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- (71) **Applicant:** JANSSEN PHARMACEUTICA NV
[BE/BE]; Turnhoutseweg 30, B-2340 Beerse (BE).
- (72) **Inventors:** SANKARAN, Banumathi; 2 Bryant Road, Lexington, Massachusetts 02420 (US). DECORY, Thomas R.; 24 Round Trail Drive, Pittsford, New York 14534 (US). TUBBS, Theresa; 140 Creighton Lane, Rochester, New York 14612 (US). COLT, Linda; 151 Saint Rita Drive, Rochester, New York 14606 (US). VliegEn, Maarten; Turnhoutseweg 30, B-2340 Beerse (BE). HASPELAGH, Pieter Rik; Turnhoutseweg 30, B-2340 Beerse (BE). HRYHORENKO, Eric; 45 Fraser Drive, Hilton, New York 14468 (US).
- (74) **Agents:** SHIRTZ, Joseph F. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933 (US).
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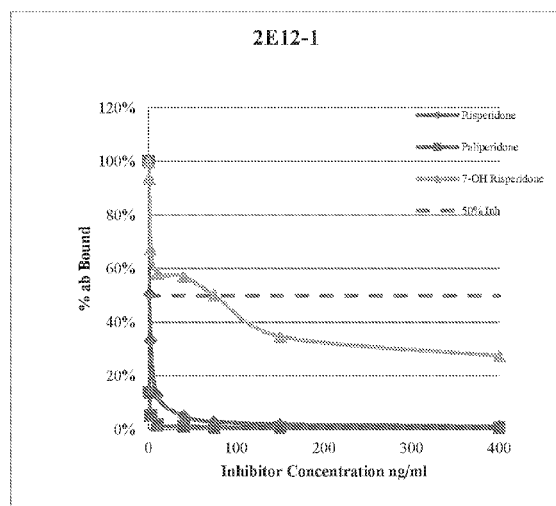
(54) **Title:** ANTIBODIES TO RISPERIDONE AND USE THEREOF

FIG. 1

(57) **Abstract:** Disclosed is an antibody or a binding fragment thereof that binds to risperidone, which can be used to detect risperidone in a sample such as in a competitive immunoassay method. The antibody or fragment thereof can be used in a lateral flow assay device for point-of-care detection of risperidone, including multiplex detection of aripiprazole, quetiapine, olanzapine, and risperidone in a single lateral flow assay device.

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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- *with sequence listing part of description (Rule 5.2(a))*

ANTIBODIES TO RISPERIDONE AND USE THEREOF

SEQUENCE LISTING

[0001] This application contains a Sequence Listing that has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created December 12, 2016, is named PRD3397USNP_SL.txt and is 8,453 bytes in size.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims benefit of U.S. Provisional Application Serial No. 62/268,898, filed December 17, 2015, the entire contents of which is incorporated by reference herein.

FIELD OF THE INVENTION

[0003] The present invention relates to the field of immunoassays, and in particular to antibodies that bind to risperidone which can be used in immunoassays for detection of risperidone.

BACKGROUND

[0004] Schizophrenia is a chronic and debilitating psychiatric disorder affecting approximately 0.45-1 % of the world's population (van Os, J.; Kapur, S. "Schizophrenia" *Lancet* 2009, 374, 635-645). The principal goals of treatment are to achieve sustained remission from psychotic symptoms, reduce the risk and consequences of relapse, and improve patient functioning and overall quality of life. While many patients with schizophrenia are able to achieve symptom stability with the available antipsychotic medications, poor adherence to medication is a common reason for relapse with daily administered oral medications. Several studies (Abdel-Baki, A.; Ouellet-Plamondon, C.; Malla, A. "Pharmacotherapy Challenges in Patients with First-Episode Psychosis" *Journal of Affective Disorders* 2012, 138, S3-S14) investigating the outcomes of non-compliance have shown that patients with schizophrenia who do not take their medication as prescribed have

higher rates of relapse, hospital admission and suicide as well as increased mortality. It is estimated that 40 to 75% of patients with schizophrenia have difficulty adhering to a daily oral treatment regimen (Lieberman, J. A.; Stroup, T. S.; McEvoy, J. P.; Swartz, M. S.; Rosenheck, R. A.; Perkins, D. O.; Keefe, R. S. E.; Davis, S. M.; Davis, C. E.; Lebowitz, B. D.; Severe, J.; Hsiao, J. K. "Effectiveness of Antipsychotic Drugs in Patients with Chronic Schizophrenia" *New England Journal of Medicine* 2005, 353(12), 1209-1223).

[0005] Therapeutic drug monitoring (TDM) is the quantification of serum or plasma concentrations of drugs, including anti-psychotic drugs, for treatment monitoring and optimization. Such monitoring permits, for example, the identification of patients that are not adhering to their medication regimen, that are not achieving therapeutic doses, that are non-responsive at therapeutic doses, that have suboptimal tolerability, that have pharmacokinetic drug-drug interactions, or that have abnormal metabolism resulting in inappropriate plasma concentrations. Considerable individual variability exists in the patient's ability to absorb, distribute, metabolize, and excrete anti-psychotic drugs. Such differences can be caused by concurrent disease, age, concomitant medication or genetic peculiarities. Different drug formulations can also influence the metabolism of anti-psychotic drugs. TDM permits dose optimization for individual patients, improving therapeutic and functional outcomes. TDM further permits a prescribing clinician to ensure compliance with prescribed dosages and achievement of effective serum concentrations.

[0006] To date, methods for determining the levels of serum or plasma concentrations of anti-psychotic drugs involve the use of liquid chromatography (LC) with UV or mass spectrometry detection, and radioimmunoassays (see, for example, Woestenborghs et al., 1990 "On the selectivity of some recently developed RIA's" in *Methodological Surveys in Biochemistry and Analysis* 20:241-246. *Analysis of Drugs and Metabolites, Including Anti-infective Agents*; Heykants et al., 1994 "The Pharmacokinetics of Risperidone in Humans: A Summary," *J Clin Psychiatry* 55/5, suppl:13-17; Huang et al., 1993 "Pharmacokinetics of the novel anti-psychotic agent risperidone and the prolactin response in healthy subjects," *Clin Pharmacol Ther* 54:257-268). Radioimmunoassays detect one or both of risperidone and paliperidone. Salamone et al. in US Patent No. 8,088,594 disclose a competitive immunoassay for risperidone using antibodies that detect both risperidone and paliperidone but not pharmacologically inactive metabolites. The antibodies used in the competitive immunoassay are developed against a particular immunogen. ID Labs Inc. (London, Ontario,

Canada) markets an ELISA for olanzapine, another anti-psychotic drug, which also utilizes a competitive format. The Instructions For Use indicate that the assay is designed for screening purposes and intended for forensic or research use, and is specifically not intended for therapeutic use. The Instructions recommend that all positive samples should be confirmed with gas chromatography/mass spectrometry (GC-MS), and indicate that the antibody used detects olanzapine and clozapine (see ID Labs Inc., "Instructions For Use Data Sheet IDEL-F083," Rev. Date Aug. 8, 2011). Some of these methods, namely HPLC and GC/MS, can be expensive and labor-intensive, and are generally only performed in large or specialty labs having the appropriate equipment.

[0007] A need exists for other methods for determining the levels of anti-psychotic drugs, particularly methods that can be performed in a prescribing clinician's office (where the treatment for an individual patient can be adjusted accordingly in a much more timely manner) and in other medical settings lacking LC or GC/MS equipment or requiring rapid test results.

SUMMARY OF THE INVENTION

[0008] The present invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and which is an isolated antibody or a binding fragment thereof selected from the group consisting of: a) an isolated antibody or a binding fragment thereof comprising a heavy chain variable region, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:7; b) an isolated antibody or a fragment thereof comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:8, and a light chain variable region; c) an isolated antibody or a fragment thereof comprising a light chain variable region having an amino acid sequence of SEQ ID NO:3 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:4; and d) an isolated antibody or a fragment thereof comprising a light chain variable region having an amino acid sequence of SEQ ID NO:7 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:8.

[0009] The present invention further is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and competes for an epitope that is capable of

binding the isolated antibody or binding fragment thereof identified above, and which is the same as an epitope bound by the antibody or binding fragment thereof identified above.

[0010] In an embodiment, the present invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and which comprises a light chain variable region comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO:3 or SEQ ID NO:7. In embodiments, the light chain variable region comprises an amino acid sequence having at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity with SEQ ID NO:3 or SEQ ID NO:7.

[0011] In a further embodiment, the present invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and which comprises a heavy chain variable region comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO:4 or SEQ ID NO:8. In embodiments, the heavy chain variable region comprises an amino acid sequence having at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity with SEQ ID NO:4 or SEQ ID NO:8.

[0012] Additional embodiments of the isolated antibody or binding fragment thereof of the subject invention are: an isolated antibody or binding fragment thereof which comprises a light chain variable region and a heavy chain variable region, wherein the light chain variable region is selected from the group consisting of: a) a light chain variable region having a complementarity determining region 1 (CDR1) sequence comprising amino acid residues 44 to 54 of SEQ ID NO:3, a CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:3, and a CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:3; and b) a light chain variable region having a CDR1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:7, a CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:7, and a CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:7; and wherein the heavy chain variable region is selected from the group consisting of: a) a heavy chain variable region having a CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:4, a CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:4, and a CDR3 sequence comprising amino acid residues 118 to 128 of

SEQ ID NO:4; and b) a heavy chain variable region having a CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:8, a CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:8, and a CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:8

[0013] The isolated antibodies or binding fragments thereof of the subject invention can be provided in assay kits and assay devices, with a presently preferred device being a lateral flow assay device which provides for point-of-care analysis.

[0014] In preferred embodiments, the isolated antibody is a monoclonal antibody. In some preferred embodiments, the antibody binding fragment is selected from the group of fragments consisting of Fv, F(ab'), F(ab')₂, scFv, minibody and diabody fragments.

[0015] The invention further provides a method of detecting risperidone in a sample. The method comprises: (i) contacting a sample with an isolated antibody or binding fragment thereof according to the subject invention which is labeled with a detectable marker, wherein the labeled antibody and risperidone present in the sample form a labeled complex; and (ii) detecting the labeled complex thereby detecting risperidone in the sample.

[0016] Further provided is a competitive immunoassay method for detecting risperidone in a sample. The method comprises: (i) contacting a sample with an isolated antibody or binding fragment thereof according to the subject invention, and with risperidone or a competitive binding partner of risperidone, wherein one of the antibody or binding fragment thereof and the risperidone or competitive binding partner thereof is labeled with a detectable marker, and wherein sample risperidone competes with the risperidone or competitive binding partner thereof for binding to the antibody or binding fragment thereof; and (ii) detecting the label thereby detecting risperidone in the sample.

[0017] Further objects, features and advantages of the present invention will be apparent to those skilled in the art from detailed consideration of the preferred embodiments that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1 shows Competitive ELISA results generated with risperidone clone 2E12-1.

[0019] FIGS. 2A and 2B show Competitive ELISA results generated with risperidone clone 7A8-1.

[0020] FIGS. 3A and 3B show Competitive ELISA results generated with risperidone clones 7A8-1 (FIG. 3A) and 2E12-1 (FIG. 3B).

[0021] FIG. 4 shows the competitive immunoassay format used on a lateral flow assay device.

[0022] FIG. 5 shows a typical dose response curve generated with risperidone antibodies.

[0023] FIG. 6 shows the chip design of a lateral flow assay device according to the subject invention.

[0024] FIG. 7 shows a typical dose response curve for an aripiprazole positive control generated with antibody 5C7 and a labeled aripiprazole competitive binding partner.

[0025] FIG. 8 shows a typical dose response curve for an olanzapine positive control generated with antibody 4G9-1 and a labeled olanzapine competitive binding partner.

[0026] FIG. 9 shows a typical dose response curve for a quetiapine positive control generated with antibody 11 and a labeled quetiapine competitive binding partner.

[0027] FIG. 10 shows a typical dose response curve for a risperidone positive control generated with antibody 5_9 and a labeled risperidone competitive binding partner.

[0028] FIG. 11 shows a typical dose response curve for a sample containing aripiprazole generated with aripiprazole antibody 5C7 in the presence of labeled aripiprazole competitive binding partner, with no dose response curve for olanzapine, quetiapine, or risperidone in the presence of a labeled competitive binding partner for each.

[0029] FIG. 12 shows a typical dose response curve for a sample containing olanzapine generated with olanzapine antibody 4G9-1 in the presence of a labeled olanzapine competitive binding partner, with no dose response curve for aripiprazole, quetiapine, or risperidone in the presence of a labeled competitive binding partner for each.

[0030] FIG. 13 shows a typical dose response curve for a sample containing quetiapine generated with quetiapine antibody 11 in the presence of a labeled quetiapine competitive binding partner, with no dose response curve for aripiprazole, olanzapine, or risperidone in the presence of a labeled competitive binding partner for each.

[0031] FIG. 14 shows a typical dose response curve for a sample containing risperidone generated with risperidone antibody 5_9 in the presence of a labeled risperidone competitive binding partner, with no dose response curve for aripiprazole, olanzapine, or quetiapine in the presence of a labeled competitive binding partner for each.

[0032] FIG. 15 shows a typical dose response curve for a sample containing aripiprazole generated with aripiprazole antibody 5C7 in the presence of a labeled aripiprazole competitive binding partner, with no dose response curve for olanzapine, quetiapine, or risperidone in the presence of antibody and labeled competitive binding partner for each.

[0033] FIG. 16 shows a typical dose response curve for a sample containing olanzapine generated with olanzapine antibody 4G9-1 in the presence of a labeled olanzapine competitive binding partner, with no dose response curve for aripiprazole, quetiapine, or risperidone in the presence of antibody and labeled competitive binding partner for each.

[0034] FIG. 17 shows a typical dose response curve for a sample containing quetiapine generated with quetiapine antibody 11 in the presence of labeled quetiapine competitive binding partner, with no dose response curve for aripiprazole, olanzapine, or risperidone in the presence of antibody and labeled competitive binding partner for each.

[0035] FIG. 18 shows a typical dose response curve for a sample containing risperidone generated with risperidone antibody 5_9 in the presence of a labeled risperidone competitive binding partner, with no dose response curve for aripiprazole, olanzapine, or quetiapine in the presence of antibody and labeled competitive binding partner for each.

[0036] FIG. 19 shows a comparison of the aripiprazole dose response curve generated as a positive control to the aripiprazole dose response curve generated in the multiplex format.

[0037] FIG. 20 shows a comparison of the olanzapine dose response curve generated as a positive control to the olanzapine dose response curve generated in the multiplex format.

[0038] FIG. 21 shows a comparison of the quetiapine dose response curve generated as a positive control to the quetiapine dose response curve generated in the multiplex format.

[0039] FIG. 22 shows a comparison of the risperidone dose response curve generated as a positive control to the risperidone dose response curve generated in the multiplex format.

DETAILED DESCRIPTION

[0040] It is to be understood that this invention is not limited to particular methods, reagents, compounds, compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0041] As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise.

[0042] The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: “reference sequence,” “comparison window,” “sequence identity,” “percentage of sequence identity,” “substantial identity,” “similarity,” and “homologous.” A “reference sequence” is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, a segment of a full length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence; a reference sequence may comprise a segment of a complete amino acid sequence encoding a protein as given in a sequence listing or may comprise a complete amino acid sequence encoding a protein. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotide or amino acid sequences may each (1) comprise a sequence (i.e., a portion of the complete nucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotide or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a “comparison window” to identify and compare local regions of sequence similarity.

[0043] A “comparison window,” as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein the polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acids and wherein the portion of the polynucleotide sequence or amino acid sequence in the comparison window may comprise additions, deletions, substitutions, and the like (i.e., gaps) of 20 percent or less as compared to the

reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0 (Genetics Computer Group, 575 Science Dr., Madison, WI), Geneworks or MacVector software packages), or by inspection, and the best alignment (i.e., resulting in the highest percentage of identity over the comparison window) generated by the various methods is selected.

[0044] The term “sequence identity” means that two polynucleotide or amino acid sequences are identical (i.e., on a nucleotide-by-nucleotide or amino acid residue-by-residue basis) over the comparison window. The term “percentage of sequence identity” is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, or U) or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term “substantial identity” or “substantially identical” as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid sequence comprises a sequence that has at least 85 percent sequence identity, preferably at least 85 to 99 percent sequence identity, more preferably at least 90 to 95 percent sequence identity, particularly preferable at least 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, or 95 percent sequence identity, more usually at least 96, 97, 98 or 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, particularly over a window of at least 18-48 nucleotide (6-16 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence. The term “similarity,” when used

to describe a polypeptide, is determined by comparing the amino acid sequence and the conserved amino acid substitutions of one polypeptide to the sequence of a second polypeptide. The term "homologous," when used to describe a polynucleotide, indicates that two polynucleotides, or designated sequences thereof, when optimally aligned and compared, are identical, with appropriate nucleotide insertions or deletions, in at least 70% of the nucleotides, preferably from at least 70% to 99%, usually from at least 75% to 99%, particularly at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, and more preferably at least 96%, 97%, 98%, 99% of the nucleotides.

