CLAIMS

What is claimed is:

1. A method of producing a *C. difficile* toxoid, the method comprising inactivating purified *C. difficile* Toxin A or purified *C. difficile* Toxin B by incubation with about 0.15%-0.5% formaldehyde (w/v) at about 17-32°C for about two to about 21 days.

2. The method of claim 1 wherein Toxin A is incubated with about 0.2% formaldehyde at about 25°C for about two days to produce Toxoid A.

3. The method of claim 1 or 2 wherein Toxin B is incubated with about 0.4% formaldehyde at about 25°C for about 13 days to produce Toxoid B.

4. A composition comprising Toxoid A prepared by the method of claim 1 or 2, and / or Toxoid B prepared by the method of claim 1 or 3.

5. A method for preparing an immunogenic composition comprising purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B by combining purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B with a composition comprising a residual amount of formaldehyde.

6. The method of claim 5 wherein the purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B are stable at 37°C for up to about six weeks.

7. The method of claim 5 or 6 wherein the residual amount of formaldehyde is about 0.001% - 0.025% (w/v).

8. A composition prepared using a method of any one of claims 5-7.

9. A method for preparing an immunogenic composition comprising purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B, the method comprising:

inactivating purified *C. difficile* Toxin A and purified *C. difficile* Toxin B by incubation with about 0.15%-0.5% formaldehyde (w/v) at about 17-32°C for about two to about 21 days; and,

combining purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B with a composition comprising a residual amount of formaldehyde.

10. The method of claim 9 wherein the purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B are stable at 37°C for up to about six weeks.

11. The method of claim 9 or 10 wherein the residual amount of formaldehyde is about 0.001%, 0.004%, 0.008%, or 0.016% (w/v).

12. The method of any one of claims 1-3, 5-7 or 9-13 wherein the Toxoid A and / or Toxoid B is maintained in a composition of 20 mM citrate, pH 7.5, 8% sucrose, and 0.016% formaldehyde.

13. The method of any one of claims 1-3, 5-7, or 9-13 wherein the composition comprising Toxoid A and / or Toxoid B is lyophilized.

14. A composition prepared using a method of any one of claims 9-13.

15. The method of any one of claims 1-3, 5-7, or 9-13, the method further comprising providing a *C. difficile* culture comprising Toxin A and Toxin B and purifying the Toxin A and Toxin B from the culture.

16. *C. difficile* Toxoid A produced in accordance to the method of any one of claims 1, 2, 5-7, or 9-13.

17. *C. difficile* Toxoid B produced in accordance to the method of any one of claims 1, 3, 5-7, or 9-13.

18. A vaccine composition comprising *C. difficile* Toxoid A and *C. difficile* Toxoid B, wherein the *C. difficile* Toxoid A is produced in accordance to the method of any one of claims 1, 2, 5-7, or 9-13 and Toxoid B is produced in accordance to the method of any one of claims 1, 3, 5-7, or 9-13.

19. The vaccine composition of claim 18 wherein the vaccine composition comprises about 0.001% to 0.020% formaldehyde.

20. The vaccine composition of claim 19 wherein the vaccine composition comprises about 0.004% formaldehyde.

21. The vaccine composition of claim 19 wherein the vaccine composition comprises 0.008 % formaldehyde.

22. The vaccine composition of claim 19 wherein the vaccine composition comprises about 0.016% formaldehyde.

23. A vaccine composition of any one of claims 18-22 wherein the Toxoid A and the Toxoid B are present in the composition in a A:B ratio of 5:1 to 1:5.

24. A vaccine composition of claim 23 wherein the Toxoid A and the Toxoid B are present in the composition in a ratio of A:B of 3:1 or 3:2.

25. A vaccine composition of any one of claims 18-24 wherein the vaccine composition is freeze dried, spray dried, or foam dried.

26. A vaccine composition of any one of claims 18-24 wherein the vaccine composition is in liquid form.

27. A vaccine composition of any one of claims 18-25 further comprising one or more pharmaceutically acceptable excipients.

28. A composition of any one of claims 4, 8, 14 or 18-26 comprising a citrate, phosphate, glycine, carbonate, or bicarbonate buffer, or a pH-controlled aqueous solution.

29. The composition of claim 28 further comprising a sugar, or sugar alcohol.

30. The composition of claim 29 comprising sucrose and citrate.

31. A method for converting *C. difficile* Toxin A into a toxoid (Toxoid A) by incubating the Toxin A with about 0.21% (w/v) formaldehyde at about 25°C for about six to 13 days.

32. The method of claim 31 wherein the incubating is for about six days.

33. The method of claim 31 or 32 wherein the incubating occurs in 0.21% (w/v) formaldehyde / 100 mM PO_4, pH 7.

34. A method for converting *C. difficile* Toxin B into a toxoid (Toxoid B) by incubating the Toxin B with about 0.42% (w/v) formaldehyde at about 25°C for about 13 to about 20 days.

