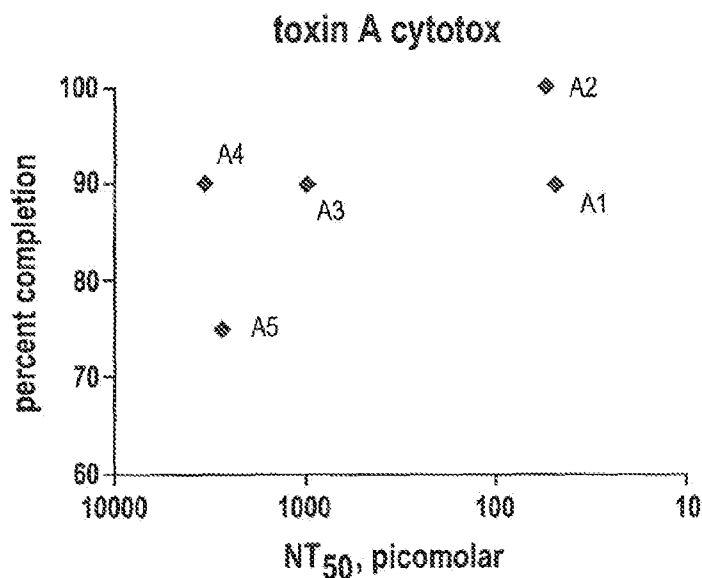




- (51) International Patent Classification:
A61K 39/40 (2006.01)
- (21) International Application Number:
PCT/US2014/028637
- (22) International Filing Date:
14 March 2014 (14.03.2014)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/794,071 15 March 2013 (15.03.2013) US
- (71) Applicants: **SANOFI PASTEUR BIOLOGICS, LLC** [US/US]; 38 Sidney Street, Cambridge, MA 02139 (US).
VALNEVA [FR/FR]; 70 Rue Saint Jean De Dieu, F-69007 Lyon (FR).
- (72) Inventors: **ANDERSON, Stephen, F.**; 7 Woodland St., Arlington, MA 02476 (US). **ANOSOVA, Natalie**; 105 Fifer Lane, Lexington, MA 02420 (US). **BELTRAM-INELLI, Nicola**; 8, Rue Des Alouettes, F-69008 Lyon (FR). **GARRONE, Pierre**; 49, Rue Saint Romain, F-69008 Lyon (FR). **KLEANTHOS, Harold**; 1 Peachtree Lane, Chelmsford, MA 01824 (US).
- (72) Inventor: **MEHTALI, Majid** (deceased).
- (72) Inventor: **ZHANG, Jianxin**; 2 Farley Lane, Acton, MA 01720 (US).
- (74) Agent: **DONALDSON, Timothy, B.**; MH2 Technology Law Group, 1951 Kidwell Drive, Suite 550, Tysons Corner, VA 22182 (US).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

[Continued on next page]

(54) Title: ANTIBODIES AGAINST *CLOSTRIDIUM DIFFICILE* TOXINS AND METHODS OF USING THE SAME**FIG. 1A**

(57) Abstract: Monoclonal antibodies, or antigen-binding fragments thereof, that bind to *Clostridium difficile* (*C. difficile*) toxin A or toxin B and methods of using the same to detect or treat *C. difficile* infections and/or *C. difficile*-associated disease.



SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

Published:

- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
- *with sequence listing part of description (Rule 5.2(a))*

ANTIBODIES AGAINST *CLOSTRIDIUM DIFFICILE*
TOXINS AND METHODS OF USING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of, and relies on the filing date of, U.S. provisional patent application number 61/794,071, filed 15 March 2013, the entire disclosure of which is incorporated herein by reference.

SEQUENCE LISTING

[002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 14, 2014, is named 0171.0002-PCT_SL.txt and is 231,155 bytes in size.

FIELD

[003] This application relates generally to antibodies against *Clostridium difficile* (*C. difficile*) toxins and methods of using the same to detect or treat *C. difficile* infections and/or *C. difficile*-associated disease.

BACKGROUND

[004] *C. difficile* is a gram positive, anaerobic bacterium that causes gastrointestinal disease in humans. The bacteria are transmitted through feces and spread to food and other surfaces when people who are infected do not thoroughly wash their hands. *C. difficile* form spores that can persist outside of a human body for weeks or even months. Symptoms of *C. difficile* infection can range from diarrhea to life-threatening inflammation of the colon. *C. difficile* infections are the most common cause of infectious diarrhea in the healthcare setting (Cohen SH et al., Infect Control Hosp Epidemiol 2010; 31:431-55).

[005] *C. difficile* infections are more frequent in older adults in a hospital or long-term care facility and commonly occur during or following antibiotic treatment, which disrupts the normal flora of the gut and permits the opportunistic *C. difficile* to colonize the gut. In more severe infections, the colon can become inflamed (colitis) or form patches of raw tissue that can bleed or produce pus (pseudomembranous colitis). Symptoms of severe *C. difficile* infection include watery diarrhea, abdominal cramping and pain, nausea, fever, dehydration, and weight loss.

[006] *C. difficile* produces two cytotoxic enterotoxins, toxin A and toxin B, that have been identified as targets for therapeutic intervention. Toxins A and B are released by the bacteria into the gut and believed to be involved in causing *C. difficile*-associated disease (CDAD) or the symptoms associated with CDAD. Symptoms of CDAD can be reproduced in animal models by transfer of the toxins. Toxins A and B have glucosyl transferase activity, which is capable of transferring glucose residues from UDP-glucose to Rho-GTPases, thereby inactivating the GTPase proteins found inside the target host cell. Inhibition of the Rho-GTPases results in depolymerization of actin filaments within the host cell, leading to dysregulation of actin cytoskeleton and tight junction integrity, which in turn produces increased cell permeability and loss of barrier function, diarrhea, inflammation, and an influx of molecules associated with the innate immune response. Toxins A and B are found in fecal samples and can be used to diagnose *C. difficile* infection.

[007] Once a *C. difficile* infection has been identified, it is best, if possible, to stop taking the antibiotic that caused the infection. The typical treatment for *C. difficile* is another antibiotic, usually metronidazole or fidaxomicin, for mild to moderate illness, or vancomycin for more severe symptoms. If effective, these antibiotics prevent *C. difficile* from growing and allow the normal flora to return and colonize the gut. However, in recent years, strains resistant to these antibiotics have been identified, as well as higher recurrence or reinfection rates. Another approach is taking probiotics. Probiotics are non-pathogenic microorganisms, such as bacteria or yeast that compete with *C. difficile* and help restore balance to the intestinal tract. For patients with severe pain or inflammation, another option is surgery to remove the diseased portion of the colon.

[008] Therapeutic antibodies have been a rapidly emerging field in recent years and provide another possible strategy for treating *C. difficile* infections. Patients infected with *C. difficile* experience a wide range of symptoms, the reasons for which are not fully understood. However, antibodies may play a role, as patients who experience milder symptoms tend to possess high titers of anti-toxin A antibody serum titers, while patients susceptible to recurring infections have demonstrated low titers of circulating anti-toxin A antibodies (Hussack and Tanha, Toxins, 2010, (2):998-1018). US2012/269841 describes murine antibodies that bind mutant *C. difficile* toxin-A or anti-toxin B. WO2011/130650 describes murine anti toxin-A and anti-toxin B antibodies that were optionally humanized to reduce their immunogenicity, including the lead anti-toxin A antibody, PA-50, and the lead anti-toxin B antibody, PA-41. U.S. Patent No. 8,257,709 describes anti toxin-A and anti-toxin B antibodies that were generated in transgenic mice, including the lead anti-toxin A antibody, 3D8, and the lead anti-toxin B

antibody, 124-152. The transgenic mice contain human immunoglobulin genes encoding certain unrearranged human heavy chain and kappa light chain sequences and, thus, are less immunogenic than murine antibodies.

[009] There remains an unmet need for effective treatment of *C. difficile* infection, particularly non-invasive treatments that are effective against antibiotic-resistant strains of *C. difficile* and/or against high-toxin producing strains, including therapeutic antibodies that present reduced immunogenicity while providing high binding affinity for *C. difficile* toxin A or toxin B and/or potent neutralization activity.

SUMMARY

[0010] The present disclosure provides antibodies that bind to *C. difficile* toxin A or *C. difficile* toxin B and can be used, for example, in methods of detecting or treating *C. difficile* infection.

[0011] One embodiment is directed to monoclonal antibodies that bind to *C. difficile* toxin A. The anti-toxin A antibodies are preferably human antibodies. In one embodiment, the anti-toxin A antibodies are recombinant antibodies.

[0012] One embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the C-terminal receptor domain (CTD) of *C. difficile* toxin A, wherein the epitope comprises the amino acid sequence X₁TGWQTI (SEQ ID NO:232), where X₁ is A or V or the amino acid sequence of X₂TGWQTIX₃GKX₄YYF (SEQ ID NO:233), where X₂ is A or V, X₃ is N or D and X₄ is K or V.

[0013] Another embodiment is directed to an isolated monoclonal antibody that binds to *C. difficile* toxin A, wherein said antibody comprises a heavy chain variable domain and a light chain variable domain, and

[0014] (a) wherein the heavy chain variable domain comprises a complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:8; and a CDR3 comprising the amino acid sequence of SEQ ID NO:10; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:13; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:17;

[0015] (b) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:24; a CDR2 comprising the amino acid sequence of SEQ ID NO:26; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID

NO:31; a CDR2 comprising the amino acid sequence of SEQ ID NO:33; and a CDR3 comprising the amino acid sequence of SEQ ID NO:35;

[0016] (c) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:44; and a CDR3 comprising the amino acid sequence of SEQ ID NO:46; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:49; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:53;

[0017] (d) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:60; a CDR2 comprising the amino acid sequence of SEQ ID NO:62; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:67; a CDR2 comprising the amino acid sequence of SEQ ID NO:69; and a CDR3 comprising the amino acid sequence of SEQ ID NO:71; or

[0018] (e) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:78; a CDR2 comprising the amino acid sequence of SEQ ID NO:80; and a CDR3 comprising the amino acid sequence of SEQ ID NO:82; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:85; a CDR2 comprising the amino acid sequence of SEQ ID NO:87; and a CDR3 comprising the amino acid sequence of SEQ ID NO:89.

[0019] Another embodiment is directed to an isolated monoclonal antibody that binds to *Clostridium difficile* toxin A, wherein said antibody comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:20, SEQ ID NO:38, SEQ ID NO:56, or SEQ ID NO:74 or a light chain variable domain comprising the amino acid sequence of SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, or SEQ ID NO:76.

[0020] Another embodiment is directed to an isolated monoclonal antibody that binds to *C. difficile* toxin A, wherein said antibody comprises a heavy chain variable domain and a light chain variable domain, and

[0021] (a) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:2 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:4;

[0022] (b) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:20 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:22;

[0023] (c) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:38 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:40;

[0024] (d) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:56 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:58; or

[0025] (e) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:74 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:76.

[0026] Yet another embodiment is directed to an isolated, human monoclonal antibody that binds to the same epitope of *C. difficile* toxin A recognized by:

[0027] (a) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:2 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:4;

[0028] (b) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:20 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:22;

[0029] (c) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:40;

[0030] (d) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:56 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:58; or

[0031] (e) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:74 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:76.

[0032] In another embodiment, the antibody is an isolated, human monoclonal antibody comprising at least one of the following characteristics:

(a) the antibody binds to *C. difficile* toxin A with a dissociation constant (K_D) equal to or less than 10 pM (10^{-11} M);

(b) the antibody neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin A in the Vero monkey kidney cell line with an NT50 equal to or less than 3000 pM;

(c) the antibody neutralizes the *C. difficile* toxin A induced loss of transepithelial electrical resistance (TEER) in the T-84 cell line with an NT50 equal to or less than 6 nM; and/or

(d) the antibody binds to toxin A produced by the strains of toxinotypes 0, III, V, XII, and XV.

[0033] The antibody may have at least two, at least three, or all four of the above-identified characteristics.

[0034] Another aspect is drawn to monoclonal antibodies that bind to *C. difficile* toxin B. The anti-toxin B antibodies are preferably human antibodies. In one embodiment, the anti-toxin B antibodies are recombinant antibodies.

[0035] One embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the glucosyl transferase domain of *C. difficile* toxin B, wherein the epitope comprises the amino acid sequence SGRNK (SEQ ID NO:234), amino acids 56-80 of SEQ ID NO:231, or amino acids 10-520 of SEQ ID NO:231.

[0036] Another embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the N-terminal translocation domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 1110-1530 of SEQ ID NO:231.

[0037] Another embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the receptor binding domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 1750-2360 of SEQ ID NO:231.

[0038] Yet another embodiment is directed to an isolated monoclonal antibody that binds to *C. difficile* toxin B, wherein said antibody comprises a heavy chain variable domain and a light chain variable domain, and

[0039] (a) wherein the heavy chain variable domain comprises a complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO:96; a CDR2 comprising the amino acid sequence of SEQ ID NO:98; and a CDR3 comprising the amino acid sequence of SEQ ID NO:100; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:103; a CDR2 comprising the amino acid sequence of SEQ ID NO:105; and a CDR3 comprising the amino acid sequence of SEQ ID NO:107;

[0040] (b) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:114; a CDR2 comprising the amino acid sequence of SEQ ID NO:116; and a CDR3 comprising the amino acid sequence of SEQ ID NO:118; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ

ID NO:121; a CDR2 comprising the amino acid sequence of SEQ ID NO:123; and a CDR3 comprising the amino acid sequence of SEQ ID NO:125;

[0041] (c) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:132; a CDR2 comprising the amino acid sequence of SEQ ID NO:134; and a CDR3 comprising the amino acid sequence of SEQ ID NO:136; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:139; a CDR2 comprising the amino acid sequence of SEQ ID NO:141; and a CDR3 comprising the amino acid sequence of SEQ ID NO:143;

[0042] (d) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:150; a CDR2 comprising the amino acid sequence of SEQ ID NO:152; and a CDR3 comprising the amino acid sequence of SEQ ID NO:154; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:157; a CDR2 comprising the amino acid sequence of SEQ ID NO:159; and a CDR3 comprising the amino acid sequence of SEQ ID NO:161;

[0043] (e) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:168; a CDR2 comprising the amino acid sequence of SEQ ID NO:170; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:175; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:179; or

[0044] (f) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:186; a CDR2 comprising the amino acid sequence of SEQ ID NO:188; and a CDR3 comprising the amino acid sequence of SEQ ID NO:190; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:193; a CDR2 comprising the amino acid sequence of SEQ ID NO:195; and a CDR3 comprising the amino acid sequence of SEQ ID NO:197.

[0045] One embodiment is directed to an isolated, monoclonal antibody that binds to *Clostridium difficile* toxin B, wherein said antibody comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:92, SEQ ID NO:110, SEQ ID NO:128, SEQ ID NO:146, SEQ ID NO:164, or SEQ ID NO:182 or a light chain variable domain comprising the amino acid sequence of SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, or SEQ ID NO:184.

[0046] Another embodiment is directed to an isolated, monoclonal antibody that binds to *C. difficile* toxin B, wherein said antibody comprises a heavy chain variable domain and a light chain variable domain, and

[0047] (a) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:92 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:94;

[0048] (b) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:110 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:112;

[0049] (c) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:128 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:130;

[0050] (d) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:146 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:148;

[0051] (e) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:164 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:166; or

[0052] (f) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:182 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:184.

[0053] Yet another embodiment is directed to an isolated monoclonal antibody that binds to the same epitope of *C. difficile* toxin B recognized by:

[0054] (a) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:92 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:94;

[0055] (b) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:110 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:112;

[0056] (c) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:128 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:130;

[0057] (d) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:146 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:148;

[0058] (e) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:164 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:166; or

[0059] (f) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:182 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:184.

[0060] In another embodiment, the antibody is an isolated, human monoclonal antibody comprising at least one of the following characteristics:

- (a) the antibody binds to *C. difficile* toxin B with a dissociation constant (K_D) equal to or less than 100 pM;
- (b) the antibody neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin B in the Vero monkey kidney cell line with an NT50 equal to or less than 1000 pM;
- (c) the antibody neutralizes the *C. difficile* toxin B induced loss of transepithelial resistance electrical (TEER) in the T-84 cell line with an NT50 equal to or less than 200 pM; and/or
- (d) the antibody binds to toxin B produced by at least the strains of toxinotypes 0, III, and V.

[0061] The antibody may have at least two, at least three, or all four of the above-identified characteristics.

[0062] In another embodiment, the antibody binds to toxin B produced by at least the strains of toxinotypes 0, III, V, and VIII. In another embodiment, the antibody binds to toxin B produced by at least the strains of toxinotypes 0, III, V, VIII, and XII. In another embodiment, the antibody binds to toxin B produced by at least the strains of toxinotypes 0, III, V, VIII, XII, and XV.

[0063] In one embodiment, the antibody is a bispecific antibody, where the bispecific antibody comprises 1) a first antigen binding site comprising the heavy chain variable domain and light chain variable domain of an antibody that binds to *C. difficile* toxin A, as described herein, and 2) a second antigen binding site comprising the heavy chain variable domain and light chain variable domain of an antibody that binds to *C. difficile* toxin B, as described herein. In an alternative embodiment, the antibody is a bispecific antibody, where the bispecific antibody comprises two antigen binding sites, each antigen binding site comprising the heavy chain

variable domain from an antibody that binds to *C. difficile* toxin A, as described herein, and the light chain variable domain from an antibody that binds to *C. difficile* B, as described herein. In a further alternative embodiment, the antibody is a bispecific antibody, where the bispecific antibody comprises two antigen binding sites, each antigen binding site comprising the heavy chain variable domain from an antibody that binds to *C. difficile* toxin B, as described herein, and the light chain variable domain from an antibody that binds to *C. difficile* toxin A, as described herein. In a further alternative embodiment, the antibody is a bispecific antibody, where the bispecific antibody comprises two antigen binding sites, each antigen binding site comprising the heavy chain variable domain from an antibody that binds to *C. difficile* toxin B, such as B1, and as further described herein, and the light chain variable domain from an antibody that binds to a different part of *C. difficile* toxin B, such as B2, and as further described herein.

[0064] In one embodiment of the bispecific antibody, the first antigen binding site comprises the heavy chain variable domain and light chain variable domain of the A1, A2, A3, A4, or A5 antibody. In another embodiment of the bispecific antibody, the second antigen binding site comprises the heavy chain variable domain and light chain variable domain of the B1, B2, B3, B4, B5, or B6 antibody. In yet another embodiment of the bispecific antibody, the first antigen binding site comprises the heavy chain variable domain and light chain variable domain of the A1, A2, A3, A4, or A5 antibody and the second antigen binding site comprises the heavy chain variable domain and light chain variable domain of the B1, B2, B3, B4, B5, or B6 antibody.

[0065] In another embodiment, the antibody is a bispecific antibody, where the bispecific antibody comprises 1) a first antigen binding site, wherein the first antigen binding site comprises the heavy chain variable domain and light chain variable domain of an antibody that binds to *C. difficile* toxin B, as described herein and 2) a second antigen binding site, wherein the second antigen binding site comprises the heavy chain variable domain and light chain variable domain of an antibody that binds to *C. difficile* toxin B, as described herein, wherein the first and second antigen binding sites are different. Preferably, the first and second antigen binding sites comprise the heavy chain variable domain and light chain variable domain of the B1, B2, B3, B4, B5, or B6 antibody, wherein the first and second antigen binding sites are different. In yet another embodiment of the bispecific antibody, the first antigen binding site comprises the heavy chain variable domain and light chain variable domain of the B1 antibody and the second antigen binding site comprises the heavy chain variable domain and light chain variable domain of the B2 antibody.

[0066] In a further embodiment, the antibody is a bispecific antibody, where the bispecific antibody comprises 1) a first antigen binding site, wherein the first antigen binding site comprises the heavy chain variable domain and light chain variable domain of an antibody that binds to *C. difficile* toxin A, as described herein and 2) a second antigen binding site, wherein the second antigen binding site comprises the heavy chain variable domain and light chain variable domain of an antibody that binds to *C. difficile* toxin A, as described herein, wherein the first and second antigen binding sites are different. Preferably, the first and second antigen binding sites comprise the heavy chain variable domain and light chain variable domain of the A1, A2, A3, A4, or A5 antibody, wherein the first and second antigen binding sites are different.

[0067] Another aspect is related to compositions comprising one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies, which compositions can be used, by way of example, for treating a *C. difficile* infection. In certain embodiments, the composition comprises one antibody that binds to *C. difficile* toxin A and one antibody that binds to *C. difficile* toxin B, as described herein. In one embodiment, the composition comprises at least one antibody that binds to *C. difficile* toxin A and at least one antibody that binds to *C. difficile* toxin B, wherein the at least one antibody that binds to *C. difficile* toxin A is preferably one or more of the A1, A2, A3, A4, and A5 antibodies. In another embodiment, the composition comprises at least one antibody that binds to *C. difficile* toxin A and at least one antibody that binds to *C. difficile* toxin B, wherein the at least one antibody that binds to *C. difficile* toxin B is preferably one or more of the B1, B2, B3, B4, B5, or B6 antibodies. In yet another embodiment, the at least one antibody that binds to *C. difficile* toxin A is preferably one or more of the A1, A2, A3, A4, and A5 antibodies and the at least one antibody that binds to *C. difficile* toxin B is preferably one or more of the B1, B2, B3, B4, B5, or B6 antibodies. These compositions can be used, by way of example, for treating a *C. difficile* infection. In one embodiment, the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody and the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, or B6 antibodies, preferably one or more of B1, B2, B4 or B6. Thus, in certain embodiments, the composition comprises the A2 and B1 antibodies, the A2 and B2 antibodies, the A2 and B4 antibodies, or the A2 and B6 antibodies. In another embodiment, the at least one antibody that binds to *C. difficile* toxin A is the A1 antibody and the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, or B6 antibodies, preferably one or more of B1, B2, B4 or B6. Thus, in certain other embodiments, the composition comprises the A1 and B1 antibodies, the A1 and B2 antibodies, the A1 and B4 antibodies, or the A1 and B6 antibodies. In other embodiments, the composition further comprises a pharmaceutically acceptable excipient.

[0068] In other embodiments, the composition comprises a combination of at least three antibodies. In one embodiment, the composition comprises two antibodies that bind to *C. difficile* toxin A, as described herein, and one antibody that binds to *C. difficile* toxin B, as described herein. Alternatively, the composition comprises one antibody that binds to *C. difficile* toxin A, as described herein, and two antibodies that bind to *C. difficile* toxin B, as described herein.

[0069] In a further embodiment the composition comprises a three antibody combination comprising one antibody that binds to *C. difficile* toxin A, as described herein, and preferably selected from the A1, A2, A3, A4, and A5 antibodies, and two antibodies that bind to *C. difficile* toxin B, as described herein, which are preferably selected from the B1, B2, B3, B4, B5, or B6 antibodies. In one embodiment, the composition comprises the A2, B1 and B2 antibodies. In another embodiment, the composition comprises the A2, B2, and B4 antibodies. In another embodiment, the composition comprises the A2, B2, and B6 antibodies.

[0070] In another embodiment, the composition comprises a first antibody that binds to *C. difficile* toxin A, as described herein, which is preferably selected from the A1, A2, A3, A4, and A5 antibodies, more preferably the A2 antibody, and a second antibody, wherein the second antibody is a bispecific antibody that binds to *C. difficile* toxin B and wherein the bispecific antibody comprises 1) a first antigen binding site, wherein the first antigen binding site comprises the heavy chain variable domain and light chain variable domain of the B1, B2, B3, B4, B5, or B6 antibody and 2) a second antigen binding site, wherein the second antigen binding site comprises the heavy chain variable domain and light chain variable domain of the B1, B2, B3, B4, B5, or B6 antibody, wherein the first and second antigen binding sites are different. In one embodiment, the composition comprises the A2 antibody and a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody. In another embodiment, the composition comprises the A1 antibody and a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody.

[0071] Another aspect is directed to methods of using antibodies that bind to *C. difficile* toxin A and/or *C. difficile* toxin B to treat *C. difficile* infection. In one embodiment, the method of treating a *C. difficile* infection comprises administering to a subject one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies or a bispecific antibody derived therefrom, including, for example, a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody in an amount effective to treat the *C. difficile* infection. In another embodiment, the method of treating a *C. difficile* infection comprises administering a composition to the subject in an amount effective to treat the *C. difficile* infection, wherein the

composition comprises at least one antibody that binds to *C. difficile* toxin A selected from the A1, A2, A3, A4, and A5 antibodies and at least one antibody that binds to *C. difficile* toxin B selected from the B1, B2, B3, B4, B5, or B6 antibodies or a bispecific antibody derived therefrom, including, for example, a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody. In one embodiment, the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody and the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, or B6 antibodies or a bispecific antibody derived therefrom, preferably one or more of B1, B2, or B4, or a bispecific antibody selected from B1 + B2 or B2 + B4.

[0072] Another aspect is directed to nucleic acids that encode an antibody of interest, or portion(s) thereof. One embodiment is directed to an isolated nucleic acid that encodes the amino acid sequence of one or more of the CDRs of the light and/or heavy chain variable regions of an A2, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibody, or a bispecific antibody derived therefrom, including, for example, a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody. Another embodiment is directed to an isolated nucleic acid that encodes an amino acid sequence of the light and/or heavy chain variable regions of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 monoclonal antibody or a bispecific antibody derived therefrom, including, for example, a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody. Other embodiments are directed to a recombinant expression vector comprising the nucleic acid or an isolated host cell comprising the recombinant expression vector.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate certain embodiments, and together with the written description, serve to explain certain principles of the antibodies and methods disclosed herein.

[0074] **Figure 1** shows the results of the Vero cell cytotoxicity assay for various antibodies with potency (NT50) represented on the x-axis and percent completion represented on the y-axis. **Figure 1A** shows the results for the anti-toxin A antibodies A1, A2, A3, A4, and A5 and **Figure 1B** shows the results for the anti-toxin B antibodies B1, B2, B3, B5, and B4 + B6 (tested as combination).

[0075] **Figure 2** shows the results of the T-84 cell TEER assay for various antibodies with potency (NT50) represented on the x-axis and percent completion represented on the y-axis. **Figure 2A** shows the results for the anti-toxin A antibodies A1, A2, A3, A4, and A5 while **Figure**

2B shows the results for the anti-toxin B antibodies B1, B2, B3, B4, B5, and B6. The asterisks (“*”) in Figure 2A indicates that the plateau for these antibodies was never reached and, thus, these values (for % completion) represent minimum values.

[0076] Figure 3 shows that *C. difficile* toxin A CTD fragments from strains of toxinotypes 0, III, V, XII, and XV inhibit the potent neutralization activity of the A2 antibody against toxin A of toxinotype 0 in Vero cells, demonstrating that the A2 antibody recognizes toxin A produced by the strains of toxinotypes 0, III, V, XII, and XV by this highly sensitive *in vitro* functional assay.

[0077] Figure 4 shows the results of the T-84 cell TEER assay. Figure 4A shows the results for the A2 antibody and *C. difficile* toxin A CTD fragments from strains of toxinotypes 0, III, V, XII, and XV, while Figure 4B shows the results of the B6 antibody and *C. difficile* toxin B CTD fragments from strains of toxinotypes 0, III, V, VIII, XII, and XV, with the CTD fragments inhibiting the potent neutralization activity of the A2 antibody against toxin A of toxinotype 0 and the potent neutralization activity of the B6 antibody against toxin B of toxinotype 0 in T-84 cells.

[0078] Figure 5 shows the therapeutic effects of antibody combinations A2 + B6 or A2 + B4 at a dosage of 50 mg/kg in a hamster model of CDAD. Figure 5A shows the effects of the antibody combinations on survival, while Figure 5B shows the effects on disease symptoms, where 0 = no illness, 1 = loose feces, 2 = wet tail and perianal region, and 3 = wet tail and lower abdomen. Figure 5C shows weight change post-challenge with *C. difficile* spores (toxinotype 0 strain 630).

[0079] Figure 6 shows the survival of hamsters treated with antibody combinations A2 + B1, A2 + B2, A2 + B4, or control (PBS) at an antibody dose of either 50 mg/kg (Figure 6A), or 6 mg/kg (Figure 6B) following an initial challenge with *C. difficile* spores (toxinotype 0 strain 630).

[0080] Figure 7 shows the disease symptoms of hamsters treated with antibody combinations A2 + B1, A2 + B2, A2 + B4, or control (PBS) at an antibody dose of either 50 mg/kg (Figure 7A), or 6 mg/kg (Figure 7B) following an initial challenge with *C. difficile* spores (toxinotype 0 strain 630), where 0 = no illness, 1 = loose feces, 2 = wet tail and perianal region, and 3 = wet tail and lower abdomen.

[0081] Figure 8 shows the mean weight change post-challenge with *C. difficile* spores (toxinotype 0 strain 630) of hamsters treated with antibody combinations A2 + B1, A2 + B2, A2 + B4, or control (PBS) at an antibody dose of either 50 mg/kg (Figure 8A), or 6 mg/kg (Figure 8B).

[0082] Figure 9 shows the *C. difficile* load in fecal pellets (CFU/g) collected from hamsters treated with antibody combinations (6 mg/kg) A2 + B1 or A2 + B2 at day 4 post challenge with *C. difficile* spores (toxintype 0 strain 630) (Figure 9A), at day 7 post challenge (Figure 9B), and as a time course (Figure 9C), showing that by day 13 none of the antibody-treated hamsters showed any detectable fecal shedding.

[0083] Figure 10 shows the therapeutic effects of antibody combinations A2 + B2 at a dosage of 6 mg/kg in a hamster model of CDAD using highly virulent *C. difficile* strains. Figure 10A shows the effects of the A2 + B2 antibody combination on survival against infection with the toxintype 0 strain VPI10463. Figure 10B shows the effects of the A2 + B2 antibody combination on survival against infection with the toxintype III (ribotype 027) strain 13695#7.

[0084] Figure 11 shows the therapeutic effects of antibody combinations A2 + B2; A2 + B1 + B2; A2 + B2 + B4; and A2 + B2 + B6 in a hamster model of CDAD using the highly virulent toxintype III (ribotype 027) strain 13695#7. Figure 11A shows the effects of antibody combinations at low dosage (6 mg/kg) on survival while Figure 11B shows the effects of antibody combinations at high dosage (50 mg/kg) on survival.

[0085] Figure 12 shows the therapeutic effects of antibody combinations A2 + B1 + B2; A2 + B2 + B4; and A2 + B2 + B6 in a hamster model of CDAD using the highly virulent toxintype III (ribotype 027) strain 13695#7. Figure 12A shows the effects of antibody combinations at low dosage (6 mg/kg) on illness while Figure 12B shows the effects of antibody combinations at high dosage (50 mg/kg) on illness, where 0 = no illness, 1 = loose feces, 2 = wet tail and perianal region, and 3 = wet tail and lower abdomen.

DETAILED DESCRIPTION

[0086] Reference will now be made in detail to various exemplary embodiments, examples of which are illustrated in the accompanying drawings. It is to be understood that the following detailed description is provided to give the reader a fuller understanding of certain embodiments, features, and details of aspects of the invention, and should not be interpreted as a limitation of the scope of the invention.

1. Definitions

[0087] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[0088] The term “antibody” as used in this disclosure refers to an immunoglobulin or an antigen-binding fragment thereof. Unless otherwise specified, the term includes, but is not limited to, polyclonal, monoclonal, monospecific, polyspecific, humanized, human, single-chain,

chimeric, synthetic, recombinant, hybrid, mutated, grafted, and *in vitro* generated antibodies. The antibody can include a constant region, or a portion thereof, such as the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes. For example, heavy chain constant regions of the various isotypes can be used, including: IgG₁, IgG₂, IgG₃, IgG₄, IgM, IgA₁, IgA₂, IgD, and IgE. By way of example, the light chain constant region can be kappa or lambda.

[0089] The terms “antigen-binding domain” and “antigen-binding fragment” refer to a part of an antibody molecule that comprises amino acids responsible for the specific binding between antibody and antigen. For certain antigens, the antigen-binding domain or antigen-binding fragment may only bind to a part of the antigen. The part of the antigen that is specifically recognized and bound by the antibody is referred to as the “epitope” or “antigenic determinant.” Antigen-binding domains and antigen-binding fragments include Fab (Fragment antigen-binding); a F(ab')₂ fragment, a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; Fv fragment; a single chain Fv fragment (scFv) *see e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883); a Fd fragment having the two V_H and C_H1 domains; dAb (Ward *et al.*, (1989) *Nature* 341:544-546), and other antibody fragments that retain antigen-binding function. The Fab fragment has V_H-C_H1 and V_L-C_L domains covalently linked by a disulfide bond between the constant regions. The F_v fragment is smaller and has V_H and V_L domains non-covalently linked. To overcome the tendency of non-covalently linked domains to dissociate, a scFv can be constructed. The scFv contains a flexible polypeptide that links (1) the C-terminus of V_H to the N-terminus of V_L, or (2) the C-terminus of V_L to the N-terminus of V_H. A 15-mer (Gly₄Ser)₃ (SEQ ID NO:331) peptide may be used as a linker, but other linkers are known in the art. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are evaluated for function in the same manner as are intact antibodies.

[0090] The terms “(cross)-block,” “(cross)-blocked,” “(cross)-blocking,” “competitive binding,” “(cross)-compete,” “(cross)-competing,” and “(cross)-competition” are used interchangeably herein to mean the ability of an antibody to interfere with the binding of other antibodies to a given target. The extent to which one antibody is able to interfere with the binding of another antibody to the target, and therefore whether it can be said to cross-block, as used herein, can be determined using competition binding assays. One particularly suitable quantitative cross-blocking assay uses a Biacore instrument which can measure the extent of interactions using surface plasmon resonance technology.

[0091] The following generally describes a suitable Biacore assay for determining whether an antibody cross-blocks or is capable of cross-blocking. It will be appreciated that the

assay can be used with any of antibodies described herein. The Biacore instrument (for example the Biacore 3000) is operated in line with the manufacturer's recommendations. Thus in one cross-blocking assay, the target protein (e.g. toxin A or toxin B) is coupled to a CMS Biacore chip using standard amine coupling chemistry to generate a surface that is coated with the target. Typically 200-800 resonance units of the target would be coupled to the chip (an amount that gives easily measurable levels of binding but that is readily saturable by the concentrations of test reagent being used). Two test binding agents {termed A* and B* } to be assessed for their ability to cross- block each other are mixed at a one to one molar ratio of binding sites in a suitable buffer to create the test mixture. When calculating the concentrations on a binding site basis the molecular weight of a binding agent is assumed to be the total molecular weight of the binding agent divided by the number of target binding sites on that binding agent. The concentration of each binding agent in the test mix should be high enough to readily saturate the binding sites for that binding agent on the target molecules captured on the Biacore chip. The binding agents in the mixture are at the same molar concentration (on a binding basis) and that concentration would typically be between 1.00 and 1.5 micromolar (on a binding site basis). Separate solutions containing A* alone and B* alone are also prepared. A* and B* in these solutions should be in the same buffer and at the same concentration as in the test mix. The test mixture is passed over the target-coated Biacore chip and the total amount of binding recorded. The chip is then treated in such a way as to remove the bound binding agents without damaging the chip-bound target. Typically this is done by treating the chip with 30 mM HCl for 60 seconds. The solution of A* alone is then passed over the target-coated surface and the amount of binding recorded. The chip is again treated to remove all of the bound binding agents without damaging the chip-bound target. The solution of B* alone is then passed over the target-coated surface and the amount of binding recorded. The maximum theoretical binding of the mixture of A* and B* is next calculated, and is the sum of the binding of each binding agent when passed over the target surface alone. If the actual recorded binding of the mixture is less than this theoretical maximum then the two binding agents are said to cross-block each other. Thus, in general, a cross-blocking antibody is one which will bind to the target in the above Biacore cross-blocking assay such that during the assay and in the presence of a second antibody the recorded binding is between 80% and 0.1% of maximum theoretical binding (as defined above) of the two antibodies in combination. Other affinity assays may also be used, including the Octet assay, as described in the examples that follow.

[0092] As used herein, a **“therapeutically effective amount”** of an antibody refers to an amount of an antibody that is effective, upon single or multiple dose administration to a subject (such as a human patient) at treating *C. difficile* infection.

[0093] The terms **“treatment of *C. difficile* infection”** or **“treating *C. difficile* infection”** and the like refer to any treatment of any disease (e.g., CDAD) or condition in a subject caused by *C. difficile* infection and includes inhibiting a disease, condition, or symptom of a *C. difficile* infection, e.g., arresting its development and/or delaying or preventing its onset or manifestation in the subject; relieving a disease, condition, or symptom of a *C. difficile* infection, e.g., causing regression of the condition or disease and/or one or more of its symptoms (e.g., diarrhea, colitis, and/or abdominal pain); or preventing or reducing the recurrence or relapse of a disease, condition, or symptom of a *C. difficile* infection.

