, PlGF, PAPP-A and FN; sFlt-1, PlGF, HPX, FN and PAPP-A; PlGF, ADAM12, FN and PAPP-A; PlGF, FN and PAPP-A; sFlt-1, PlGF and FN; PlGF, FN and PAPP-A; sFlt-1, PlGF, FN and ADAM12; sFlt-1, PlGF and FN; PlGF, sFlt-1 and FN; sFlt-1, FN and ADAM12; PlGF, FN and PAPP-A. . In some cases, the method further comprises, based upon the index, suggesting a treatment for preeclampsia, the treatment involving aspirin, preterm labor or bedrest. In some cases, the method further comprises performing replicates of identical assays for each biomarker using the sample. In some cases, the method further comprises determining a mean level for each of the biomarkers using levels derived from each of the multiple identical assays. In some cases, the method further comprises performing a $\log_2$, $\log_e$ or $\log_{10}$ transformation of the mean levels. In some cases, the method further comprises comparing the levels of each biomarker to a standard curve for that biomarker. In some cases, the method further comprises weighting each of the biomarkers, wherein the weighting includes providing numbers into a polynomial such that each marker has a distinct weight. In some cases, the calculating consisting of determining a ratio of adjusted levels of sFlt-1 and PlGF. In some cases, the calculating consisting of determining a ratio of normalized levels of sFlt-1 and PlGF. In some cases, the calculating consisting of determining a ratio of raw levels of sFlt-1 and PlGF. In some cases, the calculating consisting of determining a ratio of adjusted levels of sFlt-1 and PlGF compared to a control. In some cases, the calculating consisting of determining a ratio of normalized levels of sFlt-1 and PlGF compared to a control. In some

cases, the calculating consisting of determining a ratio of raw levels of sFlt-1 and PlGF compared to a control.

Methods of Measurement

[00131] Means for assaying protein or peptide levels include, but are not limited to, enzyme immunoassays (EIA) such as enzyme multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), sandwich ELISA, competitive ELISA, IgM antibody capture ELISA (MAC ELISA), and microparticle enzyme immunoassay (MEIA); capillary electrophoresis immunoassays (CEIA), radioimmunoassays (RIA); immunoradiometric assays (IRMA); fluorescence polarization immunoassays (FPIA), or chemiluminescence assays (CL). Such assays can be automated. Immunoassays can also be used in conjunction with laser induced fluorescence. Liposome immunoassays, such as flow-injection liposome immunoassays and liposome immunosensors, are also suitable for use in the present disclosure. In addition, nephelometry assays, in which the formation of protein/antibody complexes results in increased light scatter that is converted to a peak rate signal as a function of the marker concentration, are suitable for use in the methods of the present disclosure.

[00132] One example for ELISA assay methodology is competitive ELISA. In this methodology an antibody to the target is first exposed with a labeled target (e.g., biotinylated hemopexin). The antibody is subsequently exposed to the unlabeled target (e.g., hemopexin). When introducing both the labeled target and unmodified target to the antibodies, both sets of targets compete for binding sites on the antibody. The more targets that are available, the fewer the amount of labeled targets that bind to the antibodies. Subsequently, the detectable signal from the labeled target will be detected. The labels can include, among others, radioisotopes (for example ^{14}C , ^3H , ^{32}P , ^{33}P , ^{35}S , ^{125}I and ^{131}I), fluorescers, phosphorscers, chemiluminescers, chromogenic dyes, enzymes, antibodies, particles such as magnetic particles, quantum dots, heavy elements, nuclear magnetic resonance (NMR) detectable isotopes, molecules that can be detected by mass spectroscopy, or specific binding molecules including conjugates. The label may be directly connected to the target, or connected through a spacer arm (e.g., polyethylene glycol or hydrocarbon). Examples of conjugates include, but are not limited to calmodulin binding protein (CBP) and calmodulin, a combination of biotin and avidin, a combination of biotin and streptavidin, a combination of biotin and

NeutrAvidin®, a combination of biotin and human-derived biotin-binding molecules, a combination of biotin and Strep-Tactin®, a combination of Strep-Tag® and Strep-Tactin®, a combination of Strep-TagII® and Strep-Tactin®, a combination of S-Tag® and S-protein, a combination of Halo Ligand® and Halotag®, a combination of glutathione and glutathione S-transferase, a combination of amylose and a maltose-binding protein, a combination of appropriately designed epitope and a humanized monoclonal antibody for the epitope, and a combination of appropriately designed sugar chains and relevant sugar chain-recognizing molecules including lectin and humanized monoclonal antibodies. Antigens-antibodies conjugates include for example, digoxigenin/anti-digoxigenin, dinitrophenyl (DNP) and anti-DNP, dansyl-X-anti-dansyl, Fluorescein and anti-fluorescein, lucifer yellow and anti-lucifer yellow, rhodamine and anti-rhodamine, and other conjugates known in the art. Other suitable binding pairs may include polypeptides such as the FLAG-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255: 192-194 (1992)]; tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)] and the antibodies each thereto.

[00133] In the ELISA and ELISA-based assays and/or protocols, one or more antibodies specific for the proteins of interest may be immobilized onto a selected solid surface, preferably a surface exhibiting a protein affinity such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed material, the assay plate wells are coated with a non-specific “blocking” protein that is known to be antigenically neutral with regard to the test sample such as bovine serum albumin (BSA), casein or solutions of powdered milk. This allows for blocking of non-specific adsorption sites on the immobilizing surface, thereby reducing the background caused by non-specific binding of antigen onto the surface. After washing to remove unbound blocking protein, the immobilizing surface is contacted with the sample to be tested under conditions that are conducive to immune complex (antigen/antibody) formation. Such conditions include diluting the sample with diluents such as BSA or bovine gamma globulin (BGG) in phosphate buffered saline (PBS)/Tween or PBS/Triton-X 100, which also tend to assist in the reduction of nonspecific background, and allowing the sample to incubate for about 2-4 hours

at temperatures on the order of about 25°-27°C (although other temperatures may be used).

Following incubation, the antisera-contacted surface is washed so as to remove non immunocomplexed material. An exemplary washing procedure includes washing with a solution such as PBS/Tween, PBS/Triton-X 100 or borate buffer. The occurrence and amount of immunocomplex formation may then be determined by subjecting the bound immunocomplexes to a second antibody having specificity for the target that differs from the first antibody and detecting binding of the second antibody. In certain cases, the second antibody will have an associated enzyme, e.g., urease, peroxidase or alkaline phosphatase, which will generate a color precipitate upon incubating with an appropriate chromogenic label. For example, a urease or peroxidase-conjugated anti-human IgG may be employed, for a period of time and under conditions which favor the development of immunocomplex formation (e.g., incubation for 2 hours at room temperature in a PBS-containing solution such as PBS/Tween). After such incubation with the second antibody and washing to remove unbound material, the amount of label is quantified, for example by incubation with a chromogenic label such as urea and bromocresol purple in the case of a urease label or 2,2'-azino-di-(3-ethyl-benzthiazoline)-6-sulfonic acid (ABTS) and H₂O₂, in the case of a peroxidase label. Quantitation is then achieved by measuring the degree of color generation, e.g., using a visible spectrum spectrophotometer.

[00134] Following quantitation, the data may be expressed in optical units, for example, as optical density or OD values if data are quantified using a visible spectrum spectrophotometer. In some cases, data may be expressed as absorbance, emission, radioactivity counts or the like depending on the reactive substrate used with the second antibody as described above. In any case, the magnitude of optical units quantified from any well containing no detectable substrate, for example a blank, may be subtracted from the optical units quantified from any well containing detectable substrate. This value may be an adjusted optical unit value, or an adjusted OD and included in further calculations described herein. In some cases, the magnitude of optical units quantified from any well containing no detectable substrate, for example a blank, may not be subtracted from the optical units quantified from any well containing detectable substrate. This value may be a raw optical unit value or a raw OD and included in further calculations described herein.

[00135] Alternatively, non-ELISA based-methods for measuring the levels of one or more proteins in a sample may be employed. Representative examples include but are not limited to mass spectrometry, chromatography, proteomic arrays, xMAPTM microsphere technology, flow cytometry, western blotting, spectroscopy, nephelometry, radial immunodiffusion techniques, single radial immunodiffusion assay, protein digestion and peptide analysis (e.g., the methods and systems described by Applied Proteomics) and immunohistochemistry.

[00136] Mass spectroscopy method may include any mass spectrometric (MS) techniques that can obtain precise information on the mass of peptides, and preferably also on fragmentation and/or (partial) amino acid sequence of selected peptides (e.g., in tandem mass spectrometry, MS/MS; or in post source decay, TOF MS), are useful herein. Suitable peptide MS and MS/MS techniques and systems are well-known *per se* and may be used herein, as well as liquid chromatography coupled to mass spectroscopy (LC-MS) and two-dimensional liquid chromatography coupled to tandem mass spectroscopy (2D-LC-MS/MS). MS arrangements, instruments and systems suitable for biomarker peptide analysis may include, without limitation, matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) MS; MALDI-TOF post-source-decay (PSD); MALDI-TOF/TOF; surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF) MS; electrospray ionization mass spectrometry (ESI-MS); ESI-MS/MS; ESI-MS/(MS)ⁿ (n is an integer greater than zero); ESI 3D or linear (2D) ion trap MS; ESI triple quadrupole MS; ESI quadrupole orthogonal TOF (Q-TOF); ESI Fourier transform MS systems; desorption/ionization on silicon (DIOS); secondary ion mass spectrometry (SIMS); atmospheric pressure chemical ionization mass spectrometry (APCI-MS); APCI-MS/MS; APCI-(MS)ⁿ; atmospheric pressure photoionization mass spectrometry (APPI-MS); APPI-MS/MS; and APPI-(MS)ⁿ. Peptide ion fragmentation in tandem MS (MS/MS) arrangements may be achieved using manners established in the art, such as, e.g., collision induced dissociation (CID). Detection and quantification of biomarkers by mass spectrometry may involve multiple reaction monitoring (MRM). MS peptide analysis methods may be advantageously combined with upstream peptide or protein separation or fractionation methods, such as for example with the chromatographic and other methods.

[00137] In some cases, biomarker proteins can be derivatized or modified prior to analysis, measurement, quantification or the like. Methods known to those of ordinary skill in the art may be employed to derivatize or modify proteins. Polymorphisms or modifications to protein biomarkers listed herein may be identified using the methods described herein, using the analytical, measurement or quantification methods described herein.

[00138] In other cases, the level of at least one PE biomarker may be evaluated by detecting in a patient sample the amount or level of one or more RNA transcripts or a fragment thereof, encoded by the gene of interest to arrive at a nucleic acid marker representation. The level of nucleic acids in the sample may be detected using any convenient protocol. While a variety of different manners of detecting nucleic acids are known, such as those employed in the field of differential gene expression analysis, one representative and convenient type of protocol for generating marker representations is array-based gene expression profiling protocols. Such applications are hybridization assays in which a nucleic acid that displays “probe” nucleic acids for each of the genes to be assayed/profiled in the marker representation to be generated is employed. In these assays, a sample of target nucleic acids is first prepared from the initial nucleic acid sample being assayed, where preparation may include labeling of the target nucleic acids with a label, e.g., a member of signal producing system. Following target nucleic acid sample preparation, the sample is contacted with the array under hybridization conditions, whereby complexes are formed between target nucleic acids that are complementary to probe sequences attached to the array surface. The presence of hybridized complexes is then detected, either qualitatively or quantitatively.

[00139] Specific hybridization technology which may be practiced to generate the marker representations employed in the subject methods includes the technology described in U.S. Patent Nos.: 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,800,992; the disclosures of which are herein incorporated by reference; as well as WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280. Arrays of “probe” nucleic acids that include a probe for each of the phenotype determinative genes whose expression is being assayed can be contacted with target nucleic acids from a subject sample. Contact is carried out under hybridization conditions, e.g., stringent hybridization conditions, and unbound nucleic acid is

then removed. The term “stringent assay conditions” as used herein refers to conditions that are compatible to produce binding pairs of nucleic acids, e.g., surface bound and solution phase nucleic acids, of sufficient complementarity to provide for the desired level of specificity in the assay while being less compatible to the formation of binding pairs between binding members of insufficient complementarity to provide for the desired specificity. Stringent assay conditions are the summation or combination (totality) of both hybridization and wash conditions.

[00140] The resultant pattern of hybridized nucleic acid provides information regarding expression for each of the genes that have been probed, where the expression information is in terms of whether or not the gene is expressed and, typically, at what level, where the expression data, e.g., marker representation (e.g., in the form of a transcriptosome), may be both qualitative and quantitative.

[00141] Alternatively, non-array based methods for quantitating the level of one or more nucleic acids in a sample may be employed, including those based on amplification protocols, e.g., Polymerase Chain Reaction (PCR)-based assays, including quantitative PCR, reverse-transcription PCR (RT-PCR), real-time PCR, loop mediated isothermal amplification of DNA (LAMP), strand displacement amplification (SDA), sequence based amplification (NASBA), self-sustained sequence replication (3SR), linear amplification, and the like.

[00142] Alternatively, functional assays, methods and protocols for determining the function of a protein hypothesized to be in a sample may be employed, including any standard functional assays known to one of ordinary skill in the art which would confirm or deny the presence of a hypothesized protein in a sample. In some cases, functional assay may include enzymatic assays, substrate assays, cleavage assays, colorimetric assays, pH assays and the like. Lysosome assays may also be performed.

[00143] When protein or peptide levels are to be detected, prior to performing a protocol, the total amount protein or a portion of the total amount of protein in a sample may be determined. This can be accomplished using a colorimetric assay, such as BCA, Lowry, Bradford, Coomassie, 660nm or the like.

[00144] Any of the assays herein may involve the use of a standard curve and or quality control, e.g., a high quality control, a low quality control and at least one blank.

[00145] A PE biomarker standard may be a purified recombinant protein, a purified protein, a synthetic protein, an engineered protein or the like that is similar to or the same as a biomarker measured by the methods described herein. In an exemplary case, a biomarker standard is a purified recombinant protein that is similar to or the same as a biomarker measured by the methods described herein. A blank may be water, a buffer, more than one buffer, a chemical, more than one chemical, a reagent, more than one reagent, an antibody, more than one antibody or any component of the methods described herein. A low quality control may be a previously analyzed sample, be a purified recombinant protein, a purified protein, a synthetic protein, an engineered protein or the like which has a low level of the biomarker analyzed by the method described herein. A high quality control may be a previously analyzed sample, be a purified recombinant protein, a purified protein, a synthetic protein, an engineered protein or the like which has a high level of the biomarker analyzed by the method described herein. A curve standard may be a concentrated, diluted or purified protein supplied by the user or the manufacturer of an ELISA which may be used to analyze a level of total protein, or total biomarker levels, in order to calculate a standard curve for the method described herein, often an ELISA method.

[00146] The wells of a single plate may contain, but are not limited to, at least one sample, at least one biomarker standard, at least one curve standard, a high quality control, a low quality control and at least one blank. In some cases, the wells of a single plate may contain, but are not limited to, more than one sample, more than one biomarker standard, more than one curve standard, a high quality control, a low quality control and more than one blank. For example, the wells of a single plate may contain at least 1, 2, 3, 4, 5, 5, 6, 7, 8, 9, or 10 samples; at least 1, 2, 3, 4, 5, 5, 6, 7, 8, 9, or 10 biomarker standards; at least 1, 2, 3, 4, 5, 5, 6, 7, 8, 9, or 10 curve standards; at least 1, 2, 3, 4, 5, 5, 6, 7, 8, 9, or 10 high quality controls; at least 1, 2, 3, 4, 5, 5, 6, 7, 8, 9, or 10 low quality controls; and/or at least 1, 2, 3, 4, 5, 5, 6, 7, 8, 9, or 10 blanks. The samples may be singles, duplicates, triplicates, quadruplicates, or any further extension of replicates.

[00147] In one example, a single ELISA assay plate contains eight standard curve samples in triplicate, six high quality control samples, six low quality control samples, and three samples in triplicate arrayed across the plate to avoid variation. Often, controls may be used in place

of high quality control and/or low quality control. In some cases, controls may be used as an internal calibrator. In other cases, controls may be used in place of biomarker standards. In other cases, controls may be used in place of standards. For example, controls may be used to generate the standard curve described herein.

5 **[00148] Monoclonal Antibodies**

[00149] The disclosure further provides a method for confirming the presence, absence or severity of preeclampsia in a subject comprising: utilizing a monoclonal antibody that selectively binds fibronectin to determine the levels of fibronectin in a sample derived from the subject, generating a report indicating the presence, absence or severity of preeclampsia
10 based on the levels and containing an index; and evaluating the sample and based upon the index, suggesting a treatment for preeclampsia, the treatment selected involving aspirin, preterm labor or bedrest.

[00150] In some cases, a method of confirming, diagnosing, prognosing, monitoring, characterizing or evaluating the severity of preeclampsia in a subject comprises: deriving a
15 biological sample from the subject; diluting the biotinylated hemopexin two fold, mixing the biotinylated hemopexin with the subject's biological sample, adding this mixture to an immunoassay plate; performing an analysis of the biological sample for the presence and amount of hemopexin (HPX) in an immunoassay test, and employing the biomarker level to provide a preeclampsia diagnosis, prognosis, confirmation, monitoring, characterization or
20 evaluation of its severity. In such cases, the assay methodology exemplifies a competitive ELISA assay. The biotinylated hemopexin competes with the sample-hemopexin for antibody binding sites. The amount of biotinylated hemopexin is subsequently detected spectroscopically following the introduction of appropriate conjugate forming partners such as avidin, streptavidin, NeutrAvidin®, human-derived biotin-binding molecules, and the like.
25 Such conjugate results in turn in a detectable signal.

[00151] ELISA kits may be used for performing ELISA assays. Those may be purchased from standard commercial manufacturers such as for example, disintegrin and metalloproteinase domain-containing protein 12 (ADAM12) from Mybiosource (SD, US); hemopexin (HPX) from Abcam Inc. (MA, US); placental growth factor (PlGF) from R&D

system Inc. (MN, US); and soluble fms- like tyrosine kinase (sFlt)-1 from R&D system Inc. (MN, US).

[00152] Any commercially available kit known to one of ordinary skill in the art may be used to detect a biomarker listed herein by the ELISA method and may be used in combination with the subject methods. For example, commercially available kits can include, but are not limited to, anti-P1GF (distributed by USCN Life Science Inc., Roche and R&D Systems), anti-sFlt-1 (distributed by Boster Bio., R&D systems, MyBioSource.com, antibodies-online, Biotrend Chemikalien GmbH, and Enzo Life Sciences), anti- PAPP-A (distributed by R&D systems, RayBioTech, IBL Japan, DRG International, Abnova, USCN Life Science, Novus Bio, Rapid Test, MyBioSource, antibodies-online.com, Fisher Scientific, elabsience, Sigma Aldrich, C USA Bio, ANSH Labs, Demeditec, Alpco, AMS Bio, NovaTeinBio, Creative Biomart, Biorbyt, Biomatic Corporation), anti-VEGF (distributed by AMS Bio, Mybiosource, Abnova, antibodies-online.com, United States Biological, Biomatik Corporation, Cloud-Clone Corp, Biovendor, Boster Immunoleader, Enzo Life Sciences, Fitzgerald, Abnova, Aviva Systems Biology and Creative Biomart), anti-fibronectin (distributed by Biovendor, Boster Immunoleader, QED Bioscience, eBioscience, Biorbyt, Fitzgerald, Amsbio, MyBioSource, Nova TeinBio, Abnova, Aviva Systems Biology, Creative Biomart, antibodies-online.com, Abcam, Novus Biologicals, United States Biological, EIAAB (Hong Kong) Company Limited, Biomatik Corporation, Cloud-Clone Corp), anti-fibrinogen (distributed by Molecular Innovations, Fitzgerald, Ams Bio, Biorbyt, MyBioSource, NovaTein Bio, Abnova, Creative BioMart, Aviva Systems Biology, antibodies-online.com, Abcam, Novus Biologicals, United States Biological, EIAAB (Hong Kong) Company Limited, Biomatik Corporation, Cloud-Clone Corp) and anti-ADAM12 (distributed by Boster Immunoleader, AMS Bio, MyBioSource, Abnova, Creative Biomart, antibodies-online.com, GeneTex, Biorbyt, United States Biological, EIAAB (Hong Kong) Company Limited, R&D Systems and Cloud-Clone Corp).

[00153] Standard curve samples may be analyzed for concentration of biomarkers using the methods described herein, for example, using ELISA methods, protocols, assays and the like. Each commercially available or in-house designed ELISA method, protocol and/or assay may provide instructions containing recommended dilutions of standard curve samples prior to

performing the method, protocol and/or assay on a sample or a set of standard curve samples. In some cases, the instructions for the commercially available or in-house designed ELISA method, protocol and/or assay may be followed and standard curve samples diluted according to the instructions. In some cases, standard curve samples may be diluted to a ratio of 1:1,

5 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, 1:1000, 1:1500, 1:2000, 1:2500, 1:3000, 1:3500, 1:4000, 1:10 000, 1:15 000, 1:20 000, 1:25 000, 1:30 000, 1:35 000, 1:40 000, 1:45 000, 1:50 000, 1:55 000, 1:60 000, 1:65 000, 1:70 000, 1:75 000, 1:80 000, 1:85 000, 1:90 000, 1:95 000 or 1:100 000., 1:4500, 1:5000, 1:5500, 1:6000, 1:6500, 1:7000, 1:7500, 1:8000, 1:8500, 1:9000, 1:9500 or 1:100 000. In some cases, standard curve samples may be diluted to a ratio of about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, 1:1000, 1:1500, 1:2000, 15 1:2500, 1:3000, 1:3500, 1:4000, 1:10 000, 1:15 000, 1:20 000, 1:25 000, 1:30 000, 1:35 000, 1:40 000, 1:45 000, 1:50 000, 1:55 000, 1:60 000, 1:65 000, 1:70 000, 1:75 000, 1:80 000, 1:85 000, 1:90 000, 1:95 000 or 1:100 000., 1:4500, 1:5000, 1:5500, 1:6000, 1:6500, 1:7000, 1:7500, 1:8000, 1:8500, 1:9000, 1:9500 or about 1:100 000. In some cases, standard curve samples may be diluted to a ratio of less than 1:1, 1:2, 1:3, 1:4, 1:5, 20 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, 1:1000, 1:1500, 1:2000, 1:2500, 1:3000, 1:3500, 1:4000, 1:10 000, 1:15 000, 1:20 000, 1:25 000, 1:30 000, 1:35 000, 1:40 000, 1:45 000, 1:50 000, 1:55 000, 1:60 000, 1:65 000, 1:70 000, 1:75 000, 1:80 000, 1:85 000, 1:90 000, 25 1:95 000 or 1:100 000., 1:4500, 1:5000, 1:5500, 1:6000, 1:6500, 1:7000, 1:7500, 1:8000, 1:8500, 1:9000, 1:9500 or less than 1:100 000. In some cases, standard curve samples may be diluted to a ratio of greater than 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:150, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550, 1:600, 1:650, 1:700, 1:750, 1:800, 1:850, 1:900, 1:950, 30 1:1000, 1:1500, 1:2000, 1:2500, 1:3000, 1:3500, 1:4000, 1:10 000, 1:15 000, 1:20 000,

1:25 000, 1:30 000, 1:35 000, 1:40 000, 1:45 000, 1:50 000, 1:55 000, 1:60 000, 1:65 000, 1:70 000, 1:75 000, 1:80 000, 1:85 000, 1:90 000, 1:95 000 or 1:100 000., 1:4500, 1:5000, 1:5500, 1:6000, 1:6500, 1:7000, 1:7500, 1:8000, 1:8500, 1:9000, 1:9500 or greater than 1:100 000. For the ratios listed above, 1 is the portion of the sample and the other value represents the diluent, wherein the diluent may be provided by the commercial manufacturer or the diluent may be provided by the user.

Data Processing and Data Use

[00154] The resultant data provides information regarding levels in the sample for each of the markers that have been probed, wherein the information is in terms of whether or not the marker is present and, typically, at what level, and wherein the data may be both qualitative and quantitative. As such, where detection is qualitative, the methods provide a reading or evaluation, e.g., assessment, of whether or not the target marker, e.g., nucleic acid or protein, is present in the sample being assayed. In some cases, the methods provide a quantitative detection of whether the target marker is present in the sample being assayed, e.g., an evaluation or assessment of the actual amount or relative abundance of the target analyte, e.g., nucleic acid or protein in the sample being assayed. In such cases, the quantitative detection may be absolute or, if the method is a method of detecting plurality of different analytes, e.g., target nucleic acids or protein, in a sample, relative.

