(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2013 (21.03.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/038156 A1

(51) International Patent Classification:
C07K 16/12 (2006.01) *A61P 31/04* (2006.01)
A61K 39/40 (2006.01)

(21) International Application Number:
PCT/GB2012/052222

(22) International Filing Date:
10 September 2012 (10.09.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/535,532 16 September 2011 (16.09.2011) US
61/638,731 26 April 2012 (26.04.2012) US

(71) Applicant *(for all designated States except US)*: **UCB PHARMA S.A.** [BE/BE]; 60 Allée de la Recherche, B-1070 Brussels (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HUMPHREYS, David Paul** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **LIGHTWOOD, Daniel John** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **TYSON, Kerry Louise** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **KNIGHT, David Edward Ormonde** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **HERVÉ, Karine Jeannine Madeleine** [FR/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **COMPSON, Joanne Elizabeth** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **PAGE, Matthew Jon Timothy** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **PAYNE, Andrew Charles** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **FISHER, Nicola Louise** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **MACKENZIE, Brendon** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB). **COX, Matthew** [GB/GB]; c/o IPD, UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

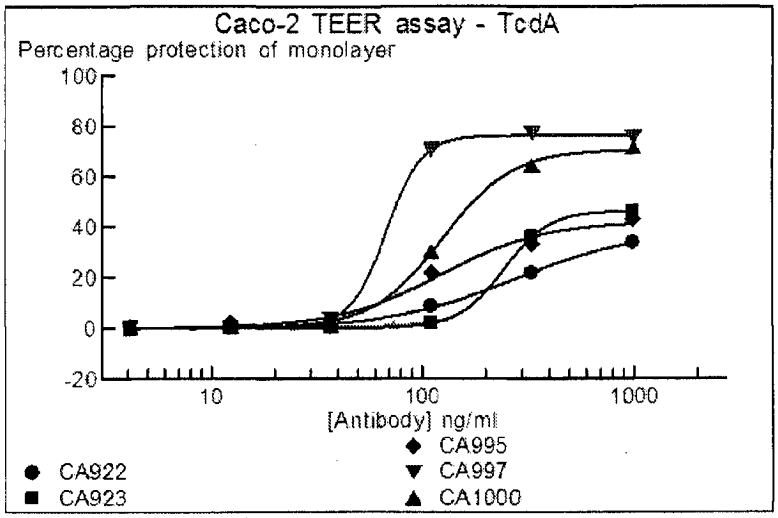
(74) Agent: **THOMPSON, John**; UCB Celltech, 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

(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, IS, JP, KE, KG, KM, 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,

[Continued on next page]

(54) Title: NEUTRALISING ANTIBODIES TO THE MAJOR EXOTOXINS TCDA AND TCDB OF CLOSTRIDIUM DIFFICILE

Figure 62A



(57) Abstract: This present invention describes the derivation and selection of antibodies capable of neutralising the major exotoxins; TcdA and TcdB of *Clostridium difficile*. The invention also describes novel neutralisation and antigen binding properties of individual Mabs and mixtures thereof.

WO 2013/038156 A1

RW, 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.

(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, 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, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

NEUTRALISING ANTIBODIES TO THE MAJOR EXOTOXINS TCDA AND TCDB OF CLOSTRIDIUM DIFFICILE

The present invention relates to antibodies to exotoxins of *Clostridium difficile*, for example TcdA and TcdB, pharmaceutical compositions comprising the same, processes of producing said antibodies and compositions and use of the antibodies and compositions in treatment and/or prophylaxis, in particular treatment or prophylaxis of *Clostridium difficile* infection, pseudomembranous colitis, fulminant colitis and/or toxic mega colon.

The two major exotoxins TcdA and TcdB have been established as the major pathogenicity determinants of *Clostridium difficile* in a large number of *in vitro* and *in vivo* studies. Non-toxigenic strains are not pathogenic to animals and man (1, 2). To date a clear understanding of the role of binary toxin has yet to be established (3).

Both toxins are entero- and cyto-toxic, but the balance of evidence suggests that TcdA is a more powerful enterotoxin than TcdB, whilst TcdB is typically observed to be ~1000x more cytotoxic than TcdA (4). Whilst both toxins are capable of inducing an inflammatory response, TcdA appears to aid the migration of the more inflammatory TcdB deeper into the gut mucosa (5).

In toto, a large collection of data generated for over 30 years support a model where both toxins are likely to be important in the human disease process. It is probable that TcdA initiates early (i.e. before TcdB) and rapid (i.e. 1-3 hours) gut damage through loss of tight junctions and destruction of villi tips and hence diarrhoea, probably through albumin driven fluid loss. This damage to the integrity of the gut lining enables TcdB to exert its superior molar potency (TcdB is typically cited as being 1000x more cytotoxic than TcdA) more rapidly and effectively (i.e. deeper into tissue, alternative cellular targets and damaging systemically accessed organs). Either toxin can be effective alone *in vitro* on human or animals cells and tissues. Either toxin can be effective alone *in vivo* in animals depending upon other eliciting factors such as mechanical damage, barrier overload and host specific sensitivities. It is now clear that in hamsters at least either TcdA or TcdB alone delivered by a *Clostridium difficile* gut infection can cause death (1). It is well established that A-B+ strains are capable of causing symptoms and death in humans (6,7). However, the majority (~95%) of clinical strains are A+B+ hence drugs aimed at treating *Clostridium difficile* infections (CDI) must be capable of neutralising the activities of and clearing both toxins effectively.

CDI is most typically a nosocomial infection of older patients or those with complicating co-morbidities. However, an increase in community acquired infections has been noted. Infection is almost always associated with or induced by use of broad spectrum

antibiotics. Healthcare associated costs are estimated to be in excess of $1bn per annum in the US alone. These costs are primarily due to patients having longer hospitals stays. Current therapies involve the use of antibiotics such as clindamycin, vancomycin or fidaxomicin which kill the *Clostridium difficile* cells within the gut. Current therapies address the bacterial infection but do not deal with or prevent directly the significant pathogenesis caused by TcdA and TcdB which are major contributors to CDI symptoms and mortality.

CDI symptoms in humans include mild to severe diarrhoea, pseudomembranous colitis (PMC) and fulminant colitis or so called toxic mega colon. Death results in 5-15% of patients receiving current best care. Thus at the present time there is no specific therapy available to patients to prevent the damage and injury caused by *C. difficile* toxins after infection.

Raising an antibody response through vaccination and parenteral administration of polyclonal and monoclonal antibodies have all been shown to be capable of protecting animals from symptoms of diarrhoea and death (8-15). Early studies in hamsters suggested that antibodies against TcdA alone were all that was necessary for protection. However, use of strains functionally deleted for TcdA or TcdB demonstrate that either toxin is capable of causing disease in hamsters, but that both toxins together are more effective (1).

For therapeutic applications, monoclonal antibodies (Mabs) can offer efficacy, safety, manufacturing and regulatory advantages over serum derived polyclonal antibodies or serum derived hyper-immune sera. For these reasons Mabs are usually the preferred option for therapeutic products.

There have been a number of attempts to generate protective Mabs against TcdA and TcdB. The most advanced of these in the clinic is a mixture of 2 IgG1 Mabs, one against each TcdA and TcdB originally called CDA1 and MDX1388 developed by MBL and Medarex. They were demonstrated to be unable to fully protect hamsters in models of acute or relapse infections (15). This Mab combination is now being developed as MK3415A by Merck Inc. In a human phase II trial MK3415A resulted in a statistically significant reduction in disease recurrence ($p = 0.006$) (see also Lowy *et al.*, NEJM (2010) 362: 197-205) but did not affect the duration / severity of diarrhoea or death rates (16). This may mean that these antibodies may only be useful for preventing recurrence of infection. Recurrence of infection results in approximately 25% of patients. Thus there likely to be a significant patient population in which these antibodies are not effective.

In order to be able to have a positive influence upon diarrhoea (for example as a result of acute damage to gut tight junctions due to TcdA) and death (for example resulting from prolonged poor nutritional status, dehydration stress and initiation of an inflammatory cascade, widespread anatomical damage to the gut lining and possibly damage to distant organs due to

systemic toxin TcdB more so than TcdA) Mabs are required with superior affinity, toxin neutralisation, superior prevention of loss of TEER (trans-epithelial electrical resistance), antigen decoration and antigen immune clearance.

Summary of the Present Invention

The present invention provide a Mab(s) with a very high level of potency *in vitro* and *in vivo* which have the potential to have an impact upon duration and severity of diarrhoea and death rate in humans suffering from *Clostridium difficile* infection (CDI).

In one embodiment there is provided a monoclonal antibody specific to antigen TcdA or TcdB, wherein the antibody has high affinity for the target antigen and is suitable for reducing the duration and/or severity of diarrhoea and morbidity in a patient with *Clostridium difficile* infection or at risk of said infection.

In one embodiment there is provided a Mab specific to TcdA or TcdB, or a population of at least two Mabs at least one of which is specific to TcdA and at least one of which is specific to TcdB, wherein the EC_{50} of the or each antibody or the combination of antibodies is 200ng/ml or less, for example 150ng/ml or less such as 100ng/ml.

The antibodies of the present disclosure are useful because they are likely to provide a means of treating the severity and duration of symptoms of a primary infection such as diarrhoea in a patient or preventing death and not just prevent the reoccurrence of disease symptoms.

In at least some embodiments the antibodies according to the present disclosure show no reduction in potency in the presence of high concentrations of toxin.

Detailed Description of the Present Invention

Specific as employed herein is intended to refer to an antibody that only recognises the antigen to which it is specific or an antibody that has significantly higher binding affinity to the antigen to which is specific compared to binding to antigens to which it is non-specific, for example 5, 6, 7, 8, 9, 10 times higher binding affinity.

Binding affinity may be measured by standard assays such as surface plasmon resonance, such as BIAcore.

In one embodiment the EC_{50} is less than 75, 70, 60, 65, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.5 ng/ml *Clostridium difficile* infection in cell culture assays and the patient. This is significantly lower (more potent) than known antibodies and is thought to be a major factor as to why the antibodies of the present disclosure have a significant and positive impact on survival of subjects receiving treatment.

As employed herein potency is the ability of the antibody to elicit an appropriate biological response, for example neutralisation of the deleterious toxin effects, at a given dose

or concentration. Examples of potency include the percent maximal neutralisation of toxin activity (extent of protection), the lowest relative concentration of Mab to antigen (e.g. EC_{50}), the speed and durability of neutralisation activity.

In cell culture assays neutralisation might be observed as one or more of the following: prevention of binding of toxin to cells, immunoprecipitation of toxin from solution, prevention of loss of cell form and shape, prevention of loss of cytoskeletal structures, prevention of loss of cell monolayer tight junctions and trans-epithelial electrical resistance, prevention of cell death, apoptosis and production of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6 and MIP1α.

In tissue section and explant assays neutralisation may, for example be observed as prevention of necrosis and/or oedematous fluid accumulation.

In *in vivo* assays neutralisation may be observed as one or more of the following: prevention of fluid accumulation in ligated ileal loops and prevention of gut tissue necrosis, diarrhoea, pseudo-membrane formation of death of animals,

Thus in one embodiment there is provided an antibody (for example an anti-toxin A antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

QASQSISNALA	SEQ ID NO: 1
SASSLAS	SEQ ID NO: 2
QYTHYSHTSKNP	SEQ ID NO: 3
GFTISSYYMS	SEQ ID NO: 4
IISSGGHFTWYANWAKG	SEQ ID NO: 5
AYVSGSSFNGYAL	SEQ ID NO: 6

In one embodiment sequences 1 to 3 are in a light chain of the antibody.

In one embodiment sequences 4 to 6 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 1 is CDR L1, SEQ ID NO: 2 is CDR L2 and SEQ ID NO; 3 is CDR L3.

In one embodiment SEQ ID NO: 4 is CDR H1, SEQ ID NO: 5 is CDR H2 and SEQ ID NO; 6 is CDR H3.

In one embodiment SEQ ID NO: 1 is CDR L1, SEQ ID NO: 2 is CDR L2, SEQ ID NO; 3 is CDR L3, SEQ ID NO: 4 is CDR H1, SEQ ID NO: 5 is CDR H2 and SEQ ID NO; 6 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 922 anti-toxin A antibody;Light chain Variable region sequence) SEQ ID NO: 7:

DPVMTQSPSTLSASVGDRVTITCQASQSISNALAWYQQKPGKAPKLLIYSASSLASGVPSRFK
GSGSGTEFTLTISSLQPDDFATYYCQYTHYSHTSKNPFGGGTKVEIK

wherein the CDRs are underlined and construct is referred to herein as 922.g1 VK (gL1).

The polynucleotide sequence encoding SEQ ID NO: 7 is shown in Figure 1 and SEQ ID NO: 8 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 922 anti-toxin A antibody heavy chain variable region sequence) SEQ ID NO: 9:

EVQLVESGGGLVQPGGSLRLSCAASGFTISSYYMSWVRQAPGKGLEWIGIISSGGHFTWYANW
AKGRFTISSDSTTVYLQMNSLRDEDTATYFCARAYVSGSSFNGYALWGQGTLVTVS

wherein the CDRs are underlined and construct is referred to herein as 922.g1 VH (gH1)

The polynucleotide sequence encoding SEQ ID NO: 9 is shown in Figure 1 and SEQ ID NO: 10 therein.

In one embodiment the antibody comprises the variable regions shown in SEQ ID NO: 7 and 9.

Thus in one embodiment there is provided an antibody (for example an anti-toxin A antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

QASQSISNYLA	SEQ ID NO: 11
SASTLAS	SEQ ID NO: 12
QYSHYGTGVFGA	SEQ ID NO: 13
AFSLSNYYMS	SEQ ID NO: 14
IISSGSNALKWYASWPKG	SEQ ID NO: 15
NYVGSGSYYGMDL	SEQ ID NO: 16

In one embodiment sequences 11 to 13 are in a light chain of the antibody.

In one embodiment sequences 14 to 16 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 11 is CDR L1, SEQ ID NO: 12 is CDR L2 and SEQ ID NO: 13 is CDR L3.

In one embodiment SEQ ID NO: 14 is CDR H1, SEQ ID NO: 15 is CDR H2 and SEQ ID NO; 16 is CDR H3.

In one embodiment SEQ ID NO: 11 is CDR L1, SEQ ID NO: 12 is CDR L2, SEQ ID NO: 13 is CDR L3, SEQ ID NO: 14 is CDR H1, SEQ ID NO: 15 is CDR H2 and SEQ ID NO; 16 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 923 anti-toxin A antibody; Light chain Variable region sequence) SEQ ID NO: 17:

DVVMTQSPSSLSASVGDRVTITCQASQSISNYLAWYQQKPGKVPKLLIYSASTLASGVPSRFK
GSGSGTQFTLTISSLQPEDVATYYCQYSHYGTGVFGAFGGGTKVEIK

wherein the CDRs are underlined and construct is referred to herein as CA923.g1 gL1

The polynucleotide sequence encoding SEQ ID NO: 17 is shown in Figure 1 and SEQ ID NO: 18 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 923 anti-toxin A antibody heavy chain variable region sequence) SEQ ID NO: 19:

EVQLVESGGGLVQPGGSLRLSCAASAFSLSNYYMSWVRQAPGKGLEWIGIISSGSNALKWYAS
WPKGRFTISKDSTTVYLQMNSLRAEDTATYFCARNYVGSGSYYGMDLWGQGTLVTVS

wherein the CDRs are underlined and construct is referred to herein as CA923.g1 gH1

The polynucleotide sequence encoding SEQ ID NO: 19 is shown in Figure 2 and SEQ ID NO: 20 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO 17: and SEQ ID NO: 19.

In one embodiment there is provided an antibody (for example an anti-toxin A antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

QASQSISSYFS	SEQ ID NO: 21
GASTLAS	SEQ ID NO: 22
QCTDYSGIYFGG	SEQ ID NO: 23
GFSLSSYYMS	SEQ ID NO: 24
IISSGSSTTFTWYASWAKG	SEQ ID NO: 25
AYVGSSSYYGFDP	SEQ ID NO: 26

In one embodiment sequences 21 to 23 are in a light chain of the antibody.

In one embodiment sequences 24 to 26 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 21 is CDR L1, SEQ ID NO: 22 is CDR L2 and SEQ ID NO; 23 is CDR L3.

In one embodiment SEQ ID NO: 24 is CDR H1, SEQ ID NO: 25 is CDR H2 and SEQ ID NO; 26 is CDR H3.

In one embodiment SEQ ID NO: 21 is CDR L1, SEQ ID NO: 22 is CDR L2, SEQ ID NO; 23 is CDR L3, SEQ ID NO: 24 is CDR H1, SEQ ID NO: 25 is CDR H2 and SEQ ID NO; 26 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 993 anti-toxin A antibody; Light chain Variable region sequence) SEQ ID NO: 27:

DVVMTQSPSTLSASVGDRVTITCQASQSISSYFSWYQQKPGKAPQLLIYGASTLASGVPSRFK
GSGSGTELTLTISSLQPDDFATYYCQCTDYSGIYFGGFGGGTKVEIK

wherein the CDRs are underlined and construct is referred to herein as CA993.g1 gL1

The polynucleotide sequence encoding SEQ ID NO: 27 is shown in Figure 2 and SEQ ID NO: 28 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 993 anti-toxin A antibody heavy chain variable region sequence) SEQ ID NO: 29:

EVQLVESGGGLVQPGGSLKLSCTASGFSLSSYYMSWVRQAPGKGLEWIGIISSGSSTTFTWYA
SWAKGRFTISKTSTTVYLQMNSLKTEDTATYFCARAYVGSSSYYGFDPWGQGTLVTVS

wherein the CDRs are underlined and construct is referred to herein as CA993.g1 gH1

The polynucleotide sequence encoding SEQ ID NO: 29 is shown in Figure 2 and SEQ ID NO: 30 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 27 and SEQ ID NO: 29.

In one embodiment there is provided an antibody (for example an anti-toxin A antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

QASQSINNYFS	SEQ ID NO: 31
GAANLAS	SEQ ID NO: 32
QNNYGVHIYGAA	SEQ ID NO: 33
GFSLSNYDMI	SEQ ID NO: 34
FINTGGITYYASWAKG	SEQ ID NO: 35
VDDYIGAWGAGL	SEQ ID NO: 36

In one embodiment sequences 31 to 33 are in a light chain of the antibody.

In one embodiment sequences 34 to 36 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 31 is CDR L1, SEQ ID NO: 32 is CDR L2 and SEQ ID NO; 33 is CDR L3.

In one embodiment SEQ ID NO: 34 is CDR H1, SEQ ID NO: 35 is CDR H2 and SEQ ID NO: 36 is CDR H3.

In one embodiment SEQ ID NO: 31 is CDR L1, SEQ ID NO: 32 is CDR L2, SEQ ID NO; 33 is CDR L3, SEQ ID NO: 34 is CDR H1, SEQ ID NO: 35 is CDR H2 and SEQ ID NO; 36 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 995 anti-toxin A antibody; Light chain Variable region sequence) SEQ ID NO: 37:

DVVMTQSPSTLSASVGDRVTITCQASQSINNYFSWYQQKPGKAPKLLIYGAANLASGVPSRFK
GSGSGTEYTLTISSLQPDDFATYSCQNNYGVHIYGAAFGGGTKVEIK

wherein the CDRs are underlined

The polynucleotide sequence encoding SEQ ID NO: 37 is shown in Figure 3 and SEQ ID NO: 38 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 995 anti-toxin A antibody heavy chain variable region sequence) SEQ ID NO: 39

EVQLVESGGGLVQPGGSLRLSCTASGFSLSNYDMIWVRQAPGKGLEYIGFINTGGITYYASWA
KGRFTISRDSSTVYLQMNSLRAEDTATYFCARVDDYIGAWGAGLWGQGTLVTVS

wherein the CDRs are underlined

The polynucleotide sequence encoding SEQ ID NO: 39 is shown in Figure 3 and SEQ ID NO: 40 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 37 and SEQ ID NO: 39.

In one embodiment there is provided an antibody (for example an anti-toxin A antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

QASQSISSYLS	SEQ ID NO: 41
RASTLAS	SEQ ID NO: 42
LGVYGYSNDDGIA	SEQ ID NO: 43
GIDLSSHHMC	SEQ ID NO: 44
VIYHFGSTYYANWATG	SEQ ID NO: 45
ASIAGYSAFDP	SEQ ID NO: 46

In one embodiment sequences 41 to 43 are in a light chain of the antibody. In one embodiment sequences 44 to 46 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 41 is CDR L1, SEQ ID NO: 42 is CDR L2 and SEQ ID NO; 43 is CDR L3.

In one embodiment SEQ ID NO: 44 is CDR H1, SEQ ID NO: 45 is CDR H2 and SEQ ID NO: 46 is CDR H3.

In one embodiment SEQ ID NO: 41 is CDR L1, SEQ ID NO: 42 is CDR L2, SEQ ID NO; 43 is CDR L3, SEQ ID NO: 44 is CDR H1, SEQ ID NO: 45 is CDR H2 and SEQ ID NO; 46 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 997 anti-toxin A antibody; Light chain Variable region sequence) SEQ ID NO: 47:

ALVMTQSPSSFSASTGDRVTITCQASQSISSYLSWYQQKPGKAPKLLIYRASTLASGVPSRFS
GSGSGTEYTLTISCLQSEDFATYYCLGVYGYSNDDGIAFGGGTKVEIK

wherein the CDRs are underlined

The polynucleotide sequence encoding SEQ ID NO: 47 is shown in Figure 3 and SEQ ID NO: 48 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 997 anti-toxin A antibody heavy chain variable region sequence) SEQ ID NO: 49:

EVQLVESGGGLVQPGGSLRLSCTVSGIDLSSHHMCWVRQAPGKGLEYIGVIYHFGSTYYANWA
TGRFTISKDSTTVYLQMNSLRAEDTATYFCARASIAGYSAFDPWGQGTLVTVS

wherein the CDRs are underlined

The polynucleotide sequence encoding SEQ ID NO: 49 is shown in Figure 4 and SEQ ID NO: 50 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 47 and SEQ ID NO: 49.

In one embodiment there is provided an antibody (for example an anti-toxin A antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

QASQSIYSYLA	SEQ ID NO: 51
DASTLAS	SEQ ID NO: 52
QGNAYTSNSHDNA	SEQ ID NO: 53
GIDLSSDAVG	SEQ ID NO: 54
IIATFDSTYYASWAKG	SEQ ID NO: 55
TGSWYYISGWGSYYYGMDL	SEQ ID NO: 56

In one embodiment sequences 51 to 53 are in a light chain of the antibody.

In one embodiment sequences 54 to 56 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 51 is CDR L1, SEQ ID NO: 52 is CDR L2 and SEQ ID NO: 53 is CDR L3.

In one embodiment SEQ ID NO: 54 is CDR H1, SEQ ID NO: 55 is CDR H2 and SEQ ID NO; 56 is CDR H3.

In one embodiment SEQ ID NO: 51 is CDR L1, SEQ ID NO: 52 is CDR L2, SEQ ID NO; 53 is CDR L3, SEQ ID NO: 54 is CDR H1, SEQ ID NO: 55 is CDR H2 and SEQ ID NO: 56 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1000 anti-toxin A antibody; Light chain Variable region sequence) SEQ ID NO: 57:

EIVMTQSPSTLSASVGDRVTITCQASQSIYSYLAWYQQKPGKAPKLLIYDASTLASGVPSRFK
GSGSGTEFTLTISSLQPDDFATYYCQGNAYTSNSHDNAFGGGTKVEIK

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 57 is shown in Figure 4 and SEQ ID NO: 58 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1000 anti-toxin A antibody heavy chain variable region sequence) SEQ ID NO: 59:

EVQLVESGGGLIQPGGSLRLSCTVSGIDLSSDAVGWVRQAPGKGLEYIGIIATFDSTYYASWA
KGRFTISKASSTTVYLQMNSLRAEDTATYFCARTGSWYYISGWGSYYYGMDLWGQGTLVTVS

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 59 is shown in Figure 4 and SEQ ID NO: 60 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 57 and SEQ ID NO: 59.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

RASKSVSTLMH	SEQ ID NO: 61
LASNLES	SEQ ID NO: 62
QQTWNDPWT	SEQ ID NO: 63
GFTFSNYGMA	SEQ ID NO: 64
SISSSGGSTYYRDSVKG	SEQ ID NO: 65
VIRGYVMDA	SEQ ID NO: 66

In one embodiment sequences 61 to 63 are in a light chain of the antibody.

In one embodiment sequences 64 to 66 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 61 is CDR L1, SEQ ID NO: 62 is CDR L2 and SEQ ID NO: 63 is CDR L3.

In one embodiment SEQ ID NO: 64 is CDR H1, SEQ ID NO: 65 is CDR H2 and SEQ ID NO: 66 is CDR H3.

In one embodiment SEQ ID NO: 61 is CDR L1, SEQ ID NO: 62 is CDR L2, SEQ ID NO; 63 is CDR L3, SEQ ID NO: 64 is CDR H1, SEQ ID NO: 65 is CDR H2 and SEQ ID NO: 66 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 926 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 67:

DTVLTQSPATLSLSPGERATLSCRASKSVSTLMHWFQQKPGQAPKLLIYLASNLESGVPARFS
GSGSGTDFTLTISSLEPEDFAVYYCQQTWNDPWTFGGGTKVEIK

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 67 is shown in Figure 5 and SEQ ID NO: 68 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 926 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 69:

EVELLESGGGLVQPGGSLRLSCEASGFTFSNYGMAWVRQAPTKGLEWVTSISSSGGSTYYRDS
VKGRFTISRDNAKSSLYLQMNSLRAEDTATYYCTTVIRGYVMDAWGQGTLVTVS

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 69 is shown in Figure 5 and SEQ ID NO: 70 therein.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

RASGSVSTLMH	SEQ ID NO: 71
KASNLAS	SEQ ID NO: 72
HQSWNSDT	SEQ ID NO: 73
GFTFSNYGMA	SEQ ID NO: 74
TINYDGRTTHYRDSVKG	SEQ ID NO: 75
ISRSHYFDC	SEQ ID NO: 76

In one embodiment sequences 71 to 73 are in a light chain of the antibody.

