METHODS OF DETERMINING IMPAIRED GLUCOSE TOLERANCE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of United States Provisional Application No. 62/959,660, filed January 10, 2020, which is incorporated by reference herein in its entirety for any purpose.

FIELD OF THE INVENTION

[0002] The present application relates generally to the detection of biomarkers and the characterization of glucose tolerance, for example, to identify subjects having or likely to have impaired glucose tolerance, which is an indication of pre-diabetes, or diabetes. In various embodiments, the invention relates to one or more biomarkers, methods, devices, reagents, systems, and kits for characterizing impaired glucose tolerance, which may indicate pre-diabetes, and/or diabetes in an individual.

BACKGROUND

[0003] Current methods for determining glucose tolerance include measuring the 2-hour plasma glucose level in individuals participating in an oral glucose tolerance test (OGTT). A typical OGTT consists of the ingestion of a 75-gram oral glucose solution, following an overnight fast, with plasma glucose measurements at baseline ("fasting plasma glucose") and at 2-hours ("2hr-OGTT glucose value") post ingestion. Both fasting plasma glucose and 2-hour OGTT plasma glucose, along with HbA1c values, are currently accepted by the American Diabetes Association for the clinical diagnosis of Type 2 diabetes. Prevention of Type 2 diabetes has primarily been evaluated based on individuals with impaired glucose tolerance, defined as 2-hour OGTT plasma glucose levels ≥ 7.8 mmol/L. *See, e.g.*, Knowler et al. *N. Engl. J. Med.* 2002; 346:393-403 and Tuomilehto et al. *N. Engl. J. Med.* 2001; 344:1343-1350. The development of a proteomic model representative of the OGTT plasma glucose threshold for impaired glucose tolerance, without the need for fasting or glucose administration, , would be highly desirable.

SUMMARY

[0004] In some examples, methods of determining whether a subject has or likely has impaired glucose tolerance are provided. In some examples, methods of identifying subjects with pre-diabetes or likely to develop pre-diabetes are provided. In some examples, methods of identifying subjects likely to develop diabetes are provided.

[0004a] In one aspect, the present disclosure refers to a method of determining whether a subject has impaired glucose tolerance or the likelihood that a subject has impaired glucose tolerance, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, wherein the biomarker panel comprises COL1A1 and at least two N biomarker proteins chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD, wherein the subject is suspected of having impaired glucose tolerance.

[0004b] In one aspect, the present disclosure refers to a method of determining whether a subject has pre-diabetes or is likely to develop pre-diabetes or diabetes, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, wherein the biomarker panel comprises COL1A1 and at least two N biomarker proteins chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD, wherein the subject is suspected of having impaired glucose tolerance.

[0004c] In one aspect, the present disclosure refers to a method of detecting the levels of N biomarker proteins in a sample, forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in the sample from the subject, wherein N is at least 3, wherein the biomarker panel comprises COL1A1 and at least two N biomarker proteins chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD, wherein the subject is suspected of having impaired glucose tolerance.

[0005] In some examples, the method herein is for determining whether a subject has impaired glucose tolerance or likely has impaired glucose tolerance, which is an indication of pre-diabetes, or diabetes, comprising obtaining a sample from the subject, forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, and wherein at least one of the N biomarker protein is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some examples, N is 3 to 41, N is 4 to 41, N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is 15 to 41, or N is 16 to 41, or N is at least 4, or N is at least 5, or N is at least 6, or N is at least 7, or N is at least 8, or N is at least 9, or N is at least 10, or N is at least 11, or N is at least 12, or N is at least 13, or N is at least 14, or N is at least 15, or N is at least 16. In some examples, N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41. In some such examples, the subject has impaired glucose tolerance. In some examples, the subject likely has impaired glucose tolerance. In some examples, the subject is likely to develop pre-diabetes. In some examples, the subject is pre-diabetic. In some examples, the subject is likely to develop diabetes. In some examples, the subject has impaired glucose tolerance and has diabetes. In some examples, a subject likely to develop diabetes undertakes preventive measures or undergoes preventive treatment to reduce the likelihood of developing diabetes.

[0006] In some examples, each of the N biomarkers is selected from Table 1. In some examples, at least one of the N biomarker proteins is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, and KIN. In some examples, one or two of the N biomarker proteins are INHBC and/or SHBG. In some examples, at least 2 or at least 3 of the N protein biomarkers are selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some examples, at least one of the N biomarker proteins is selected from FAM20B, COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4. In some examples, two of the N biomarker proteins are INHBC and ACY1, or two of the N biomarker proteins are SHBG and ACY1, or three of the N

examples, at least one aptamer is a slow off-rate aptamer. In some examples, at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications. In some examples, each slow off-rate aptamer binds to a biomarker protein with an off rate ($t_{½}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

[0009] In any of the examples described herein, the sample may be a blood sample. In any of the examples described herein, the sample may be selected from a serum sample or a plasma sample. In any of the examples described herein, the sample is a plasma sample. In some examples, the subject eats a typical diet before the sample is obtained, wherein the typical diet excludes fasting longer than usual.

