

FORMULATIONS FOR STAPHYLOCOCCUS AUREUS VACCINE**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of U.S. Provisional Application No.: 63/620,746 filed on January 12, 2024 and U.S. Provisional Application No.: 63/620,747 filed on January 12, 2024, the disclosure of each of which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] This application contains an electronic Sequence Listing which has been submitted in XML file format with this application, the entire content of which is incorporated by reference herein in its entirety. The Sequence Listing XML file submitted with this application is entitled “14197-020-228_SEQLISTING.xml”, was created on January 8, 2025, and is 79,113 bytes in size.

GOVERNMENT LICENSE RIGHTS

[0003] This invention was made with government support under IDSEP160030-02-00 awarded by Department of Health and Human Services, Administration for Strategic Preparedness and Response (HHS/ASPR). The government has certain rights in the invention.

1. FIELD

[0004] The present invention relates to lyophilized and spray freeze-dried formulations of *Staphylococcus aureus* vaccine, which have comparable biophysical attributes as the liquid formulation, and have long-term stability.

2. BACKGROUND OF THE INVENTION

[0005] *Staphylococcus aureus* is the leading cause of skin and soft tissue infections (SSTIs) in the U.S. as well as more serious invasive diseases, including bacteremia, sepsis, endocarditis, surgical site infections, osteomyelitis, and pneumonia. These infections are exacerbated by the emergence of antibiotic-resistant clinical isolates such as methicillin-resistant *S. aureus* (MRSA), highlighting the need for alternatives to antibiotics to treat bacterial infections. We have previously developed a multi-component toxoid vaccine (LTB-SA7, also referred as IBT-V02) in a liquid formulation with efficacy against multiple strains of *Staphylococcus aureus* prevalent in the industrialized world (Karauzum 2021, Front Immunol.;12:624310). However, liquid vaccine

formulations are not compatible with the paucity of cold chain storage infrastructure in many low-to-middle income countries (LMICs).

3. SUMMARY OF THE INVENTION

[0006] The present disclosure is directed to lyophilized and spray freeze-dried formulations of *Staphylococcus aureus* vaccine. Both formulations have comparable biophysical attributes as the liquid formulation, and have long-term stability. The lyophilized and spray freeze-dried formulations of *Staphylococcus aureus* vaccine (e.g. LTB-SA7 vaccine) provided herein are effective alternative to liquid vaccine formulations of *Staphylococcus aureus* vaccine (e.g. LTB-SA7 vaccine) against *S. aureus* infections, which has important implications for vaccination of patients from LMICs.

[0007] In one aspect, provided herein is a lyophilized pharmaceutical composition comprising: (1) a multicomponent vaccine, (2) a buffer, (3) sucrose, and (4) polysorbate 80, wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

[0008] In some embodiments of the lyophilized pharmaceutical composition provided herein, the composition further comprises histidine-borate.

[0009] In some embodiments of the lyophilized pharmaceutical composition provided herein, the buffer is phosphate buffer.

[0010] In some embodiments of the lyophilized pharmaceutical composition provided herein, Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1; LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2; LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3; LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SEQ ID NO:5; TBA₂₂₅ comprises a fusion protein comprising: (1) a TSST-1 toxoid with L30R, D27A, and I46A mutations, (2) a SEB toxoid with L45R, Y89A, and Y94A mutations, and (2) a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or comprising the amino acid sequence of SEQ ID NO: 6.

[0011] In some embodiments of the lyophilized pharmaceutical composition provided herein, HlaH35LH48L consists of the amino acid sequence of SEQ ID NO: 1; LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2; LukFmut1 consists of the amino acid sequence of SEQ ID NO: 3; LukABmut50 comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5; and TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.

[0012] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml after reconstitution in a liquid formulation.

[0013] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml after reconstitution in a liquid formulation.

[0014] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of sucrose is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) after reconstitution in a liquid formulation.

[0015] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) after reconstitution in a liquid formulation.

[0016] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 25mM after reconstitution in a liquid formulation.

[0017] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of phosphate buffer is about from 0.5mM to 20mM after reconstitution in a liquid formulation.

[0018] In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 2: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 1: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80

at about a ratio of 0.5: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.125: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.03125: 400: 1 by weight.

[0019] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein is about 0.2 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein is about 0.1 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein is about 0.025 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein is about 0.00625 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the

polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation.

[0020] In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein is about 200 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 40µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose.

[0021] In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein is 200 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 40µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[0022] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine has less than 15%, 10%, 5%, or 2% protein aggregation or degradation compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine has at least about 2%, at least about 5%, at

least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein aggregation or degradation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

[0023] In some embodiments of the lyophilized pharmaceutical composition provided herein, the protein aggregation or degradation is measured by SEC-HPLC, SDS-PAGE or Western blot assay.

[0024] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine has similar immunogenic activity compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation.

[0025] In some embodiments of the lyophilized pharmaceutical composition provided herein, the immunogenic activity is measured by the levels of antibody titers production in a subject induced by administering to the subject the multivalent vaccine in a lyophilized or liquid formulation. In some embodiments, the immunogenic activity of the multivalent vaccine is measured by the immune response elicited by the multivalent vaccine in a subject. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the B-cell response induced by the multivalent vaccine. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the T-cell mediated protection against *S. aureus* infection induced by the multivalent vaccine.

[0026] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0027] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at room temperature (RT) for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about

8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0028] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0029] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0030] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at RT for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0031] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0032] In another aspect, provided herein is a method of generating the lyophilized pharmaceutical composition provided herein, comprising: (1) dissolving the multicomponent vaccine, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition; (2) freezing the liquid composition to form a frozen composition; (3) drying the frozen composition under a primary reduced pressure at a primary drying temperature for a period of time effective to reduce the moisture content of the composition; and (4) further drying the frozen composition under a second reduced pressure at a second drying temperature for a period of time effective to further reduce the moisture content of the composition, thereby producing the lyophilized composition.

[0033] In some embodiments provided herein, the method comprises: (1) dissolving the multicomponent vaccine, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition; (2) freezing the liquid composition at -40°C for 2 hours to form a frozen composition; annealing the frozen composition at -25°C for 2 hours; and further freezing the frozen composition at -40°C for 1 hour; (3) drying the frozen composition under a primary reduced pressure at -40°C for 0.5 hour to reduce the moisture content of the composition; and (4) further drying the frozen composition under a second reduced pressure at 10°C for 1 hour to further reduce the moisture content of the composition, thereby producing the lyophilized composition. In some embodiments, the primary reduced pressure is the same as the second reduced pressure. In some embodiments, the pressure of the lyophilization chamber for all drying stage is set at 50mTorr.

[0034] In some embodiments provided herein, the lyophilized pharmaceutical composition has a final moisture content of between 3% and 5%.

[0035] In another aspect, provided herein is spray freeze-dried pharmaceutical composition comprising: (1) a multicomponent vaccine, (2) a buffer, (3) sucrose, and (4) polysorbate 80, wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

[0036] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the composition further comprises histidine-borate.

[0037] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the buffer is phosphate buffer.

[0038] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1; LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2; LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3; LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO:5; TBA₂₂₅ comprises a fusion protein comprising: a TSST-1 toxoid with L30R, D27A, and I46A mutations, a SEB toxoid with L45R, Y89A, and Y94A mutations, and a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or comprising the amino acid sequence of SEQ ID NO: 6.

[0039] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1; LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2; LukF_{mut1} consists of the amino acid sequence of SEQ ID NO: 3; LukAB_{mut50} comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5; and TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.

[0040] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml after reconstitution in a liquid formulation.

[0041] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml after reconstitution in a liquid formulation.

[0042] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of sucrose is from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) after reconstitution in a liquid formulation.

[0043] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) after reconstitution in a liquid formulation.

[0044] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 25mM after reconstitution in a liquid formulation.

[0045] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of phosphate buffer is about from 0.5mM to 20mM after reconstitution in a liquid formulation.

[0046] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 2: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 1: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.5: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.125: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.03125: 400: 1 by weight.

[0047] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein is about 0.2 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein is about 0.1 mg/ml, the concentration of

the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein is about 0.025 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein is about 0.00625 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation.

[0048] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein is about 200 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 40µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose.

[0049] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein is 200 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 40µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the

total protein is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[0050] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine has less than 15%, 10%, 5%, or 2% protein aggregation or degradation compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein aggregation or degradation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

[0051] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the protein aggregation or degradation is measured by SEC-HPLC, SDS-PAGE or Western blot assay.

[0052] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine has similar immunogenic activity compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation.

[0053] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the immunogenic activity is measured by the levels of antibody titers production in a subject induced by administering to the subject the multivalent vaccine in a spray freeze-dried or liquid formulation.

[0054] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months,

about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0055] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at RT for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0056] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0057] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine retains at least 70%, 80%, 90% or 95% of its initial immunogenic activity after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0058] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine retains at least 70%, 80%, 90% or 95% of its initial immunogenic activity after storage at room temperature (RT) for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0059] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

[0060] In another aspect, provided herein is a method of generating the spray freeze-dried pharmaceutical composition, comprising: dissolving the multicomponent vaccine, histidine-borate, phosphate buffer, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition; atomizing the liquid formulation into specifically sized spherical droplets; immediately freezing the droplets and locking in the size and shape of each individual particle; collecting the particles and drying the particles in bulk in a lyophilization chamber.

[0061] In another aspect, provided herein is a reconstituted pharmaceutical composition comprising: (1) a multicomponent vaccine, (2) a buffer, (3) sucrose, (4) polysorbate 80, and (5) a reconstitution liquid, wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

[0062] In some embodiments of the reconstituted pharmaceutical composition provided herein, the composition further comprises histidine-borate.

[0063] In some embodiments of the reconstituted pharmaceutical composition provided herein, the buffer is phosphate buffer.

[0064] In some embodiments of the reconstituted pharmaceutical composition provided herein, Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1; LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2; LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3; LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO:5; TBA₂₂₅ comprises a fusion protein comprising: (1) a TSST-1 toxoid with L30R, D27A, and I46A mutations, (2) a SEB toxoid with L45R, Y89A, and

Y94A mutations, and (3) a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or comprising the amino acid sequence of SEQ ID NO: 6.

[0065] In some embodiments of the reconstituted pharmaceutical composition provided herein, Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1; LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2; LukF_{mut1} consists of the amino acid sequence of SEQ ID NO: 3; LukAB_{mut50} comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5; and TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.

[0066] In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml.

[0067] In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml.

[0068] In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of sucrose is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L).

[0069] In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L).

[0070] In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 25mM.

[0071] In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of phosphate buffer is about from 0.5mM to 20mM.

[0072] In some embodiments of the reconstituted pharmaceutical composition provided herein, the reconstituted pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 2: 400: 1 by weight. In some embodiments of the reconstituted pharmaceutical composition provided herein, the reconstituted pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 1: 400: 1 by weight. In some embodiments of the reconstituted pharmaceutical composition provided herein, the reconstituted pharmaceutical composition comprises the total protein, sucrose, and polysorbate

80 at about a ratio of 0.5: 400: 1 by weight. In some embodiments of the reconstituted pharmaceutical composition provided herein, the reconstituted pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.125: 400: 1 by weight. In some embodiments of the reconstituted pharmaceutical composition provided herein, the reconstituted pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.03125: 400: 1 by weight.

[0073] In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5. In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of the total protein is about 0.2 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5. In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of the total protein is about 0.1 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5. In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of the total protein is about 0.025 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5. In some embodiments of the reconstituted pharmaceutical composition provided herein, the concentration of the total protein is about 0.00625 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5.

[0074] In some embodiments of the reconstituted pharmaceutical composition provided herein, the total protein is about 200 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, each component of the vaccine is about 40µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, the total protein is about 50 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, the total protein is about 12.5 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, the total protein is about 3.125 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose.

[0075] In some embodiments of the reconstituted pharmaceutical composition provided herein, the total protein is 200 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, each component of the vaccine is 40µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, the total protein is 50 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, the total protein is 12.5 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, the total protein is 3.125 µg per dose. In some embodiments of the reconstituted pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[0076] In another aspect, provided herein is a method of treating or preventing *Staphylococcus aureus* (SA) infection in a subject, comprising administering to the subject the reconstituted pharmaceutical composition provided herein.

[0077] In some embodiments of the method provided herein, the subject is a human subject.

[0078] In another aspect, provided herein is a composition suitable for lyophilization or spray-freeze drying, comprising: (1) a multicomponent vaccine, (2) a buffer, (3) sucrose, and (4)

polysorbate 80, wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

[0079] In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the composition further comprises histidine-borate.

[0080] In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the buffer is phosphate buffer.

[0081] In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5.

In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the concentration of the total protein is about 0.2 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the concentration of the total protein is about 0.1 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the concentration of the total protein is about 0.025 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the concentration of the total protein is about 0.00625 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the

polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation.

[0082] In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the total protein is about 200 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, each component of the vaccine is about 40µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the total protein is about 50 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the total protein is about 12.5 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the total protein is about 3.125 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, each component of the vaccine is about 0.625 µg per dose.

[0083] In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the total protein is 200 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, each component of the vaccine is 40µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the total protein is 50 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, each component of the vaccine is 10 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the total protein is 12.5 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, each component of the vaccine is 2.5 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, the total protein is 3.125 µg per dose. In some embodiments of the composition suitable for lyophilization or spray-freeze drying provided herein, each component of the vaccine is 0.625 µg per dose.

[0084] In another aspect, provided herein is a kit comprising one or more containers and instructions for use, wherein the one or more containers comprise the pharmaceutical composition provided herein.

3.1 ILLUSTRATIVE EMBODIMENTS

1. A lyophilized pharmaceutical composition comprising:
 - a. a multicomponent vaccine,
 - b. a buffer,
 - c. sucrose, and
 - d. polysorbate 80,wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.
2. The lyophilized pharmaceutical composition of paragraph 1, wherein the composition further comprises histidine-borate.
3. The lyophilized pharmaceutical composition of paragraph 1 or 2, wherein the buffer is phosphate buffer.
4. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 3, wherein
 - a. Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1;
 - b. LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2;
 - c. LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3;
 - d. LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO:5;
 - e. TBA₂₂₅ comprises a fusion protein comprising:
 - i. a TSST-1 toxoid with L30R, D27A, and I46A mutations,
 - ii. a SEB toxoid with L45R, Y89A, and Y94A mutations, and
 - iii. a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or

comprising the amino acid sequence of SEQ ID NO: 6;

5. The lyophilized pharmaceutical composition of paragraph 4, wherein
 - a. Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1;
 - b. LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2;
 - c. LukF_{mut1} consists of the amino acid sequence of SEQ ID NO: 3;
 - d. LukAB_{mut50} comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5; and
 - e. TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.
6. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 5, wherein the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml after reconstitution in a liquid formulation.
7. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 6, wherein the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml after reconstitution in a liquid formulation.
8. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 7, wherein the concentration of sucrose is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) after reconstitution in a liquid formulation.
9. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 8, wherein the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) after reconstitution in a liquid formulation.
10. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 7, wherein the concentration of histidine-borate is about from 0.1mM to 25mM after reconstitution in a liquid formulation.
11. The lyophilized pharmaceutical composition of any one of paragraphs 3 to 10, wherein the concentration of phosphate buffer is about from 0.5mM to 20mM after reconstitution in a liquid formulation.
12. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 5, wherein the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 2: 400: 1 by weight.

13. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 11, wherein the concentration of the total protein is about 0.4 mg/ml (0.2mg per dose), the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation.

14. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 9, wherein the total protein is about 200 µg per dose.

15. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 12, wherein the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein aggregation or degradation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

16. The lyophilized pharmaceutical composition of paragraph 15, wherein the protein aggregation or degradation is measured by SEC-HPLC, SDS-PAGE or Western blot assay.

17. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 14, wherein the multivalent vaccine has similar immunogenic activity compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation.

18. The lyophilized pharmaceutical composition of paragraph 17, wherein the immunogenic activity is measured by the levels of antibody titers production in a subject induced by administering to the subject the multivalent vaccine in a lyophilized or liquid formulation.

19. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 18, wherein the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

20. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 18, wherein the increase in the amount of aggregated or degraded protein in the composition is less

than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

21. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 18, wherein the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

22. The lyophilized pharmaceutical composition of any one of paragraphs 1 to 18, wherein the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

23. A method of generating the lyophilized pharmaceutical composition of any one of paragraphs 1 to 22, comprising:

- a. dissolving the multicomponent vaccine, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition;
- b. freezing the liquid composition to form a frozen composition;
- c. drying the frozen composition under a primary reduced pressure at a primary drying temperate for a period of time effective to reduce the moisture content of the composition; and
- d. further drying the frozen composition under a second reduced pressure at a second drying temperature for a period of time effective to further reduce the moisture content of the composition, thereby producing the lyophilized composition.

24. The method of paragraph 23, wherein the method comprises:

- a. dissolving the multicomponent vaccine, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition;
- b. freezing the liquid composition at -40°C for 2 hours to form a frozen composition; annealing the frozen composition at -25°C for 2 hours; and further freezing the frozen composition at -40°C for 1 hour.
- c. drying the frozen composition under a primary reduced pressure at -40°C for 0.5 hour to reduce the moisture content of the composition; and
- d. further drying the frozen composition under a second reduced pressure at 10°C for 1 hour to further reduce the moisture content of the composition, thereby producing the lyophilized composition.

25. The method of paragraph 23 or 24, wherein the lyophilized pharmaceutical composition has a final moisture content of between 3% and 5%.

26. A spray freeze-dried pharmaceutical composition comprising:

- a. a multicomponent vaccine,
- b. a buffer,
- c. sucrose, and
- d. polysorbate 80,

wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

27. The spray freeze-dried pharmaceutical composition of paragraph 26, wherein the composition further comprises histidine-borate.

28. The spray freeze-dried pharmaceutical composition of paragraph 26 or 27, wherein the buffer is phosphate buffer.

29. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 28, wherein:

- a. Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1;
- b. LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2;
- c. LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3;

- d. LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO:5;
- e. TBA₂₂₅ comprises a fusion protein comprising:
- a TSST-1 toxoid with L30R, D27A, and I46A mutations,
 - a SEB toxoid with L45R, Y89A, and Y94A mutations, and
 - a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or comprising the amino acid sequence of SEQ ID NO: 6.
30. The spray freeze-dried pharmaceutical composition of paragraph 29, wherein:
- Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1;
 - LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2;
 - LukF_{mut1} consists of the amino acid sequence of SEQ ID NO: 3;
 - LukAB_{mut50} comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5; and
 - TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.
31. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 30, wherein the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml after reconstitution in a liquid formulation.
32. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 31, wherein the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml after reconstitution in a liquid formulation.
33. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 32, wherein the concentration of sucrose is from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) after reconstitution in a liquid formulation.
34. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 33, wherein the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) after reconstitution in a liquid formulation.

35. The spray freeze-dried pharmaceutical composition of any one of paragraphs 27 to 32, wherein the concentration of histidine-borate is about from 0.1mM to 25mM after reconstitution in a liquid formulation.

36. The spray freeze-dried pharmaceutical composition of any one of paragraphs 28 to 35, wherein the concentration of phosphate buffer is about from 0.5mM to 20mM after reconstitution in a liquid formulation.

37. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 36, wherein the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at the ratio of 2: 400: 1 in weight.

38. The spray freeze-dried pharmaceutical composition of any one of paragraphs 28 to 36, wherein the concentration of the total protein is about 0.4 mg/ml (0.2 mg per dose), the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation.

39. The spray freeze-dried pharmaceutical composition of any one of paragraphs 28 to 36, wherein the total protein is about 200 µg per dose.

40. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 39, wherein the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein aggregation or degradation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

41. The spray freeze-dried pharmaceutical composition of paragraph 40, wherein the protein aggregation or degradation is measured by SEC-HPLC, SDS-PAGE or Western blot assay.

42. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 39, wherein the multivalent vaccine has similar immunogenic activity compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation.

43. The spray freeze-dried pharmaceutical composition of paragraph 42, wherein the immunogenic activity is measured by the levels of antibody titers production in a subject induced

by administering to the subject the multivalent vaccine in a spray freeze-dried or liquid formulation.

44. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 43, wherein the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

45. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 43, wherein the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

46. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 43, wherein the multivalent vaccine retains at least 70%, 80%, 90% or 95% of its initial immunogenic activity after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

47. The spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 43, wherein the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

48. A method of generating the spray freeze-dried pharmaceutical composition of any one of paragraphs 26 to 47, comprising:

- a. dissolving the multicomponent vaccine, histidine-borate, phosphate buffer, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition;
- b. atomizing the liquid formulation into specifically sized spherical droplets;
- c. immediately freezing the droplets and locking in the size and shape of each individual particle;
- d. collecting the particles and drying the particles in bulk in a lyophilization chamber.

49. A reconstituted pharmaceutical composition of any one of paragraphs 1 to 22, or 26 to 47 comprising:

- a. a multicomponent vaccine,
- b. a buffer,
- c. sucrose,
- d. polysorbate 80, and
- e. a reconstitution liquid

wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

50. The reconstituted pharmaceutical composition of paragraph 49, wherein the composition further comprises histidine-borate.

51. The reconstituted pharmaceutical composition of paragraph 49 or 50, wherein the buffer is phosphate buffer.

52. The reconstituted pharmaceutical composition of any one of paragraphs 49 to 51, wherein

- a. Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1;
- b. LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2;
- c. LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3;
- d. LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the

amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO:5;

- e. TBA₂₂₅ comprises a fusion protein comprising:
 - i. a TSST-1 toxoid with L30R, D27A, and I46A mutations,
 - ii. a SEB toxoid with L45R, Y89A, and Y94A mutations, and
 - iii. a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or comprising the amino acid sequence of SEQ ID NO: 6.

53. The reconstituted pharmaceutical composition of paragraph 52, wherein

- a. Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1;
- b. Luk_{S_{mut9}} consists of the amino acid sequence of SEQ ID NO: 2;
- c. Luk_{F_{mut1}} consists of the amino acid sequence of SEQ ID NO: 3;
- d. LukAB_{mut50} comprises a first polypeptide consisting of the amino acid

sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5; and

- e. TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.

54. The reconstituted pharmaceutical composition of any one of paragraphs 49 to 53, wherein the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml.

55. The reconstituted pharmaceutical composition of any one of paragraphs 49 to 54, wherein the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml.

56. The reconstituted pharmaceutical composition of any one of paragraphs 49 to 55, wherein the concentration of sucrose is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L).

57. The reconstituted pharmaceutical composition of any one of paragraphs 49 to 56, wherein the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L).

58. The reconstituted pharmaceutical composition of any one of paragraphs 50 to 55, wherein the concentration of histidine-borate is about from 0.1mM to 25mM.

59. The reconstituted pharmaceutical composition of any one of paragraphs 51 to 58, wherein the concentration of phosphate buffer is about from 0.5mM to 20mM.

60. The reconstituted pharmaceutical composition of any one of paragraphs 49 to 59, wherein the reconstituted pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at the ratio of 2: 400: 1 in weight.

61. The reconstituted pharmaceutical composition of any one of paragraphs 51 to 60, wherein the concentration of the total protein is about 0.4 mg/ml (0.2 mg per dose), the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5.

