Title: Liquid anti-rabies antibody formulations

FIELD OF THE INVENTION

The invention relates to medicine. In particular the invention is directed to stable formulations of specific anti-rabies antibodies.

BACKGROUND OF THE INVENTION

In the past ten years, advances in biotechnology have made it possible to identify, develop and produce a variety of antibodies for use in the diagnosis, prevention, and treatment of many different diseases and disorders. Examples of such antibodies are the anti-rabies virus antibodies described in WO 2005/118644. An antibody cocktail with two such antibodies, CR57 and CR4098, is particularly advantageous and can be used in rabies post-exposure prophylaxis. In WO 2005/118644, these antibodies were prepared and used in PBS.

Like any protein, the biological activity of an antibody, such as its binding affinity or neutralizing activity, depends upon the conformational integrity of at least a core sequence of amino acids remaining intact while protecting the protein's multiple functional groups from degradation. Chemical and physical instability can each contribute to degradation of an antibody. Because antibodies are larger and more complex than traditional organic and inorganic drugs, the formulation of such antibodies poses special problems. Antibody stability can be affected by such factors as ionic strength, pH, temperature, repeated cycles of freeze/thaw, antibody concentration and shear forces. Active antibodies may be lost as a result of physical instabilities, including denaturation, aggregation (both soluble and insoluble aggregate formation), precipitation and adsorption as well as chemical instabilities, including, for example, racemization, beta-elimination or disulfide exchange, hydrolysis, deamidation, and oxidation, to name just a few. Any of these instabilities can potentially result in the formation of antibody by-products or derivatives having lowered biological activity, increased toxicity, and/or increased immunogenicity.

While the prior art indicates numerous examples of excipients that can be suitably employed to create antibody formulations for specific antibodies, it is impossible to predict which excipients should be added and in what amount they should be added to overcome the particular instability problems that a particular antibody may have. Furthermore, it is difficult

to find optimal conditions, such as antibody concentration, pH and storage temperature, that keep a particular antibody chemically and biologically stable within a particular formulation. In view of all the factors that can be varied, finding suitable excipients and optimal conditions for formulating a single monoclonal antibody is fraught with challenges. Obviously, finding suitable excipients and optimal conditions for formulating two different monoclonal antibodies in a single formulation is even more difficult and problematic. Notably, the art does not provide a long-term stable pharmaceutical preparation containing two different recombinant monoclonal antibodies.

Accordingly, there existed a need in the art to find formulations wherein not only a single monoclonal antibody, but even two different specific monoclonal antibodies against rabies virus, are stable on storage over a prolonged period of time. The storage stability should also be retained in the case of shear forces acting during transport and under modified climatic conditions, in particular at elevated temperature and atmospheric humidity. Furthermore, the formulation should be suitable for the intended route of administration, should be well tolerated and should have a simple structure.

It is an object of the invention to provide such formulations.

SUMMARY OF THE INVENTION

Formulations that meet the requirements of the object of the invention have surprisingly been found in the form of aqueous solutions that in addition to the two different monoclonal antibodies, comprise citrate buffer, a tonicity agent and a surfactant. Phosphate buffer was surprisingly found to lead to instability of the specific antibodies, which instability was even increased by addition of surfactant. The invention thus provides formulations for the specific anti-rabies antibodies CR57 and CR4098, or functional variants thereof. The invention also pertains to antibody formulations comprising both CR57 and CR4098, or functional variants thereof. The formulations contain, besides the active ingredient (the antibody or antibodies), a citrate buffer, a tonicity agent and a surfactant. The formulations of the invention are stable for at least 1 year at –70°C and at 5°C.

DESCRIPTION OF THE FIGURES

The stability of anti-rabies virus antibody CR57 (Fig. 1), CR4098 (Fig. 2) and a cocktail of CR57 and CR4098 (Fig. 3) after storage for 0 (white columns), 2 (black columns) and 4 weeks (shaded columns) at 40±2°C/75±5% relative humidity as measured by HP-SEC is shown. From left to right the following buffer systems were tested: citrate (20 mM, pH 6.0); citrate (20 mM, pH 6.5); phosphate (20 mM, pH 7.0); phosphate (20 mM, 0.01% w/v polysorbate 80, pH 7.0).

DETAILED DESCRIPTION OF THE INVENTION

The formulations of the invention comprise at least one of, and preferably both of, antibody CR57 (heavy chain SEQ ID NO: 1 and light chain SEQ ID NO: 2) and antibody CR4098 (heavy chain SEQ ID NO: 3 and light chain SEQ ID NO: 4). Identification, isolation, preparation and characterization of the anti-rabies virus monoclonal antibodies CR57 and CR4098 has been described in detail in WO 2005/118644 which is incorporated herein by reference. Functional variants of these antibodies may have similar physicochemical properties based on their high similarity and therefore are also included within the scope of the invention. Functional variants are defined for the present invention as antibodies with an amino acid sequence that is at least 95%, preferably at least 97%, for instance at least 98% or 99% homologous to CR59 or CR4098, and capable of competing for binding to the target recognized by the parent molecule (the parent molecule being CR59 or CR4098, respectively) and having rabies virus neutralizing activity. A target for an antibody is an antigen (for the present antibodies this is rabies virus, in particular G protein thereof), and may be further defined as an epitope. The targets of the parent molecules have been disclosed in WO 2005/118644, and determining competition for binding to the target can be done by routine methods known to the skilled person. Preferably the functional variants are human antibodies, and preferably are IgG1 molecules. In preferred embodiments, a functional variant is at least 95%, 97%, 98%, or 99% identical in amino acid sequence with the parent antibody. The term "functional variant", as used herein, thus refers to a monoclonal antibody that comprises an amino acid sequence that is altered by one or more amino acids compared to the amino acid sequences of the parental monoclonal antibody. The functional variant may have conservative sequence modifications including amino acid substitutions, additions and deletions. Amino

acid modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis, molecular cloning, oligonucleotide-directed mutagenesis and random PCR-mediated mutagenesis in the nucleic acid encoding the antibodies. Conservative amino acid substitutions include the ones in which the amino acid residue is replaced with an amino acid residue having similar structural or chemical properties. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan). It will be clear to the skilled artisan that other classifications of amino acid residue families than the one used above can also be employed. Furthermore, a variant may have non-conservative amino acid substitutions, *e.g.*, replacement of an amino acid with an amino acid residue having different structural or chemical properties. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing immunological activity may be found using computer programs well known in the art. Computer algorithms such as *inter alia* Gap or Bestfit known to a person skilled in the art can be used to optimally align amino acid sequences to be compared and to define similar or identical amino acid residues.

Functional variants may have the same or different, either higher or lower, binding affinities compared to the parental antibody but are still capable of specifically binding to the rabies virus or a fragment thereof, and may have the same, higher or lower, rabies virus neutralizing activity as the parental antibody.

In a specific embodiment the formulation according to the invention comprises a first anti-rabies virus monoclonal antibody that has a kappa light chain and a second anti-rabies virus monoclonal antibody that has a lambda light chain. This allows easy determination of the antibody concentration for each antibody, as specific ELISAs can be performed for each of the kappa and the lambda light chain.

The term “monoclonal antibody” as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding

specificity and affinity for a particular epitope. The monoclonal antibodies of the invention (CR57 and CR4098 and functional variants thereof) for the formulations of the present invention are human antibodies and are in the IgG class of antibodies, preferably IgG1.

Methods for production of monoclonal antibodies are well known in the art and are described, for example, in Antibodies: A Laboratory Manual, Edited by: E. Harlow and D, Lane (1988), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, which is incorporated herein by reference.

The term "specifically binding" means immunospecifically binding to an antigen or a fragment thereof and not immunospecifically binding to other antigens. A monoclonal antibody that immunospecifically binds to an antigen may bind to other peptides or polypeptides with lower affinity as determined by, *e.g.*, radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), BIACORE, or other assays known in the art. Monoclonal antibodies or fragments thereof that immunospecifically bind to an antigen may be cross-reactive with related antigens. Preferably, monoclonal antibodies or fragments thereof that immunospecifically bind to an antigen do not cross-react with other antigens.

