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(71) Demandeur/Applicant:
NATIONAL RESEARCH COUNCIL OF CANADA, CA

(72) Inventeurs/Inventors:
MANCEUR, AZIZA, CA;
MARCIL, ANNE, CA;
ZOU, WEI, CA;
KAMEN, AMINE, CA;
GADOURY, CHRISTINE, CA

(74) Agent: THOMAS, ROULA

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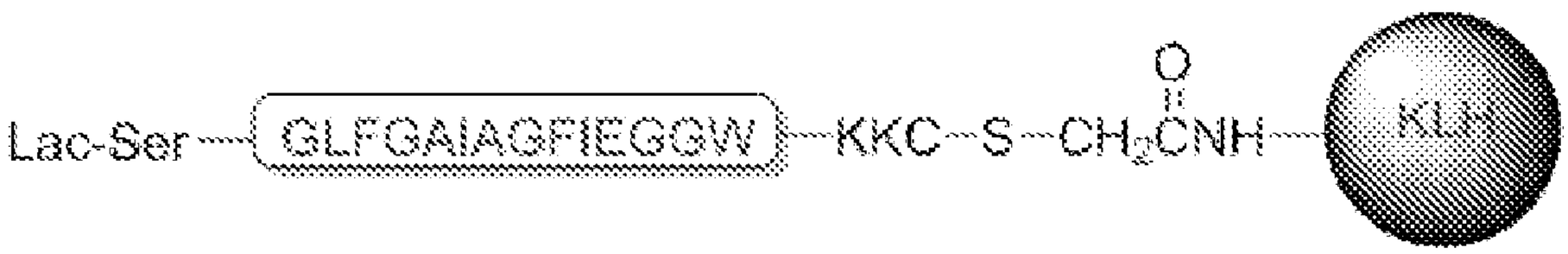

FIGURE 1A

(57) Abrégé/Abstract:
The present invention relates to hemagglutinin-specific antibodies, fragments thereof, and uses thereof. More specifically, these antibodies and fragments thereof are able to recognize antigen from multiple influenza strains.

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(71) Applicant: **NATIONAL RESEARCH COUNCIL OF CANADA** [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(72) Inventors: **MANCEUR, Aziza**; 4708 Rivard, Montreal, Québec H2J 2N4 (CA). **MARCIL, Anne**; 4801 Grier, Pierrefonds, Québec H9J 2A6 (CA). **ZOU, Wei**; 4140 Wolfe Point Way, Ottawa, Ontario K1V 1P4 (CA). **KAMEN, Amine**; 4542 Coronation, Montreal, Québec H4B 2C6 (CA). **GADOURY, Christine**; 4990 Walkley #1, Montreal, Québec H4V 2M2 (CA).

(74) Agent: **LEMAY, Catherine** et al.; National Research Council of Canada, 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

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(54) Title: HEMAGGLUTININ-SPECIFIC ANTIBODIES AND USES THEREOF

FIGURE 1A

(57) Abstract: The present invention relates to hemagglutinin-specific antibodies, fragments thereof, and uses thereof. More specifically, these antibodies and fragments thereof are able to recognize antigen from multiple influenza strains.

HEMAGGLUTININ-SPECIFIC ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates to hemagglutinin-specific antibodies, fragments thereof, and uses thereof. More specifically, the present invention relates to hemagglutinin-specific antibodies and fragments thereof able to recognize antigen from multiple influenza strains.

BACKGROUND OF THE INVENTION

[0002] Influenza is an infectious disease caused by the influenza virus which belongs to the Orthomyxoviridae family. Based on their core proteins, influenza viruses are classified into types A, B, and C. The two main types of influenza virus responsible for seasonal flu epidemics are types A and B. Influenza A virus can be further characterized by serotype based on the hemagglutinin (HA) and neuraminidase (NA) proteins on the viral surface. Currently, there are 18 known subtypes of HA and 11 subtypes of NA. Based on HA subtypes, influenza A viruses are further divided into two phylogenetic groups: group 1 (H1. H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18) and group 2 (H3, H4, H7, H10, H14 and H15). Point mutations in the viral genome RNA of a given HA subtype already in circulation, or a new subtype of HA that arises through antigenic shift (Kang et al., 2011) can result in an influenza pandemic.

[0003] According to the World Health Organization (WHO), the influenza virus is responsible for up to 500,000 deaths per year worldwide. In order to combat the potential fatal effect of influenza virus, vaccines are produced yearly and administered to global populations. However, there are thousands of influenza virus strains. Presently, each strain requires a specific antibody for detection and quantification of HA, the most abundant protein expressed at the surface of the virus. Regulatory agencies such as the WHO are responsible for producing and distributing the antibodies used to quantify new vaccine lots throughout the world. Antibody production can take from 3 up to 16 weeks, which causes significant delays for the vaccine industry.

[0004] Generally, quantification of new vaccine lots is the bottleneck in vaccine distribution. Quantification of HA is currently performed using an assay called the Single Radial Immunodiffusion (SRID) assay. However, this assay is lengthy, laborious, and highly variable depending on the operator. In addition, standardised reagents necessary for SRID (polyclonal sera and antigen), need to be updated every year, which takes 12-16 weeks and is reliant on obtaining purified HA antigen. While the SRID assay is the

only quantification method that is officially accepted by regulatory agencies, alternative quantification methods such as Enzyme-Linked Immunosorbent Assays (ELISA) could be more efficient.

[0005] Currently, specific antibodies against each strain are generated by injecting animals with isolates from each strain. However, the use of strain-specific antibodies can cause delays in releasing new vaccine lots. Using antibodies with broad specificity can speed up the detection and quantification of new influenza strains. To date, only two pan-HA antibodies are able to recognize all 3 groups of influenza commonly circulating in humans (type A group 1, type A group 2, and type B).

[0006] CR9114 is a human monoclonal antibody that was isolated using combinatorial display library derived from human B cells of subjects exposed to influenza (Dreyfus et al, 2012). CR9114 was shown to detect 10 influenza B viruses, as well as five HA belonging to influenza A (three from group 1 and two from group 2), but was not tested against all subtypes. However, its ability to recognize all HA subtypes remains untested.

[0007] Uni-1 was raised at Health Canada against a peptide sequence that is known to be highly conserved among influenza strains: GLFGAIAGFIEGGW (SEQ ID NO:29). The peptide sequence was derived from the HA fusion peptide, which was selected using bioinformatics analyses. Notably, Uni-1 was able to detect 13 different HA subtypes (H1 to H13) as well as B/Malaysia/2506/2004 by western blot (Chun et al, 2008).

[0008] Unfortunately, the main constraint with Uni-1 is that the antibodies are polyclonal rabbit antibodies, which result in high lot to lot variations. Additionally, the peptide used to raise Uni-1 was unable to elicit an immune response in mice, which has prevented production of monoclonal antibodies.

[0009] Thus, while some success has been achieved in the influenza field to generate antibodies with broad specificity to influenza HA, it is limited and not without drawbacks. The influenza community continues to seek faster and more accurate quantification methods to improve or replace the SRID assay, which could speed up the vaccine production and delivery system.

SUMMARY OF THE INVENTION

[00010] The present invention relates to hemagglutinin-specific antibodies, fragments thereof, and uses thereof. More specifically, the present invention relates to

hemagglutinin-specific antibodies and fragments thereof able to recognize antigen from multiple influenza strains.

[00011] The present invention provides an isolated or purified antibody or fragment thereof, comprising:

a) a light chain comprising a complementarity determining region (CDR) L1 sequence of $QSLLNSX_1X_2QKNX_3$ (SEQ ID NO:1) where X_1=R or D, X_2=N or T, X_3=H or F; a CDR L2 sequence of X_1AS (SEQ ID NO:35) where X_1=W or F; and a CDR L3 sequence of $QQYYX_1X_2X_3X_4T$ (SEQ ID NO:2) where X_1=T or S, X_2=Y or I, X_3=P or no amino acid, X_4=R or L,

and

b) a heavy chain comprising a complementarity determining region (CDR) H1 sequence of $GYX_1X_2TX_3DYY$ (SEQ ID NO:3) where X_1=S or T, X_2=I or F, X_3=S or no amino acid;

a CDR H2 sequence selected from the group consisting of $IGYDGX_1K$ (SEQ ID NO:4) where X_1=S or T, and IYPGNGHT (SEQ ID NO:5), and

a CDR H3 sequence selected from the group consisting of TRDRANWDDYFDY (SEQ ID NO:6) and AYDLFNY (SEQ ID NO:7).

[00012] In one non-limiting example, the isolated or purified antibody or fragment described above may comprise a CDR L1 that is selected from the group consisting of QSLLNSRNQKNH (SEQ ID NO:8) and QSLLNSDTQKNF (SEQ ID NO:9).

[00013] In another non-limiting example, the isolated or purified antibody or fragment thereof as previously described may comprise a CDR L2 that is selected from the group consisting of WAS (SEQ ID NO:36) and FAS (SEQ ID NO:37).

[00014] In another non-limiting example, the isolated or purified antibody or fragment thereof as described above may comprise a CDR L3 that is selected from the group consisting of QQYYTYXRT (SEQ ID NO:10) where X is P or no amino acid and QQYYSIPLT (SEQ ID NO:11).

[00015] In yet another non-limiting example, the isolated or purified antibody or fragment thereof as described above may comprise a CDR H1 selected from the group consisting of GYSITSDYY (SEQ ID NO:12) and GYTFTDYY (SEQ ID NO:13).

[00016] In a more specific example, the isolated or purified antibody or fragment thereof may comprise a sequence that may be selected from the group consisting of:

a) a light chain comprising CDR L1 of sequence QSLLNSRNQKNH (SEQ ID NO:8), CDR L2 of sequence WAS (SEQ ID NO:36), and CDR L3 of sequence QQYYTYRT (SEQ ID NO:14); and a heavy chain comprising CDR H1 of sequence GYSITSDYY (SEQ ID NO:12), CDR H2 of sequence IGYDGSK (SEQ ID NO:15), and CDR H3 of sequence TRDRANWDDYFDY (SEQ ID NO:6);

b) a light chain comprising CDR L1 of sequence QSLLNSRNQKNH (SEQ ID NO:8), CDR L2 of sequence WAS (SEQ ID NO:36), and CDR L3 of sequence QQYYTYRT (SEQ ID NO:14); and a heavy chain comprising CDR H1 of sequence GYSITSDYY (SEQ ID NO:12), CDR H2 of sequence IGYDGTK (SEQ ID NO:16), and CDR H3 of sequence TRDRANWDDYFDY (SEQ ID NO:6); and

c) a light chain comprising CDR L1 of sequence QSLLNSDTQKNF (SEQ ID NO:9), CDR L2 of sequence FAS (SEQ ID NO:37), CDRL3 of sequence QQYYSIPLT (SEQ ID NO:11); and a heavy chain comprising CDR H1 of sequence GYTFTDYY (SEQ ID NO:13), CDR H2 of sequence IYPGNGHT (SEQ ID NO:5), and CDR H3 of sequence AYDLFNY (SEQ ID NO:7).

[00017] More specifically, the isolated or purified antibody or fragment thereof may comprise a variable light (VL) domain having a sequence of:

DIVMX_1QSPSSLAX_2SVGX_3KVTMSCKSSQSLLNSX_4X_5QKNX_6LAWYQQKPGQS PKX_7LX_8YX_9ASTX$_{10}$ESGVPDRFX_{11}GX_{12}GSGTDFTLTIX_{13}SVX_{14}AEDLAX_{15}YX_{16}C QQYY$X_{17}X_{18}X_{19}X_{20}$TFGX_{21}GTKLEIK (SEQ ID NO:17), where X_1=S or T, X_2=V or M, X_3=E or O, X_4=R or D, X_5=N or T, X_6=H or F, X_7=L or I, X_8=I or V, X_9=W or F, X_{10}=R or K, X_{11}=S or I, X_{12}=D or S, X_{13}=S or T, X_{14}=K or Q, X_{15}=V or D, X_{16}=Y or F, X_{17}=T or S, X_{18}=Y or I, X_{19}=P or no amino acid, X_{20}=R or L, X_{21}=G or A.

[00018] In an even more specific example, the variable light (VL) domain may comprise a sequence selected from the group consisting of:

DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKL LIYWASTRESGVPDRFX$_1$GDGSGTDFTLTISSVKAEDLAVYYCQQYYTYRTFGG GTKLEIK (SEQ ID NO:18) where X_1=S or T;

DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSDTQKNFLAWYQQKPGQSPKIL VYFASTKESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQYYSIPLTFGAGT KLELK (SEQ ID NO:19); and

a sequence substantially identical thereto.

[00019] In the isolated or purified antibody or fragment as described above, the variable heavy (VH) domain may comprise a sequence selected from the group consisting of:

DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIG YDGX$_1$KNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDY FDYWGQGTTLTVSS (SEQ ID NO:20) where X_1=S or T;

QIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQRPGQGLEWIGYIYPG NGHTVYNQKFKVRATLTADNPSSTAYLQLNSLTSEDSGVYFCAYDLFNYWGQ GTLVTVSA (SEQ ID NO:21); and

a sequence substantially identical thereto.

[00020] In specific, non-limiting examples, the isolated or purified antibody or fragment thereof may comprise:

a) a variable light (V_L) domain of sequence:
DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKL LIYWASTRESGVPDRFSGDGSGTDFTLTISSVKAEDLAVYYCQQYYTYRTFGG GTKLEIK (SEQ ID NO:22);
and variable heavy (V_H) domain of sequence:
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIG YDGSKNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDYF DYWGQGTTLTVSS (SEQ ID NO:24);
or

b) a variable light (V_L) domain of sequence:
DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKL LIYWASTRESGVPDRFTGDGSGTDFTLTISSVKAEDLAVYYCQQYYTYXRTFG GGTKLEIK (SEQ ID NO: 23);
and variable heavy (V_H) domain of sequence:
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIG YDGTKNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDYF DYWGQGTTLTVSS (SEQ ID NO:25);
or

c) a variable light (V_L) domain of sequence:
DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSDTQKNFLAWYQQKPGQSPKIL VYFASTKESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQYYSIPLTFGAGT KLELK (SEQ ID NO:19);
and variable heavy (V_H) domain of sequence:

QIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQRPGQGLEWIGYIYPG
NGHTVYNQKFKVRATLTADNPSSTAYLQLNSLTSEDSGVYFCAYDLFNYWGQ
GTLVTVSA (SEQ ID NO:21);

or

d) a sequence substantially identical thereto.

[00021] The antibody or fragment thereof of the present invention, as defined above, may specifically bind hemagglutinin.

[00022] The isolated or purified antibody or fragment thereof as described herein may be a full-length IgG, Fv, scFv, Fab, or $F(ab')_2$ fragments; the antibody or fragment thereof may also comprise framework regions from IgA, IgD, IgE, IgG, or IgM. The isolated or purified antibody or fragment thereof of the present invention may be chimeric; or example, and without wishing to be limiting, such a chimeric antibody or fragment thereof may comprise the V_L and V_H domains from mouse and framework regions (constant domains) from human IgG1, more specifically human kappa 1 light chain and human IgG1 heavy chain.

[00023] The present invention also provides a nucleic acid molecule encoding the isolated or purified antibody or fragment thereof as described herein. A vector comprising the nucleic acid molecule as herein described is also provided.

[00024] The isolated or purified antibody or fragment thereof as described herein may be immobilized onto a surface, or may be linked to a cargo molecule. The cargo molecule may be a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody or fragment thereof (e.g., IgG, scFv, Fab, V_HH, etc) a chemical compound, a carbohydrate moiety, DNA-based molecules (anti-sense oligonucleotide, microRNA, siRNA, plasmid), a neutralizing agent, viral vector (adeno-, lenti-, retro-), one or more liposomes or nanocarriers loaded with any of the previously recited types of cargo molecules, or one or more nanoparticle, nanowire, nanotube, or quantum dots. In a specific, non-limiting example, the cargo molecule is a neutralizing agent.

[00025] Additionally, the present invention provides a composition comprising one or more than one isolated or purified antibody or fragment thereof as described herein and a pharmaceutically-acceptable carrier, diluent, or excipient.

[00026] An *in vitro* method of detecting hemagglutinin is also provided, the method comprising the steps of:

a) contacting a tissue sample with an (one or more than one) isolated or purified antibody or fragment thereof as described herein linked to a detectable agent; and

b) detecting the detectable agent linked to the antibody or fragment thereof bound to hemagglutinin in the tissue sample.

[00027] In the method described above, the method may detect hemagglutinin in circulating cells and the sample may be a serum sample, lung tissue sample, neuroepithelium tissue sample, or other tissue from the respiratory system. In the method as described, the step of detecting (step b)) may be performed using optical imaging, immunohistochemistry, molecular diagnostic imaging, immunoassays such as ELISA, Western blot, dot blot, slot blot, or other suitable method.

[00028] Hemagglutinin is a protein expressed at the surface of the influenza virus. Due to antigenic drift and shift, each new strain of influenza generally requires a specific antibody for its recognition. Presently, three novel antibodies [11H12 (SEQ ID NO. 36), 10A9 (SEQ ID NO. 35) and 9D1] have been identified that specifically bind hemagglutinin. The novel antibodies described herein are able to detect and bind multiple strains encompassing 13 HA subtypes. They can be used for detection, quantification, and neutralization. Furthermore, these antibodies are monoclonal, and thus can be produced in a reproducible and scalable fashion.

[00029] The antibodies of the present invention may be use to speed up or improve the accuracy of quantification methods, which would ease the bottleneck in vaccine distribution. The antibodies could also be used with in-process and crude samples in order to optimise the bioprocess used to generate vaccines.

[00030] The present invention may also provide a method for the prevention or treatment of influenza in a subject, comprising administering an (one or more than one) isolated or purified antibody or fragment thereof as described herein to the subject

[00031] Alternatively, there is also provided an isolated or purified antibody or fragment thereof as described herein for use in preventing or treating influenza in a subject.

[00032] Additional aspects and advantages of the present invention will be apparent in view of the following description. The detailed descriptions and examples, while indicating preferred embodiments of the invention, are given by way of illustration only, as various changes and modifications within the scope of the invention will become apparent to those skilled in the art in light of the teachings of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[00033] These and other features of the invention will now be described by way of example, with reference to the appended drawings, wherein:

[00034] FIGURE 1A is a schematic diagram of the conjugate structure used for mouse immunizations. The universal peptide epitope is shown in a box; Lac-Ser is O-beta-lactosyl-serine; -S- is thio-Cys; KLH is Keyhole Limpet Hemocyanin.

[00035] FIGURE 1B is a scheme representing the addition of non-immunogenic hydrophilic saccharide at one end of a linear peptide-conjugate for producing the antibodies of the invention.

[00036] FIGURES 2 are sequence alignments of the variable domains of the antibodies of the present application. A) light chains: 11H12 (SEQ ID NO:23); 10A9 (SEQ ID NO:19); and 9D1 (SEQ ID NO:22). B) heavy chains: 11H12 (SEQ ID NO:25); 10A9 (SEQ ID NO:21); and 9D1 (SEQ ID NO:24).

[00037] FIGURE 3 shows thermograms obtained by Differential Scanning Calorimetry (DSC) of the three mAb. Each mAb was scanned from 20 to 100°C.

[00038] FIGURES 4A-C shows results from ELISA analysis following one to three freeze-thaw cycles. The binding affinity of 9D1 (FIGURE 4A), 10A9 (FIGURE 4B) and 11H12 (FIGURE 4C) was measured using the peptide-conjugate shown in FIGURE 1.

[00039] FIGURES 5 show Western blot results of the reactivity of the mAb 11H12 (FIGURE 5A), 10A9 (FIGURE 5B), and 9D1 (FIGURE 5C), as well as an anti-GFP negative control (FIGURE 5D), for recombinant HA proteins. For each set of data, the purified mAb (mAb) or the unpurified hybridoma supernatant (Supernat) was used. The molecular weight of the rHA is 75KDa when uncleaved (HA_0). When cleaved, the fragments have a molecular weight of 45KDa (HA_1) and 25KDa (HA_2), as indicated in FIGURE 5A. Each mAb was assayed against H5N1/A/Indonesia/05/2005 (H5N1); H1N1

A/Puerto Rico/08/1934 (H1N1); B/Brisbane/60/2008 (B/Brisb.); and H7N7 A/Netherlands/219/2003 (H7N7).

[00040] FIGURES 6 show results of a second Western blot analysis of the reactivity of the 11H12 mAb (FIGURE 6A), the 10A9 mAb (FIGURE 6B), a mixture of the two antibodies (FIGURE 6D), and an anti-GFP negative control (FIGURE 6C). Lane 1, H1N1/A/California/06/2008; Lane 2, H3N2/A/Brisbane/10/2007; Lane 3, H5N1/A/Indonesia/05/2005; Lane 4, H5N1/A/Vietnam/1203/2004; Lane 5, H7N7/A/Netherlands/219/2003; Lane 6, H9N2/A/Hong Kong/1073/1999; Lane 7, B/Brisbane/60/2008.

[00041] FIGURES 7 show Western blot results of the reactivity of the 11H12 (FIGURE 7A) and 10A9 (FIGURE 7B) mAb with viruses produced in eggs. Full names of the virus strains are shown in Table 6.

[00042] FIGURES 8 show dot blot analysis of the 3mAbs: 9D1 (FIGURE 8A), 10A9 (FIGURE 8B) and 11H12 (FIGURE 8C), tested against HA from different strains as summarized in Table 7. Values corresponding to the optical densities of each dot are also reported in Table 7.

[00043] FIGURES 9 show the results of quantitative dot blot analysis with 11H12. A representative dot blot is shown in FIGURE 9A. H1N1 A/Puerto Rico/8/34 virus produced in HEK293 cells were quantified by generating a standard curve (FIGURE 9B). Samples were serially diluted and 4 concentrations were assayed. Sample 6 was obtained from non-infected cells and was used as a negative control (FIGURE 9C).

[00044] FIGURE 10 shows the results of ELISA analysis of 10A9, 11H12, and 9D1. A direct ELISA was performed with the antibodies against the rHA from H5N1 A/Indonesia/05/2005. A non-related antibody (anti-GFP) was used as a negative control.

[00045] FIGURES 11 show *in vivo* results of challenges with lethal concentrations of influenza viruses, along with several doses of antibodies to evaluate their protective/neutralizing capacity. In FIGURE 11A, mice were infected intra-nasally with 750 PFU of H1N1 A/Puerto Rico/8/34; antibodies were injected at three different time points: 2 hours before infection, 4 hours post-infection and 24 hours post-infection. In FIGURE 11B, mice were infected intra-nasally with 10^4 PFU of H3N2 A/Hong Kong/8/68; antibodies were injected at 4 different time points: 24 hours before infection, 2 hours before infection, 24 hours post-infection and 72 hours post-infection.

[00046] FIGURE 12. Dot blot results with a pan-HA cocktail obtained from mouse hybridomas (FIGURE 12A) and CHO pools (FIGURE 12B). Each dot represents a different strain as indicated in Table 9. Recombinant proteins and plant VLPs were loaded at a final concentration of 2.5 µg while the viruses and standard antigens were loaded at a concentration of 5 µg.

[00047] FIGURE 13. Primary amino acid sequence comparison analysis of IgGs: 11H12 (FIGURE 13A); 10A9 (FIGURE 13B); and 9D1 (FIGURE 13C). Amino acid sequences identified with a Mascot score > 30 are highlighted in bold.

[00048] FIGURE 14. Full protein sequences of recombinant monoclonal antibodies: 10A9 (FIGURE 14A); and 11H12 (FIGURE 14B).

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations

[00049] DT: diphteria toxin; HA: hemagluttinin; KLH: Keyhole limpet hemocyanin; NA: neuraminidase; TT: tetanus toxoid.

Definitions

[00050] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes “one or more than one” antibodies and reference to "the antibody" includes reference to one or more than one antibodies and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

[00051] The term “about” as used herein refers to a margin of + or – 10% of the number indicated. For sake of precision, the term about when used in conjunction with, for example: 90% means 90% +/- 9% i.e. from 81% to 99%. More precisely, the term about refer to + or - 5% of the number indicated, where for example: 90% means 90% +/- 4.5% i.e. from 86.5% to 94.5%. When used in the context of a pH, the term “about” means + / - 0.5 pH unit.

[00052] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as

"includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, un-recited elements or method steps.

[00053] As used herein, the terms "disease" may be used interchangeably or may be different in that the particular disorder, infection or condition may not have a known causative agent (so that etiology has not yet been worked out) and it is therefore not yet recognized as a disease but only as an undesirable condition or syndrome, wherein a more or less specific set of symptoms have been identified by clinicians.

[00054] The term "subject" as used herein refers to an animal, preferably a mammal or a bird, who is the object of administration, treatment, observation or experiment. "Mammal" includes humans and both domestic animals such as laboratory animals and household pets, (e.g. cats, dogs, swine, cattle, sheep, goats, horses, rabbits), and non-domestic animals such as wildlife, fowl, birds and the like. More particularly, the mammal is a rodent. Still, most particularly, the mammal is a human.

[00055] The antibody(ies) described herein can be formulated as pharmaceutical compositions by formulation with additives such as pharmaceutically acceptable excipients, pharmaceutically acceptable carriers, and pharmaceutically acceptable vehicles.

[00056] As used herein, the term "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar unwanted reaction, such as gastric upset, dizziness and the like, when administered to human. Preferably, as used herein, the term "pharmaceutically acceptable" means approved by regulatory agency of the federal or state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[00057] The terms "carrier," "diluent" or "excipient" each refers to a vehicle with which the antibodies of the present invention may be administered. Sterile water or aqueous saline solutions and aqueous dextrose and glycerol solutions may be employed as carrier, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

[00058] If administered as a medicinal preparation, the antibodies can be administered, either as a prophylaxis or treatment, to a patient by a number of methods.

The present compositions may be administered alone or in combination with other pharmaceutical antibodies and can be combined with a physiologically acceptable carrier thereof. The effective amount and method of administration and aim of the present formulation can vary based on the individual subject, the stage of the disease or condition, and other factors apparent to one skilled in the art. In the case of a pharmaceutical formulation, during the course of the treatment, the concentration of the present compositions may be monitored (for example, blood antibody levels may be monitored) to ensure that the desired response is obtained.