62. The reconstituted pharmaceutical composition of any one of paragraphs 51 to 61, wherein the total protein is about 200 µg per dose .

63. A method of treating or preventing *Staphylococcus aureus* (SA) infection in a subject, comprising administering to the subject the reconstituted pharmaceutical composition of any one of paragraphs 49 to 62.

64. The method of paragraph 62, wherein the subject is a human subject.

65. A composition suitable for lyophilization or spray-freeze drying, comprising:

- a. a multicomponent vaccine,
- b. a buffer,
- c. sucrose, and
- d. polysorbate 80,

wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

66. The composition of paragraph 65, wherein the composition further comprises histidine-borate.

67. The composition of paragraph 26 or 66, wherein the buffer is phosphate buffer.

68. The composition of any one of paragraphs 65 to 67, wherein the composition comprises the total protein, sucrose, and polysorbate 80 at the ratio of 2: 400: 1 in weight.

69. The composition of paragraph 68, wherein the concentration of the total protein is about 0.4 mg/ml (0.2 mg per dose), the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5.

70. The composition of paragraph 67, wherein the total protein is about 200 µg per dose.

71. A kit comprising one or more containers and instructions for use, wherein the one or more containers comprise the pharmaceutical composition of any one of paragraphs 1 to 22, 26 to 47.

3.2 CONVENTIONS AND ABBREVIATIONS

Abbreviation	Convention
C	Celsius
Hla	Alpha-hemolysin
LMIC	Low-to-middle income country
LukAB	Leucocidin AB
MRSA	Methicillin-resistant <i>S. aureus</i>
PLV	Panton-valentine leukocidin
RT	Room temperature
SA	<i>Staphylococcus aureus</i>
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SEA	Staphylococcal enterotoxin A
SEB	Staphylococcal enterotoxin B
SEC-HPLC	Size exclusion-high-performance liquid chromatography
SFD	Spray freeze-dried
SSTI	Skin and soft tissue infections
T _c	Collapse Temperature
T _g '	Glass Transition Temperature
TSST-1	Toxic shock syndrome toxin-1

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0085] Figures 1A-1C. Biochemical characterization of LTB-SA7 components in Lyo, SFD and Liq vaccine formulations. (Fig. 1A) SDS-PAGE and (Fig. 1B) Western Blot analysis of Lyo (Lane 2), SFD (Lane 4), Liq without desorption (Lane 6), Liq with desorption (Lane 7) and freshly prepared Liq without alhydrogel (Lane 9). Samples were loaded at 1µg for SDS-PAGE and 100ng for all antibodies in Western Blot, except anti-LukABmut50 which was loaded at 250ng. (Fig. 1C) SEC-HPLC of Liq (Top), Lyo (Middle) and SFD (Bottom).

[0086] Figures 2A-2E. Comparative immunogenicity and efficacy in a murine intradermal infection model. Mice (n=10 mice per group) were immunized with 50 µg of either Liq, Lyo, or SFD formulations of LTB-SA7 or 50 µg of BSA in 200µg of Alhydrogel (Al(OH)₃) on days 0,

14 and 28. On day 38, sera were collected to assess toxin neutralizing antibody titers in cell-based assays. On day 42, MRSA (8×10^6 CFU of the bioluminescent MRSA strain SF8300) skin infections were performed on immunized mice and bacterial burden and lesion size monitored until the experiment was arbitrarily ended after 10 days. (Fig. 2A) Toxin neutralizing antibody titers. (Fig. 2B) Representative photographs of skin lesion. (Fig. 2C) Mean total lesion size (cm²) $\pm$ SEM. (Fig. 2D) Representative in vivo BLI (photons/s) (log₁₀ scale). (Fig. 2E) Mean total flux (photons/s) $\pm$ SEM. *P < 0.05, †P < 0.01, ‡P < 0.001, as calculated by a two-way analysis of variance (ANOVA) Dunnett's multiple comparisons test (Fig. 2C) and (Fig. 2E). Results are combined from two independent experiments.

[0087] Figures 3A-3F. Comparative immunogenicity and efficacy in rabbit pyomyositis. Rabbits (n=6 rabbits per group) were immunized with 50 μ g of either Liq or Lyo formulations of LTB-SA7 or 50 μ g of BSA in 200 μ g of Alhydrogel (Al(OH)₃) on days 0, 14 and 28. On day 38, sera were collected to assess toxin binding IgG titers by Luminex, and toxin neutralizing antibody titers in cell-based assays. On day 42, MRSA ($6-8 \times 10^9$ CFU of the bioluminescent MRSA strain SF8300) pyomyositis infections were performed on immunized rabbits and skin lesions and thigh width monitored until the experiment was arbitrarily ended after 7 days, when abscesses were imaged, weighted, and harvested for ex vivo CFUs. (Fig. 3A) Toxin-binding IgG titers. (Fig. 3B) Toxin neutralizing antibody titers. (Fig. 3C) Representative photographs of skin lesion and abscess. (Fig. 3D) Mean thigh width change (% of contralateral thigh) $\pm$ SEM. (Fig. 3E) Mean tissue weight of abscess $\pm$ SEM. (Fig. 3F) Geometric mean of ex vivo CFUs $\pm$ SD. *P < 0.05, †P < 0.01, ‡P < 0.001, as calculated by a two-way analysis of variance (ANOVA) Dunnett's multiple comparisons test (Fig. 3D) or Kruskal-Wallis Dunn's multiple comparisons test (Fig. 3E) and (Fig. 3F). Results are combined from two independent experiments.

[0088] Figures 4A-4C. Characterization of LMIC isolates in vitro. (Fig. 4A) LMIC isolates characterization workflow. (Fig. 4B) PCR based toxin profiling. (Fig. 4C) Toxin release profile of the LMIC isolates used for in vivo study.

[0089] Figures 5A-5C. Protective efficacy of LTB-SA7 against LMIC isolates in a mouse bacteremia model. Mice (n=10 mice per group) were immunized with 50 μ g of either Liq or Lyo formulations of LTB-SA7 or 50 μ g of BSA in 200 μ g of Alhydrogel (Al(OH)₃) on days 0, 14 and 28. On day 42, the LMIC bacteremia infection was performed on immunized mice. (Fig. 5A) Survival (%) of mice infected with 8×10^7 CFU of KCO075. (Fig. 5B) Survival (%) of mice

infected with 4×10^7 CFU of Pyo603. (Fig. 5C) Survival (%) of mice infected with 2×10^7 CFU of Gaza209. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, BSA versus Liq or Lyo as calculated by log rank (Mantel-Cox) test. Results are from one independent experiment.

[0090] Figures 6A-6L. Protective efficacy of LTB-SA7 against LMIC isolates in a mouse intradermal infection model. Mice ($n=10$ mice per group) were immunized with 50 μg of either Liq or Lyo formulations of LTB-SA7 or 50 μg of BSA in 200 μg of Alhydrogel (Al(OH)₃) on days 0, 14 and 28. On day 42, intradermal infections with KCO075 (A-D), Pyo603 (E-H), and Gaza209 (I-L) were performed on immunized mice and skin lesions monitored until the experiment was arbitrarily ended after 10 days, when skin and kidney were harvested for ex vivo CFUs. (Fig. 6A), (Fig. 6E), and (Fig. 6I) Representative photographs of skin lesion. (Fig. 6B), (Fig. 6F), and (Fig. 6J) Mean total lesion size (cm^2) $\pm$ SEM. (Fig. 6C), (Fig. 6G), and (Fig. 6K) Geometric mean of skin lesion ex vivo CFUs $\pm$ SD. (Fig. 6D), (Fig. 6H), and (Fig. 6L) Geometric mean of kidney ex vivo CFUs $\pm$ SD. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, as calculated by a two-way analysis of variance (ANOVA) Dunnett's multiple comparisons test (Fig. 6B, Fig. 6F, Fig. 6G) or Kruskal-Wallis Dunn's multiple comparisons test (Fig. 6C, Fig. 6D, Fig. 6G, Fig. 6H, Fig. 6K, Fig. 6L). Results are combined from two independent experiments. n.s., not significant.

[0091] Figures 7A-7D. Biochemical characterization of LTB-SA7 formulations after storage for 1 and 3 months. (Figs. 7A and 7C) SDS-PAGE analysis of 1 μg Lyo LTB-SA7 stored at -20°C (Lane 2) and 30°C (Lane 3), 1 μg SFD LTB-SA7 stored at -20°C (Lane 5) and 30°C (Lane 6), 1 μg Liq LTB-SA7 stored at 30°C without desorption (Lane 8) and with desorption (Lane 9) and 1 μg freshly prepared Liq LTB-SA7 without alhydrogel without desorption (Lane 11) and with desorption (Lane 12) after storage for 1 month (Fig. 7A) and 3 months (Fig. 7C). (Fig. 7B and Fig. 7D) SEC-HPLC of Lyo (-20°C, 30°C), SFD (-20°C, 30°C), Liq (30°C, $\pm$ desorption) and freshly prepared liq ($\pm$ desorption) after storage for 1 month (Fig. 7B) and 3 months (Fig. 7D).

[0092] Figures 8A-8F. Biochemical characterization of the Lyo LTB-SA7 formulation after storage for 6, 9, and 12 months. (Fig. 8A, Fig. 8C, Fig. 8E) SDS-PAGE analysis of 1 μg Lyo LTB-SA7 stored at -20°C (Lane 2) and 30°C (Lane 3) and 1 μg freshly prepared Liq LTB-SA7 without alhydrogel (6mo: Lanes 5, 9; 12 mo: Lane 4) after storage for 6 months (Fig. 8A), 9 months (Fig. 8C), and 12 months (Fig. 8E). (Fig. 8B, Fig. 8D, Fig. 8F) SEC-HPLC of Lyo LTB-

SA7 stored at -20°C and 30°C and freshly prepared Liq without alhydrogel after storage for 6 months (Fig. 8B), 9 months (Fig. 8D), and 12 months (Fig. 8F).

[0093] Figure 9. Immunogenicity of the Lyo LTB-SA7 formulation after long-term storage. Serum toxin neutralizing titers to toxins Hla, PVL, LukAB, SEA, SEB, TSST-1 for individual BALB/c mice (n = 5/group) immunized with 40µg of Lyo LTB-SA7 on Day 0 of stability study or after 6 months storage of vaccine at either -20°C or 30°C. Bars indicate mean log-transformed ND50, the serum dilution that elicits 50% of neutralizing titer. Dotted lines indicate the limit of detection for each toxin (LOD). Statistical analysis performed with the Mann-Whitney test.

[0094] Figures 10A-10B. Toxin neutralization of LMIC supernatants in vitro. Toxicity of bacterial supernatants and neutralizing efficacy of LTB-SA7 pAbs tested with rabbit red blood cells (Fig. 10A) and induced HL-60 cells (Fig. 10B). Effective dilutions of bacterial supernatants causing 85% toxicity (ED85) against RRBCs, and HL-60 cells were calculated for each LMIC isolate. Bacterial SUP dilutions [1:17.3 (KCO075); 1:171 (pyo603); 1:32.3 (Gaza209)] resulting in ED85 were used to determine concentration of LTB-SA7 Rb pAb required to neutralize 50% of bacterial SUP toxicity (NC50).

5. DETAILED DESCRIPTION OF THE INVENTION

[0095] Provided herein are pharmaceutical compositions and their use in preventing and treating infections with *Staphylococcus aureus* (SA). In some embodiments, compositions provided in Section 5.1 are formulated such that they have one or more functional properties described in Section 5.2. In certain embodiments, the pharmaceutical compositions provided herein has various advantages, for example, improved long-term stability under various conditions. Also provided herein are methods of preparing the compositions provided in Section 5.1 (section 5.3). Also provided herein are assays that may be used in related studies (Section 5.6).

5.1 FORMULATIONS OF PHARMACEUTICAL COMPOSITION

5.1.1 LYOPHILIZED FORMULATION OF PHARMACEUTICAL COMPOSITION

[0096] The disclosure provides a lyophilized pharmaceutical composition comprising a multicomponent vaccine (e.g. such as described in Section 5.4 below), a buffer, a stabilizer, and a surfactant. In some embodiments, the lyophilized pharmaceutical composition further

comprises histidine-borate. In some embodiments, the lyophilized pharmaceutical composition comprises a multicomponent vaccine, histidine-borate, a buffer, a stabilizer, and a surfactant.

[0097] In some embodiment, the buffer is phosphate buffer. In some embodiment, the buffer is tris buffer.

[0098] In some embodiments, the stabilizer comprises sucrose.

[0099] In some embodiments, the surfactant comprises polysorbate 80. In some embodiments, the surfactant comprises polysorbate 20.

[0100] In one aspect, the disclosure provides a lyophilized pharmaceutical composition comprising a multicomponent vaccine, phosphate buffer, sucrose, and polysorbate 80.

[0101] In one aspect, the disclosure provides a lyophilized pharmaceutical composition comprising a multicomponent vaccine, histidine-borate, phosphate buffer, sucrose, and polysorbate 80.

[0102] In one aspect, the disclosure provides a lyophilized pharmaceutical composition comprising a multicomponent vaccine, tris buffer, sucrose, and polysorbate 80.

[0103] In one aspect, the disclosure provides a lyophilized pharmaceutical composition comprising a multicomponent vaccine, histidine-borate, tris buffer, sucrose, and polysorbate 80.

[0104] In some embodiments, the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

[0105] In some embodiments, Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations. In some embodiments, Hla_{H35LH48L} comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1.

[0106] In some embodiments, LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations. In some embodiments, LukS_{mut9} comprises comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2.

[0107] In some embodiments, LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation. In some embodiments, LukF_{mut1} comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, LukF_{mut1} consists of the amino acid sequence of SEQ ID NO: 3.

[0108] In some embodiments, LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB. In some embodiments, LukAB_{mut50}

comprises a variant of LukA comprising D39A mutation in LukA and a variant of LukB comprising R23E mutation in LukB. In some embodiments, LukAB_{mut50} comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SEQ ID NO:5. In some embodiments, LukAB_{mut50} comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SEQ ID NO:5.

[00109] In some embodiments, TBA₂₂₅ comprises a fusion protein comprising: (1) a TSST-1 toxoid with L30R, D27A, and I46A mutations, (2) a SEB toxoid with L45R, Y89A, and Y94A mutations, and (3) a SEA toxoid with L48R, D70R, Y92A, and H225A mutations. In some embodiments, the three toxoids are fused together via a linker. In some embodiments, the linker comprise the amino acid sequence of GGGSGGGSGGGS (SEQ ID NO: 7). In some embodiments, TBA₂₂₅ comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.

[00110] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.005 mg/ml to 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.01 mg/ml to 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.01 mg/ml to 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.05 mg/ml to 5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.05 mg/ml to 5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.1 mg/ml to 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.1 mg/ml to 1

mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.2 mg/ml to 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.2 mg/ml to 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.4 mg/ml to 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.4 mg/ml to 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.025 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.05 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 7.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 2.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine

is at most 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 1 mg/ml, 0.9 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.6 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.09 mg/ml, 0.08 mg/ml, 0.075 mg/ml, 0.07 mg/ml, 0.06 mg/ml, 0.05 mg/ml, 0.04 mg/ml, 0.03 mg/ml, 0.025 mg/ml, 0.02 mg/ml, 0.01 mg/ml, 0.009 mg/ml, 0.008 mg/ml, 0.007 mg/ml, 0.00625 mg/ml, or 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is of 1 mg/ml, 0.9 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.6 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.09 mg/ml, 0.08 mg/ml, 0.075 mg/ml, 0.07 mg/ml, 0.06 mg/ml, 0.05 mg/ml, 0.04 mg/ml, 0.03 mg/ml, 0.025 mg/ml, 0.02 mg/ml, 0.01 mg/ml, 0.009 mg/ml, 0.008 mg/ml, 0.007 mg/ml, 0.00625 mg/ml, or 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.4 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.4 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.3 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.3 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.075 mg/ml upon reconstitution in a liquid

formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.075 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.05 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.05 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.025 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.025 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.00625 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.00625 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 500 µg per dose, 450 µg per dose, 400 µg per dose, 350 µg per dose, 300 µg per dose, 250 µg per dose, 200 µg per dose, 150 µg per dose, 100 µg per dose, 50 µg per dose, 45 µg per dose, 40 µg per dose, 37.5 µg per dose, 35 µg per dose, 30 µg per dose, 25 µg per dose, 20 µg per dose, 15 µg per dose, 12.5 µg per dose, 10 µg per dose, 5 µg per dose, 4.5 µg per dose, 4 µg per dose, 3.5 µg per dose, 3.125 µg per dose, or 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 500 µg per dose,

450 µg per dose, 400 µg per dose, 350 µg per dose, 300 µg per dose, 250 µg per dose, 200 µg per dose, 150 µg per dose, 100 µg per dose, 50 µg per dose, 45 µg per dose, 40 µg per dose, 37.5 µg per dose, 35 µg per dose, 30 µg per dose, 25 µg per dose, 20 µg per dose, 15 µg per dose, 12.5 µg per dose, 10 µg per dose, 5 µg per dose, 4.5 µg per dose, 4 µg per dose, 3.5 µg per dose, 3.125 µg per dose, or 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 200 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 200 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 175 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 175 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 150 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 150 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 100 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 100 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 75 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 75 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 25 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 25 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of

the vaccine is 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 2.5 µg per dose.

[00111] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about the same. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is the same. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.001 mg/ml to 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.002 mg/ml to 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.002 mg/ml to 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.01 mg/ml to 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.01 mg/ml to 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.02 mg/ml to 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine

is from 0.02 mg/ml to 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.04 mg/ml to 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.04 mg/ml to 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.08 mg/ml to 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.08 mg/ml to 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.002 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.02 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 1.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the

concentration of each component of the vaccine is at most 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.2 mg/ml, 0.18 mg/ml, 0.16 mg/ml, 0.14 mg/ml, 0.12 mg/ml, 0.1 mg/ml, 0.08 mg/ml, 0.06 mg/ml, 0.04 mg/ml, 0.02 mg/ml, 0.018 mg/ml, 0.016 mg/ml, 0.025 mg/ml, 0.014 mg/ml, 0.012 mg/ml, 0.01 mg/ml, 0.008 mg/ml, 0.006 mg/ml, 0.005 mg/ml, 0.004 mg/ml, 0.002 mg/ml, 0.0018 mg/ml, 0.0016 mg/ml, 0.0014 mg/ml, 0.00125 mg/ml, or 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.2 mg/ml, 0.18 mg/ml, 0.16 mg/ml, 0.14 mg/ml, 0.12 mg/ml, 0.1 mg/ml, 0.08 mg/ml, 0.06 mg/ml, 0.04 mg/ml, 0.02 mg/ml, 0.018 mg/ml, 0.016 mg/ml, 0.025 mg/ml, 0.014 mg/ml, 0.012 mg/ml, 0.01 mg/ml, 0.008 mg/ml, 0.006 mg/ml, 0.005 mg/ml, 0.004 mg/ml, 0.002 mg/ml, 0.0018 mg/ml, 0.0016 mg/ml, 0.0014 mg/ml, 0.00125 mg/ml, or 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.08 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein the concentration of each component of the vaccine is 0.08 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.06 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein the concentration of each component of the vaccine is 0.06 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.04 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein the concentration of each component of the vaccine is 0.04 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.02 mg/ml upon reconstitution in a liquid formulation. In some

embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.02 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.015 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.015 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.002 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.002 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.00125 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.00125 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 100 µg per dose, 90 µg per dose, 80 µg per dose, 70 µg per dose, 60 µg per dose, 50 µg per dose, 40 µg per dose, 30 µg per dose, 20 µg per dose,

10 µg per dose, 9 µg per dose, 8 µg per dose, 7.5 µg per dose, 5 µg per dose, 6 µg per dose, 5 µg per dose, 4 µg per dose, 3 µg per dose, 2.5 µg per dose, 2 µg per dose, 1 µg per dose, 0.9 µg per dose, 0.8 µg per dose, 0.7 µg per dose, 0.625 µg per dose, or 0.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 100 µg per dose, 90 µg per dose, 80 µg per dose, 70 µg per dose, 60 µg per dose, 50 µg per dose, 40 µg per dose, 30 µg per dose, 20 µg per dose, 10 µg per dose, 9 µg per dose, 8 µg per dose, 7.5 µg per dose, 5 µg per dose, 6 µg per dose, 5 µg per dose, 4 µg per dose, 3 µg per dose, 2.5 µg per dose, 2 µg per dose, 1 µg per dose, 0.9 µg per dose, 0.8 µg per dose, 0.7 µg per dose, 0.625 µg per dose, or 0.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 40 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 35 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 35 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 30 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 30 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 20 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 20 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 15 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 15 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per

dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 μg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2 μg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2 μg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 1 μg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 μg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 μg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.5 μg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.5 μg per dose.

[00112] In some embodiments, the lyophilized pharmaceutical composition provided herein does not comprise histidine-borate. In some embodiments, the lyophilized pharmaceutical composition provided herein comprises histidine-borate. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 50mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is from 0.1mM to 50mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 40mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is from 0.1mM to 40mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 30mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is from 0.1mM to 30mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about from 5mM to 25 mM upon reconstitution in

a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is from 5mM to 25 mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about from 10mM to 25mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is from 10mM to 25mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about from 15mM to 25mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is from 15mM to 25mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is at least 0.1mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is at least 0.5mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is at least 1mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is at least 5mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is at most 50mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is at most 40mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is at most 35mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about 15mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM, or 35 mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is of 15mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27

mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM, or 35 mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is about 25mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of histidine-borate is 25mM upon reconstitution in a liquid formulation.

[00113] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about from 0.1mM to 30mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is from 0.1mM to 30mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about from 0.2mM to 20mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is from 0.2mM to 20mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about from 0.5mM to 20mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is from 0.5mM to 20mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about from 0.5mM to 10mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is from 0.5mM to 10mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about from 1mM to 5mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is from 1mM to 5mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about from 1mM to 3mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is from 1mM to 3mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized

pharmaceutical composition provided herein, the concentration of the buffer is at least 0.1mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at least 0.2mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at least 0.5mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at least 0.75mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at least 1mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at most 30mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at most 20mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at most 10mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at most 5mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is at most 3mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5 mM, 1.6 mM, 1.7 mM, 1.8 mM, 1.9 mM, 2.0 mM, 2.1 mM, 2.2 mM, 2.3 mM, 2.4 mM, 2.5 mM, 2.6 mM, 2.7 mM, 2.8 mM, 2.9 mM, or 3mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is of 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5 mM, 1.6 mM, 1.7 mM, 1.8 mM, 1.9 mM, 2.0 mM, 2.1 mM, 2.2 mM, 2.3 mM, 2.4 mM, 2.5 mM, 2.6 mM, 2.7 mM, 2.8 mM, 2.9 mM, or 3mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about 2mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the

concentration of the buffer is 2mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is about 1.2mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the buffer is 1.2mM upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the buffer is phosphate buffer. In some embodiments of the lyophilized pharmaceutical composition provided herein, the buffer is tris buffer.