[0094] The terms **“subject,” “host,” “patient,”** and **“individual”** are used interchangeably herein to refer to any mammalian subject for whom diagnosis or therapy is desired, particularly humans.

[0095] The term **“pharmaceutically acceptable excipient”** means solvents, diluents, dispersion media, coatings, antibacterial agents and antifungal agents, isotonic agents, solid and liquid fillers, and absorption delaying agents, and the like, that are suitable for administration into a human. The use of such media and agents for pharmaceutically active substances is well known in the art.

[0096] The term **“human antibody”** refers to an antibody having variable and constant regions corresponding substantially to human germline immunoglobulin sequences. A human antibody may also include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs, and in particular, CDR3.

[0097] The term **“recombinant antibody”** refers to an antibody produced or expressed using a recombinant expression vector, where the expression vector comprises a nucleic acid encoding the recombinant antibody, such that introduction of the expression vector into an appropriate host cell results in the production or expression of the recombinant antibody.

[0098] The term **“bispecific”** or **“bifunctional antibody”** refers to an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148, 1547-1553 (1992). For example, the bispecific antibody can comprises a first antigen binding site, such as a Fab' fragment, that binds to *C. difficile* toxin A

and a second antigen binding site, such as a Fab' fragment, that binds to *C. difficile* toxin B. The first and second antigen binding site may be linked using any available technique, including, for example, an immunoglobulin constant region.

[0099] The term “**neutralizing antibody**” refers to an antibody whose binding an antigen results in inhibition of the biological activity of that antigen, respectively. For example, “toxin A neutralizing antibody” or “toxin B neutralizing antibody” (or an “antibody that neutralizes toxin A or toxin B activity”) refers to an antibody whose binding to toxin A or toxin B results in the inhibition of the biological activity of toxin A or toxin B. This inhibition of the biological activity of toxin A or toxin B can be assessed by measuring one or more indicators of toxin A or toxin B biological activity, such as toxin A- or toxin B-induced cytotoxicity or loss of transepithelial electrical resistance (TEER), as demonstrated in the examples.

[00100] The term “**isolated antibody**,” refers to an antibody that is substantially free of its natural environment, including other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds *C. difficile* toxin A is substantially free of antibodies that specifically bind antigens other than *C. difficile* toxin A, unless the isolated antibody is combined with one or more isolated antibodies of interest, such as an antibody that specifically binds *C. difficile* toxin B).

[00101] The term “**isolated nucleic acid**,” as used in the context of a nucleic acid encoding an antibody, or antigen-binding fragment thereof, refers to a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody, or antigen-binding fragment thereof, are free of other nucleotide sequences encoding antibodies or portions thereof that bind antigens other than *C. difficile* toxin A or toxin B, which other sequences may naturally flank the nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid encoding a VH region of an anti-toxin A antibody contains no other sequences encoding other VH regions that bind antigens other than *C. difficile* toxin A.

[00102] The term “**identity**,” as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, “identity” also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as determined by the match between strings of such sequences. “Identity” and “similarity” can be readily calculated by known methods, including, but not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in

Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., Siam J. Applied Math., 48:1073 (1988). In addition, values for percentage identity can be obtained from amino acid and nucleotide sequence alignments generated using the default settings for the AlignX component of Vector NTI Suite 8.0 (Informax, Frederick, Md.).

[00103] Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S. F. et al., J. Molec. Biol. 215:403-410 (1990)). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBINLM NIH Bethesda, Md. 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990)). The well-known Smith Waterman algorithm may also be used to determine identity.

2. Overview

[00104] The present application provides monoclonal antibodies that bind to either *C. difficile* toxin A or *C. difficile* toxin B with high affinity and exhibit potent neutralizing activity in both *in vitro* assays (Vero cell-based toxin neutralization assay and T-84 cell-based TEER assay) and in an art-recognized, *in vivo* animal model for *C. difficile* infection. Using a unique antibody discovery strategy, tens of millions of antibody producing B lymphocytes from selected human subjects were screened for binding to and/or neutralizing activity against *C. difficile* toxin A or B, selected for cloning and recombinant expression, and further characterization to identify specific human antibodies with high binding affinity for and strong neutralizing activity against either *C. difficile* toxin A or *C. difficile* toxin B, preferably with a broad spectrum of binding to various *C. difficile* toxinotypes, such as 0, III, V, VIII, XII, and XV. The human A1, A2, A3, A4, and A5 antibodies bind and neutralize *C. difficile* toxin A of toxinotype 0 and also recognize toxin A from toxinotypes III, V, XII, and XV. The human B1, B2, B3, B4, B5, and B6 antibodies bind and neutralize *C. difficile* toxin B from toxinotype 0 and also recognize toxin B from at least toxinotype III, and, in some instances, toxin B from at least toxinotypes, III, V, and VIII. These antibodies have therapeutic activity against active disease caused by or associated with *C. difficile* and can be used either singularly, or in combination, to treat *C. difficile* infections and/or to protect against the illness.

3. Antibodies

[00105] Antibodies, also known as immunoglobulins, are typically tetrameric glycosylated proteins composed of two light (L) chains of approximately 25 kDa each and two heavy (H) chains of approximately 50 kDa each. Two types of light chain, termed lambda and kappa, may be found in antibodies. Depending on the amino acid sequence of the constant domain of heavy chains, immunoglobulins can be assigned to five major classes: A, D, E, G, and M, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. Each light chain includes an N-terminal variable (V) domain (VL) and a constant (C) domain (CL). Each heavy chain includes an N-terminal V domain (VH), three or four C domains (CHs), and a hinge region. The CH domain most proximal to VH is designated as CH1. The VH and VL domains consist of four regions of relatively conserved sequences called framework regions (FR1, FR2, FR3, and FR4), which form a scaffold for three regions of hypervariable sequences (complementarity determining regions, CDRs). The CDRs contain most of the residues responsible for specific interactions of the antibody with the antigen. CDRs are referred to as CDR1, CDR2, and CDR3. Accordingly, CDR constituents on the heavy chain are referred to as H1, H2, and H3, while CDR constituents on the light chain are referred to as L1, L2, and L3. Identification and numbering of framework and CDR residues is as described by Chothia *et al.*, Structural determinants in the sequences of immunoglobulin variable domain, *J Mol Biol* 1998, 278:457-79, which is hereby incorporated by reference in its entirety.

[00106] CDR3 is typically the greatest source of molecular diversity within the antibody-binding site. H3, for example, can be as short as two amino acid residues or greater than 26 amino acids. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in the art. For a review of the antibody structure, see *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, eds. Harlow *et al.*, 1988. One of skill in the art will recognize that each subunit structure, e.g., a CH, VH, CL, VL, CDR, FR structure, comprises active fragments, e.g., the portion of the VH, VL, or CDR subunit that binds to the antigen, i.e., the antigen-binding fragment, or, e.g., the portion of the CH subunit that binds to and/or activates, e.g., an Fc receptor and/or complement. The CDRs typically refer to the Kabat CDRs, as described in *Sequences of Proteins of Immunological Interest*, US Department of Health and Human Services (1991), eds. Kabat *et al.* Another standard for characterizing the antigen binding site is to refer to the hypervariable loops as described by Chothia. See, e.g., Chothia, D. *et al.* (1992) *J. Mol. Biol.* 227:799-817; and Tomlinson *et al.* (1995) *EMBO J.* 14:4628-4638. Still another standard is the AbM definition used by Oxford Molecular's AbM antibody modeling software. See, generally, e.g., *Protein Sequence and Structure Analysis of Antibody Variable Domains*. In: *Antibody Engineering Lab Manual* (Ed.:

Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg). Embodiments described with respect to Kabat CDRs can alternatively be implemented using similar described relationships with respect to Chothia hypervariable loops or to the AbM-defined loops. Another standard for residue numbering that can be used is IMGT (Lefranc et al., *Dev & Comp Immunol*, 27(1):55-77 (2003)).

[00107] The Fab fragment (Fragment antigen-binding) consists of V_H - C_H1 and V_L - C_L domains covalently linked by a disulfide bond between the constant regions. The F_v fragment is smaller and consists of V_H and V_L domains non-covalently linked. To overcome the tendency of non-covalently linked domains to dissociate, a single chain F_v fragment (sc F_v) can be constructed. The sc F_v contains a flexible connector, usually a polypeptide, that links (1) the C-terminus of V_H to the N-terminus of V_L , or (2) the C-terminus of V_L to the N-terminus of V_H . A 15-mer (Gly₄Ser)₃ (SEQ ID NO:331) peptide may be used as a linker, but other linkers are known in the art.

[00108] It is possible to modify an antibody to increase productivity and/or when relevant, to decrease possible immunogenicity. In addition, monoclonal antibodies may be modified at either the DNA sequence level to improve expression by removing hairpins or other secondary structure, by optimizing codon utilization, or at the amino acid level to improve expression or stability. For example, it is possible to remove residues such as unpaired cysteines to reduce aggregation, to alter glycosylation sites, or to substitute residues prone to deamidation or oxidation.

[00109] It may also be desirable to modify an antibody to improve effector function, e.g., so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antagonist. One or more amino acid substitutions or the introduction of cysteine in the Fc region may be made, thereby improving internalization capability and/or increased complement-mediated cell killing and ADCC. See Caron *et al.*, *J. Ex. Med.* 176:1191-1195 (1991) and Shopes, B.J. *Immunol.* 148:2918-2022 (1992), incorporated herein by reference in their entirety. An antibody fusion protein may be prepared that has dual Fc regions with both enhanced complement lysis and ADCC capabilities. Typical Fc receptors that bind to an Fc region of an antibody (e.g., an IgG antibody) include, but are not limited to, receptors of the Fc γ RI, Fc γ RII, and Fc γ RIII and FcRn subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc receptors are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92, 1991; Capel *et al.*, *Immunomethods* 4:25-34, 1994; and de Haas *et al.*, *J. Lab. Clin. Med.* 126:330-41, 1995). It is also possible to couple or join an antibody to another agent, such as a cytotoxic agent, drug, or therapeutic.

[00110] Anti-toxin A or anti-toxin B antibodies described in this application may optionally comprise antibody constant regions or parts thereof. For example, a V_L domain may be attached at its C-terminal end to a light chain constant domain like $C\kappa$ or $C\lambda$. Similarly, a V_H domain or portion thereof may be attached to all or part of a heavy chain like IgA, IgD, IgE, IgG, and IgM, and any isotype subclass. Constant regions are known in the art (see, for example, Kabat et al., Sequences of Proteins of Immunological Interest, No. 91-3242, National Institutes of Health Publications, Bethesda, MD (1991)).

[00111] VHH molecules (or nanobodies), as known to the skilled artisan, are heavy chain variable domains derived from immunoglobulins naturally devoid of light chains, such as those derived from Camelidae as described in WO 9404678, incorporated herein by reference. Such a VHH molecule can be derived from antibodies raised in Camelidae species, for example in camel, llama, dromedary, alpaca and guanaco and is sometimes called a camelid or camelized variable domain. See e.g., Muyldermans, *J. Biotechnology* (2001) 74(4):277-302, incorporated herein by reference. Other species besides Camelidae may produce heavy chain antibodies naturally devoid of light chain. VHH molecules are about 10 times smaller than IgG molecules. They are single polypeptides and very stable, resisting extreme pH and temperature conditions. Moreover, they are resistant to the action of proteases which is not the case for conventional antibodies. Furthermore, *in vitro* expression of VHHs produces high yield, properly folded functional VHHs. In addition, antibodies generated in Camelids will recognize epitopes other than those recognized by antibodies generated *in vitro* through the use of antibody libraries or via immunization of mammals other than Camelids (see WO 9749805, which is incorporated herein by reference).

[00112] The disclosed antibodies can be modified to alter their glycosylation; that is, at least one carbohydrate moiety can be deleted or added to the antibody. Deletion or addition of glycosylation sites can be accomplished by changing amino acid sequence to delete or create glycosylation consensus sites, which are well known in the art. Another means of adding carbohydrate moieties is the chemical or enzymatic coupling of glycosides to amino acid residues of the antibody (see WO 87/05330 and Aplin et al. (1981) *CRC Crit. Rev. Biochem.*, 22: 259-306). Removal of carbohydrate moieties can also be accomplished chemically or enzymatically (see Hakimuddin et al. (1987) *Arch. Biochem. Biophys.*, 259: 52; Edge et al. (1981) *Anal. Biochem.*, 118: 131; Thotakura et al. (1987) *Meth. Enzymol.*, 138: 350).

[00113] The antibodies of this invention may be tagged with a detectable or functional label. These labels include radiolabels (e.g., ^{131}I or ^{99}Tc), enzymatic labels (e.g., horseradish

peroxidase or alkaline phosphatase), fluorescent labels, chemiluminescent labels, bioluminescent labels, and other chemical moieties (e.g., streptavidin/biotin, avidin/biotin).

4. *C. difficile* Toxin A and Toxin B

[00114] *C. difficile* produces two cytotoxic enterotoxins, toxins A and toxin B that are released by the bacteria into the gut and believed to be involved in causing the symptoms associated with *C. difficile* infection. The genes encoding toxins A and B, *tcdA* and *tcdB*, respectively, are located in the 19.6 kb *C. difficile* pathogenicity locus (PaLoc). Toxins A and B are high molecular weight proteins (about 308 and 270 kDa, respectively) consisting of four major structural domains, the N-terminal glucosyl transferase domain, a protease domain, a central, hydrophobic translocation domain, and a C-terminal receptor binding domain. The C terminus is responsible for toxin binding to the surface of epithelial cells and contains repeating oligopeptides that mediate binding to sugar moieties on the surface of target cells. After binding the cell surface receptor, the toxins enter the target cell via receptor-mediate endocytosis. The amino terminal domain contains the glucosyl transferase active site that modifies and inactivates the Rho/Ras superfamily of GTPase proteins found inside the target host cell. Inhibition of the Rho-GTPases results in depolymerization of actin filaments within the host cell, leading to dysregulation of actin cytoskeleton and tight junction integrity, which in turn produces increased cell permeability and loss of barrier function, resulting in diarrhea, inflammation, and an influx of innate immune response molecules.

[00115] The amino acid sequences of *C. difficile* toxin A are known. For example, the amino acid sequence of toxin A from Strain VPI10463 is set forth below.

```

1  msliskeeli klaysirpre neyktitltnl deynklittnn nenkylqlkk lnesidvfmn
61  kytssrnra lenlkkdiik eviliknsnt spveknlhfv wiggevsdia leyikqwadi
121 naeyniklwy dseafivntl kkaivesstt ealqlleesi qnpqfdnmkf ykkrmefiyd
181 rqkrfinyyk sqinkptvpt iddiikshlv seynrdetvl esyrtnslrk insnhgidir
241 ansifteqel lniysqelln rgnlaaasdi vrllalknfg gvyldvdmlp gihsdlfkfi
301 srpssigldr wemikleaim kykkyinnyt senfdkldqq lkdnfkliie sksekseifs
361 klenlnvsdl eikiafalgs vinqaliskq gsyitnlvie qvknryqfin qhlnpaiesd
421 nnftdttkif hdsifnsata ensmfllkia pylqvgfmpe arstialsqp gayasayydf
481 inlqentiek tlkasdlief kfpennlsqf teqeinslws fdqasakyqf ekyvrdytgg
541 slsedngvdf nkntaldkny llnnkipsnn veeagsknyv hyliqlggdd isyeatcnlf
601 sknpknsiii qrmnesaks yflsddgesi lelnkyripe riknkekvvk tfighgkdef
661 ntsefarlsv dslsneissf ldtikldisp knvevnlhg nmfsydfnve etypgkllls
721 imdkitstlp dvnknsitig anqyevrins egrkellahs gkwinkaeai msdlskeyi
781 ffdsidnkik aksknipgla sisediktll idasvspdtk filnlnklni essigdyiyy
841 eklepvnii hnsiddlide fnllenvsde lyelkklannl dekyllsfed isknnstysv
901 rfinksnges vyvetekeif skysehitke istiknsiit dvngnlldni qldhtsqvnt

```

961 lnaafffigsi idyssnkdvl ndlstsavkvq lyaqlfstgl ntiydsiqlv nliisnavndt
 1021 invlptiteg ipivstildg inlgaaikel ldehdplikk eleakvgvla inmslsiaat
 1081 vasilvgigae vtifllpiag isagipslvn neliihdkat svvnyfnhls eskkygplkt
 1141 eddkilvpid dlvisaidfn nnsiklgtcn ilameggsggh tvtgndidhff sspsisship
 1201 slsiysaigi etenidfskk immipnaprs vfwetgavp glrslendgt rlldsirdly
 1261 pgkfywrfya ffdyaaitlk pyvedtniki kldkdtrnfi mptittneir nklsysfdga
 1321 ggtyslllss ypistnnis kddlwlfnid nevreislen gtikkkglik dlslkidink
 1381 nkliignqti dfgdidnkd ryifltceld dkisliiein lvaksyslll sgdknyllsn
 1441 lsntiekint lgldskniay nytdesnnky fgaisktsqk siihykkdsk nilefyndst
 1501 lefnskdfia edinvmkdd intitgkyv dntdksidf sislvsknqv kvnglylnes
 1561 vyssylfdvk nsdghntsn fmlfldnis fwklfgfni nfvidkyftl vgktnlgyve
 1621 ficdnknid iyfgewktss skstifsgng rnvvepiyn pdtgedists ldfsyeplgy
 1681 idryinkvii apdlytslin intnyysney ypeilvlapn tfhkkvniel dsssfeykws
 1741 tegsdfilvr yleesnkil qkirikgils ntqsfnkmsi dfkdikklsi gyimsnfksf
 1801 nseneldrdb lgfkiidnkt yyydedsklv kglininnsi fyfdpiefnl vtgwqtingk
 1861 kyyfdintga altsyking khfyfnndgv mqlgvfkpdp gfeyfapant qnnnieggai
 1921 vyqskfltin gkkyfydnns kavtgwriin nekyyfnpnn alaavglqvi dnkkyfnpd
 1981 taiiskgwqt vngsryyfdt dtaiafngyk tidgkhfyfd sdcvkvigvf stngfeyfa
 2041 pantynnnie gqaiivyqskf ltlingkkyf dnnskavtgi qtidskkyf ntntaeaatg
 2101 wqtidgkkyf fntntaeaat gwqtidgkky yfntntaiaa tgytiingkh fyfntdgimq
 2161 igvfkpnpf eyfapantda nnieggaily qnefltingk kyyfgsdka vtgwriinnk
 2221 kyyfnpnnaa aaihlctinn dkyyfsydgf lngyitier nnfyfdanne skmvtgvfkf
 2281 pngfeyfapa nthnnniegg aivyqnkflt lngkkyfydn dskavtgwqt idgkkyfni
 2341 ntaeaatgwq tidgkkyfn lntaeaatgw qtidgkkyf ntntfiastg ytsingkhfy
 2401 fntdgimqig vfkpnpfey fapantdann ieggaailyqn kfltingkky yfgsdskavt
 2461 glrtidgkky yfntntavav tgwqtingkk yfntntaiaa stgytiisgk hfyfntdgim
 2521 qigvfkpdpf feyfapantd annieggair yqnrfllyhd niyyfgnnsk aatgwvtidg
 2581 nryyfepta mgangyktid nknfyfrngl pqigvfkgsn gfeyfapant dannieggai
 2641 ryqnrflhll gkiyyfgnns kavtgwqtin gkvyfmpdt amaaagglfe idgviyffgv
 2701 dgvakpgiyy (SEQ ID NO:230)

[00116] Similarly, the amino acid sequences of *C. difficile* toxin B are known. For example, the amino acid sequence of toxin B from Strain VPII0463 is set forth below.

1 mslvnrkqlk kmanvrfrtg edeyvailda leeyhnmsen tvvekyklk dinstldiyi
 61 dtykksgrnk alkkfkeylv tevllelknkn ltpveknlfh vwiggqindt ainyinqwkd
 121 vnsdynvvnf ydsnaflint lkktvvesai ndtlesfren lndprfdynk ffrkrmeliy
 181 dkqknfinyy kaqreenpel iiddivktyl sneyskeide lntyieesln kitqnsndv
 241 rnfeefkngs sfniyeqelv erwlnlaasdl ilrisalkei ggmyldvdmf pgiqpdlfes
 301 iekpssvtvd fwemtkleai mkykeyipey tsehfmdlde evqssfesvl asksdkseif
 361 sslgdmeasp levkiafnsk giingglisv kdsycsnliv kqienrykil nnslnpaise
 421 dndfntttnt fidsimaeen adngrfmmel gkyrvrgffp dvkttinlsq peayaaayqd
 481 llmfkegsnm ihlieadlrn feisktnisq stegemaslw sfddarakaq feeykrnyfe

541 gslgeddnld fsqnivvdke yllekissla rssergyihy ivqlqgdki yeaacnlfak
 601 tpydsvlfqk niedseleyy ynpdggeiqe idkykipsii sdrpkikltf ighgkdefnt
 661 difagfdvds lsteieaaaid lakedispks ieinllgonm fsysinveet ypgklillkvk
 721 dkiselmpsi sqdsiivsan qyevrinseg rrelldhsge winkeesiik disskeyisf
 781 npkenkitvk sknlpelstl lqeirnnsns sdieleekvm lteceinvis nldtqiveer
 841 ieeaknltsd sinyikdefk liesisdalc dlkqgneled shfifedis etdegfsirf
 901 inketgesif vetektifse yanhiteeis kikgtifdtv ngklvkkvnl dtthevntln
 961 aaffiqslie ynsskeslsn lsvamkvqvy aqlfstglnt itdaakvvel vstaldetid
 1021 llptlseigp iiatiidgvs lgaaikelse tsdpllrgei eakigimavn lttattait
 1081 sslgiasgfs illvplagis agipslvnne lvlrkatkv vdyfkhvalv etegvftlld
 1141 dkimppqddl viseidfnnn sivilgkceiw rmeggsghtv tddidhffsa psityrephl
 1201 siydvlevqk eeldlskdml vlpnapnrvf awetgwtpgl rslendgtkl ldrirdnyeg
 1261 efywryfafi adalittlkp ryedtnirin ldentrsviv piitteyire klsysfygsg
 1321 gtyalslsqy nmginiele sdvwidvvn vrvdvtiesd kikkgdlied ilstlsieen
 1381 kiilnshein fsgevngsng fvsltfsile ginailevdl lksykillis gelkilmlns
 1441 nhqqkidyi gfnseiqkni pysfvdsegk engfingstk eglfvselpd vvliskvymd
 1501 dskpsfgyys nnlkdvkvit kdvniltgy ylkddikisl sltlqdekti klsvhldes
 1561 gvaailkfmn rkgnntntds lmsflesmni ksifvnflqs nikfildanf iisgttsigq
 1621 feficdendn iqpyfikfnt letnytllyvg nrqnmivepn ydlldsgdis stvinfsqky
 1681 lygidscvnk vvispniytd einitpvyet nntypevivl danyinekin vnindlsiry
 1741 vwsndgndfi lmstseenkv sqvkirfvnv fkdktlankl sfnsdkqdv pvseilslft
 1801 psyyedglig ydlglvslyn ekfyinnfgm mvsgliyind slyyfkppvn nltgfvvtvg
 1861 ddkyyfnpin ggaasigeti iddknyyfnq sgvlqtgvfs tedgfkypap antldenleg
 1921 eaaidftgkll ideniyyfdd nyrgavewke ldgemhyfsp etgkafgln qigdykyfyn
 1981 sdgvmqkgfv sindnkhyfd dsqvmkvgyt eidgkhfyfa engemqigvf ntedgfkfyfa
 2041 hhnedlgnee geeisysgil nfnkiyyfd dsftavvgwk dledgskyyf dedtaeayig
 2101 lslindgqyy fnddgimqvg fvtindkvfy fsdsgiesg vqniddnyfy iddngivqig
 2161 vfdtsdgyky fapantvndn lyggaveysg lvrvgedvyy fgetytietg wiydmenesd
 2221 kyyfnpetkk ackginlidd ikyyfdekgi mrtglisfen nnyyfnenge mqfgyinied
 2281 kmfyfgedgv mqigvfntpd gkyfahqnt ldenfegesi nytgwldlde kryyftdeyi
 2341 aatgsviidg eeyyfdpda qlvise (SEQ ID NO:231)

[00117] *C. difficile* strains are classified into variant toxinotypes according to variations in restriction sites within the DNA sequence of the PaLoc encoding toxins A and B. Currently 27 such variant toxinotypes are recognized (I to XXVII). Toxinotype 0 includes strains with restriction patterns identical to the reference laboratory strain VPI 10463 (ATCC43255) and is the most prevalent toxinotype. In a survey of strains in various culture collections worldwide, the toxinotypes 0, III, V, and VIII (toxin B only) are the most common. (Rupnik, FEMS Microbiol Rev 32 (2008) 541-555.)

5. Anti-Toxin A Antibodies

[00118] This disclosure provides antibodies that bind to *C. difficile* toxin A, including human, monoclonal antibodies having 1) high binding affinity, 2) potent *in vitro* neutralization activity, and 3) optionally with a broad spectrum of binding to various toxinotypes. Thus, in one embodiment, the antibody has at least one of the following characteristics:

- (a) the antibody binds to *C. difficile* toxin A with a dissociation constant (K_D) equal to or less than 10 pM (10^{-11} M);
- (b) the antibody neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin A in the Vero monkey kidney cell line with an NT50 equal to or less than 3000 pM;
- (c) the antibody neutralizes the *C. difficile* toxin A induced loss of transepithelial electrical resistance (TEER) in the T-84 cell line with an NT50 equal to or less than 6 nM; and/or
- (d) the antibody binds to toxin A produced by strains of toxinotypes 0, III, V, XII, and XV.

[00119] The antibody may have at least two, at least three, or all 4 of the above-identified characteristics.

[00120] In one embodiment, the human, monoclonal antibody binds to *C. difficile* toxin A with a dissociation constant (K_D) equal to or less than 500 pM, 250 pM, 200 pM, 150 pM, 100 pM (10^{-10} M), 10 pM (10^{-11} M), 1 pM (10^{-12} M), 0.1 pM (10^{-13} M), 0.01 pM (10^{-14} M), or 0.001 pM (10^{-15} M). The dissociation constant may be measured using techniques known in the art. In one embodiment, the dissociation constant is measured using biolayer interferometry, as described in the examples of this application.

[00121] In another embodiment, the human, monoclonal antibody neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin A at 2.4 ng/mL in the Vero monkey kidney cell line with an NT50 equal to or less than 3000 pM, 2000 pM, 1000 pM, 100 pM, 60 pM, or 50 pM. For the sake of consistency, when measuring the neutralizing activity in the Vero monkey kidney cell line, Vero cells (2.5×10^4 cells/well with 5% heat-inactivated FBS) are seeded in a 96-well tissue culture microtiter plates and incubated 37°C overnight. An equal volume (80 µl) of 4.8 ng/mL ($8 \times \text{MC50}$) *C. difficile* toxin A solution and individual dilutions of the antibody solutions (80 µl) in Vero cell medium are combined in a new 96-well plate, and incubated at 37°C, 5% CO₂ for 1 hour before 100 µl of the toxin/antibody solutions are added to the Vero cells, and incubated at 37°C for 72 hours. After incubating for 72 hours, the cells are washed twice with 120 µl/each of MEM medium that does not contain phenol, L-glutamine and FBS before adding 100 µl MEM medium that does not contain phenol, L-glutamine and FBS and 10 µl of Alamar Blue® (Life

Technologies) to each well. The plates are lightly mixed and incubated at 37°C for 4 hours before reading fluorescence at 560-590nm with a cut off at 590nm.

[00122] In yet another embodiment, the human, monoclonal antibody neutralizes the *C. difficile* toxin A (at 200 ng/mL applied apically) induced loss of transepithelial electrical resistance (TEER) in the T-84 cell line with an NT50 equal to or less than 6 nM, 5 nM, 2 nM, or 1.5 nM. For the sake of consistency, when measuring TEER, T-84 cells are seeded into 0.4 micron polyester transwell plates at a seeding density of 3.6×10^5 cells/cm² and maintained at 37°C, 5% CO₂ in 10% heat-inactivated FBS in DMEM/F12 culture media for 10-12 days until stable TEER achieved and media is replaced in both apical and basolateral compartments of the transwells daily from day 6 and on the day of assay. The *C. difficile* toxin A (final concentration of 200 ng/mL) is combined 1:1 with an antibody and incubated at 37°C with gentle rocking for 30 minutes before replacing the media in the apical compartment with the toxin/antibody samples. Transepithelial electrical resistance of the T-84 cells is measured at T₀ immediately before sample addition and after 2.5 hours (T₁₅₀) incubation at 37°C 5% CO₂.

[00123] In another embodiment, the human, monoclonal antibody binds to toxin A produced by the strains of toxinotypes 0, III, V, XII, and XV. Toxinotype binding may be measured using techniques known in the art, including the techniques described in the examples of this application, such as Western analysis. In another embodiment, for antibodies that bind to an epitope in the C-terminal domain (CTD) of toxin A or toxin B, the toxinotype can be measured using a CTD competition assay, as described in the examples of this application.

[00124] In another embodiment, the human, monoclonal anti-toxin A antibody has an on rate constant (K_{on}) to toxin A of at least 10⁵M⁻¹s⁻¹. In another embodiment, the human, monoclonal anti-toxin A antibody has an off rate constant (K_{off}) to toxin A of 10⁻⁴s⁻¹, 10⁻⁵s⁻¹, 10⁻⁶s⁻¹, 10⁻⁷s⁻¹, or 10⁻⁸s⁻¹, or less. The K_{on} and K_{off} may be measured using techniques known in the art. In one embodiment, the dissociation constant is measured using biolayer interferometry, as described in the examples of this application.

[00125] In one embodiment, the antibody is an isolated A1 antibody. As used herein, the term "A1" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin A, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:20 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:22; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:24, a CDR2 comprising the amino acid sequence of SEQ ID NO:26, and a CDR3 comprising the amino acid sequence of SEQ ID NO:28 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of

SEQ ID NO:31, a CDR2 comprising the amino acid sequence of SEQ ID NO:33, and a CDR3 comprising the amino acid sequence of SEQ ID NO:35. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:23, a CDR1 comprising the amino acid sequence of SEQ ID NO:24, a FR2 comprising the amino acid sequence SEQ ID NO:25, a CDR2 comprising the amino acid sequence of SEQ ID NO:26, a FR3 comprising the amino acid sequence SEQ ID NO:27, a CDR3 comprising the amino acid sequence of SEQ ID NO:28, and a FR4 comprising the amino acid sequence SEQ ID NO:29 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:30, a CDR1 comprising the amino acid sequence of SEQ ID NO:31, a FR2 comprising the amino acid sequence SEQ ID NO:32, a CDR2 comprising the amino acid sequence of SEQ ID NO:33, a FR3 comprising the amino acid sequence SEQ ID NO:34, a CDR3 comprising the amino acid sequence of SEQ ID NO:35, and a FR4 comprising the amino acid sequence SEQ ID NO:36. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin A epitope that is recognized by the A1 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the A1 antibody to *C. difficile* toxin A.

[00126] In another embodiment, the antibody is an isolated A2 antibody. As used herein, the term "A2" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin A, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:2 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:4; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a CDR2 comprising the amino acid sequence of SEQ ID NO:8, and a CDR3 comprising the amino acid sequence of SEQ ID NO:10 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:13, a CDR2 comprising the amino acid sequence of SEQ ID NO:15, and a CDR3 comprising the amino acid sequence of SEQ ID NO:17. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:5, a CDR1 comprising the amino acid sequence of SEQ ID NO:6, a FR2 comprising the amino acid sequence SEQ ID NO:7, a CDR2 comprising the amino acid sequence of SEQ ID NO:8, a FR3 comprising the amino acid sequence SEQ ID NO:9, a CDR3 comprising the amino acid sequence of SEQ ID NO:10, and a FR4 comprising the amino acid sequence SEQ ID NO:11 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:12, a CDR1 comprising the amino acid sequence of SEQ ID NO:13, a FR2 comprising the amino acid sequence SEQ ID NO:14, a CDR2 comprising the amino acid

sequence of SEQ ID NO:15, a FR3 comprising the amino acid sequence SEQ ID NO:16, a CDR3 comprising the amino acid sequence of SEQ ID NO:17, and a FR4 comprising the amino acid sequence SEQ ID NO:18. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin A epitope that is recognized by the A2 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the A2 antibody to *C. difficile* toxin A.

[00127] The A2 antibody binds to an epitope in the C-terminal receptor domain of *C. difficile* toxin A that comprises the amino acid sequence of X₁TGWQTI (SEQ ID NO:232), where X₁ is A or V or the amino acid sequence of X₂TGWQTIX₃GKX₄YYF (SEQ ID NO:233), where X₂ is A or V, X₃ is N or D and X₄ is K or V.. Thus, another embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the C-terminal receptor domain of *C. difficile* toxin A that comprises the amino acid sequence of X₁TGWQTI (SEQ ID NO:232), where X₁ is A or V or the amino acid sequence of X₂TGWQTIX₃GKX₄YYF (SEQ ID NO:233), where X₂ is A or V, X₃ is N or D and X₄ is K or V.

[00128] In another embodiment, the antibody is an isolated A3 antibody. As used herein, the term "A3" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin A, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:40; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:42, a CDR2 comprising the amino acid sequence of SEQ ID NO:44, and a CDR3 comprising the amino acid sequence of SEQ ID NO:46 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:49, a CDR2 comprising the amino acid sequence of SEQ ID NO:51, and a CDR3 comprising the amino acid sequence of SEQ ID NO:53. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:41, a CDR1 comprising the amino acid sequence of SEQ ID NO:42, a FR2 comprising the amino acid sequence SEQ ID NO:43, a CDR2 comprising the amino acid sequence of SEQ ID NO:44, a FR3 comprising the amino acid sequence SEQ ID NO:45, a CDR3 comprising the amino acid sequence of SEQ ID NO:46, and a FR4 comprising the amino acid sequence SEQ ID NO:47 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:48, a CDR1 comprising the amino acid sequence of SEQ ID NO:49, a FR2 comprising the amino acid sequence SEQ ID NO:50, a CDR2 comprising the amino acid sequence of SEQ ID NO:51, a FR3 comprising the amino acid sequence SEQ ID NO:52, a CDR3 comprising the amino acid sequence of SEQ ID NO:53, and a FR4 comprising

the amino acid sequence SEQ ID NO:54. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin A epitope that is recognized by the A3 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the A3 antibody to *C. difficile* toxin A.

[00129] In another embodiment, the antibody is an isolated A4 antibody. As used herein, the term "A4" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin A, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:56 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:58; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:60, a CDR2 comprising the amino acid sequence of SEQ ID NO:62, and a CDR3 comprising the amino acid sequence of SEQ ID NO:64 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:67, a CDR2 comprising the amino acid sequence of SEQ ID NO:69, and a CDR3 comprising the amino acid sequence of SEQ ID NO:71. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:59, a CDR1 comprising the amino acid sequence of SEQ ID NO:60, a FR2 comprising the amino acid sequence SEQ ID NO:61, a CDR2 comprising the amino acid sequence of SEQ ID NO:62, a FR3 comprising the amino acid sequence SEQ ID NO:63, a CDR3 comprising the amino acid sequence of SEQ ID NO:64, and a FR4 comprising the amino acid sequence SEQ ID NO:65 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:66, a CDR1 comprising the amino acid sequence of SEQ ID NO:67, a FR2 comprising the amino acid sequence SEQ ID NO:68, a CDR2 comprising the amino acid sequence of SEQ ID NO:69, a FR3 comprising the amino acid sequence SEQ ID NO:70, a CDR3 comprising the amino acid sequence of SEQ ID NO:71, and a FR4 comprising the amino acid sequence SEQ ID NO:72. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin A epitope that is recognized by the A4 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the A4 antibody to *C. difficile* toxin A.

[00130] In another embodiment, the antibody is an isolated A5 antibody. As used herein, the term "A5" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin A, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:74 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:76; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:78, a CDR2 comprising the amino acid

sequence of SEQ ID NO:80, and a CDR3 comprising the amino acid sequence of SEQ ID NO:82 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:85, a CDR2 comprising the amino acid sequence of SEQ ID NO:87, and a CDR3 comprising the amino acid sequence of SEQ ID NO:89. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:77, a CDR1 comprising the amino acid sequence of SEQ ID NO:78, a FR2 comprising the amino acid sequence SEQ ID NO:79, a CDR2 comprising the amino acid sequence of SEQ ID NO:80, a FR3 comprising the amino acid sequence SEQ ID NO:81, a CDR3 comprising the amino acid sequence of SEQ ID NO:82, and a FR4 comprising the amino acid sequence SEQ ID NO:83 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:84, a CDR1 comprising the amino acid sequence of SEQ ID NO:85, a FR2 comprising the amino acid sequence SEQ ID NO:86, a CDR2 comprising the amino acid sequence of SEQ ID NO:87, a FR3 comprising the amino acid sequence SEQ ID NO:88, a CDR3 comprising the amino acid sequence of SEQ ID NO:89, and a FR4 comprising the amino acid sequence SEQ ID NO:90. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin A epitope that is recognized by the A5 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the A5 antibody to *C. difficile* toxin A.