35. The method of claim 34 wherein the incubating is for about 13 days.

36. The method of claim 34 or 35 wherein the incubating occurs in 0.42% (w/v) formaldehyde / 100 mM PO_4, pH 7.

CLAIMS

What is claimed is:

1. A method of producing a *C. difficile* toxoid, the method comprising inactivating purified *C. difficile* Toxin A or purified *C. difficile* Toxin B by incubation with 0.15%-0.8% formaldehyde (w/v) at 15-32°C for two to 21 days.

2. The method of claim 1, wherein:
   (a) said incubation is with 0.15%-0.5% formaldehyde (w/v); and/or
   (b) said incubation is at 17-32^{o}C.

3. The method of claim 1 or 2, wherein:
   (a) Toxin A is incubated with 0.2% formaldehyde at 25°C for two or six days to produce Toxoid A; and/or
   (b) Toxin B is incubated with 0.4% formaldehyde at 25°C for 13 days to produce Toxoid B.

4. The method of claim 1 or 2, wherein:
   (a) *C. difficile* Toxin A is converted into a toxoid (Toxoid A) by incubating the Toxin A with 0.21% (w/v) formaldehyde at 25°C for six to 13 days*, e.g.* for six days, optionally wherein the incubating occurs in 0.21% (w/v) formaldehyde / 100 mM $P0_4$, pH 7; or
   (b) *C. difficile* Toxin B is converted into a toxoid (Toxoid B) by incubating the Toxin B with 0.42% (w/v) formaldehyde at 25°C for 13 to 20 days, *e.g.* for 13 days, optionally wherein the incubating occurs in 0.42% (w/v) formaldehyde / 100 mM $P0_4$, pH 7.

5. A method for preparing a composition, wherein either:
   (a) the composition is an immunogenic composition comprising purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B, wherein said composition is prepared by combining purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B with a composition comprising a residual amount of formaldehyde, wherein the purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B are stable at 37°C for up to six weeks, and wherein the residual amount of formaldehyde is 0.001% - 0.008% (w/v);
   (b) the composition is an immunogenic composition comprising purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B, wherein the method comprises:

(i) inactivating purified *C. difficile* Toxin A and purified *C. difficile* Toxin B by incubation with 0.15%-0.8% formaldehyde (w/v) at 15-32°C for two to 21 days; and,

(ii) combining purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B with a composition comprising a residual amount of formaldehyde; or

(c) the composition is a toxoid composition and the method comprises:

(i) inactivating purified *C. difficile* Toxin A and purified *C. difficile* Toxin B by incubation with 0.15% to 0.8% formaldehyde (w/v) at 15 to 32°C for two to 21 days; and,

(ii) combining the toxoids to produce a toxoid-containing immunological composition and/or vaccine that contains only a residual amount of formaldehyde.

6. The method of claim 5, wherein:

(a) said incubation is with 0.15%-0.5% formaldehyde (w/v); and/or

(b) said incubation is at 17-32^{o}C.

7. The method of claim 5 or 6, wherein:

(a) the *C. difficile* Toxoid A and C. difficile Toxoid B are stable at 37°C for up to six weeks;

(b) the residual amount of formaldehyde is 0.001%, 0.004%, 0.008%, or 0.016% (w/v);

(c) the composition comprises 20 mM citrate, pH 7.5, 8% sucrose, and 0.016% formaldehyde;

(d) the composition is lyophilized; and/or

(e) the method further comprises providing a *C. difficile* culture comprising Toxin A and Toxin B and purifying the Toxin A and Toxin B from the culture.

8. A composition, wherein:

(a) the composition is a composition comprising Toxoid A and/or Toxoid B prepared by the method of any one of claims 1-4; or

(b) the composition is prepared using the method of any one of claims 5-7.

9. *C. difficile* Toxoid A or B produced in accordance with the method of any one of the preceding claims.

10. A vaccine composition comprising *C. difficile* Toxoid A and *C. difficile* Toxoid B, wherein the *C. difficile* Toxoid A and Toxoid B are produced in accordance with the method of any one of claims 1-7.

11. The vaccine composition of claim 10, wherein the vaccine composition comprises:
    (a) 0.001% to 0.020% formaldehyde;
    (b) 0.004% formaldehyde;
    (c) 0.008% formaldehyde; or
    (d) 0.016% formaldehyde.

12. A vaccine composition of claim 10 or 11, wherein the Toxoid A and the Toxoid B are present in the composition in a A:B ratio of 5:1 to 1:5 or in a ratio of A:B of 3:1 or 3:2.

13. A vaccine composition of any one of claims 10-12, wherein the vaccine composition:
    (a) is freeze dried, spray dried, foam dried or in liquid form; and/or
    (b) further comprises one or more pharmaceutically acceptable excipients.

14. A composition of claim 8, or a vaccine composition of any one of claims 10-13, comprising a citrate, phosphate, glycine, carbonate, or bicarbonate buffer, or a pH-controlled aqueous solution.

15. The composition of claim 14, further comprising a sugar, or sugar alcohol, optionally wherein the composition comprises sucrose and citrate.