[00114] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is about from 1% (weight/volume, 10 g/L) to 30% (weight/volume, 300 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is from 1% (weight/volume, 10 g/L) to 30% (weight/volume, 300 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is about from 2% (weight/volume, 20 g/L) to 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is from 2% (weight/volume, 20 g/L) to 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is about from 2% (weight/volume, 20 g/L) to 15% (weight/volume, 150 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is from 2% (weight/volume, 20 g/L) to 15% (weight/volume, 150 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is about from 5% (weight/volume, 50 g/L) to 10% (weight/volume, 100 g/L) upon reconstitution in

a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is from 5% (weight/volume, 50 g/L) to 10% (weight/volume, 100 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at least 1% (weight/volume, 10 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at least 2% (weight/volume, 20 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at least 3% (weight/volume, 30 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at least 4% (weight/volume, 40 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at least 5% (weight/volume, 50 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at most 30% (weight/volume, 300 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at most 25% (weight/volume, 250 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at most 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is at most 15% (weight/volume, 150 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is about 1% (weight/volume, 10 g/L), 2% (weight/volume, 20 g/L), 3% (weight/volume, 30 g/L), 4% (weight/volume, 40 g/L), 5% (weight/volume, 50 g/L), 6% (weight/volume, 60 g/L), 7% (weight/volume, 70 g/L), 8% (weight/volume, 80 g/L), 9% (weight/volume, 90 g/L), 10% (weight/volume, 100 g/L), 11% (weight/volume, 110 g/L), 12% (weight/volume, 120 g/L), 13% (weight/volume, 130 g/L), 14% (weight/volume, 140 g/L), 15% (weight/volume, 150 g/L), 16% (weight/volume, 160 g/L), 17% (weight/volume, 170 g/L), 18% (weight/volume, 180 g/L), 19% (weight/volume, 190 g/L), or 20% (weight/volume, 200 g/L) upon reconstitution in a liquid

formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is of 1% (weight/volume, 10 g/L), 2% (weight/volume, 20 g/L), 3% (weight/volume, 30 g/L), 4% (weight/volume, 40 g/L), 5% (weight/volume, 50 g/L), 6% (weight/volume, 60 g/L), 7% (weight/volume, 70 g/L), 8% (weight/volume, 80 g/L), 9% (weight/volume, 90 g/L), 10% (weight/volume, 100 g/L), 11% (weight/volume, 110 g/L), 12% (weight/volume, 120 g/L), 13% (weight/volume, 130 g/L), 14% (weight/volume, 140 g/L), 15% (weight/volume, 150 g/L), 16% (weight/volume, 160 g/L), 17% (weight/volume, 170 g/L), 18% (weight/volume, 180 g/L), 19% (weight/volume, 190 g/L), or 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is about 8% (weight/volume, 80 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the stabilizer is 8% (weight/volume, 80 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the stabilizer is sucrose.

[00115] In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is about from 0.01% (weight/volume, 0.1 g/L) to 0.5% (weight/volume, 5 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is from 0.01% (weight/volume, 0.1 g/L) to 0.5% (weight/volume, 5 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is about from 0.01% (weight/volume, 0.1 g/L) to 0.1% (weight/volume, 1 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is from 0.01% (weight/volume, 0.1 g/L) to 0.1% (weight/volume, 1 g/L) upon reconstitution in a liquid formulation. . In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is

about from 0.01% (weight/volume, 0.1 g/L) to 0.05% (weight/volume, 0.5 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is from 0.01% (weight/volume, 0.1 g/L) to 0.05% (weight/volume, 0.5 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is at least 0.004% (weight/volume, 0.04 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is at least 0.006% (weight/volume, 0.06 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is at least 0.008% (weight/volume, 0.08 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is at least 0.01% (weight/volume, 0.1 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is at most 2% (weight/volume, 20 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is at most 1.5% (weight/volume, 15 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is at most 1% (weight/volume, 10 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is about 0.005% (weight/volume, 0.05 g/L), 0.01% (weight/volume, 0.1 g/L), 0.015% (weight/volume, 0.15 g/L), 0.02% (weight/volume, 0.2 g/L), 0.03% (weight/volume, 0.3 g/L), 0.04% (weight/volume, 0.4 g/L), 0.05% (weight/volume, 0.5 g/L), 0.06% (weight/volume, 0.6 g/L), 0.07% (weight/volume, 0.7 g/L), 0.08% (weight/volume, 0.8 g/L), 0.09% (weight/volume, 0.9 g/L), or 0.1% (weight/volume, 1.0 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is of 0.005% (weight/volume, 0.05 g/L), 0.01% (weight/volume, 0.1 g/L), 0.015% (weight/volume, 0.15 g/L), 0.02% (weight/volume, 0.2 g/L), 0.03% (weight/volume, 0.3 g/L), 0.04% (weight/volume, 0.4 g/L), 0.05% (weight/volume, 0.5 g/L), 0.06% (weight/volume, 0.6 g/L), 0.07% (weight/volume, 0.7 g/L), 0.08% (weight/volume, 0.8 g/L), 0.09% (weight/volume,

0.9 g/L), or 0.1% (weight/volume, 1.0 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is about 0.02% (weight/volume, 0.2 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the concentration of the surfactant is 0.02% (weight/volume, 0.2 g/L) upon reconstitution in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the surfactant is polysorbate 20 or polysorbate 80. In some embodiments of the lyophilized pharmaceutical composition provided herein, the surfactant is polysorbate 80.

[00116] In certain embodiments, the pH of the lyophilized pharmaceutical composition is about from 6.0 to 9.0 upon reconstitution in a liquid formulation. In certain embodiments, the pH of the lyophilized pharmaceutical composition is about 6, 6.5, 7, 7.5, 8, 8.5 or 9 upon reconstitution in a liquid formulation. In some embodiments, the pH of the lyophilized pharmaceutical composition is about 7.5 upon reconstitution in a liquid formulation. In certain embodiments, the pH of the lyophilized pharmaceutical composition is from 6.0 to 9.0 upon reconstitution in a liquid formulation. In certain embodiments, the pH of the lyophilized pharmaceutical composition is 6, 6.5, 7, 7.5, 8, 8.5 or 9 upon reconstitution in a liquid formulation. In certain embodiments, the pH of the lyophilized pharmaceutical composition is 7.5 upon reconstitution in a liquid formulation.

[00117] In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 2: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 1.5: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 1: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.75: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.5: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical

composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.25: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.125: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.03125: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.025: 400: 1 by weight.

[00118] In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 2: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 1.5: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 1: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.75: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.5: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.25: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.125: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.03125: 400: 1 by weight. In some embodiments of the lyophilized pharmaceutical composition provided herein, the lyophilized

pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.025: 400: 1 by weight.

[00119] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00120] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments

of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00121] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00122] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅

consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00123] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00124] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting

of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00125] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises:

HlaH_{35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00126] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L),

and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00127] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00128] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00129] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized

pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00130] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00131] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total

protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00132] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00133] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5,

TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00134] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises:

Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00135] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the

amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00136] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00137] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5)

polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00138] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00139] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00140] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical

composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00141] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises:

Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00142] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises:

Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some

embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00143] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00144] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of

the amino acid of sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00145] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00146] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the

amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00147] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00148] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the

concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00149] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: Hla_{H35LH48L} comprising the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} comprising the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} comprising the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of

the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00150] In some embodiments, the lyophilized pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises:

Hla_{H35LH48L} consisting of the amino acid sequence of SEQ ID NO: 1, LukS_{mut9} consisting of the amino acid sequence of SEQ ID NO: 2, LukF_{mut1} consisting of the amino acid sequence of SEQ ID NO: 3, LukAB_{mut50} comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA₂₂₅ consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the lyophilized pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00151] In some embodiments, the lyophilized pharmaceutical composition comprises a residual moisture content between about 1% and about 7%. In some embodiments, the lyophilized pharmaceutical composition comprises a residual moisture content between about 2% and about 6%. In some embodiments, the lyophilized pharmaceutical composition comprises a residual moisture content between about 3% and about 5%. In some embodiments, the lyophilized pharmaceutical composition comprises a residual moisture content about 3%. In some embodiments, the lyophilized pharmaceutical composition comprises a residual moisture content about 4%. In some embodiments, the lyophilized pharmaceutical composition comprises a residual moisture content about 5%. In some embodiments, the lyophilized pharmaceutical composition comprises a residual moisture content between 1% and 7%. In some embodiments, the lyophilized pharmaceutical composition comprises a residual moisture content between 2% and 6%. In some embodiments, the lyophilized pharmaceutical composition comprises a

residual moisture content between 3% and 5%. In some embodiments, the lyophilized pharmaceutical composition comprises a 3% residual moisture content. In some embodiments, the lyophilized pharmaceutical composition comprises a 4% residual moisture content. In some embodiments, the lyophilized pharmaceutical composition comprises a 5% residual moisture content.

[00152] As used herein and unless otherwise specified, the term “about” means within plus or minus 10% of a given value or range.

[00153] In certain embodiments, the lyophilized pharmaceutical composition is contained within a vial. The vial can be as small as 0.2 mL or less, or as large as 100 mL. Exemplar vials may be made of clear glass or amber glass, type I borosilicate glass, type II soda-silicate glass, or type III soda-silicate glass. The vial may be closed with a stopper, cap, flip cap, or a screw cap. In certain embodiments, the lyophilized pharmaceutical composition is contained within a syringe.

5.1.2 SPRAY FREEZE-DRIED FORMULATION OF PHARMACEUTICAL COMPOSITION

[00154] The disclosure provides a spray freeze-dried pharmaceutical composition comprising a multicomponent vaccine (e.g. such as described in Section 5.4 below), a buffer, a stabilizer, and a surfactant. In some embodiments, the spray freeze-dried pharmaceutical composition further comprises histidine-borate. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a multicomponent vaccine, histidine-borate, a buffer, a stabilizer, and a surfactant.

[00155] In some embodiment, the buffer is phosphate buffer. In some embodiment, the buffer is tris buffer.

[00156] In some embodiments, the stabilizer comprises sucrose.

[00157] In some embodiments, the surfactant comprises polysorbate 80. In some embodiments, the surfactant comprises polysorbate 20.

[00158] In one aspect, the disclosure provides a spray freeze-dried pharmaceutical composition comprising a multicomponent vaccine, phosphate buffer, sucrose, and polysorbate 80.

[00159] In one aspect, the disclosure provides a spray freeze-dried pharmaceutical composition comprising a multicomponent vaccine, histidine-borate, phosphate buffer, sucrose, and polysorbate 80.

[00160] In one aspect, the disclosure provides a spray freeze-dried pharmaceutical composition comprising a multicomponent vaccine, tris buffer, sucrose, and polysorbate 80.

[00161] In one aspect, the disclosure provides a spray freeze-dried pharmaceutical composition comprising a multicomponent vaccine, histidine-borate, tris buffer, sucrose, and polysorbate 80.

[00162] In some embodiments, the multicomponent vaccine comprises: HlaH35LH48L, LukSmut9, LukFmut1, TBA225 and LukABmut50.

[00163] In some embodiments, HlaH35LH48L comprises a Hla toxoid with H35L and H48L mutations. In some embodiments, HlaH35LH48L comprises the amino acid sequence of SEQ ID NO: 1. In some embodiments, HlaH35LH48L consists of the amino acid sequence of SEQ ID NO: 1.

[00164] In some embodiments, LukSmut9 comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations. In some embodiments, LukSmut9 comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, LukSmut9 consists of the amino acid sequence of SEQ ID NO: 2.

[00165] In some embodiments, LukFmut1 comprises the F subunit of a PVL toxoid with K102A mutation. In some embodiments, LukFmut1 comprises the amino acid sequence of SEQ ID NO: 3. In some embodiments, LukFmut1 consists of the amino acid sequence of SEQ ID NO: 3.

[00166] In some embodiments, LukABmut50 comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB. In some embodiments, LukABmut50 comprises a variant of LukA comprising D39A mutation in LukA and a variant of LukB comprising R23E mutation in LukB. In some embodiments, LukABmut50 comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5. In some embodiments, LukABmut50 comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5.

[00167] In some embodiments, TBA225 comprises a fusion protein comprising: (1) a TSST-1 toxoid with L30R, D27A, and I46A mutations, (2) a SEB toxoid with L45R, Y89A, and Y94A mutations, and (3) a SEA toxoid with L48R, D70R, Y92A, and H225A mutations. In some embodiments, the three toxoids are fused together via a linker. In some embodiments, the linker comprise the amino acid sequence of GGGSGGGSGGGS (SEQ ID NO: 7). In some embodiments, TBA225 comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, TBA225 consists of the amino acid sequence of SEQ ID NO: 6.

[00168] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.005 mg/ml to 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.01 mg/ml to 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.01 mg/ml to 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.05 mg/ml to 5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.05 mg/ml to 5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.1 mg/ml to 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.1 mg/ml to 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.2 mg/ml to 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.2 mg/ml to 0.5 mg/ml upon

reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about from 0.4 mg/ml to 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is from 0.4 mg/ml to 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.025 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.05 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at least 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 10 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 7.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 2.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is at most 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 1 mg/ml, 0.9 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.6 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.09 mg/ml, 0.08

mg/ml, 0.075 mg/ml, 0.07 mg/ml, 0.06 mg/ml, 0.05 mg/ml, 0.04 mg/ml, 0.03 mg/ml, 0.025 mg/ml, 0.02 mg/ml, 0.01 mg/ml, 0.009 mg/ml, 0.008 mg/ml, 0.007 mg/ml, 0.00625 mg/ml, or 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is of 1 mg/ml, 0.9 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.6 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.3 mg/ml, 0.2 mg/ml, 0.1 mg/ml, 0.09 mg/ml, 0.08 mg/ml, 0.075 mg/ml, 0.07 mg/ml, 0.06 mg/ml, 0.05 mg/ml, 0.04 mg/ml, 0.03 mg/ml, 0.025 mg/ml, 0.02 mg/ml, 0.01 mg/ml, 0.009 mg/ml, 0.008 mg/ml, 0.007 mg/ml, 0.00625 mg/ml, or 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.4 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.4 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.3 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.3 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.075 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.075 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray

freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.05 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.05 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.025 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.025 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.00625 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.00625 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is about 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the total protein of the vaccine is 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 500 µg per dose, 450 µg per dose, 400 µg per dose, 350 µg per dose, 300 µg per dose, 250 µg per dose, 200 µg per dose, 150 µg per dose, 100 µg per dose, 50 µg per dose, 45 µg per dose, 40 µg per dose, 37.5 µg per dose, 35 µg per dose, 30 µg per dose, 25 µg per dose, 20 µg per dose, 15 µg per dose, 12.5 µg per dose, 10 µg per dose, 5 µg per dose, 4.5 µg per dose, 4 µg per dose, 3.5 µg per dose, 3.125 µg per dose, or 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 500 µg per dose, 450 µg per dose, 400 µg per dose, 350 µg per dose, 300 µg per dose, 250 µg per dose, 200 µg per dose, 150 µg per dose, 100

µg per dose, 50 µg per dose, 45 µg per dose, 40 µg per dose, 37.5 µg per dose, 35 µg per dose, 30 µg per dose, 25 µg per dose, 20 µg per dose, 15 µg per dose, 12.5 µg per dose, 10 µg per dose, 5 µg per dose, 4.5 µg per dose, 4 µg per dose, 3.5 µg per dose, 3.125 µg per dose, or 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 200 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 200 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 175 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 175 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 150 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 150 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 100 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 100 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 75 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 75 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 25 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 25 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the

vaccine is 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 2.5 µg per dose.

[00169] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about the same. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is the same. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.001 mg/ml to 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.002 mg/ml to 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.002 mg/ml to 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.01 mg/ml to 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.01 mg/ml to 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.02 mg/ml to 0.2 mg/ml upon reconstitution in a liquid formulation. In some

embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.02 mg/ml to 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.04 mg/ml to 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.04 mg/ml to 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about from 0.08 mg/ml to 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is from 0.08 mg/ml to 0.1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.002 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at least 0.02 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 1.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 1 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried

pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 0.5 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is at most 0.2 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.2 mg/ml, 0.18 mg/ml, 0.16 mg/ml, 0.14 mg/ml, 0.12 mg/ml, 0.1 mg/ml, 0.08 mg/ml, 0.06 mg/ml, 0.04 mg/ml, 0.02 mg/ml, 0.018 mg/ml, 0.016 mg/ml, 0.025 mg/ml, 0.014 mg/ml, 0.012 mg/ml, 0.01 mg/ml, 0.008 mg/ml, 0.006 mg/ml, 0.005 mg/ml, 0.004 mg/ml, 0.002 mg/ml, 0.0018 mg/ml, 0.0016 mg/ml, 0.0014 mg/ml, 0.00125 mg/ml, or 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.2 mg/ml, 0.18 mg/ml, 0.16 mg/ml, 0.14 mg/ml, 0.12 mg/ml, 0.1 mg/ml, 0.08 mg/ml, 0.06 mg/ml, 0.04 mg/ml, 0.02 mg/ml, 0.018 mg/ml, 0.016 mg/ml, 0.025 mg/ml, 0.014 mg/ml, 0.012 mg/ml, 0.01 mg/ml, 0.008 mg/ml, 0.006 mg/ml, 0.005 mg/ml, 0.004 mg/ml, 0.002 mg/ml, 0.0018 mg/ml, 0.0016 mg/ml, 0.0014 mg/ml, 0.00125 mg/ml, or 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.08 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein the concentration of each component of the vaccine is 0.08 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.06 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein the concentration of each component of the vaccine is 0.06 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.04 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein the concentration of each component of the vaccine is 0.04 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments

of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.02 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.02 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.015 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.015 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.01 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.005 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.002 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.002 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.00125 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.00125 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of each component of the vaccine is about 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried

pharmaceutical composition provided herein, the concentration of each component of the vaccine is 0.001 mg/ml upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 100 µg per dose, 90 µg per dose, 80 µg per dose, 70 µg per dose, 60 µg per dose, 50 µg per dose, 40 µg per dose, 30 µg per dose, 20 µg per dose, 10 µg per dose, 9 µg per dose, 8 µg per dose, 7.5 µg per dose, 5 µg per dose, 6 µg per dose, 5 µg per dose, 4 µg per dose, 3 µg per dose, 2.5 µg per dose, 2 µg per dose, 1 µg per dose, 0.9 µg per dose, 0.8 µg per dose, 0.7 µg per dose, 0.625 µg per dose, or 0.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 100 µg per dose, 90 µg per dose, 80 µg per dose, 70 µg per dose, 60 µg per dose, 50 µg per dose, 40 µg per dose, 30 µg per dose, 20 µg per dose, 10 µg per dose, 9 µg per dose, 8 µg per dose, 7.5 µg per dose, 5 µg per dose, 6 µg per dose, 5 µg per dose, 4 µg per dose, 3 µg per dose, 2.5 µg per dose, 2 µg per dose, 1 µg per dose, 0.9 µg per dose, 0.8 µg per dose, 0.7 µg per dose, 0.625 µg per dose, or 0.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 40 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 40 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 35 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 35 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 30 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 30 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 20 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 20 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 15 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 15 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the

spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 1 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 1 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.5 µg per dose.

[00170] In some embodiments, the spray freeze-dried pharmaceutical composition provided herein does not comprise histidine-borate. In some embodiments, the spray freeze-dried pharmaceutical composition provided herein comprises histidine-borate. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 50mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is from 0.1mM to 50mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 40mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried

pharmaceutical composition provided herein, the concentration of histidine-borate is from 0.1mM to 40mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about from 0.1mM to 30mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is from 0.1mM to 30mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about from 5mM to 25 mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is from 5mM to 25 mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about from 10mM to 25mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is from 10mM to 25mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about from 15mM to 25mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is from 15mM to 25mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is at least 0.1mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is at least 0.5mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is at least 1mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is at least 5mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is at most 50mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is at most 40mM upon reconstitution in a

liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is at most 35mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about 15mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM, or 35 mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is of 15mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM, or 35 mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is about 25mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of histidine-borate is 25mM upon reconstitution in a liquid formulation.

[00171] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about from 0.1mM to 30mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is from 0.1mM to 30mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about from 0.2mM to 20mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is from 0.2mM to 20mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about from 0.5mM to 20mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is from 0.5mM to 20mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about from 0.5mM to 10mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is from 0.5mM to 10mM upon reconstitution in a liquid formulation. In some embodiments of the

spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about from 1mM to 5mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is from 1mM to 5mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about from 1mM to 3mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is from 1mM to 3mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at least 0.1mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at least 0.2mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at least 0.5mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at least 0.75mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at least 1mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at most 30mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at most 20mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at most 10mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at most 5mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is at most 3mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5 mM, 1.6 mM, 1.7 mM, 1.8 mM, 1.9 mM, 2.0 mM, 2.1 mM, 2.2

mM, 2.3 mM, 2.4 mM, 2.5 mM, 2.6 mM, 2.7 mM, 2.8 mM, 2.9 mM, or 3mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is of 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5 mM, 1.6 mM, 1.7 mM, 1.8 mM, 1.9 mM, 2.0 mM, 2.1 mM, 2.2 mM, 2.3 mM, 2.4 mM, 2.5 mM, 2.6 mM, 2.7 mM, 2.8 mM, 2.9 mM, or 3mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about 2mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is 2mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is about 1.2mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the buffer is 1.2mM upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the buffer is phosphate buffer. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the buffer is tris buffer.

[00172] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is about from 1% (weight/volume, 10 g/L) to 30% (weight/volume, 300 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is from 1% (weight/volume, 10 g/L) to 30% (weight/volume, 300 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is about from 2% (weight/volume, 20 g/L) to 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is from 2% (weight/volume, 20 g/L) to 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is about from 2% (weight/volume, 20 g/L) to 15% (weight/volume, 150 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried

pharmaceutical composition provided herein, the concentration of the stabilizer is from 2% (weight/volume, 20 g/L) to 15% (weight/volume, 150 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is about from 5% (weight/volume, 50 g/L) to 10% (weight/volume, 100 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is from 5% (weight/volume, 50 g/L) to 10% (weight/volume, 100 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at least 1% (weight/volume, 10 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at least 2% (weight/volume, 20 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at least 3% (weight/volume, 30 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at least 4% (weight/volume, 40 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at least 5% (weight/volume, 50 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at most 30% (weight/volume, 300 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at most 25% (weight/volume, 250 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at most 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of

the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is at most 15% (weight/volume, 150 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is about 1% (weight/volume, 10 g/L), 2% (weight/volume, 20 g/L), 3% (weight/volume, 30 g/L), 4% (weight/volume, 40 g/L), 5% (weight/volume, 50 g/L), 6% (weight/volume, 60 g/L), 7% (weight/volume, 70 g/L), 8% (weight/volume, 80 g/L), 9% (weight/volume, 90 g/L), 10% (weight/volume, 100 g/L), 11% (weight/volume, 110 g/L), 12% (weight/volume, 120 g/L), 13% (weight/volume, 130 g/L), 14% (weight/volume, 140 g/L), 15% (weight/volume, 150 g/L), 16% (weight/volume, 160 g/L), 17% (weight/volume, 170 g/L), 18% (weight/volume, 180 g/L), 19% (weight/volume, 190 g/L), or 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is of 1% (weight/volume, 10 g/L), 2% (weight/volume, 20 g/L), 3% (weight/volume, 30 g/L), 4% (weight/volume, 40 g/L), 5% (weight/volume, 50 g/L), 6% (weight/volume, 60 g/L), 7% (weight/volume, 70 g/L), 8% (weight/volume, 80 g/L), 9% (weight/volume, 90 g/L), 10% (weight/volume, 100 g/L), 11% (weight/volume, 110 g/L), 12% (weight/volume, 120 g/L), 13% (weight/volume, 130 g/L), 14% (weight/volume, 140 g/L), 15% (weight/volume, 150 g/L), 16% (weight/volume, 160 g/L), 17% (weight/volume, 170 g/L), 18% (weight/volume, 180 g/L), 19% (weight/volume, 190 g/L), or 20% (weight/volume, 200 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is about 8% (weight/volume, 80 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the stabilizer is 8% (weight/volume, 80 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the stabilizer is sucrose.