By "pharmaceutically acceptable excipient" is meant any inert substance that is combined with an active molecule such as monoclonal antibody for preparing an agreeable or convenient dosage form. The "pharmaceutically acceptable excipient" is an excipient that is non-toxic to recipients at the dosages and concentrations employed, and is compatible with other ingredients of the formulation comprising the monoclonal antibody.

The term "by-product" includes undesired products, which detract or diminish the proportion of therapeutic/prophylactic antibody in a given formulation. Typical by-products include aggregates of the antibody, fragments of the antibody, *e.g.* produced by degradation of the antibody by deamidation or hydrolysis, or mixtures thereof. Typically, aggregates are complexes that have a molecular weight greater than the monomer antibody. Antibody degradation products may include, for example, fragments of the antibody, for example, brought about by deamidation or hydrolysis. Typically, degradation products are complexes that have a molecular weight less than the monomer antibody. In the case of an IgG antibody, such degradation products are less than about 150 kD.

A "stable/stabilized" formulation as used herein is one in which the antibody therein essentially retains its physical stability/identity/integrity and/or chemical stability/identity/integrity and/or biological activity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N. Y., Pubs. (1991) and Jones, A. Adv. Drug Delivery Rev. 10:29-90 (1993), for example. Stability can be measured at a selected temperature and other storage conditions for a selected time period. The stability may be determined by at least one of the methods selected from the group consisting of visual inspection, SDS-PAGE, IEF, HPSEC, RFFIT, and kappa/lambda ELISA. A monoclonal antibody "retains its physical stability" in a pharmaceutical formulation, if it shows no signs of aggregation, precipitation and/or denaturation upon visual examination of colour and/or clarity, or as measured by UV light scattering, SDS-PAGE or by (high pressure) size exclusion chromatography (HPSEC). Preferably, when using the formulations according to the invention, 5% or less, typically 4% or less, preferably 3% or less, more preferably 2% or less and particularly 1% or less of the antibodies forms aggregates as measured by HPSEC or any other suitable method for measuring aggregation formation. *E.g.*, an antibody is considered stable in a particular formulation if the antibody monomer has a purity of ≥ about 90%, preferably ≥ about 95%, in particular ≥ about 98% as measured by HPSEC after a certain predetermined period of time under certain storage conditions in said particular formulation. Thus, the CR57 and CR4098 antibodies are stable in the formulations of the invention upon storage at 5±3°C for at least 18 months, i.e. the monomer peak in the HPSEC chromatogram comprises an area of >95% of the total area of all peaks (in Table 9 it can be seen that the main peak area is even >99%). Chemical stability can be assessed by detecting and quantifying chemically altered forms of the protein. Chemical alteration may involve size modification (*e.g.* clipping) which can be evaluated using (HP)SEC, SDS-PAGE and/or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS), for example. Other types of chemical alteration include charge alteration (*e.g.* occurring as a result of deamidation) which can be evaluated by ion-exchange chromatography, for example. An antibody "retains its biological activity" in a pharmaceutical formulation at a given time, if the biological activity of the antibody at a given time is at least about 90% (within the errors of the assay) of the biological activity exhibited at

the time the pharmaceutical formulation was prepared as determined in an antigen binding assay or virus neutralizing assay, for example.

"About" as used in the present application means ±10%, unless stated otherwise.

In a first aspect the invention encompasses a pharmaceutical formulation comprising at least an active ingredient, preferably in a therapeutically effective amount, and at least a pharmaceutically acceptable excipient. Preferably, the pharmaceutical formulation comprises a citrate buffer, a tonicity agent, a surfactant and two anti-rabies virus monoclonal antibodies, wherein the antibodies are different from one another. The formulation may be solid, *e.g.* frozen or lyophilised, but is preferably liquid, *e.g.* aqueous. The formulation may comprise at least two distinct anti-rabies virus monoclonal antibodies, in particular (i) CR57 (antibody with amino acid sequence of heavy chain SEQ ID NO: 1 and light chain SEQ ID NO: 2) or a functional variant thereof and (ii) CR4098 (antibody with amino acid sequence of heavy chain SEQ ID NO: 3 and light chain SEQ ID NO: 4) or a functional variant thereof.

In a specific embodiment the formulation according to the invention has a rabies virus neutralizing potency ranging from about 250 IU/ml to about 1500 IU/ml, e.g. from about 300 IU/ml to about 1400 IU/ml, typically from about 380 IU/ml to about 1350 IU/ml. It is well within the reach of a person skilled in the art to measure rabies virus neutralization. Neutralization can for instance be measured as described in Laboratory techniques in rabies, Edited by: F.-X. Meslin, M.M. Kaplan and H. Koprowski (1996), 4th edition, Chapters 15-17, World Health Organization, Geneva. A suitable and known assay for neutralizing activity is a RFFIT assay.

In an embodiment the rabies virus neutralizing potency of the formulations of the invention after 12 months of storage at 5 ± 3°C is at least 80%, preferably at least 90%, more preferably at least 95%, more preferably at least 98%, and in particular 100% of the rabies virus neutralizing potency of the formulations of the invention before storage. In certain embodiments the rabies virus neutralizing potency of the formulations of the invention after 3 months of storage at 25 ± 2°C is at least 90%, preferably at least 95%, more preferably at least 98%, and in particular 100% of the rabies virus neutralizing potency of the formulations of the invention before storage.

The formulations according to the invention comprise a surfactant, also known as stabilizer. Surfactants may include, but are not limited to, polysorbates. The skilled person is aware that other surfactants, e.g. non-ionic or ionic detergents, can be used as surfactants as long as they are pharmaceutically acceptable, i.e. suitable for administration to humans. In a preferred embodiment the invention provides a formulation according to the invention, wherein the surfactant is a polysorbate such as polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65 or polysorbate 80, with polysorbate 80 being preferred. In an embodiment the polysorbate 80 is present in the formulations in an amount from about 0.0005% w/v to about 0.05% w/v, preferably from about 0.005% w/v to about 0.03% w/v, more preferably from about 0.008% w/v to about 0.015% w/v. In a preferred embodiment polysorbate 80 is present in an amount of about 0.01% w/v.

In certain embodiments, the invention provides formulations according to the invention, wherein the citrate buffer, *e.g.* sodium citrate dehydrate (2.5 mg/ml)/citric acid monohydrate (0.3 mg/ml) buffer, is present at a concentration from about 5 mM to about 25 mM, preferably from about 7 mM to about 20 mM, more preferably from about 8 mM to about 15 mM, and particularly from about 9 mM to about 12 mM. In a preferred embodiment the citrate buffer is present at a concentration of about 10 mM.

In certain embodiments the invention is concerned with formulations according to the invention, wherein the pH ranges from about 5.2 to about 6.8, typically from about 5.5 to about 6.5, preferably from about 5.7 to about 6.3, more preferably from about 5.8 to about 6.2 and particularly from about 5.9 to about 6.1. In a preferred embodiment the pH is about 6.0.

In a specific, non-limiting, embodiment the tonicity agent is sodium chloride. Other salts can for instance also be used as tonicity agents, or for instance sugars, and the like, as long as they are pharmaceutically acceptable, as is known to the skilled person. In certain embodiments of the invention, the tonicity agent is present at a concentration from about 50 mM to about 250 mM, typically from about 75 mM to about 225 mM, preferably from about 100 mM to about 200 mM, and more preferably from about 125 mM to about 175 mM. In a preferred embodiment the tonicity agent is present at a concentration of about 150 mM. In certain embodiments the osmolality of the formulations according to the invention ranges from about 250 mOsm/kg to about 350 mOsm/kg, preferably from about 270 mOsm/kg to

about 330 mOsm/kg, more preferably from about 280 mOsm/kg to about 320 mOsm/kg, and particularly from about 290 mOsm/kg to about 310 mOsm/kg. In a preferred embodiment the osmolality is about 300 mOsm/kg. In other words, the formulations are preferably substantially isotonic, *i.e.* having substantially the same osmotic pressure as human blood. Isotonicity can be measured using vapour pressure or ice-freezing type osmometers, for example. The osmolality of the formulations of the invention can for instance be regulated by one or more tonicity agents.