[0045] A "label," "detector molecule," "reporter" or "detectable marker" as used herein is any molecule which produces, or can be induced to produce, a detectable signal. The label can be conjugated to an analyte, immunogen, antibody, or to another molecule such as a receptor or a molecule that can bind to a receptor such as a ligand, particularly a hapten or antibody. A label can be attached directly or indirectly by means of a linking or bridging moiety. Non-limiting examples of labels include radioactive isotopes (e.g., ¹²⁵I), enzymes (e.g. β -galactosidase, peroxidase), enzyme fragments, enzyme substrates, enzyme inhibitors, coenzymes, catalysts, fluorophores (e.g., rhodamine, fluorescein isothiocyanate or FITC, or Dylight 649), dyes, chemiluminescers and luminescers (e.g., dioxetanes, luciferin), or sensitizers.

[0046] The invention provides an isolated antibody which binds to risperidone. The invention further provides an assay kit and an assay device comprising the antibody. Further provided is a method of detecting risperidone in a sample, including a competitive immunoassay method.

[0047] In one embodiment, the present invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and which is an isolated antibody or a binding fragment thereof selected from the group consisting of: a) an isolated antibody or a fragment thereof comprising a heavy chain variable region, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:7; b) an isolated antibody or a fragment thereof comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:8, and a light chain variable region; c) an isolated antibody or a fragment thereof comprising a light chain variable region having an amino acid sequence of

SEQ ID NO:3 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:4; and d) an isolated antibody or a fragment thereof comprising a light chain variable region having an amino acid sequence of SEQ ID NO:7 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:8.

[0048] In another embodiment, the invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and competes for an epitope that is capable of binding: a) an isolated antibody or a binding fragment thereof comprising a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:3 and SEQ ID NO:7, and a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:8; b) an isolated antibody or a fragment thereof comprising a light chain variable region having an amino acid sequence of SEQ ID NO:3 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:4; and c) an isolated antibody or a fragment thereof comprising a light chain variable region having an amino acid sequence of SEQ ID NO:7 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:8, and which is the same as an epitope bound by those identified antibodies.

[0049] A preferred embodiment of the antibody of the subject invention is an antibody which comprises a light chain variable region having the amino acid sequence SEQ ID NO:3 and a heavy chain variable region having the amino acid sequence SEQ ID NO:4. Another preferred embodiment of the antibody of the subject invention is an antibody which comprises a light chain variable region having the amino acid sequence SEQ ID NO:7 and a heavy chain variable region having the amino acid sequence SEQ ID NO:8.

[0050] In a further embodiment, the present invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and which comprises a light chain variable region comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO:3 or SEQ ID NO:7. In embodiments, the light chain variable region comprises an amino acid sequence having at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity with SEQ ID NO:3 or SEQ ID NO:7.

[0051] In another embodiment, the present invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and which comprises a heavy chain

variable region comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO:4 or SEQ ID NO:8. In embodiments, the heavy chain variable region comprises an amino acid sequence having at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity with SEQ ID NO:4 or SEQ ID NO:8.

[0052] In another embodiment, the invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and which comprises a light chain variable region comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO:3, and a heavy chain variable region comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO:4. In embodiments, the light chain variable region comprises an amino acid sequence having at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity with SEQ ID NO:3, and the heavy chain variable region comprises an amino acid sequence having at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity with SEQ ID NO:4.

[0053] In yet another embodiment, the invention is directed to an isolated antibody or a binding fragment thereof, which binds to risperidone and which comprises a light chain variable region comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO:7, and a heavy chain variable region comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO:8. In embodiments, the light chain variable region comprises an amino acid sequence having at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity with SEQ ID NO:7, and the heavy chain variable region comprises an amino acid sequence having at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity with SEQ ID NO:8.

[0054] Further preferred embodiments of the isolated antibody or a binding fragment thereof of the subject invention are: an antibody or a binding fragment thereof which

comprises a light chain variable region and a heavy chain variable regions, wherein the light chain variable region is selected from the group consisting of: a) a light chain variable region having a complementarity determining region 1 (CDR1) sequence comprising amino acid residues 44 to 54 of SEQ ID NO:3, a CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:3, and a CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:3; and b) a light chain variable region having a CDR1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:7, a CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:7, and a CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:7; and wherein the heavy chain variable region is selected from the group consisting of : a) a heavy chain variable region having a CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:4, a CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:4, and a CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:4; and b) a heavy chain variable region having a CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:8, a CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:8, and a CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:8.

[0055] Additional preferred embodiments of the antibody or a binding fragment thereof of the subject invention are: 1) an antibody or a binding fragment thereof which comprises a light chain CDR1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:3, a light chain CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:3, and a light chain CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:3, a heavy chain CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:4, a heavy chain CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:4, and a heavy chain CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:4; and 2) an antibody or a binding fragment thereof which comprises a light chain CDR1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:7, a light chain CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:7, and a light CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:7; a heavy chain CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:8, a heavy chain CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:8, and a heavy chain CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:8.

[0056] An additional preferred embodiment of the antibody or a binding fragment thereof of the invention is an antibody or a binding fragment thereof which comprises a light chain CDR1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:3, a light chain CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:3, a light chain CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:3, a heavy chain CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:4, a heavy chain CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:4, and a heavy chain CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:4.

[0057] Another preferred embodiment of the antibody or a binding fragment thereof of the invention is an antibody or a binding fragment thereof which comprises a light chain CDR1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:7, a light chain CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:7, a light chain CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:7, a heavy chain CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:8, a heavy chain CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:8, and a heavy chain CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:8.

[0058] Further details of the antibodies or a binding fragments thereof of the subject invention are provided in the section below entitled "Antibodies."

[0059] The subject invention further provides an assay kit comprising the antibody or a binding fragment thereof, as well as an assay device comprising the antibody or a binding fragment thereof. Preferably, the assay device is a lateral flow assay device. Further details of the assay kits and assay devices are provided below in the section entitled "Assay Kits and Devices."

[0060] The invention further provides a method of detecting risperidone in a sample. The method comprises: (i) contacting a sample with an antibody or a binding fragment thereof according to the subject invention which is labeled with a detectable marker, wherein the labeled antibody or binding fragment thereof and risperidone present in the sample form a labeled complex; and (ii) detecting the labeled complex thereby detecting risperidone in the sample. Further details of the method of detecting risperidone in accordance with the subject invention are provided in the section below entitled "Immunoassays."

[0061] Further provided is a competitive immunoassay method for detecting risperidone in a sample. The method comprises: (i) contacting a sample with an antibody or a binding fragment thereof according to the subject invention, and with risperidone or a competitive binding partner of risperidone, wherein one of the antibody or binding fragment thereof and the risperidone or competitive binding partner thereof is labeled with a detectable marker, and wherein sample risperidone competes with the risperidone or competitive binding partner thereof for binding to the antibody or binding fragment thereof; and (ii) detecting the label thereby detecting sample risperidone. Further details of the competitive immunoassay method of detecting risperidone in accordance with the subject invention are provided in the section below entitled "Immunoassays."

[0062] In a preferred embodiment of the subject invention, the detection of risperidone is accompanied by the detection of one or more analytes in addition to risperidone. Preferably the one or more analytes are anti-psychotic drugs other than risperidone, and more preferably the anti-psychotic drugs other than risperidone are selected from the group consisting of: aripiprazole, paliperidone, quetiapine, olanzapine, and metabolites thereof.

[0063] As discussed above, the antibodies or binding fragments thereof of the subject invention can be used in assays to detect the presence and/or amount of the anti-psychotic drug in patient samples. Such detection permits therapeutic drug monitoring enabling all of the benefits thereof. Detection of levels of anti-psychotic drugs may be useful for many purposes, each of which represents another embodiment of the subject invention, including: determination of patient adherence or compliance with prescribed therapy; use as a decision tool to determine whether a patient should be converted from an oral anti-psychotic regimen to a long-acting injectable anti-psychotic regimen; use as a decision tool to determine if the dose level or dosing interval of oral or injectable anti-psychotics should be increased or decreased to ensure attainment or maintenance of efficacious or safe drug levels; use as an aid in the initiation of anti-psychotic drug therapy by providing evidence of the attainment of minimum pK levels; use to determine bioequivalence of anti-psychotic drug in multiple formulations or from multiple sources; use to assess the impact of polypharmacy and potential drug-drug interactions; and use as an indication that a patient should be excluded from or included in a clinical trial and as an aid in the subsequent monitoring of adherence to clinical trial medication requirements.

ANTIBODIES

[0064] The present invention provides an isolated antibody which binds to risperidone. The term “antibody” refers to a specific protein capable of binding an antigen or portion thereof (in accordance with this invention, capable of binding to an anti-psychotic drug or metabolite thereof). An antibody is produced in response to an immunogen which may have been introduced into a host, e.g., an animal or a human, by injection. The generic term “antibody” includes polyclonal antibodies, monoclonal antibodies, and antibody fragments.

[0065] “Antibody” or “antigen-binding antibody fragment” refers to an intact antibody, or a fragment thereof, that competes with the intact antibody for binding. Generally speaking, an antibody or antigen-binding antibody fragment, is said to specifically bind an antigen when the dissociation constant is less than or equal to 1 μ M, preferably less than or equal to 100 nM and most preferably less than or equal to 10 nM. Binding can be measured by methods known to those skilled in the art, an example being the use of a BIAcoreTM instrument.

[0066] Antibodies are made up of two heavy chains and two light chains. Each heavy chain has one variable domain or region (V_H) followed by a constant domain or region (C_{H1}), a hinge region, and two more constant domains or regions (C_{H2} and C_{H3}). Each light chain has one variable domain or region (V_L) and one constant domain or region (C_L). The variable domains or regions of the heavy and light chains form the paratope of the antibody (a structure analogous to a lock), which is specific for a particular epitope (similarly analogous to a key), allowing the paratope and the epitope to bind together with precision. Within the variable domain, variable loops of β -strands, three each on the light and heavy chains, are responsible for binding to the antigen. These loops are referred to as the complementarity determining regions (CDRs, namely CDR1, CDR2, and CDR3).

[0067] Antibody fragments comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Binding fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; minibodies; linear antibodies; single-chain antibody molecules (e.g., scFV); and multispecific antibodies formed from antibody fragments. An antibody other than a “bispecific” or “bifunctional” antibody is understood to have each of its binding sites identical.

[0068] As used herein, "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Two antibodies are said to "bind the same epitope" ("compete") if one antibody is shown to compete with the second antibody in a competitive binding assay, by any of the methods well known to those skilled in the art (such as the BIAcoreTM method referred to above). In reference to a hapten (such as risperidone or other anti-psychotic drug), an antibody can be generated against the non-antigenic hapten molecule by conjugating the hapten to an immunogenic carrier. An antibody is then generated which recognizes an "epitope" defined by the hapten.

[0069] "Isolated" when used in the context of an antibody means altered "by the hand of man" from any natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring antibody naturally present in a living animal in its natural state is not "isolated," but the same antibody separated from the coexisting materials of its natural state is "isolated," as the term is employed herein. Antibodies may occur in a composition, such as an immunoassay reagent, which are not naturally occurring compositions, and therein remain isolated antibodies within the meaning of that term as it is employed herein.

[0070] "Cross-reactivity" refers to the reaction of an antibody with an antigen that was not used to induce that antibody.

[0071] Monoclonal antibodies can be produced by the well-established hybridoma methods of Kohler and Milstein, e.g., *Nature* 256:495-497 (1975). Hybridoma methods typically involve immunizing a host or lymphocytes from a host, harvesting the monoclonal antibody secreting or having the potential to secrete lymphocytes, fusing the lymphocytes to immortalized cells, and selecting cells that secrete the desired monoclonal antibody.

[0072] Monoclonal antibodies can also be produced by recombinant methods such as are described in U.S. Patent No. 4,166,452. DNA encoding monoclonal antibodies can be isolated and sequenced using conventional procedures, e.g., using oligonucleotide probes that specifically bind to murine heavy and light antibody chain genes, preferably to probe DNA isolated from monoclonal antibody hybridoma cell lines secreting antibodies specific for anti-psychotic drugs.

[0073] Preferably, the antibody of the subject invention will bind to the drug and any desired pharmacologically active metabolites. By altering the location of the attachment of an immunogenic carrier in a drug conjugate, selectivity and cross-reactivity with metabolites and/or related drugs can be engineered into the antibodies. For risperidone, cross-reactivity with risperidone metabolites such as 9-hydroxyrisperidone (paliperidone, which is also administered as an anti-psychotic drug), 7-hydroxyrisperidone, and N-dealkylrisperidone may or may not be desirable. An antibody that cross-reacts with risperidone and paliperidone may be desirable, which does not react with 7-hydroxyrisperidone or N-dealkylrisperidone, thus detecting risperidone and its major pharmacologically active metabolite. Alternatively, it may be desirable to detect the pharmacologically active metabolites, risperidone and paliperidone, separately, while still not detecting the inactive metabolites, 7-hydroxyrisperidone and N-dealkylrisperidone. Antibodies may be generated that detect multiple ones of these drugs and/or metabolites, or antibodies may be generated that detect each separately (thus defining the antibody "specific binding" properties). An antibody specifically binds one or more compounds when its binding of the one or more compounds is equimolar or substantially equimolar.

[0074] The antibodies or binding fragments thereof herein are described by the nucleotide and amino acid sequences of their variable domains. Each was generated by inoculating a host with a conjugate comprising an anti-psychotic drug conjugated to an immunogenic carrier. Having now provided the nucleotide and amino acid sequences thereof, the antibodies can be produced by the recombinant methods such as are described in U.S. Patent No. 4,166,452.

[0075] Antibody fragments which contain specific binding sites for the anti-psychotic drug may also be generated. Such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse et al., *Science* 256:1270-1281 (1989)). Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *Escherichia coli*, allowing for the production of large amounts of these fragments. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., *BioTechnology* 10:163-167

(1992)). Other techniques for the production of antibody fragments are known to those skilled in the art. Single chain Fv fragments (scFv) are also envisioned (see U.S. Patent Nos. 5,761,894 and 5,587,458). Fv and sFv fragments are the only species with intact combining sites that are devoid of constant regions; thus, they are likely to show reduced non-specific binding. The antibody fragment may also be a “linear antibody” e.g., as described in U.S. Patent No. 5,642,870, for example. Such linear antibody fragments may be monospecific or bispecific.

ASSAY KITS AND DEVICES

[0076] An assay kit (also referred to as a reagent kit) can also be provided comprising an antibody as described above. A representative reagent kit may comprise an antibody or a binding fragment thereof that binds to the anti-psychotic drug, risperidone, a complex comprising an analog of an anti-psychotic drug or a derivative thereof coupled to a labeling moiety, and may optionally also comprise one or more calibrators comprising a known amount of an anti-psychotic drug or a related standard.

[0077] The phrase “assay kit” refers to an assembly of materials and reagents that is used in performing an assay. The reagents can be provided in packaged combination in the same or in separate containers, depending on their cross-reactivities and stabilities, and in liquid or in lyophilized form. The amounts and proportions of reagents provided in the kit can be selected so as to provide optimum results for a particular application. An assay kit embodying features of the present invention comprises antibodies or binding fragments thereof which bind risperidone. The kit may further comprise competitive binding partners of risperidone and calibration and control materials.

[0078] The phrase “calibration and control material” refers to any standard or reference material containing a known amount of an analyte. A sample suspected of containing an analyte and the corresponding calibration material are assayed under similar conditions. The concentration of analyte is calculated by comparing the results obtained for the unknown specimen with the results obtained for the standard. This is commonly done by constructing a calibration curve.

[0079] Antibodies embodying features of the present invention can be included in a kit, container, pack, or dispenser together with instructions for their utilization. When the

antibodies are supplied in a kit, the different components of the immunoassay may be packaged in separate containers and admixed prior to use. Such packaging of the components separately may permit long-term storage without substantially diminishing the functioning of the active components. Furthermore, reagents can be packaged under inert environments, e.g., under a positive pressure of nitrogen gas, argon gas, or the like, which is especially preferred for reagents that are sensitive to air and/or moisture.

[0080] Reagents included in kits embodying features of the present invention can be supplied in all manner of containers such that the activities of the different components are substantially preserved while the components themselves are not substantially adsorbed or altered by the materials of the container. Suitable containers include, but are not limited to, ampules, bottles, test tubes, vials, flasks, syringes, envelopes, e.g., foil-lined, and the like. The containers may be comprised of any suitable material including, but not limited to, glass, organic polymers, e.g., polycarbonate, polystyrene, polyethylene, etc., ceramic, metal, e.g., aluminum, metal alloys, e.g., steel, cork, and the like. In addition, the containers may comprise one or more sterile access ports, e.g., for access via a needle, such as may be provided by a septum. Preferred materials for septa include rubber and polytetrafluoroethylene of the type sold under the trade name TEFLON by DuPont (Wilmington, DE). In addition, the containers may comprise two or more compartments separated by partitions or membranes that can be removed to allow mixing of the components.

[0081] Reagent kits embodying features of the present invention may also be supplied with instructional materials. Instructions may be printed, e.g., on paper and/or supplied in an electronically-readable medium. Alternatively, instructions may be provided by directing a user to an internet website, e.g., specified by the manufacturer or distributor of the kit and/or via electronic mail.

[0082] The antibody or binding fragment thereof may also be provided as part of an assay device. Such assay devices include lateral flow assay devices. A common type of disposable lateral flow assay device includes a zone or area for receiving the liquid sample, a conjugate zone, and a reaction zone. These assay devices are commonly known as lateral flow test strips. They employ a porous material, e.g., nitrocellulose, defining a path for fluid flow capable of supporting capillary flow. Examples include those shown in US Patent Nos.

5,559,041, 5,714,389, 5,120,643, and 6,228,660 all of which are incorporated herein by reference in their entireties.

