

METHODS FOR DETECTING A NEUTRALIZING ANTIBODY (NAB)
AGAINST AN ANTI-TNF ALPHA ANTIBODY

CROSS-REFERENCE TO RELATED APPLICATION(S)

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/506,911, filed June 8, 2023. The contents of this provisional applications are incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] This application contains an ST.26 compliant Sequence Listing, which was submitted in xml format via Patent Center and is hereby incorporated by reference in its entirety. The .xml copy, created on May 17, 2024, is named 140505-8006WO00_SL.xml and is 20,939 bytes in size.

TECHNICAL FIELD

[0003] The present technology includes *in vitro* assays for identifying neutralizing antibodies (NAbs).

BACKGROUND

[0004] Anti-Tumor Necrosis Factor-alpha (TNF α) antibodies are novel biologics that may potentially be used for the treatment of a variety of diseases. One hurdle to the development and implementation of anti-TNF α antibodies as a treatment is the potential for a subject administered such anti-TNF α antibodies to elicit an immune response against them. A subject's immunogenicity toward the anti-TNF α antibodies, particularly the subject's development of antibodies that neutralize the anti-TNF α antibody, would thereby reduce efficacy of the anti-TNF α antibody to treat the subject's disease. To advance the development of treatments that use anti-TNF α antibodies, there is need for assays that reliably determine the presence of anti-TNF α neutralizing antibodies and amounts thereof in subjects receiving or having been administered the anti-TNF α antibody.

SUMMARY

[0005] In some embodiments, the present technology comprises methods for detecting a neutralizing antibody (NAb) against a target antibody in a sample comprising: (i) disassociating the NAb from the target antibody by adding an acid wash to the sample; (ii) purifying the NAb

in the sample; (iii) contacting the purified NAb with a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; (iv) contacting the purified NAb and the detection antibody with a TNF α -coated substrate; and (v) detecting the NAb when the detection antibody binds the NAb instead of the TNF α -coated substrate, wherein the target antibody comprises an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20.

[0006] In some embodiments, the method further comprises detecting the detection label before detecting the NAb.

[0007] In some embodiments, the method further comprises washing the TNF α -coated substrate before detecting the NAb.

[0008] In some embodiments, the acid wash comprises acetic acid.

[0009] In some embodiments, purifying the NAb of step (ii) comprises (a) contacting the sample with an affinity antibody comprising anti-TNF α antibody conjugated to a first affinity label; (b) contacting the sample with a first solid phase coated with a second affinity label, wherein the first affinity label binds to the second affinity label; (c) washing the first solid phase; and (d) eluting the NAb from the first solid phase.

[0010] In some embodiments, the first affinity label is biotin.

[0011] In some embodiments, the first solid phase is a microbead. In some embodiments, the first solid phase is a magnetic microbead.

[0012] In some embodiments, the second affinity label is streptavidin.

[0013] In some embodiments, the NAb is purified using an automated purification system.

[0014] In some embodiments, the detection label includes a luminescent compound. In some embodiments, the present technology comprises a method, wherein the detection label includes an enzyme configured to convert a chromogenic substrate into a pigment. In some embodiments, the present technology comprises a method, wherein the detection label is a third affinity label.

[0015] In some embodiments, detecting the detection label further comprises contacting the third affinity label with a fourth affinity label conjugated to a luminescent compound or enzyme configured to convert a chromogenic substrate into a pigment, wherein the third affinity label binds to the fourth affinity label.

[0016] In some embodiments, the third affinity label is biotin, and the fourth affinity label is streptavidin.

[0017] In some embodiments, detecting the detection label comprises detecting the luminescent compound. In some embodiments, detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting the pigment. In some embodiments, detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting an optical density of the sample.

[0018] In some embodiments, the enzyme is a horseradish peroxidase (HRP).

[0019] In some embodiments, the chromogenic substrate is 3,3',5,5'-tetramethylbenzidine (TMB).

[0020] In some embodiments, steps (i) to (v) are performed on a negative control sample having no detectable NAb.

[0021] In some embodiments, the detection antibody binding the NAb instead of the TNF α -coated substrate is detected when the detection label is present at a level below a predetermined level. In some embodiments, the detection label is present at the level below the predetermined level when an optical density of the sample is above a predetermined cut-point.

[0022] In some embodiments, the predetermined cut-point is the level corresponding to a false-positive rate of 3% or less. In some embodiments, the predetermined cut-point is the level corresponding to a 1% false-positive rate. In some embodiments, the predetermined cut-point is 0.5-2. In some embodiments, the predetermined cut-point is 0.91.

[0023] In some embodiments, a sensitivity of detecting the NAb is less than 500ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than 250ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than 150ng/mL.

[0024] In some embodiments, a target tolerance is about 1000ng/mL.

[0025] In some embodiments, the target antibody comprises SEQ ID NOs: 2 and 5. In some embodiments, the target antibody comprises SEQ ID NOs: 1 and 4.

[0026] In some embodiments, the present technology comprises a method for detecting a NAb (neutralizing antibody) against a target antibody in a sample comprising: (i) purifying the NAb in the sample; (ii) determining whether the NAb is present in the sample by performing a competitive binding assay, wherein the NAb is present if the NAb binds the anti-TNF α antibody

with a higher affinity than the affinity of the anti-TNF α antibody to TNF α ; and (iii) detecting the NAb; wherein a sensitivity of the method is 250ng/mL or less, wherein the target antibody comprises an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20.

[0027] In some embodiments, before the NAb is purified, the method further comprises disassociating the NAb from the target antibody by adding an acid wash to the sample.

[0028] In some embodiments, the competitive binding assay comprises: (a) contacting the purified NAb with a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; (b) contacting the purified NAb and the detection antibody with a TNF α -coated substrate; (c) washing the TNF α -coated substrate; and (d) detecting the detection label.

[0029] In some embodiments, detecting the NAb comprises determining whether the detection label is present at a level below a predetermined level.

[0030] In some embodiments, the detection label is present at the level below the predetermined level when an optical density is above a predetermined cut-point.

[0031] In some embodiments, the acid wash comprises acetic acid.

[0032] In some embodiments, purifying the NAb comprises: (a) contacting the sample with an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label; (b) contacting the sample with a first solid phase coated with a second affinity label, wherein the first affinity label binds to the second affinity label; (c) washing the first solid phase; and (d) eluting the NAb from the first solid phase.

[0033] In some embodiments, the first affinity label is biotin.

[0034] In some embodiments, the first solid phase is a microbead. In some embodiments, the first solid phase is a magnetic microbead.

[0035] In some embodiments, the second affinity label is streptavidin.

[0036] In some embodiments, the NAb is purified using an automated purification system.

[0037] In some embodiments, the detection label comprises a luminescent compound.

[0038] In some embodiments, the detection label comprises an enzyme configured to convert a chromogenic substrate into a pigment. In some embodiments, the detection label is a third affinity label.

[0039] In some embodiments, detecting the detection label further comprises contacting the third affinity label with a fourth affinity label conjugated to a luminescent compound or enzyme configured to convert a chromogenic substrate into a pigment, wherein the third affinity label binds to the fourth affinity label.

[0040] In some embodiments, the third affinity label is biotin, and the fourth affinity label is streptavidin.

[0041] In some embodiments, detecting the detection label comprises detecting the luminescent compound. In some embodiments, detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting the pigment. In some embodiments, detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting an optical density of the sample.

[0042] In some embodiments, the enzyme is a horseradish peroxidase (HRP).

[0043] In some embodiments, the chromogenic substrate is 3,3',5,5'-tetramethylbenzidine (TMB).

[0044] In some embodiments, steps (i) to (iii) are performed on a negative control sample having no detectable NAb.

[0045] In some embodiments, the predetermined cut-point is a level corresponding to a 1% false-positive rate. In some embodiments, the predetermined cut-point is 0.91.

[0046] In some embodiments, the sensitivity of detecting the NAb is less than 500ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than 250ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than 150ng/mL.

[0047] In some embodiments, a target tolerance is about 1000ng/mL.

[0048] In some embodiments, the target antibody comprises SEQ ID NOs: 2 and 5. In some embodiments, the target antibody comprises SEQ ID NOs: 1 and 4.

[0049] In some embodiments, the present technology comprises a kit for detecting a neutralizing antibody (NAb) against a target antibody comprising an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20 in a sample comprising: (i) an affinity antibody comprising the target antibody conjugated to a first affinity label; (ii) a first solid phase coated with a second affinity label; (iii) a detection antibody comprising the target antibody conjugated to a detection

label; (iv) a substrate; and (v) TNF α , wherein the target antibody comprises an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20.

[0050] In some embodiments, the first affinity label is biotin.

[0051] In some embodiments, the second affinity label is streptavidin.

[0052] In some embodiments, the detection label is a luminescent compound, an enzyme configured to convert a chromogenic substrate into a pigment, or biotin.

[0053] In some embodiments, the first solid phase is a microbead. In some embodiments, the first solid phase is a magnetic microbead.

[0054] In some embodiments, the detection label is biotin.

[0055] In some embodiments, the kit further comprising an acid wash buffer.

[0056] In some embodiments, the kit further comprising a washing buffer.

[0057] In some embodiments, the kit further comprising an elution buffer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0058] FIG. 1 shows a flow diagram of an example method of the present technology.

[0059] FIG. 2 shows a flow diagram of another example method of the present technology.

[0060] FIG. 3 shows an example normal distribution of 295 log-transformed normalized values from control samples prepared according to the methods of the present technology.

DETAILED DESCRIPTION

[0061] The present technology comprises methods for detecting a neutralizing antibody (NAb) against a target antibody. The target antibody may comprise an anti-TNF α antibody. In some embodiments, the anti-TNF α antibody is a chimeric human-murine monoclonal IgG1-kappa antibody. In some embodiments, the target antibody comprises heavy chain complementarity-determining regions (CDRs) of SEQ ID NOs: 9-11 and light chain CDRs of SEQ ID NOs: 12-14. In some embodiments, the target antibody comprises heavy chain CDRs of SEQ ID NOs: 15-17 and light chain CDRs of SEQ ID NOs: 18-20. In some embodiments, the target antibody comprises a heavy chain variable region of SEQ ID NO: 2 and a light chain variable region of SEQ ID NO: 5. In some embodiments, the target antibody comprises a heavy chain of SEQ ID NOs: 1 and light chain of SEQ ID NO: 4, in a sample obtained from a subject

receiving or having been administered the target antibody. The present technology also comprises a kit for detecting a NAb in the sample according to the methods of the present technology.

Definitions

[0062] The term “about” means a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by acceptable levels in the art. Typically, such variation may be as much 10% above and below a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length and such variation may be influenced by standard applicable measurement practices. When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth.

[0063] As used herein, an “affinity antibody” comprises an anti-TNF α antibody. In some embodiments, the affinity antibody comprises the target antibody. The affinity antibody may also comprise an affinity label that has a high affinity with a separate label. For example, the affinity label may be a first affinity label (i.e., biotin) that has a high affinity with a second affinity label (i.e., streptavidin). In some embodiments, the affinity antibody comprises an anti-TNF α antibody and an affinity label. In some embodiments, the affinity antibody comprises the target antibody and an affinity label.

[0064] As used herein, a “detection antibody” comprises a TNF α antibody. In some embodiments, the detection antibody comprises the target antibody. The detection antibody may also comprise a detection label that may be directly or indirectly detected. In some embodiments, the detection antibody comprises an anti-TNF α antibody and a detection label. In some embodiments, the detection antibody comprises the target antibody and a detection label.

[0065] In some embodiments, the method for detecting an NAb against the target antibody in the sample comprises (i) disassociating the NAb from the target antibody by adding an acid wash to the sample; (ii) purifying the NAb from the sample; (iii) contacting the purified NAb with an anti-TNF α antibody conjugate comprising the anti-TNF α antibody conjugated to a detection label (i.e., a “detection antibody”); (iv) contacting the purified NAb and the detection antibody with a TNF α -coated substrate; and (v) detecting the NAb by determining whether the detection label is present at a level above a predetermined cut-point.

[0066] In some embodiments, the method for detecting an NAb against the target antibody comprises (i) purifying the NAb in the sample; (ii) determining whether the NAb is present in the sample by performing a competitive binding assay, wherein the NAb is present if it binds the detection antibody with a higher affinity than a binding affinity of the detection antibody to TNF α ; and (iii) detecting the NAb; wherein a sensitivity of the method is about 250ng/mL or less.

[0067] In some embodiments, the competitive binding assay comprises (a) contacting the purified NAb with the detection antibody; (b) contacting the purified NAb and the detection antibody with the TNF α -coated substrate; (c) washing the TNF α -coated substrate; and (d) detecting the detection label.

[0068] The sample may be a sample obtained from a mammal, including human, rat, mouse, rabbit, or other animal. In some embodiments, the sample is a blood, plasma, or serum sample. In some embodiments, the sample is plasma. In some embodiments, the sample is serum. In some embodiments, the sample is pre-processed before the NAb is detected according to the methods of the present technology. As used herein, "pre-processing" includes extracting tissue from a subject, and identifying the sample (e.g., drawing a blood sample and identifying or isolating a plasma or serum sample).

Antibodies

Neutralizing Antibodies (NAbs)

[0069] During or after administration of a drug (e.g., a therapeutic drug) to a subject, the subject's immune system may respond by generating NAbs. NAbs may bind at least a portion of the drug, thereby sequestering, neutralizing, and/or reducing efficacy of the drug. In some embodiments, the drug is a drug antibody. When NAbs are present, the NAb may bind at least a portion of the drug antibody, including, but not limited to, binding to one or more amino acids of a heavy chain, a light chain, a variable region (e.g., a heavy chain variable region domain (V_H) or a light chain variable region domain (V_L)), a constant region, an Fc domain, an Fab domain, a hinge region, a disulfide bond, or a complementarity-determining region (CDR) of the drug antibody.

[0070] NAbs may be naturally produced as part of a subject's innate immune response. The NAb may be a natural antibody produced by the subject, for example by lymphocytes using variable (diversity) joining (V(D)J) recombination. For example, the NAb may be an

immunoglobulin. In some embodiments, the NAb is an IgG, IgA, IgE, monoclonal antibody, polyclonal antibody, or any other kind of antibody that is capable of binding to the target antibody.

Target Antibodies

[0071] Presence of NAb and/or binding of NAb to the drug antibodies may be detected using antibodies which are the same as or mimic the drug antibodies (i.e., “target antibodies”). In some embodiments, the target antibodies comprise one or more anti-TNF α antibodies. The anti-TNF α antibodies are antibodies which bind at least a portion of a TNF α protein or peptide.

[0072] In some embodiments, the target antibodies comprise a monoclonal antibody.

[0073] In some embodiments, the target antibodies of the present technology are chimeric antibodies. Nonlimiting examples of chimeric antibodies include human-murine antibodies, human-rat antibodies, human-hamster antibodies, human-rabbit antibodies, human-bovine antibodies, and human-goat antibodies. In some embodiments, the chimeric antibodies comprise a humanized antibody (e.g., a full humanized antibody or a partially humanized antibody).

[0074] In some embodiments, the target antibodies of the present technology may comprise an isotype that influences functional properties and/or structural features of the antibody. In some embodiments the target antibodies comprise an isotype selected from the group consisting of IgG (e.g., IgG1, IgG2, IgG3, IgG4), IgM, IgA, IgD, and IgE. The light chain may comprise isotype kappa (κ) or isotype lambda (λ). In some embodiments, the target antibody comprises an IgG1-kappa antibody.

Target Antibody Sequences

[0075] The target antibodies of the present technology may comprise an anti-TNF α antibody or at least a portion of the anti-TNF α antibody. For example, the target antibodies may comprise an amino acid sequence derived from one or more of a heavy chain, a light chain, a variable region (e.g., V_H or V_L domains), a constant region, an Fc domain, a Fab domain, a hinge region, a disulfide bond, or a CDR of an anti-TNF α antibody. The NAb of the present technology may bind to one or more amino acid residues of the amino acid sequence.

[0076] In some embodiments, the portion of the anti-TNF α antibody comprises a sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to an amino acid sequence in Table 1.

[0077] In some embodiments, the portion of the anti-TNF α antibody comprises a sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to an amino acid sequence in Table 1.

[0078] In some embodiments, the portion of the anti-TNF α antibody comprises a sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to an amino acid sequence in Table 1.

Heavy Chain:

[0079] The target antibodies of the present technology comprise a heavy chain having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 1.

[0080] The target antibodies of the present technology comprise a heavy chain having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 1.

[0081] The target antibodies of the present technology comprise a heavy chain having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 1.

[0082] The heavy chain of the target antibodies may comprise a variable region domain (V_H) having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 2.

[0083] The heavy chain of the target antibodies may comprise a V_H having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 2.

[0084] The heavy chain of the target antibodies may comprise a V_H having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 2.

[0085] The heavy chain of the target antibodies may comprise a constant region having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 3.

[0086] The heavy chain of the target antibodies may comprise a constant region having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 3.

[0087] The heavy chain of the target antibodies may comprise a constant region having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 3.

[0088] The target antibodies of the present technology comprise a heavy chain having at least one Complementarity Determining Region (CDR) sequence. The at least one CDR sequence confers binding to a specific antigen (e.g., a TNF α peptide).

[0089] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 9.

[0090] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 9.

[0091] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 9.

[0092] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 10.

[0093] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 10.

[0094] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 10.

[0095] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 11.

[0096] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 11.

[0097] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 11.

[0098] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 15.

[0099] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 15.

[0100] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 15.

[0101] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 16.

[0102] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 16.

[0103] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 16.

[0104] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 17.

[0105] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 17.

[0106] In some embodiments, the target antibodies comprise a heavy chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 17.

[0107] In some embodiments, the target antibodies comprise a heavy chain comprising (1) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 9, (2) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 10, and (3) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 11.

[0108] In some embodiments, the target antibodies comprise a heavy chain comprising (1) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 9, (2) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 10, and (3) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 11.

[0109] In some embodiments, the target antibodies comprise a heavy chain comprising (1) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 9, (2) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 10, and (3) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 11.

[0110] In some embodiments, the target antibodies comprise a heavy chain comprising (1) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 15, (2) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 16, and (3) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 17.

[0111] In some embodiments, the target antibodies comprise a heavy chain comprising (1) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 15, (2) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 16, and (3) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 17.

[0112] In some embodiments, the target antibodies comprise a heavy chain comprising (1) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%,

or 100% identical to SEQ. ID NO: 15, (2) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 16, and (3) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 17.

[0113] In some embodiments, the heavy chain of the target antibodies of the present technology is encoded by a nucleotide sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 8.

[0114] In some embodiments, the heavy chain of the target antibodies of the present technology is encoded by a nucleotide sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 8.

[0115] In some embodiments, the heavy chain of the target antibodies of the present technology is encoded by a nucleotide sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 8.

Light Chain:

[0116] The target antibodies of the present technology comprise a light chain having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 4.

[0117] The target antibodies of the present technology comprise a light chain having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 4.

[0118] The target antibodies of the present technology comprise a light chain having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 4.

[0119] The light chain of the target antibodies may comprise a variable region domain (V_L) having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 5.