In one embodiment sequences 74 to 76 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 71 is CDR L1, SEQ ID NO: 72 is CDR L2 and SEQ ID NO: 73 is CDR L3.

In one embodiment SEQ ID NO: 74 is CDR H1, SEQ ID NO: 75 is CDR H2 and SEQ ID NO: 76 is CDR H3.

In one embodiment SEQ ID NO: 71 is CDR L1, SEQ ID NO: 72 is CDR L2, SEQ ID NO; 73 is CDR L3, SEQ ID NO: 74 is CDR H1, SEQ ID NO: 75 is CDR H2 and SEQ ID NO: 76 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 927 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 77:

DTQMTQSPSTLSASVGDRVTITCRASGSVSTLMHWYQQKPGKAPKLLIYKASNLASGVPSRFS
GSGSGTEFTLTISSLQPDDFATYYCHQSWNSDTFGQGTRLEIK

wherein the CDRs are underlined

The polynucleotide sequence encoding SEQ ID NO: 77 is shown in Figure 5 and SEQ ID NO: 78 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 927 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 79:

EVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMAWVRQAPGKGLEWVATINYDGRTTHYRDS
VKGRFTISRDNSKSTLYLQMNSLRAEDTAVYYCTSISRSHYFDCWGQGTLVTVS

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 79 is shown in Figure 5 and SEQ ID NO: 80 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 77 and SEQ ID NO: 79.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

KASKSISNHLA	SEQ ID NO: 81
SGSTLQS	SEQ ID NO: 82
QQYDEYPYT	SEQ ID NO: 83
GFSLQSYTIS	SEQ ID NO: 84
AISGGGSTYYNLPLKS	SEQ ID NO: 85
PRWYPRSYFDY	SEQ ID NO: 86

In one embodiment sequences 81 to 83 are in a light chain of the antibody.

In one embodiment sequences 84 to 86 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 81 is CDR L1, SEQ ID NO: 82 is CDR L2 and SEQ ID NO: 83 is CDR L3.

In one embodiment SEQ ID NO: 84 is CDR H1, SEQ ID NO: 85 is CDR H2 and SEQ ID NO: 86 is CDR H3.

In one embodiment SEQ ID NO: 81 is CDR L1, SEQ ID NO: 82 is CDR L2, SEQ ID NO; 83 is CDR L3, SEQ ID NO: 84 is CDR H1, SEQ ID NO: 85 is CDR H2 and SEQ ID NO: 86 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1099 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 87:

DVQLTQSPSFLSASVGDRVTITCKASKSISNHLAWYQEKPGKANKLLIHSGSTLQSGTPSRFS
GSGSGTEFTLTISSLQPEDFATYYCQQYDEYPYTFGQGTRLEIKRT

wherein the CDRs are underlined.

In one embodiment the last two amino acids (RT) of SEQ ID NO: 87 are omitted.

The polynucleotide sequence encoding SEQ ID NO: 87 is shown in Figure 6 and SEQ ID NO: 88 therein. In one embodiment the codons encoding the last two amino acids (RT) are omitted.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1099 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 89:

EVQLQESGPGLVKPSETLSLTCTVSGFSLQSYTISWVRQPPGKGLEWIAAISGGGSTYYNLPL
KSRVTISRDTSKSQVSLKLSSVTAADTAVYYCTRPRWYPRSYFDYWGRGTLVTVS

wherein the CDRs are underlined

The polynucleotide sequence encoding SEQ ID NO: 89 is shown in Figure 6 and SEQ ID NO: 90 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO 87: and SEQ ID NO: 89.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

RASQRISTSIH	SEQ ID NO: 91
YASQSIS	SEQ ID NO: 92
QQSYSSLYT	SEQ ID NO: 93
GFTFSDSYMA	SEQ ID NO: 94
SISYGGTIIQYGDSVKG	SEQ ID NO: 95
RQGTYARYLDF	SEQ ID NO: 96

In one embodiment sequences 91 to93 are in a light chain of the antibody.

In one embodiment sequences 94 to 96 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 91 is CDR L1, SEQ ID NO: 92 is CDR L2 and SEQ ID NO; 93 is CDR L3.

In one embodiment SEQ ID NO: 94 is CDR H1, SEQ ID NO: 95 is CDR H2 and SEQ ID NO: 96 is CDR H3.

In one embodiment SEQ ID NO: 91 is CDR L1, SEQ ID NO: 92 is CDR L2, SEQ ID NO; 93 is CDR L3, SEQ ID NO: 94 is CDR H1, SEQ ID NO: 95 is CDR H2 and SEQ ID NO: 96 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1102 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 97:

NIVLTQSPATLSLSPGERATLSCRASQRISTSIHWYQQKPGQAPRLLIKYASQSISGIPARFS
GSGSGTDFTLTISSLEPEDFAVYYCQQSYSSLYTFGQGTKLEIK

wherein the CDRs are underlined

The polynucleotide sequence encoding SEQ ID NO: 97 is shown in Figure 6 and SEQ ID NO: 98 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1102 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 99:

EVQLVESGGGLVQPGGSLRLSCAVSGFTFSDSYMAWVRQAPGKGLEWIASISYGGTIIQYGDS
VKGRFTISRDNAKSSLYLQMNSLRAEDTAVYYCARRQGTYARYLDFWGQGTLVTVS

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 99 is shown in Figure 7 and SEQ ID NO: 100 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO 97: and SEQ ID NO: 99.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

RASESVSTLLH	SEQ ID NO: 101
KASNLAS	SEQ ID NO: 102
HQSWNSPPT	SEQ ID NO: 103
GFTFSNYGMA	SEQ ID NO: 104
IINYDASTTHYRDSVKG	SEQ ID NO: 105
YGRSHYFDY	SEQ ID NO: 106

In one embodiment sequences 101 to 103 are in a light chain of the antibody.

In one embodiment sequences 104 to 106 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 101 is CDR L1, SEQ ID NO: 102 is CDR L2 and SEQ ID NO: 103 is CDR L3.

In one embodiment SEQ ID NO: 104 is CDR H1, SEQ ID NO: 105 is CDR H2 and SEQ ID NO: 106 is CDR H3.

In one embodiment SEQ ID NO: 101 is CDR L1, SEQ ID NO: 102 is CDR L2, SEQ ID NO; 103 is CDR L3, SEQ ID NO: 104 is CDR H1, SEQ ID NO: 105 is CDR H2 and SEQ ID NO; 106 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1114 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 107:

ATQMTQSPSSLSASVGDRVTITCRASESVSTLLHWYQQKPGKAPKLLIYKASNLASGVPSRFS
GSGSGTDFTLTISSLQPEDFATYYCHQSWNSPPTFGQGTKLEIK

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 107 is shown in Figure 7 and SEQ ID NO: 108 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1114 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 109:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYGMAWVRQAPGKGLEWVAIINYDASTTHYRDS
VKGRFTISRDNAKSSLYLQMNSLRAEDTAVYYCTRYGRSHYFDYWGQGTLVTVS

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 109 is shown in Figure 7 and SEQ ID NO: 110 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 107 and SEQ ID NO: 109.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

RASESVSTLLH	SEQ ID NO: 111
KASNLAS	SEQ ID NO: 112
HQSWNSPPT	SEQ ID NO: 113
GFTFSNYGMA	SEQ ID NO: 114
IINYDASTTHYRDSVK	SEQ ID NO: 115
YGRSHYFDY	SEQ ID NO: 116

In one embodiment sequences 111 to 113 are in a light chain of the antibody.

In one embodiment sequences 114 to 116 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 111 is CDR L1, SEQ ID NO: 112 is CDR L2 and SEQ ID NO: 113 is CDR L3.

In one embodiment SEQ ID NO: 114 is CDR H1, SEQ ID NO: 115 is CDR H2 and SEQ ID NO: 116 is CDR H3.

In one embodiment SEQ ID NO: 111 is CDR L1, SEQ ID NO: 112 is CDR L2, SEQ ID NO; 113 is CDR L3, SEQ ID NO: 114 is CDR H1, SEQ ID NO: 115 is CDR H2 and SEQ ID NO: 116 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1114 graft 8 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 117:

DTVLTQSPSSLSASVGDRVTITCRASESVSTLLHWYQQKPGKAPKLLIYKASNLASGVPSRFS
GSGSGTDFTLTISSLQPEDFATYYCHQSWNSPPTFGQGTKLEIK

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 117 is shown in Figure 8 and SEQ ID NO: 118 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1114 graft 8 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 119:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYGMAWVRQAPGKGLEWVAIINYDASTTHYRDS
VKGRFTISRDNAKSSLYLQMNSLRAEDTAVYYCTRYGRSHYFDYWGQGTLVTVS

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 119 is shown in Figure 8 and SEQ ID NO: 120 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 117 and SEQ ID NO: 119.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

KASQNIYMYLN	SEQ ID NO: 121
NTNKLHT	SEQ ID NO: 122
LQHKSFPYT	SEQ ID NO: 123
GFTFRDSFMA	SEQ ID NO: 124
SISYEGDKTYYGDSVKG	SEQ ID NO: 125
LTITTSGDS	SEQ ID NO: 126

In one embodiment sequences 121 to 123 are in a light chain of the antibody.

In one embodiment sequences 124 to 126 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 121 is CDR L1, SEQ ID NO: 122 is CDR L2 and SEQ ID NO: 123 is CDR L3.

In one embodiment SEQ ID NO: 124 is CDR H1, SEQ ID NO: 125 is CDR H2 and SEQ ID NO: 126 is CDR H3.

In one embodiment SEQ ID NO: 121 is CDR L1, SEQ ID NO: 122 is CDR L2, SEQ ID NO: 123 is CDR L3, SEQ ID NO: 124 is CDR H1, SEQ ID NO: 125 is CDR H2 and SEQ ID NO: 126 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1125 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 127:

DIQMTQSPSSLSASVGDRVTITCKASQNIYMYLNWYQQKPGKAPKRLIYNTNKLHTGVPSRFS
GSGSGTEYTLTISSLQPEDFATYYCLQHKSFPYTFGQGTKLEIK

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 127 is shown in Figure 8 and SEQ ID NO: 128 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1125 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 129:

EVQLVESGGGLVQPGGSLRLSCAASGFTFRDSFMAWVRQAPGKGLEWVASISYEGDKTYYGDS
VKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARLTITTSGDSWGQGTMVTVSS

wherein the CDRs are underlined.

In one embodiment the last amino acid (S) of SEQ ID NO: 129 is omitted.

The polynucleotide sequence encoding SEQ ID NO: 129 is shown in Figure 9 and SEQ ID NO: 130 therein. In one embodiment the codon AGC encoding the last amino acid S is omitted.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 127 and SEQ ID NO: 129.

In one embodiment there is provided antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

KASQHVGTNVD	SEQ ID NO: 131
GASIRYT	SEQ ID NO: 132
LQYNYNPYT	SEQ ID NO: 133
GFIFSNFGMS	SEQ ID NO: 134
SISPSGGNAYYRDSVKG	SEQ ID NO: 135
RAYSSPFAF	SEQ ID NO: 136

In one embodiment sequences 131 to 133 are in a light chain of the antibody.

In one embodiment sequences 134 to 136 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 131 is CDR L1, SEQ ID NO: 132 is CDR L2 and SEQ ID NO: 133 is CDR L3.

In one embodiment SEQ ID NO: 134 is CDR H1, SEQ ID NO: 135 is CDR H2 and SEQ ID NO: 136 is CDR H3.

In one embodiment SEQ ID NO: 131 is CDR L1, SEQ ID NO: 132 is CDR L2, SEQ ID NO: 133 is CDR L3, SEQ ID NO: 134 is CDR H1, SEQ ID NO: 135 is CDR H2 and SEQ ID NO: 136 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1129 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 137:

DTQMTQSPSSLSASVGDRVTITCKASQHVGTNVDWYQQKPGKVPKLLIYGASIRYTGVPDRFT
GSGSGTDFTLTISSLQPEDVATYYCLQYNYNPYTFGQGTKLEIK

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 137 is shown in Figure 8 and SEQ ID NO: 138 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1129 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 139:

EVQLVESGGGVVQPGRSLRLSCATSGFIFSNFGMSWVRQAPGKGLEWVASISPSGGNAYYRDS
VKGRFTISRDNSKTTLYLQMNSLRAEDTAVYYCTRRAYSSPFAFWGQGTLVTVSS

wherein the CDRs are underlined.

In one embodiment the last amino acid (S) of SEQ ID NO: 139 is omitted.

The polynucleotide sequence encoding SEQ ID NO: 139 is shown in Figure 8 and SEQ ID NO: 140 therein. In one embodiment the codon AGC encoding the last amino acid S is omitted.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 137 and SEQ ID NO: 139.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

KASKSISNHLA	SEQ ID NO: 141
SGSTLQP	SEQ ID NO: 142
QQYDEYPYT	SEQ ID NO: 143
GFSLNSYTIT	SEQ ID NO: 144
AISGGGSTYFNSALKS	SEQ ID NO: 145
PRWYPRSYFDY	SEQ ID NO: 146

In one embodiment sequences 141 to 143 are in a light chain of the antibody.

In one embodiment sequences 144 to 146 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 141 is CDR L1, SEQ ID NO: 142 is CDR L2 and SEQ ID NO: 143 is CDR L3.

In one embodiment SEQ ID NO: 144 is CDR H1, SEQ ID NO: 145 is CDR H2 and SEQ ID NO: 146 is CDR H3.

In one embodiment SEQ ID NO: 141 is CDR L1, SEQ ID NO: 142 is CDR L2, SEQ ID NO: 143 is CDR L3, SEQ ID NO: 144 is CDR H1, SEQ ID NO: 145 is CDR H2 and SEQ ID NO: 146 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1134 anti-toxin B antibody; Light chain Variable region sequence):

DVQLTQSPSFLSASVGDRVTITCKASKSISNHLAWYQEKPGKANKLLIHSGSTLQPGT PSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYDEYPYTFGQGTRLEIK

SEQ ID NO: 147

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 147 is shown in Figure 9 and SEQ ID NO: 148 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1134 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 149:

EVQLQESGPGLVKPSETLSLTCTVSGFSLNSYTITWVRQPPGKGLEWIAAISGGGSTYFNSAL KSRVTISRDTSKSQVSLKLSSVTAADTAVYYCTRPRWYPRSYFDYWGRGTLVTVS

wherein the CDRs are underlined

The polynucleotide sequence encoding SEQ ID NO: 149 is shown in Figure 9 and SEQ ID NO: 150 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO 147: and SEQ ID NO: 149.

In one embodiment there is provided antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

KASQNVGNNVA	SEQ ID NO: 151
YASNRFT	SEQ ID NO: 152
QRVYQSTWT	SEQ ID NO: 153
GFSLTSYYVH	SEQ ID NO: 154
CIRTGGNTEYQSEFKS	SEQ ID NO: 155
GNYGFAY	SEQ ID NO: 156

In one embodiment sequences 151 to 153 are in a light chain of the antibody.

In one embodiment sequences 154 to 156 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 151 is CDR L1, SEQ ID NO: 152 is CDR L2 and SEQ ID NO: 153 is CDR L3.

In one embodiment SEQ ID NO: 154 is CDR H1, SEQ ID NO: 155 is CDR H2 and SEQ ID NO: 156 is CDR H3.

In one embodiment SEQ ID NO: 151 is CDR L1, SEQ ID NO: 152 is CDR L2, SEQ ID NO; 153 is CDR L3, SEQ ID NO: 154 is CDR H1, SEQ ID NO: 155 is CDR H2 and SEQ ID NO; 156 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1151 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 157:

AIQMTQSPSSLSASVGDRVTITCKASQNVGNNVAWYQHKPGKAPKLLIYYASNRFTGVPSRFT GGGYGTDFTLTISSLQPEDFATYYCQRVYQSTWTFGQGTKVEIK

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 157 is shown in Figure 9 and SEQ ID NO: 158 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1151 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 159:

EVQLQESGPGLVKPSETLSLTCTVSGFSLTSYYVHWVRQPPGKGLEWMGCIRTGGNTEYQSEF KSRVTISRDTSKNQVSLKLSSVTAADTAVYYCARGNYGFAYWGQGTLVTVS

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 159 is shown in Figure 9 and SEQ ID NO: 160 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 157 and SEQ ID NO: 159.

In one embodiment there is provided an antibody (for example an anti-toxin B antibody) comprising a CDR, such as 1, 2, 3, 4, 5 or 6 CDRs, selected from:

KASQNINKYLD	SEQ ID NO: 161
NIQSLHT	SEQ ID NO: 162
FQHNSGW	SEQ ID NO: 163
GFTFTQAAMF	SEQ ID NO: 164
RISTKSNNFATYYPDSVKG	SEQ ID NO: 165
PAYYYDGTVPFAY	SEQ ID NO: 166

In one embodiment sequences 161 to 163 are in a light chain of the antibody.

In one embodiment sequences 164 to 166 are in a heavy chain of the antibody.

In one embodiment SEQ ID NO: 161 is CDR L1, SEQ ID NO: 162 is CDR L2 and SEQ ID NO: 163 is CDR L3.

In one embodiment SEQ ID NO: 164 is CDR H1, SEQ ID NO: 165 is CDR H2 and SEQ ID NO: 166 is CDR H3.

In one embodiment SEQ ID NO: 161 is CDR L1, SEQ ID NO: 162 is CDR L2, SEQ ID NO: 163 is CDR L3, SEQ ID NO: 164 is CDR H1, SEQ ID NO: 165 is CDR H2 and SEQ ID NO: 166 is CDR H3.

In one embodiment there is provided a variable region, such as a light chain variable region with the following sequence (Antibody 1153 anti-toxin B antibody; Light chain Variable region sequence) SEQ ID NO: 167:

DIQMTQSPSSLSASVGDRVTITCKASQNINKYLDWYQQKPGKVPKLLIYNIQSLHTGIPSRFS
GSGSGTDFTLTISSLQPEDVATYYCFQHNSGWTFGQGTRLEIK

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 167 is shown in Figure 10 and SEQ ID NO: 168 therein.

In one embodiment there is provided a variable region, such as a heavy chain variable region with the following sequence (Antibody 1153 anti-toxin B antibody heavy chain variable region sequence) SEQ ID NO: 169:

EVQLVESGGGLVQPGGSLKLSCAASGFTFTQAAMFWVRQASGKGLEGIARISTKSNNFATYYP
DSVKGRFTISRDDSKNTVYLQMNSLKTEDTAVYYCTAPAYYYDGTVPFAYWGQGTLVTVS

wherein the CDRs are underlined.

The polynucleotide sequence encoding SEQ ID NO: 169 is shown in Figure 10 and SEQ ID NO: 170 therein.

In one embodiment an antibody according to the invention comprises variable regions shown in SEQ ID NO: 167 and SEQ ID NO: 169.

In one embodiment there is provided antibody comprising 6 CDRs independently selected from SEQ ID NOs 1, 2, 3, 4, 5, 6, 11, 12, 13, 14, 15, 16, 21, 22, 23, 24, 25, 26, 31, 32, 33, 34, 35, 36, 41, 42, 43, 44, 45, 46, 51, 52, 53, 54, 55, 56, 61, 62, 63, 64, 65, 66, 71, 72, 73, 74, 75, 76, 81, 82, 83, 84, 85, 86, 91, 92, 93, 94, 95, 96, 101, 102, 103, 104, 105, 106, 111, 112, 113, 114, 115, 116, 121,122, 123, 124, 125, 126, 131, 132, 133, 134, 135, 136, 141, 142, 143, 144, 145, 146, 151, 152, 153, 154, 155, 156, 161, 162, 163, 164, 165 and 166.

In one embodiment there is provided an anti-TcdA antibody comprising 6 CDRs independently selected from SEQ ID NOs 1, 2, 3, 4, 5, 6, 11, 12, 13, 14, 15, 16, 21, 22, 23, 24, 25, 26, 31, 32, 33, 34, 35, 36, 41, 42, 43, 44, 45, 46, 51, 52, 53, 54, 55 and 56.

In one embodiment there is provided an anti-TcdB antibody comprising 6 CDRs independently selected from SEQ ID NOs 61, 62, 63, 64, 65, 66, 71, 72, 73, 74, 75, 76, 81, 82, 83, 84, 85, 86, 91, 92, 93, 94, 95, 96, 101, 102, 103, 104, 105, 106, 111, 112, 113, 114, 115, 116, 121,122, 123, 124, 125, 126, 131, 132, 133, 134, 135, 136, 141, 142, 143, 144, 145, 146, 151, 152, 153, 154, 155, 156, 161, 162, 163, 164, 165 and 166.

In one embodiment there is provided an antibody which comprises two variable regions independently selected from SEQ ID NOs: 7, 9, 17, 19, 27, 29, 37, 39, 47, 49, 57, 59, 67, 69, 77, 79, 87, 89, 97, 99, 107, 109, 117, 119, 127, 129, 137, 139, 147, 149, 157 and 159.

In one embodiment there is provided an antibody which comprises two variable regions independently selected from SEQ ID NOs: 7, 9, 17, 19, 27, 29, 37, 39, 47, 49, 57 and 59.

In one embodiment there is provided an antibody which comprises two variable regions independently selected from SEQ ID NOs: 67, 69, 77, 79, 87, 89, 97, 99, 107, 109, 117, 119, 127, 129, 137, 139, 147, 149, 157 and 159.

In one embodiment the antibodies according to the invention are humanized.

In one embodiment the antibody or antibodies are directed to the C terminal "cell binding" portion of the TcdA and/or TcdB toxin.

In one embodiment an antibody according to the invention is suitable for neutralising toxin A or toxin B.

Neutralising as employed herein is intended to refer to the elimination or reduction of harmful/deleterious effects of the target toxin, for example at least a 50% reduction in the relevant harmful effect.

The inventors have established by using internal comparisons between antibodies discovered in this application and by comparison against antibodies well described in the art (Babcock et al. 2006; Lowy et al., 2010) that some antibodies have the desirable characteristic of maintaining effective neutralization (for example low EC_{50} and high % protection) even at high toxin concentrations. Other antibodies including those described in the art do not maintain effective toxin neutralization at high toxin concentrations.

Effective toxin concentrations can be defined as a 'lethal dose' (LD) in titration studies in the absence of neutralizing antibodies. Neutralisation assays are typically conducted at an LD of 50% of complete cell killing (*i.e.* an LD_{50}) but may be more rigorously conducted at an LD_{80}.

Assays may also be performed under considerably more challenging conditions such as LD_{90}, LD_{95} and/or LD_{max} (LD_{max} is the maximal toxin quantity which can be included in an assay as constrained by assay volume and maximum toxin concentration / solubility). Such assays aim to mimic the early stages of infection of humans when *C. difficile* growth in the

bowel is rampant and diarrhea and other symptoms lead one to hypothesise that toxin concentrations are at their highest. Antibodies which effectively neutralize damaging toxin activities under high toxin concentration conditions are thought by the present inventors to have special clinical value for the control of symptoms in human infections. In one embodiment the antibody or antibodies of the present disclosure have useful, for example low EC_{50} values and/or high % protection from cell death for one or more the LD_{80}, LD_{90}, LD_{95} and/or LD_{max}. In one embodiment the EC_{50} in the one or more of the latter situations is 15ng/ml or less, for example 10ng/ml or less, such as 5ng/ml or less, in particular 1ng/ml or less. In one embodiment the % protection from cell death is >90%, or >75% or >50%.

Thus in one embodiment the present disclosure provides an antibody or a combination of antibodies which maintain toxin neutralization even in the presence of high levels of toxin, for example as measured in an assay provided herein.

The harmful effect of toxin may, for example be measured in a suitable in vitro assay. In one embodiment the neutralization is measured in an assay given in Example 1 below. Also provided is an antibody or antibodies identified in a neutralization assay, for example wherein the potency of the antibody is maintained in the presence of high levels of toxin.

Toxin A is used interchangeably with TcdA.

Toxin B is used interchangeably with TcdB.

In one embodiment an antibody according to the invention is a monoclonal antibody or binding fragment thereof.

In one embodiment a monoclonal antibody according to the invention is capable of neutralising TcdA with very high potency and affinity.

In one embodiment a monoclonal antibody according to the invention is capable of neutralising TcdA with very high potency and affinity and high avidity.

Avidity as employed herein refers to the combined strength of multiple binding affinities.

In one embodiment a monoclonal antibody according to the invention is capable of neutralising TcdA with very high potency and affinity and high avidity and high valency of binding.

Valency of binding as employed herein refers to the ability for a monoclonal antibody to bind to an antigen multiple times. High valency of binding hence results in high levels of decoration of antigen with antibodies and / or high levels of cross-linking of toxin molecules, which is thought to be advantageous.

Anti-TcdA Mabs according to the present disclosure may be suitable for neutralising the early effects of TcdA, for example on cells such as loss of tight junctions.

Tight junction as employed herein is intended to refer to impermeable zone of connection between cells within a monolayer or anatomical tissue structure. Fluid loss does not occur when tight junctions retain their structural and functional integrity. Loss of tight junctions is an indication that the cell has been compromised by toxin and is well documented as being an early step in the toxic effects of TcdA and TcdB (25) and results in loss of fluid containing serum, immunoglobulin and ions (26, 3). Loss of tight junctions is thought to be a first step on the onset of diarrhoea in humans.

The TEER assay system, can be used to measure the loss of tight junction *in vitro*. TEER is an acronym for trans epithelial electric resistance assay and it is generally employed to measure the permeability of a differentiated cell layer representative of a gut endothelial lining. However, in the context of screening for antibodies TEER loss can be employed to identify antibodies that slow or prevent damage to the tight junctions and hence is a surrogate for protection against tissue damage leading to diarrhoea.