[0083] Another type of assay device is a non-porous assay device having projections to induce capillary flow. Examples of such assay devices include the open lateral flow device as disclosed in PCT International Publication Nos. WO 2003/103835, WO 2005/089082, WO 2005/118139, and WO 2006/137785, all of which are incorporated herein by reference in their entireties.

[0084] In a non-porous assay device, the assay device generally has at least one sample addition zone, at least one conjugate zone, at least one reaction zone, and at least one wicking zone. The zones form a flow path by which sample flows from the sample addition zone to the wicking zone. Also included are capture elements, such as antibodies, in the reaction zone, capable of binding to the analyte, optionally deposited on the device (such as by coating); and a labeled conjugate material also capable of participating in reactions that will enable determination of the concentration of the analyte, deposited on the device in the conjugate zone, wherein the labeled conjugate material carries a label for detection in the reaction zone. The conjugate material is dissolved as the sample flows through the conjugate zone forming a conjugate plume of dissolved labeled conjugate material and sample that flows downstream to the reaction zone. As the conjugate plume flows into the reaction zone, the conjugated material will be captured by the capture elements such as via a complex of conjugated material and analyte (as in a “sandwich” assay) or directly (as in a “competitive” assay). Unbound dissolved conjugate material will be swept past the reaction zone into the at least one wicking zone. Such devices can include projections or micropillars in the flow path.

[0085] An instrument such as that disclosed in US Patent Publication Nos. US20060289787A1 and US20070231883A1, and US Patent Nos. 7,416,700 and 6,139,800, all of which are incorporated herein by reference in their entireties, is able to detect the bound conjugated material in the reaction zone. Common labels include fluorescent dyes that can be detected by instruments which excite the fluorescent dyes and incorporate a detector capable of detecting the fluorescent dyes.

IMMUNOASSAYS

[0086] The antibodies or binding fragments thereof thus produced can be used in immunoassays to recognize/bind to the anti-psychotic drug, thereby detecting the presence and/or amount of the drug in a patient sample. Preferably, the assay format is a competitive immunoassay format. Such an assay format and other assays are described, among other places, in Hampton et al. (*Serological Methods, A Laboratory Manual*, APS Press, St. Paul, MN 1990) and Maddox et al. (*J. Exp. Med.* 158:12111, 1983).

[0087] The term “analyte” refers to any substance or group of substances, the presence or amount of which is to be determined. Representative anti-psychotic drug analytes include, but are not limited to, risperidone, paliperidone, olanzapine, aripiprazole, and quetiapine.

[0088] The term “competitive binding partner” refers to a substance or group of substances, such as may be employed in a competitive immunoassay, which behave similarly to an analyte with respect to binding affinity to an antibody. Representative competitive binding partners include, but are not limited to, anti-psychotic drug derivatives and the like.

[0089] The term “detecting” when used with an analyte refers to any quantitative, semi-quantitative, or qualitative method as well as to all other methods for determining an analyte in general, and an anti-psychotic drug in particular. For example, a method that merely detects the presence or absence of an anti-psychotic drug in a sample lies within the scope of the present invention, as do methods that provide data as to the amount or concentration of the anti-psychotic drug in the sample. The terms “detecting,” “determining,” “identifying,” and the like are used synonymously herein, and all lie within the scope of the present invention.

[0090] A preferred embodiment of the subject invention is a competitive immunoassay wherein antibodies or binding fragments thereof which bind the anti-psychotic drug, or the drug or competitive binding partner thereof, are attached to a solid support (such as the reaction zone in a lateral flow assay device) and labeled drug or competitive binding partner thereof, or labeled antibody, respectively, and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of drug in the sample.

[0091] Any sample that is suspected of containing an analyte, e.g., an anti-psychotic drug, can be analyzed in accordance with the methods of the presently preferred

embodiments. The sample can be pretreated if desired and can be prepared in any convenient medium that does not interfere with the assay. Preferably, the sample comprises an aqueous medium such as a body fluid from a host, most preferably plasma or serum.

[0092] It is to be understood that all manner of immunoassays employing antibodies are contemplated for use in accordance with the presently preferred embodiments, including assays in which antibodies are bound to solid phases and assays in which antibodies are in liquid media. Methods of immunoassays that can be used to detect analytes using antibodies or binding fragments thereof embodying features of the present invention include, but are not limited to, competitive (reagent limited) assays wherein labeled analyte (analyte analog) and analyte in a sample compete for antibodies and single-site immunometric assays wherein the antibody is labeled; and the like.

[0093] All examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following examples can be carried out as described in standard laboratory manuals, such as Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

[0094] Related applications all incorporated herein by reference in their entireties include: “Haptens of Aripiprazole” (US Provisional Patent Appl. No. 61/691,450, filed August 21, 2012, and US 20140163206, filed August 20, 2013); “Haptens of Olanzapine” (US Provisional Patent Appl. No. 61/691,454, filed August 21, 2012, and US 20140213766, filed August 20, 2013); “Haptens of Paliperidone” (US Provisional Patent Appl. No. 61/691,459, filed August 21, 2012, and US 20140213767, filed August 20, 2013); “Haptens of Quetiapine” (US Provisional Patent Appl. No. 61/691,462, filed August 21, 2012, and US 20140221616, filed August 20, 2013); “Haptens of Risperidone and Paliperidone” (US Provisional Patent Appl. No. 61/691,469, filed August 21, 2012, and US 20140155585, August 20, 2013, now US Patent 9012648, issued April 21, 2015); “Antibodies to Aripiprazole Haptens and Use Thereof” (US Provisional Patent Appl. No. 61/691,544, filed August 21, 2012, and US 20140057299, filed August 20, 2013); “Antibodies to Olanzapine Haptens and Use Thereof” (US Provisional Patent Appl. No. 61/691,572, filed August 21, 2012, and US 20140057303, filed August 20, 2013); “Antibodies to Paliperidone Haptens and Use Thereof” (US Provisional Patent Appl. No. 61/691,634, filed August 21, 2012, and US 20140057297, filed August 20, 2013); “Antibodies to Quetiapine Haptens and Use

Thereof" (US Provisional Patent Appl. No. 61/691,598, filed August 21, 2012, and US 20140057305, filed August 20, 2013); "Antibodies to Risperidone Haptens and Use Thereof" (US Provisional Patent Appl. No. 61/691,615, filed August 21, 2012, and US 20140057301, filed August 20, 2013); "Antibodies to Aripiprazole and Use Thereof" (US Provisional Patent Appl. No. 61/691,522, filed August 21, 2012, and US 20140057300, filed August 20, 2013); "Antibodies to Olanzapine and Use Thereof" (US Provisional Patent Appl. No. 61/691,645, filed August 21, 2012, and US 20140057304, filed August 20, 2013); "Antibodies to Paliperidone and Use Thereof" (US Provisional Patent Appl. No. 61/691,692, filed August 21, 2012, and US 20140057298, filed August 20, 2013); "Antibodies to Risperidone and Use Thereof" (US Provisional Patent Appl. No. 61/691,675, filed August 21, 2012, and US 20140057302, filed August 20, 2013); "Antibodies to Quetiapine and Use Thereof" (US Provisional Patent Appl. No. 61/691,659, filed August 21, 2012, and US 20140057306, filed August 20, 2013); "Antibodies to Risperidone and Use Thereof" (US Provisional Patent Appl. No. 61/790,880, filed March 15, 2013); and "Antibodies to Quetiapine and Use Thereof" (US Provisional Patent Appl. No. 62/268,924, filed December 17, 2015).

EXAMPLES

[0095] The invention can be further understood in view of the following non-limiting examples.

EXAMPLE 1

Preparation of Antibodies to Risperidone

[0096] The antibodies designated 7A8-1 and 2E12-1 were produced by standard hybridoma methods.

[0097] Materials and Methods

[0098] Hybridoma cells were generated from immunizations with risperidone/paliperidone immunogens. TRIzol® Reagent was obtained from Invitrogen/Ambion (Grand Island, NY; Cat. No. : 15596-026). PrimeScript™ 1st Strand cDNA Synthesis Kit was obtained from Takara Bio/Clontech Laboratories (Mountain View, CA; Cat. No. 6110A). SuperScript® III 1st Strand Synthesis System was obtained from

Invitrogen (Grand Island, NY; Cat. No. 18080-051). DNA Marker III was obtained from Tiangen Biotech (Beijing, China; Cat. No. MD103).

[0099] *Total RNA extraction:* Total RNA was isolated from the hybridoma cells following the technical manual of TRIzol® Reagent. The total RNA was analyzed by agarose gel electrophoresis.

[0100] *RT-PCR:* Total RNA was reverse transcribed into cDNA using isotype-specific anti-sense primers or universal primers following the technical manual of PrimeScript™ 1st Strand cDNA Synthesis Kit. The antibody fragments of V_H and V_L were amplified according to the standard operating procedure of RACE of GenScript.

[0101] *Cloning of antibody genes:* Amplified antibody fragments were separately cloned into a standard cloning vector using standard molecular cloning procedures.

[0102] *Screening and sequencing:* Colony PCR screening was performed to identify clones with inserts of correct sizes. No less than five single colonies with inserts of correct sizes were sequenced for each antibody fragment.

[0103] **Results**

[0104] *Total RNA Extraction* – The isolated total RNA of the sample was run alongside a DNA marker Marker III (TIANGEN, Cat. No. MD103) on a 1.5% agarose/GelRed™ gel.

[0105] *PCR Product* – Four microliters of PCR products of each sample were run alongside the DNA marker Marker III on a 1.5% agarose/GelRed™ gel. The PCR products were purified and stored at -20°C.

EXAMPLE 2

Antibodies to Risperidone

[0106] Antibody Fusion 22.3 Subclone 7A8-1

[0107] The hybridoma designated Fusion 22.3 Subclone 7A8-1 secretes a monoclonal antibody (mAb) specific for risperidone, and its metabolite paliperidone. The antibody is designated Fusion 22.3 Subclone 7A8-1 ("7A8-1"). The nucleotide sequence of mAb 7A8-1's light chain variable region (V_L) is designated SEQ ID NO:1 and that of the heavy chain variable region (V_H) is designated SEQ ID NO:2. Within mAb 7A8-1's V_L, nucleotides 130-

162 of SEQ ID NO:1 represent the first complementarity determining region (CDR1); nucleotides 208-228 of SEQ ID NO:1 represent the second complementarity determining region (CDR2); and nucleotides 325-351 of SEQ ID NO:1 represent the third complementarity determining region (CDR3). Within mAb 7A8-1's V_H, nucleotides 148-162 of SEQ ID NO:2 represent the CDR1; nucleotides 205-225 of SEQ ID NO:2 represent the CDR2; and nucleotides 352-384 of SEQ ID NO:2 represent the CDR3.

[00108] The corresponding predicted amino acid sequences of mAb 7A8-1's variable chain regions were also determined, and are designated SEQ ID NO:3 (light chain) and SEQ ID NO:4 (heavy chain). Within mAb 7A8-1's V_L, amino acid residues 44-54 of SEQ ID NO:3 represent the CDR1; amino acid residues 70-76 of SEQ ID NO:3 represent the CDR2; and amino acid residues 109-117 of SEQ ID NO:3 represent the CDR3. Within mAb 7A8-1's V_H, amino acid residues 50-54 of SEQ ID NO:4 represent the CDR1; amino acid residues 69-85 of SEQ ID NO:4 represent the CDR2; and amino acid residues 118-128 of SEQ ID NO:4 represent the CDR3.

[00109] Antibody 2E12-1

[00110] The hybridoma designated 2E12-1 secretes a monoclonal antibody specific for risperidone (and its metabolite paliperidone). The antibody is designated 2E12-1. The nucleotide sequence of mAb 2E12-1's V_L is designated SEQ ID NO:5 and that of the V_H is designated SEQ ID NO:6. Within mAb 2E12-1's V_L, nucleotides 130-162 of SEQ ID NO:5 represent the CDR1; nucleotides 208-228 of SEQ ID NO:5 represent the CDR2; and nucleotides 325-351 of SEQ ID NO:5 represent the CDR3. Within mAb 2E12-1's V_H, nucleotides 148-162 of SEQ ID NO:6 represent the CDR1; nucleotides 205-255 of SEQ ID NO:6 represent the CDR2; and nucleotides 352-384 of SEQ ID NO:6 represent the CDR3.

[00111] The corresponding predicted amino acid sequences of mAb 2E12-1's variable chain regions were also determined, and are designated SEQ ID NO:7 (light chain) and SEQ ID NO:8 (heavy chain). Within mAb 2E12-1's V_L, amino acid residues 44-54 of SEQ ID NO:7 represent the CDR1; amino acid residues 70-76 of SEQ ID NO:7 represent the CDR2; and amino acid residues 109-117 of SEQ ID NO:7 represent the CDR3. Within mAb 2E12-1's V_H, amino acid residues 50-54 of SEQ ID NO:8 represent the CDR1; amino acid residues 69-85 of SEQ ID NO:8 represent the CDR2; and amino acid residues 118-128 of SEQ ID NO:8 represent the CDR3.

EXAMPLE 3

Competitive Immunoassays for Risperidone/Paliperidone and Multiplex Competitive Immunoassay for Aripiprazole, Olanzapine, Quetiapine, and Risperidone/Paliperidone

[00112] Following a series of immunizations with risperidone/paliperidone immunogens, such immunogens are found in applications US 2014/0155585 and US 2014/0057301 (e.g., Compound 13), mouse tail bleeds were tested for reactivity using an ELISA. Hybridoma supernatants were also tested. ELISA data shown in Tables 1 and 2 below shows reactivity for several hybridomas (fusion partner was NSO cells).

TABLE 1

| | | | | | | |
|----------|--------|--------|--------|--------|--------|---------------------|
| dilution | | | | | | |
| 400 | | | | | | Ag = Bt-Compound#13 |
| 1200 | 2E7 | 2E12 | 5C10 | 7A8 | 7B1 | |
| 3600 | | | | | | |
| 10800 | | | | | | |
| 400 | | | | | | Ag = Bt-Compound#13 |
| 1200 | 2E7 | 2E12 | 5C10 | 7A8 | 7B1 | |
| 3600 | | | | | | |
| 10800 | | | | | | |
| 400 | 2.4719 | 3.4313 | 1.9083 | 2.6063 | 1.9269 | Ag = Bt-Compound#13 |
| 1200 | 1.5547 | 2.6345 | 1.0973 | 1.8504 | 1.0166 | |
| 3600 | 0.6642 | 1.472 | 0.5059 | 1.082 | 0.4518 | |
| 10800 | 0.2825 | 0.7352 | 0.1906 | 0.3904 | 0.1492 | |
| 400 | 2.7653 | 3.6679 | 2.0108 | 2.3865 | 1.8712 | |
| 1200 | 1.4502 | 2.7465 | 1.0691 | 1.8339 | 1.1452 | |
| 3600 | 0.6476 | 1.4073 | 0.4994 | 1.0258 | 0.4514 | |
| 10800 | 0.2924 | 0.7347 | 0.193 | 0.4187 | 0.1829 | |

TABLE 2

| dilution | 3E11 | 5C11 | 5G4 | 6H10 | 6E1 |
|----------|-------------|-------------|------------|-------------|------------|
| 400 | 1.6368 | 1.7113 | 1.35065 | 0.6388 | 0.3005 |
| 1200 | 0.7453 | 1.6610 | 0.59715 | 0.2137 | 0.0893 |
| 3600 | 0.3034 | 0.9507 | 0.23615 | 0.0817 | 0.0798 |
| 10800 | 0.1267 | 0.4219 | 0.10590 | 0.0346 | 0.0213 |
| 400 | 2.4964 | 0.4817 | 2.2200 | 2.1009 | 1.7941 |
| 1200 | 1.8422 | 0.1706 | 1.8347 | 1.6300 | 1.8345 |
| 3600 | 1.0539 | 0.0604 | 0.9171 | 0.7303 | 1.1697 |
| 10800 | 0.4046 | 0.0295 | 0.4091 | 0.3249 | 0.5512 |
| | 7A8 | 7B5 | 7D8 | 7E8 | 7H4 |

[00113] After clones were identified via ELISA reactivity, competition ELISAs were run to approximate affinity and cross-reactivity with similar compounds. Figs. 1 and 2 show the ELISA cross-reactivity results from hybridoma subclones 2E12-1 and 7A8-1. Data shows reactivity to risperidone, as well as its metabolites paliperidone and 7-hydroxyrisperidone.

[00114] Supernatants were also tested by competition ELISA to determine if the signals were specific to either risperidone or paliperidone. Figs. 3A and 3B show the results from hybridoma subclone 7A8-1 and 2E12-1. Data shows reactivity to both risperidone and paliperidone.

[00115] Fig. 4 shows the competitive immunoassay format used on a lateral flow assay device in which the capture antibody (such as risperidone/paliperidone clone 2E12-1 or 7A8-1) was deposited on a chip along with a detection conjugate consisting of risperidone conjugated to a fluorophore. In this competitive format as show in Fig. 4, a low level of analyte (risperidone or paliperidone) results in high signal, whereas a high level of analyte (risperidone or paliperidone) results in low signal. The amount of risperidone in the sample can be calculated from the loss of fluorescence compared to a control sample with no drug present. A typical dose response curve generated with risperidone/paliperidone is shown in Fig. 5.