[0010] In some examples, a level of at least one biomarker selected from SHBG, COL1A1, CRLF1:CLCF1 complex, FAM20B, COL15A1, KIN, SERPINA11, PELI2, MARCKSL1, CHAD, IL20RB, MYOC, WFDC11, MAP2K4, CALB1, FGFR4, OSTM1, ITIH1, CFAP45, and SFTPD that is higher than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has pre-diabetes, and/or is likely to develop pre-diabetes or diabetes.

[0011] In some examples, a level of at least one biomarker selected from INHBC, ACY1, RTN4R, CBX7, TFF3, HTRA1, FABP12, GAD1, CPM, SVEP1, SOCS7, F9, DLK1, HERC1, STC1, CCL16, SMCO2, GPX2, CCL23, RNASE10, and ZNF134 that is lower than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has pre-diabetes, and/or is likely to develop pre-diabetes or diabetes.

[0012] In some examples, a method described herein is for the purpose of determining a medical insurance premium or life insurance premium. In some examples, a method further comprises determining a medical insurance premium or life insurance premium. In some examples, a method described herein further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.

[0013] In some examples, kits are provided. In one aspect, the present disclosure refers to a kit comprising N biomarker protein capture reagents, wherein N is at least 3, wherein one of the N biomarker proteins capture reagents specifically binds to COL1A1, and at least two N biomarker protein capture reagents specifically bind to a biomarker protein chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some examples, a kit comprises N biomarker protein capture reagents, wherein N is at least 3, and wherein at least one of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some examples, N is 3 to 41, N is 4 to 41, N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is

CLAIMS

1. A method of determining whether a subject has impaired glucose tolerance or the likelihood that a subject has impaired glucose tolerance, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, wherein the biomarker panel comprises COL1A1 and at least two N biomarker proteins chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD, wherein the subject is suspected of having impaired glucose tolerance.

2. A method of determining whether a subject has pre-diabetes or is likely to develop pre-diabetes or diabetes, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, wherein the biomarker panel comprises COL1A1 and at least two N biomarker proteins chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD, wherein the subject is suspected of having impaired glucose tolerance.

3. A method of detecting the levels of N biomarker proteins in a sample, forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in the sample from the subject, wherein N is at least 3, wherein the biomarker panel comprises COL1A1 and at least two N biomarker proteins chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD, wherein the subject is suspected of having impaired glucose tolerance.

4. The method of any one of the preceding claims, wherein

(a) N is 3 to 41, or N is 4 to 41, or N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is 15 to 41, or N is 16 to 41; and

(b) when N is more than 3, the biomarker proteins are chosen from INHBC, SHBG, ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, FAM20B, COL15A1, KIN, SERPINA11, PELI2, TFF3, MARCKSL1, HTRA1, FABP12,

GAD1, CHAD, CPM, SVEP1, SOCS7, F9, DLK1, HERC1, IL20RB, STC1, MYOC, WFDC11, MAP2K4, CALB1, CCL16, SMCO2, GPX2, CCL23, FGFR4, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

5. The method of any one of the preceding claims, wherein

(a) N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41; and

(b) when N is more than 3, the biomarker proteins are chosen from INHBC, SHBG, ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, FAM20B, COL15A1, KIN, SERPINA11, PELI2, TFF3, MARCKSL1, HTRA1, FABP12, GAD1, CHAD, CPM, SVEP1, SOCS7, F9, DLK1, HERC1, IL20RB, STC1, MYOC, WFDC11, MAP2K4, CALB1, CCL16, SMCO2, GPX2, CCL23, FGFR4, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

6. The method of any one of the preceding claims, wherein at least one of the N biomarker proteins is chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, and ACY1.

7. The method of any one of the preceding claims, wherein one or two of the N biomarker proteins are INHBC and/or SHBG.

8. The method of any one of the preceding claims, wherein at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 of the N protein biomarkers are chosen from COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

9. The method of any one of the preceding claims, wherein at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 of the N biomarker proteins is chosen from FAM20B, COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4.

10. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and ACY1, or two of the N biomarker proteins are SHBG and ACY1, or three of the N biomarker proteins are INHBC, SHBG, and ACY1.

11. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and COL1A1, or two of the N biomarker proteins are SHBG and COL1A1, or wherein three of the N biomarker proteins are INHBC, SHBG, and COL1A1.

12. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and RTN4R, or two of the N biomarker proteins are SHBG and RTN4R, or wherein three of the N biomarker proteins are INHBC, SHBG, and RTN4R.

13. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and CRLF1:CLCF1 complex, or two of the N biomarker proteins are SHBG and CRLF1:CLCF1 complex, or wherein three of the N biomarker proteins are INHBC, SHBG, and CRLF1:CLCF1 complex.

14. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and CBX7, or two of the N biomarker proteins are SHBG and CBX7, or wherein three of the N biomarker proteins are INHBC, SHBG, and CBX7.

15. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and KIN, or two of the N biomarker proteins are SHBG and KIN, or wherein three of the N biomarker proteins are INHBC, SHBG, and KIN.

16. The method of any one of the preceding claims, wherein N is at least five and five of the N biomarker proteins are INHBC, SHBG, COL1A1, RTN4R, and ACY1.

17. The method of any one of the preceding claims, wherein N is at least 16, and wherein 16 of the N biomarker proteins are COL1A1, RTN4R, CRLF1, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, ACY1, INHBC, SHBG, FAM20B, COL15A1, MARCKSL1, and HTRA1.

18. The method of any one of the preceding claims, wherein the sample is a blood sample, a plasma sample, or a serum sample.

19. The method of any one of the preceding claims, wherein the subject continues eating a typical diet and a glucose solution is not administered to the subject before the sample is obtained.

20. The method of any one of the preceding claims, wherein a level of at least one biomarker chosen from SHBG, COL1A1, CRLF1:CLCF1 complex, FAM20B, COL15A1, KIN, SERPINA11, PELI2, MARCKSL1, CHAD, IL20RB, MYOC, WFDC11, MAP2K4, CALB1, FGFR4, OSTM1, ITIH1, CFAP45, and SFTPD that is higher than a control level of the respective biomarker, indicates that the subject has or likely has

impaired glucose tolerance, has or is likely to develop pre-diabetes, and/or is likely to develop diabetes.

21. The method of any one of the preceding claims, wherein a level of at least one biomarker chosen from INHBC, RTN4R, CBX7, TFF3, HTRA1, FABP12, GAD1, CPM, SVEP1, SOCS7, F9, ACY1, DLK1, HERC1, STC1, CCL16, SMCO2, GPX2, CCL23, RNASE10, and ZNF134 that is lower than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has or is likely to develop pre-diabetes, and/or is likely to develop diabetes.

22. The method of any one of the preceding claims, wherein the subject is at risk of developing diabetes.

23. The method of any one of the preceding claims, comprising determining whether the subject has or likely has impaired glucose tolerance.

24. The method of claim 23, wherein the subject likely has impaired glucose tolerance.

25. The method of claim 23, wherein the subject has impaired glucose tolerance.

26. The method of any one of claims 2 to 25, comprising determining whether the subject is likely to develop or has pre-diabetes.

27. The method of claim 26, wherein the subject is likely to develop pre-diabetes.

28. The method of claim 26, wherein the subject has pre-diabetes.

29. The method of any one of claims 24, 25, 27, and 28, wherein the subject is likely to develop diabetes.

30. The method of claim 29, wherein the diabetes is type 2 diabetes.

31. The method of any one of claims 20-22, 24, 25, and 27-30, comprising the subject implementing a weight loss program, implementing dietary restrictions, implementing caloric restrictions and/or implementing an exercise program.

32. The method of any one of the preceding claims, wherein the method comprises contacting biomarker proteins of the sample or samples with a set of biomarker capture reagents, wherein each biomarker capture reagent of the set of biomarker capture reagents specifically binds to a different biomarker protein being detected.

33. The method of claim 32, wherein each biomarker capture reagent is an antibody or an aptamer.

34. The method of claim 33, wherein each biomarker capture reagent is an aptamer.

35. The method of claim 34, wherein at least one aptamer is a slow off-rate aptamer.

36. The method of claim 35, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.

37. The method of claim 35 or claim 36, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{½}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

38. The method of any one of the preceding claims, wherein if the subject has or likely has impaired glucose tolerance, has pre-diabetes, or is likely to develop pre-diabetes or diabetes, the subject is recommended a regimen comprising weight loss, blood sugar control, and/or drug treatment.

39. The method of any one of the preceding claims, wherein the determining comprises analyzing the levels of the N biomarker protein levels using a classification model or an elastic net logistic regression model.

40. The method of any one of the preceding claims, wherein the method comprises determining whether a subject has or likely has impaired glucose tolerance, has pre-diabetes, or is likely to develop pre-diabetes or diabetes for the purpose of determining a medical insurance premium or life insurance premium.