Often Caco-2 cells are employed since they are derived from human colon cells and are known to form differentiated monolayers with cells connected by tight junctions. A kit is commercially available from Becton-Dickinson named the Caco-2 BioCoat HTS plate system (BD Biosciences/ 354802). The instructions in the kit are suitable for testing in the present context. The resistance of the membrane changes when the membrane has been compromised.

Generally the antibody will be pre-incubated with the toxin before addition to the TEER system to establish if the antibody can prevent or slow the damage to the membrane caused by the toxin. The assay may be performed over a suitable period, for example 24 hours taking measurements at certain time-points. The present inventors have established that the 4 hour time point is particularly discriminating for therapeutically useful antibodies.
The concentration of toxin employed in the TEER assay is generally in the range 100-200ng/ml, most preferably 125ng/ml

The concentration of antibody (for example IgG1) employed in the TEER assay is generally in the range of 4 to 2000ng/ml, for example 50 to 1000ng/ml, such as 100 to 500ng/ml.

In one embodiment the EC_{50} of the antibody in the TEER assay employed in said condition is at least 200ng/ml, for example less than 100ng/ml, such as about 60-80ng/ml.

In one embodiment there is provided an anti-TcdA antibody or an anti-TcdB antibody suitable for use as a therapeutic agent in the treatment or prevention of *C. difficile* infection, wherein said antibody was screened and selected employing a TEER assay.

In one aspect there is provided a method of screening an antibody in a TEER assay for the ability to slow or prevent loss of tight junctions. In one embodiment the antibody or

antibodies screened are anti-TcdA antibodies. In one embodiment the antibody or antibodies screened are anti-TcdB antibodies. In one embodiment the antibody or antibodies screened are a combination of anti-TcdA and anti-TcdB antibodies. In one embodiment the method comprises the step of identifying an appropriate antibody or antibodies and expressing suitable quantities of same. In one embodiment the method comprises the further step of formulating said antibody or antibodies in a pharmaceutical formulation. In one embodiment the method comprises the further step of administering said antibody or antibodies or said formulation to a patient in need thereof.

In one embodiment multiple antibodies of the disclosure may be capable of binding to the target toxin (TcdA or TcdB), which may aid immune clearance of the toxin.

Multiple antibodies as employed herein is intended to refer to multiple copies of an antibody with the same sequence or an antibody with the same amino acid sequence or an antibody specific to the same target antigen but with a different amino acid sequence.

In one embodiment the antibodies according to the invention are specific to the target antigen, for example specific to an epitope in the target antigen.

In one embodiment the antibodies of the invention are able to bind to the target antigen in two or more locations, for example two or three locations, such as four, five, six, seven, eight, nine, ten or more locations, for example the toxin may comprise repeating domains and thus an antibody may be specific to an epitope and in fact that epitope may be present in the antigen several times i.e. in more than one location. Thus given antibodies may bind the same epitope multiple times in different locations in the antigen.

In one embodiment the antibody binds to the given antigen multiple times, for example 2 to 20 times such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 times. In one embodiment the antibody binds the given antigen at least 3 times. This multiple binding is thought to be important in neutralisation and/or clearance of the toxin. Whilst not wishing to be bound by theory it is thought that multiple binding, for example 3 more times, i.e. by decoration with 3 or more Fc fragments is important in triggering rapid clearance of the toxin (24) primarily via the liver and spleen (27, 28).

In one embodiment the anti-TcdA antibody binds 3 or more times, for example 3 to 16 times.

In one embodiment the anti-TcdA antibody binds 12 times.

In one embodiment the anti-TcdA antibody binds 2 times.

In one embodiment an anti-TcdA antibody binds in the catalytic-terminal cell binding domain of TcdA.

In one embodiment the anti-Tcd B antibody binds 2 or more times, for example 2 times.

In one embodiment an anti-TcdB antibody binds in the catalytic-terminal cell binding domain of TcdB.

In one embodiment the antibody or antibodies according to disclosure are capable of cross-linking toxin molecules, for example one arm of the antibody molecule binds one toxin molecule and another of the antibody binds a epitope in a different toxin molecule, thereby forming a sort of immune complex. The formation of the latter may also facilitate activation of the immune system to clear the relate toxin and thereby minimise the deleterious *in vivo* effects of the same.

In one embodiment an innate immune response, such as complement is activated.

In one embodiment the antibody or antibodies of the disclosure have high potency against toxins derived from strains of different ribotypes, for example 003, 027, 078.

In one embodiment antibodies against TcdA may have an EC_{50} in the range of 0.1 – 100ng/ml, such as 1 to 10ng/ml and a maximal inhibition in the range of 50-100% at toxin concentrations of LD_{80-95}, for example against toxins from strains of ribotypes 003, 027 and 078.

In one embodiment antibodies against TcdA may have an EC_{50} in the range of 0.1 – 100ng/ml, such as 1 to 10ng/ml and a maximal inhibition in the range of 60-100%, 70-100%, 80-100% or 90-100% at toxin concentrations of LD_{80-95}, for example against toxins from strains of ribotypes 003, 027 and 078.

In one embodiment antibodies against TcdB may have EC_{50} in the range of 0.1 – 100ng/ml, such as 1 to 10ng/ml and a maximal inhibition in the range of 50-100% at toxin concentrations of LD_{80-95}, for example against toxins from strains of ribotype 003.

In one embodiment antibodies against TcdB may have EC_{50} in the range of 0.1 – 100ng/ml, such as 1 to 10ng/ml and a maximal inhibition in the range of 60-100%, 70-100%, 80-100% or 90-100% at toxin concentrations of LD_{80-95}, for example against toxins from strains of ribotype 003.

In one embodiment there are provided combinations of antibodies according to the invention, for example combinations of antibodies specific to TcdA, combinations of antibodies specific to TcdB or combinations of antibodies to specific to TcdA and antibodies specific to TcdB.

Combinations of antibodies specific to TcdA will generally refer to combinations of antibodies directed to different epitopes on the target antigen TcdA, or at least with different binding properties.

Combinations of antibodies specific to TcdB will generally refer to combinations of antibodies directed to different epitopes on the target antigen TcdB, or at least with different binding properties.

The combinations may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 distinct antibodies, for example 2, 3, 4 or 5 antibodies.

In one embodiment there is provided a combination of one anti-TcdA antibody and two anti-TcdB, for example wherein the anti-TcdA antibody is 997 and where the anti-TcdB antibodies are 1125 and 1151

In particular there is provided a combination of one anti-TcdA antibody comprising a heavy variable region with a sequence as shown in SEQ ID NO:49 and a light variable region with a sequence shown in SEQ ID NO: 47 and two anti-TcdB antibodies the first with a heavy variable region shown in SEQ ID NO: 129 and a light variable region shown in SEQ ID NO: 127, and the second with a heavy variable region shown in SEQ ID NO: 159 and light variable region shown in SEQ ID NO: 157.

Distinct antibodies as employed herein is intended to refer to antibodies with different amino acid sequences, which may bind the same epitope or different epitopes on the target antigen.

Also provided by the present invention is a specific region or epitope of TcdA which is bound by an antibody provided by the present invention, in particular an antibody comprising the heavy chain sequence given in SEQ ID NO:49 and the light chain sequence given in SEQ ID NO:47.

Also provided by the present invention is a specific region or epitope of TcdB which is bound by an antibody provided by the present invention, in particular an antibody comprising the heavy chain sequence given in SEQ ID NO:129 and the light chain sequence given in SEQ ID NO:127 or an antibody comprising the heavy chain sequence given in SEQ ID NO:159 and the light chain sequence given in SEQ ID NO:157.

This specific region or epitope of the TcdA or TcdB toxins can be identified by any suitable epitope mapping method known in the art in combination with any one of the antibodies provided by the present invention. Examples of such methods include screening peptides of varying lengths derived from the toxins for binding to the antibody of the present invention with the smallest fragment that can specifically bind to the antibody containing the sequence of the epitope recognised by the antibody. The peptides may be produced synthetically or by proteolytic digestion of the toxin polypeptide. Peptides that bind the antibody can be identified by, for example, mass spectrometric analysis. In another example, NMR spectroscopy or X-ray crystallography can be used to identify the epitope bound by an antibody of the present invention.

Once identified, the epitopic fragment which binds an antibody of the present invention can be used, if required, as an immunogen to obtain additional antagonistic antibodies which bind the same epitope.

Antibodies which cross-block the binding of an antibody according to the present invention may be similarly useful in neutralizing toxin activity. Accordingly, the present invention also provides a neutralizing antibody having specificity for TcdA or TcdB, which cross-blocks the binding of any one of the antibodies described above to TcdA or TcdB and/or is cross-blocked from binding these toxins by any one of those antibodies. In one embodiment, such an antibody binds to the same epitope as an antibody described herein above. In another embodiment the cross-blocking neutralising antibody binds to an epitope which borders and/or overlaps with the epitope bound by an antibody described herein above. In another embodiment the cross-blocking neutralising antibody of this aspect of the invention does not bind to the same epitope as an antibody of the present invention or an epitope that borders and/or overlaps with said epitope.

Cross-blocking antibodies can be identified using any suitable method in the art, for example by using competition ELISA or BIAcore assays where binding of the cross blocking antibody to TcdA or TcdB prevents the binding of an antibody of the present invention or *vice versa*.

In one embodiment there is provided a method of generating an anti-TcdA or anti-TcdB antibody, in particular a neutralizing antibody and/or an antibody which cross-blocks the binding of an antibody described herein, said method comprising the steps of immunizing a host with a suitable antigen, for example an antigen shown in any one of SEQ ID Nos 173 to 194 or a combination thereof. The said method may also comprise one or more the following steps, for example identifying an antibody of interest (in particular using a functional assay such as TEER assay), expressing the antibody of interest, and optionally formulating the antibody as a pharmaceutically acceptable composition.

Thus in one aspect the present disclosure provides a method of immunizing a host with an amino acid sequence shown in SEQ ID Nos 173 to 194 or a combination thereof.

In one embodiment the antibodies according to the invention have an affinity to the target antigen of 10nM or less, for example 1nM or less such as 900pM, in particular 800pM, 700pM, 600pM or500pM, such as 60pM.

In one embodiment the affinity is for TcdA or TcdB or a fragment thereof. In one example the fragment is TcdA123 corresponding to residues S1827-D2249 of TcdA. In one

example the fragment is TcdA456 corresponding to residues G2205-R2608. In one embodiment the fragment is TcdB1234 corresponding to residues S1833-E2366 of TcdB.

In one embodiment antibodies according to the invention or a combination thereof have an EC_{50} of 200ng/ml or less, for example 150ng/ml or less such as 100ng/ml or less, such as in the range 0.1 to 10ng/ml.

The individual component antibodies of mixtures are not required to have an EC_{50} in said range provided that when they are used in combination with one or more antibodies the combination has an EC_{50} in said range.

Advantageously, the antibodies of the invention are stable, for example are thermally stable at temperatures above 50°C such as 60 or 70°C.

The antibodies and combinations according to the present invention also have one or more of the following advantageous properties: slow off rate, high affinity, high potency, the ability to bind multiple times to the target antigen, to neutralise the toxin by a mechanism which reduces the loss of measurable TEER activity, to stimulate or assist the hosts natural immune response, to catalyse or assist in immune clearance of the pathogen (or toxin) and/or to educate the immune system to respond appropriately to the pathogen (or toxin).

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat *et al.* This system is set forth in Kabat et al., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat et al. (supra)"). This numbering system is used in the present specification except where otherwise indicated.

The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (Chothia, C. and Lesk, A.M. J. Mol. Biol., 196, 901-917 (1987)), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus unless indicated otherwise 'CDR-H1' as employed herein is intended to refer to residues 26 to

35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.

Antibodies for use in the present invention may be obtained using any suitable method known in the art. The toxin A and/or toxin B polypeptide/protein including fusion proteins, for example toxin-Fc fusions proteins or cells (recombinantly or naturally) expressing the polypeptide (such as activated T cells) can be used to produce antibodies which specifically recognise the target toxins. The toxin polypeptide may be the full length polypeptide or a biologically active fragment or derivative thereof.

Polypeptides may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems or they may be recovered from natural biological sources. In the present application, the term "polypeptides" includes peptides, polypeptides and proteins. These are used interchangeably unless otherwise specified. The sequence for TcdA from ribotype 027 is given in SEQ ID NO: 171 (Uniprot accession number C9YJ37) and the sequence for TcdB from ribotype 027 is given is SEQ ID NO: 172 (Uniprot accession number C9YJ35).

The antigen polypeptide may in some instances be part of a larger protein such as a fusion protein for example fused to an affinity tag.

Antibodies generated against the antigen polypeptide may be obtained, where immunisation of an animal is necessary, by administering the polypeptides to an animal, preferably a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986). Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized. However, mice, rabbits, pigs and rats are generally most suitable.

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today, 4:72) and the EBV-hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, pp77-96, Alan R Liss, Inc., 1985).

Antibodies for use in the invention may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by, for

example, the methods described by Babcook, J. et al., 1996, Proc. Natl. Acad. Sci. USA 93(15):7843-78481; WO92/02551; WO2004/051268 and International Patent Application number WO2004/106377.

Humanised antibodies (which include CDR-grafted antibodies) are antibody molecules having one or more complementarity determining regions (CDRs) from a non-human species and a framework region from a human immunoglobulin molecule (see, e.g. US 5,585,089; WO91/09967). It will be appreciated that it may only be necessary to transfer the specificity determining residues of the CDRs rather than the entire CDR (see for example, Kashmiri et al., 2005, Methods, 36, 25-34). Humanised antibodies may optionally further comprise one or more framework residues derived from the non-human species from which the CDRs were derived.

As used herein, the term 'humanised antibody molecule' refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody). For a review, see Vaughan et al, Nature Biotechnology, 16, 535-539, 1998. In one embodiment rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs described herein above are transferred to the human antibody framework (see for example, Kashmiri et al., 2005, Methods, 36, 25-34). In one embodiment only the specificity determining residues from one or more of the CDRs described herein above are transferred to the human antibody framework. In another embodiment only the specificity determining residues from each of the CDRs described herein above are transferred to the human antibody framework.

When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Suitably, the humanised antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs provided herein.

Thus, provided in one embodiment is a humanised antibody which binds toxin A or toxin B wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat et al., supra). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and

POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at: http://vbase.mrc-cpe.cam.ac.uk/

In a humanised antibody of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

Also, in a humanised antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann et al., 1998, Nature, 332, 323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967.

Generally the antibody sequences disclosed in the present specification are humanised.

The invention also provides sequences which are 80%, 90%, 91%, 92%, 93% 94%, 95% 96%, 97%, 98% or 99% similar to a sequence or antibody disclosed herein.

"Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains).

Degrees of identity and similarity can be readily calculated (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 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987, Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991, the BLAST™ software available from NCBI (Altschul, S.F. et al., 1990, J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. et al.,

1996, Meth. Enzymol. 266:131-141; Altschul, S.F. et al., 1997, Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656,).

The antibody molecules of the present invention include a complete antibody molecule having full length heavy and light chains or a fragment thereof and may be, but are not limited to Fab, modified Fab, Fab', modified Fab', F(ab')2, Fv, Fab-Fv, Fab-dsFv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma et al., 1998, Journal of Immunological Methods, 216, 165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO2005/003169, WO2005/003170 and WO2005/003171. Multi-valent antibodies may comprise multiple specificities e.g bispecific or may be monospecific (see for example WO 92/22853 and WO05/113605). Bispecific and multispecific antibody variants are especially considered in this example since the aim is to neutralise two independent target proteins: TcdA and TcdB. Variable regions from antibodies disclosed herein may be configured in such a way as to produce a single antibody variant which is capable of binding to and neutralising TcdA and TcdB.

In one embodiment the antibody according to the present disclosure is provided as TcdA or TcdB binding antibody fusion protein which comprises an immunoglobulin moiety, for example a Fab or Fab' fragment, and one or two single domain antibodies (dAb) linked directly or indirectly thereto, for example as described in WO2009/040562.

In one embodiment the fusion protein comprises two domain antibodies, for example as a variable heavy (VH) and variable light (VL) pairing, optionally linked by a disulphide bond, for example as described in WO2010/035012.

In one embodiment the Fab or Fab' element of the fusion protein has the same or similar specificity to the single domain antibody or antibodies. In one embodiment the Fab or Fab' has a different specificity to the single domain antibody or antibodies, that is to say the fusion protein is multivalent. In one embodiment a multivalent fusion protein according to the present invention has an albumin binding site, for example a VH/VL pair therein provides an albumin binding site.

In one embodiment the multivalent fusion protein according to the invention binds TcdA and TcdB.

In one embodiment the multivalent fusion protein according to the invention binds TcdB in multiple positions, for example has distinct binding regions specific for two different epitopes.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simply neutralising or agonising an antigen. It will be appreciated that sequence variants of these constant region domains may also be used. For example IgG4 molecules in which the serine at position 241 has been changed to proline as described in Angal et al., Molecular Immunology, 1993, 30 (1), 105-108 may be used. It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. Journal of Chromatography 705:129-134, 1995).

In one embodiment the antibody heavy chain comprises a CH1 domain and the antibody light chain comprises a CL domain, either kappa or lambda.

Biological molecules, such as antibodies or fragments, contain acidic and/or basic functional groups, thereby giving the molecule a net positive or negative charge. The amount of overall "observed" charge will depend on the absolute amino acid sequence of the entity, the local environment of the charged groups in the 3D structure and the environmental conditions of the molecule. The isoelectric point (pI) is the pH at which a particular molecule or solvent accessible surface thereof carries no net electrical charge. In one example, the antibody and fragments of the invention may be engineered to have an appropriate isoelectric point. This may lead to antibodies and/or fragments with more robust properties, in particular suitable solubility and/or stability profiles and/or improved purification characteristics.

Thus in one aspect the invention provides a humanised antibody engineered to have an isoelectric point different to that of the originally identified antibody from which it is derived.

The antibody may, for example be engineered by replacing an amino acid residue such as replacing an acidic amino acid residue with one or more basic amino acid residues. Alternatively, basic amino acid residues may be introduced or acidic amino acid residues can be removed. Alternatively, if the molecule has an unacceptably high pI value acidic residues may be introduced to lower the pI, as required. It is important that when manipulating the pI care must be taken to retain the desirable activity of the antibody or fragment. Thus in one embodiment the engineered antibody or fragment has the same or substantially the same activity as the "unmodified" antibody or fragment.

Programs such as ** ExPASY http://www.expasy.ch/tools/pi_tool.html, and http://www.iut-arles.up.univ-mrs.fr/w3bb/d_abim/compo-p.html, may be used to predict the isoelectric point of the antibody or fragment.

It will be appreciated that the affinity of antibodies provided by the present invention may be altered using any suitable method known in the art. The affinity of the antibodies or variants thereof may be measured using any suitable method known in the art, including BIAcore, using an appropriate isolated natural or recombinant protein or a suitable fusion protein/polypeptide.

The present invention therefore also relates to variants of the antibody molecules of the present invention, which have an improved affinity for TcdA or TcdB, as appropriate. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang et al., J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks et al., Bio/Technology, 10, 779-783, 1992), use of mutator strains of E. coli (Low et al., J. Mol. Biol., 250, 359-368, 1996), DNA shuffling (Patten et al., Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson et al., J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Crameri et al., Nature, 391, 288-291, 1998). Vaughan et al. (supra) discusses these methods of affinity maturation.

Improved affinity as employed herein in this context refers to an improvement refers to an improvement over the starting molecule.

If desired an antibody for use in the present invention may be conjugated to one or more effector molecule(s). It will be appreciated that the effector molecule may comprise a single effector molecule or two or more such molecules so linked as to form a single moiety that can be attached to the antibodies of the present invention. Where it is desired to obtain an antibody fragment linked to an effector molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector molecule. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom et al., Controlled Drug Delivery, 2nd

Ed., Robinson et al., eds., 1987, pp. 623-53; Thorpe et al., 1982 , Immunol. Rev., 62:119-58 and Dubowchik et al., 1999, Pharmacology and Therapeutics, 83, 67-123). Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 89/00195, WO 89/01476 and WO03031581. Alternatively, where the effector molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO 86/01533 and EP0392745.

The term effector molecule as used herein includes, for example, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

Other effector molecules may include chelated radionuclides such as 111In and 90Y, Lu177, Bismuth213, Californium252, Iridium192 and Tungsten188/Rhenium188; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as insulin, tumour necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g. angiostatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor and immunoglobulins.

Other effector molecules may include detectable substances useful for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine

fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include 125I, 131I, 111In and 99Tc.

In another example the effector molecule may increase the half-life of the antibody in vivo, and/or reduce immunogenicity of the antibody and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin binding proteins or albumin binding compounds such as those described in WO05/117984.

Where the effector molecule is a polymer it may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or hetero- polysaccharide.

Specific optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.

Specific examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

Specific naturally occurring polymers include lactose, amylose, dextran, glycogen or derivatives thereof.

"Derivatives" as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, for example from 5000 to 40000Da such as from 20000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product for example ability to localize to certain tissues such as tumors or extend circulating half-life (for review see Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545). Thus, for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the product remains in the circulation, it may be advantageous to use a

higher molecular weight polymer, for example having a molecular weight in the range from 20000Da to 40000Da.

Suitable polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 15000Da to about 40000Da.

In one example antibodies for use in the present invention are attached to poly(ethyleneglycol) (PEG) moieties. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see for example US 5,219,996; US 5,667,425; WO98/25971, WO2008/038024). In one example the antibody molecule of the present invention is a modified Fab fragment wherein the modification is the addition to the C-terminal end of its heavy chain one or more amino acids to allow the attachment of an effector molecule. Suitably, the additional amino acids form a modified hinge region containing one or more cysteine residues to which the effector molecule may be attached. Multiple sites can be used to attach two or more PEG molecules.

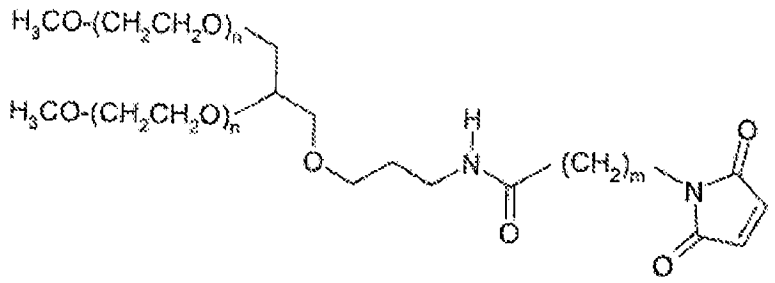
Suitably PEG molecules are covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond. Where a thiol group is used as the point of attachment appropriately activated effector molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used. An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an α-halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

In one embodiment, the antibody is a modified Fab fragment or diFab which is PEGylated, i.e. has PEG (poly(ethyleneglycol)) covalently attached thereto, e.g. according to

the method disclosed in EP 0948544 or EP1090037 [see also "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews 2002, 54:531-545]. In one example PEG is attached to a cysteine in the hinge region. In one example, a PEG modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue may be covalently linked to the maleimide group and to each of the amine groups on the lysine residue may be attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000Da. The total molecular weight of the PEG attached to the Fab fragment may therefore be approximately 40,000Da.

Particular PEG molecules include 2-[3-(N-maleimido)propionamido]ethyl amide of N,N'-bis(methoxypoly(ethylene glycol) MW 20,000) modified lysine, also known as PEG2MAL40K (obtainable from Nektar, formerly Shearwater).

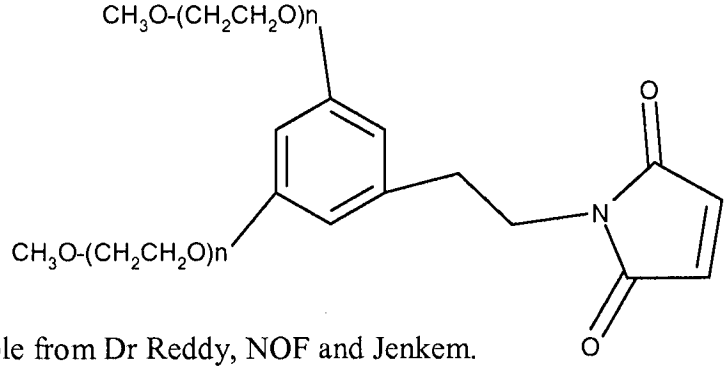
Alternative sources of PEG linkers include NOF who supply GL2-400MA2 (wherein m in the structure below is 5) and GL2-400MA (where m is 2) and n is approximately 450:



m is 2 or 5

That is to say each PEG is about 20,000Da.

Further alternative PEG effector molecules of the following type:



are available from Dr Reddy, NOF and Jenkem.

In one embodiment there is provided an antibody which is PEGylated (for example with a PEG described herein), attached through a cysteine amino acid residue at or about amino acid 226 in the chain, for example amino acid 226 of the heavy chain (by sequential numbering).

In one embodiment one certain antibodies according to the present disclosure have the following properties:

Antibody	Affinity (pM)		Valency of binding	EC_{50} (ng/ml)
	$TcdA_{123}$	$TcdA_{456}$	TcdA, est.	
CA922	4.06	2.59	16	1.21
CA923	64.7	312	12	160.42
CA995	nil	119	1	37.64
CA997	132	66.8	12	6.25
CA1000	73.3	84.1	2	19.73

The present invention also provides compositions such as a pharmaceutical composition of antibody or combination of antibodies defined herein.

The present invention also provides a composition that comprises at least two antibodies according to the invention, for example wherein at least one antibody therein is specific to TcdA and at least one antibody therein is specific to TcdB or alternatively at least two antibodies specific to TcdA or at least two antibodies specific to TcdB.

In one embodiment there is provided a composition that comprises multiple antibodies specific to TcdA and optionally one or more antibodies specific to TcdB.

In one embodiment there is provided a composition that comprises multiple antibodies specific to TcdB and optionally one or more antibodies specific to TcdA.

