(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 March 2020 (12.03.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/049151 A1

(51) International Patent Classification:
C12N 7/00 (2006.01) *A61K 39/275* (2006.01)

(21) International Application Number:
PCT/EP2019/073825

(22) International Filing Date:
06 September 2019 (06.09.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
18192857.3 06 September 2018 (06.09.2018) EP

(71) Applicant: **BAVARIAN NORDIC A/S** [DK/DK]; Hejreskovvej 10 A, 3490 Kvistgaard (DK).

(72) Inventor: **KJAER, Katrine**; Gaunøvej 3, 2700 Brønshøj (DK).

(74) Agent: **PREUSS, Ilka**; Bavarian Nordic GmbH, Fraunhoferstrasse 13, 82152 Martinsried (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

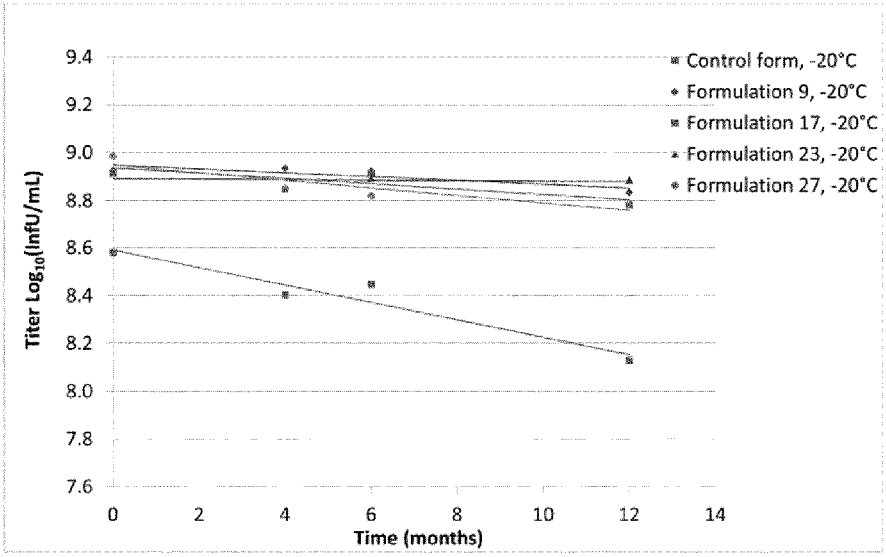
Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

(54) Title: STORAGE IMPROVED POXVIRUS COMPOSITIONS

Figure 2/3



(57) Abstract: The present invention provides storage optimized aqueous compositions comprising a poxvirus suitable as poxvirus vaccine and pharmaceutical compositions, in particular liquid compositions or liquid frozen compositions, and methods of making them.

WO 2020/049151 A1

[Continued on next page]

Published:
— *with international search report (Art. 21(3))*

STORAGE IMPROVED POXVIRUS COMPOSITIONS

FIELD OF THE INVENTION

[001] The present invention relates to stabilization of poxvirus compositions for storage at refrigerator temperature and/or -20 degrees C for longer periods of time in particular at least for 12 months. The invention further relates to stabilized poxvirus compositions for storage at elevated temperatures and humidity. The invention also relates to methods for the formulation of such compositions for long-term storage.

BACKGROUND OF THE INVENTION

[002] Poxviruses including vaccinia virus and in particular modified vaccinia Ankara (MVA) virus have been developed as a vector for vaccines against infectious diseases such as HIV, influenza, malaria and respiratory syncytial virus (RSV) and for immunotherapies and oncolytic therapies against cancer (Choi and Chang *Clin Exp Vaccine Res* 2013, 2: 97-105; Rezaee et al. *Curr Opin Virol* 2017, 24: 70-78; Al Yaghchi et al. *Immunotherapy* 2015, 7: 1249-1258; Verheust et al. *Vaccine* 2012, 30: 2623-2632; Mastrangelo et al. *J Clin Invest* 2000, 105: 1031-1034). Several unique features make them ideal candidates for vaccine development or gene delivery: (i) large packaging capacity for recombinant DNA; (ii) precise recombinant DNA expression regulated by a strong poxviral promoter; (iii) lack of persistence or genomic integration in the host due to their cytoplasmic replication; (iv) high immunogenicity as vaccine; and (v) ease of vector and vaccine production (Verheust et al. *Vaccine* 2012, 30: 2623-2632).

[003] Live, attenuated vaccines however form a formulation challenge because of its complex macromolecular structure of the virus. This is even more challenging with large enveloped viruses such as poxviruses. For examples, vaccinia virus such as MVA are very large (about 200 - 300 nm) enveloped double-stranded DNA viruses of about 192 kbps in size consisting of a core region composed of viral DNA and various enzymes encased in a lipoprotein core membrane. The outer layer consists of a double lipid

membrane envelope (Al Yaghchi et al. *Immunotherapy* 2015, 7: 1249-1258). There are two major morphologically distinct infectious forms of virions, the intracellular mature virus (IMV) and extracellular enveloped virus (EEV). IMVs represent the majority of infectious particles which remain in the cytoplasm until lysis of the cells. EEVs are released from the cell and possess an extra lipid envelope with at least 10 associated proteins absent from IMV. The lipid membrane is very fragile and an important consideration since loss of the viral envelope results in viral inactivation. The stability can further vary considerably dependent on the preparations and excipients used for preparation of the purified viruses and its storage.

[004] One difficulty is storage below the freezing point of water to avoid destabilization and/or disruption of the virus during freezing and thawing. In order to ensure stability, stocks of purified infective virus in the past were generally stored below minus 60 degrees centigrade. One problem of storing at such low temperatures is the potential to thaw and re-freeze during transit or at the site of administration.

[005] The limited stability of live viruses in aqueous composition is well known, and most of the attenuated viruses are freeze-dried products such as for example the fully replication competent vaccinia virus ACAM2000. ACAM2000 was approved as a lyophilized preparation containing 6-8mM HEPES (pH 6.5-7.5), 2% human serum albumin, 0.5 - 0.7% sodium chloride, 5% mannitol, and trace amounts of neomycin and polymyxin B. The lyophilized vaccinia virus was reconstituted in 50% (v/v) glycerol, 0.25% (v/v) Phenol in water for injection (Berhanu et al. *Vaccine* 2010, 29: 289-303).

[006] Hekker et al. described freeze-dried smallpox vaccine compositions comprising pepton-sorbitol combinations with 2% haemaccel or 2% polyvinylpyrrolidone (Hekker et al. *Journal of Biological Standardization* 1973, 1: 21-32, summarized in Burke et al. *Crit Rev Ther Drug Carrier Syst* 1999, 16: 1-83). Further freeze-dried compositions comprising MVA or ALVAC are described in WO 03/053463, WO 05/066333, WO 07/056847, WO 2011/121306, WO 2014/053571, and Zhang et al. *Chemical Research in Chinese Universities* 2007, 23: 329-332. WO 2010/135495 describes methods for stabilizing viruses in a spray dry powder composition comprising mannitol.

[007] Just and Finke analyzed lyophilized MVA compositions comprising stabilizers including albumin, sorbitol, dextran, cysteine or haemaccel and described 5% (w/v) sorbitol and 1% (w/v) human albumin superior for lyophilized compositions when stored at +4 degrees C (Just and Finke *Zentralbl Bakteriol Orig A* 1979, 245: 276-282). They also analyzed stability for non-lyophilized MVA suspensions containing 1% (w/v) human albumin but a loss of virus titer was observed for these formulations when stored at -70 degrees C over 8 months.

[008] Prabhu et al. describe three freeze-dried vaccine formulations of camelpox (Prabhu et al. *Biologicals* 2014, 42: 169-175) one containing 3.5% hydrolyzed gelatin and 3.5% sorbitol in potassium phosphate buffer pH 6.2, which after reconstitution showed a loss in virus titer even at 4 degrees C.

[009] Although freeze-dried vaccines are typically more heat-stable than non-lyophilized alternatives, lyophilization has some disadvantages, including costs, reconstitution before use, instability once reconstituted, and freezing and drying stress to the viral particles (Capelle et al. *Eur J Pharm Biopharm* 2018, 129: 215-221). Further, lyophilized vaccines are more prone to administration and dosing errors compared to liquid vaccines due to the need for reconstitution, which may lead to vaccine wastage or an ineffective vaccine dose (Capelle et al. *Eur J Pharm Biopharm* 2018, 129: 215-221).

[010] There has also been an attempt to use vaccinia virus for oral vaccine application (US 6,969,345). The compositions described comprise mannitol with other ingredients such as hydroxyethyl starch, fish oil, glycerol, and gelatin.

[011] Moreover, liquid stabilization of live attenuated viral vaccines is the most challenging as degradation kinetics and dynamic processes are more favorable (Tlaxca et al. *Adv Drug Deliv Rev* 2015, 93: 56-78).

[012] WO 2010/056991 describes liquid or liquid-frozen compositions comprising a MVA virus and mannitol, wherein mannitol is the sole stabilization agent of the composition.

[013] A beneficial effect on poxvirus stability using a chelating agent and ethanol when stored *e.g.*, at +5 degrees C for 12 to 24 months is disclosed in WO 2016/087457.

[014] WO 2011/121301 describes the use of N,N-dimethylglycine or N,N,N-trimethylglycine for stabilization of MVA in a liquid setting at 37 degrees C for one week.

[015] However, there remains a need for new formulations allowing stabilization of poxvirus-based materials allowing large scale industrial applications, providing compositions for storage without affecting biological activity of the product and preserving desired characteristics of the virus, more particularly to avoid or reduce virus titer loss. There is in particular a need for liquid pharmaceutical poxvirus preparations that need not be stored below minus 60 degrees C providing stability for extended periods of time. It is further desirable to provide high-titer low volume compositions suitable for storage at refrigerator temperature and/or at a temperature at about -20 degrees C.

[016] In particular, a need remains for the development of a poxvirus liquid composition that is stable for approximately one year or longer at about -20 degrees C followed by storage at +2 to +8 degrees C (preferably for at least 6 or 9 months) and compatible with subcutaneous, intramuscular and/or intranasal administration. Also desirable are liquid poxvirus compositions that are stable for approximately one year or longer at +2 to +8 degrees C. Such liquid compositions offer advantages including lower cost of goods, decreased development and/or production time and convenience for the user. The present invention addresses and meets these needs by disclosing improved poxvirus compositions, in particular MVA compositions, which show enhanced stability for longer periods of time at temperatures in the range of -20 degrees C and/or +2 to +8 degrees C. Further disclosed are poxvirus compositions which show enhanced stability at +25 degrees C and 60% relative humidity.

BRIEF SUMMARY OF THE INVENTION

[017] The present invention provides aqueous compositions with improved stability of a live poxvirus such as MVA. The inclusion of at least one disaccharide, a sugar alcohol, gelatin, albumin, a pharmaceutical acceptable buffer and at least one monovalent salt results in improved stability in both the liquid and liquid frozen state.

[018] Accordingly, in one aspect the present invention provides an aqueous composition comprising at least one poxvirus, a disaccharide, a sugar alcohol, gelatin, albumin, a pharmaceutical acceptable buffer and at least one monovalent salt, wherein said composition has a pH ranging between pH 7.0 and pH 8.5.

[019] Another aspect of the present invention provides a vaccine or pharmaceutical composition comprising the aqueous composition of the present invention.

[020] Another aspect of the present invention provides a method of making an aqueous live poxvirus composition, the method comprising the steps of:

a) providing a preparation comprising at least one poxvirus in a pharmaceutical acceptable buffer, and

b) combining the poxvirus preparation of step a) with a solution comprising at least one disaccharide, a sugar alcohol, gelatin, albumin, a pharmaceutical acceptable buffer and at least one monovalent salt,

wherein said buffer of a) and b) have a pH ranging between pH 7.0 and pH 8.5.

BRIEF DESCRIPTION OF THE DRAWINGS

[021] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description serve to explain the principles of the invention.

[022] Figure 1: Potency titer in $\log_{10}$ (InfU/mL) of formulations (F17, F23, F9, F27) at -20 degrees C compared to a control formulation (10mM Tris, 140mM NaCl, pH 7.7). Mean potency (infectivity) was determined by Fluorescence Activated Cell Sorter (FACS) assay of example 1.

[023] Figure 2: Potency titer in $\log_{10}$ (InfU/mL) of formulations (F17, F23, F9, F27) at +5 degrees C compared to a control formulation (10mM Tris, 140mM NaCl, pH 7.7). Mean potency (infectivity) was determined by the Fluorescence Activated Cell Sorter (FACS) assay of example 1.

[024] Figure 3: Virus titer, i.e. potency titer in log_{10} (InfU/mL) of formulations (F23, F55, F62, F66) at +25 degrees C/60% relative humidity compared to a control formulation (10mM Tris, 140mM NaCl, pH 7.7). Mean potency (infectivity) was determined by the Fluorescence Activated Cell Sorter (FACS) assay of example 1.

DETAILED DESCRIPTION OF THE INVENTION

[025] It was found that long-term stability, including preserved biological function of the poxvirus, in particular MVA, present in the aqueous composition of the present invention was provided by a combination of excipients including at least one disaccharide, a sugar alcohol, gelatin, albumin, a pharmaceutical acceptable buffer and at least one monovalent salt. Superior protection could be obtained by those excipients contained in the aqueous composition having a pH ranging between pH 7.0 and pH 8.5.

[026] Therefore, one aspect of the present invention provides an aqueous composition comprising at least one poxvirus, at least one disaccharide, a sugar alcohol, gelatin, albumin, a pharmaceutical acceptable buffer and at least one monovalent salt, wherein said composition has a pH ranging between pH 7.0 and pH 8.5.

Poxvirus

[027] Poxviruses are large viruses that are generally enveloped viruses and carry double-stranded DNA. Poxviruses belong to the Poxviridae family and include 71 species of viruses which are divided among 16 genera (Virus Taxonomy: 2017 Release). Two of the most well-known orthopoxviruses are the variola virus, the causative agent for small pox, and vaccinia virus, whose conversion to a vaccine enabled the eradication of smallpox.

[028] Poxviruses, such as a vaccinia virus, are known to the skilled person and have been used to generate recombinant vaccines in the fight against infectious organisms and more recently cancers (Mastrangelo et al. *J Clin Invest* 2000, 105: 1031-1034).

[029] Within the context of present disclosure, poxviruses preferably include orthopoxviruses or avipoxviruses. In preferred embodiments of the present invention, the poxvirus is an orthopoxvirus.

[030] Orthopoxviruses include, but are not limited to, variola virus, vaccinia virus, cowpox virus, and monkeypox virus. Preferably, the orthopoxvirus is a vaccinia virus.

[031] The term "vaccinia virus" can refer to the various strains or isolates of replicating vaccinia virus (VACV) including, for example, Ankara, VACV Western Reserve (WR), VACV Copenhagen (VACV-COP), Temple of Heaven, Paris, Budapest, Dairen, Gam, MRIVP, Per, Tashkent, TBK, Tian Tan, Tom, Bern, Patwadangar, BIEM, B-15, EM-63, IHD-J, IHD-W, Ikeda, DryVax (also known as VACV Wyeth or New York City Board of Health [NYCBH] strain), NYVAC, ACAM1000, ACAM2000, Vaccinia Lister (also known as Elstree), LC16mO or LC16m8.

[032] In further embodiments, the poxvirus of the invention is an MVA virus.

[033] MVA virus was generated by 516 serial passages on chicken embryo fibroblasts of the Ankara strain of vaccinia virus (CVA) (for review see Mayr et al. *Infektion* 1975, 3: 6-14). As a consequence of these long-term passages, the genome of the resulting MVA virus had about 31 kilobases of its genomic sequence deleted and, therefore, was described as highly host cell restricted for replication to avian cells (Meyer et al. *J Gen Virol* 1991, 72 (Pt 5): 1031-1038). It was shown in a variety of animal models that the resulting MVA was significantly avirulent compared to the fully replication competent starting material (Mayr and Danner *Dev Biol Stand* 1978, 41: 225-234).

