CLAIMS

1. A method for the manufacture of a squalene-containing oil-in-water emulsion, the method comprising the steps of:

(b) microfluidizing a first emulsion formed using a mechanical homogenizer, wherein the first emulsion has a first average oil droplet size,

(c) circulating the first emulsion by transferring from a first emulsion container through a first microfluidization device to a second emulsion container, and then again through the same microfluidization device, so as to form a second emulsion having a second average oil droplet size which is less than the first average oil droplet size; and

(d) filtering the second emulsion.

2. The method according to claim 1, comprising before step (b):

(a) forming a first emulsion having a first average oil droplet size.

3. The method according to claim 1, wherein substantially all of the emulsion components from the first container are passed through the microfluidization device into the second container, and then substantially all of the emulsion components from the second container are passed through the microfluidization device back into the first container.

4. A method for the manufacture of a squalene-container oil-in-water emulsion, the method comprising the steps of:

(i) performing a type II circulation comprising transferring first emulsion components from a first container to a second container through a homogenizer, and then returning them from the second container to the first container through the same homogenizer, so as to form a first emulsion having a first average droplet size, wherein the homogenizer provides a shear rate of up to $1x10^6$ s^{-1}; and

(ii) microfluidizing the first emulsion to form a second emulsion having a second average oil droplet size which is less than the first average oil droplet size, wherein the microfluidizing occurs in an interaction chamber that provides a shear rate $>2.5x10^6 s^{-1}$.

5. The method according to claim 4, wherein substantially all of the emulsion components from the first container are passed through the homogenizer into the second container, and then substantially all of the emulsion components from the second container are passed through the homogenizer back into the first container.

6. The method according to claim 4, further comprising:

(iii) filtering the second emulsion.

7. The method of claim 4, wherein step (i) comprises two or more cycles of transferring the first emulsion's components from the first container to the second container and back again.

8. The method of claim 4, wherein during step (ii), the second emulsion is formed by

circulating the second emulsion components through a microfluidization device a plurality of times.

9. The method of claim 8, wherein the circulation of the second emulsion components comprises transferring the second emulsion components between a first emulsion container and a microfluidization device.

10. The method of claim 8, wherein the circulation of the second emulsion components comprises transferring the second emulsion components from a first emulsion container, through a first microfluidization device to a second emulsion container, and then through a second microfluidization device, wherein the first and second microfluidization devices are the same.

11. The method of claim 8, wherein after passing through the second microfluidization device, the second emulsion components are returned to the first emulsion container and the circulation of claim 8 is repeated one or more times.

12. A method for the manufacture of a squalene-containing oil-in-water emulsion, the method comprising steps of:

providing a first emulsion having a first average oil droplet size of 5000 nm or less, and wherein the number of oil droplets having a size of >1.2 μm in the first emulsion is $5x10^{11}$/ml or less;

circulating the first emulsion by transferring from a first emulsion container through a first microfluidization device to a second emulsion container, and then again through the same microfluidization device, so as to form a second emulsion having a second average oil droplet size which is less than the first average oil droplet size; and

filtering the second emulsion.

13. The method according to claim 1, wherein the number of oil droplets having a size of 1.2 μm in the first emulsion of $5x10^{11}$/ml or less.

14. The method of claim 1, wherein the second average oil droplet size is 500 nm or less.

15. The method according to claim 1 or 12, wherein the number of oil droplets having a size of > 1.2 μm in the second emulsion is $5x10^{10}$/ml or less.

16. A method of preparing a vaccine composition, comprising preparing the oil-in-water emulsion according to claim 1 or 12 and combining the emulsion with an antigen.

17. A method for preparing a vaccine kit comprising preparing the oil-in-water emulsion according claim 1 or 12 and packaging the emulsion into a kit as a kit component together with an antigen components.

18. The method of claim 17, wherein the kit components are in separate vials.

19. The method of claim 18, wherein the vials are made from borosilicate glass.

20. The method of claim 17, wherein the oil-in-water emulsion is a bulk adjuvant and the method comprises extracting unit doses from the bulk adjuvant for packaging as kit components.

21. The method of claim 17, wherein the antigen is an influenza virus antigen.

22. the method of claim 21, wherein the combination of the emulsion and the antigen forms a vaccine composition and wherein the vaccine composition includes about 15 µg, about 10 µg, about 7.5 µg, about 5 µg, about 3.8 µg, about 1.9 µg, or about 1.5 µg of hemagglutinin per influenza virus strain.

23. The method of claim 21, wherein the combination of the emulsion and the antigen forms a vaccine composition and wherein the vaccine composition includes a thiomersal or 2-phenoxyethanol preservative.

24. The method of any one of claims 1, 4 and 12, wherein the squalene-containing oil-in-water emulsion comprises polysorbate 80 and sorbitan trioleate.

25. The method of claim 24, wherein the squalene-containing oil-in-water emulsion comprises about 4.3% squalene, about 0.5% polysorbate 80 and about 0.48% sorbitan trioleate by weight.

26. The method of claim 16, wherein the squalene-containing oil-in-water emulsion comprises polysorbate 80 and sorbitan trioleate.

27. The method of claim 26, wherein the squalene-containing oil-in-water emulsion comprises about 4.3% squalene, about 0.5% polysorbate 80 and about 0.48% sorbitan trioleate by weight.

28. The method of claim 4, wherein step (i) further comprises first perfomrming a type I circulation of transferring the first emulsion components between a first container and a homogenizer before performing the type II circulation.