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(54) **Titre : IDENTIFICATION D'ANTICORPS SPECIFIQUES DES LYSSAVIRUS ET LEURS PROCEDES D'UTILISATION**
(54) **Title: IDENTIFICATION OF ANTIBODIES SPECIFIC FOR LYSSAVIRUSES AND METHODS OF THEIR USE**

(57) **Abrégé/Abstract:**
Described herein is a method of identifying a monoclonal antibody (or antigen- binding fragment thereof) that specifically binds a plurality of lyssaviruses for use in post-exposure rabies prophylaxis or in the treatment of clinical rabies. The method includes using a naive antibody phage display library to screen for phage clones that bind whole recombinant rabies virus or cells expressing glycoprotein from multiple lyssaviruses (such as RABV, MOKV and WCBV) and/or specifically bind recombinant glycoprotein from different lyssaviruses.

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(54) **Title**: IDENTIFICATION OF ANTIBODIES SPECIFIC FOR LYSSAVIRUSES AND METHODS OF THEIR USE

(57) **Abstract**: Described herein is a method of identifying a monoclonal antibody (or antigen- binding fragment thereof) that specifically binds a plurality of lyssaviruses for use in post-exposure rabies prophylaxis or in the treatment of clinical rabies. The method includes using a naive antibody phage display library to screen for phage clones that bind whole recombinant rabies virus or cells expressing glycoprotein from multiple lyssaviruses (such as RABV, MOKV and WCBV) and/or specifically bind recombinant glycoprotein from different lyssaviruses.

IDENTIFICATION OF ANTIBODIES SPECIFIC FOR LYSSAVIRUSES AND METHODS OF THEIR USE

FIELD

This disclosure concerns methods of identifying lyssavirus-specific antibodies and the use of such antibodies in the treatment and prophylaxis of rabies.

BACKGROUND

Rabies is an inevitably fatal but preventable disease. In developing countries rabies remains a significant endemic disease burden. World-wide approximately 55,000 people die of rabies each year (WHO Expert Consultation on Rabies, 2004). Rabies is preventable with proper early post-exposure treatment. Currently, post-exposure prophylaxis includes thorough wound-washing with soap and water followed by administration of vaccine and anti-rabies virus immunoglobulin (RIG) of human or equine origin. RIG administered shortly after exposure at the wound site provides passive immunity which neutralizes rabies virus and prevents its spread until the patient's immune response following vaccination is elicited. Deaths due to post-exposure prophylaxis failure are most commonly attributed to deviations from the recommended regimen such as late initiation of post-exposure prophylaxis or no administration of RIG (Wilde, *Vaccine*, 25:7605-7609, 2007). In developing countries, availability of RIG is extremely low with only 1-2% of post-exposure prophylaxis being performed using RIG (Sudarshan *et al.*, *Int J Infect Dis*, 11:29-35, 2007; WHO Consultation on a Monoclonal Antibody Cocktail for Rabies Post Exposure Treatment, 2002). In the United States, only human derived RIG is administered due to the risk of anaphylactic shock from exposure to equine immunoglobulins, but the concern of blood-born pathogen transmission in human RIG remains.

Human monoclonal antibodies (mAbs) that neutralize rabies virus have long been recognized as an alternative to overcome the limitations of RIG (Dietzschold *et*

al., *J Virol*, 64:3087-3090, 1990). Adequate supplies of cell-cultured human mAbs could be produced in a cost-effective manner (Prosniak *et al.*, *J Infect Dis*, 188:53-56, 2003). In addition, the use of human mAbs reduces the likelihood of an adverse immune response (Weiner, *J Immunother*, 29:1-9, 2006) and has been shown to be as effective as RIG in preventing rabies in animals (de Kruif *et al.*, *Annu Rev Med*, 58:359-368, 2007). When a cocktail of two rabies-neutralizing, human mAbs was given with a rabies vaccine to animals experimentally infected with rabies, dose-dependent survival was observed, and all animals receiving the highest dose survived (Goudsmit *et al.*, *J Infect Dis*, 193:796-801, 2006). The same cocktail was recently shown to be safe to administer to healthy humans in two phase-one clinical trials (Bakker *et al.*, *Vaccine*, 26:5922-5927, 2008). However, selection of human monoclonal antibodies to include in such a cocktail has some limitations. The diversity of mAbs produced depends significantly on the diversity of viral antigens used to immunize human donors.

Rabies virus is a member (genotype 1) of the genus *Lyssavirus*. This genus also includes rabies-like viruses (genotypes 2-7) which can cause rabies disease in humans (Bourhy *et al.*, *Virology*, 194:70-81, 1993; Gould *et al.*, *Virus Res*, 54:165-187 1998). These viruses have a non-segmented, negative-sense, single-stranded RNA genome that encodes five proteins (Sokol *et al.*, *Virology*, 38:651-665, 1969; Sokol *et al.*, *J Virol*, 7:241-249, 1971). In the mature virion, RNA-dependent RNA polymerase, phosphoprotein, and nucleocapsid protein are associated with the genomic RNA while matrix protein and glycoprotein (G protein) surround it (Wiktor *et al.*, *J Immunol*, 110:269-276, 1973). Trimeric G protein "spikes" coat the surface of the virion and as the only surface exposed protein, is responsible for attachment and entry into host cells. This also makes G protein the primary antigen for induction of virus-neutralizing antibodies, and G protein-specific mAbs are included in the cocktails currently being developed for post-exposure prophylaxis (Dietzschold *et al.*, *J Virol*, 64:3087-3090, 1990; Kramer *et al.*, *Eur J Immunol*, 35:2131-2145, 2005; Wunner *et al.*, "Rabies Virus" in Rabies (Second Edition)," pp. 23-68, Academic Press, Oxford, 2007).

SUMMARY

Disclosed herein is a method of identifying an antibody (or antigen-binding fragment thereof) that specifically binds a plurality of lyssaviruses. Antibodies

identified by the methods provided herein can be used, for example, for post-exposure rabies prophylaxis or in the treatment of clinical rabies.

Provided herein is a method of identifying a monoclonal antibody or antigen-binding fragment thereof that specifically binds at least two different lyssaviruses. In some embodiments, the method includes screening a naïve antibody phage display library with at least two different lyssavirus glycoproteins, such as by panning the library against a recombinant virus expressing at least two different lyssavirus glycoproteins, panning the library against recombinant glycoprotein from at least two different lyssaviruses, panning the library against at least two different cell lines expressing different lyssavirus glycoproteins, or any combination thereof.

In particular embodiments, the method includes screening a naïve antibody phage display library with (1) a recombinant rabies virus expressing glycoprotein from rabies virus (RABV), Mokola virus (MOKV) and West Caucasian bat virus (WCBV); (2) recombinant glycoprotein from at least two of RABV, MOKV, WCBV, Lagos bat virus (LBV) and Duvenhage virus (DUVV); and/or (3) at least two different cell lines expressing different lyssavirus glycoproteins selected from RABV, MOKV, WCBV, LBV and DUVV glycoprotein. The method further includes selecting a phage display clone that specifically binds to at least two different lyssaviruses, at least two different lyssavirus glycoproteins, or both.

In some embodiments, the phage display library is a naïve human V_H domain library.

Also provided are isolated monoclonal antibodies (or antigen-binding fragments thereof) identified according to the methods disclosed herein and their use in the treatment or prophylaxis of rabies.

Further provided are isolated monoclonal antibodies (or antigen-binding fragments thereof) that specifically bind at least two different lyssaviruses or that specifically bind recombinant glycoprotein from at least two different lyssaviruses. In some embodiments, the V_H domain of the antibody is encoded by a nucleotide sequence at least 85% identical to any one of SEQ ID NOs: 1-110. Also provided are methods of treating rabies in a subject by administering to the subject a monoclonal antibody disclosed herein.

Also provided is an isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds at least two different lyssaviruses or that specifically binds glycoprotein from at least two different lyssaviruses, wherein the at least two different lyssaviruses are selected from rabies virus (RABV), Mokola virus (MOKV), West Caucasian bat virus (WCBV), Lagos bat virus (LBV) and Duvenhage virus (DUVV), and wherein the V_H domain of the antibody is encoded by the nucleotide sequence comprising any one of SEQ ID NOs: 1-110.

In embodiments, the antibody specifically binds rabies virus (RABV), Mokola virus (MOKV) or West Caucasian bat virus (WCBV) glycoprotein when encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 1-43.

In further embodiments, the antibody specifically binds Lagos bat virus (LBV), MOKV, WCBV or Duvenhage virus (DUVV) glycoprotein when encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 44-110.

Also provided is an isolated variable heavy (VH) domain monoclonal antibody that specifically binds lyssavirus glycoprotein, wherein the antibody is encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 1-110.

Also provided is an isolated immunoconjugate comprising a monoclonal antibody described herein and a fusion partner.

Also provided is a composition comprising a monoclonal antibody or immunoconjugate described herein, and a pharmaceutically acceptable carrier.

Also provided is a composition comprising a monoclonal antibody and a monoclonal antibody specific for RABV or RABV glycoprotein.

Also provided is an isolated nucleic acid molecule encoding a monoclonal antibody described herein.

Also provided is an expression vector comprising a nucleotide sequence at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the nucleotide sequence of any one of SEQ ID NOs: 1-110.

Expression vectors, such as an Fc IgG expression vector, comprising the nucleotide sequence of any one of SEQ ID NOs: 1-110 are also provided by the present disclosure. In some embodiments, the expression vector further comprises the nucleotide sequence of a variable light (V_L) domain from a lyssavirus-specific (such as rabies virus-specific) monoclonal antibody. A cell comprising an expression vector disclosed herein is further provided. Also provided are monoclonal antibodies encoded by the expression vectors.

A method of treating rabies in a subject by administering to the subject a monoclonal antibody expressed by an expressed vector disclosed herein is also provided.

Also provided is a use of a monoclonal antibody, immunoconjugate, or composition described herein, for treating rabies in a subject.

Also provided is a use of a monoclonal antibody, immunoconjugate, or composition described herein, for the preparation of a medicament for treating rabies in a subject.

Also provided is a monoclonal antibody, immunoconjugate, or composition described herein, for use in treating rabies in a subject.

The foregoing and other objects, features, and advantages of the invention will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1: Construction of ERA-3G. The G333 mutation is introduced into the ERA backbone and two transcriptional (trans) units are added. The transcriptional units are introduced between the P and M genes and between the G and L genes. The MOKV and WCBV G genes are cloned into the transcriptional units to form a recombinant ERA rabies virus with three glycoprotein genes (ERA-3G).