[00173] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried

pharmaceutical composition provided herein, the concentration of the surfactant is about from 0.01% (weight/volume, 0.1 g/L) to 0.5% (weight/volume, 5 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is from 0.01% (weight/volume, 0.1 g/L) to 0.5% (weight/volume, 5 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is about from 0.01% (weight/volume, 0.1 g/L) to 0.1% (weight/volume, 1 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is from 0.01% (weight/volume, 0.1 g/L) to 0.1% (weight/volume, 1 g/L) upon reconstitution in a liquid formulation. . In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is about from 0.01% (weight/volume, 0.1 g/L) to 0.05% (weight/volume, 0.5 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is from 0.01% (weight/volume, 0.1 g/L) to 0.05% (weight/volume, 0.5 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is at least 0.004% (weight/volume, 0.04 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is at least 0.006% (weight/volume, 0.06 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is at least 0.008% (weight/volume, 0.08 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is at least 0.01% (weight/volume, 0.1 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is at most 2% (weight/volume, 20 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is at most 1.5% (weight/volume, 15 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the

surfactant is at most 1% (weight/volume, 10 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is about 0.005% (weight/volume, 0.05 g/L), 0.01% (weight/volume, 0.1 g/L), 0.015% (weight/volume, 0.15 g/L), 0.02% (weight/volume, 0.2 g/L), 0.03% (weight/volume, 0.3 g/L), 0.04% (weight/volume, 0.4 g/L), 0.05% (weight/volume, 0.5 g/L), 0.06% (weight/volume, 0.6 g/L), 0.07% (weight/volume, 0.7 g/L), 0.08% (weight/volume, 0.8 g/L), 0.09% (weight/volume, 0.9 g/L), or 0.1% (weight/volume, 1.0 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is of 0.005% (weight/volume, 0.05 g/L), 0.01% (weight/volume, 0.1 g/L), 0.015% (weight/volume, 0.15 g/L), 0.02% (weight/volume, 0.2 g/L), 0.03% (weight/volume, 0.3 g/L), 0.04% (weight/volume, 0.4 g/L), 0.05% (weight/volume, 0.5 g/L), 0.06% (weight/volume, 0.6 g/L), 0.07% (weight/volume, 0.7 g/L), 0.08% (weight/volume, 0.8 g/L), 0.09% (weight/volume, 0.9 g/L), or 0.1% (weight/volume, 1.0 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is about 0.02% (weight/volume, 0.2 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the concentration of the surfactant is 0.02% (weight/volume, 0.2 g/L) upon reconstitution in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the surfactant is polysorbate 20 or polysorbate 80. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the surfactant is polysorbate 80.

[00174] In certain embodiments, the pH of the spray freeze-dried pharmaceutical composition is about from 6.0 to 9.0 upon reconstitution in a liquid formulation. In certain embodiments, the pH of the spray freeze-dried pharmaceutical composition is about 6, 6.5, 7, 7.5, 8, 8.5 or 9 upon reconstitution in a liquid formulation. In some embodiments, the pH of the spray freeze-dried pharmaceutical composition is about 7.5 upon reconstitution in a liquid formulation. In certain embodiments, the pH of the spray freeze-dried pharmaceutical composition is from 6.0 to 9.0 upon reconstitution in a liquid formulation. In certain embodiments, the pH of the spray freeze-dried pharmaceutical composition is 6, 6.5, 7, 7.5, 8, 8.5 or 9 upon reconstitution in a liquid formulation. In certain embodiments, the pH of the spray freeze-dried pharmaceutical composition is 7.5 upon reconstitution in a liquid formulation.

[00175] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 2: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 1.5: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 1: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.75: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.5: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.25: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.125: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.03125: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 0.025: 400: 1 by weight.

[00176] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 2: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 1.5: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose,

and polysorbate 80 at a ratio of 1: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.75: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.5: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.25: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.125: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.03125: 400: 1 by weight. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at a ratio of 0.025: 400: 1 by weight.

[00177] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: H1aH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per

dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00178] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00179] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total

protein of the vaccine is about 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00180] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises:

HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00181] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first

polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00182] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00183] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after

reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00184] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.4mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 0.2 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.04 mg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 0.2 mg per dose. In some embodiments of the spray

freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.04 mg per dose.

[00185] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00186] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some

embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00187] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00188] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid

sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00189] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00190] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the

multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00191] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00192] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.1mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 10 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 50 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 10 µg per dose.

[00193] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each

component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00194] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00195] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the

total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00196] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00197] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ

ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00198] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00199] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate

buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SES ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00200] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SES ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.025mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 2.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided

herein, the total protein of the vaccine is 12.5 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 2.5 µg per dose.

[00201] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00202] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) tris buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the spray freeze-dried pharmaceutical

composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00203] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00204] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) tris buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of

SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00205] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00206] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) phosphate buffer at the concentration of 1.2mM, (3) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (4) polysorbate 80 at the concentration of

0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00207] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises: HlaH35LH48L comprising the amino acid sequence of SEQ ID NO: 1, LukSmut9 comprising the amino acid sequence of SEQ ID NO: 2, LukFmut1 comprising the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5, TBA225 comprising the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray

freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00208] In some embodiments, the spray freeze-dried pharmaceutical composition consists of: (1) a multicomponent vaccine, (2) histidine-borate at the concentration of 25mM, (3) phosphate buffer at the concentration of 1.2mM, (4) sucrose at the concentration of 8% (weight/volume, 80 g/L), and (5) polysorbate 80 at the concentration of 0.02% (weight/volume, 0.2 g/L) after reconstitution in a liquid formulation, wherein the multicomponent vaccine comprises:

HlaH35LH48L consisting of the amino acid sequence of SEQ ID NO: 1, LukSmut9 consisting of the amino acid sequence of SEQ ID NO: 2, LukFmut1 consisting of the amino acid sequence of SEQ ID NO: 3, LukABmut50 comprising a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5, TBA225 consisting of the amino acid sequence of SEQ ID NO: 6, and wherein the total protein of the multicomponent vaccine is at the concentration of 0.00625mg/ml. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is about 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is about 0.625 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the total protein of the vaccine is 3.125 µg per dose. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, each component of the vaccine is 0.625 µg per dose.

[00209] In some embodiments, the spray freeze-dried pharmaceutical composition comprises a residual moisture content between about 1% and about 7%. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a residual moisture content between about 2% and about 6%. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a residual moisture content between about 3% and about 5%. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a residual moisture content about 3%. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a residual moisture content about 4%. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a residual moisture content about 5%. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a residual moisture content between 1% and 7%. In some embodiments, the spray freeze-dried pharmaceutical

composition comprises a residual moisture content between 2% and 6%. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a residual moisture content between 3% and 5%. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a 3% residual moisture content. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a 4% residual moisture content. In some embodiments, the spray freeze-dried pharmaceutical composition comprises a 5% residual moisture content.

[00210] As used herein and unless otherwise specified, the term “about” means within plus or minus 10% of a given value or range.

[00211] In certain embodiments, the spray freeze-dried pharmaceutical composition is contained within a vial. The vial can be as small as 0.2 mL or less, or as large as 100 mL. Exemplar vials may be made of clear glass or amber glass, type I borosilicate glass, type II soda-silicate glass, or type III soda-silicate glass. The vial may be closed with a stopper, cap, flip cap, or a screw cap. In certain embodiments, the spray freeze-dried pharmaceutical composition is contained within a syringe.

5.2 FUNCTIONAL PROPERTIES

5.2.1 FUNCTIONAL PROPERTIES OF LYOPHILIZED FORMULATION OF PHARMACEUTICAL COMPOSITION

[00212] The lyophilized compositions provided herein and described in Section 5.1.1 provide several advantages, one of which is maintaining the stability of the protein for a defined amount of time at a defined temperature. The defined amount of time after which stability is measured can be at least 14 days, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 13 months, at least 14 months, at least 15 months, at least 16 months, at least 17 months, at least 18 months, at least 19 months, at least 20 months, at least 21 months, at least 22 months, at least 23 months, at least 24 months, at least 36 months or more. The temperature at which the samples may be kept when assessing stability can be any temperature from about -80°C to about 50°C, e.g., storage at about -80°C, about -30°C, about -20°C, about 0°C, about 4°C-8°C, about 5°C, about 20°C-30°C, about 25°C, about 30°C, about 35°C, about 37°C or other physiological temperatures, about 45°C or about 50°C.

[00213] The term “stability” refers to the retention of an acceptable degree of physical structure or biological function (e.g. immunogenic activity) of the protein after storage in a relevant environment or under certain conditions. The protein may be stable even though it does not maintain 100% of its physical structure, or biological function after storage for a certain amount of time.

[00214] Stability can be measured, inter alia, by determining the percentage of native molecule that remains in a formulation after storage for a defined amount of time at a defined temperature or after delivery to a patient. The percentage of protein that retains its native form (e.g. , the portion of native species relative to total protein, including high molecular weight and low molecular weight species) can be determined by inter alia SEC-HPLC, SDS-PAGE, Western Blot. The lyophilized formulation is first solubilized and then the protein of the composition is subjected to testing. Native protein includes protein that is not aggregated (e.g, in high molecular weight forms of a protein) or otherwise degraded (e.g, in low molecular weight forms of a protein). In certain embodiments, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the native form of the protein can be detected in the lyophilized formulation after storage for a defined amount of time at a defined temperature. The defined amount of time after which stability is measured can be at least 14 days, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 13 months, at least 14 months, at least 15 months, at least 16 months, at least 17 months, at least 18 months, at least 19 months, at least 20 months, at least 21 months, at least 22 months, at least 23 months, at least 24 months, or more. The temperature at which the samples may be kept when assessing stability can be any temperature from about - 80°C to about 50°C, e.g. , storage at about - 80°C, about -30°C, about -20°C, about 0°C, about 4°-8°C, about 5°C, about 25°C, about 30°C, about 35°C, about 37°C or other physiological temperatures, about 45 °C or about 50°C.

[00215] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of aggregated protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9

months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00216] In some embodiments of the lyophilized pharmaceutical composition provided herein, the protein population present in a high molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at -20°C for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00217] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of aggregated protein in the composition is less than 15%, 10%, 5%, or 2% after storage at room temperature (RT) for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00218] In some embodiments of the lyophilized pharmaceutical composition provided herein, the protein population present in a high molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at room temperature (RT) for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00219] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of aggregated protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00220] In some embodiments of the lyophilized pharmaceutical composition provided herein, the protein population present in a high molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at 30°C for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00221] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00222] In some embodiments of the lyophilized pharmaceutical composition provided herein, the protein population present in a low molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at -20°C for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9

months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00223] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at at room temperature (RT) for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00224] In some embodiments of the lyophilized pharmaceutical composition provided herein, the protein population present in a low molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at at room temperature (RT) for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00225] In some embodiments of the lyophilized pharmaceutical composition provided herein, the increase in the amount of degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00226] In some embodiments of the lyophilized pharmaceutical composition provided herein,

the protein population present in a low molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at 30°C for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00227] Other methods may be used to assess the stability of the lyophilized protein such as, e.g. , differential scanning calorimetry (DSC) to determine thermal stability, controlled agitation to determine mechanical stability, and absorbance at about 350 nm or about 405 nm to determine solution turbidities.

[00228] Stability may also be assessed by measuring the biological activity of the multivalent vaccine of the composition, e.g. the immunogenic activity of the multivalent vaccine.

[00229] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00230] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at at room temperature (RT) for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than

18 months.

[00231] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00232] The phrase “room temperature” may be used interchangeably with the phrase “ambient temperature.” Room temperature encompasses “controlled room temperature”, which indicates the temperature of a usual working environment of 20°C to 25°C , with transient deviations (excursions) between 15° and 30° that may be experienced in pharmacies, hospitals, and warehouses.

[00233] The immunogenic activity of the multivalent vaccine can be measured by the immune response elicited by the the multivalent vaccine in a subject. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the toxin neutralizing antibody production and/or B-cell response induced by the multivalent vaccine. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the T-cell mediated protection against *S. aureus* infection induced by the multivalent vaccine.

[00234] In another aspect, lyophilized pharmaceutical composition provided herein maintains the stability of the protein in the composition during the lyophilization process. The term “stability” refers to the retention of an acceptable degree of physical structure, or biological function of the protein after the lyophilization process compared with the liquid formulation freshly prepared. The protein may be stable even though it does not maintain 100% of its physical structure, or biological function after the lyophilization process.

[00235] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine has less than 15%, 10%, 5%, or 2% protein aggregation compared with a multivalent vaccine with the same components that is formulated at the same concentration in a

liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein aggregation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

[00236] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine has less than 15%, 10%, 5%, or 2% protein degradation compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation. In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein degradation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

[00237] The protein aggregation (or protein in high molecular weight form) or degradation (or protein in low molecular weight form) can be measured by SEC-HPLC, SDS-PAGE or Western blot assay.

[00238] In some embodiments of the lyophilized pharmaceutical composition provided herein, the multivalent vaccine has similar immunogenic activity compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation.

[00239] The immunogenic activity of the multivalent vaccine can be measured by the immune response elicited by the the multivalent vaccine in a subject. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the toxin neutralizing antibody production and/or B-cell response induced by the multivalent vaccine. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the T-cell mediated protection against *S. aureus* infection induced by the multivalent vaccine.

5.2.2 FUNCTIONAL PROPERTIES OF SPRAY FREEZE-DRIED FORMULATION OF PHARMACEUTICAL COMPOSITION

[00240] The spray freeze-dried compositions provided herein and described in Section 5.1.2 provide several advantages, one of which is maintaining the stability of the protein for a defined

amount of time at a defined temperature. The defined amount of time after which stability is measured can be at least 14 days, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 13 months, at least 14 months, at least 15 months, at least 16 months, at least 17 months, at least 18 months, at least 19 months, at least 20 months, at least 21 months, at least 22 months, at least 23 months, at least 24 months, at least 36 months or more. The temperature at which the samples may be kept when assessing stability can be any temperature from about -80°C to about 50°C, e.g., storage at about -80°C, about -30°C, about -20°C, about 0°C, about 4°C-8°C, about 5°C, about 20°C-30°C, about 25°C, about 30°C, about 35°C, about 37°C or other physiological temperatures, about 45 °C or about 50°C.

[00241] The term “stability” refers to the retention of an acceptable degree of physical structure or biological function (e.g. immunogenic activity) of the protein after storage in a relevant environment or under certain conditions. The protein may be stable even though it does not maintain 100% of its physical structure, or biological function after storage for a certain amount of time.

[00242] Stability can be measured, inter alia, by determining the percentage of native molecule that remains in a formulation after storage for a defined amount of time at a defined temperature or after delivery to a patient. The percentage of protein that retains its native form (e.g., the portion of native species relative to total protein, including high molecular weight and low molecular weight species) can be determined by inter alia SEC-HPLC, SDS-PAGE, Western Blot. The spray freeze-dried formulation is first solubilized and then the protein of the composition is subjected to testing. Native protein includes protein that is not aggregated (e.g. in high molecular weight forms of a protein) or otherwise degraded (e.g. in low molecular weight forms of a protein). In certain embodiments, at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the native form of the protein can be detected in the spray freeze-dried cake after storage for a defined amount of time at a defined temperature. The defined amount of time after which stability is measured can be at least 14 days, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at

least 13 months, at least 14 months, at least 15 months, at least 16 months, at least 17 months, at least 18 months, at least 19 months, at least 20 months, at least 21 months, at least 22 months, at least 23 months, at least 24 months, or more. The temperature at which the samples may be kept when assessing stability can be any temperature from about -80°C to about 50°C, e.g., storage at about -80°C, about -30°C, about -20°C, about 0°C, about 4°C-8°C, about 5°C, about 25°C, about 30°C, about 35°C, about 37°C or other physiological temperatures, about 45°C or about 50°C.

[00243] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the increase in the amount of aggregated protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00244] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the protein population present in a high molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at -20°C for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00245] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the increase in the amount of aggregated protein in the composition is less than 15%, 10%, 5%, or 2% after storage at room temperature (RT) for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15

months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00246] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the protein population present in a high molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at room temperature (RT) for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00247] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the increase in the amount of aggregated protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00248] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the protein population present in a high molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at 30°C for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00249] In some embodiments of the spray freeze-dried pharmaceutical composition provided

herein, the increase in the amount of degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00250] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the protein population present in a low molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at -20°C for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00251] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the increase in the amount of degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at room temperature (RT) for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00252] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the protein population present in a low molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at room temperature (RT) for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about

8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00253] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the increase in the amount of degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00254] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the protein population present in a low molecular weight in the composition is less than 15%, 10%, 5%, or 2% of the total protein in the composition after storage at 30°C for about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00255] Other methods may be used to assess the stability of the spray freeze-dried protein such as, e.g., differential scanning calorimetry (DSC) to determine thermal stability, controlled agitation to determine mechanical stability, and absorbance at about 350 nm or about 405 nm to determine solution turbidities.

[00256] Stability may also be assessed by measuring the biological activity of the multivalent vaccine of the composition, e.g. the immunogenic activity of the multivalent vaccine.

[00257] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine retains at least 70%, 80%, 90% or 95% of its initial immunogenic activity after storage at -20°C for a period of time, wherein the period of time is

about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00258] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at room temperature for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00259] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 16 months, about 17 months, about 18 months, about 19 months, about 20 months, about 21 months, about 22 months, about 23 months, about 24 months, about 36 months or greater than 18 months.

[00260] The phrase “room temperature” may be used interchangeably with the phrase “ambient temperature.” Room temperature encompasses “controlled room temperature”, which indicates the temperature of a usual working environment of 20°C to 25°C , with transient deviations (excursions) between 15° and 30° that may be experienced in pharmacies, hospitals, and warehouses.

[00261] The immunogenic activity of the multivalent vaccine can be measured by the immune response elicited by the the multivalent vaccine in a subject. In some embodiments, the

immunogenic activity of the multivalent vaccine can be assessed by measuring the toxin neutralizing antibody production and/or B-cell response induced by the multivalent vaccine. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the T-cell mediated protection against *S. aureus* infection induced by the multivalent vaccine.

[00262] In another aspect, spray freeze-dried pharmaceutical composition provided herein maintains the stability of the protein in the composition during the spray freeze-dry process. The term “stability” refers to the retention of an acceptable degree of physical structure, or biological function of the protein after the spray freeze-dry process compared with the liquid formulation freshly prepared. The protein may be stable even though it does not maintain 100% of its physical structure, or biological function after the spray freeze-dry process.

[00263] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine has less than 15%, 10%, 5%, or 2% protein aggregation compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein aggregation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

[00264] In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine has less than 15%, 10%, 5%, or 2% protein degradation compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation. In some embodiments of the spray freeze-dried pharmaceutical composition provided herein, the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein degradation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

[00265] The protein aggregation (or protein in high molecular weight form) or degradation (or protein in low molecular weight form) can be measured by SEC-HPLC, SDS-PAGE or Western blot assay.

[00266] In some embodiments of the spray freeze-dried pharmaceutical composition provided

herein, the multivalent vaccine has similar immunogenic activity compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation.

[00267] The immunogenic activity of the multivalent vaccine can be measured by the immune response elicited by the the multivalent vaccine in a subject. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the toxin neutralizing antibody production and/or B-cell response induced by the multivalent vaccine. In some embodiments, the immunogenic activity of the multivalent vaccine can be assessed by measuring the T-cell mediated protection against *S. aureus* infection induced by the multivalent vaccine.

5.3 PREPARATION OF FORMULATIONS OF PHARMACEUTICAL COMPOSITION

5.3.1 PREPARATION OF LYOPHILIZED FORMULATION OF PHARMACEUTICAL COMPOSITION

[00268] In one embodiment, lyophilization starts with a pre-lyophilized liquid composition containing the multivalent vaccine and excipients, as described above in Section 5.1 above.

[00269] In some embodiments, the pre-lyophilized liquid composition is suitable for lyophilization. In some embodiments, the pre-lyophilized liquid composition comprises a multicomponent vaccine, a buffer, sucrose, and polysorbate 80. In some embodiments of the pre-lyophilized liquid composition, the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}. In some embodiments, the multicomponent vaccine is as described in Section 5.4 below. In some embodiments, the pre-lyophilized liquid composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 1: 400: 1 by weight. In some embodiments, the pre-lyophilized liquid composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 2: 400: 1 by weight. In some embodiments, the composition further comprises histidine-borate. In some embodiments of the pre-lyophilized liquid composition, the buffer is phosphate buffer. In some embodiments of the pre-lyophilized liquid composition, the concentration of the total protein of the vaccine is about from 0.02 mg/ml to 10 mg/ml. In some embodiments of the pre-lyophilized liquid composition, the concentration of each component of the vaccine is about from 0.004 mg/ml to 2 mg/ml. In some embodiments

of the pre-lyophilized liquid composition, the concentration of sucrose is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L). In some embodiments of the pre-lyophilized liquid composition, the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L). In some embodiments of the pre-lyophilized liquid composition, the concentration of histidine-borate is about from 0.1mM to 20mM. In some embodiments of the pre-lyophilized liquid composition, the concentration of phosphate buffer is about from 0.5mM to 20mM. In some embodiments of the pre-lyophilized liquid composition, the concentration of the total protein is about 0.2 mg/ml, the concentration of the histidine-borate is about 20mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5. In some embodiments of the pre-lyophilized liquid composition, the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 20mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5.

[00270] The pre-lyophilized liquid composition is placed in an open container (e.g., vial) and the open container is placed into a lyophilization chamber onto a shelf.

[00271] The lyophilization process comprises three basic steps: (1) freezing, with optional annealing cycles, (2) primary drying, and (3) secondary drying.

[00272] The first step is freezing the liquid composition. Ice crystals form within the formulation during the freezing step and the remainder of the formulation becomes more concentrated and viscous. The concentrated remainder solidifies to form an amorphous, crystalline, or combined crystalline/amorphous state.. In some embodiments, the freezing step starts with setting the program at 10°C for 0.5 hour to stabilize shelf temperature and homogeneous vial temperature. In some embodiments, the freezing step follows by setting the temperature at -15°C for 1 hour to generate homogeneous liquid temperatures before freezing the formulation. In some embodiments, the freezing step follows by setting the temperature at -40°C for 2 hours for initial freezing of the composition. In some embodiments, the frozen composition is subjected to annealing to enhance crystallization. In some embodiments, the annealing is set

for temperature at -25°C for 2 hours. In some embodiments, the freezing step comprises freezing the composition at -40°C for 1 hour for final freezing and initial drying as the last step.

[00273] In some embodiments, the freezing step comprises (1) stabilizing shelf and equilibrating the vials containing the liquid formulation at 10°C for 0.5 hour; (2) generating homogeneous liquid at -15°C for 1 hour before freezing the formulation; (3) initial freezing the composition at -40°C for 2 hours; (4) annealing at -25°C for 2 hours; and (5) final freezing and initial drying at -40°C for 1 hour.

