ASSAYS FOR THE DETECTION OF ANTI-TNF DRUGS AND AUTOANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of PCT/US2010/054125, filed October 26, 2010, which application claims priority to U.S. Provisional Application No. 61/255,048, filed October 26, 2009, U.S. Provisional Application No. 61/262,877, filed November 19, 2009, U.S. Provisional Application No. 61/324,635, filed April 15, 2010, U.S. Provisional Application No. 61/345,567, filed May 17, 2010, U.S. Provisional Application No. 61/351,269, filed June 3, 2010, U.S. Provisional Application No. 61/389,672, filed October 4, 2010, and U.S. Provisional Application No. 61/393,581, filed October 15, 2010, the disclosures of which are hereby incorporated by reference in their entirety for all purposes.

BACKGROUND OF THE INVENTION

Reference to a "Sequence Listing," a Table, or a Computer Program Listing Appendix Submitted as an ASCII Text File

[0002] The Sequence Listing written in file -182-7.TXT, created on Jul. 16, 2012, 4,096 bytes, machine format IBM-PC, MS-Windows operating system, is hereby incorporated by reference in its entirety for all purposes.

Autoimmune disorders are a significant and widespread medical problem. For example, rheumatoid arthritis (RA) is an autoimmune disease affecting more than two million people in the United States. RA causes chronic inflammation of the joints and typically is a progressive illness that has the potential to cause joint destruction and functional disability. The cause of rheumatoid arthritis is unknown, although genetic predisposition, infectious agents and environmental factors have all been implicated in the etiology of the disease. In active RA, symptoms can include fatigue, lack of appetite, low grade fever, muscle and joint aches and stiffness. Also during disease flare ups, joints frequently become red, swollen, painful and tender, due to inflammation of the synovium. Furthermore, since RA is a systemic disease, inflammation can affect organs and areas of the body other than the joints, including glands of the eyes and mouth, the lung lining, the pericardium, and blood vessels.

[0003] Traditional treatments for the management of RA and other autoimmune disorders include fast acting “first line drugs” and slower acting “second line drugs.” The first line

drugs reduce pain and inflammation. Example of such first line drugs include aspirin, naproxen, ibuprofen, etodolac and other non-steroidal anti-inflammatory drugs (NSAIDs), as well as corticosteroids, given orally or injected directly into tissues and joints. The second line drugs promote disease remission and prevent progressive joint destruction and are also referred to as disease-modifying anti-rheumatic drugs or DMARDs. Examples of second line drugs include gold, hydrochloroquine, azulfidine and immunosuppressive agents, such as methotrexate, azathioprine, cyclophosphamide, chlorambucil and cyclosporine. Many of

[0073] In certain embodiments, "TNF-α" is an "antigen," which is a molecule or a portion of the molecule capable of being bound by an anti- TNF-α antibody. TNF-α can have one or more than one epitope. In certain instances, TNF-α will react, in a highly selective manner, with an anti- TNF-α antibody. Preferred antigens that bind antibodies, fragments and regions of anti-TNF antibodies of the present invention include at least 5 amino acids of SEQ ID NO:-1. In certain instances, TNF-α is a sufficient length having an epitope of TNF α that is capable of binding anti-TNF-α antibodies, fragments and regions thereof.

[0074] In certain embodiments, the length of TNF-α having an epitope of sufficient length to bind an anti-TNF-α antibody, fragments and regions thereof is at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220 amino acids in length. In one embodiment, the TNF-α used includes residues 77-233 of SEQ ID NO:-1.

[0075] In certain instances, at least one amino acid in the soluble portion of TNF-α is labeled i.e., residues 77-233 of SEQ ID NO:-1. In certain instances, an amine reactive fluorophore derivative is used. In most instances, the amine reactive group is an acylating reagent that forms carboxamides, sulfonamides or thioureas upon reaction with amines. Virtually all proteins have lysine residues, and most have a free amine at the N-terminus and thus can be used as a point of attachment to label. In a preferred embodiment, residue 77 is labeled, and the TNF-α used includes residues 77-233 of SEQ ID NO:-1. In other embodiments, many primary amines are labeled and the TNF-α is multiply labeled.