FIG. 2: Results of whole virus ELISA. Relative binding of selected human variable heavy (V_H) domain phage display clones to recombinant ERA RABV expressing glycoprotein (G) from RABV, MOKV and WCBV. The left bar for each clone shown indicates clones selected using the whole recombinant virus ERA RABV expressing glycoprotein (G) from RABV, MOKV and WCBV. The right bar for each clone shown indicates clones selected using glycoproteins purified from the recombinant ERA RABV expressing glycoprotein (G) from RABV, MOKV and WCBV. Using a standard ELISA method, raw absorbance (420nm-650nm) was fixed relative to a known clone binding to its cognate antigen (positive control).

FIG. 3: Results of glycoprotein ELISA. Relative binding of selected human variable heavy (V_H) domain phage display clones to glycoproteins purified from recombinant ERA RABV expressing glycoprotein (G) from RABV, MOKV and WCBV. Left bars for each clone indicate clones selected using the whole recombinant virus ERA RABV expressing glycoprotein (G) from RABV, MOKV and WCBV. Right bars for each clone indicate clones selected using glycoproteins purified from the

recombinant ERA RABV expressing glycoprotein (G) from RABV, MOKV and WCBV. Using a standard ELISA method, raw absorbance (420nm-650nm) was fixed relative to a known clone binding to its cognate antigen (positive control).

FIG. 4: Results of rapid fluorescent focus inhibition test (RFFIT). Rabies virus ERA3G was incubated with standard dilutions of soluble domain antibodies from selected clones for 20 hours. Virus neutralization was assessed using direct immunofluorescent staining. Results of three biological replicates are shown with titers less than 0.02 IU/ml indicated by the symbol (<).

SEQUENCES

The nucleotide and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence listing:

SEQ ID NOs: 1-43 are the nucleotide sequences of human variable heavy (V_H) domain phage display clones selected using a recombinant ERA RABV expressing glycoprotein (G) from RABV, MOKV and WCBV.

SEQ ID NOs: 44-110 are the nucleotide sequences of human variable heavy (V_H) domain phage display clones identified by sequential selection on cells expressing LBV glycoprotein, cells expressing MOKV glycoprotein, cells expressing WCBV glycoprotein and cells expressing DUVV glycoprotein.

SEQ ID NOs: 111 and 112 are the nucleotide sequences of RT-PCR primers for amplification of the MOKV G gene.

SEQ ID NOs: 113 and 114 are the nucleotide sequences of RT-PCR primers for amplification of the WCBV G gene.

SEQ ID NO: 115 is the nucleotide sequence of MOKV G.

SEQ ID NO: 116 is the nucleotide sequence of WCBV G.

SEQ ID NO: 117 is the nucleotide sequence of a transcription unit for incorporating heterologous ORFs.

SEQ ID NO: 118 is the nucleotide sequence of LBV G.

SEQ ID NO: 119 is the nucleotide sequence of DUVV G.

DETAILED DESCRIPTION

I. Abbreviations

ABLV	Australian bat lyssavirus
ARAV	Aravan virus
BBLV	Bokeloh bat lyssavirus
CDR	complementarity determining region
dAb	domain antibody
DUVV	Duvenhage virus
EBLV-1	European bat lyssavirus-1
EBLV-2	European bat lyssavirus-2
ELISA	enzyme-linked immunosorbent assay
ERA	Evelyn-Rokitnicki-Abelseth
G	glycoprotein
IRKV	Irkut virus
KHUV	Khujand virus
L	RNA-dependent RNA polymerase
LBV	Lagos bat virus
M	matrix protein
mAb	monoclonal antibody
MOKV	Mokola virus
N	nucleoprotein
P	phosphoprotein
RABV	rabies virus
RIG	anti-rabies virus immunoglobulin
RNP	ribonucleoprotein
RABV	rabies virus
RFFIT	rapid fluorescent focus inhibition test
SHIBV	Shimoni bat virus
V_H	variable heavy
V_L	variable light
WCBV	West Caucasian bat virus

II. Terms and Methods

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

Administer: As used herein, administering a composition, such as an antibody, to a subject means to give, apply or bring the composition into contact with the subject. Administration can be accomplished by any of a number of routes, such as, for example, topical, oral, subcutaneous, intramuscular, intraperitoneal, intravenous, intrathecal and intramuscular.

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. The term "animal" includes both human and veterinary subjects, for example, humans, non-human primates, dogs, cats, horses, raccoons, bats, rats, mice, foxes, squirrels, opossum, coyotes, wolves and cows.

Antibody: A protein (or protein complex) that includes one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

The basic immunoglobulin (antibody) structural unit is generally a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" (about 50-70 kDa) chain. The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms "variable light chain"

(V_L) and "variable heavy chain" (V_H) refer, respectively, to these light and heavy chains.

As used herein, the term "antibody" includes intact immunoglobulins as well as a number of well-characterized fragments. For instance, Fabs, Fvs, and single-chain Fvs (scFvs) that bind to target protein (or epitope within a protein or fusion protein) would also be specific binding agents for that protein (or epitope). These antibody fragments are as follows: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (3) $(Fab')_2$, the fragment of the antibody obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; (4) $F(ab')_2$, a dimer of two Fab' fragments held together by two disulfide bonds; (5) Fv, a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (6) single chain antibody, a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Methods of making these fragments are routine (see, for example, Harlow and Lane, *Using Antibodies: A Laboratory Manual*, CSHL, New York, 1999).

Each heavy and light chain contains a constant region and a variable region (the regions are also known as "domains"). In combination, the heavy and the light chain variable regions specifically bind the antigen. Light and heavy chain variable regions contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs." The extent of the framework region and CDRs has been defined (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991). The Kabat database is now maintained online. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species, such as humans. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. An antibody that binds a specific antigen will have a specific V_H region and the V_L region sequence, and thus specific CDR sequences. Antibodies with different specificities (*i.e.* different combining sites for different antigens) have different CDRs. Although it is the CDRs that vary from antibody to antibody, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. These positions within the CDRs are called specificity determining residues (SDRs).

References to "V_H" or "VH" refer to the variable region of an immunoglobulin heavy chain, including that of an Fv, scFv, dsFv or Fab. References to "V_L" or "VL" refer to the variable region of an immunoglobulin light chain, including that of an Fv, scFv, dsFv or Fab.

A "monoclonal antibody" is an antibody produced by a single clone of B lymphocytes or by a cell into which the light and heavy chain genes of a single antibody have been transfected. Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells. Monoclonal antibodies include humanized monoclonal antibodies. As used herein "monoclonal antibodies" further includes antigen-binding fragments, such as Fv, scFv, dsFv or Fab fragments.

A "chimeric antibody" has framework residues from one species, such as human, and CDRs (which generally confer antigen binding) from another species, such as a murine antibody.

A "humanized" immunoglobulin is an immunoglobulin including a human framework region and one or more CDRs from a non-human (for example a mouse, rat, or synthetic) immunoglobulin. The non-human immunoglobulin providing the CDRs is termed a "donor," and the human immunoglobulin providing the framework is termed an "acceptor." In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are,

they must be substantially identical to human immunoglobulin constant regions, *i.e.*, at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A "humanized antibody" is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. A humanized antibody binds to the same antigen as the donor antibody that provides the CDRs. The acceptor framework of a humanized immunoglobulin or antibody may have a limited number of substitutions by amino acids taken from the donor framework. Humanized or other monoclonal antibodies can have additional conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. Humanized immunoglobulins can be constructed by means of genetic engineering (see for example, U.S. Patent No. 5,585,089).

A "human" antibody (also called a "fully human" antibody) is an antibody that includes human framework regions and all of the CDRs from a human immunoglobulin. In one example, the framework and the CDRs are from the same originating human heavy and/or light chain amino acid sequence. However, frameworks from one human antibody can be engineered to include CDRs from a different human antibody. All parts of a human immunoglobulin are substantially identical to corresponding parts of natural human immunoglobulin sequences.

Antibody binding affinity: The strength of binding between a single antibody binding site and a ligand (*e.g.*, an antigen or epitope). The affinity of an antibody binding site X for a ligand Y is represented by the dissociation constant (K_d), which is the concentration of Y that is required to occupy half of the binding sites of X present in a solution. A smaller K_d indicates a stronger or higher-affinity interaction between X and Y and a lower concentration of ligand is needed to occupy the sites. In general, antibody binding affinity can be affected by the alteration, modification and/or substitution of one or more amino acids in the epitope recognized by the antibody paratope. Binding affinity can be measured using any technique known in the art, such as end-point titration in an Ag-ELISA assay.

Antigen: A compound, composition, or substance that can stimulate the production of antibodies or a T-cell response in an animal, including compositions that

are injected or absorbed into an animal. An antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens.

Complementarity determining region (CDR): Amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native Ig binding site. The light and heavy chains of an Ig each have three CDRs, designated L-CDR1, L-CDR2, L-CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively.

Effector molecule (EM): The portion of a chimeric molecule that is intended to have a desired effect on a cell or system or substance to which the chimeric molecule is targeted. The term effector molecule is interchangeable with effector moiety, therapeutic agent, diagnostic agent, and similar terms.

Therapeutic agents include such compounds as nucleic acids, proteins (including monoclonal antibodies and antigen-binding fragments of monoclonal antibodies), peptides, amino acids or derivatives, glycoproteins, radioisotopes, lipids, carbohydrates, recombinant viruses or toxins. Nucleic acid therapeutic and diagnostic moieties include antisense nucleic acids, derivatized oligonucleotides for covalent cross-linking with single or duplex DNA, and triplex forming oligonucleotides. Diagnostic agents or moieties include radioisotopes and other detectable labels. Detectable labels useful for such purposes are also well known in the art, and include radioactive isotopes such as ^{32}P, ^{125}I, and ^{131}I, fluorophores, chemiluminescent agents, and enzymes.

Evelyn-Rokitnicki-Abelseth (ERA): The ERA strain of rabies virus was derived from the Street-Alabama-Dufferin (SAD) strain, first isolated from a rabid dog in Alabama (USA) in 1935. The ERA strain was derived after multiple passages of SAD rabies virus in mouse brains, baby hamster kidney (BHK) cells, and chicken embryos.

Framework region: Amino acid sequences interposed between CDRs (or hypervariable regions). Framework regions include variable light and variable heavy framework regions. Each variable domain comprises four framework regions, often referred to as FR1, FR2, FR3 and FR4. The framework regions serve to hold the CDRs in an appropriate orientation for antigen binding. Framework regions typically form β-sheet structures.

Fusion partner: Refers to any molecule that is fused (such as covalently linked) to another molecule. In the context of the present disclosure, an

immunoconjugate includes an antibody linked to a fusion partner. In some examples, the fusion partner is an effector molecule, a label (such as a detectable label), a heterologous polypeptide or a drug.

Fusion protein: A protein generated by expression of a nucleic acid sequence engineered from nucleic acid sequences encoding at least a portion of two different (heterologous) proteins. To create a fusion protein, the nucleic acid sequences must be in the same reading frame and contain no internal stop codons in that frame.

