

METHODS FOR DETECTING AN ANTI-DRUG ANTIBODY (ADA)
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. 63/460,821, filed on April 20, 2023, U.S. Provisional Patent Application No. 63/461,131, filed on April 21, 2023, and U.S. Provisional Patent Application No. 63/508,436, filed on June 15, 2023. The contents of each of these 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 April 16, 2024, is named 140505_8005_WO00_SL.xml and is 20,939 bytes in size.

TECHNICAL FIELD

[0003] The present technology includes an *in vitro* assay for identifying antibodies, such as an anti-drug antibody (ADA) that bind an anti-TNF α antibody.

SUMMARY

[0004] In some embodiments, the present technology generally relates to methods for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising: (i) incubating the sample with a composition comprising: (a) a first affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; (ii) isolating ADAs that form a complex with both the first affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; and (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point.

[0005] In some embodiments, isolating the ADAs comprises: (a) providing a solid phase comprising the second affinity label; (b) contacting the sample and the composition with the solid phase; and (c) washing the solid phase.

[0006] In some embodiments, the target antibody, the affinity antibody, and/or the detection antibody comprise a chimeric human-murine monoclonal IgG1-kappa antibody. In some embodiments, the target antibody, the affinity antibody, and/or the detection antibody comprise SEQ ID NOs: 1 and 4.

[0007] In some embodiments, the solid phase is a plate.

[0008] In some embodiments, the first affinity label is biotin. In some embodiments, the second affinity label is streptavidin.

[0009] In some embodiments, the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody.

[0010] In some embodiments, measuring the level of the detection label comprises: (1) contacting the solid phase with a marker configured to produce a chemiluminescent or electrochemiluminescent signal when the marker is proximal to the detection label; and (2) detecting the chemiluminescent or electrochemiluminescent signal.

[0011] In some embodiments, the marker is tripropylamine (TPA), and measuring the level of the detection label further comprises applying an electrical current to the marker to produce the chemiluminescent or electrochemiluminescent signal.

[0012] In some embodiments, the solid phase is blocked with a blocking buffer before the sample and the composition are contacted with the solid phase.

[0013] In some embodiments, the methods further comprise performing steps (i) to (iii) on a control sample. In some embodiments, the control sample is incubated with the composition and contacted with the second affinity label.

[0014] In some embodiments, the predetermined cut-point corresponds to a level with a 5% false-positive rate. In some embodiments, the 5% false-positive rate is determined by measuring the detection label in a plurality of control samples and calculating the 5% false-positive rate.

[0015] In some embodiments, the chemiluminescent or electrochemiluminescent signal is measured as electrochemiluminescence (ECL).

[0016] In some embodiments, the predetermined cut-point is an ECL value of at least 1. In some embodiments, the predetermined cut-point is an ECL value of about 1.10.

[0017] In some embodiments, the methods have a sensitivity that is less than 6ng/mL. In some embodiments, the sensitivity is less than 4ng/mL. In some embodiments, the methods have a drug tolerance of about 100ng/mL.

[0018] In some embodiments, the present technology generally relates to methods for detecting an ADA that binds a target antibody comprising an anti-TNF α antibody in a sample comprising: (i) contacting a sample with one or more anti-TNF α conjugates to form a complex between the ADA and the anti-TNF α conjugates; and (ii) measuring a level of anti-TNF α conjugates in the complex; wherein the methods detect the ADA with a sensitivity less than 5ng/mL.

[0019] In some embodiments, the one or more anti-TNF α conjugates comprise an affinity antibody comprising an anti-TNF α antibody conjugated to an affinity label.

[0020] In some embodiments, the one or more anti-TNF α conjugates comprise a detection antibody comprising an anti-TNF α antibody conjugated to a detection label.

[0021] In some embodiments, the affinity label comprises biotin. In some embodiments, the detection label comprises a sulfo-Tag.

[0022] In some embodiments, the methods detect the ADA with a sensitivity less than 3ng/mL. In some embodiments, the methods detect the ADA with a sensitivity of 2.77ng/mL or less.

[0023] In some embodiments, the present technology generally relates to methods for estimating a quantity of an ADA that binds a target antibody comprising an anti-TNF α antibody in a sample comprising: (i) incubating the sample with a composition comprising: (a) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point; (iv) performing

steps (i)-(iii) on a known sample having a known concentration of ADA; and (v) comparing the level of the detection label in the sample to the level of the detection label in the known sample.

[0024] In some embodiments, the known sample is serially diluted into a plurality of titer samples, wherein comparing the level of the detection label in the sample to the level of the detection label in the known sample comprises generating a curve of the levels of the detection label in each of the titer samples and comparing the detection label in the sample to the curve.

[0025] In some embodiments, the present technology generally relates to a methods of confirming that an ADA binds to a target antibody comprising an anti-TNF α antibody in a sample by performing a competitive binding assay comprising (i) incubating the sample with a composition comprising: (a) a first affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; (ii) isolating ADAs that form a complex with both the first affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; and (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point, wherein the composition further comprises an unlabeled anti-TNF α antibody.

[0026] In some embodiments, the ADA is confirmed in the sample by measuring a level of the detection label that is lower by a predetermined factor than the level of the detection label when the unlabeled anti-TNF α antibody is not present.

[0027] In some embodiments, the present technology generally relates to kits for detecting an ADA that binds a target antibody comprising an anti-TNF α antibody in a sample comprising: (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label; (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; and (iii) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label.

[0028] In some embodiments, the solid phase is a plate. In some embodiments, the second affinity label is streptavidin. In some embodiments, the first affinity label is biotin. In some embodiments, the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody.

[0029] In some embodiments, the kit further comprises a marker configured to produce a chemiluminescent or electrochemiluminescent signal where the marker is proximal to the detection label. In some embodiments, the marker is tripropylamine (TPA).

[0030] In some embodiments, the kit further comprises a blocking buffer. In some embodiments, the kit further comprises a wash buffer.

[0031] In some embodiments, the present technology generally relates to a kit for estimating a quantity of an ADA that binds a target antibody comprising an anti-TNF α antibody in a sample comprising: (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label; (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; (iii) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label; and (iv) a positive control. In some embodiments, the positive control is a plurality of titer samples.

[0032] In some embodiments, the present technology generally relates to a kit for confirming that an ADA binds a target antibody comprising an anti-TNF α antibody comprising: (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label; (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; (iii) an unlabeled anti-TNF α antibody; and (iv) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label.

BACKGROUND

[0033] Anti-TNF α (Tumor Necrosis Factor alpha) antibodies are novel biologics that may be used for the treatment of various 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 exhibit an immune response against them. A subject's immunogenicity toward the anti-TNF α antibodies, particularly the subject's development of anti-drug antibodies (ADAs) that bind 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 ADAs and amounts thereof in subjects receiving or having been administered the anti-TNF α antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. 1 shows a flow diagram of an example method according to the present technology.

[0035] FIGS. 2A and 2B show normality of a set of values of percent inhibition that were tested according to the Shapiro-Wilk W Test in an example screening and titration data normality test (FIG. 2A) and in an example confirmatory data normality test (FIG. 2B) according to the methods of the present technology.

DETAILED DESCRIPTION

[0036] The present technology comprises methods for detecting an anti-drug antibody (ADA) 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 NO: 1 and a light chain of SEQ ID NO: 4. The present technology also includes a kit for detecting the target antibody in the sample according to the methods of the present technology.

[0037] 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 technology is to be considered as an exemplification of the 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.

[0038] The use of numerical values in the various quantitative values specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges were both preceded by the word "about." It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about." It is to be understood that such range format is used for convenience and brevity

and should be understood flexibly to include numerical values explicitly specified as limits of a range, but also to include all individual numerical values or sub-ranges encompassed within that range, as if each numerical value and sub-range is explicitly specified. For example, a ratio in the range of about 1 to about 200 should be understood to include the explicitly recited limits of about 1 and about 200, but also to include individual ratios, such as about 2, about 3, and about 4, and sub-ranges, such as about 10 to about 50, about 20 to about 100, and so forth. It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0039] To the extent any materials incorporated by reference herein conflict with the present technology, the present technology controls.

Definitions

[0040] The term "about," as used herein when referring to a measurable value, such as an amount or concentration and the like, is meant to encompass variations of 20%, 10%, 5%, 1%, 0.5%, or even 0.1% of the specified amount.

[0041] 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.

[0042] 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.

[0043] In some embodiments, the method for detecting an ADA that binds the target antibody in a sample comprises: (i) contacting a sample with one or more anti-TNF α conjugates to form a complex between the ADA and the anti-TNF α conjugates; and (ii) measuring a level of anti-TNF α

conjugates in the complex; wherein the method detects the ADA with a sensitivity less than 5ng/mL. In some embodiments, the one or more anti-TNF α conjugates comprise a detection antibody. In some embodiments, measuring a level of anti-TNF α conjugates comprises measuring the level of the detection label. In some embodiments, the one or more anti-TNF α conjugates comprise an affinity antibody.

[0044] As used herein, a “screening assay” generally refers to a method of detecting an ADA that binds the target antibody. For example, a screening assay includes a method of detecting an ADA that binds a target antibody comprising: (i) incubating the sample with a composition comprising: (a) an anti-TNF α antibody conjugated to a first affinity label (affinity antibody), and (b) an anti-TNF α antibody conjugated to a detection label (detection antibody); (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; and (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point. A screening assay may also include a method of detecting an ADA comprising: (i) contacting a sample with one or more anti-TNF α conjugates to form a complex between the ADA and the anti-TNF α conjugates; and (ii) measuring a level of anti-TNF α conjugates in the complex; wherein the method detects the ADA with a sensitivity less than 5ng/mL.

[0045] In some embodiments, measuring the level of the detection label comprises contacting the solid phase with a marker configured to produce a chemiluminescent or electrochemiluminescent (ECL) signal when the marker is proximal to the detection label and detecting the chemiluminescent or ECL signal. As used herein, the marker is “proximal” to the detection label when the marker is at a distance from the detection label that is small enough to cause a reaction that produces a chemiluminescent or ECL signal. In some embodiments, the ECL is produced when the marker is proximal to the detection label and an electrode.

[0046] 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 ADA 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, or separating the blood sample into the plasma sample and/or the serum sample).

Anti-Drug Antibodies (ADAs)

[0047] During or after administration of a drug (e.g., a therapeutic drug) to a subject, the subject's immune system may respond by generating anti-drug antibodies (ADAs). ADAs may bind at least a portion of the drug, thereby sequestering, neutralizing, and/or reducing efficacy of the drug.

[0048] ADAs may be naturally produced as part of a subject's innate immune response. The ADA may be a natural antibody produced by the subject, for example by lymphocytes using variable (diversity) joining (V(D)J) recombination. For example, the ADA may be an immunoglobulin. In some embodiments, the ADA 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

[0049] During or after administration of a drug (i.e., a therapeutic drug), ADAs which bind at least a portion of the drug may develop. In some embodiments, the drug comprises antibodies, and, when ADAs develop, the ADAs may bind the drug antibodies. In some embodiments, the drug antibodies comprise an anti-TNF α antibody.

[0050] Presence of ADAs and/or binding of ADAs 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.

[0051] In some embodiments, the target antibodies comprise a monoclonal antibody.

[0052] 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).

[0053] In some embodiments, the target antibodies (e.g., anti-TNF α 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 (c.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.

[0054] Target Antibody Sequences

[0055] *Heavy Chain:*

[0056] 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.

[0057] 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.

[0058] 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.

[0059] 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.

[0060] 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.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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).

[0066] 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.

[0067] 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.

[0068] 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.

[0069] 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.

[0070] 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.

[0071] 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.

[0072] 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.

[0073] 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.

[0074] 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.

[0075] 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.

[0076] 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.

[0077] 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.

[0078] 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.

[0079] 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.

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] *Light Chain:*

[0094] 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.

[0095] 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.

[0096] 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.

[0097] 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.

[0098] 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.

[0099] 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.

[0100] 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.

[0101] 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.

[0102] 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.

[0103] 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).

[0104] 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.

[0105] 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.

[0106] 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.

[0107] 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.

[0108] 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.

[0109] 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.

[0110] 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.

[0111] 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.

[0112] 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.

[0113] 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.

[0114] 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.

[0115] 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.

[0116] 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.

[0117] 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.

[0118] 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.

[0119] 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.

[0120] 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.

[0121] 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.

[0122] 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.

[0123] 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.

[0124] 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.

[0125] 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.

[0126] 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.

[0127] 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.

[0128] 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.

[0129] 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.

[0130] 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-TNFα Antibodies of the Present Technology

SEQ ID NO:	Description	Sequence
1	aa sequence of heavy chain of anti-TNFα antibody (XTMAB-16, target antibody)	EVKLEESGGGLVQPGGSMKLSCLVSGFIFSNHWMNWVRQ SPEKGLEWVAEIRSKSINSATHYAESVKGRFTISRDDSKSAV YLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL SPGK
2	aa sequence of heavy chain variable region of anti-TNFα antibody (XTMAB-16, target antibody)	EVKLEESGGGLVQPGGSMKLSCLVSGFIFSNHWMNWVRQ SPEKGLEWVAEIRSKSINSATHYAESVKGRFTISRDDSKSAV YLQMTDLRTEDTGVYYCSRNYYGSTYDYWGQGTTLTV
3	aa sequence of heavy chain constant region of anti-TNFα antibody (XTMAB-16, target antibody)	SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPGK
4	aa sequence of light chain of anti-TNFα antibody (XTMAB-16, target antibody)	DILLTQSPAILSVPGERVSFSCRASQFVGSSIHWHYQQRTNG SPRLLIKYASEMSGIPSRFSGSGSGTDFTLSINTVESEDIAD YYCQQSHSWPFTFGSGTNLEVKRTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPV TQKSFNRGEC
5	aa sequence of light chain variable region of anti-TNFα	DILLTQSPAILSVPGERVSFSCRASQFVGSSIHWHYQQRTNG SPRLLIKYASEMSGIPSRFSGSGSGTDFTLSINTVESEDIAD YYCQQSHSWPFTFGSGTNLEV

	antibody (XTMAB-16, target antibody)	
6	aa sequence of light chain constant region of anti-TNF α antibody (XTMAB-16, target antibody)	KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC
7	Nucleotide sequence encoding of light chain of anti- TNF α antibody (XTMAB-16, target antibody)	GCTAGCGAATTCCACCATGGGCTGGTCCTGCATCATCCT GTTTCTGGTGGCCACCGCCACCGGCGTGCCTCCGACAT CCTGCTGACCCAGTCCCCCGCCATCCTGTCCGTGTCTCCC GGCGAGCGGGTGTCTTCTCCTGCCGGGCTCCAGTTC GTGGGCTCCTCCATCCACTGGTATCAGCAGCGGACCAAC GGCTCCCCCTCGGCTGCTGATCAAGTACGCCTCCGAGTCC ATGTCCGGCATCCCTTCCCGGTTCTCCGGCTCCGGCTCTG GCACCGACTTCACCTGTCCATCAACACCGTGGAGTCCG AGGATATTGCCGACTACTACTGCCAGCAGTCCCCTCCT GGCCTTTCACCTTCGGCTCCGGCACCAACCTGGAGGTGA AGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCC GCC ATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGT GTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGT ACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTC CCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAG ACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC ATCAGGGCTGAGCTCGCCCGTCACAAAGAGCTTCAACA GGGGAGAGTGTAA
8	Nucleotide sequence encoding of heavy chain of anti-TNF α antibody (XTMAB-16, target antibody)	GCTAGCGATATCGAATTCTTTTCTTTTGTGTTTGTTCACC ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTGGCCACCG CCACCGGCGTGCCTCCGAGGTGAAGCTGGAGGAGTCTG GCGGCGGACTGGTGCAGCCTGGCGGCTCCATGAAGCTGT CCTGCGTGGCCTCCGGCTTCATCTTCTCCAACCACTGGAT GAACTGGGTGCGCCAGTCCCCTGAGAAGGGCCTGGAGT GGGTGGCCGAGATCCGGTCCAAGTCCATCAACTCCGCCA CCCACTACGCCGAGTCCGTGAAGGGCCGGTTACCATCT CCCGGGACGACTCCAAGTCCGCCGTGTACCTGCAGATGA CCGACCTGCGGACCGAGGACACCGGAGTGTACTACTGCT CCCGGAACTACTACGGCTCCACCTACGACTACTGGGGCC AGGGCACCACTGACCGTGAGCTCCGCCTCCACCAAGG GCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCA CCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG ACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTCAAG GCGCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCC TACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGA CCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTCGAGCCCAATCTTGTGACAAAACCTCACAC

		ATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACC GTCAGTCTTCCTCTTCCCCCAAAACCCAAGGACACCCT CATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGT GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACT GGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGT GGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAA TGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAG GGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGATGAGCTGACCAAGAACCAGGTACGCTGACCT GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGG AGTGGGAGAGTAATGGGCAGCCGGAGAACAATACTACAAG ACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCT CCGGGTAAATAA
9	aa sequence of heavy chain CDR1 predicted by CDR-IMGT of anti-TNF α antibody (XTMAB-16, target antibody)	GFIFSNHW
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	QFVGSS

	CDR-IMGT of anti-TNF α antibody (XTMAB-16, target antibody)	
	aa sequence of light chain CDR2 predicted by CDR-IMGT of anti-TNF α antibody (XTMAB-16, target antibody)	YA
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

[0131] Antibody Labels

[0132] The target 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.

[0133] In some embodiments, the target antibodies are conjugated to an additional molecule or compound, forming an antibody conjugate. The conjugation may occur via a covalent linkage.

[0134] In some embodiments, the target 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. 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.

[0135] 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.

[0136] 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).

[0137] 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.

[0138] 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.

[0139] 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.

Compositions

[0140] The present technology comprises compositions having antibodies for detecting ADAs. In some embodiments, the compositions comprise one or more target antibodies that are bound by ADAs. The one or more target antibodies bound by ADAs may include an affinity antibody and/or a detection antibody. Both the affinity antibody and the detection antibody may be anti-TNF α antibodies and/or may comprise one or more of a heavy chain, a light chain, a variable region (e.g., V_H or V_L), a constant region (e.g., a heavy chain constant region or a light chain constant region), or a CDR of the present technology.

[0141] In some embodiments, the affinity antibody comprises an affinity label. The affinity antibody may be labeled with the affinity label directly or indirectly. Nonlimiting examples of affinity labels include fluorescent labels, enzyme labels (e.g., horseradish peroxidase and alkaline phosphatase), biotin labels, streptavidin labels, and radioisotopes. In some embodiments, the detection antibody is conjugated to a detection label. Nonlimiting examples of detection labels include

a biotin label, a fluorescent label, an enzyme label (e.g., horseradish peroxidase and alkaline phosphatase), gold nanoparticles, and radioisotopes.

[0142] In some embodiments, the compositions further comprise a second affinity antibody. The second affinity antibody may bind an affinity label, such as an affinity label conjugated to the first affinity antibody described in supra. In some embodiments, the second affinity antibody binds the affinity label when the first affinity antibody and/or the detection antibody are bound by one or more ADAs. The second affinity antibody may be an unlabeled antibody. Nonlimiting examples of unlabeled antibodies include an antibody that is not conjugated to an affinity label or a detection label.

[0143] In some embodiments, the compositions comprise a single composition having a first affinity antibody of the present technology, a detection antibody of the present technology, and a second affinity antibody of the present technology. In some embodiments, the compositions comprise (1) a first composition having a first affinity antibody and a detection antibody and (2) a second composition having a second affinity antibody. In some embodiments, the compositions comprise (1) a first composition having a first affinity antibody, (2) a second composition having a detection antibody, and (3) a third composition having a second affinity antibody. In some embodiments, the compositions comprise (1) a first composition having a first affinity antibody and (2) a second composition having a detection antibody and a second affinity antibody. In some embodiments, the compositions comprise (1) a first composition having a first affinity antibody and a second affinity antibody and (2) a second composition having a detection antibody. In some embodiments, the first and second compositions are incubated with a sample simultaneously or sequentially. In some embodiments, the first and third compositions are incubated with a sample simultaneously or sequentially. In some embodiments, the second and third compositions are incubated with a sample simultaneously or sequentially. In some embodiments, the first, second, and third compositions are each incubated with a sample simultaneously or sequentially.

[0144] Samples may comprise biological samples. Nonlimiting examples of biological samples include serum, blood, synovial fluid, plasma, saliva, and respiratory secretions.

Methods of Detecting ADAs

[0145] The present technology comprises methods for detecting the ADAs of the present technology in a sample. ADAs may be detected and/or isolated using the compositions of the present technology.

[0146] In some embodiments, detecting the ADA comprises incubating the sample with a composition of the present technology (e.g., a composition comprising one or more of an affinity antibody, a detection antibody, or an unlabeled antibody). In some embodiments, the affinity antibody is conjugated to the detection label.

[0147] In some embodiments, detecting the ADA comprises contacting the sample with a composition comprising a first affinity antibody of the present technology. In some embodiments, the affinity antibody is added at a concentration of about 1ng/mL to about 10ng/mL, about 10ng/mL to about 20ng/mL, about 20ng/mL to about 50ng/mL, or about 50ng/mL about 100ng/mL.

[0148] In some embodiments, the affinity antibody is added at a concentration of at least 1ng/mL to at least 10ng/mL, at least 10ng/mL to at least 20ng/mL, at least 20ng/mL to at least 50ng/mL, or at least 50ng/mL at least 100ng/mL.

[0149] In some embodiments, the affinity antibody is added at a concentration of at least about 1ng/mL to at least about 10ng/mL, at least about 10ng/mL to at least about 20ng/mL, at least about 20ng/mL to at least about 50ng/mL, or at least about 50ng/mL at least about 100ng/mL.