[034] An MVA virus useful in the practice of the present invention can include, but is not limited to, MVA-572 (deposited as ECACC V94012707 on January 27, 1994); MVA-575 (deposited as ECACC V00120707 on December 7, 2000), MVA-I721 (referenced in Suter et al. *Vaccine* 2009, 27: 7442-7450), NIH clone 1 (deposited as ATCC® PTA-5095 on March 27, 2003) and MVA-BN (deposited at the European Collection of Cell Cultures (ECACC) under number V00083008 on Aug. 30, 2000).

[035] More preferably the MVA used in accordance with the present invention includes MVA-BN and MVA-BN derivatives. MVA-BN has been described in

International PCT publication WO 02/042480. "MVA-BN derivatives" refer to any virus exhibiting essentially the same replication characteristics as MVA-BN, as described herein, but exhibiting differences in one or more parts of their genomes.

[036] MVA-BN, as well as MVA-BN derivatives, is replication incompetent, meaning a failure to reproductively replicate in vivo and *in vitro*. More specifically *in vitro*, MVA-BN or MVA-BN derivatives have been described as being capable of reproductive replication in chicken embryo fibroblasts (CEF), but not capable of reproductive replication in the human keratinocyte cell line HaCat (Boukamp et al (1988), *J. Cell Biol.* 106:761-771), the human bone osteosarcoma cell line 143B (ECACC Deposit No. 91112502), the human embryo kidney cell line 293 (ECACC Deposit No. 85120602), and the human cervix adenocarcinoma cell line HeLa (ATCC Deposit No. CCL-2). Additionally, MVA-BN or MVA-BN derivatives have a virus amplification ratio at least two-fold less, more preferably three-fold less than MVA-575 in Hela cells and HaCaT cell lines. Tests and assay for these properties of MVA-BN and MVA-BN derivatives are described in WO 02/42480 (U.S. Patent application No. 2003/0206926) and WO 03/048184 (U.S. Patent application No. 2006/0159699).

[037] The term "not capable of reproductive replication" or "no capability of reproductive replication" in human cell lines *in vitro* as described in the previous paragraphs is, for example, described in WO 02/42480, which also teaches how to obtain MVA having the desired properties as mentioned above. The term applies to a virus that has a virus amplification ratio *in vitro* at 4 days after infection of less than 1 using the assays described in WO 02/42480 or in U.S. Patent No. 6,761,893.

[038] The term "failure to reproductively replicate" refers to a virus that has a virus amplification ratio in human cell lines *in vitro* as described in the previous paragraphs at 4 days after infection of less than 1. Assays described in WO 02/42480 or in U.S. Patent No. 6,761,893 are applicable for the determination of the virus amplification ratio.

[039] The amplification or replication of a virus in human cell lines *in vitro* as described in the previous paragraphs is normally expressed as the ratio of virus produced from an infected cell (output) to the amount originally used to infect the cell in the first

place (input) referred to as the "amplification ratio". An amplification ratio of "1" defines an amplification status where the amount of virus produced from the infected cells is the same as the amount initially used to infect the cells, meaning that the infected cells are permissive for virus infection and reproduction. In contrast, an amplification ratio of less than 1, *i.e.*, a decrease in output compared to the input level, indicates a lack of reproductive replication and therefore attenuation of the virus.

[040] In another embodiment, the poxvirus of the present invention is an avipoxvirus, such as (but not limited to) a fowlpox virus.

[041] The term "avipoxvirus" refers to any avipoxvirus, such as Fowlpoxvirus, Canarypoxvirus, Uncopoxvirus, Mynahpoxvirus, Pigeonpoxvirus, Psittacinepoxvirus, Quailpoxvirus, Peacockpoxvirus, Penguinpoxvirus, Sparrowpoxvirus, Starlingpoxvirus and Turkeypoxvirus. Preferred avipoxviruses are Canarypoxvirus and Fowlpoxvirus.

[042] Avipoxvirus is a genus of *Poxviridae* whose viruses are able to infect and replicate in birds, however are unable to replicate in non-avian species (Vanderplasschen and Pastoret *Curr Gene Ther* 2003, 3: 583-595). Avipoxviruses, such as fowlpox virus, have been shown to be a safe and efficacious non-replicating vector when used in non-avian species. *Id.*

[043] An example of a canarypox virus is strain Rentschler. A plaque purified Canarypox strain termed ALVAC (U.S. Pat. No. 5,766,598) was deposited under the terms of the Budapest treaty with the American Type Culture Collection (ATCC), accession number VR-2547. Another Canarypox strain is the commercial canarypox vaccine strain designated LF2 CEP 524 24 10 75, available from Institute Merieux, Inc.

[044] Examples of a Fowlpox virus are strains FP-1, FP-5, TROVAC (U.S. Pat. No. 5,766,598), POXVAC-TC (U.S. Patent 7,410,644), TBC-FPV (Therion Biologics-FPV). FP-1 is a Duvette strain modified to be used as a vaccine in one-day old chickens. The strain is a commercial fowlpox virus vaccine strain designated O DCEP 25/CEP67/2309 October 1980 and is available from Institute Merieux, Inc. FP-5 is a commercial fowlpox virus vaccine strain of chicken embryo origin available from American Scientific Laboratories (Division of Schering Corp.) Madison, Wis., United States Veterinary License No. 165, serial No. 30321.

[045] In another embodiment, the poxvirus or any of the preferred poxviruses of any of the embodiments of the present invention is a live virus.

[046] The poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) is preferably present in the aqueous compositions or the methods of the present invention at a titer of at least 10^7 InfU/mL, preferably of at least 2 x 10^7 InfU/mL, at least 3 x 10^7 InfU/mL, at least 5 x 10^7 InfU/mL, at least 6 x 10^7 InfU/mL, at least 7 x 10^7 InfU/mL, at least 8 x 10^7 InfU/mL, at least 9 x 10^7 InfU/mL, at least 1 x 10^8 InfU/mL, at least 2 x 10^8 InfU/mL, at least 3 x 10^8 InfU/mL, at least 4 x 10^8 InfU/mL, at least 5 x 10^8 InfU/mL, at least 6 x 10^8 InfU/mL, at least 7 x 10^8 InfU/mL, at least 8 x 10^8 InfU/mL, at least 9 x 10^8 InfU/mL, at least 1 x 10^9 InfU/mL, at least 2 x 10^9 InfU/mL, at least 3 x 10^9 InfU/mL, at least 4 x 10^9 InfU/mL, at least 5 x 10^9 InfU/mL, at least 6 x 10^9 InfU/mL, or at least 7 x 10^9 InfU/mL. For practical reasons, the poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) is present in the aqueous compositions or the methods of the present invention at a titer of at most 1 x 10^{11} InfU/mL, at most 5 x 10^{10} InfU/mL, or preferably at most 1 x 10^{10} InfU/mL.

[047] In particular, the poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) is present in the aqueous compositions or the methods of the present invention at a titer of between about 1 x 10^7 InfU/mL to 1 x 10^{11} InfU/mL.

[048] In particular, the poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) is present in the aqueous compositions or the methods of the present invention at a titer of between about 1 x 10^7 InfU/mL to 5 x 10^{10} InfU/mL.

[049] In particular, the poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) is present in the aqueous compositions or the methods of the present invention at a titer of between about 1 x 10^7 InfU/mL to 1 x 10^{10} InfU/mL.

[050] In particular, the poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) is present in the aqueous compositions or the methods of the present invention at a titer of between about 1 x 10^7 InfU/mL to 6 x 10^9 InfU/mL.

[051] In certain embodiments, the aqueous compositions provided herein are administered to the subject in a single dose, or in multiple (*i.e.*, 2, 3, 4, etc.) doses, preferably in a volume of 0.1 to 0.5 ml. In certain embodiments, the aqueous compositions

provided herein are administered to the subject in a dose of 10^7 to 10^{10} InfU of the virus in the aqueous composition, preferably in a volume of 0.1 to 0.5 ml. In certain other embodiments, the aqueous compositions are administered in a first (priming) inoculation and one or more subsequent boosting administrations. In certain embodiments, the first dose comprises 10^7 to 10^{10} InfU of the poxvirus in the aqueous composition and the second dose comprises 10^7 to 10^{10} InfU of the virus of the aqueous composition, preferably in a volume of 0.1 to 0.5 ml.

[052] In certain embodiments, the one or more subsequent boosting administrations comprise the same recombinant poxvirus as previously administered, and the methods comprise a homologous prime-boost vaccination. In certain embodiments, the one or more subsequent boosting administrations comprise a different recombinant poxvirus than previously administered, and the methods comprise a heterologous prime-boost vaccination.

[053] In certain embodiments, the one or more subsequent administrations (*i.e.*, the one or more boosting vaccinations) are administered at intervals comprising days, weeks or months after administration of the initial priming vaccination. In certain embodiments, the one or more subsequent administrations of a recombinant poxvirus (*i.e.*, the one or more boosting vaccinations) are administered at intervals of 1, 2, 3, 4, 5, 6, 7 or more weeks after administration of the initial amount of a recombinant poxvirus (*i.e.*, the priming vaccination). In certain embodiments, the one or more subsequent administrations of a recombinant poxvirus (*i.e.*, the one or more boosting vaccinations) are administered at intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more months after administration of the initial priming vaccination.

[054] The poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) comprised in the compositions or methods of the present invention may be a wild-type poxvirus, an attenuated poxvirus or a recombinant poxvirus.

[055] The term "recombinant" virus of any of the embodiments as described herein refers to a virus, more particularly a poxvirus, comprising an exogenous nucleic acid sequence inserted in its genome, which is not naturally present in the parent virus. A recombinant virus (*e.g.*, in particular the orthopoxvirus, more particular a vaccinia virus

or preferably MVA), thus refers to a virus made by an artificial combination of two or more segments of nucleic acid sequence of synthetic or semisynthetic origin which does not occur in nature or is linked to another nucleic acid in an arrangement not found in nature. The artificial combination is most commonly accomplished by artificial manipulation of isolated segments of nucleic acids, using well-established genetic engineering techniques. Generally, a "recombinant" poxvirus as described herein refers to a poxvirus that is produced by standard genetic engineering methods, *e.g.*, a MVA virus of the present invention is thus a genetically engineered or a genetically modified MVA virus. The term "recombinant MVA" thus includes a MVA virus (*e.g.*, MVA-BN) which has integrated at least one recombinant nucleic acid, preferably in the form of a transcriptional unit, in its genome. A transcriptional unit may include a promoter, enhancer, terminator and/or silencer. Recombinant MVA viruses of the present invention may express heterologous antigenic determinants, polypeptides or proteins (antigens) upon induction of the regulatory elements *e.g.*, the promoter.

Methods for production of recombinant poxviruses

[056] Methods to obtain recombinant poxviruses (*e.g.*, VACV or MVA) or to insert exogenous coding sequences into a poxvirus (*e.g.*, VACV or MVA) genome are well known to the person skilled in the art. For example, methods for standard molecular biology techniques such as cloning of DNA, DNA and RNA isolation, Western blot analysis, RT-PCR and PCR amplification techniques are described in Molecular Cloning, A laboratory Manual 2nd Ed. (J. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)), and techniques for the handling and manipulation of viruses are described in Virology Methods Manual (B.W.J. Mahy et al. (*eds.*), Academic Press (1996)). Similarly, techniques and know-how for the handling, manipulation and genetic engineering of poxviruses are described in Molecular Virology: A Practical Approach (A.J. Davison & R.M. Elliott (Eds.), The Practical Approach Series, IRL Press at Oxford University Press, Oxford, UK (1993), see, *e.g.*, Chapter 9: Expression of genes by Vaccinia virus vectors); Current Protocols in Molecular Biology (John Wiley & Son, Inc. (1998), see, *e.g.*, Chapter 16, Section IV: Expression of proteins in mammalian cells using vaccinia viral vector);

and Genetic Engineering, Recent Developments in Applications, Apple Academic Press (2011), Dana M. Santos, see, *e.g.*, Chapter 3: Recombinant-mediated Genetic Engineering of a Bacterial Artificial Chromosome Clone of Modified Vaccinia Virus Ankara (MVA)). Construction and isolation of recombinant MVA are also described in Methods and Protocols, Vaccinia Virus and Poxvirology, ISBN 978-1-58829-229-2 (Staib et al.), Humana Press (2004) see, *e.g.*, Chapter 7.

[057] Methods for producing larger amounts of recombinant poxvirus and purifying virus-based material such as viral vectors and/or viruses used according to the present invention are known by the person skilled in the art. Available methods comprise the replication of the virus in CEF cells or cell lines in particular DF-1 (US 5,879,924), EBx chicken cell line (WO 2005/007840), EB66 duck cells (WO 08/129058), or Cairina moschata immortalized avian cells (WO 2007/077256 or WO 2009/004016). They can be cultivated under conditions well known to the person skilled in the art. Serum-free methods for virus cultivation and virus amplification are preferred. Particular, serum-free methods for virus cultivation and virus amplification in CEF cells are described for example in WO 2004/022729. Upstream and downstream processes for production of virus are well known to the skilled person. They may be obtained from WO 2012/010280 or WO 2016/087457. Methods as useful for purifying viruses of the present application are disclosed in WO 03/054175, WO 07/147528, WO 2008/138533, WO 2009/100521 and WO 2010/130753. Exemplary methods for propagation and purification of recombinant poxvirus in duck embryo-derived cell are described in Leon et al. *Vaccine* 2016, 34: 5878-5885.

Exemplary Generation of a recombinant MVA virus

[058] For the generation of the various recombinant MVA viruses disclosed herein, different methods may be applicable. The DNA sequence to be inserted into the virus can be placed into an *E. coli* plasmid construct into which DNA homologous to a section of DNA of the poxvirus has been inserted. Separately, the DNA sequence to be inserted can be ligated to a promoter. The promoter-gene linkage can be positioned in the plasmid construct so that the promoter-gene linkage is flanked on both ends by DNA

homologous to a DNA sequence flanking a region of poxvirus DNA containing a non-essential locus. The resulting plasmid construct can be amplified by propagation within *E. coli* bacteria and isolated. The isolated plasmid containing the DNA gene sequence to be inserted can be transfected into a cell culture, *e.g.*, of chicken embryo fibroblasts (CEFs), at the same time the culture is infected with MVA virus. Recombination between homologous MVA viral DNA in the plasmid and the viral genome, respectively, can generate a poxvirus modified by the presence of foreign DNA sequences.

[059] According to a preferred embodiment, a cell of a suitable cell culture as, *e.g.*, CEF cells, can be infected with a MVA virus. The infected cell can be, subsequently, transfected with a first plasmid vector comprising a foreign or heterologous gene or genes, such as one or more of the nucleic acids provided in the present disclosure; preferably under the transcriptional control of a poxvirus expression control element. As explained above, the plasmid vector also comprises sequences capable of directing the insertion of the exogenous sequence into a selected part of the MVA viral genome. Optionally, the plasmid vector also contains a cassette comprising a marker and/or selection gene operably linked to a poxvirus promoter. The use of selection or marker cassettes simplifies the identification and isolation of the generated recombinant poxvirus. However, a recombinant poxvirus can also be identified by PCR technology. Subsequently, a further cell can be infected with the recombinant poxvirus obtained as described above and transfected with a second vector comprising a second foreign or heterologous gene or genes. In case, this gene shall be introduced into a different insertion site of the poxvirus genome, the second vector also differs in the poxvirus-homologous sequences directing the integration of the second foreign gene or genes into the genome of the poxvirus. After homologous recombination has occurred, the recombinant virus comprising two or more foreign or heterologous genes can be isolated. For introducing additional foreign genes into the recombinant virus, the steps of infection and transfection can be repeated by using the recombinant virus isolated in previous steps for infection and by using a further vector comprising a further foreign gene or genes for transfection.

[060] In other embodiments, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the

embodiments of the present invention comprises a nucleic acid encoding an antigen, preferably at least one antigen.