Immunoconjugate: A covalent linkage of a fusion partner, such as an effector molecule, label, heterologous polypeptide or other moiety, to an antibody or antigen binding fragment thereof. The linkage can be by chemical or recombinant means, for instance. In some cases, the linkage is chemical, wherein a reaction between the antibody moiety and the fusion partner has produced a covalent bond formed between the two molecules to form one molecule. A peptide linker (short peptide sequence) can optionally be included between the antibody and the effector molecule.

Inhibiting or treating a disease: Inhibiting the full development of a disease or condition, for example, in a subject who is at risk for a disease. One specific example of a disease is rabies. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. As used herein, the term "ameliorating," with reference to a disease, pathological condition or symptom, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the number of relapses of the disease, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease.

Isolated: An "isolated" or "purified" biological component (such as a nucleic acid, peptide, protein, protein complex, antibody or particle) has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, that is, other chromosomal and extra-chromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins that have been "isolated" or "purified" thus include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic

acids, peptides and proteins prepared by recombinant expression in a host cell, as well as chemically synthesized nucleic acids or proteins. The term "isolated" or "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, an isolated biological component is one in which the biological component is more enriched than the biological component is in its natural environment within a cell, or other production vessel. Preferably, a preparation is purified such that the biological component represents at least 50%, such as at least 70%, at least 90%, at least 95%, or greater, of the total biological component content of the preparation.

Label: A detectable compound or composition that is conjugated directly or indirectly to another molecule to facilitate detection of that molecule. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes.

***Lyssavirus*:** A genus of viruses that is part of the *Rhabdoviridae* family within the order Mononegavirales (viruses with a single-stranded, negative sense genome). Lyssaviruses are the etiological agents of rabies encephalitis in warm-blooded animals and humans. *Lyssavirus* species include rabies virus (RABV; genotype 1), Lagos bat virus (LBV; genotype 2), Mokola virus (MOKV; genotype 3), Duvenhage virus (DUVV; genotype 4), European bat lyssavirus-1 (EBLV-1; genotype 5), European bat lyssavirus-2 (EBLV-2; genotype 6) Australian bat lyssavirus (ABLV; genotype 7) and six additional species isolated from bats: four in central Asia and Russia (Aravan virus – ARAV; Khujand virus – KHUV; Irkut virus – IRKV; and West Caucasian bat virus – WCBV), one in Africa (Shimoni bat virus - SHIBV) and one in Europe (Bokeloh bat lyssavirus – BBLV) (Kuzmin *et al.*, *Emerg. Infect. Dis.* 14(12):1887-1889, 2008; Weyer *et al.*, *Epidemiol. Infect.* 136:670-678, 2007; Kuzmin *et al.*, *Virus Res.* 149(2):197-210, 2010; Freuling *et al.*, *Emerg. Infect. Dis.* 17(8):1519-22, 2011; Kuzmin and Rupprecht, "Bat rabies" *In* Rabies, 2nd Edition, New York, Academic Press, 2007, pages 259-307, Jackson and Wunner, eds.).

Neutralizing antibody: An antibody that is capable of protecting a subject (or cells) against infection.

ORF (open reading frame): A series of nucleotide triplets (codons) coding for amino acids without any termination codons. These sequences are usually translatable into a peptide.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame. If introns are present, the operably linked DNA sequences may not be contiguous.

Phage display: A method for the study of protein-protein, protein-peptide, and protein-DNA interactions that uses bacteriophages to connect proteins with the genetic information that encodes them. Antibody phage display libraries, and methods of generating such libraries, are well known in the art (see, for example, Famm *et al.*, *J. Mol. Biol.* 376:926-931, 2008; Carmen and Jermutus, *Brief Funct Genomic Proteomic* 1(2):189-203, 2002; and U.S. Patent Nos. 6,828,422 and 7,195,866). In the context of the present disclosure, an antibody phage display library is a library of any type of antigen-binding antibody fragment displayed on phage. In particular examples, the antibody phage display library is a V_H domain phage display library, or a scFv phage display library. As used herein, a "naïve" antibody (or antibody domain) phage display library refers to a library constructed using subjects that have not been exposed to lyssavirus.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers useful in this disclosure are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compounds or molecules, proteins or antibodies that bind these proteins, viruses or vectors, and additional pharmaceutical agents.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition

to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Polypeptide: A polymer in which the monomers are amino acid residues joined together through amide bonds. When the amino acids are alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used, the L-isomers being preferred for many biological uses. The terms "polypeptide" or "protein" as used herein are intended to encompass any amino acid molecule and include modified amino acid molecules. The term "polypeptide" is specifically intended to cover naturally occurring proteins, as well as those which are recombinantly or synthetically produced. Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, that is, the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. Examples of conservative substitutions are shown below.

Original Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical

conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

Amino acids are typically classified in one or more categories, including polar, hydrophobic, acidic, basic and aromatic, according to their side chains. Examples of polar amino acids include those having side chain functional groups such as hydroxyl, sulfhydryl, and amide, as well as the acidic and basic amino acids. Polar amino acids include, without limitation, asparagine, cysteine, glutamine, histidine, selenocysteine, serine, threonine, tryptophan and tyrosine. Examples of hydrophobic or non-polar amino acids include those residues having nonpolar aliphatic side chains, such as, without limitation, leucine, isoleucine, valine, glycine, alanine, proline, methionine and phenylalanine. Examples of basic amino acid residues include those having a basic side chain, such as an amino or guanidino group. Basic amino acid residues include, without limitation, arginine, homolysine and lysine. Examples of acidic amino acid residues include those having an acidic side chain functional group, such as a carboxy group. Acidic amino acid residues include, without limitation aspartic acid and glutamic acid. Aromatic amino acids include those having an aromatic side chain group. Examples of aromatic amino acids include, without limitation, biphenylalanine, histidine, 2-napthylalananine, pentafluorophenylalanine, phenylalanine, tryptophan and tyrosine. It is noted that some amino acids are classified in more than one group, for example, histidine, tryptophan, and tyrosine are classified as both polar and aromatic amino acids. Additional amino acids that are classified in each of the above groups are known to those of ordinary skill in the art.

Substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative, for instance changes in which (a) a hydrophilic residue, for example, seryl or threonyl, is substituted for (or by) a hydrophobic residue, for example, leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, for example, lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, for example, glutamyl or aspartyl; or (d) a residue having a bulky side chain, for example, phenylalanine, is substituted for (or by) one not having a side chain, for example, glycine.

Purified: The term "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified peptide, protein, virus, or

other active compound is one that is isolated in whole or in part from naturally associated proteins and other contaminants. In certain embodiments, the term "substantially purified" refers to a peptide, protein, virus or other active compound that has been isolated from a cell, cell culture medium, or other crude preparation and subjected to fractionation to remove various components of the initial preparation, such as proteins, cellular debris, and other components.

Rabies: A viral disease that causes acute encephalitis (inflammation of the brain) in warm-blooded animals. Rabies is zoonotic (transmitted by animals), most commonly by a bite from an infected animal but occasionally by other forms of contact. Rabies is almost frequently fatal if post-exposure prophylaxis is not administered prior to the onset of severe symptoms. Rabies is caused by viruses of the *Lyssavirus* genus.

Rabies virus (RABV or RV): A member of the Rhabdoviridae family having a non-segmented RNA genome with negative sense polarity. Rabies virus is the prototype of the *Lyssavirus* genus. The rabies virus Evelyn-Rokitnicki-Abelseth (ERA) strain is a strain derived from the Street-Alabama-Dufferin (SAD) strain, first isolated from a rabid dog in Alabama (USA) in 1935. The ERA strain was derived after multiple passages of SAD RABV in mouse brains, baby hamster kidney (BHK) cells, and chicken embryos. The complete genomic sequence of the ERA strain is disclosed in PCT Publication No. WO 2007/047459.

Recombinant: A recombinant nucleic acid, protein or virus is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. In some embodiments, recombinant rabies virus is generated using reverse genetics, such as the reverse genetics system described in PCT Publication No. WO 2007/047459. In some examples, the recombinant rabies viruses comprise one or more mutations in a viral virulence factors, such as glycoprotein. In other examples, the recombinant rabies viruses comprise a heterologous gene, such as a sequence encoding a glycoprotein from another lyssavirus (such as Mokola virus, West Caucasian bat virus or Lagos bat virus).

Sequence identity: The similarity between two nucleic acid sequences, or two amino acid sequences, is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in

terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman (*Adv. Appl. Math.*, 2:482, 1981); Needleman and Wunsch (*J. Mol. Biol.*, 48:443, 1970); Pearson and Lipman (*Proc. Natl. Acad. Sci.*, 85:2444, 1988); Higgins and Sharp (*Gene*, 73:237-44, 1988); Higgins and Sharp (*CABIOS*, 5:151-53, 1989); Corpet *et al.* (*Nuc. Acids Res.*, 16:10881-90, 1988); Huang *et al.* (*Comp. Appls. Biosci.*, 8:155-65, 1992); and Pearson *et al.* (*Meth. Mol. Biol.*, 24:307-31, 1994). Altschul *et al.* (*Nature Genet.*, 6:119-29, 1994) presents a detailed consideration of sequence alignment methods and homology calculations.

The alignment tools ALIGN (Myers and Miller, *CABIOS* 4:11-17, 1989) or LFASTA (Pearson and Lipman, 1988) may be used to perform sequence comparisons (Internet Program © 1996, W. R. Pearson and the University of Virginia, "fasta20u63" version 2.0u63, release date December 1996). ALIGN compares entire sequences against one another, while LFASTA compares regions of local similarity. These alignment tools and their respective tutorials are available on the Internet at the NCSA website. Alternatively, for comparisons of amino acid sequences of greater than about 30 amino acids, the "Blast 2 sequences" function can be employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the "Blast 2 sequences" function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). The BLAST sequence comparison system is available, for instance, from the NCBI web site; see also Altschul *et al.*, *J. Mol. Biol.*, 215:403-10, 1990; Gish and States, *Nature Genet.*, 3:266-72, 1993; Madden *et al.*, *Meth. Enzymol.*, 266:131-41, 1996; Altschul *et al.*, *Nucleic Acids Res.*, 25:3389-402, 1997; and Zhang and Madden, *Genome Res.*, 7:649-56, 1997.

Orthologs (equivalent to proteins of other species) of proteins are in some instances characterized by possession of greater than 75% sequence identity counted over the full-length alignment with the amino acid sequence of specific protein using ALIGN set to default parameters. Proteins with even greater similarity to a reference sequence will show increasing percentage identities when assessed by this method,

such as at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, or at least 98% sequence identity. In addition, sequence identity can be compared over the full length of one or both binding domains of the disclosed fusion proteins.