[0150] In some embodiments, the affinity antibody is added at a concentration of about 25ng/mL.

[0151] In some embodiments, the affinity antibody is added at a concentration of at least 25ng/mL.

[0152] In some embodiments, the affinity antibody is added at a concentration of at least about 25ng/mL.

[0153] In some embodiments, detecting the ADA comprises incubating the sample with a composition comprising a detection antibody.

[0154] In some embodiments, the detection antibody is added at a concentration of about 0.001mg/mL to about 0.01mg/mL, about 0.01mg/mL to about 0.1mg/mL, about 0.1mg/mL to about 1mg/mL, about 1mg/mL to about 10mg/mL. In some embodiments, the affinity antibody is added at

a concentration of about 1.2mg/mL. In some embodiments, the detection antibody comprises a ruthenium label (i.e., Ru-XTMAB-16), and it is added at a concentration of about 1.2mg/mL.

[0155] In some embodiments, the detection antibody is added at a concentration of at least 0.001mg/mL to at least 0.01mg/mL, at least 0.01mg/mL to at least 0.1mg/mL, at least 0.1mg/mL to at least 1mg/mL, at least 1mg/mL to at least 10mg/mL. In some embodiments, the affinity antibody is added at a concentration of at least 1.2mg/mL. In some embodiments, the detection antibody comprises a ruthenium label (i.e., Ru-XTMAB-16), and it is added at a concentration of at least 1.2mg/mL.

[0156] In some embodiments, the detection antibody is added at a concentration of at least about 0.001mg/mL to at least about 0.01mg/mL, at least about 0.01mg/mL to at least about 0.1mg/mL, at least about 0.1mg/mL to at least about 1mg/mL, at least about 1mg/mL to at least about 10mg/mL. In some embodiments, the affinity antibody is added at a concentration of at least about 1.2mg/mL. In some embodiments, the detection antibody comprises a ruthenium label (i.e., Ru-XTMAB-16), and it is added at a concentration of at least about 1.2mg/mL.

[0157] In some embodiments, the methods further comprise isolating an ADA that binds a target antibody of the present technology (e.g., an affinity antibody or a detection antibody). As used herein, “isolating” ADAs that form a complex with both the affinity antibody and the detection antibody includes identifying the ADAs that form a complex and separating them from ADAs that do not form a complex. Isolating ADAs also includes identifying and separating ADAs from other compounds that bind the target antibody. Isolating ADAs may be performed using any technique known to one of skill for isolating or purifying an antibody. For example, the ADAs may be isolated using antibody pull-down or purification techniques. In some embodiments, the ADAs are isolated by contacting the ADA complex with a solid phase comprising a second affinity label, such that the first affinity label of the ADA complex binds to the second affinity label on a solid phase.

[0158] 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 solid phase is a microbead, plate, or column. In some embodiments,

the solid phase is a plate. In some embodiments, the plate is coated with a second affinity label. In some embodiments, the solid phase is blocked with a blocking buffer before the sample and the composition are contacted with the solid phase.

[0159] In some embodiments, isolating the ADA complex comprises: (a) providing a solid phase comprising the second affinity label; (b) contacting the sample and the composition with the solid phase; and (c) washing the solid phase. In some embodiments, the solid phase is washed with a wash buffer that does not disrupt the interaction between the ADA and the affinity antibody or the interaction between the ADA and the detection 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 0.05% Tween-20. In some embodiments, the wash buffer comprises 50mM Tris. In some embodiments, the wash buffer comprises 0.14 M NaCl. In some embodiments, the wash buffer has a pH of about 8.0. In some embodiments, the wash buffer comprises 50mM Tris, 0.14 M NaCl, 0.05% Tween-20, and has a pH of about 8.0.

[0160] 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 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 of the sample. In some embodiments, detecting the detection label comprises contacting a sulfo-Tag (Ru-tag) detection label with a marker comprising TPA) and the level of the detection label may be detected by applying an electrical current to the marker in the presence of the detection label to produce a detectable ECL signal. As such, a higher level of the detection label correlates with a higher amount of ADAs in the sample.

[0161] In some embodiments, the ADA is detected by determining whether the detection label is present at a level above a predetermined level. In some embodiments, the ADA is detected by determining that the ECL of the sample is above a predetermined cut-point.

[0162] 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 ADA.

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) incubating the control sample with a composition comprising an affinity antibody and a detection antibody; (ii) contacting the complex with a second affinity label that binds to the first affinity label; and (iii) measuring a level of the detection label.

[0163] 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 an ADA 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.

[0164] 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 20, 30, 40, or 50 negative control samples. In some embodiments, the ECL 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.

[0165] In some embodiments, the cut-point is identified based on about a 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%-1.0%, 1%-2%, 2%-5%, or 5%-10% false-positive rate. In some embodiments, the cut-point is identified based on about a 0.1%, 1%, or 5% false-positive rate. In some embodiments, the cut-point is identified based on about a 5% false-positive rate. In some embodiments, the cut-point is identified based on about a 1% false-positive rate. In some embodiments, the cut-point is identified based on about a 0.1% false-positive rate.

[0166] In some embodiments, the cut-point is identified based on at least a 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%-1.0%, 1%-2%, 2%-5%, or 5%-10% false-positive rate. In some embodiments, the cut-point is identified based on at least a 0.1%, 1%, or 5% false-positive rate. In

some embodiments, the cut-point is identified based on at least a 5% false-positive rate. In some embodiments, the cut-point is identified based on at least a 1% false-positive rate. In some embodiments, the cut-point is identified based on at least a 0.1% false-positive rate.

[0167] In some embodiments, the cut-point is identified based on at least about a 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.5%, 0.5%-1.0%, 1%-2%, 2%-5%, or 5%-10% false-positive rate. In some embodiments, the cut-point is identified based on at least about a 0.1%, 1%, or 5% false-positive rate. In some embodiments, the cut-point is identified based on at least about a 5% false-positive rate. In some embodiments, the cut-point is identified based on at least about a 1% false-positive rate. In some embodiments, the cut-point is identified based on at least about a 0.1% false-positive rate.

[0168] 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 ADA), 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.

[0169] In some embodiments, the predetermined cut-point is an electrochemiluminescence (ECL) of about 0.1-1, 1-2, 2-5, or 5-10. In some embodiments, the predetermined cut-point is about 1. In some embodiments, the predetermined cut-point is about 1.1.

[0170] In some embodiments, the predetermined cut-point is an ECL of at least 0.1-1, 1-2, 2-5, or 5-10. In some embodiments, the predetermined cut-point is at least 1. In some embodiments, the predetermined cut-point is at least 1.1.

[0171] In some embodiments, the predetermined cut-point is an ECL of at least about 0.1-1, 1-2, 2-5, or 5-10. In some embodiments, the predetermined cut-point is at least about 1. In some embodiments, the predetermined cut-point is at least about 1.1.

[0172] In some embodiments, the sensitivity of detecting the ADA is less than about 3ng/mL, less than about 5ng/mL, less than about 10ng/mL, less than about 50ng/mL, or less than about 100ng/mL. In some embodiments, the sensitivity of detecting the ADA is less than about 3ng/mL. In some embodiments, the sensitivity of detecting the ADA is less than about 2.77ng/mL. In some

embodiments, the sensitivity of detecting the ADA is about 4ng/mL. In some embodiments, the sensitivity of detecting the ADA is about 6ng/mL.

[0173] In some embodiments, the sensitivity of detecting the ADA is less than at least 3ng/mL, less than at least 5ng/mL, less than at least 10ng/mL, less than at least 50ng/mL, or less than at least 100ng/mL. In some embodiments, the sensitivity of detecting the ADA is less than at least 3ng/mL. In some embodiments, the sensitivity of detecting the ADA is less than at least 2.77ng/mL. In some embodiments, the sensitivity of detecting the ADA is at least 4ng/mL. In some embodiments, the sensitivity of detecting the ADA is at least 6ng/mL.

[0174] In some embodiments, the sensitivity of detecting the ADA is less than at least about 3ng/mL, less than at least about 5ng/mL, less than at least about 10ng/mL, less than at least about 50ng/mL, or less than at least about 100ng/mL. In some embodiments, the sensitivity of detecting the ADA is less than at least about 3ng/mL. In some embodiments, the sensitivity of detecting the ADA is less than at least about 2.77ng/mL. In some embodiments, the sensitivity of detecting the ADA is at least about 4ng/mL. In some embodiments, the sensitivity of detecting the ADA is at least about 6ng/mL.

[0175] In some embodiments, the drug tolerance of detecting the ADA is about 1ng/mL to about 10ng/mL, about 10ng/mL to about 50ng/mL, about 50ng/mL to about 100ng/mL, about 100ng/mL to about 1000ng/mL, or about 1000ng/mL to about 5000ng/mL. In some embodiments, the drug tolerance is about 100ng/mL.

[0176] In some embodiments, the drug tolerance of detecting the ADA is at least 1ng/mL to at least 10ng/mL, at least 10ng/mL to at least 50ng/mL, at least 50ng/mL to at least 100ng/mL, at least 100ng/mL to at least 1000ng/mL, or at least 1000ng/mL to at least 5000ng/mL. In some embodiments, the drug tolerance is at least 100ng/mL.

[0177] In some embodiments, the drug tolerance of detecting the ADA is at least about 1ng/mL to at least about 10ng/mL, at least about 10ng/mL to at least about 50ng/mL, at least about 50ng/mL to at least about 100ng/mL, at least about 100ng/mL to at least about 1000ng/mL, or at least about 1000ng/mL to at least about 5000ng/mL. In some embodiments, the drug tolerance is at least about 100ng/mL.

[0178] In some embodiments, the method comprises disassociating the ADA 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. In some embodiments, the acid wash comprises about 2M acetic acid. In some embodiments, the acid wash is added to the sample before an anti-TNF α conjugate is added. In some embodiments, the acid wash comprises about 300mM acetic acid. In some embodiments, the acid wash comprises about 0.1M glycine-HCl. In some embodiments, the acid has a pH of about 2.5.

[0179] In some embodiments, all or a portion of the method is performed using an automated system. As used herein, an “automated system” refers to a system that processes samples according to steps that are in part performed by a robotic or mechanical platform. The automated system may be completely automated or partially automated. In some embodiments, a human “loads” the automated system with the samples and reagents of the present technology and the automated system performs the remaining steps. For example, the automated system may be a KingFisher™ Flex Purification System. Other automated systems having at least the foregoing features and known to one of skill in the art are within the scope of this technology.

[0180] In some embodiments, the method for detecting an ADA that binds the target antibody in a sample comprises: (i) incubating the sample with a composition comprising: (a) an affinity antibody comprising a first affinity label and (b) a detection antibody comprising a detection label; (ii) providing a solid phase comprising a second affinity label, wherein the second affinity label is configured to bind to the first affinity label; (iii) contacting the sample and the composition with the solid phase; (iv) washing the solid phase; and (v) detecting the ADA by measuring a level of the detection label above a predetermined cut-point.

[0181] In some embodiments, the method for detecting an ADA that binds the target antibody in a sample comprises: (i) incubating the sample with a composition comprising an affinity antibody comprising a first affinity label; (ii) incubating the sample with a detection antibody comprising a detection label; (iii) providing a solid phase comprising a second affinity label, wherein the second affinity label is configured to bind to the first affinity label; (iv) contacting the sample and the composition with the solid phase; (v) washing the solid phase; and (vi) detecting the ADA by measuring a level of the detection label above a predetermined cut-point.

[0182] In some embodiments, the method for detecting an ADA that binds the target antibody in a sample comprises: (i) contacting a sample with an affinity antibody comprising a first affinity label and a detection antibody comprising a detection label; and (ii) measuring the ADA that forms a complex with the affinity antibody and the detection antibody by detecting the level of the detection label in the complex; wherein the method detects the ADA with a sensitivity less than 5ng/mL.

[0183] The present technology also includes a method for estimating a quantity of an ADA that binds a target antibody in a sample. The method comprises: (i) incubating the sample with a composition comprising an affinity antibody and a detection antibody; (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point; (iv) performing steps (i)-(iii) on a known sample having a known concentration of target antibody; and (v) comparing the level of the detection label in the sample to the level of the detection label in the known sample. As used herein, estimating “a quantity” of an ADA includes estimating a concentration of an ADA. As used herein, a “titration assay” generally refers to a method for estimating a quantity of an ADA that binds a target antibody.

[0184] As used herein, a “known sample” includes a positive control where the concentration of ADAs has been previously determined. In some embodiments, the known sample is serially diluted into a plurality of titer samples. As used herein, a “titer sample” is one of a plurality of titer samples that are diluted from a known sample. As such, the titer samples create a dilution curve.

[0185] In some embodiments, the titer samples have a concentration of, for example, between about 0.49ng/mL and about 4000ng/mL. In some embodiments, the titer samples have a concentration of, for example, about 4000ng/mL, about 2000ng/mL, about 1000ng/mL, about 500ng/mL, about 250ng/mL, about 125ng/mL, about 62.5ng/mL, about 31.25ng/mL, about 15.63ng/mL, about 7.81ng/mL, about 3.91ng/mL, about 1.95ng/mL, about 0.98ng/mL, and about 0.49ng/mL.

[0186] In some embodiments, the titer samples have a concentration of, for example, between at least 0.49ng/mL and at least 4000ng/mL. In some embodiments, the titer samples have a concentration of, for example, at least 4000ng/mL, at least 2000ng/mL, at least 1000ng/mL, at least 500ng/mL, at least 250ng/mL, at least 125ng/mL, at least 62.5ng/mL, at least 31.25ng/mL, at least

15.63ng/mL, at least 7.81ng/mL, at least 3.91ng/mL, at least 1.95ng/mL, at least 0.98ng/mL, and at least 0.49ng/mL.

[0187] In some embodiments, the titer samples have a concentration of, for example, between at least about 0.49ng/mL and at least about 4000ng/mL. In some embodiments, the titer samples have a concentration of, for example, at least about 4000ng/mL, at least about 2000ng/mL, at least about 1000ng/mL, at least about 500ng/mL, at least about 250ng/mL, at least about 125ng/mL, at least about 62.5ng/mL, at least about 31.25ng/mL, at least about 15.63ng/mL, at least about 7.81ng/mL, at least about 3.91ng/mL, at least about 1.95ng/mL, at least about 0.98ng/mL, and at least about 0.49ng/mL.

[0188] In some embodiments, the quantity or concentration of the sample is estimated by comparing the level of the detection label in the sample to the level of the detection label in the known sample or the titer samples. In some embodiments, the method further comprises generating a curve of the levels of the detection label in each of the titer samples and comparing the detection label in the sample to the curve.

[0189] The present technology also includes a method for estimating a quantity of an ADA that binds a target antibody in a sample. The method comprises: (i) incubating the sample with a composition comprising an affinity antibody comprising a first affinity label and a detection antibody comprising a detection label; (ii) providing a solid phase comprising a second affinity label, wherein the second affinity label is configured to bind to the first affinity label; (iii) contacting the sample and the composition with the solid phase; (iv) washing the solid phase; (v) detecting the ADA by measuring a level of the detection label above a predetermined cut-point; (vi) performing steps (i)-(v) on a known sample having a known concentration of target antibody; and (vii) comparing the level of the detection label in the sample to the level of the detection label in the known sample. Step (vi) may be performed in parallel with steps (i)-(v).

[0190] The present technology also includes a method of confirming that an ADA compound binds a target antibody in a sample by performing a competitive binding assay comprising performing any of the methods described in this technology for detecting an ADA within the sample, wherein the composition further comprises unlabeled anti-TNF α antibody (unlabeled antibody). As used herein, a "confirmation assay" or "confirmatory assay" generally refers to a method of confirming that an ADA component binds a target antibody.

[0191] In some embodiments, the method of confirming that an ADA compound binds a target antibody comprises: (i) incubating the sample with a composition comprising: an affinity antibody, a detection antibody, and an unlabeled antibody; (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody and/or unlabeled antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; and (iii) confirming the ADA by measuring a level of the detection label that is lower by a predetermined factor than the level of the detection label when the unlabeled antibody is not present.

[0192] In some embodiments, the unlabeled antibody will be present at an excess concentration in the composition. For example, the excess concentration of the unlabeled antibody may be about 150 μ g/mL or more, about 175 μ g/mL or more, about 200 μ g/mL or more, or about 250 μ g/mL or more. In some embodiments, the excess concentration of the unlabeled antibody may be about 200 μ g/mL or more.

[0193] In some embodiments, the excess concentration of the unlabeled antibody may be at least 150 μ g/mL or more, at least 175 μ g/mL or more, at least 200 μ g/mL or more, or at least 250 μ g/mL or more. In some embodiments, the excess concentration of the unlabeled antibody may be at least 200 μ g/mL or more.

[0194] In some embodiments, the excess concentration of the unlabeled antibody may be at least about 150 μ g/mL or more, at least about 175 μ g/mL or more, at least about 200 μ g/mL or more, or at least about 250 μ g/mL or more. In some embodiments, the excess concentration of the unlabeled antibody may be at least about 200 μ g/mL or more.

[0195] In some embodiments, ADA is confirmed in a sample by measuring a level of the detection label that is lower by a predetermined factor of about 10% or more, about 12% or more, about 14% or more, about 16% or more, about 18% or more, or about 20% or more than the level of the detection label when the unlabeled antibody is not present. In some embodiments, the predetermined factor is about 16% or more.

[0196] In some embodiments, ADA is confirmed in a sample by measuring a level of the detection label that is lower by a predetermined factor of at least 10% or more, at least 12% or more, at least 14% or more, at least 16% or more, at least 18% or more, or at least 20% or more than the

level of the detection label when the unlabeled antibody is not present. In some embodiments, the predetermined factor is at least 16% or more.

[0197] In some embodiments, ADA is confirmed in a sample by measuring a level of the detection label that is lower by a predetermined factor of at least about 10% or more, at least about 12% or more, at least about 14% or more, at least about 16% or more, at least about 18% or more, or at least about 20% or more than the level of the detection label when the unlabeled antibody is not present. In some embodiments, the predetermined factor is at least about 16% or more.

[0198] In some embodiments, the level of the detection label when the unlabeled antibody is not present is above the pre-determined cut-point.

[0199] In some embodiments, the method of confirming that an ADA compound binds a target antibody comprises: (i) incubating the sample with a composition comprising: an affinity antibody, a detection antibody, and an unlabeled antibody; (ii) providing a solid phase comprising a second affinity label, wherein the second affinity label is configured to bind to the first affinity label; (iii) contacting the sample and the composition with the solid phase; (iv) washing the solid phase; and (v) confirming the ADA by measuring a level of the detection label that is lower by a predetermined factor than the level of the detection label when the unlabeled antibody is not present.

[0200] The methods of the present technology may further comprises steps for assessing one or more of hook effects, assay selectivity, drug tolerance, intra-assay precision, inter-assay precision, and sample, control, and/or antibody stability.

[0201] The hook (prozone) effect is a phenomenon in which the signal of the assay changes from increasing to decreasing when the ADA concentration increases. The hook effect may not be critical to ADA detection if all concentrations of positive antibody yield positive results.

[0202] Stability assessments may comprise quantifying the ability of the methods to detect ADAs in the presence of other matrix components.

[0203] Drug tolerance assessments may comprise evaluating the ability of the methods to correctly detect ADAs. The presence of drug antibodies in samples may interfere with the detection of ADAs.

[0204] Stability assessments may comprise assessing positive controls. Positive controls may be assessed, for example, in a biological sample such as human serum. The ADAs of the present technology may be stable for up to six freeze/thaw cycles from about -70°C (nominal) to ambient temperature and may be stable for up to about 24.5 hours at ambient surroundings.

Kits

[0205] The present technology includes a kit for detecting an ADA against the target antibody. In some embodiments, the technology includes a kit for estimating the quantity of ADA in a sample. In some embodiments, the technology includes a kit for confirming that an ADA binds the target antibody.

[0206] In some embodiments, the kit comprises an affinity antibody comprising a first affinity label, a detection antibody, and a solid phase comprising a second affinity label. In some embodiments, the kit comprises a composition comprising the affinity antibody and the detection antibody.

[0207] In some embodiments, the solid phase is a plate. In some embodiments, the second affinity label is streptavidin. In some embodiments, the first affinity label is biotin. In some embodiments, the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody. In some embodiments, the kit further comprises a marker configured to produce a chemiluminescent or ECL signal in the presence of the detection label. In some embodiments, the marker is TPA.

[0208] In some embodiments, the kit includes an undetected antibody. In some embodiments, the undetected antibody is at an excess concentration, for example, about 200 μ g/mL or more. The kit may also comprise a composition comprising an affinity antibody, a detection antibody, and an unlabeled antibody.

[0209] 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.

[0210] 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 ADA. 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 ADA. In some embodiments, the kit may comprise ADA or ADA solution and instructions for making a positive control. For example, the kit may comprise an ADA solution and instructions to “spike” a plasma or serum sample with the ADA solution to prepare the positive control.

[0211] 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 500ng/mL to about 1000ng/mL, about 1000ng/mL to about 2000ng/mL, about 2000ng/mL to about 5000ng/mL, or about 5000ng/mL to about 10000ng/mL. In some embodiments, the HPC has an ADA concentration of about 4000ng/mL. The concentration of the LPC may be less than about 1ng/mL, about 1ng/mL to about 10ng/mL, about 10ng/mL to about 100ng/mL, about 100ng/mL to about 200ng/mL, about 200ng/mL to about 300ng/mL, or about 300ng/mL to about 500ng/mL. In some embodiments, the LPC has an ADA concentration of about 9.7ng/mL.