[0120] The light chain of the target antibodies may comprise a V_L having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 5.

[0121] The light chain of the target antibodies may comprise a V_L having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 5.

[0122] The light chain of the target antibodies may comprise a constant region having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 6.

[0123] The light chain of the target antibodies may comprise a constant region having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 6.

[0124] The light chain of the target antibodies may comprise a constant region having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 6.

[0125] The target antibodies of the present technology comprise a light chain having at least one CDR sequence. The at least one CDR sequence confers binding to a specific antigen (e.g., a TNF α peptide).

[0126] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 12.

[0127] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 12.

[0128] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 12.

[0129] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 13.

[0130] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 13.

[0131] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 13.

[0132] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 14.

[0133] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 14.

[0134] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 14.

[0135] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 18.

[0136] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 18.

[0137] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 18.

[0138] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 19.

[0139] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 19.

[0140] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 19.

[0141] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 20.

[0142] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 20.

[0143] In some embodiments, the target antibodies comprise a light chain having a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 20.

[0144] In some embodiments, the target antibodies comprise a light chain comprising (1) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 12, (2) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 13, and (3) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 14.

[0145] In some embodiments, the target antibodies comprise a light chain comprising (1) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 12, (2) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 13, and (3) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 14.

[0146] In some embodiments, the target antibodies comprise a light chain comprising (1) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 12, (2) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 13, and (3) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 14.

[0147] In some embodiments, the target antibodies comprise a light chain comprising (1) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 18, (2) a CDR having an amino acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 19, and (3) a CDR having an amino

acid sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 20.

[0148] In some embodiments, the target antibodies comprise a light chain comprising (1) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 18, (2) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 19, and (3) a CDR having an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 20.

[0149] In some embodiments, the target antibodies comprise a light chain comprising (1) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 18, (2) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 19, and (3) a CDR having an amino acid sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 20.

[0150] In some embodiments, the light chain of the target antibodies of the present technology is encoded by a nucleotide sequence that is about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 7.

[0151] In some embodiments, the light chain of the target antibodies of the present technology is encoded by a nucleotide sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 7.

[0152] In some embodiments, the light chain of the target antibodies of the present technology is encoded by a nucleotide sequence that is at least about 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to SEQ. ID NO: 7.

Table 1: Sequences of Example Anti-TNF α Antibodies of the Present Technology

SEQ ID NO:	Description	Sequence
1	aa sequence of Heavy Chain of anti-TNF α antibody	EVKLEESGGGLVQPGGSMKLSKVASGFIFSNHWMNWVRQ SPEKGLEWVAEIRSKSINSATHYAESVKGRFTISRDDSKSAV YLQMTDLRTEDTGVIYCSRNIYGSTYDYGQGTTLTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGSVF

	(XTMAB-16, target antibody)	LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
2	aa sequence of Heavy chain variable region of anti-TNF α antibody (XTMAB-16, target antibody)	EVKLEESGGGLVQPGGSMKLSCLVSGFIFSNHWMNWVRQ SPEKGLEWVAEIRSKSINSATHYAESVKGRFTISRDDSKSAV YLQMTDLRTEDTGYYCSRNYGSTDYWGQGTTLTV
3	aa sequence of Heavy chain constant region of anti-TNF α antibody (XTMAB-16, target antibody)	SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK
4	aa sequence of Light chain of anti-TNF α antibody (XTMAB-16, target antibody)	DILLTQSPAILSVPGERVSFSCRASQFVGSSIHWHYQQRRTNG SPRLLIKYASEMSGIPSRFSGSGSGTDFTLSINTVESEDIAD YYCQQSHSWPFTFGSGTNLEVKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSSTYSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
5	aa sequence of Light chain variable region of anti-TNF α antibody	DILLTQSPAILSVPGERVSFSCRASQFVGSSIHWHYQQRRTNG SPRLLIKYASEMSGIPSRFSGSGSGTDFTLSINTVESEDIAD YYCQQSHSWPFTFGSGTNLEV

	(XTMAB-16, target antibody)	
6	aa sequence of Light chain constant region of anti-TNF α antibody (XTMAB-16, target antibody)	KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
7	Nucleotide sequence encoding light chain of anti- TNF α antibody (XTMAB-16, target antibody)	GCTAGCGAATTCCACCATGGGCTGGTCCTGCATCATCCT GTTTCTGGTGGCCACCGCCACCGGCGTGACTIONCCGACAT CCTGCTGACCCAGTCCCCCGCCATCCTGTCCGTGTCTCCC GGCGAGCGGGTGTCTTCTCTGCGGGGCTCCAGTTC GTGGGCTCCTCCATCCACTGGTATCAGCAGCGGACCAAC GGCTCCCCCTCGGCTGCTGATCAAGTACGCCTCCGAGTCC ATGTCCGGCATCCCTTCCCGGTTCTCCGGCTCCGGCTCTG GCACCGACTTCACCCTGTCCATCAACACCGTGGAGTCCG AGGATATTGCCGACTACTACTGCCAGCAGTCCCACTCCT GGCCTTTCACCTTCGGCTCCGGCACCAACCTGGAGGTGA AGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCC ATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGT GTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAAGT ACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAATC CCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAG ACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCAACC ATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACA GGGGAGAGTGTTAA
8	Nucleotide sequence encoding heavy chain of anti- TNF α antibody	GCTAGCGATATCGAATTCTTTTCTTTTGTGTTTGTTCACC ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTGGCCACCG CCACCGGCGTGACTIONCCGAGGTGAAGCTGGAGGAGTCTG GCGGCGGACTGGTGCAGCCTGGCGGCTCCATGAAGCTGT CCTGCGTGGCCTCCGGCTTCATCTTCTCCAACCACTGGAT GAACTGGGTGCGCCAGTCCCTGAGAAGGGCCTGGAGT GGGTGGCCGAGATCCGGTCCAAGTCCATCAACTCCGCCA

	(XTMAB-16, target antibody)	CCCCTACGCCGAGTCCGTGAAGGGCCGGTTCACCATCT CCCGGGACGACTCCAAGTCCGCCGTGTACCTGCAGATGA CCGACCTGCGGACCGAGGACACCGGAGTGTACTACTGCT CCCGGAACTACTACGGCTCCACCTACGACTACTGGGGCC AGGGCACCACACTGACCGTGAGCTCCGCCTCCACCAAGG GCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCA CCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAATCAG GCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCC TACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGA CCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTCGAGCCCAAATCTTGTGACAAAACCTCACAC ATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACC GTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCT CATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACT GGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGT GGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAA TGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTCAAAGCCAAAG GGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCT GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGG AGTGGGAGAGTAATGGGCAGCCGGAGAACAACACTACAAG ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT CCGGGTAAATAA
9	aa sequence of Heavy Chain CDR1 predicted by CDR-IMGT	GFIFSNHW

	of anti-TNF α antibody (XTMAB-16, target antibody)	
10	aa sequence of Heavy Chain CDR2 predicted by CDR-IMGT of anti-TNF α antibody (XTMAB-16, target antibody)	IRSKSINSAT
11	aa sequence of Heavy Chain CDR3 predicted by CDR-IMGT of anti-TNF α antibody (XTMAB-16, target antibody)	SRNYYGSTYDY
12	aa sequence of Light Chain CDR1 predicted by CDR-IMGT of anti-TNF α antibody (XTMAB-16, target antibody)	QFVGSS
	aa sequence of Light Chain CDR2 predicted by CDR-IMGT of anti-TNF α	YA

	antibody (XTMAB-16, target antibody)	
14	aa sequence of Light Chain CDR3 predicted by CDR-IMGT of anti-TNF α antibody (XTMAB-16, target antibody)	QQSHSWPFT
15	aa sequence of Heavy Chain CDR1 predicted by CDR-Kabat of anti-TNF α antibody (XTMAB-16, target antibody)	NHWMN
16	aa sequence of Heavy Chain CDR2 predicted by CDR-Kabat of anti-TNF α antibody (XTMAB-16, target antibody)	EIRSKSINSATHYAESVKG
17	aa sequence of Heavy Chain CDR3 predicted by CDR-Kabat of anti-TNF α antibody	NYYGSTYDY

	(XTMAB-16, target antibody)	
18	aa sequence of Light Chain CDR1 predicted by CDR-Kabat of anti-TNF α antibody (XTMAB-16, target antibody)	RASQFVGSSIH
19	aa sequence of Light Chain CDR2 predicted by CDR-Kabat of anti-TNF α antibody (XTMAB-16, target antibody)	YASESMS
20	aa sequence of Light Chain CDR3 predicted by CDR-Kabat of anti-TNF α antibody (XTMAB-16, target antibody)	QQSHSWPFT

Dissociating NABs

Acid Wash

[0153] In some embodiments, the method comprises disassociating the NAb from the target by adding an acid wash to the sample. The acid wash may be any acid wash known to one of skill in the art to disassociate an antibody from its target. In some embodiments, the acid wash may comprise acetic acid.

[0154] In some embodiments, the acid wash comprises about 2M acetic acid. In some embodiments, the acid wash comprises about 300mM acetic acid. In some embodiments, the acid wash comprises 0.1M glycine-HCl.

[0155] In some embodiments, the acid wash comprises about 10mM, about 50mM, about 100mM, about 150 mM, about 200mM, about 250 mM, about 300 mM, about 350 mM, about 400 mM, about 500 mM, about 600 mM, about 700 mM, about 800 mM, about 900 mM, about 1M, about 1.5M, about 2M, about 2.5M, about 3M, about 3.5M, about 4M, about 4.5M, or about 5M acetic acid.

[0156] In some embodiments, the acid wash comprises at least 10mM, at least 50mM, at least 100mM, at least 150 mM, at least 200mM, at least 250 mM, at least 300 mM, at least 350 mM, at least 400 mM, at least 500 mM, at least 600 mM, at least 700 mM, at least 800 mM, at least 900 mM, at least 1M, at least 1.5M, at least 2M, at least 2.5M, at least 3M, at least 3.5M, at least 4M, at least 4.5M, or at least 5M acetic acid.

[0157] In some embodiments, the acid wash comprises at least about 10mM, at least about 50mM, at least about 100mM, at least about 150 mM, at least about 200mM, at least about 250 mM, at least about 300 mM, at least about 350 mM, at least about 400 mM, at least about 500 mM, at least about 600 mM, at least about 700 mM, at least about 800 mM, at least about 900 mM, at least about 1M, at least about 1.5M, at least about 2M, at least about 2.5M, at least about 3M, at least about 3.5M, at least about 4M, at least about 4.5M, or at least about 5M acetic acid.

[0158] In some embodiments, the acid wash comprises about 0.01M, about 0.02M, about 0.03M, about 0.04M, about 0.05M, about 0.06M, about 0.07M, about 0.08M, about 0.09M, about 0.1M, about 0.2M, about 0.3M, about 0.4M, about 0.5M, about 0.6M, about 0.7M, about 0.8M, about 0.9M, or about 1.0M glycine-HCl.

[0159] In some embodiments, the acid wash comprises at least 0.01M, at least 0.02M, at least 0.03M, at least 0.04M, at least 0.05M, at least 0.06M, at least 0.07M, at least 0.08M, at least 0.09M, at least 0.1M, at least 0.2M, at least 0.3M, at least 0.4M, at least 0.5M, at least 0.6M, at least 0.7M, at least 0.8M, at least 0.9M, or at least 1.0M glycine-HCl.

[0160] In some embodiments, the acid wash comprises at least about 0.01M, at least about 0.02M, at least about 0.03M, at least about 0.04M, at least about 0.05M, at least about 0.06M, at least about 0.07M, at least about 0.08M, at least about 0.09M, at least about 0.1M, at least about

0.2M, at least about 0.3M, at least about 0.4M, at least about 0.5M, at least about 0.6M, at least about 0.7M, at least about 0.8M, at least about 0.9M, or at least about 1.0M glycine-HCl.

[0161] In some embodiments, the acid wash has a pH of about 0.5, about 1.0, about 1.5, about 2.0, about 2.5, about 3.0, about 3.5, or about 4.0.

[0162] In some embodiments, the acid wash has a pH of at least 0.5, at least 1.0, at least 1.5, at least 2.0, at least 2.5, at least 3.0, at least 3.5, or at least 4.0.

[0163] In some embodiments, the acid wash has a pH of at least about 0.5, at least about 1.0, at least about 1.5, at least about 2.0, at least about 2.5, at least about 3.0, at least about 3.5, or at least about 4.0.

Purifying NABs

[0164] In some embodiments, the method comprises purifying a NAB. In some embodiments, the NAB is purified using affinity chromatography. In some embodiments, the NAB is purified using a microbead-based purification technology. For example, the NAB may be purified using a magnetic bead purification system. In some embodiments, antibody purification comprises the addition of chaotropic ions.

Affinity Antibodies

[0165] The antibodies of the present technology may be labeled, such as with an affinity label or a detection label. Nonlimiting examples of antibody labels include a biotin label, a fluorescent label, an enzyme label (e.g., horseradish peroxidase (HRP) and alkaline phosphatase (AP)), gold nanoparticles, radioisotopes, and drug molecules.

[0166] In some embodiments, the antibodies are conjugated to an additional molecule or compound, forming an antibody conjugate. The conjugation may occur via a covalent linkage.

In some embodiments, the antibodies of the present technology are directly (e.g., labeled via conjugation) or indirectly labeled with an affinity label or a detection label. A detection label may be any label that may be detected directly or indirectly. As used herein, "direct" detection of the detection label includes measuring the presence of the detection label or inducing the detection label to produce a detectable substance.

[0167] For example, the detection label may be a luminescent compound. In some embodiments, the detection is configured to produce a chemiluminescent or ECL signal in the presence of a marker. In some embodiments, the detection label is an enzyme configured to

convert a chromogenic marker into a pigment. For example, the enzyme is a HRP and the chromogenic marker may be 3,3',5,5'-tetramethylbenzidine (TMB). In some embodiments, the detection label comprises a sulfo-Tag. The sulfo-Tag (Ru-tag) may be contacted with a marker comprising tripropylamine (TPA), and the level of the detection label may be measured by applying an electrical current to the marker in the presence of the detection label to produce the ECL signal.

[0168] In some embodiments, the detection label is detected by a meso scale discovery (MSD) assay. In some embodiments, the detection label is detected by ECL. In some embodiments, the detection label is a ruthenium (Ru) metal ion. In some embodiments, the Ru ion is detected when the ion comes in close proximity with an electrode. As used herein, a “close proximity” between the Ru ion and the electrode is a distance between the Ru ion and the electrode that is small enough to cause a detectable oxidation-reduction reaction by ECL as understood by a person of skill in the art. In some embodiments, the solid phase comprises an electrode. In some embodiments, a Ru ion is detected by a charge-coupled device (CCD) camera when it is in close proximity with the electrode.

[0169] In some embodiments, the detection label is detected indirectly. As used herein, “indirect” detection of the detection antibody includes adding a separate component that interacts with the detection label, where the separate component is capable of being detected. For example, a secondary antibody may be added, where the secondary antibody is conjugated to a detectable luminescent probe or enzyme configured to convert a chromogenic substrate into a pigment (e.g., a detection antibody conjugated to a detection label).

[0170] An affinity label may be any molecular label that has affinity with the second affinity label. In some embodiments, a first affinity label is configured to make a covalent bond with the second affinity label. For example, the first affinity label may be biotin and the second affinity label may be streptavidin or vice versa. The first and second affinity labels may be any pairing of affinity labels known to one of ordinary skill in the art. In some embodiments, the first affinity label may be glutathione and the second affinity label may be glutathione S-transferase or vice versa. In some embodiments, the first affinity label may be maltose and the second affinity label may be maltose-binding protein or vice versa. In some embodiments, the first affinity label is chitin and the second affinity label is chitin-binding protein or vice versa.

[0171] In some embodiments, the detection label may be a third affinity label and detecting the detection label further comprises contacting the third affinity label with a fourth

affinity label conjugated to a detectable compound, where the third affinity label binds to the fourth affinity label. For example, the fourth affinity label may be conjugated to a luminescent compound or enzyme configured to convert a chromogenic substrate into a pigment.

[0172] The third affinity label may be any molecular label that has affinity with the fourth affinity label. In some embodiments, the third affinity label is configured to make a covalent bond with the fourth affinity label. For example, the third affinity label may be biotin and the fourth affinity label may be streptavidin or vice versa. The third and fourth affinity labels may be any pairing of affinity labels known to one of ordinary skill in the art. In some embodiments, the third affinity label may be glutathione and the fourth affinity label may be glutathione S-transferase or vice versa. In some embodiments, the third affinity label may be maltose and the fourth affinity label may be maltose-binding protein or vice versa. In some embodiments, the third affinity label is chitin and the fourth affinity label is chitin-binding protein or vice versa.

Methods of Purification

[0173] In some embodiments, purifying the NAb comprises contacting the sample with an anti-TNF α antibody conjugated to a first affinity label (i.e., “affinity antibody”) and contacting the sample containing the NAb and the affinity antibody with a first solid phase coated with a second affinity label. In some embodiments, the affinity antibody is added at a concentration of about 10,000ng/mL-20,000ng/mL, about 20,000ng/mL-30,000ng/mL, about 30,000ng/mL-40,000ng/mL, or about 40,000ng/mL-50,000ng/mL. In some embodiments, the affinity antibody is added at a concentration of about 30,000ng/mL.

[0174] In some embodiments, the affinity antibody is added at a concentration of at least 10,000ng/mL-20,000ng/mL, at least 20,000ng/mL-30,000ng/mL, at least 30,000ng/mL-40,000ng/mL, or at least 40,000ng/mL-50,000ng/mL. In some embodiments, the affinity antibody is added at a concentration of at least 30,000ng/mL.

[0175] In some embodiments, the affinity antibody is added at a concentration of at least about 10,000ng/mL-20,000ng/mL, at least about 20,000ng/mL-30,000ng/mL, at least about 30,000ng/mL-40,000ng/mL, or at least about 40,000ng/mL-50,000ng/mL. In some embodiments, the affinity antibody is added at a concentration of at least about 30,000ng/mL.

[0176] As used herein, “solid phase” may be any medium that remains stationary or temporarily stationary as a mobile phase moves across or through the solid phase, such that components of the mobile phase may interact with the solid phase. A solid phase may include a

plate, chromatography column, microbead, magnetic microbead, or any other solid phase known to one of ordinary skill in the art. In some embodiments, the first solid phase is a microbead, plate, or column. In some embodiments, the first solid phase is a microbead. In some embodiments, the first solid phase is a magnetic microbead.

[0177] In some embodiments, purifying the NAb comprises contacting the sample with the affinity antibody; contacting the sample containing the NAb and the affinity antibody with a first solid phase coated with a second affinity label; and washing the first solid phase. In some embodiments, the solid phase is washed with a wash buffer that does not disrupt the interaction between the NAb and the affinity antibody. In some embodiments, the wash buffer comprises phosphate-buffered saline (PBS). In some embodiments, the wash buffer comprises a phosphate-buffered solution with a surfactant. In some embodiments, the wash buffer comprises Tween-20. In some embodiments, the wash buffer comprises 1x PBS with about 0.05% Tween-20. In some embodiments, the wash buffer comprises about 50mM Tris. In some embodiments, the wash buffer comprises about 0.14 M NaCl. In some embodiments, the wash buffer has a pH of about 8.0. In some embodiments, the wash buffer comprises about 50mM Tris, about 0.14 M NaCl, about 0.05% Tween-20, and has a pH of about 8.0.