When significantly less than the entire sequence is being compared for sequence identity, homologous sequences will typically possess at least 80% sequence identity over short windows of 10-20, and may possess sequence identities of at least 85%, at least 90%, at least 95%, or at least 99% depending on their similarity to the reference sequence. Sequence identity over such short windows can be determined using LFASTA; methods are described at the NCSA website. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided. Similar homology concepts apply for nucleic acids as are described for protein. An alternative indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences, due to the degeneracy of the genetic code. It is understood that changes in nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that each encode substantially the same protein.

Subject: Living multi-cellular vertebrate organisms, a category that includes both human and non-human mammals.

Therapeutically effective amount: A quantity of a specified agent sufficient to achieve a desired effect in a subject being treated with that agent. For example, this may be the amount of a lyssavirus-specific monoclonal antibody useful for treating rabies. The effective amount of a lyssavirus-specific monoclonal antibody useful for treating rabies in a subject will be dependent on, for example, the subject being treated, the manner of administration of the composition, and other factors.

Vector: A nucleic acid molecule that can be introduced into a host cell, thereby producing a transformed host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication (DNA sequences that participate in initiating DNA synthesis). A vector may also include one or more

selectable marker genes and other genetic elements known in the art. In some embodiments herein, the vector is an Fc IgG expression vector.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Hence "comprising A or B" means including A, or B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

III. Overview of Several Embodiments

Currently available anti-rabies immunoglobulins do not neutralize lyssaviruses of other genotypes, such as Lagos bat virus (LBV), Mokola virus (MOKV), and West Caucasian bat virus (WCBV). The use of immunized humans for immune library construction biases libraries towards neutralization of genotype 1 rabies viruses with lower cross-reactivity towards other genotypes. To circumvent this limitation, disclosed herein is the use of a naïve human phage display library to identify and characterize novel monoclonal antibodies (mAbs) that broadly neutralize lyssaviruses. As described in Examples 2 and 3 below, this library displays variable heavy (V_H) domain antibodies (dAbs) with diversity added to the complement determining regions (CDR). Antibodies identified by the methods provided herein can be used, for example, for post-exposure rabies prophylaxis or in the treatment of clinical rabies.

Provided herein is a method of identifying a monoclonal antibody or antigen-binding fragment thereof that specifically binds at least two different lyssaviruses. In some embodiments, the method includes screening a naïve antibody phage display

library with at least two different lyssavirus glycoproteins, such as by screening the library against a recombinant virus expressing at least two different lyssavirus glycoproteins, screening the library against recombinant glycoprotein from at least two different lyssaviruses, screening the library against cells expressing at least two different lyssavirus glycoproteins, or any combination thereof. In some embodiments, the method further includes selecting a phage display clone that specifically binds to at least two different lyssaviruses, at least two different lyssavirus glycoproteins, or both.

The at least two different lyssaviruses can be selected from any known lyssavirus, such as, for example, rabies virus (RABV), Mokola virus (MOKV), West Caucasian bat virus (WCBV), Lagos bat virus (LBV), Duvenhage virus (DUVV), European bat lyssavirus-1 (EBLV-1), European bat lyssavirus-2 (EBLV-2), Australian bat lyssavirus (ABLV), Aravan virus (ARAV), Khujand virus (KHUV) and Irkut virus (IRKV). In some embodiments, the antibody or antigen-binding fragment specifically binds at least three, at least four or at least five different lyssaviruses, or at least three, at least four or at least five different lyssavirus glycoproteins.

In particular embodiments, the method includes screening a naïve antibody phage display library with (1) a recombinant rabies virus expressing glycoprotein from RABV, MOKV and WCBV; (2) at least two different recombinant lyssavirus glycoproteins selected from the RABV glycoprotein, the MOKV glycoprotein, the WCBV glycoprotein, the LBV glycoprotein and the DUVV glycoprotein; or (3) at least two different cell lines expressing a lyssavirus glycoprotein selected from the RABV glycoprotein, the MOKV glycoprotein, the WCBV glycoprotein, the LBV glycoprotein and the DUVV glycoprotein; and selecting a phage display clone that specifically binds to at least two different lyssaviruses, at least two different lyssavirus glycoproteins, or both. In some examples, the at least two different lyssaviruses are selected from RABV, MOKV, WCBV, LBV and DUVV. In particular non-limiting examples, the antibody or antigen-binding fragment specifically binds whole virus and/or glycoprotein of RABV, MOKV and WCBV; RABV, MOKV, WCBV and LBV; RABV, MOKV, WCBV, LBV and DUVV; or MOKV, WCBV, LBV and DUVV.

In some embodiments, the phage display library is a naïve human V_H domain library. In other embodiments, the phage display library is a naïve human scFv library or a naïve human Fab library. However, other naïve human antibody libraries can be used and an appropriate library can be selected by one of skill in the art.

In some embodiments, the antigen-binding fragment comprises a V_H domain. In other embodiments, the antigen-binding fragment comprises a scFV. In yet other embodiments, the antigen-binding fragment comprises an Fab.

In some embodiments, the method further includes cloning the antigen-binding fragment (for example, a fragment comprising a V_H domain, a scFV or a Fab) into an Fc IgG expression vector to generate an immunoglobulin molecule containing the fragment (such as an Fc IgG1 expression vector, for example pNUT-Cγ1 (Boel, *et al., J. Immunol. Methods.* 239:153-166, 2000)). In some examples, the Fc IgG expression vector further includes nucleic acid sequence encoding a variable light (V_L) domain from a rabies virus-specific antibody.

Selecting a phage display clone that specifically binds to a lyssavirus (for example, whole virus) or lyssavirus glycoprotein can be performed using any assay known in the art for evaluating antigen binding. In some embodiments, the selecting step includes an ELISA to detect specific binding to lyssavirus glycoprotein, whole virus, or both.

In some embodiments, the method further includes screening the phage display clone for lyssavirus neutralization. Assays for evaluating virus neutralization are well known in the art and include, for example, fluorescent focus assays, including the rapid fluorescent focus inhibition test (RFFIT).

Also provided are isolated monoclonal antibodies, and antigen-binding fragments thereof, identified according to the methods disclosed herein. In some embodiments, the antibody neutralizes infectivity of the lyssaviruses.

In some embodiments, the V_H domain of the antibody is encoded by a nucleotide sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to any one of SEQ ID NOs: 1-110. In some examples, the V_H domain of the antibody is encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 1-110.

In some embodiments, any in-frame "TAG" (stop codons) in the V_H domain sequence are changed to "GAG" (glutamate codons) to allow expression in eukaryotic cells.

SEQ ID NOs: 1-43 show the nucleotide sequences of 43 different clones of a naïve human V_H domain library that were selected using a recombinant rabies virus expressing glycoprotein from RABV, MOKV and WCBV. These clones bind whole

virus and/or bind glycoprotein from RABV, MOKV and WCBV. Each clone includes nucleic acid sequence encoding the V_H complementarity determining regions (CDRs). Identifying CDR sequences of an antibody or antibody fragment, given the nucleotide or amino acid sequence of the antibody or antibody fragment, is within the capabilities of one of skill in the art, such as by using the Kabat method (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991; the Kabat database is now maintained online) or the International ImMunoGeneTics Information System™ (IMGT, available online). Thus, also provided herein is a monoclonal antibody, or antigen-binding fragment thereof, comprising at least one CDR sequence, such as two CDR sequences or all three CDR sequences, encoded by a portion of any one of SEQ ID NOs: 1-43.

SEQ ID NOs: 44-110 represent the nucleotide sequences of 67 different clones of a naïve human V_H domain library that were selected by sequential panning on cell lines expressing glycoprotein from LBV, MOKV, WCBV and DUVV. CDR1, CDR2 and CDR3 sequences of each V_H domain clone are provided in Table 2. Thus, provided herein is a monoclonal antibody, or antigen-binding fragment thereof, comprising at least one, at least two or all three CDR sequences from any one of SEQ ID NOs: 44-110.

Also provided is an isolated monoclonal antibody, or antigen-binding fragment thereof, that specifically binds at least two different lyssaviruses; or that specifically binds recombinant glycoprotein from at least two different lyssaviruses. In some embodiments, the V_H domain of the antibody is encoded by a nucleotide sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the nucleotide sequence of any one of SEQ ID NOs: 1-110. In some embodiments, the V_H domain of the antibody is encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 1-110. In some embodiments, the at least two lyssaviruses are selected from RABV, MOKV, WCBV, LBV and DUVV. In some examples, the isolated monoclonal antibody specifically binds RABV, MOKV and WCBV; or specifically binds recombinant glycoprotein from RABV, MOKV and WCBV. In other examples, the isolated monoclonal antibody specifically binds RABV, MOKV, WCBV and LBV; or specifically binds recombinant glycoprotein from RABV, MOKV, WCBV and LBV. In other examples, the isolated monoclonal antibody specifically binds RABV, MOKV, WCBV, LBV and DUVV; or specifically

binds recombinant glycoprotein from RABV, MOKV, WCBV, LBV and DUVV. In yet other examples, the isolated monoclonal antibody specifically binds MOKV, WCBV, LBV and DUVV; or specifically binds recombinant glycoprotein from MOKV, WCBV, LBV and DUVV. In particular examples, the antibody neutralizes infectivity of the lyssaviruses.

In some embodiments, the monoclonal antibody comprises a V_L domain from a rabies virus-specific antibody.

In some embodiments disclosed herein, the antibody is an IgG. In other embodiments, the antibody is an IgM. In some embodiments, the antibody is a human antibody or a humanized antibody. In some embodiments, the antibody is a Fab fragment, a Fab' fragment, a $F(ab)'_2$ fragment, a single chain Fv protein (scFv), or a disulfide stabilized Fv protein (dsFv).

Further provided herein is an isolated immunoconjugate comprising a monoclonal antibody disclosed herein and a fusion partner. In some embodiments, the fusion partner is an effector molecule, a label or a heterologous polypeptide.

Also provided are compositions comprising the monoclonal antibodies or immunoconjugates disclosed herein and a pharmaceutically acceptable carrier.

Compositions comprising more than one type of anti-rabies antibody are also provided herein. In some embodiments, the composition includes (1) a monoclonal antibody that specifically binds at least two lyssaviruses as disclosed herein, and (2) a monoclonal antibody specific for RABV or RABV glycoprotein. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

Further provided is an isolated nucleic acid molecule encoding any one of the monoclonal antibodies disclosed herein.

Also provided is a method of treating rabies in a subject, comprising administering to the subject a monoclonal antibody or antigen-binding fragment thereof, an immunoconjugate or a composition disclosed herein.