Detailed description of particular embodiments

[00059] The present invention relates to hemagglutinin-specific antibodies, fragments thereof, and uses thereof. More specifically, the present invention relates to hemagglutinin-specific antibodies and fragments thereof able to recognize antigen from multiple influenza strains.

[00060] The present invention provides an isolated or purified antibody or fragment thereof, comprising:

a) a light chain comprising a complementarity determining region (CDR) L1 sequence of $QSLLNSX_1X_2QKNX_3$ (SEQ ID NO:1) where X_1=R or D, X_2=N or T, X_3=H or F; a CDR L2 sequence of X_1AS (SEQ ID NO:35) where X_1=W or F; and a CDR L3 sequence of $QQYYX_1X_2X_3X_4T$ (SEQ ID NO:2) where X_1=T or S, X_2=Y or I, X_3=P or no amino acid, X_4=R or L,

and

b) a heavy chain comprising a complementarity determining region (CDR) H1 sequence of $GYX_1X_2TX_3DYY$ (SEQ ID NO:3) where X_1=S or T, X_2=I or F, X_3=S or no amino acid;

a CDR H2 sequence selected from the group consisting of $IGYDGX_1K$ (SEQ ID NO:4) where X_1=S or T and IYPGNGHT (SEQ ID NO:5); and

a CDR H3 sequence selected from the group consisting of TRDRANWDDYFDY (SEQ ID NO:6) and AYDLFNY (SEQ ID NO:7).

[00061] The term “antibody”, also referred to in the art as “immunoglobulin” (Ig), as used herein refers to a protein constructed from paired heavy and light polypeptide chains; various Ig isotypes exist, including IgA, IgD, IgE, IgG, and IgM. When an antibody is correctly folded, each chain folds into a number of distinct globular domains joined by more linear polypeptide sequences. For example, the immunoglobulin light

chain folds into a variable (V_L) and a constant (C_L) domain, while the heavy chain folds into a variable (V_H) and three constant (C_H, C_{H2}, C_{H3}) domains. Interaction of the heavy and light chain variable domains (V_H and V_L) results in the formation of an antigen binding region (Fv). Each domain has a well-established structure familiar to those of skill in the art.

[00062] The light and heavy chain variable regions are responsible for binding the target antigen and can therefore show significant sequence diversity between antibodies. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. The variable region of an antibody contains the antigen-binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The majority of sequence variability occurs in six hypervariable regions, three each per variable heavy (V_H) and light (V_L) chain; the hypervariable regions combine to form the antigen-binding site, and contribute to binding and recognition of an antigenic determinant. The specificity and affinity of an antibody for its antigen is determined by the structure of the hypervariable regions, as well as their size, shape, and chemistry of the surface they present to the antigen. Various schemes exist for identification of the regions of hypervariability, the two most common being those of Kabat and of Chothia and Lesk. Kabat et al (1991) define the "complementarity-determining regions" (CDR) based on sequence variability at the antigen-binding regions of the V_H and V_L domains. Chothia and Lesk (1987) define the "hypervariable loops" (H or L) based on the location of the structural loop regions in the V_H and V_L domains. As these individual schemes define CDR and hypervariable loop regions that are adjacent or overlapping, those of skill in the antibody art often utilize the terms "CDR" and "hypervariable loop" interchangeably, and they may be so used herein. A more recent scheme is the IMGT numbering system (Lefranc et al., 2003), which was developed to facilitate comparison of variable domains. In this system, conserved amino acids (such as Cys23, Trp41, Cys104, Phe/Trp118, and a hydrophobic residue at position 89) always have the same position. Additionally, a standardized delimitation of the framework regions (FR1: positions 1 to 26; FR2: 39 to 55; FR3: 66 to 104; and FR4: 118 to 129) and of the CDR (CDR1: 27 to 38, CDR2: 56 to 65; and CDR3: 105 to 117) is provided.

[00063] The CDR/loops are referred to herein according to the IMGT numbering system for all CDR. The CDR of the antibodies of the present invention are referred to herein as CDR L1, L2, L3 for CDR in the light chain, and CDR H1, H2, H3 for CDR in the heavy chain.

[00064] An "antibody fragment" as referred to herein may include any suitable antigen-binding antibody fragment known in the art. The antibody fragment may be a naturally-occurring antibody fragment, or may be obtained by manipulation of a naturally-occurring antibody or by using recombinant methods. For example, an antibody fragment may be selected from the group consisting of, but is not limited to a Fv, single-chain Fv (scFv; a molecule consisting of V_L and V_H connected with a peptide linker), Fab, $F(ab')_2$, and multivalent presentations of any of these. Antibody fragments such as those herein described may require linker sequences, disulfide bonds, or other type of covalent bond to link different portions of the fragments; those of skill in the art will be familiar with various approaches.

[00065] The terms "antibody" and "antibody fragment" ("fragment thereof") as defined above may be from any selected from the group consisting of source, human, mouse, or other; may be any isotype selected from the group consisting of IgA, IgD, IgE, IgG, and IgM; and may be any type of fragment, including but not limited to Fv, scFv, Fab, and $F(ab')_2$.

[00066] In one non-limiting example, the isolated or purified antibody or fragment described above may comprise a CDR L1 that is selected from the group consisting of QSLLNSRNQKNH (SEQ ID NO:8) and QSLLNSDTQKNF (SEQ ID NO:9).

[00067] In another non-limiting example, the isolated or purified antibody or fragment thereof as previously described may comprise a CDR L2 that is selected from the group consisting of WAS (SEQ ID NO:36) and FAS (SEQ ID NO:37).

[00068] In another non-limiting example, the isolated or purified antibody or fragment thereof as described above may comprise a CDR L3 that is selected from the group consisting of QQYYTYXRT (SEQ ID NO:10) where X is P or no amino acid, and QQYYSIPLT (SEQ ID NO:11).

[00069] In yet another non-limiting example, the isolated or purified antibody or fragment thereof as described above may comprise a CDR H1 is selected from the group consisting of GYSITSDYY (SEQ ID NO:12) and GYTFTDYY (SEQ ID NO:13).

[00070] In a more specific example, the isolated or purified antibody or fragment thereof may be selected from the group consisting of:

a) a light chain comprising CDR L1 of sequence QSLLNSRNQKNH (SEQ ID NO:8), CDR L2 of sequence WAS (SEQ ID NO:36), and CDR L3 of

sequence QQYYTYRT (SEQ ID NO:14); and a heavy chain comprising CDR H1 of sequence GYSITSDYY (SEQ ID NO:12), CDR H2 of sequence IGYDGSK (SEQ ID NO:15), and CDR H3 of sequence TRDRANWDDYFDY (SEQ ID NO:6);

b) a light chain comprising CDR L1 of sequence QSLLNSRNQKNH (SEQ ID NO:8), CDR L2 of sequence WAS (SEQ ID NO:36), and CDR L3 of sequence QQYYTYRT (SEQ ID NO:17); and a heavy chain comprising CDR H1 of sequence GYSITSDYY (SEQ ID NO:12), CDR H2 of sequence IGYDGTK (SEQ ID NO:16), and CDR H3 of sequence TRDRANWDDYFDY (SEQ ID NO:6); and

c) a light chain comprising CDR L1 of sequence QSLLNSDTQKNF (SEQ ID NO:9), CDR L2 of sequence FAS (SEQ ID NO:37), CDRL3 of sequence QQYYSIPLT (SEQ ID NO:11); and a heavy chain comprising CDR H1 of sequence GYTFTDYY (SEQ ID NO:13), CDR H2 of sequence IYPGNGHT (SEQ ID NO:5), and CDR H3 of sequence AYDLFNY (SEQ ID NO:7).

[00071] More specifically, the isolated or purified antibody or fragment thereof may comprise a variable light (VL) domain having a sequence of:

DIVMX_1QSPSSLAX_2SVGX_3KVTMSCKSSQSLLNSX_4X_5QKNX_6LAWYQQKPGQS PKX_7LX_8YX_9ASTX$_{10}$ESGVPDRFX_{11}GX_{12}GSGTDFTLTIX_{13}SVX_{14}AEDLAX_{15}YX_{16}C QQYY$X_{17}X_{18}X_{19}X_{20}$TFGX_{21}GTKLEIK (SEQ ID NO:17) where X_1=S or T, X_2=V or M, X_3=E or O, X_4=R or D, X_5=N or T, X_6=H or F, X_7=L or I, X_8=I or V, X_9=W or F, X_{10}=R or K, X_{11}=S or I, X_{12}=D or S, X_{13}=S or T, X_{14}=K or Q, X_{15}=V or D, X_{16}=Y or F, X_{17}=T or S, X_{18}=Y or I, X_{19}=P or no amino acid, X_{20}=R or L, X_{21}=G or A.

[00072] In a more specific example, the variable light (VL) domain may comprise a sequence selected from the group consisting of:

a) DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQS PKLLIYWASTRESGVPDRFX$_1$GDGSGTDFTLTISSVKAEDLAVYYCQQYYTY RTFGGGTKLEIK (SEQ ID NO:18) where X_1=S or T;

b) DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSDTQKNFLAWYQQKPGQS PKILVYFASTKESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQYYSIPL TFGAGTKLELK (SEQ ID NO:19); and

c) a sequence substantially identical thereto.

[00073] In the isolated or purified antibody or fragment as described above, the variable heavy (VH) domain may comprise a sequence selected from the group consisting of:

a) DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMA YIGYDGX$_1$KNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRAN WDDYFDYWGQGTTLTVSS (SEQ ID NO:20) where X_1=S or T;

b) QIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQRPGQGLEWIGYI YPGNGHTVYNQKFKVRATLTADNPSSTAYLQLNSLTSEDSGVYFCAYDLFN YWGQGTLVTVSA (SEQ ID NO:21); and

c) a sequence substantially identical thereto.

[00074] In specific, non-limiting examples, the isolated or purified antibody or fragment thereof may comprise:

a) a variable light (V_L) domain of sequence:
DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQS PKLLIYWASTRESGVPDRFSGDGSGTDFTLTISSVKAEDLAVYYCQQYYTY RTFGGGTKLEIK (SEQ ID NO:22)
and variable heavy (V_H) domain of sequence:
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIG YDGSKNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDYF DYWGQGTTLTVSS (SEQ ID NO:24);
or

b) a variable light (V_L) domain of sequence:
DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKL LIYWASTRESGVPDRFTGDGSGTDFTLTISSVKAEDLAVYYCQQYYTYXRTFG GGTKLEIK (SEQ ID NO: 23);
and variable heavy (V_H) domain of sequence:
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIG YDGTKNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDYF DYWGQGTTLTVSS (SEQ ID NO:25);
or

c) a variable light (V_L) domain of sequence:
DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSDTQKNFLAWYQQKPGQSPKIL VYFASTKESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQYYSIPLTFGAGT KLELK (SEQ ID NO:19);
and variable heavy (V_H) domain of sequence:

QIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQRPGQGLEWIGYIYPG
NGHTVYNQKFKVRATLTADNPSSTAYLQLNSLTSEDSGVYFCAYDLFNYWGQ
GTLVTVSA (SEQ ID NO:21);

or

d) a sequence substantially identical thereto.

[00075] A substantially identical sequence may comprise one or more conservative amino acid mutations. It is known in the art that one or more conservative amino acid mutations to a reference sequence may yield a mutant peptide with no substantial change in physiological, chemical, physico-chemical or functional properties compared to the reference sequence; in such a case, the reference and mutant sequences would be considered “substantially identical” polypeptides. A conservative amino acid substitution is defined herein as the substitution of an amino acid residue for another amino acid residue with similar chemical properties (e.g. size, charge, or polarity). These conservative amino acid mutations may be made to the framework regions of the antibody or fragment thereof while maintaining the CDR sequences listed above and the overall structure of the antibody or fragment; thus, the specificity and binding of the antibody are maintained.

[00076] In a non-limiting example, a conservative mutation may be an amino acid substitution. Such a conservative amino acid substitution may substitute a basic, neutral, hydrophobic, or acidic amino acid for another of the same group. By the term “basic amino acid" it is meant hydrophilic amino acids having a side chain pK value of greater than 7, which are typically positively charged at physiological pH. Basic amino acids include histidine (His or H), arginine (Arg or R), and lysine (Lys or K). By the term “neutral amino acid” (also “polar amino acid”), it is meant hydrophilic amino acids having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Polar amino acids include serine (Ser or S), threonine (Thr or T), cysteine (Cys or C), tyrosine (Tyr or Y), asparagine (Asn or N), and glutamine (Gln or Q). The term “hydrophobic amino acid” (also “non-polar amino acid”) is meant to include amino acids exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of Eisenberg (1984). Hydrophobic amino acids include proline (Pro or P), isoleucine (Ile or I), phenylalanine (Phe or F), valine (Val or V), leucine (Leu or L), tryptophan (Trp or W), methionine (Met or M), alanine (Ala or A), and glycine (Gly or G). "Acidic amino acid" refers to hydrophilic amino acids having a side

chain pK value of less than 7, which are typically negatively charged at physiological pH. Acidic amino acids include glutamate (Glu or E), and aspartate (Asp or D).

[00077] Sequence identity is used to evaluate the similarity of two sequences; it is determined by calculating the percent of residues that are the same when the two sequences are aligned for maximum correspondence between residue positions. Any known method may be used to calculate sequence identity; for example, computer software is available to calculate sequence identity. Without wishing to be limiting, sequence identity can be calculated by software such as NCBI BLAST2 service maintained by the Swiss Institute of Bioinformatics (and as found at ca.expasy.org/tools/blast/), or any other appropriate software that is known in the art.

[00078] The substantially identical sequences of the present invention may be at least 90% identical; in another example, the substantially identical sequences may be at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical, or any percentage therebetween, at the amino acid level to sequences described herein. Importantly, the substantially identical sequences retain the activity and specificity of the reference sequence. In a non-limiting embodiment, the difference in sequence identity may be due to conservative amino acid mutation(s). In a non-limiting example, the present invention may be directed to an antibody or fragment thereof comprising a sequence at least 95%, 98% or 99% identical to that of the antibodies described herein.

[00079] The antibodies as described herein may comprise the V_L and V_H domains as described above and one or more than one constant regions from mouse IgG2a.

[00080] The present invention further encompasses an antibody or fragment thereof that is chimeric (or chimerized), veneered, or humanized. The antibody or fragment thereof as described herein may be chimeric, in that the antibody or fragment thereof is a combination of protein sequences originating from more than one species. As is known to those of skill in the art, a chimeric antibody is produced by combining genetic material from a nonhuman source (for example but not limited to a mouse) with genetic material from a human. For example, and without wishing to be limiting, human constant domains can be fused to mouse V_H and V_L sequences (see Gonzales et al 2005). Veneering, also referred to in the art as "variable region resurfacing", of antibodies involves replacing solvent-exposed residues in the framework region of the native antibody or fragment thereof with the amino acid residues in their human counterpart (Padlan, 1991; Gonzales et al 2005); thus, buried non-humanized residues,

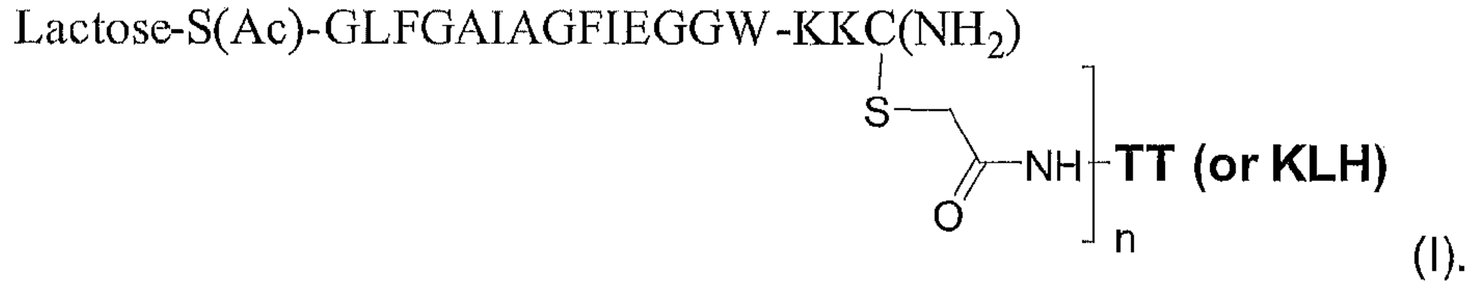
which may be important for CDR conformation, are preserved while the potential for immunological reaction against solvent-exposed regions is minimized. Humanization of an antibody or antibody fragment comprises replacing an amino acid in the sequence with its human counterpart, as found in the human consensus sequence, without loss of antigen-binding ability or specificity; this approach reduces immunogenicity of the antibody or fragment thereof when introduced into human subjects. In this process, one or more than one of the CDR defined herein may be fused or grafted to a human variable region (V_H, or V_L), to other human antibody (IgA, IgD, IgE, IgG, and IgM), to human antibody fragment framework regions (Fv, scFv, Fab), or to human proteins of similar size and nature onto which CDR can be grafted (Nicaise et al, 2004). In such a case, the conformation of said one or more than one hypervariable loop is likely preserved, and the affinity and specificity of the sdAb for its target (i.e., *C. difficile* LTA) is likely minimally affected. As is known by those of skill in the art, it may be necessary to incorporate certain native amino acid residues into the human framework in order to retain binding and specificity. Humanization by CDR grafting is known in the art (for example, see Tsurushita et al, 2005; Jones et al, 1986; Tempest et al, 1991; Riechmann et al, 1988; Queen et al, 1989; all reviewed in Gonzales et al, 2005 – see also references cited therein), and thus persons of skill would be amply familiar with methods of preparing such humanized antibody or fragments thereof.

[00081] The isolated or purified antibody or fragment thereof of the present invention may be a chimeric antibody or fragment thereof may comprise the V_L and V_H domains from mouse and framework regions (constant domains) from human IgG1, more specifically human kappa 1 light chain and human IgG1 heavy chain.

[00082] The antibody or fragment thereof of the present invention specifically binds to the influenza hemagglutinin (HA) protein. The hemagglutinin protein binds sialic acid present on the surface of target host cells and plays a key role in entry of the viral genome into the target cell. Currently, 18 types HA protein are known. The antibody or fragment thereof of the present invention may bind one subtype or multiple subtypes of HA; when binding multiple subtypes of HA, the antibody or fragment thereof may bind different subtypes with varying affinity.

[00083] The present application further provides a novel influenza HA antigen. The HA antigen comprises a peptide originating from the N-terminus of HA2, GLFGAIAGFIEGGW (SEQ ID NO:26) functionalized with O-beta-lactosyl-serine and its N-terminus and thio-Cys at its C-terminus; the C-terminus of the functionalized antigen

is conjugated to Keyhole Limpet Hemocyanin (KLH), which is one of a plurality of carrier molecules that can be used for conjugating to antigenic epitope to provoke or increase the immune response thereof (such as, for example the tetanus toxin (TT)). The carrier protein, such as KLH in this case, may also comprises multiple epitopes conjugated to its surface. A schematic of the antigen is shown in FIGURE 1A and in the following formula (I):



(I).

[00084] The antibody or fragment thereof of the present invention may bind to the novel antigen as described above.

[00085] The antibody or fragment thereof of the present invention may also comprise additional sequences to aid in expression, detection or purification of a recombinant antibody or fragment thereof. Any such sequences or tags known to those of skill in the art may be used. For example, and without wishing to be limiting, the antibody or fragment thereof may comprise a targeting or signal sequence, a detection/purification tag (for example, but not limited to c-Myc, His_5, His_6, or His_8G), or a combination thereof. In another example, the signal peptide may be MVLQTQVFISLLLWISGAYG (SEQ ID NO.27) or MDWTWRILFLVAAATGTHA (SEQ ID NO.28). In a further example, the additional sequence may be a biotin recognition site such as that described by Cronan et al in WO 95/04069 or Voges et al in WO/2004/076670. As is also known to those of skill in the art, linker sequences may be used in conjunction with the additional sequences or tags, or may serve as a detection/purification tag.

[00086] The antibody or fragment thereof of the present invention may also be in a multivalent display format, also referred to herein as multivalent presentation. Multimerization may be achieved by any suitable method of known in the art. For example, and without wishing to be limiting in any manner, multimerization may be achieved using self-assembly molecules such as those described in WO2003/046560, where pentabodies are produced by expressing a fusion protein comprising the antibody or fragment thereof of the present invention and the pentamerization domain of the B-subunit of an AB_5 toxin family (Merritt & Hol, 1995). A multimer may also be formed using the multimerization domains described by Zhu et al. (2010); this form, referred to

herein as a "combody" form, is a fusion of the antibody or fragment of the present invention with a coiled-coil peptide resulting in a multimeric molecule. Other forms of multivalent display are also encompassed by the present invention. For example, and without wishing to be limiting, the antibody or fragment thereof may be presented as a dimer, a trimer, or any other suitable oligomer. This may be achieved by methods known in the art, for example direct linking connection (Nielsen et al, 2000), c-jun/Fos interaction (de Kruif & Logtenberg, 1996), "Knob into holes" interaction (Ridgway et al, 1996).

[00087] Each subunit of the multimers described above may comprise the same or different antibodies or fragments thereof of the present invention, which may have the same or different specificity. Additionally, the multimerization domains may be linked to the antibody or antibody fragment using a linker, as required; such a linker should be of sufficient length and appropriate composition to provide flexible attachment of the two molecules, but should not hamper the antigen-binding properties of the antibody. For example, and without wishing to be limiting in any manner, the antibody or fragments thereof may be presented in a bi-specific antibody.

[00088] The invention also encompasses the antibody or fragment thereof as described above linked to a cargo molecule. The cargo molecule may be any suitable molecule. For example, and without wishing to be limiting in any manner, the cargo molecule may be a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody or fragment thereof (e.g., IgG, scFv, Fab, V_HH, V_H, V_L, etc) a chemical compound, a carbohydrate moiety, DNA-based molecules (anti-sense oligonucleotide, microRNA, siRNA, plasmid), a neutralizing agent, viral vector (adeno-, lenti-, retro-), one or more liposomes or nanocarriers loaded with any of the previously recited types of cargo molecules, or one or more nanoparticle, nanowire, nanotube, or quantum dots. The antibody or fragment thereof may be linked to the cargo molecule using any method known in the art (recombinant technology, chemical conjugation, etc.).

[00089] In one non-limiting example, the cargo molecule may be a detectable label, a radioisotope, a paramagnetic label such as gadolinium or iron oxide, a fluorophore, a fluorescent agent, Near Infra-Red (NIR) fluorochrome or dye (such as Cy5.5), an echogenic microbubble, an affinity label (for example biotin, avidin, etc), a detectable protein-based molecule, nucleotide, quantum dot, nanoparticle, nanowire, or nanotube or any other suitable agent that may be detected by imaging methods. In a

specific, non-limiting example, the anti-hemagglutinin or fragment thereof may be linked to a near infrared fluorescence (NIRF) imaging dye, for example and not wishing to be limiting Cy5.5, Alexa680, Dylight680, or Dylight800.

[00090] In another specific, non-limiting embodiment, the antibody or fragment thereof as described herein is linked to a drug, thus providing an antibody-drug conjugate (ADC). The drug may be any type of drug, for example but not limited to a neutralizing agent. The neutralizing agent may include, but is not limited to anti-microtubule agents (such as taxanes, maytansines and auristatins), DNA damaging agents (such as calicheamicin and duocarmydin), RNA polymerase inhibitors (such as alpha-amantin), and other potent neutralizing drugs (such as anthracyclines). As is known to those of skill in the art, the antibody-drug conjugate allows for targeted delivery of a drug, thus limiting systemic exposure. In this construct, the antibody or fragment thereof as described herein binds to the extracellular domain of hemagglutinin; the drug linked to the antibody or fragment thereof is thus internalized. Upon internalization DM1 is released within the target cells upon degradation of the human hemagglutininantibody-DM1 complex in lysosomes. Depending on the intracellular concentration of DM1 accumulated in cancer cells, rapid apoptosis occurs.

[00091] The cargo molecule as described herein may be linked, also referred to herein as “conjugated”, to the antibody or fragment thereof by any suitable method known in the art. For example, and without wishing to be limiting, the cargo molecule may be linked to the peptide by a covalent bond or ionic interaction. The linkage may be achieved through a chemical cross-linking reaction, or through fusion using recombinant DNA methodology combined with any peptide expression system, such as bacteria, yeast or mammalian cell-based systems. When conjugating the cargo molecule to the antibody or fragment thereof, a suitable linker may be used. Methods for linking an antibody or fragment thereof to a cargo molecule such as a therapeutic or detectable agent would be well-known to a person of skill in the art.

[00092] The present invention also encompasses nucleic acid sequences encoding the molecules as described herein. Given the degeneracy of the genetic code, a number of nucleotide sequences would have the effect of encoding the desired polypeptide, as would be readily understood by a skilled artisan. The nucleic acid sequence may be codon-optimized for expression in various micro-organisms. The present invention also encompasses vectors comprising the nucleic acids as just

described. Furthermore, the invention encompasses cells comprising the nucleic acid and/or vector as described.

[00093] The present invention further encompasses the isolated or purified antibody or fragments thereof immobilized onto a surface using various methodologies; for example, and without wishing to be limiting, the antibody or fragment may be linked or coupled to the surface via His-tag coupling, biotin binding, covalent binding, adsorption, and the like. Immobilization of the antibody or fragment thereof of the present invention may be useful in various applications for capturing, purifying or isolating proteins. The solid surface may be any suitable surface, for example, but not limited to the well surface of a microtiter plate, channels of surface plasmon resonance (SPR) sensorchips, membranes, beads (such as magnetic-based or sepharose-based beads or other chromatography resin), glass, plastic, stainless steel, a film, biosensors (such as those used in Biolayer Interferometry), or any other useful surface such as nanoparticles, nanowires and cantilever surfaces. A purified antibody or fragment thereof immobilized onto a surface may be used in a variety of methods, including diagnostic methods.

