Reformulation of the immunogenic compositions with a non-ionic tonicity agent addresses the antigen stability problems. Additionally, Examples 3, 4, 13 and 16 demonstrate the removal of substantially all NaCl from the immunogenic formulation and its replacement with sorbitol as a tonicity agent does not have a detrimental impact on the stimulation of T cell responses.

Stability of immunogenic compositions is key and may be particularly challenging when in isolated locations were refrigeration may not be readily accessible. By reducing the presence of salts in the immunogenic compositions, the present inventors have been able to reduce the extent of changes observed when the immunogenic compositions are stored.

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps.

~~All documents referred to herein, including patents and patent applications, are incorporated by reference in their entirety.~~

CLAIMS

1. An immunogenic composition comprising an Rv1196 related antigen which comprises a sequence having at least 90% identity to SEQ ID No: 1 or a fragment of SEQ ID No: 1 which is at least 350 amino acids in length, wherein:
(i) the conductivity of the composition is 13 mS/cm or lower; and/or
(ii) the concentration of salts in said composition is 130 mM or lower; and/or
(iii) the concentration of sodium chloride in said composition is 130 mM or lower.

2. The immunogenic composition according to claim 1, wherein the conductivity of the composition is 10 mS/cm or lower.

3. The immunogenic composition according to claim 2, wherein the conductivity of the composition is 3 mS/cm or lower.

4. The immunogenic composition according to any one of claims 1 to 3, wherein the concentration of salts in said composition is 100 mM or lower.

5. The immunogenic composition according to claim 4, wherein the concentration of salts in said composition is 40 mM or lower.

6. The immunogenic composition according to any one of claims 1 to 5, wherein the concentration of sodium chloride in said composition is 100 mM or lower.

7. The immunogenic composition according to claim 6, wherein the concentration of sodium chloride in said composition is 40 mM or lower.

8. The immunogenic composition according to any one of claims 1 to 7, wherein the concentration of $CaCl_2$ in the immunogenic composition is 30 mM or lower.

9. The immunogenic composition according to any one of claims 1 to 8, wherein the concentration of $MgSO_4$ in the immunogenic composition is 60 mM or lower.

10. The immunogenic composition according to any one of claims 1 to 9, total concentration of NH_4^+, Mg^{2+} and Ca^{2+} ions is 40 mM or lower.

11. The immunogenic composition according to claim any one of claims 1 to 10, further comprising a non-ionic tonicity agent.

12. The immunogenic composition according to claim 11, wherein the non-ionic tonicity agent is a polyol.

13. The immunogenic composition according to claim 12, wherein the polyol is sorbitol.

14. The immunogenic composition according to claim 13, wherein the concentration of sorbitol is between about 4 and about 6% (w/v).

15. The immunogenic composition according to any one of claims 1 to 13, wherein the concentration of sucrose is between about 4 and about 6% (w/v).

16. The immunogenic composition according to any one of claims 1 to 15, further comprising one or more immunostimulants.

17. The immunogenic composition according to claim 16, wherein the immunostimulant is a saponin and/or a TLR4 agonist.

18. The immunogenic composition according to claim 17, wherein the immunostimulant(s) is/are in the form of liposomes.

19. The immunogenic composition according to either claim 17 or 18, wherein the saponin is QS21.

20. The immunogenic composition according to any one of claims 17 to 19, wherein the TLR4 agonist is 3-de-O-acylated monophoshoryl lipid A.

21. The immunogenic composition according to any one of claims 1 to 20, wherein the osmolality is 250 to 750 mOsm/kg.

22. The immunogenic composition according to any one of claims 1 to 21, wherein the composition is provided as a unit dose of between 50 ul and 1 ml.

23. The immunogenic composition according to claim 22, wherein the unit dose is between 100 ul and 750 ul.

24. The immunogenic composition according to claim 22 or 23, wherein a unit dose contains 1 to 100 ug of Rv1196 related protein.

25. The immunogenic composition according to claim 24, wherein a unit dose contains 5 to 50 ug of Rv1196 related protein.

26. The immunogenic composition according to any one of claims 22 to 25, wherein the unit dose is a human dose and contains between 1 and 100 ug of 3D-MPL.

27. The immunogenic composition according to any one of claims 22 to 26, wherein the unit dose is a human dose and contains between 1 and 100 ug of QS21.

~~26~~28. The immunogenic composition according to any one of claims 1 to ~~25~~27, wherein the pH of said composition is in the range 7.0 to 9.0.

