CLAIMS

What is claimed is:

1. A method for the production of purified *C. difficile* toxin, the method comprising:
   a) applying an impure aqueous solution comprising *C. difficile* toxin to a hydrophobic interaction support to bind *C. difficile* toxin thereto;
   b) eluting the bound *C. difficile* toxin from the hydrophobic interaction support;
   c) applying the eluted *C. difficile* toxin from step (b) to an anion-exchange support selected from the group consisting of: (i) a polymethacrylate resin with quaternary amine functional groups; and (ii) a polyethersulfone membrane with quaternary amine functional groups, to bind *C. difficile* toxin thereto; and,
   d) eluting the bound *C. difficile* toxin from the anion-exchange support.
2. The method of claim 1 further comprising subjecting the impure aqueous solution comprising *C. difficile* toxin to tangential flow filtration before step (a).
3. The method of claim 1 or 2 further comprising recovering purified *C. difficile* toxin following step (d).
4. The method of any one of claims 1-3 wherein the toxin is *C. difficile* toxin A.
5. The method of any one of claims 1-3 wherein the toxin is *C. difficile* toxin B.
6. The method of any one of claims 1-5 wherein the hydrophobic interaction support is a matrix with attached butyl S groups or a matrix with attached propyl groups.
8. The method of claim 6 wherein the hydrophobic interaction support is a matrix with attached propyl groups and the impure aqueous solution is applied to the hydrophobic interaction support in the presence of ammonium sulphate.
9. The method of claim 8 wherein the impure aqueous solution is applied to the hydrophobic interaction support in the presence of about 0.8 to about 1.0M ammonium sulphate.
10. The method of any one of claims 1-5, 8 or 9 wherein step (b) is performed in the absence of an organic solvent.

11. The method of claim 6 wherein the hydrophobic interaction support is a Sepharose resin with attached butyl S groups.

12. The method of claim 6 or 11 wherein about 70% of the toxin A and / or about 90% of the toxin B in the impure aqueous solution is recovered after steps (a) and (b).

13. The method of any one of claims 6, 11 or 12 wherein step (b) is performed in the absence of an organic solvent.

14. The method of claim 1 wherein the aqueous solution comprising *C. difficile* toxin comprises *C. difficile* toxin A and *C. difficile* toxin B and, in step (c), *C. difficile* toxin A and *C. difficile* toxin B are individually eluted from the anion-exchange support.

15. The method of any one of claims 1-14 wherein the anion exchange support is a non-polysaccharide-based material.

16. The method of any one of claims 15 wherein the anion exchange support is a polymethacrylate resin with bound quaternary amine functional groups.

17. The method of any one of claims 16 wherein the anion exchange support comprises a strong anion exchanger, the moiety $-O\text{-}R\text{-}N^{+}\text{-}(CH_3)_3$ wherein R is a polymer; and / or, the anion exchange support is Tosoh Super Q 650M.

18. The method of any one of claims 14-17 wherein toxin A is eluted using a low salt buffer.

19. The method of claim 19 wherein toxin A the low salt buffer comprises 2 to about 32 mM $MgCl_2$ or about 125 to about 160 mM NaCl.

20. The method of any one of claims 14-17 wherein toxin B is eluted using a high salt buffer.

21. The method of claim 20 wherein the high salt buffer comprises about 120 to about 150 mM $MgCl_2$ or about 450 to about 550 mM NaCl.

22. The method of any one of claims 1 to 21 wherein the impure aqueous solution of *C. difficile* toxin is obtained by growing cells of *C. difficile* in a growth medium to provide a culture broth containing *C. difficile* toxin and grown cells, and separating

the culture broth from the grown cells to provide the impure aqueous solution of *C. difficile* toxin.

23. The method of claim 22 wherein the growth medium comprises sorbitol.

24. A purified *C. difficile* toxin obtained in accordance to the method of any one of claims 1 to 12 wherein the purified *C. difficile* toxin has a purity of about 90% or greater.