[00094] Thus, the present invention further provides an *in vitro* method of detecting influenza HA, comprising contacting a tissue sample with one or more than one isolated or purified antibody or fragment thereof of the present invention linked to a detectable agent. The HA-antibody complex can then be detected using detection and/or imaging technologies known in the art. The tissue sample in the method as just described may be any suitable tissue sample, for example but not limited to a serum sample, a vascular tissue sample such as lung tissue sample, neuroepithelium tissue sample, nasal aspirates, nasopharyngeal aspirates or swabs, nasal washes or swabs, throat swabs, endotracheal aspirates, bronchoalveolar lavage, or other tissue from the respiratory system; the tissue sample may be from a human or animal subject. The step of contacting is done under suitable conditions, known to those skilled in the art, for formation of a complex between the antibody or fragment thereof and HA. The step of detecting may be accomplished by any suitable method known in the art, for example, but not limited to optical imaging, immunohistochemistry, molecular diagnostic imaging, ELISA, or other suitable method. For example, and without wishing to be limiting in any manner, the isolated or purified antibody or fragment thereof linked to a detectable agent may be used in immunoassays (IA) including, but not limited to enzyme IA (EIA), ELISA, “rapid antigen capture”, “rapid chromatographic IA”, and “rapid EIA”. (For example, see Planche et al, 2008; Sloan et al, 2008; Rüssmann et al, 2007; Musher et al, 2007;

Turgeon et al, 2003; Fenner et al, 2008). Other immunoassay techniques in which the antibodies of the present invention may be used include Western blot, dot blot, and slot blot analysis. In a specific, non-limiting embodiment, the *in vitro* method is for detection of HA in nasal wash or swab. The one or more than one isolated or purified antibody or fragment thereof of the present invention could be used for detection in a Rapid Influenza Diagnosis Test (RIDT), also known as Point-of-Care Test (POCT) or dipsticks; these assays are used in the clinic to provide rapid diagnosis (less than 15 minutes) of patients with flu-like symptoms. The presence of viral antigens (HA) in a specimen (nasal wash or swab) is analysed in a lateral flow immunoassay and result in a colorimetric change when detected by the anti-HA antibodies.

[00095] The present invention also provides a method of preventing or treating influenza in a subject. The method comprises administering one or more than one isolated or purified antibody or fragment thereof as described herein to the subject. The one or more than one isolated or purified antibody or fragment thereof may be linked to one or more than one cargo molecule, as described herein. The subject may be a human or animal subject. The administration may be by any suitable method, for example parenteral administration, including but not limited to intravenous (iv), subcutaneous (sc), and intramuscular (im) administration.

[00096] The present invention also encompasses a composition comprising one or more than one isolated or purified antibody or fragment thereof as described herein. The composition may comprise a single antibody or fragment as described above, or may be a mixture of antibodies or fragments. Furthermore, in a composition comprising a mixture of antibodies or fragments of the present invention, the antibodies may have the same specificity, or may differ in their specificities; for example, and without wishing to be limiting in any manner, the composition may comprise antibodies or fragments thereof specific to hemagglutinin (same or different epitope). The composition may also comprise one or more than one antibody or fragments of the present invention linked to one or more than one cargo molecule.

[00097] The composition may also comprise a pharmaceutically acceptable diluent, excipient, or carrier. The diluent, excipient, or carrier may be any suitable diluent, excipient, or carrier known in the art, and must be compatible with other ingredients in the composition, with the method of delivery of the composition, and is not deleterious to the recipient of the composition. The composition may be in any suitable form; for example, the composition may be provided in suspension form, powder form

(for example, but limited to lyophilised or encapsulated), capsule or tablet form. For example, and without wishing to be limiting, when the composition is provided in suspension form, the carrier may comprise water, saline, a suitable buffer, or additives to improve solubility and/or stability; reconstitution to produce the suspension is effected in a buffer at a suitable pH to ensure the viability of the antibody or fragment thereof. Dry powders may also include additives to improve stability and/or carriers to increase bulk/volume; for example, and without wishing to be limiting, the dry powder composition may comprise sucrose or trehalose. In a specific, non-limiting example, the composition may be so formulated as to deliver the antibody or fragment thereof to the gastrointestinal tract of the subject. Thus, the composition may comprise encapsulation, time-release, or other suitable technologies for delivery of the antibody or fragment thereof. It would be within the competency of a person of skill in the art to prepare suitable compositions comprising the present antibodies or fragments thereof.

[00098] The present invention further provides an isolated or purified antibody or fragment thereof as described herein for use in preventing or treating influenza in a subject.

[00099] The present invention also encompasses a cocktail comprising both mAbs 10A9 (SEQ ID NO. 35) and 11H12 (SEQ ID NO.36) for the detection, prevention or treatment of influenza.

[000100] The present invention further provides a kit for the identification of influenza in a sample, the kit comprising a support (such as nitrocellulose) and one or more than one isolated or purified antibody or fragment thereof as described herein. The one or more than one isolated or purified antibody or fragment thereof may be immobilized onto the nitrocellulose. The nitrocellulose support may be placed inside a plastic housing (also referred to herein as cassette), or may be adhered to a paper support (also referred to herein as card). The kit may also contain a swab for collecting the sample.

[000101] Particularly, the sample is a biological sample such as for example, blood, serum, nasal wash, nasal swab, saliva or sputum.

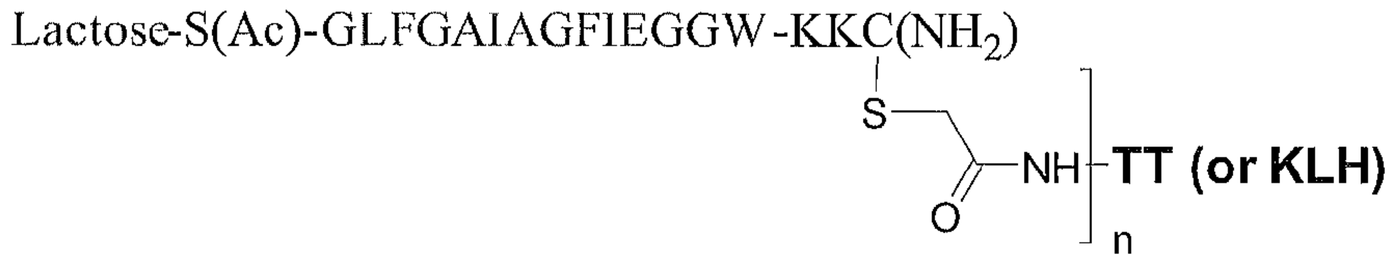
[000102] The present invention also provides a kit for the prevention or treatment of influenza in a subject, the kit comprising a container; and an isolated or purified antibody or fragment thereof contained therein. The kit may also contain a syringe for injecting the antibodies or fragments thereof to the subject.

[000103] The present invention will be further illustrated in the following examples. However, it is to be understood that these examples are for illustrative purposes only and should not be used to limit the scope of the present invention in any manner.

Example 1: Production and purification of antigen

[000104] A fusion peptide was prepared to use for immunization of mice. The fusion protein comprised a conserved peptide sequence from the N-terminal region of HA2 (GLFGAIAGFIEGGW; SEQ ID NO:26), functional groups on the peptide sequence, and keyhole limpet hemocyanin (KLH).

[000105] *Conjugate structure.* The peptide conjugate shown in FIGURE 1A, was designed by using a conserved peptide sequence at the N-terminus of HA2 that was previously identified: GLFGAIAGFIEGGW (SEQ ID NO:26). The peptide epitope was functionalized with lactose and conjugated to Keyhole Limpet Hemocyanin (KLH) to obtain a conjugate of formula (I):



(I).

[000106] The KLH portion of the conjugate also comprised multiple epitopes conjugated to its surface.

[000107] *Peptide conjugate synthesis.* The peptide conjugate was prepared according to FIGURE 1B via a thio-ether bond between terminal Cys of the (glyco)peptide antigen and bromoacetyl KLH.

[000108] *Bromoacetylation of KLH.* Typically, 20 mg of KLH (Sigma-Aldrich H7017) was solubilized in 2 mL of deionized water at room temperature, and buffer-exchanged to 10 x PBS by an Amicon Ultra centrifugal filter (MWC 30K). To the above solution of KLH in 10 X PBS (2 mL) was added 9 mg of bromoacetic acid N-hydroxysuccinimide ester in DMSO (0.18 mL) and incubated overnight at 4°C. The product was purified by a G-25 column (50 x 1.6 cm) with PBS as eluent and bromoacetyl KLH obtained was stored in PBS buffer. Similarly, bromoacetyl BSA was also made in parallel and MALDI indicated 9-10 bromoacetyl groups per BSA, we obtained similar ratio of bromoacetyl group present in KLH.

[000109] *Conjugation*: (glyco)peptide antigen with terminal Cys and bromoacetyl KLH are dissolved under the conditions of 0.1M phosphate buffer with 5 mM EDTA-0.01% sodium azide at pH 8.0-8.5 overnight at room temperature. As a reference to estimate the peptide antigen on KLH, bromoacetyl BSA was also coupled with the peptide to give a conjugate with a ratio of peptide:BSA 6-7:1. The peptide antigen coupled to KLH carrier protein was found to have a similar w/w ratio as BSA.

[000110] *Purification*. Typically, a (glyco)peptide antigen with terminal Cys was mixed with equivalent amount of biotin-maleimide (B1267, Sigma), in DMSO at room temperature and the solution was kept for 5 hours, which was diluted with water and lyophilized to give product. The product was characterized by MALDI and no further purification was needed for ELISA.

Example 2: Generation of anti-influenza antibodies

[000111] To produce antibodies that target the influenza virus, mice were immunized with the peptide conjugate obtained in Example 1. Hybridomas (monoclonal antibodies) were also prepared and evaluated by ELISA.

[000112] *Immunizations*. 6-week old A/J mice were bled (pre-immune serum) and immunized i.p. and s.c. with 100 µg of antigen (Example 1) in Titermax adjuvant. Three weeks later, a second injection of 100 µg of antigen in Titermax adjuvant was performed and mice were bled 7-10 days later. The serum titer was measured by ELISA. Two months later, a final i.p. booster injection using 100 µg of antigen was performed 4 days prior to fusion experiment.

[000113] *Fusion of the harvested spleen cells*. All manipulations were carried out under sterile conditions. Spleen cells were harvested from immunized mice in IMDM (Hy-Clone) and fused to NS0 myeloma cell line using PEG fusion protocol. To this end, spleen cells and myeloma cells were washed in IMDM, counted in RBC lysing buffer (Sigma) and mixed together at a 5:1 ratio. Pelleted cells were fused together by adding 1 ml of a 50% solution of PEG 4000 (EMD-Millipore) in PBS preheated at 37°C drop-wise over one minute, and incubated at 37°C for an additional 90 sec. The reaction was stopped by addition of 30 ml of IMDM at 22°C over 2 min. After a 10 min incubation, freshly fused cells were spun at 233 x g for 10 min. Cells were washed once in IMDM supplemented with 10% heat inactivated FBS (Sigma), and suspended at a concentration of $2x10^5$ input myeloma cells per ml in HAT selection medium (IMDM containing 20% heat inactivated FBS, penicillin-streptomycin (Sigma), 1 ng/ml mouse IL-

6 (Biosource), HAT media supplement (Sigma) and L-glutamine) and incubated at 37°C, 5% CO_2. The next day, hybridoma cells were washed and suspended at a concentration of $2x10^5$ input myeloma cells per ml in semi-solid medium D (StemCell) supplemented with 5% heat inactivated FBS, 1 ng/ml mouse IL-6 and 10 µg/ml FITC- Fab'2 Goat anti-mouse IgG (H+L) (Jackson). The cell mixture was plated in OmniTrays (Nunc) and further incubated for 6-7 days at 37°C, 5% CO_2. Secretor clones were then transferred using a mammalian cell clone picker (ClonepixFL, Molecular Devices) into sterile 96-well plates (Costar) containing 200 µl of IMDM supplemented with 20% heat inactivated FBS, penicillin-streptomycin (Sigma), 1 ng/ml mouse IL-6 (Biosource), HT media supplement (Sigma) and L-glutamine and incubated for 2-3 days at 37°C, 5% CO_2.

[000114] *Hybridoma selection*. Hybridoma supernatant were screened by ELISA to detect specific binders. To this end, 96-well half-area plates (Costar) were coated with 25 µl of neutravidinat 10 µg/ml in 50mM carbonate buffer at pH9.8 and incubated 2 hours at room temperature. Microplates were washed 3 times with PBS, blocked with PBS-BSA 1%, washed once with PBS and 25 µl of biotinylated peptide at 5 µg/ml was added and incubated overnight at 4°C. Microplates were washed 4 times with PBS-Tween 20 0.05% and 25 µl of hybridoma supernatant were added and incubated at 37°C, 5% CO_2 for 2 hours. Plates were washed 4 times with PBS-Tween 20 0,05% and incubated for 1h at 37°C, 5% CO_2 with 25 µl of secondary antibody alkaline phosphatase conjugated $F(ab)'_2$ goat anti-mouse IgG (Fcγ fragment specific) (Jackson Immunoresearch) diluted 1/000 in blocking buffer. After 4 washes with PBS-Tween 20 0,05%, 25 µl of a 1 mg/ml pNPP substrate solution was added and further incubated for one hour at 37°C. OD_{405nm} measurements were taken using a microplate reader (Spectramax 340 PC, Molecular Devices).

[000115] From fusions of mouse spleen cells, anti-influenza mAb-producing hybridomas were identified, from which conditioned medium (CM) was collected and evaluated for binding to the peptide (Example 1) by ELISA. Results for the selected clones are shown in Table 1.

Table 1. Evaluation of the CM collected from mAb-producing hybridomas by ELISA.

Clone	Species	Isotype	ELISA on peptide
F211-11H12	mouse	IgG2A,κ	+++
F211-9D1	mouse	IgG2A,κ	+++
F211-10A9	mouse	IgG2A,κ	+++

[000116] All mAbs were then purified via Protein A spin column, dialyzed twice against PBS and concentrated using an Amicon filter (cut-off MW 30,000). The final concentration of the antibody solutions was determined by nano-drop (280 nm). Clones 11H12, 9D1 and 10A9 were subsequently sequenced using art-known methods.

[000117] Sequences are as follows:

11H12 light chain:
DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKLLIYWA STRESGVPDRFTGDGSGTDFTLTISSVKAEDLAVYYCQQYYTYRTFGGGTKLEIK (SEQ ID NO:23);

11H12 heavy chain:
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIGYDGT KNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDYFDYWGQGTT LTVSS (SEQ ID NO:25).

10A9 light chain:
DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSDTQKNFLAWYQQKPGQSPKILVYFAS TKESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQYYSIPLTFGAGTKLELK (SEQ ID NO:19);

10A9 heavy chain:
QIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQRPGQGLEWIGYIYPGNGHT VYNQKFKVRATLTADNPSSTAYLQLNSLTSEDSGVYFCAYDLFNYWGQGTLVTVSA (SEQ ID NO:21).

9D1 light chain:
DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKLLIYWA STRESGVPDRFSGDGSGTDFTLTISSVKAEDLAVYYCQQYYTYRTFGGGTKLEIK (SEQ ID NO:22);

9D1 heavy chain:
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIGYDGS KNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDYFDYWGQGTT LTVSS (SEQ ID NO:24).

[000118] FIGURES 2 show the sequence alignments of the variable domains of the 11H12, 9D1 and 10A9 antibodies.

Example 3: Biophysical characterization of the anti-HA mAb

[000119] The anti-HA mAb of Example 2 were characterized using Differential Scanning Calorimetry (DSC) and surface plasmon resonance (SPR). MAb resistance to freeze-thaw cycles were also evaluated.

[000120] *Thermal stability of the mAb measured by DSC*: The mAb were analysed by DSC to determine at which temperature unfolding of the mAb is induced. Each purified mAb was diluted to 0.4 mg/mL in PBS, and a total of 400 uL was used for DSC analysis with a VP-Capillary DSC (Malvern Inc.). At the start of each DSC run, 5 buffer blank injections are performed to stabilize the baseline and a buffer injection precede each mAb injection for referencing. Each sample was scanned from 20 to 100°C at a 60°C /hr rate, with low feedback, 8 sec filter, 5 min preTstat, and 70 psi nitrogen pressure. The mAb thermograms were referenced and analyzed using Origin 7 software. The melting points of the mAb were found to be between 71.28 and 72.90°C as reported in Table 2. Thermograms are also shown in FIGURE 3.

Table 2: Melting points (TM) measured by DSC for each of the 3 mAb

Sample	**Tm (melting point in °C)**
F211-11H12	72.68
F211-10A9	71.28
F211-9D1	72.90

[000121] *Surface Plasmon Resonance (SPR).* The binding of the peptide of Example 1 to captured antibody (9D1, 10A9 or 11H12) was determined by SPR using a BIACORE T200 (GE Healthcare) using PBS containing 0.05% Tween 20 (Teknova Inc.) and 3.4 mM EDTA as a running buffer. An anti-mouse-Fc surface was immobilized to approximately 2200 RUs using the Immobilization Wizard within the T200 control software set to a 2000 RU target. Standard amine coupling of 20 ug/mL anti-mouse Fc solution in 10 mM NaOAc pH 4.5 was used. For the binding assay, approximately 350 RUs of antibody to be tested (9D1, 10A9 or 11H12) was captured onto a sheep anti-mouse Fc antibody surface by injecting 20 ug/mL solution for 300 seconds. This was followed by single cycle kinetics injection of peptide using a 2-fold dilution series with a top-nominal concentration of 250 nM for antibody 9D1 and 11H2, and 15 nM for antibody 10A9, or PBST running buffer only for referencing. 180 second injections of each peptide or buffer blank were used at a flow rate of 100 uL/min and with a 2700

second dissociation. Surfaces were regenerated with 10mM glycine pH 1.5 with a contact time of 120 seconds. Sensorgrams were double referenced and data were analyzed within Biacore T200 evaluation software v3.0 (GE Healthcare). Results of binding affinity for each mAb are shown in Table 3.

Table 3: Binding affinity measured by surface plasmon resonance for each of the mAb.

mAb	**KD (M)**
F211-9D1-2	3.93×10^{-10}
F211-10A9-2	3.38×10^{-10}
F211-11H12-3	4.65×10^{-10}

[000122] *Resistance to freeze-thaw cycles measured by ELISA.* The binding affinity of the three mAb following 1, 2 and 3 freeze-thaw cycles was assessed by ELISA. The freeze-thaw cycles were performed by freezing the mAb at -80°C and thawing at room temperature 1 to 3 times; the mAb were kept at 4°C after the last thaw. Plates were coated with 1µg/ml peptide-conjugate (Example 1) overnight at 4°C. After washing, the mAb were serially diluted and added to the plate in concentrations ranging from 2 to 480ng/ml. AP-conjugated secondary antibodies and p-nitrophenyl phosphate tablets were used for detection. Results shown in FIGURE 4 indicate that 1 to 3 freeze-thaw cycles have no detrimental effect on the binding affinity to the peptide-conjugate. KD values are summarized in Table 4.

Table 4: KD values obtained after 1 to 3 freeze-thaw cycles with the 4 mAb

	Cycle 1	Cycle 2	Cycle 3
F211-8C4-2	3.25×10^{-8}	2.28×10^{-8}	2.50×10^{-8}
F211-9D1-2	3.57×10^{-8}	3.28×10^{-8}	2.81×10^{-8}
F211-10A9-2	5.62×10^{-8}	3.79×10^{-8}	3.38×10^{-8}
F211-11H12-3	2.98×10^{-8}	2.67×10^{-8}	2.22×10^{-8}

Example 4: Immunoreactivity of anti-influenza mAb

[000123] The immunoreactivity of the anti-influenza monoclonal antibodies obtained in Example 2 was assessed by Western blot using recombinant HA proteins and viruses. Dot blot assays to determine reactivity of the mAb with multiple strains were also performed.

Western blot using recombinant protein. Four recombinant HA (rHA) proteins from influenza strains were tested:

- H5N1/A/Indonesia/05/2005 (Protein Sciences)
- H1N1 A/Puerto Rico/08/1934 (Protein Sciences)
- B/Brisbane/60/2008 (Protein Sciences)
- H7N7/A/Netherlands/219/2003 (Protein Sciences)

[000124] The rHA were used to assess the specificity of the monoclonal antibodies of Example 2. The rHA were heat-denatured at 95°C for 10min, resolved on a 4-15% gel and transferred to a nitrocellulose membrane. The membranes were blocked in 5% milk and probed overnight at 4°C with purified primary antibodies (11H12; 10A9, 9D1; anti-GFP negative control) at 2 μg/mL or hybridoma supernatant diluted 1 in 5. Membranes were washed and incubated with infrared-conjugated secondary antibodies and scanned using an Odyssey scanner (LICOR Biosciences, Lincoln, NE). Results of the Western blot analysis are shown in FIGURES 5. 11H12 and 9D1 recognized H5N1/A/Indonesia/05/200, H1N1 A/Puerto Rico/08/1934, and B/Brisbane/60/2008. Only the 10A9 mAb recognized the rHA from H7N7/A/Netherlands/219/2003; however, it did not recognize any other rHA. Identical results were obtained from the hybridoma supernatant and purified mAb for each clone, indicating that the purification process did not affect the integrity of the antibodies.

The Western blot analysis was repeated and expanded using the following rHA proteins:

- H1N1/A/California/06/2008 (Immune Technology);
- H3N2/A/Brisbane/10/2007 (BEI Resources);
- H5N1/A/Indonesia/05/2005 (Protein Sciences);
- H5N1/A/Vietnam/1203/2004 (Protein Sciences);
- H7N7/A/Netherlands/219/2003 (Protein Sciences);
- H9N2/A/HongKong/1073/1999 (Protein Sciences); or
- B/Brisbane/60/2008 (Protein Sciences).

[000125] In this assay, 300ng rHA were heat-denatured at 95°C for 10min in sample buffer (20% SDS), loaded on a 4-15% gel, resolved by SDS-PAGE, and transferred on a nitrocellulose membrane. The membranes were blocked in 5% non-fat milk and further incubated with 2μg/ml primary antibodies overnight at 4°C. The membranes were probed with either the purified mAb (11H12; 10A9; anti-GFP) or a mixture of 11H12 and 10A9 antibodies. Membranes were washed and incubated with

infrared-conjugated secondary antibodies at a dilution of 1:15000, and scanned using an Odyssey scanner (LICOR Biosciences, Lincoln, NE). Results are shown in FIGURE 6 and Table 5.

Table 5. Reactivity of antibodies with rHA proteins. "Lane" refers to the lane on the Western blot shown in FIGURE 6. "+++" indicates that the strain was highly detected by the specified antibody, "++" indicates that the strain was well detected by the specified antibody, "+" indicates that the strain was weakly detected by the specified antibody, while "-" indicates that the strain was not detected by the antibody.

Lane	rHA	11H12	10A9	Both mAb	Anti-GFP
1	H1N1 A/California/06/2008	+	++	+++	-
2	H3N2/A/Brisbane/10/2007	-	++	+++	-
3	H5N1 A/Indonesia/05/2005	+++	+	+++	-
4	H5N1/A/Vietnam/1203/2004	+++	+	+++	-
5	H7N7/A/Netherlands/219/2003	-	+++	+++	-
6	H9N2/A/Hong Kong/1073/1999	+	-	++	-
7	B/Brisbane/60/2008	+++	-	+++	-

[000126] In agreement with the previous Western blot analysis, only the 10A9 mAb recognized the H7N7/A/Netherlands and H3N2/A/Brisbane. 11H12 consistently recognized all other rHA. Use of a cocktail containing both mAb resulted in the detection of all 7 rHA demonstrating that there is no negative interactions (competition) when the mAb are combined. Thus, the antibodies can be used together in a cocktail.

[000127] *Western blot using viruses.* Thirty strains of influenza virus (see Table 6) were produced in eggs or mammalian cells using art-known methods (Chun et al., 2008). Samples were heat-denatured at 95°C for 10min, loaded and electrophoresed on a 4-15% gel then transferred to a nitrocellulose membrane. The membranes were blocked in 5% milk and probed with primary antibodies overnight at 4°C at a concentration of 2µg/ml. Membranes were next incubated with HRP-conjugated secondary antibodies and signal was revealed by chemiluminescence. Representative results with strains belonging to 13 different HA subtypes are shown in FIGURE 7. Results obtained from 40 strains produced using different platforms tested are summarized in Table 6. All of the influenza virus HA subtypes were recognized by at least one of mAb 11H12 or 10A9 demonstrating the universality of the antibodies. In addition, the mAb are able to detect HA from viruses produced in eggs as well as mammalian cells (Table 6).

Table 6 Reactivity of antibodies with 40 different influenza strains. "Yes" indicates that the strain was detected by the specified antibody, while "No" indicates that it was not.