The concentration of each antibody in the formulations of the invention preferably is between about 0.1 and 2.0 mg/ml, typically between about 0.1 and 1 mg/ml. In certain non-limiting embodiments, the concentration of each antibody is 0.15 (± 20%) mg/ml. In other non-limiting embodiments each antibody is present in a concentration of 0.3 (± 20%) mg/ml (i.e. total 0.6 mg/ml for two antibodies).

In certain embodiments, the (protein) ratio of the two antibodies is between 5:1 and 1:5, preferably between 2:1 and 1:2 and particularly about 1:1.

Furthermore, the formulation according to the invention may comprise other excipients including, but not limited to, amino acids and salts thereof, sugars, proteins, diluents, solubilizing agents, pH-modifiers, soothing agents, additional buffers, other inorganic or organic salts, antioxidants, or the like. Preferably, however, the formulations of the present invention comprise no other excipients next to a citrate buffer, a tonicity agent and a surfactant.

In the formulations according to the invention the anti-rabies virus monoclonal antibodies CR57 and CR4098 are stable at about 2°C to about 8°C for at least about 1 year, typically at least about 18 months. Preferably they may be stable at about 2-8°C for at least about 2 years, more preferably 3 years.

Furthermore, the anti-rabies virus monoclonal antibodies are stable in the formulations according to the invention at about 25 ± 2°C for at least about 2 months. Besides that, the anti-rabies virus monoclonal antibodies are stable in the formulations according to the invention at about 40 ± 2°C for at least 2 weeks.

In certain embodiments the formulations are suitable for administering intramuscularly, intradermally, subcutaneously, injected locally into a wound, or a combination thereof. Therefore, the formulations are preferably sterile. Methods for making formulations sterile are well known in the art and include filtration through sterile filtration membranes or autoclaving the ingredients of the formulation, with the exception of the antibodies, at about 120°C for about 30 minutes, for example.

In preferred embodiments the formulations are substantially free of endotoxin. Endotoxins are low molecular weight complexes of about 10 kDa that are associated with the outer cell wall of gram-negative bacteria that can produce pyrogenic reactions upon parenteral administration to a patient. Accordingly, the FDA has set an upper limit of 5 EU per dose per kilogram body weight in a single one-hour period for intravenous drug applications (see, *e.g.*, The United States Pharmacopeial Convention (USP), Pharmacopeial Forum 26 (1):223 (2000)). In certain embodiments, the formulation has a concentration of endotoxin of less than about 5.0 endotoxin units per milliliter (EU/ml) (a concentration of less than about 5.0 EU/ml is referred to herein as substantially free of endotoxin), preferably less than about 2.5 EU/ml, more preferably less than about 1.0 EU/ml, even more preferably less than about 0.5 EU/ml and particularly less than about 0.30 EU/ml. In certain embodiment, the formulation has a concentration of endotoxin that ranges from about 0.001 EU/ml to about 5.0 EU/ml. Methods for measuring endotoxins are known to a person skilled in the art and include, but are not limited to, gel-clot assays, turbidimetric (spectrophotometric) assays and chromogenic assays.

"Post exposure prophylaxis" (PEP) is indicated for persons possibly exposed to a rabid animal. Possible exposures include bite exposure (*i.e.* any penetration of the skin by teeth) including animal bites, and non-bite exposure. The formulations according to the invention can be administered to a subject in need thereof for use in prevention and/or treatment, e.g. post exposure prophylaxis, of a rabies virus infection. The formulations of the invention may be employed in conjunction with other molecules useful in diagnosis, prophylaxis and/or treatment of rabies virus. For instance, they can be co-administered with a vaccine against rabies virus. Alternatively, the vaccine may also be administered before or after

administration of the formulations of the invention. Administration of the formulations of the invention with a vaccine is suitable for post exposure prophylaxis. Rabies vaccines include, but are not limited to, purified chick embryo cell (PCEC) vaccine (RabAvert, Rabipur), human diploid cell vaccine (HDCV; Imovax vaccine) or rabies vaccine adsorbed (RVA). Preferably, a single bolus of the formulations of the invention are administered. The dosing regimen of post exposure prophylaxis is administration of five doses of rabies vaccine intramuscularly in the deltoid muscle on days 0, 3, 7, 14 and 28 after exposure in individuals not previously immunized against rabies virus. The formulations according to the invention should be administered into and around the wounds on day 0 or otherwise as soon as possible after exposure, with the remaining volume given intramuscularly at a site distant from the vaccine. Non-vaccinated individuals are advised to be administered anti-rabies virus antibodies. A therapeutically effective amount of antibody or antibodies is administered, which amount is effective or at least partially effective for PEP of rabies, i.e. rabies virus is neutralized.

In a further aspect the invention provides a pharmaceutical unit dosage form comprising an effective amount of a formulation according to the invention for post exposure prophylaxis treatment of a subject through administration of the dosage form to the subject. In a preferred embodiment the subject is a human. The human may be an adult or may be an infant. The term "pharmaceutical unit dosage form" as used herein refers to a physically discrete unit suitable as unitary dosages for the subjects to be treated, each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic/prophylactic effect in association with the required pharmaceutical carrier, diluent, or excipient.

The unit dosage form may be a container comprising the formulation. Suitable containers include, but are not limited to, sealed ampoules, vials, bottles, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic and may have a sterile access port (for example the container may be a vial having a stopper pierceable by a hypodermic injection needle). In a preferred embodiment the container is a vial. The vial preferably comprise a volume from about 0.3 ml to about 3 ml. Preferably, the vial contains anti-rabies virus antibodies in an amount from about 0.1 mg to about 2.0 mg. In an embodiment the vial contains a total of 750-2000 IU of rabies virus neutralizing

monoclonal antibodies per vial. This type of vial can suitably be used for administration to an adult, while a vial containing a total of 250-750 IU of rabies virus neutralizing monoclonal antibodies per vial can suitable be used for administration to an infant. The antibodies are typically formulated in the formulations of the invention in a therapeutically effective amount. Dosage regimens can be adjusted to provide the optimum desired response (*e.g.* a therapeutic response). A suitable dosage range may for instance be 10-30 IU/kg body weight, such as about 20 IU/kg body weight.

The pharmaceutical unit dosage form may be present in a kit, further comprising a instructions for use. The kit may further comprise more containers comprising pharmaceutically acceptable excipients and include other materials desirable from a commercial and user standpoint, including filters, needles, syringes. Associated with the kits can be instructions customarily included in commercial packages of therapeutic, prophylactic or diagnostic products, that contain information about for example the indications, usage, dosage, manufacture, administration, contra-indications and/or warnings concerning the use of such therapeutic, prophylactic or diagnostic products. In certain embodiments the kit comprises instructions to use the appropriate volume necessary to achieve a dose of about 5 IU/kg to about 40 IU/kg, e.g. 20 IU/kg.

Furthermore, the present invention is concerned with a method for improving the storage of two anti-rabies virus monoclonal antibodies in one, *e.g.* a single, formulation by formulating the antibodies (CR57 and CR4098 or functional variants) in a liquid pharmaceutical formulation according to the invention. The formulation may be stored at a temperature from about 2°C to about 40°C, e.g. between about 2-8°C. However, the formulations may also be stored at temperatures below 2°C, e.g. at about –20°C, -70°C, etc. By storing the antibodies in the specific formulations according to the invention the amount of by-product formation of the antibodies is reduced. For practical reasons, it is preferred to store the individual antibodies CR57 and CR4098 frozen, e.g. at -70±10°C, before they are mixed, while the final product (cocktail of CR57 and CR4098) is preferably stored in liquid form at 5±3°C.

In a further aspect the invention also pertains to liquid pharmaceutical formulations comprising a single anti-rabies virus monoclonal antibody, i.e. either CR57 or CR4098 or a functional variant of one of these. Preferably, these formulations comprise all features and excipients as described hereinabove. Thus, in preferred embodiments they contain citrate buffer (5-25 mM) and have pH 5.5-6.5, e.g. about 6.0; contain a tonicity agent (e.g. sodium chloride, 50-250 mM, e.g. about 150 mM); comprise a surfactant, e.g. polysorbate 80 (0.0005%-0.05%, e.g. about 0.01%), and are preferably substantially isotonic, sterile and substantially free of endotoxin Features and excipients that might differ from those described above for formulations comprising two different anti-rabies virus monoclonal antibodies are indicated below.