[0212] In some embodiments, the HPC may have a concentration of at least 500ng/mL to at least 1000ng/mL, at least 1000ng/mL to at least 2000ng/mL, at least 2000ng/mL to at least 5000ng/mL, or at least 5000ng/mL to at least 10000ng/mL. In some embodiments, the HPC has an ADA concentration of at least 4000ng/mL. The concentration of the LPC may be less than at least 1ng/mL, at least 1ng/mL to at least 10ng/mL, at least 10ng/mL to at least 100ng/mL, at least 100ng/mL to at least 200ng/mL, at least 200ng/mL to at least 300ng/mL, or at least 300ng/mL to at least 500ng/mL. In some embodiments, the LPC has an ADA concentration of at least 9.7ng/mL.

[0213] In some embodiments, the HPC may have a concentration of at least about 500ng/mL to at least about 1000ng/mL, at least about 1000ng/mL to at least about 2000ng/mL, at least about 2000ng/mL to at least about 5000ng/mL, or at least about 5000ng/mL to at least about 10000ng/mL. In some embodiments, the HPC has an ADA concentration of at least about 4000ng/mL. The concentration of the LPC may be less than at least about 1ng/mL, at least about 1ng/mL to at least about 10ng/mL, at least about 10ng/mL to at least about 100ng/mL, at least about 100ng/mL to at least about 200ng/mL, at least about 200ng/mL to at least about 300ng/mL, or at least about 300ng/mL

to at least about 500ng/mL. In some embodiments, the LPC has an ADA concentration of at least about 9.7ng/mL.

[0214] In some embodiments, the kit comprises a known sample. In some embodiments, the known sample has a concentration of ADAs of about 4000ng/mL or more, about 40μg/mL or more, about 400μg/mL or more, or about 4000μg/mL or more. The known sample may have a concentration of ADAs of about 4000ng/mL or more. The kit may also comprise one or more titer samples. For example, the kit may comprise a titer sample having an ADA concentration of about 4000ng/mL, about 2000ng/mL, about 1000ng/mL, about 500ng/mL, about 250ng/mL, about 125ng/mL, about 62.5ng/mL, about 31.25ng/mL, about 15.63ng/mL, about 7.81ng/mL, about 3.91ng/mL, about 1.95ng/mL, about 0.98ng/mL, and about 0.49ng/mL.

[0215] In some embodiments, the kit comprises a known sample. In some embodiments, the known sample has a concentration of ADAs of at least 4000ng/mL or more, at least 40μg/mL or more, at least 400μg/mL or more, or at least 4000μg/mL or more. The known sample may have a concentration of ADAs of at least 4000ng/mL or more. The kit may also comprise one or more titer samples. For example, the kit may comprise a titer sample having an ADA concentration of at least 4000ng/mL, at least 2000ng/mL, at least 1000ng/mL, at least 500ng/mL, at least 250ng/mL, at least 125ng/mL, at least 62.5ng/mL, at least 31.25ng/mL, at least 15.63ng/mL, at least 7.81ng/mL, at least 3.91ng/mL, at least 1.95ng/mL, at least 0.98ng/mL, and at least 0.49ng/mL.

[0216] In some embodiments, the kit comprises a known sample. In some embodiments, the known sample has a concentration of ADAs of at least about 4000ng/mL or more, at least about 40μg/mL or more, at least about 400μg/mL or more, or at least about 4000μg/mL or more. The known sample may have a concentration of ADAs of at least about 4000ng/mL or more. The kit may also comprise one or more titer samples. For example, the kit may comprise a titer sample having an ADA concentration of at least about 4000ng/mL, at least about 2000ng/mL, at least about 1000ng/mL, at least about 500ng/mL, at least about 250ng/mL, at least about 125ng/mL, at least about 62.5ng/mL, at least about 31.25ng/mL, at least about 15.63ng/mL, at least about 7.81ng/mL, at least about 3.91ng/mL, at least about 1.95ng/mL, at least about 0.98ng/mL, and at least about 0.49ng/mL.

[0217] 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.

[0218] 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.

[0219] 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.

[0220] The kit may include about 50 μ g to about 100 μ g, about 100 μ g to about 500 μ g, about 500 μ g to about 1000 μ g, about 1mg to about 10mg, about 10mg to about 50mg, about 50mg to about 100mg, about 100mg to about 500mg, or about 500mg to about 1000mg of the affinity antibody and/or the detection antibody. The kit may include about 50 μ g to about 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%.

[0221] The kit may include at least 50 μ g to at least 100 μ g, at least 100 μ g to at least 500 μ g, at least 500 μ g to at least 1000 μ g, at least 1mg to at least 10mg, at least 10mg to at least 50mg, at least 50mg to at least 100mg, at least 100mg to at least 500mg, or at least 500mg to at least 1000mg of the affinity antibody and/or the detection antibody. The kit may include at least 50 μ g to at least 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%.

[0222] The kit may include at least about 50 μ g to at least about 100 μ g, at least about 100 μ g to at least about 500 μ g, at least about 500 μ g to at least about 1000 μ g, at least about 1mg to at least about 10mg, at least about 10mg to at least about 50mg, at least about 50mg to at least about 100mg, at least about 100mg to at least about 500mg, or at least about 500mg to at least about 1000mg of the affinity antibody and/or the detection antibody. The kit may include at least about 50 μ g to at least about 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%.

[0223] In some embodiments, the lyophilized affinity antibody and/or detection antibody may be reconstituted in about 50 μ g to about 100 μ L, about 100 μ g to about 150 μ L, about 150 μ g to about 200 μ L, about 200 μ g to about 250 μ L, about 250 μ g to about 300 μ L, about 300 μ g to about 350 μ L, about 350 μ g to about 400 μ L, about 400 μ g to about 450 μ g, about 350 μ g to about 400 μ L, about 400 μ g to about 450 μ L, or about 450 μ g to about 500 μ L of a reconstitution buffer.

[0224] In some embodiments, the lyophilized affinity antibody and/or detection antibody may be reconstituted in at least 50 μ g to at least 100 μ L, at least 100 μ g to at least 150 μ L, at least 150 μ g to at least 200 μ L, at least 200 μ g to at least 250 μ L, at least 250 μ g to at least 300 μ L, at least 300 μ g to at least 350 μ L, at least 350 μ g to at least 400 μ L, at least 400 μ g to at least 450 μ g, at least 350 μ g to at least 400 μ L, at least 400 μ g to at least 450 μ L, or at least 450 μ g to at least 500 μ L of a reconstitution buffer.

[0225] In some embodiments, the lyophilized affinity antibody and/or detection antibody may be reconstituted in at least about 50 μ g to at least about 100 μ L, at least about 100 μ g to at least about 150 μ L, at least about 150 μ g to at least about 200 μ L, at least about 200 μ g to at least about 250 μ L, at least about 250 μ g to at least about 300 μ L, at least about 300 μ g to at least about 350 μ L, at least about 350 μ g to at least about 400 μ L, at least about 400 μ g to at least about 450 μ g, at least about 350 μ g to at least about 400 μ L, at least about 400 μ g to at least about 450 μ L, or at least about 450 μ g to at least about 500 μ L of a reconstitution buffer.

[0226] 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. The reconstitution buffer may comprise about 10% to about 15%, about 15% to about 20%, about 20% to about 25%, about 25% to about 30%, or about 30% to about 35% glycerol. The reconstitution buffer may comprise about 0.02% to about 0.04%, about 0.04% to about 0.06%, about 0.06% to about 0.08%, about 0.08% to about 0.1%, about 0.1% to about 0.12%, about 0.12% to about 0.14%, or about 0.14% to about 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.

[0227] The reconstitution buffer may comprise at least 10% to at least 15%, at least 15% to at least 20%, at least 20% to at least 25%, at least 25% to at least 30%, or at least 30% to at least 35% glycerol. The reconstitution buffer may comprise at least 0.02% to at least 0.04%, at least 0.04% to at least 0.06%, at least 0.06% to at least 0.08%, at least 0.08% to at least 0.1%, at least 0.1% to at least 0.12%, at least 0.12% to at least 0.14%, or at least 0.14% to at least 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.

[0228] The reconstitution buffer may comprise at least about 10% to at least about 15%, at least about 15% to at least about 20%, at least about 20% to at least about 25%, at least about 25% to at least about 30%, or at least about 30% to at least about 35% glycerol. The reconstitution buffer may comprise at least about 0.02% to at least about 0.04%, at least about 0.04% to at least about 0.06%, at least about 0.06% to at least about 0.08%, at least about 0.08% to at least about 0.1%, at least about 0.1% to at least about 0.12%, at least about 0.12% to at least about 0.14%, or at least about 0.14% to at least about 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.

[0229] In some embodiments, the kit includes a reconstitution buffer. In some embodiments, the kit includes an affinity antibody and/or a detection antibody that is reconstituted.

[0230] The kit may include about 1µg/mL to about 10µg/mL, about 10µg/mL to about 50µg/mL, about 50µg/mL to about 100µg/mL, about 100µg/mL to about 500µg/mL, or about 500µg/mL to about 1000µg/mL, about 1mg/mL to about 10mg/mL, about 10mg/mL to about 50mg/mL, about 50mg/mL to about 100mg/mL, about 100mg/mL to about 1000mg/mL, or about 1000mg/mL to about 10000mg/mL of the detection antibody and/or affinity antibody in solution.

[0231] The kit may include at least 1µg/mL to at least 10µg/mL, at least 10µg/mL to at least 50µg/mL, at least 50µg/mL to at least 100µg/mL, at least 100µg/mL to at least 500µg/mL, or at least 500µg/mL to at least 1000µg/mL, at least 1mg/mL to at least 10mg/mL, at least 10mg/mL to at least 50mg/mL, at least 50mg/mL to at least 100mg/mL, at least 100mg/mL to at least 1000mg/mL, or at least 1000mg/mL to at least 10000mg/mL of the detection antibody and/or affinity antibody in solution.

[0232] The kit may include at least about 1µg/mL to at least about 10µg/mL, at least about 10µg/mL to at least about 50µg/mL, at least about 50µg/mL to at least about 100µg/mL, at least about 100µg/mL to at least about 500µg/mL, or at least about 500µg/mL to at least about 1000µg/mL, at least about 1mg/mL to at least about 10mg/mL, at least about 10mg/mL to at least about 50mg/mL, at least about 50mg/mL to at least about 100mg/mL, at least about 100mg/mL to at least about 1000mg/mL, or at least about 1000mg/mL to at least about 10000mg/mL of the detection antibody and/or affinity antibody in solution.

[0233] In some embodiments, the second affinity label is streptavidin. In some embodiments, the kit includes a solid phase, a second affinity label, and instructions for coating the solid phase with the second affinity label.

[0234] The solid phase may be any material known to one of skill that is imageable and capable of binding a second affinity label, including streptavidin. For example, the substrate may be a slide, plate, dish, or flask. In some embodiments, the kit comprises a solid phase that is coated with streptavidin. In some embodiments, the kit comprises a solid phase, streptavidin, and instructions for coating the solid phase with streptavidin.

[0235] 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 comprises a blocking 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.

[0236] 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 includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more than twenty assay plates.

[0237] The kit may also include instructions for performing all or part of the methods described in the present technology. In some embodiments, the kit includes 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.

[0238] 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.

[0239] In some embodiments, the kit comprises: (i) an affinity antibody comprising biotin; (ii) a detection antibody comprising a sulfo-Tag; (iii) a plate comprising streptavidin; (iv) TPA; and (v) a wash buffer.

[0240] As used herein, the word "about" when immediately preceding a numerical value means a range of plus or minus 10 % of that value, e.g., "about 50" means 45 to 55, "about 10" means "9 to 11", etc. Furthermore, the phrases "less than about" a value or "greater than about" a value should be understood in view of the definition of the term "about" provided herein.

[0241] In another embodiment, the kit includes one or more of the components, consumables, or reagents described in the examples below.

EXAMPLES

[0242] Examples provided in this technology include example methods for detecting ADAs in human samples with an ECL assay, methods of validating the assay, and results from the validation.

[0243] *List of abbreviations and definitions:* ADA: Anti-Drug Antibody; A.T.: Ambient Temperature; Bio-XTMAB-16: Biotin Labeled XTMAB-16; CCP: Confirmatory Cut-Point; CP: Cut-Point; CV: Coefficient of Variation; diH₂O: Deionized Water; ECL: Electrochemiluminescence;

ECLIA: Electrochemiluminescence Immunoassay; HPC: High Positive Control; iNHS: Individual Naïve Human Serum; LPC: Low Positive Control; MRD: Minimum Required Dilution; 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-XTMAB-16: Ruthenium Labeled XTMA B-16; SCP: Screening Cut-Point; S-CPF: Screening Cut-Point Factor; SD: Standard Deviation; TCP: Titration Cut-Point; T-CPF: Titration Cut-Point Factor.

EXAMPLE 1: Methods for detecting ADAs in a subject by an ECL assay and methods of validating the same

[0244] *Overview:* FIG. 1 illustrates an exemplary method (method 100) for detecting an ADA that binds the target antibody in a sample. Method 100 may be used for immunogenicity assessments of various antibodies, including, but not limited to, anti-TNF α antibodies. Method 100 comprises: (i) incubating the sample with a composition comprising: (a) an anti-TNF α antibody conjugated to a first affinity label (affinity antibody), and (b) an anti-TNF α antibody conjugated to a detection label (detection antibody) **110**; (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label **120**; and (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point **130**. Isolating the ADAs may comprise: (a) providing a solid phase comprising the second affinity label; (b) contacting the sample and the composition with the solid phase; and (c) washing the solid phase.

Validation Summary: To support an immunogenicity assessment of XTMA B-16 in human samples, methods of detecting antibodies in human serum samples were assessed.

[0245] An MSD streptavidin plate was blocked to prevent non-specific antibody binding. The control and experimental samples were incubated with Bio-XTMA B-16 and transferred to the blocked MSD streptavidin plate. Following incubation, the plate was washed and then incubated with Ru-XTMA B-16. After Ru-XTMA B-16 incubation, the MSD streptavidin plate was washed again and read with plate reader (MSD Sector Imager S600). Screening, confirmatory, and titration steps were also incorporated into the validation methods.

[0246] Screening methods were used to detect potentially positive ADAs to XTMA B-16 in human serum samples. Confirmatory methods were used to determine whether the potential ADA-

positive samples were specific to XTMA B-16. Titration methods were used to provide an estimate of ADA levels in human serum samples that were confirmed to be positive for antibodies to XTMA B-16.

[0247] *Materials:* The validation methods used the reagents and controls listed in Table 2

Table 2: Reagents and controls in ECL validation

Reagent	Storage Condition
Anti-XTMA B-16 antibody (91F9D4) (0.781mg/mL)	-70°C ± 10°C
XTMA B-16 Drug (100mg/vial)	-70°C ± 10°C
Bio-XTMA B-16 (1.234mg/mL)	-70°C ± 10°C
Ru-XTMA B-16 (1.212mg/mL)	-70°C ± 10°C
MSD Streptavidin Plate	2°C-8°C
pNHS	-70°C ± 10°C
HPC 4000.0ng/mL	-70°C ± 10°C
LPC 9.70ng/mL	-70°C ± 10°C
iNHS	-70°C ± 10°C
NC 0ng/mL	-70°C ± 10°C
PBS-Tween tablet	A.T.
0.1M Glycine Buffer pH 3.0	A.T.
Next Generation Buffer I (NGBI)	2°C-8°C
PBS	2°C-8°C

MSD Read Buffer 4X	A.T.
MSD Streptavidin Plate	2°C- 8°C

[0248] Negative Controls: A pooled lot of pNHS was prepared by pooling together equal volumes of 40 lots of iNHS (29 male lots and 11 female lots).

[0249] High and Low Positive Controls: An ECL assay was validated for detection of anti-XTMAB-16 antibodies in human serum and to determine whether potentially ADA-positive samples were specific to anti-XTMAB-16. A titration method was incorporated to provide a quasi-quantitative estimate of ADA level in human serum samples that were confirmed as positive for having anti-XTMAB-16. Preparation of positive controls is further detailed in Table 3.

Table 3: Preparation of Positive Controls

ADA Tube ID	ADA Concentration	Stock Volume (μL)	NC Volume (μL)	Total Volume (μL)	Resulting Tube	Resulting Concentration Volume (ng/mL)
PC Stock	781,000	5.0	73.1	78.1	Int-1	50,000
Int-1	50,000	48	552	600	HPC	4,000
HPC	4,000	5.1	2095	2100	LPC	9.70

[0250] Reagent Preparation: The validation PCs were prepared by adding the anti-XTMAB-16 antibody to the pNHS at high (4,000.0ng/mL) and low (9.70ng/mL) concentrations for HPC and LPC, respectively.

[0251] Reagents were prepared, including MSD 2X Read Buffer (4x MSD Read Buffer diluted with diH₂O), Wash Buffer (1x PBS with 0.05% Tween-20), Blocking Buffer (Protein-Free), Assay Buffer (NGBI), and XTMAB-16 Drug Stock Solution (XTMAB-16 dissolved in PBS to 10mg/mL).

[0252] QC preparation included preparation of an NC (pNHS made from at least 40 individual lots) and LPC and HPC positive controls. HPC concentration was 4,000ng/mL and the LPC final concentration was prepared at a 99.9% confidence interval.

[0253] Establishment of LPC Concentration: The LPC concentration was calculated in both screening and confirmatory assay using the mean of the corresponding sensitivity + $t_{0.99, df} \times SD$ (sensitivity). The final LPC concentration was the higher LPC concentration between the screening LPC and the confirmatory LPC. Each sensitivity will be obtained from a separate sensitivity run. The screening sensitivity concentration will be interpolated from the screening sensitivity curve at the SCP. The confirmatory sensitivity concentration will be interpolated from the confirmatory sensitivity curve at the CCP. Once the LPC has been finalized, at least two sets of LPCs and HPCs and at least four sets of NCs will be included as plate controls in each run.

[0254] System Suitability Criteria: System suitability will be assessed from all accepted validation runs (except the robustness run and the long-term stability run, if any) to set up assay acceptance ranges for the NC, LPC, and HPC. The control ranges will be set using the response values of NC, LPC, and HPC, as detailed below.

[0255] Examples of control range calculations:

[0256] *Upper limit of NC = Mean of NC responses + 3xSD of NC responses*

[0257] *LPC range = Mean of LPC responses $\pm$ 3xSD of LPC responses*

[0258] *Lower limit of HPC = Mean of HPC responses - 3xSD of HPC responses*

[0259] In cut-point and sensitivity runs, at least 50% of each level of HPC must have %CV of the replicates $\leq 20.0\%$.

[0260] After LPC concentration is finalized, at least 50% of each level of plate controls must have: %CV of $\leq 20.0\%$.

[0261] The relationship of the accepted control samples should be as follows:

[0262] For screening and titration assays, the mean ECL: NC < SCP (if applicable) $\leq$ LPC (if applicable) < HPC

[0263] For confirmatory assay, the %inhibition: NC < CCP (if applicable) $\leq$ LPC (if applicable) and HPC.

[0264] To accept a run, at least 50% of each level of control samples and at least 66.7% of total control samples should meet the above criteria.

[0265] Screening Assay Plate Control Acceptance Criteria: For the screening assay, the CV of each plate control was 20.0% or less, and plate controls were present in duplicates. The plate NC was considered the mean of all accepted NCs, and %CV failure and outliers were excluded. The relationship of accepted control samples in the screening assays were mean response (ECL): $NC < SCP \leq LPC < HPC$, and the ECL was within the range established during validation:

[0266] NC: $ECL \text{ mean} \leq 158.8$; LPC: $95.5 \leq ECL \text{ mean} \leq 156.1$; HPC: $4761.7 \leq ECL \text{ mean}$.

[0267] The S-CPF was 1.10, and the plate-specific SCP was the value calculated from the NC multiplied by the S-CPF.

[0268] Confirmatory Assay Plate Control Acceptance Criteria: For the confirmatory assay, the CV of each plate control was 20.0% or less, and plate controls were present in duplicates. The relationship of the accepted control samples were %inhibition: $NC < CCP \leq LPC$ and HPC. Plate NC was the mean of all accepted NCs, and %CV failure and outliers were excluded. The CCP was 16.45% in the presence of XTMA-16, and %inhibition was calculated by the following formula:

[0269] $\%inhibition = 100 * (1 - [Mean \ ECL \ of \ spiked \ samples / Mean \ ECL \ of \ unspiked \ samples])$

[0270] Titration Assay Plate Control Acceptance Criteria: For the titration assay, the CV of each plate control was 20.0% or less, and plate controls were present in duplicates. The plate NC was considered the mean of all accepted NCs, and %CV failure and outliers were excluded. The relationship of accepted control samples in the titration assays were mean response (ECL): $NC < SCP \leq LPC < HPC$, and the ECL was within the range established during validation:

[0271] NC: $ECL \text{ mean} \leq 158.8$; LPC: $95.5 \leq ECL \text{ mean} \leq 156.1$; HPC: $4761.7 \leq ECL \text{ mean}$.

[0272] Titer control was $25600 \leq \text{titer for XTMA-16 ADA PC at } 4,000\text{mg/mL} \leq 102,400$.

[0273] The T-CPF was 1.20, and the plate-specific TCP was calculated by multiplying the T-CPF by the plate NC.

[0274] Screening Assay Sample Acceptance Criteria: The mean ECL values for screening samples had a %CV of 20.0% or less. Samples with mean ECL values $\geq$ plate-specific SCP were

classified as potentially positive. Samples with mean ECL values less than the plate-specific SCP were classified as negative.