Strain	Type of Ag	Production platform	11H12	10A9
H1N1 A/Puerto Rico/8/1934	rHA	Insect cells	Yes	No
	Virus	Eggs	Yes	Yes
	Virus	MDCK cells	Yes	Yes
	Virus	HEK293 cells	Yes	Yes
	VLP	HEK293 cells	Yes	Yes
H1N1 A/California/07/2009	rHA	Insect cells	Yes	Yes
	Virus	Egg	Yes	Yes (low signal)
	Virus	MDCK	Yes	
	Virus	HEK293	Yes	Yes (low signal)
	Virus	Avian	Yes	
	VLP	Plant	Yes	Yes (low signal)
				No
				Yes
H1N1 A/Wilson Smith/33	Virus	HEK293 cells	Yes	No
H1N1 Avian like Swine	Virus	MDCK cells	Yes	Yes
H1N2 Reassortant human like A/Scotland/410440/94	Virus	MDCK cells	Yes	Yes
H2N2 A/Singapore/1/57	Virus	Eggs	Yes	Yes
H3N1 A/Victoria/361/2011	VLP	Plant	No	Yes
H3N2 A/Wisconsin/67/2005 [2]	rHA	Insect cells	Yes	Yes
H3N2 A/Aichi/2/1968 [2]	Virus	HEK293 cells	Yes	Yes
H3N2 A/Hong Kong/8/1968 [2]	Virus	HEK293 cells	Yes	Yes
H3N2 A/Brisbane/10/2007	rHA	Insect cells	No	Yes
H3N2 A/New York/55/01	Virus	Eggs	Yes	Yes
H3N2 A/Texas/50/2012	Virus	HEK293	Yes	Yes (low signal)
H3N2 A/Panama/2007/99	Virus	Egg	Yes	Yes
H4N6 A/Duck/Czechoslovakia/56	Virus	Eggs	No	Yes
H5N1 A/Vietnam/1203/2004	rHA	Insect cells	Yes	No

Strain	Type of Ag	Production platform	11H12	10A9
H5N1 A/Indonesia/5/2005	rHA VLP	Insect cells Plant	Yes Yes	No Yes
H5N2 A/Finch/England	Virus	MDCK cells	Yes	Yes
H5N9 A/Turkey/Wisconsin/68	Virus	Eggs	Yes	Yes
H6N2 A/Turkey/Massachusetts/3740/65	Virus	Eggs	Yes	Yes
H7N7 A/Netherlands/219/2003	rHA	Insect cells	No	Yes
H7N7 A/Equine/Prague/1/56	Virus	Eggs	Yes	Yes
H7N9 A/Anhui/1/2013	rHA	Human cells	No	Yes
H7N9 A/Shanghai/2/2013	rHA	Human cells	No	Yes
H7N9 A/Pigeon/Shanghai/S1069/2013	rHA	Human cells	No	Yes
H7N1 A/Turkey/Italy/977/1999	Virus	MDCK cells	Yes	Yes
H7N3/A/Turkey/Oregon/71	Virus	Eggs	No	Yes
H8N4 A/Turkey/Ontario/6118/68	Virus	Eggs	Yes	Yes
H9N2 A/Hong Kong/1073/1999	rHA	Insect cells	Yes	No
H9N2 A/Turkey/Wisconsin/1/66	Virus	Eggs	Yes	Yes
H10N8 A/Quail/Italy/1117/65	Virus	Eggs	Yes	Yes
H11N6 A/Duck/England/56	Virus	Eggs	Yes	Yes
H12N6 A/Duck/Wisconsin/480/79	Virus	Eggs	Yes	Yes
H12N5 A/Duck/Alberta/60/76	Virus	MDCK cells	Yes	Yes
H13N6 A/Gull/Maryland/704/77	Virus	Eggs	Yes	Yes
B/Malaysia/2506/2004	Virus	Eggs	Yes	No
B/Brisbane/60/2008	rHA	Insect cells	Yes	No
B/Lee/40	Virus	HEK293 cells	Yes	No
B/Massachusetts/2/2012	Virus Virus	MDCK HEK293	Yes Yes	Yes (low signal) Yes (low signal)
B/Florida/04/2006	Virus Virus	MDCK cells Egg	Yes Yes	Yes Yes

[000128] *Dot blot using rHA and viruses.* Detection of 19 strains belonging to 6 different HA subtypes was also assessed by dot blot. Representative results are shown in FIGURES 8A-C. Labels at the bottom right of each dot correspond to the strain listed in Table 7. As opposed to Western blot analysis where samples are fully denatured with SDS, samples detected by dot blot are mildly denatured with 4M urea for 30min according to Li et al, 2010. The samples are then placed in a dot blot apparatus and fixed onto a nitrocellulose membrane by applying vacuum. The membranes were blocked in 5% milk and probed overnight at 4°C with purified primary antibodies (11H12; 10A9, 9D1) at 6 μg/mL. Membranes were washed and incubated with infrared-conjugated secondary antibodies and scanned using an Odyssey scanner (LICOR Biosciences, Lincoln, NE).

Table 7: List of the strains tested by dot blot (FIGURE 8) with mAb 9D1, 10A9 and 11H12, and signal intensity obtained with each mAb (arbitrary fluorescent units)

Label (FIG. 8)	**Type of Antigen**	**Production platform/ Company**	**Strain full name**	**9D1**	**10A9**	**11H12**
1	rHA	Prot. Sc.[1]	H1N1 A/puerto rico/8/34	21363	2522	20304
2	rHA	Imm. Techn.[2]	H1N1 A/California/06/2008	5778	2782	5119
3	rHA	Sino Biol.[3]	H3N2 A/Aichi/2/1968	842	18095	995
4	rHA	Prot. Sc.[1]	H3N2 A/Wisconsin67/05	70	791	176
5	rHA	Imm. Techn.[2]	H3N2 A/Hong Kong/8/68	651	5668	464
6	rHA	BEI Res.[4]	H3N2/A/Brisbane/10/2007	362	11515	280
7	rHA	Prot. Sc.[1]	H5N1 A/Indonesia/05/2005	10080	165	22168
8	rHA	Prot. Sc.[1]	H5N1/A/Vietnam/1203/2004	46752	8262	45928
9	rHA	Prot. Sc.[1]	H7N7/A/Netherlands/219/2003	425	42049	488
11	rHA	Prot. Sc.[1]	B/Brisbane/60/2008	41083	25825	38483
10	rHA	Prot. Sc.[1]	H9N2/A/HongKong/1073/1999	3759	2969	3573
12	Inactivated virus	Egg/NIBSC[5]	H1N1 A/California/06/2008	17370	227	14413
13	Inactivated virus	Egg/NIBSC[5]	H3N2/A/Texas	238	412	201
14	Inactivated	Egg/NIBSC[5]	B/Massachusetts	10499	240	9927

	virus					
15	Negative ctrl	NRC[6]	Buffer only	93	43	91
16	Negative ctrl	NRC[6]	Non-related protein	47	34	38
17	virus	HEK293/NRC[6]	H1N1 A/puerto rico/8/34	7808	7451	6537
18	virus	HEK293/NRC[6]	H1N1 A/Wilson Smith/33	6006	343	5057
19	virus	HEK293/NRC[6]	H3N2 A/Hong Kong/8/68	108	319	201
20	virus	HEK293/NRC[6]	H3N2 A/Aichi/2/1968	231	833	341
21	virus	HEK293/NRC[6]	B/Lee	7195	1054	6423
22	VLP	Plant/MedG[7]	H3N1/A/Victoria/361/2011	4292	34000	3695
23	VLP	Plant/ MedG[7]	H5N1 A/Indonesia/05/2005	46669	28633	43052
24	VLP	Plant/ MedG[7]	H1N1 A/California/06/2008	55423	55141	54122

[1]Prot. Sc: Protein Sciences; [2]Imm. Techn: Immune Technology; [3]Sino Biol.: Sino Biologicals; [4] BEI Ress.: BEI Resources; [5]NIBSC: The National Institute for Biological Standards and Control. [6]NRC: National Research Council Canada; [7]MedG: Medicago Inc.

[000129] The specificity of the antibodies is illustrated by the lack of signal with the negative controls (dots number 15 and 16). Overall, mAb 10A9 has a higher binding affinity for strains of subtype H3 (dots 3-6) and H7 (dot 9) whereas mAb 11H12 and 9D1 have a higher binding affinity for strains of subtype H1 (dots 1-2, 12 and 16) and H5 (dots 7-8).

Example 5: Quantification of HA by dot-blot and ELISA

[000130] The anti-HA monoclonal antibodies obtained in Example 2 were used to quantify viruses by dot blot, and rHA by ELISA.

[000131] *Dot blot for quantification.* Following the same denaturation and probing procedure described in Example 4, the dot blot technique was used to quantify H1N1 A/Puerto Rico/8/34 viruses produced in HEK293 cells (FIGURE 9A). A standard (virus previously quantified by SRID) was used to establish a standard curve ranging from 160 ng/mL to 20 µg/mL HA. The linear portion of the curve was used to generate an equation of the type y=ax+b (FIGURE 9B). Using this equation, six samples were quantified. Samples were run on the same blot at four different dilutions (FIGURE 9C) to ensure that they fall within the dynamic range of the standard curve. Samples 1 to 5 were produced by infecting HEK293 cells with H1N1 A/Puerto Rico/8/34 at an MOI of 0.01 and collecting the supernatant. Sample 6 was collected from non-infected cells and

was used as a negative control. This example shows that the mAb can be used in conjunction with a dot blot apparatus to quantify viruses given that the appropriate standard is used.

[000132] *Direct ELISA*. Recombinant HA from H5N1 A/Indonesia/05/2005 (Protein Science) was denatured in 8M urea supplemented with 10mM DTT for 30 minutes with shaking. Plates (96-wells flat-bottom from Costar) were coated with 0.25 to 16µg rHA overnight at 4°C. Antibodies 11H12, 10A9, 9D1, and anti-GFP (negative control) were added at a concentration of 100ng/mL and incubated at 37°C for 1 hour. HRP-conjugated secondary antibodies and TMB One component (Bethyl Laboratories) were used to reveal the signal. Results are shown in FIGURE 10 and show that 11H12 and 9D1 have the same binding affinity for H5N1/A/Indonesia/05/2005, while 10A9 has a negligible binding affinity to this strain. This example demonstrates that a standard curve can be generated with the mAb in an ELISA assay, indicating applicability for quantification purposes.

Example 6: In vivo neutralization by anti-HA antibodies

[000133] The ability of the anti-influenza mAb obtained in Example 2 to neutralize influenza *in vivo* was evaluated.

[000134] BALB/C mice (n=5 per group) were infected intra-nasally with lethal concentrations of influenza viruses, and mAb were injected at different time points (15 µg mAb per gram of body weight in 100µl). Mice treated with an anti-HA antibody named Uni-1 produced in rabbits (gift from Xuguang (Sean) Li from Health Canada) was used as a positive control. In the case where the mice were infected with 750 Plaque Forming Units (PFU) of H1N1 A/Puerto Rico/8/34, the mAb were injected 2 hours before infection, 4 hours post-infection and 24 hours post-infection (FIGURE 11A). By day 8 post-infection, none of the untreated mice had survived whereas 80% of the mice treated with mAb 11H12 survived. The antibody 10A9 was the least protective against H1N1 A/Puerto Rico/8/34 with only 11% of the mice still alive by day 9 post-infection. In the second case, mice were infected with 10^4 PFU of H3N2 A/Hong Kong/8/68, and mAb were injected 24 hours before infection, 2 hours before infection, 24 hours post-infection and 72 hours post-infection (FIGURE 11B). As a negative control, an isotype control was also included. Untreated mice and mice treated with the isotype control were all dead by day 9 post-infection. On the other hand, mice treated with mAb 10A9 or Uni-1 survived the viral challenge for all the duration of the experiment (14 days). This

example suggests that the mAb have a neutralizing capacity *in vivo*, and could be used as a therapeutic.

Example 7: Production of MAbs in Chinese Hamster Ovary (CHO) cells

[000135] The antibodies from Example 2 were also expressed in CHO cells to facilitate large-scale production using bioreactor technologies that might be required to meet industrial needs for quantification reagents.

[000136] After selecting two pan-HA antibodies (11H12 and 10A9), these were sequenced and then plasmids were generated and transfected in CHO cells. The binding affinity and specificity of antibodies produced in CHO pools (FIGURE 12B) is similar to the ones of antibodies produced from mouse hybridomas (FIGURE 12A), strongly indicating possibillity of large scale production.

[000137] *Antibody production in Chinese Hamster Ovary (CHO) pools.* F211-11H12 and F211-10A9 were sequenced by qRT-PCR and plasmids were synthesized (BIO BASIC Inc.). A CHO cell line (CHOBRI55E1-JN, proprietary to Applicant), which is inducible with cumate, was transfected with linear polyethylenimine (PEIMax, Polysciences) at a DNA/PEI ratio of 1 in 5. The resulting stable transfected pools produced antibodies named CHO-11H12 and CHO-10A9. Cells were maintained in PowerCHO media (Lonza) supplemented with 50µM MSX, and antibody production was performed in BalanCD CHO growth media (Irvine Scientific) supplemented with 50µM MSX at 32C.

[000138] *Dot blot*. In brief, samples were denatured in 4M urea for 30min with shaking at room temperature. After serial dilutions, 100ul was loaded into the dot-blot wells in duplicate, along with a standard of known concentration previously quantified by SRID. Samples were then filtered through a nitrocellulose membrane using vacuum on a bio-dot apparatus (BioRad). The membrane was blocked in 5% non-fat dry milk in PBS for 1 hour at room temperature with shaking, followed by overnight incubation with 6µg/ml antibody diluted in Odyssey blocking buffer at 4C. Infrared-conjugated secondary antibodies were used for detection, along with the Odyssey scanner (LI-COR Biosciences).

[000139] *Surface Plasmon Resonance (SPR).* A BIACORE T200 (GE Healthcare) was used to determine the binding affinity (KD) of antibodies F211-10A9, CHO-10A9, F211-11H12 and CHO-11H12 to the peptide immunogen. All SPR experiments were run

in PBS containing 0.05% Tween 20 (Teknova Inc.) and 3.4 mM EDTA as a running buffer. An anti-mouse-Fc surface was immobilized to approximately 2200 RUs using the Immobilization Wizard within the T200 control software set to a 2000 RU target.

[000140] Standard amine coupling of 20µg/mL anti-mouse Fc solution in 10 mM NaOAc pH 4.5 was used. For the binding assay, approximately 350 RUs of antibody to be tested (-10A9 or -11H12) was captured onto a sheep anti-mouse Fc antibody surface by injecting 20µg/mL solution for 300 seconds. This was followed by a single cycle kinetics injection of peptide using a 2-fold dilution series with a top-nominal concentration of 250nM for antibodies -11H2, and 15nM for antibodies -10A9, or PBST running buffer only for referencing. Using a flow rate of 100 µL/min with a 2700 second dissociation, 180 second injections of each peptide or buffer blank were used. The SPR surfaces were regenerated with 10mM glycine pH 1.5 with a contact time of 120 seconds. Sensorgrams were double referenced and data were analyzed within Biacore T200 evaluation software v3.0 (GE Healthcare).

[000141] *Hybridoma versus CHO-produced mAb*. In order to scale up the production of pan-HA mAb, a different antibody production platform was explored. The variable regions of heavy and light chains of F211-11H12 and F211-10A9 hybridomas were sequenced by qRT-PCR and confirmed by mass spectrometry analysis of the purified mAbs (results not shown). DNA corresponding to the sequences were synthesized, cloned into vectors for full-length mouse IgG expression and transfected in a cumate-inducible CHO cell line using PEI in order to produce stable CHO pools. The two antibodies generated were named **CHO-11H12** and **CHO-10A9**. Their binding affinity was tested by SPR and compared to their hybridomas counterpart; similar KD values were measured for the 4 mAbs, ranging from 3.38E-10 to 6.39E-10 M (Table 8).

Table 8. KD values (M) measured by SPR for pan-HA antibodies produced in mouse hybridomas and CHO pools.

mAb	KD (M)	
	Average	SD
F211-10A9	$3.38\ E^{-10}$	$7.85E^{-11}$
CHO-10A9	$3.65\ E^{-10}$	$4.21E^{-11}$
F211-11H12	$4.65\ E^{-10}$	$7.17E^{-11}$
CHO-11H12	$6.39\ E^{-10}$	$1.16E^{-10}$

[000142] The reactivity panel of the CHO-produced mAb cocktail is similar to the one generated by hybridoma-mAbs as demonstrated by dot blot against 18 strains listed

in Table 9 (FIGURE 12). The intensity of the signal correlates with the binding affinity of the antibody with a given strain. All the strains tested were recognized, except for a recombinant protein from H3N2 A/Wisconsin/67/05 (spot 4) for unknown reason; all other H3N2 strains were efficiently detected. Again, a variation due to the production platform was observed. For instance, the detection differs for the strain expressed as a recombinant protein (spot 5) compared to a virus (spot 19).

[000143] The first generation of antibodies used in this study were produced from mouse hybridomas with a yield of about 100mg/L of culture, but higher levels of production are achievable using mammalian expression systems such as CHO cells. The pan-HA antibodies have therefore been sequenced and expressed in CHO cells. Similar binding affinities and specificities were observed between both types of antibodies. As a result, production can easily be scaled up using CHO cells in large bioreactors if needed.

Table 9. List of different strains used in dot blot of FIGURE 12.

	Dot #	Subtype	Strain
rHA	1	H1N1	A/Puerto Rico/8/34
	2	H1N1	A/California/06/2008
	3	H3N2	A/Aichi/2/1968
	4	H3N2	A/Wisconsin/67/05
	5	H3N2	A/Hong Kong/8/68
	6	H3N2	A/Brisbane/10/2007
	7	H5N1	A/Indonesia/05/2005
	8	H5N1	A/Vietnam/1203/2004
	9	H7N7	A/Netherlands/219/2003
	10	H7N9	A/Anhui/1/2013
	11	H7N9	A/Shanghai/2/2013
	12	H7N9	A/Pigeon/Shanghai/S1069/2013
rHA	13	H9N2	A/Hong Kong/1073/1999
	14	B	Brisbane/60/2008
Sd	15	H1N1	A/California/06/2008
	16	B	Massachusetts/02/2016
Virus	17	H1N1	A/Puerto Rico/8/34
	18	H1N1	A/Wilson Smith/33
	19	H3N2	A/Hong Kong/8/68
	20	B	Lee/1940
VLP	21	H3N1	A/Victoria/361/2011
	22	H5N1	A/Indonesia/05/2005
	23	H1N1	A/California/06/2008
	24		Negative control (non-related protein)

Legend: rHA = recombinant hemagglutinin, Sd = cell-produced calibrating standards (NIBSC), Virus = viruses produced in-house in HEK293 cells, VLP = viral like particles produced in plants (Medicago, Québec, QC).

Example 8 - Primary sequence confirmation of Influenza mAbs

[000144] We confirmed the amino acid sequence of the following 3 mAbs (produced from mouse hybridomas): F211-**11H12**-2; F211-**10A9**-2; and F211-**9D1**-2.

[000145] IgGs were reduced, alkylated, and digested with trypsin. The resulting peptides were analyzed by nano-C18-LC and data-dependent MS-MS/MS on an LTQ-Orbitrap XL mass spectrometer. Peptides were identified by Mascot search on a

database containing all 4 mAb sequences. Amino acid sequences identified with a Mascot score > 30 are highlighted in bold red on the sequences of FIGURES 13.

[000146] The data suggest that the predicted sequences are likely to be correct for all 4 IgGs, especially when combined with the supportive intact mass data. We obtained sequence coverage averaging 75% which is standard in this type of analysis. Most areas that were not covered are areas that produce tryptic peptides that are either too large or too small to be identified under standard MS conditions. There are no obvious differences between 11H12 and 8C4.

Example 9 – Delivery of antibodies through recombinant Adeno-Associated Virus (rAAV)

[000147] Multiple repeated administrations of antibodies in at-risk population would be impractical and too expensive. Therefore, recombinant Adeno-Associated-Virus (rAAV) is being considered as a delivery system. Using rAAV would allow delivering the genes encoding for the mAb and ensuring their long-term expression. The heavy chain and light chain of the antibodies can be expressed on the same cassette and transfected into cells, along with two other plasmids (Rep/Cap and helper plasmids) to form the rAAV particles. After purification, the AAV can be injected for long term production of the antibodies. This would represent a cost-effective delivery route, especially in immunocompromised or elderly individuals.

Conclusion

[000148] The approach explored here is to use anti-stem antibodies to address the enduring quantification issue. The pan-HA antibodies have been tested against a large panel of strains including 13 HA influenza A subtypes as well as B subtypes. In addition, the pan-HA cocktail can detect egg-produced viruses, but also new-generation vaccines such as VLPs. Indeed, the host can also have an impact on detection due to different glycosylation patterns.

[000149] The monoclonal pan-HA antibodies were generated in mice against a highly conserved sequence found in the fusion peptide. Issues related to heterogeneity such as the ones observed with polyclonal antibodies are thus avoided.

[000150] Alternatively, large quantities can be produced in largescale bioreactors from CHO-producing cells to respond to industrial demand. This could be critical in case of a pandemic but could also considerably speed up process development.

[000151] The embodiments and examples described herein are illustrative and are not meant to limit the scope of the invention as claimed. Variations of the foregoing embodiments, including alternatives, modifications and equivalents, are intended by the inventors to be encompassed by the claims. Furthermore, the discussed combination of features might not be necessary for the inventive solution.

LISTING OF SEQUENCES

SEQ ID NO:	Sequence	Description
1	QSLLNSX_1X_2QKNX_3 where X_1=R or D, X_2=N or T, X_3=H or F;	CDR L1 consensus
35	X_1AS where X_1=W or F;	CDR L2
2	QQYY$X_1X_2X_3X_4$T where X_1=T or S, X_2=Y or I, X_3=P or no amino acid, X_4=R or L,	CDR L3
3	GYX_1X_2TX_3DYY where X_1=S or T, X_2=I or F, X_3=S or no amino acid;	CDR H1 consensus
4	IGYDGX_1K Where X_1=S or T,	9D1 and 11H12 CDR H2
5	IYPGNGHT	10A9 CDR H2
6	TRDRANWDDYFDY	9D1 and 11H12 CDR H3
7	AYDLFNY	10A9 CDR H3
8	QSLLNSRNQKNH	9D1 and 11H12 CDR L1
9	QSLLNSDTQKNF	10A9 CDR L1
36	WAS	9D1 and 11H12 CDR L2
37	FAS	10A9 CDR L2
10	QQYYTYXRT where X is P or no amino acid	9D1 and 11H12 CDR L3
11	QQYYSIPLT	10A9 CDR L3
12	GYSITSDYY	9D1 and 11H12 CDR H1
13	GYTFTDYY	10A9 CDR H1
14	QQYYTYRT	9D1 and 11H12 CDR L3
15	IGYDGSK	9D1 CDR H2
16	IGYDGTK	11H12 CDR H2
17	DIVMX_1QSPSSLAX_2SVGX_3KVTMSCKSSQSLLNSX_4X_5QK NX_6LAWYQQKPGQSPKX_7LX_8YX_9ASTX$_{10}$ESGVPDRFX$_{11}$G X$_{12}$GSGTDFTLTIX$_{13}$SVX$_{14}$AEDLAX$_{15}$YX$_{16}$CQQYYX$_{17}X_{18}X_{19}$ X$_{20}$TFGX$_{21}$GTKLEIK where X_1=S or T, X_2=V or M, X_3=E or O, X_4=R or D, X_5=N or T, X_6=H or F, X_7=L or I, X_8=I or V, X_9=W or F, X_{10}=R or K, X_{11}=S or I, X_{12}=D or S, X_{13}=S or T, X_{14}=K or Q, X_{15}=V or D, X_{16}=Y or F, X_{17}=T or S, X_{18}=Y or I, X_{19}=P or no amino acid, X_{20}=R or L, X_{21}=G or A.	Light chain consensus sequence
18	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHL AWYQQKPGQSPKLLIYWASTRESGVPDRFX$_1$GDGSGTDF	9D1 and 11H12 light chain

	TLTISSVKAEDLAVYYCQQYYTYRTFGGGTKLEIK where X_1=S or T	
19	DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSDTQKNFL AWYQQKPGQSPKILVYFASTKESGVPDRFIGSGSGTDFT LTITSVQAEDLADYFCQQYYSIPLTFGAGTKLELK	10A9 light chain
20	DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIR QFPGNKLEWMAYIGYDGX$_1$KNYNPSLKNRISITRDTSKNQ FFLKLNSVTTDDTATYYCTRDRANWDDYFDYWGQGTTLT VSS where X_1=S or T	9D1 and 11H12 heavy chain
21	QIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQR PGQGLEWIGYIYPGNGHTVYNQKFKVRATLTADNPSSTA YLQLNSLTSEDSGVYFCAYDLFNYWGQGTLVTVSA	10A9 heavy chain
22	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHL AWYQQKPGQSPKLLIYWASTRESGVPDRFSGDGSGTDF TLTISSVKAEDLAVYYCQQYYTYRTFGGGTKLEIK	9D1 light chain
23	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHL AWYQQKPGQSPKLLIYWASTRESGVPDRFTGDGSGTDF TLTISSVKAEDLAVYYCQQYYTYRTFGGGTKLEIK	11H12 light chain
24	DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIR QFPGNKLEWMAYIGYDGSKNYNPSLKNRISITRDTSKNQF FLKLNSVTTDDTATYYCTRDRANWDDYFDYWGQGTTLTV SS	9D1 heavy chain
25	DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIR QFPGNKLEWMAYIGYDGTKNYNPSLKNRISITRDTSKNQF FLKLNSVTTDDTATYYCTRDRANWDDYFDYWGQGTTLTV SS	11H12 heavy chain
26	GLFGAIAGFIEGGW	Conserved peptide sequence
27	MVLQTQVFISLLLWISGAYG	Signal peptide
28	MDWTWRILFLVAAATGTHA	Signal peptide
29	FIGURE 13A UPPER	F211-11H12-2 V_L
30	FIGURE 13A LOWER	F211-11H12-2 V_H
31	FIGURE 13B UPPER	F211-10A9-2 V_L
32	FIGURE 13B LOWER	F211-10A9-2 V_H
33	FIGURE 13C UPPER	F211-9D1-2 V_L
34	FIGURE 13C LOWER	F211-9D1-2 V_H
35	FIGURE 14A	10A9 full sequence
36	FIGURE 14B	11H12 full sequence

REFERENCES

All patents, patent applications and publications referred to throughout the application are listed below.

Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. J Mol Biol. 1987 Aug 20;196(4):901-17.

Chun S, Li C, Van Domselaru· G, Wang J, Farnsworth A, Cui X, Rode H, Cyr TD, He R, and Li X. (2008) Universal antibodies and their applications to the quantitative determination of virtually all subtypes of the influenza A viral hemagglutinins. Vaccine 26(48): 6068-76.

de Kruif J, Logtenberg T. Leucine zipper dimerized bivalent and bispecific scFv antibodies from a semi-synthetic antibody phage display library. J Biol Chem. 1996 Mar 29;271(13):7630-4.

Dreyfus C, Laursen NS, Kwaks T, Zuijdgeest D, K.hayat R, Ekiert DC, Lee JH, Metlagel Z, Bujny MV, Jongeneelen M, van der Vlugt R, Lamrani M, Korse HJ, Geelen E, Sabin 0, Sieuwerts M, Brakenhoff JP, Vogels R, Li OT, Poon LL, Peiris M, Koudstaal W, Ward AB, Wilson IA, Ooudsmit J, and Friesen RH. (2012) Highly conserved protective epitopes on influenza B viruses. Science 337(6100): 1343-8.