In certain embodiments, formulations according to the invention may comprising a single anti-rabies virus monoclonal antibody in an amount from about 0.1 mg/ml to about 6.0 mg/ml, typically from about 1.0 mg/ml to about 4.0 mg/ml, e.g. from about 2.0 mg/ml to about 3.0 mg/ml. In a specific embodiment the formulation has a rabies virus neutralizing potency ranging from about 300 IU/mg to about 1600 IU/mg, e.g. from about 500 IU/mg to about 1250 IU/mg. Formulations comprising a single antibody as described above may be combined/mixed with one another to obtain the formulations of the invention comprising two antibodies, *i.e.* an antibody cocktail.

EXAMPLES

To illustrate the invention, the following examples are provided. The examples are not intended to limit the scope of the invention in any way. In general, the practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, recombinant DNA technology, immunology such as antibody technology and standard techniques of polypeptide preparation as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: Cold Spring Harbor Laboratory Press (1989); Antibody Engineering Protocols (Methods in Molecular Biology), volume 51, Ed.: Paul S., Humana Press (1996); Antibody Engineering: A Practical Approach (Practical Approach Series, 169), Eds.: McCafferty J. *et al.*, Humana Press (1996); Antibodies: A Laboratory Manual, Harlow and Lane, Cold Spring Harbor Laboratory Press (1999); and Current Protocols in Molecular Biology, Eds. Ausubel *et al.*, John Wiley & Sons (1992), for example.

The amino acid sequences of the CR57 and CR4098 antibodies is shown in Table 10. Identification, cloning, preparation and characterization of these neutralizing anti-rabies virus antibodies has been described in detail in WO 2005/118644. The antibodies were manufactured on a large scale in an essentially similar way. A starting culture of PER.C6 cells stably expressing an anti-rabies virus antibody was thawed, the cells were cultured and cultures were expanded and used to inoculate a bioreactor. The bioreactor was first operated in batch mode followed by fed-batch mode. Medium containing the respective antibody was harvested and clarified by centrifugation and filtrated before further downstream processing. The downstream purification process consisted of standard chromatographic and filtration steps followed by a buffer exchange to formulation buffer lacking polysorbate 80 and concentration to obtain the desired antibody concentration. After addition of polysorbate 80 and filtration, the obtained drug substance (either antibody CR57 or antibody CR4098) was stored at –80°C until further use. For the manufacture into drug product the drug substances were diluted with formulation buffer, antibody concentrations were measured, and both antibody dilutions were mixed and filtered before final filling.

For stability studies different formulations of the drug substances (single antibodies) and the drug product (cocktail of antibodies) were prepared and analysed. Samples of the different formulations were analysed at different time points and temperatures using various analytical methods well known in the art.

HPSEC, SDS-PAGE (reduced and non-reduced), protein concentration (A280), IEF, appearance, pH, and osmolality were used to evaluate the stabilising effects of the different formulation buffers on the CR57 antibody, the CR4098 antibody and mixtures thereof.

HPSEC was used in part to assess the presence of degradation products of the antibodies due to aggregation or proteolysis. SDS-PAGE was used in part to assess the integrity of the intact antibody and the presence of impurities and potential degradation products. Protein concentration was measured to assess the maintenance of the formulation's protein concentration within an acceptable range. IEF was used to assess the presence and integrity of antibody isoforms that may be present in the formulations and monitor them over time to assess changes that may occur due to deamidation or loss of sialic acid. Appearance of the formulations was conducted based on visual inspection for clarity, colour and the presence of particulates. pH was measured to assess the maintenance of the formulation's pH within an

acceptable range of about 5.5 to about 6.5. Osmolality was measured to assess the maintenance of the formulation's osmolality within an acceptable range of about 250 mOsm/kg to about 350 mOsm/kg.

In a first study different buffer systems were tested. For that purpose anti-rabies virus antibody formulations formulated in citrate buffers were compared to anti-rabies virus antibody formulations formulated in phosphate buffers. Formulations comprising either CR57 (0.1 mg/ml), CR4098 (0.15 mg/ml) or a mixture/cocktail (1:1.5 mixture) of CR57 (0.1 mg/ml) and CR4098 (0.15 mg/ml) in a 20 mM citrate buffer (pH 6.0) or a 20 mM citrate buffer (pH 6.5) were stable upon storage up to 4 weeks at 5±3°C/ambient relative humidity, 25±2°C/60±5% relative humidity and 40±2°C/75±5% relative humidity as indicated by HPSEC analysis. All formulations had a purity of antibody monomer (area %) of >96% as determined by HPSEC (see Figures 1-3). Formulations comprising either CR57 (0.1 mg/ml), CR4098 (0.15 mg/ml) or a mixture (1:1.5 mixture) of CR57 (0.1 mg/ml) and CR4098 (0.15 mg/ml) in a 20 mM phosphate buffer (pH 7.0) had a purity of antibody monomer that was significantly lower upon storage for up to 4 weeks at 40±2°C/75±5% relative humidity compared to formulations in the citrate buffers for the same time period and under the same temperature conditions (see Figures 1-3). When polysorbate 80 (0.01% w/v) was added to the phosphate buffer, the purity of antibody monomer decreased further to values of about 70% for CR4098 and about 85% for CR57 and the mixture upon storage for up to 4 weeks at 40±2°C/75±5% relative humidity (see Figures 1-3). The results clearly show that stability of the separate antibodies as well as the mixture of antibodies is superior in citrate buffers compared to phosphate buffers. In phosphate buffers the antibodies are degraded through fragmentation. The antibodies were equally stable in formulations comprising citrate buffers of pH 6.0 and pH 6.5. Furthermore, it was concluded that addition of polysorbate 80 in phosphate buffers causes additional antibody impurities. The results found with HPSEC were confirmed by other analysis methods including IEF and SDS-PAGE (reduced and non-reduced)(data not shown). Based on the study citrate was used as a buffer system.

To determine optimal surfactant concentration of the formulations, citrate-based formulations comprising different polysorbate 80 concentrations were analysed (see Table 1).

The formulations were prepared as follows. The antibodies CR57 and CR4098 (drug substances) were prepared essentially as described above. They were filtered with a 0.1 μm filter. Protein concentration was the same before and after filtration as measured by A280 protein concentration determination. The concentration of CR57 was 2.5 mg/ml and the concentration of CR4098 was 1.0 mg/ml. Next, a buffer containing 10 mM citrate (pH 6.0) and 50 mM sodium chloride and a buffer containing 10 mM citrate (pH 6.0), 50 mM sodium chloride and 5% polysorbate 80 were prepared. The formulations were prepared as described in Table 2. The final volume was reached with a buffer containing 10 mM citrate (pH 6.0) and 50 mM sodium chloride. The osmolality of all formulations was determined and sodium chloride was added to bring the formulations to an osmolality of about 300 mOsm/kg (iso-osmotic), *i.e.* the final concentration of sodium chloride in the formulations was 150 mM. Finally, all formulations were filtered with 0.22 μm filters and filled out (400 μl) into 2 ml Eppendorf cups for all tests with the exception of the appearance test, shake study and pH analysis, wherein use was made of 5 ml injection vials (filled with 2 ml sample) capped with 20 mm stoppers and sealed with aluminium caps. The formulations were stored in stability cabinets at 5±3°C/ambient relative humidity, 25±2°C/60±5% relative humidity, or 40±2°C/75±5% relative humidity. At indicated time points two samples were taken and analysed *in monoplo* according to the schedule as shown in Table 3.

As already indicated above, protein concentration was the same before and after filtration as measured by A280 protein concentration determination.

