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, the purified *C. difficile* Toxin A or purified *C. difficile* Toxin B having a purity of about 75% or more.

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, the purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B having a purity of about 75% or more, wherein said composition is prepared by combining the purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B with a composition comprising formaldehyde, and wherein the immunogenic composition comprises 0.008% to 0.025% formaldehyde (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 the purified C. difficile Toxin A and purified C. difficile Toxin B having a purity of about 75% or more; and,

(ii) combining purified *C. difficile* Toxoid A and purified *C. difficile* Toxoid B with a composition comprising formaldehyde, wherein the immunogenic composition comprises 0.008% to 0.025% formaldehyde (w/v); 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, the purified C. difficile Toxin A and the purified C. difficile Toxin B having a purity of about 75% or more; and,

(ii) combining the toxoids to produce a toxoid-containing immunological composition and/or vaccine that contains 0.008% to 0.025% formaldehyde (w/v).

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 0.008%, 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:

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

(b) 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) 0.008% to 0.025% formaldehyde (w/v);
    (b) 0.008% formaldehyde (w/v); or
    (c) 0.016% formaldehyde (w/v).

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.

16. An immunogenic composition comprising *C. difficile* Toxoid A and Toxoid B and 0.004% formaldehyde, wherein:
    the *C. difficile* Toxoid A is produced by:
        incubation with 0.2% formaldehyde (w/v) at 15°C for 13 days; or,
        incubation with 0.2% formaldehyde (w/v) at 25°C for six days; and,
    the *C. difficile* Toxoid B is produced by:
        incubation with 0.8% formaldehyde (w/v) at 15°C for 13 days; or,
        incubation with 0.4% formaldehyde (w/v) at 25°C for 13 days.

17. The composition of claim 16 wherein the *C. difficile* Toxoid A is produced by incubation with 0.2% formaldehyde (w/v) at 15°C for 13 days, and the *C. difficile* Toxoid B is produced by incubation with 0.8% formaldehyde (w/v) at 15°C for 13 days.

18. The composition of claim 16 wherein the *C. difficile* Toxoid A is produced by incubation with 0.2% formaldehyde (w/v) at 25°C for six days, and the *C. difficile* Toxoid B is produced by incubation with 0.4% formaldehyde (w/v) at 25°C for 13 days.