CLAIMS (Article 34 PCT, March 2012)

1. A method for the manufacture of an oil-in-water emulsion adjuvant, comprising:

   (i) preparing squalene from a composition comprising squalene from an animal source by a process comprising steps of: (a) a purification distillation carried out at a temperature T_1; and (b) a denaturing distillation carried out at a temperature T_2; wherein steps (a) and (b) may be performed in either order; T_1 and T_2 are sufficient to cause squalene to boil; $T_2 > T_1$; and $T_2 \geq 200°C$.

   (ii) preparing an emulsion using the squalene prepared in step (i).

2. A method for the manufacture of an oil-in-water emulsion adjuvant, comprising:

   (i) preparing squalene from squalene-containing composition by a process comprising steps of: (a) a purification distillation carried out at a temperature T_1; and (b) a denaturing distillation carried out at a temperature T_2; wherein steps (a) and (b) may be performed in either order; T_1 and T_2 are sufficient to cause squalene to boil; $T_1 < 140°C$ and $T_2 \geq 200°C$.

   (ii) preparing an emulsion using the squalene prepared in step (i).

3. The method of claim 1, wherein $T_1 < 140°C$.
4. The method of any preceding claim, wherein the squalene prepared in step (i) is kept sterile following distillation treatment and prior to the preparation of the emulsion in step (ii).
5. The method of claim 1 or claim 2, wherein the composition used in step (i) comprises one or more proteins.
6. The method of claim 5, wherein the composition comprises parvalbumin.
7. The method of any one of the preceding claims, wherein the denaturing distillation is carried out at a pressure of from 0.5 mm Hg to 5.0 mm Hg.
8. The method of any one of the preceding claims, wherein the purification distillation is carried out at a temperature of from 70 to 100°C.
9. The method of any one of the preceding claims, wherein the purification distillation is carried out at a pressure of from 0.5 μm Hg to 5 μm Hg.
10. The method of any one of the preceding claims, wherein the purification distillation is carried out prior to the denaturing distillation.
11. The method of any one of the preceding claims, wherein the composition used in step (i) is subjected to saponification.
12. The method of claim 11, wherein saponification comprises the addition of NaOH or KOH to the composition comprising squalene.

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13. A method for preparing a vaccine composition, comprising preparing an emulsion according to any preceding claim and combining the emulsion with an antigen.

14. A method for preparing a vaccine kit, comprising preparing an emulsion according to any one of claims 1 to 12 and packaging the emulsion into a kit as a kit component together with an antigen component.

15. The method of claim 14, wherein the kit components are in separate vials.

16. The method of claim 15, wherein the vials are made from borosilicate glass.

17. The method of any one of claims 14 to 16, wherein the antigen is an influenza virus antigen.

18. The method of claim 17, wherein the combination of the emulsion and the antigen forms a vaccine composition and wherein the vaccine composition includes about 15µg, about 10µg, about 7.5µg, about 5µg, about 3.8µg, about 3.75µg, about 1.9µg, or about 1.5µg of hemagglutinin per influenza virus strain.

19. The method of claim 17 or claim 18, 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.

20. An oil-in-water emulsion obtained by the method of any one of claims 1-19.

21. A method for re-distillation of a composition comprising 99% squalene or more, said re-distillation being a denaturing distillation carried out at a temperature T_2; wherein $T_2 \geq 200°C$.

22. A method for the manufacture of an oil-in-water emulsion adjuvant, comprising:

(i) obtaining squalene prepared by a process as defined in step (i) of claim 1 or claim 2

(ii) preparing an emulsion using the squalene prepared in step (i).

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CLAIMS

1. A method for the manufacture of an oil-in-water emulsion adjuvant, comprising:

   (i) preparing squalene from a composition comprising squalene from an animal source by a process comprising steps of: (a) a purification distillation carried out at a temperature T_1; and (b) a denaturing distillation carried out at a temperature T_2; wherein steps (a) and (b) may be performed in either order; T_1 and T_2 are sufficient to cause squalene to boil; $T_2 > T_1$; and $T_2 \geq 200°C$.

   (ii) preparing an emulsion using the squalene prepared in step (i).

2. The method of claim 1, wherein $T_1 < 140°C$.

3. The method of any preceding claim, wherein the squalene prepared in step (i) is kept sterile following distillation treatment and prior to the preparation of the emulsion in step (ii).

4. The method of claim 1, wherein the composition used in step (i) comprises one or more proteins.

5. The method of claim 4, wherein the composition comprises parvalbumin.

6. The method of any one of the preceding claims, wherein the denaturing distillation is carried out at a pressure of from 0.5 mm Hg (66.7 Pa) to 5.0 mm Hg (666.6 Pa).

7. The method of any one of the preceding claims, wherein the purification distillation is carried out at a temperature of from 70 to 100°C.

8. The method of any one of the preceding claims, wherein the purification distillation is carried out at a pressure of from 0.5 μm Hg (66.7 mPa) to 5 μm Hg (666.6 mPa).

9. The method of any one of the preceding claims, wherein the purification distillation is carried out prior to the denaturing distillation.

10. The method of any one of the preceding claims, wherein the composition used in step (i) is subjected to saponification.

11. The method of claim 10, wherein saponification comprises the addition of NaOH or KOH to the composition comprising squalene.

12. A method for preparing a vaccine composition, comprising preparing an emulsion according to any preceding claim and combining the emulsion with an antigen.

13. A method for preparing a vaccine kit, comprising preparing an emulsion according to any one of claims 1 to 11 and packaging the emulsion into a kit as a kit component together with an antigen component.

14. The method of claim 13, wherein the kit components are in separate vials.

15. The method of claim 14, wherein the vials are made from borosilicate glass.

16. The method of any one of claims 13 to 15, wherein the antigen is an influenza virus antigen.

17. The method of claim 16, wherein the combination of the emulsion and the antigen forms a vaccine composition and wherein the vaccine composition includes 15μg, 10μg, 7.5μg, 5μg, 3.8μg, 3.75μg, 1.9μg, or 1.5μg of hemagglutinin per influenza virus strain.

18. The method of claim 16 or claim 17, 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.