Also provided herein is an expression vector comprising a nucleotide sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the nucleotide sequence of any one of SEQ ID NOs: 1-110. In some embodiments, the expression vector comprises the nucleotide sequence of any one of SEQ ID NOs: 1-110. In some embodiments, the expression vector further comprises the nucleotide sequence of a V_L domain from a lyssavirus-specific

monoclonal antibody. In particular examples, the V_L domain is from a RABV-specific monoclonal antibody. In some embodiments, the expression vector is an Fc IgG expression vector. A cell comprising an expression vectors disclosed herein is also provided. Further provided is a monoclonal antibody encoded by the Fc IgG expression vector. A monoclonal antibody expressed from such an Fc IgG expression vector can be used for rabies prophylaxis and clinical rabies treatment.

IV. Lyssavirus

The genus *Lyssavirus* is a member of the *Rhabdoviridae* family within the order Mononegavirales (viruses with a single-stranded, negative sense genome). Lyssaviruses are the etiological agents of rabies encephalitis in warm-blooded animals and humans (Tordo *et al.*, "Lyssaviruses" *In* Fauquet *et al.* eds. *Virus taxonomy: the classification and nomenclature of viruses. The 8th Report of the International Committee on Taxonomy of Viruses.* San Diego: Oxford Academic, 2006, pages 623-629; World Health Organization Expert Consultation on Rabies, 5-8 October 2004, first report, World Health Organization Technical report series 931, Geneva: World Health Organization, 2005, pages 15-19). *Lyssavirus* species include rabies virus (RABV; genotype 1), Lagos bat virus (LBV; genotype 2), Mokola virus (MOKV; genotype 3), Duvenhage virus (DUVV; genotype 4), European bat lyssavirus-1 (EBLV-1; genotype 5), European bat lyssavirus-2 (EBLV-2; genotype 6), Australian bat lyssavirus (ABLV; genotype 7) six additional species isolated from bats: four in central Asia and Russia (Aravan virus – ARAV; Khujand virus – KHUV; Irkut virus – IRKV; and West Caucasian bat virus – WCBV), one in Africa (Shimoni bat virus - SHIBV) and one in Europe (Bokeloh bat lyssavirus – BBLV) (Kuzmin *et al.*, *Emerg. Infect. Dis.* 14(12):1887-1889, 2008; Weyer *et al.*, *Epidemiol. Infect.* 136:670-678, 2007; Kuzmin *et al.*, *Virus Res.* 149(2):197-210, 2010; Freuling *et al.*, *Emerg. Infect. Dis.* 17(8):1519-22, 2011; Kuzmin and Rupprecht, "Bat rabies" *In* Rabies, 2nd Edition, New York, Academic Press, 2007, pages 259-307, Jackson and Wunner, eds.).

Based on phylogeny, immunogenicity and virulence of lyssavirus isolates, two lyssavirus phylogroups have been proposed (Badrane *et al.*, *J. Virol.* 75:3268-3276, 2001). The division into phylogroups generally correlates with the pattern of vaccine cross-protection observed for lyssaviruses (Badrane *et al.*, *J. Virol.* 75:3268-3276, 2001; Hanlon *et al.*, *Virus Res.* 111:44-54, 2005; Nel *et al.*, *Expert Rev. Vaccines*

4:553-540, 2005). Phylogroup1 includes genotypes 1, 4, 5, 6 and 7, as well as ARAV, KHUV IRKV and BBLV (Kuzmin *et al.*, *Virus Res.* 97:65-79, 2003; Kuzmin *et al.*, *Virus Res.* 111:28-43, 2005; Hanlon *et al.*, *Virus Res.* 111:44-54, 2005; Freuling *et al.*, *Emerg. Infect. Dis.* 17(8):1519-22, 2011). Currently available commercial vaccines and biologicals are considered to be effective against infections of viruses from this phylogroup (Nel *et al.*, *Expert Rev. Vaccines* 4:553-540, 2005). However, these vaccines and biologics for rabies do not offer full protection against infection with viruses outside of lyssavirus phylogroup 1 (*i.e.*, genotypes 2, 3 and SHIBV). In addition, WCBV is recognized as the most divergent lyssavirus and exhibits limited relatedness to genotype 2 and 3 viruses. Previous studies have demonstrated little or no cross-neutralization of anti-RABV sera with WCBV (Botvinkin *et al.*, *Emerg. Infect. Dis.* 9:1623-1625, 2003; Hanlon *et al.*, *Virus Res.* 111:44-54, 2005).

Lyssaviruses are composed of two major structural components, a nucleocapsid or ribonucleoprotein (RNP), and an envelope in the form of a bilayer membrane surrounding the RNP core. The infectious component of all rhabdoviruses is the RNP core, which consists of the negative strand RNA genome encapsidated by nucleoprotein (N) in combination with RNA-dependent RNA-polymerase (L) and phosphoprotein (P). The membrane surrounding the RNP contains two proteins, the trans-membrane glycoprotein (G) and the matrix (M) protein, located at the inner side of the membrane. Thus, the viral genome codes for these five proteins: the three proteins in the RNP (N, L and P), the matrix protein (M), and the glycoprotein (G).

The molecular determinants of pathogenicity of various rabies virus strains have not been fully elucidated. RABV pathogenicity was attributed to multigenic events (Yamada *et al.*, *Microbiol. Immunol.* 50:25-32, 2006). For example, some positions in the RABV genome if mutated, affect viral transcription or replication, reducing virulence. Mutations at serine residue 389 of the phosphorylation site in the N gene (Wu *et al.*, *J. Virol.* 76:4153-4161, 2002) or GDN core sequence of the highly conserved C motif in the L gene (Schnell and Conzelmann, *Virol.* 214:522-530, 1995) dramatically reduced RABV transcription and replication.

The G protein, also referred to as spike protein, is involved in cell attachment and membrane fusion of RABV. The amino acid region at position 330 to 340 (referred to as antigenic site III) of the G protein has been identified as important for virulence of certain strains of RABV. Several studies support the concept that the

pathogenicity of fixed RABV strains is determined by the presence of arginine or lysine at amino acid residue 333 of the glycoprotein (Dietzschold *et al.*, *Proc. Natl. Acad. Sci. USA* 80: 70-74, 1983; Tuffereau *et al.*, *Virology* 172: 206-212, 1989).

This phenomenon seems to apply at least to fixed rabies viruses such as CVS, ERA, PV, SAD-B19 and HEP-Flury strains (Anilionis *et al.*, *Nature* 294:275-278, 1981; Morimoto *et al.*, *Virology* 173:465-477, 1989). For example, rabies vaccine viruses possessing an amino acid differing from Arg at position 333 of the glycoprotein are described, for instance, in PCT Publication No. WO 00/32755 (describing RABV mutants in which all three nucleotides in the G protein Arg_{333} codon are altered compared to the parent virus, such that the Arg at position 333 is substituted with another amino acid); European Patent No. 350398 (describing an avirulent RABV mutant SAG1 derived from the Bern SAD strain of RABV, in which the Arg at position 333 of the glycoprotein has been substituted to Ser); and European patent application 583998 (describing an attenuated RABV mutant, SAG2, in which the Arg at position 333 in the G protein has been substituted by Glu).

Other strains, such as the RC-HL strain, possess an arginine residue at position 333 of the G, but do not cause lethal infection in adult mice (Ito *et al.*, *Microl. Immunol.* 38:479-482, 1994; Ito *et al.*, *J. Virol.* 75:9121-9128, 2001). As such, the entire G may contribute to the virulence of RABV, although the determinants or regions have not been fully elucidated.

The G gene encodes the only protein that induces viral neutralizing antibody. At least three states of RABV glycoprotein are known: the native state (N) being responsible for receptor binding; an active hydrophobic state (A) necessary in the initial step in membrane fusion process (Gaudin, *J. Cell Biol.* 150:601-612, 2000), and a fusion inactive conformation (I). Correct folding and maturation of the G protein play important roles for immune recognition. The three potential glycosylated positions in ERA G extracellular domain occur at Asn^{37}, Asn^{247} and Asn^{319}residues (Wojczyk *et al.*, *Glycobiology*. 8: 121-130, 1998). Nonglycosylation of G not only affects conformation, but also inhibits presentation of the protein at the cell surface.

V. Antibody Compositions and Therapeutic Methods

Standard treatment for post-exposure rabies prophylaxis includes thorough wound-washing with soap and water followed by administration of vaccine and anti-

rabies virus immunoglobulin (RIG) of human or equine origin. RIG administered shortly after exposure at the wound site provides passive immunity which neutralizes rabies virus and prevents its spread until the patient's immune response following vaccination is elicited. Deaths due to post-exposure prophylaxis failure are most commonly attributed to deviations from the recommended regimen such as late initiation of post-exposure prophylaxis or no administration of RIG (Wilde, *Vaccine*, 25:7605-7609, 2007). Provided herein are human monoclonal antibodies that specifically bind at least two different lyssaviruses. Thus, the disclosed antibodies are useful for the treatment of clinical rabies and/or for post-exposure rabies prophylaxis.

Compositions are provided that include a monoclonal antibody, or antigen-binding fragment thereof, that specifically binds at least two different lyssaviruses (such as whole virus or lyssavirus glycoprotein, or both). Compositions comprising immunoconjugates or immunotoxins are also provided. The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating physician to achieve the desired purposes. For example, the compositions can be administered to a subject exhibiting clinical signs of rabies, or can be administered to a subject that has been exposed to or bitten by a rabid animal (or an animal suspected of being rabid). The antibody can be formulated for systemic or local (such as at a wound site) administration.

The compositions for administration can include a solution of the antibody (or antigen-binding fragment thereof) that specifically binds lyssavirus dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, for example, buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antibody in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs.

Doses of lyssavirus mAb generally range from about 1 to about 50 IU/kg, such as about 5 to about 40 IU/kg, or about 5 to about 20 IU/kg. In particular examples, the dose is about 5 IU/kg, about 10 IU/kg or about 20 IU/kg. However, the dose of lyssavirus mAb will vary depending upon the antibody (or antibody cocktail) selected and the particular subject to be treated. An appropriate does can be determined by a medical practitioner.

Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science, 19th ed.*, Mack Publishing Company, Easton, PA (1995).

Antibodies may be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of known concentration. Antibodies can be administered by slow infusion, or by intravenous push or bolus. Antibody compositions can also be administered topically.

The antibody that specifically binds lyssavirus can be administered to prevent the development of rabies disease and/or slow the spread of lyssavirus from a wound site to distant sites in the body. In these applications, a therapeutically effective amount of an antibody is administered to a subject in an amount sufficient to inhibit virus replication or spread, or to inhibit a sign or a symptom of rabies. Suitable subjects may include those diagnosed with rabies, or subjects recently exposed to (such as bitten by) animal infected with a lyssavirus, or suspected of being infected with a lyssavirus.