25. The purified *C. difficile* toxin of claim 24 wherein the toxin has a purity of about 94% or greater.

26. The purified *C. difficile* toxin of claim 25 wherein the toxin has a purity of about 98% or greater.

27. The purified *C. difficile* toxin A of claim 26 having a purity of about 99% or greater.

28. The purified *C. difficile* toxin of claim 24 wherein the toxin is *C. difficile* Toxin A.

29. The purified *C. difficile* toxin of claim 24 wherein the toxin is *C. difficile* Toxin B.

30. A purified *C. difficile* toxin obtained in accordance to the method of any one of claims 1-23 wherein the purified *C. difficile* toxin is substantially free of solvent.

31. A purified *C. difficile* toxin obtained in accordance to any one of claims 1-23 wherein the purified *C. difficile* toxin has no detectable alcohol dehydrogenase.

32. The method of any one of claims 1-23 further comprising inactivating recovered purified *C. difficile* toxin with a chemical agent to provide a *C. difficile* toxoid.

33. The method of claim 32 wherein the recovered purified *C. difficile* toxin is inactivated with formaldehyde to provide a *C. difficile* toxoid.

34. A *C. difficile* toxoid obtained in accordance to the method of claim 32 or 33.

35. The *C. difficile* toxoid of claim 34 wherein the toxoid is toxoid A or toxoid B.

36. A composition comprising the *C. difficile* toxoid of claim 34 or 35.

37. A composition comprising the *C. difficile* toxoid of claim 34 or 35 and a pharmaceutically acceptable excipient.

38. The composition of claim 36 or 37 wherein the *C. difficile* toxoid is toxoid A or toxoid B.

39. The composition of claim 36 or 37 comprising *C. difficile* Toxoid A and *C. difficile* B at a ratio of 3:2 by weight.

40. A method of eliciting an immune response in a subject, the method comprising administering to the subject a composition of any one of claims 36-39.

41. A method of preventing or treating symptomatic *C. difficile* infection in a subject, the method comprising administering to the subject a composition of any one of claims 36-39.

42. The method of claim 41 wherein the subject does not have symptoms of but is at risk of developing symptomatic *C. difficile* infection.

43. The method of claim 41 wherein the subject has symptomatic *C. difficile* infection.

CLAIMS

What is claimed is:

1. A method for the production of purified *C. difficile* toxin, the method comprising:

    a) applying an impure aqueous solution comprising *C. difficile* toxin to a hydrophobic interaction support to bind *C. difficile* toxin thereto;

    b) eluting the bound *C. difficile* toxin from the hydrophobic interaction support;

    c) applying the eluted *C. difficile* toxin from step (b) to an anion-exchange support selected from the group consisting of: (i) a polymethacrylate resin with quaternary amine functional groups; and (ii) a polyethersulfone membrane with quaternary amine functional groups, to bind *C. difficile* toxin thereto; and,

    d) eluting the bound *C. difficile* toxin from the anion-exchange support.

2. The method of claim 1 further comprising subjecting the impure aqueous solution comprising *C. difficile* toxin to tangential flow filtration before step (a).

3. The method of claim 1 or 2 further comprising recovering purified *C. difficile* toxin following step (d).

4. The method of any one of claims 1-3 wherein the toxin is *C. difficile* toxin A.

5. The method of any one of claims 1-3 wherein the toxin is *C. difficile* toxin B.

6. The method of any one of claims 1-5 wherein the hydrophobic interaction support is a matrix with attached butyl S groups or a matrix with attached propyl groups.

7. The method of claim 6 wherein the hydrophobic interaction support is a matrix with attached propyl groups and the impure aqueous solution is applied to the hydrophobic interaction support in the presence of ammonium sulphate.

8. The method of claim 7 wherein the impure aqueous solution is applied to the hydrophobic interaction support in the presence of about 0.8 to about 1.0M ammonium sulphate.

9. The method of any one of claims 1-5, 7 or 8 wherein step (b) is performed in the absence of an organic solvent.

10. The method of claim 6 wherein the hydrophobic interaction support is a Sepharose resin with attached butyl S groups.

11. The method of claim 6 or 9 wherein about 70% of the toxin A and / or about 90% of the toxin B in the impure aqueous solution is recovered after steps (a) and (b).