[061] Suitable antigens according to the invention for instance may include one or more transgene(s) with an open reading frame encoding for one or more polypeptide(s) against which an immune response is desired when the virus is used for vaccination purposes. Examples may include for instance a transgene or several transgenes suitable to generate an immune response against a virus or a pathogen including but not limited to RSV, HIV, HPV, HBV, Malaria, Ebola, MARV, FMDV, Dengue, an Equine encephalitis virus or any combination thereof.

[062] In a preferred embodiment, the antigen is a viral antigen, a costimulatory molecule and/or a Tumor Associated antigen (TAA).

[063] In preferred embodiments of the present invention, the viral antigen is an immunogenic antigen selected from a filovirus, a picornavirus, a papillomavirus, a hepatitis virus, a flavivirus, a retrovirus, an orthomyxovirus, an equine encephalitis virus, a paramyxovirus, and/or a combination thereof.

[064] In preferred embodiments of the present invention, the immunogenic antigen is a protein, preferably a full-length protein.

[065] In a preferred embodiment of the invention, the paramyxovirus is a respiratory syncytial virus (RSV) *e.g.*, as described in WO 2014/019718.

[066] In another preferred embodiment, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the embodiments of the present invention comprises a nucleic acid encoding a RSV antigen.

[067] In another preferred embodiment, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the embodiments comprises a nucleic acid encoding a RSV protein.

[068] In another preferred embodiment, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the embodiments comprises a nucleic acid encoding a RSV F glycoprotein.

[069] In another preferred embodiment, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the

embodiments comprises a nucleic acid encoding a RSV F glycoprotein and a RSV G glycoprotein.

[070] In another preferred embodiment, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the embodiments comprises a nucleic acid encoding a RSV F glycoprotein and two RSV G glycoproteins.

[071] In another preferred embodiment, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the embodiments comprises a nucleic acid encoding a RSV F glycoprotein, two RSV G glycoproteins and a RSV N protein.

[072] In another preferred embodiment, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the embodiments comprises a nucleic acid encoding a RSV F glycoprotein, two RSV G glycoproteins, a RSV N protein and a RSV matrix protein.

[073] In another preferred embodiment, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the embodiments comprises a nucleic acid encoding a RSV F glycoprotein, two RSV G glycoproteins, a RSV N protein and a RSV M2-1 protein.

[074] In a preferred embodiment of the invention, the filovirus is an Ebolavirus and/or a Marburg virus (MARV) *e.g.*, as described in WO 2016/036955, WO 2016/036971 or WO 2016/034678.

[075] In a preferred embodiment of the invention, the picornavirus is a Foot and Mouth disease virus (FMDV) *e.g.*, as described in WO 2016/202828.

[076] In a preferred embodiment of the invention, the papillomavirus is a human papilloma virus *e.g.*, as described in WO 2017/192418, WO 90/10459, WO 05/09241, WO 98/04705, WO 99/03885 or WO 2007/121894.

[077] In a preferred embodiment of the invention, the hepatitis virus is selected from the group of hepatitis A virus, hepatitis B virus, a hepatitis C virus and hepatitis E virus *e.g.*, as described in WO 2004/111082.

[078] In a preferred embodiment of the invention, the flavivirus is a dengue virus (DENV).

[079] In a preferred embodiment of the invention, the retrovirus is HIV-1.

[080] In a preferred embodiment of the invention, the orthomyxovirus is an influenza virus.

[081] In a preferred embodiment of the invention, the equine encephalitis virus (EEV) is an eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV) and/or Venezuelan equine encephalitis virus (VEEV) *e.g.*, as described in WO 2017/129765.

[082] In preferred embodiments of the present invention, the viral antigen is an immunogenic antigen selected from the group of RSV, Ebola virus, MARV, FMDV, HPV, HBV, HIV, influenza virus, DENV, RSV, EEV and any combination thereof.

[083] Various costimulatory molecules are known to the skilled person. They include but are not limited to ICAM-1, LFA-3, CD72, B7-1, B7-2, CD40, CD40 ligand (CD40L) or other B7 related molecules or combinations thereof such as TRICOM.

[084] "TRICOM." Triad of COstimlatory Molecules (also known as TRICOM) includes B7-1 (also known as B7.1 or CD80), intracellular adhesion molecule-1 (ICAM-1, also known as CD54) and lymphocyte function-associated antigen-3 (LFA-3, also known as CD58), and is commonly included in recombinant viral vectors (*e.g.*, poxviral vectors) expressing a specific antigen in order to increase the antigen-specific immune response. The individual components of TRICOM can be under the control of the same or different promoter(s) and can be provided on the same vector with the specific antigen or on a separate vector. Exemplary vectors are disclosed, for example, in Hodge et al. *Cancer Res* 1999, 59: 5800-5807 et al., "A Triad of Costimulatory Molecules Synergize to Amplify T-Cell Activation," Cancer Res. 59:5800-5807 (1999) and U.S. Patent No. 7,211,432 B2, both of which are incorporated herein by reference.

[085] A TAA is well known to the skilled person and refers to an autologous cellular antigen detected at a higher frequency or density in tumor tissue or on tumor cells compared to non-tumor tissue or non-tumor cells.

[086] In a preferred embodiment, the TAA is selected from the group of CEA, MUC-1, TRP-1, NY-ESO-1, TRP-2, p53, PSA, HER-2, PAP, survivin, TYRP1, TYRP2, or Brachyury or in any combination thereof.

[087] In other embodiments, the recombinant poxvirus (in particular the orthopoxvirus, more particular a vaccinia virus or preferably MVA) of any of the embodiments of the present invention comprises a nucleic acid encoding a combination of TAAs. Such exemplary combination may include HER2 and Brachyury, CEA and MUC-1, or PAP and PSA.

Stabilizers of the compositions

[088] The disaccharide according to the present invention may be trehalose, sucrose or a combination thereof. In preferred embodiments of the present invention, the disaccharide is trehalose or sucrose.

[089] In other embodiment, the aqueous compositions or methods according to the invention comprise a disaccharide (preferably trehalose or sucrose) at a concentration ranging between 2% (w/v) and 12% (w/v), preferably between 4% (w/v) and 12% (w/v). In particular, the aqueous compositions or methods according to the invention comprises the disaccharide (preferably trehalose or sucrose) at a concentration is ranging between 2% (w/v) and 11% (w/v), between 2% (w/v) and 10% (w/v), between 2% (w/v) and 9% (w/v), between 2% (w/v) and 8% (w/v), between 2% (w/v) and 7% (w/v), between 2% (w/v) and 6% (w/v), between 2% (w/v) and 5% (w/v), between 4% (w/v) and 12% (w/v), between 4% (w/v) and 11% (w/v), between 4% (w/v) and 10% (w/v), between 4% (w/v) and 9% (w/v), between 4% (w/v) and 8% (w/v), between 4% (w/v) and 7% (w/v), between 5% (w/v) and 12% (w/v), between 5% (w/v) and 11% (w/v), between 5% (w/v) and 10% (w/v), between 5% (w/v) and 9% (w/v), between 5% (w/v) and 8% (w/v), between 6% (w/v) and 12% (w/v), between 6% (w/v) and 11% (w/v), between 6% (w/v) and 10% (w/v), between 6% (w/v) and 9% (w/v), between 6% (w/v) and 8% (w/v), between 7% (w/v) and 12% (w/v), between 7% (w/v) and 11% (w/v), between 7% (w/v) and 10% (w/v), between 7% (w/v) and 9% (w/v), or between 7% (w/v) and 8% (w/v).

[090] In other embodiments, the aqueous compositions or methods according to the invention comprise a disaccharide at a concentration of 10% (w/v).

[091] In other embodiments, the aqueous compositions or methods according to the invention comprise trehalose at a concentration ranging between 4% (w/v) and 12% (w/v).

[092] In other embodiments, the aqueous compositions or methods according to the invention comprise trehalose at a concentration of 10% (w/v).

[093] In other embodiments, the aqueous compositions or methods according to the invention comprise sucrose at a concentration ranging between 4% (w/v) and 12% (w/v).

[094] In other embodiments, the aqueous compositions or methods according to the invention comprise sucrose at a concentration of 10% (w/v).

[095] In other embodiments, the aqueous compositions or methods according to the invention comprise sorbitol.

[096] In other embodiments, the aqueous compositions or methods according to the invention comprise sorbitol at a concentration ranging between 0.2% (w/v) and 5% (w/v).

[097] In other embodiments, the aqueous compositions or methods according to the invention comprises sorbitol at a concentration ranging between 0.2% (w/v) and 4% (w/v).

[098] In other embodiments, the aqueous compositions or methods according to the invention comprise sorbitol at a concentration ranging between 0.5% (w/v) and 4% (w/v), preferably at a concentration ranging between 0.5% (w/v) and 3% (w/v).

[099] In other embodiments, the aqueous compositions or methods according to the invention comprise sorbitol at a concentration ranging between 0.2% (w/v) and 2.2% (w/v), preferably at a concentration ranging between 0.5% (w/v) and 2.2% (w/v).

[0100] In other embodiments, the aqueous compositions or methods according to the invention comprise sorbitol at a concentration ranging between 1% (w/v) and 4% (w/v), preferably at a concentration ranging between 1% (w/v) and 3% (w/v).

[0101] In other embodiments, the aqueous compositions or methods according to the invention comprise sorbitol at a concentration of 2% (w/v).

[0102] In other embodiments, the aqueous compositions or methods according to the invention comprise sorbitol at a concentration of 2% (w/v).

[0103] Gelatin as used for the present invention is well known to a skilled person. Gelatin is a natural, water-soluble protein, gelling or non-gelling, which may be obtained by the partial hydrolysis of collagen produced from bones, hides and skins, tendons and sinews of animals including pig, cow, fish, and poultry. Whereas type A gelatin is produced by an acid processing of collagenous raw materials, type B is produced by the alkaline processing of collagenous raw materials. As gelatin for pharmaceutical preparations, there can be mentioned, for example, a purified gelatin described in the European Pharmacopoeia (Ph.Eur.) or U.S. Pharmacopoeia (USP).

[0104] The term “gelatin hydrolysate” according to the present invention is also called hydrolyzed gelatin, hydrolyzed collagen, collagen hydrolysate, collagen peptide, gelatine hydrolysate and hydrolyzed gelatine. The terms can be used interchangeable.

[0105] The terminology "gelatin hydrolysate" means either a hydrolyzed polypeptide obtained by subjecting gelatin to degradation through hydrolytic cleavage or a polypeptide obtained by polymerizing the above-mentioned hydrolyzed polypeptides. Gelatin hydrolysate is water-soluble and has preferably has a molecular weight of about 35,000 or less. As illustrative examples of gelatin usable in the present invention, there can be mentioned commercially available products, such as VacciPro® (tradename of hydrolyzed gelatin or chemical derivative thereof manufactured and sold by Gelita®, AG, Germany), Gelysate® (tradename of hydrolyzed gelatin or chemical derivative thereof manufactured and sold by BBL Co., Ltd., USA), and Rousselot® pharmaceutical gelatin (tradename of hydrolyzed gelatin or chemical derivative thereof manufactured and sold by Rousselot B.V, NL).

[0106] In other embodiments, the gelatin of the aqueous compositions or methods of the invention is preferably bovine or porcine gelatin, preferably porcine or bovine gelatin hydrolysate. Porcine gelatin is preferably used.

[0107] In other embodiments of the present invention, the gelatin is porcine type A gelatin. In other embodiments of the present invention, the gelatin is porcine type B gelatin.

[0108] In other embodiments, the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration from between about 0.02% (w/v) and 5% (w/v). In further embodiments the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration from between about 0.1% (w/v) and 5% (w/v). In other embodiments, the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration from between about 0.2% (w/v) and 5% (w/v). In other embodiments, the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration from between about 0.25% (w/v) and 5% (w/v). In other embodiments, the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration from between about 0.25% (w/v) and 4% (w/v). In other embodiments, the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration from between about 0.2% (w/v) and 3.2% (w/v). In other embodiments, the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration from between about 1% (w/v) and 3.2% (w/v), preferably 2.5% (w/v) and 3% (w/v). In other embodiments, the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration from between about 0.2 (w/v) and 1.2% (w/v). In other embodiments, the aqueous compositions or methods of the invention comprise gelatin or gelatin hydrolysate or any of the preferred gelatin at a concentration of 0.25% (w/v), 0.5% (w/v), 1% (w/v), 2% (w/v) or 3% (w/v).

[0109] In other embodiments, the aqueous compositions or methods according to the invention comprise albumin. Albumin is a protein naturally found in the blood plasma of mammals where it is the most abundant protein. It has important roles in maintaining the desired osmotic pressure of the blood and also in transport of various substances in the

blood stream. The albumin used for the present invention is preferably a human, pig, mouse, rat, rabbit or goat albumin but also includes variants such as described in WO 2011/051489, WO 2011/051489, WO2010/092135, WO 2012/150319, WO 2014/072481, WO 2011124718, WO 2015/036579, WO 2018/065491, WO 2017/029407, WO 2013/075066, or Otagiri and Chuang *Biol Pharm Bull* 2009, 32: 527-534. Those skilled in the art will also recognize that modifications can be made to albumin by any means known in the art, for example, by recombinant DNA technology, by posttranslational modification, by proteolytic cleavage and/or by chemical means. Those substitutions and alterations to albumin that provide essentially equivalent stabilizing function to albumin without substitutions or alterations are contemplated herein. Preferably the albumin is human serum albumin. In other embodiments, the albumin is a recombinant albumin, preferably a recombinant human serum albumin expressed and purified from *Pichia pastoris*, *Saccharomyces cerevisiae* or *Oryza sativa*. Preferably the albumin as used according to the present invention is Recombumin® expressed in *Saccharomyces cerevisiae*. However, other recombinant albumins are suitable for the present invention such as for example human recombinant albumin expressed in *Pichia pastoris* (*e.g.* Albagen™, rHSA, CAS number 70024-90-7, Sigma-Aldrich).

[0110] In other embodiments, the aqueous compositions or methods according to the invention comprise albumin (preferably any of the preferred albumins mentioned herein) at a concentration ranging between 0.02% (w/v) and 3% (w/v), preferably at a concentration ranging between 0.02% (w/v) and 2% (w/v).

[0111] In other embodiments, the aqueous compositions or methods according to the invention comprise albumin (preferably any of the preferred albumins mentioned herein) at a concentration ranging between 0.2% (w/v) and 2% (w/v), preferably at a concentration ranging between 0.2% (w/v) and 1.5% (w/v).

[0112] In other embodiments, the aqueous compositions or methods according to the invention comprise albumin (preferably any of the preferred albumins mentioned herein) at a concentration ranging between 0.5% (w/v) and 2% (w/v), preferably at a concentration ranging between 0.5% (w/v) and 1.5% (w/v).

[0113] In other embodiments, the aqueous compositions or methods according to the invention comprise albumin (preferably any of the preferred albumins mentioned herein) at a concentration ranging between 0.8% (w/v) and 1.2% (w/v).

[0114] In other embodiments, the aqueous compositions or methods according to the invention comprise albumin (preferably any of the preferred albumins mentioned herein) at a concentration of 1% (w/v).

Buffer and pH

[0115] The aqueous compositions or methods according to any of the embodiments of the invention preferably have a pH ranging between pH 7.0 and pH 8.5.

[0116] In other embodiment of the invention, the aqueous compositions or methods according to any of the embodiments have a pH ranging between pH 7.3 and pH 8.1.

[0117] In other embodiment of the invention, the aqueous compositions or methods according to any of the embodiments have a pH of 7.7.

[0118] The skilled person familiar with pharmaceutical development is well aware of buffers which can be used to achieve a pH between *e.g.,* pH 7.0 and pH 8.5. Such buffers preferably are selected from the group of phosphate buffer, Tris (Tris(hydroxymethyl)aminomethane), Tris-HCl (Tris(hydroxymethyl)aminomethane-HCl), Tricine (N-[tris(hydroxymethyl)methyl)-methyl]-glycine), and HEPES (4-2-hydroxyethyl-1-piperazineethansulfonic acid). The phosphate buffer preferably comprises a mixture of Na_2HPO_4 and KH_2PO_4 or a mixture of Na_2HPO_4 and NaH_2PO_4. In certain embodiments, the buffer of the aqueous composition is a Tris buffer, preferably a Tris-HCl buffer.