[00116] Fig. 6 shows the chip design of a lateral flow assay device according to one embodiment of the subject invention. The device includes a zone or area for receiving the sample, a conjugate zone (which contains desired labeled competitive binding partner(s)), and a reaction zone (eight areas within the reaction zone are indicated; each area can contain

a separate desired antibody). Sample flows from the sample zone through the conjugate zone and to the reaction zone.

[00117] Figs. 7-10 show typical dose response curves for an aripiprazole positive control (sample containing aripiprazole) generated with antibody 5C7 deposited in reaction zone 2 and a labeled aripiprazole competitive binding partner in the conjugate zone (Fig. 7), an olanzapine positive control (sample containing olanzapine) generated with antibody 4G9-1 deposited in reaction zone 4 and a labeled olanzapine competitive binding partner in the conjugate zone (Fig. 8), a quetiapine positive control (sample containing quetiapine) generated with antibody 11 deposited in reaction zone 6 and a labeled quetiapine competitive binding partner in the conjugate zone (Fig. 9), and a risperidone positive control (sample containing risperidone) generated with antibody 5-9 deposited in reaction zone 8 and a labeled risperidone competitive binding partner in the conjugate zone (Fig. 10). The labeled competitive binding partners in the conjugate zone compete with the drugs present in the samples for binding to the antibodies. The amount of label is detected and is an indication of the amount of drug present in the sample (the amount of signal being inversely proportional to the amount of drug in the sample - see Fig. 4).

[00118] In order to confirm that conjugates of labeled competitive binding partners do not bind to antibodies deposited in the reaction zones, negative controls were conducted by using samples containing no drugs. Referring to Table 3, a sample containing no aripiprazole is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled olanzapine, labeled quetiapine, and labeled risperidone, but no labeled aripiprazole) and to the reaction zone. The reaction zone again contains aripiprazole antibody (5C7) in reaction zone 2. Table 3 below shows the results, confirming that there is no dose response and the olanzapine, quetiapine, and risperidone conjugates that move by capillary action through the reaction zone do not bind to the aripiprazole antibody.

Table 3

| Aripiprazole-Clone 5C7-Math Model 1 (0ng/mL Conc.) | | | | | | |
|--|------------------|---------------|---------------|----------------|------------------|-----------------|
| Assay-MM | Conj | Reaction Zone | Read Position | Peak Mean Area | Peak Mean Height | Mean Background |
| ARIP-MM1 | OLAN, QUET, RISP | ARIP | 2 | 0.77 | 1.56 | 3.99 |
| ARIP-MM1 | OLAN, QUET, RISP | | 4 | -0.02 | 0.06 | 4.14 |
| ARIP-MM1 | OLAN, QUET, RISP | | 6 | 0.09 | 0.10 | 4.29 |
| ARIP-MM1 | OLAN, QUET, RISP | | 8 | 0.13 | 0.12 | 4.61 |
| Other Conjugates do not bind to Aripiprazole | | | | | | |

[00119] Referring to Table 4, a sample containing no olanzapine is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled aripiprazole, labeled quetiapine, and labeled risperidone, but no labeled olanzapine) and to the reaction zone. The reaction zone again contains olanzapine antibody (4G9-1) in reaction zone 4. Table 4 below shows the results, confirming that there is no dose response and the aripiprazole, quetiapine, and risperidone conjugates that move by capillary action through the reaction zone do not bind to the olanzapine antibody.

Table 4

| OLAN-Clone 4G9-1-Math Model 1 (0ng/mL Conc.) | | | | | | |
|--|----------------|---------------|---------------|----------------|------------------|-----------------|
| Assay-MM | Conj | Reaction Zone | Read Position | Peak Mean Area | Peak Mean Height | Mean Background |
| OLAN-MM1 | ARIP,QUET,RISP | | 2 | -0.03 | 0.05 | 4.38 |
| OLAN-MM1 | ARIP,QUET,RISP | OLAN | 4 | 0.74 | 1.10 | 4.56 |
| OLAN-MM1 | ARIP,QUET,RISP | | 6 | 0.06 | 0.09 | 4.79 |
| OLAN-MM1 | ARIP,QUET,RISP | | 8 | 0.11 | 0.13 | 5.17 |
| Other Conjugates do not bind to Olanzapine | | | | | | |

[00120] Referring to Table 5, a sample containing no quetiapine is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled aripiprazole, labeled olanzapine, and labeled risperidone, but no labeled quetiapine) and to the reaction zone. The reaction zone again contains quetiapine antibody (11) in reaction zone 6. Table 5 below shows the results, confirming that there is no dose response and the aripiprazole, olanzapine, and risperidone conjugates that move by capillary action through the reaction zone do not bind to the quetiapine antibody.

Table 5

| Quetiapine-Clone 11-Math Model 1 (0ng/mL Conc.) | | | | | | |
|---|----------------|---------------|---------------|----------------|------------------|-----------------|
| Assay-MM | Conj | Reaction Zone | Read Position | Peak Mean Area | Peak Mean Height | Mean Background |
| QUET-MM1 | ARIP,OLAN,RISP | | 2 | -0.01 | 0.07 | 3.85 |
| QUET-MM1 | ARIP,OLAN,RISP | | 4 | 0.01 | 0.12 | 4.01 |
| QUET-MM1 | ARIP,OLAN,RISP | QUET | 6 | 0.03 | 0.08 | 4.24 |
| QUET-MM1 | ARIP,OLAN,RISP | | 8 | 0.04 | 0.07 | 4.56 |
| Other Conjugates do not bind to Quetiapine | | | | | | |

[00121] Referring to Table 6, a sample containing no risperidone is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled aripiprazole, labeled olanzapine, and labeled quetiapine, but no labeled risperidone) and to the reaction zone. The reaction zone again contains risperidone antibody (5-9) in reaction zone 8. Table 6 below shows the results, confirming that there is no dose response and the aripiprazole, olanzapine, and quetiapine conjugates that move by capillary action through the reaction zone do not bind to the risperidone antibody.

Table 6

| Risperidone-Clone 5-9-Math Model 1 (0ng/mL Conc.) | | | | | | |
|---|-----------------|---------------|---------------|----------------|------------------|-----------------|
| Assay-MM | Conj | Reaction Zone | Read Position | Peak Mean Area | Peak Mean Height | Mean Background |
| RISP-MM1 | ARIP,OLAN, QUET | | 2 | 0.02 | 0.11 | 7.43 |
| RISP-MM1 | ARIP,OLAN, QUET | | 4 | 0.05 | 0.14 | 7.73 |
| RISP-MM1 | ARIP,OLAN, QUET | | 6 | 0.20 | 0.19 | 8.11 |
| RISP-MM1 | ARIP,OLAN, QUET | RISP | 8 | 1.97 | 3.23 | 8.85 |
| Other Conjugates do not bind to Risperidone | | | | | | |

[00122] In order to confirm that conjugates of labeled competitive binding partners bind only to their respective antibodies deposited in the reaction zones, additional negative controls were conducted by again using samples containing no drugs. Referring to Table 7, a sample containing no aripiprazole is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled aripiprazole) and to the reaction zone. The reaction zone again contains aripiprazole antibody (5C7) in reaction zone 2, as well as olanzapine antibody (4G9-1) in reaction zone 4, quetiapine antibody (11) in reaction zone 6, and risperidone antibody (5-9) in reaction zone 8. Table 7 below shows the

results, confirming that there is no dose response except to the aripiprazole antibody 5C7 (in reaction zone 2).

Table 7

| Aripiprazole-Clone 5C7-Math Model 1 (0ng/mL Conc.) | | | | | | |
|--|---------------------|---------------|---------------|----------------|------------------|-----------------|
| Assay-MM | Conj | Reaction Zone | Read Position | Peak Mean Area | Peak Mean Height | Mean Background |
| ARIP-MM1 | ARIP,OLAN,QUET,RISP | ARIP | 2 | 60.34 | 97.53 | 5.44 |
| ARIP-MM1 | ARIP,OLAN,QUET,RISP | | 4 | 2.86 | 3.91 | 11.66 |
| ARIP-MM1 | ARIP,OLAN,QUET,RISP | | 6 | 1.12 | 1.23 | 11.03 |
| ARIP-MM1 | ARIP,OLAN,QUET,RISP | | 8 | 3.14 | 4.19 | 12.94 |
| Only the Aripiprazole Reaction Zone is binding | | | | | | |

[00123] Referring to Table 8, a sample containing no olanzapine is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled olanzapine) and to the reaction zone. The reaction zone again contains aripiprazole antibody (5C7) in reaction zone 2, as well as olanzapine antibody (4G9-1) in reaction zone 4, quetiapine antibody (11) in reaction zone 6, and risperidone antibody (5-9) in reaction zone 8. Table 8 below shows the results, confirming that there is no dose response except to the olanzapine antibody 4G9-1 (in reaction zone 4).

Table 8

| OLAN-Clone 4G9-1-Math Model 1 (0ng/mL Conc.) | | | | | | |
|--|---------------------|---------------|---------------|----------------|------------------|-----------------|
| Assay-MM | Conj | Reaction Zone | Read Position | Peak Mean Area | Peak Mean Height | Mean Background |
| OLAN-MM1 | ARIP,OLAN,QUET,RISP | | 2 | 0.02 | 0.08 | 4.86 |
| OLAN-MM1 | ARIP,OLAN,QUET,RISP | OLAN | 4 | 34.23 | 51.80 | 5.39 |
| OLAN-MM1 | ARIP,OLAN,QUET,RISP | | 6 | 0.22 | 0.32 | 5.39 |
| OLAN-MM1 | ARIP,OLAN,QUET,RISP | | 8 | 0.15 | 0.17 | 5.59 |
| Only the Olanzapine Reaction Zone is binding | | | | | | |

[00124] Referring to Table 9, a sample containing no quetiapine is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled quetiapine) and to the reaction zone. The reaction zone again contains aripiprazole antibody (5C7) in reaction zone 2, as well as olanzapine antibody (4G9-1) in reaction zone 4, quetiapine antibody (11) in reaction zone 6, and risperidone antibody (5-9) in reaction zone 8.

Table 9 below shows the results, confirming that there is no dose response except to the quetiapine antibody 11 (in reaction zone 6).

Table 9

| Quetiapine-Clone 11-Math Model 1 (0ng/mL Conc.) | | | | | | |
|---|---------------------|---------------|---------------|----------------|------------------|-----------------|
| Assay-MM | Conj | Reaction Zone | Read Position | Peak Mean Area | Peak Mean Height | Mean Background |
| QUET-MM1 | ARIP,OLAN,QUET,RISP | | 2 | 0.13 | 0.41 | 10.02 |
| QUET-MM1 | ARIP,OLAN,QUET,RISP | | 4 | 0.08 | 0.23 | 10.47 |
| QUET-MM1 | ARIP,OLAN,QUET,RISP | QUET | 6 | 140.35 | 181.33 | 7.91 |
| QUET-MM1 | ARIP,OLAN,QUET,RISP | | 8 | 1.58 | 2.61 | 11.53 |
| Only the Quetiapine Reaction Zone is binding | | | | | | |

[00125] Referring to Table 10, a sample containing no risperidone is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled risperidone) and to the reaction zone. The reaction zone again contains aripiprazole antibody (5C7) in reaction zone 2, as well as olanzapine antibody (4G9-1) in reaction zone 4, quetiapine antibody (11) in reaction zone 6, and risperidone antibody (5-9) in reaction zone 8. Table 10 below shows the results, confirming that there is no dose response except to the risperidone antibody 5-9 (in reaction zone 8).

Table 10

| Risperidone-Clone 5-9-Math Model 1 (0ng/mL Conc.) | | | | | | |
|---|---------------------|---------------|---------------|----------------|------------------|-----------------|
| Assay-MM | Conj | Reaction Zone | Read Position | Peak Mean Area | Peak Mean Height | Mean Background |
| RISP-MM1 | ARIP,OLAN,QUET,RISP | | 2 | 1.03 | 1.51 | 9.07 |
| RISP-MM1 | ARIP,OLAN,QUET,RISP | | 4 | 0.65 | 0.91 | 9.60 |
| RISP-MM1 | ARIP,OLAN,QUET,RISP | | 6 | 2.61 | 6.39 | 10.48 |
| RISP-MM1 | ARIP,OLAN,QUET,RISP | RISP | 8 | 55.98 | 100.91 | 11.58 |
| Only the Risperidone Reaction Zone is binding | | | | | | |

[00126] The results shown above confirm that conjugates of labeled competitive binding partners bind only to their respective antibodies in the reaction zone.

[00127] Figs. 11-14 show typical dose response curves in specific antibody reaction zones, and proof of dose response low/high concentration for each specific assay in the

presence of other conjugates. In Fig. 11, a sample containing aripiprazole is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled aripiprazole, labeled olanzapine, labeled quetiapine, and labeled risperidone) and to the reaction zone. The reaction zone again contains aripiprazole antibody (5C7) in reaction zone 2. A typical dose response curve was generated as is shown in Fig. 11 only for aripiprazole, and not for olanzapine, quetiapine, or risperidone.

[00128] In Fig. 12, a sample containing olanzapine is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled aripiprazole, labeled olanzapine, labeled quetiapine, and labeled risperidone) and to the reaction zone. The reaction zone again contains olanzapine antibody (4G9-1) in reaction zone 4. A typical dose response curve was generated as is shown in Fig. 12 only for olanzapine, and not for aripiprazole, quetiapine, or risperidone.

[00129] In Fig. 13, a sample containing quetiapine is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled aripiprazole, labeled olanzapine, labeled quetiapine, and labeled risperidone) and to the reaction zone. The reaction zone again contains quetiapine antibody (11) in reaction zone 6. A typical dose response curve was generated as is shown in Fig. 13 only for quetiapine, and not for aripiprazole, olanzapine, or risperidone.

[00130] In Fig. 14, a sample containing risperidone is deposited in the sample zone and moves by capillary action through the conjugate zone (this time containing labeled aripiprazole, labeled olanzapine, labeled quetiapine, and labeled risperidone) and to the reaction zone. The reaction zone again contains risperidone antibody (5-9) in reaction zone 8. A typical dose response curve was generated as is shown in Fig. 14 only for risperidone, and not for aripiprazole, olanzapine, or quetiapine.

[00131] Figs. 15-18 show typical dose response curves for each assay in the presence of other conjugates and antibodies. In Fig. 15, a sample containing aripiprazole is deposited in the sample zone and moves by capillary action through the conjugate zone (again containing labeled aripiprazole, labeled olanzapine, labeled quetiapine, and labeled risperidone) and to the reaction zone. The reaction zone again contains aripiprazole antibody (5C7) in reaction zone 2, as well as olanzapine antibody (4G9-1) in reaction zone 4, quetiapine antibody (11) in reaction zone 6, and risperidone antibody (5-9) in reaction zone 8. A typical dose response curve was generated for aripiprazole, as is shown in Fig. 15. When a sample containing

olanzapine was deposited in the sample zone of this chip, a typical dose response curve was generated for olanzapine as shown in Fig. 16. When a sample containing quetiapine was deposited in the sample zone of this chip, a typical dose response curve for quetiapine was generated as shown in Fig. 17. When a sample containing risperidone was deposited in the sample zone of this chip, a typical dose response curve for risperidone was generated as shown in Fig. 18.

[00132] Figs. 19-22 show comparisons of dose response curves generated as positive controls (Figs. 7-10) to dose response curves generated in the multiplex format (Figs. 15-18). The comparison for aripiprazole is shown in Fig. 19; for olanzapine in Fig. 20; for quetiapine in Fig. 21; and for risperidone in Fig. 22. These figures show that the positive control curves are similar to the multiplex curves.

[00133] These data show that a lateral flow assay device of the subject invention can be used to detect multiple anti-psychotic drugs using a single sample from a patient on one portable, point-of-care device.

[00134] In describing the present invention and its various embodiments, specific terminology is employed for the sake of clarity. However, the invention is not intended to be limited to the specific terminology so selected. A person skilled in the relevant art will recognize that other equivalent components can be employed and other methods developed without departing from the broad concepts of the current invention. All references cited anywhere in this specification are incorporated by reference as if each had been individually incorporated.

What is claimed is:

1. An isolated antibody or a binding fragment thereof, which binds to risperidone comprising:
 - a) the isolated antibody or the fragment thereof comprising a light chain variable region having an amino acid sequence of SEQ ID NO:3 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:4; or
 - b) the isolated antibody or the fragment thereof comprising a light chain variable region having an amino acid sequence of SEQ ID NO:7 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:8.
2. The antibody of claim 1, wherein the antibody or the fragment thereof comprises the light chain variable region having the amino acid sequence SEQ ID NO:3 and the heavy chain variable region having the amino acid sequence SEQ ID NO:4.
3. The antibody of claim 1, wherein the antibody or the fragment thereof comprises the light chain variable region having the amino acid sequence SEQ ID NO:7 and the heavy chain variable region having the amino acid sequence SEQ ID NO:8.
4. The antibody of claim 1, wherein the antibody or the fragment thereof comprises:
 - a) a light chain complementarity determining region (CDR) 1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:3,
 - b) a light chain CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:3,
 - c) a light chain CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:3,
 - d) a heavy chain CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:4,
 - e) a heavy chain CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:4, and

f) a heavy chain CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:4.

5. The antibody of claim 1, wherein the antibody or the fragment thereof comprises:

a) a light chain complementarity determining region (CDR) 1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:7,

b) a light chain CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:7,

c) a light chain CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:7,

d) a heavy chain CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:8,

e) a heavy chain CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:8, and

f) a heavy chain CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:8.