[00131] Whether an antibody competitively inhibits the binding of an antibody to *C. difficile* toxin A can be assessed using routine methods in the art, including, for example, the Octet methods described in the examples of this application and other routine quantitative methods, such as the Biacore assay. In one embodiment, competitive binding is measured using biolayer interferometry.

[00132] The amino acid and nucleotide sequences for the V_H and V_L domains of the A1 antibody are as follows:

A1 heavy chain nucleic acid	ATGAACATCTGTGGTTCTTCCTTCTCCTGGTGGCAGCCCCAGATGGGTCTGTCCAGGTGCACCTG CAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCCTGTCCCTCACCTGCACTGTCTCCGGTGAC TCCATCAGTACTTACTACTGGAGCTGGATCCGGCAGCCCCAGGGAAGGGACTGGAGTGGATTGGGTAT GTCTATTACACTGGGAGCACCAACTACAGCCCTTCCCTCGAGGGTCGAGTCACCTTATCAGTAGACACG TCCAAGAACCAGTTCTCCCTGAAGTTGAATTCTGTGAGTGCTGCGGACACGCCCCGTATTACTGTGCG AGAGGCGCGCGGAGTGGCTACGATTACAGGGGGTTCTTTGACTACTGGGGCCAGGGAATCCTGGTCTCC GTCTCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCGTGCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCA GGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCC AGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCCGTGC CCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCTCATG ATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC
---	---

	ACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAACCACAGGTGTACACCCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTACAGCTGACCTGC CTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCCGAGAACAAC TACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:19)
A1 light chain nucleic acid	ATGGAAACCCAGCGCAGCTTCTCTTCTCTCTCTGCTACTCTGGCTCCCAGATACCACCGGAGAAGTTGTG TTGACGCACTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTGTAGGGCCAGT CAGAGTGTATACCAACGGCTTCTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGGGTCTCTATC TATGGTGGCTCCAGCAGGGCCACTGGCATCCCAGACAGGTTCACTGGCAGTGGGTCTGGGACAGACTTC ACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAATGTATTACTGTGACAGTATGGTCTCTCA GGGACTTTTGGCCAGGGGACCAAGCTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCA GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAG AAACACAAAGTCTACGCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC AGGGGAGAGTGTAG (SEQ ID NO:21)
A1 heavy chain amino acid	MKHLWFFLLVAAFRWVLSQVHLQESGPGLVKPSSETLSLTCTVSGDSISTYYWSWIRQPPGKGLEWIGY VYYTGSTNYSPLGRVTLSDVTSKNQFSLKLNSVSAADTAVYYCARGAAEWLRFGRFFDYWGQILVS VSSASTKGFSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSL SVVTVFSSSLGTQTYICNVNHKPSNTKVDKKVERPKSCKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTFPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:20)
A1 light chain amino acid	METPAQLIFLLLLWLPDITGEVVLTSQSPGTLSPGERATLSCRASQSVTNGFLAWYQQKPGQAPRVLI YGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAMYYCQQYGLSGTFGQGTGLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:22)

[00133] The amino acid sequences for the FR and CDR sequences of the A1 antibody are as follows:

FRH1: QVHLQESGPGLVKPSSETLSLTCTVS (SEQ ID NO:23)
 CDRH1: GDSISTYYWS (SEQ ID NO:24)
 FRH2: WIRQPPGKGLEWIG (SEQ ID NO:25)
 CDRH2: YVYYTGSTN (SEQ ID NO:26)
 FRH3: YSPSLEGRVTLSDVTSKNQFSLKLNSVSAADTAVYYCAR (SEQ ID NO:27)
 CDRH3: GAAEWLRFGRFFDY (SEQ ID NO:28)
 FRH4: WGQGILVSVSS (SEQ ID NO:29)
 FRL1: EVVLTQSPGTLSPGERATLSC (SEQ ID NO:30)
 CDRL1: RASQSVTNGFLA (SEQ ID NO:31)
 FRL2: WYQQKPGQAPRVLIY (SEQ ID NO:32)
 CDRL2: GASSRAT (SEQ ID NO:33)

FRL3: GIPDRFSGSGSGTDFTLTISRLEPEDFAMYYC (SEQ ID NO:34)

CDRL3: QQYGLSGT (SEQ ID NO:35)

FRL4: FGQGTKLEIK (SEQ ID NO:36)

[00134] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the A1 antibody (i.e., one or more of SEQ ID NOs. 24, 26, 28, 31, 33, or 35). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin A, including, but not limited to an anti-toxin A antibody.

[00135] The amino acid and nucleotide sequences for the V_H and V_L domains of the A2 antibody are as follows:

A2 heavy chain nucleic acid	ATGAAACATCTGTGGTTCTTCCTTCTCCTGGTGGCAGCTCCAGATGGGTCTGTCCAGGTGCAGCTG CAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCCTGTCCCTCACCTGCAGTGTCTCTGGTGGC TCCATCAGTACTTACTACTGGAGCTGGATCCGGCAGTCCCCAGGGAAGGGACTGGAGTGGATGGGGTAT ATCTATTATAGTGGGAGCACCAACTACAACCCCTCCCTCGAGAGTCGGGTACCATAGCAGTGGACACG TCCAAGAATCAGTTCTCCCTGCAGTTGACCTCTGTGACTGCTCCGACACGGCCGTGATTACTGTGCG AGAGGAGCGGCGGAGTGGCTACGGTTCAGGGGGTCTTTGACTCCTGGGGCCAGGGAACCCCTGGTCACC GTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCA GGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCC AGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCCGTGC CCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTTCTTCCCCCAAAACCCAGGACACCCCTCATG ATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC ACGTACCGGGTGGTCAGCGTCTTCAACGTCCTGCACAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGA GAACCACAGGTGTACACCCCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGCCTGACCTGC CTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCTCCCGTGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:1)
A2 light chain nucleic acid	ATGGAACCCAGCGCAGCTTCTCTTCTCCTCCTGCTACTCTGGCTCCAGAGACCACCGGAGAAAATGTG TTGACGCAGTCTCCAGGGACCCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCCTCCTGACAGGGCCAGT CACAGTGTACCAACAACCTTCTTAGCCTGGTACCAGCAAAAACCTGGCCAGGCTCCAGGCTCCTCATC TATGGTGTCTCCAGCAGGGCCACTGGCATCCAGACAGGTTCAAGTGGCAGTGGGTCTGGGACAGACTTC ACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTGAGCAATATGGTGTCTCA GGGACTTTTGGCCAGGGGACCAAGCTGGAGATCAAACGAACCTGTGGCTGCACCATCTGTCTTCTATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCAATCGGTAACCTCCAGGAGAGTGTACACA GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAGCAGACTACGAG AAACACAAAGTCTACGCTTCCGAAGTCAACCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC AGGGGAGAGTGTAG (SEQ ID NO:3)
A2 heavy chain amino	MKHLWFFLLLVAAAPRWVLSQVQLQESGPGLVKPSSETLSLTCTVSGGSISTYYWSWIRQSPGKGLEWNGY IYYSGSTNYPNPSLESRTVIAVDTSKNQFSLQLTSVTAADTAVYYCARGAAEWLRFGRFFDSWQGLTIVT VSSASTKGPSVFPLAPSSKSTSGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKCDKHTHTCPPCPAPPELLGGPSVFLFPPKPKDITLM ISRTPFVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC

acid	KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:2)
A2 light chain amino acid	METPAQLLFLLLLLWLPETTGENVLTQSPGTLSPGERATLSCRASHSVTNNFLAWYQQKPGQAPRLLI YGVSSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGVSGTFTGQGTKEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:4)

[00136] The amino acid sequences for the FR and CDR sequences of the A2 antibody are as follows:

FRH1:	QVQLQESGPGLVKPSSETLSLTCTVS	(SEQ ID NO:5)
CDRH1:	GGSTSTYYWS	(SEQ ID NO:6)
FRH2:	WIRQSPGKGLEWMG	(SEQ ID NO:7)
CDRH2:	YIYYSGSTN	(SEQ ID NO:8)
FRH3:	YNPSLESRVTIADVTSKNQFSLQLTSVTAADTAVYYCAR	(SEQ ID NO:9)
CDRH3:	GAAEWLRFGRFFDS	(SEQ ID NO:10)
FRH4:	WGQGTLLVTVSS	(SEQ ID NO:11)
FRL1:	ENVLTQSPGTLSPGERATLSC	(SEQ ID NO:12)
CDRL1:	RASHSVTNNFLA	(SEQ ID NO:13)
FRL2:	WYQQKPGQAPRLLIY	(SEQ ID NO:14)
CDRL2:	GVSSRAT	(SEQ ID NO:15)
FRL3:	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	(SEQ ID NO:16)
CDRL3:	AAWDDSLTGLYV	(SEQ ID NO:17)
FRL4:	FGTGTKVTVLGQPKANPTVT	(SEQ ID NO:18)

[00137] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the A2 antibody (i.e., one or more of SEQ ID NOs. 6, 8, 10, 13, 15, or 17). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin A, including, but not limited to an anti-toxin A antibody.

[00138] The amino acid and nucleotide sequences for the V_H and V_L domains of the A3 antibody are as follows:

A3 heavy chain nucleic acid	ATGCAACTGCTGGAGTCTGGGGGAGGCTTGGTGAAGCCTGGGGGGTCCCTTAGACTCTCCTGTGCAGCC TCTGGATTCACTTTCAGTAACGCCTGGATGAGTTGGGTCCGCCAGGGTCCAGGGAAGGGGCTGGAATGG GTTGGCCGTATTAAAAGTAAACTGATGGTGGGACACAGACTACGCTGCACCCGTGAAAGGCAGATTC AGCATCTCAAGAAATGATTCAAATAACACGCTGTTCTGCAAATGAACAGCCTGAAAACCGAGGACACA GCCGTATATTACTGTACCACAGGTCCTCAAATTGTAGTTGTAGCAGGTGCTACCACTCGGGACCAGCCT AACTACTACTACTACGGTTTGGACGTCCTGGGGCCTAGGGACCACGGTCACCGTCTCGTCAGCCTCCACC AAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC TGCCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTAGCGGCCCTGACCAGCGGC GTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
---	--

	TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCCAGCACCTGAACTCCTG GGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAG GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC GTGGAGGTGCATAATGCCAAGACAAAGCCCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGC GTCCTCACCGTCTGACACAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAACAAAGCC CTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACACAGGTGTACACC CTGCCCCCATCCCGGGATGAGCTGACCAAGAACAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTAT CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCAACGCTCCC GTGCTGGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGCTCTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCC CTGTCTCCGGTAAATGA (SEQ ID NO:37)
A3 light chain nucleic acid	ATGGCCAGCTTCCCTCTCCTCCTCACCTCCTCACTCACTGTGCAGGGTCTGGGGCCAGTCTGTGCTG ACTCAGCCACCCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCTCTTGTTCGGAAGCAGCTCC AACATCGGCATTAATACTGTAACTGGTACCAGCAGCTCCCAGGAACGGCCCCCAACTCCTCATATAT AAGAGTAATCTGCGACCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCC CTGGCCATCAGTGGGCTCCGGTCTGAGGATGAGGCTGATTATTACTGTGCGGCATGGGATGACAGCCTG ACTGGTCTTTATGTCTTCGGAACCTGGGACCAAGGTACCGTCTTAGGTGAGCCCAAGGCCAACCCCACT GTCACCTCTGTTCCTCCCTCCTCTGAGGAGCTCCAAGCCAAACAAGGCCACACTAGTGTGTCTGATCAGT GACTTCTACCCGGGAGCTGTGACAGTGGCTTGAAGGCAGATGGCAGCCCCGTCAAGCCGGGAGTGGAG ACGACCAAAACCCCTCAAACAGAGCAACAACAGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCGAG CAGTGGAGTCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGTGGAGAAGACAGTG GCCCCACAGAATGTTTCATAG (SEQ ID NO:39)
A3 heavy chain amino acid	MQLLESGLLVKPGGSLRLSCAASGFTFSNAWMSWVRQGPGLVWVGRIKSKTDGGTTDYAAPVKGRF SISRNDNNNTLFLQMNSLKTEDTAVYYCTTGPIVIVVAGATSRDQPNYYYYGLDVWGLGTTVTVSSAST KGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTP SSSLGTQTYICNVNHKPSNTKVDKVEPKSCDKHTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LRPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP VLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:38)
A3 light chain amino acid	MASEPLLLLTLLTHCAGSWAQSVLTQPPSASGTPGQRVTISCSGSSSNIGINTVNWYQQLPGTAPKLLIY KSNLRPSGVPDRFSGSKSGTSASLAIISGLRSEDEADYYCAAWDDSLTGLYVFGTGKTVTLGQPKANPT VTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNKNKYAASSYLSTPE QWKSHRYSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:40)

[00139] The amino acid sequences for the FR and CDR sequences of the A3 antibody are as follows:

FRH1:	MQLLESGLLVKPGGSLRLSCAAS	(SEQ ID NO:41)
CDRH1:	GFTFSNAWMS	(SEQ ID NO:42)
FRH2:	WVRQGPGLVWVG	(SEQ ID NO:43)
CDRH2:	RIKSKTDGGTTD	(SEQ ID NO:44)
FRH3:	YAAPVKGRFSISRNDNNNTLFLQMNSLKTEDTAVYYCTT	(SEQ ID NO:45)
CDRH3:	GPQIVVVA	(SEQ ID NO:46)
FRH4:	GATSRDQPNYYYYGLDVWGLGTTVTVSS	(SEQ ID NO:47)

FRL1: QSVLTQPPSASGTPGQRTVISC (SEQ ID NO:48)
 CDRL1: SGSSSNIGINTVN (SEQ ID NO:49)
 FRL2: WYQQLPGTAPKLLIY (SEQ ID NO:50)
 CDRL2: KSNLRPS (SEQ ID NO:51)
 FRL3: GVPDRFSGSKSGTSASLAISGLRSEDEADYYC (SEQ ID NO:52)
 CDRL3: AAWDDSLTGLYV (SEQ ID NO:53)
 FRL4: FGTGKVTVLGQPKANPTVT (SEQ ID NO:54)

[00140] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the A3 antibody (i.e., one or more of SEQ ID NOs. 42, 44, 46, 49, 51, or 53). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin A, including, but not limited to an anti-toxin A antibody.

[00141] The amino acid and nucleotide sequences for the V_H and V_L domains of the A4 antibody are as follows:

A4 heavy chain nucleic acid	ATGGAGTTTGGGCTGAGCTGGGTTTCTCTCGTTGCTCTTTTAAGAGGTGTCCAGTGTACAGGTGCACCTG GTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAACCTTTGGACTC AACTTCAGTGACTATGGTTTTCAGTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTT ACATCATATGATGGAAGCAACAATACTACGCAGAATTCGTGAAGGGCCGATTACCATCTCCAGAGAC AATTACAAGAATACGGTGTATCTGCAATGAACAGCCTGAGACTTGAGGACACGGCTGTGTATTACTGT GCGAGAGATCTCGCCCCATACAATTTTGGAGTGGTTATGGGAATAATTGGTTTCGACCCCTGGGGCCAG GGAACCTGGTCAACGTCCTCTCAGCCTCCACCAAGGGCCATCGGTCTTCCCCCTGGCACCCCTCCTCC AAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCGTGGTCAAGGACTACTTCCCGAACCCTGGTACG GTGTCGTGGAACTCAGGCGCCCTGACAGCGGGCTGCACACCTTCCCGGCTGTCTACAGTCTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACCGTGGCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACCAAGGTGGACAAGAAAGTTGAGCCCAATCTTGTGACAAAACCTCAC ACATGCCACCCGTGCCAGCACCTGAACTCCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCC AAGGACACCCCTCATGATCTCCCGAACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGAC CCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCACGTACCGGGTGGTCAGCCTCTCACCCTGCTGCACCCAGGACTGGCTGAATGGC AAGGAGTACAAGTCAAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGGCC AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGATGAGCTGACCAAGAACCAG GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG CAGCCGAGAGAACTACAAGACCAGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:55)
A4 light chain nucleic acid	ATGGAAACCCAGCGCAGCTTCTCTCTCTCTGCTACTCTGGCTCCCAGATACCAACCGGAGAAATGTG TTGACGCACTCTCCAGGCACCCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCCTCTCTGACGGGCCAGT CAGAGTGTACTGGCACCTCCTTAGCCTGGTTCCAGCAGAAACCTGGCCAGGCTCCCCGGCTCCTCATC TATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAAGTGGCAGTGGGTCTGGGACAGACTTC ACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTGACAGTATGGTAGCTCA CCTAGACTCACTTTCCGGCGAGGGACCAAGGTGGAGATCAAACGAAGTGTGCTGCACCATCTGTCTTC ATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCTGCTGAATAACTTC TATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTCAAATCGGGTAACCTCCAGGAGAGT GTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGAC TACGAGAAACACAAAGTCTACGCCTGCGAAGTCAACCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGC

	TTCAACAGGGGAGAGTGTTAG (SEQ ID NO:57)
A4 heavy chain amino acid	MEFGLSWVFLVALLRGVQCQVHLVESGGGVVQPGRSRLRLSCATFGLNFSYDYGFWVRQAPGKGLEWVAV TSYDGSNKYYAEFVKGRFTISRDNKYKNTVYLQMNLSRLLEDVAVYYCARDLAPYNFWSGYGNNWFDWQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVFSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:56)
A4 light chain amino acid	METPAQLLEFLLLWLPDTTGEIVLTQSPGTLSSLSPGERATLSCRASQSVTGTSLAWFQQKPGQAPRLLI YGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPRLTFGGGTKVEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:58)

[00142] The amino acid sequences for the FR and CDR sequences of the A4 antibody are as follows:

FRH1: QVHLVESGGGVVQPGRSRLRLSCATF (SEQ ID NO:59)
 CDRH1: GLNFSYDYGFW (SEQ ID NO:60)
 FRH2: WVRQAPGKGLEWVA (SEQ ID NO:61)
 CDRH2: VTSYDGSNK (SEQ ID NO:62)
 FRH3: YYAEFVKGRFTISRDNKYKNTVYLQMNLSRLLEDVAVYYCAR (SEQ ID NO:63)
 CDRH3: DLAPYNFWSGYGNNWFDW (SEQ ID NO:64)
 FRH4: WGQGTILVTVSS (SEQ ID NO:65)
 FRL1: EIVLTQSPGTLSSLSPGERATLSC (SEQ ID NO:66)
 CDRL1: RASQSVTGTSLA (SEQ ID NO:67)
 FRL2: WFQQKPGQAPRLLIY (SEQ ID NO:68)
 CDRL2: GASSRAT (SEQ ID NO:69)
 FRL3: GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC (SEQ ID NO:70)
 CDRL3: QQYGSSPRLT (SEQ ID NO:71)
 FRL4: FGGGTKVEIK (SEQ ID NO:72)

[00143] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the A4 antibody (i.e., one or more of SEQ ID NOs. 60, 62, 64, 67, 69, or 71). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin A, including, but not limited to an anti-toxin A antibody.

[00144] The amino acid and nucleotide sequences for the V_H and V_L domains of the A5 antibody are as follows:

A5 heavy chain nucleic acid	ATGGAGTTTGGGCTGAGCTGGGTTTTCTCGTTGCTCTTTTAAAGAGGTGTCCAGTGTCCAGGTGCACCTG GTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCTGTGCAACCTTTGCACTC AACTTCAGTGACTATGGTTTTCTAGTGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTT ACATCATATGATGGAAGCAACAAATACTACGCAGAATTCGTGAAGGGCCGATTACCATCTCCAGAGAC AATTACAAGAATACGGTGTATCTGCAAAATGAACAGCCTGAGACTTGAGGACACGGCTGTGTATTACTGT GCGAGAGATCTCGCCCCATACAATTTTTGGAGTGGTTATGGGAATAATTGGTTTCGACCCCTGGGGCCAG GGAACCCTGGTCAACGTCTCTCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCTCTC AAGAGCACCTCTCGGGGACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCCGGTGACG GTGTCTGTGGAACFCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTTACAGTCTCTCAGGA CTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGACCCAGACCTACATCTGCAAC GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCAC ACATGCCCCACCGTGCCCGAGCAGCTGAACTCCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCC AAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGAC CCTGAGGTCAAGTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCAGCTACCGGGTGGTACGCGTCTCACCCTCTGCACCAGGACTGGCTGAATGGC AAGGAGTACAAGTGAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAGCC AAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAG GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG CAGCCGGAGAACAATAACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTACAGC AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:73)
A5 light chain nucleic acid	ATGGAAGCCCCAGCGCAGCTTCTCTTCTCTCTGCTACTCTGGCTCCAGATACCACTGGAGAAATAGTG ATGACGCAGTCTCCAGCCACCCTGTCTGTCTCTCCAGGAGAAAGAGCCACCCTCTCTGCAAGGGCCAGT CAGAGTATTAGCAGCAACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAGACTCCTCATCTAT GATGCATCCACCAGGGCCACTGGTATCCAGCCAGGTTCACTGGCAGTGGGTCTGGGACAGAGTTCACT CTCACCATCAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTGACCAATACAATGACTGGCTT GTGACGTCGCGCAAGGGACCAAAGTGGAAATCAAACGAACGTGGCTGCACCATCTGTCTTCTATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCC AGAGAGGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCAATCGGGTAACCTCCAGGAGAGTGTACA GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAG AAACACAAAGTCTACGCCTGCGAAGTCAACCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAAC AGGGGAGAGTCTTAG (SEQ ID NO:75)
A5 heavy chain amino acid	MEFGLSWVFLVALLRGVQCQVHLVESGGGVVQPGRSRLSLSCATFGLNFSYGFHWVRQAPGKLEWVAV TSYDGSNKYYAEFVKGRFTISRNYKNTVYLQMNSLRLEDVAVYYCARDLAPYFWSGYGNWFDPWGQ GTLVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVFSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKP KDTILMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHLEAHNHYTQKSLSLSPGK (SEQ ID NO:74)
A5 light chain amino acid	MEAPAQLLFLLLLWLPDITTEIVMTQSPATLSVSPGERATLSCRASQSISSNLAWYQQKPGQAPRLTIY DASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQYNDWLVTFGQGTKVELKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:76)

[00145] The amino acid sequences for the FR and CDR sequences of the A5 antibody are as follows:

FRH1: QVHLVESGGGVVQPGRSRLRLSCATF (SEQ ID NO:77)
 CDRH1: GLNFSYDGFH (SEQ ID NO:78)
 FRH2: WVRQAPGKGLEWVA (SEQ ID NO:79)
 CDRH2: VTSYDGSNK (SEQ ID NO:80)
 FRH3: YYAEFVKGRFTISRDNKYKNTVYLOMNSLRLEDTAVYYCAR (SEQ ID NO:81)
 CDRH3: DLAPYNFWSGYGNNWFDP (SEQ ID NO:82)
 FRH4: WGQGTLLVTVSS (SEQ ID NO:83)
 FRL1: EIVMTQSPATLSVSPGERATLSC (SEQ ID NO:84)
 CDRL1: RASQSISSNLA (SEQ ID NO:85)
 FRL2: WYQQKPGQAPRLLIY (SEQ ID NO:86)
 CDRL2: DASTRAT (SEQ ID NO:87)
 FRL3: GIPARFSGSGSGTEFTLTISLQSEDFAVYYC (SEQ ID NO:88)
 CDRL3: QQYNDWLVT (SEQ ID NO:89)
 FRL4: FGQGTKVEIK (SEQ ID NO:90)

[00146] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the A5 antibody (i.e., one or more of SEQ ID NOs. 78, 80, 82, 85, 87, or 89). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin A, including, but not limited to an anti-toxin A antibody.

[00147] The SEQ ID NOs corresponding to the sequences of the A1, A2, A3, A4, and A5, antibodies are listed in Table 1.

Table 1: SEQ ID NOs of Anti-Toxin A Antibodies

Region	Type	A1	A2	A3	A4	A5
VH	DNA	19	1	37	55	73
VH	AA	20	2	38	56	74
VL	DNA	21	3	39	57	75
VL	AA	22	4	40	58	76
FRH1	AA	23	5	41	59	77
CDRH1	AA	24	6	42	60	78
FRH2	AA	25	7	43	61	79
CDRH2	AA	26	8	44	62	80
FRH3	AA	27	9	45	63	81
CDRH3	AA	28	10	46	64	82
FRH4	AA	29	11	47	65	83
FRL1	AA	30	12	48	66	84
CDRL1	AA	31	13	49	67	85
FRL2	AA	32	14	50	68	86
CDRL2	AA	33	15	51	69	87
FRL3	AA	34	16	52	70	88
CDRL3	AA	35	17	53	71	89
FRL4	AA	36	18	54	72	90

6. Anti-Toxin B Antibodies

[00148] This disclosure provides antibodies that bind to *C. difficile* toxin B, including human, monoclonal antibodies having 1) high binding affinity, 2) potent *in vitro* neutralization activity, and 3) optionally a broad spectrum of binding to the toxins of various toxinotypes.

Thus, in one embodiment, the antibody has at least one of the following characteristics:

- (a) the antibody binds to *C. difficile* toxin B with a dissociation constant (K_D) equal to or less than 100 pM;
- (b) the antibody neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin B in the Vero monkey kidney cell line with an NT50 equal to or less than 1000 pM;
- (c) the antibody neutralizes the *C. difficile* toxin B induced loss of transepithelial electrical resistance (TEER) in the T-84 cell line with an NT50 equal to or less than 200 pM; and/or
- (d) the antibody binds to toxin B produced by at least the strains of toxinotypes 0, III, and V.

[00149] The antibody may have at least two, at least three, or all 4 of the above-identified characteristics.

[00150] In one embodiment, the human, monoclonal antibody binds to *C. difficile* toxin B with a dissociation constant (K_D) equal to or less than 500 pM, 250 pM, 200 pM, 150 pM, 100 pM (10^{-10} M), 50 pM, 30 pM, 10 pM (10^{-11} M), or 1 pM (10^{-12} M). The dissociation constant may be measured using techniques known in the art. In one embodiment, the dissociation constant is measured using biolayer interferometry, as described in the examples of this application.

[00151] In another embodiment, the human, monoclonal antibody neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin B at 17 pg/mL in the Vero monkey kidney cell line with an NT50 of equal to or less than 1000 pM, 500 pM, 100 pM, 60 pM, or 50 pM. For the sake of consistency, when measuring the neutralizing activity in the Vero monkey kidney cell line, Vero cells (2.5×10^4 cells/well with 5% heat-inactivated FBS) are seeded in 96-well tissue culture microtiter plates and incubated 37°C overnight. An equal volume (80 µl) of 34.4 pg/mL ($8 \times \text{MC50}$) *C. difficile* toxin B solution and individual dilutions of the antibody solutions (80 µl) in Vero cell medium are combined in a new 96-well plate, and incubated at 37°C, 5% CO₂ for 1 hour before 100 µl of the toxin/antibody solutions are added to the Vero cells, and incubated at 37°C for 72 hours. After incubating for 72 hours, the cells are washed twice with 120 µl/each of MEM medium that does not contain phenol, L-glutamine and FBS before adding 100 µl MEM medium that does not contain phenol, L-glutamine and FBS and 10 µl of Alamar Blue® (Life

Technologies) to each well. The plates are lightly mixed and incubated at 37°C for 4 hours before reading fluorescence at 560-590nm with a cut off at 590nm.

[00152] In yet another embodiment, the human, monoclonal antibody neutralizes the *C. difficile* toxin B (at 75 ng/mL, applied basolaterally) induced loss of transepithelial electrical resistance (TEER) in the T-84 cell line with an NT50 equal to or less than 200 pM, 150 pM, 100 pM, or 70 pM. For the sake of consistency, when measuring TEER, T-84 cells are seeded into 0.4 micron polyester transwell plates at a seeding density of 3.6×10^5 cells/cm² and maintained at 37°C, 5% CO₂ in 10% heat-inactivated FBS in DMEM/F12 culture media for 10-12 days until stable TEER is achieved and media is replaced in both apical and basolateral compartments of the transwells daily from day 6 and on the day of assay. The *C. difficile* toxin B (final concentration of 75 ng/mL) is combined 1:1 with an antibody and incubated at 37°C with gentle rocking for 30 minutes before replacing the media in the basolateral compartment with the toxin/antibody samples. Transepithelial electrical resistance of the T-84 cells is measured at T₀ immediately before sample addition and after 2.5 hours (T₁₅₀) incubation at 37°C 5% CO₂.

[00153] In another embodiment, the human, monoclonal anti-toxin B antibody binds to toxin B produced by strains of at least toxinotypes 0, III, and V, toxin B produced by strains of at least toxinotypes 0, III, V, and VIII, toxin B produced by the strains of at least toxinotypes 0, III, V, VIII, and XII, or toxin B produced by the strains of at least toxinotypes 0, III, V, VIII, XII, and XV. Toxinotype binding may be measured using techniques known in the art, including the techniques described in the examples of this application, such as Western analysis. In another embodiment, for antibodies that bind to an epitope in the C-terminal domain (CTD) of toxin A or toxin B, the toxinotype can be measured using a CTD competition assay, as described in the examples of this application.

[00154] In another embodiment, the human, monoclonal anti-toxin B antibody has an on rate constant (K_{on}) to toxin B of at least 10⁵M⁻¹s⁻¹. In another embodiment, the human, monoclonal anti-toxin B antibody has an off rate constant (K_{off}) to toxin B of 10⁻⁴s⁻¹, 10⁻⁵s⁻¹, 10⁻⁶s⁻¹, 10⁻⁷s⁻¹, or 10⁻⁸s⁻¹, or less. The K_{on} and K_{off} may be measured using techniques known in the art. In one embodiment, the dissociation constant is measured using biolayer interferometry, as described in the examples of this application.

[00155] In one embodiment, the antibody is an isolated B1 antibody. As used herein, the term "B1" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin B, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:110 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:112; or 2) a heavy chain variable domain comprising a

CDR1 comprising the amino acid sequence of SEQ ID NO:114, a CDR2 comprising the amino acid sequence of SEQ ID NO:116, and a CDR3 comprising the amino acid sequence of SEQ ID NO:118 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:121, a CDR2 comprising the amino acid sequence of SEQ ID NO:123, and a CDR3 comprising the amino acid sequence of SEQ ID NO:125. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:113, a CDR1 comprising the amino acid sequence of SEQ ID NO:114, a FR2 comprising the amino acid sequence SEQ ID NO:115, a CDR2 comprising the amino acid sequence of SEQ ID NO:116, a FR3 comprising the amino acid sequence SEQ ID NO:117, a CDR3 comprising the amino acid sequence of SEQ ID NO:118, and a FR4 comprising the amino acid sequence SEQ ID NO:119 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:120, a CDR1 comprising the amino acid sequence of SEQ ID NO:121, a FR2 comprising the amino acid sequence SEQ ID NO:122, a CDR2 comprising the amino acid sequence of SEQ ID NO:123, a FR3 comprising the amino acid sequence SEQ ID NO:124, a CDR3 comprising the amino acid sequence of SEQ ID NO:125, and a FR4 comprising the amino acid sequence SEQ ID NO:126. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin B epitope that is recognized by the B1 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the B1 antibody to *C. difficile* toxin B.

[00156] In another embodiment, the antibody is an isolated B2 antibody. As used herein, the term "B2" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin B, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:92 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:94; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:96, a CDR2 comprising the amino acid sequence of SEQ ID NO:98, and a CDR3 comprising the amino acid sequence of SEQ ID NO:100 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:103, a CDR2 comprising the amino acid sequence of SEQ ID NO:105, and a CDR3 comprising the amino acid sequence of SEQ ID NO:107. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:95, a CDR1 comprising the amino acid sequence of SEQ ID NO:96, a FR2 comprising the amino acid sequence SEQ ID NO:97, a CDR2 comprising the amino acid sequence of SEQ ID NO:98, a FR3 comprising the amino acid sequence SEQ ID NO:99, a CDR3 comprising the amino acid sequence of SEQ ID NO:100, and a FR4 comprising

the amino acid sequence SEQ ID NO:101 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:102, a CDR1 comprising the amino acid sequence of SEQ ID NO:103, a FR2 comprising the amino acid sequence SEQ ID NO:104, a CDR2 comprising the amino acid sequence of SEQ ID NO:105, a FR3 comprising the amino acid sequence SEQ ID NO:106, a CDR3 comprising the amino acid sequence of SEQ ID NO:107, and a FR4 comprising the amino acid sequence SEQ ID NO:108. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin B epitope that is recognized by the B2 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the B2 antibody to *C. difficile* toxin B.

[00157] In another embodiment, the antibody is an isolated B3 antibody. As used herein, the term "B3" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin B, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:164 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:166; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:168, a CDR2 comprising the amino acid sequence of SEQ ID NO:170, and a CDR3 comprising the amino acid sequence of SEQ ID NO:172 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:175, a CDR2 comprising the amino acid sequence of SEQ ID NO:177, and a CDR3 comprising the amino acid sequence of SEQ ID NO:179. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:167, a CDR1 comprising the amino acid sequence of SEQ ID NO:168, a FR2 comprising the amino acid sequence SEQ ID NO:169, a CDR2 comprising the amino acid sequence of SEQ ID NO:170, a FR3 comprising the amino acid sequence SEQ ID NO:171, a CDR3 comprising the amino acid sequence of SEQ ID NO:172, and a FR4 comprising the amino acid sequence SEQ ID NO:173 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:174, a CDR1 comprising the amino acid sequence of SEQ ID NO:175, a FR2 comprising the amino acid sequence SEQ ID NO:176, a CDR2 comprising the amino acid sequence of SEQ ID NO:177, a FR3 comprising the amino acid sequence SEQ ID NO:178, a CDR3 comprising the amino acid sequence of SEQ ID NO:179, and a FR4 comprising the amino acid sequence SEQ ID NO:180. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin B epitope that is recognized by the B3 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the B3 antibody to *C. difficile* toxin B.

[00158] In another embodiment, the antibody is an isolated B4 antibody. As used herein, the term “B4” refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin B, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:146 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:148; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:150, a CDR2 comprising the amino acid sequence of SEQ ID NO:152, and a CDR3 comprising the amino acid sequence of SEQ ID NO:154 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:157, a CDR2 comprising the amino acid sequence of SEQ ID NO:159, and a CDR3 comprising the amino acid sequence of SEQ ID NO:161. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:149, a CDR1 comprising the amino acid sequence of SEQ ID NO:150, a FR2 comprising the amino acid sequence SEQ ID NO:151, a CDR2 comprising the amino acid sequence of SEQ ID NO:152, a FR3 comprising the amino acid sequence SEQ ID NO:153, a CDR3 comprising the amino acid sequence of SEQ ID NO:154, and a FR4 comprising the amino acid sequence SEQ ID NO:155 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:156, a CDR1 comprising the amino acid sequence of SEQ ID NO:157, a FR2 comprising the amino acid sequence SEQ ID NO:158, a CDR2 comprising the amino acid sequence of SEQ ID NO:159, a FR3 comprising the amino acid sequence SEQ ID NO:160, a CDR3 comprising the amino acid sequence of SEQ ID NO:161, and a FR4 comprising the amino acid sequence SEQ ID NO:162. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin B epitope that is recognized by the B4 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the B4 antibody to *C. difficile* toxin B.

[00159] In another embodiment, the antibody is an isolated B5 antibody. As used herein, the term “B5” refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin B, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:182 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:184; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:186, a CDR2 comprising the amino acid sequence of SEQ ID NO:188, and a CDR3 comprising the amino acid sequence of SEQ ID NO:190 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:193, a CDR2 comprising the amino acid sequence of SEQ ID NO:195, and a CDR3 comprising the amino acid sequence of SEQ ID NO:197. In one embodiment, the

monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:185, a CDR1 comprising the amino acid sequence of SEQ ID NO:186, a FR2 comprising the amino acid sequence SEQ ID NO:187, a CDR2 comprising the amino acid sequence of SEQ ID NO:188, a FR3 comprising the amino acid sequence SEQ ID NO:189, a CDR3 comprising the amino acid sequence of SEQ ID NO:190, and a FR4 comprising the amino acid sequence SEQ ID NO:191 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:192, a CDR1 comprising the amino acid sequence of SEQ ID NO:193, a FR2 comprising the amino acid sequence SEQ ID NO:194, a CDR2 comprising the amino acid sequence of SEQ ID NO:195, a FR3 comprising the amino acid sequence SEQ ID NO:196, a CDR3 comprising the amino acid sequence of SEQ ID NO:197, and a FR4 comprising the amino acid sequence SEQ ID NO:198. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin B epitope that is recognized by the B5 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the B5 antibody to *C. difficile* toxin B.