[0076] In certain instances, a portion of TNF-α is not labeled, i.e., portions that are recognized by antibodies, and fragments and regions thereof. In other words, certain portions of TNF-α antigens provide a topographical or three dimensional epitope of TNF which is recognized by, and/or binds with anti-TNF activity, an antibody, and fragments, and variable regions thereof. These portions are preferably free to bind, and thus are not labeled. They include residues 136-157 and 164-185 of SEQ ID NO:1:

[0077] Tyr-Ser-Gln-Val-Leu-Phe-Lys-Gly-Gln-Gly-Cys-Pro-Ser-Thr-His-Val-Leu-Leu-Thr-His-Thr-Ile (SEQ ID NO:2); and

[0078] Tyr-Gln-Thr-Lys-Val-Asn-Leu-Leu-Ser-Ala-Ile-Lys-Ser-Pro-Cys-Gln-Arg-Glu-Thr-Pro-Glu-Gly (SEQ ID NO:3).

[0079] TNFα or an anti-TNFα drug can be labeled with a variety of detectable group(s). Preferably, TNFα or an anti-TNFα drug is labeled with a fluorophore or a fluorescent dye. Exemplary fluorophores suitable for use in the present invention include those listed in the

WHAT IS CLAIMED IS:

1. A method for determining the presence or level ~~an effective amount~~ of an anti-TNFα drug in a sample~~for a subject receiving therapy with said anti-TNFα drug~~, the method comprising:

(a) contacting a labeled TNFα with a sample having an anti-TNFα drug to form a labeled complex with the anti-TNFα drug;~~measuring the level of said anti-TNFα drug in a first sample from said subject, comprising:~~

~~(i) contacting said first sample with an amount of a labeled TNFα to form a first complex comprising said labeled TNFα with said anti-TNFα drug; and~~

~~(ii) detecting said first complex by size exclusion chromatography, thereby measuring said level of said anti-TNFα drug;~~

(b) subjecting the labeled complex to size exclusion chromatography to separate the labeled complex from free labeled TNFα and to detect an amount of the labeled complex and an amount of the free labeled TNFα ; and

~~measuring the level of an autoantibody to said anti-TNFα drug in a second sample from said subject, comprising:~~

~~(i) contacting said second sample with an amount of a labeled anti-TNFα drug to form a second complex comprising said labeled anti-TNFα drug with said autoantibody; and~~

~~(ii) detecting said second complex by size exclusion chromatography, thereby measuring said level of said autoantibody; and~~

(c) comparing the amount of the labeled complex and the amount of the free labeled TNFα detected in step (b) to a standard curve of known amounts of the anti-TNFα drug, thereby determining the presence or level of the anti-TNFα drug.~~subtracting the level of said autoantibody measured in step (b) from the level of said anti-TNFα drug measured in step (a),~~

~~thereby determining the effective amount of said anti-TNFα drug.~~

2. The method of claim 1, wherein the standard curve is generated by incubating the labeled TNFα with known amounts of the anti-TNFα drug.~~said detection in step (a)(ii) comprises:~~

~~(1) integrating the area-under-the curve for a peak of said labeled TNFα from a first plot of signal intensity as a function of elution time from said size exclusion chromatography;~~

~~(2) integrating the area-under-the curve for a peak of said first complex from said first plot;~~

~~(3) determining a ratio by dividing the resultant integration from step (2) by the resultant integration from step (1); and~~

~~(4) multiplying said amount of said labeled TNFα by said ratio of step (3~~).

3. The method of claim 1, wherein the sample is serum. ~~or 2, wherein said detection in step (b)(ii) comprises:~~

~~(1) integrating the area-under-the curve for a peak of said labeled anti-TNFα drug from a second plot of signal intensity as a function of elution time from said size exclusion chromatography;~~

~~(2) integrating the area-under-the curve for a peak of said second complex from said second plot;~~

~~(3) determining a ratio by dividing the resultant integration from step (2) by the resultant integration from step (1); and~~

~~(4) multiplying said amount of said labeled anti-TNFα drug by said ratio of step (3).~~

4. The method of claim 1, wherein the anti-TNFα drug is a member selected from the group consisting of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), CIMZIA® (certolizumab pegol), and combinations thereof.~~The method of any one of claims 1 to 3, wherein both of said first and second samples are serum samples.~~

5. The method of claim 1, wherein the size exclusion chromatography is size exclusion-high performance liquid chromatography (SE-HPLC).~~The method of any one of claims 1 to 4, wherein said detection in step (a)(ii) and/or step (b)(ii) comprises fluorescence label detection.~~