6 The antibody of claim 1, wherein the antibody binding fragment is selected from the group of fragments consisting of Fv, F(ab'), F(ab')₂, scFv, minibody and diabody fragments.

7. The antibody of claim 1, wherein the antibody is a monoclonal antibody.

8. An assay kit comprising the antibody of claim 1.

9. An assay device comprising the antibody of claim 1.

10. The assay device of claim 9, wherein the device is a lateral flow assay device.

11. A method of detecting risperidone in a sample, the method comprising:

- (i) contacting a sample with the antibody of claim 1 labeled with a detectable marker, wherein the labeled antibody and risperidone present in the sample form a labeled complex; and
- (ii) detecting the labeled complex so as to detect risperidone in the sample.

12. A competitive immunoassay method for detecting risperidone in a sample, the method comprising:

- (i) contacting a sample with the antibody of claim 1, and with risperidone or a competitive binding partner of risperidone, wherein one of the antibody and the risperidone or competitive binding partner thereof is labeled with a detectable marker, and wherein sample risperidone competes with the risperidone or competitive binding partner thereof for binding to the antibody; and
- (ii) detecting the label so as to detect sample risperidone.

13. The method of claim 12, wherein the risperidone or competitive binding partner thereof is labeled with the detectable marker.

14. The method of claim 12, wherein the antibody is labeled with a detectable marker.

15. The method of claim 12, wherein the immunoassay is performed on a lateral flow assay device and the sample is applied to the device.

16. The method of claim 11 or 12, further comprising detecting the presence of one or more analytes in addition to risperidone.

17. The method of claim 16, wherein the one or more analytes are anti-psychotic drugs other than risperidone.

18. The method of claim 17, wherein the anti-psychotic drugs other than risperidone are selected from the group consisting of: paliperidone, quetiapine, olanzapine, aripiprazole, and metabolites thereof.

19. An isolated antibody or a binding fragment thereof, which binds to quetiapine comprising:

a) a light chain CDR 1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:3,

b) a light chain CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:3,

c) a light chain CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:3,

d) a heavy chain CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:4,

e) a heavy chain CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:4, and

f) a heavy chain CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:4.

20. An isolated antibody or a binding fragment thereof, which binds to quetiapine comprising:

a) a light chain CDR 1 sequence comprising amino acid residues 44 to 54 of SEQ ID NO:7,

b) a light chain CDR2 sequence comprising amino acid residues 70 to 76 of SEQ ID NO:7,

c) a light chain CDR3 sequence comprising amino acid residues 109 to 117 of SEQ ID NO:7,

d) a heavy chain CDR1 sequence comprising amino acid residues 50 to 54 of SEQ ID NO:8,

e) a heavy chain CDR2 sequence comprising amino acid residues 69 to 85 of SEQ ID NO:8, and

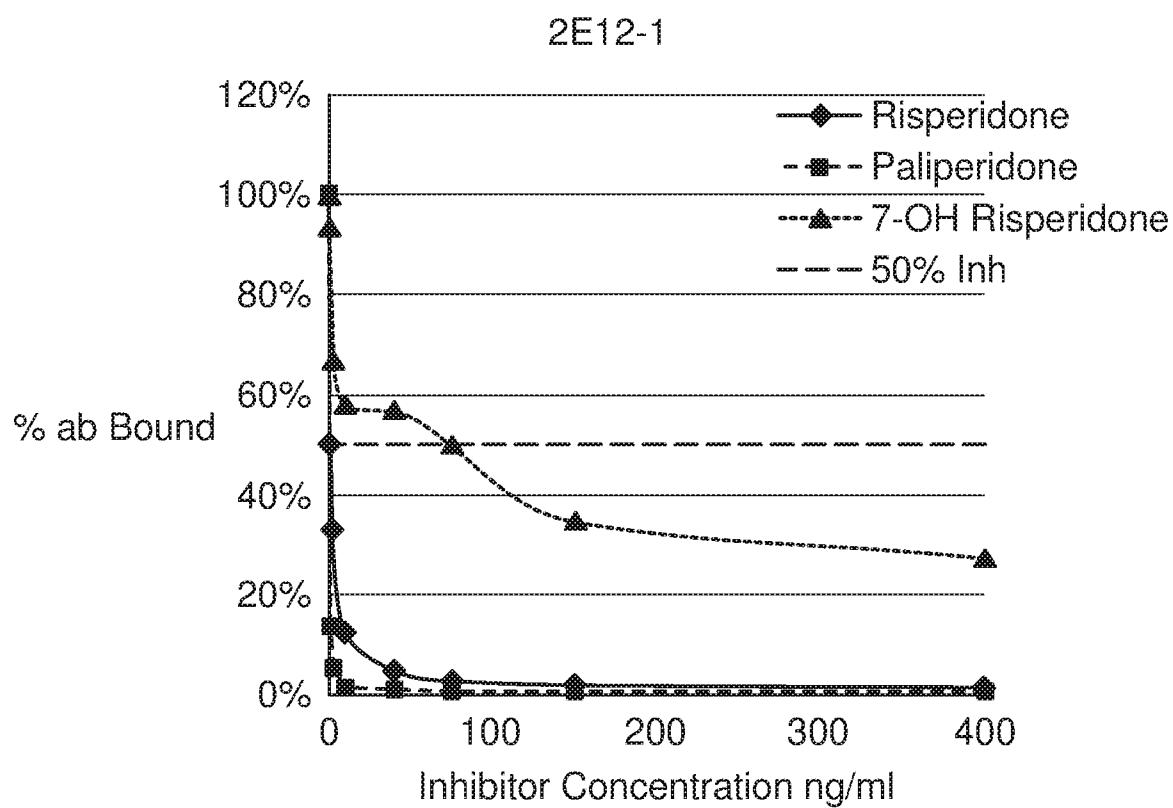
f) a heavy chain CDR3 sequence comprising amino acid residues 118 to 128 of SEQ ID NO:8.

21. The antibody of claim 19 or claim 20, wherein the antibody binding fragment is selected from the group of fragments consisting of Fv, F(ab'), F(ab')₂, scFv, minibody and diabody fragments.

22. The antibody of claim 19 or claim 20, wherein the antibody is a monoclonal antibody.
23. An assay kit comprising the antibody of claim 19 or claim 20.
24. An assay device comprising the antibody of claim 19 or claim 20.
25. The assay device of claim 24, wherein the device is a lateral flow assay device.
26. A method of detecting risperidone in a sample, the method comprising:
- (i) contacting a sample with the antibody of claim 19 or claim 20 labeled with a detectable marker, wherein the labeled antibody and risperidone present in the sample form a labeled complex; and
 - (ii) detecting the labeled complex so as to detect risperidone in the sample.
27. A competitive immunoassay method for detecting risperidone in a sample, the method comprising:
- (i) contacting a sample with the antibody of claim 19 or claim 20, and with risperidone or a competitive binding partner of risperidone, wherein one of the antibody and the risperidone or competitive binding partner thereof is labeled with a detectable marker, and wherein sample risperidone competes with the risperidone or competitive binding partner thereof for binding to the antibody; and
 - (ii) detecting the label so as to detect sample risperidone.
28. The method of claim 27, wherein the risperidone or competitive binding partner thereof is labeled with the detectable marker.
29. The method of claim 27, wherein the antibody is labeled with a detectable marker.
30. The method of claim 27, wherein the immunoassay is performed on a lateral flow assay device and the sample is applied to the device.

31. The method of claim 26 or 27, further comprising detecting the presence of one or more analytes in addition to risperidone.
32. The method of claim 31, wherein the one or more analytes are anti-psychotic drugs other than risperidone.
33. The method of claim 32, wherein the anti-psychotic drugs other than risperidone are selected from the group consisting of: paliperidone, quetiapine, olanzapine, aripiprazole, and metabolites thereof.

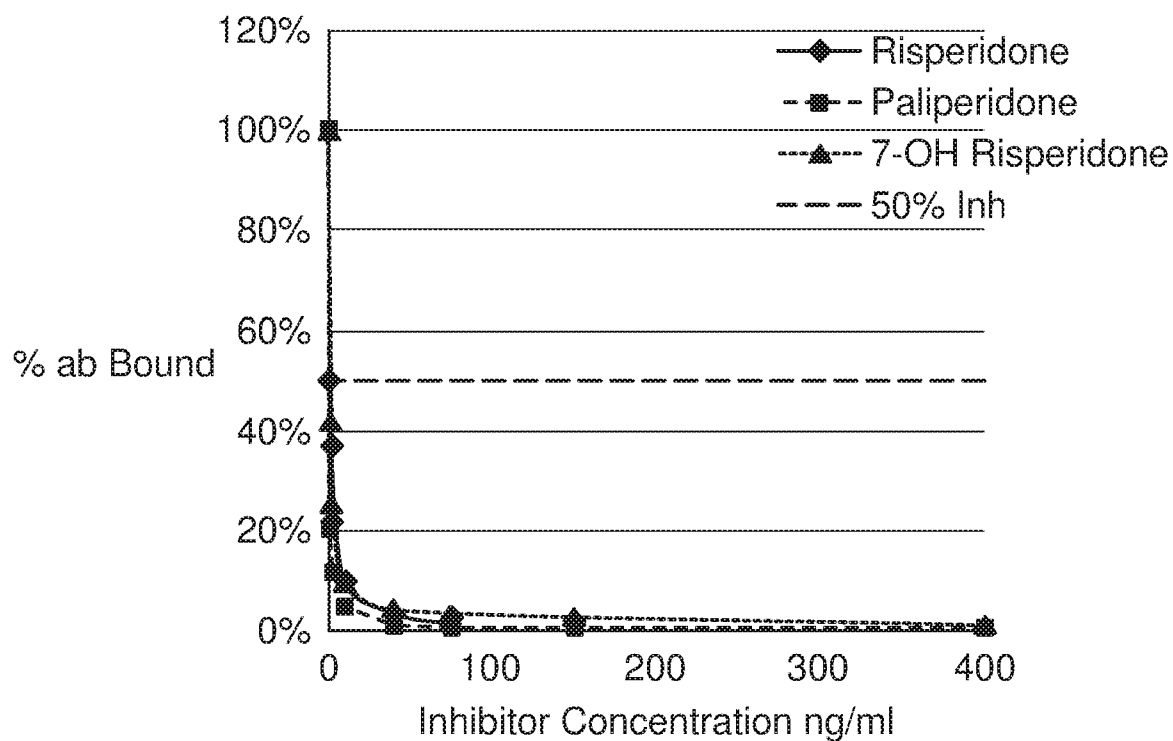
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FIG. 1

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FIG. 2A

7A8-1

**FIG. 2B**

7A8-1

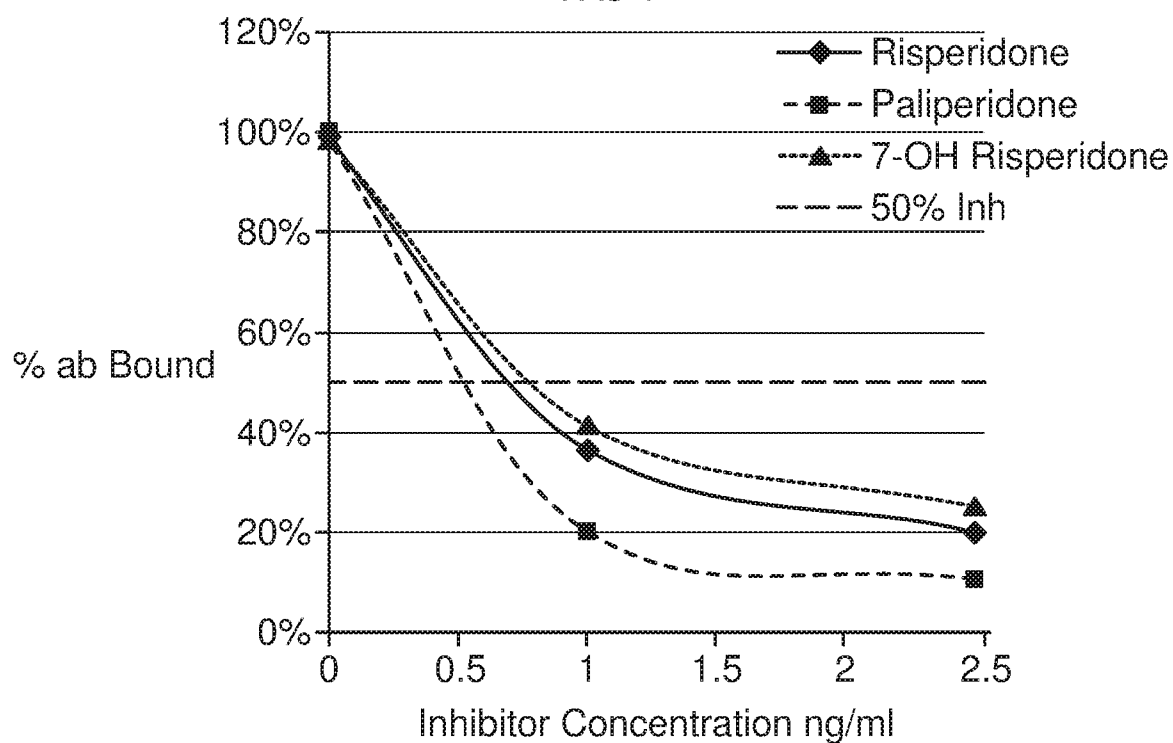
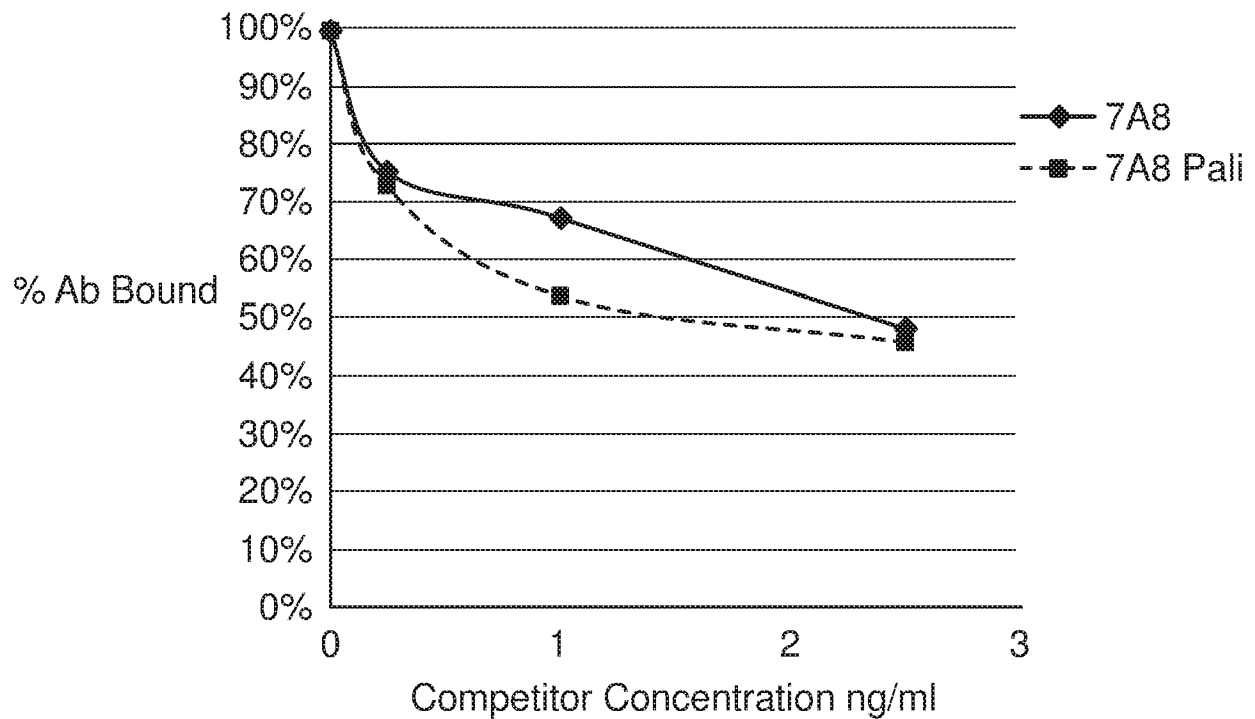
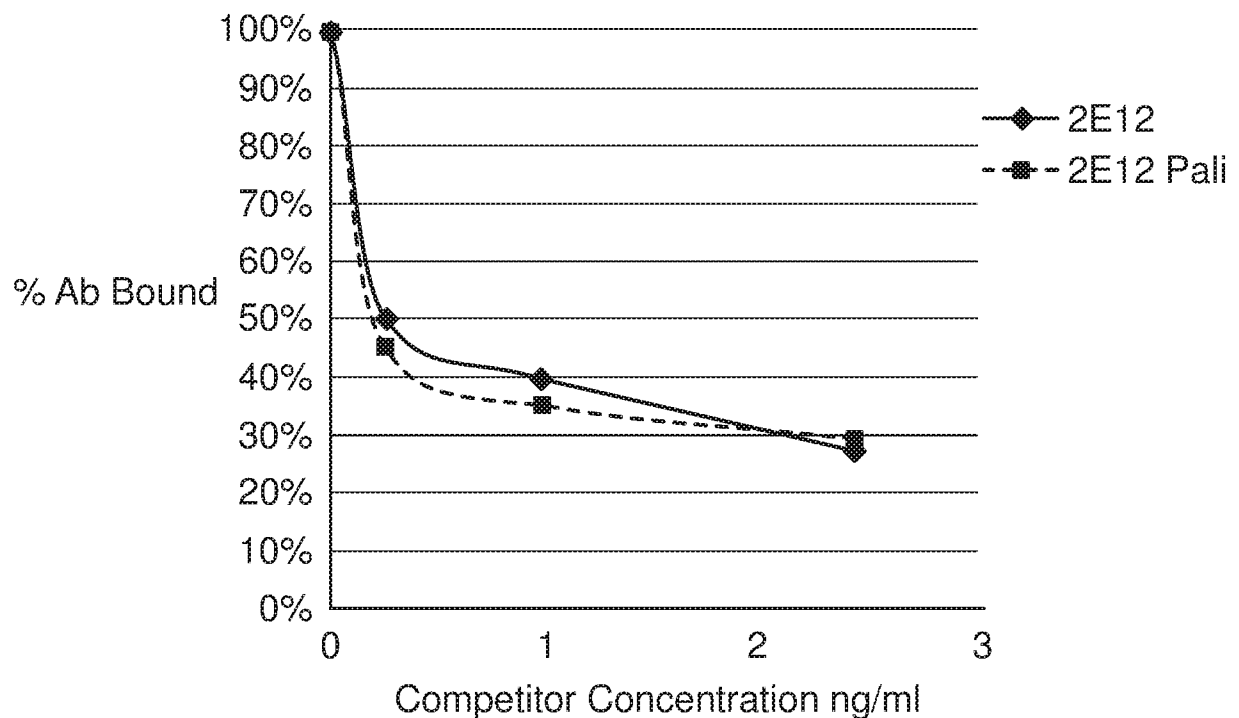