[00155] Once the level of the one or more preeclampsia markers has been determined, the measurement(s) may be analyzed in any of a number of ways to obtain a preeclampsia marker level representation. By a “biomarker level representation” or “gene representation” it is meant a representation of the levels, e.g., RNA, DNA or protein levels, of one or more preeclampsia markers and/or protein cofactors of interest.

[00156] For example, the preeclampsia marker measurements may be analyzed to produce a preeclampsia score or index which may be calculated using a logistic regression analysis. In some cases, a ratio of at least two biomarkers may be calculated and combined with additional explanatory variables for use in a model. Often the ratio of biomarkers may be the ratio of sFlt-1 and PlGF. In some cases, the model generated may be a penalized model. In other cases, the model generated may be an un-penalized model. A sample may contribute to an inaccurate model and as such, the out-of-sample performance may be evaluated to

determine if a sample contributes to an inaccurate model. In some cases, the out-of-sample performance may be evaluated using any number of approaches, including but not limited to, a cross-validation approach. In some instances, the accuracy of the model depends on a number of parameters including, but not limited to, how representative the training sample is of the target population, the number of variables included in the model, biomarker measurement uncertainty, etc. The out-of-sample performance of the model is often evaluated to estimate the error of the model in a sample set that has not been used for training.

[00157] As an example, the preeclampsia marker measurements may be analyzed as a biomarker panel. Predictive members of the biomarker panel may be selected by statistical feature selection process. For example, the panel of analytes may be selected by combining genetic algorithm (GA) and all paired (AP) support vector machine (SVM) methods for preeclampsia classification analysis. Predictive features are automatically determined, e.g. through iterative GA/SVM, leading to very compact sets of non-redundant preeclampsia-relevant analytes with the optimal classification performance. It is possible that these different classifier sets harbor only modest overlapping gene, protein fragment or protein features, but have similar levels of accuracy.

[00158] As an example, the preeclampsia marker measurements may be analyzed to generate a preeclampsia signature. A preeclampsia signature for a patient sample may be calculated by any of a number of methods known in the art for calculating biomarker signatures. For example, the levels of each of the one or more preeclampsia markers in a patient sample may be $\log_2$, $\log_e$ or $\log_{10}$ transformed, and normalized, e.g., as described above for generating a preeclampsia marker profile. The normalized expression levels for each marker is then weighted by multiplying the normalized level to a weighting factor, or weight, to arrive at weighted expression levels for each of the one or more markers. The weighted levels are then totaled and in some cases averaged to arrive at a single weighted level for the one or more preeclampsia markers analyzed.

[00159] In some cases, a PE test can confirm whether or not a subject suspected of having PE actually does have PE. The single weighted level for the one or more preeclampsia markers analyzed can confirm the severity of PE. For example, the single weighted level can yield high, medium or low weighted scores which can confirm high, medium or low severity of PE

in a subject. In some instances, a high single weighted level confirms a high severity of PE in a subject. In some instances, a medium single weighted level confirms a high severity of PE. In some instances, a low single weighted level confirms a high severity of PE. In some instances, a high single weighted level confirms a medium severity of PE. In some instances, a medium single weighted level confirms a medium severity of PE. In some instances, a low single weighted level confirms a medium severity of PE. In some instances, a high single weighted level confirms a low severity of PE. In some instances, a medium single weighted level confirms a low severity of PE. In some instances, a low single weighted level confirms a low severity of PE in a subject.

[00160] The single weighted level for the one or more preeclampsia markers analyzed can indicate the likelihood that a subject suspected of having PE has or doesn't have PE. For example, the single weighted level can yield high, medium, and low weighted scores which can indicate the likelihood of a subject having or not having PE. In some instances, a high single weighted level indicates a high likelihood of a subject having or not having PE. In some instances, a medium single weighted level indicates a high likelihood of a subject having or not having PE. In some instances, a low single weighted level indicates a high likelihood of a subject having or not having PE. In some instances, a high single weighted level indicates a medium likelihood of a subject having or not having PE. In some instances, a medium single weighted level indicates a medium likelihood of a subject having or not having of PE. In some instances, a low single weighted level indicates a medium likelihood of a subject having or not having PE. In some instances, a high single weighted level indicates a low likelihood of a subject having or not having PE. In some instances, a medium single weighted level indicates a low likelihood of a subject having or not having PE. In some instances, a low single weighted level indicates a low likelihood of a subject having or not having PE.

[00161] The single weighted level for the one or more preeclampsia markers analyzed can indicate the likelihood that a subject will develop preeclampsia. For example, the single weighted level can yield high, medium, and low weighted scores which can indicate the likelihood that a subject will develop preeclampsia prior to having any symptoms. In some instances, a high single weighted level indicates a high likelihood that a subject will develop

preeclampsia. In some instances, a medium single weighted level indicates a high likelihood that a subject will develop preeclampsia. In some instances, a low single weighted level indicates a high likelihood that a subject will develop preeclampsia. In some instances, a high single weighted level indicates a medium likelihood that a subject will develop preeclampsia. In some instances, a medium single weighted level indicates a medium likelihood that a subject will develop preeclampsia. In some instances, a low single weighted level indicates a medium likelihood that a subject will develop preeclampsia. In some instances, a high single weighted level indicates a low likelihood that a subject will develop preeclampsia. In some instances, a medium single weighted level indicates a low likelihood that a subject will develop preeclampsia. In some instances, a low single weighted level indicates a low likelihood that a subject will develop preeclampsia.

[00162] The weighting factor, or weight, may be determined by any statistical machine learning methodology, for example, Principle Component Analysis (PCA), linear regression, support vector machines (SVMs), and/or random forests of the dataset from which the sample was obtained may be used. For example, the analyte level of each preeclampsia marker may be $\log_2$, $\log_e$ or $\log_{10}$ transformed and weighted either as 1 (for those markers that are increased in level in preeclampsia) or -1 (for those markers that are decreased in level in preeclampsia), and the ratio between the sum of increased markers as compared to decreased markers determined to arrive at a preeclampsia signature. A preeclampsia signature is an example of a preeclampsia marker level representation.

[00163] In certain cases the relative weight of plurality of markers is used to determine a biomarker signature, biomarker score or biomarker index. Markers whose weight may be compared include any of the markers described herein or those selected from a group comprising HPX, sFlt-1, PAPP-A, FN, FG, VEGF (excluding VEGF-R1), PlGF and ADAM12. For example, in some instances a PE signature, PE score or PE index involves comparing the levels of sFlt-1 and PlGF in combination with at least one other biomarker and determining if their relative weight is at least 1.5:1, 2:1, 2.5:1, 3:1 or 3.5:1 respectively, wherein such a determination is indicative of PE or likelihood of PE.

[00164] The PE score or PE index can be further categorized into values that confirms the severity of PE in a subject. For example, the PE score or PE index can yield high, medium or

low values which can confirms the severity of PE in a subject. In some instances, a high PE score or PE index can confirms a high severity of PE in a subject. In some instances, a medium PE score or PE index confirms a high severity of PE. In some instances, a low PE score or PE index confirms a high severity of PE. In some instances, a high PE score or PE index confirms a medium severity of PE. In some instances, a medium PE score or PE index confirms a medium severity of PE. In some instances, a low PE score or PE index confirms a medium severity of PE. In some instances, a high PE score or PE index confirms a low severity of PE. In some instances, a medium PE score or PE index confirms a low severity of PE. In some instances, a low PE score or PE index confirms a low severity of PE in a subject.

[00165] The PE score or PE index can be further categorized into values that indicate the likelihood that a subject suspected of having PE has or does not have PE. For example, PE score or PE index can yield high, medium, and low values which can indicate the likelihood of a subject having or not having PE. In some instances, a high PE score or PE index indicates a high likelihood of a subject having or not having PE. In some instances, a medium PE score or PE index indicates a high likelihood of a subject having or not having PE. In some instances, a low PE score or PE index indicates a high likelihood of a subject having or not having PE. In some instances, a high PE score or PE index indicates a medium likelihood of a subject having or not having PE. In some instances, a medium PE score or PE index indicates a medium likelihood of a subject having or not having of PE. In some instances, a low PE score or PE index indicates a medium likelihood of a subject having or not having PE. In some instances, a high PE score or PE index indicates a low likelihood of a subject having or not having PE. In some instances, a medium PE score or PE index indicates a low likelihood of a subject having or not having PE. In some instances, a low PE score or PE index indicates a low likelihood of a subject having or not having PE.

[00166] The PE score or PE index can be further categorized into values that can predict the likelihood that a subject will develop preeclampsia prior to the onset of symptoms (e.g., high, medium or low likelihood). In some instances, a high PE score or PE index indicates a high likelihood that a subject will develop preeclampsia. In some instances, a medium PE score or PE index indicates a high likelihood that a subject will develop preeclampsia. In some

instances, a low PE score or PE index indicates a high likelihood that a subject will develop preeclampsia. In some instances, a high PE score or PE index indicates a medium likelihood that a subject will develop preeclampsia. In some instances, a medium PE score or PE index indicates a medium likelihood that a subject will develop preeclampsia. In some instances, a low PE score or PE index indicates a medium likelihood that a subject will develop preeclampsia. In some instances, a high PE score or PE index indicates a low likelihood that a subject will develop preeclampsia. In some instances, a medium PE score or PE index indicates a low likelihood that a subject will develop preeclampsia. In some instances, a low PE score or PE index indicates a low likelihood that a subject will develop preeclampsia.

[00167] In some cases, the data may be processed and/or further analyzed using any number of methods, algorithms and calculations in order to prioritize the individual data points, data sets and prevent over-fitting of false positive or falsely correlated results. As described and shown herein, some data may not be corrected, for example training data, which may be at times an optimistic estimate of the data. In addition, some data may be corrected, for example, corrected data, which may be at times a conservative estimate of the data. In some cases, the corrected data are a cross-validated estimate of a value, for example the value may be the area under the curve (AUC). Sometimes, the performance of the model is evaluated on the training data providing an optimistic estimate. Occasionally, a correction for optimism estimate of performance is calculated for different performance metrics. In some cases, the corrected performance estimate is calculated using cross-validation. In some examples, the performance metric is the area under the curve (AUC) or ROC value.

[00168] Data may be used to prepare models. In some cases, the models are penalized or optimized. Models derived from using penalized data treat biomarkers individually and prevents the data acquired for each individual biomarker, or replicate data measurement for each biomarker, from controlling the preeclampsia index, preeclampsia score or preeclampsia profile. In some cases, the data used in penalized models controls the preeclampsia index, preeclampsia score or preeclampsia profile by improperly weighting the preeclampsia index, preeclampsia score or preeclampsia profile to one direction or another. In some cases, the data is used in a penalized model such that the preeclampsia index, preeclampsia score or preeclampsia profile is not improperly weighted and prevents the preeclampsia index,

preeclampsia score or preeclampsia profile from being determined on an unrelated factor, such as a damaged sample, a sample not reflective of the subject, etc. In some cases, optimized models are derived from data such that correction may prevent the acquired data value for an individual marker from falsely contributing to the equation. In some cases, corrected and/or optimized models are derived from data that may be more predictive of a preeclampsia index, preeclampsia score or preeclampsia profile compared to the training numbers. In an exemplary case, a model is constructed using a penalized logistic regression approach, wherein the penalized approach fits a regression model while adding a constraint on the sum of the absolute values of the coefficients of the model described herein. The constraint may prevent variables that might be highly associated with the outcome in this sample set from falsely affecting the model.

[00169] In some cases, the data calculation to determine the preeclampsia index, preeclampsia score or preeclampsia profile includes the ratio of sFlt-1 and PlGF. This ratio may or may not be normalized along with the assay result. As described herein, the preeclampsia index, preeclampsia score or preeclampsia profile is a combination of biomarkers that may be determined using the analysis methods described herein.

[00170] The data may fit into three separate categories, Tier I, Tier II and Tier III. Tier I corresponds to ROC values of at least 0.98 or more. Tier II corresponds to ROC values between 0.92 and 0.98, and Tier III corresponds to ROC values of 0.92 or less. In some cases, ROC values greater than 0.850 may be clinically valuable. In some cases, ROC values greater than 0.90 or 0.950 may be clinically valuable. In some instances, there is no statistically significant difference between Tier I and Tier II. In some examples, there is statistically significant difference between Tier I and Tier II. In some cases, the tier values may be ROC values which may indicate the sensitivity and/or specificity of a method and or a data point for a particular biomarker or set of biomarkers. In some cases, the data outputs may be classified using at least one algorithm, at least one threshold value, at least directional change over time, comparisons within a single subject, comparisons within a group of subjects or comparisons to a reference standard. Often, the at least one algorithm may be an algorithm described herein or at least one known to one of ordinary skill in the art. Often the at least one threshold value may be described herein or known to one of ordinary skill in the

art. In some cases, the threshold value may be set based on a single parameter or a set of parameters; either the single or the set may be defined by the user. Often the directional change may be described herein or known to one of ordinary skill in the art. In some cases, the directional change may be based on a single parameter or a set of parameters; either the single or the set may be defined by the user. Often the comparisons within a single subject may be described herein or known to one of ordinary skill in the art. In some cases, the comparisons within a single subject (which may be the same or different from the tested subject) may be based on a single parameter or a set of parameters; either the single or the set may be defined by the user. Often the comparisons within a group of subjects may be described herein or known to one of ordinary skill in the art. In some cases, the comparisons within a group of subjects may be based on a single parameter or a set of parameters; either the single or the set may be defined by the user. Often the comparisons to a reference standard may be described herein or known to one of ordinary skill in the art. In some cases, the comparisons to a reference standard may be based on a single parameter or a set of parameters; either the single or the set may be defined by the user. The reference standard may be based on actual test subject or subjects, or other experimental values, or a theoretical value derived from a model, or on any combination thereof.

[00171] In some cases the disclosure provides for a method for confirming the presence or the absence of preeclampsia in a subject comprising: evaluating a plurality of biomarkers in a sample derived from the subject to confirm if the subject has or does not have preeclampsia wherein the confirmation has a specificity of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or more, which is used to calculate an index. In some instances, the confirmation has an AUC of at least 0.9, 0.91, 0.92, 0.93, 0.95, 0.96, 0.97, 0.980, 0.985, 0.988, 0.990, 0.995, 0.999 or more, which is used to calculate an index. For example, a sample derived from more than subject is evaluated to confirm the presence or the absence of PE, at a specificity described herein, for examples samples from more than 5, 10, 50, 100, 130, 135, 138, 150, 200, 220, 247, 250, 300, 350, 400, 450, 500, or more than 1000 subjects are evaluated to confirm the presence or the absence of PE at a specificity described herein. In some instances, the subject is evaluated as having PE using traditional methods. In some examples, the subject actually experiences PE. Traditional methods involve measuring proteinuria, blood pressure, weight

gain, blood glucose, platelet count and any other method traditionally used to evaluate PE known in the art.

[00172] The disclosure further provides a method for distinguishing a subject having preeclampsia from a subject having symptoms suggestive of preeclampsia but who does not have preeclampsia, the method distinguishing preeclampsia from complication of pregnancy symptoms, chronic hypertension, gestational hypertension, autoimmune disorders and/or gestational diabetes, wherein the method has a specificity of at least 95%, or has an AUC of at least 0.9, comprising: evaluating the level of a plurality of different biomarkers from a sample derived from the subject, generating an index indicative of the presence of preeclampsia, absence of preeclampsia, severity of preeclampsia. In some cases, the method further comprises based upon the index, suggesting a treatment for preeclampsia, the treatment involving aspirin, preterm labor or bedrest. In some cases, the autoimmune disorder is SLE or lupus. In some cases, the method further comprises weighting each of the plurality of biomarkers, wherein the weighting includes providing numbers into a polynomial such that each marker has a distinct weight.

[00173] The disclosure provides a method for confirming the presence or the absence of preeclampsia in a subject comprising: evaluating a plurality of biomarkers in a sample derived from the subject to confirm if the subject has preeclampsia wherein the confirmation has a specificity of greater than 95%, or has an AUC greater than 0.9 and is used to calculate an index.

[00174] The disclosure further provides a method for diagnosing or confirming a presence of preeclampsia in a subject comprising: (a) performing at least two different assays that determine a level of fibronectin in a sample derived from the subject; and (b) evaluating the sample and using the levels from the two different assays to diagnose or confirm the presence of preeclampsia and calculate an index.

[00175] In some cases, the disclosure provides a method for diagnosing or confirming a presence of preeclampsia in a subject comprising: (a) performing at least one assay which utilizes an antibody which binds fibronectin or an antibody that selectively binds a same antigen of fibronectin as the antibody, wherein the binding of the antibody determines a level of fibronectin in a sample derived from the subject; and (b) evaluating the sample and using

the level of fibronectin from the at least one assay to diagnose or confirm the presence of preeclampsia and calculating an index.

[00176] The disclosure also describes a method for diagnosing or confirming a presence of preeclampsia or the absence of preeclampsia in a subject comprising: (a) evaluating a level of a ratio of sFlt-1 and PlGF and a level a plurality of different biomarkers in a sample derived from the subject, wherein the different biomarker is not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) or heme; and (b) evaluating the sample and using the levels from step (a) to determine an index to diagnose or confirm the existence of preeclampsia and calculate an index.

[00177] The disclosure further provides a method for distinguishing a subject having preeclampsia from a subject having symptoms suggestive of preeclampsia but who does not have preeclampsia, the method distinguishing preeclampsia from complication of pregnancy symptoms, chronic hypertension, gestational hypertension, autoimmune disorders and/or gestational diabetes, wherein the method has a specificity of at least 90% or AUC of at least 0.9 comprising: evaluating the level of a plurality of different biomarkers from a sample derived from the subject, generating an index indicative of the presence of PE, absence of PE, characteristics of PE, severity of PE, diagnosis of PE or prognosis of PE.

[00178] In some cases, the disclosure further describes a method for analyzing the diagnosis, prognosis, characteristics, presence, absence or severity of preeclampsia in a subject comprising: (a) utilizing a monoclonal antibody that selectively binds fibronectin to determine the levels of fibronectin in a sample derived from the subject, (b) generating a report indicating the presence, absence or severity of preeclampsia based on the levels and containing an index; and (c) evaluating the sample and based upon the index, suggesting a treatment for preeclampsia, the treatment involving aspirin, preterm labor or bedrest.

[00179] The disclosure also describes a method for analyzing the diagnosis, prognosis, characteristics, presence, absence or severity of preeclampsia in a sample derived from a

subject comprising: utilizing an antibody directed to the antigen of the fibronectin antibody in at least one fibronectin ELISA kit to analyze and evaluate a sample from the subject.

[00180] The disclosure further provides a method for diagnosing or confirming a presence of preeclampsia in a subject comprising: (a) performing at least one assay which utilizes an antibody that selectively binds fibronectin, a portion of fibronectin, a part of fibronectin or a fragment of fibronectin, wherein the binding of the antibody determines the level of fibronectin in a sample derived from the subject; and (b) evaluating the sample and using the level of fibronectin from the one assay to diagnose or confirm the existence of preeclampsia and calculate an index.

[00181] In some cases, the disclosure describes a method for confirming that a subject does not have preeclampsia comprising: evaluating a plurality of biomarkers in a sample derived from the subject to confirm the subject does not have preeclampsia wherein the confirmation has a specificity of greater than 95% or has an 0.9 AUC and is used to calculate an index.

[00182] In some cases a PE signature, PE score or PE index involves comparing the levels of FN, FG, and another biomarkers selected from the following: HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), PlGF and ADAM12; and determining if their relative weight is at least 3:1, 5:1, 10:1, 20:1, 30:1, 50:1, 50:1, 100:1, respectively, wherein such a determination is indicative of PE or likelihood of PE.

[00183] Other combinations of interest include comparing sFlt-1 and another biomarkers selected from the following: HPX, FN, FG, PAPP-A, VEGF (excluding VEGF-R1), PlGF and ADAM12 and determining if their relative weight is at least 2:1, 2.5:1 3:1, 5:1, 10:1, 20:1, 30:1, 50:1, 50:1, 100:1, respectively, wherein such a determination is indicative of PE or likelihood of PE.

[00184] Other combinations of interest include comparing PlGF and another biomarkers selected from the following: HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12 and determining if their relative weight is at least 2:1, 2.5:1 3:1, 5:1, 10:1, 20:1, 30:1, 50:1, 50:1, 100:1, respectively, wherein such a determination is indicative of PE or likelihood of PE.

[00185] Other combinations of interest include comparing VEGF (excluding VEGF-R1) and another biomarkers selected from the following: HPX, sFlt-1, PAPP-A, PlGF, FN, FG, and

ADAM12; and determining if their relative weight is at least 2:1, 2.5:1, 3:1, 5:1, 10:1, 20:1, 30:1, 50:1, 100:1, respectively, wherein such a determination is indicative of PE or likelihood of PE.

[00186] As an example, the preeclampsia marker measurements may be analyzed to produce a preeclampsia score. Like a preeclampsia signature, a preeclampsia score is a single metric value that represents the sum of the weighted levels of one or more preeclampsia markers in a patient sample. A preeclampsia score may be determined by methods very similar to those described above for a preeclampsia signature, e.g. the levels of each of the one or more preeclampsia markers in a patient sample may be $\log_2$, $\log_e$ or $\log_{10}$ transformed and normalized, e.g., as described above for generating a preeclampsia profile; the normalized expression levels for each marker is then weighted by multiplying the normalized level to a weighting factor, or weight, to arrive at weighted levels for each of the one or more markers; and the weighted levels are then totaled and in some cases averaged to arrive at a single weighted level for the one or more preeclampsia markers analyzed. Occasionally, the weighted levels for the one or more preeclampsia markers may be subsequently transformed, for example using a logarithm-like inverse functions such as double logarithm $\ln(\ln(x))$, super-4-logarithm (i.e. tetra logarithm), hyper-4-logarithm (i.e. tetration), iterated logarithm, Lambert W function, or logit.

[00187] In contrast to a preeclampsia signature, the weighted levels are defined by a reference dataset, or training dataset. Thus, the preeclampsia score is defined by a reference dataset.

[00188] A preeclampsia index is an example of a preeclampsia marker level representation. A PE index is a metric system that indicates severity of PE or the degree of likelihood of developing PE. It is used to determine in what class the female subject is in. The PE index is calculated from the PE score, using a classification algorithm. Examples for classification algorithms are well known in the art. These algorithms can be formed using any suitable statistical classification (or “learning”) method that attempts to segregate bodies of data into classes based on objective parameters present in the data. Classification methods may be either supervised or unsupervised. Examples of supervised and unsupervised classification processes are described in Jain, “Statistical Pattern Recognition: A Review,” IEEE

Transactions on Pattern Analysis and Machine Intelligence, Vol. 22, No. 1, January 2000. In supervised classification, training data containing examples of known categories are presented to a learning mechanism, which learns one or more sets of relationships that define each of the known classes. New data may then be applied to the learning mechanism, which then classifies the new data using the learned relationships. Examples of supervised classification processes include linear regression processes (e.g., multiple linear regression (MLR), partial least squares (PLS) regression and principal components regression (PCR)), binary decision trees (e.g., recursive partitioning processes such as CART-classification and regression trees), artificial neural networks such as back propagation networks, discriminant analyses (e.g., Bayesian classifier or Fischer analysis), logistic classifiers, and support vector classifiers (support vector machines). An additional classification method is a recursive partitioning process. Recursive partitioning processes use recursive partitioning trees to classify spectra derived from unknown samples. Further details about recursive partitioning processes are provided in U.S. Patent Application No. 2002/0138208 A1 to Paulse et al., “Method for Analyzing Mass Spectra.”

[00189] In some embodiments, the classification of PE that is used to provide the preeclampsia index described herein is not based on at least one of the factors in the group comprising blood pressure, weight gain, water retention, hereditary factors, proteinuria, headache, edema, protein/creatinine ratio, platelet count, stress, PE in prior pregnancies, nulliparity, age, age less than 20 years, age greater than 35, race, African-American and Filipino decent, serotype, Papanicolaou test results (Pap smear), prior preeclampsia episodes, familial history, number of pregnancies, number of miscarriages, body mass index (BMI), gestational diabetes, type I diabetes, obesity, glucose level, current and past medications, stress, PE in prior pregnancies (of the subject or her family members), chronic hypertension, renal disease or thrombophilia. In some embodiments, the classification of PE that is used to provide the preeclampsia index described herein is not based on any of the characteristics just delineated in this paragraph.