6. The method of claim 1, wherein the labeled TNFα is a fluorophore-labeled TNFα.~~A method for optimizing the therapeutic amount of an anti-TNFα drug in a subject receiving therapy with said anti-TNFα drug, said method comprising:~~

~~(a) determining an effective amount of said anti-TNFα drug in accordance with the method of any one of claims 1 to 5;~~

~~(b) comparing said effective amount of said anti-TNFα drug with said level of said anti-TNFα drug; and~~

~~(c) determining a subsequent dose of said anti-TNFα drug for said subject based upon the comparison of step (b),~~

~~thereby optimizing the therapeutic amount of said anti-TNFα drug.~~

7. The method of claim 6, wherein the fluorophore is an Alexa Fluor® dye. ~~method further comprises:~~

~~(d) increasing the subsequent dose of said anti-TNFα drug when the~~

~~effective amount of said anti-TNFα drug is less than said level of said anti-TNFα drug.~~

8. The method of claim 1, wherein the labeled complex is eluted first, followed by the free labeled TNFα.~~The method of claim 7, wherein the subsequent dose of said anti-TNFα drug is increased such that the effective amount of said anti-TNFα drug is about equal to said level of said anti-TNFα drug.~~

9. The method of claim 1, wherein the sample is obtained from a subject receiving therapy with the anti-TNFα drug.~~The method of any one of claims 1 to 8, wherein said anti-TNFα drug is a member selected from the group consisting of REMICADE™ (infliximab), ENBREL™~~

~~(etanercept), HUMIRA™ (adalimumab), CIMZIA® (certolizumab pegol), and combinations thereof.~~

10. A method for determining the presence or level of an autoantibody to an anti-TNFα drug in a sample, the method comprising:

(a) contacting a labeled anti-TNFα drug with the sample to form a labeled complex with the autoantibody;

(b) subjecting the labeled complex to size exclusion chromatography to separate the labeled complex from free labeled anti-TNFα drug and to detect an amount of the labeled complex and an amount of the free labeled anti-TNFα drug; and

(c) comparing the amount of the labeled complex and the amount of the free labeled anti-TNFα drug detected in step (b) to a standard curve of known amounts of the autoantibody, thereby determining the presence or level of the autoantibody.

~~The method of any one of claims 1 to 8, wherein said anti-TNFα drug is infliximab (REMICADE™).~~

11. The method of claim 10, wherein the standard curve is generated by incubating the labeled anti-TNFα drug with serum positive for the autoantibody.~~The method of any one of claims 1 to 8, wherein said anti-TNFα drug is adalimumab (HUMIRA™).~~

12. The method of claim 10, wherein the sample is serum.~~The method of any one of claims1 to8, wherein said anti-TNFα drug is etanercept (ENBREL™).~~

13. The method of claim 10, wherein the anti-TNFα drug is a member selected from the group consisting of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), CIMZIA® (certolizumab pegol), and combinations thereof.~~The method of any one of claims 1 to 8, wherein said anti-TNFα drug is CIMZIA® (certolizumab pegol).~~

14. The method of claim 10, wherein the autoantibody is a member selected from the group consisting of a human anti-mouse antibody (HAMA), a human anti-chimeric antibody (HACA), a human anti-humanized antibody (HAHA), and combinations thereof.~~The method of any one of claims 1 to 13, wherein said labeled TNFα is a fluorophore labeled TNFα.~~

15. The method of claim 10, wherein the size exclusion chromatography is size exclusion-high performance liquid chromatography (SE-HPLC).~~The method of any one of claims 1 to 14, wherein said measured anti-TNFα drug is quantitated.~~

16. The method of claim 10, wherein the labeled anti-TNFα drug is a fluorophore-labeled anti-TNFα drug.~~The method of any one of claims 1 to 15, wherein said first complex is eluted first, followed by free labeled TNFα.~~

17. The method of claim 16, wherein the fluorophore is an Alexa Fluor® dye. ~~The method of any one of claims 1 to 16, wherein said second complex is eluted first, followed by free labeled anti-TNFα drug.~~

18. The method of claim 10, wherein the labeled complex is eluted first, followed by the free labeled anti-TNFα drug.~~The method of any one of claims 1 to 17, wherein said size exclusion chromatography is size exclusion-high performance liquid chromatography (SE-HPLC).~~