12. The method of any one of claims 6, 10 or 11 wherein step (b) is performed in the absence of an organic solvent.

13. The method of claim 1 wherein the aqueous solution comprising *C. difficile* toxin comprises *C. difficile* toxin A and *C. difficile* toxin B and, in step (c), *C. difficile* toxin A and *C. difficile* toxin B are individually eluted from the anion-exchange support.

14. The method of any one of claims 1-13 wherein the anion exchange support is a non-polysaccharide-based material.

15. The method of any one of claims 14 wherein the anion exchange support is a polymethacrylate resin with bound quaternary amine functional groups.

16. The method of any one of claims 15 wherein the anion exchange support comprises a strong anion exchanger, the moiety $–O\text{-}R\text{-}N^{+}\text{-}(CH_3)_3$ wherein R is a polymer; and / or, the anion exchange support is Tosoh Super Q 650M.

17. The method of any one of claims 13-16 wherein toxin A is eluted using a low salt buffer.

18. The method of claim 17 wherein toxin A the low salt buffer comprises 2 to about 32 mM $MgCl_2$ or about 125 to about 160 mM NaCl.

19. The method of any one of claims 13-16 wherein toxin B is eluted using a high salt buffer.

20. The method of claim 19 wherein the high salt buffer comprises about 120 to about 150 mM $MgCl_2$ or about 450 to about 550 mM NaCl.

21. The method of any one of claims 1 to 20 wherein the impure aqueous solution of *C. difficile* toxin is obtained by growing cells of *C. difficile* in a growth medium to provide a culture broth containing *C. difficile* toxin and grown cells, and separating the culture broth from the grown cells to provide the impure aqueous solution of *C. difficile* toxin.

22. The method of claim 21 wherein the growth medium comprises sorbitol.

23. A purified *C. difficile* toxin obtained in accordance to the method of any one of claims 1 to 11 wherein the purified *C. difficile* toxin comprises 90% or greater *C. difficile* toxin A and *C. difficile* toxin B.

24. The purified *C. difficile* toxin of claim 23 wherein the toxin comprises 94% or greater *C. difficile* toxin A and *C. difficile* toxin B.

25. The purified *C. difficile* toxin of claim 24 wherein the toxin comprises 98% or greater *C. difficile* toxin A and *C. difficile* toxin B.

26. The purified *C. difficile* toxin of claim 25 comprises 99% or greater *C. difficile* toxin A and *C. difficile* toxin B.

27. A purified *C. difficile* toxin obtained in accordance to the method of any one of claims 1-22 wherein the purified *C. difficile* toxin is free of solvent.

28. A purified *C. difficile* toxin obtained in accordance to any one of claims 1-22 wherein the purified *C. difficile* toxin has no detectable alcohol dehydrogenase as determined by the lack of a 100 kDa protein in the toxin as determined by SDS-PAGE.

29. The method of any one of claims 1-22 further comprising inactivating recovered purified *C. difficile* toxin with a chemical agent to provide a *C. difficile* toxoid.

30. The method of claim 29 wherein the recovered purified *C. difficile* toxin is inactivated with formaldehyde to provide a *C. difficile* toxoid.

31. A composition comprising *C. difficile* toxoid obtained in accordance to the method of claim 30, and 0.004% or 0.008% formaldehyde.

32. The composition of claim 31 wherein the toxoid is toxoid A or toxoid B.

33. The composition of claim 31 and a pharmaceutically acceptable excipient.

34. The composition of claim 33 wherein the *C. difficile* toxoid is toxoid A or toxoid B.

35. The composition of claim 31 comprising *C. difficile* Toxoid A and *C. difficile* B at a ratio of 3:2 by weight.

36. Use of a composition of any one of claims 31-35 in the manufacture of a medicament for eliciting an immune response in a subject.

37. Use of a composition of any one of claims 31-35 in the manufacture of a medicament for preventing or treating symptomatic *C. difficile* infection in a subject.

38. The use of claim 37 wherein the subject does not have symptoms of but is at risk of developing symptomatic *C. difficile* infection.

39. The use of claim 37 wherein the subject has symptomatic *C. difficile* infection.