[0178] In some embodiments, purifying the NAb comprises contacting the sample with the affinity antibody; contacting the sample containing the NAb and the affinity antibody with a first solid phase coated with a second affinity label; washing the first solid phase; and eluting the NAb from the first solid phase. In some embodiments, the NAb is eluted using an elution buffer that disrupts the interaction between the NAb and the affinity antibody. In some embodiments, the elution buffer comprises glycine and sodium chloride (NaCl). In some embodiments, the elution buffer comprises about 0.1M glycine and about 0.15M NaCl. In some embodiments, the elution buffer is acidic. In some embodiments, the elution buffer has a pH of about 2 to about 3. In some embodiments, the elution buffer has a pH of about 2.2. In some embodiments, the elution buffer has a pH of about 2.8.

[0179] In some embodiments, the NAb purification is performed using an automated purification system. As used herein, an “automated purification system” refers to a system that purifies an antibody according to steps that are in part performed by a robotic or mechanical platform. The automated purification system may be completely automated or partially automated. In some embodiments, a human “loads” the automated purification system with the samples and reagents of the present technology and the automated purification system performs

the remaining steps. For example, the automated purification system may be a KingFisher™ Flex Purification System or another automated purification system that uses magnetic particle separation technology. Other automated purification systems having at least the foregoing features and known to one of skill in the art are within the scope of this technology.

[0180] The method further comprises contacting the purified NAb with the detection antibody, which is an anti-TNF α antibody conjugated to a detection label. The detection label may be any label that may be detected directly or indirectly. As used herein, “direct” detection of the detection label includes measuring the presence of the detection label or inducing the detection label to produce a detectable substance. For example, the detection label may be a luminescent compound. In some embodiments, the detection label comprises an enzyme configured to convert a chromogenic substrate into a pigment. For example, the enzyme is a horseradish peroxidase (HRP) and the chromogenic substrate may be 3,3',5,5'-tetramethylbenzidine (TMB). In some embodiments, the detection label is detected by a meso scale discovery (MSD) assay. In some embodiments, the detection label is detected by electrochemiluminescence. In some embodiments, the detection label is a ruthenium (Ru) metal ion. In some embodiments, the Ru ion is detected when the ion comes in close proximity with an electrode. As used herein, a “close proximity” between the Ru ion and the electrode is a distance between the Ru ion and the electrode that is small enough to cause a detectable oxidation-reduction reaction by electrochemiluminescence as understood by a person of skill in the art. In some embodiments, the TNF α -coated substrate comprises an electrode. In some embodiments, a Ru ion is detected by a CCD camera when it is in close proximity with the electrode.

[0181] In some embodiments, the detection antibody is added at a concentration of about 10,000ng/mL-20,000ng/mL, about 20,000ng/mL-30,000ng/mL, about 30,000ng/mL-40,000ng/mL, or about 40,000ng/mL-50,000ng/mL. In some embodiments, the detection antibody is added at a concentration of about 30,000ng/mL.

[0182] In some embodiments, the detection antibody is added at a concentration of at least 10,000ng/mL-20,000ng/mL, at least 20,000ng/mL-30,000ng/mL, at least 30,000ng/mL-40,000ng/mL, or at least 40,000ng/mL-50,000ng/mL. In some embodiments, the detection antibody is added at a concentration of at least 30,000ng/mL.

[0183] In some embodiments, the detection antibody is added at a concentration of at least about 10,000ng/mL-20,000ng/mL, at least about 20,000ng/mL-30,000ng/mL, at least about 30,000ng/mL-40,000ng/mL, or at least about 40,000ng/mL-50,000ng/mL. In some

[0184] The method of the present technology comprises contacting the purified NAb and the anti-TNF α antibody conjugate with a TNF α -coated substrate. The TNF α -coated substrate may comprise any substrate coated with TNF α by methods known to one of ordinary skill in the art. In some embodiments, the substrate is coated with human TNF α .

[0185] The substrate may be coated with about 50 μ g/mL-200 μ g/mL, about 75 μ g/mL-150 μ g/mL, or about 90 μ g/mL-110 μ g/mL TNF α . In some embodiments, the substrate is coated with about 100 μ g/mL TNF α .

[0186] The substrate may be coated with at least 50 μ g/mL-200 μ g/mL, at least 75 μ g/mL-150 μ g/mL, or at least 90 μ g/mL-110 μ g/mL TNF α . In some embodiments, the substrate is coated with at least 100 μ g/mL.

[0187] The substrate may be coated with at least about 50 μ g/mL-200 μ g/mL, at least about 75 μ g/mL-150 μ g/mL, or at least about 90 μ g/mL-110 μ g/mL TNF α . In some embodiments, the substrate is coated with at least about 100 μ g/mL.

[0188] In some embodiments, the TNF α substrate is blocked to prevent non-specific binding to the substrate. The TNF α substrate may be blocked using any blocking buffer or reagent known to one of ordinary skill in the art. In some embodiments, the substrate is a plate.

[0189] In some embodiments, after the purified NAb and detection antibody are contacted with the TNF α -coated substrate, the substrate is washed. In some embodiments, the TNF α -coated substrate is washed with a wash buffer that does not disrupt interactions between the NAb and the TNF α -coated substrate or interactions between the NAb and the detection antibody. The wash buffer may be configured to remove the NAb that binds the detection antibody from the substrate, but it does not remove the NAb that binds the TNF α -coated substrate. In some embodiments, the substrate is a plate.

[0190] In some embodiments, the method further comprises detecting the detection label. In some embodiments, the detection label is detected by measuring a luminescent compound. In some embodiments, the detection label is detected by inducing an enzyme to convert a chromogenic substrate into the pigment and then detecting the pigment and measuring the presence of the pigment. In some embodiments, detecting the detection label comprises

converting the chromogenic substrate into the pigment and detecting the optical density (OD) of the sample. In some embodiments, the detection label is a Ru metal ion and detecting the detection label comprises using a camera to detect light emitted by a Ru metal ion that is in close proximity to a TNF α -coated substrate comprising an electrode.

[0191] The method further comprises detecting the NAb when the detection antibody binds the NAb instead of the TNF α -coated substrate. When the detection antibody binds an NAb instead of the TNF α -coated substrate, then less of the detection antibody will bind to the substrate, and therefore the detection label will be lower when the NAb is present. In some embodiments, the substrate is a plate. In embodiments where the detection label is detected with a luminescent compound, the presence of the NAb may be measured by lower luminescent intensity. In embodiments where the detection label is detected with a pigment, the presence of the NAb may be measured by a lower density of pigment. In embodiments where the detection label is detected with a pigment, the presence of the NAb may be measured by a higher OD (i.e., more light passes through the sample because there is less pigment). In embodiments where the detection label is an Ru-metal ion, the presence of the NAb may be measured by a higher intensity of light emitted when the Ru-metal ion is in close proximity to the TNF α -coated substrate.

[0192] In some embodiments, the NAb is detected by determining whether the detection label is present at a level below a predetermined level. In some embodiments, the NAb is detected by determining that the OD of the sample is above a predetermined cut-point.

Control Samples

[0193] In some embodiments, the method further comprises performing the method on a negative control sample. In some embodiments, the negative control sample has no detectable NAb. In some embodiments, the negative control sample is a drug-naïve sample from a healthy subject who has not received the target antibody. In some embodiments, the method is performed on the negative control sample in parallel with the sample. As used herein, performing the method “in parallel” includes performing the method on the negative control sample at the same time and/or on the same plate as the sample. Performing the method on the negative control sample comprises (i) treating the negative control sample with an acid wash; (ii) performing the purification steps on the negative control sample; (iii) contacting the negative control sample with the detection antibody; (iv) contacting the negative control sample having the detection antibody with a TNF α -coated substrate; and (v) detecting the absence of NAb by measuring anti-TNF α antibody conjugate in contact with the TNF α -coated substrate.

[0194] In some embodiments, the method further comprises performing the method on a positive control sample. In some embodiments, the positive control sample is a sample from a subject who has received the target antibody. In some embodiments, the positive control sample is a sample that has been shown to contain NAb in a previous assay. The method may be performed on the positive control sample in parallel with the sample. The method may be performed on a sample, a negative control sample, and a positive control sample in parallel.

[0195] In some embodiments, the cut-point is determined by performing the method on a plurality of negative control samples. In some embodiments, the negative control samples are drug-naïve samples from healthy subjects who have never received the target antibody. In some embodiments, the cut-point is determined by performing the method on at least 50 negative control samples. In some embodiments, the OD signal from the 50 negative control samples is normalized by the mean of all negative control samples. In some embodiments, outliers are identified and removed. In some embodiments, the cut-point is identified based on the 0.5%-1%, 1%-1.5%, 1.5%-2%, 2%-2.5%, or 2.5%-3% false-positive rate. In some embodiments, the cut-point is identified based on the 3% false-positive rate. In some embodiments, the cut-point is identified based on the 1% false-positive rate.

[0196] In some embodiments, the method further comprises performing the method on a quality control (QC) sample. One or more QC samples may be included to assess the performance of the method. In some embodiments, the QC sample is a blank sample, matrix spike sample (i.e., a plasma or serum sample spiked with NAb), field duplicate sample, replicate sample, and/or reference material sample. The method may be performed on the QC sample in parallel with the sample. The method may be performed on a sample, a negative control sample, a positive control sample, and a QC sample in parallel.

[0197] In some embodiments, the predetermined cut-point is an OD of 0.5-2, 0.5-1, 1-1.5, or 1.5-2. In some embodiments, the predetermined cut-point is about 1. In some embodiments, the predetermined cut-point is about 0.91.

Sensitivity and Target Tolerance

[0198] In some embodiments, the sensitivity of detecting the NAb is less than about 150ng/mL, less than about 200ng/mL, less than about 250ng/mL, less than about 300ng/mL, less than about 350ng/mL, less than about 400ng/mL, less than about 450ng/mL, less than about 500ng/mL, less than about 550ng/mL, or less than about 600ng/mL. In some embodiments, the

sensitivity of detecting the NAb is less than about 500ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than about 250ng/mL.

[0199] In some embodiments, the sensitivity of detecting the NAb is less than at least 150ng/mL, less than at least 200ng/mL, less than at least 250ng/mL, less than at least 300ng/mL, less than at least 350ng/mL, less than at least 400ng/mL, less than at least 450ng/mL, less than at least 500ng/mL, less than at least 550ng/mL, or less than at least 600ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than at least 500ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than at least 250ng/mL.

[0200] In some embodiments, the sensitivity of detecting the NAb is less than at least about 150ng/mL, less than at least about 200ng/mL, less than at least about 250ng/mL, less than at least about 300ng/mL, less than at least about 350ng/mL, less than at least about 400ng/mL, less than at least about 450ng/mL, less than at least about 500ng/mL, less than at least about 550ng/mL, or less than at least about 600ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than at least about 500ng/mL. In some embodiments, the sensitivity of detecting the NAb is less than at least about 250ng/mL.

[0201] In some embodiments, the target tolerance of detecting the NAb is about 500ng/mL-750ng/mL, about 750ng/mL-1000ng/mL, about 1000ng/mL-1250ng/mL, or about 1250ng/mL-1500ng/mL. In some embodiments, the target tolerance of detecting the NAb is about 1000ng/mL.

[0202] In some embodiments, the target tolerance of detecting the NAb is at least 500ng/mL-750ng/mL, at least 750ng/mL-1000ng/mL, at least 1000ng/mL-1250ng/mL, or at least 1250ng/mL-1500ng/mL. In some embodiments, the target tolerance of detecting the NAb is at least 1000ng/mL.

[0203] In some embodiments, the target tolerance of detecting the NAb is at least about 500ng/mL-750ng/mL, at least about 750ng/mL-1000ng/mL, at least about 1000ng/mL-1250ng/mL, or at least about 1250ng/mL-1500ng/mL. In some embodiments, the target tolerance of detecting the NAb is at least about 1000ng/mL.

Kits

[0204] The present technology further comprises a kit for detecting an NAb against the target antibody comprising the affinity antibody; a first solid phase coated with a second affinity label; the detection antibody; a substrate; and TNF α . The concentration of the reagents in the kit

is formulated to enable a person of ordinary skill in the art to perform the methods of the present technology.

[0205] In some embodiments, the affinity antibody comprises a first affinity label. In some embodiments, the first affinity label is biotin. In some embodiments, the detection antibody comprises a detection label. In some embodiments, the detection label is a luminescent compound, an enzyme configured to convert a chromogenic substrate into a pigment, or biotin. In some embodiments, the detection label is biotin.

[0206] In some embodiments, the kit comprises a negative control. The negative control may be a sample from a drug-naïve subject. In some embodiments, the negative control is a sample that is known to contain no NAb. In some embodiments, the kit comprises a positive control. The positive control may be a sample from a subject that has received the target antibody. In some embodiments, the positive control is a sample known to contain NAb. In some embodiments, the kit may comprise NAb or NAb solution and instructions for making a positive control. For example, the kit may comprise a NAb solution and instructions to “spike” a plasma or serum sample with the NAb solution to prepare the positive control.

[0207] In some embodiments, the kit comprises a “high” positive control (HPC) and a “low” positive control (LPC), where the HPC has a higher concentration than the LPC. For example, the HPC may have a concentration of about 300ng/mL-400ng/mL, about 400ng/mL-500ng/mL, about 500ng/mL-600ng/mL, about 600ng/mL-700ng/mL, about 700ng/mL-800ng/mL, about 800ng/mL-900ng/mL, about 900ng/mL-1000ng/mL, or greater than about 1000ng/mL. The concentration of the LPC may be less than about 1ng/mL, about 1ng/mL-10ng/mL, about 10ng/mL-100ng/mL, about 100ng/mL-200ng/mL, about 200ng/mL-300ng/mL, or about 300ng/mL-500ng/mL.

[0208] In some embodiments, the HPC has a concentration of at least 300ng/mL-400ng/mL, at least 400ng/mL-500ng/mL, at least 500ng/mL-600ng/mL, at least 600ng/mL-700ng/mL, at least 700ng/mL-800ng/mL, at least 800ng/mL-900ng/mL, at least 900ng/mL-1000ng/mL, or greater than at least 1000ng/mL. The concentration of the LPC may be less than at least 1ng/mL, at least 1ng/mL-10ng/mL, at least 10ng/mL-100ng/mL, at least 100ng/mL-200ng/mL, at least 200ng/mL-300ng/mL, or at least 300ng/mL-500ng/mL.

[0209] In some embodiments, the HPC has a concentration of at least about 300ng/mL-400ng/mL, at least about 400ng/mL-500ng/mL, at least about 500ng/mL-600ng/mL, at least about 600ng/mL-700ng/mL, at least about 700ng/mL-800ng/mL, at least about 800ng/mL-

900ng/mL, at least about 900ng/mL-1000ng/mL, or greater than at least about 1000ng/mL. The concentration of the LPC may be less than at least about 1ng/mL, at least about 1ng/mL-10ng/mL, at least about 10ng/mL-100ng/mL, at least about 100ng/mL-200ng/mL, at least about 200ng/mL-300ng/mL, or at least about 300ng/mL-500ng/mL.

[0210] The affinity antibody and/or the detection antibody may be affinity purified. In some embodiments, the affinity antibody and/or the detection antibody may be stored as a lyophilized powder. In some embodiments, the affinity antibody and/or detection antibody may be stored as a reconstituted liquid antibody.

[0211] Any of the components of the kit may be provided at a concentration that is more concentrated than the concentration at which it is used. For example, the components of the kit may be provided as a "stock solution" that must be diluted to the desired concentration of the "working solution." In some embodiments, the stock solution may be about 2X, 5X, 10X, 50X, 100X, 500X, 1000X, 5000X, or 10000X more concentrated than the working solution.

[0212] The affinity antibody and/or the detection antibody may be lyophilized using methods known to one of skill in the art. One or more lyophilizers may be included. Examples of suitable lyophilizers include, for example, Tris-EDTA and sucrose. Any other lyophilizer commonly used in the art is also available. Determining the appropriate lyophilizer as well as the effective amount of other lyophilizers is within the scope of routine skill in the art.

[0213] The kit may include about 50 μ g-100 μ g, about 100 μ g-500 μ g, about 500 μ g-1000 μ g, about 1 μ g-10mg, about 10 μ g-50mg, about 50 μ g-100mg, about 100 μ g-500mg, or about 500 μ g-1000mg of the affinity antibody and/or the detection antibody. The kit may include about 50 μ g-200 μ g of the affinity antibody and/or the detection antibody. The kit may include about 100mg of the affinity antibody and/or the detection antibody. The kit may include about 1mg of the affinity antibody and/or the detection antibody. The purity of the affinity antibody and/or the detection antibody may be about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99%.

[0214] The kit may include at least 50 μ g-100 μ g, at least 100 μ g-500 μ g, at least 500 μ g-1000 μ g, at least 1 μ g-10mg, at least 10 μ g-50mg, at least 50 μ g-100mg, at least 100 μ g-500mg, or at least 500 μ g-1000mg of the affinity antibody and/or the detection antibody. The kit may include at least 50 μ g-200 μ g of the affinity antibody and/or the detection antibody. The kit may include at least 100mg of the affinity antibody and/or the detection antibody. The kit may include at least 1mg of the affinity antibody and/or the detection antibody. The purity of the affinity

antibody and/or the detection antibody may be at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99%.

[0215] The kit may include at least about 50 μ g-100 μ g, at least about 100 μ g-500 μ g, at least about 500 μ g-1000 μ g, at least about 1 μ g-10mg, at least about 10 μ g-50mg, at least about 50 μ g-100mg, at least about 100 μ g-500mg, or at least about 500 μ g-1000mg of the affinity antibody and/or the detection antibody. The kit may include at least about 50 μ g-200 μ g of the affinity antibody and/or the detection antibody. The kit may include at least about 100mg of the affinity antibody and/or the detection antibody. The kit may include at least about 1mg of the affinity antibody and/or the detection antibody. The purity of the affinity antibody and/or the detection antibody may be at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater than 99%.

[0216] In some embodiments, the lyophilized affinity antibody and/or detection antibody may be reconstituted in about 50 μ g-100 μ L, about 100 μ g-150 μ L, about 150 μ g-200 μ L, about 200 μ g-250 μ L, about 250 μ g-300 μ L, about 300 μ g-350 μ L, about 350 μ g-400 μ L, about 400 μ g-450 μ L, about 350 μ g-400 μ L, about 400 μ g-450 μ L, or about 450 μ g-500 μ L of a reconstitution buffer.

[0217] In some embodiments, the lyophilized affinity antibody and/or detection antibody may be reconstituted in at least 50 μ g-100 μ L, at least 100 μ g-150 μ L, at least 150 μ g-200 μ L, at least 200 μ g-250 μ L, at least 250 μ g-300 μ L, at least 300 μ g-350 μ L, at least 350 μ g-400 μ L, at least 400 μ g-450 μ L, at least 350 μ g-400 μ L, at least 400 μ g-450 μ L, or at least 450 μ g-500 μ L of a reconstitution buffer.