~~27. The immunogenic composition according to any one of claims 1 to 26, wherein the Rv1196 related antigen comprises a sequence having at least 70% identity to SEQ ID No: 1.~~

~~28. The immunogenic composition according to claim 27, wherein the Rv1196 related antigen comprises a sequence having at least 90% identity to SEQ ID No: 1.~~

29. The immunogenic composition according to any one of claims 1 to ~~26~~28, wherein the Rv1196 related antigen comprises a fragment of SEQ ID No: 1 which is at least 350 amino acids in length.

30. The immunogenic composition according to any one of claims 1 to ~~26~~28, wherein the Rv1196 related antigen comprises a sequence having at least 90% identity to SEQ ID No: 7.

31. The immunogenic composition according to any one of claims 1 to ~~26~~28, wherein the Rv1196 related antigen consists of a sequence having at least 90% identity to SEQ ID No: 7.

32. The immunogenic composition according to claim 31, wherein the Rv1196 related antigen comprises the amino acid sequence of SEQ ID No: 7.

33. The immunogenic composition according to claim 32, wherein the Rv1196 related antigen consists of the amino acid sequence of SEQ ID No: 7.

~~34. The immunogenic composition according to any one of claims 1 to 33, wherein at least 80% of the antigenicity of the immunogenic composition remains after storage at 25 °C for a period of 24 hours.~~

~~35~~34. The immunogenic composition according to any one of claims 1 to ~~34~~33, for use in medicine.

~~35~~36 Use of an immunogenic composition according to any one of claims 1 to ~~33~~34 in the manufacture of a medicament.

~~37. A method~~ for the prophylaxis, treatment or amelioration of infection by mycobacteria, such as infection by *Mycobacterium tuberculosis*, ~~comprising the administration of a safe and effective amount of an immunogenic composition according to any one of claims 1 to 34~~.

~~38~~36. A process for the making an immunogenic composition according to any one of claims 1 to ~~34~~33 comprising the steps:

a) lyophilising an Rv1196 related antigen which comprises a sequence having at least 90% identity to SEQ ID No: 1 or a fragment of SEQ ID No: 1 which is at least 350 amino acids in length; and

b) reconstituting the lyophilised Rv1196 related antigen of step a) with an aqueous solution wherein:

(i) the conductivity of the solution is 13 mS/cm or lower; and/or

(ii) the concentration of salts in said solution is 130 mM or lower; and/or

(iii) the concentration of sodium chloride in said solution is 130 mM or lower.

~~39. A method for the manufacture of a stable immunogenic composition according to any one of claims 1 to 34 comprising the steps:~~

~~a) lyophilising an Rv1196 related antigen; and~~

~~b) reconstituting the lyophilised Rv1196 related antigen of step a) with an aqueous solution wherein:~~

~~(i) the conductivity of the solution is 13 mS/cm or lower; and/or~~

~~(ii) the concentration of salts in said solution is 130 mM or lower; and/or~~

~~(iii) the concentration of sodium chloride in said solution is 130 mM or lower;~~

~~wherein at least 80% of the antigenicity of the immunogenic composition remains after storage at 25 °C for a period of 24 hours.~~

~~40~~37. A kit comprising:

a) a lyophilised Rv1196 related antigen which comprises a sequence having at least 90% identity to SEQ ID No: 1 or a fragment of SEQ ID No: 1 which is at least 350 amino acids in length; and

b) an aqueous solution wherein:

(i) the conductivity of the solution is 13 mS/cm or lower; and/or

(ii) the concentration of salts in said solution is 130 mM or lower; and/or

(iii) the concentration of sodium chloride in said solution is 130 mM or lower.

~~41~~38. The process according to claim 36~~38, method according to claim 39~~ or kit according to claim ~~40~~37 wherein the aqueous solution comprises an immunostimulant.

~~42~~39. The process~~, method~~ or kit according to claim ~~41~~38, wherein the aqueous solution comprises a saponin and a TLR4 agonist, in a liposomal formulation, and a non-ionic tonicity agent.

~~43~~40. The process~~, method~~ or kit according to claim ~~42~~39, wherein the non-ionic tonicity agent is sorbitol.

~~44~~41. The process~~, method~~ or kit according to claim ~~43~~40, wherein the concentration of sorbitol is between about 2.5 and about 15% (w/v).

~~45~~42. The process~~, method~~ or kit according to any one of claims ~~42~~ 39 to ~~44~~41, wherein the saponin is QS21.

~~46~~43. The process~~, method~~ or kit according to any one of claims ~~42~~39 to ~~45~~42, wherein the TLR4 agonist is 3-de-O-acylated monophoshoryl lipid A.