[00190] In some examples, the classification of PE that is used to provide the preeclampsia index described herein is based on at least one of the factors in the group comprising blood pressure, weight gain, water retention, hereditary factors, proteinuria, headache, edema,

protein/creatinine ratio, platelet count, stress, PE in prior pregnancies, nulliparity, age, age less than 20 years, age greater than 35, race, African-American and Filipino decent, serotype, Papanicolaou test results (Pap smear), prior preeclampsia episodes, familial history, number of pregnancies, number of miscarriages, body mass index (BMI), gestational diabetes, type I diabetes, obesity, glucose level, current and past medications, stress, PE in prior pregnancies (of the subject or her family members), chronic hypertension, renal disease and thrombophilia.

Analysis of Data and Determination of Phenotype

[00191] In certain cases, the expression (e.g., polypeptide level) of only one marker is evaluated to produce a marker level representation. In some cases, the expression of plurality of markers (e.g., at least 1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, or more markers) is evaluated. Accordingly, in the subject methods, the expression of at least one marker in a sample is evaluated. In certain cases, the evaluation that is made may be viewed as an evaluation of the proteome, as that term is employed in the art.

[00192] The marker level representation arrived at in this manner finds many uses in diagnosing, prognosing, characterizing, evaluating the severity of preeclampsia, or in confirming the presence or the absence of preeclampsia. For example, the marker level representation may be employed to predict if a subject will develop preeclampsia, to diagnose preeclampsia in a subject, to characterize a diagnosed preeclampsia, or to monitor the responsiveness of the subject to treatment for preeclampsia. In some instances, the measurement of particular combinations of preeclampsia markers disclosed herein provides for a preeclampsia prognosis that has an improved accuracy over a preeclampsia prognosis made using standard methods known in the art, e.g. VEGF-R1 (e.g., sFLT-1) and PlGF.

[00193] In one case, the marker level representation may be employed in a method for diagnosing, prognosing, monitoring, characterizing or evaluating the severity of preeclampsia, or for confirming the presence or the absence of preeclampsia in a subject based on relative weights of biomarkers. Such method comprises: deriving a biological sample from a subject; performing an analysis of the subject's biological sample for the presence and amount of PlGF, HPX, sFlt-1 (i.e. VEGF-R1), PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12 employing the biomarker level to provide a preeclampsia diagnosis or

prognosis; wherein the relative weight of FN, to the biomarker is at least 1.5, 2, 2.5, 3, 3.5, 4, 4.5, or at least 5, and employing the biomarker level to provide a preeclampsia diagnosis or prognosis; wherein the weight comprises: generating a biomarker profile, and determining a single weighted level of the biomarker; wherein the profile comprises expression $\log_2$, $\log_e$ or $\log_{10}$ transformation and normalization; wherein weight level comprises multiplying the profile with a weighting factor, and wherein the weighting factor is calculated by a method comprising statistical machine learning method which may include, for example, any of the following: Principle Component Analysis (PCA), linear regression, support vector machines (SVMs), and random forests analysis.

[00194] In some cases, the marker level representation may be employed in a method diagnosing, prognosing, monitoring, characterizing, evaluating the severity of preeclampsia, or confirming the presence or the absence of preeclampsia in a subject based on relative weights of biomarkers. Such method comprises: deriving a biological sample from the subject; performing an analysis of her biological sample for the presence and amount P1GF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), and ADAM12; employing the biomarker level to provide a preeclampsia diagnosis or prognosis; wherein the relative level of FN, FG, to the number of biomarkers is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50 or more.

[00195] The disclosure provides a method for confirming if a subject does not have preeclampsia comprising: evaluating a sample derived from the subject to determine level of plurality of biomarkers in the sample, using the levels of the plurality of biomarkers to calculate an index representative of a likelihood that the subject does not have preeclampsia; and based upon the index, confirming if the subject does not have preeclampsia. In some cases, the evaluating does not comprise comparing a sample derived from the subject at a first time point and a sample derived from the same subject at a second time point. In some instances, the evaluating does comprise comparing a sample derived from the subject at a first time point and a sample derived from the same subject at a second time point. In some cases, the method further comprises, based upon the index, suggesting a treatment for preeclampsia, the treatment involving aspirin, preterm labor or bedrest. Sometimes, the plurality of biomarkers are selected from the group comprising sFlt-1, P1GF, FN and PAPP-

A; sFlt-1, PlGF, FN, ADAM12 and PAPP-A; sFlt-1, PlGF, PAPP-A and FN; sFlt-1, PlGF, HPX, FN and PAPP-A; PlGF, ADAM12, FN and PAPP-A; PlGF, FN and PAPP-A; sFlt-1, PlGF and FN; PlGF, FN and PAPP-A; sFlt-1, PlGF, FN and ADAM12; sFlt-1, PlGF and FN; PlGF, sFlt-1 and FN; sFlt-1, FN and ADAM12; or PlGF, FN and PAPP-A. In some cases, the calculating further comprises determining a ratio of levels of sFlt-1 and PlGF. In some cases, the levels are adjusted, normalized or raw levels, or any combination thereof.

[00196] In some cases, the marker level representation is employed by comparing it to a phenotype determination element, e.g., a preeclampsia phenotype determination element, to identify similarities or differences with the phenotype determination element, where the similarities or differences that are identified are then employed to predict if a subject will develop preeclampsia, to diagnose preeclampsia in a subject, to characterize a diagnosed preeclampsia, to monitor the responsiveness of the subject to treatment for preeclampsia, to evaluate the severity of preeclampsia, etc. For example, a preeclampsia phenotype determination element may be a sample derived from an individual that has or does not have preeclampsia. Such sample may be used, for example, as a reference or control in the experimental determination of the marker representation for a given subject. As an example, a preeclampsia phenotype determination element may be a marker level representation (e.g., marker profile, signature, score or index) that is representative of a preeclampsia state and may be used as a reference or control to interpret the marker level representation of a given subject. The phenotype determination element may be a positive reference or control. The positive reference or control may be a sample or marker level representation thereof from a subject that has preeclampsia, or that will develop preeclampsia, or that has preeclampsia that is manageable by known treatments, or that has preeclampsia that has been determined to be responsive only to the delivery of the baby. Alternatively, the phenotype determination element may be a negative reference or control. The negative reference or control may be a sample or marker level representation thereof from a subject that has not developed preeclampsia, or a subject that is not pregnant. In some instances, the marker representations are obtained from the same type of sample as the sample that was employed to generate the marker representation for the individual being monitored. In such instances, the phenotype determination elements are obtained from the same type of sample. For example, if the

serum of an individual is being evaluated, the reference or control would preferably be of serum.

[00197] In certain cases, the obtained marker level representation is compared to a single phenotype determination element to obtain information regarding the individual being tested for preeclampsia. In certain cases, the obtained marker level representation is compared to plurality of phenotype determination elements. For example, the obtained marker level representation may be compared to a negative reference and a positive reference to obtain confirmed information regarding if the individual will develop preeclampsia. In some examples, the obtained marker level representation may be compared to a reference that is representative of a preeclampsia which is responsive to treatment, and a reference that is representative of a preeclampsia that is not responsive to treatment, in order to obtain information as to whether or not the patient will be responsive to treatment.

[00198] The comparison of the obtained marker level representation and the one or more phenotype determination elements may be performed using any convenient methodology known to those of skill in the art. For example, those of skill in the art of arrays will know that array profiles may be compared by, e.g., comparing digital images of the expression profiles, by comparing databases of expression data, etc. Patents describing ways of comparing expression profiles include, but are not limited to, U.S. Patent Nos. 6,308,170 and 6,228,575, the disclosures of which are herein incorporated by reference. Methods of comparing marker level profiles are also described above. Similarly, those of skill in the art of ELISAs will know that ELISA data may be compared by, e.g. normalizing to standard curves, comparing normalized values, etc. The comparison step results in information regarding how similar or dissimilar the obtained marker level profile is to the control or reference profile(s), and which similarity or dissimilarity information is employed to diagnose, prognose, monitor, characterize or evaluate the severity of preeclampsia, or confirm the presence or absence of preeclampsia in order to predict the onset of a preeclampsia, diagnose preeclampsia, monitor a preeclampsia patient, evaluate the severity of PE, characterize PE, confirm the presence of PE or confirm the absence of PE. Similarity may be based on relative marker levels, absolute marker levels or a combination of both. In certain cases, a similarity determination is made using a computer having a program stored

thereon that is designed to receive input for a marker level result obtained from a subject, e.g., from a user, determine similarity to one or more reference profile, and return a preeclampsia prognosis, e.g., to a user (e.g., lab technician, physician, lay person, pregnant female, etc.). Further descriptions of computer-implemented cases of the disclosure are described below.

[00199] Depending on the type and nature of the reference or control profile(s) to which the obtained marker level profile is compared, the above comparison step yields a variety of different types of information regarding the cell or bodily fluid that is assayed. As such, the above comparison step can yield a positive or negative prediction of the onset of preeclampsia. Alternatively, such a comparison step can yield a positive or negative diagnosis of preeclampsia. Alternatively, such a comparison step can provide a characterization or an evaluation of the severity of a preeclampsia.

[00200] In some cases, the PE marker level representation may be based on a threshold value. The method may also involve obtaining levels of one or more biomarkers and comparing the levels to a pre-determined threshold (e.g. a standard value). Such threshold may be determined according to the concentration of a biomarker.

[00201] In an example, the threshold for prediction and/or confirmation of PE may be determined according to the relative concentration of a biomarker in a subject tested for PE or having PE as compared to a control (e.g., the same subject pre-pregnancy or at an earlier stage in pregnancy or another female in the same or another gestation period without PE).

For example, an indication of PE or likelihood of PE, or severity of PE may be a FN, concentration that is increased by a factor of at least, 100, at least 500, at least 1,000, at least 2,000, at least 3,000, at least 4,000, at least 5,000, at least 10,000, at least 12,000, at least 15,000, at least 20,000, at least 30,000, at least 40,000, at least 50,000, at least 100,000 or at least 20,000 relative to control; and/or VEGF concentration increased a factor of at least 2, at least 4, at least, at least 8, at least 10, at least 15, at least 20, at least 30, at least 40 or at least 50 relative to control; and/or concentration decreased by a factor of at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140 or at least 150 relative to a control; and/or Fms-like tyrosine kinase 1 (sFlt1) concentration increased by a factor of at least 5, at least 10, at least

20, at least 30, at least 40, at least 40, at least 50, at least 60, at least 70 or at least 80 relative to control; and/or Placental growth factor (PlGF) concentration decreased by a factor of at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 100, at least 1100, at least 1200, at least 1300, at least 1400 or at least 1500 relative to control; and/or ADAM metalloproteinase domain 12 (ADAM12) concentration increased by a factor of at least 2, at least 5, at least 10, at least 15, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 relative to control.

[00202] In certain cases, no physical characteristics aside from biomarker level is taken into account when diagnosing, prognosing, monitoring, characterizing or evaluating the severity of preeclampsia, or when confirming the presence or absence of PE in a subject. In some instances, at least one of gestation period, blood pressure, weight gain, water retention, hereditary factors, proteinuria, headache, edema, protein/creatinine ratio, platelet count, stress, PE in prior pregnancies (of the subject or her family members), nulliparity, age, age less than 20 years, age greater than 35, race, African-American and Filipino decent, serotype, Papanicolaou test results (Pap smear), prior preeclampsia episodes, familial history, number of pregnancies, number of miscarriages, body mass index (BMI), gestational diabetes, type I diabetes, obesity, glucose level, current and past medications, chronic hypertension, renal disease and thrombophilia, are not taken into account when confirming, diagnosing, prognosing, characterizing or evaluating the severity of PE in a subject.

[00203] In some instances, at least one of gestation period, blood pressure, weight gain, water retention, hereditary factors, proteinuria, headache, edema, protein/creatinine ratio, platelet count, stress, PE in prior pregnancies (of the subject or her family members), nulliparity, age, age less than 20 years, age greater than 35, race, African-American and Filipino decent, serotype, Papanicolaou test results (Pap smear), prior preeclampsia episodes, familial history, number of pregnancies, number of miscarriages, body mass index (BMI), gestational diabetes, type I diabetes, obesity, glucose level, current and past medications, chronic hypertension, renal disease and thrombophilia, are taken into account when confirming, diagnosing, prognosing, characterizing or evaluating the severity of PE in a subject.

[00204] In some cases, gestation period is taken into account when confirming, diagnosing, prognosing, monitoring, characterizing or evaluating the severity of preeclampsia. In some cases, gestational period may be divided into early and late gestational period. In some cases, in addition to the patient's gestation period, other elements may be taken to account comprising the subject's blood pressure, familial history and urine protein index.

[00205] In some cases, other analysis may be employed in conjunction with the aforementioned marker level representation to provide a preeclampsia prognosis for the individual. Such analyses are well known in the art, and take into account, for example, blood pressure, weight gain, water retention, hereditary factors, proteinurea, headache, edema, protein/creatinine ratio, platelet count, stress, PE in prior pregnancies (of the subject or her family members), nulliparity, age, age less than 20 years, age greater than 35, race, African-American and Filipino decent, serotype, Papanicolaou test results (Pap smear), prior preeclampsia episodes, familial history, number of pregnancies, number of miscarriages, body mass index (BMI), gestational diabetes, type I diabetes, obesity, glucose level, current and past medications, chronic hypertension, renal disease, thrombophilia well as other characteristics of the pregnancy.

[00206] A test for PE measuring biomarkers from a subject's biological sample, may provide predictive performance of each biomarker panel analysis, as evaluated by ROC curve analysis (Zweig et al. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clinical chemistry 1993;39:561-77; Sing et al. ROCR: visualizing classifier performance in R. Bioinformatics 2005;21:3940-1). In certain cases, the PE signature, score or index may have a cumulative ROC value of at least 0.8, 0.85, 0.9, 0.95, 0.96, 0.97, 0.980, 0.985, 0.988, 0.990, 0.995, 0.998 or more. In certain cases, the PE signature, score or index may have a cumulative ROC value of at most 0.9, 0.95, 0.96, 0.97, 0.980, 0.985, 0.988, 0.990, 0.995, 0.998 or less. Alternatively or additionally, the PE threshold, signature, score or index can have a sensitivity of at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%; and/or a specificity of at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. Alternatively or additionally, the PE threshold, signature, score or index can have a sensitivity of at most 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%,

96%, 97%, 98% or 99%; and/or a specificity of at most 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. Such PE signature, score or index can be for prognosis, diagnosis, monitoring, characterization or evaluating the severity of PE, confirming the absence of PE, or confirming the presence of PE, early PE, or late PE.

5 Such PE signature, score or index preferably comprises up to 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 biomarkers. ROC values may be applicable to biomarker signature, score, threshold or index.

[00207] In some cases, the prediction, diagnosis, prognosis, monitoring, characterization, evaluating the severity of PE, or confirming the presence or absence of PE may be provided
10 by providing, e.g., generating, a written report that includes the artisan's monitoring assessment, e.g., the artisan's prediction of the onset of preeclampsia (a "preeclampsia prediction"), the artisan's diagnosis of preeclampsia (a "preeclampsia diagnosis"), the artisan's confirmation of the presence of preeclampsia (a "preeclampsia positive confirmation"), the artisan's confirmation of the absence of preeclampsia (a "preeclampsia
15 negative confirmation"), the artisan's monitoring of the subject's preeclampsia (a "preeclampsia monitor"), the artisan's characterization of the subject's preeclampsia (a "preeclampsia characterization"), or the artisan's determination of the severity of the subject's preeclampsia (a "evaluating the severity of preeclampsia"). Thus, a subject method may further include a step of generating or outputting a report providing the results of an
20 assessment, which report can be provided in the form of an electronic medium (e.g., an electronic display on a computer monitor or an electronic file which may be transferable), or in the form of a tangible medium (e.g., a report printed on paper or other tangible medium).

Computer Systems and Software

[00208] These methods of analysis may be readily performed by one of ordinary skill in the
25 art by employing a computer-based system, e.g., using any hardware, software and data storage medium as is known in the art, and employing any algorithms convenient for such analysis. For example, data mining algorithms can be applied through "cloud computing", smartphone based or client-server based platforms, and the like.

[00209] The present disclosure contemplates the use of a computer system, computer
30 readable medium, or software, with an input module for collecting input on levels of a

plurality of biomarkers; a processor for performing an algorithm for performing a $\log_2$, $\log_e$ or $\log_{10}$ transformation of the biomarker levels, thus obtaining log transformed levels; a processor for performing an algorithm for normalizing each of the log transformed levels to normalized levels; a processor for performing an algorithm for adjusting each of the
5 normalized levels to a weighted normalized level; a processor for an algorithm for totaling and optionally averaging each of the adjusted levels; a processor for an algorithm for providing a PE score based on the total amount, and a processor for optionally performing an algorithm for providing a PE index based on the preeclampsia score. The computer preferably generates a report that can be provided to the caregiver and/or the subject female
10 (e.g., pregnant female). The report can include in it none, any or all of the following: name of patient or subject, gestation period at time of testing, list of markers analyzed, levels of each marker as measured in the sample, direct comparison of level of biomarkers to those in the training set, log transformed and normalized level of biomarkers as compared to log transformed and normalized levels in the training set, log transformed, normalized and
15 weighted numbers of biomarkers, a PE score, a PE index, and recommended course of action for the subject.

[00210] In some cases, the disclosure includes a system for diagnosing, prognosing, monitoring, characterizing, or evaluating the severity of preeclampsia, confirming the presence or the absence of PE in a female subject comprising: (a) an input module for
20 receiving as input levels of one or more biomarkers, such as sFLT-1, PlGF and at least two other different biomarkers, (b) a processor optionally configured to perform algorithms such as (i) a $\log_2$, $\log_e$ or $\log_{10}$ transformation of the levels to obtain log transformed levels, (ii) normalizing each of the log transformed levels to normalized levels, (iii) adjusting each of said normalized levels to a weighted normalized level, (iv) totaling each of the adjusted
25 levels, (v) averaging each of the adjusted levels; and (c) an output module for outputting a preeclampsia index based on a score wherein the index score comprises sFLT-1/PlGF and an addition of two other different biomarkers. In some cases, the processor may perform an algorithm adjusting the levels of one or more biomarkers to a training set or a control value, thereby providing one or more adjusted biomarker levels. The processor may further perform
30 another algorithm that applies at least one binary operation using the adjusted biomarker

levels, adds or subtracts the one or more adjusted biomarker level, calculates a ratio between two adjusted biomarker levels, and/or manipulates the one or more adjusted biomarker levels by multiplying one or more variables by one or more corresponding weight factors, wherein the level of each of the one or more adjusted biomarker levels is input into a specific variable, wherein the corresponding weight factor is unique for each specific variable, wherein at least one of the corresponding weight factors is not one. In some cases, the algorithm is a real function.

[00211] In some cases, the disclosure includes a computer readable medium containing instructions which, when executed by a computer system, cause the computer system to receive a first data set pertaining to levels of PE biomarkers in a biological sample derived from a subject, and perform an analysis on those levels to obtain an assessment of PE in the subject. In some cases, the instructions, when executed by a computer system, can cause the computer system to perform those steps a second time (e.g., receive a second data set pertaining to levels of PE biomarkers, and perform a second analysis to obtain a second assessment). In some cases, those steps may be performed at different points in time. In some cases, the instructions cause the computer system to compare the first assessment with the second assessment and confirm PE or the lack thereof based on the comparison.

Reports

[00212] The report may include information about the testing facility, which information is relevant to the hospital, clinic, or laboratory in which sample gathering and/or data generation was conducted. Sample gathering can include deriving a fluid sample, e.g. blood, saliva, urine etc.; a tissue sample, e.g. a tissue biopsy, etc. from a subject. Data generation can include measuring the level of polypeptide concentration for one or more genes that are differentially expressed or present at different levels in preeclampsia patients versus healthy individuals, e.g., individuals that do not have and/or do not develop preeclampsia. This information can include one or more details relating to, for example, the name and location of the testing facility, the identity of the lab technician who conducted the assay and/or who entered the input data, the date and time the assay was conducted and/or analyzed, the location where the sample and/or result data is stored, or the lot number of the reagents (e.g.,

kit, etc.) used in the assay. Report fields with this information can be populated using information provided by the user.

[00213] The report may include information about the service provider, which may be located outside the healthcare facility at which the user is located, or within the healthcare facility.

5 Examples of such information can include the name and location of the service provider, the name of the reviewer, and where necessary or desired the name of the individual who conducted sample gathering and/or data generation. Report fields with this information can be populated using data entered by the user, which can be selected from among pre-scripted selections (e.g., using a drop-down menu). Other service provider information in the report
10 can include contact information for technical information about the result and/or about the interpretive report.

[00214] The report may include a patient data section. The patient data section may include one or more items of the list consisting of patient medical history and symptoms (which can include, e.g., gestational period, blood pressure, proteinurea, diabetes, glucose level, body
15 mass index, age, race, serotype, Papanicolaou test results (Pap smear), prior preeclampsia episodes, familial history, number of pregnancies, number of miscarriages, weight gain, water retention, hereditary factors, headache, edema, protein/creatinine ratio, current medications or past medications, stress, PE in prior pregnancies (of the subject or her family members), nulliparity, chronic hypertension, renal disease or thrombophilia, and any other
20 characteristics of the pregnancy), administrative patient data such as information to identify the patient (e.g., name, patient date of birth (DOB), gender, mailing and/or residence address, medical record number (MRN), room and/or bed number in a healthcare facility, insurance information, and the like), the name of the patient's physician or other health professional who ordered the monitoring assessment, and (if different from the ordering physician) the
25 name of a staff physician who is responsible for the patient's care (e.g., primary care physician).

[00215] The report may include a sample data section, which may provide information about the biological sample analyzed in the monitoring assessment, such as the source of biological sample derived from the patient (e.g., blood, saliva, or type of tissue, etc.), how the sample
30 was handled (e.g., storage temperature, preparatory protocols) or the date and time collected.

Report fields with this information can generally be populated using data entered by the user, some of which may be provided as pre-scripted selections (e.g., using a drop-down menu).

[00216] The report may include an assessment report section, which may include information generated after processing of the data as described herein. The interpretive report can include a prediction of the likelihood that the subject will develop PE, diagnosis of PE, a confirmation of that the subject has PE, a confirmation that the subject does not have PE, monitoring of the subject's PE (e.g., whether the level of biomarkers remains stable or is altered), the characteristics of PE, the level of severity of PE, or any combination thereof. If the biomarker level is altered, the report may include the degree of such alteration, and the alteration's significance in terms of PE occurrence, forecast or severity. The interpretive report can include, for example, the results of a protein level determination assay (e.g., "1.5 nmol/liter ADAM12 in serum"); and interpretation of that biomarker level, e.g., prediction, diagnosis, monitoring, characterization, evaluation of the severity of PE, or confirmation of the presence or absence of PE. In some examples, the assessment portion of the report includes a recommendation(s). For example, where the results indicate that preeclampsia is likely, the recommendation includes a recommendation that diet be altered, blood pressure medicines administered, bed-rest is recommended, pre-term labor recommended, diabetes medicines administered, etc., as recommended in the art.

[00217] The report may include at least one of diagnosis, prognosis, characteristics, monitor, severity of PE, or confirmation of the presence or absence of PE; a biomarker index value based on the analysis of one or more biomarkers detected in a biological sample from the pregnant subject. The report may include the predictive performance of each biomarker panel analysis. In some instances, the predictive performance is evaluated by ROC curve analysis.

[00218] It will also be readily appreciated that the reports can include additional elements or modified elements. For example, where electronic, the report can contain hyperlinks which point to internal or external databases which provide more detailed information about selected elements of the report. For example, the patient data element of the report can include a hyperlink to an electronic patient record, or a site for accessing such a patient record, which patient record is maintained in a confidential database. In some instances, the

inclusion of a hyperlink may be of interest in an in-hospital system or in-clinic setting. In some instances, the inclusion of a hyperlink may be of interest in a home or work setting. When in electronic format, the report is recorded on a suitable physical medium, such as a computer readable medium, e.g., in a computer memory, zip drive, floppy disc, USB chip, CD, DVD, or any other storage media capable of storing magnetic or electronic information that is retrievable.