[0275] Confirmatory Assay Sample Acceptance Criteria: The mean ECL values for confirmatory samples had a %CV of 20.0% or less. Samples were classified as positive for anti-XTMAB-16 antibodies if the following conditions were met: (1) the mean ECL for the sample in the screening assay was $\geq$ plate-specific SCP and (2) the %inhibition in the confirmatory assay was $\geq$ CCP. Samples were classified as negative for anti-XTMAB-16 antibodies if any of the following conditions were met: (1) the mean ECL in the screening assay $<$ plate-specific SCP and (2) the %inhibition in the confirmatory assay $<$ CCP.

[0276] Titration Assay Sample Acceptance Criteria: The titer of undiluted samples with an ECL below plate TCP were reported as MRD. The titer of undiluted samples with an ECL above the TCP, but where the first dilution (minimum dilution) had an ECL below the plate TCP, were reported as MRD. For samples with at least one dilution that reaches an ECL below the TCP, the diluted sample must have at least two dilutions spanning the TCP with a %CV $\leq$ 20.0%.

[0277] The titer of a diluted sample with ECL above the plate-specific TCP was considered the dilution factor of the last dilution sample with ECL above the plate-specific TCP.

[0278] Determination of Cut-Point: SCP: The ECL signal of each individual sample was normalized by the plate NC (expressed as S/N). The data was then log-transformed. After outlier removal, normality test was performed on the data using JMP. The S-CPF was calculated based on 5% false-positive rate using the equation below based on the data distribution.

[0279] $S\text{-}CPF = \text{“Mean} + 1.645 \times SD\text{”}$ (if data normally distributed) or “95th percentile” (if data not normally distributed)

[0280] A plate-specific SCP will be calculated using the following formula:

[0281] $SCP = \text{Mean NC ECL values in a plate} * S\text{-}CPF$

[0282] CCP: CCP was defined by the inhibition rate from individual samples with and without spiking reference drug. The %inhibition and CCP, using a 1% false-positive rate, are calculated using the equation below based on the data distribution by JMP.

[0283] $\%inhibition = 100 * (ECL_{\text{without drug}} - ECL_{\text{with drug}}) / ECL_{\text{without drug}}$

[0284] $CCP = \text{“Mean \%inhibition} + 2.33 * SD \%inhibition\text{”}$ (if data normally distributed) or “99th Percentile” (if data not normally distributed)

[0285] TCP: T-CPF will be calculated using the S-CPF normalized data. T-CPF will be calculated based on 0.1% false-positive rate using the equation below based on the data distribution. T-CPF will be reported to two decimal places.

[0286] $T-CPF = \text{“Mean} + 3.09 * SD\text{”}$ (if data normally distributed) or “99.9th percentile” (if data not normally distributed)

[0287] $\text{Plate TCP} = \text{Mean NC ECL values in a plate} * T-CPF$

[0288] Experimental design and target specifications for determining cut-point are outlined in Table 4.

Table 4: Experimental Design and Target Specifications for Determining Cut-Point

	Experimental Design	Target Specifications
S-CPF/T-CPF	At least 50 individual serum samples n: 6 minimum Replicates: 2 Analysts: 2 minimum Days: 2 minimum	- Individual samples demonstrating a %CV > 20.0% will be excluded from the final data set and will not be included in the assay cut-point calculations - At least 66.7% of the data for individual lots of sera should be included in the assay cut-point factor calculations. Failed lots due to %CV > 20.0% will be reanalyzed until at least 66.7% of the data for individual lots of sera have valid results
CCP	At least 50 individual serum samples incubated with the drug (XTMAB-16). n: 6 minimum Replicates: 2	Individual samples demonstrating a %CV > 20.0% will be excluded from the final data set and will not be included in the assay cut-point calculations

	Analysts: 2 minimum Days: 2 minimum	- Calculate the %inhibition using the following formula: $\%Signal\ Inhibition = 100*[1-(Response\ of\ sample\ competed\ with\ drug/Response\ of\ sample)]$ - At least 66.7% of the data for individual lots of sera should be included in the assay cut-point factor calculations. Failed lots due to %CV > 20.0% will be reanalyzed until at least 66.7% of the data for individual lots of sera have valid results
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[0289] Sensitivity: Sensitivity of ADA assays may be defined by the lowest concentration at which a positive control antibody preparation consistently provides a positive signal in the assay. Assay sensitivity was determined in the presence and absence of drug. The experimental design for the sensitivity assay for the screening assay is outlined in Table 5, and for the confirmatory assay in Table 6.

Table 5: Screening Assay Experimental Design and Target Specifications for Sensitivity Assay

	Experimental Design	Target Specifications
Assay sensitivity in the screening assay	ADA PCs (4000ng/mL) prepared by spiking ADA PC into pooled matrix and then two-fold serially diluted. At least 6 dilutions are suggested. At least one dilution with ECL below the plate SCP will be included in the curve fitting for assay sensitivity evaluation. Replicates: 2 n: 1 minimum for 3 individuals, 3 minimum for 1 pooled matrix Analysts: 2 minimum Runs: 2 minimum	- Plate acceptance criteria is met - At least 66.7% of sensitivity runs will be used in estimating the assay screening sensitivity - Assay screening sensitivity will be the mean

Table 6: Confirmatory Assay Experimental Design and Target Specifications for Sensitivity Assay

	Experimental Design	Target Specifications
Assay sensitivity in the confirmatory assay	ADA PCs (4000ng/mL) prepared by spiking ADA PC into pooled matrix and then two-fold serially diluted. At least 6 dilutions are suggested. At least one dilution with %inhibition below the CCP will be included in the curve fitting for confirmatory sensitivity evaluation. Replicates: 2 n: 1 minimum for 3 individuals, 3 minimum for 1 pooled matrix Analysts: 2 minimum Runs: 2 minimum	• Plate acceptance criteria is met • At least 66.7% of sensitivity runs will be used in estimating the assay confirmatory sensitivity • Assay confirmatory sensitivity will be the mean

[0290] Hook Effect: Positive control antibody samples used for prozone assessment was prepared at a concentration level higher than HPC to demonstrate that higher concentrations may not cause a decrease in response. The prepared samples were frozen for a minimum of 12 hours before serial dilution and analysis. On the day of the assay, the sample was diluted using negative serum as diluent before analysis to give concentrations above and at the assay sensitivity level. At least 80% of the validation sample used in hook effect evaluation had a %CV $\geq$ 20.0%.

[0291] Intra-Assay and Inter-Assay Precision: Intra-assay precision was evaluated based on the %CV of the NC, LPC, and HPC using a minimum of six independent preparations of the same sample in one validation run, using both the screening and confirmatory assays. The experimental design and target specifications for the intra-assay precision are outlined in Table 7.

Table 7: Intra-Assay Precision in Screening and Confirmatory Assays

	Experimental Design	Target Specifications
Intra-assay precision in screening assay	NC, LPC, HPC Replicates: 2 n: $\geq$ 6 sets each for NC, LPC, and HPC Analysts: 1 minimum Days: 1 minimum	• CV $\leq$ 20% between replicates • Mean ECL: NC < SCP $\leq$ LPC < HPC • At least 6 sets of each plate controls will be included in the intra-assay precision %CV calculation • Intra-assay CV $\leq$ 20% for HPC and LPC

		No intra CV criteria for NC
Intra-assay precision in confirmatory assay	NC, LPC, HPC Replicates: 2 n: ≥ 6 sets each for NC, LPC, and HPC Analysts: 1 minimum Days: 1 minimum	- CV ≤ 20% between replicates - Mean ECL: NC < CCP ≤ LPC < HPC - At least 6 sets of each plate controls will be included in the intra-assay precision %CV calculation - Intra-assay CV ≤ 20% for HPC and LPC - No intra CV criteria for NC

[0292] Inter-assay precision was evaluated based on the %CV of the NC, LPC, and HPC results across all applicability accepted validation runs, except for the test of robustness and long-term stability. The experimental design and target specifications for the inter-assay precision are outlined in Table 8.

Table 8: Inter-Assay Precision in Screening and Confirmatory Assays

	Experimental Design	Target Specifications
Inter-assay precision in screening assay	All NC, LPC, HPC accepted in validation runs, except robustness runs Replicates: 2 Runs: 6 minimum Analysts: 2 minimum Days: 2 minimum	- CV ≤ 20% between replicates - Mean ECL: NC < SCP ≤ LPC < HPC - CV ≤ 20% for HPC and LPC - No inter CV criteria for NC

Intra-assay precision in confirmatory assay	All NC, LPC, HPC accepted in validation runs, except robustness runs Replicates: 2 Runs: 6 minimum Analysts: 2 minimum Days: 2 minimum	• $CV \leq 20\%$ between replicates • Mean ECL: $NC < SCP \leq LPC < HPC$ • $CV \leq 20\%$ for HPC and LPC • No inter CV criteria for NC
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[0293] Selectivity and Matrix Interference: The selectivity and matrix interference for individual normal matrix was evaluated by spiking ADA at LPC level to at least 10 individual normal matrixes in both screening and confirmatory tests. The selectivity and matrix interference for individual diseased matrix was evaluated by spiking ADA at LPC level to at least 10 individual diseased matrixes in both screening and confirmatory tests. In the screening assay, ADA spiked and non-ADA spiked matrix samples were analyzed in comparison to SCP. In the confirmatory assay, the analyzed samples will be compared with CCP. Samples may be frozen prior to analysis. The selectivity samples confirmed with pre-existing antibodies were excluded in evaluating assay selectivity. The selectivity evaluation experimental design and target specifications are outlined in Table 9.

Table 9: Selectivity Evaluation Experimental Design and Target Specifications

	Experimental Design	Target Specifications
Selectivity	Minimum of 10 individual samples of normal matrix unspiked (blank) and spiked with ADA PC at LPC level. An LPC spiked normal pooled serum may be used as a reference. N: minimum 10 samples (spiked and unspiked) Replicates: 2 Runs: 1 minimum Minimum of 10 individual samples of diseased matrix unspiked (blank) and spiked with ADA PC at LPC level. An LPC spiked normal pooled serum may be used as a reference.	• Plate acceptance criteria is met • The method selectivity is considered acceptable if no more than 20% of the unspiked samples report as positive, and at least 80% of the spiked samples report as positive • Samples are reported as positive only if the below criteria are met: (1) screening assay $ECL \geq SCP$, (2) confirmatory assay $\%inhibition \geq CCP$, (3) precision (expressed as $\%CV$) has CV value $\leq$

		20.0% between replicates
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[0294] Haemolyzed and Lipemic Matrix Interference: The selectivity assessment includes haemolyzed (2%) and lipemic (150mg/dL) matrix assessment. A minimum of three control matrix pooled serum samples for each condition were assessed unspiked and spiked at LPC level. The spiked matrix samples will be analyzed in comparison to plate SCP. In confirmatory assays, the analyzed samples were compared with CCP. Samples may be frozen prior to analysis. The matrix interference experimental design and target specifications are detailed in Table 10.

Table 10: Matrix Interference Experimental Design and Target Specifications

	Experimental Design	Target Specifications
Matrix interference	Minimum of 3 pooled lot of 2% haemolyzed sample, minimum of 3 pooled lot of 150mg/dL lipemic sample, unspiked (blank) and spiked with ADA PC at LPC level n: minimum 3 for haemolyzed and lipemic, respectively Replicates: 2 Runs: 1 minimum	• Plate acceptance criteria is met • Method selectivity is considered acceptable if (1) no more than 20% of the unspiked samples report as negative, and (2) at least 80% of the spiked samples report as positives • Samples are reported as positive if the below criteria are met: (1) screening assay ECL ≥ SCP, (2) confirmatory assay %inhibition ≥ CCP, (3) precision (expressed as %CV) ≤ 20.0% between replicates

[0295] Determination of Drug Tolerance: Drug tolerance is to determine the highest level of drug that may be added to a sample spiked with the positive control antibodies without interfering

with its detection as positive antibody response. Various drug concentrations were spiked into matrix samples containing various concentrations of positive control antibodies.

[0296] Drug tolerance limit may be defined as the highest level of drug in the presence of which ADA is still detected. ADA PC at 100ng/mL and LPC level will be tested. The highest drug concentration that retains a positive response in the screening assay for each ADA level tested will be defined as the drug tolerance level of the screening assay. The experimental design and target specifications for testing drug tolerance are outlined in Table 11.

Table 11: Experimental Design and Target Specifications for Testing Drug Tolerance

	Experimental Design	Target Specifications
Selectivity	Positive control samples at various ADA concentrations mixed with drug samples at various concentration levels, including a 0ng/mL drug level. ADA PC at 100ng/mL and LPC level with different concentration levels of drug (400, 200, 100, 50, 20, 10, 5, and 0µg/mL). n: 1 minimum Replicates: 2 Runs: 1 minimum	Plate acceptance criteria is met At least 80% of samples above the SCP have CV ≤ 20% between replicates The samples with highest drug concentration that still gives ECL result above SCP will be reported as drug tolerance

[0297] Stability Test: Combined stressed samples with bench-top (24±6 hours or 8±2 hours) and freeze/thaw (6 or 3 cycles from -70°C ± 10°C to ambient temperature) were tested. The samples were prepared and stored at -70°C ± 10°C for overnight (at least 12 hours) before being thawed at ambient temperature 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 bench-top for 2±1 hours before refreezing. The experimental design and target specifications are outlined in Table 12.

Table 12: Experimental Design and Target Specifications for Bench-Top and Freeze/Thaw Stability Assays

Parameter	Experimental Design	Target Specification
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Bench-top, freeze/thaw stability	NC, LPC, and HPC $n: \geq 3$	System acceptance criteria is met Plate acceptance criteria is met $\%CV \leq 20.0\%$ between replicates Confirmatory assay: $\%inhibition \text{ of NC} < CCP \leq \text{LPC and HPC}$ Screening assay: $\text{Mean ECL of NC} < SCP \leq \text{LPC} < \text{HPC}$ At least 66.7% stability samples at each level must meet above acceptance criteria.
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[0298] Data Evaluation: ECL count raw data was captured using the MSD Sector Imager S600 and Discovery Workbench Software Version 4.0. $\%CV$, SD, and $\%inhibition$ were each calculated. Statistical evaluation of cut-point factors was conducted using JMP software.

[0299] Assay Performance Characteristics: All samples and controls, including two sets of LPC and HPC and at least four sets of NC, were evaluated in duplicate. The text file imported from the MSD Sector Imager S600 was used as the source data for analysis and verified against the screen capture of the original data. The source data was processed in Microsoft Excel to calculate mean of ECL values, $\%CV$, and $\%inhibition$. The S-CPF, T-CPF, and CCPs were calculated using JMP 14 statistical software. All mean duplicate or quadruplicate ECL values were assessed in the analysis.

[0300] Failed tests: Plate control testing resulting in failed runs was repeated twice. If the repeated run failed again and failure was not due to the experimental error, the method was investigated and the method was revised or reoptimized with runs repeated, where necessary.

EXAMPLE 2: Validation of method of detecting ADAs in serum samples using an electrochemiluminescence immunoassay

[0301] Cut-Point Determination: To determine the screening, confirmatory, and titration assay cut-point factors for the detection of anti-XTMAB-16 antibodies, two serum samples from 54 lots of naïve male and female human serum were tested over five days. Each sample was tested six times across 18 individual runs.

[0302] Cut-point statistical outliers were determined using quantile range outlier's method, and identified outliers were removed. Cut-point normality values were tested by Shapiro-Wilk W Test using JMP software.

[0303] For each run, the mean ECL signal of each individual serum sample was normalized using the mean ECL of a plate NC. The normalized ECL (rECL) was log-transformed. Examples of data analyzed according to the present technology are shown in Tables 12-14, for example. Table 12 shows raw data from one run of samples that were tested according to the methods of the present technology. Table 13 shows raw data for S-CPF and T-CPF for the runs in Table 12. Table 14 illustrates an example of the summarized normalized data for S-CPF and T-CPF calculation.

Table 12: Example raw data from a single run

Example Run	Validation Parameter	Result
34	Screening/Confirmation Cut Point (S01-S18)	Pass
35	Screening/Confirmation Cut Point (S19-S36)	Pass
36	Screening/Confirmation Cut Point (S37-S54)	Pass
37	Screening/Confirmation Cut Point (S01-S18)	Pass
38	Screening/Confirmation Cut Point (S19-S36)	Pass
39	Screening/Confirmation Cut Point (S37-S54)	Pass
40	Screening/Confirmation Cut Point (S01-S18)	Pass
41	Screening/Confirmation Cut Point (S19-S36)	Pass
42	Screening/Confirmation Cut Point (S37-S54)	Pass
43	Screening/Confirmation Cut Point (S01-S18)	Pass
44	Screening/Confirmation Cut Point (S19-S36)	Pass
45	Screening/Confirmation Cut Point (S37-S54)	Pass
46	Screening/Confirmation Cut Point (S01-S18)	Void
47	Screening/Confirmation Cut Point (S19-S36)	Void
48	Screening/Confirmation Cut Point (S37-S54)	Void
49	Screening/Confirmation Cut Point (S01-S18)	Pass
50	Screening/Confirmation Cut Point (S19-S36)	Pass
51	Screening/Confirmation Cut Point (S37-S54)	Pass
52	Screening/Confirmation Cut Point (S01-S18)	Pass
53	Screening/Confirmation Cut Point (S19-S36)	Pass
54	Screening/Confirmation Cut Point (S37-S54)	Pass
55	Individual Sensitivity-01	Pass
56	Individual Sensitivity-02	Pass
57	Individual Sensitivity-03	Pass

58	Pooled Sensitivity-01	Pass
59	Pooled Sensitivity-02	Pass
60	Pooled Sensitivity-03	Pass
61	Robustness-Maximum	Pass
62	Robustness-Minimum	Pass
63	Intra-Run Precision	Pass
64	Hook Effect	Pass
65	Drug Tolerance	Pass
66	Bench-Top Stability	Pass
67	Freeze-Thaw Stability	Pass
68	Selectivity-1	Pass
69	Selectivity-2	Pass
70	Selectivity-3	Pass
71	Selectivity-4	Pass

Table 13: S-CPF and T-CPF raw data

Example Run*	Rep 1	Rep 2	Mean	%CV	Plate NC	rECL
34	94	88	91.0	4.7	89.4	1.0179
	86	83	84.5	2.5		0.9452
	93	86	89.5	5.5		1.0011
	91	86	88.5	4.0		0.9899
	82	80	81.0	1.7		0.9060
	95	91	93.0	3.0		1.0403
	77	73	75.0	3.8		0.8389
	79	76	77.5	2.7		0.8669
	79	76	77.5	2.7		0.8669
	79	82	80.5	2.6		0.9004
	78	85	81.5	6.1		0.9116
	92	90	91.0	1.6		1.0179
	89	81	85.0	6.7		0.9508
	88	82	85.0	5.0		0.9508
	81	81	81.0	0.0		0.9060
	89	84	86.5	4.1		0.9676
	83	80	81.5	2.6		0.9116
	87	84	85.5	2.5		0.9564
35	81	83	82.0	1.7	80.3	1.0212
	76	77	76.5	0.9		0.9527
	84	88	86.0	3.3		1.0710
	85	87	86.0	1.6		1.0710

	83	89	86.0	4.9		1.0710
	83	88	85.5	4.1		1.0648
	80	77	78.5	2.7		0.9776
	80	77	78.5	2.7		0.9776
	78	73	75.5	4.7		0.9402
	81	82	81.5	0.9		1.0149
	80	87	83.5	5.9		1.0399
	79	82	80.5	2.6		1.0025
	78	82	80.0	3.5		0.9963
	85	88	86.5	2.5		1.0772
	76	74	75.0	1.9		0.9340
	79	75	77.0	3.7		0.9589
	75	75	75.0	0.0		0.9340
	81	81	81.0	0.0		1.0087
36	78	79	78.5	0.9	77.8	1.0090
	67	76	71.5	8.9		0.9190
	88	84	86.0	3.3		1.1054
	86	81	83.5	4.2		1.0733
	83	82	82.5	0.9		1.0604
	83	79	81.0	3.5		1.0411
	76	71	73.5	4.8		0.9447
	74	71	72.5	2.9		0.9319
	73	69	71.0	4.0		0.9126

*Each row represents a different lot

Table 14: S-CPF and T-CPF raw data

Screening 1*	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6
1.0179	0.9988	0.9880	1.0205	0.9793	0.9721
0.9452	1.0229	0.9398	1.3406	1.1463	1.0510
1.0011	1.0349	1.1325	1.0266	1.0023	1.1543
0.9899	1.0168	1.0843	1.0145	0.9735	1.0875
0.9060	0.9687	1.0361	0.9541	0.9620	1.0510
1.0403	1.0048	1.0241	1.0326	1.0023	1.0693
0.8389	0.9146	0.9880	0.9722	1.0023	0.9235
0.8669	0.9386	0.9819	1.0447	1.0138	0.9781
0.8669	0.9446	0.9880	0.9783	1.0484	0.9781
0.9004	0.9807	0.9880	0.8877	1.0426	1.0207
0.9116	0.9868	1.0783	0.9541	0.9562	1.0085
1.0179	1.0650	1.1024	1.0507	1.1290	1.0693