19. The method of claim 10, wherein the sample is obtained from a subject receiving therapy with the anti-TNFα drug.~~The method of any one of claims 1 to 18, wherein said autoantibody is a member selected from the group consisting of human anti-mouse antibody (HAMA), human anti-chimeric antibody (HACA), human anti-humanized antibody (HAHA), and combinations thereof.~~

20. The method of claim 10, wherein a ratio of the free labeled anti-TNFα drug to an internal control is determined and used to extrapolate the level of the autoantibody from the standard curve.~~The method of any one of claims 1 to 19, wherein said measured autoantibody is quantitated.~~

21.~~ The method of any one of claims 1 to 20, wherein both of said first and second samples are obtained from said subject during therapy with said anti-TNFα drug.~~

22. A method for optimizing therapy and/or reducing toxicity to an anti-TNFα drug in a subject receiving therapy with said anti-TNFα drug, said method comprising:

(a) measuring the level of said anti-TNFα drug in a first sample from said subject;

(b) measuring the level of an autoantibody to said anti-TNFα drug in a second sample from said subject; and

(c) determining a subsequent course of therapy for said subject based upon the levels of said anti-TNFα drug and said autoantibody,

thereby optimizing therapy and/or reducing toxicity to said anti-TNFα drug.

23. The method of claim 22, wherein the subsequent course of therapy comprises co-administering an immunosuppressive drug with said anti-TNFα drug when said level of said anti-TNFα drug is a high level and said level of said autoantibody is a low level.

24. The method of claim 22, wherein the subsequent course of therapy comprises increasing the level of said anti-TNFα drug and co-administering an immunosuppressive drug when said level of said anti-TNFα drug is a medium level and said level of said autoantibody is a low level.

25. The method of claim 22, wherein the subsequent course of therapy comprises administering a different anti-TNFα drug when said level of said anti-TNFα drug is a medium level and said level of said autoantibody is a medium level.

26. The method of claim 22, wherein the subsequent course of therapy comprises administering a different anti-TNFα drug when said level of said anti-TNFα drug is a low level and said level of said autoantibody is a high level.

27. The method of claim 25 or 26, wherein adalimumab (HUMIRA™) is administered instead of infliximab (REMICADE™).

28. The method of any one of claims 22 to 27, wherein said anti-TNFα drug is measured with an assay comprising:

(i) contacting said first sample with an amount of a labeled TNFα to form a first complex comprising said labeled TNFα with said anti-TNFα drug; and

(ii) detecting said first complex by size exclusion chromatography,

thereby measuring said level of said anti-TNFα drug.

~~29. The method of any one of claims 22 to 28, wherein said autoantibody is measured with an assay comprising:~~

~~(i) contacting said second sample with an amount of a labeled anti-TNFα drug to form a second complex comprising said labeled anti-TNFα drug with said autoantibody; and~~

~~(ii) detecting said second complex by size exclusion chromatography, thereby measuring said level of said autoantibody.~~

~~30. The methods of any one of claims 22 to 29, wherein said anti-TNFα drug is a member selected from the group consisting of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), CIMZIA® (certolizumab pegol), and combinations thereof.~~

~~31. The method of any one of claims 22 to 29, wherein said anti-TNFα drug is infliximab (REMICADE™).~~

~~32. The method of any one of claims 22 to 29, wherein said anti-TNFα drug is adalimumab (HUMIRA™).~~

~~33. The method of any one of claims 22 to 29, wherein said anti-TNFα drug is etanercept (ENBREL™).~~

~~34. The method of any one of claims 22 to 29, wherein said anti-TNFα drug is CIMZIA® (certolizumab pegol).~~

~~35. The method of any one of claims 22 to 34, wherein said measured anti-TNFα drug is quantitated.~~

~~36. The method of any one of claims 22 to 35, wherein said measured autoantibody is quantitated.~~

~~37. The method of any one of claims 22 to 36, wherein both of said first and second samples are serum samples.~~

~~38. The method of any one of claims 22 to 37, wherein both of said first and second samples are obtained from said subject during therapy with said anti-TNFα drug.~~

39. The method of any one of claims 22 to 38, wherein said autoantibody is a member selected from the group consisting of human anti-mouse antibody (HAMA), human anti-chimeric antibody (HACA), human anti-humanized antibody (HAHA), and combinations thereof.