[00160] In another embodiment, the antibody is an isolated B6 antibody. As used herein, the term "B6" refers to a monoclonal antibody, or antigen-binding fragment thereof, that binds to *C. difficile* toxin B, wherein the antibody comprises 1) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:128 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:130; or 2) a heavy chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:132, a CDR2 comprising the amino acid sequence of SEQ ID NO:134, and a CDR3 comprising the amino acid sequence of SEQ ID NO:136 and a light chain variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:139, a CDR2 comprising the amino acid sequence of SEQ ID NO:141, and a CDR3 comprising the amino acid sequence of SEQ ID NO:143. In one embodiment, the monoclonal antibody comprises a heavy chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:131, a CDR1 comprising the amino acid sequence of SEQ ID NO:132, a FR2 comprising the amino acid sequence SEQ ID NO:133, a CDR2 comprising the amino acid sequence of SEQ ID NO:134, a FR3 comprising the amino acid sequence SEQ ID NO:135, a CDR3 comprising the amino acid sequence of SEQ ID NO:136, and a FR4 comprising the amino acid sequence SEQ ID NO:137 and a light chain variable domain comprising a FR1 comprising the amino acid sequence SEQ ID NO:138, a CDR1 comprising the amino acid sequence of SEQ ID NO:139, a FR2 comprising the amino acid sequence SEQ ID NO:140, a CDR2 comprising the amino acid sequence of SEQ ID NO:141, a FR3 comprising the amino acid sequence SEQ ID NO:142, a CDR3 comprising the amino acid sequence of SEQ ID

NO:143, and a FR4 comprising the amino acid sequence SEQ ID NO:144. In yet another embodiment, the antibody is a monoclonal antibody that binds to a *C. difficile* toxin B epitope that is recognized by the B6 antibody, such that the monoclonal antibody competitively inhibits, or cross-blocks, the binding of the B6 antibody to *C. difficile* toxin B.

[00161] Whether an antibody competitively inhibits the binding of an antibody to *C. difficile* toxin B can be assessed using routine methods in the art, including, for example, the Octet methods described in the examples of this application or other routine quantitative binding assays, such as the Biacore assay. In one embodiment, competitive binding is measured using biolayer interferometry.

[00162] The B1, B2, and B3 antibodies bind to an epitope within amino acids 10-520 of SEQ ID NO:231. Thus, one embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the glucosyl transferase domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 10-520 of SEQ ID NO:231. More specifically, the B1 and B3 antibodies bind to an epitope comprising the amino acid sequence SGRNK (SEQ ID NO:234) or amino acids 56-80 of SEQ ID NO:231. Thus, another embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the glucosyl transferase domain of *C. difficile* toxin B, wherein the epitope comprises the amino acid sequence SGRNK (SEQ ID NO:234) or amino acids 56-80 of SEQ ID NO:231.

[00163] The B4 antibody binds to an epitope in the N-terminal translocation domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 1110-1530 of SEQ ID NO:231. Thus, another embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the N-terminal translocation domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 1110-1530 of SEQ ID NO:231.

[00164] The B6 antibody binds to an epitope in the receptor binding domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 1750-2360 of SEQ ID NO:231. Thus, another embodiment is directed to an isolated monoclonal antibody that binds to an epitope in the receptor binding domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 1750-2360 of SEQ ID NO:231.

[00165] The amino acid and nucleotide sequences for the V_H and V_L domains of the B1 antibody are as follows:

B1 heavy chain nucleic	ATGGAGTTTGGGCTGAGCTGGGTTTCTTGGTTGCCATTTTAAAGGTGTCCAGTGTGAGGTGCAGCTG GTGGAGTCCGGGGGAGGCTTAGTTTCAGCCTGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTG ACTTTCAGAAGTTACTGGATGCACTGGGTCCGCCAAGTTCCAGGGAAGGGCTGGTGTGGGTGTCATGT ATTAATAAAGAAGGGAGTAGCACAACCTACGCGGACTCCGTGAAGGGCCGATTACCATCTCCAGAGAC AACGCCAAGAACACGCTGTATTGGAAATGAACAGTCTGAGAGCCGACGACACGGCTGTGTATTATTGT CTAAGGGGATACGATGTTGACTACTGGGGCCAGGGAACGCTGGTCACCGTCTCCTCAGCCTCCACCAAG
------------------------	---

acid	GGCCCATCGGTCTTCCCCCTGGCACCTCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTGACCAGCGGCGTG CACACCTTCCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAG AAAGTTGAGCCCAAATCTTGTGACAAACTCACACATGCCCCACCGTGCCAGCACCTGAACTCCTGGGG GGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTC CTCACCGTCTTCGACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAACAAAGCCCTC CCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG CCCCCATCCCGGATGAGCTGACCAAGAACAGGTGAGCCTGACCTGCCTGGTCAAAGCCTTCTATCCC AGCGACATCGCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAATAACAAGACCAGCCTCCCGTG CTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGAACAAGAGCAGGTGGCAGCAGGGG AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG TCTCCGGGTAAATGA (SEQ ID NO:109)
BI light chain nucleic acid	ATGGCCTGGACTCCTCTCCTCCTCTGTTCTCTCTCACTGCACAGGTTCCCTCTCGCAGGCTGTGCTG ACTCAGCCGTCTCCCTCTCTGCATCTCCCGGAGCATCAGTCAGTCTCACCTGCACCTTGCGCAGTGGC ATCAATGTTGGTACCTACAGGATATACTGGTATCAGCAGAAGCCAGGGAGTCTTCCCCGTTATCTCCTG AGGTACAAATCAGGCTTAGATAAACACAGGGCTCTGGAGTCCCCAGCCGCTTCTCTGGATCCAAAGAT GATTCGGCCAATGCAGGGATTTTATTCATTTCTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGT TTGATTTGGCACAGCAGCGCTGTGGTATTCGGCGGAGGACCAAGCTGACCGTCTAGGTGAGCCCAAG GCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAAACAGGCCACACTAGTG TGTCTGATCAGTGACTTCTACCCGGAGCTGTGACAGTGGCTTGAAGGCAGATGGCAGCCCCGTCAAG GCGGGAGTGGAGACGACCAACCCCTCCAACAGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC CTGACGCCCCGAGCAGTGAAGTCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGTG GAGAAGACAGTGGCCCCCTACAGAATGTTTCATAG (SEQ ID NO:111)
BI heavy chain amino acid	MEFGLSWVFLVAILKGVQCEVQLVESGGGLVQPGGSLRLSCLASGFTFRSYWMHWVRQVPGKGLVWVSC INKEGSSTTYADSVKGRFTISRDNKNTLYLEMNSLRADDATAVYYCLRGYDVDYWGQGLVTVSSASTK GPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTIISKAKGQPRPEQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGSGFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:110)
BI light chain amino acid	MAWTFLLLLFLSHCTGSLSQAVLTQPSLSASPGASVLTCTLRSGINVTYRIYWYQKPGSPFRYLL RYKSGLDKHQSGVPSRFSKSDSANAGILFISGLQSEDEADYYCLIWSSAVVFGGGTKLTVLGQPK AAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNKYAASSYLS LTPEQWKSHRSYSQCQVTHEGSTVEKTVAPTECS (SEQ ID NO:112)

[00166] The amino acid sequences for the FR and CDR sequences of the BI antibody are as follows:

FRH1: EVQLVESGGGLVQPGGSLRLSCLAS (SEQ ID NO:113)
 CDRH1: GFTFRSYWMH (SEQ ID NO:114)
 FRH2: WVRQVPGKGLVWVSC (SEQ ID NO:115)
 CDRH2: CINKEGSSTT (SEQ ID NO:116)
 FRH3: YADSVKGRFTISRDNKNTLYLEMNSLRADDATAVYYCLR (SEQ ID NO:117)
 CDRH3: GYDVDYWG (SEQ ID NO:118)

FRH4: QGTLVTVSS (SEQ ID NO:119)
 FRL1: QAVLTQPSSLSASPGASVSLTCTLR (SEQ ID NO:120)
 CDRL1: SGINVGTYRIY (SEQ ID NO:121)
 FRL2: WYQQKPGSPFRYLL (SEQ ID NO:122)
 CDRL2: RYKSGLDKH (SEQ ID NO:123)
 FRL3: QGSGVPSRFSGSKDDSANAGILFISGLQSEDEADYYCLI (SEQ ID NO:124)
 CDRL3: WHSSAVVF (SEQ ID NO:125)
 FRL4: GGGTKLTVLGQPKAAPSVT (SEQ ID NO:126)

[00167] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the B1 antibody (i.e., one or more of SEQ ID NOs. 114, 116, 118, 121, 123, or 125). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin B, including, but not limited to an anti-toxin B antibody.

[00168] The amino acid and nucleotide sequences for the V_H and V_L domains of the B2 antibody are as follows:

B2 heavy chain nucleic acid	ATGAACACCTGTGGTTCTTCGTCCTCCTGGTGGCAGCTCCAGATGGGTCTGTCCAGGTGCAACTA CTGCAGGGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCCTGTCCCTCACGTGCGCTGTCTATGGTGGG TCCTTTAGTGAACACTATTGGAGTTGGATCCGCCAGCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAA ATCAATTATGGTGGAAACACCAACTACAACCCGTCCTCGAGAGTCAATCTCCATCTCAGTGGACACA TCAAAGAACCAGGTCTTCCTGAGAGTGAGATTTGTGACAGCTGCGGACACGGCTGTGATTTTTTGTTCG GGAGGCCGGCAGCAGCAGTACATGCCCGGACTTTTGCTATCTGGGGCCAAGGACAATGGTCACCGTC TCTTCAGCCTCCACCAAGGGGCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGC ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCGAACCAGGTGACGGTGTGCTGGAACCTCAGGC GCCCTGACCAGCGGGCTGCACACCTTCCCGGCTGTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCCA GCACCTGAACCTCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAAGGACACCCCTCATGATC TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAAC TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAGC TACCGGGTGGTCAGCGTCTCACCCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAG GTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAGAA CCACAGGTGTACACCCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTG GTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTAC AAGACCACGCCTCCCGTGTGTTGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAG AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG CAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:91)
B2 light chain nucleic acid	ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGCTCTGGGTCTCCGGGTCCAGTGGGGATATTGTG ATGACGAGTCTCCACTCTCCCTGCCGTCACCCCTGGAGAGCCGGCTCCATCTCCTGCAGGTCTAGT CAGAGCCTGCTTCATACTAATGGAAACAATAATTTGGTATGGTATCTGCAGAAGCCAGGGCAGGCTCCA CATCTCCTGATCTATCTGGGATCTAATCGGGCCTCCGGGGTCCCTGGCAGGTTTCACTGGCAGTGGATCA GGCACAGATTTTACACTGAAAATCAGCAGAGTGGAGGTGAGGATGTTGGGGTTTATTACTGCATGCAA TCTCTACAAACTCCTCCACTTTTGGCCAGGGGACCAAGCTGGAGATCAAACGAACTGTGGCTGCACCA TCTGTCTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCTGGAACGCTCTGTTGTGTGCTGCTG AATAACTTCTATCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAACTCC CAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGC

	AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC ACAAAGAGCTTCAACAGGGGAGAGTGTAG (SEQ ID NO:93)
B2 heavy chain amino acid	MKHLWFFVLLVAAPRWVLSQVQLLQGGAGLLKPSETLSLTCAVYGGSTSEHYWSWIRQPPGKGLEWIGE INYGGNTNYNPSLESRISISVDTSKNQVFLRVRFVTAADTAVYFCSGGRRRAAVHGRTFAIWGGTMVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:92)
B2 light chain amino acid	MRLPAQLLGLMLWVSGSSGDIVMTQSPLSLPVTPGEPASISCRSSQSLHTNGNNYLVWYLQKPGQAP HLLIYLGSNRASGVPGRFSGSGSGTDFTLKISRVEEDVGYYCMQSLQTPPTFGQGTKLEIKRTVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKOSTYLSSTLTLS KADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:94)

[00169] The amino acid sequences for the FR and CDR sequences of the B2 antibody are as follows:

FRH1: QVQLLQGGAGLLKPSETLSLTCAVY (SEQ ID NO:95)
 CDRH1: GGSFSEHYWS (SEQ ID NO:96)
 FRH2: WIRQPPGKGLEWIG (SEQ ID NO:97)
 CDRH2: EINYGGNTN (SEQ ID NO:98)
 FRH3: YNPSLESRISISVDTSKNQVFLRVRFVTAADTAVYFCSG (SEQ ID NO:99)
 CDRH3: GRRRAAVHGRTFAI (SEQ ID NO:100)
 FRH4: WGQGTMTVTSS (SEQ ID NO:101)
 FRL1: DIVMTQSPLSLPVTPGEPASISC (SEQ ID NO:102)
 CDRL1: RSSQSLHTNGNNYLV (SEQ ID NO:103)
 FRL2: WYLQKPGQAPHLLIY (SEQ ID NO:104)
 CDRL2: LGSNRAS (SEQ ID NO:105)
 FRL3: GVPGRFSGSGSGTDFTLKISRVEEDVGYYC (SEQ ID NO:106)
 CDRL3: MQSLQTPPT (SEQ ID NO:107)
 FRL4: FGQGTKLEIK (SEQ ID NO:108)

[00170] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the B2 antibody (i.e., one or more of SEQ ID NOs.96, 98, 100, 103, 105, or 107). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin B, including, but not limited to an anti-toxin B antibody.

[00171] The amino acid and nucleotide sequences for the V_H and V_L domains of the B3 antibody are as follows:

B3 heavy chain nucleic acid	ATGGAGTTTGGGCTGAGCTGGGTTTTCTTGTGGCATTTTAAAGGTGTCCAGTGTGAGGTGCAGCTG GTGGAGTCCGGGGGAGGCTTAGTTACAGCTGGGGGGTCCCTGAGACTCTCTGTTCAGCCTCTGGATTCT ACTTTTCAAGATTACTGGATGCAGTGGGTCCGCCAAGTTCCAGGGAAGGGGCTGGTATGGCTCTCATGT ATTAATAAAGAAGGGAGTAGCACAACCTACGCGGACTCCGTGAAGGGCCGATTCAACCATCTCCAGAGAC AACGCCAAGAACACGCTGTATTTGCAATGAACAGTCTGAGAGCCGACGACACGGCTGTGTATTACTGT CTAAGGGGATACGATGTTGACTACTGGGGCCAGGGAACCCCTGGTCAACCGTCTCCTCAGCCTCCACCAAG GGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGC CTGCTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAATCAGGCGCCCTGACCAGCGGGCTG CACACCTTCCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCAACCCAGACCTACATCTGCAACGTGAATCACAAGCCAGCAACACCAAGGTGGACAAG AAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCCACCGTGGCCAGCACCTGAACCTCCTGGGG GGACCGTCACTCTTCTCTTCCCCCAAAACCCAAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCGGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAAGCGTC CTCACCGTCTCGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAACAAAGCCCTC CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG CCCCCATCCCGGATGAGCTGACCAAGAACAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC AGCGACATCGCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCTCCCGTG CTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG TCTCCGGGTAAATGA (SEQ ID NO:163)
B3 light chain nucleic acid	ATGGCCTGGACTCCTCTCCTCCTCTGTTCTCTCTCACTGCACAGGTTCCTCTCGCAGGCTGTGCTG ACTCAGCCGTCTCCTCTCTGCTCTCCCGGAGCATCACTCAGTCTCACCTGCACCTTGCGCAGTGGC GTCAATGTTGGTCTCTACAGGATATACTGGTATCAGCAGAAGCCAGGGAGTCTCTCCCGGTATCTCCTG AGGTACAAATCAGGCTTAGATAAACACAGGGCTCTGGAGTCCCCAGCCGCTCTCTGGATCCAAAGAT GATTCGGCCAATGCAGGGATTTTATTCATTTCTGGGCTCCAGTCTGAGAATGATGCTGATTATTACTGT TTGATTTGGCACAACAGCGCTGGGTATTTCGGCGGAGGACCAAGCTGACCGTCTAGGTGAGCCCAAG GCTGCCCCCTCGGTCACTCTGTTCCTCCGCTCTCTGAGGAGCTTCAAGCCAAACAGGCCACACTGGTG TGCTGTATCAGTCACTTCTACCCGGGAGCTGTGACAGTGGCTTGAAGGCAGATGGCAGCCCCGTCAAG GCGGGAGTGGAGACGACCAACCCCTCCAAACAGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC CTGACGCCCCGAGCAGTGAAGTCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGTG GAGAAGACAGTGGCCCCCTACAGAATGTTTCATAG (SEQ ID NO:165)
B3 heavy chain amino acid	MEFGLSWVFLVAILKGVQCEVQLVESGGGLVQPGGSLRLSCSASGFTFRSYWMHWVRQVPGKGLVWVSC INKEGSSSTYADSVKGRFTISRDNKNTLYLQMNSLRADDTAVYYCLRGYDVDYWGQGLVTVSSASTK GPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNKTRPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPV LDSGDSFFLYSLKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:164)
B3 light chain amino acid	MAWTPLLLLFLSHCTGSLSQAVLTQPSLSPASPGASVLTCTLRSGVNVGSYRIYWYQKPGSPPRYLL RYKSGLDKHQSGVPSRFSKDDSANAGILFISGLQSENDADYICLIWHNSAVVFGGGTKLTVLCQPK AAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNKYAASSYL LTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:166)

[00172] The amino acid sequences for the FR and CDR sequences of the B3 antibody are as follows:

FRH1: EVQLVESGGGLVQPGGSLRLSCSAS

(SEQ ID NO:167)

CDRH1: GFTFRSYWMH (SEQ ID NO:168)
 FRH2: WVRQVPGKGLVWVS (SEQ ID NO:169)
 CDRH2: CINKEGSSTT (SEQ ID NO:170)
 FRH3: YADSVKGRFTISRDNAKNTLYLQMNSLRADDTAVYYCLR (SEQ ID NO:171)
 CDRH3: GYDVDYWG (SEQ ID NO:172)
 FRH4: QGTILTVSS (SEQ ID NO:173)
 FRL1: QAVLTQPSLSASPGASVSLTCTLR (SEQ ID NO:174)
 CDRL1: SGVNVGSYRIY (SEQ ID NO:175)
 FRL2: WYQQKPGSPFRYLL (SEQ ID NO:176)
 CDRL2: RYKSGLDKH (SEQ ID NO:177)
 FRL3: QGSGVPSRFSKDDSANAGILFISGLQSENDADYYCLI (SEQ ID NO:178)
 CDRL3: WHNSAVVF (SEQ ID NO:179)
 FRL4: GGGTKLTVLGQPKAAPSVT (SEQ ID NO:180)

[00173] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the B3 antibody (i.e., one or more of SEQ ID NOs.168, 170, 172, 175, 177, or 179). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin B, including, but not limited to an anti-toxin B antibody.

[00174] The amino acid and nucleotide sequences for the V_H and V_L domains of the B4 antibody are as follows:

B4 heavy chain nucleic acid	ATGGAACCTGGGGCTCCGCTGGGTTTCTCTGTTGCTATTTTAGAAGGTGTCCAGTGTGAGGTGCAGCTG GTGGAGTCTGGGGGAGGCCCTGGTCAAGCCTGGGGGGTCCCTGAGAGTCTCTGTGCAGCCTCTGGATTCT ACCTTCAGTAGCTATAGCATGAACCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCC ATTAGTAGTAATAGTAGTTACATATACTACGCAGACTCAGTTAAGGGCCGATTACCATCTCCAGAGAC AACGCCAAGAACTCACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTTTATTACTGT GCGAGAGATCGGGACTACAGTAACCTTACCGCTGGGGCCAGGGAACCTGGTCAACGCTCTCTCA GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCTCTCAAGAGCACTCTGGGGGCACAGCG GCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGGCGCCCTG ACCAGCGCGTGCACACCTTCCCCGGCTGTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGGTG ACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGGCCAGCACCT GAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTCCCGG ACCCCTGAGGTCAATGCGTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCACTGGTAC GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGGGAGGAGCAGTACAACAGCACGTACCGG GTGGTCAGCGTCTCACCCTCTGCACCAAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCC AACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGCGAGCCCCGAGAACCACAG GTGTACACCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGCTGACCTGCCTGGTCAAA GGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCGAGCCGAGAACAACTACAAGACC ACGCCTCCCGTGTCTGGACTCCGACGGCTCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGTAAATGA (SEQ ID NO:145)
B4 light chain	ATGGCCTGGTCTCTCTCTCTCTCACTCTCTCTGCTCACTGCACAGGGTCTGGGGCCAGTCTGTGCTG ACGCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTCACCATCTCTGCACTGGGAGCAGCTCC

nucleic acid	AACATCGGGGCAGGTTATGATGTACACTGGTACCGCCAACCTCCAGGAACAGCCCCAACTCCTCATC TATGGTAAGAACAAATCGGCCCTCAGGGGTCCCTAACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCC TCCCTGGCCATCACTGGCCTCCAGGCTGAGGATGAGGCTGATTACTGTCTAGTCTATGACAGCAGC CTGAGTGGTTCGGTATTCGGCGGAGGGACCAAGCTGACCGTCTAGGTGAGCCCAAGGCTGCCCCCTCG GTCACCTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTAGTGTGTCTGATCAGT GACTTCTACCCGGGAGCTGTGACAGTGGCTTGGAAAGGCAGATGGCAGCCCCGTCAAGCGGGGAGTGGAG ACGACCAAACCTCCAAACAGAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCGAG CAGTGGGAAGTCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGTGGAGAAGACAGTG GCCCCCTACAGAATGTTTCATAG (SEQ ID NO:147)
B4 heavy chain amino acid	MELGLRWVFLVAILEGVQCEVQLVESGGGLVLPKPGGSLRVSCAASGFTFSSYSMNWIRQAPGKGLEWVSS ISSNSSYIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARDRDYSNYLTAWGQGTLLTVSS ASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVSVLTFLVHQDWLNGKEYKCKVS NKAHPAPIEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGK (SEQ ID NO:146)
B4 light chain amino acid	MAWSPLLELLLAHCTGSAQSVLTQPPSVSGAPGQRTISCTGSSSNIGAGYDVHWYRQLPGTAPKLLI YGKNNRPSGVFNRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLGSGVFGGCTKLTVLGQPKAAPS VTLFPPSSEELQANKATLVCLISDFYPGAVTVANKADGSPVKAGVETTKPSKQSNKNKYAASSYLSTPE QWKSHRYSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO:148)

[00175] The amino acid sequences for the FR and CDR sequences of the B4 antibody are as follows:

FRH1:	EVQLVESGGGLVLPKPGGSLRVSCAAS	(SEQ ID NO:149)
CDRH1:	GFTFSSYSMN	(SEQ ID NO:150)
FRH2:	WIRQAPGKGLEWVS	(SEQ ID NO:151)
CDRH2:	SISSNSSYI	(SEQ ID NO:152)
FRH3:	YYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAR	(SEQ ID NO:153)
CDRH3:	DRDYSNYLTA	(SEQ ID NO:154)
FRH4:	WGQGTLLTVSS	(SEQ ID NO:155)
FRL1:	QSVLTQPPSVSGAPGQRTISC	(SEQ ID NO:156)
CDRL1:	TGSSSNIGAGYDVH	(SEQ ID NO:157)
FRL2:	WYRQLPGTAPKLLIY	(SEQ ID NO:158)
CDRL2:	GKNNRPS	(SEQ ID NO:159)
FRL3:	GVFNRFSGSKSGTSASLAITGLQAEDEADYYC	(SEQ ID NO:160)
CDRL3:	QSYDSSLGSGV	(SEQ ID NO:161)
FRL4:	FGGGTKLTVLGQPKAAPSVT	(SEQ ID NO:162)

[00176] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the B4 antibody (i.e., one or more of SEQ ID NOs. 150, 152, 154, 157, 159, or 161). In certain embodiments, the isolated polypeptide further comprises additional amino

acid sequences, or other molecules, to form a molecule that binds toxin B, including, but not limited to an anti-toxin B antibody.

[00177] The amino acid and nucleotide sequences for the V_H and V_L domains of the B5 antibody are as follows:

B5 heavy chain nucleic acid	ATGAAACACCTGTGGTTCTTCCTCCTCCTGGTGGCAGCTCCCAGATGGGTCTGTCTCAGGTGCATCTG CAGGAGTGGGGCCCAGGACTGGTGAAGCCTTCGGGGACCTGTCCCTCACCTGCGCTGTCTCTGGTGGC TCCATCAGTTACACTAACTGGTGGAGTTGGGTCCGCCTGCCCCAGGGAAGGGGCTGGAGTGGATAGGG GAAATCTATCATAGTAGGAGCACCAACTACAACCCGTCCCTCAAGAGTGGAGTCAACCATGTCAATAGAC AAGTCCAAGAATCTGTTCTCCCTGAAGCTGAACTCTGTGACCGCCGCGGACACGGCCATCTATTACTGT GCTAAAGCCGCTTACACAAGGGATGGAATACAGCCTTTTGACAACCTGGGGCCAGGGAACCTGGTCACC GTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCGAACCCTGACGGTGTCTGTGGAAGTCA GGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTTACAGTCTCAGGACTCTACTCCCTCAGC AGCGTGGTGAACCTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCC AGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAATCTTGTGACAAAACCTCACACATGCCACCCGTGC CCAGCACCTGAACCTCTGGGGGACCGTCACTCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATG ATCTCCCGGACCCCTGAGGTCAATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTC AACTGGTACGTGCACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC ACGTACCGGGTGGTCAAGCTCCTCACCGTCTGCACAGGACTGGCTGAATGGCAAGGAGTACAAGTGC AAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGA GAACACAGGTGTACACCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAAGCTGACCTGC CTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGAGCCCGGAGAACAC TACAAGACCAGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGAC AAGAGCAGGTGGCAGCAGGGGAACCTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTAC ACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:181)
B5 light chain nucleic acid	ATGGTGTTCAGACCCAGGTCTTCATTTCTCTGTGCTCTGGATCTCTGGTGCCTACGGGGACATCGTG ATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGTCCAGC CAGAGTGTTTTAAAGAGCTCCAACAATAAGAACTACTTAGCTTGGTACCAGCAGAAACCAGGACAGCCT CCTAAGCTGCTCATTTTCTGGGCAATGACCCGGGAATCCGGGGTCCCTGACCGATTCACTGGCAGCGGG TCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTGGCAGTTTATTACTGTGAG CAATAATCTAGTGCTCCTCGAACTTTCGGCGGAGGGACCAACGTAGAAATCAGACGAACCTGTGGCTGCA CCATCTGTCTTCTATCTCCCGCATCTGATGAGCAGTTGAAATCTGGAACCTGCTGTGTGTGCTGCTG CTGAATAACTTCTATCCAGAGAGGCCAAAGTACAGTGGGAAGGTGGATAACGCCCTCCAATCGGGTAAC TCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCTGCGAAGTACCCATCAGGGCCTGAGCTCGCCC GTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG (SEQ ID NO:183)
B5 heavy chain amino acid	MKHLWFFLLLVAAAPRWVLSQVHLQESGPGLVKPSGTLSTCAVSGGSISYTNWWSWVRLPPGKGLEWIG EIIYHSRSTNYPNPSLKSRVTMSIDKSKNLFSLKLNVSVAADTAIYYCAKAAAYTRDGIQFPDNGQGTIVT VSSASTKGPSVFFPLAPSSKSTSGGTAALGLVQDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAPIEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:182)
B5 light chain amino acid	MVLQTVVFISLLLVISGAYGDIVMTQSPDLSAVSLGERATINCKSSQSVLKSSNNKNYLAWYQQKPGQP PKLLIFWASTRESGVPRFSGSGSGTDFTLTISISLQAEDVAVYYCQYSSAPRTFGGGTNEIIRRTVAA PSVFIPTPPSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTSL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:184)

acid	
------	--

[00178] The amino acid sequences for the FR and CDR sequences of the B5 antibody are as follows:

FRH1:	QVHLQESGPGGLVKPSGTLSTCAVS	(SEQ ID NO:185)
CDRH1:	GGISYTNWWS	(SEQ ID NO:186)
FRH2:	WVRLPPGKGLEWIG	(SEQ ID NO:187)
CDRH2:	EIYHSRSTN	(SEQ ID NO:188)
FRH3:	YNPSLKSRVTMSIDKSKNLFSLKLNSVTAADTAIYYCAK	(SEQ ID NO:189)
CDRH3:	AAVTRDGIQPFDN	(SEQ ID NO:190)
FRH4:	WGQGTLLVTVSS	(SEQ ID NO:191)
FRL1:	DIVMTQSPDSLAVSLGERATINC	(SEQ ID NO:192)
CDRL1:	KSSQSVLKSSNNKNYLA	(SEQ ID NO:193)
FRL2:	WYQQKPGQPPKLLIF	(SEQ ID NO:194)
CDRL2:	WASTRES	(SEQ ID NO:195)
FRL3:	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	(SEQ ID NO:196)
CDRL3:	QQYSSAPRT	(SEQ ID NO:197)
FRL4:	FGGTINVEIR	(SEQ ID NO:198)

[00179] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the B5 antibody (i.e., one or more of SEQ ID NOs. 186, 188, 190, 193, 195, or 197). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin B, including, but not limited to an anti-toxin B antibody.

[00180] The amino acid and nucleotide sequences for the V_H and V_L domains of the B6 antibody are as follows:

B6 heavy chain nucleic acid	ATGGAGTTGGGGCTGTGCTGGGTTTCTCTGTTGCTATTTTAGAAGGTGTCCAGTGTGAGGTGCAGCTG GTGGAGTCTGGGGGAGGCTTGGTACAGCCGGGGGGGTCCCTGAGACTCTCTGTGCAGCCTCTGGATTG ACCTTCACTACCTCTACCATGAAGTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATAC ATTACTAGGACCAGCACTGTGATATACTATGCAGACTCTGTGAAGGGCCGATTACCATCTCCAGAGAC AATGCCAAGAACTCACTGTATCTGCAAAATGAGCAGCCTGAGAGCCGAGGACACGGCTGTGTATTATTGT GCGAGAGGGGTGAGGGACATTGGCGGAAACGGTTTGGACTACTGGGGCCAGGGAACCTGGTCACCGTC TCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCTGGCACCTCTCCAAAGAGCACCTCTGGGGG ACAGCGGCCCTGGGCTGCCGTTCAAGGACTACTTCCCGAACCAGGTGACGGTGTGTTGGAAGTCAAGG GCCCTGACCAAGCGGCTGCACACCTTCCCGGTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGC GTGGTGACCGTGCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCAGC AACACCAAGGTGGACAAGAAAGTTGAGCCCAATCTGTGACAAAACCTCACACATGCCACCGTGCCCA GCACCTGAAGTCTCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATC TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGAGCTGAGCCACGAAGACCTGAGGTCAAGTTCAAC TGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAGCAGTACAACAGCAGC TACCGGGTGGTCAAGCTCTCAGCGTCTGACCCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA
-----------------------------	--

	CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGAGCCTGACCTGCCTG GTCAAAGGCTTCTATCCCAGCSACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTAC AAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTCACCGTGGACAAG AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG CAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO:127)
B6 light chain nucleic acid	ATGGAAACCCAGCGCAGCTTCTCTTCTCCTGCTACTCTGGCTCCCAGATACCACCGGAGAAATTGTG TTGACGCAGTCTCCAGGCACCCTCTCTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGT CAGAGTGTAAACCAGCAGTTACTTAGCCTGGTACCAGCAGAAAAGTGGCCAGGCTCCAGGCTCCTCATC TACGGCGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCACTGGCAGTGGGTCTGGGACAGACTTC ACTCTCACCATCGCCAGACTGGAGCCTGAAGATTTTGGCGTGTATTACTGTGACAGTATGGTAGCTCG CCTCCGTACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAACGAAGTGTGGCTGCACCATCTGTCTTC ATCTTCCCGCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCTGCTGAATAACTTC TATCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTCCAATCGGSTAACTCCAGGAGAGT GTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGAC TACGAGAAACACAAAGTCTACGCCTGCGAAGTCAACCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGC TTCAACAGGGGAGAGTGTAG (SEQ ID NO:129)
B6 heavy chain amino acid	MELGLCWVFLVAILEGVQCEVQLVESGGGLVQPGGSLRLSCAASGFTFTTSTMNWVRQAPGKGLEWVS YITRTSTVIYYADSVKGRFTISRDNKNSLYLQMSLRAEDTAVYYCARGVRDIGGNGFDYWGQGLVTV SSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCPPECPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLAQDNLNGKEYCKK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDSGFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSPGK (SEQ ID NO:128)
B6 light chain amino acid	METPAQLFLLLLWLPTDTGEIVLTQSPGTLSPGERATLSCRASQSVTSSYLAWYQOKTGQAPRLLI YGASSRATGIPDRFSGSGSGTDFTLTITARLEPEDFAVYYCQYQYSSPPYTFGQGTKLEIKRTVAAPSVF IFPPSDEQLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:130)

[00181] The amino acid sequences for the FR and CDR sequences of the B6 antibody are as follows:

FRH1:	EVQLVESGGGLVQPGGSLRLSCAAS	(SEQ ID NO:131)
CDRH1:	GFTFTTSTMN	(SEQ ID NO:132)
FRH2:	WVRQAPGKGLEWVS	(SEQ ID NO:133)
CDRH2:	YITRTSTVI	(SEQ ID NO:134)
FRH3:	YYADSVKGRFTISRDNKNSLYLQMSLRAEDTAVYYCAR	(SEQ ID NO:135)
CDRH3:	GVRDIGGNGFDY	(SEQ ID NO:136)
FRH4:	WGQGLVTVSS	(SEQ ID NO:137)
FRL1:	EIVMTQSPATLSVSPGERATLSC	(SEQ ID NO:138)
CDRL1:	RASQSISSNLA	(SEQ ID NO:139)
FRL2:	WYQKPGQAPRLLIY	(SEQ ID NO:140)
CDRL2:	DASTRAT	(SEQ ID NO:141)
FRL3:	GIPARFSGSGSGTEFTLTISLQSEDFAVYYC	(SEQ ID NO:142)

CDRL3: QQYNDWLVT

(SEQ ID NO:143)

FRL4: FGQGTKVEIK

(SEQ ID NO:144)

[00182] One aspect is directed to an isolated polypeptide comprising one or more of the CDR sequences of the B6 antibody (i.e., one or more of SEQ ID NOs.132, 134, 136, 139, 141, or 143). In certain embodiments, the isolated polypeptide further comprises additional amino acid sequences, or other molecules, to form a molecule that binds toxin B, including, but not limited to an anti-toxin B antibody.

[00183] The SEQ ID NOs corresponding to the sequences of the B1, B2, B3, B4, B5, and B6 antibodies are listed in Table 2.

Table 2: SEQ ID NOs of Anti-Toxin B Antibodies

Region	Type	B1	B2	B3	B4	B5	B6
VH	DNA	109	91	163	145	181	127
VH	AA	110	92	164	146	182	128
VL	DNA	111	93	165	147	183	129
VL	AA	112	94	166	148	184	130
FRH1	AA	113	95	167	149	185	131
CDRH1	AA	114	96	168	150	186	132
FRH2	AA	115	97	169	151	187	133
CDRH2	AA	116	98	170	152	188	134
FRH3	AA	117	99	171	153	189	135
CDRH3	AA	118	100	172	154	190	136
FRH4	AA	119	101	173	155	191	137
FRL1	AA	120	102	174	156	192	138
CDRL1	AA	121	103	175	157	193	139
FRL2	AA	122	104	176	158	194	140
CDRL2	AA	123	105	177	159	195	141
FRL3	AA	124	106	178	160	196	142
CDRL3	AA	125	107	179	161	197	143
FRL4	AA	126	108	180	162	198	144

7. Modified Antibodies

[00184] Modified versions of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, and B6 antibodies are also provided. Typically modifications to an antibody can be introduced through the nucleic acids that encode the heavy or light chain variable domains of the antibody. These modifications can include deletions, insertions, point mutations, truncations, and amino acid substitutions and addition of amino acids or non-amino acid moieties. For example, random mutagenesis of the disclosed V_H or V_L sequences can be used to generate variant V_H or V_L domains still capable of binding *C. difficile* toxin A or B. A technique using error-prone PCR is described by Gram et al. (Proc. Nat. Acad. Sci. U.S.A. (1992) 89: 3576-3580). Another method uses direct mutagenesis of the disclosed V_H or V_L sequences. Such techniques are disclosed by Barbas et al. (Proc. Nat. Acad. Sci. U.S.A. (1994) 91: 3809-3813) and Schier et al. (J. Mol. Biol.

(1996) 263: 551-567). Modifications can also be made directly to the amino acid sequence, such as by cleavage, addition of a linker molecule or addition of a detectable moiety, such as biotin, addition of a fatty acid, and the like.

[00185] In one embodiment, the antibody is a monoclonal antibody that binds to *C. difficile* toxin A and comprises 1) a heavy chain variable domain that is at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to the amino acid sequence of the heavy chain variable domain of the A1, A2, A3, A4, or A5 antibody, and 2) a light chain variable domain that is at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to the amino acid sequence of the light chain variable domain of the A1, A2, A3, A4, or A5 antibody, wherein the heavy chain and light chain variable domains from the same antibody are combined as shown in Table 3.