[0218] In some embodiments, the lyophilized affinity antibody and/or detection antibody may be reconstituted in at least about 50 μ g-100 μ L, at least about 100 μ g-150 μ L, at least about 150 μ g-200 μ L, at least about 200 μ g-250 μ L, at least about 250 μ g-300 μ L, at least about 300 μ g-350 μ L, at least about 350 μ g-400 μ L, at least about 400 μ g-450 μ L, at least about 350 μ g-400 μ L, at least about 400 μ g-450 μ L, or at least about 450 μ g-500 μ L of a reconstitution buffer.

[0219] The reconstitution buffer may comprise any components known to one of skill in the art. For example, the reconstitution buffer may comprise water, PBS, DMSO, glycerol, or sodium azide.

[0220] The reconstitution buffer may comprise about 10%-15%, about 15%-20%, about 20%-25%, about 25%-30%, or about 30%-35% glycerol. The reconstitution buffer may comprise about 0.02%-0.04%, about 0.04%-0.06%, about 0.06%-0.08%, about 0.08%-0.1%,

about 0.1%-0.12%, about 0.12%-0.14%, or about 0.14%-0.16% sodium azide. In some embodiments, the reconstitution buffer has a pH of about 6 to about 7 or about 7 to about 8. In some embodiments, the reconstitution buffer has a pH of about 7.2.

[0221] The reconstitution buffer may comprise at least 10%-15%, at least 15%-20%, at least 20%-25%, at least 25%-30%, or at least 30%-35% glycerol. The reconstitution buffer may comprise at least 0.02%-0.04%, at least 0.04%-0.06%, at least 0.06%-0.08%, at least 0.08%-0.1%, at least 0.1%-0.12%, at least 0.12%-0.14%, or at least 0.14%-0.16% sodium azide. In some embodiments, the reconstitution buffer has a pH of at least 6 to at least 7 or at least 7 to at least 8. In some embodiments, the reconstitution buffer has a pH of at least 7.2.

[0222] The reconstitution buffer may comprise at least about 10%-15%, at least about 15%-20%, at least about 20%-25%, at least about 25%-30%, or at least about 30%-35% glycerol. The reconstitution buffer may comprise at least about 0.02%-0.04%, at least about 0.04%-0.06%, at least about 0.06%-0.08%, at least about 0.08%-0.1%, at least about 0.1%-0.12%, at least about 0.12%-0.14%, or at least about 0.14%-0.16% sodium azide. In some embodiments, the reconstitution buffer has a pH of at least about 6 to at least about 7 or at least about 7 to at least about 8. In some embodiments, the reconstitution buffer has a pH of at least about 7.2.

[0223] In some embodiments, the kit comprises a reconstitution buffer. In some embodiments, the kit comprises an affinity antibody and/or detection antibody that is reconstituted.

[0224] The kit may include about 1µg/mL-10µg/mL, about 10µg/mL-50µg/mL, about 50µg/mL-100µg/mL, about 100µg/mL-500µg/mL, about 500µg/mL-1000µg/mL, about 1mg/mL-10mg/mL, about 10mg/mL-50mg/mL, about 50mg/mL-100mg/mL, about 100mg/mL-1000mg/mL, or about 1000mg/mL-10000mg/mL of the detection antibody and/or affinity antibody in solution.

[0225] The kit may include at least 1µg/mL-10µg/mL, at least 10µg/mL-50µg/mL, at least 50µg/mL-100µg/mL, at least 100µg/mL-500µg/mL, at least 500µg/mL-1000µg/mL, at least 1mg/mL-10mg/mL, at least 10mg/mL-50mg/mL, at least 50mg/mL-100mg/mL, at least 100mg/mL-1000mg/mL, or at least 1000mg/mL-10000mg/mL of the detection antibody and/or affinity antibody in solution.

[0226] The kit may include at least about 1µg/mL-10µg/mL, at least about 10µg/mL-50µg/mL, at least about 50µg/mL-100µg/mL, at least about 100µg/mL-500µg/mL, at least about

500 μ g/mL-1000 μ g/mL, at least about 1mg/mL-10mg/mL, at least about 10mg/mL-50mg/mL, at least about 50mg/mL-100mg/mL, at least about 100mg/mL-1000mg/mL, or at least about 1000mg/mL-10000mg/mL of the detection antibody and/or affinity antibody in solution.

[0227] The first solid phase may include any solid phase known to one of skill in the art to be useful for antibody affinity purification. In some embodiments, the first solid phase is a microbead. In some embodiments, the first solid phase is a magnetic microbead. The kit may include 1 μ g/mL-10 μ g/mL, 10 μ g/mL-50 μ g/mL, 50 μ g/mL-100 μ g/mL, 100 μ g/mL-500 μ g/mL, or 500 μ g/mL-1000 μ g/mL, 1mg/mL-10mg/mL, 10mg/mL-50mg/mL, 50mg/mL-100mg/mL, 100mg/mL-1000mg/mL, or 1000mg/mL-10000mg/mL of microbeads in solution. The kit may include 0.5mL-1mL, 1mL-1.5mL, 1.5mL-2mL, 2mL-2.5mL, 2.5mL-3mL, 3mL-3.5mL, 3.5mL-4mL, 4mL-4.5mL, or 4.5mL-5mL of microbeads in solution.

[0228] In some embodiments, the second affinity label is streptavidin. In some embodiments, the kit comprises a solid phase, a second affinity label, and instructions for coating the solid phase with the second affinity label.

[0229] The substrate may be any material known to one of skill that is imageable and capable of binding TNF α . For example, the substrate may be a slide, plate, dish, or flask. In some embodiments, the kit comprises a substrate that is coated with TNF α . In some embodiments, the kit comprises a substrate, TNF α , and instructions for coating the substrate with TNF α . For example, the kit may comprise about 1 μ g-5 μ g, 5 μ g-10 μ g, 10 μ g-20 μ g, or 20 μ g-50 μ g of human TNF α . In some embodiments, the TNF α is a 1ng/mL-10ng/mL, 10ng/mL-100ng/mL, 100ng/mL-1000ng/mL, or 1000ng/mL-10000ng/mL solution of human TNF α . The TNF α solution may comprise any media known to one of skill in the art, for example phosphate buffered saline (PBS).

[0230] The kit may comprise one or more buffers. In some embodiments, the kit comprises an acid wash buffer. In some embodiments, the kit further comprises a washing buffer. In some embodiments, the kit further comprises an elution buffer. The buffers of the present technology may be formulated according to any of the formulations of this technology or the formulations known to one of skill in the art.

[0231] In accordance with some embodiments, the kit may contain one or more consumables for performing the methods of the present technology. For example, the kit may include one or more plates containing a plurality of wells. In some embodiments, the plate may include 6, 9, 12, 16, 24, 36, 96, 384, or 1536 wells. In some embodiments, a kit or system

comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than 20 assay plates.

[0232] The kit may also include instructions for performing all or part of the methods described in the present technology. In some embodiments, the kit comprises links (e.g., URLs or QR codes) that direct a user to instructions for performing all or part of the methods of the present technology. The kit may include software or other computer technology to facilitate performing the methods of the present technology.

[0233] The components of the kit may be stored to provide stability to the particular components. For example, components of the kit may be stored in a light-impermeable container if the component comprises a light-sensitive compound. In some embodiments, the components of the kit may be stored in an airtight container. In some embodiments, the components of the kit are stored frozen, in liquid form, or in solid form. In some embodiments, the components of the kit are stored at about -195°C, -80°C, -20°C, 4°C, or 20°C.

[0234] In another embodiment, the kit comprises one or more of the components, consumables, or reagents described in the examples below.

[0235] While the present technology is capable of being embodied in various forms, the description below of several embodiments is made with the understanding that the present disclosure is to be considered as an exemplification of the present technology and is not intended to limit the present technology to the specific embodiments illustrated. Headings are provided for convenience only and are not to be construed to limit the present technology in any manner. Embodiments illustrated under any heading may be combined with embodiments illustrated under any other heading.

[0236] From the foregoing, it will be appreciated that specific embodiments of the present technology have been described herein for purposes of illustration, but that various modifications may be made without deviating from the scope of the present technology. Accordingly, the present technology is not limited except as by the appended claims.

EXAMPLES

Abbreviations

ADA: Anti-Drug Antibody; A.T.: Ambient Temperature; Bio-MAB-16: Biotin Labeled MAB-16; CP: Cut-Point; Conc.: Concentration; %CV: Percent Coefficient of Variation; diH₂O: Deionized Water; ECL: Electrochemiluminescence; ECLIA: Electrochemiluminescence Immunoassay; FT: Freeze/Thaw; HPC: High Positive Control; LPC: Low Positive Control;

MSD: Meso Scale Discovery; NA: Not Applicable; NC: Negative Control; pNHS: Pooled Naïve Human Serum; PBS: Phosphate-Buffered Saline; QC : Quality Control; rECL: Relative ECL; Ru-MAB-16: Ruthenium Labeled MAB-16; SCP: Screening Cut-Point; S-CPF: Screening Cut-Point Factor; SD: Standard Deviation; TCP: Titration Cut-Point; T-CPF: Titration Cut-Point Factor.

Overview:

[0237] FIG. 1 illustrates a method for detecting an NAb against the target antibody in the sample comprises (i) disassociating the NAb from the target antibody by adding an acid wash to the sample 110; (ii) purifying the NAb from the sample 120; (iii) contacting the purified NAb with an anti-TNF α antibody conjugate comprising the anti-TNF α antibody conjugated to a detection label (detection antibody) 130; (iv) contacting the purified NAb and the detection antibody with a TNF α -coated substrate 140; and (v) detecting the NAb by determining whether the detection label is present at a level above a predetermined cut-point 150.

[0238] FIG. 2 illustrates a method for detecting an NAb against the target antibody comprises (i) purifying the NAb in the sample **210**; (ii) determining whether the NAb is present in the sample by performing a competitive binding assay, wherein the NAb is present if it binds the detection antibody with a higher affinity than a binding affinity of the detection antibody to TNF α **220**; and (iii) detecting the NAb **230**; wherein a sensitivity of the method is 250ng/mL or less.

Example 1: Methods for Detecting NABs

[0239] The presence of NABs against XTMAb-16 in human serum based on competitive ligand binding (CLB) assay was assessed. XTAMB-16 is an anti-TNF α monoclonal antibody for the treatment of patients with sarcoidosis. XTMAb-16 is an example of an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 1 and 4 (i.e., a “target antibody”) of the present technology.

Buffers and Solutions

[0240] Assay buffer: 33.3 mL of 30% BSA combined with 5mL of 10% Tween-20 with approximately 800 mL of DPBS. Q.S. (quantum satis) solution to 1.0 L with DPBS. Stored at 2°C-8°C for up to 6 months from the date of preparation.

[0241] Dilution/wash buffer: C16.7 mL of 30% BSA, 100 mL of 10x DPBS (pH7.4 $\pm$ 0.1), 10 mL of 10% Tween-20, 0.5 mL of ProClin 300 combined with approximately 800 mL of

deionized water until dissolved. Q.S. solution to 1.0 L with deionized water. Stored at 2°C-8°C for up to 6 months from the date of preparation.

[0242] Biotin-XTMAB-16 working solution (30,000ng/mL) in 2M Tris-HCl: Diluted Biotin-XTMAB-16 stock solution to 30,000ng/mL in 2M Tris-HCl, pH 9.6.

[0243] Coating solution (100ng/mL of Human TNF α in DPBS): Diluted Human TNF α to 100ng/mL in DPBS.

[0244] Neutralizing buffer: Added 1mL of 1M HEPES (pH 9.0) to 23 mL of Dilution/wash buffer.

[0245] Stop solution (1M Phosphoric acid solution): Diluted 68.0 mL 85% phosphoric acid to 1.0L with diH₂O.

Preparing plate controls

[0246] Preparation of Negative Control (NC): NC is pNHS.

[0247] Preparation of High Positive Controls (HPC): Anti-XTMAB-16 antibody (91F9D4, 0.781mg/mL) was used as NAb PC. The concentration of HPC was 600ng/mL. The final concentration of LPC (220.6ng/mL) was prepared at 99% confidence interval (1% failure rate) after the SCP was calculated.

Table 2: NAb concentrations for controls

	NAb Conc. (ng/ml)	Stock Vol. (μ L)	NC Vol. (μ L)	Total Vol. (μ L)	Resulting Tube	Resulting Conc. (ng/mL)
Stock	781,000.0	5.0	73.1	78.1	PC Int-A	50000.0
PC Int-A	50,000.0	6.0	494.0	500.0	HPC	600.0
HPC	600.0	36.8	63.2	100	LPC	220.6

Analytical procedure

[0248] On day 1, well plates were coated with TNF α (100 μ g/mL) and incubated at 2°C-8°C for 16 to 48 hours. On day 2, controls and samples were added to their respective wells. Dilution/wash buffer and acid wash buffer (2M Acetic Acid) were added to wells. Plates were shaken at A.T. for 30 minutes.

[0249] Add Biotin-XTMAB-16 working solution was added to each well (30,000ng/mL) and allowed to shake at A.T. for 60 minutes.

[0250] Streptavidin Magnetic Beads Solution was added to wells and plate shook at A.T. for 60 minutes. Dilution/wash buffer was added to wells of four additional well plates (further referred to as Wash 1, 2, 3, and 4 Plates).

[0251] 0.1M Glycine, 0.15M NaCl, pH 2.2 (Elution Solution) was added into all wells of the well plates (further referred to as Acid Elution Plate). Wash beads and acid elute.

[0252] Neutralization Buffer was added to wells and mixed. 100 μ L/well of eluted controls/samples was added to a new non-binding plate, and 120 μ L/well of Biotin-XTMAB-16 working solution was added. Plates were shaken at A.T. for 60 $\pm$ 10 mins at 450rpm.

[0253] Wells were washed with wash buffer. Strong Block III was added to each well and plates were shaken at A.T. for 1-2 hours at 450rpm. Coated plates were washed with wash buffer.

[0254] 100 μ L/well of eluted controls/samples were transferred to a TNF α coated plate. Plates were shaken at A.T. for 60 $\pm$ 10 mins at 450rpm and washed with wash buffer. 100 μ L/well of streptavidin-HRP working solution (1:50000) was added to each well and plates were shaken at A.T. for 60 $\pm$ 10 mins at 450rpm.

[0255] Plates were then washed with wash buffer and TMB solution was added to wells.

[0256] Stop Solution was added to each well and plates were read on a platereader at 450nm with a reference filter of 630nm.

[0257] Data Analysis: The raw data (OD) were captured using a SpectraMax M2e plate reader. JMP software was used for the statistical evaluations of the cut-point factor.

Plate control acceptance criteria

[0258] Mean response (OD): NC < Plate Assay Cut-Point $\leq$ LPC < HPC.

[0259] The %CV of the average response value of NC, HPC, and LPC must be $\leq$ 25%. Plate NC is the mean of all accepted NCs. NC identified as outliers (out of the range of Mean $\pm$ 3*SD) were excluded from plate NC calculation.

Table 3: Ranges of assay responses

HPC Lower Limit	0.03
LPC Lower Limit	0.56

LPC Upper Limit	1.27
NC Upper Limit	1.92
(LPC/NC) Lower Limit	0.57
(LPC/NC) Upper Limit	0.85

[0260] The assay cut-point factor was 0.91. A plate-specific assay cut-point was calculated using the following formula:

Plate-specific assay cut-point = the mean OD value of all NC in a plate x the assay cut-point factor

[0261] Two-thirds of the QC samples on every plate, at least half (50%) at each level, were required to meet the acceptance criteria.

Sample acceptance criteria

[0262] The %CV of the average response value of samples was $\leq 25\%$. Samples with mean OD values $\leq$ the plate-specific assay cut-point were classified as positive. Samples with mean OD values $>$ the plate-specific assay cut-point and %CVS 25% were classified as negative.

Validation

[0263] A CLB assay was developed and validated for the detection of NABs against XTMA-B-16 in human serum. Validation parameters for this study included cut-point determination, sensitivity, drug tolerance, intra-assay precision and inter-assay precision, selectivity, matrix interference, specificity, stability, and robustness.

[0264] NC, HPC, and LPC were included in each run. LPC were not included for cut-point and sensitivity runs. Two sets of HPC and LPC, and four sets of NC were included in each NAb assay plate.

[0265] Assay validation included assessment of the following parameters: assay cut-point, establishment of LPC concentration, assay sensitivity and drug tolerance, intra- and inter-assay precision, system suitability (control acceptance criteria), selectivity and matrix interference, specificity, stability, and robustness.

Determination of cut-point

[0266] Assay cut-point data from multiple runs analyzing drug-naïve samples were generated. At least 50 lots of individual human serum samples of healthy volunteers (50

Lots) were screened in a total of six different panels. The experimental design is outlined in Table 4.

[0267] The OD signal was normalized by plate NC mean. After outlier identification and removal, normality tests were performed on the data using JMP software. The assay cut-point factor (ACPF) was calculated based on 1% false-positive rate using the equation below based on the data distribution.

$$ACPF = \text{"Mean"} - 2.33 \times SD \text{ (if data normally distributed) or "1st percentile" (if data not normally distributed)}$$

[0268] A plate-specific screening cut-point was calculated using the following formula:

$$\text{Plate Assay Cut-Point (ACP)} = \text{Mean NC OD values in a plate} \times A\text{-CPF}$$

Table 4: Experimental design and target specifications for cut-point assay

	Experimental Design	Target Specifications
Cut-Point	At least 50 unspiked individual human sera of healthy volunteers matrix samples n: 6 minimum Replicates: 2 Analysts: 2 minimum Days: 3 minimum	Report data - Individual samples demonstrating a %CV > 25.0% will be excluded from the final data set and will not be included in the assay cut-point calculations - At least 80% of the individual lots of sera should be included in the assay cut-point factor calculations. Failed lots due to %CV > 25.0% will be reanalyzed until at least 80% of the individual lots of sera have valid results

Sensitivity

[0269] Sensitivity of competitive ligand binding assay was defined by the lowest concentration at which a positive control antibody preparation consistently provided a positive signal in the assay. The experimental design is outlined in Table 5.

Table 5: Experimental design and target specification for sensitivity assay

	Experimental Design	Target Specifications
--	---------------------	-----------------------

Assay sensitivity in the assay	NAb PCs (600ng/mL) prepared by spiking NAb PC into pooled matrix and then 1.5-fold serially diluted, followed by drug removal treatment. These samples will be measured in the assay. At least 7 dilutions are suggested. At least one dilution point above the plate-specific cut-point will be included in the curve fitting for assay sensitivity evaluation. Replicates: 2 n: 6 minimum Analysts: 2 minimum Days: 3 minimum	• Plate acceptance criteria is met. • Sensitivity is calculated using the linear responses from a diluted NAb PC series to interpolate the expected concentration of NAb PC equal to the ACP. • If the PC titer dilutions do not fall above the assay cut-point or have dilutions that fall above, and subsequent dilutions rise below the assay cut-point, the PC titer for that curve is indeterminate. • The mean concentration from all acceptable assays will be used to describe the relative sensitivity of the assay.
--------------------------------	---	---

The LPC concentration was defined considering the sensitivity values obtained during validation.