[0119] In certain embodiments the aqueous compositions or methods according to any of the embodiments does not comprise citrate or citrate buffer.

[0120] In certain embodiments, the buffer of the aqueous compositions or methods according to any of the embodiments is preferably present at a concentration ranging between 1mM and 50mM, preferably ranging between 1mM and 25mM.

[0121] In certain embodiments, the buffer of the aqueous compositions or methods according to any of the embodiments is present at a concentration ranging between 1mM and 15mM, preferably ranging between 5mM and 11mM, more preferably ranging between 7mM and 10mM.

[0122] In certain embodiments, the buffer of the aqueous compositions or methods according to any of the embodiments is present at a concentration of 10mM.

[0123] In certain embodiments, the buffer of the aqueous compositions or methods according to any of the embodiments is present at a concentration of 7.5mM.

Salts

[0124] In other embodiments, the aqueous compositions or methods according to any of the embodiments comprise a monovalent salt. Said monovalent salt is preferably sodium chloride (NaCl) or potassium chloride (KCl), preferably NaCl. Said NaCl is preferably present at a concentration of between 40mM and 200mM, preferably between 40mM and 150mM.

[0125] In another embodiment, NaCl may be present at a concentration of between 40mM and 140mM, 40mM and 130mM, 40mM and 120mM, 40mM and 110mM, 40mM and 100mM, 40mM and 90mM, 40mM and 80mM, 50mM and 150mM, 50mM and 140mM, 50mM and 130mM, 50mM and 120mM, 50mM and 110mM, 50mM and 100mM, 50mM and 90mM, 50mM and 80mM, 60mM and 150mM, 60mM and 140mM, 60mM and 130mM, 60mM and 120mM, 60mM and 110mM, 60mM and 100mM, 60mM and 90mM, 60mM and 80mM, 70mM and 150mM, 70mM and 140mM, 70mM and 130mM, 70mM and 120mM, 70mM and 110mM, 70mM and 100mM, 70mM and 90mM, or 70mM and 80mM.

[0126] In another embodiment, NaCl may be present at a concentration of between 60mM and 80mM.

[0127] In another embodiment, NaCl may be present at a concentration of between 70mM.

[0128] In other embodiments, the aqueous compositions or methods according to any of the embodiments comprise a divalent salt. Said divalent salt is preferably

magnesium chloride ($MgCl_2$). Said $MgCl_2$ is preferably present at a concentration of between 1mM and 300mM.

[0129] In another embodiment, $MgCl_2$ may be present at a concentration of between 2mM and 300mM, 5mM and 300mM, 10mM and 300mM, 20mM and 300mM, 30mM and 300mM, 40mM and 300mM, 50mM and 300mM, 60mM and 300mM, 70mM and 300mM, 80mM and 300mM, 90mM and 300mM, 100mM and 300mM, 150mM and 300mM, 180mM and 300mM, 200mM and 300mM, 2mM and 260mM, 5mM and 260mM, 10mM and 260mM, 20mM and 260mM, 30mM and 260mM, 40mM and 260mM, 50mM and 260mM, 60mM and 260mM, 70mM and 260mM, 80mM and 260mM, 90mM and 260mM, 100mM and 260mM, 150mM and 260mM, 180mM and 260mM, 200mM and 260mM, 75mM and 300mM, 75mM and 260mM, 100mM and 260mM, 120mM and 260mM, 150mM and 260mM, 200mM and 260mM, 2mM and 200mM, 5mM and 200mM, 10mM and 200mM, 20mM and 200mM, 30mM and 200mM, 40mM and 200mM, 50mM and 200mM, 60mM and 200mM, 70mM and 200mM, 80mM and 200mM, 90mM and 200mM, 2mM and 150mM, 5mM and 150mM, 10mM and 150mM, 20mM and 150mM, 30mM and 150mM, 40mM and 150mM, 50mM and 150mM, 60mM and 150mM, 70mM and 150mM, 80mM and 150mM, 90mM and 150mM, 2mM and 100mM, 5mM and 100mM, 10mM and 100mM, 20mM and 100mM, 30mM and 100mM, 40mM and 100mM, 50mM and 100mM, 60mM and 100mM, 70mM and 100mM, 80mM and 100mM, 90mM and 100mM, 2mM and 75mM, 5mM and 75mM, 10mM and 75mM, 20mM and 75mM, 30mM and 75mM, 40mM and 75mM, 50mM and 75mM, 60mM and 75mM, 70mM and 75mM, 2mM and 50mM, 5mM and 50mM, 10mM and 50mM, 20mM and 50mM, 30mM and 50mM, 40mM and 50mM, 2mM and 40mM, 5mM and 40mM, 10mM and 40mM, 20mM and 40mM, or 30mM and 40mM.

[0130] In another embodiment, $MgCl_2$ may be present at a concentration of between 75mM and 300mM.

[0131] In another embodiment, $MgCl_2$ may be present at a concentration of between 100mM and 300mM.

[0132] In another embodiment, $MgCl_2$ may be present at a concentration of between 200mM and 300mM.

[0133] In another embodiment, $MgCl_2$ may be present at a concentration of between 220mM and 260mM.

[0134] In another embodiment, $MgCl_2$ may be present at a concentration of between 240mM and 260mM.

[0135] In other embodiments, the aqueous compositions or methods according to any of the embodiments comprise a monovalent salt and a divalent salt, preferably in any concentration as indicated above.

[0136] In another embodiment, the aqueous compositions or methods according to any of the embodiments comprise a monovalent salt at a concentration of between 60mM and 80mM and a divalent salt at a concentration of between 220 and 260mM.

[0137] In another embodiment, the aqueous compositions or methods according to any of the embodiments comprise NaCl at a concentration of between 40mM and 150mM and $MgCl_2$ at a concentration of between 220mM and 260mM.

[0138] In another embodiment, the aqueous compositions or methods according to any of the embodiments comprise NaCl at a concentration of between 70mM and 150mM and $MgCl_2$ at a concentration of between 220mM and 260mM.

[0139] In another embodiment, the aqueous compositions or methods according to any of the embodiments comprise NaCl at a concentration of between 60mM and 80mM and $MgCl_2$ at a concentration of between 220mM and 260mM.

Further preferred embodiments

[0140] The aqueous compositions or methods according to any of the embodiments described herein may contain one or more amino acid(s). Preferred amino acids are histidine, arginine, lysine glycine and/or glutamic acid or salts thereof, in particular the L-isomer L-histidine, L-arginine, L-lysine, L-glycine and/or L-glutamic acid or salts thereof. Said amino acid(s) is/are not an amino acid encoded by the recombinant or non-recombinant virus of the present invention. Thus, the amino acid is not contained in the composition through the process of purification of the virus (*e.g.*, MVA) but added during the generation of the composition for manufacturing a vaccine.

[0141] The amino acid is preferably present at a concentration below 150mM, below 130mM, below 120mM, preferably below 110mM.

[0142] In further embodiments, the amino acid is present at a concentration ranging between 10mM and 110mM, 20mM and 110mM, 30mM and 110mM, 40mM and 110mM, 50mM and 110mM, 60mM and 110mM, 70mM and 110mM, or 80mM and 110mM.

[0143] In further embodiments, the amino acid is present at a concentration ranging between 40mM and 110mM.

[0144] In further embodiments, the amino acid is present at a concentration ranging between 90mM and 110mM.

[0145] In further embodiments, the amino acid is present at a concentration of 100mM.

[0146] In further embodiments, the amino acid is present at a concentration of 50mM.

[0147] In further embodiments, the aqueous composition of the invention may contain histidine (preferably L-histidine) at a concentration of between 40mM and 60mM.

[0148] In further embodiments, the aqueous composition of the invention may contain arginine (preferably L-arginine) at a concentration of between 40mM and 60mM.

[0149] In further embodiments, the aqueous composition of the invention may contain lysine (preferably L-lysine) at a concentration of between 40mM and 60mM.

[0150] In further embodiments, the aqueous composition of the invention may contain glycine (preferably L-glycine) at a concentration of between 40mM and 60mM.

[0151] In further embodiments, the aqueous composition of the invention may contain histidine, arginine, lysine and glycine, preferably each at a concentration of between 40mM and 60mM.

[0152] In further embodiments, the aqueous composition of the invention may contain histidine, arginine, lysine and glycine, preferably each at a concentration of between 40mM and 60mM.

[0153] The concentrations of the amino acid described above may be used for any of the specific amino acids mentioned in the section above (*e.g.*, histidine, arginine, lysine and/or glycine).

[0154] Glutamic acid or salts thereof (*e.g.*, monosodium glutamate or monosodium glutamate monohydrate) are preferably present at a concentration of between 2.5mM and 7.5mM.

[0155] In further embodiments, glutamic acid or salts thereof (*e.g.*, monosodium glutamate monohydrate or monosodium glutamate monohydrate) are present at a concentration ranging between 3mM and 6mM. Preferably, glutamic acid or salts thereof (*e.g.*, monosodium glutamate monohydrate or monosodium glutamate monohydrate)) are present at a concentration of 5mM.

[0156] The aqueous compositions or methods according to any of the embodiments described herein may further comprise octanoate. Preferably the aqueous compositions or methods according to any of the embodiments comprises octanoate ion at a concentration of less than or equal to 5mM, preferably of less or equal than 1mM, more preferably 0.001 to 1mM.

[0157] The aqueous compositions or methods according to any of the embodiments described herein may further comprise one or more additional carrier, additive, antibiotic, preservative, adjuvant, and/or diluent, preferably, any of the additional carrier, additive, antibiotic, preservative, adjuvant, and/or diluent is pharmaceutical acceptable.

[0158] In other embodiments, aqueous composition of the present invention is a vaccine or pharmaceutical composition.

[0159] In further embodiments, the aqueous composition of the present invention is substantially free of HPBCD.

[0160] In further embodiments, the aqueous composition of the present invention is free of HPBCD.

[0161] In further embodiments, the aqueous composition of the present invention is substantially free of mannitol.

[0162] In further embodiments, the aqueous composition of the present invention is free of mannitol.

[0163] In further embodiments, the aqueous composition of the present invention is substantially free of citrate.

[0164] In further embodiments, the aqueous composition of the present invention is free of citrate.

[0165] In further embodiments, the aqueous composition of the present invention is substantially free of a chelating agent. Examples of chelating agents include ethylenediaminetetraacetic acid (EDTA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), ethylene glycol tetraacetic acid (EGTA), dimercaptosuccinic acid (DMSA), diethylene triamine pentaacetic acid (DTPA), and 2,3-Dimercapto-1 - propanesulfonic acid (DMPS). "Substantially free of a chelating agent" according to the present invention means less than 50µM of the chelating agent.

[0166] In further embodiments, the aqueous composition of the present invention is free of a chelating agent.

[0167] In further embodiments, the aqueous composition of the present invention is substantially free of polysorbate. Examples of polysorbate include polysorbate 80.

[0168] In further embodiments, the aqueous composition of the present invention is free of polysorbate.

[0169] In further embodiments, the aqueous composition of the present invention is substantially free of a C_2-C_3 alcohol, wherein the C_2-C_3 alcohol is ethanol and/or isopropanol. "Substantially free of a C_2-C_3 alcohol" according to the present invention means less than 0.05 (v/v) of ethanol or isopropanol.

[0170] In further embodiments, the aqueous composition of the present invention is free of a C_2-C_3 alcohol, wherein the C_2-C_3 alcohol is ethanol and/or isopropanol.

[0171] In further embodiments, the aqueous composition of the present invention is substantially free of mannitol, citrate, a chelating agent, C_2-C_3 alcohol (*i.e.*, ethanol and/or isopropanol) and/or polysorbate.

[0172] In further embodiments, the aqueous composition of the present invention is free of mannitol, citrate, a chelating agent, C_2-C_3 alcohol (*i.e.*, ethanol and/or isopropanol) and/or polysorbate.

[0173] In further embodiments, the aqueous composition of the present invention is substantially free of HPBCD and mannitol.

[0174] In further embodiments, the aqueous composition of the present invention is free of HPBCD and mannitol.

[0175] In further embodiments, the aqueous composition of the present invention is substantially free of HPBCD, mannitol and citrate.

[0176] In further embodiments, the aqueous composition of the present invention is free of HPBCD, mannitol and citrate.

[0177] In further embodiments, the aqueous composition of the present invention is substantially free of HPBCD, mannitol, citrate, a chelating agent, and C_2-C_3 alcohol (*i.e.*, ethanol and/or isopropanol).

[0178] In further embodiments, the aqueous composition of the present invention is free of HPBCD, mannitol, citrate, a chelating agent, and C_2-C_3 alcohol (*i.e.*, ethanol and/or isopropanol).

[0179] In further embodiments, the aqueous composition of the present invention is substantially free of HPBCD, mannitol, citrate, a chelating agent, C_2-C_3 alcohol (*i.e.*, ethanol and/or isopropanol) and polysorbate.

[0180] In further embodiments, the aqueous composition of the present invention is free of HPBCD, mannitol, citrate, a chelating agent, C_2-C_3 alcohol (*i.e.*, ethanol and/or isopropanol) and polysorbate.

[0181] In other embodiments the aqueous composition according to the present invention is contained in a vial. The term "vial" refers to any container, vessel, cartridge, device, glass ampoule, or syringe capable for storage of active pharmaceutical ingredients such as the viruses as disclosed herein. The terms vial, container, vessel, cartridge, device, glass ampoule, or syringe can thus be used interchangeably. The vial is usually made of inert material, in particular glass (such as DIN 2R type I borosilicate glass viral) or polymeric material. In a preferred embodiment the composition is contained in DIN 2R

type I borosilicate glass viral. In a preferred embodiment the composition is contained in a syringe.

[0182] The composition of the present invention can be administered to the subject preferably a human by any means known in the art. The routes of administration include but are not limited to intramuscular injection, subcutaneous injection, intradermal injection, intravenous application, intranasal administration, transdermal administration, transcutaneous administration, or percutaneous administration. The mode of administration, the dose and the number of administrations can be optimized by those skilled in the art in a known manner. In a preferred embodiment, the aqueous composition of the present invention is suitable for parenteral administration or application. In other preferred embodiments, the aqueous composition of the present invention is suitable for intranasal administration or application. In other preferred embodiments, the aqueous composition of the present invention is suitable for intramuscular or subcutaneous administration or application.

[0183] In certain embodiments, the aqueous composition of any of the embodiments of the present invention is further defined as having an infectivity of at least 50%, 60%, 79%, 80% or 90% of the starting infectivity (at day 0) when stored for three months at +5 degrees C.

[0184] In certain embodiments, the aqueous composition of any of the embodiments of the present invention is further defined as having an infectivity of at least 70%, 80% or 90% of the starting infectivity (at day 0) when stored for three months at -20 degrees C.

[0185] In certain embodiments, the aqueous composition of any of the embodiments of the present invention is further defined as having an infectivity of at least 70%, 80% or 90% of the starting infectivity (at day 0) when stored for six months at +5 degrees C.

[0186] In certain embodiments, the aqueous composition of any of the embodiments of the present invention is further defined as having an infectivity of at least 70%, 80% or 90% of the starting infectivity (at day 0) when stored for six months at -20 degrees C.

[0187] In certain embodiments, the aqueous composition of any of the embodiments of the present invention is further defined as having an infectivity of at least 70%, 80% or 90% of the starting infectivity (at day 0) when stored for five months at +25 degrees C/60% relative humidity.

[0188] According to particular embodiments, the aqueous composition of the present invention is stable.