Table 3: Modified Anti-Toxin A Antibodies

<i>C. difficile</i> Toxin A Antibody	
V _H	V _L
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:20 (A1)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:22 (A1)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:2 (A2)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:4 (A2)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:38 (A3)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:40 (A3)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:56 (A4)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:58 (A4)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:74 (A5)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:76 (A5)

[00186] In another embodiment, the antibody is a monoclonal antibody binds to *C. difficile* toxin B and comprises 1) a heavy chain variable domain that is at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to the amino acid sequence of the heavy chain variable domain of the B1, B2, B3, B4, B5, or B6 antibody, and 2) a light chain variable domain that is at least about 70%, at least about 80%, at

least about 90%, at least about 95%, at least about 98% identical, or 100% identical to the amino acid sequence of the light chain variable domain of the B1, B2, B3, B4, B5, or B6 antibody, wherein the heavy chain and light chain variable domains from the same antibody are combined as shown in Table 4.

Table 4: Modified Anti-Toxin B Antibodies

<i>C. difficile</i> Toxin B Antibody	
V _H	V _L
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:110 (B1)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:112 (B1)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:92 (B2)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:94 (B2)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:164 (B3)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:166 (B3)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:146 (B4)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:148 (B4)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:182 (B5)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:184 (B5)
At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:128 (B6)	At least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98% identical, or 100% identical to SEQ ID NO:130 (B6)

[00187] In another embodiment, the monoclonal antibody binds to *C. difficile* toxin A and comprises six CDRs (H1, H2, H3, L1, L2, and L3) that are at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 98% identical to the amino acid sequences of the six CDRs (H1, H2, H3, L1, L2, and L3) of the heavy and light chain variable domains of the A1, A2, A3, A4, , or A5 antibody.

[00188] In yet another embodiment, the monoclonal antibody binds to *C. difficile* toxin B and comprises six CDRs (H1, H2, H3, L1, L2, and L3) that are at least about 70%, at least about 80%, at least about 90%, at least about 95% or at least about 98% identical to the amino acid sequences of the six CDRs (H1, H2, H3, L1, L2, and L3) of the heavy and light chain variable domains of the B1, B2, B3, B4, B5, or B6 antibody.

[00189] In another embodiment, the monoclonal antibody binds to *C. difficile* toxin A and comprises a heavy chain variable domain identical to SEQ ID NO:20 (A1), SEQ ID NO:2 (A2), SEQ ID NO:38 (A3), SEQ ID NO:56 (A4), or SEQ ID NO:74 (A5) except for 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, and in certain cases, up to 10 amino acid substitutions in the CDR sequences. In another embodiment, the monoclonal antibody binds to *C. difficile* toxin A and comprises a light chain variable domain identical to SEQ ID NO:22 (A1), SEQ ID NO:4 (A2), SEQ ID NO:40 (A3), SEQ ID NO:58 (A4), or SEQ ID NO:76 (A5) except for 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, and in certain cases, up to 10 amino acid substitutions in the CDR sequences.

[00190] In yet another embodiment, the monoclonal antibody binds to *C. difficile* toxin B and comprises a heavy chain variable domain identical to SEQ ID NO:110 (B1), SEQ ID NO:92 (B2), SEQ ID NO:164 (B3), SEQ ID NO:146 (B4), SEQ ID NO:182 (B5), or SEQ ID NO:128 (B6) except for 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, and in certain cases, up to 10 amino acid substitutions in the CDR sequences. In another embodiment, the monoclonal antibody binds to *C. difficile* toxin A and comprises a light chain variable domain identical to SEQ ID NO:112 (B1), SEQ ID NO:94 (B2), SEQ ID NO:166 (B3), SEQ ID NO:148 (B4), SEQ ID NO:184 (B5), or SEQ ID NO:130 (B6) except for 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, and in certain cases, up to 10 amino acid substitutions in the CDR sequences.

[00191] The specific amino acid positions that can be substituted in a CDR, as well as the donor amino acid that can be substituted into those positions can be readily determined by one of skill in the art using known methods, such as those disclosed in published U.S. Application 2006/0099204, the disclosure of which is hereby incorporated by reference in its entirety. Typically, this involves substitution of an amino acid with an amino acid having similar charge, hydrophobic, or stereochemical characteristics. More drastic substitutions in FR regions, in contrast to CDR regions, may also be made as long as they do not adversely affect (e.g., reduce affinity by more than 50% as compared to unsubstituted antibody) the binding properties of the antibody.

[00192] Modified versions of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, and B6 antibodies can also be screened to identify which mutation provides a modified antibody that retains a desired property, such as high affinity binding of the parent antibody for either *C. difficile* toxin A or B and/or potent *in vitro* neutralizing activity.

[00193] Thus, in one embodiment, the modified antibody, including those described in Table 3, binds to *C. difficile* toxin A with a dissociation constant (K_D) equal to or less than 10 pM (10^{-11} M), 1pM (10^{-12} M), 0.1 pM (10^{-13} M), 0.01 pM (10^{-14} M), or 0.001 pM (10^{-15} M). In another

embodiment, the modified antibody, including those described in Table 4, binds to *C. difficile* toxin B with a dissociation constant (K_D) equal to or less than 250 pM, 200 pM, 150 pM, 100 pM, 50 pM, 30 pM, 10 pM, 1 pM (10^{-12} M), or 0.1 pM (10^{-13} M). The dissociation constant may be measured using techniques known in the art, including biolayer interferometry, as described in the examples of this application.

[00194] In one embodiment, the modified antibody, including those described in Table 3, neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin A at 2.4 ng/mL in the Vero monkey kidney cell line with an NT50 equal to or less than 3000 pM, 2000 pM, 1000 pM, 100 pM, 60 pM, or 50 pM. In another embodiment, the modified antibody, including those described in Table 4, neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin B at 17 pg/mL in the Vero monkey kidney cell line with an NT50 of equal to or less than 1000 pM, 100 pM, 60 pM, or 50 pM. For the sake of consistency, when measuring the neutralizing activity in the Vero monkey kidney cell line, Vero cells (2.5×10^4 cells/well with 5% heat-inactivated FBS) are seeded in a 96-well tissue culture microtiter plates and incubated 37°C, 5% CO₂ overnight. An equal volume (80 µl) of 4.8 ng/mL (8×MC50) *C. difficile* toxin A solution or 34.4 pg/mL (8×MC50) *C. difficile* toxin B solution and individual dilutions of the antibody solutions (80 µl) in Vero cell medium are combined in a new 96-well plate, and incubated at 37°C, 5% CO₂ for 1 hour before 100 µl of the toxin/antibody solutions are added to the Vero cells, and incubated at 37°C for 72 hours. After incubating for 72 hours, the cells are washed twice with 120 µl/each of MEM medium that does not contain phenol, L-glutamine and FBS before adding 100 µl MEM medium that does not contain phenol, L-glutamine and FBS and 10 µl of Alamar Blue® (Life Technologies) to each well. The plates are lightly mixed and incubated at 37°C for 4 hours before reading fluorescence at 560-590nm with a cut off at 590nm.

[00195] In one embodiment, the modified antibody, including those described in Table 3, neutralizes the *C. difficile* toxin A (at 200 ng/mL, applied apically) induced loss of transepithelial resistance (TEER) in the T-84 cell line with an NT50 equal to or less than 6 nM, 5 nM, 2 nM, or 1.5 nM. In another embodiment, the modified antibody, including those described in Table 4, neutralizes the *C. difficile* toxin B (at 75 ng/mL, applied basolaterally) induced loss of TEER in the T-84 cell line with an NT50 equal to or less than 200 pM, 150 pM, 100 pM, or 70 pM. For the sake of consistency, when measuring TEER, T-84 cells are seeded into 0.4 micron polyester transwell plates at a seeding density of 3.6×10^5 cells/cm² and maintained at 37°C, 5% CO₂ in 10% heat-inactivated FBS in DMEM/F12 culture media for 10-12 days until stable TEER is achieved and media is replaced in both apical and basolateral compartments of the transwells daily from day 6 and on the day of assay. The *C. difficile* toxin A (final concentration 200

ng/mL) or toxin B (final concentration of 75 ng/mL) is combined 1:1 with an antibody and incubated at 37°C with gentle rocking for 30 minutes before replacing the media in the apical compartment with the toxin A/antibody samples or the media in the basolateral compartment with the toxin B/antibody samples. Transepithelial electrical resistance of the T-84 cells is measured at T_0 immediately before sample addition and after 2.5 hours (T_{150}) incubation at 37°C 5% CO₂.

[00196] In one embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of at least one of the A1, A2, A3, A4, or A5 antibodies to toxin A, using a routine quantitative cross-blocking assay, such as the Biacore assay discussed above. In one embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the A1 antibody to toxin A. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the A2 antibody to toxin A. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the A3 antibody to toxin A. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the A4 antibody to toxin A. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the A5 antibody to toxin A.

[00197] In one embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of at least one of the B1, B2, B3, B4, B5, or B6 antibodies to toxin B, using a routine quantitative cross-blocking assay, such as the Biacore assay discussed above. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the B1 antibody to toxin B. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the B2 antibody to toxin B. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the B3 antibody to toxin B. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the B4 antibody to toxin B. In another embodiment, the modified antibody is a monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the B5 antibody to toxin B. In another embodiment, the modified antibody is a

monoclonal antibody, including, but not limited to, a bispecific antibody, that cross-blocks the binding of the B6 antibody to toxin B. .

8. Nucleic Acids, Cloning and Expression Systems

[00198] The present disclosure further provides isolated nucleic acids encoding the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, and B6 antibodies or portions thereof. The nucleic acids may comprise DNA or RNA and may be wholly or partially synthetic or recombinant. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

[00199] The nucleic acids provided herein encode at least one CDR, all six CDRs (i.e., H1, H2, H3, L1, L2, and L3), a V_H domain, and/or a V_L domain of one of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies.

[00200] The present disclosure also provides expression vectors (or plasmids) comprising at least one nucleic acid encoding a CDR, all six CDRs (i.e., H1, H2, H3, L1, L2, and L3), a V_H domain, and/or a V_L domain of one of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies, as well as other nucleic acid sequences useful for regulating polypeptide expression. Suitable expression vectors can be chosen or constructed, so that they contain appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate.

[00201] The expression vectors can be introduced into a host cell to produce the desired antibody. Systems for cloning and expression of a polypeptide in a variety of different host cells are well known in the art. For cells suitable for producing antibodies, see *Gene Expression Systems*, Academic Press, eds. Fernandez *et al.*, 1999. Any protein compatible expression system may be used to produce the disclosed antibodies. Suitable expression systems include transgenic animals described in *Gene Expression Systems*, Academic Press, eds. Fernandez *et al.*, 1999.

[00202] A further aspect of the disclosure provides an isolated host cell comprising a nucleic acid (or expression vector) as disclosed herein. A still further aspect provides a method comprising introducing such nucleic acid (or expression vector) into a host cell. The introduction may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g., vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. The introduction of the nucleic acid into

the cells may be followed by causing or allowing expression from the nucleic acid, e.g., by culturing host cells under conditions for expression of the gene. Following production by expression an antibody may be isolated and/or purified using any suitable technique, then used as appropriate.

9. Methods of Making Antibodies

[00203] Numerous methods known to those skilled in the art are available for obtaining antibodies or antigen-binding fragments thereof. Antibodies can also be produced using recombinant DNA methods. See, e.g., U.S. Patent 4,816,567; EPO 8430268.0; EPO 85102665.8; EPO 85305604.2; PCT/GB 85/00392; EPO 85115311.4; PCT/US86/002269; and Current Trends in Monoclonal Antibody Development (Steven Shire et al., Eds. Springer, 2010), the disclosures of which are incorporated herein by reference in their entirety. Given the disclosure in this application of specific nucleic acid sequences and the V_H and V_L (or CDR) amino acid sequences encoded thereby, it is possible, using recombinant DNA techniques, to insert a nucleic acid of interest into an expression vector or otherwise express the nucleic acid of interest in a host cell to produce the desired antibody. In addition, as disclosed elsewhere in this application, modified versions of the antibodies described herein can be produced using known techniques, including, for example, random mutagenesis, error-prone PCR, and direct mutagenesis.

[00204] Monoclonal antibodies may also be produced by preparing immortalized cell lines capable of producing antibodies having desired specificity, for example against an antigen expressing a desired epitope, such as the specific *C. difficile* toxin A and B epitopes disclosed in this application. Such immortalized cell lines may be produced in a variety of ways.

Conveniently, a small non-human animal, such as a mouse, is hyperimmunized with the desired immunogen. The vertebrate is then sacrificed, usually several days after the final immunization, the spleen cells removed, and the spleen cells immortalized. The most common technique is fusion with a myeloma cell fusion partner, as first described by Kohler and Milstein (1975) Nature 256:495-497. Other techniques, including EBV transformation, transformation with bare DNA, e.g., oncogenes, retroviruses, etc., or any other method which provides for stable maintenance of the cell line and production of monoclonal antibodies. Specific techniques for preparing monoclonal antibodies are described in Antibodies: A Laboratory Manual, Harlow and Lane, eds., Cold Spring Harbor Laboratory, 1988, the full disclosure of which is incorporated herein by reference.

[00205] In one embodiment, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer transgenic mouse strains that express human heavy and light chain genes, but are incapable of expressing the endogenous

mouse immunoglobulin heavy and light chain genes. Using the hybridoma technology, antigen-specific monoclonal antibodies derived from the genes with the desired specificity may be produced and selected. See, e.g., XENOMOUSE™, Green *et al.* (1994) *Nature Genetics* 7:13-21, US 2003-0070185, U.S. Patent No. 5,225,539, WO 96/34096, published Oct. 31, 1996, and PCT Application No. PCT/US96/05928, filed Apr. 29, 1996, the disclosures of which are incorporated herein by reference in their entirety.

[00206] Immortalized cell lines can be screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA) or surface plasmon resonance analysis, to identify one or more hybridomas that produce an antibody that specifically binds with a specified antigen and/or epitope. Any form of the specified antigen may be used as the immunogen, e.g., recombinant antigen, naturally occurring forms, any variants or fragments thereof, as well as antigenic peptide thereof.

[00207] Another exemplary method of making antibodies includes screening protein expression libraries, e.g., phage or ribosome display libraries. Phage display technology mimics the mammalian immune system by cloning large libraries of antibody genes and selecting for binding to a desired target, such as the specific *C. difficile* toxin A and B epitopes disclosed in this application. Phage display is described, for example, in Ladner *et al.*, U.S. Patent No. 5,223,409; Smith (1985) *Science* 228:1315-1317; Clackson *et al.* (1991) *Nature*, 352: 624-628; Marks *et al.* (1991) *J. Mol. Biol.*, 222: 581-597; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; and WO 90/02809, the disclosures of which are incorporated herein by reference in their entirety. It is also possible to produce antibodies that bind a specific antigen, such as one of the specific *C. difficile* epitopes disclosed in this application, by using a variable heavy domain (e.g., SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, SEQ ID NO:76, SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, or SEQ ID NO:184) and screening a library of complementary variable domains to identify antibodies that retain the desired binding specificity. See Portolano *et al.*, *The Journal of Immunology* (1993) 150:880-887 and Clarkson *et al.*, *Nature* (1991) 352:624-628, the disclosures of which are incorporated herein by reference in their entirety.

10. Methods of Use

[00208] The antibodies described in this application that bind to *C. difficile* toxin A or toxin B can be used in a variety of research and medical applications. In one aspect, the disclosure provides a method of treating a *C. difficile* infection in a subject, comprising administering to the subject one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6

antibodies in an amount effective to treat the *C. difficile* infection. In another embodiment, the method of treating a *C. difficile* infection in a subject, comprises administering at least one antibody that binds to *C. difficile* toxin A selected from the A1, A2, A3, A4, and A5 antibodies and at least one antibody that binds to *C. difficile* toxin B selected from the B1, B2, B3, B4, B5, and B6 antibodies. In one embodiment, the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody and the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, and B6 antibodies, preferably one or more of B1, B2, or B4. In another embodiment, the method comprises administering the A2 antibody and at least two antibodies that binds to *C. difficile* toxin B, wherein the at least two antibodies that binds to *C. difficile* toxin B are the B1 and B2 antibodies, the B2 and B4 antibodies, or the B2 and B6 antibodies. In another embodiment, the method comprises administering the 1) A2 antibody and 2) a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody. The antibodies may be administered at the same time or sequentially.

[00209] In another embodiment, the method of treating a *C. difficile* infection comprises administering a composition to the subject in an amount effective to treat the *C. difficile* infection, wherein the composition comprises at least one antibody that binds to *C. difficile* toxin A selected from the A1, A2, A3, A4, and A5 antibodies and at least one antibody that binds to *C. difficile* toxin B selected from the B1, B2, B3, B4, B5, and B6 antibodies. In another embodiment, the composition comprises at least one antibody that binds to *C. difficile* toxin A and at least one antibody that binds to *C. difficile* toxin B selected from the B1, B2, B3, B4, B5, and B6 antibodies. In another embodiment, the composition comprises at least one antibody that binds to *C. difficile* toxin A selected from the A1, A2, A3, A4, and A5 antibodies and at least one antibody that binds to *C. difficile* toxin B.

[00210] In one embodiment, the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody and the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, and B6 antibodies, preferably one or more of B1, B2, or B4. In one embodiment, the composition comprises the A2 antibody and the B4 antibody. In another embodiment, the composition comprises the A2 antibody and the B2 antibody. In another embodiment, the composition comprises the A2 antibody and the B1 antibody.

[00211] In another embodiment, the composition comprises the A2 antibody and two antibodies that bind to *C. difficile* toxin B, wherein the two antibodies that bind to *C. difficile* toxin B are selected from the B1, B2, B3, B4, B5, and B6 antibodies. In one embodiment, the composition comprises the A2, B1, and B2 antibodies. In another embodiment, the composition comprises the A2, B2, and B4 antibodies. In another embodiment the composition comprises the

A2, B2, and B6 antibodies.

[00212] In yet another embodiment, the composition comprises the 1) A2 antibody and 2) a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody.

[00213] Subjects that can be treated with the antibodies disclosed in this application include humans and non-human mammals, including, but not limited to, non-human primates, dogs, cats, horses, cows, sheep, pigs, goats, mice, rats, hamsters, and guinea pigs.

[00214] In addition, one or more of the A1, A2, A3, A4, and A5 antibodies can be used to detect *C. difficile* toxin A in a sample, while one or more of the B1, B2, B3, B4, B5, or B6 antibodies can be used to detect *C. difficile* toxin B in a sample. In one embodiment, the method comprises contacting one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies with the sample and analyzing the sample to detect binding of the antibody to toxin A or toxin B in the sample, wherein binding of the antibody to toxin A or toxin B in the sample indicates the presence of *C. difficile* in the biological sample. In one embodiment, the sample comprises a non-biological sample, such as soil, water, or food products such as meat. In other embodiments, the sample comprises a biological sample, such as blood, serum, tissue, or stool. Such methods can be used to detect a *C. difficile* infection in a patient, wherein binding of the antibody to toxin A or toxin B in a sample from the patient indicates the presence of the *C. difficile* infection in the patient.

[00215] Any appropriate label may be used in the detection methods and compositions described herein. A label is any molecule or composition bound to an antibody, or a secondary molecule that is conjugated thereto, and that is detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Examples of labels, including enzymes, colloidal gold particles, colored latex particles, have been disclosed (U.S. Patents No. 4,275,149; 4,313,734; 4,373,932; and 4,954,452, each incorporated by reference herein). Additional examples of useful labels include, without limitation, haptens (e.g., biotin, digoxigenin (DIG), dinitrophenol (DNP), etc.), radioactive isotopes, co-factors, ligands, chemiluminescent or fluorescent agents, protein-adsorbed silver particles, protein-adsorbed iron particles, protein-adsorbed copper particles, protein-adsorbed selenium particles, protein-adsorbed sulphur particles, protein-adsorbed tellurium particles, protein-adsorbed carbon particles, and protein-coupled dye sacs. The attachment of a compound to a label can be through any means, including covalent bonds, adsorption processes, hydrophobic and/or electrostatic bonds, as in chelates and the like, or combinations of these bonds and interactions and/or may involve a linking group.

II. Formulations and Administration

[00216] The disclosure provides compositions comprising an antibody described herein that binds to *C. difficile* toxin A or toxin B. In certain embodiments, the compositions are suitable for pharmaceutical use and administration to patients. These compositions comprise one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies and a pharmaceutically acceptable excipient. In one embodiment, the composition comprises at least one antibody that binds to *C. difficile* toxin A selected from the A1, A2, A3, A4, and A5 antibodies and at least one antibody that binds to *C. difficile* toxin B. In another embodiment, the composition comprises at least one antibody that binds to *C. difficile* toxin A and at least one antibody that binds to *C. difficile* toxin B, wherein the at least one antibody that binds to *C. difficile* toxin B is preferably one or more of the B1, B2, B3, B4, B5, and B6 antibodies.

[00217] In one embodiment, the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody or A1 antibody and the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, or B6 antibodies, preferably one or more of B1, B2, or B4. In one embodiment, the composition comprises the A2 antibody and the B4 antibody. In another embodiment, the composition comprises the A2 antibody and the B2 antibody. In another embodiment, the composition comprises the A2 antibody and the B1 antibody. In another embodiment, the composition comprises the A1 antibody and the B1 antibody. In another embodiment, the composition comprises the A1 antibody and the B2 antibody. In another embodiment, the composition comprises the A1 antibody and the B4 antibody. In another embodiment, the composition comprises the A1 antibody and the B6 antibody.

[00218] In another embodiment, the composition comprises the A2 antibody or A1 antibody and two antibodies that bind to *C. difficile* toxin B, wherein the two antibodies that bind to *C. difficile* toxin B are selected from the B1, B2, B3, B4, B5, and B6 antibodies. In one embodiment, the composition comprises the A2, B1, and B2 antibodies. In another embodiment, the composition comprises the A2, B2, and B4 antibodies. In another embodiment the composition comprises the A2, B2, and B6 antibodies. In yet another embodiment, the composition comprises the 1) A2 antibody and 2) a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody. In one embodiment, the composition comprises the A1, B1, and B2 antibodies. In another embodiment, the composition comprises the A1, B2, and B4 antibodies. In another embodiment the composition comprises the A1, B2, and B6 antibodies. In yet another embodiment, the composition comprises the 1) A1 antibody and 2) a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody.

[00219] In one embodiment, the composition comprises one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies for use in treating a *C. difficile* infection.

Preferably, the composition comprises at least one antibody that binds to *C. difficile* toxin A selected from the A1, A2, A3, A4, and A5 antibodies and at least one antibody that binds to *C. difficile* toxin B selected from the B1, B2, B3, B4, B5, and B6 antibodies for use in treating a *C. difficile* infection. In one embodiment, the composition comprises the A2 antibody or A1 antibody and the at least one antibody that binds to *C. difficile* toxin B selected from the B1, B2, B3, B4, B5, or B6 antibodies, preferably one or more of B1, B2, or B4 for use in treating a *C. difficile* infection. In one embodiment, the composition comprises the A2 antibody and the B4 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A2 antibody and the B2 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A2 antibody and the B1 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A1 antibody and the B4 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A1 antibody and the B2 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A1 antibody and the B1 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A1 antibody and the B6 antibody for use in treating a *C. difficile* infection.

[00220] In yet another embodiment, the composition comprises the A2 or A1 antibody and two antibodies that bind to *C. difficile* toxin B for use in treating a *C. difficile* infection, wherein the two antibodies that bind to *C. difficile* toxin B are selected from the B1, B2, B3, B4, B5, and B6 antibodies. In one embodiment, the composition comprises the A2 antibody, the B1 antibody, and the B2 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A2 antibody, the B2 antibody, and the B4 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A2 antibody, the B2 antibody, and the B6 antibody for use in treating a *C. difficile* infection. In yet another embodiment, the composition comprises the 1) A2 antibody and 2) a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody for use in treating a *C. difficile* infection. . In one embodiment, the composition comprises the A1 antibody, the B1 antibody, and the B2 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A1 antibody, the B2 antibody, and the B4 antibody for use in treating a *C. difficile* infection. In another embodiment, the composition comprises the A1 antibody, the B2 antibody, and the B6 antibody for use in treating a *C. difficile* infection. In yet another embodiment, the composition comprises the 1) A1 antibody and 2) a bispecific B1 + B2 antibody, a bispecific B2 + B4 antibody, or a bispecific B2 + B6 antibody for use in treating a *C. difficile* infection.

[00221] The compositions may also contain other active compounds providing supplemental, additional, or enhanced therapeutic functions. In one embodiment, the other active compound is an antibiotic, including, but not limited to, metronidazole, fidaxomicin, or vanomycin. The pharmaceutical compositions may also be included in a container, pack, or dispenser together with instructions for administration.

[00222] Pharmaceutically acceptable excipients include, but are not limited to a carrier or diluent, such as a gum, a starch (e.g. corn starch, pregeletanized starch), a sugar (e.g. lactose, mannitol, sucrose, dextrose), a cellulosic material (e.g. microcrystalline cellulose), an acrylate (e.g. polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof; a binder (e.g. acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone); a disintegrating agent (e.g. cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate), a buffer (e.g. Tris-HCl, acetate, phosphate) of various pH and ionic strength; and additive such as albumin or gelatin to prevent absorption to surfaces; a detergent (e.g. Tween 20, Tween 80, Pluronic F68, bile acid salts); a protease inhibitor; a surfactant (e.g. sodium lauryl sulfate); a permeation enhancer; a solubilizing agent (e.g. glycerol, polyethylene glycerol); an anti-oxidants (e.g. ascorbic acid, sodium metabisulfite, butylated hydroxyanisole); a stabilizer (e.g. hydroxypropyl cellulose, hydroxypropylmethyl cellulose); a viscosity increasing agent (e.g. carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum); a sweetener (e.g. aspartame, citric acid); a preservative (e.g. Thimerosal, benzyl alcohol, parabens); a lubricant (e.g. stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate); a flow-aid (e.g. colloidal silicon dioxide), a plasticizer (e.g. diethyl phthalate, triethyl citrate); an emulsifier (e.g. carbomer, hydroxypropyl cellulose, sodium lauryl sulfate); a polymer coating (e.g. poloxamers or poloxamines); a coating and film forming agent (e.g. ethyl cellulose, acrylates, polymethacrylates); an adjuvant; a pharmaceutically acceptable carrier for liquid formulations, such as an aqueous (water, alcoholic/aqueous solution, emulsion or suspension, including saline and buffered media) or non-aqueous (e.g., propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate) solution, suspension, emulsion or oil; and a parenteral vehicle (for subcutaneous, intravenous, intraarterial, or intramuscular injection), including but not limited to, sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils.

[00223] Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically

acceptable adjuvants. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions. Examples of oils are those of animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, olive oil, sunflower oil, fish-liver oil, another marine oil, or a lipid from milk or eggs.

[00224] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Methods to accomplish the administration are known to those of ordinary skill in the art. This includes, for example, injections, by parenteral routes such as intravenous, intravascular, intraarterial, subcutaneous, intramuscular, intraperitoneal, intraventricular, intracerebral, or others as well as oral, nasal, ophthalmic, rectal, or topical. Sustained release administration is also specifically contemplated, by such means as depot injections or erodible implants. Localized delivery is particularly contemplated, by such means as delivery via a catheter to one or more arteries, such as the renal artery or a vessel supplying a localized site of interest.

[00225] In one embodiment a subject antibody is administered to a patient by intravenous, intramuscular or subcutaneous injection. An antibody may be administered within a dose range between about 1 $\mu\text{g/kg}$ to about 100 mg/kg. A therapeutically effective amount of antibody may include, but is not limited to, dosage ranges of about 0.1 mg/kg to about 100 mg/kg; 0.1 mg/kg to about 10 mg/kg; about 0.5 mg/kg to 75 mg/kg; 1 mg/kg to about 50 mg/kg; 1 mg/kg to about 10 mg/kg; 0.5 mg/kg to about 25 mg/kg; or about 1 mg/kg to about 5 mg/kg. The antibody may be administered, for example, by bolus injection or by slow infusion. The dosage may depend on the type and severity of the infection and/or on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs and should be adjusted, as needed, according to individual need and professional judgment. The dosage may also vary depending upon factors, such as route of administration, target site, or other therapies administered. The skilled artisan will be able to determine appropriate doses depending on these and other factors.

[00226] Toxicity and therapeutic efficacy of the composition can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio $\text{LD}_{50}/\text{ED}_{50}$. Antibodies that exhibit large therapeutic indices may be less toxic and/or more therapeutically effective.

12. Kits

[00227] In some embodiments, at least one antibody described herein that binds to *C. difficile* toxin A or toxin B is supplied in the form of a kit useful, for example, for performing the treatment or diagnostic methods described in this application. In one embodiment, an appropriate amount of one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies is provided in one or more containers. In other embodiments, one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies is provided suspended in an aqueous solution or as a freeze-dried or lyophilized powder, for instance. The container(s) in which the at least one antibody is supplied can be any conventional container that is capable of holding the supplied form, for instance, microfuge tubes, ampoules, or bottles. The amount of antibody supplied can be any appropriate amount.

[00228] Other kit embodiments include means for detecting one or more of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies, such as secondary antibodies. In some such instances, the secondary antibody is directly labeled with a detectable moiety (as described elsewhere in this disclosure). In other instances, the primary or secondary (or higher-order) antibody is conjugated to a hapten (such as biotin, DNP, DIG, etc.), which is detectable by a detectably labeled cognate hapten-binding molecule (e.g., streptavidin (SA)-horse radish peroxidase, SA-alkaline phosphatase, SA-QDot® (Invitrogen, Carlsbad, CA), etc.). In some embodiments, the primary or secondary antibody is conjugated with a fluorescent detection moiety (e.g., FITC, rhodamine, ALEXA FLUOR® (Invitrogen, Carlsbad, CA) dyes, Cy designated fluorophores, etc.). Some kit embodiments may include colorimetric reagents (e.g., DAB, AEC, etc.) in suitable containers to be used in concert with primary or secondary (or higher-order) antibodies that are labeled with enzymes for the development of such colorimetric reagents.

[00229] In one embodiment, a kit includes instructional materials disclosing methods of use of the kit contents (e.g., an antibody described herein that binds to *C. difficile* toxin A or toxin B) in a disclosed method. The instructional materials may be provided in any number of forms, including, but not limited to, written form (e.g., hardcopy paper, etc.), in an electronic form (e.g., computer diskette or compact disk) or may be visual (e.g., video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

[00230] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although methods

and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

[00231] Example 1. Antibody Screening

[00232] **Blood donor selection.** Serum was collected from 3000 healthy donors and assessed for its capacity to neutralize *C. difficile* toxin A and/or toxin B by cytotoxicity assay on IMR90 cells as described by Babcock *et al.* (Infection and Immunity, Nov. 2006, p. 6339-6347). Briefly, IMR90 cells were seeded in a 96 well plate (half size well plates) at a cell density of 1×10^4 cells/well in a 50 μ l volume. The plates were incubated for 24 hours at 37°C, 5% CO₂, before removing the supernatant from the wells. Sera were diluted in IMR90 cell culture medium 1/25 and 1/100 for toxin A and 1/100 and 1/500 for toxin B and incubated for 60 minutes with either 4x MC50 of toxin A or 2x MC50 of toxin B. This mixture was then added to the wells of the 96 well plate and incubated for 16-24 hours at 37°C, 5% CO₂ before assessing the cytopathic effect. The cytopathic effect was determined microscopically and scored as 0 (0% rounded cells), 1 (25% rounded cells), 2 (50% rounded cells), 3 (75% rounded cells), 4 (100% rounded cells). Sera exhibiting a neutralizing activity were further tested with the same assay in a series of dilutions ranging from 1/25 to 1/3200. In parallel, neutralizing sera were also tested by ELISA to determine their titers against both toxins, as well as their cross-reactivity for the toxinotypes 0, III, V, VIII, XII, XIV, and XV. Peripheral Blood Mononuclear Cells (PBMCs) from the 12 best donors were used for the screening campaigns to maximize the probability to isolate B cells secreting high quality antibodies.

[00233] **Antigens.** Untoxoided *C. difficile* toxins A and B were purified from the supernatant of a culture of strain VPI10463 (ATCC 43255).

[00234] **Antibody Screening.** The A2, A4, A5, B6, B4 antibodies were obtained by implementing the method described in Jin *et al.* (2011, Nature Protocols Vol.6, N°5 pp668-676) and named ISAAC (ImmunoSpot Array Assay on a Chip). In brief, *C. difficile* toxin A or B antigen was coated on the chip. The anti-toxin A or B human antibodies, secreted from wells containing one human B cell, diffuse onto the chip surface and bind to the antigen coated on the chip surface. Bound antibodies were visualized using Cyanine 3 anti-human IgG monoclonal

antibody. The B cells secreting specific anti-toxin A or B antibodies were isolated and the heavy and light chains of human monoclonal antibodies were obtained by single cell reverse transcriptase polymerase chain reaction (RT-PCR). The amplified VH and VL fragments were subsequently cloned into expression vectors for production and recombinant antibody testing.

[00235] The A1, A3, B1, B2, B3, B5 antibodies were obtained by implementing the method described in the patent application WO2013/000982 named VIVASCREEN. B-lymphocytes from the best donors were isolated, activated and expanded *in vitro*. Supernatants from expanded B-lymphocyte pools were then screened using a binding assay against *C. difficile* toxin A or toxin B. B-cell pools that secreted antibodies against toxin A or B were further screened with a functional assay. Notably, the functional hits were identified using a cytotoxicity assay on IMR90 cells as described by Babcock *et al.* 2006. Briefly, IMR90 cells were seeded in a 96 well plate (half size well plates) at a cell density of 1×10^4 cells/well in a 50 μ l volume. The plates were incubated during 24 hours at 37°C, 5% CO₂, before removing the supernatant from the wells. 150 μ l of supernatants from each of the expanded B cell pools were incubated for 60 minutes with either 8x MC50 of toxin A or 2x MC50 of B. This mixture was then added to the wells of the 96 well plate and incubated for 16-24 hours at 37°C + CO₂ before assessing the cytopathic effect. The cytopathic effect was determined microscopically and scored as 0 (0% rounded cells), 1 (25% rounded cells), 2 (50% rounded cells), 3 (75% rounded cells), 4 (100% rounded cells).

[00236] The single B cells secreting specific anti-toxin A or B antibodies from each relevant B-cell pool were further isolated by the ISAAC (ImmunoSpot Array Assay on a Chip) method on toxin A- or toxin B-coated chips, and the heavy and light chains of the monoclonal antibodies were obtained by single cell reverse transcriptase polymerase chain reaction (RT-PCR). The amplified VH and VL fragments were subsequently cloned into expression vectors for production and further recombinant antibody characterization. Using these processes, tens of millions of antibody producing B lymphocytes were screened and characterized to identify extremely rare antibodies having high binding affinity for and strong neutralizing activity against either *C. difficile* toxin A or *C. difficile* toxin B, and preferably with a broad spectrum of binding to strains of various *C. difficile* toxinotypes, such as 0, III, V, VIII, XII, and XV.

[00237] Example 2. Binding Affinity of Anti-Toxin A and B Antibodies

[00238] The binding affinities of all antibodies were determined by Bio-Layer Interferometry using a Octet® Red96 (FortéBio). Bio-Layer Interferometry is an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein (e.g., antibody) on the biosensor tip, and an internal

reference layer. Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time. Abdiche et al., *Analytical Biochemistry*, 2009, 386(2), 172-180.

[00239] Sensors coated with Protein A were first wet for 10 minutes in FortéBio kinetics buffer (KB), PBS pH 7.4 containing 0.002% Tween-20 and 0.1 mg/ml BSA. The sensors were then transferred to wells containing antibody at a concentration of 10 µg/ml in KB and the accumulation of antibody on the sensors was measured for 300 seconds. The sensors were then transferred to wells containing KB for 300 seconds to wash off contaminants. Binding of toxin to the antibody was initiated by transferring the antibody-coated sensors into wells containing *C. difficile* toxin A or B at concentrations ranging from 0.7 to 20 µg/ml. After 400 seconds, the sensors were moved into KB buffer for 900 seconds to monitor the dissociation of the bound toxin. Throughout the experiment, samples were agitated at 30°C and 1000 rpm. The changes in thickness of the protein layer over time at four different concentrations of toxin were fit to the equations of equilibrium kinetics to calculate association and dissociation constants. The dissociation constants, on rate constants, and off rate constants of the anti-toxin A and anti-toxin B antibodies are provided in Table 5.