The results of the HPSEC analysis are shown in Table 4. The protein components were separated through HPSEC using an isocratic elution method, which allows rapid analysis and high resolution of protein components and also has an improved reproducibility. The results show that at t=0 weeks all formulations had a purity as determined by HPSEC of 98-100%. All formulations showed a comparable purity at t=13 weeks (and all intermediate time points between t=0 and t=13 weeks) compared to t=0 weeks at 5±3°C/ambient relative humidity (*i.e.* 2-8°C/ambient relative humidity), indicating stability at this temperature for at least 13 weeks. Only for formulation 2 the purity at t=8 and t=13 weeks was just below 98%. Moreover, it was concluded from Table 4 that at 25±2°C/60±5% relative humidity all formulations at all time points showed a purity higher than 95%, indicating that the antibodies are also stable for at least 13 weeks at this temperature. At an elevated temperature of

40±2°C/75±5% relative humidity all formulations showed a purity of >95% in the first two weeks, indicating that the antibodies are stable for at least two weeks at this elevated temperature. At all time points beyond two weeks all formulations showed a purity higher than about 90%, indicating that the antibodies are relatively stable for at least 13 weeks at this elevated temperature. The impurities found with this method included aggregates and fragments of the antibody monomers. The results further indicated that formulations with 0.01% (w/v) polysorbate 80 had a higher purity than similar formulations with 0.03% (w/v) polysorbate 80 (compare purity of formulation 1 with 2, formulation 3 with 4, and formulation 5 with 6). The same impurities were observed for both polysorbate concentrations.

Results of the SDS-PAGE analysis were consistent with the data found with HPSEC. On the basis of non-reduced and reduced SDS-PAGE analysis, the formulations stored at 5±3°C/ambient relative humidity and 25±2°C/60±5% relative humidity showed no signs of significant degradation at all time points when compared to a reference standard, while some minor degradation was found at the different time points of the formulations stored at 40±2°C/75±5% relative humidity (data not shown). No differences for all formulations were observed between the different polysorbate 80 concentrations.

The identification of the antibody samples through SDS-PAGE only confirms the integrity of the antibodies, but it does not illustrate their native or denatured state. IEF illustrates the pI of the antibodies and is also helpful in indicating the conformational microheterogenicity of the antibodies. The combination of IEF with SDS-PAGE is a powerful tool for the detection of even small differences in antibody structures and properties. Based on the IEF results, for all formulations no significant differences were found between the different polysorbate 80 concentrations (data not shown). When the formulations were stored at 40±2°C/75±5% relative humidity, minor degradation (most likely due to deamidation) was observed from time point t=6 weeks on.

The visual inspection of the clarity and colour of all formulations stored at 5±3°C/ambient relative humidity, 25±2°C/60±5% relative humidity and 40±2°C/75±5% relative humidity showed that the formulations were practically free of particles up to t=13 weeks, although it was observed that the amount of formulations with particles increased slightly when stored at higher temperatures. The formulations comprising CR57 and CR4098

and 0.01% (w/v) polysorbate 80 contained less particles compared to the formulations comprising CR57 and CR4098 and 0.03% (w/v) polysorbate 80. The shake study indicated no differences between each of the formulations with respect to appearance. The pH values of all formulations did not significantly change during storage at the indicated temperatures and time periods.

The osmolality values of all formulations showed a very small increase during the stability study when kept at 25±2°C/60±5% relative humidity and 40±2°C/75±5% relative humidity. There was no significant difference between formulations comprising 0.01% (w/v) polysorbate 80 compared to formulations comprising 0.03% (w/v) polysorbate 80.

Overall, the results from the study show that single anti-rabies virus antibodies as well as mixtures/cocktails of anti-rabies virus antibodies have the best stability after 13 weeks at 5±3°C/ambient relative humidity, 25±2°C/60±5% relative humidity and 40±2°C/75±5% relative humidity in citrate-based formulations comprising 0.01% (w/v) polysorbate 80.

In a further study formulations comprising citrate (10 mM, pH 6.0), sodium chloride (150 mM), 0.01% (w/v) polysorbate 80 and the single antibody CR57 (1.2 mg/ml) or CR4098 (1.2 mg/ml) were studied when stored under the following two temperatures, 5±3°C and -70±10°C. Formulations were filled out (250 µl) into 1.2 ml tubes for IEF, SDS-PAGE (reduced and non-reduced) and HPSEC analyses. For pH and appearance analyses 2 ml tubes filled with 2 ml formulation were used. The formulations were stored in stability cabinets at 5±3°C or -70±10°C. At indicated time points (1, 2 and 3 months) samples were taken and analysed. The results of the study can be found in Tables 5 and 6.

SDS-PAGE analysis (both reduced and non-reduced) of CR57 and CR4098 formulations at both temperatures indicated that the integrity of the antibodies remained intact for a period of at least 3 months, as no additional degradation bands were observed compared to t=0 months. These results were confirmed by both IEF and HPSEC analysis showing that the antibody structure and aggregate level, respectively, after 3 months at 5±3°C/ambient relative humidity or -70±10°C was no different from t=0 months. In addition, protein concentration and pH did not significantly change over time. Visual inspection of each formulation showed a clear colourless liquid, practically free from particles.

Analysis of formulations stored at -70±10°C that were subjected to an additional freeze/thaw cycle after 1 month or 3 months storage showed no differences compared to t=0 months based on the above-mentioned assays, *i.e.* SDS-PAGE (reduced and non-reduced), HPSEC, IEF, appearance, OD280, and pH (data not shown).

In summary, the results indicate that the antibodies CR57 and CR4098 are stable at 5±3°C/ambient relative humidity and at -70±10°C in formulations comprising citrate buffer (10 mM, pH 6.0), polysorbate 80 (0.01% w/v) and sodium chloride (150 mM), thereby confirming the results described above. In addition, an additional freeze/thaw cycle after long-term storage (t=1 or t=3 months) of antibody samples stored at -70±10°C has no influence on the stability of antibody CR57 or CR4098.

In a similar stability study formulations comprising citrate (10 mM, pH 6.0), sodium chloride (150 mM), 0.01% (w/v) polysorbate 80 and the single antibody CR57 (2.47 mg/ml) or CR4098 (2.48 mg/ml) were studied when stored under two different temperatures, *i.e.* 5±3°C and -70±10°C, for an even longer period than 3 months. Formulations were filtered through a 0.22 µm filter and filled out in polypropylene tubes (4 ml). In addition, the combination of CR57 and CR4098 at a 1:1 ratio based on protein content (0.3 mg/ml of each antibody resulting in overall protein concentration of 0.6 mg/ml) was studied when stored under two different temperatures, *i.e.* 5±3°C and -70±10°C, for up to 6 months. The formulations comprising the cocktail/mixture of antibodies were filtered through a 0.22 µm filter and filled out in glass vials (2.6 ml). The formulations were tested using the following analysis methods: SDS-PAGE (reduced and non-reduced), IEF, HPSEC, RFFIT, appearance, pH (see Tables 7 and 8). Formulations with the combination CR57/CR4098 were also tested by kappa/lambda ELISA and osmolality (see Table 9). Moreover, at t=0 months the endotoxin levels of the formulations comprising the single antibodies or the cocktail of antibodies were determined. Endotoxin levels were assessed with a Limulus Amoebocyte Lysate (LAL) assay using a gel-clot technique. The formulation comprising CR57 contained < 0.30 EU/ml, the formulation comprising CR4098 contained < 0.30 EU/ml and the formulation containing the cocktail of both antibodies contained < 0.24 EU/ml. The formulations were stored in stability cabinets at -70±10°C, 5±3°C, 25±2°C or 40±2°C for the indicated time periods.

Formulations containing single antibody were analysed over a 6 months period and compared to the initial results obtained at t=0 months. IEF and SDS-PAGE band patterns of CR57 and CR4098 formulations stored at –70±10°C and 5±3°C for 1, 2, 3, and 6 months were comparable to the band patterns of the CR57 and CR4098 formulations at t=0 months, respectively (see Tables 7 and 8). No additional bands were detected. The HPSEC patterns of both antibodies stored at –70±10°C and 5±3°C for 6 months compared well with storage at t=0 months. The target specification of "main peak area > 95%" was met in all cases. The dimer peak remained < 1% (surface area) and no degradation peaks were detected in any of the samples tested.

Based on the results obtained with these three methods, it was concluded that no degradation of both CR57 and CR4098 occurred during 6 months storage at –70±10°C and 5±3°C in the indicated formulations.