[0189] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at +5 degrees C for at least 3 months is less than 0.5 $\log_{10}$ InfU/mL, preferably less than 0.4 $\log_{10}$ InfU/mL, more preferably less than 0.3 $\log_{10}$ InfU/mL, most preferably less than 0.2 $\log_{10}$ InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0190] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at +5 degrees C for at least 6 months is less than 0.5 $\log_{10}$ InfU/mL, preferably less than 0.4 $\log_{10}$ InfU/mL, more preferably less than 0.3 $\log_{10}$ InfU/mL, most preferably less than 0.2 $\log_{10}$ InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0191] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at +5 degrees C for at least 9 months is less than 0.5 $\log_{10}$ InfU/mL, preferably less than 0.4 $\log_{10}$ InfU/mL, more preferably less than 0.3 $\log_{10}$ InfU/mL, most preferably less than 0.2 $\log_{10}$ InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0192] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at +5 degrees C for at least 12 months is less than 0.5 $\log_{10}$ InfU/mL, preferably less than 0.4 $\log_{10}$ InfU/mL, more preferably less than 0.3 $\log_{10}$ InfU/mL, most preferably less than 0.2 $\log_{10}$ InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0193] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at -20 degrees C for at least 3 months is less than 0.5 log_{10} InfU/mL, preferably less than 0.4 log_{10} InfU/mL, more preferably less than 0.3 log_{10} InfU/mL, most preferably less than 0.2 log_{10} InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0194] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at -20 degrees C for at least 6 months is less than 0.5 log_{10} InfU/mL, preferably less than 0.4 log_{10} InfU/mL, more preferably less than 0.3 log_{10} InfU/mL, most preferably less than 0.2 log_{10} InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0195] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at -20 degrees C for at least 9 months is less than 0.5 log_{10} InfU/mL, preferably less than 0.4 log_{10} InfU/mL, more preferably less than 0.3 log_{10} InfU/mL, most preferably less than 0.2 log_{10} InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0196] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at -20 degrees C for at least 12 months is less than 0.5 log_{10} InfU/mL, preferably less than 0.4 log_{10} InfU/mL, more preferably less than 0.3 log_{10} InfU/mL, most preferably less than 0.2 log_{10} InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0197] According to particular embodiments, the aqueous composition of the present invention is stable when the overall loss of virus titer at +25 C/60% relative humidity for at least 5 months is less than 0.5 log_{10} InfU/mL, preferably less than 0.4 log_{10} InfU/mL, more preferably less than 0.3 log_{10} InfU/mL, most preferably less than 0.2 log_{10} InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0198] According to particular embodiments, the aqueous composition of the present invention is stable for a time period of at least 12 months at about -20 degrees C followed by storage at +2 to +8 degrees C for 3 months, wherein the overall loss of virus titer at -20 degrees C for the specified time period of at least 12 months followed by storage at +2 to +8 degrees C for at least 3 months is less than 0.5 $\log_{10}$ InfU/mL. Preferably the overall loss of virus titer is less than 0.4 $\log_{10}$ InfU/mL, more preferably less than 0.3 $\log_{10}$ InfU/mL, most preferably less than 0.2 $\log_{10}$ InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0199] According to particular embodiments, the aqueous composition of the present invention is stable for a time period of at least 12 months at about -20 degrees C followed by storage at +2 to +8 degrees C for 9 months, wherein the overall loss of virus titer at -20 degrees C for the specified time period of at least 12 months followed by storage at +2 to +8 degrees C for at least 9 months is less than 0.5 $\log_{10}$ InfU/mL. Preferably the overall loss of virus titer is less than 0.4 $\log_{10}$ InfU/mL, more preferably less than 0.3 $\log_{10}$ InfU/mL, most preferably less than 0.2 $\log_{10}$ InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0200] According to particular embodiments, the aqueous composition of the present invention is stable for a time period of at least 24 months at about -20 degrees C followed by storage at +2 to +8 degrees C for 9 months, wherein the overall loss of virus titer at -20 degrees C for the specified time period of at least 24 months followed by storage at +2 to +8 degrees C for at least 9 months is less than 0.5 $\log_{10}$ InfU/mL. Preferably the overall loss of virus titer is less than 0.4 $\log_{10}$ InfU/mL, more preferably less than 0.3 $\log_{10}$ InfU/mL, most preferably less than 0.2 $\log_{10}$ InfU/mL, preferably as determined by the Fluorescence Activated Cell Sorter (FACS) assay according to example 1.

[0201] The "overall loss of virus titer" according to the present invention is defined as the cumulative loss in virus titer measured during storage of the composition at the indicated temperature n (*e.g.*, at *+5 degrees* C) and time t (*e.g.,* for 6 months) given as $\log_{10}$InfU/mL. The overall loss of virus titer is given as x $\log_{10}$ (*e.g.*, as 0.5 $\log_{10}$ at +5 degrees C for six months).

[0202] According to particular embodiments, the aqueous composition of the present invention is stable, wherein the composition exhibits a potency loss of less than 0.3 $\log_{10}$ InfU/mL when stored for a period of 12 months at -20 degrees C.

[0203] According to particular embodiments, the aqueous composition of the present invention is stable, wherein the composition exhibits a potency loss of less than 0.3 $\log_{10}$ InfU/mL when stored for a period of 12 months at +4 degrees C to +8 degrees C.

[0204] According to particular embodiments, the aqueous composition of the present invention is stable, wherein the composition exhibits a potency loss of less than 0.2 $\log_{10}$ InfU/mL when stored for a period of 12 months at -20 degrees C.

[0205] According to particular embodiments, the aqueous composition of the present invention is stable, wherein the composition exhibits a potency loss of less than 0.2 $\log_{10}$ InfU/mL when stored for a period of 12 months at +4 degrees C to +8 degrees C.

[0206] According to particular embodiments, the aqueous composition of the present invention is stable, wherein the composition exhibits a potency loss of less than 0.1 $\log_{10}$ InfU/mL when stored for a period of 12 months at -20 degrees C.

[0207] According to particular embodiments, the aqueous composition of the present invention is stable, wherein the composition exhibits a potency loss of less than 0.1 $\log_{10}$ InfU/mL when stored for a period of 12 months at +4 degrees C to +8 degrees C.

[0208] According to particular other embodiments, the aqueous composition of the present invention is a liquid composition or a liquid frozen composition.

[0209] According to particular other embodiments, the aqueous composition of the present invention is an aqueous frozen composition.

[0210] According to particular other embodiments, the aqueous composition of the present invention is an aqueous liquid composition.

[0211] According to particular other embodiments, the aqueous composition of the present invention is not a dried composition, preferably not a freeze-dried or lyophilized composition.

[0212] Another aspect provides the aqueous composition of the present invention for use as a medicament or vaccine.

[0213] Another aspect provides the aqueous composition of the present invention for treating or preventing a disease, preferably an infectious disease or cancer.

[0214] Another aspect provides a use of the aqueous composition of the present invention for manufacturing a medicament or vaccine for treating or preventing an infectious disease or cancer.

[0215] Another aspect provides a method of treating or preventing an infectious disease, administering to the subject the composition of any of the embodiments of the invention.

[0216] The invention provides also the following non-limiting embodiments:

1. An aqueous composition comprising at least one poxvirus, at least one disaccharide, sorbitol, gelatin, albumin, a pharmaceutical acceptable buffer and at least one monovalent salt, wherein said composition has a pH ranging between pH 7.0 and pH 8.5.
2. The composition of embodiment 2, wherein the buffer is a Tris buffer or phosphate buffer.
3. The composition of embodiment 2, comprising the Tris buffer at a concentration ranging between 1mM and 50mM.
4. The composition of embodiment 2, comprising the Tris buffer at a concentration ranging between 1mM and 15mM.
5. The composition of embodiment 2, comprising the Tris buffer at a concentration ranging between 5mM and 11mM.
6. The composition of embodiment 2, comprising the Tris buffer at a concentration ranging between 7mM and 10mM.
7. The composition of any one of embodiments 1to 6, wherein the disaccharide is trehalose, sucrose or a combination thereof.
8. The composition of any one of embodiments 1 to 6, wherein the disaccharide is trehalose.
9. The composition of any one of embodiments 1 to 6, wherein the disaccharide is sucrose.

10. The composition of any one of embodiments 1 to 9, comprising the disaccharide at a concentration ranging between 2% (w/v) and 12% (w/v), between 4% (w/v) and 12% (w/v), or between 7% (w/v) and 11% (w/v).
11. The composition of any one of embodiments 1 to 10, comprising the disaccharide at a concentration ranging between 7% (w/v) and 11% (w/v).
12. The composition of any of embodiments 1 to 11, comprising the disaccharide at a concentration ranging between 7% (w/v) and 11% (w/v).
13. The composition of any of embodiments 1 to 12, comprising sorbitol at a concentration ranging between 0.2% (w/v) and 5% (w/v), between 0.1% (w/v) and 3% (w/v), preferably between 1% (w/v) and 3% (w/v).
14. The composition of embodiment 12, comprising sorbitol at a concentration of 2% (w/v).
15. The composition of any one of embodiments 1 to 14, wherein said composition has a pH ranging between pH 7.3 and pH 8.1.
16. The composition of any one of embodiments 1 to 14, wherein said composition has a pH of 7.7.
17. The composition of any one of embodiments 1 to 16, comprising albumin at a concentration ranging between 0.1% (w/v) and 5% (w/v), between 0.1% (w/v) and 1.2% (w/v), preferably between 0.2% (w/v) and 1.2% (w/v).
18. The composition of any one of embodiments 1 to 17, wherein the gelatin is gelatin hydrolysate.
19. The composition of any one of embodiments 1 to 18, comprising gelatin at a concentration ranging between 0.25% (w/v) and 5% (w/v), between 0.2% (w/v) and 4% (w/v), between 0.5% (w/v) and 3% (w/v), between 0.5% (w/v) and 2.2% (w/v), or between 1% (w/v) and 3% (w/v), preferably at a concentration of 2% (w/v).
20. The composition of embodiment 1, wherein said composition comprises a disaccharide at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.8% (w/v) and 1.2% (w/v), gelatin hydrolysate at a concentration between 1% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a

concentration ranging between 5 and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

21. An aqueous composition comprising at least one poxvirus, wherein said composition comprises a disaccharide selected from trehalose and sucrose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 0.5% (w/v) and 3% (w/v), gelatin hydrolysate at a concentration between 0.2% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

22. An aqueous composition comprising at least one poxvirus, wherein said composition comprises trehalose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 0.5% (w/v) and 3% (w/v), gelatin hydrolysate at a concentration between 0.2% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

23. An aqueous composition comprising at least one poxvirus, sucrose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 0.5% (w/v) and 3% (w/v), gelatin hydrolysate at a concentration between 0.2% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

24. An aqueous composition comprising at least one poxvirus, wherein said composition comprises a disaccharide selected from trehalose and sucrose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v), gelatin hydrolysate at a concentration between 1% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

25. An aqueous composition comprising at least one poxvirus, wherein said composition comprises trehalose at a concentration ranging between 4% (w/v) and 12% (w/v),

sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v), gelatin hydrolysate at a concentration between 1% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

26. An aqueous composition comprising at least one poxvirus, sucrose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v), gelatin hydrolysate at a concentration between 1% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

27. An aqueous composition comprising at least one poxvirus, wherein said composition comprises a disaccharide selected from trehalose and sucrose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 0.5% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.2% (w/v) and 1.2% (w/v), gelatin hydrolysate at a concentration between 0.2% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

28. An aqueous composition comprising at least one poxvirus, wherein said composition comprises trehalose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 0.5% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.2% (w/v) and 1.2% (w/v), gelatin hydrolysate at a concentration between 0.2% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

29. An aqueous composition comprising at least one poxvirus, sucrose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 0.5% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.2% (w/v) and 1.2% (w/v), gelatin hydrolysate at a concentration between 0.2% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration

ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

30. An aqueous composition comprising at least one poxvirus, wherein said composition comprises a disaccharide selected from trehalose and sucrose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.8% (w/v) and 1.2% (w/v), gelatin hydrolysate at a concentration between 1% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

31. An aqueous composition comprising at least one poxvirus, wherein said composition comprises trehalose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.8% (w/v) and 1.2% (w/v), gelatin hydrolysate at a concentration between 1% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

32. An aqueous composition comprising at least one poxvirus, sucrose at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.8% (w/v) and 1.2% (w/v), gelatin hydrolysate at a concentration between 1% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

33. An aqueous composition comprising at least one poxvirus, wherein said composition comprises a disaccharide selected from trehalose and sucrose at a concentration of 10% (w/v), sorbitol at a concentration ranging of 2% (w/v), albumin at a concentration 1% (w/v), gelatin hydrolysate at a concentration of 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

34. An aqueous composition comprising at least one poxvirus, wherein said composition comprises a disaccharide selected from trehalose at a concentration of 10% (w/v), sorbitol at a concentration ranging of 2% (w/v), albumin at a concentration 1% (w/v), gelatin hydrolysate at a concentration of 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

35. An aqueous composition comprising at least one poxvirus, wherein said composition comprises sucrose at a concentration of 10% (w/v), sorbitol at a concentration ranging of 2% (w/v), albumin at a concentration 1% (w/v), gelatin hydrolysate at a concentration of 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

36. The composition of any one of embodiments 1 to 35, wherein the composition comprises sodium chloride or potassium chloride, preferably sodium chloride.

37. The composition of embodiment 36 comprising sodium chloride at a concentration ranging between 50mM and 150mM.

38. The composition of embodiment 36 comprising sodium chloride at a concentration ranging between 50mM and 110mM.

39. The composition of any of the embodiments 36 to 38, further comprising magnesium chloride.

40. The composition of embodiment 39 comprising magnesium chloride at a concentration ranging between 1mM and 300mM.

41. The composition of embodiment 39 comprising magnesium chloride at a concentration ranging between 100mM and 300mM.

42. The composition of embodiment 39 comprising magnesium chloride at a concentration ranging between 200mM and 300mM.

43. The composition of any one of embodiments 1 to 42 further comprising up to four distinct types of amino acids selected from arginine, serine, histidine, lysine, and glycine.

44. The composition of embodiment 44, wherein the amino acid is present in the composition at a concentration of less than 120mM, preferably less than 60mM.
45. The composition of any one of embodiments 1 to 44 further comprising glutamic acid or a salt thereof.
46. The composition of embodiment 45, wherein glutamic acid or the salt thereof is present in said composition at a concentration of less than 12mM, preferably less than 6mM.
47. The composition of any one of embodiments 1 to 46, wherein the composition is suitable for parenteral use.
48. The composition of any one of embodiments 1 to 46, wherein the composition is suitable for intranasal use.
49. The composition of any one of embodiments 1 to 46, wherein the composition is suitable for intramuscular or subcutaneous use.
50. The composition of any one of embodiments 1 to 49, wherein the composition is a liquid composition or a liquid frozen composition.
51. The composition of any one of embodiments 1 to 49, wherein the composition is an aqueous frozen composition.
52. The composition of any one of embodiments 1 to 51, wherein the poxvirus is a vaccinia virus.
53. The composition of any one of embodiments 1 to 52, wherein the poxvirus is a modified vaccinia Ankara virus (MVA).
54. The composition of any one of embodiments 1 to 53, wherein the virus is a recombinant virus.
55. The composition of embodiment 54, wherein the recombinant virus comprises a nucleic acid expressing an antigen, preferably a viral antigen.
56. The composition of embodiment 54 or 55, wherein the recombinant virus comprises a nucleic acid encoding a respiratory syncytial virus (RSV) antigen.
57. The composition of any one of embodiments 54 to 56, wherein the recombinant virus comprises a nucleic acid encoding a respiratory syncytial virus (RSV) protein.
58. The composition of any one of embodiments 54 to 57, wherein the recombinant virus comprises a nucleic acid encoding a RSV F glycoprotein.

59. The composition of any one of embodiments 54 to 58, wherein the recombinant virus comprises a nucleic acid encoding a RSV F glycoprotein, two RSV G glycoproteins, a RSV N protein and a RSV M2-1 protein.