Determination of drug tolerance

[0270] XTMAb-16 was prepared in the pNHS at 2X the desired concentration of the sample. Antibody samples were prepared in the pNHS at 2X levels. Each 2X sample was combined 1:1 with each 2X drug sample. The samples were incubated for at least 1 hour at room temperature with agitation prior to analysis. Samples were frozen prior to analysis. The mean response of the PC samples preincubated with drug were compared to the response of matching PC samples. The experimental design is outlined in Table 6.

Table 6: Experimental design and target specifications for drug tolerance assay

Experimental Design	Target Specifications
HPC, LPC, and NC with different concentration levels of XTMAb-16 (200, 100, 50, 25, 12.5, and 0µg/mL). n: 1 minimum	• Plate acceptance criteria is met. • The obtained results will be used to evaluate the effect of XTMAb-16.

Replicates: 2	
Runs: 1 minimum	

Intra-assay and inter-assay precision

[0271] Intra-assay precision was evaluated by analyzing multiple samples of each run acceptance control level in one validation run. Intra-assay precision was calculated using NC, LPC, and HPC responses. Positive control preparations were made from six aliquots each of LPC and HPC and analyzed at 2 replicates each. The NC was analyzed from 6 aliquots and analyzed at 2 replicates each. The experimental design is outlined in Table 7.

Table 7: Experimental design and target specifications for intra-assay precision

	Experimental Design	Target Specifications
Intra-assay precision	NC, LPC, HPC n: 6 each for NC, LPC, and HPC Replicates: 2 Runs: 1 minimum Analysts: 1 minimum Days: 1 minimum	NC - The NC responses will be reported, but no intra-assay precision acceptance criteria will be applied. LPC and HPC - CV ≤ 25.0% between replicates - HPC signal < LPC signal ≤ ACP

[0272] Inter-assay precision was evaluated by analyzing samples of each run acceptance control level across all applicably accepted validation runs. Inter-assay precision was calculated using the LPC/NC ratio. The experimental design is outlined in Table 8.

Table 8: Experimental design and target specifications for inter-assay precision

Validation Items	Experimental Design	Target Specifications
Inter-assay precision	NC, LPC, HPC All NC, LPC, HPC in accepted validation runs. In addition to inter-assay %CV from precision runs, overall statistics for PCs from all validation runs will be presented.	.NC The NC responses will be reported, but no intra-assay precision acceptance criteria will be applied. LPC and HPC

	Runs: 6 minimum Days: 3 minimum	• The CV of LPC/NC ratio, HPC/NC ratio must be $\leq 25.0\%$ across runs (outlier criteria may be applied)
--	--	---

Selectivity and matrix interference

[0273] The selectivity was evaluated by spiking NAb LPC to at least 20 individual sera of sarcoidosis patients. NAb spiked and unspiked matrix samples were analyzed in comparison to the ACP. Once prepared, the samples were preincubated for at least 1 hour at room temperature with agitation, and then frozen at -70°C (-80°C to -60°C) prior to analysis. The experimental design is outlined in Table 9.

Table 9: Experimental design and target specifications for matrix selectivity assays

Experimental Design	Target Specifications
Minimum of 20 individual samples of sarcoidosis patients unspiked (blank) and spiked at LPC level. n: minimum 20 samples (LPC spiked and unspiked) Replicates: 2 Runs: 1 minimum	The method selectivity is considered acceptable if at least 80% of the unspiked samples screen negative, and at least 80% of the spiked samples screen positive (above the cut-point). Samples are positive only if the criteria below are met: • Assay: The mean OD Signal $\leq$ ACP. • Precision (expressed as CV): CV $\leq 25.0\%$ between replicated Samples are negative only if the criteria below are met: • Assay: The mean OD Signal $>$ ACP. If the above criteria are not met, the experiment may be repeated with a larger number of samples and/or different level(s) of NAb, while considering relative sensitivity of assay.

[0274] The selectivity assessment included hemolyzed and Lipemia matrix assessment. A minimum of 6 sets of plate controls at NC and LPC were assessed unspiked and spiked with interferences (hemolyzed whole blood and lipids). The experimental design is outlined in Table 10.

Table 10: Experimental design and target specifications for interference assays

Parameter	Experimental Design	Target Specifications
Hemolysis interference	Minimum of 6 sets of NC, LPC, and HPC unspiked and spiked with 2% hemolyzed whole blood n: minimum 6 Replicates: 2 Runs: 1 minimum	- Plate acceptance criteria is met - The hemolyzed interference is considered acceptable if 80% of the unspiked samples screen negative, and at least 80% of the NAb PC spiked samples screen positive.
Lipemia interference	Minimum of 6 sets of NC, LPC, and HPC unspiked and spiked with 300mg/dL lipids. n: 6 minimum Replicates: 2 Runs: 1 minimum	- Plate acceptance criteria is met - The lipemic interference is considered acceptable if 80% of the unspiked samples screen negative, and at least 80% of the NAb PC spiked samples screen positive.

[0275] Specificity refers to the ability of detecting only the NABs, but not other components in the matrix. In this assay, the tolerance of target (TNF α) interference was determined. Various target concentrations covering the anticipated target levels in the samples were spiked into matrix samples at HPC, LPC, and NC levels. The experimental design is outlined in Table 11.

Table 11: Experimental design and target specifications for specificity assays

Experimental Design	Target Specifications
HPC, LPC, and NC with different concentration levels of target: TNF α (1, 0.5, 0.25 and 0 μ g/mL) n: 1 minimum Replicates: 2 Runs: 1 minimum	- Plate acceptance criteria is met. - The obtained results will be used to evaluate the effect of TNFα.

Stability test

[0276] Combined stressed samples with benchtop (24 ± 6 hours or 8 ± 2 hours) and freeze/thaw (6 or 3 cycles from $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ to A.T.) were tested. The samples were prepared and stored at $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ overnight (at least 12 hours) before being thawed at A.T. for the first cycle. For the subsequent freeze/thaw cycles, samples were frozen for at least 12 hours under the same conditions and then thawed. Samples were left at the benchtop for 2 ± 1 hours before refreezing. The experimental design is outlined in Table 12.

Table 12: Experimental design and target specifications for benchtop freeze/thaw stability assays

Parameter	Experimental Design	Target Specification
Benchtop freeze/thaw stability	NC, LPC, and HPC $n: \geq 3$	%CV $\leq 25.0\%$ between replicates for LPC and HPC - OD response: NC > ACP $\geq$ LPC > HPC At least 66.7% stability samples at each level must meet above acceptance criteria.

Evaluation of assay robustness

[0277] The robustness test included incubation time range. Incubation times are shown in Table 13. Three sets of HPC, LPC, and NC were applied for the robustness assay. Only qualified robustness parameters were used in sample analysis. Acceptance criteria were as follows:

NC, LPC, and HPC: %CV $\leq 25\%$ for each replicate

The mean LPC signal and mean HPC signal must be $\leq$ the assay cut-point calculated for the corresponding plate, the mean NC signal > plate assay cut-point.

All robustness samples at each level must be above acceptance criteria.

Table 13: Incubation times for each incubation step

Incubation Step	Reference Incubation Time (minutes)	Min Incubation Time (minutes)	Max Incubation Time (minutes)
TNF α Coat Incubation	16-48 hours	16 hours	48 hours
Acid disassociation	30 $\pm$ 5 minutes	25 minutes	35 minutes
Biotin-XTMAB-16 incubation with acid treated samples	60 $\pm$ 10 minutes	50 minutes	70 minutes

Streptavidin-coated beads incubation	60±10 minutes	50 minutes	70 minutes
Sample/drug incubation	60±10 minutes	50 minutes	70 minutes
Sample/Biotin-XTMAB-16 incubation	60±10 minutes	50 minutes	70 minutes
Anti-SA HRP conjugate incubation	60±10 minutes	50 minutes	70 minutes

All minimum and maximum incubation times listed above are target times; the actual minimum and maximum times evaluated may be adjusted.

[0278] Results from a minimum of 12 acceptable validation runs were used to determine assay control acceptance criteria to be applied to the assay controls in sample analysis.

Lower limit for HPC = overall mean response -3xSD

Acceptance range for LPC = overall mean response ±3xSD

Upper limit for NC= overall mean response +3xSD

[0279] Acceptance ranges for the ratio of the LPC/NC responses were determined post-validation and will not be applied to validation runs. These acceptance ranges were set to achieve a 1% failure rate.

[0280] The LPC/NC ratio is based on mean responses, where

LPC/NC Ratio Acceptance Range = Ratio Mean ±2.33 x SD

[0281] An assay control table for all acceptable validation runs, as well as the LPC/NC ratio acceptance range table, will be included in the validation report.

Determination of an assay cut-point according to the present technology

[0282] Fifty individual human serum samples were screened six times by two analysts to calculate an assay cut-point factor. For each run, the mean OD signal of each individual serum sample was normalized by dividing the sample mean OD by the plate negative control mean OD. The normalized OD was then log-transformed.

[0283] No sample's %CV was higher than 25.0% and all 300 individual human serum values were used for the screening cut-point analysis.

[0284] Box-plot analysis was performed on all 300 normalized values. The formulas are shown in Table 14. Five (5) analytical outliers were identified.

Table 14: Formulas for identifying outliers

Analytical Outlier	
Q1 (25% quartile)	-0.0129
Q3 (75% quartile)	0.0014
Q3-Q1	0.0143
High end ($Q3+3*(Q3-Q1)$)	0.0443
Low end ($Q1-3*(Q3-Q1)$)	-0.0558

Identification of biological outliers

[0285] After analytical outliers were identified and excluded from analysis, the normalized and log-transformed data for each individual serum sample (295 values) were averaged across the 6 determinations, if available. Box-plot analysis was performed on the 295 averaged normalized values. The formulae for identifying biological outliers is provided in Table 15. No biological outliers were identified.

Table 15: Formulas for identifying biological outliers

Biological Outlier	
Q1 (25% quartile)	-0.0099
Q3 (75% quartile)	-0.0023
Q3-Q1	0.0076
High end ($Q3+3*(Q3-Q1)$)	0.0205
Low end ($Q1-3*(Q3-Q1)$)	-0.0327

Screening data normality test

[0286] The normality of 295 log-transformed normalized values was tested by JMP 14.0 software. FIG. 3 showed that the data is not normally distributed by the Shapiro-Wilk Test. Therefore, a non-parametric approach was used to calculate a cut-point.

Table 16: Calculations for non-parametric assay cut-point factors

Log(rOD) distribution (Not Normally Distributed)	
S-CPF Calculation	
Log(rOD, 0.99)	-0.0400
S-CPF	0.91

Formulas:

Log (Δ CPF, 1% false positive) = 1% percentile of Log(rOD) values

*Plate Assay Cut-Point (ACP) = Mean NC OD values in a plate * ACPF*

Summary

[0287] No %CV failure was identified, and all 300 individual human serum values were used for the assay cut-point analysis. Five (5) analytical outliers and no biological outliers were identified during the assay cut-point determination. The final assay ACPF were determined as 0.91, using a non-parametric approach based on 295 log-transformed normalized data values.

[0288] An example of data analyzed according to the present technology is shown in Tables 17-20, for example. Table 17 shows raw data from one run of samples that were tested according to the methods of the present technology. Table 18 shows log-transformed OD data from 6 screenings of the samples that was used to identify analytical outliers. Table 19 shows the OD data that was used to identify biological outliers after analytical outliers were removed (no biological outliers were identified). Table 20 shows the data that was used to identify the cut-point after all outliers were removed.

Table 17: Raw data

Rep 1	Rep2	Mean	%CV	Plate NC	OD Ratio
1.4054	1.4188	1.4121	0.7	1.4687	0.9615
1.3957	1.3596	1.3777	1.9		0.9380
1.3584	1.3716	1.3650	0.7		0.9294
1.3528	1.3522	1.3525	0.0		0.9209
1.4321	1.3759	1.4040	2.8		0.9559
1.4396	1.4136	1.4266	1.3		0.9713
1.4512	1.4323	1.4418	0.9		0.9817
1.4515	1.4428	1.4472	0.4		0.9854
1.4155	1.4039	1.4097	0.6		0.9598
1.4069	1.393	1.4000	0.7		0.9532
1.4128	1.4164	1.4146	0.2		0.9632
1.391	1.4129	1.4020	1.1		0.9546
1.4182	1.4075	1.4129	0.5		0.9620

Rep 1	Rep2	Mean	%CV	Plate NC	OD Ratio
1.4061	1.3978	1.4020	0.4		0.9546
1.3775	1.3745	1.3760	0.2		0.9369
1.449	1.4306	1.4398	0.9		0.9803
1.3751	1.4128	1.3940	1.9		0.9491
1.3851	1.4074	1.3963	1.1		0.9507
1.4256	1.4293	1.4275	0.2		0.9719
1.4064	1.4246	1.4155	0.9		0.9638
1.4293	1.4322	1.4308	0.1		0.9742
1.3578	1.3535	1.3557	0.2		0.9231
1.436	1.4327	1.4344	0.2		0.9766
1.4722	1.4492	1.4607	1.1		0.9946
1.3534	1.4007	1.3771	2.4		0.9376
1.3941	1.4251	1.4096	1.6		0.9598
1.4258	1.42	1.4229	0.3		0.9688
1.3501	1.3994	1.3748	2.5		0.9361
1.439	1.4404	1.4397	0.1		0.9803
1.3811	1.4222	1.4017	2.1		0.9544
1.4444	1.4407	1.4426	0.2		0.9822
1.4678	1.4518	1.4598	0.8		0.9939

*Each row represents an individual sample

Table 18: Log-transformed OD data

Screening 1	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6
-0.0171	-0.0026	-0.0188	-0.0229	0.0058	-0.0098
-0.0278	-0.0725	0.0154	0.0037	0.0173	-0.0097
-0.0318	0.0023	0.0039	-0.0082	0.0102	-0.0129
-0.0358	-0.0724	0.0086	-0.0396	0.0092	-0.0092
-0.0196	-0.0108	0.0063	-0.0167	0.0251	-0.0099
-0.0126	-0.0013	-0.0028	-0.0121	0.0190	-0.0105
-0.0080	0.0048	0.0181	-0.0024	0.0240	0.0032
-0.0064	-0.0063	0.0050	-0.1006	0.0286	-0.0067
-0.0178	0.0025	-0.0232	-0.0135	-0.0165	-0.0030
-0.0208	0.0109	0.0033	-0.0015	-0.0088	0.0021
-0.0163	0.0075	0.0020	0.0033	-0.0071	-0.0018
-0.0202	0.0003	0.0019	-0.0034	0.0106	-0.0022
-0.0168	0.0101	0.0045	0.0028	0.0057	0.0085
-0.0202	0.0125	-0.0146	-0.0127	0.0039	0.0040
-0.0283	-0.0015	-0.0070	-0.0070	-0.0126	0.0083
-0.0086	0.0044	-0.0064	-0.0166	-0.0160	-0.0013
-0.0227	-0.0063	-0.0309	-0.0159	-0.0254	-0.0016
-0.0220	0.0057	-0.0044	0.0053	-0.0027	0.0007
-0.0124	-0.0066	-0.0013	0.0023	-0.0010	0.0047
-0.0160	0.0016	0.0109	-0.0081	-0.0041	-0.0031
-0.0114	0.0044	0.0140	-0.0004	0.0093	-0.0007
-0.0348	-0.2314	-0.0071	-0.0089	0.0089	0.0012
-0.0103	-0.0037	-0.0049	-0.0017	0.0036	-0.0072
-0.0024	0.0124	0.0009	-0.0049	0.0129	-0.0056
-0.0280	-0.0153	0.0046	-0.0243	-0.0074	-0.0129
-0.0178	-0.0015	0.0221	-0.0104	-0.0042	-0.0156
-0.0138	-0.0037	0.0183	-0.0105	-0.0046	-0.0007
-0.0287	-0.0350	0.0141	-0.0139	-0.0075	-0.0179
-0.0086	-0.0018	0.0148	-0.0033	-0.0519	-0.0101
-0.0203	-0.0018	0.0153	-0.0113	-0.0192	-0.0101

Screening 1	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6
-0.0078	0.0069	0.0144	-0.0173	-0.0185	-0.0013
-0.0027	0.0156	0.0216	-0.0014	0.0038	-0.0036
-0.0200	-0.0097	-0.0101	-0.0088	-0.0164	-0.0018
-0.0049	-0.0027	0.0003	0.0253	-0.0032	0.0038
-0.0122	-0.0057	-0.0017	0.0021	-0.0023	-0.0003
-0.0120	-0.0075	0.0013	-0.0008	0.0008	-0.0123
-0.0085	-0.0158	-0.0105	-0.0010	-0.0106	-0.0146
-0.0033	-0.0037	-0.0023	0.0074	0.0032	-0.0005
0.0007	-0.0067	0.0067	0.0044	-0.0089	-0.0116
0.0036	-0.0113	0.0038	-0.0064	-0.0027	-0.0112
-0.0525	-0.0094	-0.0175	-0.0171	-0.0567	-0.0114
-0.0067	-0.0094	-0.0070	-0.0005	-0.0202	0.0013
-0.0162	-0.0061	0.0026	-0.0062	-0.0253	0.0006
-0.0168	-0.0175	-0.0013	-0.0069	-0.0185	-0.0045
-0.0131	-0.0084	0.0043	-0.0071	-0.0161	-0.0008
-0.0032	-0.0012	0.0013	-0.0027	-0.0096	-0.0002
-0.0067	-0.0141	0.0081	0.0081	-0.0018	0.0003
-0.0137	-0.0207	-0.0035	0.0040	-0.0070	-0.0276
-0.0361	-0.0194	-0.0121	-0.0263	-0.0458	-0.0129