[00219] It will be readily appreciated that the report can include all or some of the elements above, with the proviso that the report generally includes at least the elements sufficient to provide the analysis requested by the user (e.g., prediction, diagnosis, monitoring, characterization, evaluation of the severity of preeclampsia, or confirmation of the absence or presence of preeclampsia).

[00220] The disclosure further provides a business method comprising the step of determining presence, absence, forecast, severity, characteristics, of preeclampsia in a subject, or confirming the absence or presence of preeclampsia in a subject. The method comprises the steps of: evaluating levels of sFLT-1, PlGF and a plurality of biomarkers in a sample derived from the subject, wherein the plurality of biomarkers are not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) or heme, determining a biomarker index value, the index comprises sFLT-1/PlGF and the addition of the a plurality of biomarkers, employing the biomarker index to provide a preeclampsia determination, confirmation, absence, diagnosis, prognosis, severity or characteristics, and providing a report in exchange for a fee, wherein the report indicates an index value based on the analysis of the biomarkers, and an indication of a range specifying whether the subject is at low risk of PE, high risk of preeclampsia, or experiencing preeclampsia. In some cases, the business method further comprises transmitting a report. In some cases, the report contains information about the subject including blood pressure, age, weight, gestational age, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history, proteinuria, body mass index, swelling, abdominal pressure, uterine pulsatility index, thrombocytopenia,

previous history of preeclampsia, previous history of eclampsia, first birth, multiple births, circulating free DNA, circulating fetal DNA, free DNA, fetal DNA, history of smoking or a family history of a related complicating factor. In some cases, the a plurality of biomarkers excludes endoglin, fibrinopeptide A, antithrombin III, IGFBP-5, TGF-B1, fibrinopeptideA:D-dimer, ficolin-2, ficolin-3, creatinine, clusterin, H2 relaxin, P1GF-2, P1GF-3 and human chorionic gonadotropin. In some cases, the report is electronic. In some cases, the index is unaffected by at least one of blood pressure, age, weight, gestational age, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history and a family history. In some cases, the index is unaffected by all of blood pressure, weight, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history and a family history. In some cases, the index is unaffected by age. Sometimes, the index is unaffected by gestational age. [00221] In some cases, the disclosure further provides a business method comprising the step of determining the presence, absence, forecast, severity, or characteristics, of preeclampsia in a subject, or confirming the absence or presence of preeclampsia in a subject. The method can comprise the steps of: (a) evaluating levels of sFLT-1, PlGF and at least two other different biomarkers in a sample derived from the subject, wherein the at least two other biomarkers are not ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic gonadotropin (free beta hCG) or heme, (b) determining a biomarker index value, the index comprising sFLT-1/PlGF and the addition of the at least two other different biomarkers, (c) employing the biomarker index to provide a preeclampsia determination, confirmation, absence, diagnosis, prognosis, severity or characteristic, and (d) providing a report in exchange for a fee, wherein the report indicates an index value based on the analysis of the biomarkers, and at least one of: specifying whether the subject is at low risk of preeclampsia, specifying whether the subject is at high risk of preeclampsia, specifying whether the subject has preeclampsia, specifying whether the subject does not have preeclampsia, characterizing preeclampsia, and indicating the severity of preeclampsia.

Reagents, Systems and Kits

[00222] Also provided are reagents, systems and kits thereof for practicing one or more of the above-described methods. The subject reagents, systems and kits thereof may vary greatly.

Variation may include alterations in incubation time and temperature. Reagents of interest include reagents specifically designed for use in producing the above-described marker level representations of preeclampsia markers from a sample, for example, one or more detection elements. The detection elements may be antibodies or peptides for the detection of protein, protein fragments. The detection elements can be oligonucleotides for the detection of nucleic acids. In some instances, the detection element comprises a reagent to detect the expression of a single preeclampsia marker, for example, the detection element may be a dipstick, a plate, an array, or cocktail that comprises one or more detection elements (e.g. one or more antibodies, one or more oligonucleotides, one or more sets of PCR primers, one or more sets of or isothermal polynucleotide amplification primers, etc.) which may be used to detect the expression of at least two preeclampsia markers simultaneously.

[00223] In one case, a kit for detecting the presence, absence, forecast, severity or character of preeclampsia in subject is provided. Such kit includes a plurality of detection elements (analytes) used for measuring the plurality of biomarkers selected from the group consisting of P1GF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12. In other instances, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 (or more) different reagents are utilized to measure at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 (or more) different biomarkers. When referring to a biomarker, for example P1GF, unless explicitly stated, it is understood that all isoforms of the biomarker, for example, P1GF, which are known or to be discovered are referred to by the biomarker term, for example, P1GF (e.g., hPA18788.6 [152 aa], hPA18788.1 [1017 aa], hPA18788.2 [1009 aa], hPA18788.3 [775 aa], hPA18788.4 [403 aa], hPA18788.5 [375 aa] or hPA18788.9 [463 aa]). (P1GF as used herein is equivalent to P1GF or PLGF). When referring to hemopexin (HPX), it is understood that both heme bound and heme unbound forms of hemopexin are referred to.

[00224] In one embodiment, one type of reagent specifically tailored for generating marker level representations (e.g., preeclampsia markers) is a collection of antibodies that bind specifically to the protein markers. In some examples, such antibodies may be employed in

an ELISA (such as competitive or sandwich ELISA format). Other analytic methodologies that may be employed include xMAP™ microsphere format, a proteomic array, suspension for analysis by flow cytometry, western blotting, dot blotting or immunohistochemistry. Methods for using the same are well understood in the art. These antibodies can be provided
5 in solution. Alternatively, they may be provided pre-bound to a solid matrix, for example, the wells of a multi-well dish or the surfaces of xMAP microspheres.

[00225] In some embodiments, an array of probe nucleic acids in which the genes of interest are represented, may be employed as reagents. A variety of different array formats are known in the art, with a wide variety of different probe structures, substrate compositions and
10 attachment technologies (e.g., dot blot arrays, microarrays, etc.). Representative array structures of interest include those described in U.S. Patent Nos.: 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,800,992; the disclosures of which are herein incorporated by reference; as well as WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373
15 203; and EP 785 280.

[00226] A type of reagent that is specifically tailored for generating marker level representations of genes, e.g. preeclampsia genes, is a collection of gene specific primers that is designed to selectively amplify such genes (e.g., using a PCR-based technique, e.g., real-time RT-PCR, or isothermal amplification techniques such as loop mediated isothermal
20 amplification of DNA (LAMP), strand displacement amplification (SDA), sequence based amplification (NASBA), self-sustained sequence replication (3SR) and the like). Gene specific primers and methods for using the same are described in U.S. Patent No. 5,994,076, the disclosure of which is herein incorporated by reference.

[00227] In some cases, the kit may include polynucleotide primers that selectively hybridize
25 polynucleotide sequences encoding selected biomarkers. In certain cases, the primers selectively hybridize at least two polynucleotide sequences encoding proteins that are selected from the group consisting of P1GF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12. Such primers may be DNA or RNA primers.

[00228] Of interest are also arrays of probes, collections of primers, or collections of
30 antibodies that include probes, primers or antibodies (also called reagents) that are specific

for at least one gene or protein selected from the group consisting P1GF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12. In some instances for a plurality of these genes, e.g., at least 2, 3, 4, 8 or more may be selected.

[00229] In certain cases, the collection of probes, primers or antibodies include reagents

specific for one or more of P1GF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12. The subject probe, primer, or antibody collections or reagents may include reagents that are specific only for the genes, proteins or cofactors that are listed above, or they may include reagents specific for additional genes, proteins or cofactors that are not listed above, such as probes, primers, or antibodies specific for genes, proteins or cofactors whose expression pattern are known in the art to be associated with preeclampsia, e.g. sFLT-1 (VEGF-R1) and P1GF.

[00230] The systems and kits of the subject disclosure may include the above-described arrays, gene-specific primer collections, or protein-specific antibody collections. In the case of protein-specific antibody kit, the systems and kits may further include one or more

additional reagents, such as bovine serum albumin (BSA), casein, solutions of powdered milk, bovine gamma globulin (BGG) in phosphate buffered saline (PBS)/Tween or PBS/Triton-X 100, PBS/Tween, PBS/Triton-X 100, or borate buffer; selected solid surface, preferably a surface exhibiting a protein affinity such as the wells of a polystyrene microtiter plate; second antibody will have an associated enzyme, e.g. urease, peroxidase, or alkaline phosphatase, and an appropriate chromogenic substrate. For example, a urease or peroxidase-conjugated anti-human IgG may be employed, PBS-containing solution such as PBS/Tween), a chromogenic substrate such as urea and bromocresol purple in the case of a urease label or 2,2'-azino-di-(3-ethyl-benzthiazoline)-6-sulfonic acid (ABTS) and H₂O₂, in the case of a peroxidase label. Quantitation is then achieved by measuring the degree of color generation, e.g., using a visible spectrum spectrophotometer.

[00231] The systems and kits may further include one or more additional reagents employed in the various methods, such as primers for generating target nucleic acids, dNTPs and/or rNTPs, which may be either premixed or separate, one or more uniquely labeled dNTPs and/or rNTPs, such as biotinylated or Cy3 or Cy5 tagged dNTPs, gold or silver particles with different scattering spectra, or other post synthesis labeling reagent, such as chemically active

derivatives of fluorescent dyes, enzymes, such as reverse transcriptases, DNA polymerases, RNA polymerases, and the like, various buffer mediums, e.g. hybridization and washing buffers, prefabricated probe arrays, labeled probe purification reagents and components, like spin columns, etc., signal generation and detection reagents, e.g. labeled secondary
5 antibodies, streptavidin-alkaline phosphatase conjugate, chemifluorescent or chemiluminescent substrate, and the like.

[00232] The subject systems and kits may also include a preeclampsia phenotype determination element, which element is, in many cases, a reference or control sample or marker representation that can be employed, e.g., by a suitable experimental or computing
10 means, to make a preeclampsia prognosis based on an “input” marker level profile, e.g., that has been determined with the above described marker determination element. Representative preeclampsia phenotype determination elements include samples from an individual known to have or not have preeclampsia, databases of marker level representations, e.g., reference or control profiles, and the like, as described above.

[00233] In addition to the above components, the subject kits will further include instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, one or more of which may be present in the kit. One form in which these instructions may be present is as printed information on a suitable medium or substrate, e.g., a piece or pieces of paper on which the information is printed, in the packaging of the kit, in a
20 package insert, etc. A means would be a computer readable medium, e.g., diskette, floppy disc, USB device, CD, etc., on which the information has been recorded. Some means may be present is a website address which may be used via the internet (i.e. via the cloud) to access the information at a removed site. Any convenient means may be present in the kits.

[00234] In certain cases, the present disclosure provides a business method for determining
25 presence, absence, forecast, severity, monitor or character of PE in a subject. Such method includes: performing an analysis of the subject’s biological sample for the presence and amount of one or more biomarkers selected from a group consisting of P1GF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12; determining a weight, signature, score or index value of the biomarkers; employing the biomarker weight, signature,
30 score or index value to provide a diagnosis, prognosis, severity, confirmation of presence,

confirmation of absence, or characteristics of PE, and providing a report in exchange for a fee.

[00235] In other cases, the present disclosure provides a business method for determining presence, determining absence, determining forecast, monitoring, determining severity, confirming presence of, confirming absence of or characterizing PE in a subject. Such method includes: performing an analysis of the subject's biological sample for the presence and amount of one or more biomarkers selected from a group consisting of P1GF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12. In certain cases, the collection of probes, primers, or antibodies includes reagents specific for P1GF, HPX, sFlt-1, PAPP-A, VEGF (excluding VEGF-R1), FN, FG, and ADAM12; determining a weight, signature, score or index value of the biomarkers; employing the biomarker weight, signature, score or index value to provide a diagnosis, prognosis, characteristic, confirmation presence, confirmation absence, or determination of the severity of PE and providing a report in exchange for a fee.

[00236] In certain cases, the index value is based on the analysis of biomarkers. In some examples, an indication of a range or threshold value is provided specifying whether the subject is at low risk of PE, high risk of PE, or experiencing PE. In other cases, the index value is based on the analysis of the biomarkers, and an indication of a range specifying whether the subject has mild PE, moderate PE, or severe PE. In some cases an indication of the reliability or certainty of the confirmation (whether the female subject has or does not have PE) is provided.

[00237] Any method, kit, composition, business method, computer system disclosed herein may be used with a sample wherein the sample is a serum sample. In some cases, the sample is derived from blood, plasma, serum, urine, cells or body fluids. In some cases, the sample is derived from the mother or the fetus. In some cases, the sample is a vaginal swab. In some cases, the sample is not a urine sample. In some cases, the biomarker is a peptide. In some cases, the biomarker in a sample is a peptide, a portion of the peptide, a fragment of the peptide, a peptide containing an antigen, a portion of the peptide wherein the portion of the peptide contains an antigen, a fragment of the peptide wherein the fragment of the peptide contains an antigen. In some cases, evaluating comprises measuring a level of at least one

RNA molecule. In some cases, evaluating comprises performing at least one sequencing reaction. In some cases, the index is unaffected by blood pressure, age, weight, gestational age, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history or a family history. In some cases, evaluating does not comprise comparing a sample derived from a
5 subject at a first time point and a sample derived from the same subject at a second time point. In some cases, evaluating comprises comparing a sample derived from a subject at a first time point and a sample derived from the same subject at a second time point. In some cases, the evaluating step comprises determining the levels of a biomarker selected from the group consisting of sFlt-1, PlGF, VEGF (excluding VEGF-R1), ADAM12, HPX, PAPP-A
10 and FN. In some cases, the plurality of biomarkers excludes endoglin, fibrinopeptide A, antithrombin III, IGFBP-5, TGF- β 1, fibrinopeptide A:D-dimer, ficolin-2, ficolin-3, creatinine, clusterin, H2 relaxin, PlGF-2, PlGF-3 and human chorionic gonadotropin. In some cases, the measuring is conducted using at least one antibody which recognizes a biomarker selected from the group consisting of sFlt-1, PlGF, VEGF (excluding
15 VEGF-R1), ADAM12, HPX, PAPP-A and FN. In some cases, the method does not include predicting preeclampsia in a subject that is asymptomatic of preeclampsia. In some cases, the evaluating does not comprise Doppler screening. In some cases, the method does not include detecting the presence of microvesicles or exosomes in the sample. In some cases, the method is greater than 85% accurate. In some cases, the method is greater than 85% sensitive. In
20 some cases, the method is greater than 85% specific. In some cases, the method is greater than 85% accurate. In some cases, the method has a positive predictive value greater than 85%. In some cases, the method has a negative predictive value greater than 85%. In some cases, the biomarker correlates with blood pressure, age, weight, gestational period, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history, proteinuria, body mass
25 index, swelling, abdominal pressure, uterine pulsatility index, thrombocytopenia, previous history of preeclampsia, previous history of eclampsia, the number of previous births given by the subject, circulating free DNA, circulating fetal DNA, free DNA, fetal DNA, history of smoking or a family history of a related complicating factor. In some cases, the biomarker does not correlate with blood pressure, age, weight, gestational age, ethnicity, diabetes,
30 kidney disease, autoimmune disease, maternal history, proteinuria, body mass index,

swelling, abdominal pressure, uterine pulsatility index, thrombocytopenia, previous history of preeclampsia, previous history of eclampsia, the number of previous births given by the subject, circulating free DNA, circulating fetal DNA, free DNA, fetal DNA, history of smoking or a family history of a related complicating factor.

5 **[00238]** The disclosure further provides a test for excluding diagnosis of preeclampsia, wherein said test measures one or more biomarkers from a sample derived from a subject and has an overall ROC value of at least 0.8, 0.85, 0.9, 0.95, 0.980, 0.981, 0.982, 0.983, 0.984, 0.985, 0.986, 0.987, 0.989, 0.990, 0.991, 0.992, 0.993, 0.994, 0.995, 0.996, 0.997, 0.998, 0.999 or more. In some cases, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 biomarkers are
10 assayed. In some cases, at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 biomarkers are assayed. In some cases, the overall ROC value is unaffected by blood pressure, age, weight, gestational period, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history or a family history. In some cases, the sample is a serum sample. In some cases, the sample is derived from blood, plasma, serum, urine, cells or body fluids. In some cases, the sample is
15 derived from the mother or the fetus. In some cases, the sample is a vaginal swab. In some cases, the sample is not a urine sample. In some cases, the biomarker is a peptide. In some cases, the biomarker in a sample is a peptide, a portion of the peptide, a fragment of the peptide, a peptide containing an antigen, a portion of the peptide wherein the portion of the peptide contains an antigen, a fragment of the peptide wherein the fragment of the peptide
20 contains an antigen. In some cases, the result of the test is unaffected by blood pressure, age, weight, gestational period, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history or family history. In some cases, the test does not comprise comparing a sample derived from a subject at a first point in time and a sample derived from the same subject at a second point in time. In some cases, the test consists of comparing a sample derived from a
25 subject at a first point in time and a sample derived from the same subject at a second point in time. In some cases, the test comprises determining the levels of a biomarker selected from the group consisting of sFlt-1, P1GF, VEGF (excluding VEGF-R1), ADAM12, HPX, PAPP-A and FN. In some cases, the biomarkers excludes endoglin, fibrinopeptide A, antithrombin III, IGFALS, FLT4, IGFBP-5, TGF-B1, fibrinopeptideA:D-dimer, ficolin-2, ficolin-3,
30 creatinine, clusterin, H2 relaxin, P1GF-2, P1GF-3 and human chorionic gonadotropin. In

some cases, the test is conducted using at least one antibody which recognizes a biomarker selected from the group consisting of sFlt-1, PlGF, VEGF (excluding VEGF-R1), ADAM12, HPX, PAPP-A and FN. In some cases, the test does not include predicting preeclampsia in a subject that is asymptomatic. In some cases, the test does not consist of Doppler screening. In some cases, the test does not include detecting the presence of microvesicles or exosomes in the sample. In some cases, the test is at least 85, 90, 95% or more accurate. In some cases, the test is at least 85, 90, 95% or more sensitive. In some cases, the test is at least 85, 90, 95% or more specific. In some cases, the test has a positive predictive value of at least 85, 90, 95%. In some cases, the test has a negative predictive value of at least 85, 90, 95% or more. In some cases, the biomarker correlates with blood pressure, age, weight, gestational period, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history, proteinuria, body mass index, swelling, abdominal pressure, uterine pulsatility index, thrombocytopenia, previous history of preeclampsia, previous history of eclampsia, the number of previous births given by the subject, circulating free DNA, circulating fetal DNA, free DNA, fetal DNA, history of smoking or a family history of a related complicating factor. In some cases, the biomarker does not correlate with blood pressure, age, weight, gestational age, ethnicity, diabetes, kidney disease, autoimmune disease, maternal history, proteinuria, body mass index, swelling, abdominal pressure, uterine pulsatility index, thrombocytopenia, previous history of preeclampsia, previous history of eclampsia, the number of previous births given by the subject, circulating free DNA, circulating fetal DNA, free DNA, fetal DNA, history of smoking or a family history of a related complicating factor. In some cases, the test includes performing a biological assay, a functional assay, an ELISA, an immunological assay, mass spectrometry, chromatography, nephelometry, radial immunodiffusion or single radial immunodiffusion. In some cases, the test includes digesting and measuring a biomarker. In some cases, the test includes derivatizing and measuring a biomarker. In some cases, the test includes isolating peptides from at least one cell in the sample. In some cases, the test further includes detecting polymorphisms or modifications to the biomarker. In some cases, the test further includes detecting RNA and/or DNA. In some cases, the test further includes detecting RNA and/or DNA associated with the biomarker. In some cases, the test further includes detecting transcription factors and/or transcription factor co-factors. In some cases,

the test further includes detecting transcription factors and/or transcription factor co-factors associated with the biomarker. In some cases, the test further includes use of an algorithm, a threshold value, a directional change over time, comparing the index to a single patient, comparing the index to a control group or comparing the index to a reference standard. In some cases, the test is used to confirm the presence of preeclampsia in a subject wherein the subject has at least one symptom associated with preeclampsia. In some cases, the test is used to provide a diagnosis of preeclampsia in a subject wherein the subject has hypertension and proteinuria. In some instances, the test is used to provide a diagnosis of preeclampsia in a subject wherein the subject has hypertension.

[00239] In some cases, the test is used to provide a diagnosis of preeclampsia in a subject wherein the subject has one risk factor associated with preeclampsia. In some cases, the test is used to determine if a subject is at risk for preeclampsia. In some cases, the test is used to quantify a risk that a subject will develop preeclampsia. In some cases, the test is used to predict a time at which a subject will develop preeclampsia. In some cases, the test is used to predict maternal and/or fetal outcomes of preeclampsia. In some cases, the test is used to distinguish between mild, moderate and severe preeclampsia. In some cases, the test is used to determine whether HELLP syndrome, preterm birth, interuterine growth restriction, placental abruption, placental accrete, low fetal birth weight, low size for gestational period, gestational hypertension, chronic hypertension, gestational diabetes, Type I diabetes, Type II diabetes or risk of spontaneous abortion are a result of the subject having preeclampsia. In some cases, the test further includes creating an electronic or non-electronic report.

[00240] In some embodiments, the disclosure includes a test for excluding diagnosis of preeclampsia, wherein said test measures one or more biomarkers from a sample taken from a subject and has an overall ROC value of at least 0.8. In some examples the test has a ROC value of at least 0.8, 0.85, 0.9, 0.95, 0.980, 0.981, 0.982, 0.983, 0.984, 0.985, 0.986, 0.987, 0.989, 0.990, 0.991, 0.992, 0.993, 0.994, 0.995, 0.996, 0.997, 0.998, 0.999 or more.

[00241] The disclosure further provides a kit for confirming the presence of PE, confirming the absence of PE, diagnosing, prognosing, monitoring, characterizing, or determining the severity of preeclampsia in a subject, said kit comprising at least two reagents that are specific for determining level of fibronectin in a sample from the subject. In some cases, the

at least two reagents comprise two different antibodies that selectively bind fibronectin. In some cases, the kit further comprises reagents that are specific for determining levels of sFlt-1 and PlGF in the sample. In some cases, the sample is a serum sample. In some cases, the sample is a blood sample. In some cases, the sample is not a urine sample. In some cases, the kit does not include a reagent to detect IGFBP1, FLT4, PlGF, PlGF-2, PlGF-3 or sFlt-1.

[00242] The disclosure further provides a kit for diagnosing, prognosing, monitoring, characterizing, determining the severity of preeclampsia, or confirming the presence or absence of preeclampsia in a subject, said kit comprising a first reagent specific for determining level of PAPP-A and a second reagent specific for determining ADAM12. In some cases, the kit does not include a reagent to detect IGFBP1, FLT4, PlGF, PlGF-2, PlGF-3 or sFlt-1. In some cases, the kit cannot be used to prognose, diagnose, screen for, determine or confirm Down's syndrome. In some cases, the kit cannot be used to prognose, diagnose, screen for, determine or confirm cardiovascular disease. In some cases, the biomarker is not PlGF-2 or PlGF-3.

[00243] Often, the disclosure further provides a kit for confirming the presence of PE, confirming the absence of PE, diagnosing, prognosing, monitoring, characterizing or determining the severity of PE in a subject, said kit comprising at least two reagents that are specific for determining level of fibronectin in a sample from the pregnant woman.

[00244] In some cases, the evaluating includes performing a biological assay, a functional assay, an ELISA, an immunological assay, mass spectrometry, chromatography, nephelometry, radial immunodiffusion or single radial immunodiffusion. In some cases, the evaluating includes digesting and measuring a biomarker. In some cases, the evaluating includes derivatizing and measuring a biomarker. In some cases, the evaluating includes isolating peptides from at least one cell in the sample. In some cases, the method further includes detecting polymorphisms or modifications to the biomarker. In some cases, the method further includes detecting RNA and/or DNA. In some cases, the method further includes detecting RNA and/or DNA associated with the biomarker. In some cases, the method further includes detecting transcription factors and/or transcription factor co-factors. In some cases, the method further includes detecting transcription factors and/or transcription factor co-factors associated with the biomarker. In some cases, the method further includes

use of an algorithm, a threshold value, a threshold range, a directional change over time, comparing the index to a single patient, comparing the index to a control group or comparing the index to a reference standard. In some cases, the method is used to confirm a diagnosis of preeclampsia in a subject wherein the subject has at least one symptom associated with preeclampsia. In some cases, the method is used to provide a diagnosis of preeclampsia in a subject wherein the subject has hypertension and proteinuria. In some cases, the method is used to provide a diagnosis of preeclampsia in a subject wherein the subject has hypertension. In some cases, the method is used to provide a diagnosis of preeclampsia in a subject wherein the subject has one risk factor associated with preeclampsia. In some cases, the method is used to determine if a subject is at risk for preeclampsia. In some cases, the method is used to quantify a risk that a subject will develop preeclampsia. In some cases, the method is used to predict a time at which a subject will develop preeclampsia. In some cases, the method is used to predict maternal and/or fetal outcomes of preeclampsia. In some cases, the method is used to distinguish between mild, moderate and severe preeclampsia. In some cases, the method is used to determine whether HELLP syndrome, preterm birth, interuterine growth restriction, placental abruption, placental accrete, low fetal birth weight, low size for gestational age, gestational hypertension, chronic hypertension, gestational diabetes, Type I diabetes, Type II diabetes or risk of spontaneous abortion are a result from a subject having preeclampsia. In some cases, the method further includes creating an electronic report.