A therapeutically effective amount of a lyssavirus-specific antibody will depend upon the severity of the disease and the general state of the patient's health. A therapeutically effective amount of the antibody is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer. These compositions can be administered in conjunction with other therapeutic agents (such as a rabies vaccine), either simultaneously or sequentially.

Single or multiple administrations of the compositions are administered depending on the dosage and frequency as required and tolerated by the patient. In any event, the composition should provide a sufficient quantity of at least one of the antibodies disclosed herein to effectively treat the patient. The dosage can be administered once but may be applied periodically until either a therapeutic result is

achieved or until side effects warrant discontinuation of therapy. Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the patient.

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure to the particular features or embodiments described.

EXAMPLES

Example 1: Construction and characterization of recombinant rabies virus with three glycoprotein genes

This example describes the generation and characterization of a recombinant ERA strain rabies virus encoding three different glycoprotein genes. The recombinant virus, referred to as ERA-3G, comprises rabies virus (RABV) glycoprotein, Mokola virus (MOKV) glycoprotein and West Caucasian bat virus (WCBV) glycoprotein. The cloning strategy for ERA-3G is shown in FIG. 1. The rabies virus reverse genetics system used to generate this virus in described in detail in PCT Publication No. WO 2007/047459. ERA-3G includes the attenuating mutation in the RABV glycoprotein gene that results in an arginine to glutamic acid change at amino acid residue 333 of the protein.

The G genes from MOKV and WCBV were cloned into the ERA backbone (see SEQ ID NO: 7 of PCT Publication No. WO 2007/047459) by RT-PCR using viral genomic RNA from virus-infected cells as template. The following primers were used for amplification of the glycoprotein genes:

MokolaG5 - CGACTGCAGATGAATATACCTTGCTTTGTTGTGATTC (SEQ ID NO: 111)

MokolaG3 - CGTGGTACCTCATGTACCTGGAAGCCCTTTATAGGACTC (SEQ ID NO: 112)

WCBVG5 - CATCTGCTAGCAATGGCTTCCTACTTTGCGTTG (SEQ ID NO: 113)

WCBVG3 - TTCAATGGTACCTTATTGGGCAGTTTGTCCCTT (SEQ ID NO: 114)

The amplified G genes for MOKV (SEQ ID NO: 115) and WCBV (SEQ ID NO: 116) were confirmed by sequencing. Two extra transcription units were

synthesized (each with the sequence of SEQ ID NO: 117) and introduced into the gene junctions between the phosphoprotein (P) and the matrix protein (M), and the G and the RNA dependent RNA polymerase (L) (FIG. 1). The MOKV G was cloned into the gene junction between the P and M, and WCBV G into the gene junction between the G and L in the ERA genome backbone.

Recombinant virus was recovered by transfection of the above described construct into BSR cells using the method described in PCT Publication No. WO 2007/047459.

Example 2: Identification of Domain Antibodies Specific for Lyssaviruses

Current anti-rabies immune globulins do not neutralize all lyssaviruses. The use of immunized humans for immune library construction biases libraries towards neutralization of RABV genotype 1 with lower cross-reactivity towards other *Lyssavirus* genotypes. One method to circumvent this limitation is selecting monoclonal antibodies (mAbs) from naïve immune libraries, which theoretically contain binders to any antigen.

This example describes the use of a naïve, human-heavy domain, phage display library (Famm *et al.*, *J. Mol. Biol.* 376:926-931, 2008) to identify and characterize novel anti-*Lyssavirus* mAbs. The phage display library (3×10^9 clones) is based on human V_H framework with diversity introduced into CDR1, CDR2 and CDR3 by PCR mutagenesis. The library was panned using a recombinant ERA RABV expressing three different G proteins (see Example 1) – the G proteins from rabies virus (RABV), Mokola virus (MOKV) and West Caucasian Bat virus (WCBV) – following established methods (Kramer *et al.*, *Eur. J. Immunol.* 35:2131-2145, 2005; Lee *et al.*, *Nat. Protoc.* 2:3001-3008, 2007).

Library panning

For the first two times the library was panned, either purified G protein or whole virus was used to select binders for three rounds and then switched antigens for three more rounds so that binders selected for G protein were then panned against whole virus and vice versa. After the last round of selection, 552 individual clones were picked (276 from each panning scheme) and potential high affinity domain antibodies (dAb) were identified by ELISA.

ELISA

The ELISA procedure was standardized using the soluble dAb fragments from these clones and the same antigens used to pan the library. Approximately 5-10% of selected clones bind to the original antigen and 2-3% bind to the secondary antigen (Table 1).

Table 1. Results of Panning Phage Display Library

Scheme	Screened	Binding* whole virus	Binding* G protein
Whole virus→G Protein	276	30 (10.9%)	4 (1.4%)
G Protein→Whole virus	276	8 (2.9%)	13 (4.7%)
Total	**552**	**38 (6.9%)**	**17 (3.1%)**

* Number of clones with mean ELISA binding ≥ positive control from three biological replicates

Relative binding (as assessed by ELISA) of each of the clones to whole virus and glycoprotein is shown in FIG. 2 and FIG. 3, respectively. Representative clones from groups 1 and 17 appear to bind to whole virus, whereas clones from groups 1, 9, 23, 30 and 31 appear to bind glycoprotein. A modified rapid fluorescent focus inhibition test (RFFIT) was used to screen dAbs for virus neutralization. The results are shown in FIG. 4. Observed weak neutralization may be due to poor expression of soluble antibodies due to in-frame stop codons.

Sequencing of clones

Sixty-nine percent of the clones selected for binding to whole virus and 37% of the clones selected for binding to glycoprotein were successfully sequenced. The sequences of each clone are provided in the Sequence Listing as SEQ ID NOs: 1-43 as follows:

Clone	SEQ ID NO:	Clone	SEQ ID NO:
H01_plate2_group1	1	E04_plate11_group7	23
B05_plate4_group2	2	H08_plate13_group15	24
B08_plate4_group3	3	F12_plate13_group18	25

Clone	SEQ ID NO:	Clone	SEQ ID NO:
C10_plate4_group4	4	B03_plate14_group19	26
G05_plate2_group5	5	H05_plate13_group21	27
G04_plate2_group6	6	G06_plate14_group22	28
C02_plate4_group7	7	F05_plate11_group23	29
A06_plate4_group8	8	D11_plate11_group24	30
H12_plate1_group9	9	H01_plate13_group25	31
F09_palte4_group10	10	C10_plate11_group26	32
H09_plate4_group11	11	D11_plate14_group27	33
G04_plate4_group12	12	B08_plate11_group28	34
F05_plate4_group13	13	H08_plate14_group29	35
E01_plate2_group14	14	H06_plate14_group30	36
E07_plate2_group15	15	H07_plate11_group31	37
F03_plate1_group16	16	E12_plate14_group32	38
E02_plate2_group17	17	A11_plate11_group33	39
A05_plate1_group18	18	B04_plate13_group34	40
C08_plate1_group19	19	A08_plate11_group35	41
B02_plate1_group20	20	B05_plate13_group36	42
G09_plate11_group1	21	E04_plate14_group37	43
H07_plate14_group2	22		

Example 3: Lyssavirus Domain Antibodies Selected by Sequential Panning of Glycoprotein Expressing Cells

This example describes the identification of V_H domain antibodies specific for lyssavirus glycoprotein by sequential panning of a naïve, human V_H domain phage display library (Famm *et al.*, *J. Mol. Biol.* 376:926-931, 2008) on cell lines expressing glycoprotein (G) from several different lyssaviruses.

Flp-In-BHK cells (Invitrogen) were transfected with a pEF5/FRT/V5 plasmid (Invitrogen) encoding glycoprotein from LBV (SEQ ID NO: 118), MOKV (SEQ ID NO: 115), WCBV (SEQ ID NO: 116), or DUVV (SEQ ID NO: 119). The library was panned sequentially on cells that express LBV G protein, followed by MOKV G protein, followed by WCBV G protein and finally DUVV G protein. Selection on cells was carried out using the method described by Lee *et al.* (*Nature Protocols* 2(11):3001-3008, 2007).

Using this method, 67 unique nucleotide sequences were identified (Table 2). Each V_H sequence has at least one change in amino acid sequence when translated. In-frame "TAG" (stop codons) will be changed to "GAG" (glutamate codons) to allow expression in eukaryotic cell line. V_H sequences will be cloned into the pEF5/FRT/V5 plasmid to transfect Flp-In-BHK cells for expression of antibodies.

Table 2. Unique V_H clones identified by sequential panning of cells

V_H Clone Name	SEQ ID NO:	CDR1 nucleotide positions[1]	CDR2 nucleotide positions[1]	CDR2 nucleotide positions[1]
plate11_G09_group1	44	76-111	148-193	295-336
plate14_H07_group2	45	76-111	148-193	295-336
plate4_B08_group3	46	76-111	148-193	295-330
plate4_C10_group4	47	76-111	148-193	295-333
plate2_G05_group5	48	76-111	148-193	295-351
plate2_G04_group6	49	76-111	148-193	295-339
plate4_C02_group7	50	76-111	148-193	295-339
plate4_A06_group8	51	76-111	148-193	295-351
plate1_H12_group9	52	76-111	148-193	295-339
palte4_F09_group10	53	76-111	148-193	295-336
plate4_H09_group11	54	76-111	148-193	295-333
plate4_G04_group12	55	76-111	148-193	295-342
plate4_F05_group13	56	76-111	148-193	295-354
plate2_E01_group14	57	76-111	148-193	295-360
plate13_H08_group15	58	76-111	148-193	295-348
plate4_B05_group16	59	76-111	148-193	295-336
plate2_E02_group17	60	76-111	148-193	295-336
plate1_A05_group18	61	76-111	148-193	295-336
plate14_B03_group19	62	76-111	148-193	295-333
plate1_B02_group20	63	76-111	148-193	295-351
plate13_F12_group21	64	76-111	148-193	295-336
plate14_G06_group22	65	76-111	148-193	295-351
plate11_F05_group23	66	76-111	148-193	295-339
plate11_D11_group24	67	76-111	148-193	295-330
plate13_H01_group25	68	76-111	148-193	295-339
plate11_C10_group26	69	76-111	148-193	295-336
plate14_D11_group27	70	76-111	148-193	295-330
plate11_B08_group28	71	76-111	148-193	295-330
plate14_H08_group29	72	76-111	148-193	295-330
plate14_H06_group30	73	76-111	148-193	295-345
plate11_H07_group31	74	76-111	148-193	295-333
plate14_E12_group32	75	76-111	148-193	295-336
plate11_A11_group33	76	76-111	148-193	295-333