60. The composition of any one of embodiments 1 to 59, wherein the virus titer in said composition is comprised between 1 x 10^6 InfU/mL and 1 x 10^{10} InfU/mL.

61. The composition of any one of embodiments 1 to 60, wherein the composition is substantially free of citrate.

62. The composition of any one of embodiments 1 to 61, wherein the composition is substantially free of mannitol.

63. The composition of any one of embodiments 1 to 60, wherein the composition is free of citrate.

64. The composition of any one of embodiments 1 to 61, wherein the composition is free of mannitol.

65. The composition of any of the embodiments 1 to 64, wherein the composition exhibits a loss of potency of less than 0.3 (preferably 0.2, most preferably 0.1) $\log_{10}$ InfU/mL when stored for a period of 12 months at +4 degrees C to +8 degrees C.

66. A vaccine or pharmaceutical composition comprising the composition of any one of the embodiments 1 to 65.

67. The composition of any one of embodiments 1 to 65 for use as a medicament or vaccine.

68. The composition of any one of embodiments 1 to 65 for treating or preventing a disease, preferably an infectious disease.

69. Use of the composition of any one of embodiments 1 to 65 of the present invention for manufacturing a medicament or vaccine for treating or preventing an infectious disease.

70. A method of treating or preventing an infectious disease administering to the subject a therapeutically effective amount of the composition of any of embodiments 1 to 65.

71. A method of making an aqueous live poxvirus composition, the method comprising the steps of:

a. providing a preparation comprising at least one live poxvirus in a pharmaceutical acceptable buffer, and
b. combining the poxvirus preparation of step a) with a solution comprising a disaccharide, sorbitol, gelatin, albumin, and a pharmaceutical acceptable buffer and at least one monovalent salt,

wherein said buffer of a) and b) have a pH ranging between pH 7.0 and pH 8.5.

72. The method of embodiment 71, wherein the buffer is Tris buffer or phosphate buffer.
73. The method of embodiment 72, wherein the Tris buffer has a concentration ranging between 1mM and 25mM.
74. The method of any one of embodiments 72 or 73, wherein the Tris buffer has a concentration ranging between 1mM and 15mM.
75. The method of any one of embodiments 72 to 73, wherein the Tris buffer has a concentration ranging between 5mM and 11mM.
76. The method of any one of embodiments 72 to 73, wherein the Tris buffer has a concentration ranging between 7mM and 10mM.
77. The method any one of embodiments 71 to 76, wherein the buffer of a) and b) have a pH ranging between 7.3 and 8.1.
78. The method of any one of embodiments 71 to 77, wherein the disaccharide is trehalose, sucrose or a combination thereof.
79. The method of any one of embodiments 71 to 77, wherein the disaccharide is trehalose.
80. The method of any one of embodiments 71 to 77, wherein the disaccharide is sucrose.
81. The method of any one of embodiments 71 to 80, wherein after combining a) and b) the combined composition comprises the disaccharide at a concentration ranging between 4% (w/v) and 12% (w/v).
82. The method any one of embodiments 71 to 80, wherein after combining a) and b) the combined composition comprises the disaccharide at a concentration of 10% (w/v).
83. The method any one of embodiments 71 to 82, wherein after combining a) and b) the combined composition comprises sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v).

84. The method of any one of embodiments 71 to 82, wherein after combining a) and b) the combined composition comprises sorbitol at a concentration of 2% (w/v).

85. The method any one of embodiments 71 to 84, wherein after combining a) and b) the combined composition comprises gelatin at a concentration ranging between 0.2% (w/v) and 3% (w/v), preferably at a concentration ranging between 1% (w/v) and 3% (w/v).

86. The method any one of embodiments 71 to 85, wherein after combining a) and b) the combined composition comprises gelatin (preferably gelatin hydrolysate) at a concentration ranging between 0.2% (w/v) and 3% (w/v), preferably at a concentration ranging between 1% (w/v) and 3% (w/v).

87. The method any one of embodiments 71 to 86, wherein after combining a) and b) the combined composition comprises albumin at a concentration ranging between 0.2% (w/v) and 3% (w/v), preferably at a concentration ranging between 0.2% (w/v) and 1.2% (w/v).

88. The method of embodiments 71, wherein after combining a) and b) the combined composition comprises the disaccharide (preferably trehalose, sucrose or a combination thereof) at a concentration ranging between 2% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 0.5% (w/v) and 3% (w/v), gelatin hydrolysate at a concentration between 0.2% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.2% (w/v) and 1.2% (w/v), and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

89. The method of any one of embodiments 71 to 88, wherein the monovalent salt is sodium chloride or potassium chloride, preferably wherein after combining a) and b) the combined composition comprises sodium chloride at a concentration ranging between 50mM and 150mM.

90. The method of embodiment 89, further comprising magnesium chloride, preferably wherein after combining a) and b) the combined composition comprises magnesium chloride at a concentration ranging between 1mM and 300mM.

91. The method of embodiment 89, further comprising magnesium chloride, preferably wherein after combining a) and b) the combined composition comprises magnesium chloride at a concentration ranging between 200mM and 300mM.
92. The method of any one of embodiments 71 to 91, wherein the poxvirus is a vaccinia virus.
93. The method of any one of embodiments 71 to 91, wherein the poxvirus is a modified vaccinia Ankara (MVA) virus.
94. The method of any one of embodiments 71 to 93, wherein the virus is recombinant virus.
95. The method of embodiment 94, wherein the recombinant virus comprises a nucleic acid expressing an antigen, preferably a viral antigen.
96. The method of embodiment 94, wherein the recombinant virus comprises a nucleic acid encoding a respiratory syncytial virus (RSV) antigen.
97. The method of embodiment 94, wherein the recombinant virus comprises a nucleic acid encoding a respiratory syncytial virus (RSV) protein.
98. The method of embodiment 96 or 97, wherein the recombinant virus comprises a nucleic acid encoding a RSV F glycoprotein.
99. The method of embodiment 98, wherein the recombinant virus comprises a nucleic acid encoding a RSV F glycoprotein, two RSV G glycoproteins, a RSV N protein and a RSV M2-1 protein.

DEFINITIONS AND TERMINOLOGY

[0217] It is to be understood that both the foregoing summary and the detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. It is to be understood that this invention is not limited to a particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and

scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

[0218] Terms are defined and explained so that the invention may be understood more readily. Additional definitions are set forth throughout the detailed description.

[0219] It must be noted that, as used herein, the singular forms "a", "an", and "the", include plural references unless the context clearly indicates otherwise. Thus, for example, reference to "a nucleic acid" includes one or more nucleic acid sequences and reference to "the method" includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

[0220] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the present invention.

[0221] As used herein, the conjunctive term "and/or" between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by "and/or", a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or" as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or."

[0222] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used in the context of an aspect or embodiment in the description of the

present invention the term “comprising” can be amended and thus replaced with the term “containing” or “including” or when used herein with the term “having.” Similarly, any of the aforementioned terms (comprising, containing, including, having), whenever used in the context of an aspect or embodiment in the description of the present invention include, by virtue, the terms “consisting of” or “consisting essentially of,” which each denotes specific legal meaning depending on jurisdiction.

[0223] When used herein “consisting of” excludes any element, step, or ingredient not specified in the claim element. When used herein, “consisting essentially of” does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim.

[0224] The term “substantially free of” an ingredient as used herein does not exclude trace amounts of the ingredient which does not materially affect the stability of the composition of the present if not stated otherwise herein. The term “free of” in front of for example mannitol means that the aqueous composition of the present invention does not contain mannitol.

[0225] "About" as used in the present application means ±10%, unless stated otherwise. It must also be noted that unless otherwise stated, any numerical value, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term “about.” Through the specification the term “about” with respect to any quantity or concentration is contemplated to include that quantity. For example, “about 5mM” is contemplated herein to include 5mM as well as values understood to be approximately 5mM with respect to the entity described. As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise. Likewise, the term “about” preceding any numerical value or range used herein in the context of the invention can be deleted and be replaced by the numerical value or range without the term “about” though less preferred.

[0226] The term "nucleic acid", “nucleotide sequence”, “nucleic acid sequence” and "polynucleotide" can be used interchangeably and refers to RNA or DNA that is linear

or branched, single or double stranded, or a hybrid thereof. The polynucleotides can be obtained by chemical synthesis or derived from a microorganism. The term "exogenous" nucleic acid sequences when used in connection with a recombinant virus means a foreign nucleic acid sequence, a nucleic acid sequence not contained in the non-recombinant virus used for generating the recombinant virus or inserted into the virus genome while generating the recombinant virus.

[0227] "Pharmaceutically acceptable" means that the carrier, additive, antibiotic, preservative, adjuvant, diluent, stabilizer or excipient, at the dosages and concentrations employed, will substantially not cause an unwanted or harmful effect(s) in the subject(s) to which they are administered. A "pharmaceutically acceptable" excipient is any inert substance that is combined with an active molecule such as a virus for preparing an agreeable or convenient dosage form. The "pharmaceutically acceptable" excipient is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation comprising the viral preparation. Examples of excipients are cryoprotectants, non-ionic detergents, buffers, salts and inhibitors of free radical oxidation. "Pharmaceutically acceptable carriers" are for example described in Remington's Pharmaceutical Sciences, 18th edition, A. R. Gennaro, Ed., Mack Publishing Company (1990); Pharmaceutical Formulation Development of Peptides and Proteins, S. Frokjaer and L. Hovgaard, *eds*., Taylor & Francis [2000]; and Handbook of Pharmaceutical Excipients, 3rd edition, A. Kibbe, Ed., Pharmaceutical Press (2000).

[0228] By "stable", "stabilized", "stability" or "stabilizing", which can all be used interchangeable, it is understood that the poxvirus contained in the composition of the present invention essentially retains its physical stability, identity, integrity, and/or chemical stability, identity, integrity, particle morphology and/or biological activity or potency upon storage required for shelf-life of a pharmaceutical composition. As used herein, the term "shelf-life" means the time that a product remains active and/or stable according to the product characteristics under specified storage conditions (*e.g*., storage at +2 degrees C to +8 degrees C) for use as a human medication. Shelf-lives correspond to the time points for which the lower limit or upper limit of a given specification is exceeded.

[0229] Stability can be assessed by determining different characteristics such as the quantity (of poxviruses in a formulation), the potency, and/or other quality aspects of the poxvirus (*e.g.*, MVA) in the formulation over a period of time and under certain storage conditions. These characteristics of a poxvirus (*e.g.*, MVA) formulation can be measured at elevated temperatures (predictive for real-time temperatures) or under other stress conditions, for instance formulations can be subjected to +20 degrees C incubation, at +25 degrees C, at -20 degrees C or +5 degrees C or subjected to freeze/thaw cycles and agitation in order to study effects of different formulations maximizing shelf-life. Methods to determine stability of the poxvirus (*e.g.*, MVA) are well known to the skilled person and may be determined by at least one method selected from the group of visual inspection, pH measurement, turbidity assay, particle morphology and potency (infectivity) assay.

[0230] Turbidimetry measures the loss of intensity of transmitted light due to scattering of particles in samples (apparent absorbance), detected at a wavelength where the molecules in the sample do not absorb light (*e.g.*, 350 nm for samples in which proteins are the main chromophore). When molecules aggregate or form supramolecular complexes, the light scattering, which was random when coming from the separate particles, now becomes coherent, and thereby the measured intensity increases. This makes light scattering and turbidimetry useful techniques for detecting aggregation and complex formation or dissociation.

[0231] In the turbidity assay, samples are transferred in triplicate to a UV-transparent, flat-bottom microplate. Absorbance spectra are recorded by a microplate reader between 230 and 500 nm, and the absorbance at 975 nm is measured to determine and possibly correct for differences in optical path length. Control samples consisting of the formulations without MVA were included in the assay to correct for scattering or absorbing matrix components if required. The apparent absorbance at 350 nm was used as a quantitative measure for turbidity.

[0232] The turbidity assay is stability-indicating for MVA samples. MVA aggregation leads to an increase in turbidity and capsid dissociation to a decrease. The assay precision is < 5% (CV%) at turbidity values > 1 NTU.

[0233] Methods to determine particle morphology are well known to the skilled person. For example, particle morphology can be determined using transmission electron microscopy and immunoelectron microscopy (immune-EM) as for example described in Schweneker et al. *J Virol* 2017, 91: e00343-00317. Alternative methods to determine particle morphology is the Nanoparticle Tracking Analysis (NTA) described for example in Filipe et al. *Pharm Res* 2010, 27: 796-810. Nanoparticle tracking analysis (NTA) is a method for the direct and real-time visualization and analysis of particle size distribution and aggregation in liquids. Based on a laser illuminated microscopic technique, Brownian motion of nanoparticles is analyzed in real-time by a charge-couple device (CCD) camera, each particle being simultaneously but separately visualized and tracked by a dedicated particle tracking image-analysis program. The ability of NTA to measure simultaneously particle size and particle scattering intensity allows heterogeneous particle mixtures to be resolved and particle concentration to be estimated directly.

[0234] The term "potency" or "infectivity", when used in relation to a virus as used herein refers to the ability of the virus to infect cells, referring to the invasion and multiplication of the virus in a cell or organism. Infectivity thus refers to the activity of the poxvirus (*e.g.,* vaccinia virus or MVA) expressed as infectious units (InfU) usually given as InfU/mL. Both terms "potency" and "infectivity" can be used interchangeably in the present invention. The potency of a poxvirus such as MVA can be determined using various methods known to the skilled person such as for example determining the percentage of virus-positive cells such as Baby Hamster Kidney Cells 21 (BHK-21) after infection with the virus. A preferred assay is for example the Fluorescence Activated Cell Sorter (FACS) assay as described in the examples.

[0235] The terms "subject" and "patient" are used interchangeably. As used herein, a subject is typically a mammal, such as a non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats, etc.) or a primate (*e.g.*, monkey and human), and in some preferred embodiments a human.

[0236] According to the present invention, "virus" means viruses, virus particles and viral vectors. The terms can all be used interchangeably. This term includes wild-type

viruses, recombinant and non-recombinant viruses, live viruses and live-attenuated viruses.

[0237] According to the present invention, a concentration given in % (w/v) means weight in gram (g) per volume in 100 mL for example 20% (w/v) means 20 g/100mL. A concentration given in % (v/v) means weight in mL per volume in 100mL for example 20% (v/v) means 20 mL/100mL.

[0238] Throughout the specification, except where stated otherwise, values of physical parameters such as pH are those measured at +25 degrees C.

[0239] Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. To the extent, the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0240] The practice of the invention will employ, if not otherwise specified, conventional techniques of immunology, molecular biology, microbiology, cell biology, and recombinant technology, which are all within the skill of the art. See *e.g.* Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual, 2nd edition, 1989; Current Protocols in Molecular Biology, Ausubel FM, et al., *eds*, 1987; the series Methods in Enzymology (Academic Press, Inc.); PCR2: A Practical Approach, MacPherson MJ, Hams BD, Taylor GR, *eds*, 1995; Antibodies: A Laboratory Manual, Harlow and Lane, *eds*, 1988.

EXAMPLES

[0241] The following examples illustrate the invention but should not be construed as in any way limiting the scope of the claims. They merely serve to clarify the invention.

Example 1: Potency (Infectivity) assay

[0242] The titer (InfU/mL) of recombinant or non-recombinant MVA was determined by a Fluorescence Activated Cell Sorter (FACS) assay. MVA infected Baby Hamster Kidney Cells 21 (BHK-21) cells were immune-stained with a fluorochrome-conjugated antibody specific for vaccinia virus (VACV) which were subsequently acquired and quantified using the FACSVerse™ (BD Bioscience) instrument equipped with a BD Flow Sensor for quantitative cell counting.