[00274] After freezing step, evaporation of solvent from the ice occurs via sublimation process. The pressure of the lyophilization chamber and the shelf temperature are adjusted below the triple point of water.

[00275] The primary drying step involves raising the shelf temperature below the critical temperature (glass transition temperature (Tg') and collapse temperature (Tc)) of the frozen product and reducing the chamber pressure to allow the frozen water in the composition to sublime. This difference between chamber pressure and ice vapor pressure serve is the driving force for ice sublimation. In some embodiment, the Tg' of the composition is between -29°C to -33°C. In some embodiment, the Tg' of the composition is between -30°C and -31°C. In some embodiment, the Tg' of the composition is between -31°C and -32°C. In some embodiment, the Tg' of the composition is about -30°C. In some embodiment, the Tg' of the composition is about -31°C. In some embodiment, the Tc of the composition is between -33°C and -35°C. In some embodiment, the Tc of the composition is between -34°C and -35°C. In some embodiment, the Tc of the composition is about -33°C. In some embodiment, the Tc of the composition is about -34°C. In some embodiment, the primary drying is set at -40°C for 0.5 hour. In some embodiments, the pressure of the lyophilization chamber is set at 50mTorr.

[00276] Some water may remain trapped within the matrix after the primary drying step. The remaining water is removed through the process of desorption during the secondary drying step. Here, the shelf temperature is increased to accelerate desorption and to attain the optimal moisture content in the lyophilized product. In some embodiment, the secondary drying is set at 10°C for 0.5 hour. In some embodiments, the pressure of the lyophilization chamber is set at 50mTorr.

[00277] In some embodiment, the drying step further comprises water vapor desorption set at 15°C for 2 hours. In some embodiment, the drying step further comprises final drying set at 25°C

for 2 hours. In some embodiment, the drying step is set at 35°C for 1 hour to achieve homogeneous glass temperature before stoppering.

[00278] In some embodiments, the drying step comprises (1) primary drying at -40°C for 0.5 hour; (2) secondary drying at 10°C for 1 hour; (3) water vapor desorption at 15°C for 2 hours; (4) final drying at 25°C for 2 hours; (5) achieving homogeneous glass temperature at 30°C for 1 hour.

[00279] In some embodiments, the final moisture content is not less than about 0.5% and not more than about 10%. In another embodiment, the final moisture content is not less than about 3% and not more than about 6%. In a specific embodiment, the moisture content is more than 3% but less than 5%. In another specific embodiment, the moisture content is about 3%. In another specific embodiment, the moisture content is about 3.5%. In another specific embodiment, the moisture content is about 4%. In another specific embodiment, the moisture content is about 4.5%. In yet another specific embodiment, the moisture content is about 5%.

[00280] In some embodiments, the invention provide lyophilization methods in which a process for producing a lyophilized formulation of the pharmaceutical composition comprises: (1) stabilizing shelf and equilibrating the vials containing a liquid formulation of the pharmaceutical composition at 10°C for 0.5 hour; (2) generating homogeneous liquid at -15°C for 1 hour; (3) initial freezing the liquid formulation at -40°C for 2 hours; (4) annealing at -25°C for 2 hours; (5) final freezing and initial drying at -40°C for 1 hour; (6) primary drying at -40°C for 0.5 hour; (7) secondary drying at 10°C for 1 hour; (8) water vapor desorption at 15°C for 2 hours; (9) final drying at 25°C for 2 hours; (10) achieving homogeneous glass temperature at 30°C for 1 hour.

5.4 MULTICOMPONENT STAPHYLOCOCCUS AUREUS VACCINE

[00281] In some embodiments, the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

[00282] In some embodiments, Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations. In some embodiments, LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations. In some embodiments, LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation. In some embodiments, LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB. In some

embodiments, TBA₂₂₅ comprises a fusion protein comprising: (1) a TSST-1 toxoid with L30R, D27A, and I46A mutations, (2) a SEB toxoid with L45R, Y89A, and Y94A mutations, and (3) a SEA toxoid with L48R, D70R, Y92A, and H225A mutations. In some embodiments, the three toxoids are fused together via a linker. In some embodiments, the linker comprises the amino acid sequence of GGGGSGGGGSGGGGS (SEQ ID NO:7).

5.4.1 Hla_{H35LH48L}

[00283] In some embodiments, the multicomponent vaccine provided herein comprises an alpha-hemolysin (Hla) toxoid. In some embodiments, the Hla toxoid is referred as Hla_{H35LH48L}. In some embodiments, Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations in the N-terminal domain of the Hla. In some embodiments, Hla_{H35LH48L} comprising H35L and H48L mutations are unable to heptamerize and is non-toxic.

[00284] In some embodiments, Hla_{H35LH48L} comprises the following amino acid sequence: MADSDINIKTGTTDIGSNTTVKTGDLVTYDKENGMLKKVFYSFIDDKNLNKKLLVIRTK GTIAGQYRVYSEEGANKSGLAWPSAFKVQLQLPDNEVAQISDYYPNRSIDTKEYMSTLT YGFNGNVTGDDTGKIGGLIGANVSIGHTLKYVQPDFKTILESPTDKKVGWKVIFNNMVN QNWGPYDRDSWNPVYGNQLFMKTRNGSMKAADNFLDPNKASSLLSSGFSPDFATVIT MDRKASKQQTNIDVIYERVRDDYQLHWTSTNWKGNTKDKWIDRSSERYKIDWEKEE MTN (SEQ ID NO:1)

[00285] In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to Hla_{H35LH48L}. In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to SEQ ID NO: 1.

5.4.2 LukS_{mut9}

[00286] In some embodiments, the multicomponent vaccine provided herein comprises the S subunit of a Pantone-Valentine Leukocidin (PLV) toxoid. In some embodiments, the S subunit of the PLV toxoid is referred as LukS_{mut9}. In some embodiments, LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A and S209A mutations. In some embodiments, LukS_{mut9} comprising T28F, K97A and S209A mutations is fully attenuated in combination with the wild type or mutant PVL counterpart or non-canonical partners.

[00287] In some embodiments, LukS_{mut9} comprises the following amino acid sequence:
 DNNIENIGDGAEEVVKRTEDTSSDKWGVFQNIQFDFVKDKKYNKDALILKMQGFINSKTT
 YYNYKNTDHIKAMRWPFQYNIGLKTNDPNVDLINYLPAKIDSVNVSQTLGYNIGGNF
 NSGPSTGGNGSFNYSKTISYNQQNYISEVEHQNSKSVQWGIKANSFITS LGKMSGHDPNL
 FVGYPYSQNP RDYFVPDNE LPLVHSGFNPAFIATVSHEKGS GDTSEFEITYGRNMDVT
 HATRRTTHYGNSYLEGSRIHNAFVNRNYTVKYE VNWKT HEIKVKGHN (SEQ ID NO:2)

[00288] In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to LukS_{mut9}. In some embodiments the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to SEQ ID NO: 2.

5.4.3 LukF_{mut1}

[00289] In some embodiments, the multicomponent vaccine provided herein comprises the F subunit of a Pantone-Valentine Leukocidin (PLV) toxoid. In some embodiments, the F subunit of the PLV toxoid is referred as LukF_{mut1}. In some embodiments, LukF_{mut1} comprises the F subunit of a PLV toxoid with K102A mutation. In some embodiments, LukF_{mut1} comprising K102A mutation is fully attenuated in combination with the wild type or mutant PVL counterpart or non-canonical partners.

[00290] In some embodiments, LukF_{mut1} comprises the following amino acid sequence:
 AQHITPVSEKKVDDKITLYKTTATSDSDKLKISQILTFNFIKDKSYDKDTLILKAAGNIYS
 GYTKPNPKDTISSQFYWGSKYNISINSDSNDVNVVDYAPANQNEEFQVQQTVGYSGG
 DINISNGLSGGGNGSKSFSETINYKQESYRTSLDKRTNFKKIGWDVEAHKIMNNGWGPY
 GRDSYHSTYGNEMFLGSRQSNLNAGQNFLEYHKMPVLSRGNFNPEFIGVLSRKQNAAK
 KSKITVTYQREMDRYTNFWNQLHWIGNNYKDENRATHTSIYEVDWENHTV KLIDTQSK
 EKNPMS (SEQ ID NO:3)

[00291] In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to LukF_{mut1}. In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to SEQ ID NO: 3.

5.4.4 LukAB_{mut50}

[00292] In some embodiments, the multicomponent vaccine provided herein comprises a dimeric toxoid form of leucocidin AB (LukAB). In some embodiments, the dimeric toxoid form of LukAB is referred as LukAB_{mut50}. In some embodiments, LukAB_{mut50} comprises a variant of LukA and a variant of LukB. In some embodiment, the variant of LukA comprises a LukA toxoid with D39A mutation. In some embodiment, the variant of LukB comprises a LukB toxoid with R23E mutation. In some embodiments, the variant of LukA and the variant of LukB render the protein unable to form a pore in the plasma membrane of target cells.

[00293] In some embodiments, the variant of LukA comprises the following amino acid sequence:

MKNKKRVLIASSLSCAILLLSAATTQANSAHKDSQDQNKKEHVDKSQQKDKRNVTNKD
KNSTAPDDIGKNGKITKRTETVYDEKTNILQNLQFDFIDDPTYDKNVLLVKKQGSIHNL
KFESHKEEKNSNWLYPSEYHVDFQVKNRNRKTEILDQLPKNKISTAKVDSTFSYSSGGK
FDSTKGIGRTSSNSYSKTISYNQQNYDTIASGKNNNWHVHWSVIANDLKYGGGEVKNRN
DELLFYRNTRIATVENPELSFASKYRYPALVRSGFNPEFLTYLSNEKSNEKTQFEVITYTR
NQDILKNRPGIHYAPPILEKNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKPYPKEG
(SEQ ID NO:4)

[00294] In some embodiments, the variant of LukB comprises the following amino acid sequence:

MIKQLYKNITICTLALSTTFTVLPATSF AKINSEIKQVSEKNLDGDTKMYTRTATTSDSQK
NITQSLQFNFLT EPNYDKETVFIKAKGTIGSGLRILDPNGYWNSTLRWPGSYSVSIQNVD
DNNNTNVTDFAPKNQDESREVKYTYGYKTGGDFSINRGGLTG NITKESNYSETISYQQP
SYRTLDDQSTSHKGVGWKVEAHLINNMGHDHTRQLTNDSDNRTKSEIFSLTRNGNLWA
KDNFTPKDKMPVTVSEGFNPEFLAVMSHDKKDKGKSQFVVHYKRSMDEFKIDWNRHG
FWGYWSGENHVDKKEEKL SALYEVDWKTHNVKFVKVLNDNEKK (SEQ ID NO:5)

[00295] In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that comprises an amino acid sequence that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to the variant of LukA. In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that comprises an amino acid sequence that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to SEQ ID NO: 4.

[00296] In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that comprises an amino acid sequence that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to the variant of LukA. In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that comprises an amino acid sequence that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to SEQ ID NO: 5.

5.4.5 TBA₂₂₅

[00297] In some embodiments, the multicomponent vaccine provided herein comprises a fusion protein comprising a toxoid for toxic shock syndrome toxin-1 (TSST-1), a toxoid for staphylococcal enterotoxin B (SEB), and a toxoid for staphylococcal enterotoxin A (SEA). In some embodiments, the fusion protein comprising a toxoid for TSST-1, a toxoid for SEB, and a toxoid for SEA is referred to as TBA₂₂₅. In some embodiments, the toxoid for TSST-1 comprises a variant of TSST-1 with L30R, D27A, and I46A mutations. In some embodiments, the toxoid for SEB comprises a variant of SEB with L45R, Y89A, and Y94A mutations. In some embodiments, the toxoid for SEA comprises a variant of SEA with L48R, D70R, Y92A, and H225A mutations. In some embodiments, the toxoid for TSST-1, the toxoid for SEB, and the toxoid for SEA are mutated in the MHC Class II binding sites, and therefore unable to crosslink T cells and antigen presenting cells, resulting in a loss of superantigenicity.

[00298] In some embodiments, TBA₂₂₅ comprises the following amino acid sequence:
 HMSTNDNIKDLLDWYSSGSDTFTNSEVLANSRGSMRIKNTDGSISLIAFPSPYYSPAFTK
 GEKVDLNTKRTKKSQHTSEGTYIHFQISGVTNTEKLPTPIELPLKVKVHGKDSPLKYWPK
 FDKKQLAISTLDFEIRHQLTQIHGLYRSSDKTGGYWKITMNDGSTYQSDLSKKFEYNTEK
 PPINIDEIKTIEAEINGGGGSGGGGSGGGGSESQDPKPDELHKSSKFTGLMENMKVLYD
 DNHVSAINVKSIDQFRYFDLIYSIKDTKLGNYDNVRVEFKNKDLADKYKDKYVDVFGA
 NAYYQCAFSKKTNDINSHQTDKRKTCMYGGVTEHNGNQLDKYRSITVRVFEDGKNLLS
 FDVQTNKKKVTAQELDYLTRHYLVKNKKLYEFNNSPYETGYIKFIENENSWYDMMPA
 PGDKFDQSKYLMMYNDNKMVDSKDVKIEVYLTTKKKGGGGSGGGGSGGGGSEKSEEI
 NEKDLRKKSELQGTALGNLKQIYYYNEKAKTENKESHDQFRQHTILFKGFFTDHSWYN
 DLLVRFDISKDIVDKYKGKKVDLYGAYAGYQCAGGTPNKTACMYGGVTLHDNNRLTEE
 KKVPINLWLDGKQNTVPLETVKTNKKNVTVQELDLQARRYLQEKYNLYNSDVFDDGKV
 QRGLIVFHTSTEPSVNYDLFGAQQQYSNTLLRIYRDNKTINSENMAIDIYLYTS (SEQ ID

NO:6)

[00299] In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that comprises an amino acid sequence that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to TBA₂₂₅. In some embodiments, the multicomponent vaccine provided herein comprises a polypeptide, peptide, or protein that comprises an amino acid sequence that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to SEQ ID NO:6.

5.5 DOSAGE AND MODE OF ADMINISTRATION

[00300] In some embodiments, the lyophilized formulation as described in Section 5.1.1 is reconstituted to a liquid formulation before being administered to a subject. In some embodiments, the spray-freezed dried formulation as described in Section 5.1.2 is reconstituted to a liquid formulation before being administered to a subject.

[00301] The term “reconstituted” or “reconstitution” used herein refers to the restoration of a lyophilized form or spray-freezed dried form to a liquid form, e.g., by rehydration, of a substance previously altered for preservation and/or storage, e.g., the restoration to a liquid state of a lyophilized formulation or spray-freezed dried form of the application that has been stored. A lyophilized composition of the present application can be reconstituted in any aqueous solution which produces a stable, aqueous solution suitable for administration. Such an aqueous solution includes, but is not limited to, sterile water, TE (Tris EDTA), phosphate buffered saline (PBS), Tris buffer or normal saline. A lyophilized or spray-freezed dried sample can be reconstituted with a lower, the same or higher volume than was used to lyophilize the sample.

[00302] It should be understood that a dose of a reconstituted lyophilized or spray-freezed dried formulation of the application can be determined in light of various relevant factors including the conditions to be treated, the chosen route of administration, the age, sex and body weight of the individual patient, and the severity of the patient's symptom, and can be administered in a single dose, divided dose or multiple doses.

[00303] The multicomponent vaccine of the pharmaceutical composition is administered to a subject in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of 2 µg to 500 µg of protein per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection

desired. In some embodiments, the total protein of the multicomponent vaccine of the pharmaceutical composition is at least about 2.5 µg, 3.125 µg, 10 µg, 12.5 µg, 25 µg, 50 µg, 75 µg, 100 µg, 125 µg, 150 µg, 200 µg, 225 µg, 250 µg, 275 µg, or 300 µg protein per dose. In some embodiments, the total protein of the multicomponent vaccine of the pharmaceutical composition is about 2.5 µg, 3.125 µg, 10 µg, 12.5 µg, 25 µg, 50 µg, 75 µg, 100 µg, 125 µg, 150 µg, 200 µg, 225 µg, 250 µg, 275 µg, or 300 µg protein per dose. In some embodiments, the total protein of the multicomponent vaccine of the pharmaceutical composition is 2.5 µg, 3.125 µg, 10 µg, 12.5 µg, 25 µg, 50 µg, 75 µg, 100 µg, 125 µg, 150 µg, 200 µg, 225 µg, 250 µg, 275 µg, or 300 µg protein per dose. In some embodiments, the total protein of the multicomponent vaccine is 0.2mg per dose. In some embodiments, the total protein of the multicomponent vaccine 50 µg per dose. In some embodiments, the total protein of the multicomponent vaccine is 12.5 µg, per dose. In some embodiments, the total protein of the multicomponent vaccine is 3.125 µg, per dose.

[00304] Dosage can be by a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes e.g. a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, etc. Multiple doses will typically be administered at least 1 week apart (e.g. about 2 weeks, about 3 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 10 weeks, about 12 weeks, about 16 weeks, etc.). In some embodiments, multiple doses will be administered at least 1 month apart. In some embodiments, multiple doses will be administered at least 2 month apart. In some embodiments, multiple doses will be administered at least 3 month apart. In some embodiments, multiple doses will be administered at least 4 month apart. In some embodiments, multiple doses will be administered at least 5 month apart. In some embodiments, multiple doses will be administered at least 6 month apart.

[00305] Vaccines prepared according to the invention may be used to treat both children and adults. Thus a human patient may be less than 1 year old, 1-5 years old, 5-15 years old, 15-55 years old, or at least 55 years old. Vaccines produced by the invention may be administered to patients at substantially the same time as (e.g. during the same medical consultation or visit to a healthcare professional or vaccination centre) other vaccines.

[00306] In certain aspects, disclosed herein is a method of preventing or treating *Staphylococcus aureus* infection, comprising administering to the subject the reconstituted liquid

formulation of the pharmaceutical composition by intravenous administration, intravenous administration, subcutaneous administration, intramuscular injection, suprachoroidal injection (for example, via a suprachoroidal drug delivery device such as a microinjector with a microneedle), subretinal injection via transvitreal approach (a surgical procedure), subretinal administration via the suprachoroidal space (for example, a surgical procedure via a subretinal drug delivery device comprising a catheter that can be inserted and tunneled through the suprachoroidal space toward the posterior pole, where a small needle injects into the subretinal space), and/or a posterior juxtasceral depot procedure (for example, via a juxtasceral drug delivery device comprising a cannula whose tip can be inserted and kept in direct apposition to the scleral surface)). In certain embodiments, the concentration of the multicomponent vaccine of the pharmaceutical composition is about 0.002 mg/ml, 0.004 mg/ml, 0.006 mg/ml, 0.008 mg/ml, 0.01 mg/ml, 0.02 mg/ml, 0.03 mg/ml, 0.04 mg/ml, 0.05 mg/ml, 0.06 mg/ml, 0.07 mg/ml, 0.08 mg/ml, 0.09 mg/ml, 0.1 mg/ml.

[00307] In certain aspects, disclosed herein is a kit comprising one or more containers and instructions for use, wherein the one or more containers comprise the lyophilized formulation as described in Section 5.1.1. In certain aspects, disclosed herein is a kit comprising one or more containers and instructions for use, wherein the one or more containers comprise the spray freeze-dried formulation as described in Section 5.1.2.

5.6 ASSAYS

[00308] The skilled artisan may use the assays as described herein and/or techniques known in the art to study the composition and methods described herein, for example to test the formulations provided herein. The examples provided in Section 6 also demonstrate in more detail how such assays can be used to test the formulations provided herein.

5.6.1 Biochemical/biophysical characterization of lyophilized vaccine by BCA, SDS-PAGE, Western blot (WB), and size exclusion high performance liquid chromatography (SEC-HPLC).

[00309] In some embodiments, the lyophilized and spray freeze-dried (SFD) formulations are characterized by several biophysical/biochemical assays. Briefly, Bicinchoninic acid protein assay (BCA) is used to confirm the protein concentrations after reconstitution of lyophilized/SFD formulation in sterile water. SDS-PAGE is run at protein loads using a 12% Bis-Tris gel. For

WB, primary antibodies which are antigen-specific and goat anti-rabbit IgG alkaline phosphatase conjugate (Bio-Rad) secondary antibody (1:3000, v/v) are used. For SEC-HPLC, 50µg of Lyo, SFD, or liquid LTB-SA7 are injected in an Agilent Technologies 1260 Infinity Series instrument using an AdvanceBio SEC 300Å 7.8x300 mm LC column with a mobile phase of 50 mM sodium phosphate buffer + 150 mM NaCl, pH 7.0 running at a flow rate of 0.5 mL/min. The chromatogram generated by the Agilent OpenLabs software plots absorbance at 280 nm as a function of retention time. All analysis of the peaks is performed by the auto-integrate function in the OpenLabs software.

[00310] Other assays that could be used for characterization of the formulations provided herein include toxin neutralization assays, and potency in mice. More details regarding how such assays can be used to test the formulations are provided herein in section 6.

6. EXAMPLES

[00311] The examples in this section (i.e., section 6) are offered by way of illustration, and not by way of limitation.

6.1 SOLID AND LIQUID LTB-SA7 FORMULATIONS HAVE COMPARABLE PROTEIN CHARACTERIZATION.

[00312] To determine the biochemical characterization of the LTB-SA7 toxoid vaccine components in our solid lyophilized (Lyo) and spray freeze-dried (SFD) formulations compared to liquid (Liq) formulation, SDS-PAGE, Western Blot, and SEC-HPLC analyses (Fig. 1A) were performed. First, the formulations by SDS-PAGE was examined and found that overall protein integrity was intact in the Lyo (lane 2), SFD (lane 4), Liq without desorption (lane 6), Liq with desorption (Lane 7), and freshly prepared Liq without alhydrogel (Lane 9) formulations (Fig. 1A). Next, a Western blot on vaccine toxoid components from the same conditions described above was performed and verified that neutralizing antibody binding specificity was maintained in all the formulations tested (Fig. 1B). Finally, SEC-HPLC was performed, which confirmed the

SDS-PAGE and Western blot results that protein constitution of the LTB-SA7 vaccine toxoid components is comparable between the Liq, Lyo, and SFD formulations tested (Fig. 1C).

6.2 SOLID LTB-SA7 FORMULATIONS MAINTAIN LONG-TERM STABILITY AT ROOM TEMPERATURE STORAGE.

[00313] Given the difficulty in maintaining cold chain storage in LMICs (Reza HM et al., 2023, *Lancet Reg Health Southeast Asia*.;11:100158), the stability of the Lyo and SFD LTB-SA7 formulations at varying storage times and temperatures was examined, and compared the stability to the freshly prepared Liq formulation. First, the storage stability of the LTB-SA7 formulations at -20°C and 30°C for 1- and 3-month durations was tested. Excitingly, vaccine components of both Lyo and SFD formulations had comparable stability to the freshly prepared Liq formulation by SDS-PAGE (Fig. 7A) and SEC-HPLC (Fig. 7B) analyses. The storage stability of the Lyo formulation at -20°C and 30°C for 6-, 9-, and 12-month durations was tested. Similar to the findings at 1 and 3 months, the Lyo formulation had comparable biochemical stability as freshly prepared Liq LTB-SA7 formulation at 6-9 months and 12 months of storage (Figs. 8A-8F). Collectively, the findings suggested that the Lyo and SFD LTB-SA7 formulations effectively maintain long-term stability at room temperature storage.