FIG. 3A**FIG. 3B**

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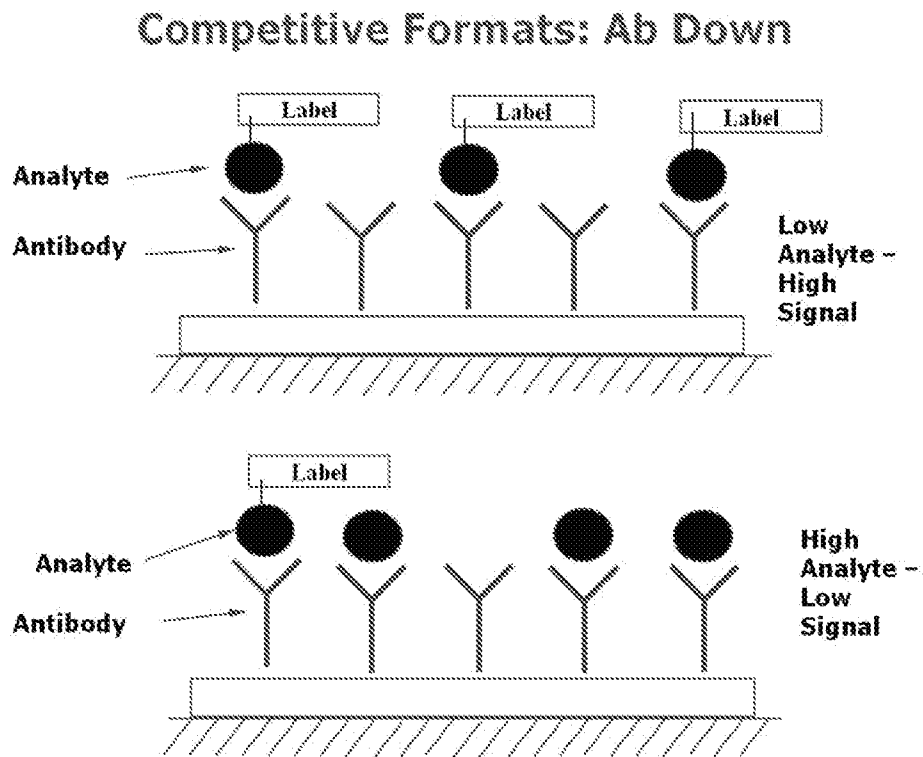


FIG. 4

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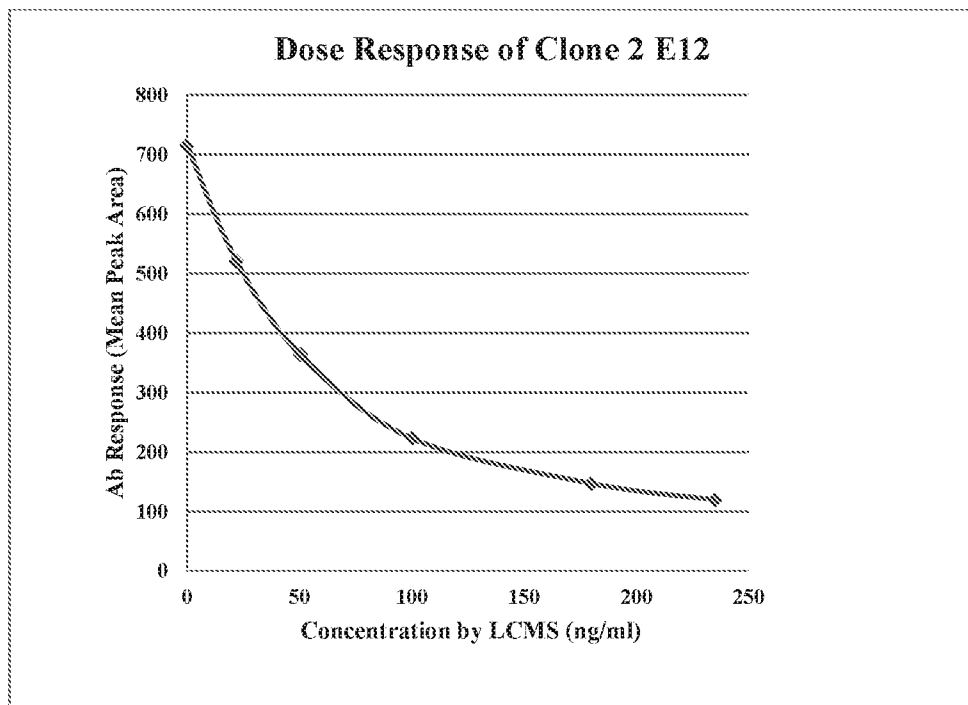


FIG. 5

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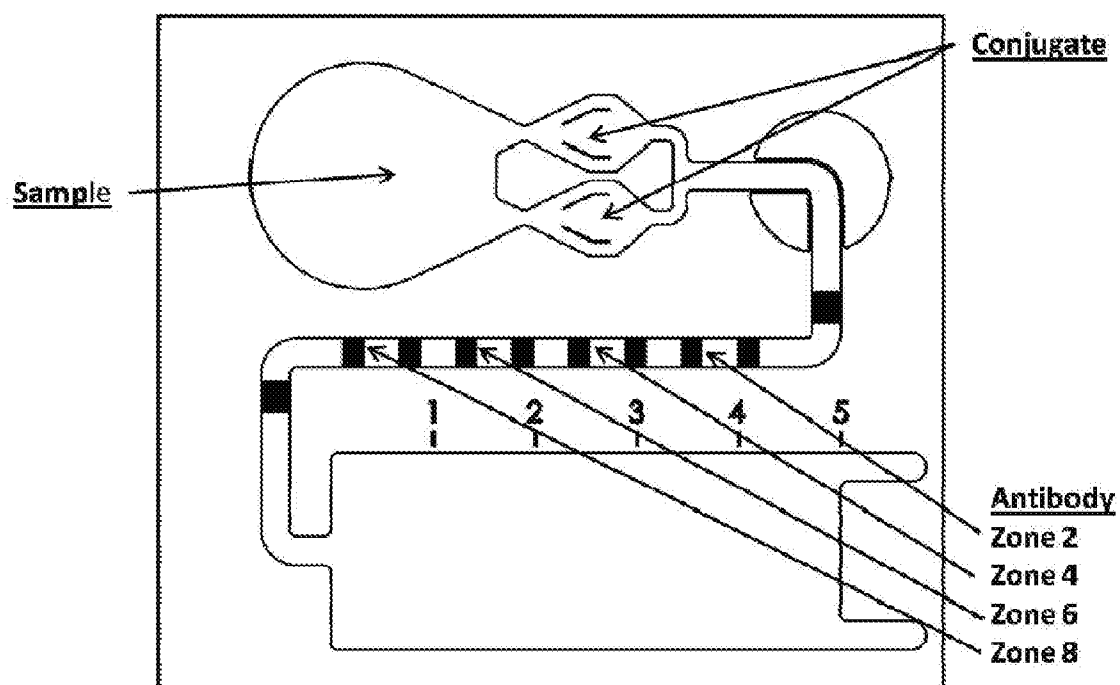


FIG. 6

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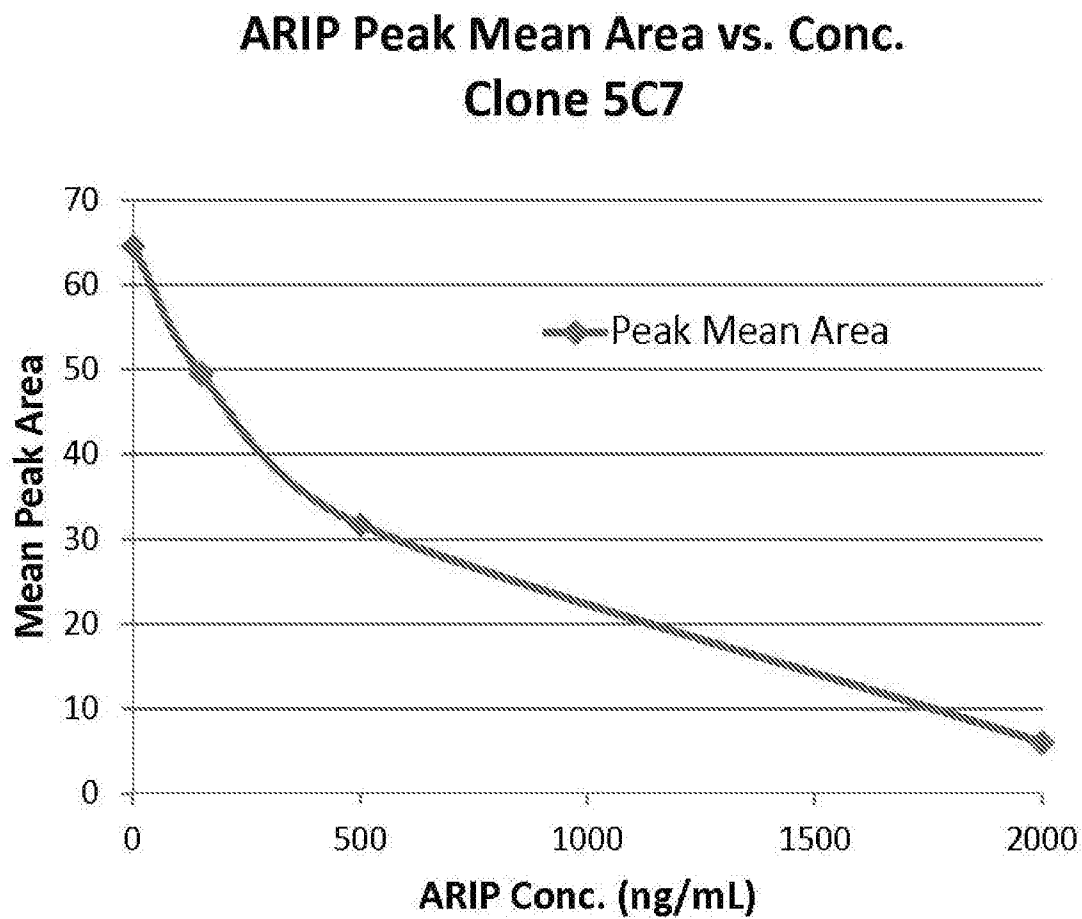


FIG. 7

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**OLAN Peak Mean Area vs. Conc.
Clone 4G9-1**

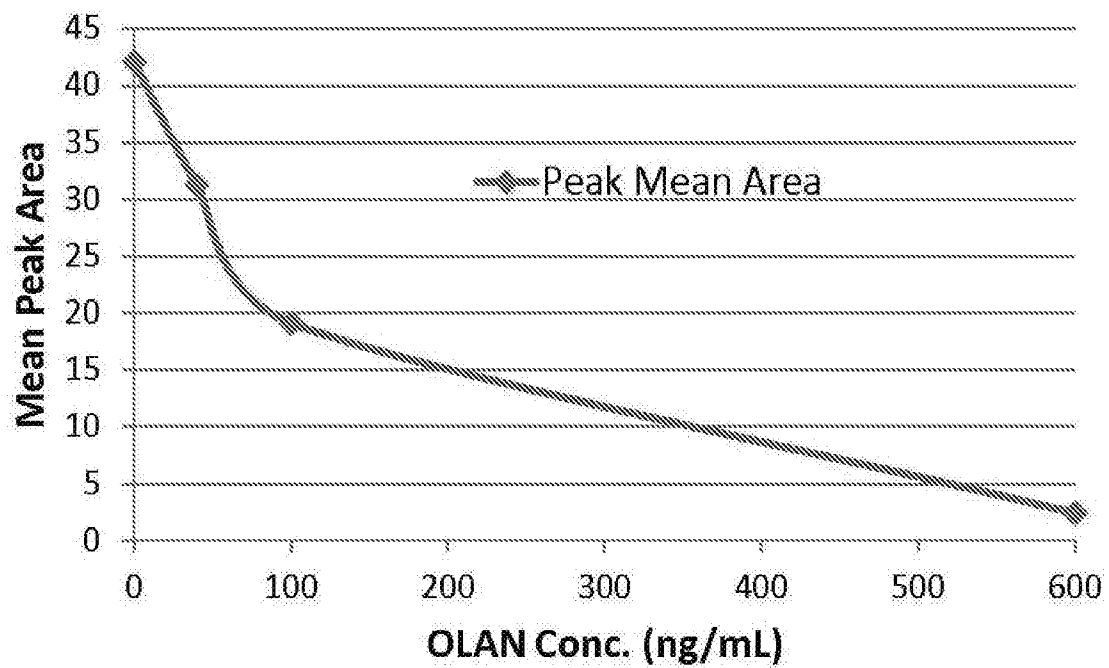


FIG. 8

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**QUET Peak Mean Area vs. Conc.
Clone 11**

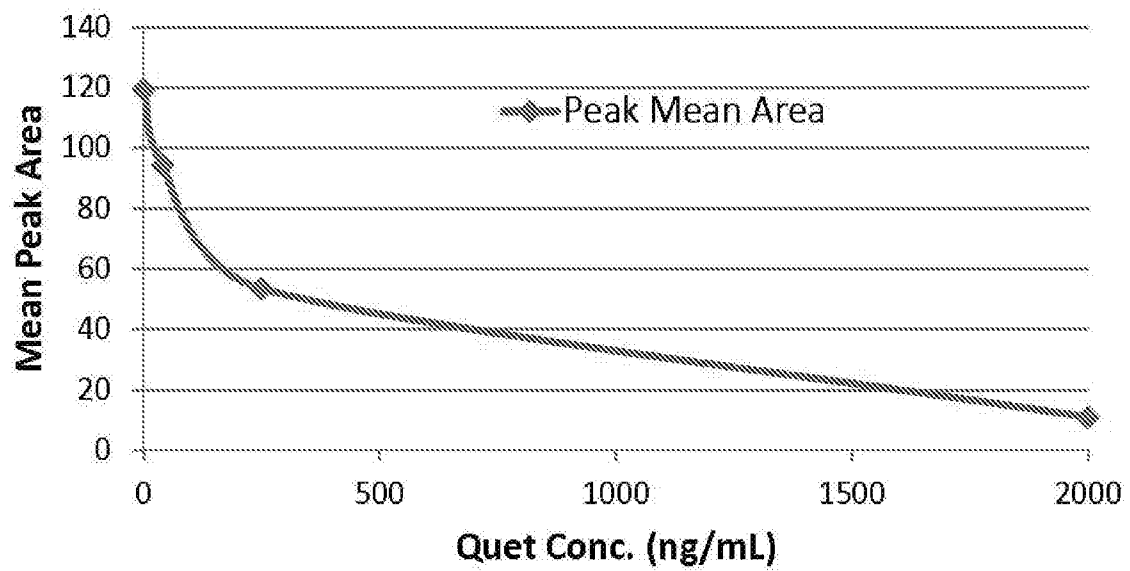


FIG. 9

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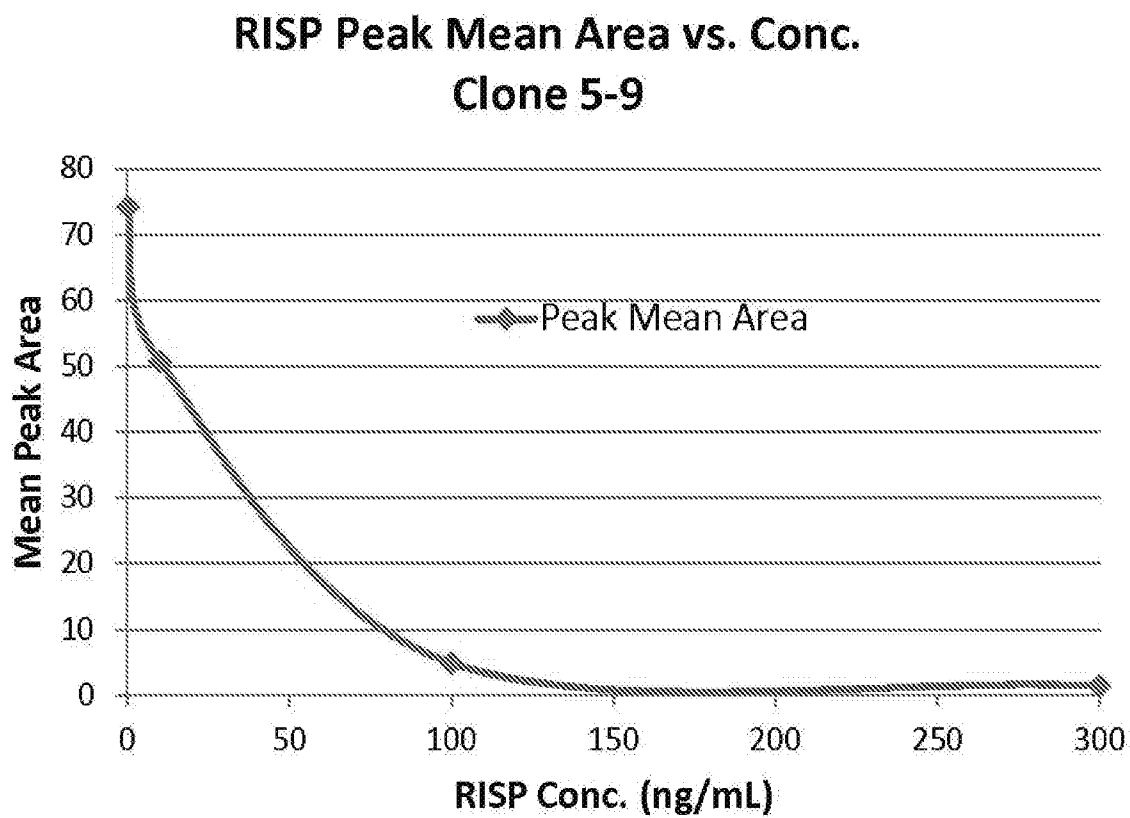