*Each row represents an individual sample

Table 19: Log-transformed OD data with outliers removed

Screening 1	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6	Mean
-0.0171	-0.0026	-0.0188	-0.0229	0.0058	-0.0098	-0.0109
-0.0278	NA	0.0154	0.0037	0.0173	-0.0097	-0.0002
-0.0318	0.0023	0.0039	-0.0082	0.0102	-0.0129	-0.0061
-0.0358	NA	0.0086	-0.0396	0.0092	-0.0092	-0.0133
-0.0196	-0.0108	0.0063	-0.0167	0.0251	-0.0099	-0.0043
-0.0126	-0.0013	-0.0028	-0.0121	0.0190	-0.0105	-0.0034
-0.0080	0.0048	0.0181	-0.0024	0.0240	0.0032	0.0066
-0.0064	-0.0063	0.0050	NA	0.0286	-0.0067	0.0029
-0.0178	0.0025	-0.0232	-0.0135	-0.0165	-0.0030	-0.0119
-0.0208	0.0109	0.0033	-0.0015	-0.0088	0.0021	-0.0025
-0.0163	0.0075	0.0020	0.0033	-0.0071	-0.0018	-0.0021
-0.0202	0.0003	0.0019	-0.0034	0.0106	-0.0022	-0.0022
-0.0168	0.0101	0.0045	0.0028	0.0057	0.0085	0.0025
-0.0202	0.0125	-0.0146	-0.0127	0.0039	0.0040	-0.0045
-0.0283	-0.0015	-0.0070	-0.0070	-0.0126	0.0083	-0.0080
-0.0086	0.0044	-0.0064	-0.0166	-0.0160	-0.0013	-0.0074
-0.0227	-0.0063	-0.0309	-0.0159	-0.0254	-0.0016	-0.0171
-0.0220	0.0057	-0.0044	0.0053	-0.0027	0.0007	-0.0029
-0.0124	-0.0066	-0.0013	0.0023	-0.0010	0.0047	-0.0024
-0.0160	0.0016	0.0109	-0.0081	-0.0041	-0.0031	-0.0031
-0.0114	0.0044	0.0140	-0.0004	0.0093	-0.0007	0.0025
-0.0348	NA	-0.0071	-0.0089	0.0089	0.0012	-0.0081
-0.0103	-0.0037	-0.0049	-0.0017	0.0036	-0.0072	-0.0040
-0.0024	0.0124	0.0009	-0.0049	0.0129	-0.0056	0.0022
-0.0280	-0.0153	0.0046	-0.0243	-0.0074	-0.0129	-0.0139
-0.0178	-0.0015	0.0221	-0.0104	-0.0042	-0.0156	-0.0046
-0.0138	-0.0037	0.0183	-0.0105	-0.0046	-0.0007	-0.0025
-0.0287	-0.0350	0.0141	-0.0139	-0.0075	-0.0179	-0.0148
-0.0086	-0.0018	0.0148	-0.0033	-0.0519	-0.0101	-0.0102

Screening 1	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6	Mean
-0.0203	-0.0018	0.0153	-0.0113	-0.0192	-0.0101	-0.0079
-0.0078	0.0069	0.0144	-0.0173	-0.0185	-0.0013	-0.0039
-0.0027	0.0156	0.0216	-0.0014	0.0038	-0.0036	0.0055
-0.0200	-0.0097	-0.0101	-0.0088	-0.0164	-0.0018	-0.0112
-0.0049	-0.0027	0.0003	0.0253	-0.0032	0.0038	0.0031
-0.0122	-0.0057	-0.0017	0.0021	-0.0023	-0.0003	-0.0034
-0.0120	-0.0075	0.0013	-0.0008	0.0008	-0.0123	-0.0051
-0.0085	-0.0158	-0.0105	-0.0010	-0.0106	-0.0146	-0.0102
-0.0033	-0.0037	-0.0023	0.0074	0.0032	-0.0005	0.0001
0.0007	-0.0067	0.0067	0.0044	-0.0089	-0.0116	-0.0026
0.0036	-0.0113	0.0038	-0.0064	-0.0027	-0.0112	-0.0040
-0.0525	-0.0094	-0.0175	-0.0171	NA	-0.0114	-0.0216
-0.0067	-0.0094	-0.0070	-0.0005	-0.0202	0.0013	-0.0071
-0.0162	-0.0061	0.0026	-0.0062	-0.0253	0.0006	-0.0084
-0.0168	-0.0175	-0.0013	-0.0069	-0.0185	-0.0045	-0.0109
-0.0131	-0.0084	0.0043	-0.0071	-0.0161	-0.0008	-0.0069
-0.0032	-0.0012	0.0013	-0.0027	-0.0096	-0.0002	-0.0026
-0.0067	-0.0141	0.0081	0.0081	-0.0018	0.0003	-0.0010
-0.0137	-0.0207	-0.0035	0.0040	-0.0070	-0.0276	-0.0114
-0.0361	-0.0194	-0.0121	-0.0263	-0.0458	-0.0129	-0.0254

*Each row represents an individual sample

Table 20: Data used to identify cut-point

Screening 1	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6
-0.0171	-0.0026	-0.0188	-0.0229	0.0058	-0.0098
-0.0278	NA	0.0154	0.0037	0.0173	-0.0097
-0.0318	0.0023	0.0039	-0.0082	0.0102	-0.0129
-0.0358	NA	0.0086	-0.0396	0.0092	-0.0092
-0.0196	-0.0108	0.0063	-0.0167	0.0251	-0.0099
-0.0126	-0.0013	-0.0028	-0.0121	0.0190	-0.0105
-0.0080	0.0048	0.0181	-0.0024	0.0240	0.0032
-0.0064	-0.0063	0.0050	NA	0.0286	-0.0067
-0.0178	0.0025	-0.0232	-0.0135	-0.0165	-0.0030
-0.0208	0.0109	0.0033	-0.0015	-0.0088	0.0021
-0.0163	0.0075	0.0020	0.0033	-0.0071	-0.0018
-0.0202	0.0003	0.0019	-0.0034	0.0106	-0.0022
-0.0168	0.0101	0.0045	0.0028	0.0057	0.0085
-0.0202	0.0125	-0.0146	-0.0127	0.0039	0.0040
-0.0283	-0.0015	-0.0070	-0.0070	-0.0126	0.0083
-0.0086	0.0044	-0.0064	-0.0166	-0.0160	-0.0013
-0.0227	-0.0063	-0.0309	-0.0159	-0.0254	-0.0016
-0.0220	0.0057	-0.0044	0.0053	-0.0027	0.0007
-0.0124	-0.0066	-0.0013	0.0023	-0.0010	0.0047
-0.0160	0.0016	0.0109	-0.0081	-0.0041	-0.0031
-0.0114	0.0044	0.0140	-0.0004	0.0093	-0.0007
-0.0348	NA	-0.0071	-0.0089	0.0089	0.0012
-0.0103	-0.0037	-0.0049	-0.0017	0.0036	-0.0072
-0.0024	0.0124	0.0009	-0.0049	0.0129	-0.0056
-0.0280	-0.0153	0.0046	-0.0243	-0.0074	-0.0129
-0.0178	-0.0015	0.0221	-0.0104	-0.0042	-0.0156
-0.0138	-0.0037	0.0183	-0.0105	-0.0046	-0.0007
-0.0287	-0.0350	0.0141	-0.0139	-0.0075	-0.0179
-0.0086	-0.0018	0.0148	-0.0033	-0.0519	-0.0101
-0.0203	-0.0018	0.0153	-0.0113	-0.0192	-0.0101

Screening 1	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6
-0.0078	0.0069	0.0144	-0.0173	-0.0185	-0.0013
-0.0027	0.0156	0.0216	-0.0014	0.0038	-0.0036
-0.0200	-0.0097	-0.0101	-0.0088	-0.0164	-0.0018
-0.0049	-0.0027	0.0003	0.0253	-0.0032	0.0038
-0.0122	-0.0057	-0.0017	0.0021	-0.0023	-0.0003
-0.0120	-0.0075	0.0013	-0.0008	0.0008	-0.0123
-0.0085	-0.0158	-0.0105	-0.0010	-0.0106	-0.0146
-0.0033	-0.0037	-0.0023	0.0074	0.0032	-0.0005
0.0007	-0.0067	0.0067	0.0044	-0.0089	-0.0116
0.0036	-0.0113	0.0038	-0.0064	-0.0027	-0.0112
-0.0525	-0.0094	-0.0175	-0.0171	NA	-0.0114
-0.0067	-0.0094	-0.0070	-0.0005	-0.0202	0.0013
-0.0162	-0.0061	0.0026	-0.0062	-0.0253	0.0006
-0.0168	-0.0175	-0.0013	-0.0069	-0.0185	-0.0045
-0.0131	-0.0084	0.0043	-0.0071	-0.0161	-0.0008
-0.0032	-0.0012	0.0013	-0.0027	-0.0096	-0.0002
-0.0067	-0.0141	0.0081	0.0081	-0.0018	0.0003
-0.0137	-0.0207	-0.0035	0.0040	-0.0070	-0.0276
-0.0361	-0.0194	-0.0121	-0.0263	-0.0458	-0.0129

*Each row represents an individual sample

Validation summary

Assay performance characteristics

[0289] All samples and controls, two sets of LPC and HPC, and at least four sets of NC were evaluated in duplicate. The text files imported from the SpectraMax M2e reader were used as the source data for analysis. The assay cut-point was calculated using JMP 14.0 statistical software. The study-phase bioanalysis acceptance criteria for the OD ranges of HPC, LPC, and NC were calculated by the Responsible Scientist (RS). All mean duplicate (or quadruplicate, where applicable) OD values were assessed in the analysis.

Assay cut-point

[0290] To determine the assay cut-point for the detection of NAb to XTMA16, two analysts tested 50 individual naïve human serum samples. Each sample was tested six times across 12 individual runs. The assay cut-point factor (0.91) was calculated as described in the present technology.

[0291] This cut-point factor was used to calculate a plate-specific assay cut-point for each assay run as follows: plate-specific assay cut-point = 0.91 * mean OD value of NC on each plate. Samples were classified as presumptive positive when their OD value $\leq$ plate-specific assay cut-point.

Sensitivity

[0292] The assay sensitivity was evaluated 12 times. Serial 1.5-fold dilutions of the spiked samples (from 600.00ng/mL to 52.67ng/mL) in pNHS were analyzed. The assessment of sensitivity in human serum was based on the sample with the lowest concentration of ADA that consistently produced a result above the plate-specific assay cut-point. The assay sensitivity was determined for each sensitivity set using a point-to-point linear regression, by interpolation at the assay cut-point. The final assay sensitivity was calculated using a concentration among all the qualified curves. The results showed that the assay sensitivity of the assay to detect anti-XTMA16 antibodies was 102.7ng/mL in human serum.

[0293] The sensitivity data was also used to determine the LPC of the assay. LPC for assay was calculated based on the corresponding sensitivity with a 1% false-positive rate, which is Mean (Sensitivity)+99%, df x SD (Sensitivity), where 99%, df = 2.821 (df= 9). The final LPC concentration for the assay was calculated to be 220.6ng/mL (Table 21).

Table 21: Sensitivity Data

Assay	Sensitivity 1						Sensitivity 2						Sensitivity 3					
	OD	%CV	Condi- tion	Plate NC	Plate ACP	Sensiti- vity	OD	%CV	Condi- tion	Plate NC	Plate ACP	Sensiti- vity	OD	%CV	Condi- tion	Plate NC	Plate ACP	Sensiti- vity
600.00	0.2604	1.6	Pass	1.4687	1.337	NA	0.3387	1.1	Pass	1.4564	1.325	75.36	0.2918	3.2	Pass	1.3510	1.229	56.30
400.00	0.6690	4	Pass				0.6620	1.0	Pass				0.6094	1.0	Pass			
266.67	0.8813	2.8	Pass				0.9692	1.3	Pass				0.9454	0.5	Pass			
177.78	1.0626	0.1	Pass				1.1320	1.1	Pass				1.0566	0.4	Pass			
118.52	1.1970	1.8	Pass				1.2520	0.6	Pass				1.1944	1.9	Pass			
79.01	1.0306	1.3	Pass				1.3164	0.4	Pass				1.1326	2.9	Pass			
52.67	1.2807	1.9	Pass				1.3784	0.1	Pass				1.2444	0.4	Pass			

Assay	Sensitivity 4						Sensitivity 5						Sensitivity 6					
NAb Cone. (ng/mL)	OD	%CV	Condi tion	Plate NC	Plate ACP	Sensiti vity	OD	%CV	Condi tion	Plate NC	Plate ACP	Sensiti vity	OD	%CV	Condi tion	Plate NC	Plate ACP	Sensiti vity
600.00	0.2635	0.4	Pass	1.3421	1.221	60.95	0.3024	1.1	Pass	0.9590	0.873	189.62	0.2988	1.4	Pass	0.9670	0.880	126.88
400.00	0.6370	0.3	Pass				0.6009	0.1	Pass				0.5553	0.4	Pass			
266.67	0.9093	0.8	Pass				0.7891	0.5	Pass				0.7253	0.2	Pass			
177.78	1.0843	0.5	Pass				0.8859	0.9	Pass				0.8435	0.1	Pass			
118.52	1.1730	2.2	Pass				0.9203	0.4	Pass				0.8860	1.5	Pass			
79.01	1.1592	1.4	Pass				0.9759	0.5	Pass				0.9118	1.1	Pass			
52.67	1.2493	0.8	Pass				1.0007	1.8	Pass				0.9309	1.0	Pass			
Assay	Sensitivity 7						Sensitivity 8						Sensitivity 9					
NAb Cone. (ng/mL)	OD	%CV	Condi tion	Plate NC	Plate ACP	Sensiti vity	OD	%CV	Condi tion	Plate NC	Plate ACP	Sensiti vity	OD	%CV	Condi tion	Plate NC	Plate ACP	Sensiti vity
600.00	0.3392	0.1	Pass	1.5392	1.401	NA	0.2718	0.2	Pass	1.5802	1.438	93.39	0.3471	1.8	Pass	1.3061	1.189	142.51
400.00	0.7880	0.3	Pass				0.7035	0.4	Pass				0.7176	3.0	Pass			
266.67	1.0809	0.6	Pass				1.0677	2.4	Pass				0.9649	0.5	Pass			
177.78	1.2406	2.1	Pass				1.2807	1.6	Pass				1.1230	0.7	Pass			
118.52	1.3682	2.2	Pass				1.3702	0.4	Pass				1.2339	1.6	Pass			
79.01	1.4133	0.9	Pass				1.4768	0.1	Pass				1.2570	0.6	Pass			
52.67	1.3692	0.6	Pass				1.4983	3.1	Pass				1.2992	2.4	Pass			

Selectivity

[0294] Selectivity is the ability of the assay to detect anti-XTMAB-16 NAb in the presence of other matrix components. NAb at the LPC level (220.6ng/mL) was spiked into 20 lots of individual diseased human serum. One unspiked sample out of 20 samples tested positive. All 20 NAb spiked samples at the LPC level were confirmed as positive. These results meet the acceptance criteria for selectivity (Table 22).

Table 22: Exemplary Run Selectivity

OD mean	%CV	Plate NC	Plate ACP	Condition	Sample Condition	LPC Spiked Individuals	OD mean	%CV	Condition	Sample Condition
1.2268	4.2	1.2338	1.123	>ACP	Negative	LPC-Indi 01	0.7528	2.0	<ACP	Positive
1.2228	3.5			>ACP	Negative	LPC-Indi 02	0.7745	2.5	<ACP	Positive
1.2613	2.5			>ACP	Negative	LPC-Indi 03	0.8372	2.0	<ACP	Positive
1.2767	0.8			>ACP	Negative	LPC-Indi 04	0.8487	0.1	<ACP	Positive
1.2374	2.1			>ACP	Negative	LPC-Indi 05	0.8643	2.4	<ACP	Positive
1.2552	2.9			>ACP	Negative	LPC-Indi 06	0.8178	0.7	<ACP	Positive
1.2516	0.9			>ACP	Negative	LPC-Indi 07	0.8551	0.9	<ACP	Positive
1.1529	1.0			>ACP	Negative	LPC-Indi 08	0.8557	2.9	<ACP	Positive
1.1208	3.0			<ACP	Positive	LPC-Indi 09	0.7180	3.6	<ACP	Positive
1.2044	2.8			>ACP	Negative	LPC-Indi 10	0.8229	0.8	<ACP	Positive
1.2258	0.8			>ACP	Negative	LPC-Indi 11	0.8200	1.8	<ACP	Positive

OD mean	%CV	Plate NC	Plate ACP	Condition	Sample Condition	LPC Spiked Individuals	OD mean	%CV	Condition	Sample Condition
1.2111	1.5			>ACP	Negative	LPC-Indi 12	0.8056	2.8	<ACP	Positive
1.2248	1.4			>ACP	Negative	LPC-Indi 13	0.8889	1.6	<ACP	Positive
1.2372	0.2			>ACP	Negative	LPC-Indi 14	0.8669	1.1	<ACP	Positive
1.2021	0.2			>ACP	Negative	LPC-Indi 15	0.8745	0.2	<ACP	Positive
1.1847	0.9			>ACP	Negative	LPC-Indi 16	0.8265	0.2	<ACP	Positive
1.1679	0.0			>ACP	Negative	LPC-Indi 17	0.8572	0.7	<ACP	Positive
1.2208	4.1			>ACP	Negative	LPC-Indi 18	0.8451	3.2	<ACP	Positive
1.2226	0.3			>ACP	Negative	LPC-Indi 19	0.8601	3.1	<ACP	Positive
1.2397	0.1			>ACP	Negative	LPC-Indi 20	0.8348	1.8	<ACP	Positive

*each row represents an individual unspiked sample

[0295] Matrix interference was evaluated in 300mg/dL lipemia or 2% hemolyzed samples. Six pooled serum samples with 300mg/dL triglycerides were assessed unspiked and spiked at LPC level (220.6ng/mL) and HPC level (600ng/mL). As shown in FIG. 7, all the unspiked samples were confirmed negative, and all the LPC and HPC spiked samples were confirmed positive. These results meet the acceptance criteria, indicating that 300mg/dL lipemia matrix do not interfere with the assay results.

[0296] Six pooled serum samples with 2% hemolyzed serum were assessed unspiked and spiked at LPC level (220.6ng/mL) and HPC level (600ng/mL). As shown in Table 23, all the unspiked samples were confirmed negative, and all the LPC and HPC spiked samples were confirmed positive. These results meet the acceptance criteria, indicating that 2% hemolyzed matrix do not interfere with the assay results.

Table 23: Matrix Interference Data

Example Run: Lipemia Matrix Interference						Example Run: Hemolyzed Matrix Interference				
Samples	Samples ID	OD mean	%CV	Condition	Sample Condition	Samples ID	OD mean	%CV	Condition	Sample Condition
Unspiked Samples	1	1.4978	3.3	>ACP	Negative	1	1.5336	0.9	>ACP	Negative
	2	1.4829	0.1	>ACP	Negative	2	1.5391	0.3	>ACP	Negative
	3	1.5208	0.4	>ACP	Negative	3	1.5387	0.9	>ACP	Negative
	4	1.5706	0.3	>ACP	Negative	4	1.5860	1.0	>ACP	Negative
	5	1.5369	1.0	>ACP	Negative	5	1.5636	0.3	>ACP	Negative
	6	1.5489	0.0	>ACP	Negative	6	1.5692	0.1	>ACP	Negative
LPC Spiked Samples	1	1.0047	1.1	<ACP	Positive	1	1.0238	1.4	<ACP	Positive
	2	1.0258	2.4	<ACP	Positive	2	1.0360	2.6	<ACP	Positive
	3	1.0292	1.2	<ACP	Positive	3	1.0258	2.5	<ACP	Positive
	4	1.0263	0.4	<ACP	Positive	4	1.0340	0.6	<ACP	Positive
	5	1.0516	0.2	<ACP	Positive	5	1.0778	0.9	<ACP	Positive

Example Run: Lipemia Matrix Interference						Example Run: Hemolyzed Matrix Interference				
Samples	Samples ID	OD mean	%CV	Condition	Sample Condition	Samples ID	OD mean	%CV	Condition	Sample Condition
	6	1.0692	2.3	<ACP	Positive	6	1.0691	2.0	<ACP	Positive
HPC Spiked Samples	1	0.2197	2.3	<ACP	Positive	1	0.2326	1.1	<ACP	Positive
	2	0.2269	1.7	<ACP	Positive	2	0.2111	1.9	<ACP	Positive
	3	0.2161	1.5	<ACP	Positive	3	0.2266	0.7	<ACP	Positive
	4	0.2185	2.5	<ACP	Positive	4	0.2258	3.4	<ACP	Positive
	5	0.2349	10.8	<ACP	Positive	5	0.2505	0.3	<ACP	Positive
	6	0.2266	3.7	<ACP	Positive	6	0.2728	1.5	<ACP	Positive
Plate NC	1.5428					1.5486				
CPF	0.91					0.91				
Plate ACP	1.404					1.409				

Drug tolerance

[0297] The presence of drug (XTMAB-16) in the samples may interfere with the detection of anti-XTMAB-16 NAb and, hence, the ability of the assay system to correctly detect the NAb must be evaluated. Drug tolerance is defined as the highest level of drug that does not change the samples/controls from positive to negative or negative to positive.