0.9508	0.9807	1.0060	0.9481	1.0138	1.1908
0.9508	1.0289	1.0060	0.9783	1.0484	1.0510
0.9060	0.9206	0.9277	0.9722	1.0253	0.9842
0.9676	0.9627	0.9096	1.0266	1.0311	0.9903
0.9116	0.8905	0.8795	0.8937	0.9505	0.9538
0.9564	0.9507	0.9458	0.8877	0.9389	0.9781
1.0212	1.0025	0.9615	1.0453	1.0313	1.0012
0.9527	0.9774	0.9505	0.9466	0.9881	0.9595
1.0710	1.0276	1.0495	1.0395	1.0583	0.9893
1.0710	1.0338	1.0330	1.1556	1.0745	0.9833
1.0710	1.0276	0.9945	1.1150	1.0205	0.9535
1.0648	1.0526	1.0165	1.1556	1.0529	1.0012
0.9776	0.9774	1.0165	1.0337	0.9665	0.9118
0.9776	0.9837	0.9890	1.0511	0.9233	0.9297
0.9402	0.9586	1.0055	1.0046	0.9341	0.8880
1.0149	0.9900	1.0330	1.1150	1.0205	0.9058
1.0399	0.9712	1.0165	0.8827	1.0853	0.8164
1.0025	0.9649	1.0110	1.0453	1.1015	0.9178
0.9963	0.9524	0.9945	0.9233	1.0259	0.8701
1.0772	1.0464	0.9780	0.9233	1.0313	0.8284
0.9340	0.9398	0.9835	0.9988	0.9395	0.9416
0.9589	1.0088	1.0330	1.0046	1.0097	0.8522
0.9340	0.9524	1.0165	0.9872	0.9233	0.8582
1.0087	1.0088	1.0714	0.9640	0.9935	0.8760
1.0090	1.0053	1.0244	0.9378	1.0409	0.9712
0.9190	0.9987	0.9022	1.0044	0.9386	0.8960
1.1054	1.0185	1.0303	0.9267	1.1131	1.0589
1.0733	0.9656	0.9837	0.9378	1.0590	0.9586
1.0604	0.9458	0.9895	0.9378	1.0890	0.9962
1.0411	0.9392	1.0070	0.8824	1.0229	0.9461
0.9447	0.9458	1.0012	0.9323	0.9988	0.9336
0.9319	0.9458	1.0361	0.9822	0.9928	0.8835
0.9126	0.9392	0.9837	0.9323	1.0289	0.8897
0.9769	1.0053	1.0419	0.9545	1.0289	1.0150
1.0411	0.9193	1.0536	0.8491	1.0710	0.9398
1.0990	0.9524	1.1176	0.9212	1.1312	0.9900
1.0347	0.9590	1.0768	0.8602	1.0770	0.9336
1.0540	0.9458	1.0885	0.8879	1.1071	0.9273
0.9447	0.9590	0.9837	0.8491	1.0229	0.9085

1.0476	0.9722	1.0361	0.8879	1.0710	0.9649
0.9769	0.9656	1.0070	0.8435	1.0229	0.9336
1.0347	0.9921	1.0303	0.8879	1.0409	0.9336

*Each row represents a different lot

[0304] Analytical outliers were identified using box-plot analysis on the normalized and log-transformed serum values (Table 15). Biological outliers were identified and excluded from analysis, and the normalized and log-transformed data for each individual serum sample was averaged across the six determinations, if available. Box-plot analysis was then performed to identify biological outliers (Table 16). Examples of the outliers analyzed are shown in Tables 15-17, for example. Tables 15, 16, 18, and 19 show examples of the analytical and biological outlier identification from the lots assessed in Tables 12-14. Table 17 illustrates an example of the summarized data of log-transformed rECL for S-CPF and T-CPF calculation after the removal of the biological and analytical outliers, such as those shown in Tables 15, 16, 18, and 19.

Table 15: Analytical and Biological Outlier Identification (Example A)

Screening 1*	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6
1.0179	0.9988	0.9880	1.0205	0.9793	0.9721
0.9452	1.0229	0.9398	1.3406	1.1463	1.0510
1.0011	1.0349	1.1325	1.0266	1.0023	1.1543
0.9899	1.0168	1.0843	1.0145	0.9735	1.0875
0.9060	0.9687	1.0361	0.9541	0.9620	1.0510
1.0403	1.0048	1.0241	1.0326	1.0023	1.0693
0.8389	0.9146	0.9880	0.9722	1.0023	0.9235
0.8669	0.9386	0.9819	1.0447	1.0138	0.9781
0.8669	0.9446	0.9880	0.9783	1.0484	0.9781
0.9004	0.9807	0.9880	0.8877	1.0426	1.0207
0.9116	0.9868	1.0783	0.9541	0.9562	1.0085
1.0179	1.0650	1.1024	1.0507	1.1290	1.0693
0.9508	0.9807	1.0060	0.9481	1.0138	1.1908
0.9508	1.0289	1.0060	0.9783	1.0484	1.0510
0.9060	0.9206	0.9277	0.9722	1.0253	0.9842
0.9676	0.9627	0.9096	1.0266	1.0311	0.9903
0.9116	0.8905	0.8795	0.8937	0.9505	0.9538
0.9564	0.9507	0.9458	0.8877	0.9389	0.9781
1.0212	1.0025	0.9615	1.0453	1.0313	1.0012
0.9527	0.9774	0.9505	0.9466	0.9881	0.9595

1.0710	1.0276	1.0495	1.0395	1.0583	0.9893
1.0710	1.0338	1.0330	1.1556	1.0745	0.9833
1.0710	1.0276	0.9945	1.1150	1.0205	0.9535
1.0648	1.0526	1.0165	1.1556	1.0529	1.0012
0.9776	0.9774	1.0165	1.0337	0.9665	0.9118
0.9776	0.9837	0.9890	1.0511	0.9233	0.9297
0.9402	0.9586	1.0055	1.0046	0.9341	0.8880
1.0149	0.9900	1.0330	1.1150	1.0205	0.9058
1.0399	0.9712	1.0165	0.8827	1.0853	0.8164
1.0025	0.9649	1.0110	1.0453	1.1015	0.9178
0.9963	0.9524	0.9945	0.9233	1.0259	0.8701
1.0772	1.0464	0.9780	0.9233	1.0313	0.8284
0.9340	0.9398	0.9835	0.9988	0.9395	0.9416
0.9589	1.0088	1.0330	1.0046	1.0097	0.8522
0.9340	0.9524	1.0165	0.9872	0.9233	0.8582
1.0087	1.0088	1.0714	0.9640	0.9935	0.8760
1.0090	1.0053	1.0244	0.9378	1.0409	0.9712
0.9190	0.9987	0.9022	1.0044	0.9386	0.8960
1.1054	1.0185	1.0303	0.9267	1.1131	1.0589
1.0733	0.9656	0.9837	0.9378	1.0590	0.9586
1.0604	0.9458	0.9895	0.9378	1.0890	0.9962
1.0411	0.9392	1.0070	0.8824	1.0229	0.9461
0.9447	0.9458	1.0012	0.9323	0.9988	0.9336
0.9319	0.9458	1.0361	0.9822	0.9928	0.8835
0.9126	0.9392	0.9837	0.9323	1.0289	0.8897
0.9769	1.0053	1.0419	0.9545	1.0289	1.0150
1.0411	0.9193	1.0536	0.8491	1.0710	0.9398
1.0990	0.9524	1.1176	0.9212	1.1312	0.9900
1.0347	0.9590	1.0768	0.8602	1.0770	0.9336
1.0540	0.9458	1.0885	0.8879	1.1071	0.9273
0.9447	0.9590	0.9837	0.8491	1.0229	0.9085
1.0476	0.9722	1.0361	0.8879	1.0710	0.9649
0.9769	0.9656	1.0070	0.8435	1.0229	0.9336
1.0347	0.9921	1.0303	0.8879	1.0409	0.9336

*Each row represents a different lot

Table 16: Analytical and Biological Outlier Identification (Example B)

Screening 1*	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6	Mean
1.0179	0.9988	0.9880	1.0205	0.9793	0.9721	-0.0018
0.9452	1.0229	0.9398	NA	1.1463	1.0510	0.0078

1.0011	1.0349	1.1325	1.0266	1.0023	1.1543	0.0240
0.9899	1.0168	1.0843	1.0145	0.9735	1.0875	0.0115
0.9060	0.9687	1.0361	0.9541	0.9620	1.0510	-0.0095
1.0403	1.0048	1.0241	1.0326	1.0023	1.0693	0.0123
0.8389	0.9146	0.9880	0.9722	1.0023	0.9235	-0.0277
0.8669	0.9386	0.9819	1.0447	1.0138	0.9781	-0.0137
0.8669	0.9446	0.9880	0.9783	1.0484	0.9781	-0.0151
0.9004	0.9807	0.9880	0.8877	1.0426	1.0207	-0.0140
0.9116	0.9868	1.0783	0.9541	0.9562	1.0085	-0.0083
1.0179	1.0650	1.1024	1.0507	1.1290	1.0693	0.0301
0.9508	0.9807	1.0060	0.9481	1.0138	1.1908	0.0052
0.9508	1.0289	1.0060	0.9783	1.0484	1.0510	0.0043
0.9060	0.9206	0.9277	0.9722	1.0253	0.9842	-0.0199
0.9676	0.9627	0.9096	1.0266	1.0311	0.9903	-0.0086
0.9116	0.8905	0.8795	0.8937	0.9505	0.9538	-0.0396
0.9564	0.9507	0.9458	0.8877	0.9389	0.9781	-0.0257
1.0212	1.0025	0.9615	1.0453	1.0313	1.0012	0.0044
0.9527	0.9774	0.9505	0.9466	0.9881	0.9595	-0.0167
1.0710	1.0276	1.0495	1.0395	1.0583	0.9893	0.0166
1.0710	1.0338	1.0330	1.1556	1.0745	0.9833	0.0242
1.0710	1.0276	0.9945	1.1150	1.0205	0.9535	0.0124
1.0648	1.0526	1.0165	1.1556	1.0529	1.0012	0.0237
0.9776	0.9774	1.0165	1.0337	0.9665	0.9118	-0.0089
0.9776	0.9837	0.9890	1.0511	0.9233	0.9297	-0.0111
0.9402	0.9586	1.0055	1.0046	0.9341	0.8880	-0.0203
1.0149	0.9900	1.0330	1.1150	1.0205	0.9058	0.0049
1.0399	0.9712	1.0165	0.8827	1.0853	0.8164	-0.0159
1.0025	0.9649	1.0110	1.0453	1.1015	0.9178	0.0024
0.9963	0.9524	0.9945	0.9233	1.0259	0.8701	-0.0182
1.0772	1.0464	0.9780	0.9233	1.0313	0.8284	-0.0101
0.9340	0.9398	0.9835	0.9988	0.9395	0.9416	-0.0196
0.9589	1.0088	1.0330	1.0046	1.0097	0.8522	-0.0106
0.9340	0.9524	1.0165	0.9872	0.9233	0.8582	-0.0251
1.0087	1.0088	1.0714	0.9640	0.9935	0.8760	-0.0064
1.0090	1.0053	1.0244	0.9378	1.0409	0.9712	-0.0011
0.9190	0.9987	0.9022	1.0044	0.9386	0.8960	-0.0259
1.1054	1.0185	1.0303	0.9267	1.1131	1.0589	0.0171
1.0733	0.9656	0.9837	0.9378	1.0590	0.9586	-0.0022
1.0604	0.9458	0.9895	0.9378	1.0890	0.9962	0.0007

1.0411	0.9392	1.0070	0.8824	1.0229	0.9461	-0.0126
0.9447	0.9458	1.0012	0.9323	0.9988	0.9336	-0.0182
0.9319	0.9458	1.0361	0.9822	0.9928	0.8835	-0.0174
0.9126	0.9392	0.9837	0.9323	1.0289	0.8897	-0.0238
0.9769	1.0053	1.0419	0.9545	1.0289	1.0150	0.0015
1.0411	0.9193	1.0536	0.8491	1.0710	0.9398	-0.0108
1.0990	0.9524	1.1176	0.9212	1.1312	0.9900	0.0136
1.0347	0.9590	1.0768	0.8602	1.0770	0.9336	-0.0057
1.0540	0.9458	1.0885	0.8879	1.1071	0.9273	-0.0008
0.9447	0.9590	0.9837	0.8491	1.0229	0.9085	-0.0255
1.0476	0.9722	1.0361	0.8879	1.0710	0.9649	-0.0023
0.9769	0.9656	1.0070	0.8435	1.0229	0.9336	-0.0194
1.0347	0.9921	1.0303	0.8879	1.0409	0.9336	-0.0066

*Each row represents a different lot

Table 17: log-transformed rECL for S-CPF and T-CPF

Screening 1*	Screening 2	Screening 3	Screening 4	Screening 5	Screening 6
1.0179	0.9988	0.9880	1.0205	0.9793	0.9721
0.9452	1.0229	0.9398	NA	1.1463	1.0510
1.0011	1.0349	1.1325	1.0266	1.0023	1.1543
0.9899	1.0168	1.0843	1.0145	0.9735	1.0875
0.9060	0.9687	1.0361	0.9541	0.9620	1.0510
1.0403	1.0048	1.0241	1.0326	1.0023	1.0693
0.8389	0.9146	0.9880	0.9722	1.0023	0.9235
0.8669	0.9386	0.9819	1.0447	1.0138	0.9781
0.8669	0.9446	0.9880	0.9783	1.0484	0.9781
0.9004	0.9807	0.9880	0.8877	1.0426	1.0207
0.9116	0.9868	1.0783	0.9541	0.9562	1.0085
1.0179	1.0650	1.1024	1.0507	1.1290	1.0693
0.9508	0.9807	1.0060	0.9481	1.0138	1.1908
0.9508	1.0289	1.0060	0.9783	1.0484	1.0510
0.9060	0.9206	0.9277	0.9722	1.0253	0.9842
0.9676	0.9627	0.9096	1.0266	1.0311	0.9903
0.9116	0.8905	0.8795	0.8937	0.9505	0.9538
0.9564	0.9507	0.9458	0.8877	0.9389	0.9781
1.0212	1.0025	0.9615	1.0453	1.0313	1.0012
0.9527	0.9774	0.9505	0.9466	0.9881	0.9595
1.0710	1.0276	1.0495	1.0395	1.0583	0.9893
1.0710	1.0338	1.0330	1.1556	1.0745	0.9833
1.0710	1.0276	0.9945	1.1150	1.0205	0.9535

1.0648	1.0526	1.0165	1.1556	1.0529	1.0012
0.9776	0.9774	1.0165	1.0337	0.9665	0.9118
0.9776	0.9837	0.9890	1.0511	0.9233	0.9297
0.9402	0.9586	1.0055	1.0046	0.9341	0.8880
1.0149	0.9900	1.0330	1.1150	1.0205	0.9058
1.0399	0.9712	1.0165	0.8827	1.0853	0.8164
1.0025	0.9649	1.0110	1.0453	1.1015	0.9178
0.9963	0.9524	0.9945	0.9233	1.0259	0.8701
1.0772	1.0464	0.9780	0.9233	1.0313	0.8284
0.9340	0.9398	0.9835	0.9988	0.9395	0.9416
0.9589	1.0088	1.0330	1.0046	1.0097	0.8522
0.9340	0.9524	1.0165	0.9872	0.9233	0.8582
1.0087	1.0088	1.0714	0.9640	0.9935	0.8760
1.0090	1.0053	1.0244	0.9378	1.0409	0.9712
0.9190	0.9987	0.9022	1.0044	0.9386	0.8960
1.1054	1.0185	1.0303	0.9267	1.1131	1.0589
1.0733	0.9656	0.9837	0.9378	1.0590	0.9586
1.0604	0.9458	0.9895	0.9378	1.0890	0.9962
1.0411	0.9392	1.0070	0.8824	1.0229	0.9461
0.9447	0.9458	1.0012	0.9323	0.9988	0.9336
0.9319	0.9458	1.0361	0.9822	0.9928	0.8835
0.9126	0.9392	0.9837	0.9323	1.0289	0.8897
0.9769	1.0053	1.0419	0.9545	1.0289	1.0150
1.0411	0.9193	1.0536	0.8491	1.0710	0.9398
1.0990	0.9524	1.1176	0.9212	1.1312	0.9900
1.0347	0.9590	1.0768	0.8602	1.0770	0.9336
1.0540	0.9458	1.0885	0.8879	1.1071	0.9273
0.9447	0.9590	0.9837	0.8491	1.0229	0.9085
1.0476	0.9722	1.0361	0.8879	1.0710	0.9649
0.9769	0.9656	1.0070	0.8435	1.0229	0.9336
1.0347	0.9921	1.0303	0.8879	1.0409	0.9336

*Each row represents a different lotTable 18: Calculation of SCP and TCP Analytical Outliers

Measurement	Value
Q1 (25% quartile)	-0.0243
Q3 (75% quartile)	0.0135
Q3-Q1	0.0378

High end ($Q3+3*[Q3-Q1]$)	0.1269
Low end ($Q1-3*[Q3-Q1]$)	-0.1377

Table 19: Calculation of SCP and TCP Biological Outliers

Measurement	Value
Q1 (25% quartile)	-0.0172
Q3 (75% quartile)	0.0044
Q3-Q1	0.0216
High end ($Q3+3*[Q3-Q1]$)	0.0692
Low end ($Q1-3*[Q3-Q1]$)	-0.0820

[0305] Screening and Titration Data Normality Test: The normality of 323 log-transformed normalized values were tested by Shapiro-Wilk W Test using JMP software (FIG. 2A). Normal distribution was confirmed, and a parametric approach was used to calculate S-CPF and T-CPF (Table 20).

Table 20: Calculated Parametric S-CPF and T-CPF

Measurement	Value
Mean	-0.0056
SD	0.0283
Log(S-CPF)	0.04
S-CPF	1.10
Log(T-CPF)	0.08
T-CPF	1.20

[0306] CCP Determination: For each run, %inhibition for each sample was calculated using the following formula:

[0307] $\%inhibition = 100 * (ECL_{without\ drug} - ECL_{with\ drug}) / ECL_{without\ drug}$

[0308] SCPs and TCPs were removed from the CCP analysis.

[0309] CCP analytical outliers were identified and excluded from analysis using box-plot analysis on the %inhibition values (Table 21).

Table 21: Calculation of CCP Analytical Outliers

Measurement	Value
Q1 (25% quartile)	0.5600
Q3 (75% quartile)	8.2100
Q3-Q1	7.6500
High end ($Q3+3*[Q3-Q1]$)	31.1600
Low end ($Q1-3*[Q3-Q1]$)	-22.3900

[0310] After identification and exclusion of analytical outliers, %inhibition data for each individual serum sample was averaged across the six determinations, if available. Box-plot analysis was performed on the values to identify biological outliers (Table 22).

Table 22: Calculation of CCP Biological Outliers

Measurement	Value
Q1 (25% quartile)	1.5850
Q3 (75% quartile)	6.1625
Q3-Q1	4.5775
High end ($Q3+3*[Q3-Q1]$)	19.8950
Low end ($Q1-3*[Q3-Q1]$)	-12.1475

[0311] Confirmatory Data Normality Test: Normality of 323 values of %inhibition were tested by Shapiro-Wilk W Test using JMP software (FIG. 2B). Normal distribution was confirmed, and a parametric approach was used to calculate S-CPF and T-CPF (Table 23).

Table 23: Calculated Parametric CCP Factors

Measurement	Value
Mean	3.8557

SD	6.1523
CCP (%)	16.45

[0312] Cut-point values were determined for application of the assay to detect ADAs to XTMAB-16 in human serum (Table 24). Examples of the CCP values assessed are shown in Tables 25-28. An example of the raw data values assessed is shown in Table 25, and examples of these values after the removal of outliers are shown in Tables 26-28, no outliers were found in the example.