FIG. 10

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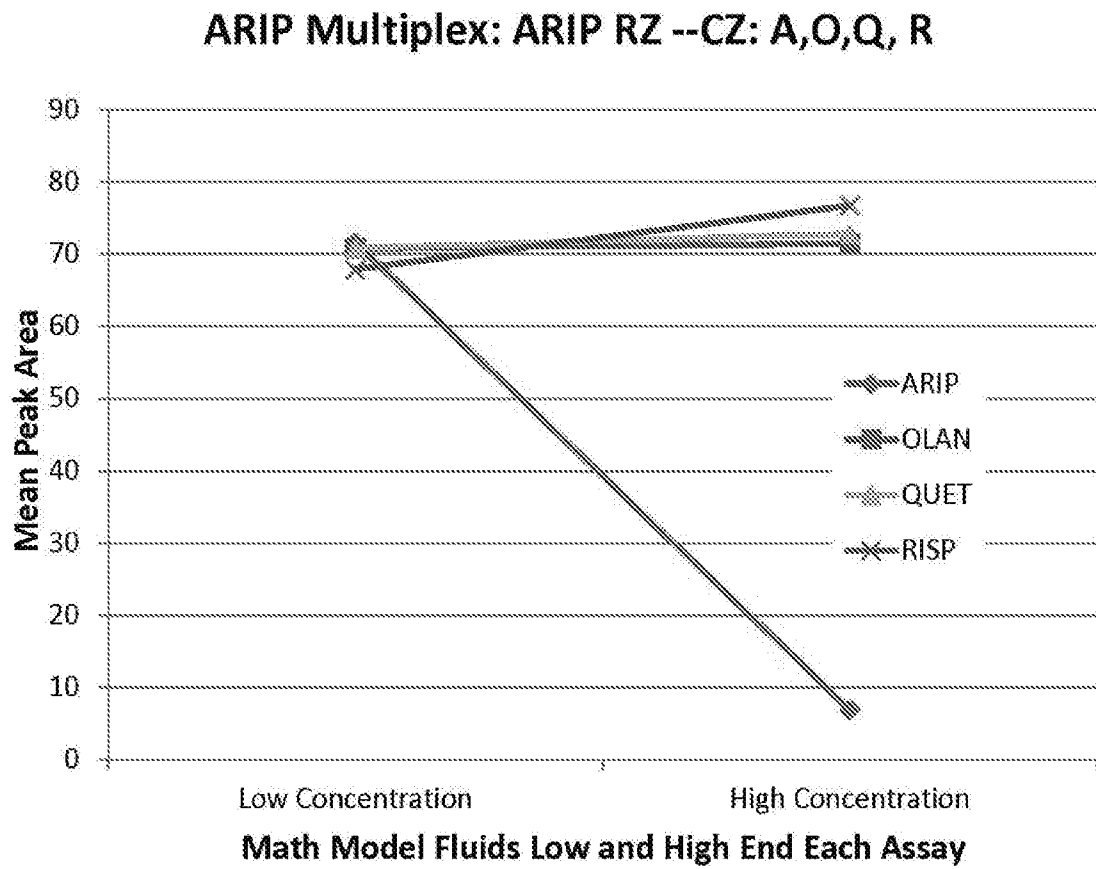


FIG. 11

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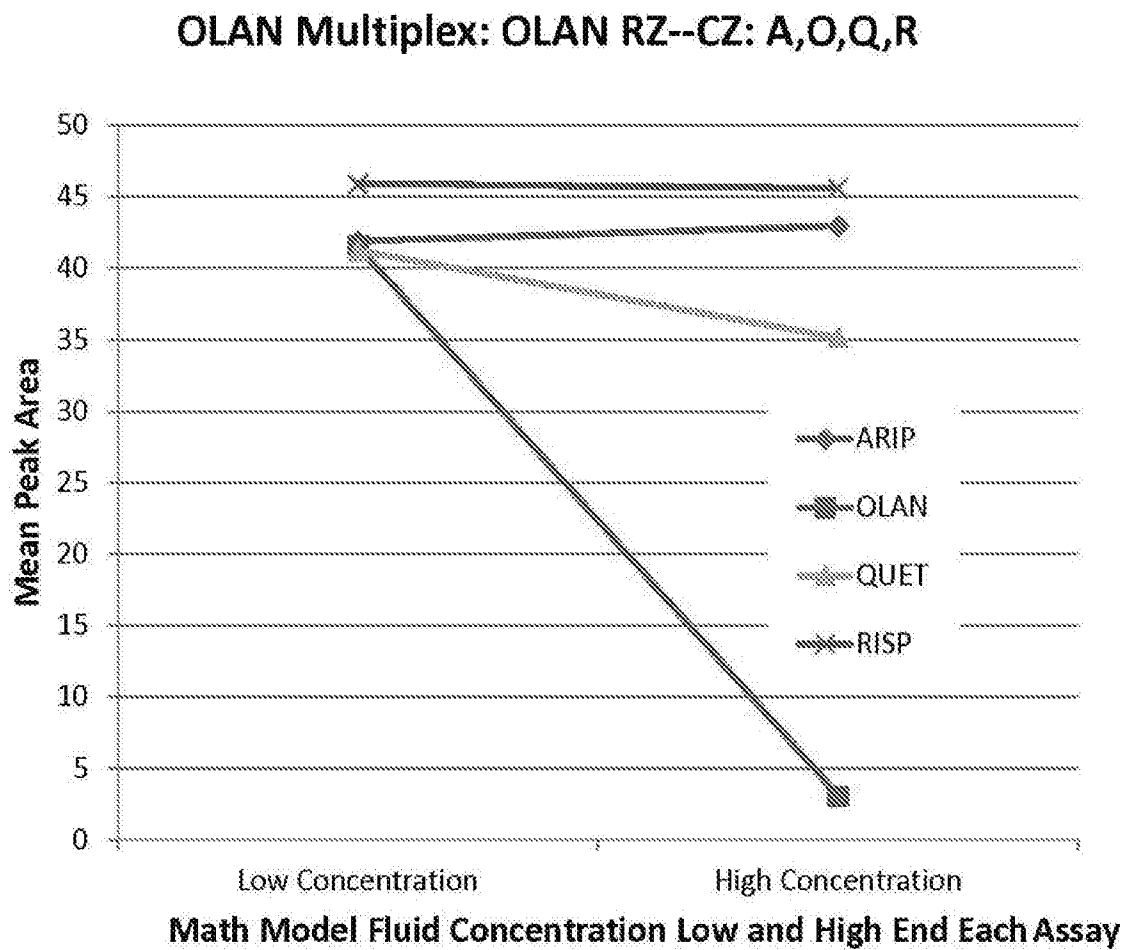


FIG. 12

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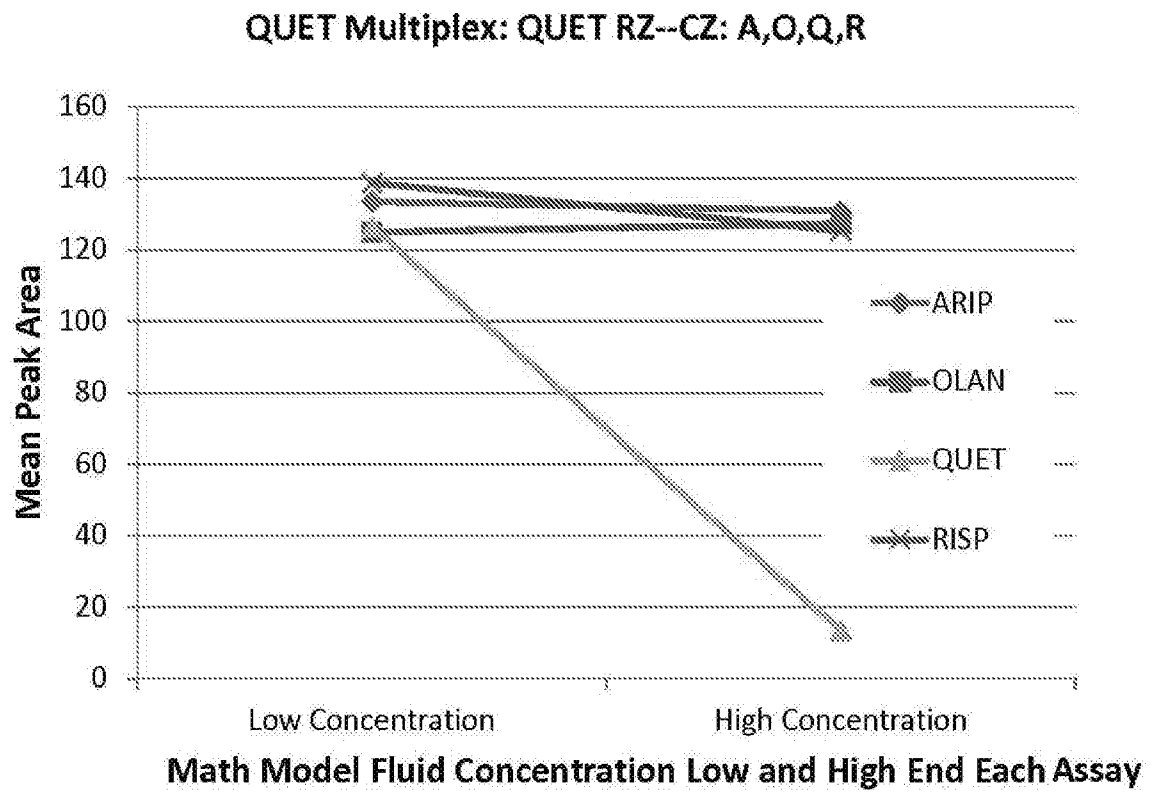


FIG. 13

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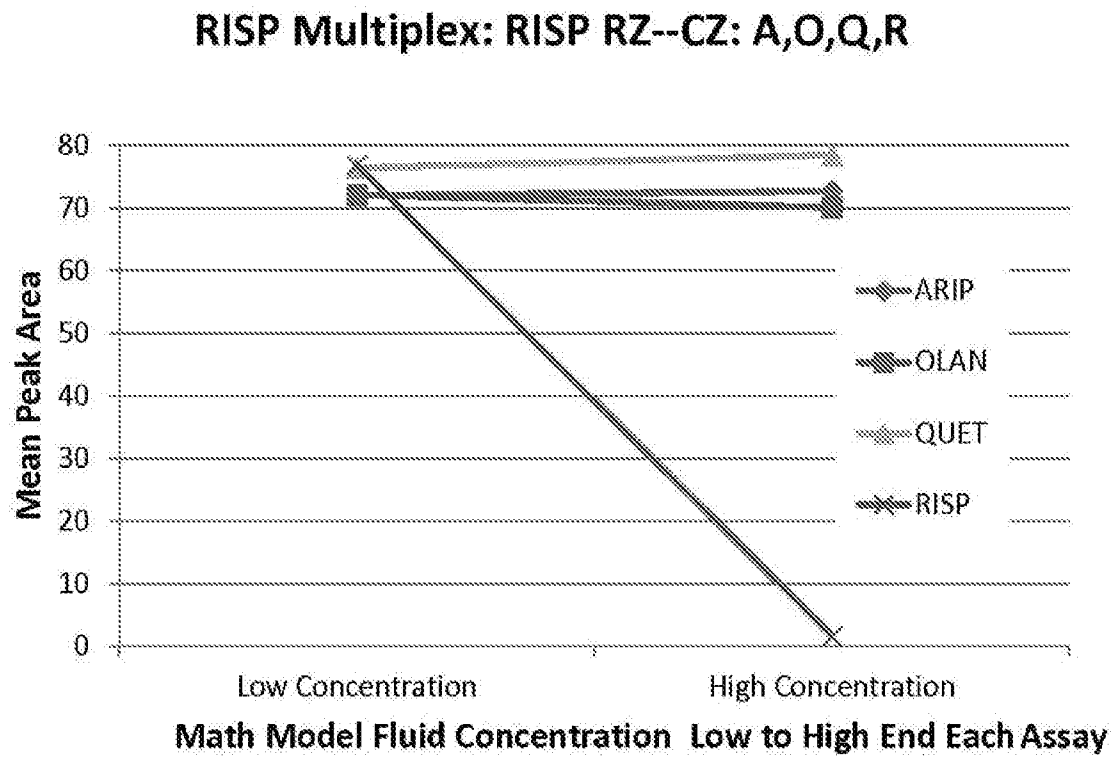


FIG. 14

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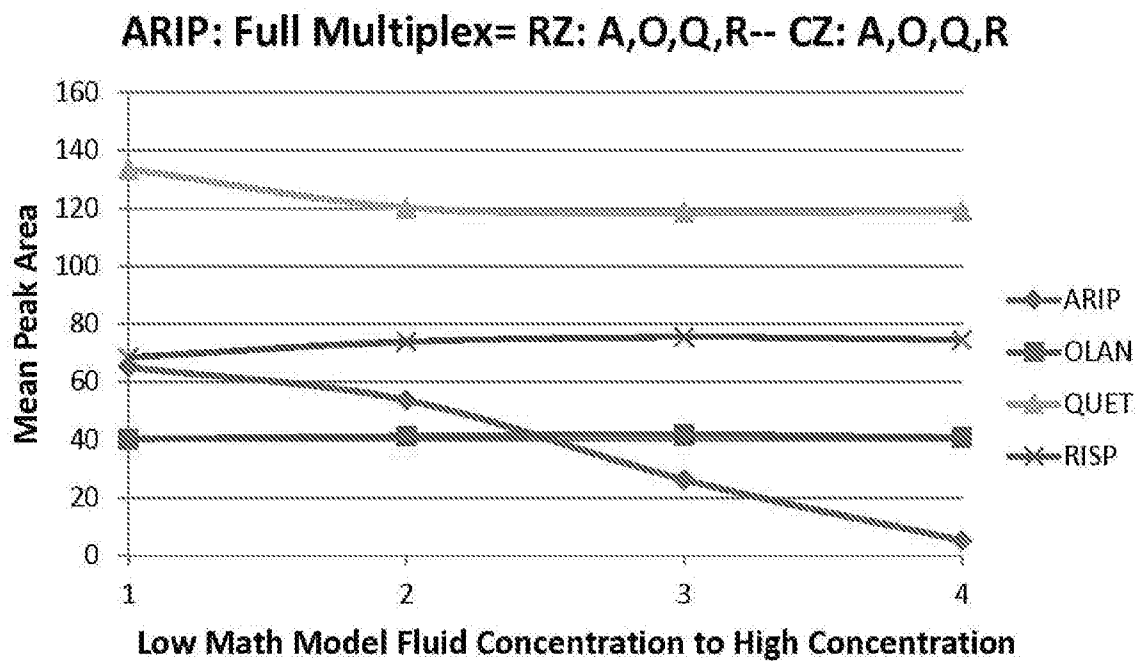