6.3 COMPARATIVE IMMUNOGENICITY OF SOLID AND LIQUID LTB-SA7 FORMULATIONS IN VACCINATED MICE.

[00314] After testing the long-term biochemical stability of the solid formulations, the next step is set out to compare the immunogenicity of the solid and liquid LTB-SA7 formulations in vaccinated mice. To this end, mice were immunized with 50 µg of either Liq, Lyo, or SFD formulations in 200µg of Alhydrogel (Al(OH)₃) on days 0, 14 and 28. On day 38, 10 days after the final immunization, sera were collected to assess toxin binding IgG titers by Luminex, and toxin neutralizing antibody titers in cell-based assays, as previously described (Karauzum H et al., 2021, *Front Immunol.*;12:624310). All three formulations of LTB-SA7 induced similar levels of toxin neutralizing capacity when tested against target antigens, which were increased over BSA-controls (Fig. 2A). Importantly, LTB-SA7 immunogenicity was maintained in the Lyo formulation after long-term storage (6 months) at -20°C and 30°C compared to fresh

preparations (Fig. 9), indicating the viability of the solid formulations as an alternative to LTB-SA7 vaccination with the liquid formulation in mice.

6.4 COMPARATIVE EFFICACY OF SOLID AND LIQUID LTB-SA7 FORMULATIONS IN A MURINE INTRADERMAL INFECTION MODEL.

[00315] The protective efficacy was compared of Liq, Lyo, and SFD LTB-SA7 formulations compared to BSA controls in a mouse model of MRSA skin infection, whereby mice were injected i.d. with 8×10^6 CFU of the bioluminescent MRSA strain, SF8300, and bacterial burden and lesion size monitored until the experiment was arbitrarily ended after 10 days. *In vivo* bioluminescence imaging (BLI) was used to non-invasively monitor bacterial burden, which has been previously shown to correlate with *ex vivo* CFUs ($R^2 = 0.9996$) (Guo Y et al., 2013, Antimicrob Agents Chemother.;57(2):855-63). Liq, Lyo, and SFD vaccinated mice had significantly reduced lesion sizes (Figs. 2B and 2C) and bacterial burdens (Figs. 2D and 2E) compared to BSA-treated controls. Taken together, the findings indicated that Liq, Lyo, and SFD LTB-SA7 formulations had significant efficacy against MRSA skin infections in mice.

6.5 COMPARATIVE IMMUNOGENICITY OF SOLID AND LIQUID LTB-SA7 FORMULATIONS IN VACCINATED RABBITS.

[00316] Since *S. aureus* pore-forming toxins that are neutralized by the LTB-SA7 vaccine have higher affinity to rabbit immune cells than mice (Diep BA et al., 2016, Antimicrob Agents Chemother.;60(10):6333-40), the next step was to compare the immunogenicity of the dry and liquid LTB-SA7 formulations in vaccinated rabbits. Rabbits were immunized with 50 µg of either Liq or Lyo formulations in 200µg of Alhydrogel (Al(OH)₃) on days 0, 14 and 28. On day 38, 10 days after the final immunization, sera were collected to assess toxin binding IgG titers by Luminex, and toxin neutralizing antibody titers in cell-based assays, as previously described (Karauzum H et al., 2021, Front Immunol.;12:624310). The Liq and Lyo LTB-SA7 formulations

had similar trends in the increased levels of toxin-binding IgG titers (Fig. 3A) and toxin-neutralizing antibodies (Fig. 3B) compared to BSA-treated rabbits.

6.6 COMPARATIVE EFFICACY OF SOLID AND LIQUID LTB-SA7 FORMULATIONS IN A NOVEL RABBIT PYOMYOSITIS INFECTION MODEL.

[00317] Pyomyositis is a major health burden in LMICs and increasing in prevalence in high-income countries (Ngor C et al., 2021, Trop Med Int Health.;26(10):1210-9), with *S. aureus* as the predominant etiological agent (Habeych ME et al., 2020, J Neurol Sci.;413:116767). Therefore, a rabbit pyomyositis infection model was developed to compare the protective efficacy of Lyo and liquid LTB-SA7 formulations whereby rabbit thighs were injected intramuscularly (i.m.) with $6-8 \times 10^9$ CFU of *S. aureus* strain SF8300 and skin lesions and thigh width monitored until the experiment was arbitrarily ended after 7 days, when abscesses were imaged, weighted, and harvested for ex vivo CFUs. Lyo and Liq treated rabbits had marked reductions in gross skin lesion and abscess sizes (Fig. 3C) and thigh widths (Fig. 3D) compared to BSA-treated rabbits, which correlated with significant reductions in abscess weights (Fig. 3E) and ex vivo CFUs (Fig. 3F). Thus, the data suggested that Lyo and Liq LTB-SA7 formulations had comparable and significant efficacy in a novel rabbit pyomyositis infection model.

6.7 LMIC ISOLATE DOWN-SELECTION STRATEGY VIA IN VITRO TOXIN CHARACTERIZATION.

[00318] Since only efficacy of LTB-SA7 against *S. aureus* isolates from the U.S was tested in the preclinical infection models (Karauzum H et al., 2021, Front Immunol.;12:624310), the next step is to confirm the efficacy of the Lyo and Liq LTB-SA7 formulations against *S. aureus* isolates from LMICs. Thus, a total of 83 clinical isolates of *S. aureus* from pyomyositis patients in the Democratic Republic of Congo, Palestine, and Cambodia were acquired. Since testing 83 clinical isolates in vivo is cost-prohibitive, a rational multi-dimensional down-selection strategy to screen isolates that exhibited broad toxin production was created for testing the efficacy of the vaccine formulations (Fig. 4A). First, toxin release was measured via Western blot to down-select to 29 clinical isolates.

[00319] Next, a toxin profile PCR assay was performed to identify isolates that were positive for toxins neutralized by LTB-SA7 (Fig. 4B and Table 1). 100 % of the isolates had the hla,

hlgA, B, C, and hlb genes, 90% of the isolates had the LukE and tst, 60-70% of isolates had the PVL and LukD genes, and less than 40% of isolates had the Sea, Seb, and Sec genes (Fig. 4B). However, Sed and See were not detected in any of the isolates (Fig. 4B). Out of the 29 isolates analyzed, 25 of them had more than 4 leukotoxins genes other than PVL. Furthermore, 22 of the isolates had 1-2 superantigen (SAg) genes (Table 1). Only one isolate had 4 SAg genes, 4 isolates had 3, and 3 isolates did not have any SAg genes. Furthermore, these isolates were typed using 18 different genes as typing markers. Among them, 14 were toxin genes, and 4 (SPA type, ST, CC, and SCCmec) were the established epidemiological typing genes. Out of 29 isolates, 15 were MSSA isolates, and 14 were MRSA isolates. Among MRSA isolates, SCCmec type IVA was the most predominant (12 out of 14), and 2 were SCCmec type III. The most common CC types were CC22 and CC121 whereas the SPA types were highly diverse.

Table 1 Characterization of LMIC isolates in vitro

Country	Strain ID	SP A type	S T	CC	SCC mec	h l a	h l b	Bicomponent Leukocidins						Superantigens (SAgs)						SAG#	Hla	PVL	# of Other Leukocidin
								P V L	h l g A	h l g B	h l g C	l u k D	l u k E	t s t	s e a	s e b	s e c	s e d	s e e				
Kinshasa	KCO 075	t590	3509	CC 1	abse nt	P	P	P	P	P	P	P	P	N	P	N	N	N	N	1	+	+	5
	KCO 079	t304	6	CC 5	abse nt	P	P	P	P	P	P	P	P	P	P	N	N	N	N	2	+	+	5
	KCO 005	t2034	8	CC 8	abse nt	P	P	N	P	P	P	P	P	P	P	N	N	N	N	2	+	-	5
	KCO 009	t314	121	CC 121	abse nt	P	P	P	P	P	P	P	P	P	N	P	N	N	N	2	+	+	5
	KCO 020	t8096	121	NA	abse nt	P	P	P	P	P	P	P	P	P	N	P	N	N	N	2	+	+	5
	KCO 048	t10600	3511	CC 45	abse nt	P	P	P	P	P	P	P	P	P	N	P	P	N	N	3	+	+	5
Cambodia	bact1 900	t314	121	CC 121	abse nt	P	P	P	P	P	P	P	P	P	P	P	N	N	N	3	+	+	5
	bact2 537	t1379	834	CC 1	IVA	P	P	N	P	P	P	P	P	P	N	N	P	N	N	2	+	-	5
	cop11 38	t127	N S T	CC 1	abse nt	P	P	N	P	P	P	P	P	P	P	P	N	N	N	3	+	-	5
	ns195	t1379	834	CC 1	IVA	P	P	N	P	P	P	P	P	P	N	N	P	N	N	2	+	-	5
	pyo10 07	t19346	1420	nd	abse nt	P	P	P	P	P	P	P	P	P	N	N	N	N	N	1	+	+	5
	pyo49 0	t272	121	CC 121	abse nt	P	P	P	P	P	P	P	P	P	N	P	N	N	N	2	+	+	5
	pyo60 3	t159	121	CC 121	abse nt	P	P	P	P	P	P	P	P	N	N	P	N	N	N	1	+	+	5
	pyo89 1	2421	88	nd	abse nt	P	P	P	P	P	P	P	P	P	P	P	N	N	N	3	+	+	5
	pyo15 44	t159	121	CC 121	abse nt	P	P	P	P	P	P	P	P	P	N	P	N	N	N	2	+	+	5
	ns102 0	t1379	834	CC 1	IVA	P	P	P	P	P	P	P	P	P	P	P	P	N	N	4	+	+	5

	bact1 442	t31 4	12 1	CC 121	III	P	P	P	P	P	P	P	P	P	N	P	N	N	N	2	+	+	5
Gaza	Gaza 75	t30 9	22	CC 22	IVA	P	P	N	P	P	P	N	P	P	N	N	N	N	1	+	-	4	
	Gaza 64A	t70 63	22	CC 22	III	P	P	N	P	P	P	N	P	P	N	N	N	N	1	+	-	4	
	Gaza 74	t54 85	22	CC 22	IVA	P	P	N	P	P	P	N	P	P	N	N	N	N	1	+	-	4	
	Gaza 209	t22 3	22	CC 22	IVA	P	P	P	P	P	P	N	P	P	N	N	N	N	1	+	+	4	
	Gaza 208	t22 3	22	CC 22	IVA	P	P	P	P	P	P	N	P	P	P	N	N	N	2	+	+	4	
	Gaza 384	t54 1	22	CC 22	IVA	P	P	N	P	P	P	N	N	P	N	N	N	N	1	+	-	3	
	Gaza 42	t22 3	22	CC 22	IVA	P	P	N	P	P	P	N	N	P	N	N	N	N	1	+	-	3	
	Gaza 419	NT	N S T	NA	IVA	P	P	P	P	P	P	P	P	P	N	N	N	N	1	+	+	4	
	Gaza 91	t00 5	22	CC 22	abse nt	P	P	P	P	P	P	N	N	N	N	N	N	N	0	+	+	3	
	Gaza 414	t01 9	N S T	NA	abse nt	P	P	P	P	P	P	P	P	P	N	N	N	N	0	+	+	5	
	Gaza 405	t31 8	30	CC 30	IVA	P	P	P	P	P	P	P	P	P	P	N	N	N	1	+	+	5	
	Gaza 182	t31 8	30	CC 30	IVA	P	P	P	P	P	P	P	N	P	N	N	P	N	2	+	+	5	
	Control Strains	SF83 00	t00 8	8	CC 8	IVA	P	P	P	P	P	P	P	P	N	N	N	N	N	0	+	+	5
		MNH OCH	t02 4	8	CC 8	abse nt	P	P	N	P	P	P	P	P	N	N	P	N	N	1	+	-	5
MN8		t01 2	30	CC 30	III	P	P	N	P	P	P	P	P	P	P	N	N	N	2	+	-	5	
New man		t00 8	25 4	CC 8	abse nt	P	P	N	P	P	P	P	P	P	P	N	N	N	2	+	-	5	

(P=POSITIVE, N=NEGATIVE, NA= CC not available, “-“ and “0”: NEW SEQUENCE TYPE, NT= NON-TYPEABLE)

[00320] To confirm toxin protein production by the down-selected isolates, in vitro hemolytic, leukocytic, and superantigen activity assays using human red blood cells, neutrophils, and PBMCs, were performed respectively. From the multi-dimensional screen, 3 clinical isolates were identified to use in in vivo studies, one from each of the Democratic Republic of Congo, Palestine, and Cambodia that expressed the lytic toxins and at least one superantigen. These strains were named KCO075, Pyo603, and Gaza209, which in addition to the lytic toxins produced SEA, SEB, and TSST-1, respectively (Fig. 4C).

6.8 IN VITRO NEUTRALIZATION OF CULTURE SUPERNATANTS BY LTB-SA7 PABS.

[00321] To assess the potential efficacy of LTB-SA7 against the down-selected isolates from LMICs, the neutralizing potential was explored via in vitro hemolytic, leukocytic, and superantigen activity assays. The toxicity of each bacterial supernatant (SUP) was first determined in rabbit red blood cells and HL-60 cells (Figs. 10A and 10B). SUPs were prepared

as described previously (Han X et al., 2022, Front Immunol.;13:893921). Starting at neat, cells were incubated with 11-point dilutions of bacterial SUPs and toxicity was measured. Data were analyzed using a 4-parameter (4PL) curve fit in XLFit (Microsoft) and the effective dilution at the point of the 4PL curve where ED₈₅ toxicity occurred was calculated. Cells were then incubated with the ED₈₅ dilution of SUPs in the presence or absence of decreasing concentrations of LTB-SA7 rabbit pAbs. The neutralizing concentration (NC) at the point of the 4PL curve where 50% neutralization of SUP toxicity occurred (NC₅₀) was then calculated. As shown in Figs. 10A and 10B, pAbs raised against LTB-SA7 showed neutralizing capacity towards the SUPs of all three LMIC isolates were at different concentrations. For RRBCs the NC₅₀ ranged from 19.3-26.4µg/ml and for HL60 cells from 20.5-60.9µg/ml.

6.9 PROTECTIVE EFFICACY OF LTB-SA7 FORMULATIONS AGAINST LMIC ISOLATES IN A BACTEREMIA INFECTION MODEL IN MICE.

[00322] *S. aureus* bacteremia has a 20-30% mortality rate in high-income countries (Kaasch AJ et al., 2014, J Infect.;68(3):242-51, Anantha RV et al., 2014, CMAJ Open.;2(4):E352-9), which is elevated to over 50% in LMICs (Nickerson EK et al., 2009, PLoS One.;4(1):e4308., Eshwara VK et al., 2013, Int J Infect Dis.;17(11):e1051-5.). Thus, to evaluate the in vivo efficacy of the LTB-SA7 formulations against LMIC isolates, a *S. aureus* bacteremia infection model in mice was performed whereby KCO075, Pyo603 and Gaza209 isolates were injected intraperitoneally into BSA, Liq, or Lyo treated mice and survival monitored over time until day 14 when the experiments were arbitrarily ended. Mice infected with the KCO075 isolate had comparably low reductions in survival rates between all groups tested (Fig. 5A). However, mice treated with the Liq and Lyo LTB-SA7 formulations had significant increases in survival compared to BSA-treated mice when infected with the Pyo603 isolate (Fig. 5B). Lastly, a similar trend was found in mice infected with the Gaza209 isolate, whereby Liq and Lyo vaccinated mice had markedly increased survival rates compared to BSA control mice (Fig. 5C).

Collectively, the data suggested that Liq and Lyo LTB-SA7 formulations have protective efficacy against LMIC isolates in a *S. aureus* bacteremia infection model.

6.10 PROTECTIVE EFFICACY OF LTB-SA7 FORMULATIONS AGAINST LMIC ISOLATES IN A MURINE INTRADERMAL INFECTION MODEL.

[00323] The efficacy of the Liq and Lyo LTB-SA7 formulations against LMIC isolates KCO075, Pyo603 and Gaza 209 was tested in the mouse intradermal infection model as described. Mice infected with *S. aureus* isolate KCO075 and vaccinated with Liq or Lyo formulations had significant reductions in lesions sizes compared to BSA-treated mice (Figs. 6A and 6B). These results corresponded with markedly reduced weight loss and *ex vivo* CFUs from the skin and kidneys of Liq and Lyo treated mice compared with BSA-treated mice (Figs. 6C and 6D). Similar to mice infected with KCO075, Liq and Lyo treated mice infected with Pyo603 had markedly reduced lesion sizes, weight loss, and *ex vivo* CFUs from skin compared to BSA controls (Figs. 6E-6H). However, Pyo603-infected mice did not have differences in *ex vivo* CFUs from kidneys between all the groups tested (Fig. 6H). Lastly, Liq and Lyo treated mice infected with Gaza209 had significantly dampened lesion sizes and *ex vivo* skin CFUs compared to BSA-treated mice (Figs. 6I-6L), with *ex vivo* kidney CFUs significantly lessened only in the Lyo treated mice (Fig. 6L). Taken together, Liq and Lyo LTB-SA7 formulations were effective against *S. aureus* isolates from LMICs in a mouse intradermal infection model.

6.11 Materials & Methods

[00324] Bacterial strains and cell lines. Bioluminescent *S. aureus* strain USA300 (SF8300) was kindly provided by Dr. Binh Diep at UCSF (Wang Y et al., 2023, Front Immunol.;14:1171934). *S. aureus* strains referred to as ‘Cambodia isolates’ were kindly provided by Dr. Catherine Moore at University of Oxford, UK. *S. aureus* strains referred to as ‘Kinshasa isolates’ were kindly provided by Dr. Frieder Schaumburg at University Hospital of Munster, Germany. *S. aureus* strains referred to as ‘Gaza isolates’ were kindly provided by Dr. Barry Kreiswirth at Center for Discovery & Innovation, HMH, NJ, USA.

[00325] The HL-60 cells, from ATCC (atcc.org), Manassas, VA were cultured in RPMI media containing 82 U/ml of penicillin and streptomycin with 16% FBS. Cells were differentiated in media with 1.5% dimethyl sulfoxide (DMSO), as previously described (Han X et al., 2022, Front Immunol.;13:893921).

[00326] Preparation of inoculum for infection. For mouse infections with USA300 (SF8300) and LMIC isolates (KCO075, Pyo603, or Gaza209) and for rabbit infections with USA300 (SF8300), *S. aureus* strains were cultured in tryptic soy broth (TSB) as previously described (Wang Y et al., 2021, J Orthop Res., Wang Y et al., 2017, Proc Natl Acad Sci U S A.;114(26):E5094-E102). Briefly, *S. aureus* was streaked onto a tryptic soy agar (TSA) plate (TSB plus 1.5% bacto agar (BD Biosciences)) and grown overnight at 37°C in a bacterial incubator. Two-to-three colonies were picked and cultured in TSB at 37°C in a shaking incubator (MaxQ HP 420, ThermoFisher) (240 rpm) overnight (16 h), followed by a 1:50 subculture at 37°C for 2 h to obtain mid-logarithmic phase bacteria. The bacteria were pelleted and washed in sterile phosphate-buffered saline (PBS). The absorbance at 600 nm (A₆₀₀) was measured to estimate the number of colony-forming units (CFU) for inoculation, which was verified in each experiment after overnight culture on TSA plates.

[00327] Animals. For mouse experiments, 6-week-old female BALB/c mice obtained from Charles River were used. For rabbit experiments, 16-week-old female Dutch Belted Rabbits obtained from Robinson Services were used. All animals were maintained under the same specific pathogen-free conditions, with air-isolated cages at an American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility at Johns Hopkins University and Integrated BioTherapeutics. They were handled according to procedures described in the Guide for the Care and Use of Laboratory Animals as well as Johns Hopkins University's policies and procedures as outlined in the Johns Hopkins University Animal Care and Use Training Manual. Studies at JHU were approved by the Johns Hopkins Animal Care and Use Committee (Protocol #: MO21M378 for mice, Protocol#: RB22M400 for rabbit). Mouse studies at IBT were approved by institutional animal care and use committees (IACUC) (Immunogenicity Protocol# AP160805 and Efficacy against bacterial challenge Protocol#: AP-161007).

[00328] Vaccination protocol. Mice and rabbits were immunized intramuscularly (IM) or subcutaneously (SC) on each side of the tail base or thigh muscle (50 µl each side) three times, two weeks apart, with a total of 50 µg of LTB-SA7 (10 µg each antigen) or 50 µg of BSA in 200 µg Alhydrogel in 100 µl histidine-borate buffer (buffer reconstituted in sterile water in the case of lyophilized vaccine) for each immunization. For mouse serological analyses, mice were bled via the retro-orbital (RO) route prior to and 10 days after the final immunization. For rabbit

serological analyses, rabbits were bled via the central ear artery prior to, 10 days after the final immunization, and 7 days after infection.

[00329] Intradermal infection model in mice. Two weeks after the last immunization, the ID infection was performed as previously described (Karauzum H et al., 2021, Front Immunol.;12:624310, Youn C et al., 2023, Sci Adv.;9(24):eadf8748., Marchitto MC et al., 2019, Proc Natl Acad Sci U S A.;116(22):10917-26.). Briefly, the dorsal backs of anesthetized mice (2% isoflurane) were shaved and intradermally injected with *S. aureus* (8×10^6 CFU of SF8300, 1×10^9 CFU of KCO075, 3×10^8 CFU of Pyo609, 2×10^8 CFU of Gaza209) in 100 μ l of PBS using a 29-gauge insulin syringe. Inocula were chosen to achieve an ~ 2 cm² lesion size for each *S. aureus* isolate. Total lesion size (cm²) was measured by analyzing digital photographs using Image J (<https://imagej.nih.gov/ij/>) and a millimeter ruler as a reference. For the mice infected with bioluminescent SF8300, in vivo bioluminescence (BLI) was performed using a Lumina III IVIS (PerkinElmer) and total flux (photons/s) was measured within a 1.78×1.78 cm circular region of interest using Living Image software (PerkinElmer). Ex vivo CFU were enumerated from overnight cultures of serially diluted 10 mm lesioned skin punch biopsy specimen homogenized at 4 °C (Pro200 Series homogenizer; Pro Scientific).

[00330] Pyomyositis infection model in rabbits. Two weeks after the last immunization, the IM infection was performed on the right thigh. Rabbits were anesthetized with IM ketamine (20-30 mg/kg) and xylazine (1-2 mg/kg) and general anesthesia was maintained with inhalation isoflurane ($\sim 1.5\%$). Sterile ophthalmic ointment (Rugby, Livonia, MI, USA) was applied to the eyes. Metoclopramide (Teva) (0.3 mg/kg) was injected subcutaneously as a gastrointestinal promotility agent and sustained-release buprenorphine (ZooPharm) (0.2 mg/kg) was injected subcutaneously for analgesia. For infection, $6-8 \times 10^9$ CFU of bioluminescent SF8300 in 100 μ l of PBS was injected into the muscle located in the middle of femur with depth of 0.5 cm using a 29-gauge insulin syringe. In vivo BLI was performed using a Lumina III IVIS (PerkinElmer) and total flux (photons/s) was measured within an 8.1×6.4 cm circular region of interest using Living Image software (PerkinElmer). Thigh width was measured by Pittsburgh digital caliper (Harbor Freight Tools). Ex vivo CFU was enumerated from overnight cultures of serially diluted muscle abscess specimen homogenized at 4 °C (Pro200 Series homogenizer; Pro Scientific).