40. The method of claim 23 or 24, wherein said immunosuppressive drug is selected from the group consisting of methotrexate, azathioprine, metabolites thereof, and combinations thereof.

41. A method for determining the presence or level of an anti-TNFα drug with reference to an internal control in a sample having or suspected of having said anti-TNFα drug, the method comprising:

(a) contacting an amount of a labeled TNFα and an amount of a labeled internal control with said sample to form a complex of said labeled TNFα and said anti-TNFα drug;

(b) detecting said labeled TNFα and said labeled internal control by size exclusion chromatography;

(c) integrating the area-under-the curve for a peak of said labeled TNFα from a plot of signal intensity as a function of elution time from said size exclusion chromatography;

(d) integrating the area-under-the curve for a peak of said labeled internal control from said plot of signal intensity as a function of elution time from said size exclusion chromatography;

(e) determining a first ratio by dividing said amount of labeled TNFα by said amount of labeled internal control;

(f) determining a second ratio by dividing the resultant integration from step (c) by the resultant integration from step (d); and

(g) comparing the first ratio determined in step (e) with the second ratio determined in step (f),

thereby determining the presence or level of said anti-TNFα drug with reference to an internal control.

42. The method of claim 41, wherein the first ratio determined in step (e) is from about 80 to about 100.

~~43. The method of claim 41, wherein the first ratio determined in step (e) is about 100.~~

~~44. The method of any one of claims 41 to 43, wherein said labeled internal control is Biocytin-Alexa 488.~~

~~45. The method of any one of claims 41 to 44, wherein said amount of said labeled internal control is from about 1 to about 25 ng per 100 µL of sample analyzed.~~

~~46. A method for optimizing the therapeutic amount of an anti-TNFα drug in a subject receiving therapy with said anti-TNFα drug, said method comprising:~~

~~determining a subsequent dose of said anti-TNFα drug for said subject based upon the comparison of said first and second ratios in accordance with the method of any one of claims 41 to 44;~~

~~thereby optimizing the therapeutic amount of said anti-TNFα drug.~~

~~47. The method of claim 46, wherein the method further comprises:~~

~~increasing the subsequent dose of said anti-TNFα drug when the first ratio is about 100:1 and the second ratio is less than about 95:1,~~

~~thereby optimizing the therapeutic amount of said anti-TNFα drug.~~

~~48. The method of any one of claims 41 to 47, wherein said anti-TNFα drug is a member selected from the group consisting of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), CIMZIA® (certolizumab pegol), and combinations thereof.~~

~~49. The method of any one of claims 41 to 47, wherein said anti-TNFα drug is infliximab (REMICADE™).~~

~~50. The method of any one of claims 41 to 47, wherein said anti-TNFα drug is adalimumab (HUMIRA™).~~

~~51. The method of any one of claims 41 to 47, wherein said anti-TNFα drug is etanercept (ENBREL™).~~

~~52. The method of any one of claims 41 to 47, wherein said anti-TNFα drug is CIMZIA® (certolizumab pegol).~~

53. The method of any one of claims 41 to 52, wherein said labeled TNFα is a fluorophore labeled TNFα.

54. The method of any one of claims 41 to 53, wherein said detected labeled anti-TNFα and labeled internal control are quantitated.

55. The method of any one of claims 41 to 54, wherein said complex is eluted first, followed by free labeled TNFα.

56. The method of any one of claims 41 to 55, wherein said sample is serum.

57. The method of any one of claims 41 to 56, wherein said sample is obtained from a subject receiving therapy with said anti-TNFα drug.

58. The method of any one of claims 41 to 57, wherein said size exclusion chromatography is size exclusion-high performance liquid chromatography (SE-HPLC).

59. A method for determining the presence or level of an autoantibody to an anti-TNFα drug with reference to an internal control in a sample having or suspected of having said autoantibody, the method comprising:

(a) contacting an amount of labeled anti-TNFα drug and an amount of labeled internal control with said sample to form a complex of said labeled anti-TNFα drug and said autoantibody;

(b) detecting said labeled anti-TNFα drug and said labeled internal control by size exclusion chromatography;

(c) integrating the area-under-the curve for a peak of said labeled anti-TNFα drug from a plot of signal intensity as a function of elution time from said size exclusion chromatography;

(d) integrating the area-under-the curve for a peak of said labeled internal control from said plot of signal intensity as a function of elution time from said size exclusion chromatography;

(e) determining a first ratio by dividing said amount of labeled anti-TNFα drug by said amount of labeled internal control; and

(f) determining a second ratio by dividing the resultant integration from step (c) and (d); and

(g) comparing the first ratio determined in step (e) with said second ratio determined in step (f),

thereby determining the presence or level of said autoantibody with reference to an internal control.