[0243] In more detail, $2.5x10^5$ BHK 21 cells (source ATCC) were seeded in GMEM / 9% FBS/ 1.8% Ala-Gln into 12 well plates. Cells were infected on the following day with a serial dilution of the MVA virus stock of interest. Following 1 h of incubation at 37 degrees Rifampin (100 µg/mL in GMEM / 9% FBS/ 1.8% Ala-Gln) was added. Cells were harvested 19 ± 2 h after infection and fixed and permeabilized with the BD Perm/Wash™ kit prior to antibody staining. Fixed cells were incubated with anti-vaccinia FITC (Fitzgerald Industries International, Cat#60-v68) for 60 - 90 minutes. Then, the percentage of virus-positive cells was determined by flow cytometry using the BD FACSVerse™ cytometer. The total cell count was determined by using the BD FACSVerse™ Flow Sensor on unstained cells that were fixed in parallel. The calculation of the virus titer (InfU/mL) was based on the percentage of virus-positive cells, the virus dilution used during infection, the infection volume and the average cell number per well. To limit the effect of well to well variability of the cell count, the cell number was established by averaging the cell count of multiple wells. For calculation of the InfU/mL of the virus sample, only dilutions containing 2 to 35% VACV-positive cells were included. The calculation of the InfU/mL per sample dilution was done according to the following formulas:

$$Inf.U/ml = \text{average cell number} * \left[-LN\left(1 - \frac{\%\ VACV\ pos.\ cells}{100}\right)\right] * \frac{virus\ dilution}{infection\ volume}$$

Example 2: Generation of recombinant MVA

[0244] Recombinant MVA used for the stability study in the examples was MVA-BN RSV (MVA-mBN294B) as described in WO 2015/136056 incorporated by reference herewith. MVA-mBN294B encodes for an RSV -F(Along) protein, a G(A) and G(B) protein, as well as an N and M2-1 protein, wherein the N and M2-1 sequences are encoded by a single open reading frame separated by a 2A self-cleavage protease domain of the FMDV virus. As integration site for the antigens IGR 64/65 and IGR 148/149 were used. The genes were expressed under the poxvirus promoters Pr7.5e/l (Cochran et al. *J Virol* 1985, 54: 30-37), PrS (Chakrabarti et al. *Biotechniques* 1997, 23: 1094-1097), PrLE1 (Baur et al. *J Virol* 2010, 84: 8743-8752) and PrH5m (Wennier et al. *PLoS One* 2013, 8: e73511) as described in WO 2015/136056.

[0245] MVA-BN RSV (MVA-mBN294B) was produced in chicken embryonic fibroblast (CEF) cells. CEF cells were produced using standard methods well known to the skilled person such as described in WO 2012/010280. Several methods are available to amplify the recombinant MVA virus and to purify bulk drug substance which are also well known to the skilled person (*e.g.* as described in WO 2006/052826). One exemplary method used is described as follows.

[0246] MVA-BN RSV (MVA-mBN294B) was produced in CEF cells using a 50L wave bag cultivation in VP-SFM medium supplemented with 4mM L-Glutamine, 0.01% Pluronic and antibiotic (*e.g.* Gentamycin 100 µg/mL). Cells were recovered and homogenized by ultrasonification to lyse the cells and release virus particles produced. After centrifugation, depth filtration and Benzonase treatment the virus preparation was further purified and concentrated by ultrafiltration and diafiltration to finally obtain the purified bulk drug substance in Tris 10mM, 140mM NaCl with a pH of 7.7 ±0.4. The BDS was stored at -80 degrees C ±10 degrees C.

Example 3: Vaccine formulation

[0247] As starting material, a blend of two MVA-BN RSV batches was used (BDS). The two batches were up-concentrated to a final titer of 9.129 Log_{10} (InfU/mL)

corresponding to 1.35 x 10^9 InfU/mL and formulated in diafiltration buffer containing 10mM Tris and 140mM sodium chloride at pH 7.7 ±0.4. The residual host cell DNA was 46 µg/mL.

[0248] Such concentrated BDS of MVA-BN RSV was mixed into two-fold concentrated formulation buffers (final one-fold concentration 10mM Tris, 70mM NaCl) containing the various stabilizers which were filtered using a 0.2 µm syringe before use. The samples were filled into DIN 2-R glass vials (0.25mL). Details of the liquid formulation and the ingredients used are presented in Table 1. The following raw materials were used for preparing the two-fold formulation buffers: NEOSORB® PF pyrogen-free sorbitol, GMP, Roquette, FR; Sucrose, Sigma Aldrich; Trehalose, GMP, Pfanstiehl GmbH; Magnesium chloride hexahydrate, Ph. Eur. Sigma; Glycine, Pharmagrade, USP, Sigma Aldrich; L-Glutamic acid, monosodium salt, PharmaGrage (MSG), Sigma; Recombinant Human Albumin, Recombumin®, Albumedix, Hydrolysed gelatin, Vaccipro®, Gelita; L-Arginine, PharmaGrade, USP, Sigma Aldrich; L-Histidine, PharmaGrade, USP, Sigma Aldrich; Dextran 70, USP, Pharmacosmos; Sodium Chloride BioXtra Sigma; Tris hydrochloride, PharmaGrade, Sigma Aldrich. MVA-mBN294B in 10mM Tris and 140mM sodium chloride at pH 7.7 ±0.4 was used as a control.

[0249] For each of the formulations, samples have been aliquoted for storage and analysis for various time points at +5 ±3 degrees C (0, 3, 5, 6, 7, 8 and 12 months) or -20 degrees C (0, 4, 6 and 12 months) using triplicates. At each time points vials are filled with 0.25 mL each of the corresponding MVA-BN RSV formulation stored under respective storage conditions for potency analysis using the Fluorescence Activated Cell Sorter (FACS) assay as stability indicating test according to example 1. The pH and appearance were also tested.

Example 4: Linear Regression analysis – +5 and -20 degrees C

[0250] Stability slopes of formulations were analyzed by linear regression, listed in descending order (*i.e.*, smallest absolute slope value/most stable first) for +5 ±3 degrees C (see Table 1) and -20 ± 3 degrees C (see Table 2) over 12 months.

Table 1 – Stability slopes (+5 degrees C) analyzed by linear regression

Formulation	Slope, +5°C	Excipients beyond NaCl and Tris
F17	-0.0069	10% Trehalose, 2% Sorbitol, 3% Gelatine, 1% rHSA, 50mM Glycine
F23	-0.0116	10% Sucrose, 2% Sorbitol, 3% Gelatine, 1% rHSA
F9	-0.0126	10% Sucrose, 2% Sorbitol, 3% Gelatine, 1% rHSA, 246mM $MgCl_2$, 50mM Glycine, 5mM Monosodium glutamate
F27	-0.0315	10% Sucrose, 2% Dextran70, 3% Gelatine, 1% rHSA, 50mM Arginine, 246mM $MgCl_2$
Control	-0.0487	-

Table 2 - Stability slopes (-20 degrees C) analyzed by linear regression

Formulation	Slope, -20°C	Excipients beyond NaCl and Tris
F23	-0.0026	10% Sucrose, 2% Sorbitol, 3% Gelatine, 1% rHSA
F9	-0.0079	10% Sucrose, 2% Sorbitol, 3% Gelatine, 1% rHSA, 246mM $MgCl_2$, 50mM Glycine, 5mM Monosodium glutamate
F17	-0.0111	10% Trehalose, 2% Sorbitol, 3% Gelatine, 1% rHSA, 50mM Glycine
F27	-0.0162	10% Sucrose, 2% Dextran70, 3% Gelatine, 1% rHSA, 50mM Arginine, 246mM $MgCl_2$
Control	-0.0365	-

Summary of the results

[0251] Real time stability data have shown that the aqueous composition of F23, F9 and F17 showed improved stability over previous prior art compositions (Control) and Dextran based formulations such as F27 when stored at refrigerator temperature (*i.e.*., +5 degrees C). Gelatin hydrolysate and albumin added to disaccharide, in particular trehalose or sucrose, sorbitol compositions provided the best stability results with limited virus loss over up to at least 12 months when stored at between +5 degrees C (Table 1). This enables supply of non-frozen vaccine product at +2 degrees C to +8 degrees C. Further formulation

development studies showed that the stability of aqueous MVA-mBN294B could be further improved by storing frozen at -20 degrees C for at least 12 months after drug product manufacturing and provided sufficient storage when thawed and stored at a temperature between +2 degrees C and +8 degrees C for a limited time of up to 9 months. This was not expected based on the results described by Prabhu et al. who observed that freeze-dried vaccine formulations of camelpox containing 3.5% hydrolyzed gelatin and 3.5% sorbitol after reconstitution showed an immediate loss in virus titer at 4 degrees C and within ten days a 2 $\log_{10}$ $TCID_{50}$ virus loss (Prabhu et al. Biologicals 2014, 42: 169-175).

Example 5: Linear Regression analysis – +25 degrees C/60% relative humidity

[001] Virus formulations as specified in Table 3 were exposed to +25 ± 2 degrees C at 60% ± 5% relative humidity. At the study start samples corresponding to time point zero were transferred to -80 degrees C. After five weeks (35 days) of incubation samples of each formulation were obtained for determining the virus titer.

[002] The incubation of formulations was continued for a total of 10 weeks. The pH was measured at the start of the study and after 10 weeks to evaluate the pH stability of virus formulations.

[003] A FACS assay was used for determining the virus titer as described in example 1. Samples were analyzed in parallel (same assay). The virus titer drop was determined in triplicates, with each replicate pair being analyzed on different days.

[004] Stability slopes of formulations incubated at +25 ± 2 degrees C and 60% ± 5% relative humidity (RH) for 5 weeks (wk) were determined by linear regression analysis and listed in Table 3 in descending order (*i.e.*, smallest absolute slope value/most stable first). Table 3 also shows the decreases of titer $\log_{10}$ (InfU/mL) and pH decreases.

Table 3 – Stability slope and titer and pH decrease (+25 degrees C/60% RH)

Formulation	Slope, +25°C/60% RH, 5 wk	Titer decrease +25°C/60% RH, 5 wk	pH decrease +25°C/60% RH, 10 wk	Excipients beyond NaCl and Tris
F23	-0.012	-0.4	-0.24	10% Sucrose, 2% Sorbitol, 3% Gelatine, 1% rHSA
F66	-0.028	-1.0	-0.79	10% Trehalose, 2% Sorbitol, 1% rHSA
F55	-0.042	-1.5	-0.77	5% Trehalose, 2% Sorbitol, 0.5% rHSA
F62	-0.046	-1.6	-0.84	10% Trehalose, 2% Sorbitol
Control	-0.048	-1.7	-0.78	-

Summary of the results

[005] As described in example 4, formulation F23 showed an improved stability of MVA-BN-RSV when stored at +5 degrees C or -20 degrees C for 12 months as compared to the control. Additionally, the stability of formulation F23 was improved over the control even after 26 months at -20 degrees C (data not shown).

[006] An improved stability was also seen when MVA-BN-RSV formulated in F23 was stored at +25 ± 2 degrees C/60% ± 5% relative humidity for 5 weeks (see Table 3, Figure 3). Similarly, the pH was more stable in F23 than in the control formulation when stored at +25°C/60% relative humidity for 10 weeks (see Table 3).

[007] More particularly, in comparison to the other formulations, formulation F23 showed the best results in terms of titer decrease and slope (Table 3, Figure 3), and pH decrease (Table 3).

[008] While formulations F66, F55 and F62 contained trehalose, F23 contained sucrose instead. This difference, however, is considered not to be relevant in respect of virus stability.

[009] Furthermore, F23 differed from the other formulations in that it contained gelatin. When comparing F23 and F66 it was apparent that gelatin and albumin provided for a better stability than albumin alone.

[010] Thus, gelatin and albumin added to compositions containing sorbitol and a disaccharide (e.g. sucrose) provided the best stability results with limited virus loss over up to at least 5 weeks when stored at +25 degrees C and 60% relative humidity. This permits shipping and storage of the non-frozen vaccine product under conditions of elevated ambient temperatures and humidity, for example in regions where warm, humid climate prevails.

[011] While a number of aspects of the invention herein are exemplified or illustrated with MVA-BN and in particular with MVA-mBN294B, the principles embodied by the invention are applicable to other poxvirus strains as well as other recombinant poxvirus strains and compositions comprising them as well and should not necessarily be limited to particular strains/viruses as used herein. Thus, other recombinant and non-recombinant poxviruses and compositions comprising them are also within the purview of the invention.

[012] It will be apparent that the precise details of the methods or compositions described herein may be varied or modified without departing from the spirit of the described invention. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

References

Al Yaghchi, C., Zhang, Z., Alusi, G., Lemoine, N.R., and Wang, Y. (2015). Vaccinia virus, a promising new therapeutic agent for pancreatic cancer. *Immunotherapy* 7: 1249-1258.

Baur, K., Brinkmann, K., Schweneker, M., Patzold, J., Meisinger-Henschel, C., Hermann, J., Steigerwald, R., Chaplin, P., Suter, M., and Hausmann, J. (2010). Immediate-early expression of a recombinant antigen by modified vaccinia virus ankara breaks the immunodominance of strong vector-specific B8R antigen in acute and memory CD8 T-cell responses. *J Virol* 84: 8743-8752.

Berhanu, A., King, D.S., Mosier, S., Jordan, R., Jones, K.F., Hruby, D.E., and Grosenbach, D.W. (2010). Impact of ST-246(R) on ACAM2000 smallpox vaccine reactogenicity, immunogenicity, and protective efficacy in immunodeficient mice. *Vaccine* 29: 289-303.

Burke, C.J., Hsu, T.A., and Volkin, D.B. (1999). Formulation, stability, and delivery of live attenuated vaccines for human use. *Crit Rev Ther Drug Carrier Syst* 16: 1-83.

Capelle, M.A.H., Babich, L., van Deventer-Troost, J.P.E., Salerno, D., Krijgsman, K., Dirmeier, U., Raaby, B., and Adriaansen, J. (2018). Stability and suitability for storage and distribution of Ad26.ZEBOV/MVA-BN(R)-Filo heterologous prime-boost Ebola vaccine. *Eur J Pharm Biopharm* 129: 215-221.

Chakrabarti, S., Sisler, J.R., and Moss, B. (1997). Compact, synthetic, vaccinia virus early/late promoter for protein expression. *Biotechniques* 23: 1094-1097.

Choi, Y., and Chang, J. (2013). Viral vectors for vaccine applications. *Clin Exp Vaccine Res* 2: 97-105.

Cochran, M.A., Puckett, C., and Moss, B. (1985). In vitro mutagenesis of the promoter region for a vaccinia virus gene: evidence for tandem early and late regulatory signals. *J Virol* 54: 30-37.

Filipe, V., Hawe, A., and Jiskoot, W. (2010). Critical evaluation of Nanoparticle Tracking Analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. *Pharm Res* 27: 796-810.

Hekker, A.C., J.M., B., and Smith, L. (1973). A stable freeze-dried smallpox vaccine made in monolayer cultures of primary rabbit kidney cells. *Journal of Biological Standardization* 1: 21-32.

Hodge, J.W., Sabzevari, H., Yafal, A.G., Gritz, L., Lorenz, M.G., and Schlom, J. (1999). A triad of costimulatory molecules synergize to amplify T-cell activation. *Cancer Res* 59: 5800-5807.

Just, I., and Finke, H. (1979). [A contribution to the stabilisation of the MVA-vaccine (author's transl)]. *Zentralbl Bakteriol Orig A* 245: 276-282.

Leon, A., David, A.L., Madeline, B., Guianvarc'h, L., Dureau, E., Champion-Arnaud, P., Hebben, M., Huss, T., Chatrenet, B., and Schwamborn, K. (2016). The EB66(R) cell line as a valuable cell substrate for MVA-based vaccines production. *Vaccine* 34: 5878-5885.

Mastrangelo, M.J., Eisenlohr, L.C., Gomella, L., and Lattime, E.C. (2000). Poxvirus vectors: orphaned and underappreciated. *J Clin Invest* 105: 1031-1034.

Mayr, A., and Danner, K. (1978). Vaccination against pox diseases under immunosuppressive conditions. *Dev Biol Stand* 41: 225-234.