[00331] Bacteremia infection model in mice. Lethal and sublethal doses of the three LMIC strains in BALB/c mice were first established prior to testing the efficacy of LTB-SA7 in a

systemic infection model. Mice were challenged with different doses of KCO075, pyo603 or Gaza209 either via the intraperitoneal (IP) route and infective dose resulting in 60-80% mortality was selected for testing efficacy of LTB-SA7 in the IP infection model. For bacteremia, six weeks after the last immunization, mice were infected intraperitoneally (IP) with a 200 μ l suspension of *S. aureus* (8×10^7 CFU/mouse of KCO075, 4×10^7 CFU/mouse of pyo603 or 2×10^7 CFU/mouse of Gaza209), as modified from previously described protocols (Han X et al., 2022, Front Immunol.;13:893921). The animals were monitored daily for 14 days for survival.

[00332] Lyophilization of LTB-SA7. Differential Scanning Calorimetry was performed to determine the glass transition temperature (T_g'), collapse temperature (T_c), and impact of freezing rate for the liquid formulation (Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50} formulated at total protein 0.2mg/ml in 20mM histidine-borate, 1.2mM phosphate buffer with 8% sucrose and 0.02% Tween 80). The average values for T_g' and T_c obtained from several experiments were used to design the lyophilization process, with primary drying set at -40°C (5°C below T_c) and annealing at -25°C (at least 5°C above the T_g'). Traditional lyophilization was accomplished by freezing the liquid vaccine and then reducing the surrounding pressure and applying enough heat to allow the frozen water in the material to sublime. During primary drying, the pressure in the surrounding environment was lowered to facilitate sublimation. In secondary drying the remaining water molecules were removed, bringing the lyophilized vaccine down to a final moisture content of between 3 and 5%. The result of the process was a “cake” of powder that could be reconstituted in water at time of use. In addition to traditional lyophilization, a spray freeze-drying process was also incorporated where a carefully formulated liquid solution was atomized into specifically sized spherical droplets (determined by spray nozzle orifice) and immediately frozen, locking in the size and shape of each individual particle. The particles were collected and dried in bulk in a standard lyophilization chamber. Both traditional lyophilization and spray freeze-drying processes were run at Engineered BioPharmaceuticals to produce small-scale batches to determine feasibility followed by production of large-scale batches.

[00333] Biochemical/biophysical characterization of lyophilized vaccine by BCA, SDS-PAGE, Western blot (WB), and size exclusion high performance liquid chromatography (SEC-HPLC). The lyophilized and spray freeze-dried (SFD) LTB-SA7 were characterized by several biophysical/biochemical assays. Briefly, Bicinchoninic acid protein assay (BCA) was

used to confirm the protein concentrations after reconstitution of lyophilized/SFD LTB-SA7 in sterile water. SDS-PAGE was run at protein loads as indicated in Fig. 1A using a 12% Bis-Tris gel. For WB, primary antibodies which were antigen-specific and goat anti-rabbit IgG alkaline phosphatase conjugate (Bio-Rad) secondary antibody (1:3000, v/v) were used. For SEC-HPLC, 50µg of Lyo, SFD, or liquid LTB-SA7 were injected in an Agilent Technologies 1260 Infinity Series instrument using an AdvanceBio SEC 300Å 7.8x300 mm LC column with a mobile phase of 50 mM sodium phosphate buffer + 150 mM NaCl, pH 7.0 running at a flow rate of 0.5 mL/min. The chromatogram generated by the Agilent OpenLabs software plots absorbance at 280 nm as a function of retention time. All analysis of the peaks was performed by the auto-integrate function in the OpenLabs software.

[00334] Preparation of Supernatants. Bacterial cells were streaked on sheep blood agar plates and incubated at 37°C overnight for 16 hours. Next, 7ml of BHI broth were inoculated with 4-6 colonies from each plate, incubated at 37°C at 230rpm for 16 hours, and OD600 was measured. Cells were centrifuged at 3500rpm, and supernatant was collected. Supernatants were normalized to an OD600 of 6, sterile-filtered and stored at -80°C.

[00335] Determination of Hemolytic Activities on Blood Agar Plates. Qualitative α -, β - and δ -hemolysin production was evaluated on sheep blood agar plates, as previously described (Adhikari RP et al., 2007, Infect Immun.;75(9):4534-40). Briefly, bacterial strains were streaked perpendicular to a β -hemolysin producing tester strain, RN4220. The inoculated plate was incubated at 37°C for 16-18 hours. Results were determined as follows: δ -hemolysin and β -hemolysin act synergistically, which produces a clearing zone at their intersection. α -hemolysin, and β -hemolysin inhibit each other, which results in a V-shaped zone where they intersect. When a strain is β -hemolytic, the clearing blends in with the tester strain. When a strain is both α - and δ -hemolytic, there is a stronger clearing on the characteristic V-shaped zone.

[00336] HL-60 Cytotoxicity Assay. Induced HL-60 cells were harvested by centrifugation at 1200 rpm for 10 minutes at 20°C. Cells were washed and resuspended with phenol red-free RPMI 1640 (Gibco) supplemented with 2% FBS to a final concentration of 5×10^6 cells/ml. Supernatants were serially diluted 2-fold across 96-well plates (50 µl/well) and 100µl of cells were added to each well. Plates were incubated at 37°C, 5% CO₂, 95% humidity for 3 hours. After 3 hours, 50 µl of reconstituted CellTiter Glo reagent (Promega) was added to each well. The plate was shaken on an orbital shaker for 10-15 min at room temperature followed by

measurement of luminescence (emission at 560 nm) using Cytation 5 imaging reader (Biotek) and Gen5 2.09 software to determine cell viability.

[00337] Toxin Neutralization Assay in HL-60 Cells. Polyclonal rabbit antibody against LTB-SA7 was diluted 2-fold in phenol-red RPMI across 96-well plate starting at 500µg/ml (25 µl/well). Equal volumes of supernatants were added at determined concentrations. 100µl of prepared induced HL-60 cells were added to each well and incubated at 37°C, 5% CO₂, 95% humidity. Cell viability was determined with CellTiter Glo reagent, as described above.

[00338] Toxicity in Rabbit and Human Red Blood Cells. 10ml PBS was added to 5ml of rabbit (Colorado Springs) or human blood (BioIVT) and centrifuged at 1500rpm for 10 minutes at 20°C. Supernatant was discarded and pellet was washed with 13ml PBS. The final pellet was weighed and diluted in PBS to 8% w/v for rabbit and 4% w/v for human. Bacterial supernatants were serially diluted 2-fold in PBS across 96-well plates (100 µl/well). 100 µl of prepared red blood cells were added to each well. The plate was incubated at 37°C for 30 minutes and then centrifuged at 3500rpm for 3 minutes at 20°C. Supernatants were transferred to 96-well plates compatible with plate reader. Toxicity was determined by measuring absorbance at 416nm using Spectramax 190 plate reader (Molecular Devices) and Softmax 5.4.5 software.

[00339] Toxin Neutralization Assay in Rabbit Red Blood Cells. Polyclonal rabbit antibody against LTB-SA7 was diluted 2-fold in PBS across 96-well plate starting at 500µg/ml (50 µl/well). Equal volumes of supernatants were added at determined concentrations. 100µl of prepared rabbit red blood cells were added to each well and incubated at 37°C, 5% CO₂, 95% humidity. Neutralization was determined by measuring absorbance at 416nm using Spectramax 190 plate reader (Molecular Devices) and Softmax 5.4.5 software.

[00340] Western Blots for bacterial supernatants. Supernatant dilutions consisted of 75% supernatant and 25% 4X Laemli's reducing buffer (Boston BioProducts). Dilutions were denatured at 70°C for 10 minutes or at 100°C for 5 minutes. Gels were run for 35 minutes at 165V. Blots were transferred using a standard 7-minute procedure using iBlot 2 (Thermo Fisher Scientific). Blots were blocked with StartingBlock (TBS) blocking buffer (Thermo Fisher Scientific) for 10 minutes and incubated in primary antibodies specific to different Staphylococcal toxins: Hla (Cat# Hla-ID-P), LukS-PV(Cat# LukS-ID-P), LukAB (cat# LukAB-ID-P), LukF-PV (Cat# LukF-IP-T01), TSST-1 (cat# TSST-1-ID-P-02), SEB (Cat-SEB-ID-P), and SEA (cat# SEA_ID-02) (all generated by IBT Bioservice) for 18 +/- 2 hours. This was

followed by 2 rapid washes and a third 5-minute wash in 1X TBS-T (Thermo Fisher Scientific). Secondary antibody was diluted at 1:3000, and blots were incubated in it for 50-60 minutes. The blots were finally washed three times as described earlier. Blots incubated in AP- or HRP-conjugated secondary antibodies were washed in 1X TBS for 5 minutes. When using AP-conjugated secondary antibody (Bio-Rad), blots were developed using AP detection buffer (Bio-Rad) and imaged with a camera (Azure system). The blots incubated in HRP-conjugated secondary antibodies (KPL) were developed with ECL reagent (Azure) and imaged with Azure 600 imager.

[00341] Genotyping of isolates from low to middle income countries (LMICs). QiAmp DNA Mini Kit (Qiagen, Cat# 51306) was used to extract and purify DNA from the bacterial isolates. A small number of pure colonies (5-6) bacteria were mixed with 200 uL of purified water as a starting material. The DNA was extracted by following the manufacture's protocol. The following are the PCR reagents and conditions used for PCRs. PCR Reagents: 15ul of HotStarTaq™ Master Mix, 1ul of Forward primer (20 pmol/ul), 1ul of Reverse primer (20 pmol/ul), 3ul of extracted DNA for the template, 0.6 ul of 50 mM Mg2+, 9.4 ul of dH2O PCR Conditions: i.) Initial denaturation 95°C for 15 minutes; ii.) 35 cycles of Denaturation 94°C for 30 seconds, Annealing 55°C for 30seconds, and Elongation 72°C for 45 seconds; iii.) Final Elongation 72°C for 10 minutes; and iv.) Hold at 4°C. Genotyping primers are listed in Table 2.

Table 2. List of PCR targets and primers.

Target	Primer Sequence (5' to 3')	Expected PCR Product Size (bp)	SEQ ID Nos
<u>Enterotoxin primers</u>			
SEA-3	5' - CCT TTG GAA ACG GTT AAA ACG	127	SEQ ID NO:8
SEA-4	5' - TCT GAA CCT TCC CAT CAA AAA C		SEQ ID NO:9
SEB-1	5' - TCG CAT CAA ACT GAC AAA CG	477	SEQ ID NO:10
SEB-4	5' - GCA GGT ACT CTA TAA GTG CCT GC		SEQ ID NO:11
SEC-3	5' - CTC AAG AAC TAG ACA TAA AAG CTA GG	271	SEQ ID NO:12
SEC-4	5' - TCA AAA TCG GAT TAA CAT TAT CC		SEQ ID NO:13
SED-3	5' - CTA GTT TGG TAA TAT CTC CTT TAA ACG	319	SEQ ID NO:14

SED-4	5' - TTA ATG CTA TAT CTT ATA GGG TAA ACA TC		SEQ ID NO:15
SEE-3	5' - CAG TAC CTA TAG ATA AAG TTA AAA CAA GC	178	SEQ ID NO:16
SEE-2	5' - TAA CTT ACC GTG GAC CCT TC		SEQ ID NO:17
TST-3	5' - AAG CCC TTT GTT GCT TGC G	445	SEQ ID NO:18
TST-6	5' - ATC GAA CTT TGG CCC ATA CTT T		SEQ ID NO:19
HLA-F	5' - CAA TCA AAC CGC CAA TTT TT	174	SEQ ID NO:20
HLA-R	5' - CCT GGC CTT CAG CAT TTA AG		SEQ ID NO:21
HLB-F	5' - TAT CCA AAC TGG GGG CAA TA	292	SEQ ID NO:22
HLB-R	5' - AAC CGC TTT TGA AAA CAT GC		SEQ ID NO:23
HLD-F	5' - TAA TTA AGG AAG GAG TGA TTT CAA TG	100	SEQ ID NO:24
HLD-R	5' - TTT TTA GTG AAT TTG TTC ACT GTG TC		SEQ ID NO:25
HLGB-F	5' - GGC AGA CAA AGC AGT GCA TA	202	SEQ ID NO:26
HLGB-R	5' - CTG CCC AGT AGA AGC CAT TC		SEQ ID NO:27
HLGA-F	5'-- CACAAGACCCAACTGGTCCAGCAGC	202	SEQ ID NO:28
HLGA-R	5'-- GCTAAACGATGTCTTGTACGTAAGC		SEQ ID NO:29
HLGC-F	5'-GGAAGCGATATAGAAATTATC	510	SEQ ID NO:30
HLGC-R	5'-TAAATCGCTATCAAAGGCTG		SEQ ID NO:31
LUKD-L	5' - TTG CAC TGC TTT TGC TAT CG	675	SEQ ID NO:32
LUKD-R	5' - GCA TTT GAT GTG TTG GCA AG		SEQ ID NO:33
LUKE-L	5' - GAT TGC GCC TTT AGC ATC TC	612	SEQ ID NO:34
LUKE-R	5' - GCT GAA CCT GTT GGA CCA TT		SEQ ID NO:35
LUK-PV-1	5' - ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A	433	SEQ ID NO:36
LUK-PV-2	5' - GCA TCA AST GTA TTG GAT AGC AAA AGC		SEQ ID NO:37
<u>Spa Primers</u>			
SPAR1	5' - CAG CAG TAG TGC CGT TTG C	200-600	SEQ ID NO:38
SPAF2	5' - GAA CAA CGT AAC GGC TTC ATC C		SEQ ID NO:39
<u>SCCmec PCR Primers</u>			
TYPE I-F	5' - GCT TTA AAG AGT GTC GTT ACA GG	613	SEQ ID NO:40
TYPE I-R	5' - GTT CTC TCA TAG TAT GAC GTC C		SEQ ID NO:41
TYPE II-F	5' - CGT TGA AGA TGA TGA AGC G	398	SEQ ID NO:42
TYPE II-R	5' - CGA AAT CAA TGG TTA ATG GAC C		SEQ ID NO:43

TYPE III-F	5' - CCA TAT TGT GTA CGA TGC G	280	SEQ ID NO:44
TYPE III-R	5' - CCT TAG TTG TCG TAA CAG ATC G		SEQ ID NO:45
TYPE IVA-F	5' - GCC TTA TTC GAA GAA ACC G	776	SEQ ID NO:46
TYPE IVA-R	5' - CTA CTC TTC TGA AAA GCG TCG		SEQ ID NO:47
TYPE IVB-F	5' - TCT GGA ATT ACT TCA GCT GC	493	SEQ ID NO:48
TYPE IVB-R	5' - AAA CAA TAT TGC TCT CCC TC		SEQ ID NO:49
TYPE IVC-F	5' - ACA ATA TTT GTA TTA TCG GAG AGC	200	SEQ ID NO:50
TYPE IVC-R	5' - TTG GTA TGA GGT ATT GCT GG		SEQ ID NO:51
TYPE IVD-F5	5' - CTC AAA ATA CGG ACC CCA ATA CA	881	SEQ ID NO:52
TYPE IVD-R6	5' - TGC TCC AGT AAT TGC TAA AG		SEQ ID NO:53
TYPE V-F	5' - GAA CAT TGT TAC TTA AAT GAG CG	325	SEQ ID NO:54
TYPE V-R	5' - TGA AAG TTG TAC CCT TGA CAC C		SEQ ID NO:55
MECA147-F	5' - GTG AAG ATA TAC CAA GTG ATT	147	SEQ ID NO:56
MECA147-R	5' - ATG CGC TAT AGA TTG AAA GGA T		SEQ ID NO:57
MLST primers			
ARCC UP	5' - TTG ATT CAC CAG CGC GTA TTG TC	569	SEQ ID NO:58
ARCC DN	5' - AGG TAT CTG CTT CAA TCA GCG		SEQ ID NO:59
AROE UP	5' - ATC GGA AAT CCT ATT TCA CAT TC	535	SEQ ID NO:60
AROE DN	5' - GGT GTT GTA TTA ATA ACG ATA TC		SEQ ID NO:61
GLPF UP	5' - CTA GGA ACT GCA ATC TTA ATC C	575	SEQ ID NO:62
GLPF DN	5' - TGG TAA AAT CGC ATG TCC AAT TC		SEQ ID NO:63
GMK UP	5' - ATC GTT TTA TCG GGA CCA TC	487	SEQ ID NO:64
GMK DN	5' - CGCGCTCTCTTTTAAAGTGC		SEQ ID NO:65
PTAUP	5' - GTT AAA ATC GTA TTA CCT GAA GG	574	SEQ ID NO:66
PTA DN	5' - GAC CCT TTT GTT GAA AAG CTT AA		SEQ ID NO:67
TPI UP	5' - TCG TTC ATT CTG AAC GTC GTG AA	470	SEQ ID NO:68
TPI DN	5' - TTT GCA CCT TCT AAC AAT TGT AC		SEQ ID NO:69
YQIL UP	5' - CAG CAT ACA GGA CAC CTA TTG GC	597	SEQ ID NO:70
YOIL DN	5' - CGT TGA GGA ATC GAT ACT GGA AC		SEQ ID NO:71

[00342] Serum total antibody titers. A multiplex assay to detect serum IgG titers to *S. aureus* antigens has been previously developed at IBT using the Luminex[®] xMAP[®] technology. Briefly, LTB-SA7 target antigens Hla, LukS-PV, LukF-PV, LukAB, SEA, SEB, and TSST-1 were coupled to carboxylated MagPlex microsphere beads with distinct spectral regions via a carbodiimide reaction. Antigen-coupled beads were incubated with serum samples (mouse or rabbit) at a starting dilution of 1:40 in a two-fold 8-point dilution series at room temperature (RT) for 2 hours. Samples were washed and incubated with a PE-conjugated goat anti-mouse or donkey anti-rabbit IgG Antibody (Biolegend, San Diego, CA.) for one hour at RT. The samples were washed and acquired using a Luminex200. Data were analyzed using a 4-parameter (4PL) curve fit in XLFit (Microsoft). IgG titers were expressed as the effective dilution at the point of the 4PL curve where 50% (ED50) of antigen was detected by toxin-specific antibodies present in the serum sample.

[00343] Serum total neutralizing titers. Hla TNAs were performed as previously described (Adhikari RP et al., 2012, PLoS One.;7(6):e38567). In brief, 4% rabbit red blood cells (RRBCs) were co-cultured with wild-type Hla $\pm$ serially diluted serum samples. Cells were centrifuged after 30 min at 37°C, and absorbance determined at OD416 nm. PVL and LukAB TNAs were performed with human promyelocytic leukemia (HL-60) cells as previously described (Kailasan S et al., 2019, Toxins (Basel).;11(6)). In brief, differentiated HL-60 cells were incubated with either PVL or LukAB $\pm$ serially diluted serum samples for 3 hours at 37°C, and CellTiter Glo reagent (Promega) was added to the culture to measure cell viability. Sag TNAs were performed with human PBMCs isolated from leukopaks (StemExpress). Cells were co-cultured with SEA, SEB, or TSST-1 in the presence or absence of serially diluted serum samples for 48 hours; supernatants were collected, and IFN γ was measured in the supernatants as a readout of superantigenicity as we previously described (Venkatasubramaniam A et al., 2019, Sci Rep.;9(1):3279). Data was analyzed using a 4-parameter (4PL) curve fit with XLFit (Microsoft). Toxin neutralizing activity was defined as the effective dilution of sera at the point of the 4PL curve at which 50% of toxin activity was neutralized (ND50).

[00344] Statistical analyses. Data between more than two groups for longitudinal comparisons (in vivo BLI, lesion size) were compared using a two-way analysis of variance (ANOVA) multiple comparisons test with Dunnett correction. For single time point comparisons

(abscess weight and ex vivo CFU), groups were compared using a one-way ANOVA-Kruskal-Wallis multiple comparisons test with Dunn's correction, and survival rates were compared by log rank (Mantel-Cox) test, as indicated in the figure legends. All statistical analyses were calculated with Prism software (GraphPad 10.3 Software, La Jolla, California). Data are presented as mean or geometric mean (for ex vivo CFU data) $\pm$ standard error of the mean (SEM) and values of $P < 0.05$ were considered statistically significant.

EQUIVALENTS

[00345] Although the invention is described in detail with reference to specific embodiments thereof, it will be understood that variations which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims. 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 following claims.

[00346] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference in their entireties.