Moreover, the protein content and the pH of CR57 and CR4098 were stable in the formulations over the tested time period of 6 months, both at –70±10°C and at 5±3°C. Analysis of potency (RFFIT-assay) indicated an increased potency value for both antibodies at the 2, 3, and 6 month time points compared to t=0 months and t=1 month time point under both conditions tested (*i.e.*, –70±10°C and 5±3°C). This apparent increase in potency was caused by an unstable SRIG control sample that was used as a positive reference in the assay (see Laboratory techniques in rabies, Edited by: F.-X. Meslin, M.M. Kaplan and H. Koprowski (1996), 4th edition, Chapters 15-17, World Health Organization, Geneva.). The effect was eliminated by expressing the results as 50% neutralizing end-point titers (data not shown). Based on these results, it was concluded that CR57 and CR4098 show stable end-point titers, hence stable potency, up to at least 6 months at both storage conditions in the indicated formulations.

In summary, based on the results, CR57 and CR4098 are considered to be stable for at least 6 months at the real time storage condition of –70±10°C as well as at least 6 months at the accelerated condition of 5±3°C in formulations comprising citrate (10 mM, pH 6.0), sodium chloride (150 mM) and 0.01% (w/v) polysorbate 80.

The stability was analysed after longer periods. Based on the stability results obtained with SDS-PAGE (NR+R), IEF, HP-SEC, RFFIT, and OD280 it was concluded that both CR57 and CR4098 are stable for at least 18 months at the storage condition of –70±10°C as

well as at for least 12 months (similar results were found after 9 months, data not shown) at the accelerated condition of 5±3°C in formulations comprising citrate (10 mM, pH 6.0), sodium chloride (150 mM) and 0.01% (w/v) polysorbate 80.

Formulations containing the cocktail of antibodies CR57 and CR4098 were analysed over a 6 months time period and compared to the initial results obtained at t=0 months. After 6 months, the appearance of the cocktail stored at 5±3°C and 25±2°C remained within target specifications, *i.e.* the cocktail/mixture was a clear and colourless liquid, practically free from particles (see Table 9). Furthermore, it was observed that the antibody cocktail remained within target specifications when stored up to 3 months at 40±2°C.

The pH and osmolality were monitored at t=0 and t=6 months for 5±3°C and at 25±2°C and at the 1 and 3 month time point for the study at 40°C±2°C. All data were within target specifications.

Furthermore, the antibody cocktail showed stable potency up to 6 months at 5±3°C and 25±2°C (see Table 9). A slightly lower potency value was obtained after 3 months at 40±2°C. All data were within the target specification of about 380 to about 1350 IU/ml.

The amount of total protein present in the antibody cocktail as determined by OD280 was stable over the tested time period of 3 months at 40±2°C, and 6 months at 5±3°C and 25±2°C.

The IgG kappa and lambda ELISA results (*i.e.* presented as the ratio of both antibodies in the antibody cocktail) remained within the target specifications for up to 6 months at 5±3°C and 25±2°C and for up to 3 months at 40±2°C. Based on these results it was concluded that the CR57 and CR4098 content in the antibody cocktail does not change over a time period of 6 months at 5±3°C and 25±2°C and 3 months at 40±2°C. The IEF gel pattern of antibody cocktail stored at 5±3°C for 6 months was comparable to that at t=0 months (see Table 9). An additional band was observed after 3 and 6 months in the samples stored at 25±2°C and several additional bands were detected after 1 and 3 months in the samples stored at 40±2°C (see Table 9). These additional bands obtained at elevated storage temperatures might be first indications for degradation.

After 6 months, the SDS-PAGE (reduced and non-reduced) banding patterns of the antibody cocktail stored at 5±3°C were comparable to the banding pattern at t=0 months (see

Table 9). No additional bands were detected. Antibody cocktail stored at 25±2°C for up to 3 months showed under reducing SDS-PAGE conditions a banding pattern identical to the banding pattern at t=0 months. Under non-reducing conditions, a weak band with a size of approximately 43 kDa was observed after storage of 3 months at 25±2°C. After 6 months storage at 25±2°C reducing and non-reducing SDS-PAGE analysis showed weak bands at ~40 kDa. A similar result was obtained by SDS-PAGE analysis (both reduced and non-reduced) for the antibody cocktail stored at 40±2°C. Furthermore, smaller bands with a size of 10-15 kDa were detected under this storage condition. Based on these results, it was concluded that at 25±2°C and 40±2°C some degradation of the heavy and/or light chains of CR57 and CR4098 might take place over time.

The HPSEC patterns of the antibody cocktail stored at 5±3°C and at 25±2°C for up to 6 months compare well with the pattern at t=0 months. The target specification of "main peak area > 95%" was met in all cases (see Table 9). The dimer peak remained < 1% (surface area) and no degradation peaks were detected in any of the samples tested. Antibody cocktail stored at 40±2°C showed minor degradation after 3 months as well as an slightly increased dimer peak (2.6% (surface area)) compared to antibody cocktails stored at the two lower temperatures. However, even at 40±2°C the target specification of "main peak area > 95%" was met at all storage time periods tested (see Table 9).

Based on the results obtained with the three latter methods it was concluded that no significant degradation of the antibodies in the antibody cocktail occurred during storage for 6 months at 5±3°C or 25±2°C, while some minor aggregation and degradation was observed after storage for up to 3 months at 40±2°C.

In summary, it was concluded that the antibody cocktail is stable for at least 6 months at the storage condition of 5±3°C and 25±2°C and for at least 3 months at the storage condition of 40±2°C.

Based on the results after 12 months (similar results were obtained after 9 months, data not shown) obtained with SDS-PAGE (NR+R), IEF, HP-SEC, RFFIT, kappa/lambda ELISA it was concluded that the antibody cocktail is stable for at least 12 months at the storage condition of 5±3°C.

Based on the results after 18 months obtained with the analytical assays (SDS-PAGE, IEF, HP-SEC, ELISA; data not shown), it was concluded that the antibody cocktail is stable for at least 18 months at the storage condition of 5±3°C.

Table 1: Composition of the antibody formulations.

Number	Antibody	citrate buffer (mM)	NaCl (mM)	Polysorbate 80 (% w/v)	pH
1	0.3 mg/ml CR57	10	150	0.01	6.0
2	0.3 mg/ml CR57	10	150	0.03	6.0
3	0.3 mg/ml CR4098	10	150	0.01	6.0
4	0.3 mg/ml CR4098	10	150	0.03	6.0
5	0.3 mg/ml CR57 + 0.3 mg/ml CR4098	10	150	0.01	6.0
6	0.3 mg/ml CR57 + 0.3 mg/ml CR4098	10	150	0.03	6.0

Table 2. Preparation of the formulations.

Number	Antibody CR57 (2.5 mg/ml) (ml)	Antibody CR4098 (1.0 mg/ml) (ml)	5% w/v polysorbate 80 (ml)	Final volume (ml)
1	4.20	0.00	0.70	35.00
2	4.20	0.00	2.10	35.00
3	0.00	10.50	0.70	35.00
4	0.00	10.50	2.10	35.00
5	4.20	10.50	0.70	35.00
6	4.20	10.50	2.10	35.00

Table 3. Analysis of samples of the various formulations at the indicated time points and with the indicated methods.

Time points (weeks)	5±3°C/ambient relative humidity	25±2°C/60±5% relative humidity	40±2°C/75±5% relative humidity

0	A-G	A-G	A-G
2	B,E	B,E	B,E
6	B-E	B-E	B-E
8	B-E	B-E	B-E
13	C-E	C-E	C-E

A: Protein concentration (A280)
B: HPSEC
C: SDS-PAGE (reduced and non-reduced)
D: IEF
E: Appearance
F: pH
G: Osmolality

Table 4. Purity of antibody monomer (area %) as determined by HPSEC.