Mayr, A., Hochstein-Mintzel, V., and Stickl, H. (1975). Abstammung, Eigenschaften und Verwendung des attenuierten Vaccinia-Stammes MVA. *Infektion* 3: 6-14

Meyer, H., Sutter, G., and Mayr, A. (1991). Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. *J Gen Virol* 72 (Pt 5): 1031-1038.

Otagiri, M., and Chuang, V.T. (2009). Pharmaceutically important pre- and posttranslational modifications on human serum albumin. *Biol Pharm Bull* 32: 527-534.

Prabhu, M., Bhanuprakash, V., Venkatesan, G., Yogisharadhya, R., Bora, D.P., and Balamurugan, V. (2014). Evaluation of stability of live attenuated camelpox vaccine stabilized with different stabilizers and reconstituted with various diluents. *Biologicals* 42: 169-175.

Rezaee, F., Linfield, D.T., Harford, T.J., and Piedimonte, G. (2017). Ongoing developments in RSV prophylaxis: a clinician's analysis. *Curr Opin Virol* 24: 70-78.

Schweneker, M., Laimbacher, A.S., Zimmer, G., Wagner, S., Schraner, E.M., Wolferstatter, M., Klingenberg, M., Dirmeier, U., Steigerwald, R., Lauterbach, H., *et al.*

(2017). Recombinant Modified Vaccinia Virus Ankara Generating Ebola Virus-Like Particles. *J Virol* 91: e00343-00317.

Suter, M., Meisinger-Henschel, C., Tzatzaris, M., Hulsemann, V., Lukassen, S., Wulff, N.H., Hausmann, J., Howley, P., and Chaplin, P. (2009). Modified vaccinia Ankara strains with identical coding sequences actually represent complex mixtures of viruses that determine the biological properties of each strain. *Vaccine* 27: 7442-7450.

Tlaxca, J.L., Ellis, S., and Remmele, R.L., Jr. (2015). Live attenuated and inactivated viral vaccine formulation and nasal delivery: potential and challenges. *Adv Drug Deliv Rev* 93: 56-78.

Vanderplasschen, A., and Pastoret, P.P. (2003). The uses of poxviruses as vectors. *Curr Gene Ther* 3: 583-595.

Verheust, C., Goossens, M., Pauwels, K., and Breyer, D. (2012). Biosafety aspects of modified vaccinia virus Ankara (MVA)-based vectors used for gene therapy or vaccination. *Vaccine* 30: 2623-2632.

Wennier, S.T., Brinkmann, K., Steinhausser, C., Maylander, N., Mnich, C., Wielert, U., Dirmeier, U., Hausmann, J., Chaplin, P., and Steigerwald, R. (2013). A novel naturally occurring tandem promoter in modified vaccinia virus ankara drives very early gene expression and potent immune responses. *PLoS One* 8: e73511.

Zhang, Y.-z., Jiang, C.-l., Yu, X.-h., Lou, C.-p., Zhao, D.-h., Wu, Y.-g., Jin, Y.-h., Liu, C.-s., and Kong, W. (2007). Immunogenicity of Lyophilized MVA Vaccine for HIV-1 in Mice Model. *Chemical Research in Chinese Universities* 23: 329-332.

CLAIMS

1. An aqueous composition comprising at least one poxvirus, at least one disaccharide, sorbitol, gelatin, albumin, a pharmaceutical acceptable buffer and at least one monovalent salt, wherein said composition has a pH ranging between pH 7.0 and pH 8.5.
2. The composition of claim 2, wherein the buffer is a Tris buffer or phosphate buffer.
3. The composition of claim 2, comprising the Tris buffer at a concentration ranging between 1mM and 50mM.
4. The composition of any one of claims 1 to 3, wherein the disaccharide is trehalose, sucrose or a combination thereof.
5. The composition of any one of claims 1 to 4, comprising the disaccharide at a concentration ranging between 2% (w/v) and 12% (w/v).
6. The composition of any one of claims 1 to 5, comprising sorbitol at a concentration ranging between 0.2% (w/v) and 5% (w/v).
7. The composition of any one of claims 1 to 6, wherein the gelatin is gelatin hydrolysate, preferably at a concentration ranging between 0.25% (w/v) and 5% (w/v).
8. The composition of any one of claims 1 to 7, comprising albumin at a concentration ranging between 0.1% (w/v) and 5% (w/v).
9. The composition of claim 1, wherein said composition comprises a disaccharide at a concentration ranging between 4% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 1% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.2% (w/v) and 1.2% (w/v), gelatin hydrolysate at a concentration between 0.5% (w/v) and 3% (w/v) and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5 and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.
10. The composition of any one of claims 1 to 9, wherein the composition comprises sodium chloride or potassium chloride, preferably sodium chloride.
11. A method of making an aqueous live poxvirus composition, the method comprising the steps of:

a. providing a preparation comprising at least one live poxvirus in a pharmaceutical acceptable buffer, and

b. combining the poxvirus preparation of step a) with a solution comprising at least one disaccharide, sorbitol, gelatin, albumin, and a pharmaceutical acceptable buffer and at least one monovalent salt,

wherein said buffer of a) and b) have a pH ranging between pH 7.0 and pH 8.5.

12. The method of claim 11, wherein the buffer is Tris buffer or phosphate buffer.

13. The method of claims 11, wherein after combining a) and b) the combined composition comprises the disaccharide (preferably trehalose, sucrose or a combination thereof) at a concentration ranging between 2% (w/v) and 12% (w/v), sorbitol at a concentration ranging between 0.5% (w/v) and 3% (w/v), gelatin hydrolysate at a concentration between 0.2% (w/v) and 3% (w/v), albumin at a concentration ranging between 0.2% (w/v) and 1.2% (w/v), and a pharmaceutical acceptable buffer (preferably Tris buffer at a concentration ranging between 5mM and 25mM), wherein said composition has a pH ranging between 7.3 and 8.1.

14. The composition of any one of claims 1 to 10 or the method of any one of claims 11 to 13, wherein the poxvirus is a vaccinia virus, preferably modified vaccinia Ankara (MVA) virus.

15. The composition of any one of claims 1 to 10 or 14 or the method of any one of claims 11 to 14, wherein the virus is a recombinant virus, preferably wherein the recombinant virus comprises a nucleic acid encoding a respiratory syncytial virus (RSV) antigen.

Figure 1/3

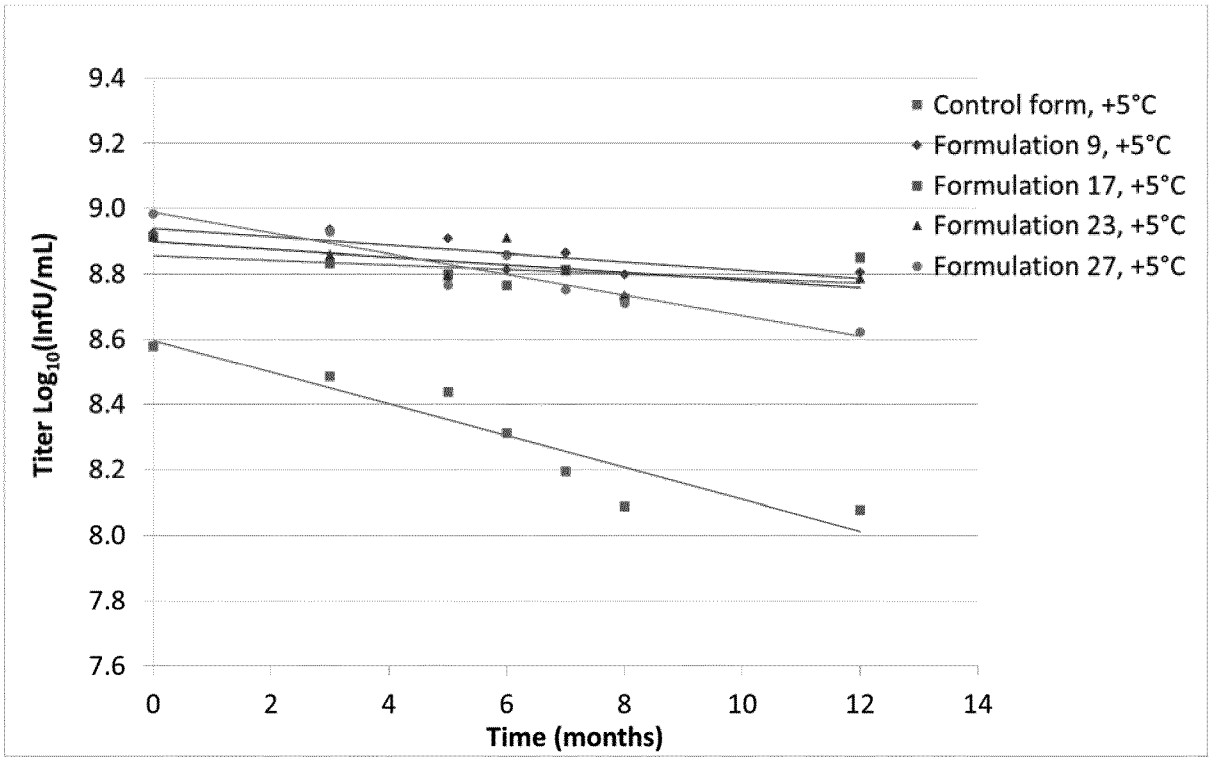

9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
Titer Log10(InfU/mL)
0
2
4
6
8
10
12
14
Time (months)
Control form, +5°C
Formulation 9, +5°C
Formulation 17, +5°C
Formulation 23, +5°C
Formulation 27, +5°C

Figure 2/3

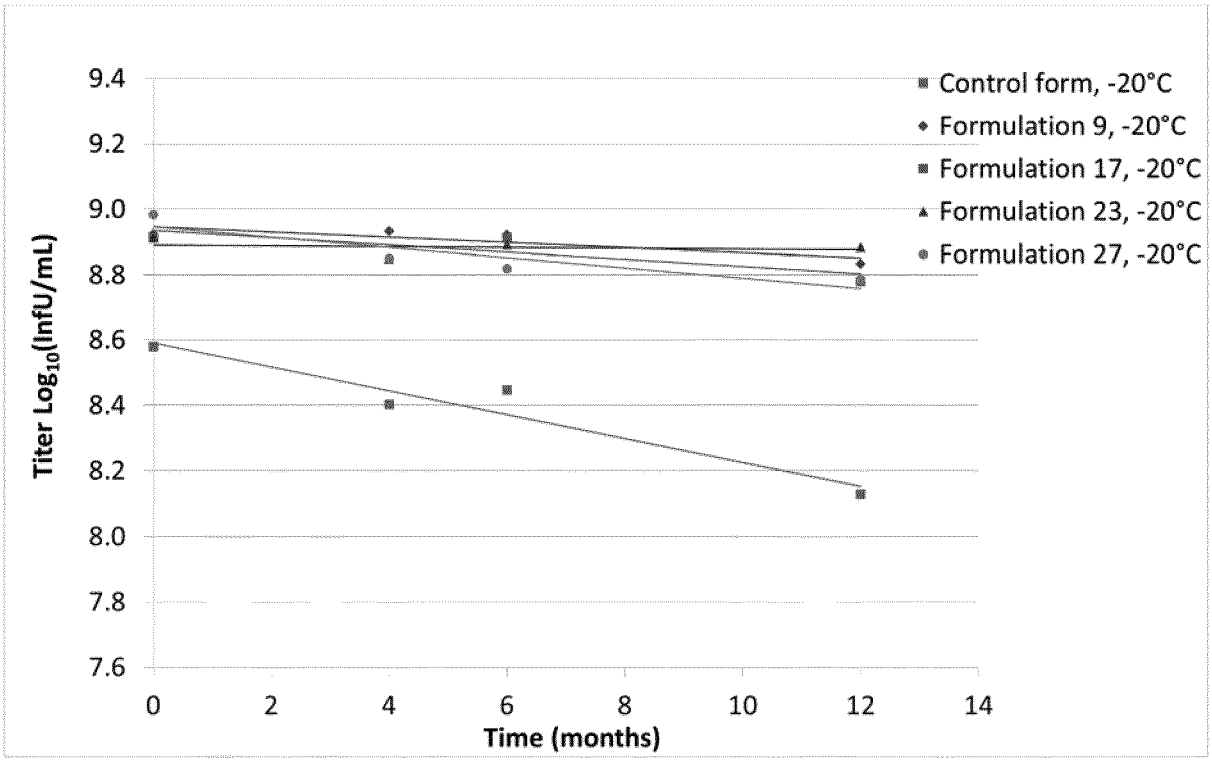
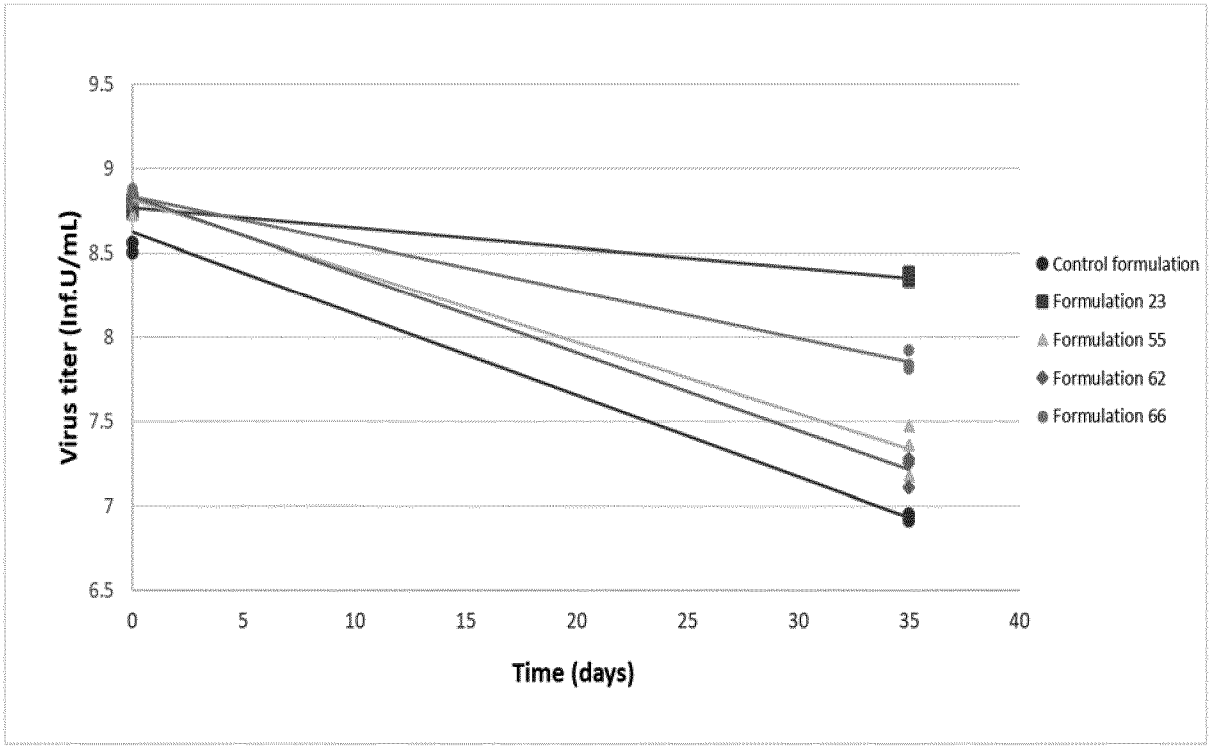

9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
Titer Log10(InfU/mL)
0
2
4
6
8
10
12
14
Time (months)
Control form, -20°C
Formulation 9, -20°C
Formulation 17, -20°C
Formulation 23, -20°C
Formulation 27, -20°C

Figure 3/3


9.5
9
8.5
8
7.5
7
6.5
Virus titer (Inf.U/mL)
0
5
10
15
20
25
30
35
40
Time (days)
Control formulation
Formulation 23
Formulation 55
Formulation 62
Formulation 66