[00245] The disclosure further provides a kit for confirming the presence of PE, confirming the absence of PE, diagnosing, prognosing, monitoring, characterizing, or determining the severity of preeclampsia in a subject, said kit comprising a first reagent specific for determining level of PAPP-A and a second reagent specific for determining level of ADAM12. In some cases, the kit does not include a reagent to detect IGFBALS, FLT4, P1GF, P1GF-2, P1GF-3 or sFlt-1. In some cases, the kit cannot be used to prognose, diagnose, screen for, determine or confirm Down's syndrome. In some cases, the kit cannot be used to prognose, diagnose, screen for, determine or confirm cardiovascular disease. In some cases, the biomarker is not P1GF-2 or P1GF-3.

Applications

[00246] The methods, compositions and reagents provided herein may be used for diagnosing, analyzing, distinguishing or confirming the presence or absence of preeclampsia (PE) in a female subject. Additionally, the methods, compositions and reagents provided herein may be used for diagnosing, analyzing, distinguishing or confirming preeclampsia (PE) in a female subject wherein the female subject also has other symptoms as described herein. The methods, compositions and reagents find use in a number of applications, including, for example, predicting if an individual will develop preeclampsia, diagnosing preeclampsia, confirming the presence, absence or severity of preeclampsia, and monitoring an individual with preeclampsia.

[00247] Additionally, the methods, compositions and reagents find use in a number of applications, including, for example, predicting if an individual will develop preeclampsia who has other symptoms as described herein, diagnosing preeclampsia in an individual who has other symptoms as described herein, confirming the presence, absence or severity of preeclampsia in an individual who has other symptoms as described herein, and monitoring an individual with preeclampsia in an individual who has other symptoms as described herein. In some cases, the other symptoms may include hypertension and proteinuria. In other cases, the other symptoms may include hypertension.

[00248] The methods, compositions and reagents may also find use in identifying an individual at risk for preeclampsia and quantifying the risk of preeclampsia in an individual.

Often, the methods, compositions and reagents find use in predicting the time of the onset of preeclampsia. In some cases, the methods, compositions and reagents find use in predicting the timeline of progression of preeclampsia. The methods, compositions and reagents provided herein may find use to predict an outcome of preeclampsia, wherein the outcome affects the fetus or wherein the outcome affects the subject, often the mother. In some cases, the methods, compositions and reagents provided herein may find use to distinguish a stage of preeclampsia from another stage of preeclampsia. For example, a stage of preeclampsia may be mild or severe. In some cases, the methods, compositions and reagents provided herein may find use to distinguish preeclampsia from eclampsia. Often, the methods, compositions and reagents provided herein may find use to determine whether HELLP syndrome, risk of or ongoing process of preterm birth, risk of or ongoing interuterine growth

restriction, risk of spontaneous abortion, risk of or ongoing placental abruption, risk of or ongoing placental accrete, risk of low or high fetal birth weight and risk of small or large size relative to gestational age are a result of the subject having preeclampsia. The methods, compositions and reagents provided herein may further find use to distinguish patients having preeclampsia from patients not having preeclampsia but having symptoms associated with preeclampsia, such symptoms including complication of pregnancy symptoms, gestational hypertension, chronic hypertension, gestational diabetes, Type I diabetes and/or Type II diabetes.

[00249] The methods, compositions and reagents provided herein also find use in creating a report, often an electronic report, to communicate the outcomes of the methods, compositions and reagents described herein. In some cases, the report may communicate preeclampsia score, preeclampsia index and/or preeclampsia profile of a subject.

[00250] The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

[00251] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present disclosure, and are not intended to limit the scope of the disclosure nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

EXAMPLE 1- Analysis of Samples from Normal Subjects and Subjects with Preeclampsia by ELISA

[00252] This example demonstrates use of the disclosure described herein for performing an analysis of a set of samples derived from pregnant females, some of which did not have preeclampsia and some of which had preeclampsia using an ELISA method. Use of the biomarkers demonstrated herein is a practical advancement for diagnosing, prognosing,

monitoring, characterizing, predicting preeclampsia, or confirming the presence or absence of preeclampsia in a female subject.

Equipment

[00253] The equipment used to perform ELISA assays included Eppendorf Research[®] plus calibrated single and multi-channel pipettes, Accu-Jet[®] pro pipettor, 2°-8°C deli refrigerator or cold room, -80°C freezer, non-humidified laboratory incubator set to 37°C, orbital microplate shaker, automated microplate washer (Titretek[™], M384 Washer with Stacker), microplate spectrophotometer (Molecular Devices, SpectraMax[®] 340PC), SoftMax[®] Pro software and an Alarm timer. Other equivalent equipment may be substituted for the above and achieve the same quality results.

Materials

[00254] Materials and reagents needed to perform the method include 0.5 mL and 2 mL deep well polypropylene dilution blocks, 15 mL and 50 mL polypropylene tubes, 5 mL, 10 mL and 25 mL disposable pipettes, and reagent troughs. 10X PBST (i.e. Phosphate Buffered Saline with Tween[®]) (1 L) was used for washing and was prepared by combining 80 g NaCl, 2 g KCl, 11.5 g Na₂HPO₄, 2 g KH₂PO₄, 5 ml Tween[®]-20 to 1 L in distilled water and 1 N sulfuric acid (0.1% Proclin 300 may be added to wash buffer as a preservative). Calibrator and assay diluent includes either 1% BSA or animal serum in phosphate buffered saline. As known by those skilled in the art, diluents generally need to be optimized to maximize the detection of the marker of interest in complex matrices like serum and were optimized as such for the experiments described herein.

Clinical Samples

[00255] Serum samples derived from pregnant patient with or without preeclampsia and/or with a variety of comorbidities were obtained from typical sources including ProMedDx[®], Discovery Life Sciences, Ortho Clinical Diagnostics, SeraCare[®], etc. Samples were stored at -80°C, thawed at 4°C before use and snap frozen in LN2 after use. Stability of some markers tested was confirmed by accelerated freeze/thaw experiments. To reduce biological variability of clinical samples, templates of 20 to 32 serum samples (and quality control (QC) samples) were thawed at 4°C the night before an assay was run and tested in all assays on the same day.

Templates

[00256] Multiple different 96-well plate templates containing clinical samples, QC controls and standards can be used (see FIG. 3 and FIG. 4). In all cases, columns 1 and 2 contained duplicate, 8-point standard curves with 2-fold dilutions. Column 12 contained an inverted 8-point standard curve or an inverted duplicate 4 point standard curve to address right to left and top to bottom variation. 20 (triplicates) or 32 (duplicates) clinical serum samples were arrayed on the plate in a randomized fashion. For triplicates, samples were interleaved by column across the plate and for duplicates, samples were interleaved by row down the plate. Each plate contained 4 or 6 replicates of Hi QC and Lo QC control samples arrayed strategically across the plate to assess top to bottom and left to right variation.

Experimental Procedures

[00257] Preparation of Master Blocks. The front side of 0.5 ml or 2 mL deep well block was labeled with template number (e.g. "T1 Master"). Each thawed clinical sample was mixed with a pipette and sufficient sample for all the assays being run (typically 30-500 microliter (μl) including 20% average) was added to the master block. Hi and Lo QC are not added to the master block (only the dilution blocks) so these wells remained empty in Master Block. The master block was sealed with the plate sealer and stored at 4°C when not being used to make dilution blocks.

[00258] Preparation of Dilution Blocks. All necessary reagents and samples were warmed for 30 minutes at room temperature before use. The front of a 2 mL deep well block was labeled as "TX-Marker" as a human-readable barcode for tracking where "X" was the Template being prepared and "Marker" was the protein marker being assayed. Lyophilized marker standards were reconstituted with 1-2 ml of deionized water or calibrator diluent, mixed gently by agitation, and allowed to reconstitute for at least 5 minutes but up to 15 minutes to make 10X or 1X concentrated stock solutions. Serum samples were used undiluted or diluted between 1:2 and 1:1,000 (typically 1:4, 1:10, 1:15, 1:20, or 1:1000) with assay diluent depending on the concentration range of the biomarker of interest in the samples (based on historical results). For example, for a 1:2 dilution, 120 μl of serum from the master block was added to 120 μl of assay diluent in the dilution block. Standard solutions were diluted 1:10 if necessary for the first concentration and then a standard curve was made by making

1:2 serial dilutions into calibrator diluent. Typically, standard curves started at 50, 20, 10, 5, 2 or 1 nanograms per milliliter (ng/ml) for low concentration markers, but could start as high as 0.8, 2.5, or 5 micrograms per milliliter ($\mu\text{g/ml}$) if the marker is at a high concentration in serum. Hi and Lo QC controls were diluted similarly to serum samples or, sometimes, received a lower dilution due to the stock concentration of the QC controls. QC controls were set up by spiking purified protein into normal or synthetic serum so that the OD of the QC control was at or near the second or fifth point on the standard curve.

[00259] ELISA protocol. Assay plates pre-coated with capture antibody against the marker of interest were labeled with template number and marker as "TX-Marker" for tracking. Assay plates were filled with 50 or 100 μl of assay diluent prior to adding sample. Samples and standard curve samples were added to the plate as shown in the templates above. Plates were covered with an adhesive strip and incubated for 1, 2 or 3 hours at room temperature or 37°C (depending on the marker being tested). Plates were then washed on a plate washer with 3-6 washes of 300-400 μl of wash buffer each then blotted on a paper towel. 50 to 200 μl of an HRP-conjugated secondary antibody against the antigen of interest in assay diluent was added to the whole plate, covered with an adhesive strip, and incubated for 30 minutes to 2 hours at room temperature or 37°C depending on the marker being tested. The plate was washed again with 3-6 washes of 300-400 μl of wash buffer each then blotted on a paper towel. While washing, a two-component TMB reagent (i.e. 3,3',5,5'-Tetramethylbenzidine) was mixed together (equal volumes of both reagents) and 100 to 200 μl of this reagent was added to the plate. Plates were incubated in the dark for 5 to 28 minutes depending on the marker being tested. At the end of this incubation, 50 μl of 1N sulfuric acid was added to the whole plate to stop the HRP (i.e. horseradish peroxidase) enzymatic reaction. Plates were read on a spectrophotometer at 450 nm (subtracting 570 nm reading).

Data Calculation, Acceptance and Retesting

[00260] Calculation of marker concentrations. All data calculations to convert optical density (OD) to concentration were carried out in SoftMax® pro using preformatted protocols. These protocols fit the standard curves and then the final concentrations calculated taking into account dilutions to 4 significant digits. Analyte concentrations were flagged with "R" if the

measured OD values are outside range of standard curve. Data were exported to *.TXT format using SoftMax® Pro for further analysis.

[00261] Assay acceptance criteria. The coefficient of determination of the standard curve can be $R^2 \geq 0.95$. Hi and Lo QC control values were tracked over time with a 3s control chart.

5 QC control results may be used to alert the technical team that additional scrutiny may be appropriate. Any other variability in standard curve or QC controls (edge effects, left to right variation, etc.) are assessed by a statistician and reported in the study report.

[00262] Sample acceptance criteria. A logarithmic transformation was applied to all the standard curve measurements prior to their use for calculating the overall standard deviation
10 of replicates. Any individual measurement that exceeded 4 standard deviations (SDs) was removed and the mean at each concentration calculated using the non-excluded points.

[00263] Sample retesting. If sample mean concentration is not in the quantifiable range of the standard curve, samples were re-tested with necessary dilution changes. See FIG. 1 and FIG. 2 for exemplary data.

15 [00264] The preceding merely illustrates the principles of the disclosure. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the disclosure and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the
20 principles of the disclosure, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, cases, and cases of the disclosure as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents
25 developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present disclosure, therefore, is not intended to be limited to the exemplary cases shown and described herein. Rather, the scope and spirit of the present disclosure is embodied by the appended claims.

[00265] While preferred cases of the present disclosure have been shown and described
30 herein, it will be obvious to those skilled in the art that such cases are provided by way of

example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the cases of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[00266] Table 2: A listing of various PE biomarkers.

Renin (REN), human chorionic gonadotropin (HCG), alpha fetoprotein (AFP), inhibin A (INHA), activin A (INHBA), sex hormone-binding globulin (SHBG), adiponectin (ADIPOQ), antithrombin III (SERPINC1), plasminogen activator inhibitor-1 (PAI-1/SERPINE1), plasminogen activator inhibitor-2 (PAI-2/SERPINE2), apolipoprotein A-I (APOA1), apolipoprotein B-100 (APOB), apolipoprotein C-II (APOC2), apolipoprotein C-III (APOC3), apolipoprotein E (APOE), endothelin (EDN), prostacyclin, thromboxane, placenta growth factor-1 (PlGF-1), placenta growth factor-2 (PlGF-2), placenta growth factor-3 (PlGF-3), vascular endothelial growth factor (VEGF), FMS-like tyrosine kinase (Flt1), soluble FMS-like tyrosine kinase (sFlt1), vascular endothelial growth factor receptor 3 (Flt4), endoglin (ENG), soluble endoglin (sENG), endothelial PAS domain-containing protein 1 (EPAS1), neurokinin B, metalloproteinase inhibitor 1 (TIMP1), metalloproteinase inhibitor (TIMP-2), metalloproteinase inhibitor 3 (TIMP3), angiopoietin 2 (ANGPT2), decorin (DCN), heparin-binding EGF-like growth factor (HBEGF), amiloride-binding protein-1 (ABP1), solute carrier family 21 (prostaglandin transporter) member 2 (SLC21A2), palladin (KIAA0992), lipoprotein lipase (LPL), insulin-like growth factor-binding protein complex acid labile subunit (IGFBP3), selenoprotein P (SEPP1), sulfhydryl oxidase 1 (QSOX1), peroxiredoxin-1 (PRDX1), peroxiredoxin-2 (PRDX2), lysosomal pro-X carboxypeptidase (PRCP), leucyl-cystinyl aminopeptidase (LNPEP), tenascin-X (TNXB), basement membrane-specific heparan sulfate proteoglycan core protein (HSPG2), cell surface glycoprotein MUC18 (MCAM), phosphatidylinositol-glycan-specific phospholipase D (GPLD1), Kunitz-type serine protease inhibitor 1 (SPINT1), G-protein-coupled receptor 126 (GPR126), C-reactive protein (CRP),

phosphatidylcholine-sterol acyltransferase (LCAT), roundabout homolog 4 (ROBO4),
 ectonucleotide pyrophosphatase/phosphodiesterase family member 2 (ENPP2),
 protein S100-A9 (S100-A9), fatty acid-binding protein 4 (FABP4), enoyl-CoA
 hydratase (ECHS1), $\Delta^3,5$ - $\Delta^2,4$ -dienoyl-CoA isomerase (ECH1), peroxidase 6 (PER6),
 5 heat shock protein beta-1 (HSP27), stathmin (STMN), annexin A1 (ANXA), annexin
 A2 (ANXA2), annexin A4 (ANXA4), prostaglandin dehydrogenase 1 (HPGD),
 proliferation-associated protein 2G4 (PA2G4), estradiol 17-beta-dehydrogenase
 (HSD17), macrophage-capping protein (CAPG), hypoxia-inducible factor 1-alpha
 (HIF1A), chaperonin (CPN), ER-60 protease, isocitrate dehydrogenase 1 (IDH1),
 10 aldehyde reductase 1 (AKR1B1), fidaresta chain B bonded to human aldose reductase,
 voltage-dependent anion-selective channel protein 1 (VDAC1), nuclear chloride
 channel, phosphoglycerate mutase 1 (PGAM1), endoplasmic reticulum protein,
 proteasome subunit alpha type-2 (PSMA2), glutathione-S-transferase (GST), Ig
 heavy-chain V region, smooth muscle myosin alkali light chain, tumor necrosis factor
 15 alpha (TNF), macrophage colony-stimulating factor (M-CSF), granulocyte colony-
 stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-
 CSF), fibroblast growth factor (FGF), relaxin H2 (RLN2), FERM and PDZ domain-
 containing protein 4 (FRMPD4), somatostatin (SST), interphotoreceptor matrix
 proteoglycan 1 (IMPG1), C-X-C motif chemokine 9 (CXCL9), C-X-C motif
 20 chemokine 11 (CXCL11), hydroxy-delta-5-steroid dehydrogenase 3-beta and steroid
 delta-isomerase 2 (HSD3B2), partitioning defective 6 homolog beta (PARDB6), Bile
 salt export pump (ABCB11), membrane-spanning 4-domains subfamily A member 3
 (MS4A3), LOC196993, bestrophin (BEST1), glycosyl-phosphatidylinositol-anchored
 molecule-like protein (GML), cell division cycle 2-like 5 (CDC2L5), glycine receptor
 25 alpha 1 (GLRA1), dihydropyrimidinase-related protein 4 (DPYSL4), kappa-type
 opioid receptor (OPRK1), dimethylarginine dimethylaminohydrolase 1 (DDAH1),
 homeobox protein Hox-A4 (HOXA4), Homeobox protein Hox-A7 (HOXA7),
 Homeobox protein Hox-B5 (HOXB5), thyrotropin-releasing hormone receptor
 (TRHR), nuclear transition protein 2 (TNP2), vasopressin, placental protein 13 (PP13),
 30 neutrophil gelatinase-associated lipocalin (LCN2), interferon gamma-inducible

protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1), intracellular adhesion
 molecule-1 (ICAM-1), intracellular adhesion molecule-3 (ICAM-3), vascular cell
 adhesion molecule-1 (VCAM-1), interleukin-1 (IL-1), interleukin-2 (IL-2),
 interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6),
 5 interleukin-7 (IL-7), interleukin 8 (IL-8), interleukin-9 (IL-9), interleukin 10 (IL-10),
 interleukin-11 (IL-11), interleukin-12 (IL-12), interleukin 13 (IL-13), interleukin-27
 subunit beta (EBI3), lectin, platelet-derived growth factor (PDGF), matrix
 metalloprotease-2 (MMP-2), matrix metalloprotease-9 (MMP-9), matrix
 metalloprotease-12 (MMP12), matrix metalloprotease-23A (MIFR), matrix
 10 metalloprotease-23B (MIFR-2), fibrinogen, fibrinogen alpha (FGA), fibronectin-1
 (FN1), protein S (PROS1), protein C (PROC), pikachurin (EGFLAM), hemopexin
 (HPX), ADAM metallopeptidase domain 2 (ADAM2), ADAMTS3, ADAM
 metallopeptidase domain 12 (ADAM12), ADAM metallopeptidase domain 12 short
 isoform (ADAM12-S), ADAM metallopeptidase domain 12 long isoform (ADAM12-
 15 L), haptoglobin (HP), serum-alpha2-macroglobulin (A2M), retinol-binding protein 4,
 small inducible cytokine A2 (CCL2), C-C motif chemokine 5 (CCL5), cathepsin B
 (CTSB), cathepsin C (CTSC), cathepsin D chain H (CTSD), heme oxygenase-1
 (HMOX1), insulin-like growth factor-binding protein 1 (IGFBP1), insulin-like growth
 factor-binding protein 2 (IGFBP2), insulin-like growth factor-binding protein-3
 20 (IGFBP3), insulin-like growth factor-binding protein-5 (IGFBP5), insulin-like growth
 factor-binding protein-7 (IGFBP7), insulin-like growth factor-1 (IGF-1), keratin 4
 (KRT4), keratin 16 (KRT16), keratin 19 (KRT19), keratin 33A (KRT33A), keratin 40
 (KRT40), pro-platelet basic protein (PPBP), perilipin 2 (PLIN2), kininogen-1 (KNG1),
 choriogonadotropin-subunit beta (CGB), cystatin C (CST3), pappalysin-1 (PAPPA1),
 25 pappalysin-2 (PAPPA2), alpha-1 B-glycoprotein (A1BG), actin (ACTB), C4b-binding
 protein beta chain (C4BP), cholinesterase (BCHE), chorionic somatomammotropin
 hormone (CSH1), coagulation factor VII (F7), coagulation factor XI (F11), filamin A
 (FLNA), filamin B (FLNB), heparin cofactor 2 (HCII), hepatocyte growth factor-like
 protein (MST1), histidine-rich glycoprotein (HRG), laminin subunit beta-1 (LAMB1),
 30 lipopolysaccharide-binding protein (LBP), plastin-2 (LCP1), profilin-1 (PFN1),

pregnancy-specific beta-1-glycoprotein (PSG1), pregnancy-specific beta-4-
 glycoprotein (PSG4), pregnancy-specific beta-11-glycoprotein (PSG11), receptor-
 type tyrosine-protein phosphatase gamma precursor (PTPRG), pregnancy-zone
 protein (PZP), SH3 domain-binding glutamic acid-rich-like protein 3 (SH3BGRL3),
 5 transgelin-2 (TAGLN2), talin-1 (TLN-1), tropomyosin alpha-4 chain (TPM4), vasorin
 (VSN), vinculin (VCL), von Willebrand factor (VWF), ferritin (FT), ferritin light
 chain, hemoglobin (HB), heme, podocin (NPHS2), nephrin (NPHS1), podocalyxin
 (PODXL), synaptopodin (SYNPO), leptin (LEP), follistatin-like 3 protein (FSTL3),
 beta fertilin (FTNB), CD33L, neutrotrophic tyrosine kinase receptor 2 (TRKB), beta
 10 glucosidase (BGL), angiogenin (ANG), leukocyte-associated Ig-like receptor secreted
 protein (LAIR), erythroid differentiation protein, adipogenesis inhibitory factor (IL-
 11), corticotropin-releasing factor-binding protein (CRHBP), alpha 1-
 antichymotrypsin (SERPINA3), cytokine receptor-like factor 1 (CRLF1), lysyl
 hydroxylase isoform 2 (LH2), stanniocalcin precursor (STC), secreted frizzled
 15 related-protein (SFRP), galectin-3 (LGALS3), alpha neutrophil defensin 1 (DEFA1),
 cholecystokinin precursor (CCK), interferon-stimulated T-cell alpha chemoattractant
 (I-TAC), azurocidin (HBP), spermine oxidase (SMOX), UDP glycosyltransferase 2
 family polypeptide B28 (UGT2B28), neutral endopeptidase (NEP), CDC28 protein
 kinase regulatory subunit 2 (CKSHS2), lanosterol synthase (LSS),
 20 calcium/calmodulin-dependent serine protein kinase (CASK), chemokine (CX3C
 motif) receptor 1 (CX3CR1), tyrosinase-related protein 1 (TYRP1), hydroxy-delta-5-
 steroid dehydrogenase (HSD3), cytochrome P450-family 11 (CYP11), cytochrome
 P450-family 11 subfamily A polypeptide 1 (CYP11A1), cytochrome P450-family 11
 subfamily B polypeptide 1 (CYP11B1), cytochrome P450 1A1 (CYP1A1), coronin-
 25 2A (CORO2A), cytochrome P450 2J2 (CYP2J2), paralemmin (PALM),
 glyceraldehyde-3-phosphate dehydrogenase (GAPD), ATP-binding cassette sub-
 family A member 12 (ABCA12), transcription factor Eb (TFEB), transcription factor
 IIE (TFIIE), syntaxin binding protein 5-like (STXBP5L), guanylin (GUCA2A),
 ribosomal protein S6 kinase alpha-2 (RPS6KA2), protein phosphatase 1 regulatory
 30 subunit 16B (PPP1R16B), class B basic helix-loop-helix protein 2 (BHLHB2),

glycophorin E (GYPE), nebulin (NEBL), leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG1), glucose transporter 3 (GLUT3), UDP-glucuronosyltransferase 2B28 (UGT2B28), nuclear receptor subfamily 5 group A member 2 (NR5A2), neuronatin (NNAT), sodium- and chloride-dependent creatine transporter 1 (SLC6A8), receptor tyrosine-protein kinase erbB-2 (ERBB2), receptor tyrosine-protein kinase erbB-3 (ERBB3), sialic acid-binding Ig-like lectin 6 (SIGLEC6), SHC-transforming protein 3 (SHC3), neurexophilin 4 (NXPH4), lymphocyte antigen 6D (LY6D), prostacyclin synthase (PTGIS), ATP-dependent RNA helicase DDX51 (DDX51), TRAF3-interacting protein 1 (TRAF3IP1), trophoblast glycoprotein (TPBG), transforming growth factor beta-3 (TGFB3), cyclin B1 (CCNB1), kinesin family member 17 (KIF17), N-myc downstream mediated gene 1 (NDRG1), SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3 (SMARCD3), serine/threonine-protein kinase Chk2 (CHEK2), amphiregulin (AREG), minor histocompatibility antigen HA-1 (HA-1), POU domain, class 4, transcription factor 1 (POU4F1), prostate stem cell antigen (PSCA), collagen alpha-1(X) chain (COL10A1), collagen alpha-3(VI) chain (COL6A3), collagen alpha-3 (IX) chain (COL9A3), paired box gene 2 (PAX2), paired box gene 4 (PAX4), paired box gene 7 (PAX7), latrophilin 3 (LPHN3), bile acid receptor (NR1H4), empty spiracles homolog 1 (EMX1), desmoglein 3 (DSG3), DNA-binding protein Ikaros (ZNFN1A1), melanoma-associated antigen 5 (MAGEA5), melanoma-associated antigen 3 (MAGEA3), afadin- and alpha-actinin-binding protein (SSX2IP), WD repeat-containing protein 21 (WDR21), orexin receptor type 2 (HCRTR2), NKG2-D type II integral membrane protein (KLRK1), HLA class II histocompatibility antigen DP alpha 1 chain (HLA-DPA1), HLA class II histocompatibility antigen DP beta 1 chain (HLA-DPB1), HLA class II histocompatibility antigen DR alpha chain (HLA-DRA), HLA class I histocompatibility antigen, alpha chain G (HLA-G), peripheral myelin protein 2 (PMP2), guanine nucleotide-binding protein G(o) subunit alpha (GNAO1), voltage-dependent L-type calcium channel subunit beta-2 (CACNB2), c-Jun-amino-terminal kinase-interacting protein 2 (MAPK8IP2), P antigen family member 1 (PAGE1),