Thus in one embodiment there is provided a composition comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 antibodies according to the invention i.e. distinct antibodies.

The invention describes one particular mixture comprising 3 Mabs, one Mab of which is specific for TcdA and two Mabs of which are specific for TcdB. This mixture demonstrated very high levels of protection from death and gut inflammation from a lethal infective oral dose of *Clostridium difficile* in hamsters.

In particular there is provided a composition comprising a combination of one anti-TcdA antibody comprising a heavy variable region with a sequence as shown in SEQ ID NO:49 and a light variable region with a sequence shown in SEQ ID NO: 47 and two anti-

TcdB the first with a heavy variable region shown in SEQ ID NO: 129 and a light variable region shown in SEQ ID NO: 127, and the second with a heavy variable region shown in SEQ ID NO: 159 and light variable region shown in SEQ ID NO: 157.

In one embodiment wherein the composition comprises 3 antibodies, such as one anti-TcdA and two anti-TcdB antibodies the antibodies are in the ratio of 50%, 25% and 25% respectively of the total antibody content thereof.

In one embodiment there is provided a composition comprising 2, 3, 4 or 5 antibodies specific to TcdA and optionally 1, 2, 3, 4 or 5 antibodies specific to TcdB.

In one embodiment the compositions provided according to the invention are well defined, for example are mixtures of monoclonal antibodies rather than simply polyclonal compositions derived from an immunised or immune competent host.

In one embodiment the composition of antibodies has an EC_{50} of 200ng/ml or less, for example 150ng/ml or less, such as 100ng/ml or less, such as 0.1 to 10ng/ml.

Advantageously the antibodies described herein have very high levels of biophysical stability and so are suitable for inclusion in mixtures of antibodies.

In one aspect a pharmaceutical formulation or composition according to the invention further comprises a pharmaceutically acceptable excipient.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Suitable forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, in one or more embodiments the compositions are adapted for administration to human subjects.

Suitably in formulations according to the present disclosure, the pH of the final formulation is not similar to the value of the isoelectric point of the antibody or fragment, for example if the pH of the formulation is 7 then a pI of from 8-9 or above may be appropriate.

Whilst not wishing to be bound by theory it is thought that this may ultimately provide a final formulation with improved stability, for example the antibody or fragment remains in solution.

In one embodiment the composition or formulation of the present disclosure comprises 1-200mg/mL of antibodies, that this to say the combined antibody content, for example 150mg/mL or less, such as 100mg/mL or less, in particular 90, 80, 70 , 60, 50, 40, 30, 20, 10mg/mL or less.

In one embodiment a composition or formulation according to the present disclosure comprises 20mg/mL of each antibody therein.

In one embodiment there is provided a formulation comprising:

33mg/mL or less of one anti-TcdA antibody comprising a heavy variable region with a sequence as shown in SEQ ID NO: 49 and a light variable region with a sequence shown in SEQ ID NO: 47, and

28mg/mL or less of a first anti-TcdB with a heavy variable region shown in SEQ ID NO: 129 and a light variable region shown in SEQ ID NO: 127, and

25mg/mL of a second anti-TcdB with a heavy variable region shown in SEQ ID NO: 159 and light variable region shown in SEQ ID NO: 157.

In one embodiment the pharmaceutical formulation at a pH in the range of 4.0 to 7.0 comprises: 1 to 200mg/mL of an antibody according to the present disclosure, 1 to 100mM of a buffer, 0.001 to 1% of a surfactant,

a) 10 to 500mM of a stabiliser,

b) 5 to 500 mM of a tonicity agent , or

c) 10 to 500mM of a stabiliser and 5 to 500 mM of a tonicity agent.

In one embodiment the composition or formulation according to the present disclosure comprises the buffer phosphate buffered saline.

For example the formulation at approximately pH6 may comprise 1 to 50mg/mL of antibody, 20mM L-histidine HCl, 240 mM trehalose and 0.02% polysorbate 20. Alternatively a formulation at approximately pH 5.5 may comprise 1 to 50mg/mL of antibody, 20mM citrate buffer, 240mM sucrose, 20mM arginine, and 0.02% polysorbate 20.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as

injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue.

The compositions can also be administered into a lesion or directly into the gastrointestinal tract by for examples, encapsulated oral dosage for swallowing, through a nasogastric tube to the stomach or ileum, through a rectal tube or enema solutions or by rectal capsule. Dosage treatment may be a single dose schedule or a multiple dose schedule.

It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

The present invention also provides an antibody or antibody combination or a composition comprising the same, as described herein, for treatment, for example for the treatment or prophylaxis of *C. difficile* infection or complications associated with the same such as diarrhoea, colitis in particular pseudomembranous colitis, bloating, abdominal pain and toxic megacolon.

Prophylaxis can also be achieved by the administration of pre-formed complexes of inactivated toxin antigen (toxoid) and antibody in order to create a vaccine.

In one embodiment the antibodies, combinations thereof and compositions comprising the same according to the invention are suitable for treating infection with so-called super strains of *C. difficile*, i.e. hypervirulent strains such as ribotype 027.

The antibodies and compositions according to the present invention are suitable for use in the treatment or prophylaxis of acute and/or chronic effects of the relevant *C. difficile* toxins during primary infection.

The antibodies and compositions according to the present invention are suitable for use in the treatment or prophylaxis of effects of the relevant *C. difficile* toxins during secondary infection or re-infection. International guidelines enshrine time intervals after a primary infection which hence defines a secondary (or recurrent) infection as being distinct from a continuation of existing symptoms sometimes described as a relapse (29). Research has shown that secondary infections can be the result of the same strain or ribotype as the primary

infection. In such cases recurrence rather than relapse relies on agreed temporal constraints. However, research also clearly shows that secondary infection can also be the result of infection of a strain or ribotype distinct from that of the primary infection. In one study, 48% of disease recurrences were the result of a second strain distinct from that having caused the first infection (30). In another study, more than 56% of disease recurrences were the result of a second strain distinct from that having caused the first infection (31).

In one embodiment the antibodies, combinations thereof and compositions comprising the same according to the invention are suitable for use in the prevention of damage, for example long term structural damage to the epithelium of the colon.

In one embodiment the antibodies, combinations and composition are suitable for preventing *C. difficile* infection including recurrence of infection, in particular nosocomial infection.

In one embodiment the antibodies, combinations thereof and compositions comprising the same according to the invention are suitable for reducing the risk of recurrence of *C. difficile* infection.

Advantageously, the antibodies of the present disclosure can be administered prophylactically to prevent infection or re-infection because in the absence of toxin to which the antibody is specific the antibody is simply to be cleared from the body without causing adverse interactions with the subjects body tissues.

Advantageously the antibodies of the present disclosure seem to elicit a rapid response after administration, for example within one or two days of administration rapid clearance of the target toxin is invoked, this may prevent vital organs such as the lungs, heart and kidneys being damaged. This is the first time that agents have been made available with can be employed to prevent damage or injury to a patient by toxins A and/or B in the acute *C. difficile* infection stage.

Thus in one embodiment the antibodies, combinations thereof and compositions comprising the same according to the invention are suitable for preventing damage to vital organs.

In one embodiment the antibody, combinations or formulations described herein are suitable for preventing death of an infected patient, if administered within an appropriate time frame before irreparable damage has been done by the toxins.

The antibodies of the present disclosure have fast on-rates, which facilitates the rapid *effect in vivo.*

In one embodiment the patient population is over 60, such as over 65 years of age.

In one embodiment the patient population is 5 years old or less.

The antibodies according the invention may be employed in combination with antibiotic treatment for example metronidazole, vancomycin or fidaxomicin.

A range of *in vitro* data exemplify the properties of the Mabs and Mab mixtures. We show that one mixture of 3 Mabs (50% molar quantities of anti-TcdA and 50% molar quantities of anti-TcdB components) was able to protect hamsters from a lethal CDI.

In one embodiment there is provided a method of treating a patient in need thereof by administering a therapeutically effective amount of an antibody as described herein or antibody combination or a composition comprising the same, for example in the treatment or prophylaxis of *C. difficile* infection or complications associated with the same such as diarrhoea, colitis in particular pseudomembranous colitis, bloating, abdominal pain and toxic megacolon.

In one embodiment the antibody, combination or formulation is administered by a parenteral route, for example subcutaneously, intraperitoneally, intravenously or intramuscularly. The data in the Examples generated in hamsters indicates that the doses administered by this route reach the gut and thus are able to generate a therapeutic effect.

In one embodiment the antibody, combination or formulation is administered orally, for example an enterically coated formulation.

In one embodiment there is provided use of an antibody, combination or formulation as described herein for the manufacture of a medicament for the treatment or prophylaxis of *C. difficile* infection.

In one embodiment the dose administered is in the range 1 to 1000mg/Kg, for example 10 to 75mg/Kg, such 20 to 50mg/Kg.

In one embodiment the half-life of the antibody or antibodies in mice and hamsters *in vivo* is in the range 6 to 8 days in healthy (uninfected) animals and hence are expected to have half-lives in humans in the range of 14-28 days.

In one embodiment the antibody or antibodies are given as one dose only.

In one embodiment the antibody or antibodies are given as a weekly or biweekly dose.

In one embodiment the antibody or antibodies are given as once daily doses.

In one embodiment there is provided complex comprising TcdA or an immunogenic fragment thereof, complexed with one or more anti-TcdA antibodies defined herein. The complex may be employed as the antigen in a vaccine formulation, for example suitable for generating protective antibodies to toxin A in vivo after administration to a human.

In one embodiment there is provided complex comprising TcdB or an immunogenic fragment thereof, complexed with one or more anti-TcdB antibodies defined herein. The

complex may be employed as the antigen in a vaccine formulation, for example suitable for generating protective antibodies to toxin B in vivo after administration to a human.

Th1-type immunostimulants which may be formulated to produce adjuvants suitable for use in the present invention include and are not restricted to the following.

In one embodiment there is provided a complex comprising TcdA or an immunogenic fragment thereof and TcdB or an immunogenic fragment thereof, wherein each toxin or fragment is complexed with one or more antibodies specific thereto, wherein the complex is suitable for administration as a vaccine formulation.

Antibody:antigen complexes are known to be taken up by the immune system in an Fc receptor mediated process (27, 28) and pre-formed complexes of antibody:antigen complexes have been successfully use as vaccines in human clinical trials (22).

In one or more embodiments the vaccine formulation further comprises an adjuvant as an immunostimulant.

Monophosphoryl lipid A, in particular 3-de-O-acylated monophosphoryl lipid A (3D-MPL), is a preferred Th1-type immunostimulant for use in the invention. 3D-MPL is a well known adjuvant manufactured by Ribi Immunochem, Montana. Chemically it is often supplied as a mixture of 3-de-O-acylated monophosphoryl lipid A with either 4, 5, or 6 acylated chains. It can be purified and prepared by the methods taught in GB 2122204B, which reference also discloses the preparation of diphosphoryl lipid A, and 3-O-deacylated variants thereof. Other purified and synthetic lipopolysaccharides have been described (US 6,005,099 and EP 0 729 473 B1; Hilgers *et al.*, 1986, *Int.Arch.Allergy.Immunol.*, 79(4):392-6; Hilgers *et al.*, 1987, Immunology, 60(1):141-6; and EP 0 549 074 B1). A preferred form of 3D-MPL is in the form of a particulate formulation having a small particle size less than 0.2mm in diameter, and its method of manufacture is disclosed in EP 0 689 454.

Saponins are also preferred Th1 immunostimulants in accordance with the invention. Saponins are well known adjuvants and are taught in: Lacaille-Dubois, M and Wagner H. (1996. A review of the biological and pharmacological activities of saponins. Phytomedicine vol 2 pp 363-386). For example, Quil A (derived from the bark of the South American tree Quillaja Saponaria Molina), and fractions thereof, are described in US 5,057,540 and "Saponins as vaccine adjuvants", Kensil, C. R., *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1-2):1-55; and EP 0 362 279 B1. The haemolytic saponins QS21 and QS17 (HPLC purified fractions of Quil A) have been described as potent systemic adjuvants, and the method of their production is disclosed in US Patent No. 5,057,540 and EP 0 362 279 B1. Also described in these references is the use of QS7 (a non-haemolytic fraction of Quil-A) which acts as a potent adjuvant for systemic vaccines. Use of QS21 is further described in Kensil *et al.* (1991. J.

Immunology vol 146, 431-437). Combinations of QS21 and polysorbate or cyclodextrin are also known (WO 99/10008). Particulate adjuvant systems comprising fractions of QuilA, such as QS21 and QS7 are described in WO 96/33739 and WO 96/11711. One such system is known as an Iscorn and may contain one or more saponins.

Another preferred immunostimulant is an immunostimulatory oligonucleotide containing unmethylated CpG dinucleotides ("CpG"). CpG is an abbreviation for cytosine-guanosine dinucleotide motifs present in DNA. CpG is known in the art as being an adjuvant when administered by both systemic and mucosal routes (WO 96/02555, EP 468520, Davis *et al., J.Immunol*, 1998, 160(2):870-876; McCluskie and Davis, *J.Immunol.*, 1998, 161(9):4463-6). Historically, it was observed that the DNA fraction of BCG could exert an anti-tumour effect. In further studies, synthetic oligonucleotides derived from BCG gene sequences were shown to be capable of inducing immunostimulatory effects (both in vitro and in vivo). The authors of these studies concluded that certain palindromic sequences, including a central CG motif, carried this activity. The central role of the CG motif in immunostimulation was later elucidated in a publication by Krieg, Nature 374, p546 1995. Detailed analysis has shown that the CG motif has to be in a certain sequence context, and that such sequences are common in bacterial DNA but are rare in vertebrate DNA. The immunostimulatory sequence is often: Purine, Purine, C, G, pyrimidine, pyrimidine; wherein the CG motif is not methylated, but other unmethylated CpG sequences are known to be immunostimulatory and may be used in the present invention.

In certain combinations of the six nucleotides a palindromic sequence is present. Several of these motifs, either as repeats of one motif or a combination of different motifs, can be present in the same oligonucleotide. The presence of one or more of these immunostimulatory sequences containing oligonucleotides can activate various immune subsets, including natural killer cells (which produce interferon g and have cytolytic activity) and macrophages (Wooldrige et al Vol 89 (no. 8), 1977). Other unmethylated CpG containing sequences not having this consensus sequence have also now been shown to be immunomodulatory.

CpG when formulated into vaccines, is generally administered in free solution together with free antigen (WO 96/02555; McCluskie and Davis, *supra*) or covalently conjugated to an antigen (WO 98/16247), or formulated with a carrier such as aluminium hydroxide ((Hepatitis surface antigen) Davis *et al. supra* ; Brazolot-Millan *et al., Proc.Natl.Acad.Sci.*, USA, 1998, 95(26), 15553-8).

Such immunostimulants as described above may be formulated together with carriers, such as for example liposomes, oil in water emulsions, and or metallic salts, including

aluminium salts (such as aluminium hydroxide). For example, 3D-MPL may be formulated with aluminium hydroxide (EP 0 689 454) or oil in water emulsions (WO 95/17210); QS21 may be advantageously formulated with cholesterol containing liposomes (WO 96/33739), oil in water emulsions (WO 95/17210) or alum (WO 98/15287); CpG may be formulated with alum (Davis *et al. supra* ; Brazolot-Millan *supra*) or with other cationic carriers.

Combinations of immunostimulants are also preferred, in particular a combination of a monophosphoryl lipid A and a saponin derivative (WO 94/00153; WO 95/17210; WO 96/33739; WO 98/56414; WO 99/12565; WO 99/11241), more particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153. Alternatively, a combination of CpG plus a saponin such as QS21 also forms a potent adjuvant for use in the present invention. Alternatively the saponin may be formulated in a liposome or in an Iscorn and combined with an immunostimulatory oligonucleotide.

Thus, suitable adjuvant systems include, for example, a combination of monophosphoryl lipid A, preferably 3D-MPL, together with an aluminium salt.

Thus is one embodiment the adjuvant is a combination of QS21 and 3D-MPL in an oil in water or liposomal formulation.

An enhanced system involves the combination of a monophosphoryl lipid A and a saponin derivative particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a less reactogenic composition where the QS21 is quenched in cholesterol containing liposomes (DQ) as disclosed in WO 96/33739. This combination may additionally comprise an immunostimulatory oligonucleotide.

A particularly potent adjuvant formulation involving QS21, 3D-MPL & tocopherol in an oil in water emulsion is described in WO 95/17210 and is another preferred formulation for use in the invention.

Another preferred formulation comprises a CpG oligonucleotide alone or together with an aluminium salt.

In a further aspect of the present invention there is provided a method of manufacture of a vaccine formulation as herein described, wherein the method comprises admixing a polypeptide according to the invention with a suitable adjuvant.

Particularly suitable adjuvant combinations for use in the formulations according to the invention are as follows:

i) 3D-MPL + QS21 in a liposome

ii) Alum + 3D-MPL

iii) Alum + QS21 in a liposome + 3D-MPL

iv) Alum + CpG

v) 3D-MPL + QS21 + oil in water emulsion

vi) CpG

As used herein, the term "comprising" in context of the present specification should be interpreted as "including".

Embodiments and preferences may be combined as technically appropriate.

The disclosure herein describes embodiments comprising certain integers. The disclosure also extends to the same embodiments consisting or consisting essentially of said integers.

FIGURES

Fig 1-10 shows various antibody and fragment sequences

Fig 11 shows sera titres for TcdA and TcdB

Fig 12 shows anti TcdA (Ribotype 003) in-vitro neutralization data for single Mabs

Fig 13 shows anti TcdA (Ribotype 003) in-vitro neutralization data for single and paired Mabs

Fig 14-15 shows anti TcdA (Ribotype 003) in-vitro neutralization data for paired Mabs

Fig 16-18 shows anti TcdA (Ribotype 003) in-vitro neutralization data for three Mab mixtures

Fig 19-20 shows anti TcdA (Ribotype 003) in-vitro neutralization data for four and five Mab mixtures

Fig 21-22 shows anti TcdA (Ribotype 003) in-vitro neutralization data for single and paired Mabs at different TcdA concentrations

Fig 23-24 shows anti TcdA (Ribotype 003) in-vitro neutralization data for single and to five Mab mixturess at different TcdA concentrations

Fig 25-26 shows anti TcdB (Ribotype 003) in-vitro neutralization data for single Mabs

Fig 27-30 shows anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs

Fig 31-33 shows anti TcdB (Ribotype 003) in-vitro neutralization data for three Mab mixtures

Fig 34-40 shows anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

Fig 41-45 shows anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations

Fig 46-59 shows TcdB neutralisation data for single antibodies and pairs of antibodies

Fig 60 shows the amino acid sequence for TcdA

Fig 61 shows the amino acid sequence for TcdB

Fig 62 shows TEER assay data for TcdA in a histogram format

Fig 62A shows TEER assay data for TcdA in line graph format

Fig 63 shows a meier-kaplan curve for the combination of antibodies 997, 1125 and 1151, high concentration is 50mg/Kg and low concentration is 5mg/Kg 50mg/kg' dose gave 100% protection to day 11, ~82% protection to day 28. 5mg/kg' dose resulted in non-durable and incomplete protection.

Fig 64 shows bodyweight changes for vancomycin and vehicle treated hamsters

Fig 65 shows the bodyweight for low dose antibodies 5mg/Kg and high dose antibodies 50mg/Kg

Fig 66 shows photographs of a colon where the animal received treatment with antibodies according to the present disclosure vs a control

Fig 67-68 show effects of vortexing on antibody stability

Fig 69 shows a comparison of aggregation stability for various antibodies

Fig 70-73 show neutralisation of TcdA for various ribotypes

EXAMPLES

Antibody Generation

A range of different immunogens and screening reagents were either purchased or produced by conventional *E. coli* expression techniques in order to provide a diverse and broad immune response and to facilitate identification and characterisation of monoclonal antibodies (listed in Table 1). In cases where recombinant proteins or peptides were generated, sequences were based on ribotype 027. The sequence for TcdA from ribotype 027 is given in SEQ ID NO: 171 (Uniprot accession number C9YJ37) and the sequence for TcdB from ribotype 027 is given is SEQ ID NO: 172 (Uniprot accession number C9YJ35).

Sprague Dawley rats and half-lop rabbits were immunised with either synthetic peptides mapping to regions common to both TcdA and TdcdB full-length toxin, formaldehyde-inactivated toxoid A, binding domain fragments of Toxin A (CROPs1,2,3 or CROPs4,5,6) or binding domain fragment of Toxin B (CROPs1,2,3,4), or in some cases, a combination of the above. Following 2 to 6 immunisations, animals were sacrificed and PBMC, spleen and bone marrow harvested. Sera were monitored for binding to Toxin A domains, toxin B domains, toxin or toxoid by ELISA. Sera titres of 2 such immunisations are shown in figure 11.

UCB SLAM was used as a means to generate monoclonal antibodies. B cells were cultured directly from immunised animals (Zubler et al., 1985). This step enabled sampling of a large percentage of the B cell repertoire. By incorporating the selected lymphocyte antibody method (SLAM) (Babcook et al., 1996) it was possible to deconvolute positive culture wells and

identify antigen-specific antibody-secreting cells. Here we used a modified version of SLAM (UCB SLAM (Tickle et al. 2009)) that utilises a fluorescence-based method to identify antigen-specific B cells from culture wells. B cell cultures were set up and supernatants were first screened for their ability to bind a relevant purified toxin domain (binding, translocation or catalytic) in a bead-based assay using an Applied Biosystem 8200 cellular detection system. This was a homogeneous assay using B cell culture supernatant containing IgG, biotinylated toxin domains coated onto streptavidin beads and a goat anti-rat/rabbit Fc-Cy5 conjugate. Cell cultures positive for binding to TcdA or TcdB components from this assay were selected for use in cell-based functional assays to identify neutralisers of toxin-induced cytotoxicity. Approximately 12,000 toxin-specific positives were identified in the primary binding screen from a total of 40 x 50-plate experiments. This equated to the screening of approximately 0.5 billion B cells. Heavy and light variable region gene pairs were isolated from single cells harvested by micromanipulation from approximately 100 toxin-neutralising wells following reverse transcription (RT)-PCR. These V-region genes were then cloned as mouse IgG1/kappa full-length antibodies for rat variable regions and rabbit IgG/kappa full-length antibodies for rabbit variable regions. Antibodies were re-expressed in a HEK-293 transient expression system. These recombinant antibodies were then retested for their ability to neutralise toxin in cell based assays. Recombinant antibodies were also screened by BIAcore to determine affinity for a given toxin domain and to also determine the specificity and approximate the number of binding events of antibody to toxin. Based on in vitro activity in cell based assays and affinity measurements, lead candidates were selected for humanisation. Unless otherwise stated, all the data herein was generated using the humanised antibodies.

A panel of recombinant, *E. coli*-produced toxin fragments (TcdA), *C. difficile*-derived toxin or toxoid (A) and synthetic peptides (B) were generated or purchased from commercial sources.

Table 1. Toxin A (TcdA) sequence related reagents for screening and immunizations.

Fragment	Residue number	Source
TcdA catalytic	M1-E659	UCB E. coli expression
TcdA translocation	K577-D1350	UCB E. coli expression
TcdA $CROPS_{123}$ (TcdA123)	S1827-D2249	UCB E. coli expression
TcdA $CROPS_{456}$ (TcdA456)	G2205-R2608	UCB E. coli expression
TcdA $CROP_1$	S1827-N1978	UCB E. coli expression
TcdA $CROP_2$	G1966-N2133	UCB E. coli expression
TcdA $CROP_3$	G2100-D2249	UCB E. coli expression
TcdA $CROP_4$	G2213-N2381	UCB E. coli expression

TcdA $CROP_5$	G2328-N2494	UCB E. coli expression
TcdA $CROP_6$	G2462-N2609	UCB E. coli expression
TcdA $CROP_7$	R2554-D2701	UCB E. coli expression
TcdB catalytic	M1-A593	UCB E. coli expression
TcdB translocation	R576-D1349	UCB E. coli expression
TcdB binding (TcdB1234)	S1833-E2366	UCB E. coli expression
TcdB $CROP_1$	S1833-S1981	UCB E. coli expression
TcdB $CROP_2$	G1968-D2113	UCB E. coli expression
TcdB $CROP_3$	G2100-E2247	UCB E. coli expression
TcdB $CROP_4$	G2234-E2366	UCB E. coli expression
Toxin A	Full length	purchased
Toxin B	Full length	purchased
Toxoid A	Full length	purchased

Table 2. Toxin B (TcdB) sequence related reagents for screening and immunizations.

Toxin Domain	**Amino acid Sequence**	
Catalytic	SPVEKNLHFVWIGGEVSD	SEQ ID NO: 173
Catalytic	NLAAASDIVRL	SEQ ID NO: 174
Catalytic	CGGVYLDVDMLPGIH	SEQ ID NO: 175
Catalytic	CGGVYLDVDMLPGIHSDLFK	SEQ ID NO: 176
Catalytic	CWEMIKLEAIMKYK	SEQ ID NO: 177
Catalytic	CTNLVIEQVKNR	SEQ ID NO: 178
Catalytic	PEARSTISLSGP	SEQ ID NO: 179
Catalytic	CSNLIVKQIENR	SEQ ID NO: 180
Catalytic	TEQEINSLWSFDQA	SEQ ID NO: 181
Catalytic	TEQEINSLWSFDPEARSTISLSGPC	SEQ ID NO: 182
Translocation	NVEETYPGKLLLC	SEQ ID NO: 183
Translocation	Acetyl-CANQYEVRINSEGR	SEQ ID NO: 184
Translocation	VNTLNAAFFIQSLIC	SEQ ID NO: 185
Translocation	YAQLFSTGLNTIC	SEQ ID NO: 186
Translocation	CAGISAGIPSLVNNEL	SEQ ID NO: 187
Translocation	DDLVISEIDFNNNSIC	SEQ ID NO: 188
Translocation	MEGGSGHTVT	SEQ ID NO: 189
Translocation	AVNDTINVLPTITEGIPIVSTILDGINLGAAIKEL	

	SEQ ID NO: 190
Binding	CGFEYFAPANTDANNIEGQA SEQ ID NO: 191
Binding	CGYKYFAPANTVNDNIYGQA SEQ ID NO: 192
Binding	CKYYFNTNTAEA SEQ ID NO: 193
Binding	CKYYFDEDTAEA SEQ ID NO: 194

Expression and purification of *C. difficile* anti-toxin Mabs

Separate light chain and heavy chain mammalian expression plasmids were combined in equimolar ratios and used to transfect HEK-293 or CHO-S cells. For small scale expression studies lipofectamine and HEK-293 cells were used whereas for production of larger batches of IgG electroporation into CHO-S was preferred.