[00240] Table 5

mAb	K_D (M)	K_{on} (1/Ms)	K_{on} error	K_{off} (1/s)	K_{off} error
A1	6.81×10^{-14} *	3.75×10^5	2.19×10^3	2.55×10^{-8}	3.05×10^{-6}
A2	4.43×10^{-12} *	3.56×10^5	2.16×10^3	1.58×10^{-6}	3.16×10^{-6}
A3	4.61×10^{-10}	1.69×10^5	5.25×10^2	7.77×10^{-3}	1.38×10^{-6}
A4	5.57×10^{-13} *	5.55×10^5	5.07×10^3	3.09×10^{-7}	4.62×10^{-6}
A5	1.44×10^{-10}	3.12×10^5	2.90×10^3	4.49×10^{-5}	4.84×10^{-6}
B1	4.77×10^{-11}	5.85×10^5	4.34×10^3	2.79×10^{-3}	3.73×10^{-6}
B2	7.97×10^{-11}	4.20×10^5	3.18×10^3	3.35×10^{-5}	3.92×10^{-6}
B3	4.87×10^{-10}	1.93×10^5	1.45×10^3	9.40×10^{-5}	3.73×10^{-6}
B4	1.21×10^{-10}	5.31×10^5	4.46×10^3	6.44×10^{-5}	4.26×10^{-6}
B5	2.02×10^{-10}	4.77×10^5	2.15×10^3	9.62×10^{-3}	2.35×10^{-6}
B6	7.26×10^{-13} *	7.30×10^5	4.61×10^3	5.30×10^{-7}	3.13×10^{-6}

[00241] The K_D of the A3 and A5 antibodies for toxin A was 461 pM and 144 pM, respectively. The K_D of the A1, A2, and A4 antibodies for toxin A are shown in Table 5. All were less than 10 pM. The lower limit of quantification by the Octet® Red96 (FortéBio) system is about 10 pM. These results demonstrate that the A1, A2, A3, A4, and A5 antibodies bound *C. difficile* toxin A with at least picomolar affinities. Some of the antibody affinities may be in the subpicomolar range if measured with a more sensitive system.

[00242] The K_D of the B1, B2, B3, B4, and B5 antibodies for toxin B was 48, 80, 487, 121, and 202 pM, respectively. The K_D of the B6 antibody for toxin B is shown in Table 5 and was less than the lower limit of quantification by the Octet® Red96 (FortéBio) system, which is about 10 pM. These results demonstrate that the B1, B2, B3, B4, B5, and B6 antibodies bound *C. difficile* toxin B with at least picomolar affinities. Some of the antibody affinities may be in the subpicomolar range if measured with a more sensitive system.

[00243] **Example 3. *In Vitro* Vero Cell Cytotoxicity Based Toxin Neutralization Assay**

[00244] Cell-based neutralization assays in either Vero monkey kidney cells or T-84 human colon epithelial cell monolayer were used to evaluate the ability of the anti-toxin A and anti-toxin B antibodies to neutralize the activity of toxin A or toxin B. The first assay uses Vero cells, a cell line which was derived from the kidney of a normal adult African green monkey. The Vero cell assay assesses the ability of anti-toxin A or anti-toxin B antibodies to inhibit toxin A or toxin B induced killing of Vero cells. This assay uses an Alamar Blue® (Life Technologies) readout to assess cell viability. Resazurin, the active ingredient of Alamar Blue® (Life Technologies), is a non-toxic, cell permeable blue compound. Only living cells are able to reduce Resazurin to a red fluorescent compound, consequently viable cell number is directly proportional red fluorescence. Therefore the lower the fluorescence reading, the fewer viable cells present.

[00245] Vero cells (2.5×10^4 cells/well with 5% heat-inactivated FBS) were seeded in a 96-well tissue culture microtiter plates and incubated 37°C, 5% CO₂ overnight. Stock solutions of 8×MC50 (concentration inducing 50% of the maximum response) *C. difficile* toxin A or B were prepared in Vero cell medium. One MC50 dose was 0.6 ng/mL and 4.3 pg/mL for toxin A and B, respectively.

[00246] Various dilutions of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies were prepared in Vero cell medium and added to a 96-well tissue culture plate. An equal volume (80 µl) of 8×MC50 *C. difficile* toxin A or toxin B solution and individual dilutions of the antibody solutions (80 µl) were combined in a new 96-well tissue culture plate, and incubated at 37°C with 5% CO₂ and humidity for 1 hour with appropriate controls (toxin A or B

without antibody or media). The resulting toxin/antibody solution has a toxin A or B concentration of $4 \times \text{MC50}$. After incubating for 1 hour, 100 μl of the toxin/antibody solutions was added to the Vero cells in 96-well tissue culture microtiter plates. The Vero cells were incubated with the toxin/antibody solution at 37°C for 72 hours.

[00247] After incubating for 72 hours of incubation, the cells were washed twice with 120 μl /each of MEM medium that does not contain phenol, L-glutamine and FBS. Next 100 μl MEM medium that does not contain phenol, L-glutamine & FBS and 10 μl of Alamar Blue® (Life Technologies) was added to each well. The plates were lightly mixed and incubated at 37°C for 4 hours before reading fluorescence at 560-590nm with a cut off at 590nm.

[00248] Percent survival was plotted over antibody concentration. Cell survival in toxin/antibody treated cells was compared to cells treated with toxin A or B without antibody and NT50 was calculated for each antibody. NT50 is the concentration of antibody that results in 50% reduction in survival as compared to control cells treated with toxin A or B but no antibody.

[00249] The results obtained with the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, and B6 antibodies are summarized in Table 6, depicting both the potency of the neutralizing activity (Cytotox NT50) and the percent completion of the antibody-induced neutralization (% completion cytotox).

[00250] Table 6

mAb	A1	A2	A3	A4	A5	B1	B2	B3	B5	B4	B6
Cytotox NT50, pM	48	55	980	3400	2700	49	63	100	2900	70 as combo	
% completion cytotox	<90	100	90	<90	75	95	75	100	95	95 as combo	

[00251] All five anti-toxin A antibodies exhibited a cytotoxic NT50 of less than 3500 pM. The A1, A2, A3, and A5 exhibited a cytotoxic NT50 of less than 3000 pM, and remarkably A1, A2, and A3 exhibited a cytotoxic NT50 of less than 1000 pM, with A2 and A1 showing the greatest potency at 55 pM and 48 pM, respectively. All five anti-toxin A antibodies also showed a high completion percentage of at least 75%, with A2 and A3 showing the greatest percent completion of 100% and 90%, respectively.

[00252] The anti-toxin B antibodies similarly exhibited high potency in the Vero cell neutralization assay, with all but the B5 antibody having a cytotoxic NT50 of 100 pM or less (B6 and B4 were tested as a combination). All six anti-toxin B antibodies also showed a high completion percentage of at least 75%, with B1, B3, B5, and B6 and B4 (tested as a combination) having a percent completion of at least 95%. The results of the Vero cell neutralization assays

for the anti-toxin A and anti-toxin B antibodies are also graphically illustrated in Figures 1A and 1B, with potency represented on the x axis and percent completion represented on the y axis.

[00253] **Example 4. *In Vitro* TEER Based Toxin Neutralization Assay**

[00254] The second cell-based neutralization assay uses a T-84 human carcinoma cell line derived from a lung metastasis of a colon carcinoma (ATCC CCL-248). This assay assesses the ability of anti-toxin A or anti-toxin B antibodies to inhibit toxin A or toxin B induced loss of transepithelial electrical resistance (TEER) in T-84 cells.

[00255] T-84 cells were seeded into 0.4 micron polyester transwell plates at a seeding density of 3.6×10^5 cells/cm² and maintained at 37°C, 5% CO₂ in 10% heat-inactivated FBS in DMEM/F12 culture media for 10-12 days until stable TEER was achieved. Transepithelial electrical resistance was measured using Millipore Millicell® ERS-2 Volt-Ohm Meter. Media was replaced in both apical and basolateral compartments daily from day 6 and on the day of assay. Final concentration of toxin A used for challenge dose was equivalent to 6 times challenge dose required to produce loss of transepithelial resistance of 50% (TER50). One TER50 dose was 33 ng/mL and 15 ng/mL for toxin A and toxin B, respectively. Toxin A challenge was performed in the apical compartment of the transwell. The final concentration of toxin B used for challenge dose was equivalent to 5 times TER50. Toxin B challenge was performed in basolateral compartment.

[00256] Toxin A or toxin B and one of the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, or B6 antibodies were combined at 1:1 ratio and incubated at 37°C with gentle rocking for 30 minutes, with appropriate controls (toxin A or B without antibody or media). Media was removed from the appropriate apical or basolateral compartment and the toxin/antibody samples were added to the T-84 cells in the transwell plates. Transepithelial resistance of the T-84 cells is measured at T₀ immediately before sample addition and after 2.5 hours (T₁₅₀) incubation at 37°C 5% CO₂.

[00257] Percent TEER loss is calculated for each sample using the following equation: %TEER loss = $[(T_0 - T_{150}) \div T_0] * 100\%$ - %TEER loss Negative well. Percent protection for antibody is calculated for each treatment using the following equation: % Protection = $[(\% \text{ TEER loss Toxin Challenge}) - (\% \text{ TEER loss Toxin with Treatment})]$.

[00258] Percent TEER loss was plotted over antibody concentration. TEER loss in toxin/antibody treated cells was compared to cells treated with toxin A or B without antibody and NT₅₀ was calculated for each antibody. NT₅₀ is the concentration of antibody that results in 50% reduction in TEER loss as compared to control cells treated with toxin A or B but no antibody.

[00259] The results obtained with the A1, A2, A3, A4, A5, B1, B2, B3, B4, B5, and B6 antibodies are summarized in Table 7, depicting both the potency of the neutralizing activity

(TEER NT50) and the percent completion of the antibody-induced neutralization (% completion TEER).

[00260] Table 7

mAb	A1	A2	A3	A4	A5	B1	B2	B3	B5	B4	B6
TEER NT50, nM	<1.3	1.8	1.3	4.7	7.3	270	70	600	200	130	100
% completion TEER	80	75	80	ND	ND	91	98	95	70	93	92

[00261] All five anti-toxin A antibodies exhibited a TEER NT50 of less than 10 nM. The A1, A2, A3, and A4 antibodies exhibited a TEER NT50 of less than 5 nM, and remarkably A1, A2, and A3 exhibited a TEER NT50 of less than 2 nM. The A1, A2, and A3 antibodies also showed a high completion percentage (TEER) of at least 75%. The plateau for the A4 and A5 antibodies was never reached.

[00262] The anti-toxin B antibodies similarly exhibited high potency in the TEER neutralization assay, with all but the B3 antibody having a TEER NT50 of 300 pM or less, and the B2, B4, B5, and B6 antibodies having a TEER NT50 of 200 pM or less, with B6, B4, and B2 showing the greatest potency at 100 pM, 130 pM, and 70 pM, respectively. All 6 anti-toxin B antibodies also showed a high completion percentage of at least 70%, with B1, B2, B3, B4, and B6 having a percent completion of at least 90%. The results of the TEER neutralization assays for the anti-toxin A and anti-toxin B antibodies are also graphically illustrated in Figures 2A and 2B, with potency represented on the x axis and percent completion represented on the y axis.

[00263] Example 5. Toxinotype Analysis by Western Blotting

[00264] The breadth of protection against various *C. difficile* toxinotypes was assessed using two different assays. In the first, toxinotype binding was measured using Western analysis. *C. difficile* strains representative of toxinotypes 0, III, V, VIII, XII, and XV were grown anaerobically at 250 ml scale. The representative strain of toxinotype 0 is VPI10463 or ATCC 43255, the *C. difficile* reference strain. The representative toxinotype III strain (CDC# 2005099) is a hypervirulent NAP1/027 strain isolated from an outbreak in Montreal. The representative toxinotype V strain is CDC# 2004255. The representative toxinotype VIII strain is CDC# 2005195. The representative toxinotype XII strain is CDC# 2004097. The representative toxinotype XV strain is CDC# 2004012.

[00265] The supernatants were recovered by tangential flow filtration through a 0.2 µm membrane and adjusted to 0.4 M ammonium sulfate using a 3.7 M stock solution. The supernatant was loaded on a 1 ml Phenyl Sepharose FF (hi-sub) column (GE Healthcare) and the

column was washed with Buffer A (45 mM Tris-HCl, 45 mM NaCl, 0.4 M NH_4SO_4 , 1 mM DTT, 0.2 mM EDTA, pH 7.5). The crude toxins were eluted using a 200 ml gradient to Buffer B (50 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.2 mM EDTA, pH 7.5). Fractions containing toxins were identified by SDS-PAGE. Fractions were stored in SDS-PAGE loading buffer to prevent autolysis prior to Western blot analysis.

[00266] Purified toxins (about 20 ng) were analyzed using SDS-PAGE on a NuPAGE® (Life Technologies) 4-12% polyacrylamide gel run at 200V using SeeBlue2® standards (Invitrogen). The proteins in the gel were transferred to a nitrocellulose membrane in 6 min, using the iBlot® (Invitrogen) gel blotting system. The blot was blocked with PBST (10mM sodium phosphate, 2mM potassium phosphate, 2.7mM potassium chloride, 137mM sodium chloride, 0.1% Tween 20) containing 5% nonfat dry milk (NFDm) for 1h at room temperature. The blot was probed with the mAb diluted 1:5000 in 2.5% NFDm/PBST for 1h at RT, then washed 3 x 5 min with PBST. The blot was incubated with goat anti-human Alkaline Phosphatase conjugate (Sigma, A1543) [1: 6600 in 2.5% NFDm/PBST,] for 1h at RT. The blot was washed 3 x 5 min with PBST and developed with 1 BCIP/NBT tablet (Sigma, B5655) in 10ml water. Development was stopped by putting the blot into deionized water.

[00267] Each of the anti-toxin A antibodies, A1, A2, A3, A4, and A5, demonstrated binding to toxinotypes 0, III, V, XII, and XV by Western analysis. Of the anti-toxin B antibodies, B3 and B6 bound to toxinotypes 0, III, V, VIII, XII, and XV, while B1 bound to at least toxinotypes 0, III, V, VIII, XII, and B2 bound to toxinotypes 0, III, V, and VIII. B4 bound to toxinotypes 0, III, and V, while B5 bound to toxinotype 0 and III. The toxinotype binding results for the anti-toxin A and anti-toxin B antibodies are summarized below in Table 8.

[00268] Table 8

<i>Anti A mAb</i>	<i>A1</i>	<i>A2</i>	<i>A3</i>	<i>A4</i>	<i>A5</i>	
<i>Toxinotype Binding</i>	0, III, V, XII, XV	0, III, V, XII, XV	0, III, V, XII, XV	0, III, V, XII, XV	0, III, V, XII, XV	
<i>Anti B mAb</i>	<i>B1</i>	<i>B2</i>	<i>B3</i>	<i>B4</i>	<i>B5</i>	<i>B6</i>
<i>Toxinotype Binding</i>	0, III, V, VIII, XII	0, III, V, VIII	0, III, V, VIII, XII, XV	0, III, V	0, III	0, III, V, VIII, XII, XV

[00269] Example 6. Toxinotype Analysis by CTD Competition Assay

[00270] Toxinotyping by Western analysis can be biased by low toxin production in some strains. Therefore a more sensitive CTD (C Terminal Domain) competition assay was

developed. For the CTD competition assay, the CTDs of *C. difficile* toxin A and toxin B from genomic DNA of toxinotypes 0, III, V, VIII, XII and XV were cloned, expressed, and purified and combined with anti-toxin A or anti-toxin B antibodies to measure the effect on cytotoxicity or TEER in cell based neutralization assays (as described in previous example). The CTD competition assay only works for antibodies that recognize an epitope in the CTD of toxin A or toxin B.

[00271] Briefly, for toxin A, a QuickExtract™ DNA Extraction Kit (Epicentre) was used to isolate genomic DNA from 1 ml samples of cultures of each of the six *C. difficile* strains representing five toxinotypes (0, III, V, XII and XV). The following primers were used for amplification of the toxin A C-terminal domains (CTDs):

FP: 5'-CACCATGGGATTTAAAATAATAGATAATAAACTTATTAC-3' (SEQ ID NO:235)

RP: 5'-GCCATATATCCCAGGGGC-3' (SEQ ID NO:236)

[00272] The primers were designed to amplify the last 900 amino acids (amino acids 1811-2710 in the VPI10463 reference sequence), or 2700 bp of the toxin A toxinotype 0 CTD. Amplification was performed using Pfx50 DNA Polymerase and a standard touchdown PCR protocol. In the case where multiple bands were amplified, the band of the correct size (about 2700 bp for toxinotypes 0, III, V, XII and XV) was purified by excision of the band of the correct size from an agarose gel followed by gel extraction. Purified or unpurified PCR product was directionally cloned into the expression plasmid pET101-D-Topo, using a ligation-independent cloning strategy, as per the manufacturer's instructions (Invitrogen, Champion™ pET Directional TOPO® Expression Kit).

[00273] Directionality and sequence were confirmed by traditional DNA sequencing, using the forward and reverse cloning primers. Due to the highly repetitive nature of the intervening sequences the sequence of the entire CTD was not confirmed. The translation start site is at the ATG in the forward primer sequence. Expression continues through the reverse primer sequence and the C-terminal tags encoded by the expression plasmid. Recombinant expression of these proteins yields a protein of the following sequence as previously described: Met-GFKIIDNKTY-[toxinotype-specific A CTD aa's 1823-2704]-APGIYG-[V5 epitope]-RTG-[6xHis] (SEQ ID NO:237).

[00274] For toxin B, DNA samples were isolated from the six *C. difficile* strains representing six toxinotypes (0, III, V, VIII, XII and XV). The following primers were used for amplification of the toxin B CTD's:

FP: 5'-CGGATCCGAATTCATTCTTATGTCAACTAGTGAAGAAAATAAGG-3' (SEQ ID NO:238)

RP: 5'-GTGGTGGTGCTCGAGAGCTGTATCAGGATCAAAATAATAC-3' (SEQ ID NO:239)

[00275] The primers were designed to amplify the last 615 amino acids excluding the final 6 amino acids of the Toxin B CTD (aa 1752-2360), or 1827 bp of the toxin B toxinotype 0 CTD.

[00276] Amplification was performed using TaKaRa LA Taq DNA Polymerase and a standard touchdown PCR protocol. In the case where multiple bands were amplified, the band of the correct size (about 1827 bp for all toxinotypes) was purified by excision of the band of the correct size from an agarose gel followed by gel extraction. Purified or unpurified PCR product was directionally cloned into the expression plasmid pET24A+, using traditional restriction digest and ligation-dependent cloning strategy. Directionality and sequence were confirmed by traditional DNA sequencing, using the forward and reverse cloning primers. Due to the highly repetitive nature of the intervening sequences the sequence of the entire CTD was not confirmed. The translation start site is at the ATG in the forward primer sequence. Expression continues through the reverse primer sequence and the C-terminal tags encoded by the expression plasmid. Recombinant expression of these proteins yields a protein of the following sequence: Met-STSEENK-[toxinotype-specific B CTD aa's 1760-2352]-YYFDPDTA-LE-[6xHis] (SEQ ID NO:240).

[00277] The cloned toxin A and B CTD proteins were expressed as soluble full-length His-tagged proteins by recombinant expression in the *E. coli* strain BL21 Star (DE3) using the IPTG-free Overnight Express Autoinduction System 1 as per the manufacturer's instructions (Novagen). Proteins were purified under native conditions by bind-and-elute affinity chromatography on Ni-NTA resin, followed by anion exchange in the negative purification mode. Purified CTD proteins were used in cell-based, *in vitro* neutralization assays to determine the toxinotype specificity of certain antibodies.

[00278] The A2 antibody was tested in the Vero cell competition assay to measure the impact of toxin A CTDs of toxinotype 0, III, V, XII, and XV on the neutralizing activity of A2. The Vero cell neutralization was carried out as described above with varying dilutions of the antibodies, plus the addition of 1 µg/ml of toxinotype 0, III, V, XII, or XV toxin A CTDs. A2 neutralizes toxin A induced cytotoxicity in Vero cells with high potency. Toxinotype 0, III, V, XII, and XV CTDs strongly inhibited the neutralizing activity of A2 at low concentrations of antibody (0.625 µg and below) but had minimal, if any effect, at A2 concentrations above 1.25 µg/ml. Figure 3.

[00279] The A2 antibody was also tested in a T-84 cell neutralization assay to measure the impact of toxin A CTDs of toxinotypes 0, III, V, XII, and XV on TEER in T-84 cells. The T-84 cell neutralization assay was carried out as described above with varying dilutions of A2, plus the

addition of 0.4 µg/ml or 1 µg/ml of toxinotype 0, III, V, XII, and XV toxin A CTDs. A2 neutralizes toxin A induced loss of TEER in T-84 cells with high potency. CTDs from toxinotypes 0, III, V, XII, and XV strongly inhibited the neutralizing activity of A2 in the TEER assay. Figure 4A. Thus, the neutralizing activity of the A2 antibody was strongly inhibited by CTDs from five toxinotypes CTDs (0, III, V, XII, and XV), revealing a broad spectrum of protection against *C. difficile* toxinotypes with the more sensitive *in vitro* functional assays.

[00280] The B6 antibody was similarly tested in the T-84 cell neutralization assay. Toxin B CTDs from toxinotypes 0, III, V, VIII, XII, and XV strongly inhibited the neutralizing activity of B6. Figure 4B.

[00281] **Example 7. Epitope Mapping of Toxin B Antibodies by Western Blot**

[00282] Epitope mapping of anti-toxin B antibodies was conducted by Western analysis using recombinant domains from *C. difficile* toxin A and toxin B. The recombinant domains from toxin A were used as negative controls. Segments of the genes for toxin A and toxin B were cloned by PCR from *C. difficile* DNA of strain VPI10463. The amino acid sequences of toxin A and toxin B from *C. difficile* strain VPI10463 are set forth in SEQ ID NO:230 and SEQ ID NO:231, respectively. The corresponding amino acid residues for the cloned gene segments of toxin A and B are set forth in the table below:

Toxin Fragment	Amino Acid Residues	Domain
A2	300-660 of SEQ ID NO:230	Glucosyltransferase/protease
A3	660-1100 of SEQ ID NO:230	Protease/translocation
B1	510-1110 of SEQ ID NO:231	Protease
B2	1110-1530 of SEQ ID NO:231	Translocation N-terminal
B3	1750-2360 of SEQ ID NO:231	Receptor binding
B4	10-520 of SEQ ID NO:231	Glucosyltransferase
B5	1530-1750 of SEQ ID NO:231	Translocation C-terminal

[00283] A methionine start codon was added to the N-terminus and a 6xHis tag (SEQ ID NO:332) followed by a stop codon was added to the C-terminus. The resulting PCR products were ligated into the multiple cloning site of plasmid pET24+. The constructs were transformed into *E. coli* BL21(DE3) and induced by addition of IPTG.

[00284] Constructs A2 and A3 were expressed but were insoluble and were purified by denaturing chromatography, while constructs B1-B5 were at least partly soluble and purified by non-denaturing chromatography. Soluble constructs were grown to liter scale in LB medium at 37°C. Cells were pelleted by centrifugation and lysed by microfluidization (Microfluidics Corp, Newton MA) in 50 mM NaHPO₄, 300 mM NaCl, 20 mM imidazole, pH 8.0. Insoluble material was removed by centrifugation and the cleared lysate was loaded onto a Ni-NTA column

(Qiagen). The column was washed with 50 mM NaHPO₄, 300 mM NaCl, 20 mM imidazole, pH 8.0 and eluted with 50 mM NaHPO₄, 300 mM NaCl, 250 mM imidazole, pH 8.0. Insoluble constructs were grown and harvested as for soluble ones, but the cell pellet was resuspended in 8M Urea, 100mM NaHPO₄, 10mM Tris-HCl, pH 8.0 before microfluidization. Insoluble material was removed by centrifugation and the cleared lysate was loaded onto a Ni-NTA column and washed with 8M Urea, 100mM NaHPO₄, 10mM Tris-HCl, pH 6.3. The column was eluted with 8M Urea, 100mM NaHPO₄, 10mM Tris-HCl, pH 4.5 and protein-containing fractions were dialysed with multiple changes against 50 mM NaHPO₄, 300 mM NaCl, 250 mM imidazole, pH 8.0.

[00285] The binding of the B1, B2, B4, and B6 antibodies to the recombinant domains was assessed by Western analysis. Purified recombinant domains (about 400ng) were analyzed using SDS-PAGE on a NuPAGE® (Life Technologies) 4-12% polyacrylamide gel run at 200V using SeeBlue2® standards (Invitrogen). The proteins in the gel were transferred to a nitrocellulose membrane in 6 min, using the iBlot® (Invitrogen) gel blotting system. The blot was blocked with PBST (10mM sodium phosphate, 2mM potassium phosphate, 2.7mM potassium chloride, 137mM sodium chloride, 0.1% Tween 20) containing 5% nonfat dry milk (NFDm) for 1h at room temperature. The blot was probed with the antibody diluted 1:5000 in 2.5% NFDm/PBST for 1h at RT, then washed 3 x 5 min with PBST. The blot was incubated with goat anti-human Alkaline Phosphatase conjugate (Sigma, A1543) [1: 6600 in 2.5% NFDm/PBST,] for 1h at RT. The blot was washed 3 x 5 min with PBST and developed with 1 BCIP/NBT tablet (Sigma, B5655) in 10ml water. Development was stopped by putting the blot into deionized water.

[00286] Western analysis revealed that B1 and B2 bound to an epitope in the glucosyl transferase domain (amino acids 10-520 of SEQ ID NO:231) of toxin B, while B4 bound to an epitope in the N-terminal translocation domain (amino acids 1110-1530 of SEQ ID NO:231) of toxin B. The B6 antibody bound to an epitope in the receptor binding domain (amino acids 1750-2360 of SEQ ID NO:231) of toxin B.

[00287] Example 8. Epitope Mapping Using PepSet ELISA

[00288] The PepSet ELISA was used to identify linear epitopes of toxin A recognized by the A2 antibody. For the CTD, the following non-overlapping peptides of varying length were designed to cover the repetitive oligopeptide units:

[00289]	1	SGSGHLGFKIIDNKTYYYDEDSKL	(SEQ ID NO:199)
[00290]	2	SGSGVTGWQTINGKKYYFDINTGA	(SEQ ID NO:200)
[00291]	3	SGSGLTSYKIINGKHFYFNNDGVM	(SEQ ID NO:201)
[00292]	4	SGSGQSKFLTLNGKKYYFDNNSKA	(SEQ ID NO:202)

[00293]	5	SGSGVTGWRIINNERKYYFNPNNAI	(SEQ ID NO:203)
[00294]	6	SGSGAVGLQVIDNNKYYFNPDFAI	(SEQ ID NO:204)
[00295]	7	SGSGSKGWQTVNGSRYYFDTDTAI	(SEQ ID NO:205)
[00296]	8	SGSGFNGYKTIDGKHFFYFSDCVV	(SEQ ID NO:206)
[00297]	9	SGSGVTGLQTIDSKYYFNTNTAE	(SEQ ID NO:207)
[00298]	10	SGSGATGWQTIDGKKYYFNTNTAE	(SEQ ID NO:208)
[00299]	11	SGSGATGWQTIDGKKYYFNTNTAI	(SEQ ID NO:209)
[00300]	12	SGSGSTGYTIINGKHFFYFNTDGIM	(SEQ ID NO:210)
[00301]	13	SGSGQNEFLTNGKKYYFGSDSKA	(SEQ ID NO:211)
[00302]	14	SGSGVTGWRIINNKKYYFNPNNAI	(SEQ ID NO:212)
[00303]	15	SGSGAIHLCTINNDKYYFSYDGIL	(SEQ ID NO:213)
[00304]	16	SGSGQNGYITIERNNFYFDANNES	(SEQ ID NO:214)
[00305]	17	SGSGQNKFLTNGKKYYFNDNSKA	(SEQ ID NO:215)
[00306]	18	SGSGVTGWQTIDGKKYYFNLNTAE	(SEQ ID NO:216)
[00307]	19	SGSGATGWQTIDGKKYYFNLNTAE	(SEQ ID NO:217)
[00308]	20	SGSGATGWQTIDGKKYYFNTNTFI	(SEQ ID NO:218)
[00309]	21	SGSGSTGYTSINGKHFFYFNTDGIM	(SEQ ID NO:219)
[00310]	22	SGSGQNKFLTNGKKYYFGSDSKA	(SEQ ID NO:220)
[00311]	23	SGSGVTGLRTIDGKKYYFNTNTAV	(SEQ ID NO:221)
[00312]	24	SGSGVTGWQTINGKKYYFNTNTSI	(SEQ ID NO:222)
[00313]	25	SGSGSTGYTIISGKHFFYFNTDGIM	(SEQ ID NO:223)
[00314]	26	SGSGQNRFLYLHDNIYYFGNNNSKA	(SEQ ID NO:224)
[00315]	27	SGSGATGWVTIDGNRYFFEPNTAM	(SEQ ID NO:225)
[00316]	28	SGSGANGYKTIDNKNFYFRNGLPQ	(SEQ ID NO:226)
[00317]	29	SGSGQNRFLHLLGKIYYFGNNNSKA	(SEQ ID NO:227)
[00318]	30	SGSGVTGWQTINGKVVYFMPDTAM	(SEQ ID NO:228)
[00319]	31	SGSGAGGLFEIDGVIYFFGVGDKV	(SEQ ID NO:229)

[00320] 15 amino acid sequences with overlapping 5 amino acids domains and a moving window of 10 amino acids were also designed to cover the gaps between those repetitive units. All peptides were synthesized and probed for binding to A2.

[00321] Peptide binding was measured by ELISA. Briefly, 100µl of 5µg/ml streptavidin (Southern Biotech) in a sodium carbonate/sodium bicarbonate coating buffer solution (pH 9.6) was added to each well of NUNC Maxisorp® (eBiosciences) 96 well plates and incubated at 4°C overnight. The plates were washed 4 times with PBS Tween 20 (PBST) using a volume of

300µl/well before blocking with 3% BSA solution for 60 minutes. Biotinylated *C. difficile* toxin A CTD peptides were diluted to a concentration of 100 ng/ml, in diluent (3% BSA in PBST), added to each well (100µl/well), and incubated at 25°C for 60 minutes. The plates were then washed 4 times with PBST using a volume of 300µl/well. The antibody solution was diluted to the appropriate dilution in diluent buffer (3% BSA in PBST), added to the plates (100µl/well), and incubated at 25°C for 60 minutes. Following the incubation with antibody, the plates were again washed 4 times with PBST using a volume of 300µl/well.

[00322] For the secondary antibody reaction, horseradish peroxidase (HRP)-goat anti human IgG (Jackson ImmunoResearch) was diluted to 1:2000 in diluent buffer, added to the plates (100µl/well), and incubated at 25°C for 60 minutes. Following the incubation with secondary antibody, the plates were again washed 4 times with PBST using a volume of 300µl/well. SureBlue Reserve™ TMB peroxidase substrate (KPL Inc.) was then added to each well (100 µl/well) and incubated at 25°C for 10 minutes. The reaction was stopped by adding 100 µl/well of TMB stop solution (KPL Inc.). The plates were read at a wavelength of 450 nm at 25°C using a Molecular Devices, Model Spectra Max M5.

[00323] No binding was observed between A2 and the longer, overlapping peptide sequences. The A2 antibody bound to non-overlapping peptides 2, 18, 19, 20, 24 and 30:

P2: VTGWQTINGKKYYFDINTGA (SEQ ID NO:241)

P18: VTGWQTIDGKKYYFNLNTAE (SEQ ID NO:242)

P19: ATGWQTIDGKKYYFNLNTAE (SEQ ID NO:333)

P20: ATGWQTIDGKKYYFNTNTFI (SEQ ID NO:334)

P24: VTGWQTINGKKYYFNTNTSI (SEQ ID NO:335)

P30 VTGWQTINGKVYYFMPDTAM (SEQ ID NO:243)

[00324] Thus, the A2 antibody recognizes a minimal linear epitope in the C-terminal domain of toxin A comprising the amino acid sequence X₁TGWQTI (SEQ ID NO:232), where X₁ is A or V. The A2 antibody also recognizes a longer consensus sequence comprising the amino acid sequence of: X₂TGWQTIX₃GKX₄YYF (SEQ ID NO:233), where X₂ is A or V, X₃ is N or D and X₄ is K or V.

[00325] As discussed above, it was determined by Western analysis that the B1 and B2 antibodies bound to an epitope in the glucosyl transferase domain (GTD) of toxin B. In addition, sequence analysis showed that B1 and B3 light and heavy chains differed by only 3 CDR and 2 FR mutations in the light chain and 2 FR mutations in the heavy chain and predicted that the B1 and B3 antibodies would bind to the same epitope. Thus, the PepSet peptide binding assay was also used to identify linear epitopes in the GTD of toxin B recognized by B1, B2, and B3, using

the general protocol described above for the A2 antibody. For the GTD, the following peptides with 15 amino acid sequences and 5 amino acid overlap were designed to cover the N terminal domain of the molecule:

[00326]	179	SGSGMSLVNRKQLEKMANV	(SEQ ID NO:247)
[00327]	180	SGSGRKQLEKMANVRFTQ	(SEQ ID NO:248)
[00328]	181	SGSGKMANVRFTQDEEYV	(SEQ ID NO:249)
[00329]	182	SGSGRFTQDEEYVAILDA	(SEQ ID NO:250)
[00330]	183	SGSGEDEYVAILDALEEYH	(SEQ ID NO:251)
[00331]	184	SGSGAILDALEEYHNMSSEN	(SEQ ID NO:252)
[00332]	185	SGSGLEEYHNMSSENTVVEK	(SEQ ID NO:253)
[00333]	186	SGSGNMSSENTVVEKYLKIK	(SEQ ID NO:254)
[00334]	187	SGSGTVVEKYLKIKDINSL	(SEQ ID NO:255)
[00335]	188	SGSGYLKIKDINSLTDICI	(SEQ ID NO:256)
[00336]	189	SGSGDINSLTDICIDTYKK	(SEQ ID NO:257)
[00337]	190	SGSGTDICIDTYKKSGRNK	(SEQ ID NO:258)
[00338]	191	SGSGDTYKKSGRNKALKKF	(SEQ ID NO:259)
[00339]	192	SGSGSGRNKALKKFKEYLV	(SEQ ID NO:260)
[00340]	193	SGSGALKKFKEYLVTEVLE	(SEQ ID NO:261)
[00341]	194	SGSGKEYLVTEVLELKNNN	(SEQ ID NO:262)
[00342]	195	SGSGTEVLELKNNNLTPVE	(SEQ ID NO:263)
[00343]	196	SGSGLKNNNLTPVEKNLHF	(SEQ ID NO:264)
[00344]	197	SGSGKNLHFVWIGGQINDT	(SEQ ID NO:265)
[00345]	198	SGSGVWIGGQINDTAINYI	(SEQ ID NO:266)
[00346]	199	SGSGQINDTAINYINQWKD	(SEQ ID NO:267)
[00347]	200	SGSGAINYINQWKDVNSDY	(SEQ ID NO:268)
[00348]	201	SGSGNQWKDVNSDYNVNVF	(SEQ ID NO:269)
[00349]	202	SGSGVNSDYNVNVFYDSNA	(SEQ ID NO:270)
[00350]	203	SGSGLKKTIVESAINDTLE	(SEQ ID NO:271)
[00351]	204	SGSGVESAINDTLESFREN	(SEQ ID NO:272)
[00352]	205	SGSGNDTLESFRENLDPR	(SEQ ID NO:273)
[00353]	206	SGSGSFRENLDPRFDYNK	(SEQ ID NO:274)
[00354]	207	SGSGLNDPRFDYNKFFRKR	(SEQ ID NO:275)
[00355]	208	SGSGFDYNKFFRKRMEIY	(SEQ ID NO:276)
[00356]	209	SGSGFFRKRMEIYDKQKN	(SEQ ID NO:277)

[00357] 210 SGSGMETIYDKQKNFINYY (SEQ ID NO:278)
[00358] 211 SGSGDKQKNFINYYKAQRE (SEQ ID NO:279)
[00359] 212 SGSGFINYYKAQREENPEL (SEQ ID NO:280)
[00360] 213 SGSGKAQREENPELIIDDI (SEQ ID NO:281)
[00361] 214 SGSGENPELIIDDIVKTYL (SEQ ID NO:282)
[00362] 215 SGSGIIDDIVKTYLSNEYS (SEQ ID NO:283)
[00363] 216 SGSGVKTYLSNEYSKEIDE (SEQ ID NO:284)
[00364] 217 SGSGSNEYSKEIDELNTYI (SEQ ID NO:285)
[00365] 218 SGSGKEIDELNTYIEESLN (SEQ ID NO:286)
[00366] 219 SGSGLNTYIEESLNKITQN (SEQ ID NO:287)
[00367] 220 SGSGEESLNKITQNSGNDV (SEQ ID NO:288)
[00368] 221 SGSGKITQNSGNDVRNFGE (SEQ ID NO:289)
[00369] 222 SGSGSGNDVRNFGEFKNGE (SEQ ID NO:290)
[00370] 223 SGSGRNFGEFKNGESFNLY (SEQ ID NO:291)
[00371] 224 SGSGFKNGESFNLYEQELV (SEQ ID NO:292)
[00372] 225 SGSGDVDMLPGIQPDLFES (SEQ ID NO:293)
[00373] 226 SGSGPGIQPDLFESIEKPS (SEQ ID NO:294)
[00374] 227 SGSGDLFESIEKPSSVTVD (SEQ ID NO:295)
[00375] 228 SGSGIEKPSSVTVDFWEMT (SEQ ID NO:296)
[00376] 229 SGSGSVTVDFWEMTKLEAI (SEQ ID NO:297)
[00377] 230 SGSGKLEAIMKYKEYIPEY (SEQ ID NO:298)
[00378] 231 SGSGMKYKEYIPEYTSEHF (SEQ ID NO:299)
[00379] 232 SGSGYIPEYTSEHFDMLDE (SEQ ID NO:300)
[00380] 233 SGSGTSEHFDMLDEEVQSS (SEQ ID NO:301)
[00381] 234 SGSGDMLDEEVQSSSFESVL (SEQ ID NO:302)
[00382] 235 SGSGEVQSSSFESVLASKSD (SEQ ID NO:303)
[00383] 236 SGSGFESVLASKSDKSEIF (SEQ ID NO:304)
[00384] 237 SGSGASKSDKSEIFSSLGD (SEQ ID NO:305)
[00385] 238 SGSGKSEIFSSLGDMEASP (SEQ ID NO:306)
[00386] 239 SGSGSSLGDMEASPLEVKI (SEQ ID NO:307)
[00387] 240 SGSGMEASPLEVKIAFNSK (SEQ ID NO:308)
[00388] 241 SGSGLEVKIAFNSKGIINQ (SEQ ID NO:309)
[00389] 242 SGSGAFNSKGIINQGLISV (SEQ ID NO:310)
[00390] 243 SGSGKDSYCSNLIVKQIEN (SEQ ID NO:311)

[00391] 244 SGSGKQIENRYKILNNSLN (SEQ ID NO:312)
 [00392] 245 SGSGRYKILNNSLNPAISE (SEQ ID NO:313)
 [00393] 246 SGSGNNSLNPAISEDNDNFN (SEQ ID NO:314)
 [00394] 247 SGSGPAISEDNDNFNTTTNT (SEQ ID NO:315)
 [00395] 248 SGSGDNDNFNTTTNTFIDSI (SEQ ID NO:316)
 [00396] 249 SGSGTTNTTFIDSIMAEAN (SEQ ID NO:317)
 [00397] 250 SGSGFIDSIMAEANADNGR (SEQ ID NO:318)
 [00398] 251 SGSGMAEANADNGRFMMEL (SEQ ID NO:319)
 [00399] 252 SGSGADNGRFMMELGKYLR (SEQ ID NO:320)
 [00400] 253 SGSGLLMFKEGSMNIHLIE (SEQ ID NO:321)
 [00401] 254 SGSGEGSMNIHLIEADLRN (SEQ ID NO:322)
 [00402] 255 SGSGIHLIEADLRNFEISK (SEQ ID NO:323)
 [00403] 256 SGSGADLRNFEISKTNISQ (SEQ ID NO:324)
 [00404] 257 SGSGFEISKTNISQSTEQE (SEQ ID NO:325)
 [00405] 258 SGSGTNISQSTEQEMASLW (SEQ ID NO:326)
 [00406] 259 SGSGSTEQEMASLWSFDDA (SEQ ID NO:327)
 [00407] 260 SGSGMASLWSFDDARAKAQ (SEQ ID NO:328)
 [00408] 261 SGSGSFDDARAKAQFEEYK (SEQ ID NO:329)
 [00409] 262 SGSGRAKAQFEEYKRNIFE (SEQ ID NO:330)

[00410] All peptides were synthesized and probed for binding to B1, B2, and B3.