Table 24: Cut-Point Values Determined for Detection Assay

Description	Units	False-Positive Rate	Parametric/Non-Parametric	Result
S-CPF	NA	5%	Non-Parametric	1.10
T-CPF	NA	0.1%	Non-Parametric	1.20
CCP	%	1%	Non-Parametric	16.45

Table 25: Exemplary Raw Data

Example Run*	Drug Unspiked				Drug Spiked (MAB-16 at 200 ug/mL)				
	Rep 1	Rep 2	Mean	%CV	Rep 1	Rep 2	Mean	%CV	%Inhibition
34	94	88	91.0	4.7	82	80	81.0	1.7	10.99
	86	83	84.5	2.5	92	83	87.5	7.3	-3.55
	93	86	89.5	5.5	79	87	83.0	6.8	7.26
	91	86	88.5	4.0	87	86	86.5	0.8	2.26
	82	80	81.0	1.7	82	77	79.5	4.4	1.85
	95	91	93.0	3.0	96	88	92.0	6.1	1.08
	77	73	75.0	3.8	73	68	70.5	5.0	6.00
	79	76	77.5	2.7	83	76	79.5	6.2	-2.58
	79	76	77.5	2.7	82	71	76.5	10.2	1.29
	79	82	80.5	2.6	82	77	79.5	4.4	1.24
	78	85	81.5	6.1	92	88	90.0	3.1	-10.43
	92	90	91.0	1.6	92	87	89.5	4.0	1.65
	89	81	85.0	6.7	84	82	83.0	1.7	2.35
	88	82	85.0	5.0	83	78	80.5	4.4	5.29
	81	81	81.0	0.0	79	73	76.0	5.6	6.17
	89	84	86.5	4.1	78	77	77.5	0.9	10.40
	83	80	81.5	2.6	77	73	75.0	3.8	7.98
	87	84	85.5	2.5	78	76	77.0	1.8	9.94
35	81	83	82.0	1.7	81	82	81.5	0.9	0.61

	76	77	76.5	0.9	77	76	76.5	0.9	0.00
	84	88	86.0	3.3	72	72	720	0.0	16.28
	85	87	86.0	1.6	74	72	73.0	1.9	15.12
	83	89	86.0	4.9	78	70	74.0	7.6	13.95
	83	88	85.5	4.1	77	75	76.0	1.9	11.11
	80	77	78.5	2.7	70	72	710	2.0	9.55
	80	77	78.5	2.7	72	72	72.0	0.0	8.28
	78	73	75.5	4.7	72	69	70.5	3.0	6.62
	81	82	81.5	0.9	77	80	78.5	2.7	3.68
	80	87	83.5	5.9	75	85	80.0	8.8	4.19
	79	82	80.5	2.6	69	73	71.0	4.0	11.80
	78	82	80.0	3.5	68	73	70.5	5.0	11.88
	85	88	86.5	2.5	74	78	76.0	3.7	12.14
	76	74	75.0	1.9	71	80	75.5	8.4	-0.67
	79	75	77.0	3.7	76	85	80.5	7.9	-4.55
	75	75	75.0	0.0	69	76	725	6.8	3.33
	81	81	81.0	0.0	78	80	79.0	1.8	2.47
36	78	79	78.5	0.9	85	79	82.0	5.2	-4.46
	67	76	71.5	8.9	84	76	80.0	7.1	-11.89
	88	84	86.0	3.3	73	74	73.5	1.0	14.53
	86	81	83.5	4.2	76	75	75.5	0.9	9.58

*Each row represents a different lot

Table 26: Values after Outlier Removal (Example A)

Confirmatory 1	Confirmatory 2	Confirmatory 3	Confirmatory 4	Confirmatory 5	Confirmatory 6
11.0	-1.8	-5.5	-3.0	2.4	-2.5
-3.6	-5.9	-9.0	NA	-5.0	-11.6
7.3	11.1	16.5	10.0	3.5	16.3
2.3	8.3	13.9	5.4	0.6	7.3
1.9	6.8	11.1	8.2	6.0	9.8
1.1	6.6	11.8	8.2	0.0	7.4
6.0	4.0	6.1	6.2	9.2	5.9
-2.6	0.0	4.3	1.2	4.6	5.6
1.3	6.4	4.9	-0.6	4.4	5.6
1.2	1.2	-6.7	-3.4	8.8	7.1
-10.4	-6.1	0.0	-3.2	-1.2	-10.2
1.7	6.2	7.1	6.9	9.7	3.4
2.4	3.7	1.8	4.5	5.1	16.3
5.3	6.4	3.6	6.2	5.5	2.9

6.2	2.0	-12.3	4.4	5.1	-5.6
10.4	4.4	-9.9	5.9	-0.6	-4.3
8.0	0.7	-10.3	1.4	3.0	-1.9
9.9	-3.2	-7.0	-2.0	-7.4	-7.5
0.6	-3.8	0.6	-5.0	-6.8	1.2
0.0	-1.3	-2.9	-10.4	6.0	6.8
16.3	6.1	9.4	18.4	8.7	3.0
15.1	6.1	0.5	9.1	-2.0	-0.6
14.0	11.6	5.0	13.0	5.8	7.5
11.1	8.9	2.7	4.5	3.1	7.7
9.6	7.7	10.8	-2.3	2.2	7.8
8.3	1.9	3.9	1.1	-1.8	3.2
6.6	8.5	8.7	4.6	-0.6	2.0
3.7	5.7	4.8	4.2	2.7	-2.6
4.2	-6.5	-1.1	-13.2	9.5	1.5
11.8	3.3	0.5	1.1	9.3	5.8
11.9	1.3	6.6	-4.4	5.8	9.6
12.1	6.0	0.6	-3.8	6.8	15.1
-0.7	8.7	10.6	-5.2	6.3	7.0
-4.6	6.2	11.2	0.0	4.3	16.8
3.3	2.0	13.0	-2.4	3.5	9.0
2.5	5.0	10.8	-7.8	-0.5	10.2
-4.5	9.9	-1.7	8.3	-11.0	2.6
-11.9	9.3	-6.5	1.7	-9.0	-7.0
14.5	7.1	-1.7	9.6	4.9	8.9
9.6	4.1	-4.7	7.1	5.1	3.9
10.9	1.4	-4.7	10.1	8.8	5.7
12.4	0.0	-4.1	8.8	0.6	7.3
10.9	4.2	9.3	16.7	9.0	10.7
2.8	-0.7	6.7	14.1	3.0	0.0
1.4	1.4	4.1	10.7	11.7	5.6
1.3	2.0	0.6	9.9	5.3	7.4
-0.6	-7.9	-0.6	-10.5	-2.3	-10.7

*Each row represents a different lot

Table 27: Values after Outlier Removal (Example B)

Confirmatory 1*	Confirmatory 2	Confirmatory 3	Confirmatory 4	Confirmatory 5	Confirmatory 6
11.0	-1.8	-5.5	-3.0	2.4	-2.5

-3.6	-5.9	-9.0	NA	-5.0	-11.6
7.3	11.1	16.5	10.0	3.5	16.3
2.3	8.3	13.9	5.4	0.6	7.3
1.9	6.8	11.1	8.2	6.0	9.8
1.1	6.6	11.8	8.2	0.0	7.4
6.0	4.0	6.1	6.2	9.2	5.9
-2.6	0.0	4.3	1.2	4.6	5.6
1.3	6.4	4.9	-0.6	4.4	5.6
1.2	1.2	-6.7	-3.4	8.8	7.1
-10.4	-6.1	0.0	-3.2	-1.2	-10.2
1.7	6.2	7.1	6.9	9.7	3.4
2.4	3.7	1.8	4.5	5.1	16.3
5.3	6.4	3.6	6.2	5.5	2.9
6.2	2.0	-12.3	4.4	5.1	-5.6
10.4	4.4	-9.9	5.9	-0.6	-4.3
8.0	0.7	-10.3	1.4	3.0	-1.9
9.9	-3.2	-7.0	-2.0	-7.4	-7.5
0.6	-3.8	0.6	-5.0	-6.8	1.2
0.0	-1.3	-2.9	-10.4	6.0	6.8
16.3	6.1	9.4	18.4	8.7	3.0
15.1	6.1	0.5	9.1	-2.0	-0.6
14.0	11.6	5.0	13.0	5.8	7.5
11.1	8.9	2.7	4.5	3.1	7.7
9.6	7.7	10.8	-2.3	2.2	7.8
8.3	1.9	3.9	1.1	-1.8	3.2
6.6	8.5	8.7	4.6	-0.6	2.0
3.7	5.7	4.8	4.2	2.7	-2.6
4.2	-6.5	-1.1	-13.2	9.5	1.5
11.8	3.3	0.5	1.1	9.3	5.8
11.9	1.3	6.6	-4.4	5.8	9.6
12.1	6.0	0.6	-3.8	6.8	15.1
-0.7	8.7	10.6	-5.2	6.3	7.0
-4.6	6.2	11.2	0.0	4.3	16.8
3.3	2.0	13.0	-2.4	3.5	9.0
2.5	5.0	10.8	-7.8	-0.5	10.2
-4.5	9.9	-1.7	8.3	-11.0	2.6
-11.9	9.3	-6.5	1.7	-9.0	-7.0
14.5	7.1	-1.7	9.6	4.9	8.9
9.6	4.1	-4.7	7.1	5.1	3.9

10.9	1.4	-4.7	10.1	8.8	5.7
12.4	0.0	-4.1	8.8	0.6	7.3
10.9	4.2	9.3	16.7	9.0	10.7
2.8	-0.7	6.7	14.1	3.0	0.0
1.4	1.4	4.1	10.7	11.7	5.6
1.3	2.0	0.6	9.9	5.3	7.4
-0.6	-7.9	-0.6	-10.5	-2.3	-10.7
9.4	4.2	5.2	4.8	11.7	3.2
6.2	8.3	2.7	1.3	14.0	4.7
9.2	7.0	4.3	2.5	11.4	2.7
6.1	0.7	0.6	3.9	6.5	5.5
16.0	3.4	5.1	6.3	9.6	6.5
13.2	6.9	7.5	1.3	10.6	10.7
14.9	2.0	2.3	2.5	6.4	5.4

*Each row represents a different lot

Table 28: Values after Outlier Removal (Example C)

Confirmatory 1*	Confirmatory 2	Confirmatory 3	Confirmatory 4	Confirmatory 5	Confirmatory 6
11.0	-1.8	-5.5	-3.0	2.4	-2.5
-3.6	-5.9	-9.0	NA	-5.0	-11.6
7.3	11.1	16.5	10.0	3.5	16.3
2.3	8.3	13.9	5.4	0.6	7.3
1.9	6.8	11.1	8.2	6.0	9.8
1.1	6.6	11.8	8.2	0.0	7.4
6.0	4.0	6.1	6.2	9.2	5.9
-2.6	0.0	4.3	1.2	4.6	5.6
1.3	6.4	4.9	-0.6	4.4	5.6
1.2	1.2	-6.7	-3.4	8.8	7.1
-10.4	-6.1	0.0	-3.2	-1.2	-10.2
1.7	6.2	7.1	6.9	9.7	3.4
2.4	3.7	1.8	4.5	5.1	16.3
5.3	6.4	3.6	6.2	5.5	2.9
6.2	2.0	-12.3	4.4	5.1	-5.6
10.4	4.4	-9.9	5.9	-0.6	-4.3
8.0	0.7	-10.3	1.4	3.0	-1.9
9.9	-3.2	-7.0	-2.0	-7.4	-7.5
0.6	-3.8	0.6	-5.0	-6.8	1.2
0.0	-1.3	-2.9	-10.4	6.0	6.8

16.3	6.1	9.4	18.4	8.7	3.0
15.1	6.1	0.5	9.1	-2.0	-0.6
14.0	11.6	5.0	13.0	5.8	7.5
11.1	8.9	2.7	4.5	3.1	7.7
9.6	7.7	10.8	-2.3	2.2	7.8
8.3	1.9	3.9	1.1	-1.8	3.2
6.6	8.5	8.7	4.6	-0.6	2.0
3.7	5.7	4.8	4.2	2.7	-2.6
4.2	-6.5	-1.1	-13.2	9.5	1.5
11.8	3.3	0.5	1.1	9.3	5.8
11.9	1.3	6.6	-4.4	5.8	9.6
12.1	6.0	0.6	-3.8	6.8	15.1
-0.7	8.7	10.6	-5.2	6.3	7.0
-4.6	6.2	11.2	0.0	4.3	16.8
3.3	2.0	13.0	-2.4	3.5	9.0
2.5	5.0	10.8	-7.8	-0.5	10.2
-4.5	9.9	-1.7	8.3	-11.0	2.6
-11.9	9.3	-6.5	1.7	-9.0	-7.0
14.5	7.1	-1.7	9.6	4.9	8.9
9.6	4.1	-4.7	7.1	5.1	3.9
10.9	1.4	-4.7	10.1	8.8	5.7
12.4	0.0	-4.1	8.8	0.6	7.3
10.9	4.2	9.3	16.7	9.0	10.7
2.8	-0.7	6.7	14.1	3.0	0.0
1.4	1.4	4.1	10.7	11.7	5.6
1.3	2.0	0.6	9.9	5.3	7.4
-0.6	-7.9	-0.6	-10.5	-2.3	-10.7
9.4	4.2	5.2	4.8	11.7	3.2
6.2	8.3	2.7	1.3	14.0	4.7
9.2	7.0	4.3	2.5	11.4	2.7
6.1	0.7	0.6	3.9	6.5	5.5
16.0	3.4	5.1	6.3	9.6	6.5
13.2	6.9	7.5	1.3	10.6	10.7
14.9	2.0	2.3	2.5	6.4	5.4

*Each row represents a different lot

[0313] Sensitivity: The screening assay sensitivity was evaluated six times, in pooled human serum (three times) and three individual lots of human serum (one time for each lot). Each sample was spiked with anti-XTMAB-16 antibodies PC. Serial two-fold dilutions of the spiked samples (from

4,000ng/mL to 0.49ng/mL) in pNHS and individual mouse serum (iNMS) were performed. The screening sensitivity was determined for each sensitivity set using Microsoft Excel Forecast, by interpolation at the SCP (5% false-positive). The final assay sensitivity in screening assay is the mean of calculated concentration among all the qualified curves. The results showed that the screening sensitivity of the assay to detect anti-XTMAB-16 antibodies was 2.77ng/mL in human serum. An example of this result is shown in Table 29.

Table 29: Screening Sensitivity

Screening Assay		Sensitivity in Individual serum														
Run Example		Sensitivity 01 (Individual-01) Run 55					Sensitivity 02 (Individual-02) Run 56					Sensitivity 03 (Individual-03) Run 57				
Titer	ADA Cone (ng/mL)	ECL	%CV	Plate NC	Plate SCP	Plate TCP	ECL	%CV	Plate NC	Plate SCP	Plate TCP	ECL	%CV	Plate NC	Plate SCP	Plate TCP
1	4000.00	7790.5	2.5	82.8	91.0	99.3	8550.0	0.3	81.8	89.9	98.1	8489.5	1.6	88.9	97.7	106.68
2	2000.00	6952.0	3.5				7490.0	1.1				7082.0	10.3			
4	1000.00	4194.0	0.9				4613.5	1.0				4428.5	9.2			
8	500.00	1520.0	1.0				1653.5	0.7				1703.0	1.0			
16	250.00	742.5	2.2				789.5	0.6				785.0	5.0			
32	125.00	375.5	2.1				377.5	0.9				489.0	3.2			
64	62.50	200.0	9.2				248.5	3.1				283.0	1.5			
128	31.25	156.5	1.4				163.5	0.4				185.0	1.5			
256	15.63	122.5	4.0				116.5	0.6				130.5	7.0			
512	7.81	110.0	3.9				102.0	2.8				110.5	1.9			
1024	3.91	94.0	1.5				88.0	4.8				97.5	0.7			
2048	1.95	89.5	0.8				84.0	1.7				90.0	0.0			

4096	0.98	83.0	8.5				79.0	1.8				86.5	4.1			
8192	0.49	82.5	6.0				82.5	0.9				87.0	3.3			

[0314] Confirmatory assay sensitivity was performed by spiking XTMA B-16 into diluted samples prepared for screening sensitivity. The confirmatory sensitivity is determined for each sensitivity set using Microsoft Excel Forecast, by interpolation at the CCP (1% false-positive). The final assay sensitivity in confirmatory assay is the mean of calculated concentration among all the qualified curves. The results showed that the confirmatory sensitivity of the assay to detect anti-XTMA B-16 antibodies was 4.25ng/mL. An example of this result is shown in Table 30.

Table 30: Confirmatory Sensitivity

Confirmatory Sensitivity		Sensitivity in Individual serum											
Example Run		Sensitivity 01 (Individual-01) Run 55				Sensitivity 02 (Individual-02) Run 56				Sensitivity 03 (Individual-03) Run 57			
Titer	ADA Cone. (ng/mL)	EC L	%C V	%Inhibition	Condition	EC L	%C V	%Inhibition	Condition	EC L	%C V	%Inhibition	Condition
1	4000.00	74.0	9.6	99.05	>CCP	71.5	1.0	99.16	>CCP	81.0	5.2	99.05	>CCP
2	2000.00	79.0	9.0	98.86	>CCP	73.0	3.9	99.03	>CCP	85.5	2.5	98.79	>CCP
4	1000.00	74.5	10.4	98.22	>CCP	72.0	2.0	98.44	>CCP	86.0	0.0	98.06	>CCP
8	500.00	75.5	4.7	95.03	>CCP	72.5	1.0	95.62	>CCP	83.0	1.7	95.13	>CCP
16	250.00	72.0	5.9	90.30	>CCP	67.0	6.3	91.51	>CCP	83.0	3.4	89.43	>CCP
32	125.00	77.5	4.6	79.36	>CCP	70.5	5.0	81.32	>CCP	90.5	5.5	81.49	>CCP
64	62.50	76.0	5.6	62.00	>CCP	68.0	6.2	72.64	>CCP	86.5	0.8	69.43	>CCP
128	31.25	77.5	2.7	50.48	>CCP	71.0	0.0	56.57	>CCP	90.0	0.0	51.35	>CCP
256	15.63	85.5	10.8	30.20	>CCP	85.5	12.4	26.61	>CCP	90.0	7.9	31.03	>CCP
512	7.81	79.5	4.4	27.73	>CCP	80.5	2.6	21.08	>CCP	86.5	2.5	21.72	>CCP

102 4	3.91	79. 0	7.2	15.96	<CCP	72. 0	9.8	18.18	>CCP	84. 5	0.8	13.33	<CCP
204 8	1.95	73. 5	6.7	17.88	>CCP	72. 5	4.9	13.69	<CCP	81. 5	0.9	9.44	<CCP
409 6	0.98	74. 5	0.9	10.24	<CCP	73. 0	3.9	7.59	<CCP	91. 0	0.0	-5.20	<CCP
819 2	0.49	77. 0	3.7	6.67	<CCP	69. 5	3.1	15.76	<CCP	91. 5	7.0	-5.17	<CCP

[0315] The sensitivity data was also used to determine the LPC of the assay. The LPC is based on the sensitivity with a 1% false-positive rate, which was calculated in both screening and confirmatory assays using the mean of the Sensitivity + $t_{0.99, df} \times SD$ (Sensitivity). The LPC concentration was 6.80 and 9.70ng/mL for screening and confirmatory assay, respectively. Results are detailed in Table 31. The calculated LPC concentration is the higher LPC between the screening assay and the confirmatory assay, which is 9.70ng/mL.

Table 31: LPC Determination for Screening Assay and Confirmatory Assay

LPC Calculation	Screening Assay	Confirmatory Assay
Mean (Sensitivity) (ng/mL)	2.77	4.25
SD (Sensitivity) (ng/mL)	1.21	1.61
df	5	5
$t_{0.99, df}$	3.365	3.365
Intermediate LPC (ng/mL)	6.80	9.70
Final LPC (ng/mL)	9.70	9.70

[0316] Hook Effect Assay: The hook (prozone) effect is a phenomenon in which the signal of the assay changes from increasing to decreasing when the ADA concentration increases. The hook effect may not be critical to ADA detection if all concentrations of positive antibody yield positive results. The hook effect was assessed using a sample with an ultra-high concentration of anti-XTMAB-16 antibodies in pNHS (32,000ng/mL), which was prepared by spiking ADA PC into pooled serum and freezing for at least 12 hours. The sample was then serially two-fold diluted to a concentration below the plate-specific TCP. All samples with a concentration of 7.81ng/mL anti-

XTMAB-16 antibodies or greater demonstrated a positive ECL value as compared to the plate-specific TCP, indicating that no prozone effect was observed. An example of data showing this result is illustrated in Table 32.

Table 32: Hook Effect Assay

Run Example		Hook Effect & Titration				
Dilution Factor	ADAConc. (ng/mL)	ECL	%CV	Plate JC	Plate TCP	Condition
1	32000.00	11475.0	0.1	93.1	111.72	>TCP
2	16000.00	10057.5	3.1			>TCP
4	8000.00	9138.5	1.1			>TCP
8	4000.00	8216.5	2.3			>TCP
16	2000.00	7559.0	0.5			>TCP
32	1000.00	5498.0	1.2			>TCP
64	500.00	2717.5	0.2			>TCP
128	250.00	1290.0	1.3			>TCP
256	125.00	681.0	0.8			>TCP
512	62.50	390.5	1.6			>TCP
1024	31.25	238.5	4.4			>TCP
2048	15.63	172.0	0.8			>TCP
4096	7.81	127.5	2.8			>TCP
819	3.91	110.5	4.5			<TCP
16384	1.95	88.0	3.2			<TCP
32768	0.98	92.0	3.1			<TCP

[0317] *Selectivity:* Selectivity is the ability of the assay to detect anti-XTMAB-16 antibodies in the presence of other matrix components. ADA unspiked and spiked samples at LPC level (9.70ng/mL) were prepared from 10 lots of normal human serum, 10 lots of disease human serum, 3 lots of hemolyzed human serum, and 3 lots of lipemic human serum that originally tested in 4 runs with both screening and confirmatory assay. For individual lots of normal human serum, 9 out of 10 unspiked samples (90%) were confirmed as negative, while 9 out of 10 spiked LPC samples (90%) were confirmed as positive. For individual lots of disease human serum, 10 out of 10 unspiked samples (100%) were confirmed as negative, while 10 out of 10 spiked LPC samples (100%) were confirmed as positive. For individual lots of hemolyzed human serum, 3 out of 3 unspiked samples

(100%) were confirmed as negative, while 3 out of 3 spiked LPC samples (100%) were confirmed as positive. For individual lots of lipemic human serum, 3 out of 3 unspiked samples (100%) were confirmed as negative, while 3 out of 3 spiked LPC samples (100%) were confirmed as positive, which met the acceptance criteria. This suggests there is no matrix interference for detection of anti-XTMAB-16 antibodies in human serum. An example of data showing this result is illustrated in Table 33.