SEQUENCES TABLE

SEQ ID NO.	Description	Sequence
1	Hla _{H35LH48L} (Hla toxoid with H35L and H48L mutations)	MADSDINIKTGTDDIGSNTTVKTGDLVTYDKENGMLKKVFYSFI DDKNLNKKLLVIRTKGTIAGQYRVYSEEGANKSGLAWPSAFKVQ LQLPDNEVAQISDYYPNRSIDTKEYMSTLTYGFGNVTGDDTGK IGGLIGANVSIGHTLKYPVQPDFKTILESPDCKVGVKVI FNNMV NQNWGPYDRDSWNPVYGNQLFMKTRNGSMKAADNFLDPNKASSL LSSGFSPDFATVITMDRKASKQQTNIDVIYERVRDDYQLHWTST NWKGTNTKDKWIDRSSERYKIDWEKEEMTN
2	LukS _{mut9} (S subunit of a PVL toxoid with T28F, K97A, and S209A mutations)	DNNIENIGDGAEVVKRTEDTSSDKWGVFQNIQFDFVKDKKYNKD ALILKMQGFINSKTTYNYKNTDHIKAMRWPFOYNI GLKTNDPN VDLINYL PANKIDSVNVSQTLGYNIGGNFNSSGPSTGGNGSFNYS KTISYNQQNYISEVEHQNSKSVQWGIKANSFITSLGKMSGHDPN LFVGYKPYSONPRDYFVPDNEPLPLVHSGFNPAFIATVSHEKGS GDTSEFEITYGRNMDVTHATRRTTHYGNSYLEGSR IHNAFVN RN YTVKYE VNWKTHEIKVKGHN
3	LukF _{mut1} (F subunit of a PVL toxoid with K102A mutation)	AQHITPVSEKKVDDKITLYKTTATSDSDKLKISQILTFNFIKDK SYDKDTLILKAAGNIYSGYTKPNPKDTISSQFYWGSKYNISINS DSNDSVNVDYAPANQNEEFQVQQTGVGYSYGGDINISNGLSGGG NGSKSFSETINYKQESYRTSLDKRTNFKKIGWDVEAHKIMNNGW GPYGRDSYHSTYGNEMFLGSRQSNLNAGQNFLEYHKMPVLSRGN FNPEFIGVLSRKQNAAKKSKITVITYQREMDRYTNFWNLHWIGN NYKDENRATHTSIYEVDWENHTVKLIDTQSKEKNPMS
4	LukA Variant (with D39A mutation in LukA)	MKNKKRVLIASSLSCAILLLSAATTQANSAHKDSQDQNKKEHVD KSQQKDKRNVTKDKNSTAPDDIGKNGKITKRTE TVYDEKTNIL QNLQFDFIDDPYDKNVLLVKKQGS IHSNLKFESHKEEKN SNWL KYPSEYHVD FQVKRNRKTEILDQLPKNKISTAKVDSTFSYSSGG KFDSTKGIGRTSSNSYSKTI SYNQQNYDTIASGKNNNWHVHWSV IANDLKYGGEVKNRNDEL LFYRNTRIATVENPELSFASKYRYP A LVRSGFNPEFLTYLSNEKSNEKTQFEV TYTRNQDILKNRPGIHY APP ILEKNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKP YKEG
5	LukB Variant (with R23E mutation in LukB)	MIKQLYKNITICTLALSTTFTVLPATSF AKINSEIKQVSEKNLD GDTKMYTRTATTSDSQKNITQSLQFNFLTEPNYDKETVFIKAKG TIGSGLRILD PNGYWNSTLRWPGSYSVSIQNVDDNNNTNVT DFA PKNQDESREVKYTYGYKTGGDFS INRGGLTG NITKESNYSETIS YQQPSYRTLLDQSTSHKGVGWKVEAHLINNMGHDHTRQLTNDSD NRTKSEIFSLTRNGNLWAKDNFTPKDKMPVTVSEGFNPEFLAVM SHDKKDKGKSQFVVHYKRSMDEFKIDWNRHGFWGYWSGENHVDK KEEKL SALYEVDWKTHNVKFKVLNDNEKK
6	TBA ₂₂₅ (a fusion protein)	HMSTNDNIKDLLDWYSSGSDTFTNSEVLANSRGSMRIKNTDGS I SLIAFPSPYYSPAFTKGEKVLDLNTKRTKKSQHTSEGTYIHFQIS

SEQ ID NO.	Description	Sequence
	comprising: a TSST-1 toxoid with L30R, D27A, and I46A mutations, a SEB toxoid with L45R, Y89A, and Y94A mutations, and a SEA toxoid with L48R, D70R, Y92A, and H225A mutations)	GVTNTEKLPTPIELPLKVKVHGKDSPLKYWPKFDDKKQLAISTLD FEIRHQLTQIHGLYRSSDKTGGYWKITMNDGSTYQSDLSKKFEY NTEKPPINIDEIKTIEAEINGGGGSGGGGSGGGGSESQDPKPD ELHKSSKFTGLMENMKVLYDDNHVSAINVKSIDQFRYFDLIYSI KDTKLGNYDNVRVEFKNKDLADKYKDKYVDVFGANAYYQCAFSK KTNDINSHQTDKRKTCMYGGVTEHNGNQLDKYRSITVRVFEDGK NLLSFDVQTNKKKVTAQELDYLTRHYLVKNKKLYEFNNSPYETG YIKFIENENSFWYDMMPAPGDKFDQSKYLMYNDNKMVDSKDVK IEVYLTTKKKGGGSGGGGSGGGGSEKSEEINEKDLRKKSELQG TALGNLQKIYYNEKAKTENKESHQFRQHTILFKGFFTDHSWY NDLLVRFDSKDIVDKYKGGKVDLYGAYAGYQCAGGTPNKTACMY GGVTLHDNNRLTEEEKVPINLWLDGKQNTVPLETVKTNKKNVTV QELDLQARRYLQEKYNLYNSDVFDGKVQRGLIVFHTSTEPSVNY DLFGAQQGYSNTLLRIYRDNKTINSENMAIDIYLYTS
7	Linker fused the three toxoids of TBA ₂₂₅	GGGGSGGGGSGGGGS
8	SEA-3 primer sequence	CCT TTG GAA ACG GTT AAA ACG
9	SEA-4 primer sequence	TCT GAA CCT TCC CAT CAA AAA C
10	SEB-1 primer sequence	TCG CAT CAA ACT GAC AAA CG
11	SEB-4 primer sequence	GCA GGT ACT CTA TAA GTG CCT GC
12	SEC-3 primer sequence	CTC AAG AAC TAG ACA TAA AAG CTA GG
13	SEC-4 primer sequence	TCA AAA TCG GAT TAA CAT TAT CC
14	SED-3 primer sequence	CTA GTT TGG TAA TAT CTC CTT TAA ACG
15	SED-4 primer sequence	TTA ATG CTA TAT CTT ATA GGG TAA ACA TC
16	SEE-3 primer sequence	CAG TAC CTA TAG ATA AAG TTA AAA CAA GC
17	SEE-2 primer sequence	TAA CTT ACC GTG GAC CCT TC
18	TST-3 primer sequence	AAG CCC TTT GTT GCT TGC G
19	TST-6 primer sequence	ATC GAA CTT TGG CCC ATA CTT T
20	HLA-F primer sequence	CAA TCA AAC CGC CAA TTT TT

SEQ ID NO.	Description	Sequence
21	HLA-R primer sequence	CCT GGC CTT CAG CAT TTA AG
22	HLB-F primer sequence	TAT CCA AAC TGG GGG CAA TA
23	HLB-R primer sequence	AAC CGC TTT TGA AAA CAT GC
24	HLD-F primer sequence	TAA TTA AGG AAG GAG TGA TTT CAA TG
25	HLD-R primer sequence	TTT TTA GTG AAT TTG TTC ACT GTG TC
26	HLGB-F primer sequence	GGC AGA CAA AGC AGT GCA TA
27	HLGB-R primer sequence	CTG CCC AGT AGA AGC CAT TC
28	HLGA-F primer sequence	CACAAGACCCAACTGGTCCAGCAGC
29	HLGA-R primer sequence	GCTAAACGATGTCTTGTACGTAAGC
30	HLGC-F primer sequence	GGAAGCGATATAGAAATTATC
31	HLGC-R primer sequence	TAAATCGCTATCAAAGGCTG
32	LUKD-L primer sequence	TTG CAC TGC TTT TGC TAT CG
33	LUKD-R primer sequence	GCA TTT GAT GTG TTG GCA AG
34	LUKE-L primer sequence	GAT TGC GCC TTT AGC ATC TC
35	LUKE-R primer sequence	GCT GAA CCT GTT GGA CCA TT
36	LUK-PV-1 primer sequence	ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A
37	LUK-PV-2 primer sequence	GCA TCA AST GTA TTG GAT AGC AAA AGC
38	SPAR1 primer sequence	CAG CAG TAG TGC CGT TTG C
39	SPAF2 primer sequence	GAA CAA CGT AAC GGC TTC ATC C
40	TYPE I-F primer sequence	GCT TTA AAG AGT GTC GTT ACA GG
41	TYPE I-R primer sequence	GTT CTC TCA TAG TAT GAC GTC C
42	TYPE II-F primer sequence	CGT TGA AGA TGA TGA AGC G
43	TYPE II-R primer sequence	CGA AAT CAA TGG TTA ATG GAC C
44	TYPE III-F primer sequence	CCA TAT TGT GTA CGA TGC G

SEQ ID NO.	Description	Sequence
45	TYPE III-R primer sequence	CCT TAG TTG TCG TAA CAG ATC G
46	TYPE IVA-F primer sequence	GCC TTA TTC GAA GAA ACC G
47	TYPE IVA-R primer sequence	CTA CTC TTC TGA AAA GCG TCG
48	TYPE IVB-F primer sequence	TCT GGA ATT ACT TCA GCT GC
49	TYPE IVB-R primer sequence	AAA CAA TAT TGC TCT CCC TC
50	TYPE IVC-F primer sequence	ACA ATA TTT GTA TTA TCG GAG AGC
51	TYPE IVC-R primer sequence	TTG GTA TGA GGT ATT GCT GG
52	TYPE IVD-F5 primer sequence	CTC AAA ATA CGG ACC CCA ATA CA
53	TYPE IVD-R6 primer sequence	TGC TCC AGT AAT TGC TAA AG
54	TYPE V-F primer sequence	GAA CAT TGT TAC TTA AAT GAG CG
55	TYPE V-R primer sequence	TGA AAG TTG TAC CCT TGA CAC C
56	MECA147-F primer sequence	GTG AAG ATA TAC CAA GTG ATT
57	MECA147-R primer sequence	ATG CGC TAT AGA TTG AAA GGA T
58	ARCC UP primer sequence	TTG ATT CAC CAG CGC GTA TTG TC
59	ARCC DN primer sequence	AGG TAT CTG CTT CAA TCA GCG
60	AROE UP primer sequence	ATC GGA AAT CCT ATT TCA CAT TC
61	AROE DN primer sequence	GGT GTT GTA TTA ATA ACG ATA TC
62	GLPF UP primer sequence	CTA GGA ACT GCA ATC TTA ATC C
63	GLPF DN primer sequence	TGG TAA AAT CGC ATG TCC AAT TC
64	GMK UP primer sequence	ATC GTT TTA TCG GGA CCA TC
65	GMK DN primer sequence	CGCGCTCTCTTTTAAAGTGC
66	PTAUP primer sequence	GTT AAA ATC GTA TTA CCT GAA GG
67	PTA DN primer sequence	GAC CCT TTT GTT GAA AAG CTT AA
68	TPI UP primer sequence	TCG TTC ATT CTG AAC GTC GTG AA

SEQ ID NO.	Description	Sequence
69	TPI DN primer sequence	TTT GCA CCT TCT AAC AAT TGT AC
70	YQIL UP primer sequence	CAG CAT ACA GGA CAC CTA TTG GC
71	YQIL DN primer sequence	CGT TGA GGA ATC GAT ACT GGA AC

WHAT IS CLAIMED:

1. A lyophilized pharmaceutical composition comprising:
 - a. a multicomponent vaccine,
 - b. a buffer,
 - c. sucrose, and
 - d. polysorbate 80,wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.
2. The lyophilized pharmaceutical composition of claim 1, wherein the composition further comprises histidine-borate.
3. The lyophilized pharmaceutical composition of claim 1 or 2, wherein the buffer is phosphate buffer.
4. The lyophilized pharmaceutical composition of any one of claims 1 to 3, wherein
 - a. Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1;
 - b. LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2;
 - c. LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3;
 - d. LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SEQ ID NO:5;
 - e. TBA₂₂₅ comprises a fusion protein comprising:
 - i. a TSST-1 toxoid with L30R, D27A, and I46A mutations,
 - ii. a SEB toxoid with L45R, Y89A, and Y94A mutations, and
 - iii. a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or

comprising the amino acid sequence of SEQ ID NO: 6;

5. The lyophilized pharmaceutical composition of claim 4, wherein
 - a. Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1;
 - b. LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2;
 - c. LukF_{mut1} consists of the amino acid sequence of SEQ ID NO: 3;
 - d. LukAB_{mut50} comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5; and
 - e. TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.
6. The lyophilized pharmaceutical composition of any one of claims 1 to 5, wherein the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml after reconstitution in a liquid formulation.
7. The lyophilized pharmaceutical composition of any one of claims 1 to 6, wherein the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml after reconstitution in a liquid formulation.
8. The lyophilized pharmaceutical composition of any one of claims 1 to 7, wherein the concentration of sucrose is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) after reconstitution in a liquid formulation.
9. The lyophilized pharmaceutical composition of any one of claims 1 to 8, wherein the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) after reconstitution in a liquid formulation.
10. The lyophilized pharmaceutical composition of any one of claims 1 to 7, wherein the concentration of histidine-borate is about from 0.1mM to 25mM after reconstitution in a liquid formulation.

11. The lyophilized pharmaceutical composition of any one of claims 3 to 10, wherein the concentration of phosphate buffer is about from 0.5mM to 20mM after reconstitution in a liquid formulation.

12. The lyophilized pharmaceutical composition of any one of claims 1 to 5, wherein the lyophilized pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at about a ratio of 2: 400: 1 by weight.

13. The lyophilized pharmaceutical composition of any one of claims 1 to 11, wherein the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation.

14. The lyophilized pharmaceutical composition of any one of claims 1 to 9, wherein the total protein is about 200 µg per dose.

15. The lyophilized pharmaceutical composition of any one of claims 1 to 12, wherein the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein aggregation or degradation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

16. The lyophilized pharmaceutical composition of claim 15, wherein the protein aggregation or degradation is measured by SEC-HPLC, SDS-PAGE or Western blot assay.

17. The lyophilized pharmaceutical composition of any one of claims 1 to 14, wherein the multivalent vaccine has similar immunogenic activity compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation.

18. The lyophilized pharmaceutical composition of claim 17, wherein the immunogenic activity is measured by the levels of antibody titers production in a subject induced by administering to the subject the multivalent vaccine in a lyophilized or liquid formulation.

19. The lyophilized pharmaceutical composition of any one of claims 1 to 18, wherein the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

20. The lyophilized pharmaceutical composition of any one of claims 1 to 18, wherein the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

21. The lyophilized pharmaceutical composition of any one of claims 1 to 18, wherein the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

22. The lyophilized pharmaceutical composition of any one of claims 1 to 18, wherein the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months,

about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

23. A method of generating the lyophilized pharmaceutical composition of any one of claims 1 to 22, comprising:

- a. dissolving the multicomponent vaccine, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition;
- b. freezing the liquid composition to form a frozen composition;
- c. drying the frozen composition under a primary reduced pressure at a primary drying temperature for a period of time effective to reduce the moisture content of the composition; and
- d. further drying the frozen composition under a second reduced pressure at a second drying temperature for a period of time effective to further reduce the moisture content of the composition, thereby producing the lyophilized composition.

24. The method of claim 23, wherein the method comprises:

- a. dissolving the multicomponent vaccine, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition;
- b. freezing the liquid composition at -40°C for 2 hours to form a frozen composition; annealing the frozen composition at -25°C for 2 hours; and further freezing the frozen composition at -40°C for 1 hour.
- c. drying the frozen composition under a primary reduced pressure at -40°C for 0.5 hour to reduce the moisture content of the composition; and
- d. further drying the frozen composition under a second reduced pressure at 10°C for 1 hour to further reduce the moisture content of the composition, thereby producing the lyophilized composition.

25. The method of claim 23 or 24, wherein the lyophilized pharmaceutical composition has a final moisture content of between 3% and 5%.

26. A spray freeze-dried pharmaceutical composition comprising:

- a. a multicomponent vaccine,
- b. a buffer,
- c. sucrose, and
- d. polysorbate 80,

wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

27. The spray freeze-dried pharmaceutical composition of claim 26, wherein the composition further comprises histidine-borate.

28. The spray freeze-dried pharmaceutical composition of claim 26 or 27, wherein the buffer is phosphate buffer.

29. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 28, wherein:

a. Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1;

b. LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2;

c. LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3;

d. LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 5;

e. TBA₂₂₅ comprises a fusion protein comprising:

- i. a TSST-1 toxoid with L30R, D27A, and I46A mutations,
- ii. a SEB toxoid with L45R, Y89A, and Y94A mutations, and
- iii. a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or comprising the amino acid sequence of SEQ ID NO: 6.

30. The spray freeze-dried pharmaceutical composition of claim 29, wherein:

- a. Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1;
- b. LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2;
- c. LukF_{mut1} consists of the amino acid sequence of SEQ ID NO: 3;
- d. LukAB_{mut50} comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid sequence of SEQ ID NO: 5; and
- e. TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.

31. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 30, wherein the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml after reconstitution in a liquid formulation.

32. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 31, wherein the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml after reconstitution in a liquid formulation.

33. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 32, wherein the concentration of sucrose is from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L) after reconstitution in a liquid formulation.

34. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 33, wherein the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L) after reconstitution in a liquid formulation.

35. The spray freeze-dried pharmaceutical composition of any one of claims 27 to 32, wherein the concentration of histidine-borate is about from 0.1mM to 25mM after reconstitution in a liquid formulation.

36. The spray freeze-dried pharmaceutical composition of any one of claims 28 to 35, wherein the concentration of phosphate buffer is about from 0.5mM to 20mM after reconstitution in a liquid formulation.

37. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 36, wherein the spray freeze-dried pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at the ratio of 2: 400: 1 in weight.

38. The spray freeze-dried pharmaceutical composition of any one of claims 28 to 36, wherein the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5 after reconstitution in a liquid formulation.

39. The spray freeze-dried pharmaceutical composition of any one of claims 28 to 36, wherein the total protein is about 200 µg per dose.

40. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 39, wherein the multivalent vaccine has at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 50% less protein aggregation or degradation compared with a multivalent vaccine with the same composition that is formulated at the same concentration in a liquid formulation.

41. The spray freeze-dried pharmaceutical composition of claim 40, wherein the protein aggregation or degradation is measured by SEC-HPLC, SDS-PAGE or Western blot assay.

42. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 39, wherein the multivalent vaccine has similar immunogenic activity compared with a multivalent vaccine with the same components that is formulated at the same concentration in a liquid formulation.

43. The spray freeze-dried pharmaceutical composition of claim 42, wherein the immunogenic activity is measured by the levels of antibody titers production in a subject induced

by administering to the subject the multivalent vaccine in a spray freeze-dried or liquid formulation.

44. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 43, wherein the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

45. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 43, wherein the increase in the amount of aggregated or degraded protein in the composition is less than 15%, 10%, 5%, or 2% after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

46. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 43, wherein the multivalent vaccine retains at least 70%, 80%, 90% or 95% of its initial immunogenic activity after storage at -20°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

47. The spray freeze-dried pharmaceutical composition of any one of claims 26 to 43, wherein the multivalent vaccine retains at least 70% , 80%, 90% or 95% of its initial immunogenic activity after storage at 30°C for a period of time, wherein the period of time is about 1 weeks, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8

months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.

48. A method of generating the spray freeze-dried pharmaceutical composition of any one of claims 26 to 47, comprising:

- a. dissolving the multicomponent vaccine, histidine-borate, phosphate buffer, sucrose, and polysorbate 80 in a suitable solvent to form a liquid composition;
- b. atomizing the liquid formulation into specifically sized spherical droplets;
- c. immediately freezing the droplets and locking in the size and shape of each individual particle;
- d. collecting the particles and drying the particles in bulk in a lyophilization chamber.

49. A reconstituted pharmaceutical composition of any one of claims 1 to 22, or 26 to 47 comprising:

- a. a multicomponent vaccine,
- b. a buffer,
- c. sucrose,
- d. polysorbate 80, and
- e. a reconstitution liquid

wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.

50. The reconstituted pharmaceutical composition of claim 49, wherein the composition further comprises histidine-borate.

51. The reconstituted pharmaceutical composition of claim 26 or 27, wherein the buffer is phosphate buffer.

52. The reconstituted pharmaceutical composition of any one of claims 49 to 51, wherein

- a. Hla_{H35LH48L} comprises a Hla toxoid with H35L and H48L mutations, or comprises the amino acid sequence of SEQ ID NO: 1;
 - b. LukS_{mut9} comprises the S subunit of a PVL toxoid with T28F, K97A, and S209A mutations, or comprises the amino acid sequence of SEQ ID NO: 2;
 - c. LukF_{mut1} comprises the F subunit of a PVL toxoid with K102A mutation, or comprises the amino acid sequence of SEQ ID NO: 3;
 - d. LukAB_{mut50} comprises a dimeric toxoid form of LukAB with D39A mutation in LukA and R23E mutation in LukB, or comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 4 and a second polypeptide comprising the amino acid of sequence of SEQ ID NO: 5;
 - e. TBA₂₂₅ comprises a fusion protein comprising:
 - i. a TSST-1 toxoid with L30R, D27A, and I46A mutations,
 - ii. a SEB toxoid with L45R, Y89A, and Y94A mutations, and
 - iii. a SEA toxoid with L48R, D70R, Y92A, and H225A mutations; or comprising the amino acid sequence of SEQ ID NO: 6.
53. The reconstituted pharmaceutical composition of claim 52, wherein
- a. Hla_{H35LH48L} consists of the amino acid sequence of SEQ ID NO: 1;
 - b. LukS_{mut9} consists of the amino acid sequence of SEQ ID NO: 2;
 - c. LukF_{mut1} consists of the amino acid sequence of SEQ ID NO: 3;
 - d. LukAB_{mut50} comprises a first polypeptide consisting of the amino acid sequence of SEQ ID NO: 4 and a second polypeptide consisting of the amino acid of sequence of SEQ ID NO: 5; and
 - e. TBA₂₂₅ consists of the amino acid sequence of SEQ ID NO: 6.

54. The reconstituted pharmaceutical composition of any one of claims 49 to 53, wherein the concentration of the total protein of the vaccine is about from 0.005 mg/ml to 10 mg/ml.

55. The reconstituted pharmaceutical composition of any one of claims 49 to 54, wherein the concentration of each component of the vaccine is about from 0.001 mg/ml to 2 mg/ml.

56. The reconstituted pharmaceutical composition of any one of claims 49 to 55, wherein the concentration of sucrose is about from 2% (weight/volume, 20 g/L) to 10% (weight/volume, 100 g/L).

57. The reconstituted pharmaceutical composition of any one of claims 49 to 56, wherein the concentration of polysorbate 80 is about from 0.004% (weight/volume, 0.04 g/L) to 2% (weight/volume, 20 g/L).

58. The reconstituted pharmaceutical composition of any one of claims 50 to 55, wherein the concentration of histidine-borate is about from 0.1mM to 25mM.

59. The reconstituted pharmaceutical composition of any one of claims 51 to 58, wherein the concentration of phosphate buffer is about from 0.5mM to 20mM.

60. The reconstituted pharmaceutical composition of any one of claims 49 to 59, wherein the reconstituted pharmaceutical composition comprises the total protein, sucrose, and polysorbate 80 at the ratio of 2: 400: 1 in weight.

61. The reconstituted pharmaceutical composition of any one of claims 51 to 60, wherein the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5.

62. The reconstituted pharmaceutical composition of any one of claims 51 to 61, wherein the total protein is about 200 µg per dose .

63. A method of treating or preventing *Staphylococcus aureus* (SA) infection in a subject, comprising administering to the subject the reconstituted pharmaceutical composition of any one of claims 49 to 62.

64. The method of claim 62, wherein the subject is a human subject.

65. A composition suitable for lyophilization or spray-freeze drying, comprising:
- a multicomponent vaccine,
 - a buffer,
 - sucrose, and
 - polysorbate 80,
- wherein the multicomponent vaccine comprises: Hla_{H35LH48L}, LukS_{mut9}, LukF_{mut1}, TBA₂₂₅ and LukAB_{mut50}.
66. The composition of claim 65, wherein the composition further comprises histidine-borate.
67. The composition of claim 65 or 66, wherein the buffer is phosphate buffer.
68. The composition of any one of claims 65 to 67, wherein the composition comprises the total protein, sucrose, and polysorbate 80 at the ratio of 2: 400: 1 in weight.
69. The composition of claim 68, wherein the concentration of the total protein is about 0.4 mg/ml, the concentration of the histidine-borate is about 25mM, the concentration of the phosphate buffer is about 1.2mM, the concentration of the sucrose is about 8% (weight/volume, 80 g/L), the concentration of the polysorbate 80 is about 0.02% (weight/volume, 0.2 g/L), and the pH is 7.5.
70. The composition of claim 67, wherein the total protein is about 200 µg per dose.
71. A kit comprising one or more containers and instructions for use, wherein the one or more containers comprise the pharmaceutical composition of any one of claims 1 to 22, 26 to 47.