Culture supernatants were loaded onto a MabSelect SuRe column (in PBS pH 7.4). Antibody was eluted with 100% 0.1M Sodium Citrate pH 3.4 buffer. Samples were neutralized to pH7.4 with Tris.Cl pH8.0. Aggregate was removed by Superdex 200 Gel Filtration column in PBS pH 7.4.

TABLE 3

Antibody	Cell type	Volume of SN (L)	Expression type	Amount purified (mg)
CA164_00997.g1_P3	CHO	10	Transient	755.93
CA164_00922.g1_P3	CHO	0.5	Transient	129.36
CA164_01125.g2_P3	CHO	10	Transient	498.96
CA164_01151.g4_P3	CHO	5	Transient	262.43

Example 1 In-vitro neutralization of TcdA activity by purified Mabs

All neutralisation screening assays were run in 96 well polystyrene plates. The assay uses CACO-2 cells grown, and screened in MEM + 20% FCS, 2mM Q, and NEAA. Any antibody combinations are at equal molar ratios unless stated otherwise. **Day 1:** Cells were plated @ 3000 per well in 50 µl media, and incubated for 24 hrs; **Day 2:** Purified samples of humanised Mab were added to 96 well round bottomed polypropylene sterile plates; Spike PP plates with toxin A at a concentration sufficient to generated the appropriate lethal dose i.e. LD_{80} or above and incubate for 1 hr, at 37oC; Add 50 µl of this mixture to cell plates and incubate for 96 hrs; **Day 5:** Add Methylene blue (0.5% Methylene Blue 50% ethanol); Incubate for 1 hr at room temperature; Lyse the cells with 1% N-Lauryl Sarcosine, and Read on the BIOTEK Synergy2 plate reader at 405nm. The results are shown in Fig 12 to 24. EC_{50} and % maximum neutralization of TcdA activity shown confirm that the selected antibodies have very high potencies as single agents. Combinations of 2 to 5 of these did not improve upon the best EC_{50}

or % maximum neutralization. Lack of any synergy when combining Mabs CA922, 923, 995, 997 and 1000 is an important observation and may be due to the fact the each antibody alone has exceptionally high levels of affinity and potency. Supporting data in Example 5 also show that some of the Mabs (e.g. CA997) are capable of binding to TcdA subdomains many times. Hence it seems probable that these 5 Mabs represent that the maximum affinity, potency and valency that is achievable when targeting the C-terminal cell binding domain of TcdA. The antibodies were also effective at neutralising very high toxin concentrations ranging from LD80 to greater than LD_{95} (LD_{max}) but some modest increases in EC_{50} (i.e. decreases in potency) were observed with very high levels of [TcdA]. These data are also surprising since others have shown substantial reductions in potency when testing elevated TcdA concentrations (20).

The high potency and affinity of the Mabs described here, e.g. for CA997; is not due solely to their high valency of binding. Others (20 and WO06/071877) describe anti-TcdA Mabs capable of binding up to 14 times. These Mabs only had affinities in the range 0.3 to 100nM and showed incomplete protection against TcdA mediated cell killing, alone (26-63% protection) or in pairs (31-73% protection). Hence it has been demon-strated that high valency of binding to TcdA does not necessarily invoke either high affinity of binding to or neutralisation of TcdA. Neither the affinities nor valency of binding to TcdA were described for Mab CDA-1 (18 and US7625559). Thus Mabs described herein to have surprising affinity, potency and valency.

TABLE 4 Anti TcdA 1, 2 & 3 Mab combinations at a single TcdA conc. (LD_{80})

Antibody	Final (highest) Mab conc.ng/ml	EC_{50}(ng/ml)
922	500	1.21
923	500	160.42
995	500	37.64
997	500	6.25
1000	500	19.73
922+923	500	3.58
922+925	500	3.326
922+997	500	2.88
922+1000	500	2.64
923+995	500	60.23
923+997	500	7.54
923+1000	500	9.24
995+997	500	7.29
995+1000	500	19.63
997+1000	500	4.46
922+923+995	500	4.72
922+923+997	500	3.23
922+923+1000	500	3.21
922+995+997	500	2.22
922+995+1000	500	2.85
922+997+1000	500	2.22
923+995+997	500	5.04
923+995+1000	500	9.49
995+997+1000	500	5.84
922+923+995+997	500	2.75
922+923+995+1000	500	3.75
922+995+997+1000	500	3.46
923+995+997+1000	500	4.81
922+923+997+1000	500	3.06
922+923+995+997+1000	500	4.72

TABLE 5 **Anti TcdA single, paired, and triplet Mab combinations at various TcdA concentrations, where TcdA is at its LD_{80}, LD_{90}, LD_{95} and LD_{max}.**

Toxin TcdA	Sample	Final Mab conc.ng/ml	EC_{50} (ng/ml)
@ 3000 pg/ml (LD_{MAX})	922	500	4.89
	997	500	10.99
	1000	500	50.17
	922+997	500	7.18
	922+1000	500	6.99
	997+1000	500	9.437
	922+997+1000	500	10.80
	922+997+1000+995	500	15.03
	922+997+1000+995+923	500	7.16
@ 1000 pg/ml (LD_{95})	922	500	1.24
	997	500	3.42
	1000	500	9.60
	922+997	500	1.85
	922+1000	500	2.51
	997+1000	500	3.61
	922+997+1000	500	2.40
	922+997+1000+995	500	2.74
	922+997+1000+995+923	500	2.38
@ 700 pg/ml (LD_{90})	922	500	0.84
	997	500	2.40
	1000	500	6.23
	922+997	500	1.19
	922+1000	500	1.33
	997+1000	500	2.68
	922+997+1000	500	1.84
	922+997+1000+995	500	2.17
	922+997+1000+995+923	500	2.06
@ 350 pg/ml (LD_{80})	922	500	0.39
	997	500	1.18
	1000	500	2.76
	922+997	500	0.67
	922+1000	500	0.85
	997+1000	500	2.06
	922+997+1000	500	0.83
	922+997+1000+995	500	0.97
	922+997+1000+995+923	500	0.98

Example 2 Anti TcdB *in-vitro* neutralization by purified Mab.

Assay methods description:

All neutralisation screening assays were run in 96 well polystyrene plates.

The assay uses CACO-2 cells grown, and screened in MEM + 20% FCS, 2mM Q, and NEAA.Unless stated all Ab combinations are in equal ratios.

- Day 1: Cells are plated @ 3000 per well in 50 µl media, and incubated for 24 hrs
- Day 2: Purified samples of humanised Mab were added to 96 well round bottomed polypropylene sterile plates
- Spike PP plates with toxin B lot # 031 and incubate for 1 hr, at 37oC
- Add 50 µl of this mixture to cell plates
- Incubate for 96 hrs
- Day 5: Add Methylene blue (0.5%Methylene Blue 50% ethanol)
- Incubate for 1 hr at room temperature
- Lyse the cells with 1% N-Lauryl Sarcosine
- Read on the BIOTEK Synergy2 plate reader at 405nm

The data in Figures 25 to 33 show that single Mabs alone were relatively ineffective at neutralizing TcdB, both in terms of % maximum neutralization and activity (EC_{50}). However, when the antibodies were combined in two's and three's considerable improvements in both % maximum neutralization and activity (EC_{50}) were observed. 1125 and 1151 were selected as a best pairing, although other good pairings were observed: 1125+1153, 1125+1134.

The most effective pairs of Mabs were selected empirically and were found retrospectively to make unexpected and surprising combinations when regarding the individual potencies of each Mab. For example, in Table 6 only CA927 had a TcdB neutralisation potential which could result in a defined EC_{50} whilst the TcdB neutralisation potential of both CA1125 and CA1151 were insufficient under these assay conditions to result in a defined EC_{50}. However, CA927 was not found to be the most effective Mab to use within a combination. The best CA927 containing combination had an EC_{50} of 13.5ng/ml whereas other two Mab combinations had EC_{50}'s as low as 2.59 and 4.71ng/ml. In another example, in Table 8 CA1099 had the lowest TcdB neutralisation EC_{50} under the assay conditions used. However, CA1099 was not found to be the most effective Mab to use within a combination. The best CA1099 containing combination had an EC_{50} of 6ng/ml whereas other two Mab combinations had EC_{50}'s as low as 2 and 1ng/ml. We might speculate that the most effective pairings of Mabs are defined by their cooperative binding modalities especially as defined by having non-overlapping epitopes.

TABLE 6 Anti-TcdB Mab combinations and relative Mab ratios at constant toxin concentration.

Sample	Final Mab conc.ng/ml	EC_{50}(ng/ml)
1125.g2	1000	>1000
1134.g5	1000	>1000
927.g2	1000	12.89
1153.g8	1000	>1000
1102.g4	1000	>1000
927+1099	1000	>1000
927+1102	1000	>1000
927+1114	1000	>111.111
927+1125	1000	13.55
927+1134	1000	51.58
1099+1114	1000	>1000
1102+1114	1000	>333.333
1102+1125	1000	15.51
1114+1134	1000	19.70
1114+1151	1000	25.69
1114+1153	1000	27.48
1125+1134	1000	2.59
1125+1151	1000	4.71
1125+1153	1000	21.23
1125+1134+1114	1000	3.77
1125+1134+927	1000	2.63
1125+1151+1114	1000	4.90
1125+1151+927	1000	5.69
1125.g2+1134.g5+927.g2	1000	5.83
1125.g2+1134.g5+1153.g8	1000	9.89
1125.g2+1134.g5+1102.g4	1000	2.72

Example 3 Neutralisation of TcdB by combinations of purified Mab.

All neutralisation screening assays were run in 96 well polystyrene plates.

The assay uses CACO-2 cells grown, and screened in MEM + 20% FCS, 2mM Q, and NEAA.

- Day 1: Cells are plated @ 3000 per well in 50 µl media, and incubated for 24 hrs
- Day 2: Purified samples of humanised Mab were added to 96 well round bottomed polypropylene sterile plates
- Spike PP plates with toxin B (VPI 10463) and incubate for 1 hr, at 37oC
- Add 50 µl of this mixture to cell plates
- Incubate for 72 hrs
- Day 5: Add Methylene blue (0.5%Methylene Blue 50% ETOH)
- Incubate for 1 hr at room temperature
- Lyse the cells with 1% N-Lauryl Sarcosine
- Read on the BIOTEK Synergy2 plate reader at 405nm

The results are shown in Figures 34 to 45.

These data show that the best pair of Mabs for neutralizing TcdB at a range of toxin concentrations was CA1125 and CA1151. Moreover, the 1125+1151 combination was largely unaffected by changes in the relative molar ratios which is in contrast to 1125+1153.

TABLE 7 Anti-TcdB Mab combinations and relative Mab ratios at 3 different toxin concs.

	EC50 values (ng/ml)		
Antibody combination	**TcdB LD60**	**TcdB LD77**	**TcdB LD85**
1125.g2 + 927.g2 (50:50)	2.8	6	11.3
1125.g2 + 1102.g4 (50:50)	4	13	44
1125.g2 + 1114.g8 (50:50)	3.5	7.1	25.4
1125.g2 + 1134.g5 (50:50)	0.48	1.4	4
1125.g2 + 1151.g4 (50:50)	0.85	0.85	1.5
1125.g2 + 1153.g8 (50:50)	2.7	5.2	25.2
1125.g2 + 1134.g5 (25:75)	<0.15	0.84	7.2
1125.g2 + 1151.g4 (25:75)	0.73	1	2.1
1125.g2 + 1153.g8 (25:75)	7	10	27
1125.g2 + 1134.g5 (75:25)	0.66	1.2	2.5
1125.g2 + 1151.g4 (75:25)	1.4	1.2	8.3
1125.g2 + 1153.g8 (75:25)	2.9	7.5	30

The data show that even the most active specific paired combinations have surprisingly and non-predictably different properties relative to each other. The EC_{50} of the preferred combination of CA1125 and CA1151 in equimolar ratios is largely unaffected by an increasing [TcdB]. The three

relative molar ratios of Mabs tested (i.e. 25:75 vs 50:50 vs 75:25) have very similar EC_{50}'s to each other, suggesting that CA1125 and CA1151 have especially complementary modes of action. This is in contrast to the combination of CA1125 with CA1134 where the increase in EC_{50} (i.e. reduction of potency) with higher [TcdB] is more substantial and where the three Mab molar ratios are not equally effective: The CA1125:CA1134 ratio of 25:75 is notably less potent than 50:50 and 75:25. This suggests that the combined potency of CA1125+CA1134 is more dependent upon the CA1125 component. The EC_{50} of all three molar combinations of CA1125 and CA1153 is substantially affected by increasing [TcdB] suggesting that CA1153 is a less suitable partner for combination with CA1125. *In toto*, these data show that CA1125 and CA1151 are a particularly favourable combination since the highest potency is maintained across a range of Mab and TcdB molar ratios.

TABLE 8 TcdB neutralisation – 1 or 2 anti-TcdB Mabs at constant toxin dose (LD_{80}).

Antibody	IC50 (ng/ml)
1099	2
1102	N/A
1114	103
1125	N/A
1134	8
1151	182
1153	260
926	N/A
927	N/A
1099 + 1125	6
1114 + 1125	7
1151 + 1125	2
1134 + 1125	1
1102 + 1125	6
1125 + 1153	12
926 + 1125	42
927 + 1125	4

TABLE 9 TcdB neutralisation – 1 or 2 anti-TcdB Mabs at various TcdB doses.

	EC50 values (ng/ml)			Maximum neutralisation		
Antibody combination	TcdB LD75	TcdB LD86	TcdB LD90	TcdB LD75	TcdB LD86	TcdB LD90
1125.g2	n/a	n/a	n/a	40%	25%	15%
1114.g8	n/a	n/a	n/a	45%	25%	15%
1134.g5	n/a	n/a	n/a	45%	25%	15%
1151.g4	n/a	n/a	n/a	45%	25%	20%
1153.g8	28.3	n/a	n/a	65%	35%	28%
1125.g2 + 1114.g8 (50:50)	10.1	243.8	n/a	85%	65%	40%
1125.g2 + 1134.g5 (50:50)	1.7	22.6	n/a	87%	60%	40%
1125.g2 + 1153.g8 (50:50)	6.1	32.2	n/a	95%	75%	48%
1125.g2 + 1151.g4 (50:50)	0.8	2.8	19.1	85%	80%	55%
1125.g2 + 1151.g4 (25:75)	1.2	2.8	47.2	85%	75%	60%
1125.g2 + 1151.g4 (75:25)	2.9	3.8	2.6	75%	70%	60%

These data show that combination of Mabs, especially CA1125 and CA1151 improve both the potency as measured by EC_{50} but also as measured by % maximum protection. The % maximum protection is of particular relevance in this assay method since the Mab:TcdB mixture is incubated with cells for a long time (72h). Since TcdB is toxic to Caco-2 cells in the range of pg/ml in 2-4h this measure may be considered to be a very difficult test of Mab neutralisation ability and may reflect the ability of Mab mixture with regard to their binding kinetics or modalities. In turn this may reflect the ability of Mab mixtures to protect against the effects of TcdB during an established infection when there may be substantial quantities of TcdB within tissues for many hours.
Selected data from Tables 6-9 are further illustrated in Figures 46-59.

Example 4 Valency of binding of Mabs to TcdB sub-domains.

The number of moles of binding events of anti-*C. difficile* TcdB antibodies to $TcdB_{1234}$ was determined by Surface Plasmon Resonance (SPR) on a Biacore 3000 (GE Healthcare). Streptavidin was immobilized on a CM5 sensor chip (GE Healthcare) to a level of ~4000RU via amine coupling and biotinylated $TcdB_{1234}$ was bound at 500-600RU. Two 20µl injections of the same anti-TcdB antibody mixtures (final concentration of each antibody was 500nM) were injected over this surface at 10µl/min and the saturating binding response recorded. The surface was regenerated after every cycle using HCl. All the data was corrected for background binding using the response to the streptavidin only reference flowcell.

Table 10: Surface plasmon resonance analysis of the number of IgG binding sites on $TcdB_{1234}$

Antibody combination	No. of binding cycle repeats	Binding Response (RU)	Binding relative to CA927 average response
CA1125.g2	10	750	0.9
CA1151.g4	10	1232	1.6
CA1125_CA1151	4	1941	2.5
CA1125_CA927	3	1570	2.0
CA1151_CA927	3	1959	2.5
CA927	8	791	1.0

All responses have been expressed relative to a multiple of CA927 average response (final column table 10) since CA927 appears to be representative of a Mab which binds to $TcdB_{1234}$ once only.

Immobilized CA1125, when bound to $TcdB_{1234}$, does not allow CA1125 to bind further supporting the idea that CA1125 has one binding site on $TcdB_{1234}$ and that after this has been saturated that no other binding site for CA1125 can be found. However, when $TcdB_{1234}$ has been saturated by CA1125, CA1151 can still bind. This demonstrates that CA1151 binds at alternative sites to that occupied by CA1125. Together these data show that CA1125 is a single binder of $TcdB_{1234}$ whereas 1151 IgG binds to $TcdB_{1234}$ more than once, most likely twice. Hence a mixture of CA1125 and CA1151 can bind to $TcdB_{1234}$ approximately 3 times.
All antibodies combinations have an additive binding response showing that there are 2 or more non-competitive sites on $TcdB_{1234}$ bound by these combinations.

Example 5 Valency of binding of Mabs to TcdA sub-domains.

The number of moles of binding events of anti-*C.difficile* TcdA antibodies to $TcdA_{123}$ and A_{456} were determined by Surface Plasmon Resonance (SPR) on a Biacore 3000 (GE Healthcare). Streptavidin was immobilized on a CM5 sensor chip (GE Healthcare) via amine coupling to a level of ~4000RU and biotinylated $TcdA_{123}$ was bound to one flowcell and $TcdA_{456}$ was bound to a different flowcell to a response of ~500RU. Two 30µl injections of the same anti-TcdA antibody at 1µM were injected over both flowcells at 10µl/min and the saturating binding response recorded. The surface was regenerated after every cycle using HCl. All the data was corrected for background binding using the response to the streptavidin only reference flowcell.

Table 11: SPR analysis of the binding responses of IgGs to immobilised $TcdA_{123}$ and $TcdA_{456}$

	CA997	CA1000	CA997/CA1000 ratio
TcdA123	1069	166	6
TcdA456	1285	407	3

Antibodies CA997 and CA1000 bind to $TcdA_{123}$ in a ratio of six CA997's to one CA1000 whereas they bind to $TcdA_{456}$ in a ratio of three CA997's to one CA1000 (Table 2).

The maximum antibody response for CA997, corrected for molecular weight and immobilized toxin level is similar for $TcdA_{123}$ and $TcdA_{456}$. This suggests that CA997 binds $TcdA_{456}$ six times and CA1000 binds twice to $TcdA_{456}$. Hence antibody CA997 likely binds to TcdA whole toxin (TcdA) approximately 12 times.

Overall CA997 binds six times or more to A_{123} and six times or more to A_{456}, whereas CA1000 binds at least once to A_{123} and twice to A_{456}.

Increased valency of binding to TcdA and TcdB may have two important effects *in vivo*. The first is that any Mab or Mab mixture which is capable of binding TcdB more than once will have increased potential to form inter-toxin binding events and hence immunoprecipitation. Immunoprecipitation can contribute to potency by reducing the solubility of toxin and forming very large macromolecular complexes which hence reduce the effective working concentration of toxin. Such large protein complexes may be taken up by macrophages and monocytes resident in the tissue and may contribute to an augmented host immune respone. Antigen:antibody complexes bearing Fc fragments have been specifically shown to be capable of priming a host immune respone against a gut pathogen (21). Also, soluble antigen:antibody complexes have been successfully used as a vaccine directed against the antigen in human clinical trials (22). In addition, immune decoration of toxin with Fc bearing IgG may contribute to immune clearance using normal mechanisms through the liver and spleen. In general, higher levels of Fc decoration of antigen lead to faster and more complete levels of clearance (23) Critically, it may be that presence of 2 or more Mab Fc domains per toxin, especially 3 Fc domains per toxin may represent a critical number of Fcs required for very rapid and substantial clearance of toxin (24). The anti-TcdA Mab CA997 is likely capable of binding to TcdA up to 12 times and the combination of CA1125 and CA1151 is likely capable of binding to TcdB 3 times. Hence the 3 Mab mixture is very likely to be capable of providing for these kinds of additional potency mechanisms *in vivo*.

Example 6 Mab neutralisation of loss of TEER caused by TcdA.

C.difficile monolayer integrity assay is performed using the Becton-Dickinson (BD) Caco-2 BioCoat HTS plate system.

Day 1 – Caco-2 cells seeded @ $2x10^5$/ml per well of the plate insert in 500µl Basal seeding medium (provided by BD). 35ml of Basal seeding medium added to the feeder tray. Cells incubated for 24 hours at 37°C. **Day 2** – Basal seeding medium removed from inserts and feeder tray, and replaced with Entero-STIM differentiation medium (supplied by BD). 500µl added per well insert and 35ml to the feeder tray. Cells incubate for a further 72hrs at 37°C. **Day 5** – Antibodies prepared at 2x concentration relative to that to be used in the assay well in a polypropylene plate and toxin A. Toxin A added to antibodies at a concentration of 125ng/ml and plate incubated for 1hr at 37°C. 1ml of Caco-2 growth medium (MEM + 20% FCS, 2mM Q, NEAA) added to each well of a standard 24-well TC plate. BioCoat insert plate transferred to the 24-well TC plate. Entero-STIM medium removed from inserts and replaced with 400µl of toxin:Ab mixture.

Loss of tight junctions between gut cells is the key early effect of TcdA on cell monolayers and gut tissue sections and is the primary cause of diarrhoea. Albumin and other serum proteins are lost into the gut lumen along with accompanying serum fluid. The loss of trans-epithelial electrical resistance in differentiated cultured cells which have formed a monolayer is a useful surrogate for the protection against the acute effects of TcdA. Three antibodies shown have good levels of protection against TEER loss, Figure 62. It is notable and surprising that the abilities of these Mabs in TEER assays do not reflect those seen in toxin neutralization as measured in a cell proliferation assay. CA922 has the best performance in a cell proliferation assay (EC_{50} = 1.21ng/ml) and yet this is considerably out-performed in the TEER assay by an antibody (CA1000) which has >10x lower potency in a cell proliferation assay (EC_{50} = 19.73ng/ml). CA997 had the best performance in the TEER assay since it had both high levels of protection and maintained this at the lower Mab concs. CA997 had a substantial potential to neutralize TEER loss with maximal inhibition approaching 80% and an EC_{50} of approximately 80ng/ml at 4h. These observations are unexpected since the Mabs in question all had high affinities for TcdA domains (CA922 ~4pM, CA997 ~132pM, CA1000 ~73pM). These data suggest that CA997 and CA1000 recognise epitopes important in TEER loss or neutralize TcdA by different mechanism to other Mabs. Furthermore, since CA1000 is estimated to bind to holotoxin twice (once in $TcdA_{123}$ and once in $TcdA_{456}$) CA1000 may define 'TEER critical' epitopes within the TcdA cell binding regions which might have particular value for defining vaccine immunogens. Results are shown in Figures 62.

Example 7 Affinity of anti-*C. difficile* toxin antibodies for sub-domains of TcdA and TcdB: $TcdA_{123}$, $TcdA_{456}$ and $TcdB_{1234}$.

Kinetic constants for the interactions of anti-*C. difficile* TcdA and TcdB antibodies were determined by surface plasmon resonance conducted on a BIAcore 3000 using CM5 sensor chips. All experiments were performed at 25°C. Affinipure $F(ab')_2$ fragment goat anti-human IgG, Fc fragment specific (Jackson ImmunoResearch) was immobilised on a CM5 Sensor Chip (GE) via amine coupling chemistry to a capture level of ≈7000 response units (RUs). HBS-EP buffer (10mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005 % Surfactant P20, Biacore AB) was used as the running buffer with a flow rate of 10 µL/min. A 10 µL injection of each antibody at 1ug/ml or lower was used for capture by the immobilised anti-human IgG, Fc. TcdA123, TcdA456 or TcdB1234 were titrated over captured purified antibodies at doubling dilutions from 12.5nM at a flow rate of 30 µL/min. For antibodies present in culture supernatants, a single concentration of 12.5nM of TcdA123 or TcdA456 and 50nM of TcdB1234 was passed over the antibodies at 30ul/min. Kinetics were calculated on n=2 The surface was regenerated at a flowrate of 10uL/min by two 10 µL injections of 40 mM HCl, and a 5 µL injection of 5 mM NaOH.

Double referenced background subtracted binding curves were analysed using the BIAevaluation software (version 3.2) following standard procedures. Kinetic parameters were determined from the fitting algorithm.