[00411] The B1 and B3 antibodies both bound to peptides 190, 191, and 192 from the toxin B GTD:

P190: aa 56-70 TDICIDTYKKSGR^NK (SEQ ID NO:244)

P191: aa 61-75 DTYKKSGR^NKALKKF (SEQ ID NO:245)

P192: aa 66-80 SGR^NKALKKFKEYLV (SEQ ID NO:246)

[00412] Thus, the B1 and B3 antibodies both recognize a minimal linear epitope in the GTD of toxin B comprising the amino acid sequence SGR^NK (SEQ ID NO:234). This epitope maps to amino acids 56-80 of SEQ ID NO:231. The B2 antibody binds very weakly to the P190, P191, and P192 peptides but did not bind strongly to any of the GTD short repeat sequences. The B1 and B3 antibodies were also shown to bind to the same epitope by Octet analysis (data not shown).

[00413] The N-terminal 91 amino acids of the GTD shares homology with a domain found in cholera toxin and other pathogens. In cholera, this domain, referred to as the 4-helix bundle (4HB) or membrane localization domain (MLD), has been shown to be involved in

direct binding of the toxin to the cell membrane and mutagenesis of several amino acids in the MLD abolishes this function (Geissler et al, PNAS, 2010). The SGRNK (SEQ ID NO:234) sequence identified through peptide binding analysis is located in a loop between alpha helices 3 and 4 of the MLD.

[00414] Amino acids 1-91 of toxin B were cloned into a pET28a expression construct (GeneArt) with an LPETG (SEQ ID NO:336) motif (which allows for sortase-catalyzed conjugation of labels, such as biotin) and a C-terminal 6xHis tag both with the wild-type SGRNK (SEQ ID NO:234) sequence and a mutated version: AGANK (SEQ ID NO:337). These constructs had the following amino acid sequences:

[00415] WT Toxin B MLD (1-91) + LPETGG + 6X HIS

[00416] MSGLVPRGSHMSLVNRKQLEKMANVRFRTQEDEYVAILDALEEEY
HNMSENTVVEKYLKLDINSLTDIYIDTYKKSGRNKALKKKFKEYLVTEVLELKNNNLLP
ETGGHHHHHH (SEQ ID NO:338)

[00417] Mutant Toxin B MLD (1-91) + LPETGG + 6X HIS

[00418] MSGLVPRGSHMSLVNRKQLEKMANVRFRTQEDEYVAILDALEEEY
HNMSENTVVEKYLKLDINSLTDIYIDTYKKAGANKALKKKFKEYLVTEVLELKNNNLLP
ETGGHHHHHH (SEQ ID NO:339)

[00419] The GTD enzymatic domain (amino acids 95-586; "ASE") was also cloned into a pET28a expression construct (GeneArt) with an LPETG motif and a C-terminal 6xHis tag. This construct has the following amino acid sequence:

[00420] WT Toxin B ASE (95-586) + LPETGG + 6X HIS

[00421] MEKNLHFVWIGGQINDTAINYINQWKDVNSDYNVNVFYDSNAFLI
NTLKKTVVESAINDTLESFRENLDPRFDYNKFFRKRMEIHYDKQKNFINYYKAQREENP
ELIIDDIVKTYLSNEYSKEIDELNTYIEESLNKITQNSGNDVRNFEEFKNGESFNLYEQELV
ERWNLAAASDILRISALKEIGGMYLDVDMLPGIQPDLFESIEKPSSVTVDWFEMTKLEAI
MKYKEYIPEYTSEHFDMLDEEVQSSFESVLASKSDKSEIFSSLGDMEASPLEVKIAFNNSKG
IINQGLISVKDSYCSNLIVKQIENRYKILNNSLNPAISEDNDNFNTTNTFIDSIMAEANADN
GRFMMELGKYL RVGFFPDVKTINLSGPEAYAAAYQDLLMFKEGSMNIHLIEADLRNFE
ISKTNISQSTEQEMASLWSFDDARAKAQFEEYKRNYFEGSLGELPETGGHHHHHH (SEQ
ID NO:340)

[00422] Using both Western and dot blot analysis, B2 was found to bind strongly to the wild type MLD sequence and to the mutant MLD sequence to a much lesser extent. The B1 and B3 antibodies did not bind to either the wild type or mutant MLD sequence by Western or dot blot analysis. No binding to the ASE domain was observed with any of the antibodies.

[00423] Binding of the B1, B2, and B3 antibodies to the wild type and mutant toxin B MLD sequences and the wild type toxin B ASE sequence was also assessed using Bio-Layer Interferometry on a Octet® RED96 (FortéBio) at 30°C, as described above in Example 2. As expected, all three antibodies bind to the full length toxin B and toxin B GTD by Octet analysis. B2 bound to the wild type and mutant MLD sequences, while neither B1 nor B3 bound to either MLD sequence. Unexpectedly, all three antibodies were found to bind the toxin B ASE domain by Octet analysis, suggesting that the non-denatured ASE domain may possess some non-specific binding activity due to misfolding or a hydrophobic surface generated by separating the ASE domain from the MLD. In the Western analysis, under denaturing conditions, only the positive control (6xHis) antibody bound the ASE domain.

[00424] The Octet analysis was also conducted for the B1 and B2 antibodies using the cloned GTD (aa 1-586) with the wild-type SGRNK (SEQ ID NO:234) sequence and a mutated version: AGANK (SEQ ID NO:337). Both antibodies bind strongly to the wild type GTD. B2 binding was reduced by the mutations to the SGRNK motif (SEQ ID NO:234, leading to an approximately 100-fold difference in K_d . B1 binding was unaffected by the mutations.

[00425] Hydrogen-Deuterium Exchange Mass Spectroscopy shows that the binding of the B2 antibody strongly reduces solvent exchange of the N-terminal helix of the GTD, while the SGRNK (SEQ ID NO:234) sequence is barely protected by B2 binding (data not shown). Saturation binding on the Octet shows that B1 and B2 do not interfere with each other's ability to bind toxin B, thus suggesting that the two antibodies recognize different epitopes.

[00426] In summary, while PepSet ELISA showed that the B1 and B3 antibodies bind strongly to a linear epitope comprising the SRGNK (SEQ ID NO:234) motif of the MLD, neither antibody bound to the toxin B MLD by Western, dot blot, or Octet analysis. However, the B1 and B3 antibodies do bind to the toxin B GTD by Western and Octet analysis, suggesting that the conformational epitope recognized by the B1 and B3 antibodies may not be exposed or present when the MLD is expressed separately from the ASE domain. On the other hand, the B2 antibody, which binds very weakly to linear epitopes comprising the SRGNK (SEQ ID NO:234) motif, was found to bind the toxin B MLD by Western, dot blot, and Octet analysis. Mutating the SRGNK (SEQ ID NO:234) sequence in the toxin B MLD and GTD reduces the binding of the B2 antibody. Together, these observations suggest that the B2 antibody binds to a conformational epitope within the MLD. The SRGNK (SEQ ID NO:234) motif within the MLD may play a role in contributing to the tertiary structure of the epitope recognized by B2 or may interact non-specifically with the B2 antibody.

[00427] **Example 9. *In Vivo* Efficacy of Antibodies in Hamster Model**

[00428] The hamster model is widely recognized as the optimal choice for the evaluation of novel treatment strategies against *C. difficile* (Best et al. Gut, 2012, 3(2):145-167; Babcock et al. Infection & Immunity, 2006,74(11):6339-6347). Once the normal intestinal flora of these animals is compromised by antibiotic treatment, challenge with live toxigenic *C. difficile* bacteria or viable spores from a toxigenic strain leads to colonization followed by lethal colitis. Diarrhea, histological damage and colitis result from the action of *C. difficile* toxin A and B and the stimulation of local inflammation. These symptoms are very similar to the symptoms observed in human suffering from *C. difficile*-associated diarrhea (CDAD). Thus, the *in vivo* efficacy of the human anti-toxin A and anti-toxin B antibodies was evaluated in a hamster CDAD model (also known as the hamster *C. difficile* infection (CDI) model).

[00429] Female Golden Syrian hamsters (*Mesocricetus auratus*), obtained from Charles River Laboratories, were individually caged and allowed to acclimate to the animal facility for at least 48 hours prior to any treatment, challenge, or other manipulation. All procedures involving animals were conducted under protocols approved by the Institutional Animal Care and Use Committee (IACUC).

[00430] For the primary challenge, animals were intraperitoneally (IP) injected with anti-toxin A and B antibodies at doses ranging from 6-50 mg/kg, every day for 4 consecutive days on days -3, -2, -1, and 0 relative to bacterial challenge on day 0. The test antibodies were injected as a combination of one human anti-toxin A antibody and one human anti-toxin B antibody. Combinations tested included 1) A2 (anti toxin A) and B6 (anti toxin B), 2) A2 (anti toxin A) and B4 (anti toxin B), 3) A2 (anti toxin A) and B1 (anti toxin B), and 4) A2 (anti toxin A) and B2 (anti toxin B). Control animals were also injected on the same 4-day injection schedule with 2 ml of PBS. In addition, 24 hours prior to bacterial challenge all animals were weighed and IP injected with 1 ml of a 1mg/ml clindamycin solution. This antibiotic pretreatment disrupts the normal intestinal flora and facilitates gut colonization with *C. difficile*.

[00431] On the day of challenge, animals received their final IP injection of antibodies or PBS prior to intragastric (IG) challenge with a LD100 dose of *C. difficile* spores (toxinotype 0 strain 630). To prepare *C. difficile* spores, the bacteria were grown for 24 hours in thioglycollate medium. This culture was used to inoculate anaerobic blood agar plates which were incubated at 37°C until the bacterial were confluent (3-4 days). After reaching confluence, plates were incubated for an additional 3 days to induce spore formation. Spores were harvested into PBS without Ca or Mg, washed once and then heat shocked at 56°C for 10 minutes to kill the vegetative cells. The spore suspension was centrifuged at 500 g for 30 minutes and re-suspended in 20% glycerol in PBS. Spore preparations were frozen at -65°C or less for long term storage.

Viable spore counts (CFU ml⁻¹) were assessed by thawing the spore stock at 37°C and performing serial 10-fold dilutions in water. Dilutions were plated in triplicate onto pre-reduced CDSA agar plates. Plates were incubated under anaerobic conditions at 37°C for no less than 48 hours. The colonies were counted and CFU ml⁻¹ was calculated. After completion of IP injection and IG challenge, animals were housed individually in sterile caging that consists of autoclaved sterilized bedding, autoclaved sterilized water, and irradiated food.

[00432] After challenge, animals were observed at least twice a day for morbidity and mortality and were weighed as per approved protocol. Both diarrheal disease and animal behavior were assessed. Diarrheal disease was scored numerically on a scale of 0-3: 0 – no disease, 1 - loose feces, 2 - wet tail and perianal region, 3 - wet perianal region, belly and hind paws. Behavior is evaluated categorically using the following criteria: N - animal appears normal; QAR – animal appears slightly lethargic, but alert and arousable; I – animal appears severely dehydrated, immobile, and exhibits hunched posture and/or ruffled fur. If an animal received a behavior score of I, the animal was immediately euthanized via CO₂ overdose. Percent weight loss was also calculated and if the animal lost >30% of its pre-challenge body weight it was considered moribund and was immediately euthanized via CO₂ overdose. All animals in a study were observed until all animals had either died or been euthanized or there was a period of at least 48 hours with no animals displaying any diarrheal symptoms or behaviors of illness.

[00433] In initial tests, the A2 antibody was paired with either the B4 or B6 antibody. Control hamsters that did not receive an antibody usually died by day 4 of the study. With these antibody combinations, a dose of 50 mg/kg provided optimal results (data not shown). At a dose of 50 mg/kg, A2 + B4 conferred survival on all animals tested through the end of the study (15 days post challenge), whereas only 60% of animals treated with A2 and B6 survived. Figure 5A. The animals treated with A2 + B4 also showed no disease symptoms and had less weight loss than those treated with A2 + B6. Figures 5B and 5C.

[00434] Subsequent testing in the CDAD model compared A2 + B4 to A2 + B1 and A2 + B2. At the 50 mg/kg dose, both A2 + B1 and A2 + B2 conferred survival on all animals tested through the end of the study (12 days post challenge). Figure 6A. The antibody pairs were also tested at lower doses. At the 6 mg/kg dose, A2 + B2 conferred 100% survival through the end of the study (12 days post challenge), whereas A2 + B1 and A2 + B4, at the lower dose, conferred only 40% survival. Figure 6B. The animals treated with 50 mg/kg of each of the three antibody combinations showed minimal disease symptoms, with A2 + B2 showing superior protection against illness at the lower dosage (6 mg/kg). Figures 7A and 7B. The A2 + B2 combination

also conferred less weight loss than the A2 + B1 and A2 + B4 combinations. Figures 8A and 8B.

[00435] Fecal pellets were collected from challenged animals throughout the study, usually but not always on days 4, 7 and 12 post-challenge. In order to ensure collection of fresh fecal pellets, fecal matter was collected 1 day after animals were transferred into clean cages. Fecal matter was cultured to determine the *C. difficile* colonization status of animals. To culture fecal matter, fecal pellets were weighed and at least 40 mg of feces were homogenized with 5 × volumes of DPBS and 5 × volumes ethanol per mg of sample. Material was serially diluted and 100µl of diluted homogenized fecal matter was cultured on reduced *C. difficile* selective Agar plates (CDSA plates) at 37°C in an anaerobic jar. After 56-72 hours of growth, colonies, which should appear as flat to low umbonate yellow colonies with ground glass-like appearance and a slightly filamentous edge, were counted.

[00436] Treatment with 6 mg/kg A2 + B1 or A2 + B2 did not affect the initial colonization and *C. difficile* burden, as measured by a *C. difficile* fecal culture tested on Day 4 post challenge. Figure 9A. However, when measured at Day 7 post challenge, both antibody combinations reduced *C. difficile* load as measured by fecal shedding of *C. difficile*. Figure 9B. By Day 13 post challenge neither the A2 + B1 nor the A2 + B2 combination showed any detectable fecal shedding. Figure 9C.

[00437] **Example 10. In Vivo Efficacy of Antibodies Against Highly Virulent Strains**

[00438] Based on the results from Example 9, the A2 + B2 antibody combination was tested against highly virulent strains of *C. difficile*: the toxinotype 0 strain VPI10463 and toxinotype III (ribotype 027) strain 13695#7. The hamster CDAD model was used as described in Example 9. At the 6 mg/kg dose, A2 + B2 prolonged life as compared to the PBS control but resulted in 0% survival at days 6 and 4, respectively for the VPI10463 and 13695#7 strains. Figures 10A and 10B.

[00439] Adding a third antibody to the A2 + B2 combination significantly enhances survival in the hamster CDAD model. The following three antibody combinations were tested at low (6 mg/kg) and high doses (50 mg/kg) using epidemic highly virulent strain 13695#7: A2 (6 mg/kg or 50 mg/kg) + B1 (3 mg/kg or 25 mg/kg) + B2 (3 mg/kg or 25 mg/kg), A2 (6 mg/kg or 50 mg/kg) + B2 (3 mg/kg or 25 mg/kg) + B4 (3 mg/kg or 25 mg/kg), and A2 (6 mg/kg or 50 mg/kg) + B2 (3 mg/kg or 25 mg/kg) + B6 (3 mg/kg or 25 mg/kg). All three combinations conferred survival on all animals tested through the end of the study (10 days post challenge) except for the low dose of A2 + B2 + B4, which conferred 80% survival through the end of the study. Figures 11A and 11B. All three combinations showed strong protection against illness, with two combinations (A2 + B1 + B2 and A2 + B2 + B6) showing no disease symptoms and the

third combination (A2 + B2 + B4) showing no disease symptoms after day 5 post challenge. Figures 12A and 12B. Similar results were observed for the VPI10463 strain (data not shown).

[00440] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

What is claimed:

1. An isolated monoclonal antibody that binds to an epitope in the C-terminal receptor domain of *Clostridium difficile* toxin A, wherein the epitope comprises the amino acid sequence X₁TGWQTI (SEQ ID NO:232), wherein X₁ is A or V or the amino acid sequence of X₂TGWQTI X₃GKX₄YYF (SEQ ID NO:233), wherein X₂ is A or V, X₃ is N or D and X₄ is K or V.

2. An isolated monoclonal antibody that binds to *Clostridium difficile* toxin A, wherein said antibody comprises a heavy chain variable domain and a light chain variable domain, and

(a) wherein the heavy chain variable domain comprises a complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:8; and a CDR3 comprising the amino acid sequence of SEQ ID NO:10; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:13; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:17;

(b) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:24; a CDR2 comprising the amino acid sequence of SEQ ID NO:26; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:31; a CDR2 comprising the amino acid sequence of SEQ ID NO:33; and a CDR3 comprising the amino acid sequence of SEQ ID NO:35;

(c) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:44; and a CDR3 comprising the amino acid sequence of SEQ ID NO:46; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:49; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:53;

(d) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:60; a CDR2 comprising the amino acid sequence of SEQ ID NO:62; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid

sequence of SEQ ID NO:67; a CDR2 comprising the amino acid sequence of SEQ ID NO:69; and a CDR3 comprising the amino acid sequence of SEQ ID NO:71; or

(e) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:78; a CDR2 comprising the amino acid sequence of SEQ ID NO:80; and a CDR3 comprising the amino acid sequence of SEQ ID NO:82; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:85; a CDR2 comprising the amino acid sequence of SEQ ID NO:87; and a CDR3 comprising the amino acid sequence of SEQ ID NO:89.

3. An isolated monoclonal antibody that binds to *Clostridium difficile* toxin A, wherein said antibody comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:20, SEQ ID NO:38, SEQ ID NO:56, or SEQ ID NO:74 or a light chain variable domain comprising the amino acid sequence of SEQ ID NO:4, SEQ ID NO:22, SEQ ID NO:40, SEQ ID NO:58, or SEQ ID NO:76.

4. The isolated monoclonal antibody of claim 3, wherein said antibody comprises a heavy chain variable domain and a light chain variable domain, and:

(a) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:2 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:4;

(b) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:20 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:22;

(c) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:38 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:40;

(d) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:56 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:58; or

(e) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:74 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:76.

5. An isolated, human monoclonal antibody that binds to the same epitope of *Clostridium difficile* toxin A recognized by or that competitively inhibits the binding of toxin A to:

(a) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:2 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:4;

(b) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:20 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:22;

(c) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:38 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:40;

(d) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:56 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:58; or

(e) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:74 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:76.

6. The isolated antibody of any one of claims 1-5, wherein the antibody comprises at least one of the following characteristics:

- (a) the antibody binds to *C. difficile* toxin A with a dissociation constant (K_D) equal to or less than 10 pM (10^{-11} M);
- (b) the antibody neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin A in the Vero monkey kidney cell line with an NT50 equal to or less than 3000 pM;
- (c) the antibody neutralizes the *C. difficile* toxin A induced loss of transepithelial electrical resistance (TEER) in the T-84 cell line with an NT50 equal to or less than 6 nM; or
- (d) the antibody binds to toxin A produced by the strains of toxinotypes 0, III, V, XII, and XV.

7. An isolated monoclonal antibody that binds to an epitope in the glucosyl transferase domain of *C. difficile* toxin B, wherein the epitope comprises the amino acid sequence SGRNK (SEQ ID NO:234), amino acids 56-80 of SEQ ID NO:231, or amino acids 10-520 of SEQ ID NO:231.

8. An isolated monoclonal antibody that binds to an epitope in the N-terminal translocation domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 1110-1530 of SEQ ID NO:231.

9. An isolated monoclonal antibody that binds to an epitope in the receptor binding domain of *C. difficile* toxin B, wherein the epitope comprises amino acids 1750-2360 of SEQ ID NO:231.

10. An isolated monoclonal antibody that binds to *Clostridium difficile* toxin B, wherein said antibody comprises a heavy chain variable domain and a light chain variable domain, and:

(a) wherein the heavy chain variable domain comprises a complementarity determining region 1 (CDR1) comprising the amino acid sequence of SEQ ID NO:96; a CDR2 comprising the amino acid sequence of SEQ ID NO:98; and a CDR3 comprising the amino acid sequence of SEQ ID NO:100; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:103; a CDR2 comprising the amino acid sequence of SEQ ID NO:105; and a CDR3 comprising the amino acid sequence of SEQ ID NO:107;

(b) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:114; a CDR2 comprising the amino acid sequence of SEQ ID NO:116; and a CDR3 comprising the amino acid sequence of SEQ ID NO:118; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:121; a CDR2 comprising the amino acid sequence of SEQ ID NO:123; and a CDR3 comprising the amino acid sequence of SEQ ID NO:125;

(c) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:132; a CDR2 comprising the amino acid sequence of SEQ ID NO:134; and a CDR3 comprising the amino acid sequence of SEQ ID NO:136; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:139; a CDR2 comprising the amino acid sequence of SEQ ID NO:141; and a CDR3 comprising the amino acid sequence of SEQ ID NO:143;

(d) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:150; a CDR2 comprising the amino acid sequence of SEQ ID NO:152; and a CDR3 comprising the amino acid sequence of SEQ ID NO:154; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:157; a CDR2 comprising the amino acid sequence of SEQ ID NO:159; and a CDR3 comprising the amino acid sequence of SEQ ID NO:161;

(e) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:168; a CDR2 comprising the amino acid sequence of SEQ ID NO:170; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172;

and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:175; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:179; or

(f) wherein the heavy chain variable domain comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:186; a CDR2 comprising the amino acid sequence of SEQ ID NO:188; and a CDR3 comprising the amino acid sequence of SEQ ID NO:190; and wherein the light chain variable domain comprises: a CDR1 comprising the amino acid sequence of SEQ ID NO:193; a CDR2 comprising the amino acid sequence of SEQ ID NO:195; and a CDR3 comprising the amino acid sequence of SEQ ID NO:197.

11. An isolated, monoclonal antibody that binds to *Clostridium difficile* toxin B, wherein said antibody comprises a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:92, SEQ ID NO:110, SEQ ID NO:128, SEQ ID NO:146, SEQ ID NO:164, or SEQ ID NO:182 or a light chain variable domain comprising the amino acid sequence of SEQ ID NO:94, SEQ ID NO:112, SEQ ID NO:130, SEQ ID NO:148, SEQ ID NO:166, or SEQ ID NO:184.

12. The isolated monoclonal antibody of claim 11, wherein said antibody comprises a heavy chain variable domain and a light chain variable domain, and:

(a) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:92 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:94;

(b) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:110 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:112;

(c) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:128 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:130;

(d) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:146 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:148;

(e) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:164 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:166; or

(f) wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:182 and the light chain variable domain comprises the amino acid sequence of SEQ ID NO:184.

13. An isolated monoclonal antibody that binds to the same epitope of *Clostridium difficile* toxin B recognized by or that competitively inhibits the binding of toxin A to:

(a) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:92 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:94;

(b) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:110 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:112;

(c) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:128 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:130;

(d) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:146 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:148;

(e) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:164 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:166; or

(f) an antibody having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:182 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:184.

14. The isolated antibody of any one of claims 7-13, wherein the antibody comprises at least one of the following characteristics:

- (a) the antibody binds to *C. difficile* toxin B with a dissociation constant (K_D) equal to or less than 100 pM;
- (b) the antibody neutralizes the *in vitro* cytotoxicity of *C. difficile* toxin B in the Vero monkey kidney cell line with an NT50 equal to or less than 1000 pM;
- (c) the antibody neutralizes the *C. difficile* toxin B induced loss of transepithelial resistance (TEER) in the T-84 cell line with an NT50 equal to or less than 200 pM; or
- (d) the antibody binds to toxin B produced by at least the strains of toxinotypes 0, III, and V.

15. The antibody of claim 14, wherein the antibody binds to toxin B produced by at least the strains of toxinotypes 0, III, V, and VIII, toxin B produced by at least the strains of toxinotypes 0, III, V, VIII, and XII, or toxin B produced by at least the strains of toxinotypes 0, III, V, VIII, XII, and XV.

16. The antibody of any one of claims 1-15, wherein the antibody is a human antibody.

17. The antibody of any one of claims 1-16, wherein the antibody is a recombinant antibody.

18. The antibody of any of claims 1-17, wherein the antibody is a bispecific antibody, wherein the bispecific antibody comprises 1) a first antigen binding site comprising the heavy chain variable domain and light chain variable domain of the antibody of any one of claims 1-5 and 2) a second antigen binding site comprising the heavy chain variable domain and light chain variable domain of the antibody of any one of claims 7-12.

19. The antibody of any of claims 1-17, wherein the antibody is a bispecific antibody, wherein the bispecific antibody comprises 1) a first antigen binding site comprising the heavy chain variable domain and light chain variable domain of the B1 antibody and 2) a second antigen binding site comprising the heavy chain variable domain and light chain variable domain of the B2 antibody.

20. A composition comprising one or more of the antibodies of claims 1-19.

21. The composition of claim 20, comprising at least one antibody that binds to *C. difficile* toxin A, wherein the at least one antibody that binds to *C. difficile* toxin A is one or more of the A1, A2, A3, A4, and A5 antibodies and at least one antibody that binds to *C. difficile* toxin B.

22. The composition of claim 20, comprising at least one antibody that binds to *C. difficile* toxin A and at least one antibody that binds to *C. difficile* toxin B, wherein the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, and B6 antibodies.

23. The composition of claim 20, comprising at least one antibody that binds to *C. difficile* toxin A, wherein the at least one antibody that binds to *C. difficile* toxin A is one or more of the A1, A2, A3, A4, and A5 antibodies and at least one antibody that binds to *C. difficile* toxin B, wherein the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, and B6 antibodies.

24. The composition of claim 23, wherein the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody or the A1 antibody and the at least one antibody that binds to *C. difficile* toxin B is one or more of the B1, B2, B3, B4, B5, and B6 antibodies.

25. The composition of claim 24, wherein the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody or the A1 antibody and the at least one antibody that binds to *C. difficile* toxin B is the B1, B2, or B4 antibody or a B1+ B2 bispecific antibody, a B1+ B4 bispecific antibody, or a B1+ B6 bispecific antibody.

26. The composition of claim 25, wherein the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody or the A1 antibody and the at least one antibody that binds to *C. difficile* toxin B is the B2 antibody or a B1+ B2 bispecific antibody.

27. The composition of claim 24, wherein the at least one antibody that binds to *C. difficile* toxin A is the A2 antibody or the A1 antibody and wherein the at least one antibody that binds to *C. difficile* toxin B is the B2 antibody and at least one of the B1, B4, or B6 antibodies.

28. The composition of claim 27, wherein the at least one antibody that binds to *C. difficile* toxin B is the B2 antibody and the B1 antibody.

29. The composition of any one of claims 20-28, further comprising a pharmaceutically acceptable excipient.

30. A method of treating a *C. difficile* infection, comprising administering to a subject one or more of the antibodies of claims 1-19 in an amount effective to treat the *C. difficile* infection.

31. A method of treating a *C. difficile* infection, comprising administering to a subject the composition of any one of claims 20-29 in an amount effective to treat the *C. difficile* infection.

32. The method of claim 30 or 31, wherein the subject is a human.

33. An isolated nucleic acid that encodes the amino acid sequence of one or more of the complementarity determining regions (CDRs) of the light chain variable domain and/or heavy chain variable domain of the antibody of claim 4 or 12.

34. An isolated nucleic acid that encodes the amino acid sequence of the light and/or heavy chain variable regions of the antibody of claim 2 or 10.