FIG. 15

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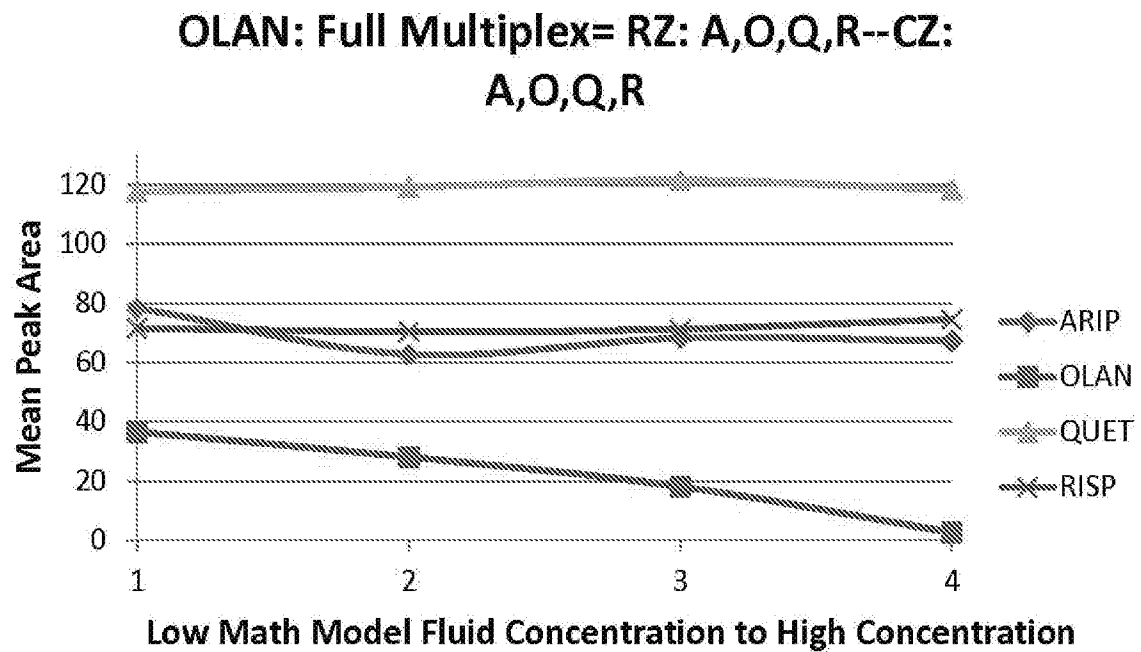


FIG. 16

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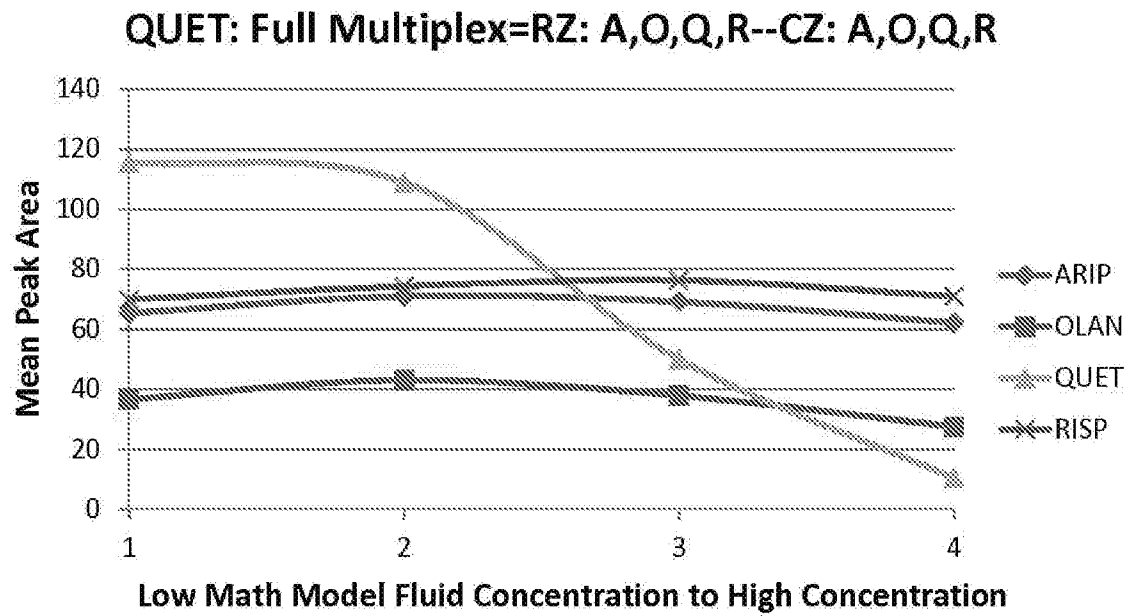


FIG. 17

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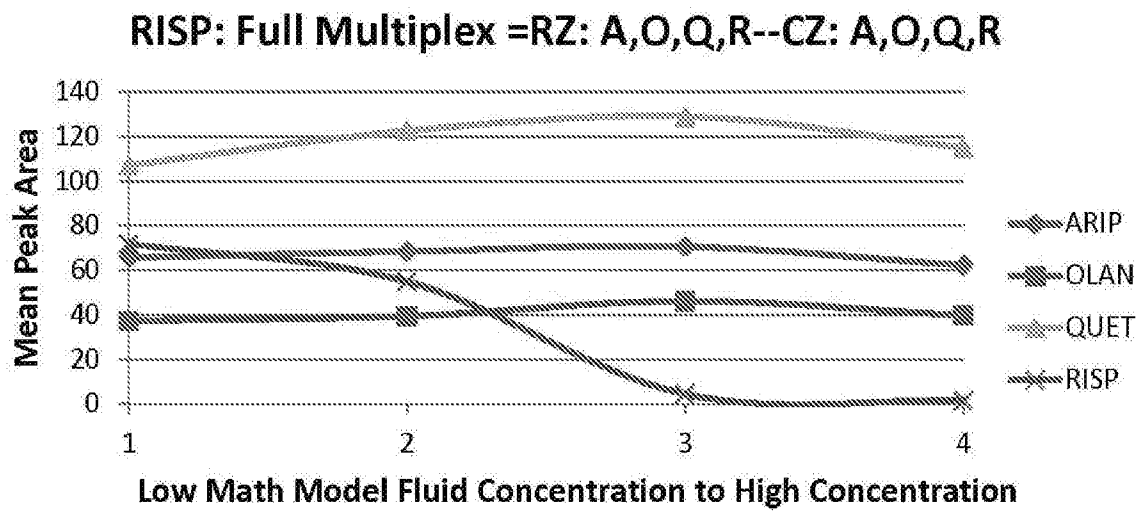


FIG. 18

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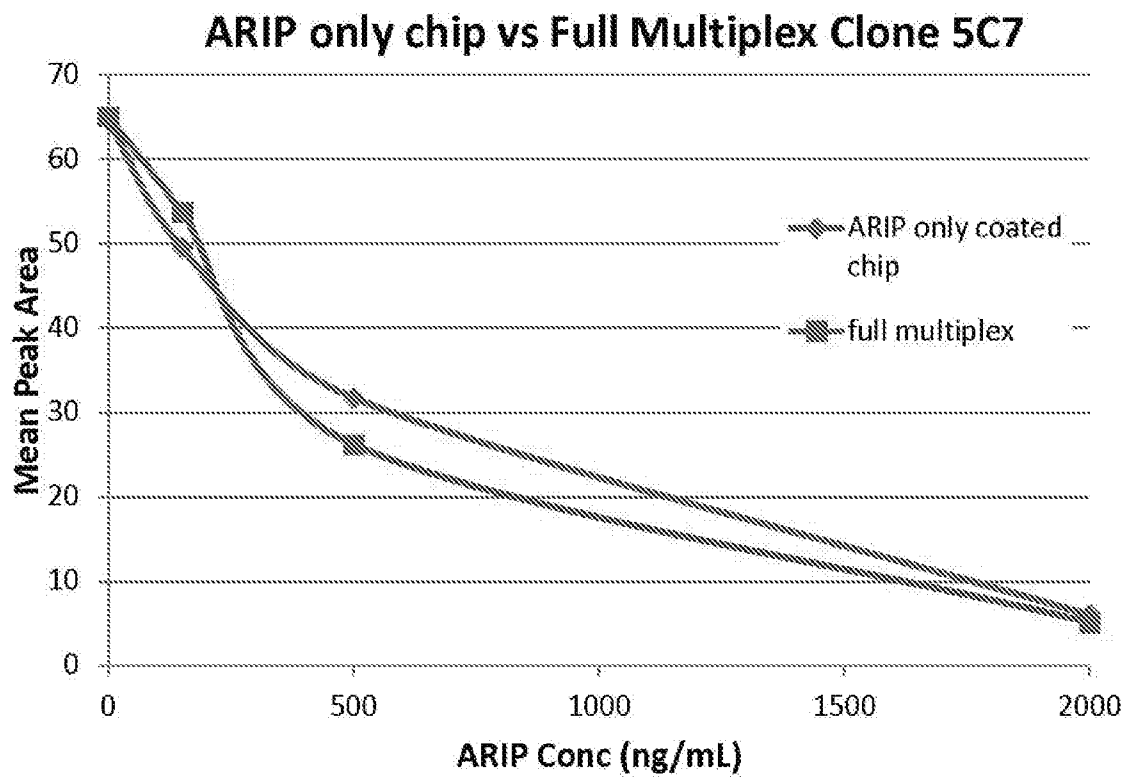


FIG. 19

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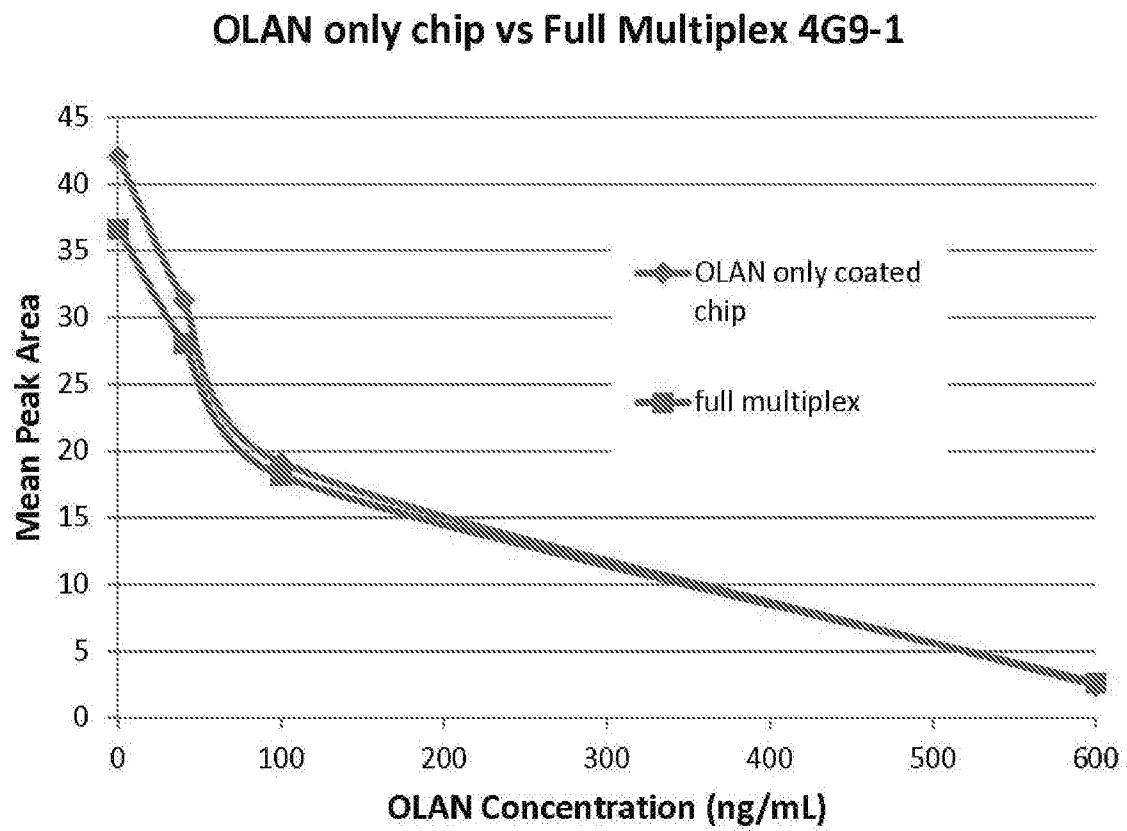


FIG. 20

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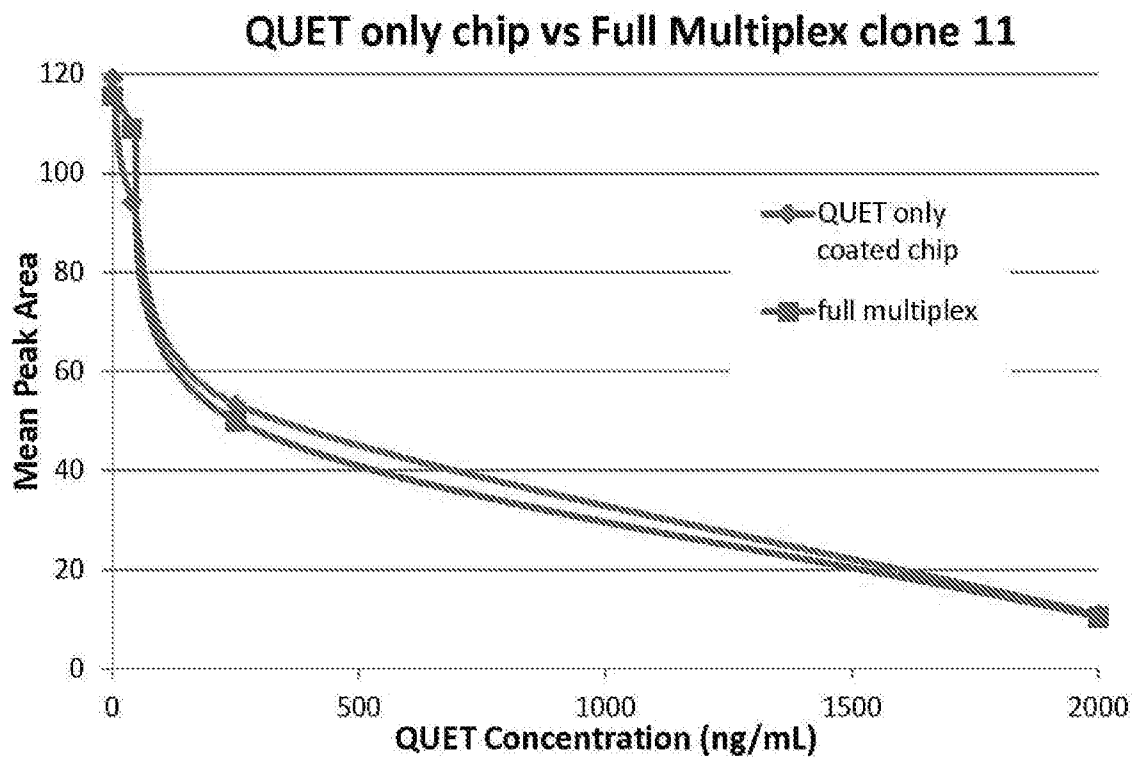


FIG. 21

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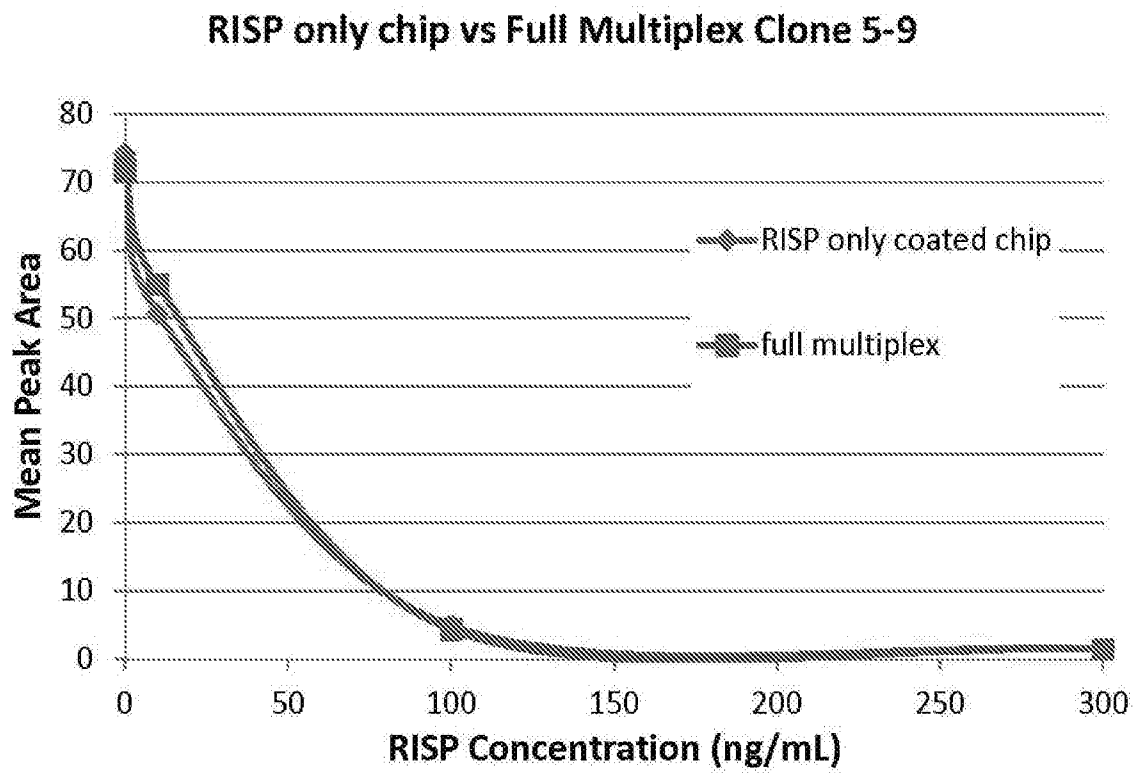


FIG. 22