[0298] Potential interference by the drug was evaluated using serum prepared at the NC level (0.0ng/mL), LPC level (220.6ng/mL), and HPC level (600.0ng/ml). All three levels of controls were spiked with XTMAB-16 at concentrations at 0.0, 12.5, 25.0, 50.0, 100.0, and 200.0µg/mL.

[0299] As shown in Table 24, no interference was found with the addition of up to 200µg/mL XTMAB-16 to NC, LPC, and HPC levels.

Table 24: Interference Data

Example Run: Drug Tolerance												
Drug Cone. (µg/mL)	NAb PC at NC level (0 ng/mL)				NAb PC at LPC level (220.6 ng/mL)				NAb PC at HPC level (600 ng/mL)			
	resulting tube ID	OD mean	%CV	Condition	resulting tube ID	OD mean	%CV	Condition	resulting tube ID	OD mean	%CV	Condition
200.00	100	1.4147	0.2	>ACP	100	1.0566	3.5	<ACP	100	0.2612	2.1	<ACP
100.00	200	1.4034	0.4	>ACP	200	1.1017	1.8	<ACP	200	0.2320	2.7	<ACP
50.00	300	1.4700	1.1	>ACP	300	1.0561	1.7	<ACP	300	0.2267	0.2	<ACP
25.00	400	1.4626	2.4	>ACP	400	1.0613	0.4	<ACP	400	0.2222	0.8	<ACP
12.50	500	1.4840	1.3	>ACP	500	1.0479	1.7	<ACP	500	0.2216	0.4	<ACP
0.00	600	1.5169	2.1	>ACP	600	1.0572	1.0	<ACP	600	0.2152	1.6	<ACP
Plate NC	1.5343											
CPF	0.91											

Example Run: Drug Tolerance												
Drug Conc. ($\mu\text{g/mL}$)	NAb PC at NC level (0 ng/mL)				NAb PC at LPC level (220.6 ng/mL)				NAb PC at HPC level (600 ng/mL)			
	resulting tube ID	OD mean	%CV	Condition	resulting tube ID	OD mean	%CV	Condition	resulting tube ID	OD mean	%CV	Condition
Plate ACP	1.396											
Drug Tolerance Conc. ($\mu\text{g/mL}$)	>200				>200				>200			

Target tolerance

[0300] The presence of target (TNF α) in the samples may interfere with the detection of anti-XTMAB-16 NAb. Target tolerance is defined as the highest level of target that does not change the samples/controls from positive to negative or negative to positive.

[0301] Potential interference from the target, TNF α , was evaluated using serum prepared at the NC level (0.0ng/mL), LPC level (220.6ng/mL), and HPC level (600.0ng/ml). Four samples of each control level were prepared and spiked with 0.0, 0.25, 0.50, or 1.0 $\mu\text{g/mL}$ TNF α . The addition of 0.96 $\mu\text{g/mL}$ of TNF α interfered with NAb detection in samples spiked at the NC level but no target interference was observed in samples spiked at the HPC and LPC level with up to 1 $\mu\text{g/mL}$ TNF α (Table 25).

Table 25: Interference Data from Target

Example Run: Target Interference						
	Samples ID	OD mean	%CV	Condition	Sample Condition	Target tolerance ($\mu\text{g/mL}$)
NC-Target	1	1.1396	2.7	<ACP	Positive	0.96
	2	1.1799	2.1	>ACP	Negative	
	3	1.1890	0.1	>ACP	Negative	
	4	1.2391	1.6	>ACP	Negative	
LPC-Target	1	0.7038	2.1	<ACP	Positive	>1.0
	2	0.7177	3.3	<ACP	Positive	
	3	0.8447	2.2	<ACP	Positive	
	4	0.8053	2.6	<ACP	Positive	
HPC-Target	1	0.1412	4.5	<ACP	Positive	>1.0
	2	0.1654	7.2	<ACP	Positive	

Example Run: Target Interference						
	Samples ID	OD mean	%CV	Condition	Sample Condition	Target tolerance (µg/mL)
	3	0.1671	5.2	<ACP	Positive	
	4	0.1183	5.6	<ACP	Positive	

Precision

[0302] Intra-assay precision, an evaluation of the within-run performance of the controls, was evaluated in one run using six sets of the HPC (600.0ng/mL), LPC (220.6ng/mL), and NC (0.0ng/mL). The controls should meet the criteria of OD of NC > Plate-specific assay cut-point $\geq$ LPC > HPC for the assay. The CV in intra-assay precision for the OD of HPC and LPC should also meet $\leq 25.0\%$. All plate control meets the above criteria. The results shown in Table 26, demonstrated that the intra-assay precision of the HPC, LPC, and NC were 21.6%, 5.6%, and 3.0%, respectively.

Table 26: Intra-Assay Precision

Example Run: Intra-Assay Precision						
Replicate	HPC		LPC		NC	
	OD mean	%CV	OD mean	%CV	OD mean	%CV
1	0.2021	2.6	0.7895	3.6	1.1375	3.3
2	0.1742	0.4	0.8175	1.1	1.2196	4.5
3	0.2466	3.2	0.8776	1.1	1.2304	4.6
4	0.1709	3.4	0.8782	4.4	1.2015	0.4
5	0.2636	0.8	0.8689	1.0	1.2134	1.1
6	0.2860	2.5	0.9244	3.3	1.2394	0.2
Mean	0.2239		0.8594		1.2070	
%CV	21.6		5.6		3.0	
Plate NC	1.2070					
CPF	0.91					
Plate ACP	1.098					

[0303] Inter-assay precision, an evaluation of the between-run performance of the controls, was evaluated based on the LPC/NC and HPC/NC across all accepted validation runs as listed in Tables 27-29. The inter-assay precision of the LPC/NC and HPC/NC were 8.5% and 24.6%, respectively, after removal of the outliers. The results shown in Tables 27-29 demonstrated that the controls met the inter-assay precision criteria.

Table 27: Inter-Assay Precision (Part 1)

Run ID	Validation Parameter	NC(OD Mean)	LPC (220.6 ng/mL)	HPC (600 ng/mL)	LPC (220.6 ng/mL)/NC	HPC (600 ng/mL)/NC	HPC (600 ng/mL)/NC (after removal of the outliers)
1	Assay Cut Point	1.4687					0.22
	/Sensitivity		NA	0.3943	NA	0.27	0.27
2	Assay Cut Point	1.4564	NA	0.3640	NA	0.25	0.25
	/Sensitivity		NA	0.3504	NA	0.24	0.24
3	Assay Cut Point	1.3510	NA	0.2774	NA	0.21	0.21
	/Sensitivity		NA	0.3482	NA	0.26	0.26
4	Assay Cut Point	1.3421	NA	0.2888	NA	0.22	0.22
	/Sensitivity		NA	0.3022	NA	0.23	0.23
5	Assay Cut Point	0.9590	NA	0.3146	NA	0.33	0.33
	/Sensitivity		NA	0.3218	NA	0.34	0.34
6	Assay Cut Point	0.9670	NA	0.3090	NA	0.32	0.32
	/Sensitivity		NA	0.3092	NA	0.32	0.32
7	Assay Cut Point	1.5392	NA	0.3179	NA	0.21	0.21
	/Sensitivity		NA	0.3903	NA	0.25	0.25
8	Assay Cut Point	1.5802	NA	0.3156	NA	0.20	0.20
	/Sensitivity		NA	0.3014	NA	0.19	0.19
9	Assay Cut Point	1.3061	NA	0.3433	NA	0.26	0.26
	/Sensitivity		NA	0.3664	NA	0.28	0.28
10	Assay Cut Point	1.3913	NA	0.2874	NA	0.21	0.21
	/Sensitivity		NA	0.2984	NA	0.21	0.21
11	Assay Cut Point	1.5605	NA	0.5377	NA	0.34	0.34
	/Sensitivity		NA	0.6667	NA	0.43	NA
12	Assay Cut Point	1.5563	NA	0.4874	NA	0.31	0.31
	/Sensitivity		NA	0.5974	NA	0.38	NA

Run ID	Validation Parameter	NC(OD Mean)	LPC (220.6 ng/mL)	HPC (600 ng/mL)	LPC (220.6 ng/mL)/NC	HPC (600 ng/mL)/NC	HPC (600 ng/mL)/NC (after removal of the outliers)
13	Drug Tolerance	1.5343	1.0383	0.3575	0.68	0.23	0.23
			1.1039	0.3803	0.72	0.25	0.25

Table 28: Inter-Assay Precision (Part 2)

Run ID	Validation Parameter	NC(OD Mean)	LPC (220.6 ng/mL)	HPC (600 ng/mL)	LPC (220.6 ng/mL)/NC	HPC (600 ng/mL)/NC	HPC (600 ng/mL)/NC (After removal of the outliers)
14	Matrix effect (Lipemia)	1.5428	1.0809	0.3461	0.70	0.22	0.22
			1.0947	0.3801	0.71	0.25	0.25
15	Matrix effect (Hemolysis)	1.5486	1.0541	0.3286	0.68	0.21	0.21
			1.0746	0.3926	0.69	0.25	0.25
16	Selectivity	1.2338	0.8508	0.2173	0.69	0.18	0.18
			0.8375	0.2975	0.68	0.24	0.24
17	Intra Assay Precision	1.2070	0.7895	0.2021	0.65	0.17	0.17
			0.8175	0.1742	0.68	0.14	0.14
			0.8776	0.2466	0.73	0.20	0.20
			0.8782	0.1709	0.73	0.14	0.14
			0.8689	0.2636	0.72	0.22	0.22
			0.9244	0.2860	0.77	0.24	0.24
18	Specificity (Target Interference)	1.2556	0.7833	0.1638	0.62	0.13	0.13
			0.8857	0.2837	0.71	0.23	0.23
19	Stability (Bench Top)	1.1412	0.7108	0.2602	0.62	0.23	0.23
			0.7766	0.2589	0.68	0.23	0.23
20	Robustness (Min time)	1.1109	0.8122	0.2194	0.73	0.20	0.20
			0.8292	0.2516	0.75	0.23	0.23
			0.8123	0.2685	0.73	0.24	0.24
21	Stability (Freeze/Thaw)	1.3686	0.8390	0.1386	0.61	0.10	0.10
			1.0212	0.1330	0.75	0.10	0.10

Run ID	Validation Parameter	NC(OD Mean)	LPC (220.6 ng/mL)	HPC (600 ng/mL)	LPC (220.6 ng/mL)/NC	HPC (600 ng/mL)/NC	HPC (600 ng/mL)/NC (After removal of the outliers)
22	Robustness (Max time)	1.2456	0.9377	0.1554	0.75	0.12	0.12
			1.0208	0.2700	0.82	0.22	0.22
			1.0738	0.2958	0.86	0.24	0.24

Table 29: Inter-Assay Precision (Part 3)

	NC	LPC (220.6 ng/mL)	HPC (600 ng/mL) (After removal of the outliers)	LPC (220.6 ng/mL)/NC	HPC (600 ng/mL)/NC	HPC/NC (After removal of the outliers)
Mean	1.3565	0.9151	0.3037	0.7100	0.2338	0.2267
S.D.	0.2	0.1	0.1	0.1	0.1	0.1
%CV	13.8	12.8	29.9	8.5	27.8	24.6

Stability

[0304] The HPC, LPC, and NC controls in pooled human serum were used to assess sample stability. Freeze/thaw (6 cycles of -70°C±10°C to A.T.) and benchtop (23 hours) stability were tested. The acceptance criteria for the stability samples were as follows:

%CV ≤ 25% for the LPC and HPC.

The mean OD: NC > ACP 2: LPC > HPC

At least 66.7% stability samples must meet the above acceptance criteria.

[0305] The tested samples were stored at -70°C (nominal) for at least 12 hours prior to first thaw and at least 12 hours prior to each additional thaw. Samples were at A.T. for 2±1 hours before refreezing. Three aliquots for each control (HPC, LPC, and NC) were tested after undergoing six freeze/thaw cycles on a plate with frozen PCs and NC, which were used for plate acceptance. All stability samples met the acceptance criteria (Table 30); therefore, anti-

XTMAB-16 NAb in human serum are stable up to six freeze/thaw cycles from -70°C (nominal) to A.T.

Table 30: Freeze/Thaw Stability

Example Run: Bench Top Stability (23 hours)				Example Run: Freeze-Thaw Stability (6 cycles)			
Sample ID	OD mean	%CV	Condition (Pass or Fail)	Sample ID	OD mean	%CV	Condition (Pass or Fail)
HPC 1	0.2326	2.7	Pass	FT-HPC 1	0.3012	2.5	Pass
HPC 2	0.2389	3	Pass	FT-HPC2	0.3146	0.4	Pass
HPC 3	0.2215	3.5	Pass	FT-HPC 3	0.3084	0.1	Pass
LPC 1	0.8199	1.6	Pass	FT-LPC 1	0.9661	0.2	Pass
LPC 2	0.8371	1.1	Pass	FT-LPC 2	1.0143	4.4	Pass
LPC 3	0.8119	1.1	Pass	FT-LPC 3	1.0274	1.6	Pass
NC 1	1.0706	4.2	Pass	FT-NC 1	1.2508	6.1	Pass
NC2	1.0743	1.1	Pass	FT-NC2	1.2512	5.7	Pass
NC 3	1.095	0.7	Pass	FT-NC 3	1.2755	2.6	Pass
Plate NC	1.1412			1.3686			
CPF	0.91			0.91			
Plate ACP	1.038			1.245			

[0306] The tested samples were stored at -70°C (nominal) overnight prior to stability testing. Three aliquots of each pre-existing frozen control (HPC, LPC, and NC) were thawed on the benchtop for 23 hours, prior to analysis with frozen PCs and NC, which were used for plate acceptance. All stability samples met the acceptance criteria (Table 30). Therefore, anti-XTMAB-16 NAb in human serum are stable up to 23 hours at A.T. (benchtop).

Study-phase bioanalysis acceptance criteria for controls

[0307] System suitability criteria were based on the data from all valid runs in the validation runs. The %CV criterion for intra-replicate measurements of controls and samples is $\leq 25\%$.

[0308] Acceptance criteria for NC:

Upper limit for NC = overall mean response + 3xSD and > ACP

The upper limit of OD value for NC is 1.92, inclusive.

[0309] Acceptance criteria for LPC:

Acceptance range for LPC = overall mean response $\pm 3xSD$ and $\leq ACP$.

Range OD values for LPC (220.6ng/mL XTMAb-16 NAb in pNHS) is 0.56 to 1.27, inclusive.

[0310] Acceptance criteria for HPC:

Lower limit for HPC = overall mean response - 3xSD and < LPC.

The lower limit of OD value for HPC (600.0ng/mL XTMAb-16 NAb in pNHS) is 0.03, inclusive.

[0311] Acceptance criteria for LPC/INC:

LPC/NC Ratio Acceptance Range = Ratio Mean $\pm 2.33 \times SD$

Range OD values for LPC/NC is 0.57 to 0.85, inclusive.

Assay robustness

[0312] To determine assay robustness, the parameters of the assay were tested. The HPC, LPC, and NC were tested in both assay and confirmatory assay with different incubation times in two runs as per the conditions noted in Table 13.

[0313] The runs met the method acceptance criteria, and the controls were within the established ranges. As shown in Table 31, the assay demonstrated robustness at the maximum and minimum incubation times.

Table 31: Robustness at Minimum and Maximum Incubation Times

Robustness	Example Run: Min Time			Example Run: Max Time		
Sample ID	OD mean	%CV	Condition	OD mean	%CV	Condition
HPC	0.2194	2.2	Pass	0.1554	1.4	Pass
	0.2516	5.2	Pass	0.2700	4.3	Pass
	0.2685	2.1	Pass	0.2958	3.8	Pass
LPC	0.8122	0.3	Pass	0.9377	5.2	Pass
	0.8292	0.4	Pass	1.0208	4.6	Pass
	0.8123	0.7	Pass	1.0738	0.3	Pass
NC	1.0522	4.2	Pass	1.2047	0.4	Pass

Robustness	Example Run: Min Time			Example Run: Max Time		
Sample ID	OD mean	%CV	Condition	OD mean	%CV	Condition
	1.0985	0.3	Pass	1.2222	0.4	Pass
	1.1820	2.3	Pass	1.3099	1.7	Pass
Plate ACP	1.011			1.133		
CPF	0.91					

Table 32: Summary of results

Validation Parameter	Results
Assay cut-point factor	0.91
Relative sensitivity	102.7ng/mL
Selectivity (20 sarcoidosis disease individual samples)	95% unspiked samples were negative 100% spiked samples at 220.6ng/mL (LPC) were positive
Matrix interference (6 hemolyzed and 6 lipemic)	100% unspiked samples were negative 100% spiked samples at 220.6ng/mL (LPC) were positive
Drug tolerance	Up to 200µg/mL at LPC and PC1000
Assay precision	Inter %CV - HPC/NC: 24.6, LPC/NC: 8.5 Intra %CV - HPC/NC: 15.7, LPC/NC: 7.2
Hook effect	No hook effect was observed at concentration up to 20ug/mL
Target interference	No interference was observed at the LPC spiked samples with 1µg/mL of TNFα
Stability	QCs are stable up to 24 hours at 2°C-8°C, and 6 freeze/thaw cycles from -70°C

Additional Embodiments

[0314] Clause 1. A method for detecting a NAb (neutralizing antibody) against a target antibody in a sample comprising: (i) disassociating the NAb from the target antibody by adding an acid wash to the sample; (ii) purifying the NAb in the sample; (iii) contacting the purified NAb with a detection antibody comprising an anti-TNFα antibody conjugated to a detection label; (iv) contacting the purified NAb and the detection antibody with a TNFα-coated substrate;

and (v) detecting the NAb when the detection antibody binds the NAb instead of the TNF α -coated substrate, wherein the target antibody comprises an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20.

[0315] Clause 2. The method of clause 1, further comprising detecting the detection label before detecting the NAb.

[0316] Clause 3. The method of clause 1, further comprising washing the TNF α -coated substrate before detecting the NAb.