V_H Clone Name	SEQ ID NO:	CDR1 nucleotide positions[1]	CDR2 nucleotide positions[1]	CDR2 nucleotide positions[1]
plate13_B04_group34	77	76-111	148-193	295-339
plate11_A08_group35	78	76-111	148-193	295-345
plate13_B05_group36	79	76-111	148-193	295-333
plate14_E04_group37	80	76-111	148-193	295-333
plate5_B02_group38	81	76-111	148-193	295-339
plate5_A08_group39	82	76-111	148-193	295-330
plate5_G03_group40	83	76-111	148-193	295-339
plate5_D02_group41	84	76-111	148-193	295-339
plate5_B11_group42	85	76-111	148-193	295-339
plate5_A05_group43	86	76-111	148-193	295-330
plate5_G09_group44	87	76-111	148-193	295-339
plate5_C01_group45	88	76-111	148-193	295-339
plate5_D01_group46	89	76-111	148-193	295-339
plate5_C10_group47	90	76-111	148-193	295-339
plate5_D10_group48	91	76-111	148-193	295-339
plate5_D03_group49	92	76-111	148-193	295-339
plate5_C03_group50	93	76-111	148-193	295-339
plate5_G10_group51	94	76-111	148-193	295-339
plate5_H06_group52	95	76-111	148-193	295-330
plate5_A06_group53	96	76-111	148-193	295-330
plate52_E08_group54	97	10-45	82-127	229-264
plate52_C06_group55	98	44-79	115-160	262-306
plate52_E01_group56	99	76-111	148-193	295-330
plate52_C08_group57	100	34-69	106-151	253-288
plate52_B09_group58	101	12-47	84-129	231-266
plate53_A03_group59	102	76-111	148-193	295-336
plate53_C12_group60	103	76-111	148-193	295-330
plate53_B08_group61	104	76-111	148-193	295-351
plate54_A03_group62	105	73-105	142-187	289-324
plate54_E11_group63	106	65-97	134-179	281-316
plate54_D09_group64	107	65-97	134-179	281-316
plate54_C01_group65	108	65-97	134-179	281-316
plate54_G05_group66	109	60-92	129-174	276-311
plate54_C12_group67	110	77-112	149-194	296-358

[1]Each CDR sequence is identified by the nucleotide positions of the provided SEQ ID NO.

Example 4: Rabies prophylaxis using lyssavirus mAbs

A subject diagnosed with rabies or at risk of developing rabies (such as a subject bitten by a potentially rabid animal) is subjected to rabies post-exposure prophylaxis, which includes administration of a rabies vaccine, such as Imovax™ or RabAvert™, according to the recommended dosing schedule. Subjects who have not

previously been vaccinated against rabies will further be treated with one or more of the lyssavirus mAbs disclosed herein.

Lyssavirus mAb is administered intramuscularly to the subject at a site distant to the site of vaccine administration. If the subject diagnosed with rabies, or at risk of developing rabies, has a bite wound, lyssavirus mAb can optionally be administered directly into the wound and to the area directly adjacent to the wound.

Doses of lyssavirus mAb generally range from 1 to 50 IU/kg. In particular examples, the dose is about 5 or about 20 IU/kg. However, the dose of lyssavirus mAb will vary depending upon the antibody (or antibody cocktail) selected and the particular subject to be treated. An appropriate does can be determined by a medical practitioner.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated embodiments are only preferred examples of the invention and should not be taken as limiting the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

CLAIMS

1. An isolated variable heavy (VH) domain monoclonal antibody that specifically binds lyssavirus glycoprotein, wherein the antibody is encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 1-110; wherein the antibody specifically binds rabies virus (RABV), Mokola virus (MOKV) or West Caucasian bat virus (WCBV) glycoprotein when encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 1-43; and wherein the antibody specifically binds Lagos bat virus (LBV), MOKV, WCBV or Duvenhage virus (DUVV) glycoprotein when encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 44-110.

2. An isolated immunoconjugate comprising the monoclonal antibody of claim 1 and a fusion partner.

3. The isolated immunoconjugate of claim 2, wherein the fusion partner is an effector molecule, a label or a heterologous polypeptide.

4. A composition comprising the monoclonal antibody of claim 1 or the immunoconjugate of claim 2 or claim 3 and a pharmaceutically acceptable carrier.

5. A composition comprising the monoclonal antibody of claim 1 and a monoclonal antibody specific for RABV or RABV glycoprotein.

6. An isolated nucleic acid molecule encoding the monoclonal antibody of claim 1.

7. The isolated nucleic acid molecule of claim 6, comprising the nucleotide sequence of any one of SEQ ID NOs: 1-110.

8. Use of the monoclonal antibody of claim 1, the immunoconjugate of claim 2 or claim 3, or the composition of claim 4 or claim 5, for treating rabies in a subject.

9. Use of the monoclonal antibody of claim 1, the immunoconjugate of claim 2 or claim 3, or the composition of claim 4 or claim 5, for the preparation of a medicament for treating rabies in a subject.

10. The monoclonal antibody of claim 1, the immunoconjugate of claim 2 or claim 3, or the composition of claim 4 or claim 5, for use in treating rabies in a subject.

11. An expression vector comprising the nucleotide sequence of any one of SEQ ID NOs: 1-110.

12. The expression vector of claim 11, further comprising the nucleotide sequence of a V_L domain from a lyssavirus-specific monoclonal antibody.

13. The expression vector of claim 12, wherein the V_L domain is from a rabies virus (RABV)-specific monoclonal antibody.

14. The expression vector of any one of claims 11-13, wherein the expression vector is an Fc IgG expression vector.

15. A cell comprising the expression vector of any one of claims 11-14.

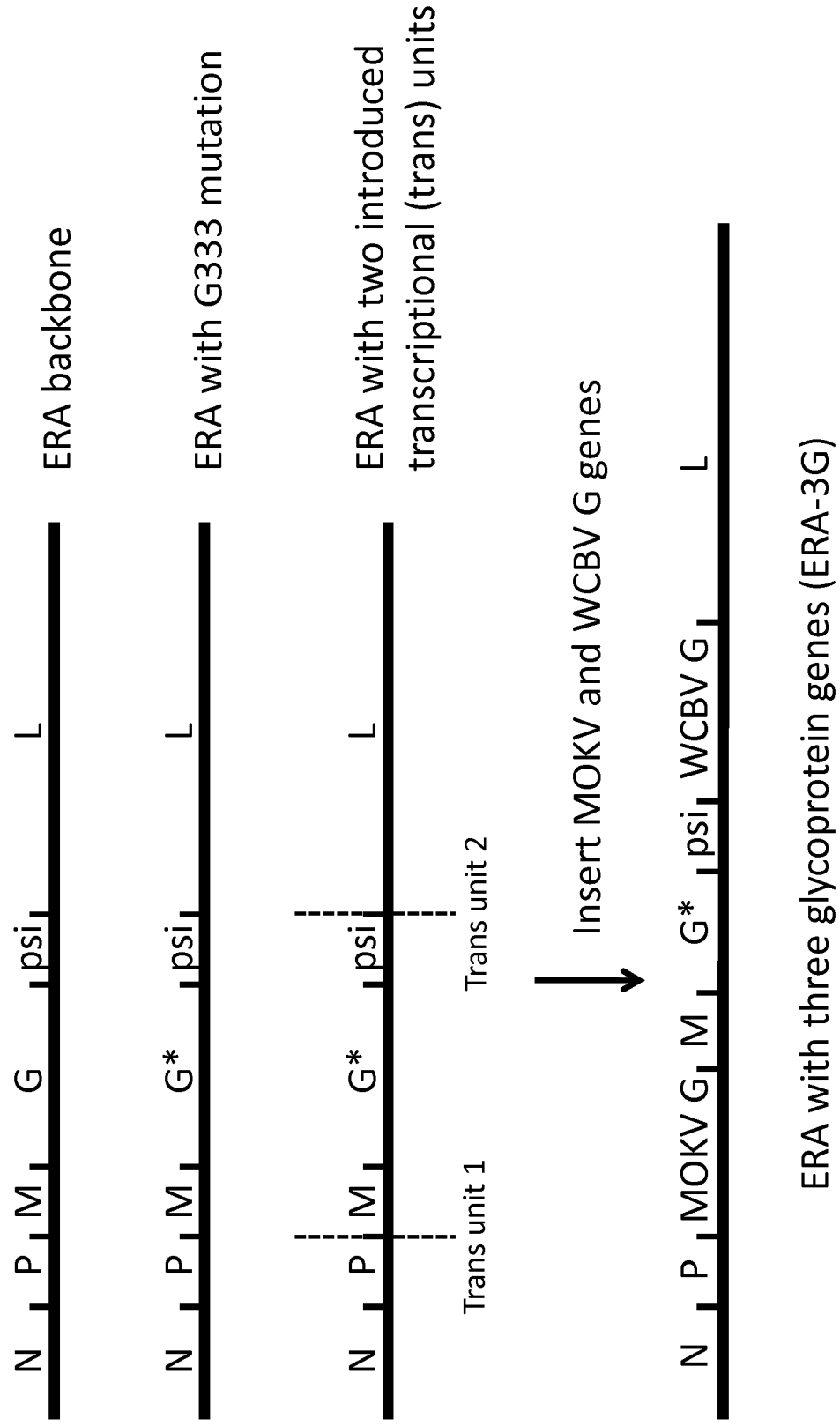
16. A monoclonal antibody encoded by the expression vector of any one of claims 11-14.