60. The method of claim 59, wherein first ratio determined in step (e) is from about 80 to about 100.

61. The method of claim 59, wherein first ratio determined in step (e) is about 100.

62. The method of any one of claims 59 to 61, wherein said labeled internal control is Biocytin-Alexa 488.

63. The method of any one of claims 59 to 62, wherein said amount of said labeled internal control is from about 50 to about 200 pg per 100 µL of sample analyzed.

64. The method of any one of claims 59 to 62, wherein said anti-TNFα drug is a member selected from the group consisting of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), CIMZIA® (certolizumab pegol), and combinations thereof.

65. The method of any one of claims 59 to 62, wherein said anti-TNFα drug is infliximab (REMICADE™).

66. The method of any one of claims 59 to 62, wherein said anti-TNFα drug is adalimumab (HUMIRA™).

67. The method of any one of claims 59 to 62, wherein said anti-TNFα drug is etanercept (ENBREL™).

68. The method of any one of claims 59 to 62, wherein said anti-TNFα drug is CIMZIA® (certolizumab pegol).

69. The method of any one of claims 59 to 68, wherein said detected labeled anti-TNFα drug and labeled internal control are quantitated.

70. The method of any one of claims 59 to 69, wherein said complex is eluted first, followed by free labeled anti-TNFα drug.

71. The method of any one of claims 59 to 70, wherein said sample is serum.

72. The method of any one of claims 59 to 71, wherein said sample is obtained from a subject receiving therapy with said anti-TNFα drug.

73. The method of any one of claims 59 to 72, wherein said size exclusion chromatography is size exclusion-high performance liquid chromatography (SE-HPLC).

74. The method of any one of claims 59 to 73, wherein said autoantibody is a member selected from the group consisting of human anti-mouse antibody (HAMA), human anti-chimeric antibody (HACA), human anti-humanized antibody (HAHA), and combinations thereof.

75. A kit for measuring the presence or level of an anti-TNFα drug and the presence or level of an autoantibody to an anti-TNFα drug in a sample, said kit comprising:

(a) a first measuring substrate comprising an amount of a labeled TNFα;

(b) a second measuring substrate comprising an amount of a labeled anti-TNFα drug;

(c) optionally a third measuring substrate comprising an amount of a labeled TNFα and an amount of a labeled internal control;

(d) optionally a fourth measuring substrate comprising an amount of a labeled anti-TNFα drug and an amount of a labeled internal control;

(e) optionally a means for extracting a sample from a subject; and

(f) optionally a pamphlet of instructions for using said kit.

76. The kit of claim 75, further comprising a means for detecting said labeled TNFα, said labeled anti-TNFα drug, and/or said labeled internal control.

77. The kit of claim 76, wherein the means for detecting comprises fluorescence label detection, UV-radiation detection, or iodine exposure.

78. The kit of any one of claims 75 to 77, further comprising a size exclusion-high performance liquid chromatography (SE-HPLC) instrument.

~~79. The kit of any one of claim 75 to 80, wherein the first, second, third, and fourth measuring substrates are selected from the group consisting of nitrocellulose, silica gel, and a size-exclusion chromatographic medium.~~

~~80. The kit of any one of claims 75 to 79, wherein said anti-TNFα drug is a member selected from the group consisting of REMICADE™ (infliximab), ENBREL™ (etanercept), HUMIRA™ (adalimumab), CIMZIA® (certolizumab pegol), and combinations thereof.~~

~~81. The kit of any one of claims 75 to 80, wherein said labeled TNFα is a fluorophore labeled TNFα.~~

~~82. The kit of any one of claims 75 to 81, wherein said sample is serum.~~

~~83. The kit of any one of claims 75 to 82, wherein said sample is obtained from a subject receiving therapy with said anti-TNFα drug.~~

~~84. The kit of any one of claims 75 to 83, wherein said autoantibody is a member selected from the group consisting of human anti-mouse antibody (HAMA), human anti-chimeric antibody (HACA), human anti-humanized antibody (HAHA), and combinations thereof.~~