41. The method of any one of the preceding claims, wherein the method further comprises determining a medical insurance premium or life insurance premium.

42. The method of any one of the preceding claims, wherein the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.

43. A kit comprising N biomarker protein capture reagents, wherein N is at least 3, wherein one of the N biomarker proteins capture reagents specifically binds to COL1A1, and at least two N biomarker protein capture reagents specifically bind to a biomarker protein chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

44. The kit of claim 43, wherein:

(a) N is 3 to 41, or N is 4 to 41, or N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is 15 to 41, or N is 16 to 41; and

(b) when N is more than 3, the biomarker proteins are chosen from INHBC, SHBG, ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, FAM20B, COL15A1, KIN, SERPINA11, PELI2, TFF3, MARCKSL1, HTRA1, FABP12, GAD1, CHAD, CPM, SVEP1, SOCS7, F9, DLK1, HERC1, IL20RB, STC1, MYOC, WFDC11, MAP2K4, CALB1, CCL16, SMCO2, GPX2, CCL23, FGFR4, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

45. The kit claim 43 or 44, wherein:

(a)N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41; and

(b) when N is more than 3, the biomarker proteins are chosen from INHBC, SHBG, ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, FAM20B, COL15A1, KIN, SERPINA11, PELI2, TFF3, MARCKSL1, HTRA1, FABP12, GAD1, CHAD, CPM, SVEP1, SOCS7, F9, DLK1, HERC1, IL20RB, STC1, MYOC, WFDC11, MAP2K4, CALB1, CCL16, SMCO2, GPX2, CCL23, FGFR4, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

46. The kit of any one of claims 43 to 45, wherein each of the N biomarker protein capture reagents specifically binds to a different biomarker protein.

47. The kit of any one of claims 43 to 46, wherein at least one of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, and ACY1.

48. The kit of any one of claims 43 to 47, wherein one or two of the N biomarker protein capture reagents specifically binds to INHBC and/or SHBG.

49. The kit of any one of claims 43 to 48, wherein at least 4 of the N protein biomarker capture reagents each specifically bind to a protein chosen from COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

50. The kit of any one of claims 43 to 49, wherein at least one of the N biomarker protein capture reagents specifically binds to a protein chosen from FAM20B,

COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4.

51. The kit of any one of claims 43 to 50, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or ACY1, or each of two of the N biomarker protein capture reagents specifically binds to SHBG or ACY1, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from INHBC, SHBG, and ACY1.

52. The kit of any one of claims 43 to 51, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or COL1A1, each of two of the N biomarker protein capture reagents specifically binds to SHBG or COL1A1, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from INHBC, SHBG, and COL1A1.

53. The kit of any one of claims 43 to 52, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or RTN4R, each of two of the N biomarker protein capture reagents specifically binds to SHBG or RTN4R, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from INHBC, SHBG, and RTN4R.

54. The kit of any one of claims 43 to 53, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or CRLF1:CLCF1 complex, each of two of the N biomarker protein capture reagents specifically binds to SHBG or CRLF1:CLCF1 complex, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from INHBC, SHBG, and CRLF1:CLCF1 complex.

55. The kit of any one of claims 43 to 54, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or CBX7, each of two of the N biomarker protein capture reagents specifically binds to SHBG or CBX7, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from INHBC, SHBG, and CBX7.

56. The kit of any one of claims 43 to 55, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or KIN, each of two of the N biomarker protein capture reagents specifically binds to SHBG or KIN, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from INHBC, SHBG, and KIN.

57. The kit of any one of claims 43 to 56, wherein N is at least five and each of five of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from INHBC, SHBG, COL1A1, RTN4R, and ACY1.

58. The kit of any one of claims 43 to 57, wherein N is at least 16, and wherein each of 16 of the N biomarker protein capture reagents specifically binds to a biomarker protein chosen from COL1A1, RTN4R, CRLF1, CBX7, KIN, SERPINA, PELI2, TFF3, FABP12, ACY1, INHBC, SHBG, FAM20B, COL15A1, MARCKSL1, and HTRA1.

59. The kit of any one of claims 43 to 58, wherein each of the N biomarker capture reagents is an antibody or an aptamer.

60. The kit of claim 59, wherein each biomarker capture reagent is an aptamer.

61. The kit of claim 60, wherein at least one aptamer is a slow off-rate aptamer.

62. The kit of claim 61, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.

63. The kit of claim 61 or claim 62, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{½}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

64. The kit of any one of claims 43 to 63, for use in detecting the N biomarker proteins in a sample from a subject.

65. The kit of claim 64, for use in determining whether the subject has or likely has impaired glucose tolerance or has pre-diabetes, or is likely to develop pre-diabetes or diabetes.