TABLE 12 Anti-TcdA Mab affinities and binding kinetics

	Antibody ID	ka (1/Ms)	kd (1/s)	KD (M)	KD(pM)	Material/Assay
TcdA123	CA164_00922.g1	1.09E+06	4.43E-06	4.06E-12	4.06	Purified Mab 5 point titration
	CA164_00923.g1	5.36E+05	3.47E-05	6.47E-11	64.7	
	CA164_00995.g1	No binding			No binding	
	CA164_00997.g1	7.84E+05	1.03E-04	1.32E-10	132	
	CA164_01000.g1	1.33E+05	9.78E-06	7.33E-11	73.3	
	CA164_00993.g1	9.00E+05	5.00E-06	5.56E-12	5.56	Supernatant 2x 1point titration
TcdA456	CA164_00922.g1	1.29E+06	3.33E-06	2.59E-12	2.59	Purified Mab 5 point titration
	CA164_00923.g1	6.16E+05	1.92E-04	3.12E-10	312	
	CA164_00995.g1	2.87E+05	3.42E-05	1.19E-10	119	
	CA164_00997.g1	9.21E+05	6.15E-05	6.68E-11	66.8	

	CA164_01000.g1	3.55E+05	2.98E-05	8.41E-11	84.1	
	CA164_00993.g1	1.25E+06	5.00E-06	4.00E-12	4.00	Supernatant 2x 1point titration

TABLE 13 Anti-TcdB Mab affinities and binding kinetics

	Antibody ID	ka (1/Ms)	kd (1/s)	KD(M)	KD (pM)	Material/Assay
TcdB1234	CA164_1125.g2	2.64E+05	3.23E-05	1.22E-10	122	Purified Mab 3 point titration
	CA164_1151.g4	7.49E+05	4.13E-04	5.51E-10	551	Purified Mab 3 point titration
	CA164_926.g1	1.38E+05	7.12E-05	5.16E-10	516	Supernatant 2x 1point titration
	CA164_927.g2	3.97E+05	3.61E-05	9.11E-11	91	Purified Mab 3 point titration
	CA164_1099.g2	5.24E+05	1.63E-05	3.10E-11	31	Purified Mab 3 point titration
	CA164_1102.g4	1.17E+05	3.78E-04	3.25E-09	3250	Supernatant 2x 1point titration
	CA164_1114.g2	2.87E+05	1.97E-03	6.87E-09	6870	Supernatant 2x 1point titration
	CA164_1114.g8	2.55E+05	1.85E-03	7.25E-09	7250	Supernatant 2x 1point titration
	CA164_1129.g1	1.89E+05	2.30E-04	1.22E-09	1220	Supernatant 2x 1point titration
	CA164_1134.g5	5.09E+05	2.45E-05	4.81E-11	48	Purified Mab 3 point titration
	CA164_1153.g8	1.43E+05	4.48E-05	3.14E-10	314	Purified Mab 3 point titration

The anti-TcdA affinities are particularly high compared to the published affinities of other Mabs. We demonstrate that affinities as low as 4pM are achievable. The preferred CA997 has an affinity of 132pM, CA1125 122pM and CA115 551pM. CA995 clearly shows that it does not bind to

CROPs A_{123} and hence that demonstrates that the Mab shown here have properties which are different from each other in surprising and unexpected ways. CA922, 923, 997 and 1000 do bind at least once to CROPs A123 and A456. Hence these 4 Mabs confirming that each must bind to holotoxin at least twice. We have been unable to derive affinities for the binding of these Mabs to holotoxin due to technical constraints. However, given the high affinities and valencies demonstrated for the anti-TcdA Mabs it is possible to speculate that the functional affinities against holotoxin may be even stronger than those illustrated for binding to toxin sub-domains.
The anti-TcdB Mabs also demonstrated strong affinities reaching as low as 31pM. In particular CA1125, 1151, 927, 1099, 1134 and 1153 show affinities which surpass those demonstrated by others.

Example 8 Biophysical characteristion of *C. difficile* anti-toxin humanised IgG1 Molecules.
Molecules analysed

Anti-TcdA IgG1:

CA164_00922.g1
CA164_0923.g1
CA164_0995.g1
CA164_0997.g1
CA164_01000.g1

Anti-TcdB IgG1

CA164_01125.g1
CA164_01125.g2
CA164_01134.g4
CA164_01134.g5
CA164_01134.g6
CA164_01102.g1
CA164_01102.g4
CA164_01151.g4

Antibody combinations need to be made up of Mabs having high levels of stability in order to mitigate potential risks of aggregation during long term storage. Thermal stability (Tm) is used as one measure. Of special value to Mab mixtures is measuring their propensity to aggregate due to physical stress such as agitation or shaking. Aggregates are undesirable components of drug

compositions since they may reduce storage life time and may pose a safety risk to patients at certain levels. The Tm data show that all 5 anti-TcdA Mabs have high Tm stability, whilst three (CA922, 923 and 997) have very high Tm's in the range of 79-81°C. Of the anti-TcdB Mabs tested all but two have very high Tm's. Of note is that CA997, CA1125 and CA1151 which were tested in the hamster infection study (Example 9) had very high Tm's (79.2°C, 79.3°C and 80.8°C respectively) which makes them suitable for use in a Mab mixture.

In the shaking aggregation assay, CA997 and 922 had the lowest propensity to aggregate of the 5 anti-TcdA Mabs. Similarly, CA115 and 1151 had the lowest aggregation propensities of the anti-TcdB Mabs. Hence the use of CA997, 1125 and 1151 as a Mab mixture may have special value since they are more likely to survive co-formulation and storage at high protein concentrations.

Estimation of isoelectric point (pI) by capillary IEF

Samples were prepared by mixing the following: 30ul Protein sample at 2mg/ml, 0.35% Methylcellulose, 4% pH3-10 ampholytes (Pharmalyte), synthetic pI markers (4.65 and 9.77), 1ul of each stock solution, and HPLC grade water to make up the final volume to 200ul. The mixture was then analysed using iCE280 IEF analyzer(pre-focusing at 1500V for 1 min followed by focusing at 3000V for 6mins). The calibrated electropherograms were then integrated using Empower software (from Waters)

Thermal stability (Tm) measured *via* Thermofluor assay.

This method uses Sypro orange fluorescent dye to monitor the unfolding process of protein domains. The dye binds to exposed hydrophobic regions that become exposed as a consequence of unfolding which results in a change to the emission spectrum.

The sample (5ul at 1mg/ml) is mixed with a 5ul of a stock solution of Sypro orange (30x) and the volume made up to 50ul with PBS,pH 7.40.

10ul aliquots of this solution is applied towells in a 384 well plate (n=4).

The plate is placed in a 7900HT fast real-time PCR system containing a heating device for accurate temperature control. The temperature is increased from 20°C to 99° C (Ramp rate of 1.1°C/min).A CCD device simultaneously monitors the fluorescence changes in the wells. An algorithm is used to process intensity data and take into account multiple transitions.

Stressing of samples by agitation.

During manufacture antibody samples are subjected to mechanical stress generated by processes such as pumping and filtration. This may cause denaturation and consequently aggregation due to exposure of the protein to air-liquid interfaces and shear forces resulting in the ultimate loss of

bioactivity. Stress by vortexing is a method to screen the robustness of the antibody samples for prediction of aggregation stability.

Both anti-TcdA and anti-TcdB IgG1 molecules were subjected to stress by agitation, by vortexing using an Eppendorf Thermomixer Comfort at 25 °C, 1400rpm. Sample size was 250uL, (x3 per sample) in a 1.5 mL conical Eppendorf-style capped tube (plastic), in PBS pH 7.4. Each sample was brought to a concentration of 1mg/ml (using extinction coefficient calculated from sequence) and aggregation was monitored by absorbance at 340nm and/or 595nm, by use of a Varian Cary 50-Bio spectrophotometer, measured at intervals for up to 24 hours.

Results Table 14 provides a summary of the measured pI and Tm data for both anti-TcdA and anti-TcdB IgG1 molecules.

Table 14 : Compilation of pI and Tm Data

	measured pI	**Tm(Fab) in PBS**	**Tm(CH2)**
Anti-TcdA IgG1			
CA164_00922.g1	8.8	81	69.2
CA164_0923.g1	9.2	79	69.3
CA164_0995.g1	8.5	71	no data*
CA164_0997.g1	8.3	79.2	68.4
CA164_01000.g1	7.74	70.5	no data*
Anti-TcdB IgG1			
CA164_01125.g1	9.2	79.3	69.4
CA164_01125.g2	9.2	79.5	69.3
CA164_01134.g4	9.3	78.4	69.4
CA164_01134.g5	9.2	76.4	69.2
CA164_01134.g6	9.2	76.6	69.6
CA164_01102.g1	9.1	69	no data*
CA164_01102.g4	9.1	69.1	no data*
CA164_01151.g4	9.2	80.8	69.8

*denotes that it was not possible to discern the Fab and CH2 domains.

Measured pI

The measured pI of the molecules were high (except for CA164_01000.g1_P3) and away from the pH of formulation buffers such as PBS, pH 7.4 and 50m sodium acetate/125mM sodium chloride,

pH 5. This may mean that buffers with pH's suitable for co-formulation of two or more Mabs can be selected.

Thermal Stability (Tm) Measured *via* Thermofluor assay

Since all of the molecules are IgG1, the Tm of the Fc domain (Tm(CH2)) are the same. The difference in thermal stability between the molecules can be determined by the Tm of the Fab' domain (Tm(Fab)).

For the anti-TcdA molecules, the rank order (most stable first) was CA922≥997>923>995>1000 and for the anti-TcdB molecules (most stable first) was CA1151.g4>1125.g1,g4>1134.g4>1134.g5≥1134.g6>1102.g1=1102.g4.

Stressing of samples by agitation

It was possible to determine different aggregation stability between the different antibodies, Figure 67 shows the effect of agitation *via* vortexing on different anti-TcdA IgG1 molecules in PBS,pH 7.4.

It was possible to determine a ranking order (most aggregation stable first) : CA922≥997>923≥995>1000

Figure 68 shows the effect of agitation *via* vortexing on different anti-TcdB molecules.

It was possible to rank the order of aggregation stability, such that the CA1125 grafts appeared more stable than the CA1134 molecules which were more stable than the CA1102 molecules.

A further study was performed to compare directly the aggregation stability of the anti-TcdB molecule (CA1151.g4) with the more stable molecule CA1125.g2 (see Figure 2) and more aggregation stable anti-TcdA molecules (CA922.g1 and CA997.g1). The results can be seen in Figure 69.

Further results for these 4 Mabs are also shown in Figures 67 and 68.

For the anti-TcdA molecules, CA922.g1 and CA977.g1, CA922 were preferable based on the analyses above, although apart from CA1000) all molecules could be considered suitable candidates for use as therapeutic IgG1.

For the anti- TcdB molecules, the biophysical characteristics could be grouped within the family of grafts based on the aggregation stability and Tm, such that the CA1125 grafts potentially proved more stable. The CA1102 grafts showed poorest Tm data and also showed the greatest tendancy to aggregate *via* stress by agitation.

A study using CA1151.g4 showed that this molecule exhibited slightly increased aggregation stability relative to CA11125.g2 and seemed equivalent to the TcdA molecules (CA922.g1 and

CA997.g1.All four molecules showed equivalent Tm values. CA997, CA1125 and CA1151 show very high levels of thermostability and very low levels of aggregate formation after agitation.

Example 9 Anti-*C. difficile* toxin Mab hamster infection study.

The hamster infection study was performed by Ricerca Biosciences LLC, Cleveland, Ohio, USA. The study protocol was approved by the Ricerca IACUC committee. Active and control components (composition and dose) were blinded to Ricerca staff until after completion of the planned 28 day study period.

Golden Syrian male hamsters (weight 82-103g, 54 days old) were individually housed in HEPA filtered disposable cages and fed Teklad Global Diet 2016 and water *ad libitum*. After acclimatisation, hamsters were pre-dosed (i.p.) with Mab mixtures or PBS (vehicle control) once a day for each of 4 days: days -3, -2, -1 and 0. Two doses of Mab were investigated: high dose = 50mg/kg each of anti-TcdA and anti-TcdB components and low dose 5mg/kg each of anti-TcdA and anti-TcdB components.

The drug combination tested was composed of one anti-TcdA antibody (CA997.g1) which constituted 50% of the injected protein and two anti-TcdB antibodies (CA1125.g2 and CA1151.g4) which together constituted 50% of the injected protein but which alone constituted 25% of the injected protein. Hamsters were sensitised (day -1) with 50mg/kg of Clindamycin phosphate in PBS (s.c.) before being challenged 1 day later (day 0) with 3.4 x 106 c.f.u. of vegetative cells from strain ATCC43596. Vancomycin was dosed at 5mg/kg twice a day for 5 days (p.o.) on days 1, 2, 3, 4, 5.

Viability checks were performed on animals twice a day, animals found to be *in extremis* were euthanised and counted as dead. Body weights were determined on each day of dosing, then twice weekly and before euthanising survivors. Gross necropsy was performed on all animals. Survival curves were created by the method of Kaplan and Meier. Survival curves were analysed using the P value from the log rank test compared to the Bonferroni corrected threshold of P = 0.005. The Vancomycin treated group were not included in the analysis. All statistical tests were done with Prism v5.04. All groups contained 11 animals, except the Vancomycin control group which contained 5 animals.

Survival curves can be seen in Figure 63. Hamsters receiving PBS (control) all died on days +2 and +3, whilst those receiving vancomycin treatment for 5 days all died on days +10 and +11. Hamsters receiving the high dose of UCB Mab mixture all survived until day +11, thereafter only two animals died until the end of the 28 day study. Hamsters receiving the low dose of UCB Mab

mixture all survived until day +3, thereafter animals were lost fairly steadily until day +16 when all had died. The data show exceptional levels and duration of protection when compared to published data for use of anti-toxin Mabs in hamsters (18). These in vivo data support the in vitro observations of very high level performance for neutralization and stability.

There is no apparent link between death and body weight during the acute phase (days 1-5) of the infection, Figures 64-65. Hence it may be supposed that hamsters die of overwhelming direct and indirect effects of TcdA and TcdB. Hamsters which survive the acute period due to partial protection (UCB low dose) of neutralizing Mabs lose weight, presumably due to gut damage and altered nutritional status. It was notable that many of the hamsters which went on to survive the 28 period of the study due to the protective effects of the UCB high dose Mabs recovered from weight loss and indeed even gained weight. This may be taken as evidence of the superior protective effects of the UCB Mabs enabling the gut to function as normal.

Table 15. Gross pathology scores

Group	Black caecum	Dark red caecum	Red caecum	Pink caecum	Normal caecum	Anogenital staining 'wet-tail'	Red small intestine
PBS control	1	9	1	0	0	1	1
UCB low	0	4	5	2	0	4	1
UCB high	0	0	1	1	9	3	0

It is clear that UCB Mabs were able to protect the large and small intestines from the bloody effusions caused by TcdA and TcdB.

The results are shown in Figures 63 to 66

The photographs in Figure 66 show typical gross pathologies for the swelling and bloody effusions of caeca caused by TcdA and TcdB (left image, PBS control, animal death on day 2) and a normal stool filled caeca after protection by UCB high dose Mabs (right image, UCB high dose, animal surviving to day 28). These data show that after protection with a high dose of UCB Mabs the large intestine can return to normal morphology and function.

Example 10 Neutralisation of TcdA from different ribotyped strains by purified Mab.

Clinical infections are caused by a variety of different strains. Strain differences are characterized using a number of different methods of which ribotyping is a key one. Different ribotype strains are observed to have different pathogenicity, infection and sporulation properties. All of the TcdA

neutralization shown above used TcdA purified from strain known as VPI10463. However, the predominant aggressively pathogenic strain associated with out-breaks is called ribotype 027. Other key ribotypes include 078, 001, 106. Amino acid sequence difference have been observed between toxins produced by different ribotypes and hence it is important that Mabs are capable of neutralizing toxin from a diverse set of clinical isolates. CA922, 997 and 1000 were tested for their ability to neutralize TcdA from strains 027 and 078 and compared to their abilities against TcdA from VPI10463. Mabs were tested at 4 [TcdA] and found to be capable of neutralizing all toxins without significant difference at LD_{80}, LD_{90} and LD_{95}

Table 16

	EC50 values (ng/ml) - TcdA strain VPI 10463			
Antibody	**LD80**	**LD90**	**LD95**	**LDmax**
CA164_922	0.27	0.9	1.2	>500
CA164_997	1	2.5	3.5	25.4
CA164_1000	3.6	13.5	19.3	>500

Table 17

	EC50 values (ng/ml) - TcdA ribotype 027			
Antibody	**LD80**	**LD90**	**LD95**	**LDmax**
CA164_922	0.19	0.25	0.41	1.46
CA164_997	0.92	1.27	1.75	7.19
CA164_1000	2.25	2.49	3.52	16.32

Table 18

	EC50 values (ng/ml) - TcdA ribotype 078			
Antibody	**LD80**	**LD90**	**LD95**	**LDmax**
CA164_922	0.11	0.12	0.25	0.68
CA164_997	0.33	0.64	1.11	2.57
CA164_1000	2.04	2.41	5.03	14.16

Example 11 PK data

A PK study of a human IgG1 (20mg/kg) in healthy hamsters. The hamster PK was found a half-life similar to Mabs in mice or rats. (t½ 6-8 days). i.p. and s.c. dosing were essentially the same.

The pharmacokinetics and distribution to the gut of a hIgG1 Mab was studied in 'normal' (non-infected) golden Syrian hamsters. Purified Mab was administered to male hamsters (120-135g) by CARE Research LLC, Fort Collins, Colorado, USA and samples were assayed by UCB Pharma. The study was approved by the CARE IACUC committee. Eight animals each received a single dose of 20 mg/kg of IgG1, four were dosed *i.p.*, four were dosed *s.c.* Blood was collected at 1, 3, 8, 24, 48, 72, 103 and 168 hours post-dose, serum was separated before storage at -80°C. Blood was also taken from two untreated hamsters in order to provide assay controls. Following euthanasia, a

2cm length of colon was cut from the caeca junction onwards from each hamster. The colon section was flushed with wash buffer (50% (v/v) PBS containing 50% (v/v) Sigma protease inhibitor cocktail (P2714) before being opened and separation and removal of the mucosa from the underlying muscle. Mucosal samples were placed in 0.5ml of wash buffer homogenized until visually uniform and stored at 4°C before immediate shipping on wet ice. For the anti-human IgG1 ELISA Nunc maxisorp 96 well plates were coated overnight in 0.1M $NaHCO_3$ pH 8.3 with Goat $F(ab')_2$ anti-human IgG-Fcγ fragment (Jackson 109-006-098), plates were washed with PBS-Tween (PBS/0.1% (v/v) Tween 20) and then blocked with 1.0% (w/v) BSA & 0.1% (v/v) Tween in PBS. Serum samples were diluted in sample-conjugate buffer (1% (w/v) BSA, 0.2% Tween in PBS) and after washing were revealed with goat anti-human kappa-HRP (Cambridge Bioscience 2060-05) in sample-conjugate buffer and TMB with a 2.5M H_2SO_4 stop solution.

Gut, Mucosa and Serum Levels:

Serum samples collected from blood taken at 168 hour time point and colon samples were removed after this.

20mg/kg IP at 168 hour

Sample	ng/mL per cm mucosa	serum µg/mL
1001	23.2	75.0
1002	13.7	90.8
1003	21.8	70.5
1004	53.8	119.4

20mg/kg SC at 168 hour

Sample	ng/mL per cm mucosa	serum µg/mL
2001	41.4	108.7
2002	62.1	76.6
2003	35.6	163.7
2004	37.3	153.3

Serum Data

		Hamster *i.p.*		Hamster *s.c.*	
		Mean	SE of mean	Mean	SE of mean
C_{max}:	µg/mL	202	12	186	21
T_{max}:	hr	36	7	76	16
$AUC_{(last)}$:	hr·µg/mL	22626	1378	22371	2258
$AUC_{(inf)}$:	hr·µg/mL	43287	7169	61290	17637
% Extrapolation:		43.7	9.2	54	11.7
CL/F	mL/hr/kg	0.50	0.07	0.43	0.13
MRT_{inf}	h	223	53	310	88
$t_{½,z}$:	h	149.2	36.9	188.5	61.9

The data is also shown in Figure 70 and 71

Hamster ID		Mean	SE
IP serum kinetics			
C_{max}:	µg/mL	202	12
T_{max}:	hr	36	7
$AUC_{(last)}$:	hr·µg/mL	22626	1378
$AUC_{(inf)}$:	hr·µg/mL	43287	7169
% Extrapolation:		43.7	9.2
CL/F	mL/hr/kg	0.50	0.07
MRT_{inf}	h	223	53
$t_{½,z}$:	h	149.2	36.9
SC serum kinetics			
Hamster ID		Mean	SE
C_{max}:	µg/mL	186	21
T_{max}:	hr	76	16

$AUC_{(last)}$:	hr·µg/mL	22371	2258
$AUC_{(inf)}$:	hr·µg/mL	61290	17637
% Extrapolation:		54	11.7
CL/F	mL/hr/kg	0.43	0.13
MRT_{inf}	h	310	88
$t_{½}$:	h	188.5	61.9

It was also shown that hIgG1 could be found in 'scrapings' of the gut i.e that hIgG1 gets into the vasculature of healthy gut – and so could be protective in 'prophylactic dosing'. This effect would be even more profound in humans since they have a cognate hFcRn.

Example 12 Serum Levels in Hamsters with C. difficile Infection

This study was to determine the serum concentration of CA725.0, CA726.0, CA997.g1 CA1125.g2, and CA01151.g4 following i.p. administration (various doses detailed below) in the Golden Syrian Hamster.

Humanised Mabs were quantified using liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis following tryptic digestion. Quantitation was achieved by comparison to authentic standard material spiked at known concentrations into blank matrix, with spiked horse myoglobin used as the internal standard.

A unique ("proteotypic") peptide common to all of the humanised Mabs investigated was selected (DTLMISR, a CH2 region peptide) and both samples and calibration samples were tryptically digested as outlined. Tryptic digest of 5 µl serum samples was performed overnight using sequencing grade modified Trypsin (Promega, Southampton, UK) following denaturation / reduction with acetonitrile / Tris (2-carboxyethyl) phosphine and carbamido-methylation with iodoacetamide (Sigma-Aldrich, Poole, UK).

The LC-MS/MS system consisted of a CTC HTS-x Autosampler (CTC Analytics, Zwingen, Switzerland), a Agilent 1290 LC system (Agilent Technologies, Stockport, UK) and a Sciex 5500 QTrap MS system (AB Sciex, Warrington, UK), equipped with a Turbo V ion source operated in electrospray mode. Analytes were separated using an Onyx Monolithic C18 column (100x4.6 mm, Phenomenex, Macclesfield, UK) with a gradient of 2 to 95 % (v/v) water/acetonitrile (0.1 %

formic acid) delivered at 1.5 mL/min over 6 minutes. The injection volume was 10 µL; all of the eluent was introduced into the mass spectrometer source. The source temperature of the mass spectrometer was maintained at 600 °C and other source parameters (e.g. collision energy, declustering potential, curtain gas pressure etc.) were optimized to achieve maximum sensitivity for the peptide of interest. Selective transitions for each proteotypic peptide of interest were monitored.

Unique ("proteotypic) peptides were selected for all of the analytes of interest; samples were analysed following tryptic digestion.

Plasma concentrations calculated based on the peptides monitored are outlined below.

For CA164_00997 and CA164_01151, interfering peaks were observed in the MRM traces. For this reason, these two analytes could not be quantified in the samples.

Total h-IgG was quantified in all samples using a peptide common to all analytes of interest. This was done using a combined standard curve of all five analytes. The validity of this approach is demonstrated by the fact that the sum of the concentrations observed for CA164_00725 and CA164_00726 are in good agreement (within experimental error) of the concentration observed for total h-IgG.

Using this approach, the total concentration of h-IgG in the samples of animals dosed with CA164_00997, CA164_01125 and CA164_01151 was determined.

Overall the data obtained indicate that the exposure of all five analytes of interest was similar for a given dose.

Study groups

Blinded labels		Actual Treatments	Dose days	Treatment components		
Grp	Treatment			Anti-toxin A	Anti-toxin B	
4	Treatment 3	Vehicle PBS 5mL/kg i.p.	3, -2, -1, 0			
2	Vancomycin	Vancomycin 5mg/kg b.i.d. p.o.	1, 2, 3, 4, 5			
1	Treatment 1	UCB LD* 5mg/kg A 5mg/kg i.p.	3, -2, -1, 0	CA997.g1_P3 5mg/kg	CA1125.g2_P3 2.5mg/kg	CA1151.g4_P3 2.5mg/kg
5	Treatment 4	UCB HD* 50mg/kg A 50mg/kg i.p.	3, -2, -1, 0	CA997.g1_P3 50mg/kg	CA1125.g2_P3 25mg/kg	CA1151.g4_P3 25mg/kg
6	Treatment 5	Competitor LD* 5mg/kg A 5mg/kg i.p.	3, -2, -1, 0	CA726_P3 5mg/kg	CA725_P3 5mg/kg	
3	Treatment 2	Competitor HD* 50mg/kg A 50mg/kg i.p.	3, -2, -1, 0	CA726_P3 50mg/kg	CA725_P3 50mg/kg	

Table 19

Group/time	Day	Animal No	Dose	Serum conc µg/mLtotal h-IgG
1	1	44	5 mg/kg 997, 2.5 mg/kg 1125, 2.5 mg/kg 1151	280
	1	45		302
	1	46		182
	6	45		61
	6	47		71
	6	49		45
3	1	60	50 mg/kg 725, 50 mg/kg 726	3040
	1	61		3330
	1	62		2990
	6	62		583
	6	63		913
	6	64		1240
	28	64		199
	28	65		36
4	1	71	Vehicle	nd
	1	72		nd
	1	73		nd
5	1	82	50 mg/kg 997, 25 mg/kg 1125, 25 mg/kg 1151	3050
	1	83		2790
	1	84		2370
	6	82		838
	6	83		645
	6	84		855
	28	82		116
	28	83		65
	28	84		66
	28	85		44
	28	86		101
	28	87		89

	28	88		27
	28	89		31
	28	90		66
6	1	93	5 mg/kg 725, 5 mg/kg 726	335
	1	94		322
	1	95		260
	6	200		103
	6	202		62
	6	203		79
	28	203		nd

nd - not detected (LOQ = 2.5 µg/mL for all analytes

na - not analysed: interference in the sample was observed for 997 and 1151

Table 20 Antibody CA725 is prior art antibody MDX1388. Antibody CA726 is prior art antibody CDA1 as described (15) A summary of this data is presented in Figure 72.