Eisenberg, D., Schwarz, E., Komaromy, M., and Wall, R. Analysis of membrane and surface protein sequences with the hydrophobic moment plot. (1984) J. Mol. Biol. 179, 125-142

Fenner, L., Widmer, A.F., Goy, G., Rudin, S., and Frei, R. Rapid and reliable diagnostic algorithm for detection of Clostridium difficile. (2008) J. Clin. Microbiol. 46, 328-330.

Gonzales NR, DePascalis R, Schlom J, Kashmiri SVS (2005) Tumor Biol 26, 31-43.

Jones PT, Dear PH, Foote J, Neuberger MS, Winter G (1986) Nature 321, 522-525.

Kabat EA, Wu TT. Identical V region amino acid sequences and segments of sequences in antibodies of different specificities. Relative contributions of VH and VL genes, minigenes, and complementarity-determining regions to binding of antibody-combining sites. J Immunol. 1991;147:1709-19

Kang SM, Song JM, Compans RW. Novel vaccines against influenza viruses. Virus Res. 2011 Dec;162(1-2):31-8.

Lefranc MP, Pommié C, Ruiz M, Giudicelli V, Foulquier E, Truong L, Thouvenin-Contet V, Lefranc G. IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev Comp Immunol. 2003 Jan;27(1):55-77. Review.

Li C, Jaentchke B, Song Y, Wang J, Cyr TD, Van Domselaar G, et al. (2010) A simple slot blot for the detection of virtually all subtypes of the influenza A viral hemagglutinins using universal antibodies targeting the fusion peptide. Nat Protoc Jan;5(1):14-9.

Merritt, E.A., and Hol, W.G. (1995) Curr. Opin. Struct. Biol. 5, 165-171.

Musher, D.M., Manhas, A., Jain, P., Nuila, F., Waqar, A., Logan, N., Marino, B., Graviss, E.A. Detection of Clostridium difficile toxin: comparison of enzyme immunoassay results with results obtained by cytotoxicity assay. (2007) J. Clin. Microbiol. 45, 2737-2739.

Nicaise M, Valeio-Lepiniec M, Minard P, Desmadril M. (2004) Affinity transfer by CDR grafting on a nonimmunoglobulin scaffold. Protein Sci. 13(7): 1882-1891.

Nielsen UB, Adams GP, Weiner LM, Marks JD. Targeting of bivalent anti-ErbB2 diabody antibody fragments to tumor cells is independent of the intrinsic antibody affinity. Cancer Res. 2000 Nov 15;60(22):6434-40.

Padlan EA (1991) A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties. Mol Immunol 28, 489-498.

Planche, T. Aghaizu, A., Holliman, R., Riley, P., Poloniecki, J., Breathnach, A., and Krishna, S. (2008) Diagnosis of Clostridium difficile infection by toxin detection kits: a systematic review. Lancet Infect. Dis. 8, 777-784.

Ridgway, J.B., Presta, L.G., and Carter, P. "'Knobs-into-holes' engineering of antibody CH3 domains for heavy chain heterodimerization. (1996) Protein Eng. 9, 617-621.

Queen C, Schneider WP, Selick HE, Payne PW, Landolfi NF, Duncan JF, Avdalovic NM, Levitt M, Junghans RP, Waldmann TA (1989) Proc Natl Acad Sci USA 86, 10029-10033.

Ridgway, J.B., Presta, L.G., and Carter, P. (1996) Protein Eng. 9, 617-621.

Riechmann L, Clark M, Waldmann H, Winter G (1988) Nature 332, 323-327.

Rüssmann, H., Panthel, K., Bader, R.C., Schmitt, C., and Schaumann, R. Evaluation of three rapid assays for detection of Clostridium difficile toxin A and toxin B in stool specimens. (2007) Eur. J. Clin. Microbiol. Infect. Dis. 26, 115-119.

Sloan, L.M., Duresko, B.J., Gustafson, D.R., and Rosenblatt, J.E. Comparison of real-time PCR for detection of the tcdC gene with four toxin immunoassays and culture in diagnosis of Clostridium difficile infection. (2008) J. Clin. Microbiol. 46, 1996-2001.

Tempest PR, Bremmer P, Lambert M, Taylor G, Furze JM, Carr FJ, Harris WJ (1991) Biotechnology 9, 266-271.

Tsurushita N, Hinton, RP, Kumar S (2005) Design of humanized antibodies: From anti-Tac to Zenapax. Methods 36, 69-83.

Turgeon, D.K., Novicki, T.J., Quick, J., Carlson, L., Miller, P., Ulness, B., Cent, A., Ashley, R., Larson, A., Coyle, M., Limaye, A.P., Cookson, B.T., and Fritsche, T.R. Six rapid tests for direct detection of Clostridium difficile and its toxins in fecal samples compared with the fibroblast cytotoxicity assay. (2003) J. Clin. Microbiol. 41, 667-670.

Zhu X, Wang L, Liu R, Flutter B, Li S, Ding J, Tao H, Liu C, Sun M, Gao B. COMBODY: one-domain antibody multimer with improved avidity. Immunol Cell Biol. 2010 Aug;88(6):667-75. doi: 10.1038/icb.2010.21. Epub 2010 Mar 9.

WO 95/04069; WO/2004/076670; WO2003/046560.

CLAIMS

1. An isolated or purified antibody or fragment thereof, comprising:

a) a light chain comprising a complementarity determining region (CDR) L1 sequence of QSLLNSX_1X_2QKNX_3 (SEQ ID NO:1) where X_1=R or D, X_2=N or T, X_3=H or F; a CDR L2 sequence of X_1AS (SEQ ID NO:35) where X_1=W or F; and a CDR L3 sequence of QQYY$X_1X_2X_3X_4$T (SEQ ID NO:2) where X_1=T or S, X_2=Y or I, X_3=P or no amino acid, X_4=R or L;

and

b) a heavy chain comprising a complementarity determining region (CDR) H1 sequence of GYX_1X_2TX_3DYY (SEQ ID NO:3) where X_1=S or T, X_2=I or F, X_3=S or no amino acid;

a CDR H2 sequence selected from the group consisting of IGYDGX_1K (SEQ ID NO:4) where X_1=S or T, and IYPGNGHT (SEQ ID NO:5); and

a CDR H3 sequence selected from the group consisting of TRDRANWDDYFDY (SEQ ID NO:6) and AYDLFNY (SEQ ID NO:7).

2. The isolated or purified antibody or fragment thereof of claim 1, wherein CDR L1 is selected from the group consisting of QSLLNSRNQKNH (SEQ ID NO:8) and QSLLNSDTQKNF (SEQ ID NO:9).

3. The isolated or purified antibody or fragment thereof of claim 1 or 2, wherein CDR L2 is selected from the group consisting of WAS (SEQ ID NO:36) and FAS (SEQ ID NO:37).

4. The isolated or purified antibody or fragment thereof of any one of claims 1 to 3, wherein CDR L3 is selected from the group consisting of QQYYTYRT (SEQ ID NO:10) wherein X is P or no amino acid; and QQYYSIPLT (SEQ ID NO:11).

5. The isolated or purified antibody or fragment thereof of any one of claims 1 to 4, wherein CDR H1 is selected from the group consisting of GYSITSDYY (SEQ ID NO:12) and GYTFTDYY (SEQ ID NO:13).

6. The isolated or purified antibody or fragment thereof of any one of claims 1 to 5, wherein the antibody or fragment thereof is selected from the group consisting of:

a) a light chain comprising CDR L1 of sequence QSLLNSRNQKNH (SEQ ID NO:8), CDR L2 of sequence WAS (SEQ ID NO:36), and CDR L3 of sequence QQYYTYRT (SEQ ID NO:14); and a heavy chain comprising CDR H1 of

sequence GYSITSDYY (SEQ ID NO:12), CDR H2 of sequence IGYDGSK (SEQ ID NO:15), and CDR H3 of sequence TRDRANWDDYFDY (SEQ ID NO:6);

b) a light chain comprising CDR L1 of sequence QSLLNSRNQKNH (SEQ ID NO:8), CDR L2 of sequence WAS (SEQ ID NO:36), and CDR L3 of sequence QQYYTYRT (SEQ ID NO:14); and a heavy chain comprising CDR H1 of sequence GYSITSDYY (SEQ ID NO:12), CDR H2 of sequence IGYDGTK (SEQ ID NO:16), and CDR H3 of sequence TRDRANWDDYFDY (SEQ ID NO:6); and

c) a light chain comprising CDR L1 of sequence QSLLNSDTQKNF (SEQ ID NO:9), CDR L2 of sequence FAS (SEQ ID NO:37), CDRL3 of sequence QQYYSIPLT (SEQ ID NO:11); and a heavy chain comprising CDR H1 of sequence GYTFTDYY (SEQ ID NO:13), CDR H2 of sequence IYPGNGHT (SEQ ID NO:5), and CDR H3 of sequence AYDLFNY (SEQ ID NO:7).

7. The isolated or purified antibody or fragment thereof of any one of claims 1 to 6, wherein the variable light (VL) domain comprises the sequence:

DIVMX_1QSPSSLAX_2SVGX_3KVTMSCKSSQSLLNSX_4X_5QKNX_6LAWYQQKPGQS PKX_7LX_8YX_9ASTX$_{10}$ESGVPDRFX_{11}GX_{12}GSGTDFTLTIX_{13}SVX_{14}AEDLAX_{15}YX_{16}C QQYY$X_{17}X_{18}X_{19}X_{20}$TFGX_{21}GTKLEIK (SEQ ID NO:17) where X_1=S or T, X_2=V or M, X_3=E or O, X_4=R or D, X_5=N or T, X_6=H or F, X_7=L or I, X_8=I or V, X_9=W or F, X_{10}=R or K, X_{11}=S or I, X_{12}=D or S, X_{13}=S or T, X_{14}=K or Q, X_{15}=V or D, X_{16}=Y or F, X_{17}=T or S, X_{18}=Y or I, X_{19}=P or no amino acid, X_{20}=R or L, X_{21}=G or A.

8. The isolated or purified antibody or fragment thereof of claim 7, wherein the variable light (VL) domain comprises a sequence selected from the group consisting of:

DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKL LIYWASTRESGVPDRFX_1GDGSGTDFTLTISSVKAEDLAVYYCQQYYTYRTFGG GTKLEIK (SEQ ID NO:18) where X_1=S or T; and

DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSDTQKNFLAWYQQKPGQSPKIL VYFASTKESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQYYSIPLTFGAGT KLELK (SEQ ID NO:19).

9. The isolated or purified antibody or fragment thereof of any one of claims 1 to 8, wherein the variable heavy (VH) domain comprises a sequence selected from the group consisting of:

DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIG YDGX_1KNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDY FDYWGQGTTLTVSS (SEQ ID NO:20) X_1=S or T; and

QIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQRPGQGLEWIGYIYPG NGHTVYNQKFKVRATLTADNPSSTAYLQLNSLTSEDSGVYFCAYDLFNYWGQ GTLVTVSA (SEQ ID NO:21).

10. The isolated or purified antibody or fragment thereof any one of claims 1 to 9, wherein the isolated or purified antibody or fragment thereof comprises a sequence selected from the group consisting of

a) a variable light (V_L) domain of sequence:
DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKL LIYWASTRESGVPDRFSGDGSGTDFTLTISSVKAEDLAVYYCQQYYTYRTFGG GTKLEIK (SEQ ID NO:22);
and variable heavy (V_H) domain of sequence:
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIG YDGSKNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDYF DYWGQGTTLTVSS (SEQ ID NO:24);

b) a variable light (V_L) domain of sequence:
DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLNSRNQKNHLAWYQQKPGQSPKLLIY WASTRESGVPDRFTGDGSGTDFTLTISSVKAEDLAVYYCQQYYTYXRTFGGGTKL EIK (SEQ ID NO: 23);
and variable heavy (V_H) domain of sequence:
DVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQFPGNKLEWMAYIGYD GTKNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDRANWDDYFDYWG QGTTLTVSS (SEQ ID NO:25);

c) a variable light (V_L) domain of sequence:
DIVMTQSPSSLAMSVGQKVTMSCKSSQSLLNSDTQKNFLAWYQQKPGQSPKILVY FASTKESGVPDRFIGSGSGTDFTLTITSVQAEDLADYFCQQYYSIPLTFGAGTKLEL K (SEQ ID NO:19);
and variable heavy (V_H) domain of sequence:
QIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQRPGQGLEWIGYIYPGNG HTVYNQKFKVRATLTADNPSSTAYLQLNSLTSEDSGVYFCAYDLFNYWGQGTLVT VSA (SEQ ID NO:21);
and
d) a sequence substantially identical thereto.

11. The isolated or purified antibody or fragment thereof of any one of claims 1 to 10, wherein the antibody or fragment thereof specifically binds to the peptide: GLFGAIAGFIEGGW (SEQ ID NO:26).

12. The isolated or purified antibody or fragment thereof any one of claims 1 to 11, wherein the antibody or fragment thereof is a full-length IgG, Fv, scFv, Fab, or $F(ab')_2$.

13. The isolated or purified antibody or fragment thereof of any one of claims 1 to 12, wherein the antibody or fragment thereof comprises framework regions from IgA, IgD, IgE, IgG, or IgM.

14. The isolated or purified antibody or fragment thereof of any one of claims 1 to 13, wherein the antibody or fragment thereof is chimeric.

15. The isolated or purified antibody or fragment thereof of claim 14, wherein the chimeric antibody or fragment thereof constant domain is from human IgG1.

16. The isolated or purified antibody or fragment thereof of claim 14, wherein the chimeric antibody or fragment thereof comprises human kappa 1 light chain and human IgG1 heavy chain constant domains.

17. A nucleic acid molecule encoding the isolated or purified antibody or fragment thereof of any one of claims 1 to 16.

18. A vector comprising the nucleic acid molecule of claim 17.

19. The isolated or purified antibody or fragment thereof of any one of claims 1 to 16, wherein the antibody or fragment thereof is immobilized onto a surface.

20. The isolated or purified antibody or fragment thereof of claim 19, wherein the surface is nitrocellulose.

21. The isolated or purified antibody or fragment thereof of any one of claims 1 to 16, wherein the antibody or fragment thereof is linked to a cargo molecule.

22. The isolated or purified antibody or fragment thereof of claim 21, wherein the cargo molecule is a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody or fragment thereof, a chemical compound, a carbohydrate moiety, DNA-based molecules, a neutralizing agent, viral vector, one or more liposomes or nanocarriers loaded with any of the previously recited types of cargo molecules, or one or more nanoparticle, nanowire, nanotube, or quantum dots.

23. The isolated or purified antibody or fragment thereof of claim 21, wherein the cargo molecule is a neutralizing agent.

24. A composition comprising one or more than one isolated or purified antibody or fragment thereof of any one of claims 1 to 16 and 21 to 23 and a pharmaceutically-acceptable carrier, diluent, or excipient.

25. An *in vitro* method of detecting influenza hemagglutinin (HA), comprising:

a) contacting a biological sample or a viral suspension, with an isolated or purified antibody or fragment thereof according to any one of claims 1 to 16 linked to a detectable agent; and

b) detecting the detectable agent linked to the antibody or fragment thereof bound to hemagglutinin in the biological sample or a viral suspension.

26. The method of claim 25, wherein the biological sample is from: blood, serum, nasal wash, nasal swab, saliva or sputum.

27. The method of claim 25 or 26, wherein the step of detecting step b) is performed using: optical imaging, immunohistochemistry, molecular diagnostic imaging, ELISA, or other suitable method.

28. A method of preventing or treating influenza in a subject, comprising administering a pharmaceutically acceptable dose of an isolated or purified antibody or fragment thereof of any one of claims 1 to 16 and 21 to 23 to the subject.

29. A kit for detecting influenza HA comprising a support and an isolated or purified labelled-antibody or -fragment thereof according to any one of claims 1 to 16.

30. The kit of claim 29, wherein the support is nitrocellulose.

31. The kit of claim 30, wherein the isolated or purified labelled-antibody or -fragment thereof is immobilized onto the nitrocellulose.

32. An *in vitro* method for quantifying influenza HA, comprising:

a) contacting a biological sample comprising an isolated or purified antibody or fragment thereof according to any one of claims 1 to 16 linked to a detectable agent; and

b) quantifying the detectable agent linked to the antibody or fragment thereof.

33. The method of claim 32, wherein the step of detecting step b) is performed using: optical imaging, immunohistochemistry (dot blot, slot blot, quantitative Western blot), molecular diagnostic imaging, ELISA (direct, indirect or competitive), or other suitable method.

34. The method of claim 33, wherein the immunohistochemistry is: dot blot, slot blot or quantitative Western blot.

35. The method of claim 33, wherein the ELISA is direct, indirect or competitive.

36. The method of claim 33. 34 or 35, wherein said biological sample is selected from: a purified vaccine or in-process supernatant, produced with a platform selected from: eggs, mammalian cells, and plant.

37. A kit for measuring influenza HA comprising: one or more than one antibody as defined in any one of claims 1 to 16; and a detection reagent for detecting the antibody bound to said influenza HA in a biological sample; a measuring reagent for measuring a level of the detection agent.

38. An isolated or purified antibody or fragment thereof, for the manufacture of a composition for the treatment or prevention of influenza in a subject.

39. An isolated or purified antibody or fragment thereof, for use in the treatment or prevention of influenza in a subject.

40. The method of claim 28, wherein the subject is a human or an animal.

41. The isolated or purified antibody or fragment thereof of claim 38 or 39, wherein the subject is a human or an animal.

42. The isolated or purified antibody or fragment thereof, wherein the antibody is selected from:

- mAb 9D1 comprising sequences SEQ ID NO. 29 and 30;
- mAb 10A9 comprising sequences SEQ ID NO. 31 and 32; and
- mAb 11H12 comprising sequences SEQ ID NO 33 and 34.

43. The isolated or purified antibody of claim 42, referred to as mAb **10A9** and defined by SEQ ID NO. 35.

44. The isolated or purified antibody of claim 42, referred to as mAb **11H12** and defined by SEQ ID NO. 36.

45. A cocktail comprising the antibodies 10A9 and 11H12, as defined in any one of claims 42, 43 or 44.

5

FIGURE 1A

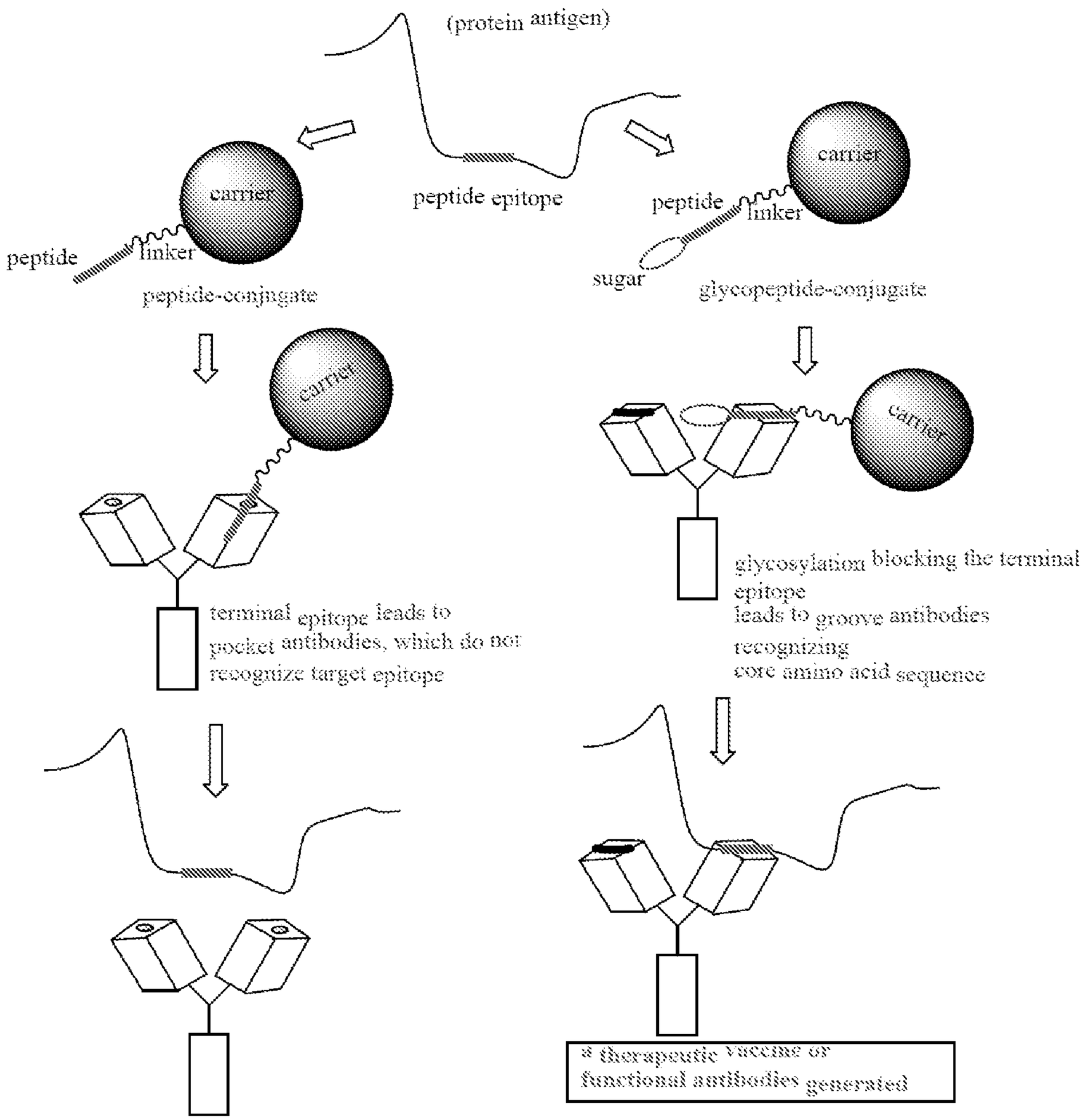


FIGURE 1B

A) Alignment Light Chains:

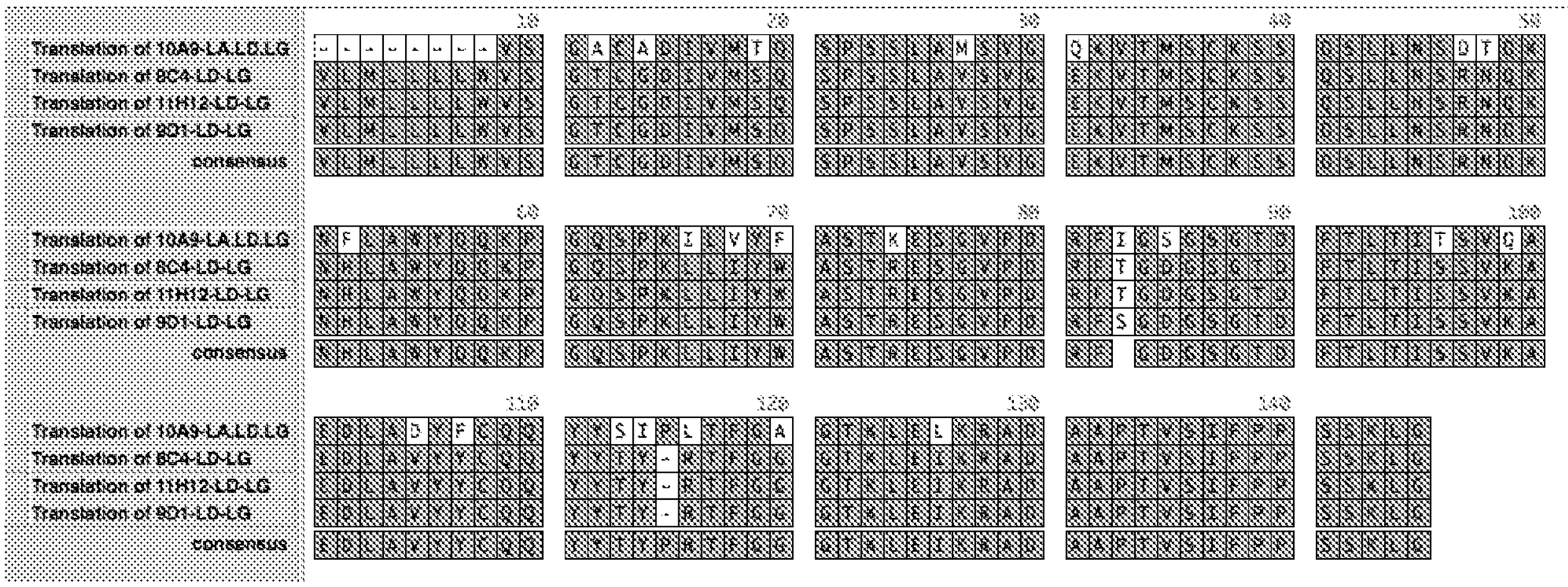


FIGURE 2A

B) Alignment Heavy Chains:

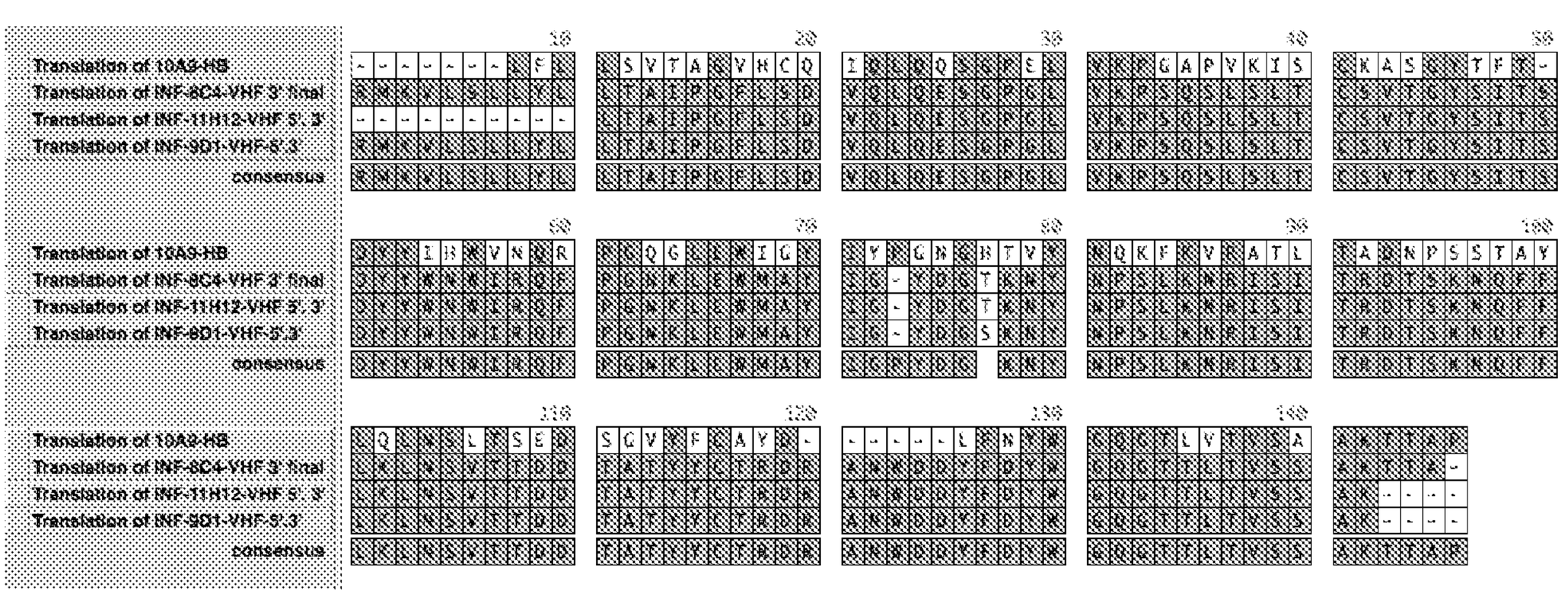


FIGURE 2B

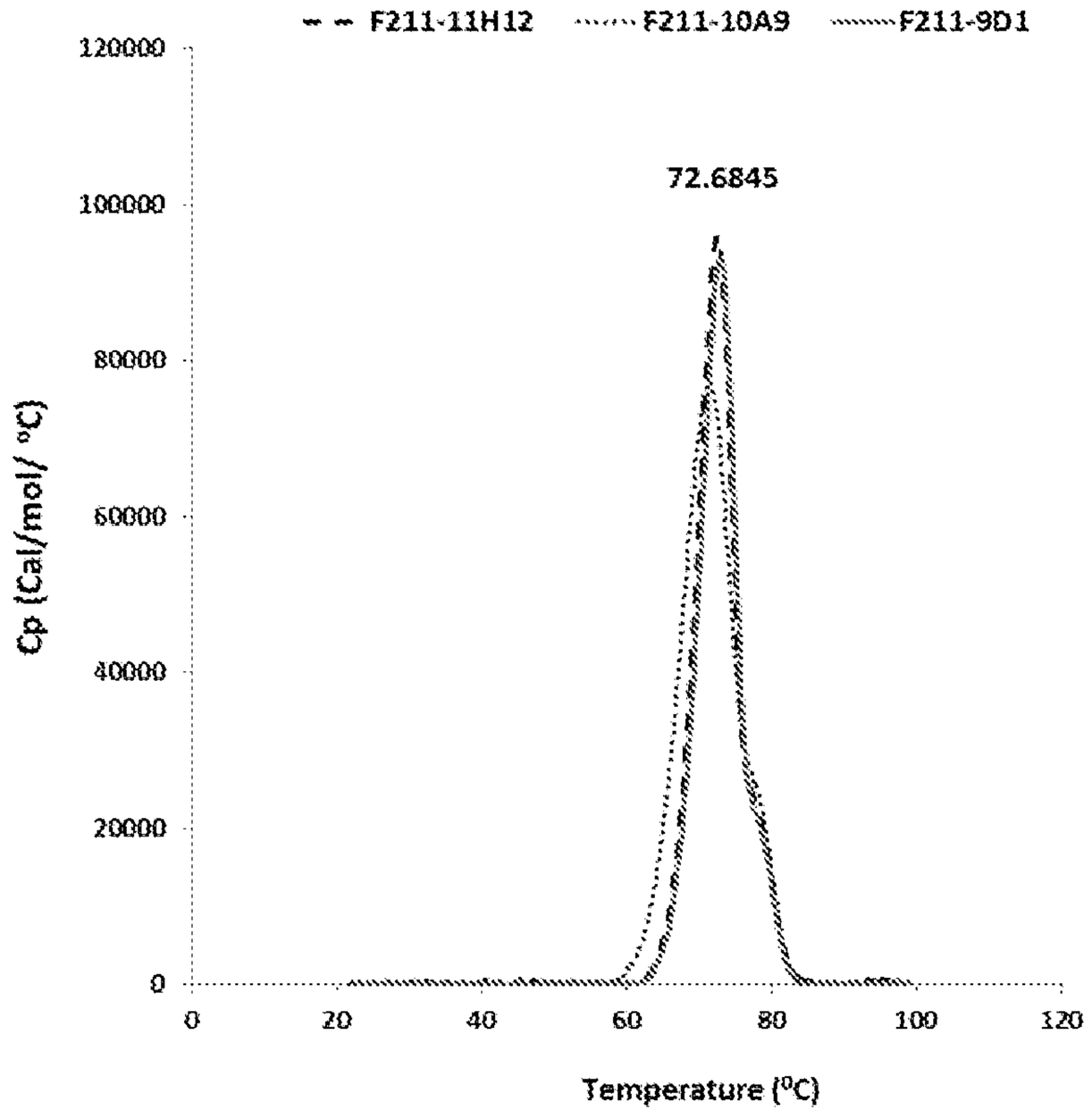


FIGURE 3

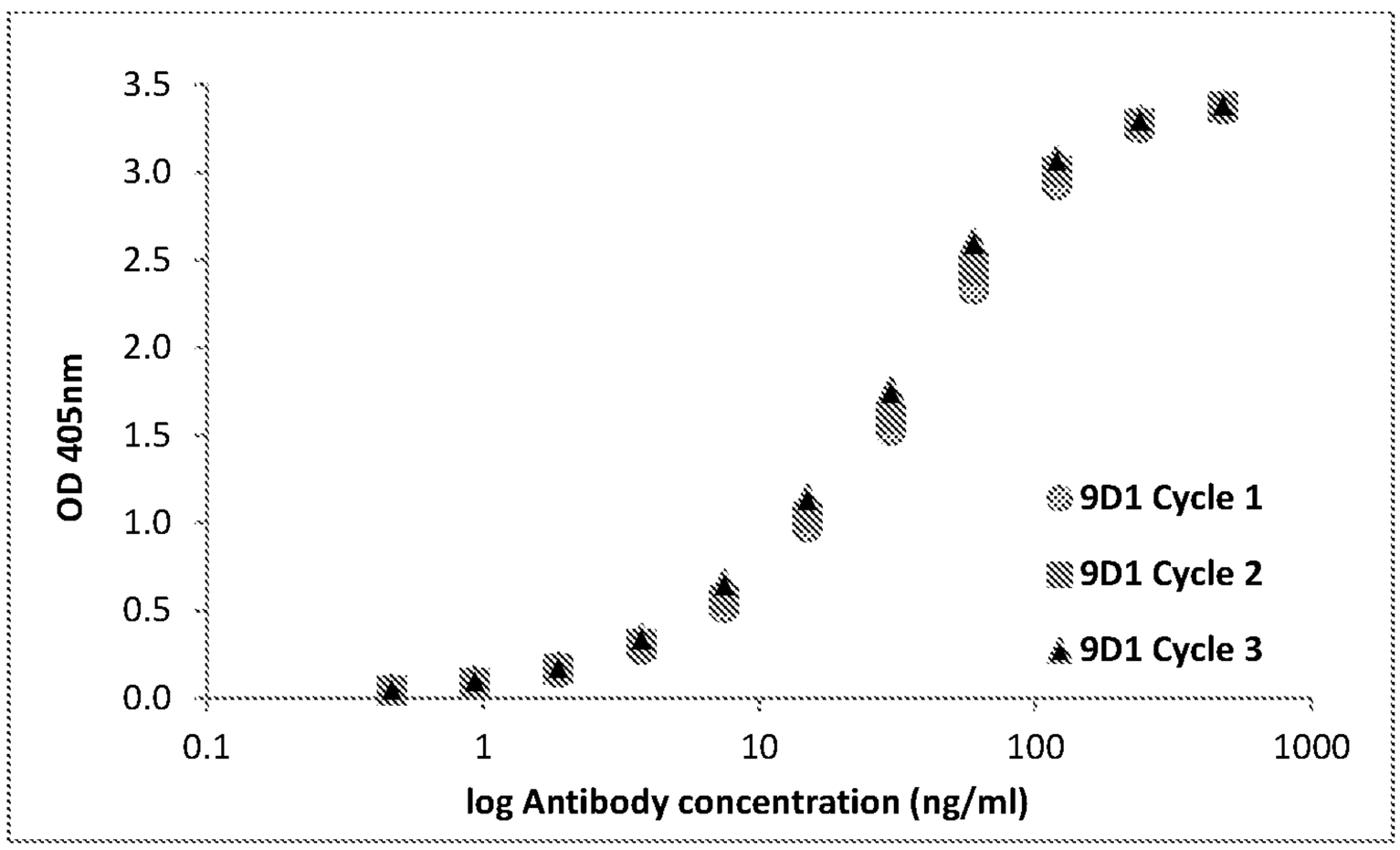


FIGURE 4A

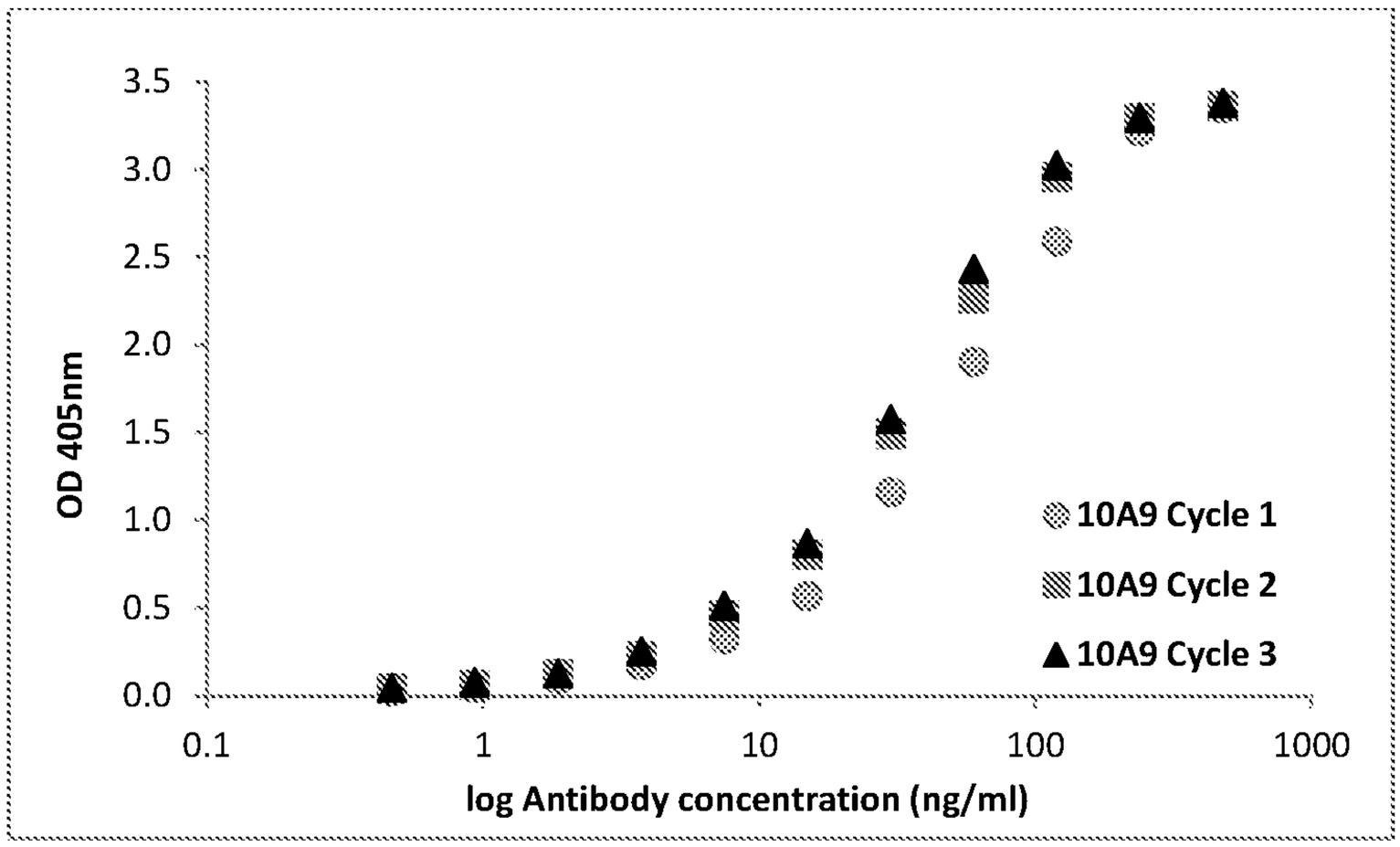


FIGURE 4B

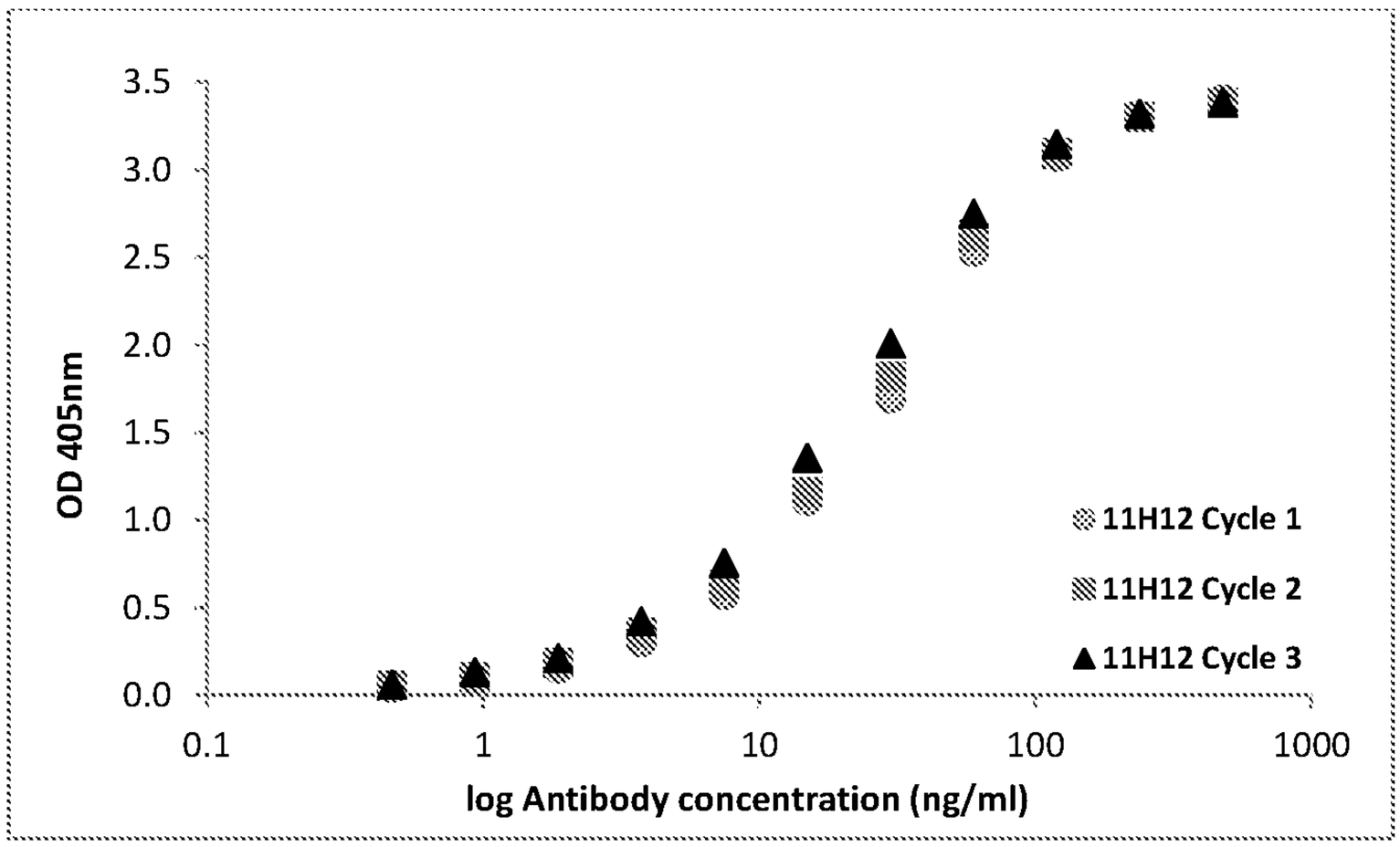


FIGURE 4C

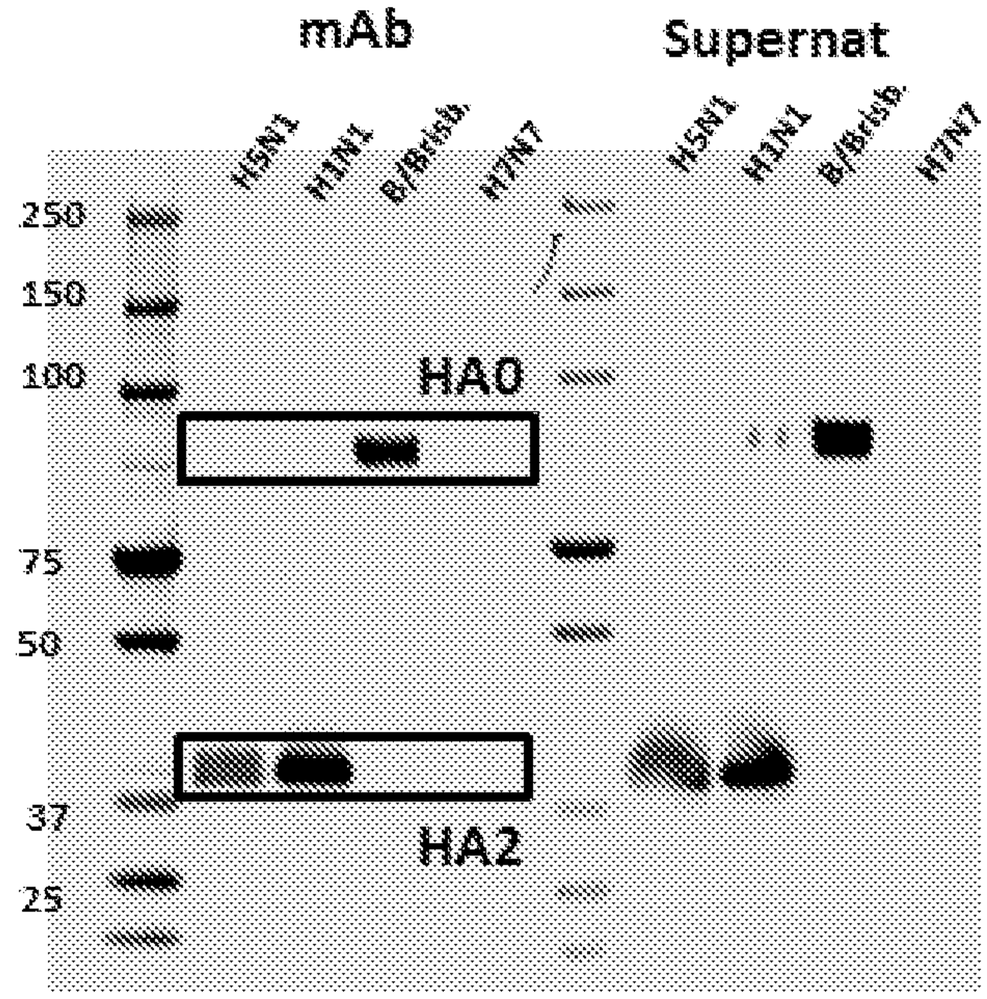


FIGURE 5A (11H12)

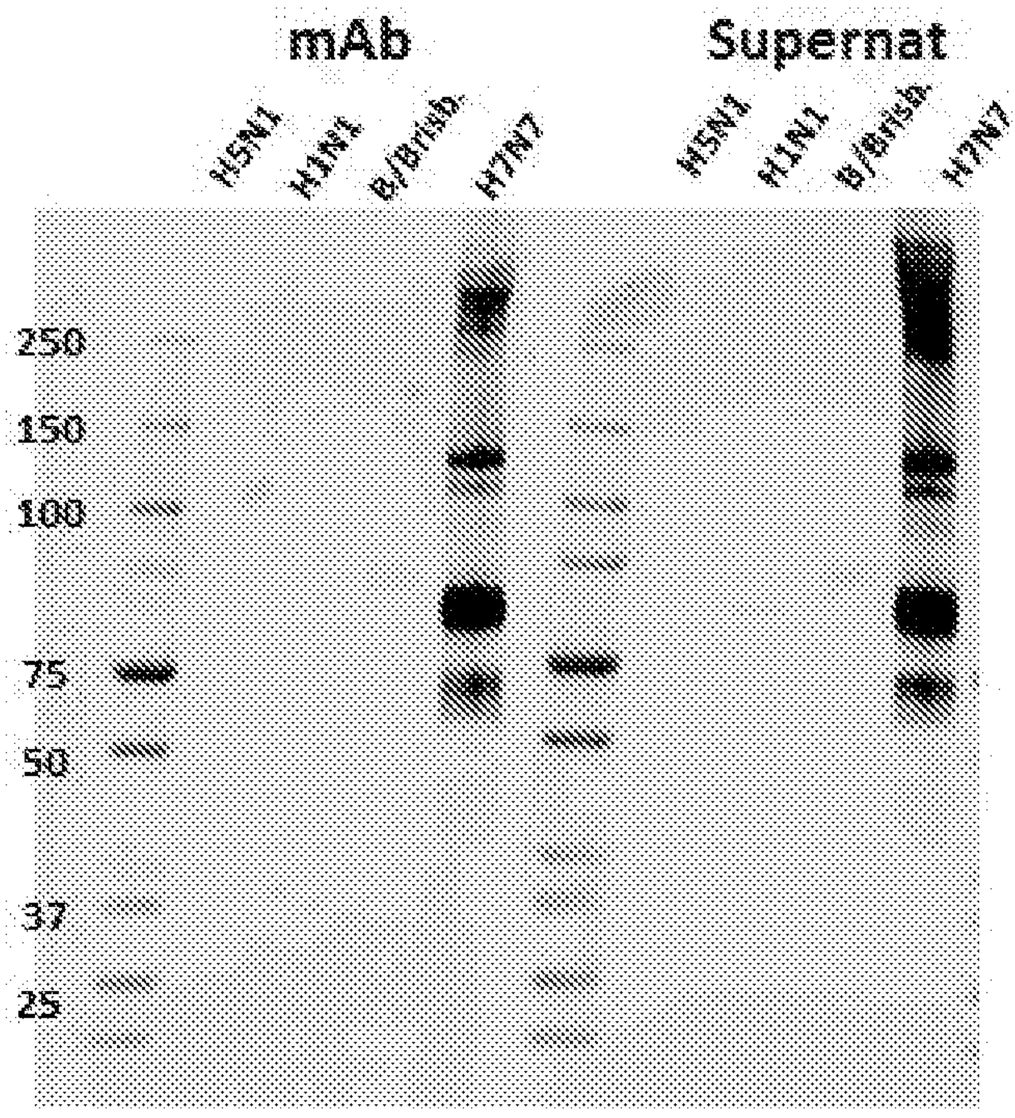


FIGURE 5B (10A9)

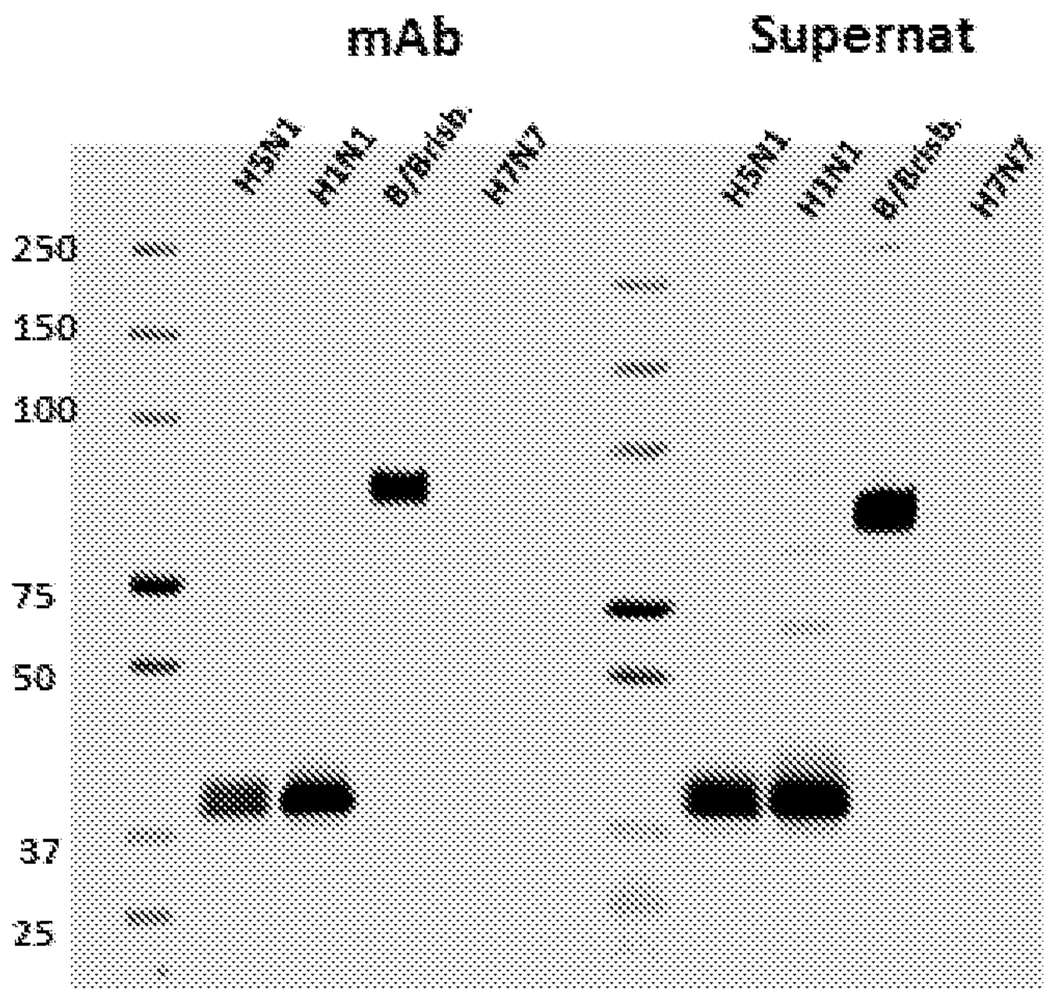


FIGURE 5C (9D1)