Table 33: Assessing Selectivity and Matrix Interference

Example Run	Serum ID		Drug Unspiked					Drug Spiked (MAB-16 at 200 µg/mL)					Sample condition (Neg/Pos/Fail)
			ECL	%CV	Plate NC	Plate SCP	Condition	ECL	%CV	%Inhibition	CCP (%)	Condition	
68	ADA Unspiked	Indi 01	83.0	3.4	82.5	90.75	<SCP	80.0	5.3	3.61	16.45	<CCP	Negative
		Indi 02	84.0	0.0			<SCP	83.0	6.8	1.19		<CCP	Negative
		Indi 03	80.5	0.9			<SCP	84.0	1.7	-4.35		<CCP	Negative
		Indi 04	85.0	1.7			<SCP	122.0	34.8	-43.53		Fail	Fail
		Indi 05	78.5	0.9			<SCP	80.0	1.8	-1.91		<CCP	Negative
		Indi 06	77.5	6.4			<SCP	82.5	9.4	-6.45		<CCP	Negative
		Indi 07	75.5	2.8			<SCP	83.0	6.8	-9.93		<CCP	Negative
		Indi 08	84.0	8.4			<SCP	88.5	7.2	-5.36		<CCP	Negative
	ADA Spiked at LPC	LPC-Indi01	109.5	4.5			>SCP	89.0	9.5	18.72		>CCP	Positive
		LPC-Indi02	115.5	5.5			>SCP	78.0	3.6	32.47		>CCP	Positive
		LPC-Indi03	115.5	4.3			>SCP	77.0	9.2	33.33		>CCP	Positive
		LPC-Indi04	117.5	1.8			>SCP	82.0	13.8	30.21		>CCP	Positive
		LPC-Indi05	110.5	1.9			>SCP	78.0	1.8	29.41		>CCP	Positive
		LPC-Indi06	111.0	8.9			>SCP	79.0	1.8	28.83		>CCP	Positive
		LPC-Indi07	104.5	2.0			>SCP	76.5	6.5	26.79		>CCP	Positive
		LPC-Indi08	113.5	0.6			>SCP	78.5	4.5	30.84		>CCP	Positive
69	ADA Unspiked	Indi 09	93.5	2.3	89.4	98.34	<SCP	81.5	6.1	12.83	16.45	<CCP	Negative
		Indi 10	87.0	1.6			<SCP	85.5	2.5	1.72		<CCP	Negative
		Disease-01	90.5	7.0			<SCP	81.5	0.9	9.94		<CCP	Negative
		Disease-02	96.5	6.6			<SCP	84.5	2.5	12.44		<CCP	Negative
		Disease-03	92.0	12.3			<SCP	83.5	11.0	9.24		<CCP	Negative
		Disease-04	91.0	0.0			<SCP	87.0	1.6	4.40		<CCP	Negative

		Disease-05	80.0	1.8			<SCP	86.0	1.6	-7.50		<CCP	Negative
		Disease-06	85.5	5.8			<SCP	92.5	3.8	-8.19		<CCP	Negative
	ADA Spiked at LPC	LPC-Indi09	120.0	7.1			>SCP	97.0	24.8	19.17		Fail	Fail
		LPC-Indi10	121.0	0.0			>SCP	81.0	8.7	33.06		>CCP	Positive
		LPC-Disease-01	116.0	0.0			>SCP	78.5	4.5	32.33		>CCP	Positive
		LPC-Disease-02	121.0	2.3			>SCP	80.0	1.8	33.88		>CCP	Positive
		LPC-Disease-03	113.5	3.1			>SCP	84.0	3.4	25.99		>CCP	Positive
		LPC-Disease-04	118.5	0.6			>SCP	86.5	7.4	27.00		>CCP	Positive
		LPG-Disease-05	109.5	0.6			>SCP	81.0	3.5	26.03		>CCP	Positive
		LPC-Disease-06	112.5	0.6			>SCP	86.0	0.0	23.56		>CCP	Positive

[0318] Drug Tolerance: The presence of drug XTMA B-16 in samples may interfere with the detection of anti-XTMA B-16 antibodies and, hence, the ability of the assay system to correctly detect the ADA was evaluated. Drug tolerance limit was defined as the highest level of drug in the presence of which ADA is still detected. Potential interference by the drug was evaluated using a positive control prepared at concentrations of 100ng/mL and 9.7ng/mL. These controls were spiked with XTMA B-16 at concentrations ranging from 5.0µg/mL to 400µg/mL. The addition of up to 100.0µg/mL XTMA B-16 had no impact on the ADA detection in samples spiked at 100ng/mL, and the addition of up to 5.0µg/mL XTMA B-16 had impact on the ADA detection in samples spiked at 9.7ng/mL, which led to a false-negative result. An example of data showing this result is illustrated in Table 34.

Table 34: Assessing Drug Tolerance

Example Run	Drug Tolerance							
Concentration (µg/mL)	ADA PC at 100 ng/mL			ADA PC at 9.70 ng/mL			Plate NC	Plate SCP
	ECL	%CV	Condition	ECL	%CV	Condition		

400.0	85.5	5.8	<SCP	91.0	4.7	<SCP	91.1	100.21
200.0	98.5	0.7	<SCP	88.0	1.6	<SCP		
100.0	104.0	5.4	>SCP	94.5	14.2	<SCP		
50.0	109.5	1.9	>SCP	92.0	4.6	<SCP		
20.0	112.0	0.0	>SCP	95.0	6.0	<SCP		
10.0	109.0	1.3	>SCP	95.5	5.2	<SCP		
5.0	103.0	4.1	>SCP	99.0	8.6	<SCP		
0.0	323.5	2.8	>SCP	127.5	2.8	>SCP		

[0319] Intra-assay precision: Intra-assay precision, an evaluation of the within-run performance of the controls, was evaluated in one run using six independent preparations of the HPC (4,000ng/mL), LPC (9.70ng/mL), and NC. The results demonstrate that the controls met the intra-assay precision criteria of $\leq 20.0\%$ for the ECL signal for both screening and confirmatory assays. The controls also met the criteria of ECL of NC < plate-specific SCP $\leq$ LPC < HPC for screening assay, and %inhibition of NC < CCP $\leq$ LPC < and HPC for confirmatory assay. The screening intra-assay precision of the HPC and LPC was 18.2% and 4.3%, respectively. The confirmatory intra-assay precision of the ECL signal for HPC and LPC was 6.3% and 4.1%, respectively. The intra-assay precision of percent for HPC and LPC was 0.2% and 9.1%, respectively. An example of data showing these results is illustrated in Table 35.

Table 35: Assessing Intra-assay Precision

Example Run	Drug Unspiked						Drug Spiked (MAB-16 at 200 μ g/mL)								
	HPC		LPC		NC		HPC			LPC			NC		
	ECL mean	%CV	ECL mean	%CV	ECL mean	%CV	ECL mean	%CV	%Inhibition	ECL mean	%CV	%Inhibition	ECL mean	%CV	%Inhibition
1	9496.5	11.8	144.5	0.5	98.5	0.7	90.0	6.3	99.05	89.5	2.4	38.06	89.0	6.4	9.64
2	12278.0	34.5	140.0	3.0	98.0	10.1	88.0	3.2	99.28	88.5	2.4	36.79	92.0	4.6	6.12
3	8407.5	1.7	137.5	3.6	92.5	2.3	100.5	3.5	98.80	90.5	2.3	34.18	102.0	9.7	-10.27
4	8686.0	0.0	136.0	0.5	94.5	6.0	85.0	0.8	99.02	80.5	5.3	40.81	82.0	0.9	13.23
5	7914.0	0.1	132.0	3.2	89.0	0.8	90.5	0.8	98.86	88.5	1.6	32.95	91.0	0.0	-2.25

6	7880.5	0.1	128.0	0.6	91.5	3.9	91.5	5.4	98.84	86.5	8.2	32.42	106.5	4.6	-16.39
Mean	9110.4	NA	136.3	NA	94.0	NA	90.9	NA	98.98	87.3	NA	35.87	93.8	NA	0.0
%CV	18.2	NA	4.3	NA	NA	NA	5.7	NA	0.2	4.1	NA	9.1	NA	NA	NA

[0320] Inter-assay precision: Inter-assay precision, an evaluation of the between-run performance of the controls, was evaluated based on the HPC (4,000ng/mL), LPC (9.7ng/mL), and NC results across all accepted validation runs, excluding PCs from the robustness run. The screening inter-assay precision of the method was 14.1% (HPC) and 7.9% (LPC). The controls met the inter-assay precision criteria of $\leq 20.0\%$ for HPC and LPC. The confirmatory inter-assay precision of the %inhibition for HPC and LPC was 0.2% and 13.3%, respectively. The controls also met the criteria of $\text{ECL NC} < \text{plate-specific SCP} \leq \text{LPC} < \text{HPC}$ for screening assay, and $\text{\%inhibition of NC} < \text{CCP} \leq \text{LPC}$ and HPC for confirmatory assay, respectively. An example of data showing this result is illustrated in Table 36.

Table 36: Assessing Inter-assay Precision

Example Run	Run Parameter	Screening Assay							
		HPC	HPC	LPC	LPC	NC	NC	NC	NC
34	CP 1	8561.0	7558.0	NA	NA	97.5	90.5	83.0	86.5
35	CP 2	7783.5	7819.0	NA	NA	82.5	80.0	76.5	82.0
36	CP 3	8032.5	8030.0	NA	NA	81.5	78.0	75.5	76.0
37	CP 4	8252.5	8187.0	NA	NA	87.0	81.0	80.5	84.0
38	CP 5	8714.0	8668.5	NA	NA	80.0	79.0	78.0	82.0
39	CP 6	8866.5	8049.0	NA	NA	77.5	71.0	77.5	76.5
40	CP 7	9051.0	8226.0	NA	NA	89.5	86.0	78.0	78.5
41	CP 8	8736.0	8364.0	NA	NA	93.0	88.0	91.5	91.5
42	CP 9	8833.0	8025.5	NA	NA	91.0	84.0	84.0	84.5
43	CP 10	10524.5	9894.0	NA	NA	85.0	83.5	81.5	81.0
44	CP 11	10455.0	10403.5	NA	NA	89.0	86.5	85.5	83.5
45	CP 12	10864.0	10656.5	NA	NA	90.0	88.5	91.0	91.0
49	CP 13	8053.0	7539.0	NA	NA	87.0	85.5	88.0	86.5
50	CP 14	9018.5	8162.5	NA	NA	102.5	93.5	85.5	89.0
51	CP 15	8498.0	7558.5	NA	NA	88.5	83.5	78.5	82.0
52	CP 16	8083.0	7484.0	NA	NA	85.0	84.0	79.5	80.5
53	CP 17	8310.5	7673.5	NA	NA	85.5	82.0	80.0	88.0
54	CP 18	8127.5	7328.5	NA	NA	82.0	79.0	77.5	80.5

55	Individual Sensitivity- 1	8187.5	7516.0	NA	NA	90.5	84.5	76.0	80.0
56	Individual Sensitivity-2	8348.5	8288.5	NA	NA	84.5	79.5	81.0	82.0
57	Individual Sensitivity-3	7817.5	6080.0	NA	NA	88.5	85.5	88.0	93.5
58	Pooled Sensitivity-1	8279.0	7531.0	NA	NA	89.0	92.5	97.0	93.0
59	Pooled Sensitivity-2	8314.5	7464.0	NA	NA	99.0	93.0	82.5	85.5
60	Pooled Sensitivity-3	8642.5	7899.0	NA	NA	103.5	102.5	93.0	93.0

[0321] *Stability:* The stability of positive controls in human serum was used to assess sample stability. Freeze/thaw (6 cycles of $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ to ambient temperature) and bench-top (24 hours at ambient temperature) stability were tested. The acceptance criteria for the stability samples were as follows: (1) $\text{NC} < \text{plate-specific SCP} \leq \text{LPC} < \text{HPC}$ for screening assay, (2) $\% \text{inhibition of NC} < \text{CCP} \leq \text{LPC}$ and HPC for confirmatory assay, (3) $\% \text{CV} \leq 20\%$ for the NC, LPC, and HPC, (4) at least 66.7% (two out of three) stability samples must meet the above acceptance criteria.

[0322] The tested samples were stored at -70°C (nominal) for at least 24 hours prior to first thaw and 12 hours prior to each additional thaw. Samples were at ambient temperature for 1-2 hours prior to refreezing. Three aliquots for each control (HPC, LPC, and NC) were tested after undergoing six freeze/thaw cycles on a plate using both screening and confirmatory assay, with frozen PCs and NC, which were used for plate acceptance. The results met the acceptance criteria, therefore, anti-XTMAB-16 antibodies in human serum are stable for up to six freeze/thaw cycles from -70°C (nominal) to ambient temperature. An example of these results is shown in Table 37.

Table 37: Assessing Stability (Example A)

Freeze/Thaw (6-Cycles)		Drug Unspiked					Drug Spiked (MAB-16 at 200 $\mu\text{g/mL}$)				
Exam ple Run	Samp le ID	ECL mean	%C V	Plat e NC	Plat e SCP	Condi tion	ECL mean	%C V	%Inhibiti on	Confirmat ory CP (%)	Condi tion
67	HPC-1	8775 .0	0.9	92. 9	102. 19	Pass	91.5	10. 0	98.96	16.45	Pass
	HPC-2	8360 .5	0.2			Pass	94.5	0.7	98.87		Pass
	HPC-3	9073 .0	3.9			Pass	106. 0	20. 0	98.83		Pass

	LPC-1	133.5	2.6			Pass	90.0	3.1	32.58		Pass
	LPC-2	128.5	0.6			Pass	88.5	0.8	31.13		Pass
	LPC-3	133.0	2.1			Pass	95.0	3.0	28.57		Pass
	NC-1	93.0	4.6			Pass	90.0	3.1	3.23		Pass
	NC-2	94.5	0.7			Pass	95.0	6.0	-0.53		Pass
	NC-3	87.5	0.8			Pass	91.0	4.7	-4.00		Pass

[0323] The tested samples were stored at -70°C (nominal) for at least 24 hours prior to first thaw. Three aliquots of each pre-existing frozen control (HPC, LPC, and NC) were thawed on the bench-top for 24.5 hours, prior to analysis on a plate using both screening and confirmatory assay with frozen PCs and NC, which were used for plate acceptance. All HPC, LPC, and NC stability samples met the acceptance criteria, therefore, anti-XTMAB-16 antibodies in human serum are stable for up to 24.5 hours at ambient surroundings. An example of these results is shown in Table 38.

Table 38: Assessing Stability (Example B)

Bench top (24.5 hours)		Drug Unspiked					Drug Spiked (MAB-16 at 200 µg/mL)				
Example Run	Sample ID	ECL mean	%CV	Plate NC	Plate SC P	Condition	ECL mean	%CV	%Inhibition	Confirmatory CP (%)	Condition
66	HPC-1	8074.5	0.5	90.1	99.11	Pass	84.5	5.9	98.95	16.45	Pass
	HPC-2	8175.5	2.8			Pass	85.0	5.0	98.96		Pass
	HPC-3	8311.5	4.8			Pass	114.0	29.8	98.63		Fail
	LPC-1	119.0	1.2			Pass	83.5	4.2	29.83		Pass
	LPC-2	121.0	2.3			Pass	76.0	7.4	37.19		Pass

	LPC-3	122.0	2.3			Pass	92.5	3.8	24.18		Pass
	NC-1	89.0	6.4			Pass	84.5	4.2	5.06		Pass
	NC-2	93.5	2.3			Pass	83.0	3.4	11.23		Pass
	NC-3	86.5	5.7			Pass	88.0	0.0	-1.73		Pass

[0324] *Study Phase Bioanalysis Acceptance Criteria for Controls:* System suitability criteria was established using data from all valid runs in the validation except robustness runs. In future sample analysis, the criteria are utilized as acceptance criteria for the acceptance or rejection of a run. The %CV criterion for intra-replicate measurements of controls and samples is $\leq 20.0\%$.

[0325] Acceptance criteria for NC included an in screening and titration assay and an ECL of NC $<$ plate-specific SCP, and an ECL of NC mean ≤ 142.2 . These results are further outlined in Table 39.

Table 39: Plate Control Acceptance Ranges

System Suitability Criteria	HPC	LPC	NC
Mean	8118.6	126.8	86.4
SD	1141.0	10.0	18.6
Upper Limit (Mean + 3xSD)	NA	156.8	142.2
Lower Limit (Mean - 3xSD)	4695.6	96.8	NA

[0326] Acceptance criteria for NC also included a confirmatory assay with %inhibition of NC $<$ CCP (16.45%).

[0327] Acceptance criteria for LPC included an in screening and titration assay and an ECL of LPC $\geq$ plate-specific SCP and an LPC where the ECL mean ≥ 96.8 or ≤ 156.8 .

[0328] Acceptance criteria for LPC also included an in confirmatory assay, and %inhibition of LPC $\geq$ CCP (16.45%).

[0329] Acceptance criteria for HPC included an in screening and titration assay with an ECL of HPC > ECL of LPC, and HPC of $4695.6 \leq$ ECL mean.

[0330] Acceptance criteria for HPC also included an in confirmatory assay with %inhibition of HPC $\geq$ CCP (16.45%).

[0331] Acceptance criteria for titer control included the titer control value equaling $\pm 2 \times$ overall mean titers from sensitivity runs (inclusive), and the titer of XTMA B-16 ADA PC at 4000ng/mL in pNHS is 25600-102400.

[0332] Assay Robustness: To determine assay robustness, the parameters of the assay were tested. The HPC, LPC, and NC were tested in both screening and confirmatory assay with different incubation times in two runs. The results of both runs met the method acceptance criteria, and the controls were within the established ranges, indicating that the assay demonstrated robustness. Examples of these results are shown in Tables 40 and 41.

Table 40: Assessing Assay Robustness (Example A)

Example Run	Sample ID	Drug Unspiked					Drug Spiked (MAB-16 at 200 µg/mL)					Run Condition
		ECL	%CV	Plate NC	Plate SCP	Condition	ECL	%CV	%Inhibition	CCP (%)	Condition	
61	HPC	8253.0	1.6	111.8	123.02	Pass	101.0	1.4	98.78	16.45	Pass	Pass
	HPC	8929.0	7.5			Pass	107.5	9.9	98.80		Pass	
	HPC	7354.5	1.6			Pass	94.5	0.7	98.72		Pass	
	LPC	132.5	10.1			Pass	106.0	4.0	20.00		Pass	
	LPC	125.0	5.7			Pass	102.5	2.1	18.00		Pass	
	LPC	120.0	5.9			Fail	90.5	0.8	24.58		Pass	
	NC	100.0	0.0			Pass	105.5	6.0	-5.50		Pass	
	NC	111.5	20.9			Fail	100.0	2.8	10.31		Fail	
	NC	113.5	15.6			Pass	97.5	0.7	14.10		Pass	

	NC	114.0	1.2			Pass	124.0	26.2	-8.77		Fail	
	NC	121.0	10.5			Pass	115.0	1.2	4.96		Pass	
	NC	111.0	2.5			Pass	128.0	3.3	-15.32		Pass	
62	HPC	7926.0	0.7	95.1	104.59	Pass	87.0	1.6	98.90		Pass	Pass
	HPC	7689.5	1.0			Pass	94.0	6.0	98.78		Pass	
	HPC	6926.0	3.2			Pass	85.5	0.8	98.77		Pass	
	LPC	109.5	0.6			Pass	88.0	3.2	19.63		Pass	
	LPC	116.5	3.0			Pass	86.5	5.7	25.75		Pass	
	LPC	103.5	3.4			Fail	81.5	6.1	21.26		Pass	
	NC	113.0	32.5			Fail	85.5	0.8	24.34		Fail	
	NC	91.0	4.7			Pass	86.0	0.0	5.49		Pass	
	NC	88.0	3.2			Pass	90.5	2.3	-2.84		Pass	
	NC	94.5	0.7			Pass	91.5	0.8	3.17		Pass	
	NC	89.0	1.6			Pass	96.5	3.7	-8.43		Pass	
	NC	95.0	3.0			Pass	101.5	7.7	-6.84		Pass	

Table 41: Assessing Assay Robustness (Example B)

Incubation Step	Min Incubation Time (Run 61)	Max Incubation Time (Run 62)
Controls/samples incubate with glycine buffer	28 min	65 min
MSD plate incubate with blocking buffer	56 min	172 min
Controls/samples incubate with Bio-MAB-16 solution in polypropylene plate	4 min	17 min
Controls/samples incubate in MSD plate	54 min	128 min
Controls/samples incubate with Ru-MAB-16 solution	49 min	134 min

Additional Embodiments

[0333] The present technology includes, but is not limited to, the following specific embodiments set forth herein below in paragraphs [0334]-[0410]:

[0334] 1. A method for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

- (i) incubating the sample with a composition comprising:
 - (a) a first affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and
 - (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (ii) isolating ADAs that form a complex with both the first affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; and
- (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point.

[0335] 2. The method of embodiment 1, wherein isolating the ADAs comprises:

- (a) providing a solid phase comprising the second affinity label;
- (b) contacting the sample and the composition with the solid phase; and
- (c) washing the solid phase.

[0336] 3. The method of embodiment 1 or 2, wherein the target antibody, the affinity antibody, and/or the detection antibody comprise a chimeric human-murine monoclonal IgG1-kappa antibody.

[0337] 4. The method of any one of embodiments 1-3, wherein the target antibody, the affinity antibody, and/or the detection antibody comprise SEQ ID NOs: 1 and 4.

[0338] 5. The method of any one of embodiments 2-4, wherein the solid phase is a plate.

[0339] 6. The method of any one of embodiments 1-5, wherein the first affinity label is biotin.

[0340] 7. The method of any one of embodiments 1-6, wherein the second affinity label is streptavidin.

[0341] 8. The method of any one of embodiments 1-7, wherein the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody.

[0342] 9. The method of any one of embodiments 2-8, wherein measuring the level of the detection label comprises:

- (1) contacting the solid phase with a marker configured to produce a chemiluminescent or electrochemiluminescent signal when the marker is proximal to the detection label; and
- (2) detecting the chemiluminescent or electrochemiluminescent signal.

[0343] 10. The method of embodiment 9, wherein the marker is tripropylamine (TPA), and measuring the level of the detection label further comprises applying an electrical current to the marker to produce the chemiluminescent or electrochemiluminescent signal.

[0344] 11. The method of any one of embodiments 2-10, wherein the solid phase is blocked with a blocking buffer before the sample and the composition are contacted with the solid phase.