Group	**Caecal pathology**					**Small intestine pathology**	
	Black	Dark Red	Red	Pink	Normal	Dark Red	Red
PBS control	1	9	1	0	0	0	1
MDX high 50mg/Kg x4	0	1	4	4	2	1	0
UCB high 50mg/Kg x4	0	0	1	1	9	0	0

References

1. Kuehne, S et al., "The role of toxin A and toxin B in *Clostridium difficile* Infection" Nature (2010) 467: 711-713.
2. Davies AH et al., "Super toxins from a super bug: structure and function of *Clostridium difficile* toxins" Biochem. J (2011) 436: 517-526.
3. Rothman, S et al., "Differential Cytotoxic Effects of Toxins A and B Isolated from *Clostridium difficile*" Infect. Imm. (1984) 46: 324-331.
4. Du, T and Alfa, MJ "Translocation of *Clostridium difficile* toxin B across polarized Caco-2 cell monolayers is enhanced by toxin A" Can J Infect Dis. (2004) 15: 83-88.

5. Kim, Iaconis and Rolfe. "Immunization of Adult Hamsters against *Clostridium diffcile*-Associated Ileocecitis and Transfer of Protection to Infant Hamsters" Infect. Imm. (1987) 55:2984-2992
6. Rupnik JCM (2003) 41:1118-1125
7. Chaves-Olarte JBC (1999) 274:11046-11052.
8. Lylerly, DM et al., "Passive Immunization of Hamsters against Disease Caused by *Clostridium difficile* by Use of Bovine Immunoglobulin G Concentrate" Infection and Immunity (1991) 59:2215-2218.
9. Lylerly, DM et al., "Vaccination against Lethal *Clostridium difficile* Enterocolitis with a Nontoxic Recombinant Peptide of Toxin A" Current Microbiology (1990) 21:29-32.
10. Lylerly, DM et al., "Characterization of Toxins A and B of *Clostridium difficile* with Monoclonal Antibodies" Infect. Imm. (1986) 54:70-76.
11. Corthier et al., "Protection against Experimental Pseudomembranous Colitis in Gnotobiotic Mice by Use of Monoclonal Antibodies against *Clostridium difficile* Toxin A" Infect. Imm. (1991) 59: 1192-1195.
12. Kink JA and Williams JA, "Antibodies to Recombinant *Clostridium difficile* Toxins A and B Are an Effective Treatment and Prevent Relapse of *C. difficile*-Associated Disease in a Hamster Model of Infection" Infect. Imm. (1998) 66:2018-2025.
13. Ma D, et al., Progenics inc. ASM Poster 27th May 2010
14. Hansen, G and Demarest, SJ. WO 2006/0718877 A2
15. Babcock GJ, et al., "Human monoclonal antibodies directed against toxins A and B prevent *Clostridium difficile*-induced mortality in hamster" Infect. Imm.(2006) 74:6339-6347.
16. Lowy I et al., "Treatment with Monoclonal Antibodies against *Clostridium difficile* Toxins" NEJM (2010) 362: 197-205.
17. Zubler, R. H., Erard, F., Lees, R. K., Van, L. M., Mingari, C., Moretta, L. & MacDonald, H. R. (1985). Mutant EL-4 thymoma cells polyclonally activate murine and human B cells via direct cell interaction. J. Immunol. 134, 3662-3668
18. Babcook, J. S., Leslie, K. B., Olsen, O. A., Salmon, R. A. & Schrader, J. W. (1996). A novel strategy for generating monoclonal antibodies from single, isolated lymphocytes producing antibodies of defined specificities. Proc. Natl. Acad. Sci. U. S. A 93, 7843-7848
19. Tickle, S., Adams, R., Brown, D., Griffiths, M., Lightwood, D. & Lawson, A. (2010). High-Throughput Screening for High Affinity Antibodies ., pp. 303-307.
20. Demarest et al., mAbs (2010) 2:190-198

21. Yoshida et al., J. Clin. Invest. (2006) 116: 2142-2151
22. Xu et al., Vaccine (2005) 23:2658-2664.
23. Yousaf et al., Clin. Exp. Immunol. (1986) 66:654-660
24. Mannik et al., J. Exp. Med. (1971) 133: 713-739
25. Nusrat et al., Infection and Immunity (2001) 69:1329-1336
26. Lima et al., Infect Immun (1988) 56:582-588
27. Ravichandran et al J of Pharmacology and Experimental Therapeutics. (2006) 318: 1343-1351
28. Takahashi et al., (2009) Vaccine 27:2616-2619
29. Cohen et al., Infect. Cont. and Hosp. Epidem. (2010) 31: 431-455
30. Barbut et al., J. Clin. Microbiol. (2000) 38: 2386-2388
31. Wilcox et al., J. Hospital Infection (1998) 38: 93-100.

Claims

1. A monoclonal antibody specific to antigen TcdA or TcdB, wherein the antibody has high affinity for the target antigen and is suitable for reducing the duration and/or severity of diarrhoea , morbidity and/or mortality in a patient with *Clostridium difficile* infection or at risk of said infection.
2. A monoclonal antibody according to claim 1, wherein the antibody has high potency, for example an EC_{50} of 200ng/ml or less such as 150ng/ml or less, in particular 100ng/ml or less when toxin is used at an LD_{80} or higher.
3. A monoclonal antibody according to claim 2, wherein the antibody EC_{50} is between 0.1 and 10ng/ml when toxin is at an LD_{80} or higher.
4. A monoclonal antibody according to claim 2 or claim 3 wherein the maximal inhibition of toxin is between 50 and 100% when toxin is used at an LD_{80} or higher.
5. A monoclonal antibody according to any one of claims 1 to 4, wherein the antibody binds the target antigen multiple times.
6. A monoclonal antibody according to claim 5, wherein the antibody binds the target antigen 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 times or more.
7. A monoclonal antibody according to any one of claims 1 to 6, wherein the antibody is specific to TcdA.
8. A monoclonal antibody according to any one of claims 1 to 6, wherein the antibody is specific to TcdB.
9. A monoclonal antibody according to any one of claims 1 to 8, wherein the antibody has an affinity of 1nM or less, for example 600pM, such as 50 to 600pM.
10. A monoclonal antibody according to any one of claims 1 to 9, wherein the antibody is a neutralizing antibody including at high concentrations of toxin, in particular effective against ribotypes 003, 012, 027 and 078.
11. A monoclonal antibody according to any one of claims 1 to 10, wherein the antibody has an EC_{50} in a TEER assay in the range of 60 to 80ng/ml when measured at 4h after initiation of the assay.
12. A monoclonal antibody according to claim 1 which specifically binds TcdA comprising a heavy chain wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:44 for CDR-H1, a CDR having the sequence given in SEQ ID NO:45 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:46 for CDR-H3.

13. A monoclonal antibody according to claim 12 further comprising a light chain wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:41 for CDR-L1, a CDR having the sequence given in in SEQ ID NO:42 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:43 for CDR-L3.
14. A monoclonal antibody according to claim 13 having a heavy chain comprising the sequence given in SEQ ID NO:49 and a light chain comprising the sequence given in SEQ ID NO:47.
15. A monoclonal antibody according to claim 1 which specifically binds TcdA comprising a heavy chain wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:54 for CDR-H1, a CDR having the sequence given in SEQ ID NO:55 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:56 for CDR-H3.
16. A monoclonal antibody according to claim 15 further comprising a light chain wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:51 for CDR-L1, a CDR having the sequence given in in SEQ ID NO:52 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:53 for CDR-L3.
17. A monoclonal antibody according to claim 16 having a heavy chain comprising the sequence given in SEQ ID NO:59 and a light chain comprising the sequence given in SEQ ID NO:57.
18. A monoclonal antibody according to claim 1 which specifically binds TcdB comprising a heavy chain wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:124 for CDR-H1, a CDR having the sequence given in in SEQ ID NO:125 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:126 for CDR-H3.
19. A monoclonal antibody according to claim 18 further comprising a light chain wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:121 for CDR-L1, a CDR having the sequence given in in SEQ ID NO:122 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:123 for CDR-L3.
20. A monoclonal antibody according to claim 19 having a heavy chain comprising the sequence given in SEQ ID NO:129 and a light chain comprising the sequence given in SEQ ID NO:127.
21. A monoclonal antibody according to claim 1 which specifically binds TcdB comprising a heavy chain wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:154 for CDR-H1, a CDR having the sequence

given in in SEQ ID NO:155 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:156 for CDR-H3.

22. A monoclonal antibody according to claim 21 further comprising a light chain wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:151 for CDR-L1, a CDR having the sequence given in in SEQ ID NO:152 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:153 for CDR-L3.
23. A monoclonal antibody according to claim 22 having a heavy chain comprising the sequence given in SEQ ID NO:159 and a light chain comprising the sequence given in SEQ ID NO:157.
24. A monoclonal antibody according to claim 1 which specifically binds TcdA having a heavy chain and a light chain wherein the heavy chain variable region comprises a sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:19, SEQ ID NO:29 and SEQ ID NO:39 and the light chain variable region comprises a sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:17, SEQ ID NO:27 and SEQ ID NO:37.
25. A monoclonal antibody according to claim 1 which specifically binds TcdB having a heavy chain and a light chain wherein the heavy chain variable region comprises a sequence selected from the group consisting of SEQ ID NO:69, SEQ ID NO:79, SEQ ID NO:89, SEQ ID NO:99, SEQ ID NO:109, SEQ ID NO:119, SEQ ID NO:139, SEQ ID NO:149 and SEQ ID NO:159 and the light chain variable region comprises a sequence selected from the group consisting of SEQ ID NO:67, SEQ ID NO:77, SEQ ID NO:87, SEQ ID NO:97, SEQ ID NO:107, SEQ ID NO:117, SEQ ID NO:137, SEQ ID NO:147 and SEQ ID NO:157.
26. A pharmaceutical composition comprising one or more antibodies as defined in any one of claims 1 to 25.
27. A pharmaceutical composition according to claim 26, comprising two or more antibodies specific to TcdB.
28. A pharmaceutical composition according to claim 26, comprising two or more antibodies specific to TcdA.
29. A pharmaceutical composition according to claim 26, wherein at least one antibody in the composition is specific to TcdA and at least one antibody in the composition is specific to TcdB.
30. A pharmaceutical composition according to claim 29, wherein the composition further comprises at least a second antibody specific to TcdB.

31. A pharmaceutical composition according to claims 26 to 30, wherein the composition comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 distinct antibodies to the target antigen or antigens, such as 2, 3, 4, or 5 antibodies.
32. A pharmaceutical composition or mixture comprising the antibodies of claims 14, 20 and 23.
33. A pharmaceutical composition according to any one of claims 26 to 32, which further comprises a pharmaceutically acceptable excipient.
34. A monoclonal antibody according to any one of claims 1 to 25 or pharmaceutical composition according to claims 26 to 33, for use in treatment, for example the treatment or prophylaxis of *Clostridium difficile* infection or complications therefrom.
35. A method of treating a patient with a *Clostridium difficile* infection or at risk therefrom comprising administering a therapeutically effective amount of a monoclonal antibody according to any one of claims 1 to 25 or a pharmaceutical composition according to any one of claims 26 to 33.
36. A method of treatment according to claim 35, wherein the treatment is employed in combination with a further treatment for *Clostridium difficile* treatment, for example selected from the group comprising metronidazole, vancomycin, clindamycin, fidaxomicin and combinations thereof.
37. Use of an antibody as defined in any one of claims 1 to 25 or a composition as defined in any one of claims 26 to 33, for the manufacture of a medicament for the treatment or prophylaxis of *Clostridium difficile* infect or complications therefrom.
38. A method of selecting an antibody as defined in any one of claims 1 to 11 using assays to measure protection against loss of TEER (trans-epithelial electrical resistance).
39. A method of selecting an antibody as defined in any one of claims 1 to 11 using assays to measure thermal stability (Tm) and resistance to shaking aggregation.
40. A method of selecting an antibody mixture for the treatment of *Clostridium difficile* infections by combining measurements of toxin neutralization, TEER measurements, thermostability measurements (Tm), shaking aggregation measurements and isoelectric point (pI) suitable for coformulation.
41. A monoclonal antibody according to any one of claims 1 to 26 combined with toxoid or pharmaceutical composition comprising same, for example for use in vaccination, such as the treatment or prophylaxis of *Clostridium difficile* infection or complications therefrom.

Figure 1

SEQ ID NO: 8 polynucleotide sequence encoding anti-toxin A antibody 922.g1 VK (gL1)

GACCCTGTGA TGACCCAGAG TCCGAGCACT CTTTCTGCCT CCGTGGGAGA CCGCGTGACC
ATTACATGTC AGGCTTCACA AAGTATCTCC AATGCTCTGG CCTGGTATCA GCAGAAACCC
GGCAAAGCCC CTAAGCTGCT CATCTACTCT GCATCAAGCC TGGCTAGCGG CGTGCCAAGC
CGATTCAAGG GGAGCGGTTC TGGCACTGAG TTTACGCTGA CCATCAGTAG CTTGCAGCCT
GACGATTTTG CAACCTATTA CTGCCAGTAC ACACACTACT CCCATACATC TAAAAACCCA
TTCGGAGGGG GTACTAAGGT CGAAATAAAG

SEQ ID NO: 10 polynucleotide sequence encoding anti-toxin A antibody 922.g1 VH (gH1)

GAAGTGCAAT TGGTGGAAAG TGGCGGAGGA CTGGTGCAAC CCGGGGGTAG TCTGCGACTG
AGCTGTGCTG CCTCCGGCTT TACCATTAGC TCCTACTATA TGAGCTGGGT TCGACAGGCC
CCTGGAAAAG GACTCGAATG GATCGGCATC ATATCTTCCG GTGGGCATTT CACCTGGTAC
GCAAACTGGG CTAAGGGGAG ATTCACGATT AGCAGCGACT CCACAACCGT GTACCTGCAA
ATGAACAGCC TGAGGGATGA GGACACTGCC ACATATTTCT GCGCACGCGC TTACGTGAGC
GGAAGCTCAT TTAATGGCTA TGCACTGTGG GGGCAAGGAA CACTCGTGAC TGTCTCG

SEQ ID NO: 18 polynucleotide sequence encoding anti-toxin A antibody CA923.g1 gL1

GACGTCGTGATGACTCAGAGCCCATCTAGTCTGAGCGCTAGCGTCGGAGACCGAGTCACAATTACC
TGTCAAGCCTCCCAGAGCATCTCCAACTACCTGGCCTGGTACCAACAGAAACCTGGCAAGGTGCCC
AAGCTGCTGATCTATAGTGCTTCCACACTCGCAAGCGGCGTTCCGTCACGCTTTAAGGGATCTGGC
TCTGGCACTCAGTTCACCTTGACGATCTCAAGCCTGCAGCCAGAAGATGTGGCCACCTATTACTGC
CAGTATTCCCACTACGGGACTGGGGTGTTCGGTGCCTTTGGAGGTGGGACCAAAGTGGAGATAAAG

Figure 2

SEQ ID NO: 20 polynucleotide sequence encoding anti-toxin A antibody CA923.g1 gH1

GAAGTTCAACTTGTGGAATCTGGAGGCGGGCTCGTGCAGCCTGGTGGAAGCCTTAGACTGAGCTGC
GCTGCATCCGCATTTTCCCTGTCCAACTACTACATGAGCTGGGTGCGACAAGCACCAGGCAAGGGA
CTGGAATGGATTGGCATCATAAGCTCCGGTTCCAATGCCCTGAAATGGTACGCATCATGGCCGAAA
GGCCGCTTTACCATAAGCAAGGACTCCACCACCGTCTATCTGCAGATGAACTCATTGCGTGCCGAG
GACACTGCAACGTACTTCTGTGCTCGCAACTACGTGGGAAGCGGATCTTATTATGGCATGGATCTG
TGGGGACAAGGTACACTCGTGACCGTCTCG

SEQ ID NO: 28 polynucleotide sequence encoding anti-toxin A antibody CA993.g1 gL1

GATGTCGTGA TGACTCAGTC CCCCTCTACA TTGAGTGCCT CTGTCGGTGA TCGAGTTACC
ATCACCTGTC AAGCAAGCCA GAGCATCAGC TCCTACTTCT CTTGGTACCA GCAAAAGCCG
GGAAAAGCCC CTCAACTGCT GATTTATGGG GCCTCAACAC TGGCTTCTGG CGTGCCATCA
AGATTCAAGG GATCTGGCTC CGGCACTGAG CTTACACTGA CCATTAGCTC CCTGCAACCT
GACGATTTTG CTACCTACTA CTGCCAGTGC ACCGACTATA GTGGGATATA TTTCGGCGGA
TTTGGGGGAG GGACGAAAGT GGAAATCAAG

SEQ ID NO: 30 polynucleotide sequence encoding anti-toxin A antibody CA993.g1 gH1

GAAGTTCAGC TGGTCGAGAG CGGAGGCGGA CTGGTGCAAC CTGGTGGTAG CCTGAAACTC
TCTTGTACTG CCTCCGGGTT TTCCCTGAGC TCTTACTATA TGTCATGGGT GAGACAGGCT
CCCGGGAAAG GATTGGAATG GATCGGGATT ATCTCCTCCG GCTCTTCCAC CACTTTCACA
TGGTACGCCT CATGGGCAAA GGGGAGGTTT ACCATAAGCA AGACAAGCAC GACCGTGTAT
CTTCAGATGA ACTCCCTGAA GACGGAGGAT ACTGCCACCT ACTTTTGCGC TCGGGCCTAT
GTGGGCTCAA GCTCTTACTA TGGCTTCGAC CCATGGGGAC AGGGCACACT TGTGACCGTC
TCG

Figure 3

SEQ ID NO: 38 polynucleotide sequence encoding anti-toxin A antibody 995.g1 VL region

GACGTCGTGA TGACACAGAG CCCTTCAACA CTGTCTGCAA GCGTGGGCGA TAGGGTCACC
ATAACGTGCC AGGCCTCTCA ATCCATCAAC AACTATTTTA GCTGGTACCA GCAGAAGCCA
GGCAAGGCTC CGAAACTTCT GATCTACGGA GCTGCCAACC TGGCAAGTGG CGTGCCATCA
CGGTTCAAGG GATCCGGGAG CGGTACTGAG TATACCCTGA CCATTTCATC TCTCCAACCC
GACGATTTCG CCACCTACTC CTGCCAGAAT AATTACGGCG TGCACATCTA TGGAGCTGCC
TTTGGCGGTG GGACAAAAGT GGAAATTAAG

SEQ ID NO: 40 polynucleotide sequence encoding anti-toxin A antibody 995.g1 VH region

GAAGTTCAGC TGGTCGAGAG TGGGGGAGGG CTTGTGCAAC CTGGTGGCTC CCTCCGTCTG
AGCTGTACTG CTTCTGGATT CTCACTGAGC AATTACGACA TGATCTGGGT GCGACAGGCA
CCCGGCAAAG GACTGGAGTA CATTGGCTTC ATCAACACCG GGGGTATAAC GTACTATGCC
TCATGGGCTA AGGGGCGCTT TACAATTAGT AGGGATTCCT CTACCGTGTA CCTGCAGATG
AACTCACTGA GAGCCGAGGA CACTGCCACA TATTTCTGCG CTCGGGTGGA TGACTATATC
GGGGCCTGGG GCGCCGGATT GTGGGGCCAA GGAACACTGG TCACCGTCTC G

SEQ ID NO: 48 polynucleotide sequence encoding anti-toxin A antibody 997.g1 VL region

GCACTCGTGATGACACAGAGCCCGAGTAGCTTTAGTGCTTCAACCGGTGATAGGGTCACTATTACT
TGCCAAGCCTCTCAGAGTATATCTAGCTATCTGAGCTGGTACCAGCAAAAGCCCGGGAAGGCTCCT
AAACTGCTGATCTACCGGGCTTCCACATTGGCCTCCGGCGTTCCCTCACGCTTTAGCGGCTCCGGA
TCCGGAACCGAGTACACCCTGACTATCTCTTGCCTGCAATCTGAGGACTTCGCAACCTACTATTGT
CTGGGCGTCTACGGATATAGCAACGATGACGGGATCGCCTTCGGCGGCGGTACCAAAGTGGAAATT
AAG

Figure 4

SEQ ID NO: 50 polynucleotide sequence encoding anti-toxin A antibody 997.g1 VH region

GAGGTGCAACTTGTGGAAAGCGGGGGAGGACTGGTGCAGCCTGGGGGCTCATTGAGACTGAGCTGC
ACCGTTTCTGGTATTGACCTGAGCTCCCATCATATGTGCTGGGTGCGCCAGGCACCCGGAAAAGGA
CTGGAATACATCGGCGTCATATACCACTTTGGCTCTACATACTATGCCAACTGGGCAACTGGGCGA
TTCACAATTAGCAAGGACTCAACTACCGTTTACCTGCAAATGAATAGCCTGAGGGCTGAGGATACT
GCCACCTATTTCTGTGCCCGGGCTTCAATCGCCGGCTATTCTGCCTTTGATCCATGGGGGCAAGGA
ACACTCGTGACCGTCTCG

SEQ ID NO: 58 polynucleotide sequence encoding anti-toxin A antibody 1000.g1 VL region

GAAATCGTGA TGACGCAGTC ACCAAGCACA CTGAGCGCTT CTGTGGGAGA TCGGGTCACA
ATAACCTGTC AGGCCTCCCA GAGCATCTAC TCTTATCTGG CATGGTACCA GCAGAAGCCA
GGGAAAGCTC CCAAGCTGCT GATTTATGAC GCCAGCACTT TGGCTTCCGG TGTTCCTAGT
AGGTTCAAAG GCTCCGGAAG CGGTACCGAG TTTACCCTGA CCATCTCATC TCTGCAACCC
GATGACTTTG CCACATACTA TTGCCAGGGG AATGCCTACA CTTCCAACTC ACACGACAAC
GCATTCGGGG GAGGCACCAA AGTCGAAATT AAG

SEQ ID NO: 60 polynucleotide sequence encoding anti-toxin A antibody 1000.g1 VH region

GAAGTTCAGC TGGTCGAGAG CGGAGGGGGT TTGATTCAGC CCGGTGGCTC ACTTAGATTG
AGCTGCACCG TGTCCGGAAT CGATCTGTCA TCTGATGCCG TGGGCTGGGT GCGACAGGCA
CCTGGGAAAG GACTGGAGTA TATAGGGATC ATCGCCACCT TCGACTCCAC ATACTACGCT
AGCTGGGCAA AAGGGCGCTT TACGATTAGC AAGGCCTCCT CTACTACCGT GTACCTCCAA
ATGAACTCAC TGAGGGCCGA GGACACTGCC ACTTATTTCT GTGCTCGGAC CGGTAGCTGG
TACTACATCT CTGGCTGGGG CTCCTACTAT TATGGCATGG ACCTGTGGGG ACAGGGGACA
CTCGTGACCG TCTCG

Figure 5

SEQ ID NO: 68 polynucleotide sequence encoding anti-toxin B antibody 926.g1 VL region

GATACCGTGCTGACCCAGAGCCCTGCTACATTGTCACTGAGCCCCGGGGAGAGGGCCACATTGAGC
TGCCGGGCTTCAAAATCCGTGTCCACCCTCATGCACTGGTTTCAGCAAAAGCCCGGGCAGGCCCCA
AAACTGCTGATCTACCTCGCATCTAACCTTGAATCTGGCGTGCCGGCCCGCTTTAGTGGCTCCGGA
AGCGGAACCGACTTCACACTGACGATTAGCTCCCTGGAGCCTGAGGATTTCGCCGTGTACTATTGC
CAGCAAACTTGGAATGACCCTTGGACTTTCGGGGGCGGTACTAAGGTCGAAATAAAG

SEQ ID NO: 70 polynucleotide sequence encoding anti-toxin B antibody 926.g1 VH region

GAGGTGGAACTGCTCGAATCTGGTGGTGGGCTGGTGCAGCCCGGTGGATCTCTGAGATTGTCATGC
GAGGCATCCGGCTTTACCTTTTCCAACTACGGAATGGCCTGGGTGAGACAGGCCCCAACGAAGGGG
CTCGAATGGGTTACAAGCATCAGCTCTTCTGGGGGATCTACTTACTATCGCGATAGCGTCAAAGGC
CGGTTTACCATTAGCCGAGATAATGCCAAATCAAGCCTGTATCTGCAAATGAACAGCCTGAGGGCT
GAGGACACCGCCACATACTATTGTACAACCGTGATAAGGGGCTACGTGATGGACGCATGGGGACAG
GGGACATTGGTTACCGTCTCG

SEQ ID NO: 78 polynucleotide sequence encoding anti-toxin B antibody 927.g2 VL region

GACACACAGA TGACCCAGAG CCCATCCACT TTGTCTGCAT CCGTGGGCGA CCGAGTGACA
ATCACCTGTA GAGCAAGCGG TTCCGTGAGC ACACTGATGC ATTGGTACCA GCAGAAGCCT
GGGAAGGCTC CCAAGCTGCT GATCTACAAA GCCAGCAACC TTGCCTCCGG CGTTCCAAGC
CGGTTTAGCG GTTCCGGATC TGGAACCGAG TTCACCCTGA CCATATCAAG CCTGCAACCC
GACGACTTCG CCACCTACTA TTGCCACCAG AGCTGGAATA GCGACACGTT CGGGCAAGGC
ACAAGGCTGG AAATCAAA

SEQ ID NO: 80 polynucleotide sequence encoding anti-toxin B antibody 927.g2 VH region