[0317] Clause 4. The method of clause 1, wherein the acid wash comprises acetic acid.

[0318] Clause 5. The method of clause 1 or 4, wherein purifying the NAb of step (ii) comprises: (a) contacting the sample with an affinity antibody comprising anti-TNF α antibody conjugated to a first affinity label; (b) contacting the sample with a first solid phase coated with a second affinity label, wherein the first affinity label binds to the second affinity label; (c) washing the first solid phase; and (d) eluting the NAb from the first solid phase.

[0319] Clause 6. The method of clause 5, wherein the first affinity label is biotin.

[0320] Clause 7. The method of clause 5 or 6, wherein the first solid phase is a microbead.

[0321] Clause 8. The method of any one of clauses 5-7, wherein the first solid phase is a magnetic microbead.

[0322] Clause 9. The method of any one of clauses 5-8, wherein the second affinity label is streptavidin.

[0323] Clause 10. The method of any one of clauses 1-9, wherein the NAb is purified using an automated purification system.

[0324] Clause 11. The method of any one of clauses 1-9, wherein the detection label comprises a luminescent compound.

[0325] Clause 12. The method of any one of clauses 1-9, wherein the detection label comprises an enzyme configured to convert a chromogenic substrate into a pigment.

[0326] Clause 13. The method of any one of clauses 1-9, wherein the detection label is a third affinity label.

[0327] Clause 14. The method of clause 13, wherein detecting the detection label further comprises contacting the third affinity label with a fourth affinity label conjugated to a luminescent compound or enzyme configured to convert a chromogenic substrate into a pigment, wherein the third affinity label binds to the fourth affinity label.

[0328] Clause 15. The method of clause 14, wherein the third affinity label is biotin, and the fourth affinity label is streptavidin.

[0329] Clause 16. The method of clause 11 or 14, wherein detecting the detection label comprises detecting the luminescent compound.

[0330] Clause 17. The method of clause 12 or 14, wherein detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting the pigment.

[0331] Clause 18. The method of clause 12 or 14, wherein detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting an optical density of the sample.

[0332] Clause 19. The method of any one of clauses 12 or 14-18, wherein the enzyme is a horseradish peroxidase (HRP).

[0333] Clause 20. The method of clause 19, wherein the chromogenic substrate is 3,3',5,5'-tetramethylbenzidine (TMB).

[0334] Clause 21. The method of any one of clauses 1-20, wherein steps (i) to (v) are performed on a negative control sample having no detectable NAb.

[0335] Clause 22. The method of any of clauses 1-21, wherein the detection antibody binding the NAb instead of the TNF α -coated substrate is detected when the detection label is present at a level below a predetermined level.

[0336] Clause 23. The method of clause 22, wherein the detection label is present at the level below the predetermined level when an optical density of the sample is above a predetermined cut-point.

[0337] Clause 24. The method of clause 23, wherein the predetermined cut-point is the level corresponding to a false-positive rate of 3% or less.

[0338] Clause 25. The method of any one of clauses 23-24, wherein the predetermined cut-point is the level corresponding to a 1% false-positive rate.

[0339] Clause 26. The method of any one of clauses 23-25, wherein the predetermined cut-point is 0.5-2.

[0340] Clause 27. The method of clause 26, wherein the predetermined cut-point is 0.91.

[0341] Clause 28. The method of any one of clauses 1-27, wherein a sensitivity of detecting the NAb is less than 500ng/mL.

[0342] Clause 29. The method of clause 28, wherein the sensitivity of detecting the NAb is less than 250ng/mL.

[0343] Clause 30. The method of any one of clauses 28 or 29, wherein the sensitivity of detecting the NAb is less than 150ng/mL.

[0344] Clause 31. The method of any one of clauses 1-30, wherein a target tolerance is about 1000ng/mL.

[0345] Clause 32. The method of any one of clauses 1-31, wherein the target antibody comprises SEQ ID NOs: 2 and 5.

[0346] Clause 33. The method of any one of clauses 1-32, wherein the target antibody comprises SEQ ID NOs: 1 and 4.

[0347] Clause 34. A method for detecting a NAb against a target antibody in a sample comprising: (i) purifying the NAb in the sample; (ii) determining whether the NAb is present in the sample by performing a competitive binding assay, wherein the NAb is present if the NAb binds the anti-TNF α antibody with a higher affinity than the affinity of the anti-TNF α antibody to TNF α ; and (iii) detecting the NAb; wherein a sensitivity of the method is 250ng/mL or less, wherein the target antibody comprises an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20.

[0348] Clause 35. The method of clause 34, wherein before the NAb is purified, the method further comprises disassociating the NAb from the target antibody by adding an acid wash to the sample.

[0349] Clause 36. The method of clause 34 or 35, wherein the competitive binding assay comprises: (a) contacting the purified NAb with a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; (b) contacting the purified NAb and the detection antibody with a TNF α -coated substrate; (c) washing the TNF α -coated substrate; and (d) detecting the detection label.

[0350] Clause 37. The method of clause 36, wherein detecting the NAb comprises determining whether the detection label is present at a level below a predetermined level.

[0351] Clause 38. The method of clause 37, wherein the detection label is present at the level below the predetermined level when an optical density is above a predetermined cut-point.

[0352] Clause 39. The method of any one of clauses 35-38, wherein the acid wash comprises acetic acid.

[0353] Clause 40. The method of any one of clauses 34-39, wherein purifying the NAb comprises: (a) contacting the sample with an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label; (b) contacting the sample with a first solid phase coated with a second affinity label, wherein the first affinity label binds to the second affinity label; (c) washing the first solid phase; and (d) eluting the NAb from the first solid phase.

[0354] Clause 41. The method of clause 40, wherein the first affinity label is biotin.

[0355] Clause 42. The method of clause 40 or 41, wherein the first solid phase is a microbead.

[0356] Clause 43. The method of any one of clauses 40-42, wherein the first solid phase is a magnetic microbead.

[0357] Clause 44. The method of any one of clauses 40-43, wherein the second affinity label is streptavidin.

[0358] Clause 45. The method of any one of clauses 34-44, wherein the NAb is purified using an automated purification system.

[0359] Clause 46. The method of any one of clauses 36-45, wherein the detection label comprises a luminescent compound.

[0360] Clause 47. The method of any one of clauses 36-45, wherein the detection label comprises an enzyme configured to convert a chromogenic substrate into a pigment.

[0361] Clause 48. The method of any one of clauses 36-45, wherein the detection label is a third affinity label.

[0362] Clause 49. The method of clause 48, wherein detecting the detection label further comprises contacting the third affinity label with a fourth affinity label conjugated to a luminescent compound or enzyme configured to convert a chromogenic substrate into a pigment, wherein the third affinity label binds to the fourth affinity label.

[0363] Clause 50. The method of clause 49, wherein the third affinity label is biotin, and the fourth affinity label is streptavidin.

[0364] Clause 51. The method of clause 46 or 49, wherein detecting the detection label comprises detecting the luminescent compound.

[0365] Clause 52. The method of clause 47 or 49, wherein detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting the pigment.

[0366] Clause 53. The method of clause 47 or 49, wherein detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting an optical density of the sample.

[0367] Clause 54. The method of any one of clauses 47 or 49-52, wherein the enzyme is a horseradish peroxidase (HRP).

[0368] Clause 55. The method of clause 54, wherein the chromogenic substrate is 3,3',5,5'-tetramethylbenzidine (TMB).

[0369] Clause 56. The method of any one of clauses 34-55, wherein steps (i) to (iii) are performed on a negative control sample having no detectable NAb.

[0370] Clause 57. The method of clause 38, wherein the predetermined cut-point is a level corresponding to a 1% false-positive rate.

[0371] Clause 58. The method of clause 38 or 57, wherein the predetermined cut-point is 0.91.

[0372] Clause 59. The method of any one of clauses 34-58, wherein the sensitivity of detecting the NAb is less than 500ng/mL.

[0373] Clause 60. The method of any one of clauses 34-59, wherein the sensitivity of detecting the NAb is less than 250ng/mL.

[0374] Clause 61. The method of any one of clauses 34-60, wherein the sensitivity of detecting the NAb is less than 150ng/mL.

[0375] Clause 62. The method of any one of clauses 34-61, wherein a target tolerance is about 1000ng/mL.

[0376] Clause 63. The method of any one of clauses 34-62, wherein the target antibody comprises SEQ ID NOs: 2 and 5.

[0377] Clause 64. The method of any one of clauses 34-63, wherein the target antibody comprises SEQ ID NOs: 1 and 4.

[0378] Clause 65. A kit for detecting a NAb against a target antibody comprising in a sample comprising: (i) an affinity antibody comprising the target antibody conjugated to a first affinity label; (ii) a first solid phase coated with a second affinity label; (iii) a detection antibody comprising the target antibody conjugated to a detection label; (iv) a substrate; and (v) TNF α , wherein the target antibody comprises an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20.

[0379] Clause 66. The kit of clause 65, wherein the first affinity label is biotin.

[0380] Clause 67. The kit of clause 65 or 66, wherein the second affinity label is streptavidin.

[0381] Clause 68. The kit of any one of clauses 65-67, wherein the detection label is a luminescent compound, an enzyme configured to convert a chromogenic substrate into a pigment, or biotin.

[0382] Clause 69. The kit of any one of clauses 65-68, wherein the first solid phase is a microbead.

[0383] Clause 70. The kit of any one of clauses 65-69, wherein the first solid phase is a magnetic microbead.

[0384] Clause 71. The kit of any one of clauses 65-70, wherein the detection label is biotin.

[0385] Clause 72. The kit of any one of clauses 65-71, further comprising an acid wash buffer.

[0386] Clause 73. The kit of any one of clauses 65-72, further comprising a washing buffer.

[0387] Clause 74. The kit of any one of clauses 65-73, further comprising an elution buffer.

Claims group

CLAIMS

We claim:

1. A method for detecting a neutralizing antibody (NAb) against a target antibody in a sample comprising:

- (i) disassociating the NAb from the target antibody by adding an acid wash to the sample;
- (ii) purifying the NAb in the sample;
- (iii) contacting the purified NAb with a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (iv) contacting the purified NAb and the detection antibody with a TNF α -coated substrate; and
- (v) detecting the NAb when the detection antibody binds the NAb instead of the TNF α -coated substrate,

wherein the target antibody is an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20.

2. The method of claim 1, further comprising detecting the detection label before detecting the NAb.

3. The method of claim 1, further comprising washing the TNF α -coated substrate before detecting the NAb.

4. The method of claim 1, wherein the acid wash comprises acetic acid.

5. The method of claim 1 or 4, wherein purifying the NAb of step (ii) comprises:

- (a) contacting the sample with an affinity antibody comprising anti-TNF α antibody conjugated to a first affinity label;
- (b) contacting the sample with a first solid phase coated with a second affinity label, wherein the first affinity label binds to the second affinity label;
- (c) washing the first solid phase; and

- (d) eluting the NAb from the first solid phase.
6. The method of claim 5, wherein the first affinity label is biotin.
7. The method of claim 5 or 6, wherein the first solid phase is a microbead.
8. The method of any one of claims 5-7, wherein the first solid phase is a magnetic microbead.
9. The method of any one of claims 5-8, wherein the second affinity label is streptavidin.
10. The method of any one of claims 1-9, wherein the NAb is purified using an automated purification system.
11. The method of any one of claims 1-9, wherein the detection label comprises a luminescent compound.
12. The method of any one of claims 1-9, wherein the detection label comprises an enzyme configured to convert a chromogenic substrate into a pigment.
13. The method of any one of claims 1-9, wherein the detection label is a third affinity label.
14. The method of claim 13, wherein detecting the detection label further comprises contacting the third affinity label with a fourth affinity label conjugated to a luminescent compound or enzyme configured to convert a chromogenic substrate into a pigment, wherein the third affinity label binds to the fourth affinity label.
15. The method of claim 14, wherein the third affinity label is biotin, and the fourth affinity label is streptavidin.
16. The method of claim 11 or 14, wherein detecting the detection label comprises

detecting the luminescent compound.

17. The method of claim 12 or 14, wherein detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting the pigment.

18. The method of claim 12 or 14, wherein detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting an optical density of the sample.

19. The method of any one of claims 12 or 14-18, wherein the enzyme is a horseradish peroxidase (HRP).

20. The method of claim 19, wherein the chromogenic substrate is 3,3',5,5'-tetramethylbenzidine (TMB).

21. The method of any one of claims 1-20, wherein steps (i) to (v) are performed on a negative control sample having no detectable NAb.

22. The method of any of claims 1-21, wherein the detection antibody binding the NAb instead of the TNF α -coated substrate is detected when the detection label is present at a level below a predetermined level.

23. The method of claim 22, wherein the detection label is present at the level below the predetermined level when an optical density of the sample is above a predetermined cut-point.

24. The method of claim 23, wherein the predetermined cut-point is the level corresponding to a false-positive rate of 3% or less.

25. The method of any one of claims 23-24, wherein the predetermined cut-point is the level corresponding to a 1% false-positive rate.

26. The method of any one of claims 23-25, wherein the predetermined cut-point is

0.5-2.

27. The method of claim 26, wherein the predetermined cut-point is 0.91.
28. The method of any one of claims 1-27, wherein a sensitivity of detecting the NAb is less than 500ng/mL.
29. The method of claim 28, wherein the sensitivity of detecting the NAb is less than 250ng/mL.
30. The method of any one of claims 28 or 29, wherein the sensitivity of detecting the NAb is less than 150ng/mL.
31. The method of any one of claims 1-30, wherein a target tolerance is about 1000ng/mL.
32. The method of any one of claims 1-31, wherein the target antibody comprises SEQ ID NOs: 2 and 5.
33. The method of any one of claims 1-32, wherein the target antibody comprises SEQ ID NOs: 1 and 4.
34. A method for detecting an NAb against a target antibody in a sample comprising:
- (i) purifying the NAb in the sample;
 - (ii) determining whether the NAb is present in the sample by performing a competitive binding assay, wherein the NAb is present if the NAb binds the anti-TNF α antibody with a higher affinity than the affinity of the anti-TNF α antibody to TNF α ; and
 - (iii) detecting the NAb;
- wherein the target antibody comprises an anti-TNF α chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20, and
- wherein a sensitivity of the method is 250ng/mL or less.

35. The method of claim 34, wherein before the NAb is purified, the method further comprises disassociating the NAb from the target antibody by adding an acid wash to the sample.
36. The method of claim 34 or 35, wherein the competitive binding assay comprises:
- (a) contacting the purified NAb with a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
 - (b) contacting the purified NAb and the detection antibody with a TNF α -coated substrate;
 - (c) washing the TNF α -coated substrate; and
 - (d) detecting the detection label.
37. The method of claim 36, wherein detecting the NAb comprises determining whether the detection label is present at a level below a predetermined level.
38. The method of claim 37, wherein the detection label is present at the level below the predetermined level when an optical density is above a predetermined cut-point.
39. The method of any one of claims 35-38, wherein the acid wash comprises acetic acid.
40. The method of any one of claims 34-39, wherein purifying the NAb comprises:
- (a) contacting the sample with an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label;
 - (b) contacting the sample with a first solid phase coated with a second affinity label, wherein the first affinity label binds to the second affinity label;
 - (c) washing the first solid phase; and
 - (d) eluting the NAb from the first solid phase.
41. The method of claim 40, wherein the first affinity label is biotin.
42. The method of claim 40 or 41, wherein the first solid phase is a microbead.
43. The method of any one of claims 40-42, wherein the first solid phase is a magnetic

microbead.

44. The method of any one of claims 40-43, wherein the second affinity label is streptavidin.

45. The method of any one of claims 34-44, wherein the NAb is purified using an automated purification system.

46. The method of any one of claims 36-45, wherein the detection label comprises a luminescent compound.

47. The method of any one of claims 36-45, wherein the detection label comprises an enzyme configured to convert a chromogenic substrate into a pigment.

48. The method of any one of claims 36-45, wherein the detection label is a third affinity label.

49. The method of claim 48, wherein detecting the detection label further comprises contacting the third affinity label with a fourth affinity label conjugated to a luminescent compound or enzyme configured to convert a chromogenic substrate into a pigment, wherein the third affinity label binds to the fourth affinity label.

50. The method of claim 49, wherein the third affinity label is biotin, and the fourth affinity label is streptavidin.

51. The method of claim 46 or 49, wherein detecting the detection label comprises detecting the luminescent compound.

52. The method of claim 47 or 49, wherein detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting the pigment.

53. The method of claim 47 or 49, wherein detecting the detection label comprises converting the chromogenic substrate into the pigment and detecting an optical density of the

sample.

54. The method of any one of claims 47 or 49-52, wherein the enzyme is a horseradish peroxidase (HRP).

55. The method of claim 54, wherein the chromogenic substrate is 3,3',5,5'-tetramethylbenzidine (TMB).

56. The method of any one of claims 34-55, wherein steps (i) to (iii) are performed on a negative control sample having no detectable NAb.

57. The method of claim 38, wherein the predetermined cut-point is a level corresponding to a 1% false-positive rate.

58. The method of claim 38 or 57, wherein the predetermined cut-point is 0.91.

59. The method of any one of claims 34-58, wherein the sensitivity of detecting the NAb is less than 500ng/mL.

60. The method of any one of claims 34-59, wherein the sensitivity of detecting the NAb is less than 250ng/mL.

61. The method of any one of claims 34-60, wherein the sensitivity of detecting the NAb is less than 150ng/mL.

62. The method of any one of claims 34-61, wherein a target tolerance is about 1000ng/mL.

63. The method of any one of claims 34-62, wherein the target antibody comprises SEQ ID NOs: 2 and 5.

64. The method of any one of claims 34-63, wherein the target antibody comprises SEQ ID NOs: 1 and 4.

65. A kit for detecting a NAb against a target antibody in a sample comprising:
- (i) an affinity antibody comprising the target antibody conjugated to a first affinity label;
 - (ii) a first solid phase coated with a second affinity label;
 - (iii) a detection antibody comprising the target antibody conjugated to a detection label;
 - (iv) a substrate; and
 - (v) $\text{TNF}\alpha$,
- wherein the target antibody is an anti- $\text{TNF}\alpha$ chimeric human-murine monoclonal IgG1-kappa antibody comprising SEQ ID NO: 9-11 or 15-17 and SEQ ID NOs: 12-14 or 18-20.
66. The kit of claim 65, wherein the first affinity label is biotin.
67. The kit of claim 65 or 66, wherein the second affinity label is streptavidin.
68. The kit of any one of claims 65-67, wherein the detection label is a luminescent compound, an enzyme configured to convert a chromogenic substrate into a pigment, or biotin.
69. The kit of any one of claims 65-68, wherein the first solid phase is a microbead.
70. The kit of any one of claims 65-69, wherein the first solid phase is a magnetic microbead.
71. The kit of any one of claims 65-70, wherein the detection label is biotin.
72. The kit of any one of claims 65-71, further comprising an acid wash buffer.
73. The kit of any one of claims 65-72, further comprising a washing buffer.
74. The kit of any one of claims 65-73, further comprising an elution buffer.