[0345] 12. The method of any one of embodiments 1-11, further comprising performing steps (i) to (iii) on a control sample.

[0346] 13. The method of embodiment 12, wherein the control sample is incubated with the composition and contacted with the second affinity label.

[0347] 14. The method of any one of embodiments 1-13, wherein the predetermined cut-point corresponds to a level with a 5% false-positive rate.

[0348] 15. The method of embodiment 14, wherein the 5% false-positive rate is determined by measuring the detection label in a plurality of control samples and calculating the 5% false-positive rate.

[0349] 16. The method of embodiment 9, wherein the chemiluminescent or electrochemiluminescent signal is measured as electrochemiluminescence (ECL).

[0350] 17. The method of embodiment 16, wherein the predetermined cut-point is an ECL value of at least 1.

[0351] 18. The method of embodiment 16 or 17, wherein the predetermined cut-point is an ECL value of about 1.10.

[0352] 19. The method of any one of embodiments 1-18, wherein the method has a sensitivity that is less than 6ng/mL.

[0353] 20. The method of embodiment 19, wherein the sensitivity is less than 4ng/mL.

[0354] 21. The method of any one of embodiments 1-20, wherein the method has a drug tolerance of about 100ng/mL.

[0355] 22. A method for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

- (i) contacting a sample with one or more anti-TNF α conjugates to form a complex between the ADA and the anti-TNF α conjugates; and
- (ii) measuring a level of anti-TNF α conjugates in the complex;

wherein the method detects the ADA with a sensitivity less than 5ng/mL.

[0356] 23. The method of embodiment 22, wherein the one or more anti-TNF α conjugates comprise an affinity antibody comprising an anti-TNF α antibody conjugated to an affinity label.

[0357] 24. The method of embodiment 22 or 23, wherein the one or more anti-TNF α conjugates comprise a detection antibody comprising an anti-TNF α antibody conjugated to a detection label.

[0358] 25. The method of embodiment 23, wherein the affinity label comprises biotin.

[0359] 26. The method of embodiment 24, wherein the detection label comprises a sulfo-Tag.

[0360] 27. The method of any one of embodiments 22-26, wherein the method detects the ADA with a sensitivity less than 3ng/mL.

[0361] 28. The method of any one of embodiments 22-27, wherein the method detects the ADA with a sensitivity of 2.77ng/mL or less.

[0362] 29. A method for estimating a quantity of an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

- (i) incubating the sample with a composition comprising:
 - (a) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and
 - (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label;
- (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point;
- (iv) performing steps (i)-(iii) on a known sample having a known concentration of ADA; and
- (v) comparing the level of the detection label in the sample to the level of the detection label in the known sample.

[0363] 30. The method of embodiment 29, wherein the known sample is serially diluted into a plurality of titer samples, wherein comparing the level of the detection label in the sample to the level of the detection label in the known sample comprises generating a curve of the levels of the detection label in each of the titer samples and comparing the detection label in the sample to the curve.

[0364] 31. A method of confirming that an anti-drug antibody (ADA) binds a target antibody comprising an anti-TNF α antibody in a sample by performing a competitive binding assay comprising performing the method of embodiment 1 on the sample, wherein the composition further comprises an unlabeled anti-TNF α antibody.

[0365] 32. The method of embodiment 31, wherein the ADA is confirmed in the sample by measuring a level of the detection label that is lower by a predetermined factor than the level of the detection label when the unlabeled anti-TNF α antibody is not present.

[0366] 33. A kit for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

- (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label;

- (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; and
- (iii) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label.

[0367] 34. The kit of embodiment 33, wherein the solid phase is a plate.

[0368] 35. The kit of embodiment 33 or 34, wherein the second affinity label is streptavidin.

[0369] 36. The kit of any one of embodiments 33-35, wherein the first affinity label is biotin.

[0370] 37. The kit of any one of embodiments 33-36, wherein the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody.

[0371] 38. The kit of any one of embodiments 33-37, further comprising a marker configured to produce a chemiluminescent or electrochemiluminescent signal where the marker is proximal to the detection label.

[0372] 39. The kit of embodiment 38, wherein the marker is tripropylamine (TPA).

[0373] 40. The kit of any one of embodiments 33-39, further comprising a blocking buffer.

[0374] 41. The kit of any one of embodiments 33-40, further comprising a wash buffer.

[0375] 42. A kit for estimating a quantity of an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

- (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label;
- (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (iii) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label; and
- (iv) a positive control.

[0376] 43. The kit of embodiment 42, wherein the positive control is a plurality of titer samples.

[0377] 44. A kit for confirming that an anti-drug antibody (ADA) binds a target antibody comprising an anti-TNF α antibody comprising:

- (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label;
- (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (iii) an unlabeled anti-TNF α antibody; and
- (iv) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label.

[0378] 45. Use of a target antibody comprising an anti-TNF α antibody for detecting an ADA that binds the target antibody in a sample comprising:

- (i) incubating the sample with a composition comprising:
 - (a) a first affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and
 - (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (ii) isolating ADAs that form a complex with both the first affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; and
- (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point.

[0379] 46. The use of embodiment 45, wherein isolating the ADAs comprises:

- (a) providing a solid phase comprising the second affinity label;
- (b) contacting the sample and the composition with the solid phase; and
- (c) washing the solid phase.

[0380] 47. The use of embodiment 45 or 46, wherein the target antibody, the affinity antibody, and/or the detection antibody comprise a chimeric human-murine monoclonal IgG1-kappa antibody.

- [0381] 48. The use of any one of embodiments 45-47, wherein the target antibody, the affinity antibody, and/or the detection antibody comprise SEQ ID NOs: 1 and 4.
- [0382] 49. The use of any one of embodiments 46-48, wherein the solid phase is a plate.
- [0383] 50. The use of any one of embodiments 45-49, wherein the first affinity label is biotin.
- [0384] 51. The use of any one of embodiments 45-50, wherein the second affinity label is streptavidin.
- [0385] 52. The use of any one of embodiments 45-51, wherein the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody.
- [0386] 53. The use of any one of embodiments 46-52, wherein measuring the level of the detection label comprises:
- (1) contacting the solid phase with a marker configured to produce a chemiluminescent or electrochemiluminescent signal when the marker is proximal to the detection label; and
 - (2) detecting the chemiluminescent or electrochemiluminescent signal.
- [0387] 54. The use of embodiment 53, wherein the marker is tripropylamine (TPA), and measuring the level of the detection label further comprises applying an electrical current to the marker to produce the chemiluminescent or electrochemiluminescent signal.
- [0388] 55. The use of any one of embodiments 46-54, wherein the solid phase is blocked with a blocking buffer before the sample and the composition are contacted with the solid phase.
- [0389] 56. The use of any one of embodiments 45-55, further comprising performing steps (i) to (iii) on a control sample.
- [0390] 57. The use of embodiment 56, wherein the control sample is incubated with the composition and contacted with the second affinity label.
- [0391] 58. The use of any one of embodiments 45-57, wherein the predetermined cut-point corresponds to a level with a 5% false-positive rate.

[0392] 59. The use of embodiment 58, wherein the 5% false-positive rate is determined by measuring the detection label in a plurality of control samples and calculating the 5% false-positive rate.

[0393] 60. The use of embodiment 53, wherein the chemiluminescent or electrochemiluminescent signal is measured as electrochemiluminescence (ECL).

[0394] 61. The use of embodiment 60, wherein the predetermined cut-point is an ECL value of at least 1.

[0395] 62. The use of embodiment 60 or 61, wherein the predetermined cut-point is an ECL value of about 1.10.

[0396] 63. The use of any one of embodiments 45-62, wherein the method has a sensitivity that is less than 6ng/mL.

[0397] 64. The use of embodiment 63, wherein the sensitivity is less than 4ng/mL.

[0398] 65. The use of any one of embodiments 45-64, wherein the method has a drug tolerance of about 100ng/mL.

[0399] 66. Use of one or more anti-TNF α conjugates for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

(i) contacting a sample with the one or more anti-TNF α conjugates to form a complex between the ADA and the anti-TNF α conjugates; and

(ii) measuring a level of anti-TNF α conjugates in the complex;

wherein the method detects the ADA with a sensitivity less than 5ng/mL.

[0400] 67. The use of embodiment 66, wherein the one or more anti-TNF α conjugates comprise an affinity antibody comprising an anti-TNF α antibody conjugated to an affinity label.

[0401] 68. The use of embodiment 66 or 67, wherein the one or more anti-TNF α conjugates comprise a detection antibody comprising an anti-TNF α antibody conjugated to a detection label.

[0402] 69. The use of embodiment 67, wherein the affinity label comprises biotin.

[0403] 70. The use of embodiment 68, wherein the detection label comprises a sulfo-Tag.

[0404] 71. The use of any one of embodiments 66-70, wherein the method detects the ADA with a sensitivity less than 3ng/mL.

[0405] 72. The use of any one of embodiments 66-71, wherein the method detects the ADA with a sensitivity of 2.77ng/mL or less.

[0406] 73. Use of a target antibody comprising an anti-TNF α antibody for estimating a quantity of an anti-drug antibody (ADA) that binds the target antibody comprising the anti-TNF α antibody in a sample comprising:

- (i) incubating the sample with a composition comprising:
 - (a) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and
 - (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label;
- (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point;
- (iv) performing steps (i)-(iii) on a known sample having a known concentration of ADA; and
- (v) comparing the level of the detection label in the sample to the level of the detection label in the known sample.

[0407] 74. The use of embodiment 73, wherein the known sample is serially diluted into a plurality of titer samples, wherein comparing the level of the detection label in the sample to the level of the detection label in the known sample comprises generating a curve of the levels of the detection label in each of the titer samples and comparing the detection label in the sample to the curve.

[0408] 75. Use of a target antibody comprising an anti-TNF α antibody for confirming that an anti-drug antibody (ADA) binds the target antibody comprising the anti-TNF α antibody in a

sample by performing a competitive binding assay comprising performing the method of embodiment 1 on the sample, wherein the composition further comprises an unlabeled anti-TNF α antibody.

[0409] 76. The use of embodiment 75, wherein the ADA is confirmed in the sample by measuring a level of the detection label that is lower by a predetermined factor than the level of the detection label when the unlabeled anti-TNF α antibody is not present.

[0410] 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.

CLAIMS

What is claimed is:

1. A method for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:
 - (i) incubating the sample with a composition comprising:
 - (a) a first affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and
 - (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
 - (ii) isolating ADAs that form a complex with both the first affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; and
 - (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point.
2. The method of claim 1, wherein isolating the ADAs comprises:
 - (a) providing a solid phase comprising the second affinity label;
 - (b) contacting the sample and the composition with the solid phase; and
 - (c) washing the solid phase.
3. The method of claim 1 or 2, wherein the target antibody, the affinity antibody, and/or the detection antibody comprise a chimeric human-murine monoclonal IgG1-kappa antibody.
4. The method of any one of claims 1-3, wherein the target antibody, the affinity antibody, and/or the detection antibody comprise SEQ ID NOs: 1 and 4.
5. The method of any one of claims 2-4, wherein the solid phase is a plate.
6. The method of any one of claims 1-5, wherein the first affinity label is biotin.

7. The method of any one of claims 1-6, wherein the second affinity label is streptavidin.
8. The method of any one of claims 1-7, wherein the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody.
9. The method of any one of claims 2-8, wherein measuring the level of the detection label comprises:
 - (1) contacting the solid phase with a marker configured to produce a chemiluminescent or electrochemiluminescent signal when the marker is proximal to the detection label; and
 - (2) detecting the chemiluminescent or electrochemiluminescent signal.
10. The method of claim 9, wherein the marker is tripropylamine (TPA), and measuring the level of the detection label further comprises applying an electrical current to the marker to produce the chemiluminescent or electrochemiluminescent signal.
11. The method of any one of claims 2-10, wherein the solid phase is blocked with a blocking buffer before the sample and the composition are contacted with the solid phase.
12. The method of any one of claims 1-11, further comprising performing steps (i) to (iii) on a control sample.
13. The method of claim 12, wherein the control sample is incubated with the composition and contacted with the second affinity label.
14. The method of any one of claims 1-13, wherein the predetermined cut-point corresponds to a level with a 5% false-positive rate.
15. The method of claim 14, wherein the 5% false-positive rate is determined by measuring the detection label in a plurality of control samples and calculating the 5% false-positive rate.

16. The method of claim 9, wherein the chemiluminescent or electrochemiluminescent signal is measured as electrochemiluminescence (ECL).

17. The method of claim 16, wherein the predetermined cut-point is an ECL value of at least 1.

18. The method of claim 16 or 17, wherein the predetermined cut-point is an ECL value of about 1.10.

19. The method of any one of claims 1-18, wherein the method has a sensitivity that is less than 6ng/mL.

20. The method of claim 19, wherein the sensitivity is less than 4ng/mL.

21. The method of any one of claims 1-20, wherein the method has a drug tolerance of about 100ng/mL.

22. A method for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

- (i) contacting a sample with one or more anti-TNF α conjugates to form a complex between the ADA and the anti-TNF α conjugates; and
 - (ii) measuring a level of anti-TNF α conjugates in the complex;
- wherein the method detects the ADA with a sensitivity less than 5ng/mL.

23. The method of claim 22, wherein the one or more anti-TNF α conjugates comprise an affinity antibody comprising an anti-TNF α antibody conjugated to an affinity label.

24. The method of claim 22 or 23, wherein the one or more anti-TNF α conjugates comprise a detection antibody comprising an anti-TNF α antibody conjugated to a detection label.

25. The method of claim 23, wherein the affinity label comprises biotin.
26. The method of claim 24, wherein the detection label comprises a sulfo-Tag.
27. The method of any one of claims 22-26, wherein the method detects the ADA with a sensitivity less than 3ng/mL.
28. The method of any one of claims 22-27, wherein the method detects the ADA with a sensitivity of 2.77ng/mL or less.
29. A method for estimating a quantity of an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:
- (i) incubating the sample with a composition comprising:
 - (a) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and
 - (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
 - (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label;
 - (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point;
 - (iv) performing steps (i)-(iii) on a known sample having a known concentration of ADA;
- and
- (v) comparing the level of the detection label in the sample to the level of the detection label in the known sample.
30. The method of claim 29, wherein the known sample is serially diluted into a plurality of titer samples, wherein comparing the level of the detection label in the sample to the level of the detection label in the known sample comprises generating a curve of the levels of the detection label in each of the titer samples and comparing the detection label in the sample to the curve.

31. A method of confirming that an anti-drug antibody (ADA) binds a target antibody comprising an anti-TNF α antibody in a sample by performing a competitive binding assay comprising performing the method of claim 1 on the sample, wherein the composition further comprises an unlabeled anti-TNF α antibody.

32. The method of claim 31, wherein the ADA is confirmed in the sample by measuring a level of the detection label that is lower by a predetermined factor than the level of the detection label when the unlabeled anti-TNF α antibody is not present.

33. A kit for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

- (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label;
- (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label; and
- (iii) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label.

34. The kit of claim 33, wherein the solid phase is a plate.

35. The kit of claim 33 or 34, wherein the second affinity label is streptavidin.

36. The kit of any one of claims 33-35, wherein the first affinity label is biotin.

37. The kit of any one of claims 33-36, wherein the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody.

38. The kit of any one of claims 33-37, further comprising a marker configured to produce a chemiluminescent or electrochemiluminescent signal where the marker is proximal to the detection label.

39. The kit of claim 38, wherein the marker is tripropylamine (TPA).
40. The kit of any one of claims 33-39, further comprising a blocking buffer.
41. The kit of any one of claims 33-40, further comprising a wash buffer.
42. A kit for estimating a quantity of an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:
- (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label;
 - (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
 - (iii) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label; and
 - (iv) a positive control.
43. The kit of claim 42, wherein the positive control is a plurality of titer samples.
44. A kit for confirming that an anti-drug antibody (ADA) binds a target antibody comprising an anti-TNF α antibody comprising:
- (i) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label;
 - (ii) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
 - (iii) an unlabeled anti-TNF α antibody; and
 - (iv) a solid phase comprising a second affinity label, wherein the second affinity label binds to the first affinity label.
45. Use of a target antibody comprising an anti-TNF α antibody for detecting an ADA that binds the target antibody in a sample comprising:
- (i) incubating the sample with a composition comprising:

- (a) a first affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and
- (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (ii) isolating ADAs that form a complex with both the first affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label; and
- (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point.

46. The use of claim 45, wherein isolating the ADAs comprises:

- (a) providing a solid phase comprising the second affinity label;
- (b) contacting the sample and the composition with the solid phase; and
- (c) washing the solid phase.

47. The use of claim 45 or 46, wherein the target antibody, the affinity antibody, and/or the detection antibody comprise a chimeric human-murine monoclonal IgG1-kappa antibody.

48. The use of any one of claims 45-47, wherein the target antibody, the affinity antibody, and/or the detection antibody comprise SEQ ID NOs: 1 and 4.

49. The use of any one of claims 46-48, wherein the solid phase is a plate.

50. The use of any one of claims 45-49, wherein the first affinity label is biotin.

51. The use of any one of claims 45-50, wherein the second affinity label is streptavidin.

52. The use of any one of claims 45-51, wherein the detection antibody comprises a sulfo-Tag labeled anti-TNF α antibody.

53. The use of any one of claims 46-52, wherein measuring the level of the detection label

comprises:

- (1) contacting the solid phase with a marker configured to produce a chemiluminescent or electrochemiluminescent signal when the marker is proximal to the detection label; and
- (2) detecting the chemiluminescent or electrochemiluminescent signal.

54. The use of claim 53, wherein the marker is tripropylamine (TPA), and measuring the level of the detection label further comprises applying an electrical current to the marker to produce the chemiluminescent or electrochemiluminescent signal.

55. The use of any one of claims 46-54, wherein the solid phase is blocked with a blocking buffer before the sample and the composition are contacted with the solid phase.

56. The use of any one of claims 45-55, further comprising performing steps (i) to (iii) on a control sample.

57. The use of claim 56, wherein the control sample is incubated with the composition and contacted with the second affinity label.

58. The use of any one of claims 45-57, wherein the predetermined cut-point corresponds to a level with a 5% false-positive rate.

59. The use of claim 58, wherein the 5% false-positive rate is determined by measuring the detection label in a plurality of control samples and calculating the 5% false-positive rate.

60. The use of claim 53, wherein the chemiluminescent or electrochemiluminescent signal is measured as electrochemiluminescence (ECL).

61. The use of claim 60, wherein the predetermined cut-point is an ECL value of at least 1.

62. The use of claim 60 or 61, wherein the predetermined cut-point is an ECL value of

about 1.10.

63. The use of any one of claims 45-62, wherein the method has a sensitivity that is less than 6ng/mL.

64. The use of claim 63, wherein the sensitivity is less than 4ng/mL.

65. The use of any one of claims 45-64, wherein the method has a drug tolerance of about 100ng/mL.

66. Use of one or more anti-TNF α conjugates for detecting an anti-drug antibody (ADA) that binds a target antibody comprising an anti-TNF α antibody in a sample comprising:

(i) contacting a sample with the one or more anti-TNF α conjugates to form a complex between the ADA and the anti-TNF α conjugates; and

(ii) measuring a level of anti-TNF α conjugates in the complex;

wherein the method detects the ADA with a sensitivity less than 5ng/mL.

67. The use of claim 66, wherein the one or more anti-TNF α conjugates comprise an affinity antibody comprising an anti-TNF α antibody conjugated to an affinity label.

68. The use of claim 66 or 67, wherein the one or more anti-TNF α conjugates comprise a detection antibody comprising an anti-TNF α antibody conjugated to a detection label.

69. The use of claim 67, wherein the affinity label comprises biotin.

70. The use of claim 68, wherein the detection label comprises a sulfo-Tag.

71. The use of any one of claims 66-70, wherein the method detects the ADA with a sensitivity less than 3ng/mL.

72. The use of any one of claims 66-71, wherein the method detects the ADA with a

sensitivity of 2.77ng/mL or less.

73. Use of a target antibody comprising an anti-TNF α antibody for estimating a quantity of an anti-drug antibody (ADA) that binds the target antibody comprising the anti-TNF α antibody in a sample comprising:

- (i) incubating the sample with a composition comprising:
 - (a) an affinity antibody comprising an anti-TNF α antibody conjugated to a first affinity label, and
 - (b) a detection antibody comprising an anti-TNF α antibody conjugated to a detection label;
- (ii) isolating ADAs that form a complex with both the affinity antibody and the detection antibody, wherein the ADA is isolated by contacting the complex with a second affinity label that binds to the first affinity label;
- (iii) detecting the ADA by measuring a level of the detection label above a predetermined cut-point;
- (iv) performing steps (i)-(iii) on a known sample having a known concentration of ADA; and
- (v) comparing the level of the detection label in the sample to the level of the detection label in the known sample.

74. The use of claim 73, wherein the known sample is serially diluted into a plurality of titer samples, wherein comparing the level of the detection label in the sample to the level of the detection label in the known sample comprises generating a curve of the levels of the detection label in each of the titer samples and comparing the detection label in the sample to the curve.

75. Use of a target antibody comprising an anti-TNF α antibody for confirming that an anti-drug antibody (ADA) binds the target antibody comprising the anti-TNF α antibody in a sample by performing a competitive binding assay comprising performing the method of claim 1 on the sample, wherein the composition further comprises an unlabeled anti-TNF α antibody.

76. The use of claim 75, wherein the ADA is confirmed in the sample by measuring a level of the detection label that is lower by a predetermined factor than the level of the detection label when the unlabeled anti-TNF α antibody is not present.