GABA receptor subunit beta-1 (GABRB1), sodium- and chloride-dependent betaine
 transporter (SLC6A12), lactadherin (MFGE8), integrin alpha-L (ITGAL),
 desmocollin 1A/1B (DSC1), villin 2 (VIL2), plectin 1 (PLEC), ankyrin 1 (ANK1),
 vimentin (VIM), osteopontin (SPP1), dynamin 2 (DNM2), muscle cadherin (CDH15),
 5 kinesin heavy chain, fatty acid synthase (FASN), alpha-adducin (ADD1), NADH-
 cytochrome B5 reductase (CYB5R), dihydrofolate reductase (DHFR), ADP-
 ribosylation factor-like protein 3 (ARL3), NADPH menadione oxidoreductase 1-
 dioxin-inducible (NQO1), CD73, ubiquitin (UB), glutathione S-transferase Mu 3
 (GSTM3), superoxide dismutase 1 (SOD1), cytochrome C oxidase subunit VIa
 10 polypeptide 1 (COX6A1), glutathione reductase (GSR), myristoylated alanine-rich C-
 kinase substrate (MARCKS), protein disulfide-isomerase A2 (PDIA2), DNA
 topoisomerase 3-alpha (TOP3A), forkhead (Drosophila)-like 7, LIM/homeobox
 protein Lhx2 (LHX2), T-box transcription factor TBX3 (TBX3), CCAAT/enhancer-
 binding protein alpha (CEBPA), CCAAT/enhancer-binding protein delta (CEBPD),
 15 disrupted in schizophrenia 1 protein (DISC1), runt-related transcription factor 1
 (RUNX1), sterol regulatory element-binding protein 2 (SREBF2), interferon-induced,
 double-stranded RNA-activated protein kinase (EIF2AK2), zinc finger protein 208
 (ZNF208), tonsoku-like protein (TONSL), signal transducer and activator of
 transcription 2 (STAT2), myocyte-specific enhancer factor 2D (MEF2D), GA-binding
 20 protein alpha chain (GABPA), dual specificity mitogen-activated protein kinase
 kinase 6 (MAP2K6), growth hormone variant (GH2), erythropoietin (EPO), ephrin
 type-A receptor 3 (EPHA3), ephrin type-A receptor 4 (EPHA4), ephrin type-A
 receptor 5 (EPHA5), granulin (GRN), granulocyte colony-stimulating factor receptor
 (CSF3R), macrophage colony-stimulating factor 1 receptor (CSF1R), receptor-type
 25 tyrosine-protein phosphatase F (PTPRF), bone morphogenetic protein 1 (BMP1),
 epithelial discoidin domain-containing receptor 1 (DDR1), transferrin receptor protein
 1 (TFRC), angiopoietin 1 receptor (TEK), insulin receptor (INSR), 78 kDa glucose-
 regulated protein (HSPA5), S-phase kinase-associated protein 1 (SPK1), Regulator of
 chromosome condensation (RCC1), caspase 6 (CASP6), heat shock 90kDa protein A
 30 (HSP90A), butanoic acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic

acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid, eicosanoic acid, docosanoic acid, tetracosanoic acid, hexacosanoic acid, pristanic acid, phytanic acid, dihydroxycholestanic acid (DHCA), and trihydroxycholestanic acid (THCA), uric acid.

	Fibronectin ("FN")	Hemopexin ("HPX")	interferon-gamma
	Fibrinogen ("FG")		TNF-alpha
	ADAM metalloproteinase	placental growth factor	beta-amyloid
	domain 12 ("ADAM12")	("P1GF")	
	pregnancy-associated plasma	soluble vascular endothelial	IL-4
	protein A ("PAPP-A")	growth factor ("sFlt-1")	
	arginine vasopressin	copeptin	IL-10
5	arginine vasopressin;		
	copeptin;		
	interferon-gamma;		
	TNF-alpha;		
	IL-10;		
10	IL-4;		
	beta-amyloid;		
	interferon-inducible protein 6-16;		
	albumin;		
	SERPINA1;		
15	Ceruloplasmin; and		
	Immunoglobulin free light chains		

Isoforms of the above biological entities are also contemplated as biomarkers.

Such isoforms include, for example, sFlt-2, sFlt-4 and sFlt-5.

20 Other isoforms include FN GenBank Accession No. NM_212474.1), FG GenBank Accession No. NM_000508.3 (FGA) and GenBank Accession No. NM_005141.4 (FGB), PAPP-A e.g., GenBank Accession No. NM_002581.3), HPX GenBank Accession No. NM_000613.2); ADAM12 Genbank Accession Nos. NM_003474.4 (isoform 1), NM_021641.3 (isoform 2);

sFlt-1; e.g., Genbank Accession Nos. NM_001159920.1 (isoform 2), NM_001160030.1 (isoform 3), and NM_001160031.1 (isoform 4)); P1GF e.g., Genbank Accession Nos. NM_002632.5 (isoform 1) and NM_001207012.1 (isoform 2)).

- 5 Fragments or portions of a PE biomarker which are recognized by a detection reagent, e.g., an antibody, are also deemed PE biomarkers herein.

CLAIMS

WHAT IS CLAIMED IS:

- 5 1. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
 - a) measuring levels of one or more biomarkers in a sample derived from the female subject;
 - b) calculating an index based on the levels of the one or more biomarkers; and
 - 10 c) confirming the presence or absence of preeclampsia in the female subject, based on the index.
- 15 2. The method of claim 1, wherein the measuring levels of one or more biomarkers comprise measuring levels of three or more biomarkers.
3. The method of claim 1, wherein the measuring levels of one or more biomarkers comprise measuring levels of four or more biomarkers.
- 20 4. The method of claim 1, wherein the measuring levels of one or more biomarkers comprise measuring levels of five or more biomarkers.
- 25 5. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
 - a) measuring levels of one or more biomarkers in a sample derived from the female subject;
 - b) comparing the levels of the one or more biomarkers to a respective recombinant protein level or to a standard value; and
 - 30 c) confirming the presence or absence of preeclampsia in the female subject, based on the comparing.

6. The method of claim 5, further comprising calculating an index based on the levels of the one or more biomarkers.
7. A method for confirming a presence or absence of preeclampsia in a female
5 subject, the method comprising:
- a) measuring levels of fibronectin (FN) and two or more biomarkers in a sample derived from the female subject, wherein at least two of the two or more biomarkers are different from fibronectin,
 - b) calculating an index based on the levels of FN and the two or more
10 biomarkers; and
 - c) confirming the presence or absence of preeclampsia in the female subject, based on the index.
8. The method of claim 7, wherein the two or more biomarkers are selected from the
15 group consisting of sFLT-1, PlGF, ADAM-12, HPX and PAPP-A.
9. The method of claim 7, wherein the biomarkers are sFLT-1, PlGF and PAPP-A.
10. The method of claim 7, wherein the biomarkers are sFLT-1, PlGF, PAPP-A and
20 ADAM-12.
11. The method of claim 7, wherein the biomarkers are sFLT-1, PlGF, PAPP-A and HPX.
12. The method of claim 7, wherein the biomarkers are PlGF, PAPP-A and ADAM-
25 12.
13. The method of claim 7, wherein the biomarkers are sFLT-1 and PlGF.
14. The method of claim 7, wherein the biomarkers are PlGF and PAPP-A.
30

15. The method of claim 7, wherein the biomarkers are sFLT-1, P1GF and ADAM-12.

16. The method of claim 7, wherein the biomarkers are sFLT-1 and ADAM-12.

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17. The method of claim 7, wherein the biomarkers are P1GF, ADAM-12, sFLT1, PAPP-A2, and HPX.

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18. The method of claim 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17, further comprising comparing the index to a threshold value.

19. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:

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a) measuring levels of fibronectin (FN) or FN fragment in a sample derived from the female subject using a monoclonal antibody that selectively binds the FN or the FN fragment;

b) comparing the levels of fibronectin (FN) or FN fragment to a respective recombinant protein level or to a standard value; and

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c) confirming the presence or the absence of preeclampsia, based on the comparing.

20. The method of claim 19, further comprising measuring levels of two or more biomarkers in a sample derived from the female subject.

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21. The method of claim 20, wherein the two or more biomarkers are selected from the group consisting of sFLT-1, P1GF, ADAM-12, HPX and PAPP-A.

22. The method of claim 20, wherein the biomarkers are sFLT-1, P1GF and PAPP-A.

23. The method of claim 20, wherein the biomarkers are sFLT-1, P1GF, PAPP-A and ADAM-12.
- 5 24. The method of claim 20, wherein the biomarkers are sFLT-1, P1GF, PAPP-A and HPX.
25. The method of claim 20, wherein the biomarkers are P1GF, PAPP-A and ADAM-12.
- 10 26. The method of claim 20, wherein the biomarkers are sFLT-1 and P1GF.
27. The method of claim 20, wherein the biomarkers are P1GF and PAPP-A.
- 15 28. The method of claim 20, wherein the biomarkers are sFLT-1, P1GF and ADAM-12.
29. The method of claim 20, wherein the biomarkers are sFLT-1 and ADAM-12.
- 20 30. The method of claim 20, wherein the biomarkers are P1GF, FN, ADAM-12, sFLT1, PAPP-A2, and HPX.
31. The method of claim 19, further comprising calculating an index based on levels of bound monoclonal antibodies.
- 25 32. The method of claim 20, 22, 23, 24, 25, 26, 27, 28 or 29 further comprising calculating an index based on levels of (1) bound monoclonal antibodies and (2) the two or more biomarkers.

33. The method of claims 31 or 32 further comprising comparing the index to a threshold value, wherein the index is indicative of the presence or absence of preeclampsia in the female subject.

5 34. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:

- a) measuring levels of sFLT, PlGF and one or more biomarkers in a sample derived from the female subject, wherein the one or more biomarker is different from VEGF, wherein VEGF excludes VEGF R-1;
- 10 b) calculating an index based on the levels of sFLT, PlGF and the one or more biomarkers; and
- c) confirming the presence or absence of preeclampsia in the female subject, based on the index.

15 35. The method of claim 34, wherein the one or more biomarkers are selected from the group consisting of fibronectin (FN), ADAM-12, HPX and PAPP-A.

36. The method of claim 34, wherein the biomarkers are ADAM-12.

20 37. The method of claim 34, wherein the biomarkers are PAPP-A.

38. The method of claim 34, wherein the biomarkers are fibronectin (FN).

25 39. The method of claim 34, wherein the biomarkers are fibronectin (FN) and PAPP-A.

40. The method of claim 34, wherein the biomarkers are fibronectin (FN) and ADAM-12.

41. The method of claim 34, wherein the biomarkers are fibronectin (FN), ADAM-12 and PAPP-A.
- 5 42. The method of claim 34, wherein the biomarkers are fibronectin (FN), HPX and PAPP-A.
43. The method of claim 34, wherein the biomarkers are FN, ADAM-12, PAPP-A2, and HPX.
- 10 44. The method of claims 34, 35, 36, 37, 38, 39, 40, 41 or 42 further comprising comparing the index to a threshold value.
45. A method for confirming a presence or absence of preeclampsia in a female subject, the method comprising:
- 15 a. measuring levels of biomarkers consisting of: sFLT and P1GF;
- b. calculating an index based on the levels of sFLT and P1GF; and
- c. confirming the presence or absence of preeclampsia in the female subject, based on the index.
- 20 46. The method of claim 45, wherein the calculating comprises multiplying each of the measured levels of sFLT and P1GF by a unique weight factor, and applying one or more binary functions to weighted measured levels of sFLT and P1GF.
- 25 47. A method for diagnosing, prognosing, characterizing, monitoring, determining a severity of, confirming a presence of, or confirming an absence of preeclampsia in a female subject, the method comprising:
- a) measuring levels of least one fibronectin (FN) fragment in two different assays, wherein the assays determine the level of FN in a sample derived from the female subject; and

- b) diagnosing, prognosing, characterizing, monitoring, determining the severity of, confirming the presence of, or confirming the absence of preeclampsia in the female subject, based on the levels of at least one FN fragment measured in the two different assays.

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48. The method of claim 47, wherein each of the two different assays utilizes a different monoclonal antibody.

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49. The method of claim 48, further comprising measuring levels of one or more biomarkers in the sample derived from the female subject, wherein the one or more biomarkers are different from fibronectin (FN).

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50. The method of claim 49, wherein the biomarkers are selected from the group consisting of sFLT-1, PlGF, ADAM-12, HPX and PAPP-A.

51. The method of claim 49, wherein the biomarkers are sFLT-1 or PlGF.

52. The method of claim 49, wherein the biomarkers are sFLT-1, PlGF or PAPP-A.

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53. The method of claim 49, wherein the biomarkers are sFLT-1, PlGF or ADAM-12.

54. The method of claim 49, wherein the biomarkers are PlGF, ADAM-12, sFLT1, PAPP-A2, and HPX.

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55. The method of claims 49, 50, 51, 52, 53, or 54, further comprising calculating an index based on the levels of (1) bound monoclonal antibodies and (2) the one or more biomarkers.

56. The method of claims 1, 5, 7, 34, or 47, wherein the measuring comprises:

coating an immunoassay plate with antibodies exhibiting an affinity for
a biomarker to be measured; and

coating the immunoassay plate with a non-specific blocking protein.

5 57. The method of claim 56, further comprising:

mixing labeled biomarkers with the sample resulting in a mixture; and
adding the mixture to an immunoassay plate.

58. The method of claim 56, further comprising:

10 introducing the sample to the immunoassay plate; and
introducing secondary conjugated antibodies to the immunoassay plate.

59. The method of claim 1, 6 or 55 further comprising comparing the index to a
threshold value.

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60. The method of claim 7, 18, 31, 32, 33, 34, 44 , or 59, wherein the index is
calculated by a real function algorithm for totaling measured levels of biomarker
levels, wherein the algorithm comprises multiplying one or more variables by one
or more corresponding weight factors,

20

wherein the level of each of the biomarker levels is input into a
specific variable of the one or more variables,

wherein a corresponding weight factor is unique for each specific
variable,

25

wherein at least one of the one or more corresponding weight factors is
different from one.

61. The method of claim 60, wherein the algorithm comprises at least one binary
operation.

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62. The method of claim 61, wherein the at least one binary operation is division.

63. The method of claim 61, wherein the at least one binary operation is addition or subtraction.

5 64. The method of claim 60, wherein the one or more weight factors is a ratio of measured levels of two biomarkers.

65. The method of claim 1, 5, 7, 19, 34 or 47, further comprising generating a report indicating the presence or absence of preeclampsia in the female subject.

10 66. The method of claim 1, 5, 7, 19, 34 or 47, wherein the method excludes consideration of blood pressure, sugar blood level, urine protein level, familial preeclampsia history, or weight gain.

15 67. The method of claim 1, 5, 7, 19, 34 or 47, wherein the female subject has at least one symptom in the group consisting of: blood pressure above 140/90 mm Hg, sugar blood level above 100 mg/dL while fasting, urine protein level more than 5 grams in a 24 hour collection or more than 3+ on two random urine samples collected at least four hours apart, weight gain of more than two pounds in a week,
20 platelets level below 155,000 (per microliter) in a second trimester or below 145,000 (per microliter) during a third trimester, oliguria of less than 400 milliliters in 24 hours, high body-mass index above 25, familial history of preeclampsia, pulmonary edema, cyanosis and change in vision.

25 68. The method of claim 1 or 5, wherein the one or more biomarkers are selected from the group consisting of sFLT-1, PlGF, fibronectin (FN), ADAM-12, HPX and PAPP-A.

30 69. The method of claim 1, 5, 7, 19, 34 or 47, wherein the biomarkers exclude ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-

macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3),
apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB),
fibrinogen alpha (FGA), pikachurin (EGFLAM), free human chorionic
gonadotropin (free beta hCG) and heme.

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70. The method of claim 18, 44 or 59, wherein the comparing comprises comparing
the biomarkers to (1) that of a single pregnant female or a group of pregnant
females having preeclampsia and (2) that of a group of pregnant females not
having preeclampsia.

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71. The method of claim 70, wherein the single pregnant female is the female subject.

72. The method of claim 70, wherein the comparing comprises comparing the
biomarkers to a respective recombinant protein index value.

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73. The method of claim 1, 5, 7, 19, 34 or 47, wherein biomarkers comprise one or
more proteins or protein fragments.

74. The method of claim 1, 5, 7, 19, 34 or 47, wherein the biomarkers comprise
polynucleotides.

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75. The method of claim 1, 5, 7, 19, 34 or 47, wherein the measuring comprises
utilizing an immunological assay, mass spectrometry, chromatography,
nephelometry, radial immunodiffusion or single radial immunodiffusion assay.

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76. The method of claim 1, 5, 7, 19, 34 or 47, wherein the measuring comprises
measuring by an immunological assay.

77. The method of claim 76, wherein the immunological assay is selected from the group consisting of ELISA, sandwich ELISA, competitive ELISA and IgM antibody capture ELISA.

5 78. A kit for diagnosing, prognosing, monitoring, characterizing, determining a severity of, confirming a presence of, or confirming an absence of preeclampsia in a female subject, the kit comprising: at least two different reagents that are specific for determining a level of fibronectin (FN) in a sample derived from the female subject.

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79. The kit of claim 78, further comprising two or more reagents for measuring levels of two or more biomarkers in the sample derived from the female subject.

80. The kit of claim 79, wherein the biomarkers are sFLT-1, PlGF and PAPP-A.

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81. The kit of claim 79, wherein the biomarkers are sFLT-1, PlGF, PAPP-A and ADAM-12.

82. The kit of claim 79, wherein the biomarkers are sFLT-1, PlGF, PAPP-A and HPX.

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83. The kit of claim 79, wherein the biomarkers are PlGF, PAPP-A and ADAM-12.

84. The kit of claim 79, wherein the biomarkers are sFLT-1 and PlGF.

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85. The kit of claim 79, wherein the biomarkers are PlGF and PAPP-A.

86. The kit of claim 79, wherein the biomarkers are sFLT-1, PlGF and ADAM-12.

87. The kit of claim 79, wherein the biomarkers are sFLT-1 and ADAM-12.

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88. The kit of claim 79, wherein the biomarkers are P1GF, FN, ADAM-12, sFLT1, PAPP-A2, and HPX.

89. The kit of claim 78 or 79, wherein the kit does not include a reagent for measuring the levels of biomarkers selected from the group consisting of ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free beta hPC and heme.

90. A kit for confirming the presence or absence of preeclampsia in a female subject, the kit comprising:

- a) a first reagent specific for determining level of PAPP-A; and
- b) a second reagent specific for determining level of ADAM12.

91. The kit of claim 90, further comprising one or more reagents for measuring levels of one or more biomarkers in a sample derived from the female subject.

92. The kit of claim 91, wherein the biomarkers are sFLT-1, P1GF and fibronectin (FN).

93. The kit of claim 91 wherein the biomarkers are P1GF and fibronectin (FN).

94. The kit of claim 90 or 91, wherein the kit does not include a reagent for measuring levels of biomarkers selected from the group consisting of ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free beta hPC and heme.

95. A kit for diagnosing, prognosing, monitoring, characterizing, determining a severity of, confirming a presence of, or confirming an absence of preeclampsia, the kit comprising:

- a) a first reagent specific for determining a level of one of sFLT-1 or PlGF;
- b) a second reagent specific for determining fibronectin (FN); and
- c) a third reagent specific for determining a level of a biomarker that is different from biomarker determined by the first and second reagent.

96. The kit of claim 95, wherein the first reagent is specific for determining the level of sFLT-1, the third reagent is specific for determining the level of PlGF, and the kit further comprises a fourth reagent specific for determining a level of a biomarker different from sFLT-1, PlGF and FN.

97. The kit of claim 96, wherein the fourth reagent is specific for determining the levels of PAPP-A, HPX or ADAM12.

98. The kit of claim 95, 96 or 97 wherein the kit does not include reagents specific for determining levels of biomarkers selected from the group consisting of ferritin (FT), cathepsin B (CTSB), cathepsin C (CTSC), haptoglobin (HP), alpha-2-macroglobulin (A2M), apolipoprotein E (ApoE), apolipoprotein C-III (Apo-C3), apolipoprotein A-1 (ApoA1), retinol binding protein 4 (RBP4), hemoglobin (HB), fibrinogen alpha (FGA), pikachurin (EGFLAM), free beta hPC and heme.

99. A test for confirming a presence or absence of preeclampsia in a female subject, wherein the test measures one or more biomarkers from a sample derived from the female subject, wherein a receiver operating characteristic (ROC) value associated with the biomarkers is at least 0.8.

100. The test of claim 99, wherein the ROC value is at least 0.9.

101. The test of claim 99, wherein the ROC value is at least 0.95.

102. The test of claim 99, wherein the ROC value is at least 0.98.

5 103. The test of claim 99, wherein the ROC value is at least 0.984.

104. A test for confirming a presence or absence of preeclampsia in a subject,
wherein the test measures one or more biomarkers from a sample derived from the
subject, wherein a receiver operating characteristic (ROC) value associated with
10 the biomarkers is greater than a ROC value associated with sFLT/P1GF.

105. The test of claim 104, wherein the female subject exhibits clinical symptoms
of preeclampsia.

15 106. The test of claim 104, wherein the test comprises measuring a ratio of
measured levels of sFLT/P1GF.

107. The test of claim 104, wherein the ratio of measured levels of sFLT/P1GF is
normalized, raw, adjusted, or a combination thereof.

20

108. A system for diagnosing, prognosing, characterizing, monitoring, determining
a severity of, confirming a presence of, or confirming an absence of preeclampsia
in a female subject, the system comprising:

(a) an input module for receiving as an input levels of one or more biomarkers;

25 (b) a processor configured to:

perform a first algorithm adjusting the levels of the one or more
biomarkers to a training set, thereby providing one or more adjusted
biomarker levels; and

perform a second algorithm that applies at least one binary operation using the adjusted biomarker levels, wherein the second algorithm is a real function that results in an index value; and

- (c) an output module for outputting the index value, wherein the index value indicates diagnosis, prognosis, characterization, a monitored aspect, determination of the severity, confirmation of the presence, or confirmation of the absence, of preeclampsia in the female subject.