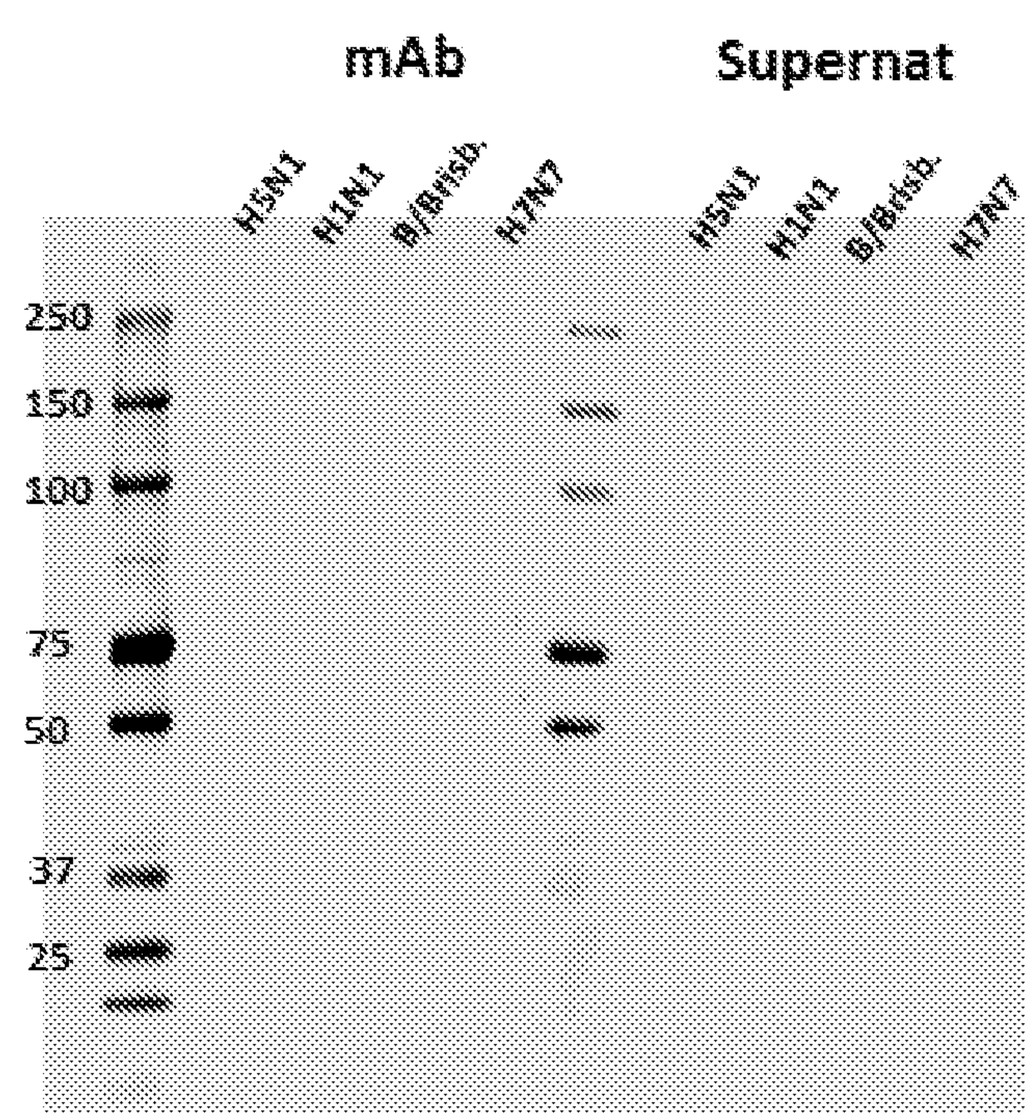


FIGURE 5D (anti-GFP)

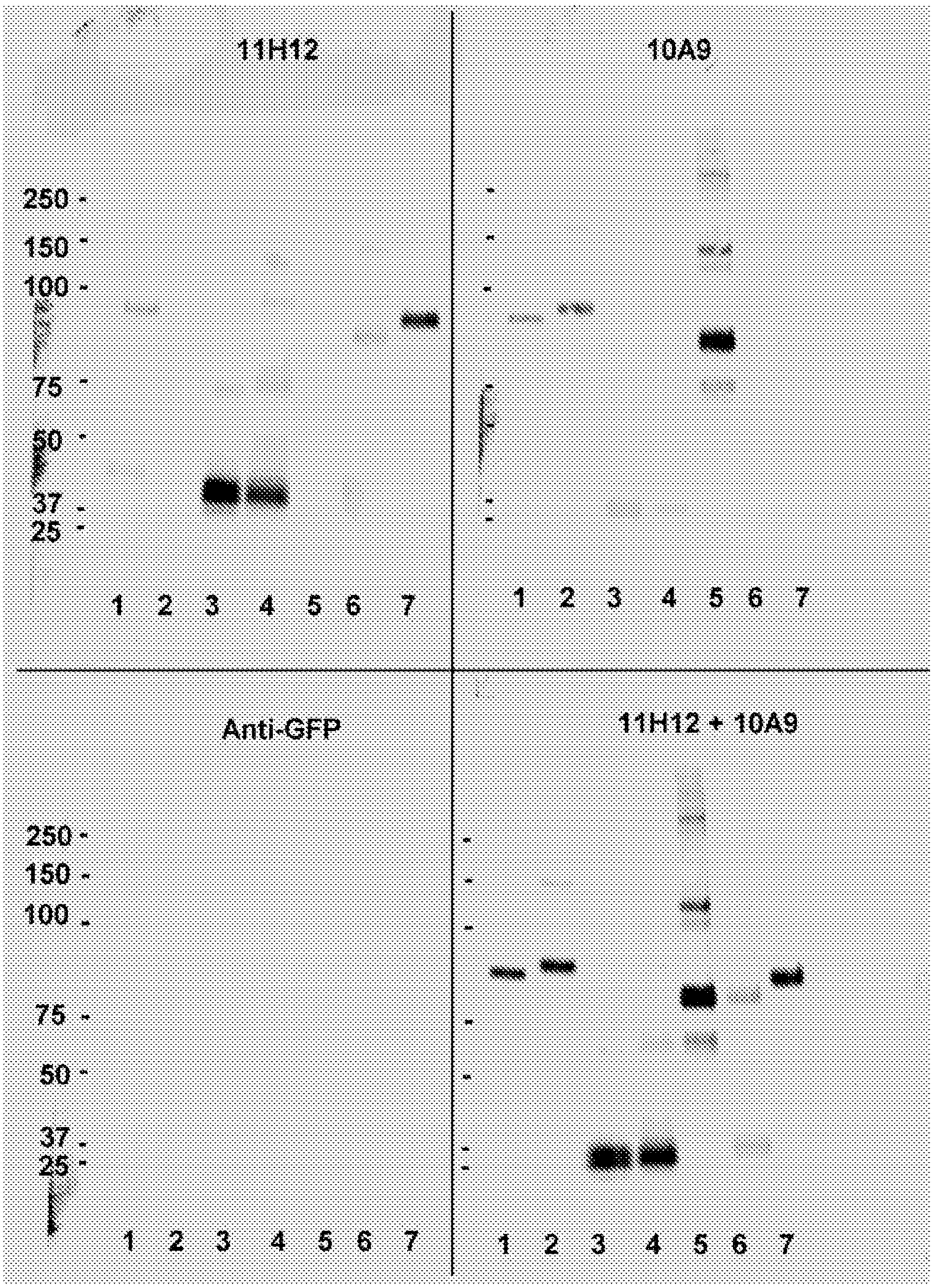

A.
B.
11H12
10A9
250
150
100
75
50
37
25
1 2 3 4 5 6 7
Anti-GFP
11H12 + 10A9
C.
D.

FIGURE 6

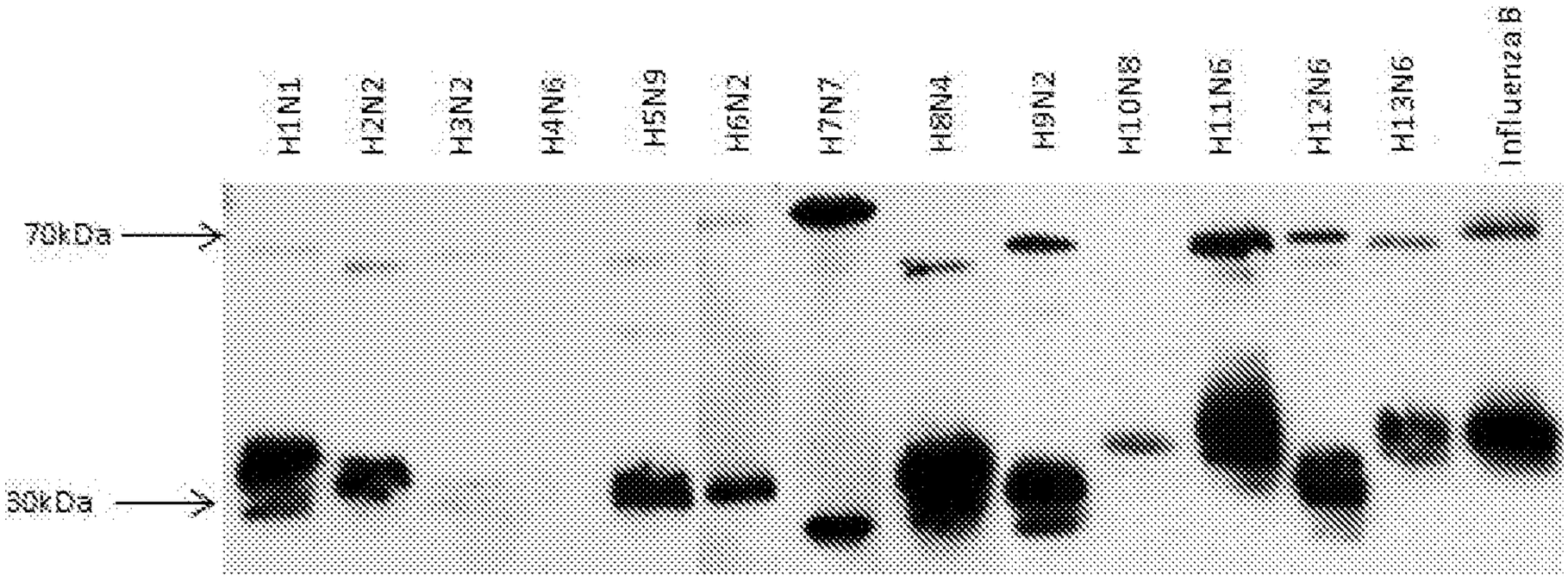


FIGURE 7A

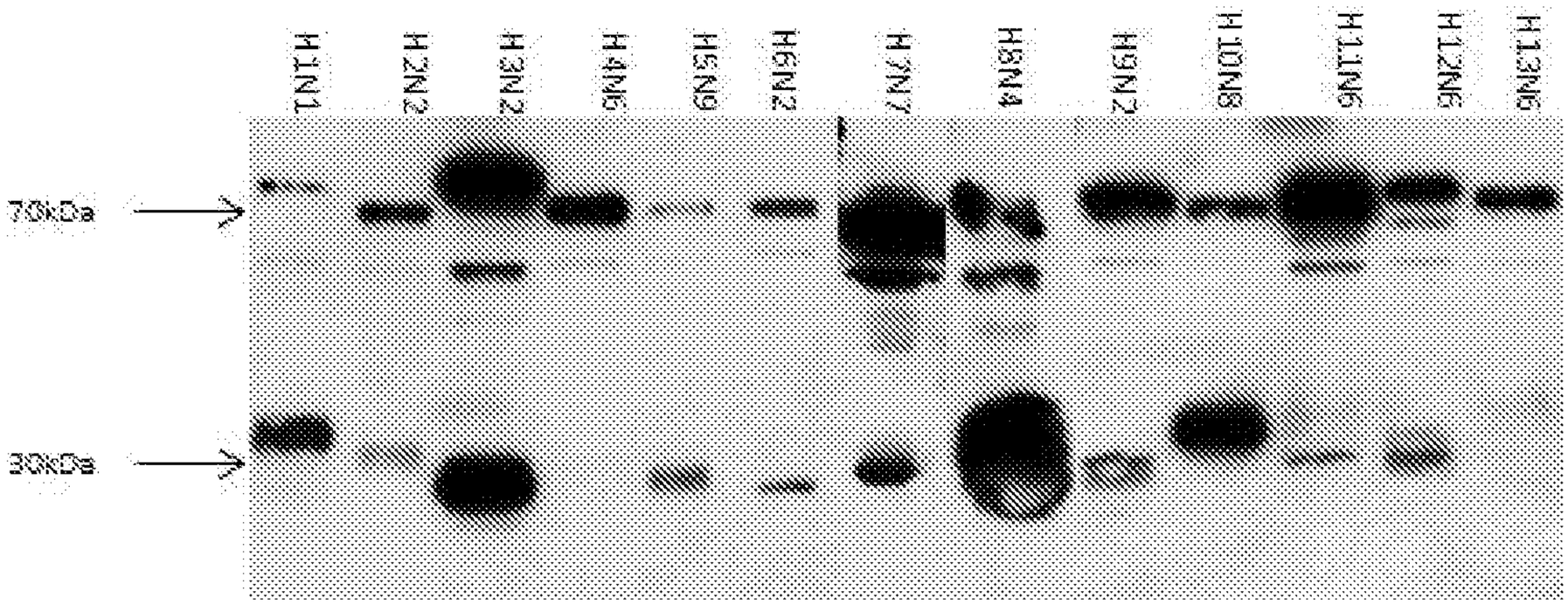
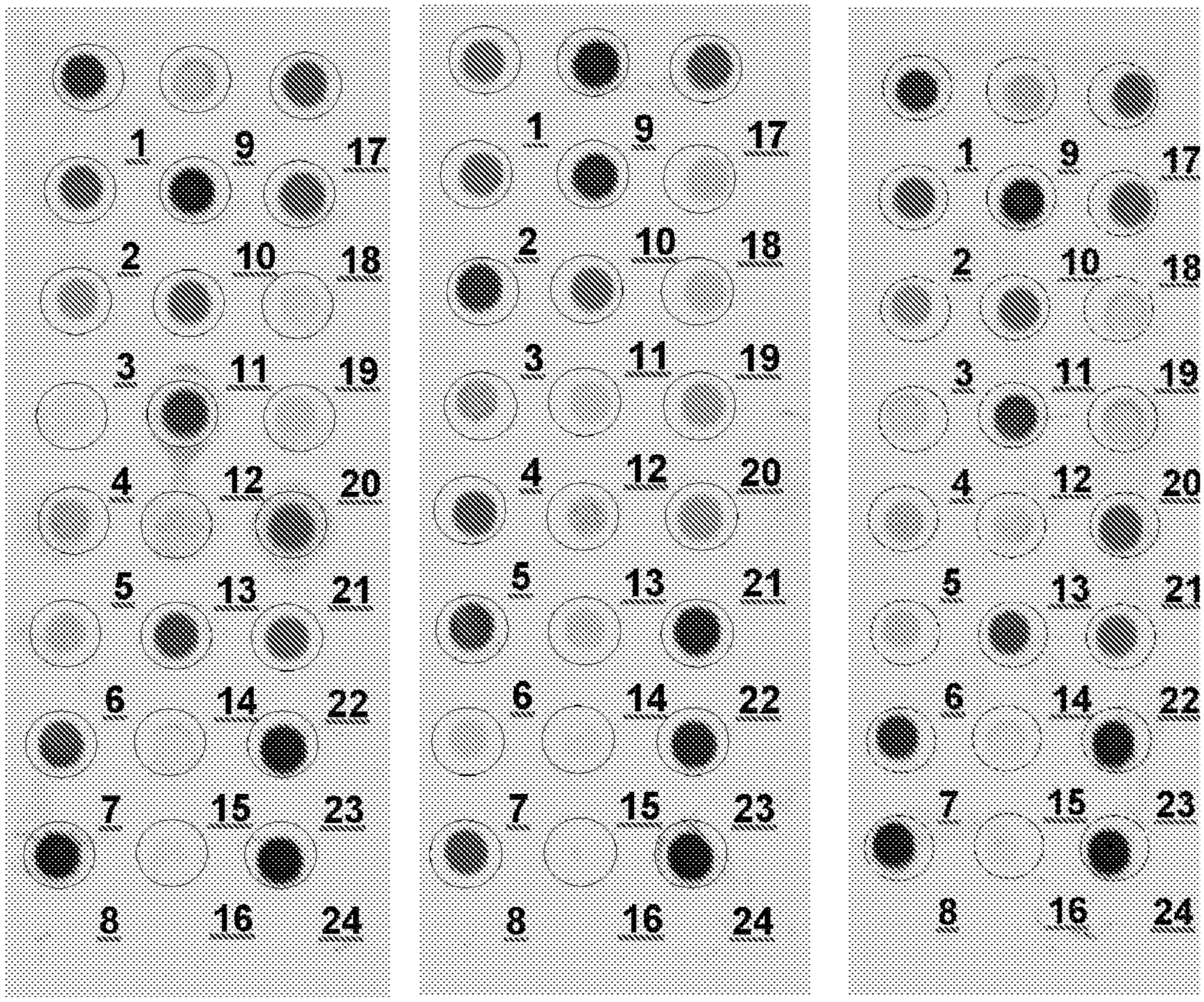


FIGURE 7B



FIGURES 8A (9D1) 8B (10A9) 8C (11H12)

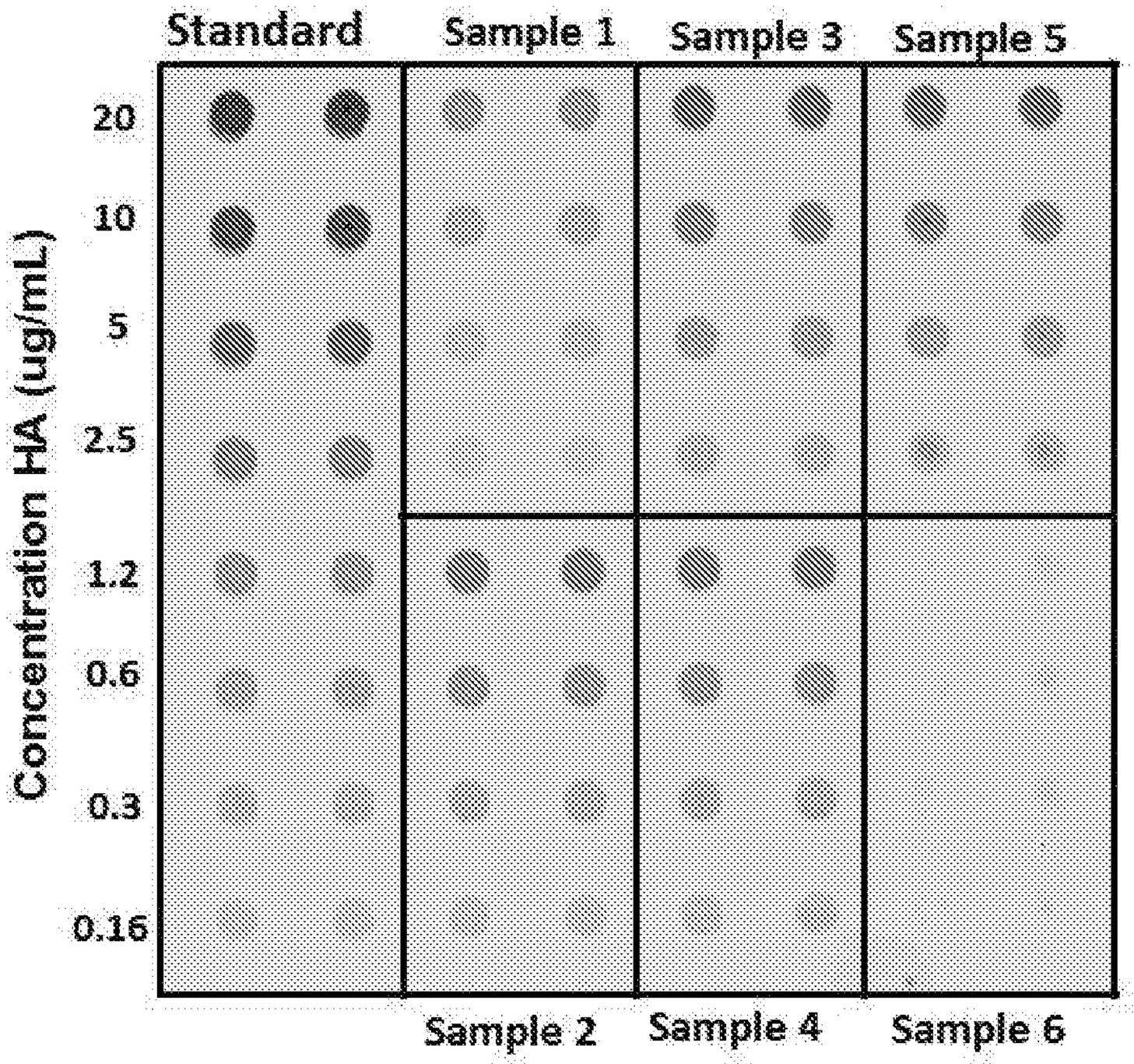


FIGURE 9A

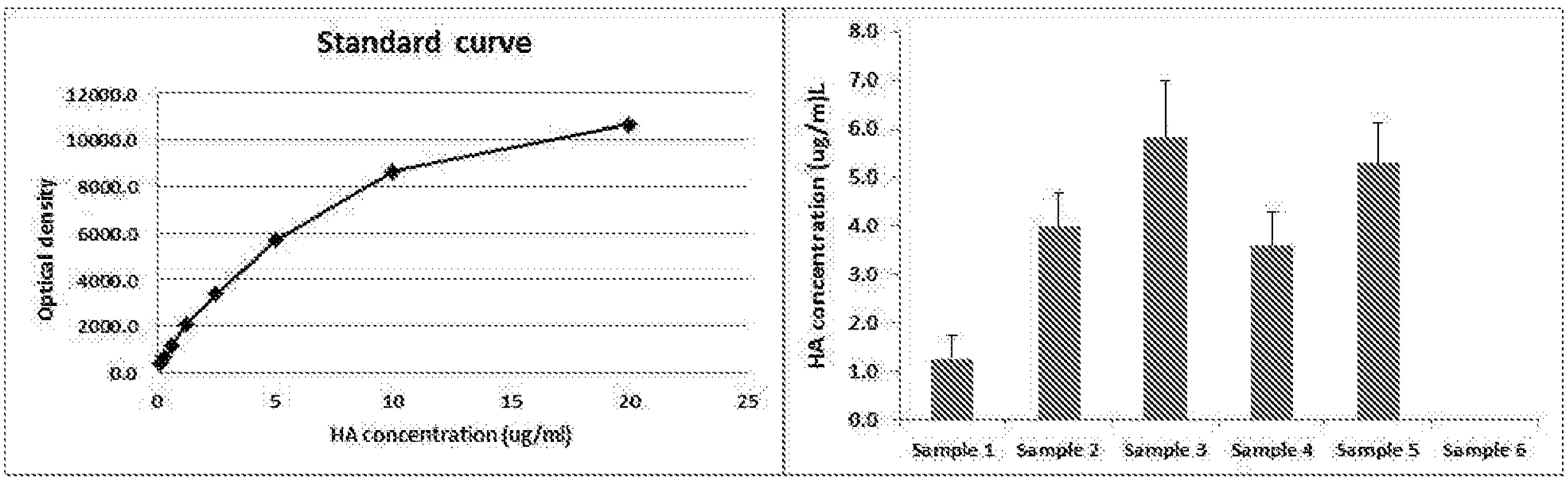


FIGURE 9B

FIGURE 9C

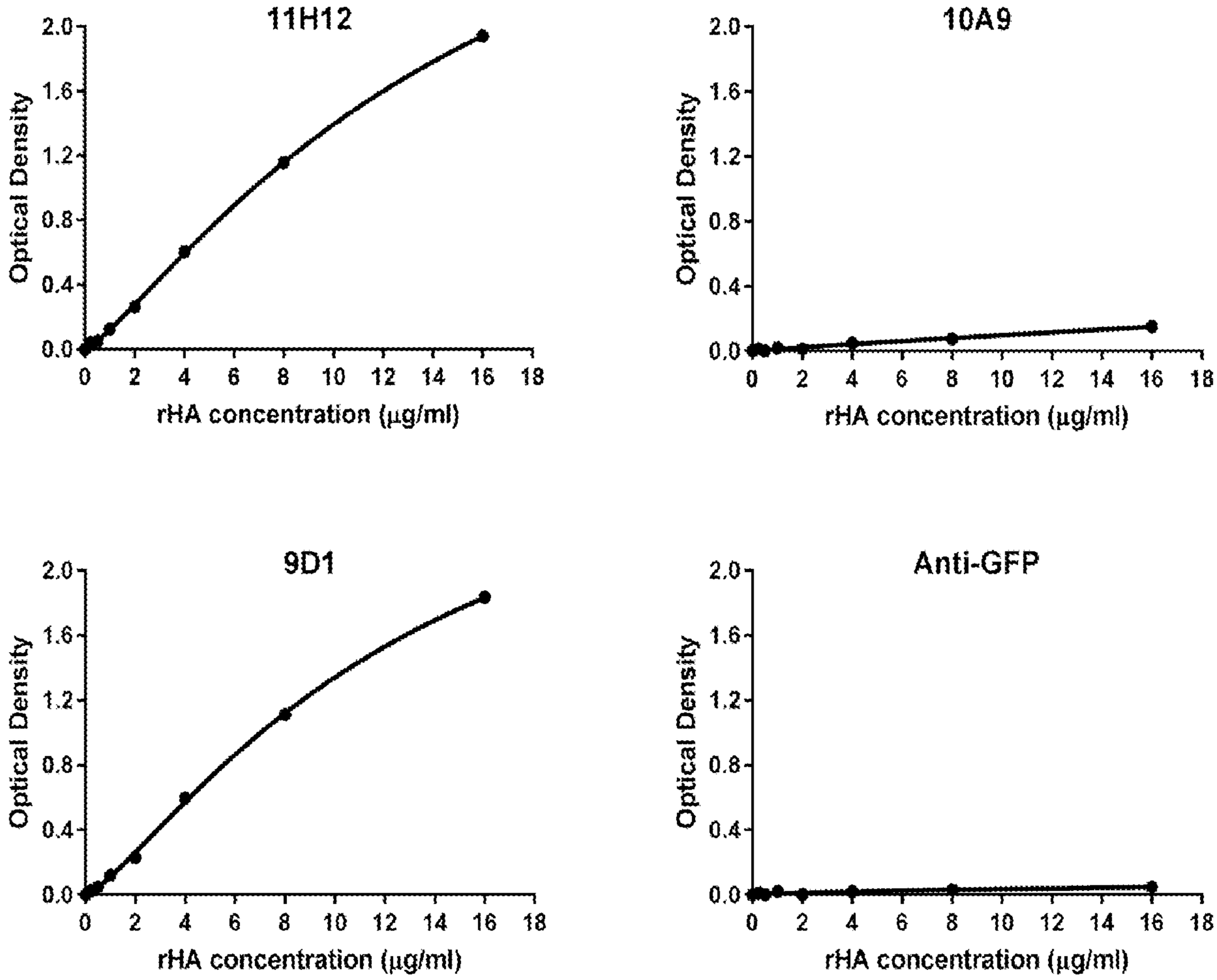

11H12
10A9
9D1
Anti-GFP
Optical Density
rHA concentration (µg/ml)
0.0
0.4
0.8
1.2
1.6
2.0
0
2
4
6
8
10
12
14
16
18