35. A recombinant expression vector comprising the nucleic acid of claim 33 or 34.

36. An isolated host cell comprising the recombinant expression vector of claim 35.

1/12

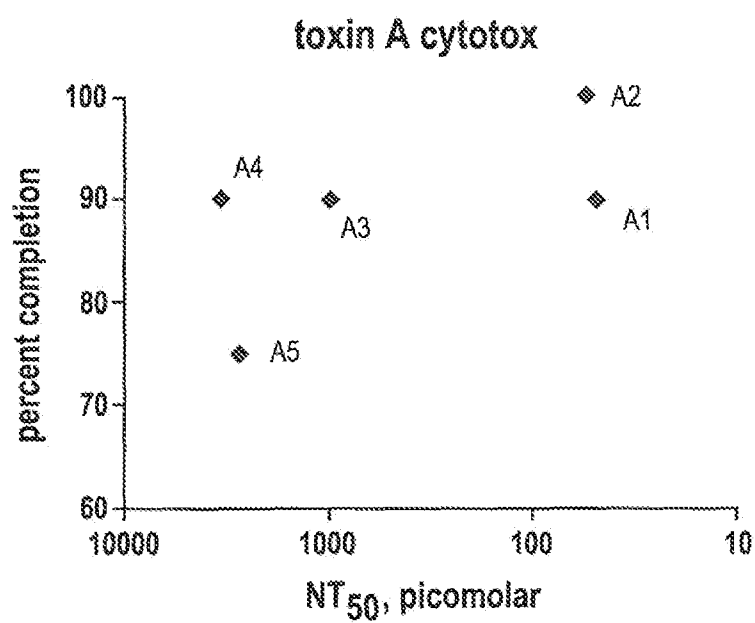


FIG. 1A

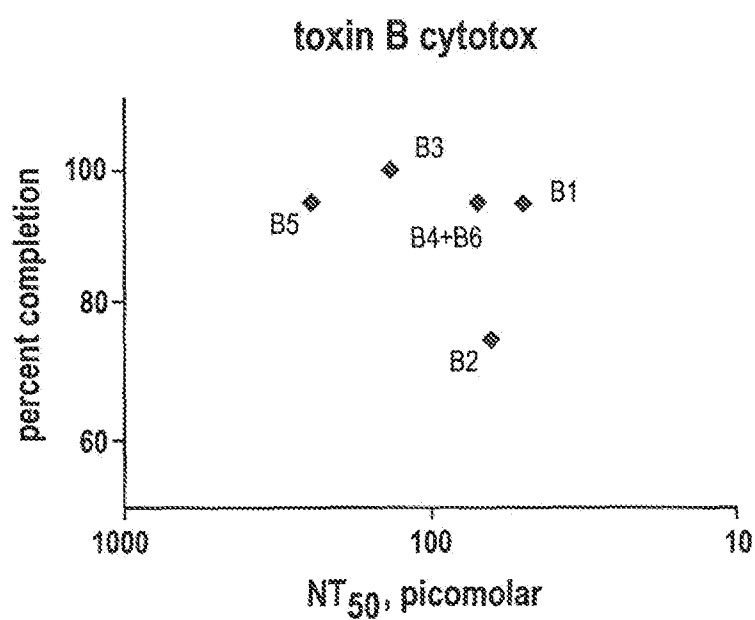


FIG. 1B

2/12

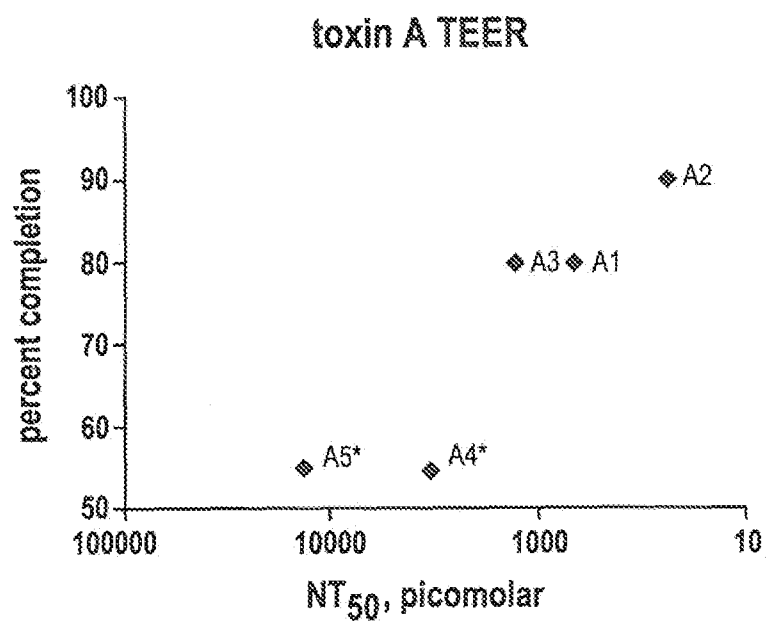


FIG. 2A

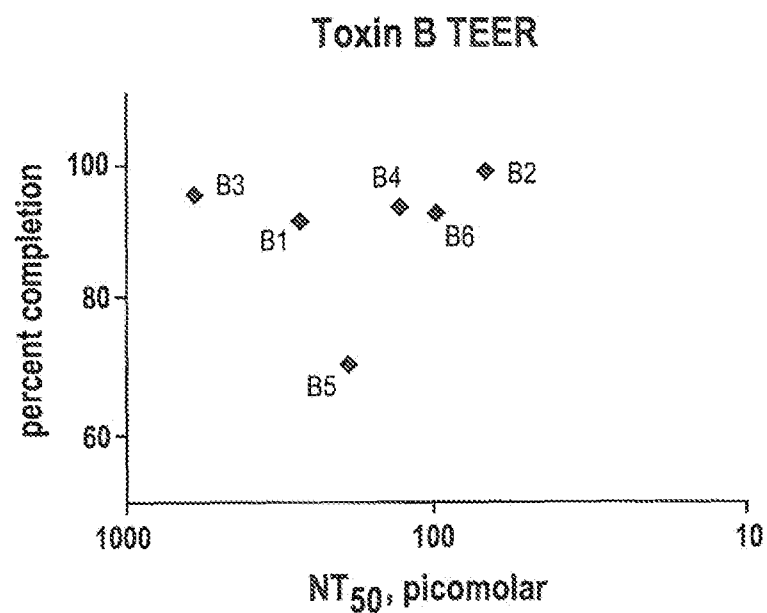
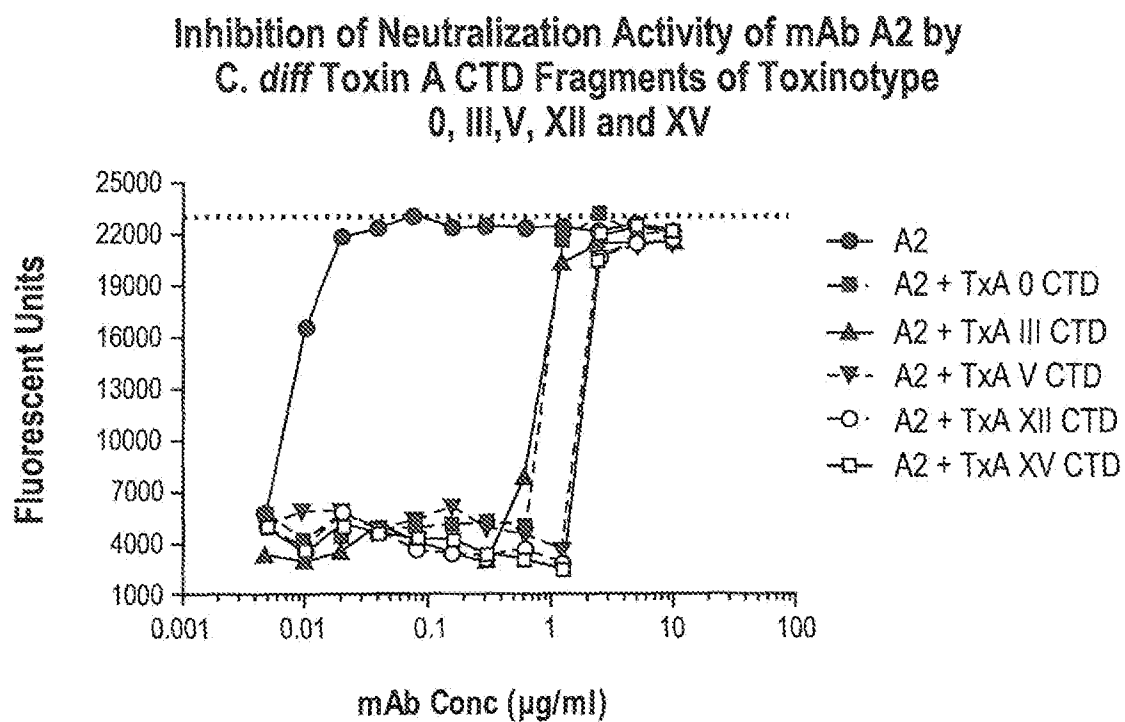
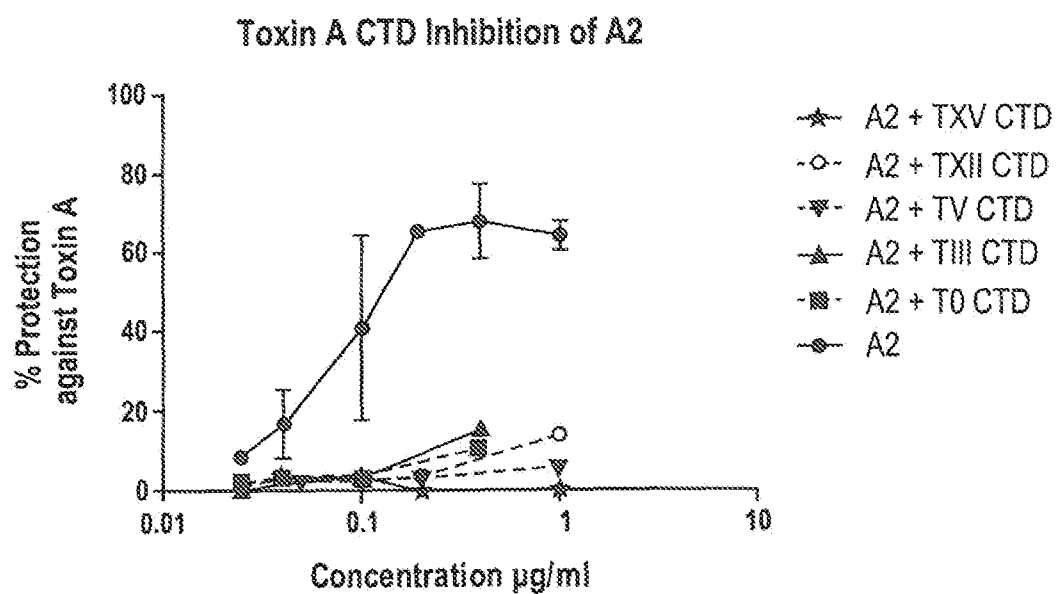
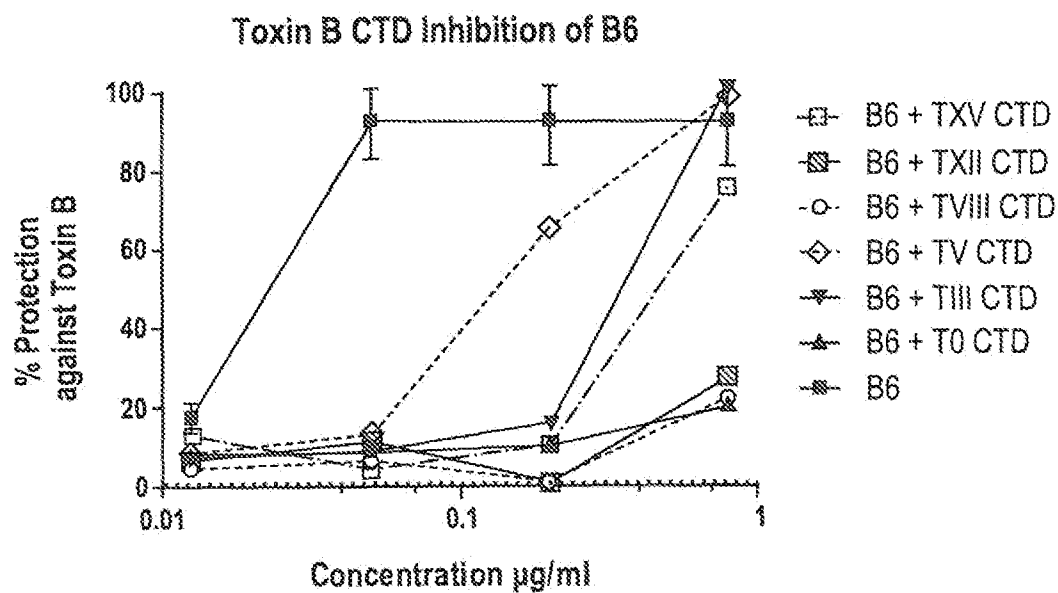


FIG. 2B

3/12

**FIG. 3**

4/12

**FIG. 4A****FIG. 4B**

5/12

SURVIVAL

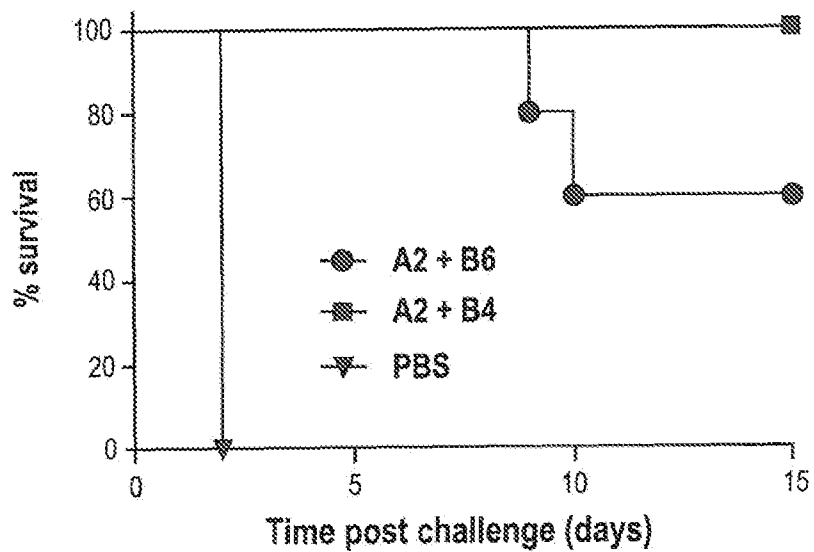


FIG. 5A

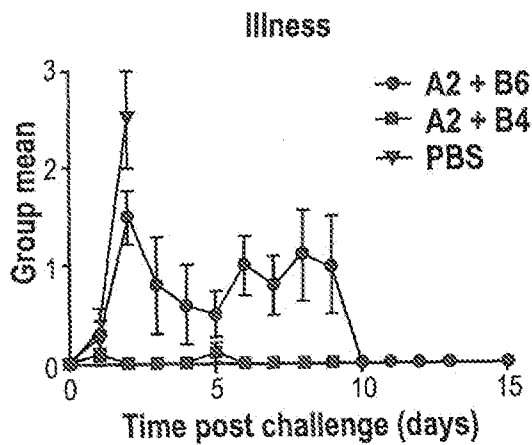


FIG. 5B

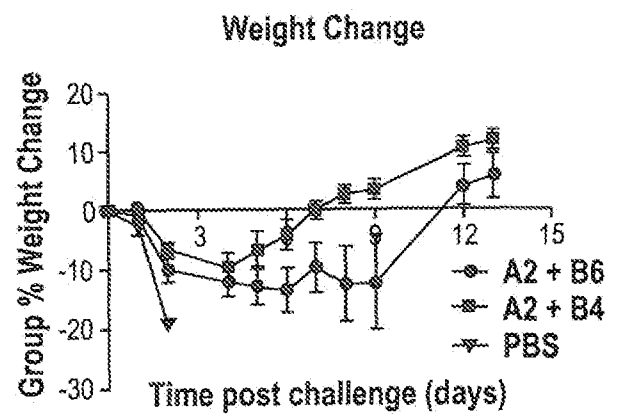


FIG. 5C

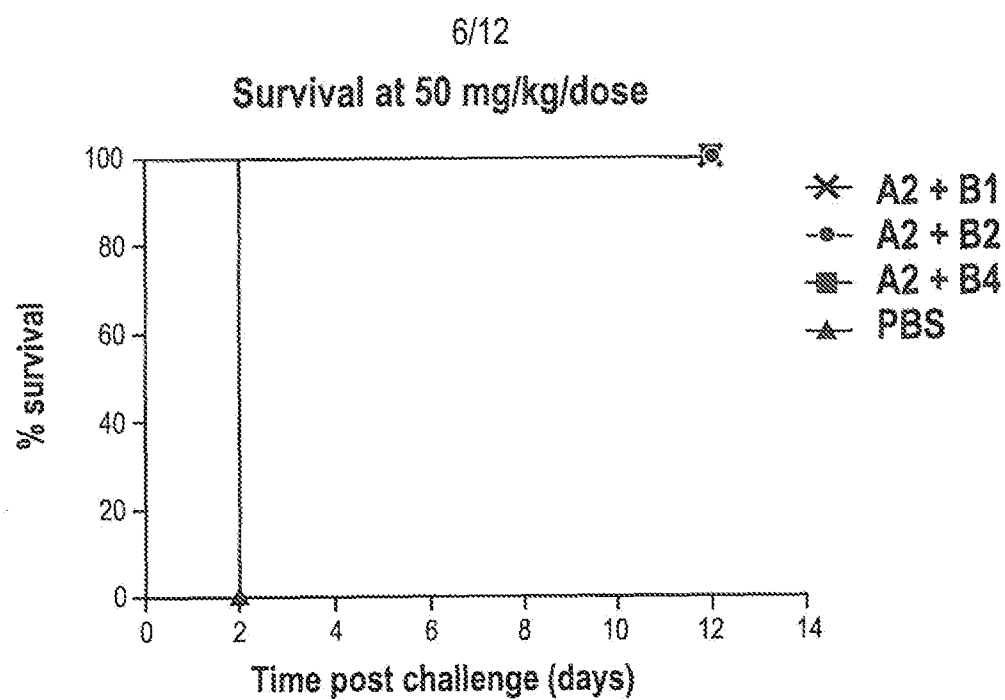


FIG. 6A

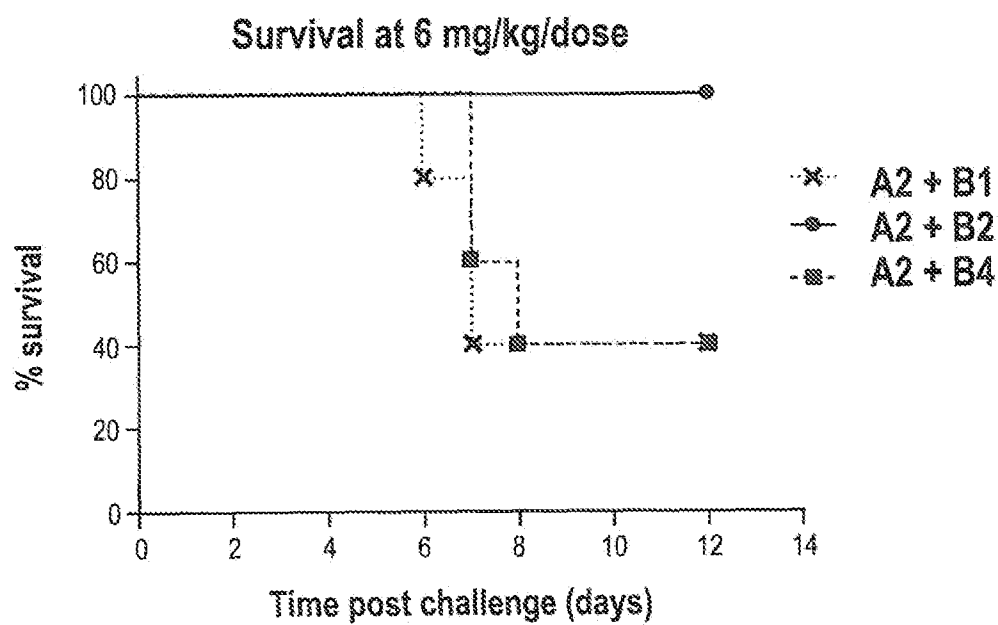


FIG. 6B

7/12

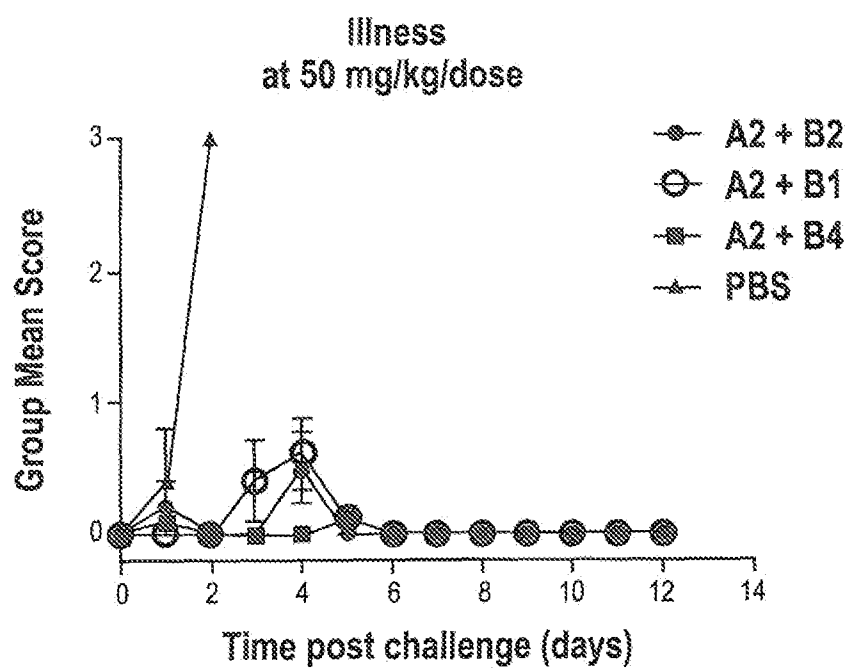


FIG. 7A

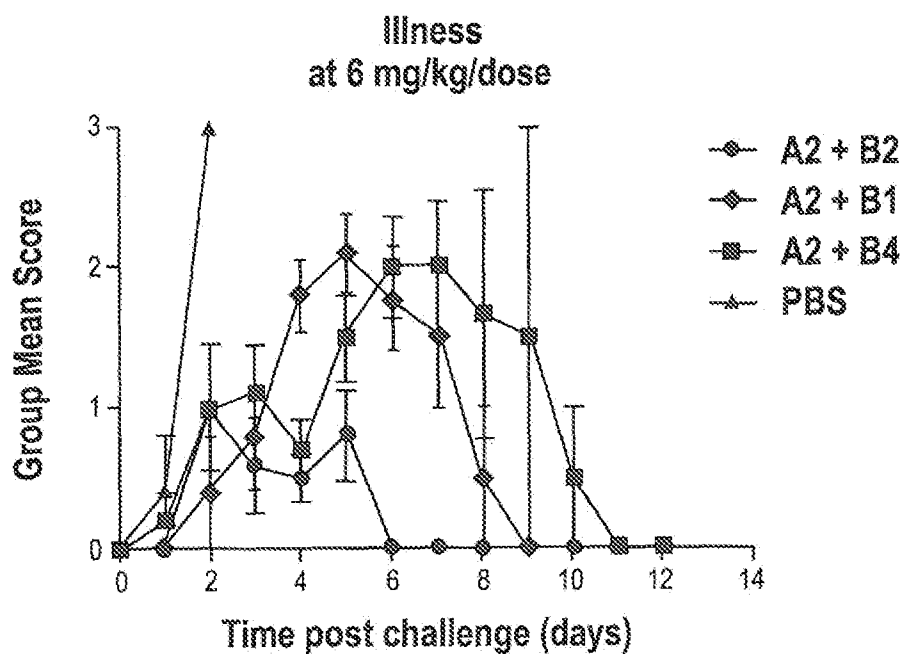


FIG. 7B

8/12

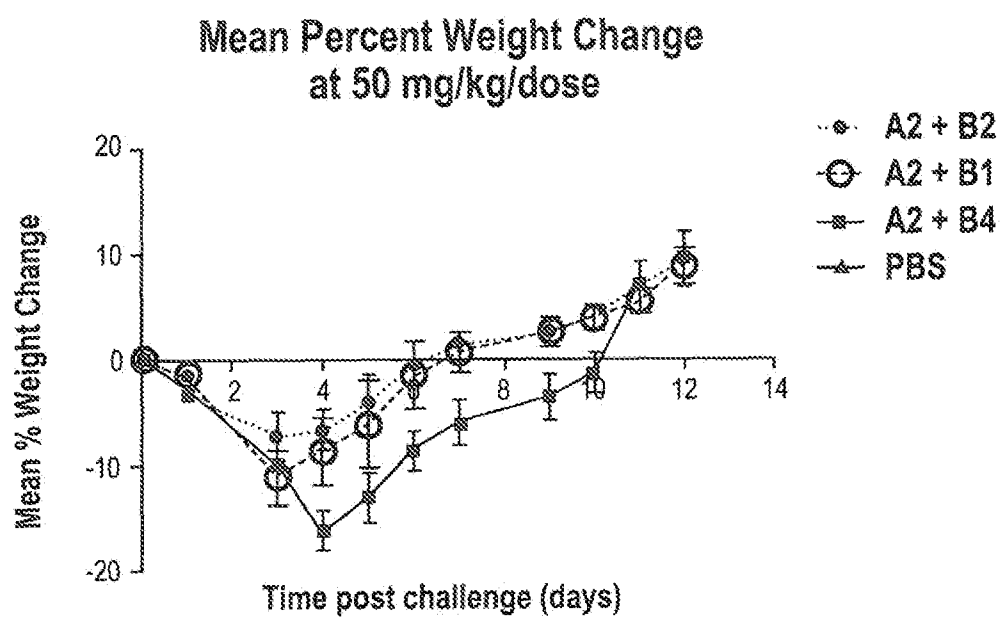


FIG. 8A

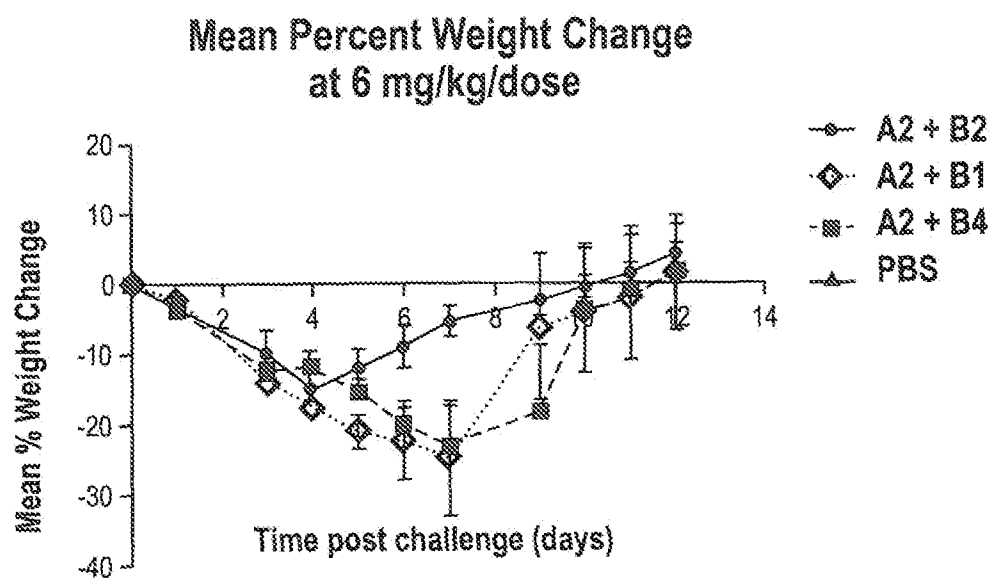


FIG. 8B

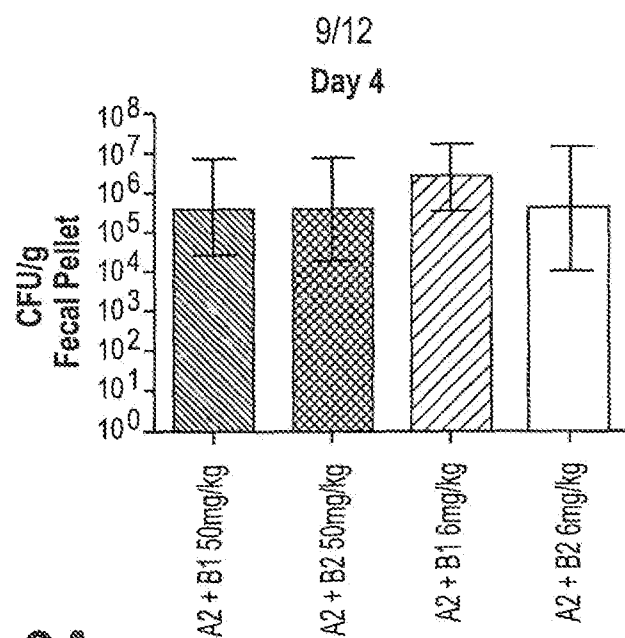


FIG. 9A

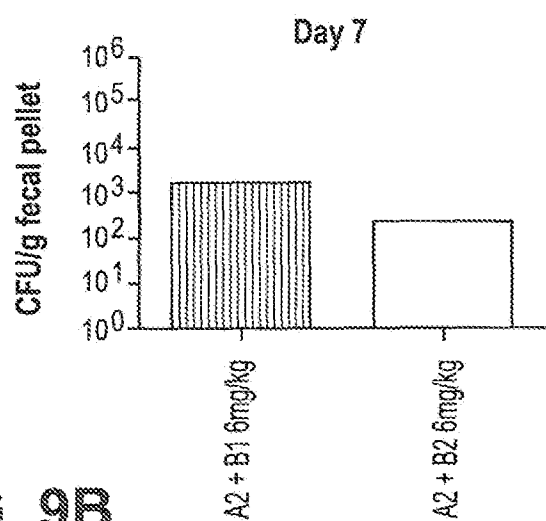


FIG. 9B

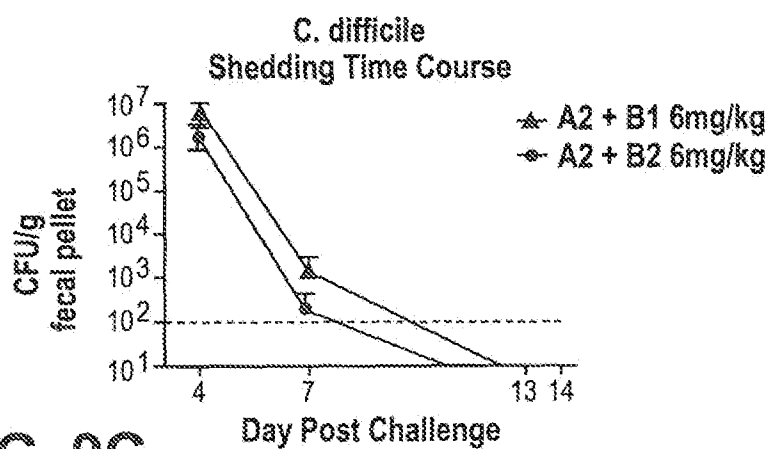
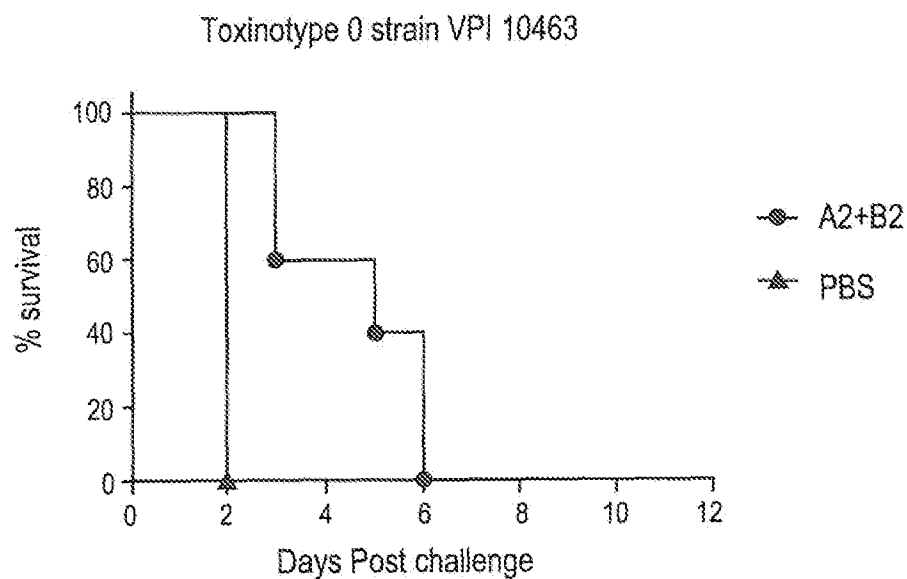
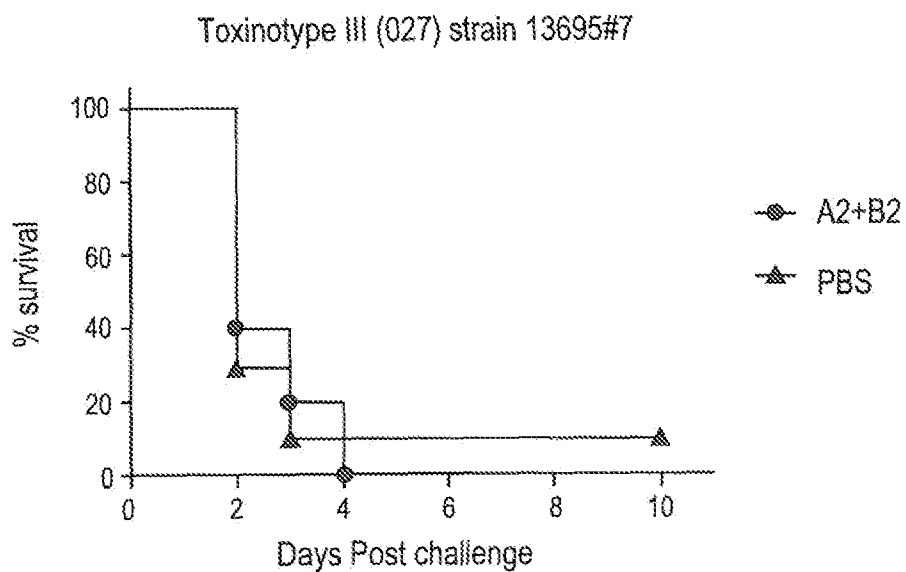


FIG. 9C

10/12

**FIG. 10A****FIG. 10B**

11/12

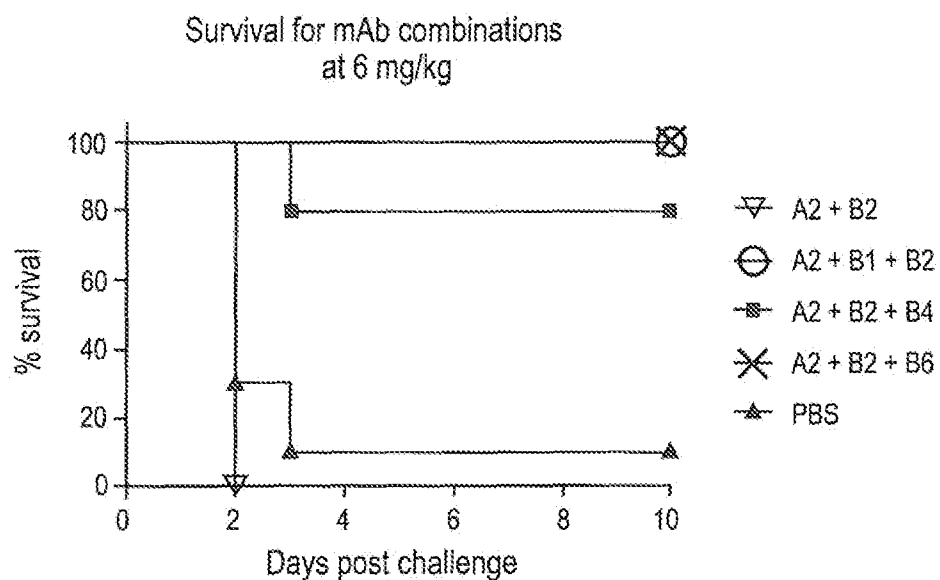


FIG. 11A

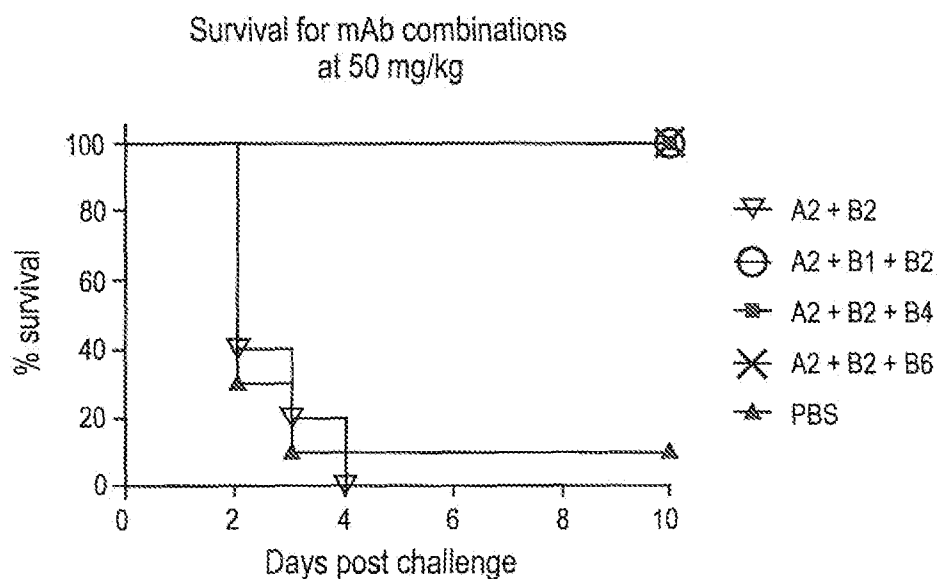
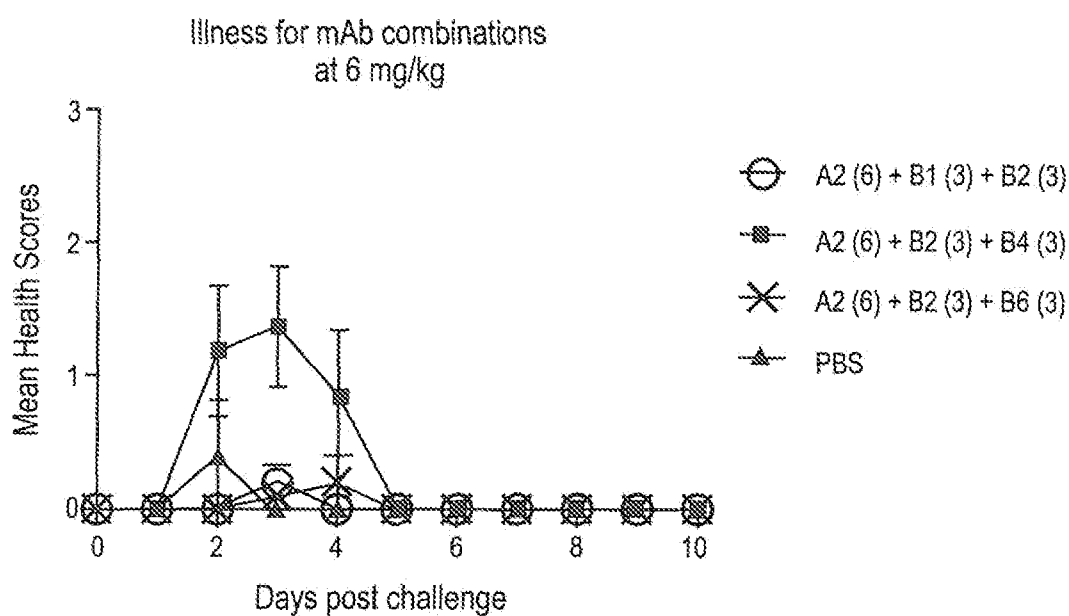
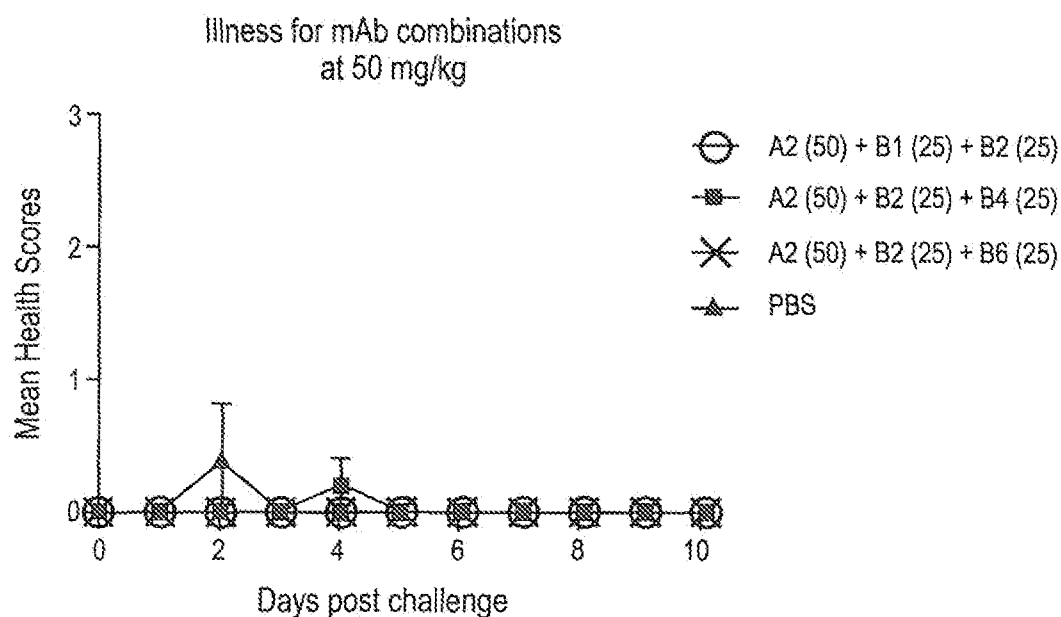


FIG. 11B

12/12

**FIG. 12A****FIG. 12B**