109. A system for confirming a presence or absence of preeclampsia in a female subject, the system comprising:

- (a) an input module for receiving as an input levels of one or more biomarkers;
(b) a processor configured to:

perform a first algorithm adjusting the levels of the one or more biomarkers to a control value; and

perform a second algorithm adding or subtracting the one or more adjusted biomarker levels, wherein the second algorithm is a real function that results in an index value; and

- (c) an output module for outputting the index value, wherein the index value indicates the absence or the presence of preeclampsia in the female subject.

110. A system for confirming a presence or absence of preeclampsia in a female subject, the system comprising:

- (a) an input module for receiving as an input levels of two or more biomarkers;
(b) a processor configured to:

perform a first algorithm adjusting the levels of the two or more biomarkers to a control value, thereby providing two or more adjusted biomarker levels; and

perform a second algorithm calculating a ratio between two of the two or more adjusted biomarker levels, wherein the second algorithm is a real function that results in an index value; and

(c) an output module for outputting the index value, wherein the index value indicates the absence or presence of preeclampsia in the female subject.

111. The system of claim 108, 109 or 110 wherein the real function comprises a complex statistical algorithm.

112. The system of claim 108, 109 or 110, wherein the real function comprises at least one binary operation.

113. The system of claim 112, wherein the real function comprises a multiplying a variable by a corresponding weight factor.

114. The system of claim 113, wherein a level of each biomarker is input into a specific variable corresponding to the biomarkers, and wherein the corresponding weight factor is unique for each variable or unique for each ratio of two variables.

115. The system of claim 108, wherein the processor is further configured to perform a third algorithm averaging each of the one or more adjusted biomarker levels.

116. The system of claim 108, wherein the processor is further configured to perform a third algorithm applying a logarithmic transformation of the levels to obtain log transformed levels; a fourth algorithm normalizing each of the log transformed levels to normalized levels; and a fifth algorithm adjusting each of the normalized levels to a weighted normalized level.

117. A system for diagnosing, prognosing, monitoring, characterizing, determining a severity of, confirming a presence of, or confirming an absence of preeclampsia in a female subject, the system comprising:

(a) an input module for receiving as an input levels of one or more biomarkers;

(b) a processor configured to:

perform an algorithm adjusting the levels of each of the one or more biomarkers to a corresponding control value, thereby providing one or more adjusted biomarker levels;

perform a real function algorithm manipulating the one or more adjusted biomarker levels by multiplying one or more variables by one or more corresponding weight factors, wherein a level of each of the one or more adjusted biomarker levels is input into a specific variable of the one or more variables, wherein the corresponding weight factor is unique for each specific variable, wherein at least one of the corresponding weight factors is different from one; and

(c) an output module for outputting an index value, wherein the index value indicates diagnosis, prognosis, characterization, a monitored aspect, determination of the severity, confirmation of the presence, or confirmation of the absence, of preeclampsia in the female subject.

118. The system of claims 109, 110 or 117 wherein the control value is established using a training set.

119. The system of claim 117, wherein the training set is based on a model.

120. The system of claim 117, wherein the training set is based on real values obtained from subjects.

121. The system of claim 120, wherein the subjects comprise at least 150 subjects.

122. The system of claim 120, wherein subjects comprise complex subjects.

123. The system of claim 122, wherein the complex subjects comprise at least 10% of all subjects used for the training set.

124. The system of claim 117, wherein the algorithm applies at least one binary operation.

5 125. The system of claim 124, wherein the at least one binary operation is division.

126. The system of claim 124, wherein the at least one binary operation is addition or subtraction.

10 127. A test for confirming preeclampsia in a subject, wherein the test is able to discern subjects that do not have preeclampsia but have one or more symptoms associated with preeclampsia, from subjects having preeclampsia, with a receiving operating characteristic (ROC) value of at least 0.8.

15 128. The test of claim 127, wherein the ROC value is of at least 0.9.

129. The test of claim 127, wherein the one or more symptoms associated with preeclampsia are selected from the group consisting of diabetes, higher than normal glucose level, hypertension, excess or sudden weight gain, overweight, 20 obesity, higher than normal body mass index, abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results, prior preeclampsia episodes, familial history of PE, renal disease and thrombophilia.

25 130. The test of claim 129, wherein the diabetes is gestational, type I or type II.

131. The test of claim 129, wherein the hypertension is chronic hypertension.

132. A test for confirming preeclampsia in a subject, wherein the test is able to discern subjects not having preeclampsia but having one or more symptoms associated with preeclampsia, from subjects having preeclampsia, with a sensitivity of at least 80% .

5

133. A test for confirming preeclampsia in a subject, wherein the test is able to discern subjects not having preeclampsia but having one or more symptoms associated with preeclampsia, from subjects having preeclampsia, with specificity of at least 80%.

10

134. A test for confirming preeclampsia in a subject, wherein the test is able to discern subjects not having preeclampsia but having one or more symptoms associated with preeclampsia, from subjects having preeclampsia, with a negative predictive value (NPV) of at least 80%.

15

135. The test of claim 132, 133 or 134, wherein the one or more symptoms associated with preeclampsia are selected from the group consisting of diabetes, higher than normal glucose level, hypertension, excess or sudden weight gain, overweight, obesity, higher than normal body mass index, abnormal weight gain, abnormal blood pressure, water retention, hereditary factors, abnormal proteinuria, headache, edema, abnormal protein/creatinine ratio, abnormal platelet count, stress, nulliparity, abnormal Papanicolaou test results, prior preeclampsia episodes, familial history of PE, renal disease and thrombophilia.

20

25

136. The test of claim 135, wherein the diabetes is gestational, type I or type II.

137. The test of claim 135, wherein the hypertension is chronic hypertension.

138. The test of claim 135, wherein the sensitivity is of at least 90%.

30

139. The test of claim 133, wherein the specificity is of at least 90%.

140. The test of claim 134, wherein the NPV is of at least 90%.

5 141. A method for confirming preeclampsia, the method comprising performing a test on a sample derived from a female subject wherein the test comprises measuring levels of a plurality of markers and using the levels of the plurality of markers to confirm preeclampsia with a receiving operating characteristic (ROC) value of at least 0.90.

10 142. The method of claim 141, wherein the ROC value is at least 0.95.

143. A method for confirming preeclampsia, the method comprising performing a test on a sample derived from a female subject wherein the test comprises
15 measuring levels of a plurality of markers and using the levels of the plurality of markers to confirm preeclampsia with a specificity of at least 80%.

144. The method of claim 143, wherein the specificity is at least 90%.

20 145. A method for confirming preeclampsia, the method comprising performing a test on a sample derived from a female subject wherein the test comprises measuring levels of a plurality of markers and using the levels of the plurality of markers to confirm preeclampsia with a sensitivity of at least 80%.

25 146. The method of claim 145, wherein the sensitivity is at least 90%.

147. A method for confirming preeclampsia, the method comprising performing a test on a sample derived from a female subject wherein the test comprises measuring levels of a plurality of markers and using said levels to confirm
30 preeclampsia with a negative predictive value of at least 80%.

148. The method of claim 147, wherein the negative predictive value is at least 90%.

5 149. The test as in any of claims 127-140, wherein the sample is selected from the group consisting of whole blood, urine, serum and plasma.

150. The method as in one of claims 141-148, wherein the sample is selected from the group consisting of whole blood, urine, serum and plasma.

10

151. The method as in any of claims 1-7, 31, 34, 60, 141, 143 or 147, wherein the biomarker comprises a biomarker of Group-1.

15

152. The test as in any of claims 30, 126 or 131-134, wherein the biomarker comprises a biomarker of Group-1.

153. The system as in any of claims 108-110 or 116, wherein the biomarker comprises a biomarker of Group-1.

20

154. The kit as in any of claims 78, 90 or 95, wherein the biomarker comprises a biomarker of Group-1.

155. A computer readable medium containing instructions which, when executed by a computer system, cause the computer system to:

25

receive a first data set pertaining to first levels of a plurality of preeclampsia biomarkers in a first biological sample derived from a subject at a first point-in-time;
perform a first analysis on the first levels to obtain a first assessment of preeclampsia in the subject;

receive a second set of data pertaining to second levels of the plurality of preeclampsia biomarkers in a second biological sample derived from the subject at a second point-in-time;

perform a second analysis on the second levels to obtain an assessment of preeclampsia in the subject;

compare the first assessment with the second assessment; and

confirm preeclampsia or lack thereof based on the comparison.

156. A method for diagnosing or confirming preeclampsia in a subject, the method comprising:

detecting protein levels of sFLT, PlGF, and a protein or protein fragment binding to placental growth factor antibody in a biological sample derived from the subject; and

calculating a preeclampsia index score using the detected protein levels, wherein the preeclampsia score is indicative of the presence or absence of preeclampsia in the subject.

157. The method of claim 156, further comprising diagnosing or confirming preeclampsia in the subject.

158. The method of claim 156, wherein the calculating comprises multiplying the detected protein levels by a unique weight factor, and applying one or more binary functions to weighted detected protein levels.

159. The method of claim 3, wherein each of the four or more biomarkers is selected from the group consisting of sFLT-1, PlGF, abfFN, PAPP-A, TakFN, ADAM-12, and HPX, wherein each of the four or more biomarkers is different from each other.

160. The method of claim 5 or 49, wherein the one or more biomarkers comprise four or more biomarkers, wherein each of the four or more biomarkers is selected from the group consisting of sFLT-1, PIGF, abFN, PAPP-A, TakFN, ADAM-12, and HPX, wherein each of the four or more biomarkers is different from each other.

5

161. The method of claim 7 or 20, wherein the two or more biomarkers comprise four or more biomarkers, wherein each of the four or more biomarkers is selected from the group consisting of sFLT-1, PIGF, abFN, PAPP-A, TakFN, ADAM-12, and HPX, wherein each of the four or more biomarkers is different from each other.

10

162. The kit of claim 79, wherein the two or more biomarkers comprise four or more biomarkers, wherein each of the four or more biomarkers is selected from the group consisting of sFLT-1, PIGF, abFN, PAPP-A, TakFN, ADAM-12, and HPX, wherein each of the four or more biomarkers is different from each other.

15

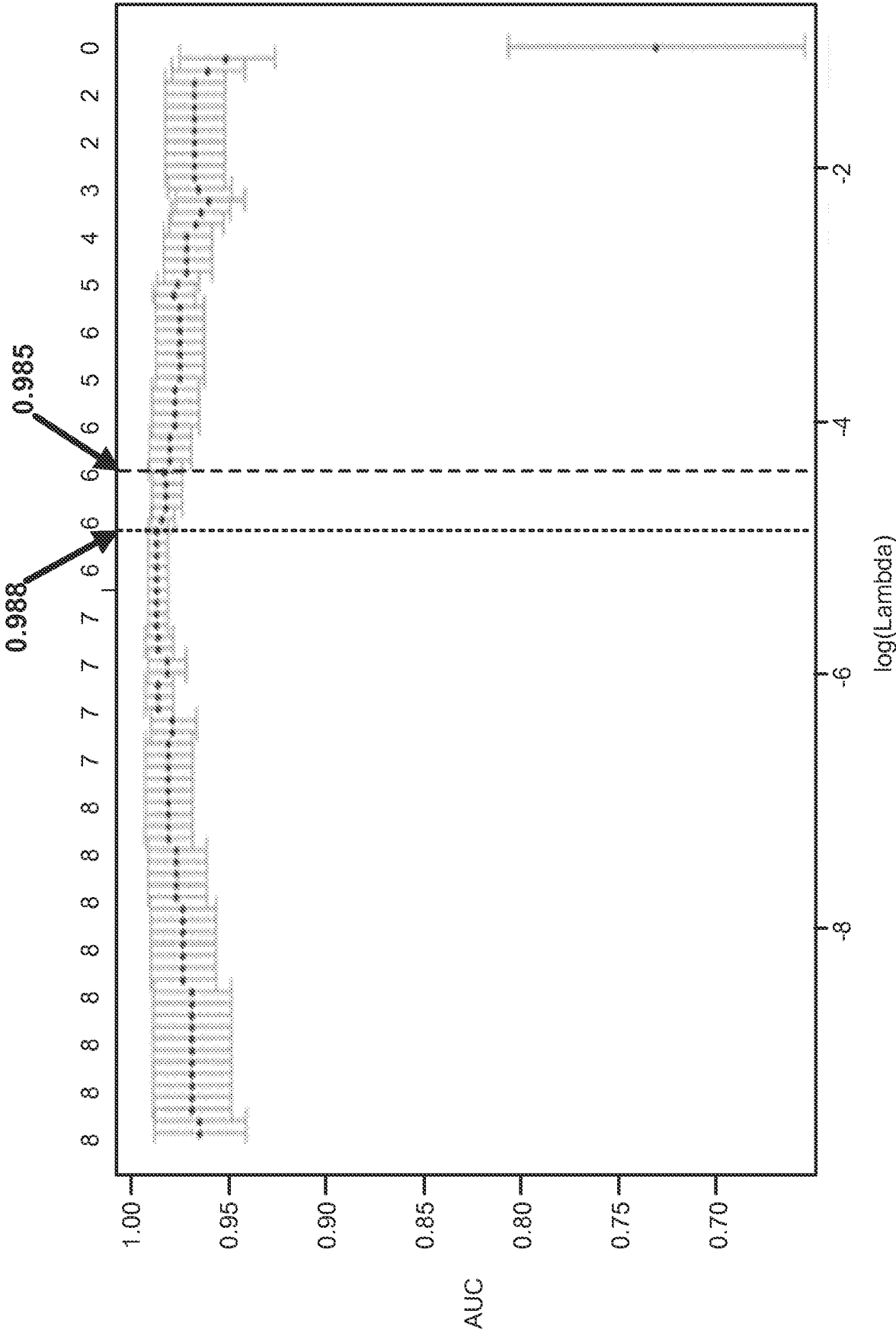


FIG. 1

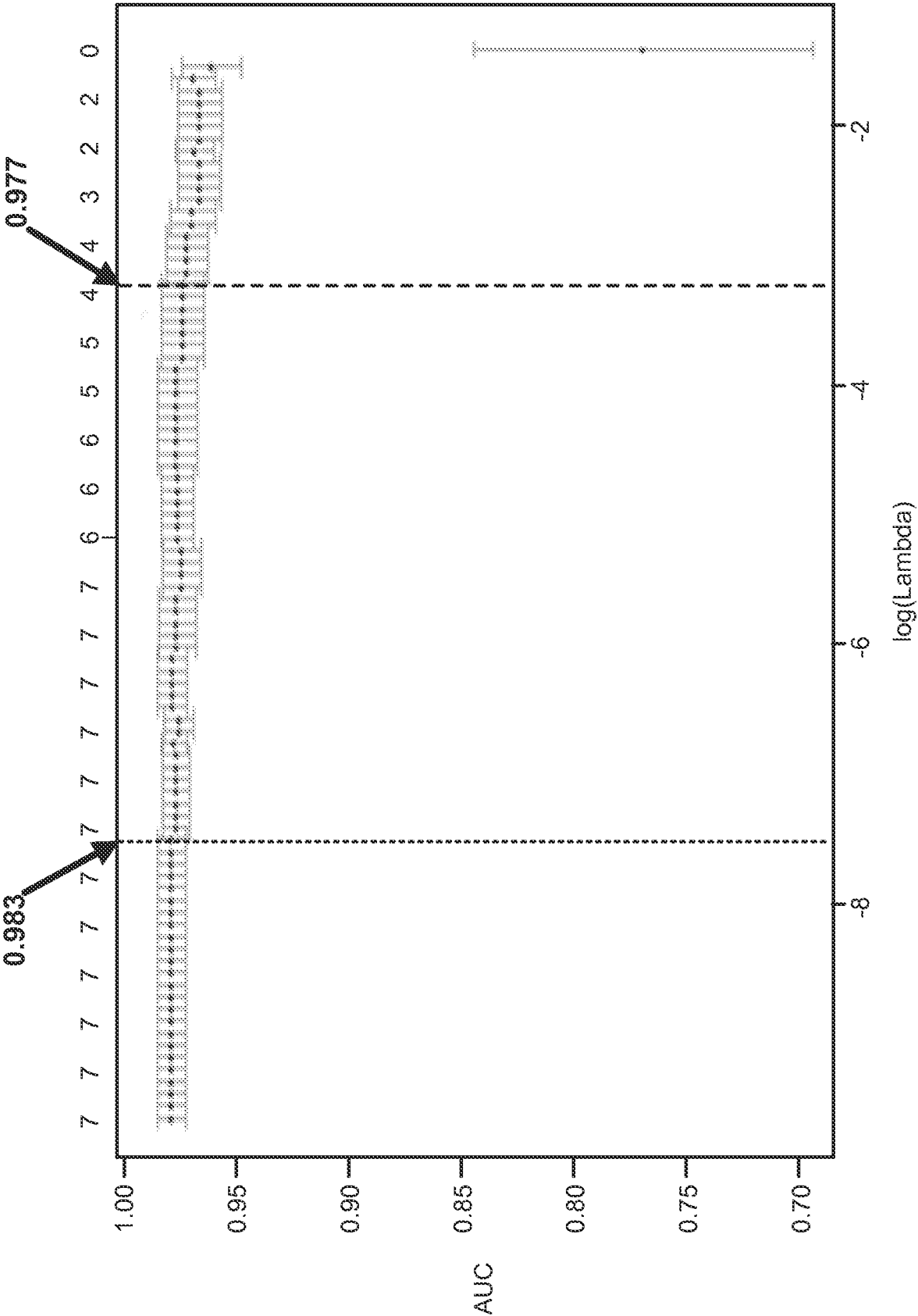


FIG. 2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Top	Top	1	Hi QC	2	3	4	5	6	Lo QC	7	0
B	Top/2	Top/2	8	9	10	11	12	13	14	15	16	Top/64
C	Top/4	Top/4	17	18	19	20	21	22	23	24	25	Top/32
D	Top/8	Top/8	Lo QC	26	27	28	29	30	Hi QC	31	32	Top/16
E	Top/16	Top/16	1	Hi QC	2	3	4	5	6	Lo QC	7	Top/8
F	Top/32	Top/32	8	9	10	11	12	13	14	15	16	Top/4
G	Top/64	Top/64	17	18	19	20	21	22	23	24	25	Top/2
H	0	0	Lo QC	26	27	28	29	30	Hi QC	31	32	Top

FIG. 3

	1	2	3	4	5	6	7	8	9	10	11	12
A	Top	Top	Hi QC	7	Lo QC	Hi QC	7	Lo QC	Hi QC	7	Lo QC	0
B	Top/2	Top/2	1	8	15	1	8	15	1	8	15	Top/64
C	Top/4	Top/4	2	9	16	2	9	16	2	9	16	Top/32
D	Top/8	Top/8	3	10	17	3	10	17	3	10	17	Top/16
E	Top/16	Top/16	4	11	18	4	11	18	4	11	18	Top/8
F	Top/32	Top/32	5	12	19	5	12	19	5	12	19	Top/4
G	Top/64	Top/64	6	13	20	6	13	20	6	13	20	Top/2
H	0	0	Lo QC	14	Hi QC	Lo QC	14	Hi QC	Lo QC	14	Hi QC	Top

FIG. 4

Renin (REN), human chorionic gonadotropin (HCG), alpha fetoprotein (AFP), inhibin A (INH A), activin A (INHBA), sex hormone-binding globulin (SHBG), adiponectin (ADIPOQ), antithrombin III (SERPINI1), plasminogen activator inhibitor-1 (PAI-1/SERPINE1), plasminogen activator inhibitor-2 (PAI-2/SERPINE2), apolipoprotein A-I (APOA1), apolipoprotein B-100 (APOB), apolipoprotein C-II (APOC2), apolipoprotein CIII (APOC3), apolipoprotein E (APOE), endothelin (EDN), prostacyclin, thromboxane, placenta growth factor-1 (PlGF-1), placenta growth factor-2 (PlGF-2), placenta growth factor-3 (PlGF-3), vascular endothelial growth factor (VEGF), FMS-like tyrosine kinase (Flt1), soluble FMS-like tyrosine kinase (sFlt1), vascular endothelial growth factor receptor 3 (Flt4), endoglin (ENG), soluble endoglin (sENG), endothelial PAS domain-containing protein 1 (EPAS1), neurokinin B, metalloproteinase inhibitor 1 (TIMP1), metalloproteinase inhibitor 2 (TIMP2), metalloproteinase inhibitor 3 (TIMP3), angiopoietin 2 (ANGPT2), decorin (DCN), heparin-binding EGF-like growth factor (HBEGF), amiloride-binding protein-1 (ABP1), solute carrier family 21 (prostaglandin transporter) member 2 (SLC21A2), palladin (KIAA0992), lipoprotein lipase (LPL), insulin-like growth factor-binding protein complex acid labile subunit (IGFBP3), selenoprotein P (SEPP1), sulfhydryl oxidase 1 (QSOX1), peroxiredoxin-1 (PRDX1), peroxiredoxin-2 (PRDX2), lysosomal pro-X carboxypeptidase (PRCP), leucyl-cystinyl aminopeptidase (LNPEP), tenascin-X (TNXB), basement membrane-specific heparan sulfate proteoglycan core protein (HSPG2), cell surface glycoprotein MUC18 (MCAM), phosphatidylinositol-glycan-specific phospholipase D (GPLD1), Kunitz-type serine protease inhibitor 1 (SPINT1), G-protein-coupled receptor 126 (GPR126), C-reactive protein (CRP), phosphatidylcholine-sterol acyltransferase (LCAT), roundabout homolog 4 (ROBO4), ectonucleotide pyrophosphatase/phosphodiesterase family member 2 (ENPP2), protein S100-A9 (S100-A9), fatty acid-binding protein 4 (FABP4), enoyl-CoA hydratase (ECHS1), $\Delta^3,5$ - $\Delta^2,4$ -dienoyl-CoA isomerase (ECH1), peroxidase 6 (PER6), heat shock protein beta-1 (HSP27), stathmin (STMN), annexin A1 (ANXA1), annexin A2 (ANXA2), annexin A4 (ANXA4), prostaglandin dehydrogenase 1 (HPGD), proliferation-associated protein 2G4 (PA2G4), estradiol 17-beta-dehydrogenase (HSD17), macrophage-capping protein (CAPG), hypoxia-inducible factor 1-alpha (HIF1A), chaperonin (CPN), ER-60 protease, isocitrate dehydrogenase 1 (IDH1), aldehyde reductase 1 (AKR1B1), fidaresta chain B bonded to human aldose reductase,

FIG. 5A

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voltage-dependent anion-selective channel protein 1 (VDAC1), nuclear chloride channel, phosphoglycerate mutase 1 (PGAM1), endoplasmic reticulum protein, proteasome subunit alpha type-2 (PSMA2), glutathione-S-transferase (GST), Ig heavy-chain V region, smooth muscle myosin alkali light chain, tumor necrosis factor alpha (TNF), macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), fibroblast growth factor (FGF), relaxin H2 (RLN2), FERM and PDZ domain-containing protein 4 (FRMPD4), somatostatin (SST), interphotoreceptor matrix proteoglycan 1 (IMPG1), C-X-C motif chemokine 9 (CXCL9), C-X-C motif chemokine 11 (CXCL11), hydroxy-delta-5-steroid dehydrogenase 3-beta and steroid delta-isomerase 2 (HSD3B2), partitioning defective 6 homolog beta (PAR6B), Bile salt export pump (ABCB11), membrane-spanning 4-domains subfamily A member 3 (MS4A3), LOC196993, bestrophin (BEST1), glycosyl-phosphatidylinositol-anchored molecule-like protein (GML), cell division cycle 2-like 5 (CDC2L5), glycine receptor alpha 1 (GLRA1), dihydropyrimidinase-related protein 4 (DPYSL4), kappa-type opioid receptor (OPRK1), dimethylarginine dimethylaminohydrolase 1 (DDAH1), homeobox protein Hox-A4 (HOXA4), Homeobox protein Hox-A7 (HOXA7), Homeobox protein Hox-B5 (HOXB5), thyrotropin-releasing hormone receptor (TRHR), nuclear transition protein 2 (TNP2), vasopressin, placental protein 13 (PP13), neutrophil gelatinase-associated lipocalin (LCN2), interferon gamma-inducible protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1), intracellular adhesion molecule-1 (ICAM-1), intracellular adhesion molecule-3 (ICAM-3), vascular cell adhesion molecule-1 (VCAM-1), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin 8 (IL-8), interleukin-9 (IL-9), interleukin 10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), interleukin 13 (IL-13), interleukin-27 subunit beta (EBI3), lectin, platelet-derived growth factor (PDGF), matrix metalloprotease-2 (MMP-2), matrix metalloprotease-9 (MMP-9), matrix metalloprotease-12 (MMP12), matrix metalloprotease-23A (MIFR), matrix metalloprotease-23B (MIFR-2), fibrinogen, fibrinogen alpha (FGA), fibronectin-1 (FN1), protein S (PROS1), protein C (PROC), pikachurin (EGFLAM), hemopexin (HPX), ADAM metalloproteinase domain 2 (ADAM2), ADAMTS3, ADAM metalloproteinase domain 12 (ADAM12), ADAM metalloproteinase domain 12 short isoform (ADAM12-S), ADAM metalloproteinase domain 12 long isoform (ADAM12-L), haptoglobin (HP), serum-alpha2-macroglobulin (A2M), retinol-binding protein 4, small inducible cytokine A2 (CCL2), C-C motif chemokine 5 (CCL5), cathepsin B (CTSB),