FIGURE 10

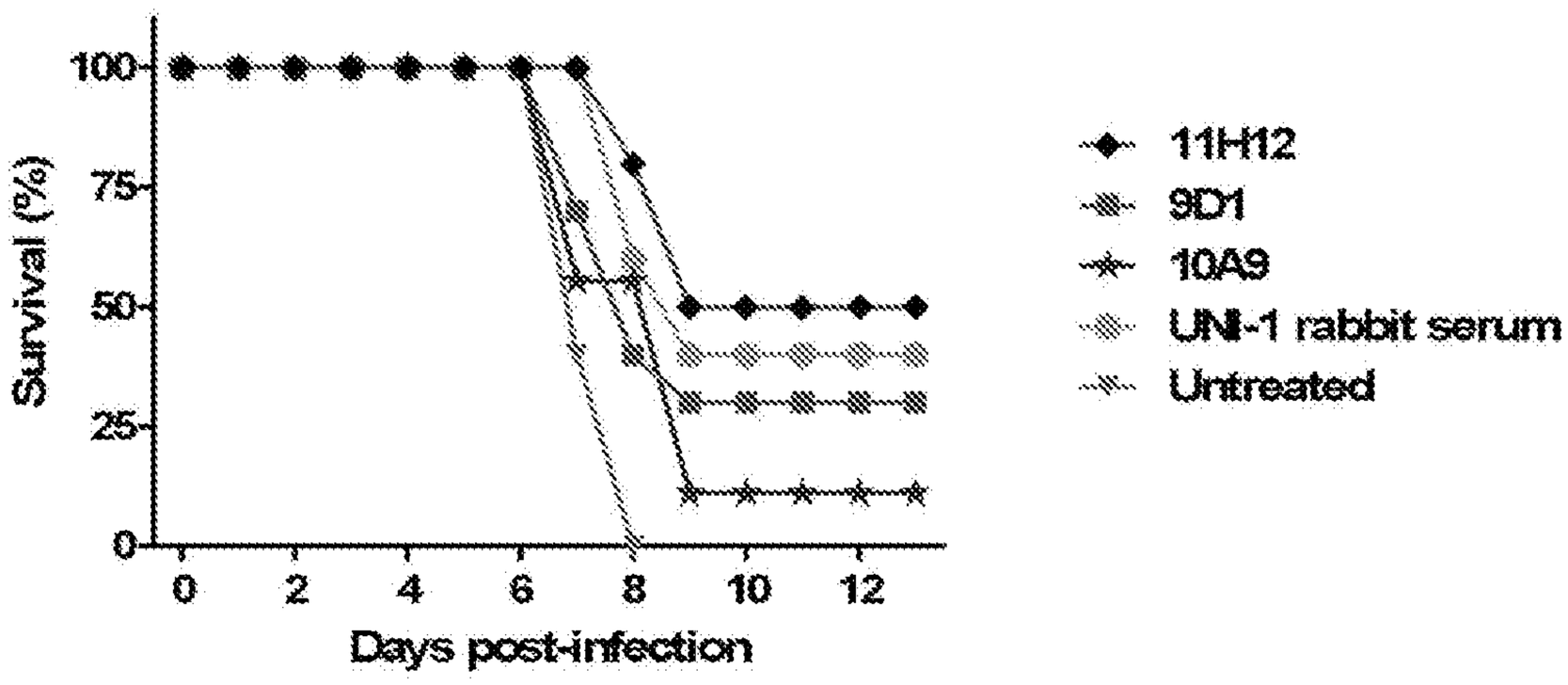


FIGURE 11A

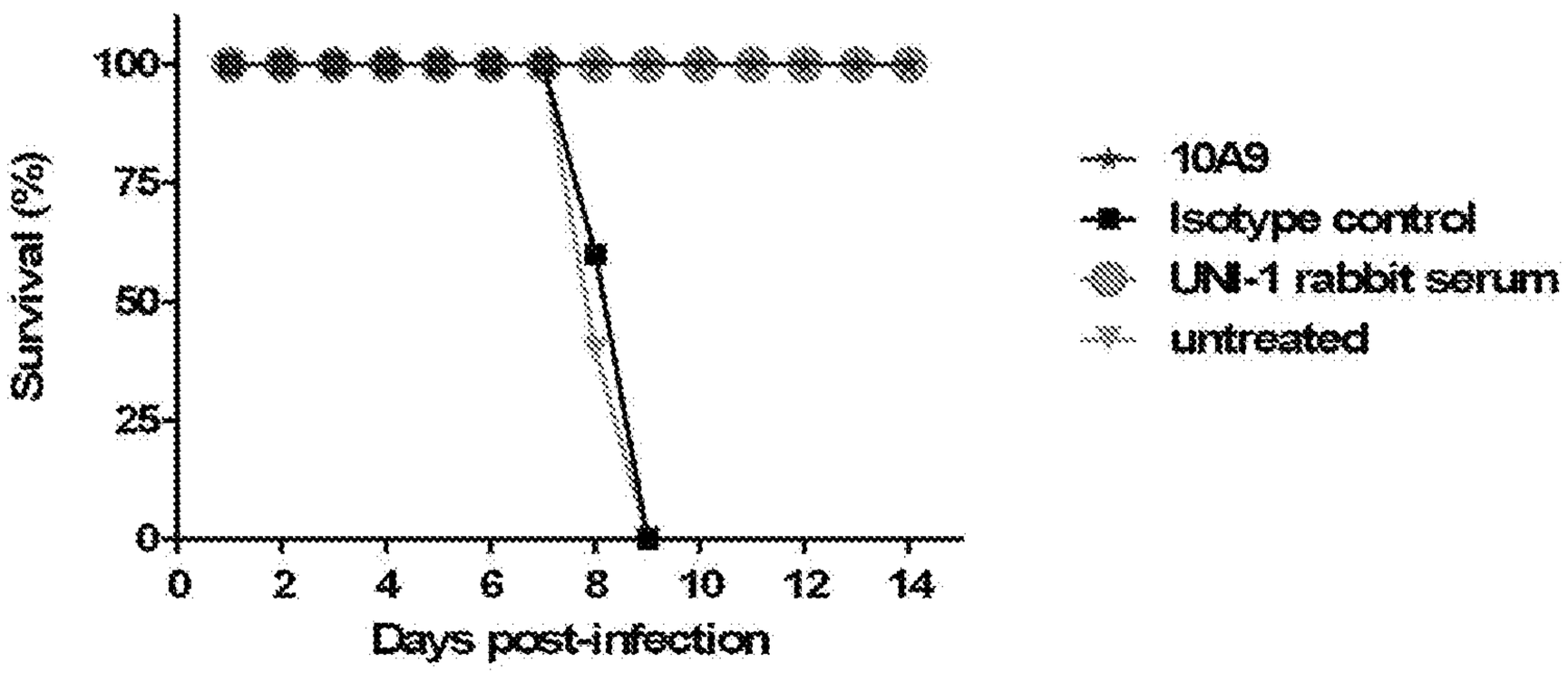


FIGURE 11B

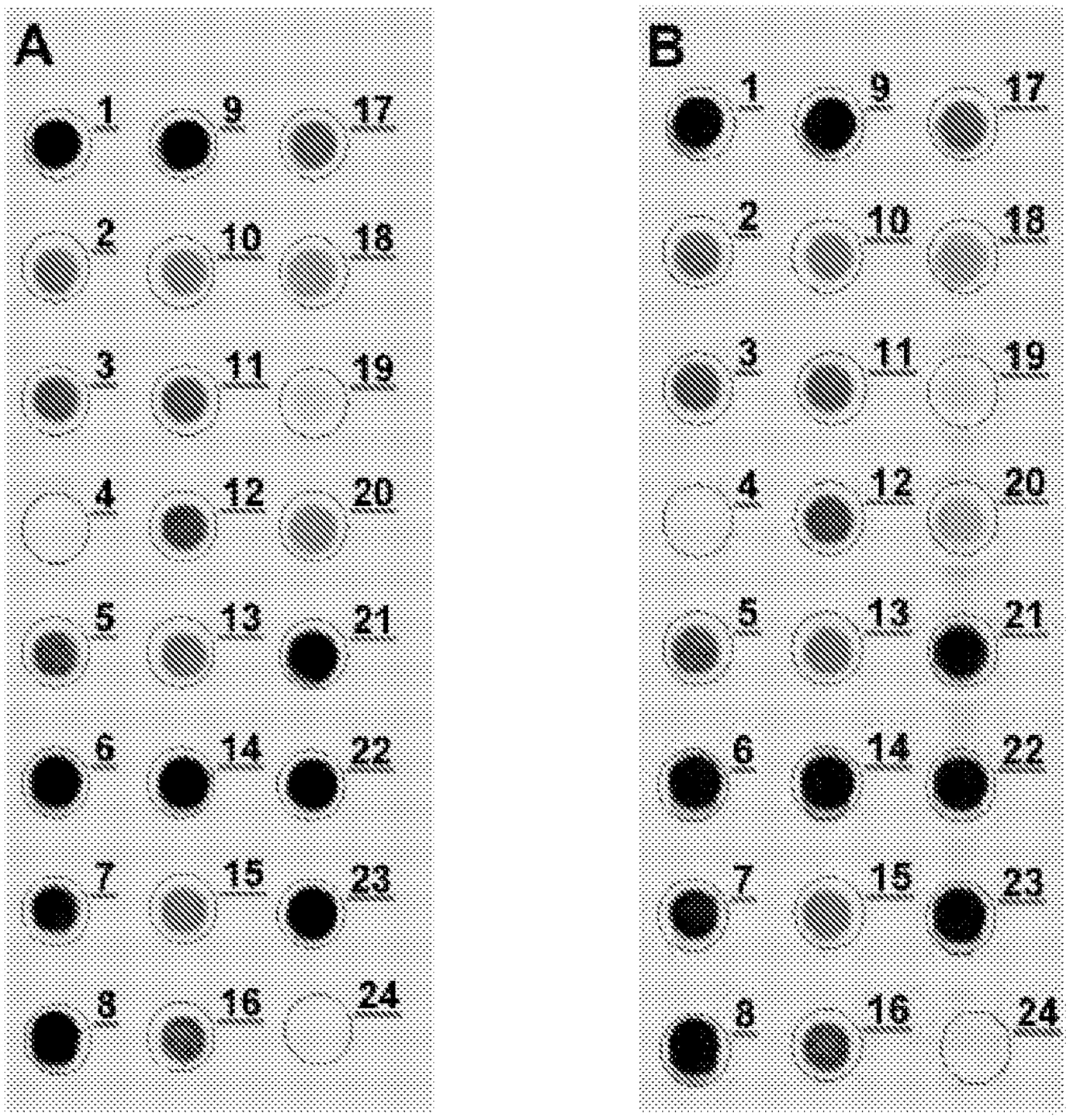

A
1
9
17
2
10
18
3
11
19
4
12
20
5
13
21
6
14
22
7
15
23
8
16
24
B
1
9
17
2
10
18
3
11
19
4
12
20
5
13
21
6
14
22
7
15
23
8
16
24

FIGURE 12

F211-11H12-2 sequence coverage: VL (SEQ ID NO. 29) and VH (SEQ ID NO.30)

Matched peptides shown in bold red.

Signal sequence

1 MMKVLSLLYL LTAIPGFLSD VQLQESGPGL VKPSQSLSLT CSVTGYSITS

51 DYYWNWIRQF PGNKLEWMAY ISYDGTNNYN PSLKNRISIT RDTSKNQFFL

101 KLNSVTTEDT ATYYCTRDRA WNDYYDYWG QGTTLTVSSA KTTAPSVYPL

151 APVCGDTTGS SVTLGCLVKG YFPEPVTLTW NSGSLSSGVH TFPAVLQSDL

201 YTLSSSVTVT SSTWPSQSIT CNVAHPASST KVDKKIEPRG PTIKPCPPCK

251 CPAPNLEGGP SVFIFPPKIK DVLMISLSPI VTCVVVDVSE DDPDVQISWF

301 VNNVEVHTAQ TQTHREDYNS TLRVVSALPI QHQDWMSGKE FKCKVNNKDL

351 PAPIERTISK PKGSVRAPQV YVLPPPEEEM TKKQVTLTCM VTDFMPEDIY

401 VEWTNNGKTE LNYKNTEPVL DSDGSYFMYS KLRVEKKNWV ERNSYSCSVV

451 HEGLHNHHTT KSFSRTPGK

Signal sequence

1 VLMLLLLWVS GTCGDIVMSQ SPSSLAVSVG EKVTMSCKSS QSLLNSRNQK

51 NYLAWYQQKP GQSPKLLIYW ASTRESGVPD RFTGSGSGTD FTLTISSVKA

101 EDLAVYYCQQ YYSYRTFGGG TKLEIKRADA APTVSIFPPS SEQLTSGGAS

151 VVCFLNNFYP KDINVKWKID GSERQNGVLN SWTDQDSKDS TYSMSSTLTL

201 TKDEYERHNS YTCEATHKTS TSPIVKSFNR NEC

FIGURE 13A

F211-10A9-2 sequence coverage: VL (SEQ ID NO. 31) and VH (SEQ ID NO.32)

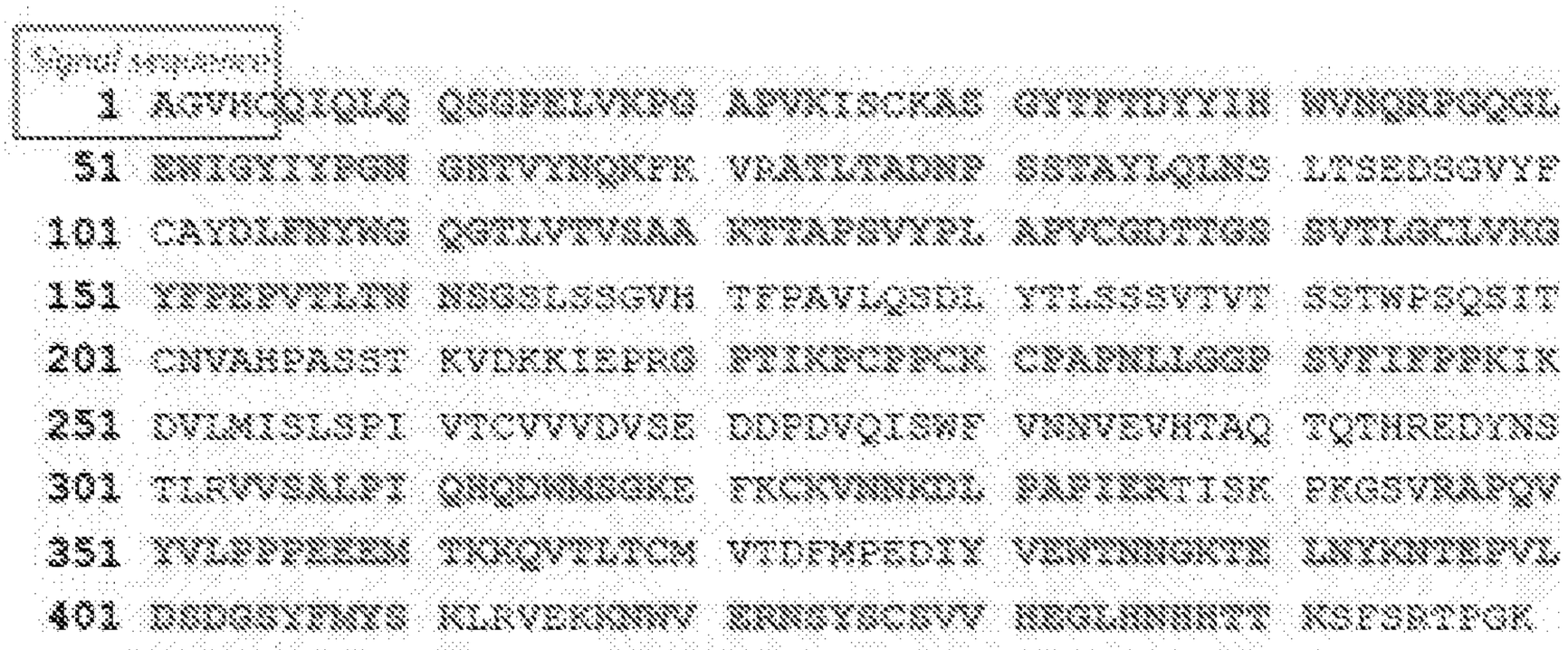


FIGURE 13B

F211-9D1-2 sequence coverage: VL (SEQ ID NO. 33) and VH (SEQ ID NO.34)

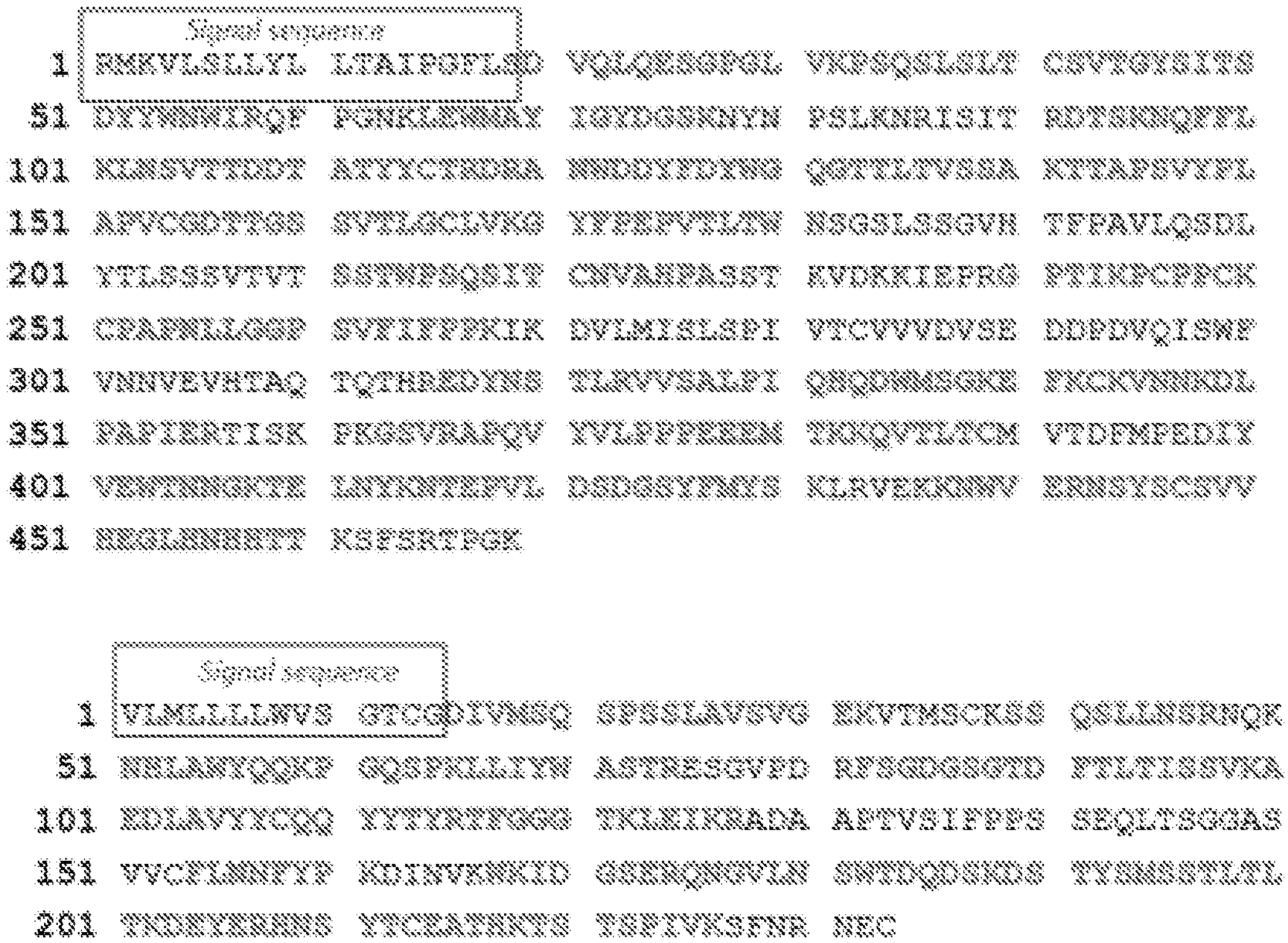


FIGURE 13C

10A9 full protein sequence

METDTLLLWVLLLWVPGSTGQIQLQQSGPELVKPGAPVKISCKASGYTFTDYYIHWVNQR
PGQGLEWIGYIYPGNGHTVYNQKFKVRATLTADNPSSTAYLQLNSLTSEDSGVYFCAYDL
FNYWGQGTLVTVSAAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSL
SSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKP
CPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISWFVNNVE
VHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSV
RAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSDGS
YFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGRKRRAPVKQTLNFDLLK
LAGDVESNPGPMETDTLLLWVLLLWVPGSTGDIVMTQSPSSLAMSVGQKVTMSCKSSQSL
LNSDTQKNFLAWYQQKPGQSPKILVYFASTKESGVPDRFIGSGSGTDFTLTITSVQAEDL
ADYFCQQYYSIPLTFGAGTKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKD
INVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTS
PIVKSFNRNEC*

FIGURE 14A

11H12 full protein sequence

METDTLLLWVLLLWVPGSTGDVQLQESGPGLVKPSQSLSLTCSVTGYSITSDYYWNWIRQ
FPGNKLEWMAYIGYDGTKNYNPSLKNRISITRDTSKNQFFLKLNSVTTDDTATYYCTRDR
ANWDDYFDYWGQGTTLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLT
WNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPR
GPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQISW
FVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPIERTIS
KPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPV
LDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGRKRRAPVKQTL
NFDLLKLAGDVESNPGPMETDTLLLWVLLLWVPGSTGDIVMSQSPSSLAVSVGEKVTMSC
KSSQSLLNSRNQKNHLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGDGSGTDFTLTISS
VKAEDLAVYYCQQYYTYRTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNN
FYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATH
KTSTSPIVKSFNRNEC*

FIGURE 14B

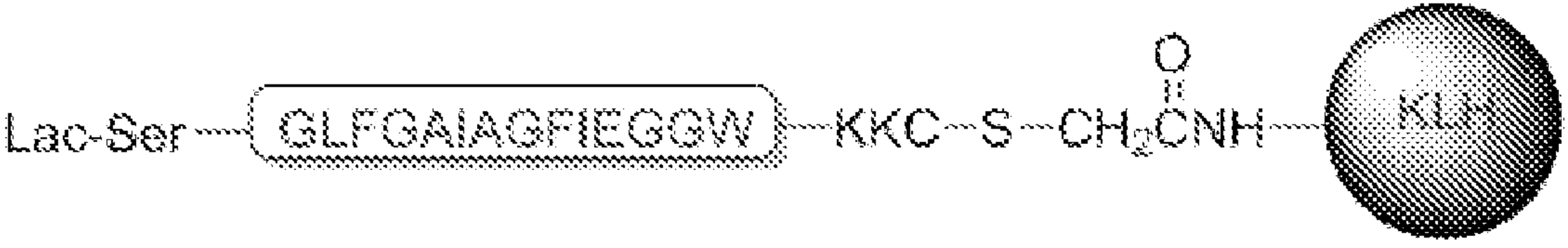


FIGURE 1A