Number	Temperature	t=0 weeks	t=2 weeks	t=6 weeks	t=8 weeks	t=13 weeks
1	5±3°C/ambient relative humidity	98.9	ND	98.7	98.5	98.6
	25±2°C/60±5% relative humidity	98.9	ND	98.4	98.2	96.7
	40±2°C/75±5% relative humidity	98.9	98.0	93.7	94.3	90.4
2	5±3°C/ambient relative humidity	98.3	ND	98.2	96.9	97.6
	25±2°C/60±5% relative humidity	98.3	ND	97.5	96.9	95.4
	40±2°C/75±5% relative humidity	98.3	97.5	93.4	94.1	89.9
3	5±3°C/ambient relative humidity	100	ND	100	100	99.9
	25±2°C/60±5% relative humidity	100	ND	99.4	99.5	97.9
	40±2°C/75±5% relative humidity	100	99.4	92.3	94.2	91.8
4	5±3°C/ambient relative humidity	98.6	ND	99.1	97.9	98.6
	25±2°C/60±5% relative humidity	98.6	ND	98.5	97.8	96.8

	40±2°C/75±5% relative humidity	98.6	98.5	91.9	94.4	91.6
5	5±3°C/ambient relative humidity	99.4	ND	99.2	99.3	99.2
	25±2°C/60±5% relative humidity	99.4	ND	98.4	98.7	97.4
	40±2°C/75±5% relative humidity	99.4	98.4	91.6	93.6	89.5
6	5±3°C/ambient relative humidity	99.3	ND	99.1	98.8	99.0
	25±2°C/60±5% relative humidity	99.3	ND	98.1	98.3	96.9
	40±2°C/75±5% relative humidity	99.3	98.4	91.6	93.8	90.6

ND: Not determined

Table 5. Analysis of samples of the various formulations at the indicated time points and with the indicated methods.

Antibody (storage temp.)	SDS-PAGE reduced				SDS-PAGE non-reduced				HPSEC % monomer				IEF			
Months	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
CR57 (5±3°C)	-	-	-	-	-	-	-	-	98.9	98.5	98.2	98.3	-	-	-	-
CR57 (-70±10°C)	-	-	-	-	-	-	-	-	98.9	98.5	98.3	98.4	-	-	-	-
CR4098 (5±3°C)	-	-	-	-	-	-	-	-	99.7	99.4	99.2	99.6	-	-	-	-
CR4098 (-70±10°C)	-	-	-	-	-	-	-	-	99.7	98.9	99.5	99.6	-	-	-	-

-: No unexpected bands detected

Table 6. Analysis of samples of the various formulations at the indicated time points and with the indicated methods.

Antibody (storage temp.)	A280 mg/ml (%A320/A280)				Appear.				pH			
Months	0	1	2	3	0	1	2	3	0	1	2	3
CR57 (5±3°C)	1.33 0.40%	1.40 0.74%	1.40 1.01%	1.42 0.59%	+	+	+	+	6.0	ND	ND	5.9
CR57 (-70±10°C)	1.33 0.40%	1.34 1.42%	1.34 0.60%	1.34 0.62%	+	+	+	+	6.0	ND	ND	6.0
CR4098 (5±3°C)	1.21 0.67%	1.22 1.02%	1.31 1.21%	1.29 0.98%	+	+	+	+	6.1	ND	ND	6.1
CR4098 (-70±10°C)	1.21 0.67%	1.21 1.49%	1.20 1.37%	1.24 0.97%	+	+	+	+	6.1	ND	ND	6.1

+: Approved (clear colourless and practically free of particles

ND: not determined

Table 7. Stability testing of CR57.

Test	Target specification	Storage condition	t=0	1 month	3 months	6 months	12 months	18 months
pH	6 ± 0.5	-70ºC	6.1	ND	ND	6.1	6.0	6.1
		5ºC	ND	ND	ND	6.1	6.0	ND
Appearance	Clear colourless liquid, practically free from particles	-70ºC	+	+	+	+	+	+
		5ºC	+	+	+	+	+	ND
Quantity by OD280	1-5 mg/ml	-70ºC	2.47	2.57	2.47	2.57	2.55	2.48
		5ºC	ND	2.55	2.27	2.68	2.59	ND
Non-reduced SDS-PAGE	Band pattern conforms to WS# and contains no additional impurity bands	-70ºC	+	+	+	+	+	+
		5ºC	ND	+	+	+	+	ND
Reduced SDS-PAGE	Band pattern conforms to WS# and contains no additional impurity bands	-70ºC	+	+	+	+	+	+
		5ºC	ND	+	+	+	+	ND
IEF	Band pattern conforms to WS# (additional bands to be reported)	-70ºC	+	+	+	+	+	+
		5ºC	ND	+	+	+	+	ND
HP-SEC	Main peak by area% > 95%	-70ºC	99.5	99.7	99.4	99.7	99.8	99.7
		5ºC	ND	99.6	99.4	99.6	99.4	ND
Potency	500-1250 IU/mg	-70ºC	937	974	1581	2130	927	1440
		5ºC	ND	922	1523	1629	1054	ND

ND: Not determined

#: CR57 Working Standard (WS) and CR4098 WS

+: Conform target specification

Table 8 Stability testing of CR4098.

Test	Target specification	Storage condition	0 months	1 month	3 months	6 months	12 months	18 months
pH	6 ± 0.5	-70ºC	6.1	ND	ND	6.1	6.0	6.1
		5ºC	ND	ND	ND	6.1	6.0	ND
Appearance	Clear colourless liquid, practically free from particles	-70ºC	+	+	+	+	+	+
		5ºC	+	+	+	+	+	ND
Quantity by OD280	1-5 mg/ml	-70ºC	2.48	2.58	2.49	2.57	2.56	2.41
		5ºC	ND	2.65	2.52	3.03	2.76	ND
Non-reduced SDS-PAGE	Band pattern conforms to WS# and contains no additional impurity bands	-70ºC	+	+	+	+	+	+
		5ºC	ND	+	+	+	+	ND
Reduced SDS-PAGE	Band pattern conforms to WS# and contains no additional impurity bands	-70ºC	+	+	+	+	+	+
		5ºC	ND	+	+	+	+	ND
IEF	Band pattern conforms to WS# (additional bands to be reported)	-70ºC	+	+	+	+	+	+
		5ºC	ND	+	+	+	+	ND
HP-SEC	Main peak by area% > 95%	-70ºC	99.6	99.7	99.7	99.8	99.7	99.7
		5ºC	ND	99.7	99.5	99.5	99.3	ND
Potency	500-1250 IU/mg	-70ºC	924	971	1698	1695	991	1093
		5ºC	ND	897	1434	1645	1093	ND

ND: Not determined
#: CR57 Working Standard (WS) and CR4098 WS
+: Conform target specification

Table 9: Stability testing of antibody cocktail.

Test	Target specification	Storage condition	t=0 months	1 month	2 months	3 months	6 months	12 months
Osmolality	300 ± 50 mOsmol/kg	5ºC	307	ND	ND	ND	310	315
		25ºC	ND	ND	ND	ND	314	ND
		40ºC	ND	308	ND	312	ND	ND
pH	6 ± 0.5	5ºC	5.7	ND	ND	ND	5.7	5.7
		25ºC	ND	ND	ND	ND	5.7	ND
		40ºC	ND	5.7	ND	5.8	ND	ND
Appearance	Clear colourless liquid, practically free from particles	5ºC	+	+	+	+	+	+
		25ºC	ND	+	+	+[1]	+	ND
		40ºC	ND	+	ND	+	ND	ND
Quantity by OD280	0.48-0.72 mg/ml	5ºC	0.60	0.60	0.60	0.61	0.60	0.61
		25ºC	ND	0.61	0.60	0.60	0.60	ND
		40ºC	ND	0.62	ND	0.60	ND	ND
CR57/ CR4098 ratio	0.6-1.4	5ºC	0.9	0.8	0.9	0.9	0.9	1.0
		25ºC	ND	0.8	0.9	0.9	0.9	ND
		40ºC	ND	0.8	ND	0.9	ND	ND
Non-reduced SDS-PAGE	Band pattern comparable to WS# and contains no additional impurity bands	5ºC	+	+	+	+	+	+
		25ºC	ND	+	+	+[2]	+[4]	ND
		40ºC	ND	+[2]	ND	+[3]	ND	ND
Reduced SDS-PAGE	Band pattern comparable to WS# and contains no additional impurity bands	5ºC	+	+	+	+	+	+
		25ºC	ND	+	+	+	+[4]	ND
		40ºC	ND	+[2]	ND	+[5]	ND	ND
HP-SEC	Main peak area % > 95%	5ºC	99.6	100	99.5	99.5	99.9	99.2
		25ºC	ND	99.6	99.5	99.5	99.4	ND
		40ºC	ND	99.0	ND	95.1 (degr. peak of 2.6%)	ND	ND
IEF	Band pattern conforms to WS# (additional bands to be reported)	5ºC	+	+	+	+	+	+
		25ºC	ND	+	+	+[6]	+[7]	ND
		40ºC	ND	+[8]	ND	+[9]	ND	ND