FIG. 5B

cathepsin C (CTSC), cathepsin D chain H (CTSD), heme oxygenase-1 (HMOX1), insulin-like growth factor-binding protein 1 (IGFBP1), insulin-like growth factor-binding protein 2 (IGFBP2), insulin-like growth factor-binding protein-3 (IGFBP3), insulin-like growth factor-binding protein-5 (IGFBP5), insulin-like growth factor-binding protein-7 (IGFBP7), insulin-like growth factor-1 (IGF-1), keratin 4 (KRT4), keratin 16 (KRT16), keratin 19 (KRT19), keratin 33A (KRT33A), keratin 40 (KRT40), pro-platelet basic protein (PPBP), perilipin 2 (PLIN2), kininogen-1 (KNG1), choriogonadotropin-subunit beta (CGB), cystatin C (CST3), pappalysin-1 (PAPPA1), pappalysin-2 (PAPPA2), alpha-1 B-glycoprotein (A1BG), actin (ACTB), C4b-binding protein beta chain (C4BP), cholinesterase (BCHE), chorionic somatomammotropin hormone (CSH1), coagulation factor VII (F7), coagulation factor XI (F11), filamin A (FLNA), filamin B (FLNB), heparin cofactor 2 (HCII), hepatocyte growth factor-like protein (MST1), histidine-rich glycoprotein (HRG), laminin subunit beta-1 (LAMB1), lipopolysaccharide-binding protein (LBP), plastin-2 (LCP1), profilin-1 (PFN1), pregnancy-specific beta-1-glycoprotein (PSG1), pregnancy-specific beta-4-glycoprotein (PSG4), pregnancy-specific beta-11-glycoprotein (PSG11), receptor-type tyrosine-protein phosphatase gamma precursor (PTPRG), pregnancy-zone protein (PZP), SH3 domain-binding glutamic acid-rich-like protein 3 (SH3BGRL3), transgelin-2 (TAGLN2), talin-1 (TLN1), tropomyosin alpha-4 chain (TPM4), vasorin (VSN), vinculin (VCL), von Willebrand factor (VWF), ferritin (FT), ferritin light chain, hemoglobin (HB), heme, podocin (NPHS2), nephrin (NPHS1), podocalyxin (PODXL), synaptopodin (SYNPO), leptin (LEP), follistatin-like 3 protein (FSTL3), beta fertilin (FTNB), CD33L, neutrotrophic tyrosine kinase receptor 2 (TRKB), beta glucosidase (BGL), angiogenin (ANG), leukocyte-associated Ig-like receptor secreted protein (LAIR), erythroid differentiation protein, adipogenesis inhibitory factor (IL-11), corticotropin-releasing factor-binding protein (CRHBP), alpha 1-antichymotrypsin (SERPINA3), cytokine receptor-like factor 1 (CRLF1), lysyl hydroxylase isoform 2 (LH2), stanniocalcin precursor (STC), secreted frizzled related-protein (SFRP), galectin-3 (LGALS3), alpha neutrophil defensin 1 (DEFA1), cholecystokinin precursor (CCK), interferon-stimulated T-cell alpha chemoattractant (I-TAC), azurocidin (HBP), spermine oxidase (SMOX), UDP glycosyltransferase 2 family polypeptide B28 (UGT2B28), neutral endopeptidase (NEP), CDC28 protein kinase regulatory subunit 2 (CKSHS2), lanosterol synthase (LSS), calcium/calmodulin-dependent serine protein kinase (CASK), chemokine (CX3C motif) receptor 1 (CX3CR1), tyrosinase-related protein 1 (TYRP1), hydroxy-delta-5-steroid dehydrogenase (HSD3), cytochrome P450-family 11 (CYP11),

FIG. 5C

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cytochrome P450-family 11 subfamily A polypeptide 1 (CYP11A1), cytochrome P450-family 11 subfamily B polypeptide 1 (CYP11B1), cytochrome P450 1A1 (CYP1A1), coronin-2A (CORO2A), cytochrome P450 2J2 (CYP2J2), paralemmin (PALM), glyceraldehyde-3-phosphate dehydrogenase (GAPD), ATP-binding cassette sub-family A member 12 (ABCA12), transcription factor Eb (TFEB), transcription factor IIE (TFIIE), syntaxin binding protein 5-like (STXBP5L), guanylin (GUCA2A), ribosomal protein S6 kinase alpha-2 (RPS6KA2), protein phosphatase 1 regulatory subunit 16B (PPP1R16B), class B basic helix-loop-helix protein 2 (BHLHB2), glycoporphin E (GYPE), nebulin (NEBL), leucine-rich repeats and immunoglobulin-like domains protein 1 (LRIG1), glucose transporter 3 (GLUT3), UDP-glucuronosyltransferase 2B28 (UGT2B28), nuclear receptor subfamily 5 group A member 2 (NR5A2), neuronatin (NNAT), sodium- and chloride-dependent creatine transporter 1 (SLC6A8), receptor tyrosine-protein kinase erbB-2 (ERBB2), receptor tyrosine-protein kinase erbB-3 (ERBB3), sialic acid-binding Ig-like lectin 6 (SIGLEC6), SHC-transforming protein 3 (SHC3), neuroligin 4 (NLGN4), lymphocyte antigen 6D (LY6D), prostacyclin synthase (PTGIS), ATP-dependent RNA helicase DDX51 (DDX51), TRAF3-interacting protein 1 (TRAF3IP1), trophoblast glycoprotein (TPBG), transforming growth factor beta-3 (TGFB3), cyclin B1 (CCNB1), kinesin family member 17 (KIF17), N-myc downstream mediated gene 1 (NDRG1), SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 3 (SMARCD3), serine/threonine-protein kinase Chk2 (CHEK2), amphiregulin (AREG), minor histocompatibility antigen HA-1 (HA-1), POU domain, class 4, transcription factor 1 (POU4F1), prostate stem cell antigen (PSCA), collagen alpha-1(X) chain (COL10A1), collagen alpha-3(VI) chain (COL6A3), collagen alpha-3 (IX) chain (COL9A3), paired box gene 2 (PAX2), paired box gene 4 (PAX4), paired box gene 7 (PAX7), latrophilin 3 (LPHN3), bile acid receptor (NR1H4), empty spiracles homolog 1 (EMX1), desmoglein 3 (DSG3), DNA-binding protein Ikaros (ZNFN1A1), melanoma-associated antigen 5 (MAGEA5), melanoma-associated antigen 3 (MAGEA3), afadin- and alpha-actinin-binding protein (SSX2IP), WD repeat-containing protein 21 (WDR21), orexin receptor type 2 (HCRTR2), NKG2-D type II integral membrane protein (KLRK1), HLA class II histocompatibility antigen DP alpha 1 chain (HLA-DPA1), HLA class II histocompatibility antigen DP beta 1 chain (HLA-DPB1), HLA class II histocompatibility antigen DR alpha chain (HLA-DRA), HLA class I histocompatibility antigen, alpha chain G (HLA-G), peripheral myelin protein 2 (PMP2),

FIG. 5D

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guanine nucleotide-binding protein G(o) subunit alpha (GNAO1), voltage-dependent L-type calcium channel subunit beta-2 (CACNB2), c-Jun-amino-terminal kinase-interacting protein 2 (MAPK8IP2), P antigen family member 1 (PAGE1), GABA receptor subunit beta-1 (GABRB1), sodium- and chloride-dependent betaine transporter (SLC6A12), lactadherin (MFGE8), integrin alpha-L (ITGAL), desmocollin 1A/1B (DSC1), villin 2 (VIL2), plectin 1 (PLEC), ankyrin 1 (ANK1), vimentin (VIM), osteopontin (SPP1), dynamin 2 (DNM2), muscle cadherin (CDH15), kinesin heavy chain, fatty acid synthase (FASN), alpha-adducin (ADD1), NADH-cytochrome B5 reductase (CYB5R), dihydrofolate reductase (DHFR), ADP-ribosylation factor-like protein 3 (ARL3), NADPH menadione oxidoreductase 1-dioxin-inducible (NQO1), CD73, ubiquitin (UB), glutathione S-transferase Mu 3 (GSTM3), superoxide dismutase 1 (SOD1), cytochrome C oxidase subunit VIa polypeptide 1 (COX6A1), glutathione reductase (GSR), myristoylated alanine-rich C-kinase substrate (MARCKS), protein disulfide-isomerase A2 (PDIA2), DNA topoisomerase 3-alpha (TOP3A), forkhead (Drosophila)-like 7, LIM/homeobox protein Lhx2 (LHX2), T-box transcription factor TBX3 (TBX3), CCAAT/enhancer-binding protein alpha (CEBPA), CCAAT/enhancer-binding protein delta (CEBPD), disrupted in schizophrenia 1 protein (DISC1), runt-related transcription factor 1 (RUNX1), sterol regulatory element-binding protein 2 (SREBF2), interferon-induced, double-stranded RNA-activated protein kinase (EIF2AK2), zinc finger protein 208 (ZNF208), tonsoku-like protein (TONSL), signal transducer and activator of transcription 2 (STAT2), myocyte-specific enhancer factor 2D (MEF2D), GA-binding protein alpha chain (GABPA), dual specificity mitogen-activated protein kinase kinase 6 (MAP2K6), growth hormone variant (GH2), erythropoietin (EPO), ephrin type-A receptor 3 (EPHA3), ephrin type-A receptor 4 (EPHA4), ephrin type-A receptor 5 (EPHA5), granulin (GRN), granulocyte colony-stimulating factor receptor (CSF3R), macrophage colony-stimulating factor 1 receptor (CSF1R), receptor-type tyrosine-protein phosphatase F (PTPRF), bone morphogenetic protein 1 (BMP1), epithelial discoidin domain-containing receptor 1 (DDR1), transferrin receptor protein 1 (TFRC), angiopoietin 1 receptor (TEK), insulin receptor (INSR), 78 kDa glucose-regulated protein (HSPA5), S-phase kinase-associated protein 1 (SPK1), Regulator of chromosome condensation (RCC1), caspase 6 (CASP6), heat shock 90kDa protein A (HSP90A), butanoic acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid, eicosanoic acid, docosanoic acid, tetracosanoic acid, hexacosanoic acid, pristanic acid, phytanic acid, dihydroxycholestanoic acid (DHCA), and trihydroxycholestanoic acid (THCA), uric acid.

FIG. 5E

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Fibronectin ("FN")	Hemopexin ("HPX")	interferon-gamma
Fibrinogen ("FG")		TNF-alpha
ADAM metalloproteinase	placental growth factor	beta-amyloid
domain 12 ("ADAM12")	("PIGF")	
pregnancy-associated plasma	soluble vascular endothelial	IL-4
protein A ("PAPP-A")	growth factor ("sFlt-1")	
arginine vasopressin	copeptin	IL-10

arginine vasopressin;
 copeptin;
 interferon-gamma;
 TNF-alpha;
 IL-10;
 IL-4;
 beta-amyloid;
 interferon-inducible protein 6-16;
 albumin;
 SERPINA1;
 Ceruloplasmin; and
 Immunoglobulin free light chains

Isoforms of the above biological entities are also contemplated as biomarkers.

Such isoforms include, for example, sFlt-2, sFlt-4 and sFlt-5.

Other isoforms include FN GenBank Accession No. NM_212474.1), FG GenBank Accession No. NM_000508.3 (FGA) and GenBank Accession No. NM_005141.4 (FGB), PAPP-A e.g., GenBank Accession No. NM_002581.3), HPX GenBank Accession No. NM_000613.2); ADAM12 Genbank Accession Nos. NM_003474.4 (isoform 1), NM_021641.3 (isoform 2); sFlt-1; e.g., Genbank Accession Nos. NM_001159920.1 (isoform 2), NM_001160030.1 (isoform 3), and NM_001160031.1 (isoform 4)); PIGF e.g., Genbank Accession Nos. NM_002632.5 (isoform 1) and NM_001207012.1 (isoform 2)).

Fragments or portions of a PE biomarker which are recognized by a detection reagent, e.g., an antibody, are also deemed PE biomarkers herein.

FIG. 5F

	1	2	3	4	5	6	7	8	9	10	11	12
A						7						
B			1			8			15			
C			2			9			16			
D			3			10			17			
E			4			11			18			
F			5			12			19			
G			6			13			20			
H						14						

FIG. 6

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-162 (partially) insofar as these claims relate to Inventions 1-32 only

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/042976

A. CLASSIFICATION OF SUBJECT MATTER G01N 33/50 (2006.01)		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Databases: TXPEA, TXPEB, TXPEC, TXPEE, TXPEF, TXPEI, TXPEP, TXPEPEA, TXPES, TXPUS01A, TXPUSE1A, TXPUSEA, TXPUSEB, TXPWOEA, WPIAP, EPODOC, CAPLUS, BIOSIS, EMBASE, MEDLINE, with KEYWORDS: pre-eclampsia, eclampsia, fibronectin, SFLT1, P1GF, ADAM12, HPX, hemopexin, PAPP-A & like terms. Databases: AusPat, The Lens, Google, PAMS NOSE, INTESS with APPLICANT / INVENTOR names.		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	
☒ Further documents are listed in the continuation of Box C ☒ See patent family annex		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search 23 November 2015	Date of mailing of the international search report 23 November 2015	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Catriona Bruce AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 7940	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2015/042976
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MYATT, L., 'Identification of different phenotypes of preeclampsia by clustering analysis of biomarker profiles', Reproductive Sciences, 2013, vol. 20, Suppl. 1, Abstract F-255. 60 th Annual Meeting, Society for Gynecologic Investigation, Orlando, FL, USA, 20-23 March 2013. Abstract	1-9, 11-16, 18-20, 22-23, 25-29, 31-34, 36-42, 44-49, 51-53, 55-81, 83-87, 89-155, 159-161
X	MYATT, L., 'Association of maternal biomarkers with obstetric outcomes', Reproductive Sciences, 2013, vol. 20, Suppl. 1, Abstract S-207. 60 th Annual Meeting, Society for Gynecologic Investigation, Orlando, FL, USA, 20-23 March 2013. Abstract	1-2, 5-8, 13, 18-21, 26, 31-35, 38, 44-49, 51-53, 55-79, 84, 89, 95-96, 98-158
X	TEJERA, E., et al., 'Preeclampsia: a bioinformatics approach through protein-protein interaction networks analysis', BMC Systems Biology, 2012, vol. 6: 97 Abstract; Table 1; Discussion	1-2, 5-8, 13, 18-21, 26, 31-35, 38, 44-49, 51-53, 55-79, 84, 89, 95-96, 98-158
X	MOLVAREC, A., et al., 'Circulating angiogenic factors determined by electrochemiluminescence immunoassay in relation to the clinical features and laboratory parameters in women with pre-eclampsia', Hypertension Research, 2010, vol. 33, pages 892-898 Abstract	1-2, 5-8, 13, 18-21, 26, 31-35, 38, 44-49, 51-53, 55-79, 84, 89, 95-96, 98-158
X	PENNINGS, J. L. A., et al., 'Integrative data mining to identify novel candidate serum biomarkers for pre-eclampsia screening', Prenatal Diagnosis, 2011, vol. 31, pages 1153-1159 Abstract; Table 1	1-2, 5-8, 14, 18-21, 27, 31-33, 44, 47-53, 55-79, 85, 89, 95, 99-103, 108-155
Y	Abstract; Table 1	3, 9, 13, 22, 26, 34-35, 37-39, 45-46, 80, 84, 96-98, 104-107, 159-162
Y	WO 2006/069373 A2 (YALE UNIVERSITY) 29 June 2006 Abstract; Figure 7	3, 9, 13, 22, 26, 34-35, 37-39, 45-46, 80, 84, 96-98, 104-107, 159-162
L	Lack of Unity	
X	WO 2009/097584 A1 (PROTEOGENIX, INC.) 06 August 2009 Abstract; Example 1; page 18, line 13; page 25, line 22 to page 26, line 11; claims; Table 3b	1, 5-8, 18-19, 31, 33, 47-48, 50, 52, 55-79, 89, 99-103, 108-155
Y	Abstract; Example 1; page 18, line 13; page 25, line 22 to page 26, line 11; claims; Table 3b	2-4, 9-17, 20-30, 32, 34-46, 49, 51, 53-54, 80-88, 90-98, 104-107, 156-162
L	Lack of Unity	
	WO 2013/169751 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 14 November 2013	
Form PCT/ISA/210 (fifth sheet) (July 2009)		

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2015/042976
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Entire document; Abstract; Figure 27; Table 7; Examples 2 and 3	2-4, 9-17, 20-30, 32, 34-46, 49, 51, 53-54, 80-88, 90-98, 104-107, 159-162
L	Lack of Unity	
L	WO 2014/036440 A2 (ANSH LABS LLC) 06 March 2014 Lack of Unity	
L	PARK, H. J., et al., 'Screening models using multiple markers for early detection of late-onset preeclampsia in low-risk pregnancy', BMC Pregnancy and Childbirth, 2014, vol. 14: 35 Lack of Unity	
L	SPENCER, K., et al., 'ADAM12s in maternal serum as a potential marker of pre-eclampsia', Prenatal Diagnosis, 2008, vol. 28, pages 212-216 Lack of Unity	
L	LIU, L. Y., et al., 'Integrating multiple 'omics' analyses identifies serological protein biomarkers for preeclampsia', BMC Medicine, 2013, vol. 11,:236 Lack of Unity	

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2015/042976
Supplemental Box	
Continuation of: Box III This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art. When there is no special technical feature common to all the claimed inventions there is no unity of invention. The present claim set is characterised by multiple inventions and multiple independent and dependent claims which in themselves embody numerous possible alternatives. The ISA considers that the presentation of so many possible alternatives makes it difficult to conduct a meaningful novelty and inventive step search across the full scope of the claims. In particular, the claims relate to methods or systems for diagnosing or prognosing preeclampsia based on calculated index levels of biomarkers, optionally using real function algorithms including weighting or comparison of levels with a standard; tests for confirming preeclampsia with one or more biomarkers with specified ROC values; tests for preeclampsia with specified results of sensitivity or specificity or negative predictive value; or computer readable media with instructions to analyse data levels of biomarkers at two time points for assessment of preeclampsia. It is apparent that the field of preeclampsia diagnosis and prognosis using biomarkers is highly regarded and well-researched. The methods, tests and computer systems of the broad claims of the current claim set are well-known. Therefore, due to the broad nature of the claims, and the large quantity of prior art in this field, it is not feasible to conduct a comprehensive search over the full scope of the general concepts covered by the claims. A comprehensive search can be carried out with regard to explicitly specified biomarkers. Thus, a comprehensive search is possible insofar as the claims are directed to specific biomarkers or combinations of biomarkers, for example the biomarkers specified in some of the claims: FN, sFLT-1, PlGF, ADAM-12, HPX, PAPP-A and pikachurin. However, many of the claims are directed to the use of specified biomarkers in combination with other, non-specified biomarkers. There may be thousands of such possible biomarkers. The specific exclusion of certain biomarkers in some of the claims does little to alter the broad scope of these claimed biomarkers. Due to the large number of possible biomarkers, and the large quantity of prior art relating to biomarkers in preeclampsia, it is not feasible to conduct a comprehensive search over the full scope of the claims with regard to any biomarkers that are not specifically defined in the claims. This Authority has found that there are different searchable inventions based on the following features that separate the claims into distinct groups insofar as the claims relate to specified biomarkers. Inventions 1-32 (group). Claims 1-162 (partially). The feature of the use of FN, alone or in combination with any one or combination of SFLT1, PlGF, ADAM-12, HPX, PAPP-A as biomarkers for preeclampsia, wherein each combination of biomarkers represents a separate invention, is specific to this group of claims. Inventions 33-41 (group). Claims 1-162 (partially). The feature of the use of both SFLT1 and PlGF, together or in further combination with any one or combination of FN, ADAM-12, HPX, PAPP-A, pikachurin as biomarkers for preeclampsia, wherein each combination of biomarkers is a separate invention, is specific to this group of claims. Invention 42. Claims 1-162 (partially). The feature of the use of both PAPP-A and ADAM12 together as biomarkers for preeclampsia, is specific to this group of claims. Further inventions (group). Whilst not explicitly specified in the current claim set, the feature of the use of any further markers disclosed in the description as biomarkers for preeclampsia, such as the 415 (approx.) biomarkers listed at Table 2, represent further inventions, wherein each specific biomarker or combination of biomarkers is a separate invention. This group includes a very large number of separate inventions each relating to specific biomarker combinations. In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to	

INTERNATIONAL SEARCH REPORT	International application No. PCT/US2015/042976
Supplemental Box	
all of the claimed inventions and which provides a technical relationship among them is the statistical analysis of biomarkers levels in the diagnosis or prognosis of preeclampsia. However this feature does not make a contribution over the prior art because it is disclosed in the following citations, which each disclose such methods comprising detection and analysis of at least one of FN, sFLT-1, P1GF, ADAM-12, HPX, PAPP-A or pikachurin, or combinations thereof, in the diagnosis or prognosis of preeclampsia. WO 2009/097584 A1 (PROTEOGENIX, INC.) 6 August 2009 WO 2006/069373 A2 (YALE UNIVERSITY) 29 June 2006 WO 2014/036440 A2 (ANSH LABS LLC) 6 March 2014 WO 2013/169751 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 14 November 2013 PARK, H. J., et al., 'Screening models using multiple markers for early detection of late-onset preeclampsia in low-risk pregnancy', BMC Pregnancy and Childbirth, 20 January 2014, vol. 14, 35 SPENCER, K., et al., 'ADAM12s in maternal serum as a potential biomarker of pre-eclampsia', Prenatal Diagnosis, 2008,, vol. 28, pages 212-216 LIU, L. Y., et al., 'Integrating multiple 'omics' analyses identifies serological protein biomarkers for preeclampsia', BMC Medicine, 2013, vol. 11, 236 Therefore in the light of these documents this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all of the claimed inventions and the requirements for unity of invention are consequently not satisfied <i>a posteriori</i>. The ISA has searched the use of FN, alone and in combination with any one or combination of SFLT1, P1GF, ADAM-12, HPX, PAPP-A as biomarkers for preeclampsia (Inventions 1-32) for the single search fee that has already been paid. Form PCT/ISA/210 (Supplemental Box) (July 2009)	

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/US2015/042976	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
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		CN 101163970 A	16 Apr 2008
		EP 1839056 A2	03 Oct 2007
		EP 1839056 B1	03 Aug 2011
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		EP 2434283 B1	26 Mar 2014
		JP 2008524635 A	10 Jul 2008
		JP 5009811 B2	22 Aug 2012
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		CN 104487593 A	01 Apr 2015
		EP 2847354 A1	18 Mar 2015
		GB 2515983 A	07 Jan 2015
		JP 2015519564 A	09 Jul 2015
		US 2015099655 A1	09 Apr 2015
WO 2014/036440 A2	06 March 2014	WO 2014036440 A2	06 Mar 2014
		EP 2890816 A2	08 Jul 2015
		US 2014141456 A1	22 May 2014
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			

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INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/US2015/042976	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
Empty space for data entry			
End of Annex			
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2009)