17. Use of the monoclonal antibody of claim 16 for treating rabies in a subject.

18. Use of the monoclonal antibody of claim 16 for the preparation of a medicament for treating rabies in a subject.

19. The monoclonal antibody of claim 16 for use in treating rabies in a subject.

FIG. 1


ERA backbone
N
P
M
G
psi
L
ERA with G333 mutation
G*
ERA with two introduced transcriptional (trans) units
Trans unit 1
Trans unit 2
Insert MOKV and WCBV G genes
MOKV G
WCBV G
ERA with three glycoprotein genes (ERA-3G)

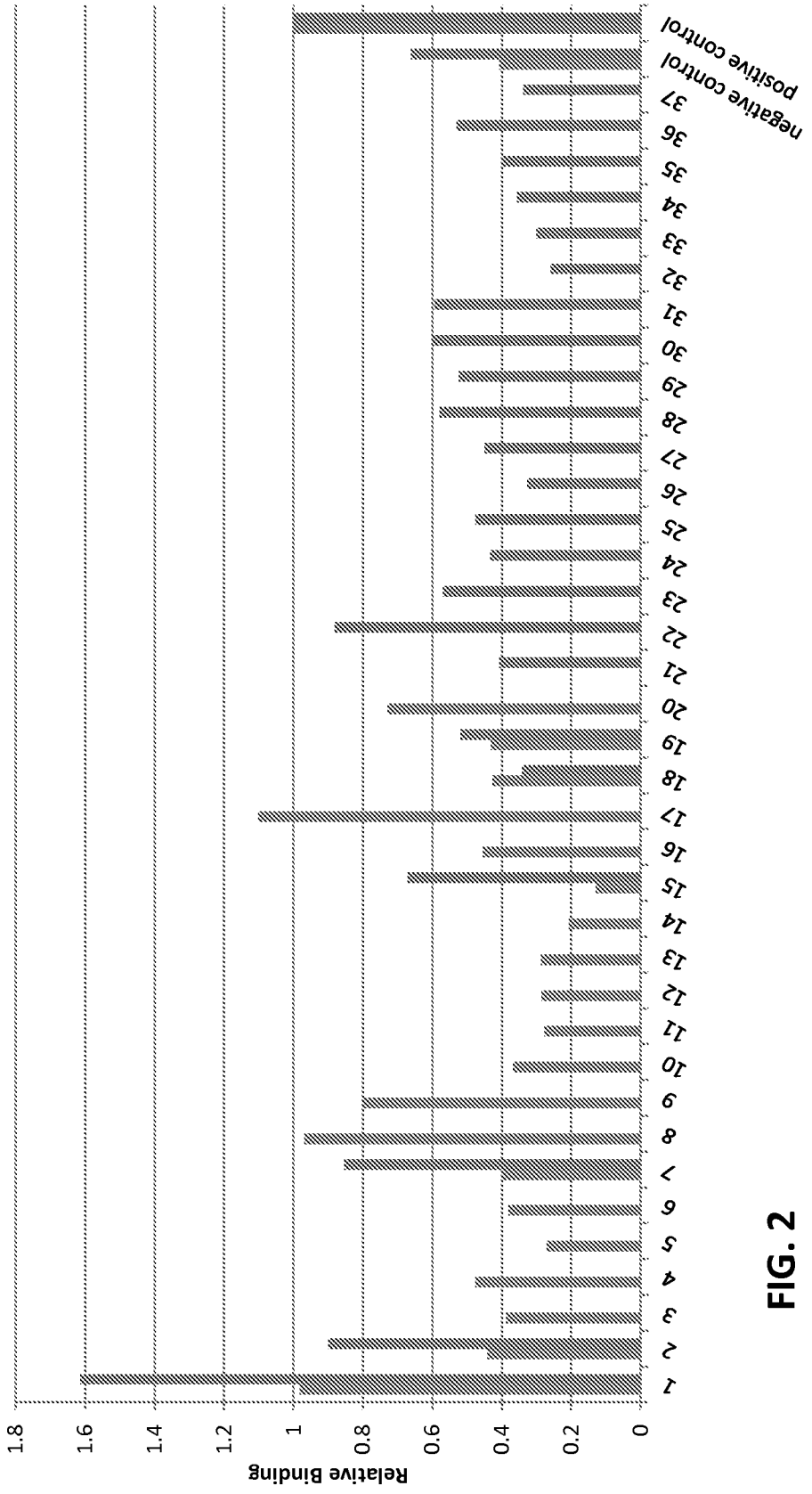
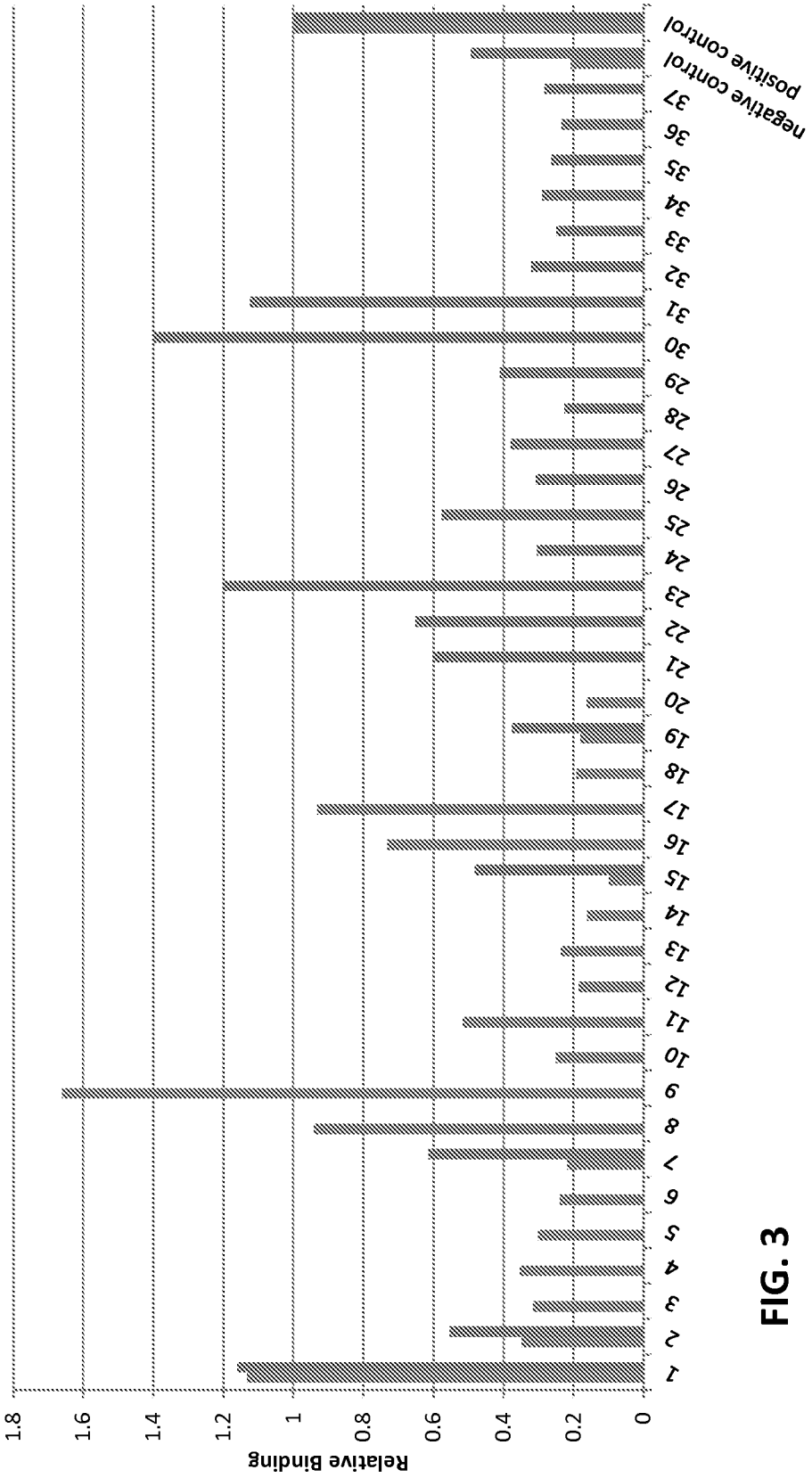

Relative Binding
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Clones/Groups
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
negative control
positive control

FIG. 2


Relative Binding
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Clones/Groups
1
2
3
4
5
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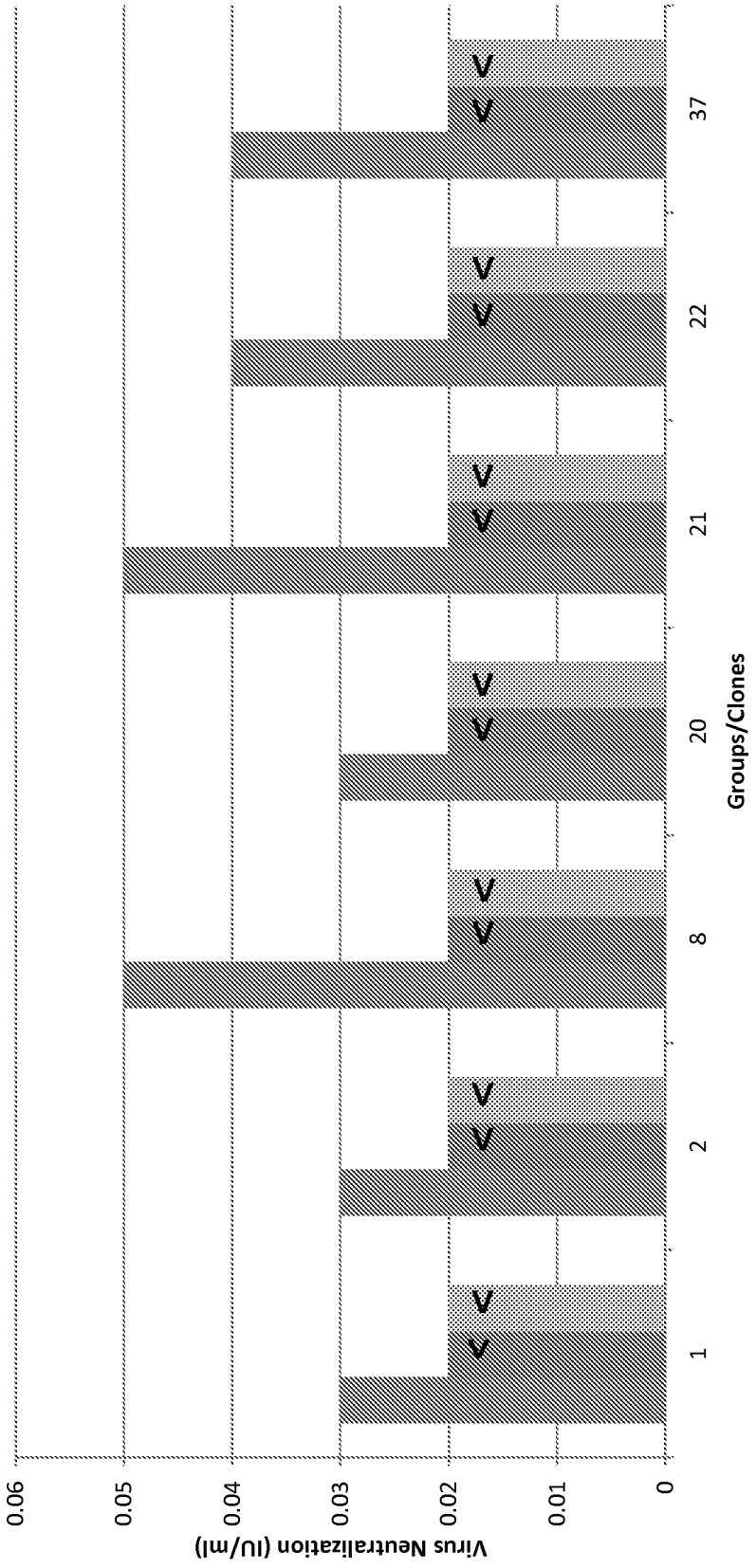
negative control
positive control

FIG. 3

FIG. 4


Virus Neutralization (IU/ml)
0.06
0.05
0.04
0.03
0.02
0.01
0
1
2
8
20
21
22
37
Groups/Clones
V