Potency	380-1350 IU/ml	5ºC	1006	933	1089	1401	999	830
		25ºC	ND	937	1015	1001	847	ND
		40ºC	ND	807	ND	621	ND	ND
Sterility	Complies	5ºC	+	ND	ND	ND	ND	+

ND: Not determined

#: Working Standard (WS) CR57 and CR4098 antibody cocktail

+: Conform target specification

+1: Clear colourless liquid, > 10 particles/ml

+2: Band pattern comparable to WS# and contains weak additional impurity band with size of about 43kDa

+3: Band pattern comparable to WS# and contains weak additional impurity bands with size of about 10, 15 and 43 kDa

+4: Deviant, weak bands appear around 40 kDa

+5: Band pattern comparable to WS# and contains weak additional impurity bands with size of about 10 kDa, 15 kDa and between 25-50 kDa

+6: Band pattern conforms to WS#; higher staining intensity of bands in the 8.0-8.5 pI-area

+7: Deviant, weak bands appear below pI 7.5, different staining intensity in the 8.0-8.5 pI-area

+8: Band pattern conforms to WS# and weak additional bands (pI 7.2, 7.3, 8.6)

+9: Band pattern conforms to WS# and weak additional bands (pI range 6.3-7.4 and pI 8.6)

Table 10. Sequences of CR57 and CR4098

A. CR57

heavy chain (SEQ ID NO: 1)

```
QVQLVQSGAE VKKPGSSVKV SCKASGGTFN RYTVNWVRQA PGQGLEWMGG IIPIFGTANY      60
AQRFQGRLTI TADESTSTAY MELSSLRSDD TAVYFCAREN LDNSGTYYYF SGWFDPWGQG     120
TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF     180
PAVLQSSGLY SLSSVVTVPS SSLGTQTYIC NVNHKPSNTK VDKRVEPKSC DKTHTCPPCP     240
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK     300
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT     360
LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL     420
TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK                              457
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light chain (SEQ ID NO: 2)

```
QSALTQPRSV SGSPGQSVTI SCTGTSSDIG GYNFVSWYQQ HPGKAPKLMI YDATKRPSGV      60
PDRFSGSKSG NTASLTISGL QAEDEADYYC CSYAGDYTPG VVFGGGTKLT VLGQPKAAPS     120
VTLFPPSSEE LQANKATLVC LISDFYPGAV TVAWKADSSP VKAGVETTTP SKQSNNKYAA     180
SSYLSLTPEQ WKSHRSYSCQ VTHEGSTVEK TVAPTECS                             218
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B. CR4098

heavy chain (SEQ ID NO: 3)

```
QVQLVESGGG AVQPGRSLRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAV ILYDGSDKFY      60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKVA VAGTHFDYWG QGTLVTVSSA     120
STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH TFPAVLQSSG     180
LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKRVEPK SCDKTHTCPP CPAPELLGGP     240
SVFLFPPKPK DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS     300
TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQV YTLPPSREEM     360
TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ     420
QGNVFSCSVM HEALHNHYTQ KSLSLSPGK                                       449
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light chain (SEQ ID NO: 4)

```
DIQMTQSPSS LSASVGDRVT ITCRASQGIR NDLGWYQQKP GKAPKLLIYA ASSLQSGVPS      60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ LNSYPPTFGG GTKVEIKTVA APSVFIFPPS     120
DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL     180
SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC                                  213
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CLAIMS

1. A pharmaceutical formulation of two anti-rabies monoclonal antibodies that is stable for at least 12 months at a temperature between about 2°C to about 8°C, comprising:
(i) anti-rabies virus monoclonal antibody CR59 (heavy chain SEQ ID NO: 1 and light chain SEQ ID NO: 2), or an antibody that is at least 95% homologous in sequence thereto and is capable of competing for binding to the target recognized by CR59 and having rabies virus neutralizing activity; and
(ii) anti-rabies virus monoclonal antibody CR4098 (heavy chain SEQ ID NO: 3 and light chain SEQ ID NO: 4), or an antibody that is at least 95% homologous in sequence thereto and is capable of competing for binding to the target recognized by CR4098 and having rabies virus neutralizing activity;
wherein the formulation comprises a citrate buffer, a tonicity agent, and a surfactant.

2. A formulation according to claim 1, wherein the citrate buffer is present at a concentration from about 5 mM to about 25 mM, preferably about 10 mM.

3. A formulation according to claim 1 or 2, wherein the pH ranges from about 5.5 to about 6.5, preferably wherein the pH is about 6.0.

4. A formulation according to any one of claims 1-3, wherein the tonicity agent is sodium chloride and is present at a concentration from about 50 mM to about 250 mM, preferably about 150 mM.

5. A formulation according to any one of claims 1-4, wherein the surfactant is a polysorbate, preferably polysorbate 80.

6. A formulation according to claim 5, wherein polysorbate 80 is present in an amount from about 0.005% w/v to about 0.05% w/v, preferably about 0.01% w/v.

7. A formulation according to any one of the preceding claims, wherein the osmolality of the formulation ranges from about 250 mOsm/kg to about 350 mOsm/kg, preferably wherein

the osmolality is about 300 mOsm/kg.

8. A formulation according to any one of the preceding claims, wherein the two anti-rabies virus monoclonal antibodies each are present in an amount from about 0.1 mg/ml to about 2.0 mg/ml.

9. A formulation according to any one of the preceding claims, wherein the formulation has a rabies virus neutralizing potency ranging from about 250 IU/ml to about 1500 IU/ml.

10. A formulation according to any one of the preceding claims, wherein the ratio of the two antibodies is between 5:1 and 1:5, preferably between 2:1 and 1:2, for instance about 1:1.

11. A formulation according to any one of the preceding claims, wherein the formulation is sterile.

12. A formulation according to any one of the preceding claims, wherein the formulation is substantially free of endotoxin.

13. A formulation according to any one of the preceding claims, wherein the formulation has a purity of antibody monomer of at least 95% as determined by HPSEC after two weeks storage at a temperature of 40±2°C and a relative humidity of 75±5%.

14. A pharmaceutical unit dosage form comprising an effective amount of a formulation according to any of the preceding claims for post-exposure prophylaxis treatment of a subject through administration of the dosage form to the subject.

15. A method for improving the storage of two anti-rabies virus monoclonal antibodies in one formulation, comprising formulating the antibodies in a pharmaceutical formulation according to any one of claims 1-13.

16. A method according to claim 15, wherein the formulation is stored at a temperature from about 2°C to about 40°C, preferably from about 2°C to about 8°C.

17. A method according to claim 15 or 16, wherein the amount of by-product formation of the antibodies is reduced.

18. A method according to claim 17, wherein 5% or less of the antibodies forms aggregates as measured by HPSEC within 12 months of storage at a temperature from about 2°C to about 8°C.

19. A pharmaceutical formulation of anti-rabies virus monoclonal antibody CR59 (heavy chain SEQ ID NO: 1 and light chain SEQ ID NO: 2), or an antibody that is at least 95% homologous in sequence thereto and is capable of competing for binding to the target recognized by CR59 and having rabies virus neutralizing activity, which formulation is stable for at least 18 months at a temperature between about 2°C to about 8°C and at a temperature between about –60°C and –80°C, wherein the formulation comprises a citrate buffer, a tonicity agent, and a surfactant.

20. A pharmaceutical formulation of anti-rabies virus monoclonal antibody CR4098 (heavy chain SEQ ID NO: 3 and light chain SEQ ID NO: 4), or an antibody that is at least 95% homologous in sequence thereto and is capable of competing for binding to the target recognized by CR4098 and having rabies virus neutralizing activity, which formulation is stable for at least 18 months at a temperature between about 2°C to about 8°C and at a temperature between about –60°C and –80°C, wherein the formulation comprises a citrate buffer, a tonicity agent, and a surfactant.