GAGGTGCAAC TTGTGGAAAG CGGAGGGGGC GTGGTCCAAC CCGGAAGAAG TCTCCGTCTT
TCTTGCGCCG CAAGTGGCTT CACCTTTTCC AACTACGGAA TGGCCTGGGT TCGACAAGCT
CCTGGGAAAG GATTGGAGTG GGTGGCCACT ATCAACTATG ACGGACGCAC GACACACTAC
CGAGACTCTG TTAAGGGGCG CTTTACGATT TCCCGCGACA ATAGCAAGAG CACCCTCTAC
CTGCAAATGA ATAGCCTCCG GGCCGAGGAT ACTGCTGTGT ACTATTGTAC CTCCATCTCA
CGGAGCCACT ACTTCGATTG CTGGGGACAA GGCACACTCG TGACTGTCTCG

Figure 6

SEQ ID NO: 88 polynucleotide sequence encoding anti-toxin B antibody 1099.g2 VL region

GACGTCCAGC TCACTCAATC TCCCTCCTTT CTGTCTGCTT CTGTGGGCGA TCGCGTGACA
ATAACCTGCA AGGCCTCCAA ATCAATTAGC AACCATCTGG CATGGTATCA GGAGAAGCCT
GGCAAAGCCA ATAAGCTGCT GATCCACTCC GGCTCAACTC TGCAATCCGG TACCCCAAGC
CGATTTAGCG GATCTGGGAG CGGAACCGAG TTCACACTTA CCATTAGCTC CCTGCAACCG
GAGGACTTCG CCACCTATTA CTGCCAGCAA TACGACGAAT ACCCCTATAC GTTCGGCCAA
GGGACAAGAT TGGAAATCAA GCGTACG

SEQ ID NO: 90 polynucleotide sequence encoding anti-toxin B antibody 1099.g2 VH region

GAAGTTCAGC TGCAGGAATC TGGACCTGGC TTGGTGAAAC CAAGCGAGAC ACTTAGTCTC
ACTTGCACCG TTTCCGGCTT CTCCCTTCAA TCCTACACGA TCTCTTGGGT GCGGCAACCA
CCCGGGAAAG GACTGGAATG GATCGCAGCC ATTAGCGGGG GAGGGAGCAC CTATTACAAC
TTGCCTCTCA AGAGCCGCGT GACCATATCC CGTGACACAA GCAAGAGCCA GGTTTCCCTG
AAGCTGAGCT CCGTGACTGC TGCCGATACG GCTGTTTACT ATTGCACCCG ACCTCGCTGG
TATCCCCGTT CCTATTTCGA CTACTGGGGA AGAGGCACAC TGGTTACCGT CTCG

SEQ ID NO: 98 polynucleotide sequence encoding anti-toxin B antibody 1102.g4 VL region

AACATCGTGC TGACACAGTC TCCTGCAACC CTTTCACTGT CTCCAGGTGA ACGAGCAACC
CTGAGTTGTA GAGCCAGTCA GAGGATCTCC ACGAGCATTC ACTGGTATCA GCAAAAGCCT
GGGCAAGCTC CCAGACTCTT GATCAAGTAC GCCTCTCAGA GCATAAGTGG CATTCCAGCT
AGGTTTAGCG GCTCAGGCTC AGGAACAGAC TTCACTCTGA CCATCAGCTC CCTGGAACCG
GAGGACTTTG CCGTCTATTA CTGCCAGCAA TCCTACTCCA GTCTGTACAC CTTCGGGCAG
GGTACTAAAC TGGAGATAAA G

Figure 7

SEQ ID NO: 100 polynucleotide sequence encoding anti-toxin B antibody 1102.g4 VH region

GAAGTGCAGC TGGTCGAATC CGGGGGAGGT TTGGTGCAAC CAGGTGGCTC ACTGAGACTG
AGCTGTGCCG TTTCCGGCTT TACGTTCTCA GACAGTTATA TGGCCTGGGT GCGTCAAGCA
CCTGGAAAAG GGCTGGAGTG GATTGCCAGT ATCAGCTATG GTGGGACCAT AATCCAGTAC
GGCGATAGCG TCAAGGGCAG GTTTACTATC TCCAGGGACA ACGCCAAGTC AAGCCTTTAC
CTGCAGATGA ATTCTCTCCG CGCAGAGGAT ACCGCTGTGT ATTACTGCGC TAGACGGCAG
GGAACCTACG CTCGATACCT GGACTTCTGG GGTCAGGGAA CACTCGTTAC AGTCTCG

SEQ ID NO: 108 polynucleotide sequence encoding anti-toxin B antibody 1114.g2 VL region

GCGACGCAAA TGACTCAGTC GCCCTCATCG CTTAGCGCGT CCGTCGGAGA TAGAGTGACG
ATCACCTGCC GCGCATCAGA GTCGGTGTCC ACACTCCTCC ACTGGTATCA GCAGAAACCG
GGGAAGGCAC CAAAACTCTT GATCTACAAA GCCAGCAACC TTGCGTCCGG TGTCCCGTCA
AGGTTCTCCG GGAGCGGTTC GGGGACAGAC TTTACTTTGA CCATTTCGTC GCTTCAGCCG
GAGGACTTCG CCACCTATTA CTGTCATCAG TCATGGAACT CACCTCCCAC ATTTGGCCAG
GGAACGAAAC TCGAAATCAA G

SEQ ID NO: 110 polynucleotide sequence encoding anti-toxin B antibody 1114.g2 VH region

GAAGTACAAC TCGTAGAGTC AGGGGGTGGG CTGGTCCAAC CTGGCGGCTC CCTTCGGCTT
TCGTGTGCCG CCTCGGGATT CACGTTTAGC AATTACGGTA TGGCCTGGGT GAGGCAGGCA
CCAGGGAAGG GTCTTGAGTG GGTAGCGATC ATCAACTATG ATGCAAGCAC CACCCACTAC
AGGGATAGCG TCAAGGGACG CTTTACTATC AGCCGGGATA ATGCGAAATC CTCGCTCTAT
CTGCAGATGA ACTCCCTCAG AGCCGAGGAC ACCGCAGTGT ACTATTGCAC ACGATACGGA
CGCTCGCACT ATTTCGACTA TTGGGGACAG GGGACGCTCG TAACTGTCTC G

Figure 8

SEQ ID NO: 118 polynucleotide sequence encoding anti-toxin B antibody 1114.g8 VL region

GACACGGTCC TGACTCAGTC GCCCTCATCG CTTAGCGCGT CCGTCGGAGA TAGAGTGACG
ATCACCTGCC GCGCATCAGA GTCGGTGTCC ACACTCCTCC ACTGGTATCA GCAGAAACCG
GGGAAGGCAC CAAAACTCTT GATCTACAAA GCCAGCAACC TTGCGTCCGG TGTCCCGTCA
AGGTTCTCCG GGAGCGGTTC GGGGACAGAC TTTACTTTGA CCATTTCGTC GCTTCAGCCG
GAGGACTTCG CCACCTATTA CTGTCATCAG TCATGGAACT CACCTCCCAC ATTTGGCCAG
GGAACGAAAC TCGAAATCAA G

SEQ ID NO: 120 polynucleotide sequence encoding anti-toxin B antibody 1114.g8 VH region

GAAGTACAAC TCGTAGAGTC AGGGGGTGGG CTGGTCCAAC CTGGCGGCTC CCTTCGGCTT
TCGTGTGCCG CCTCGGGATT CACGTTTAGC AATTACGGTA TGGCCTGGGT GAGGCAGGCA
CCAGGGAAGG GTCTTGAGTG GGTAGCGATC ATCAACTATG ATGCAAGCAC CACCCACTAC
AGGGATAGCG TCAAGGGACG CTTTACTATC AGCCGGGATA ATGCGAAATC CTCGCTCTAT
CTGCAGATGA ACTCCCTCAG AGCCGAGGAC ACCGCAGTGT ACTATTGCAC ACGATACGGA
CGCTCGCACT ATTTCGACTA TTGGGGACAG GGGACGCTCG TAACTGTCTC G

SEQ ID NO: 128 polynucleotide sequence encoding anti-toxin B antibody 1125.g2 VL region

GATATACAAA TGACTCAGAG CCCTAGCTCA CTGAGCGCTT CTGTGGGCGA TCGTGTGACA
ATCACTTGCA AAGCAAGCCA GAACATCTAT ATGTACCTGA ATTGGTACCA GCAAAAACCG
GGAAAAGCTC CCAAGCGCCT GATTTACAAC ACCAATAAGC TGCATACCGG CGTGCCAAGC
CGTTTTAGCG GATCTGGCTC TGGAACCGAA TATACACTGA CCATAAGCTC CCTGCAACCG
GAAGACTTTG CAACTTACTA TTGCCTCCAG CACAAATCCT TCCCCTATAC GTTCGGACAA
GGGACCAAAC TGGAAATCAA A

SEQ ID NO: 130 polynucleotide sequence encoding anti-toxin B antibody 1125.g2 VH region

GAAGTGCAGC TGGTCGAAAG CGGCGGAGGA TTGGTGCAAC CTGGTGGCTC TCTTCGCCTG
TCTTGCGCTG CAAGCGGCTT TACGTTCCGC GATAGCTTTA TGGCTTGGGT GCGACAAGCT
CCTGGGAAAG GGCTGGAATG GGTCGCTAGC ATAAGCTACG AAGGCGACAA GACTTACTAT
GGGGACTCTG TGAAAGGCCG ATTCACCATT AGCCGAGACA ACGCAAAGAA CTCCCTGTAC
CTGCAGATGA ACTCCCTGCG TGCCGAAGAT ACCGCCGTGT ACTATTGCGC TAGGCTGACG
ATCACTACAA GCGGAGATAG CTGGGGACAA GGGACAATGG TGACCGTCTC GAGC

SEQ ID NO: 138 polynucleotide sequence encoding anti-toxin B antibody 1129.g1 VL region

GACACCCAGA TGACTCAGTC TCCGTCAAGC CTTTCTGCCT CTGTTGGAGA TCGAGTCACA
ATTACGTGCA AGGCAAGCCA ACACGTGGGT ACCAACGTGG ACTGGTATCA ACAGAAGCCA
GGGAAGGTCC CCAAACTGCT GATCTACGGT GCCAGTATTC GCTATACCGG CGTGCCTGAT
CGCTTCACCG GAAGCGGGTC AGGGACCGAT TTCACACTGA CAATCAGCTC CCTGCAACCT
GAAGACGTGG CTACTTACTA CTGCCTGCAG TACAACTATA ATCCCTACAC CTTTGGCCAG
GGCACCAAAC TGGAGATAAA G

SEQ ID NO: 140 polynucleotide sequence encoding anti-toxin B antibody 1129.g1 VH region

GAGGTGCAAC TTGTGGAATC AGGAGGTGGC GTGGTTCAGC CCGGTAGATC ACTTCGTCTG
AGTTGTGCAA CAAGCGGCTT TATCTTCTCC AACTTCGGGA TGTCTTGGGT TAGACAGGCT
CCTGGTAAGG GCCTCGAATG GGTGGCTAGT ATTAGCCCAA GCGGGGGAAA CGCCTACTAT
AGGGACAGCG TGAAAGGACG CTTCACTATC AGCCGAGATA ACTCCAAGAC CACGCTGTAT
CTGCAGATGA ATAGTCTGAG GGCCGAGGAT ACCGCAGTGT ACTACTGCAC TCGACGGGCC
TATTCTTCCC CTTTTGCCTT TTGGGGACAG GGGACTCTGG TGACAGTCTC GAGC

Figure 9

SEQ ID NO: 148 polynucleotide sequence encoding anti-toxin B antibody 1134.g5 VL region

GACGTCCAGC TCACTCAATC TCCCTCCTTT CTGTCTGCTT CTGTGGGCGA TCGCGTGACA
ATAACCTGCA AGGCCTCCAA ATCAATTAGC AACCATCTGG CATGGTATCA GGAGAAGCCT
GGCAAAGCCA ATAAGCTGCT GATCCACTCC GGCTCAACTC TGCAACCCGG TACCCCAAGC
CGATTTAGCG GATCTGGGAG CGGAACCGAG TTCACACTTA CCATTAGCTC CCTGCAACCG
GAGGACTTCG CCACCTATTA CTGCCAGCAA TACGACGAAT ACCCCTATAC GTTCGGCCAA
GGGACAAGAT TGGAAATCAA G

SEQ ID NO: 150 polynucleotide sequence encoding anti-toxin B antibody 1134.g5 VH region

GAAGTTCAGC TGCAGGAATC TGGACCTGGC TTGGTGAAAC CAAGCGAGAC ACTTAGTCTC
ACTTGCACCG TTTCCGGCTT CTCCCTTAAT TCCTACACGA TCACTTGGGT GCGGCAACCA
CCCGGGAAAG GACTGGAATG GATCGCAGCC ATTAGCGGGG GAGGGAGCAC CTATTTCAAC
TCGGCTCTCA AGAGCCGCGT GACCATATCC CGTGACACAA GCAAGAGCCA GGTTTCCCTG
AAGCTGAGCT CCGTGACTGC TGCCGATACG GCTGTTTACT ATTGCACCCG ACCTCGCTGG
TATCCCCGTT CCTATTTCGA CTACTGGGGA AGAGGCACAC TGGTTACCGT CTCG

SEQ ID NO: 158 polynucleotide sequence encoding anti-toxin B antibody 1151.g4 VL region

GCGATTCAAA TGACTCAGTC GCCCTCATCG CTTAGCGCGT CCGTCGGAGA TAGAGTGACG
ATCACGTGCA AAGCATCACA AAATGTCGGG AACAATGTGG CATGGTATCA GCATAAACCG
GGGAAGGCAC CAAAACTCTT GATCTACTAC GCCAGCAACA GGTTTACTGG TGTCCCGTCA
AGGTTCACGG GAGGGGGTTA CGGGACAGAC TTTACTTTGA CCATTTCGTC GCTTCAGCCG
GAGGACTTCG CCACCTATTA CTGTCAGAGG GTCTACCAGT CAACGTGGAC ATTTGGCCAG
GGAACGAAAG TGGAAATCAA G

Figure 10

SEQ ID NO: 160 polynucleotide sequence encoding anti-toxin B antibody 1151.g4 VH region

GAAGTACAAC TCCAAGAGTC GGGGCCTGGT CTGGTCAAGC CGTCCGAAAC ACTTTCGCTG
ACGTGTACGG TATCAGGATT CTCACTTACA TCATACTACG TCCACTGGGT GAGGCAGCCA
CCCGGGAAGG GTCTTGAGTG GATGGGCTGC ATTAGAACCG GAGGGAATAC CGAGTACCAG
AGCGAATTTA AGAGCCGCGT CACTATCAGC CGGGATACGT CCAAAAACCA GGTGTCGCTC
AAATTGTCCT CCGTGACGGC CGCTGACACC GCAGTGTACT ATTGCGCGCG AGGAAACTAT
GGCTTTGCGT ATTGGGGACA GGGGACGCTC GTAACTGTCT CG

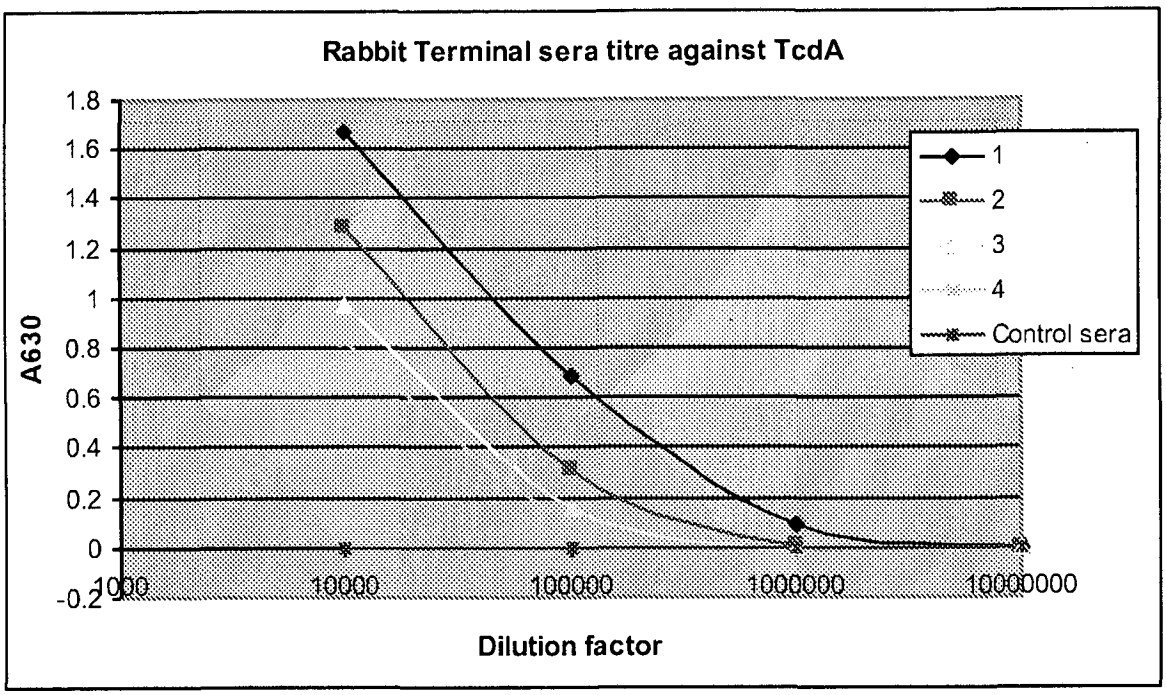
SEQ ID NO: 168 polynucleotide sequence encoding anti-toxin B antibody 1153.g8 VL region

GATATACAGA TGACTCAGTC CCCTTCTAGC CTTTCAGCTT CCGTGGGCGA TAGAGTGACT
ATCACGTGTA AGGCTAGTCA GAACATTAAC AAGTATCTGG ACTGGTACCA GCAGAAACCC
GGGAAGGTTC CCAAGCTGCT GATCTACAAC ATCCAGTCCC TGCATACAGG CATTCCTAGC
CGGTTTAGCG GATCTGGTTC AGGGACCGAC TTCACCCTGA CAATCAGCTC TCTGCAACCA
GAAGACGTGG CCACCTATTA CTGCTTCCAG CACAATAGTG GCTGGACTTT TGGACAAGGT
ACCAGGCTGG AGATCAAA

SEQ ID NO: 170 polynucleotide sequence encoding anti-toxin B antibody 1153.g8 VH region

GAGGTTCAGC TGGTGGAATC AGGAGGGGGT CTGGTGCAAC CAGGAGGCTC CCTGAAACTG
TCTTGCGCCG CAAGCGGCTT TACGTTTACC CAGGCCGCTA TGTTCTGGGT TAGGCAGGCC
AGTGGGAAGG GTCTTGAAGG CATCGCAAGA ATCAGCACCA AGAGCAACAA TTTCGCTACG
TACTATCCGG ACTCCGTGAA AGGCCGGTTT ACCATTTCTC GCGATGACAG CAAGAACACC
GTGTACCTGC AGATGAACAG TCTCAAGACC GAGGACACAG CCGTGTACTA TTGTACTGCT
CCCGCCTATT ATTACGATGG CACAGTGCCT TTCGCATACT GGGGACAGGG
TACTTTGGTG ACTGTCTCG

Figure 11
Sera titres from 4 rabbits immunised with TcdA toxoid and 5 rats immunised with TcdB binding domain (TcdB1234). ELISA data generated using TcdA toxin or TcdB binding domain coated on an ELISA plate



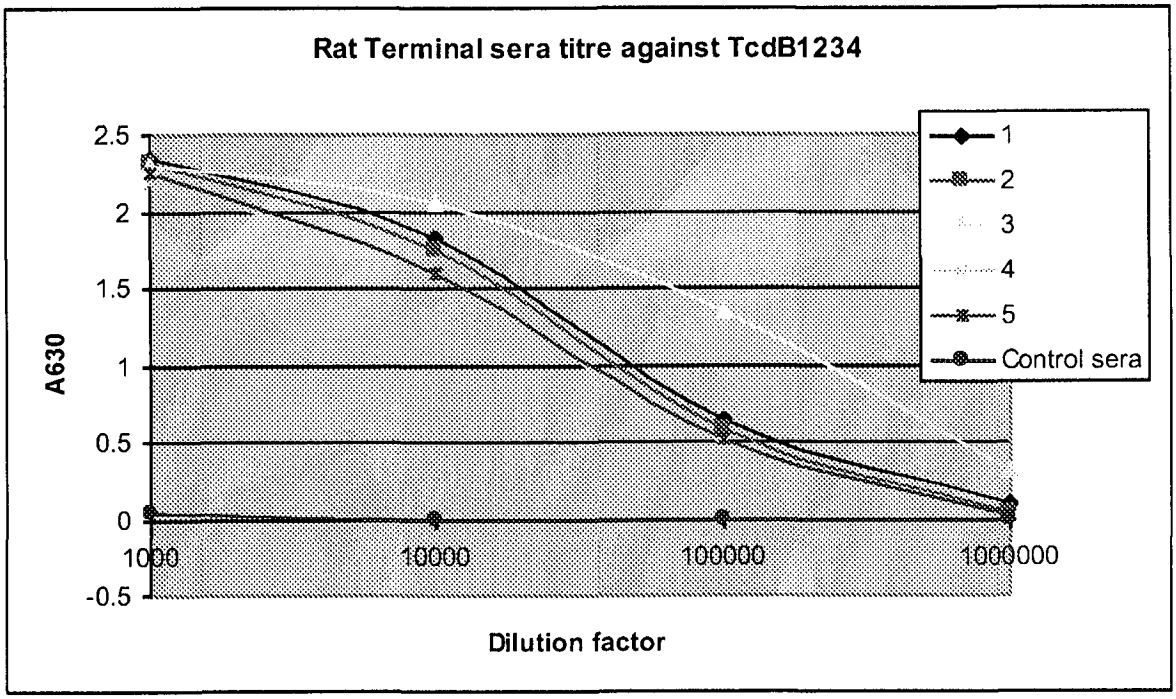


Figure 12 Anti TcdA (Ribotype 003) in-vitro neutralization data for single Mabs (X axis conc. (ng/ml) and Y axis % Neutralization)

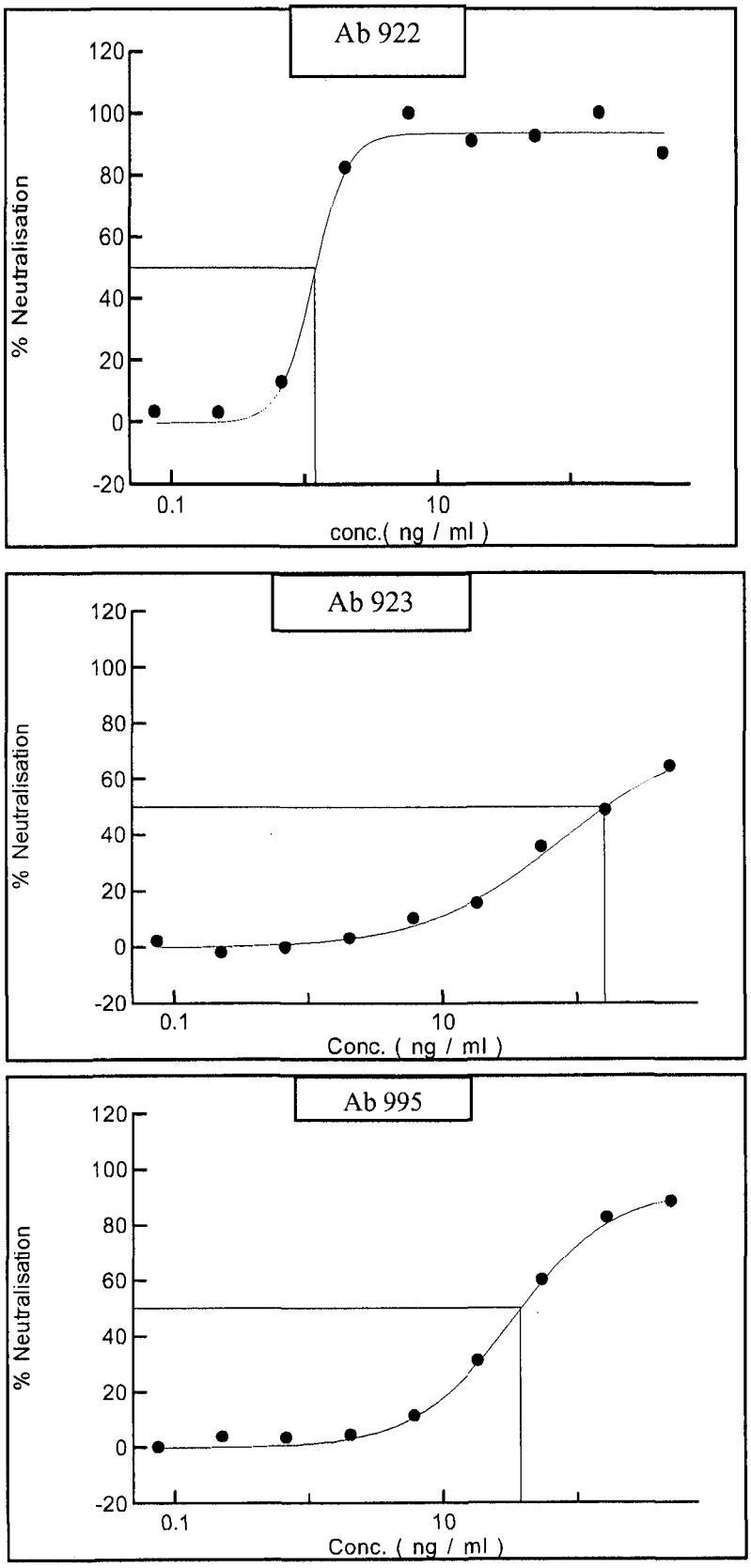


Figure 13 Anti TcdA (Ribotype 003) in-vitro neutralization data for single Mabs (X axis conc. (ng/ml) and Y axis % Neutralization)

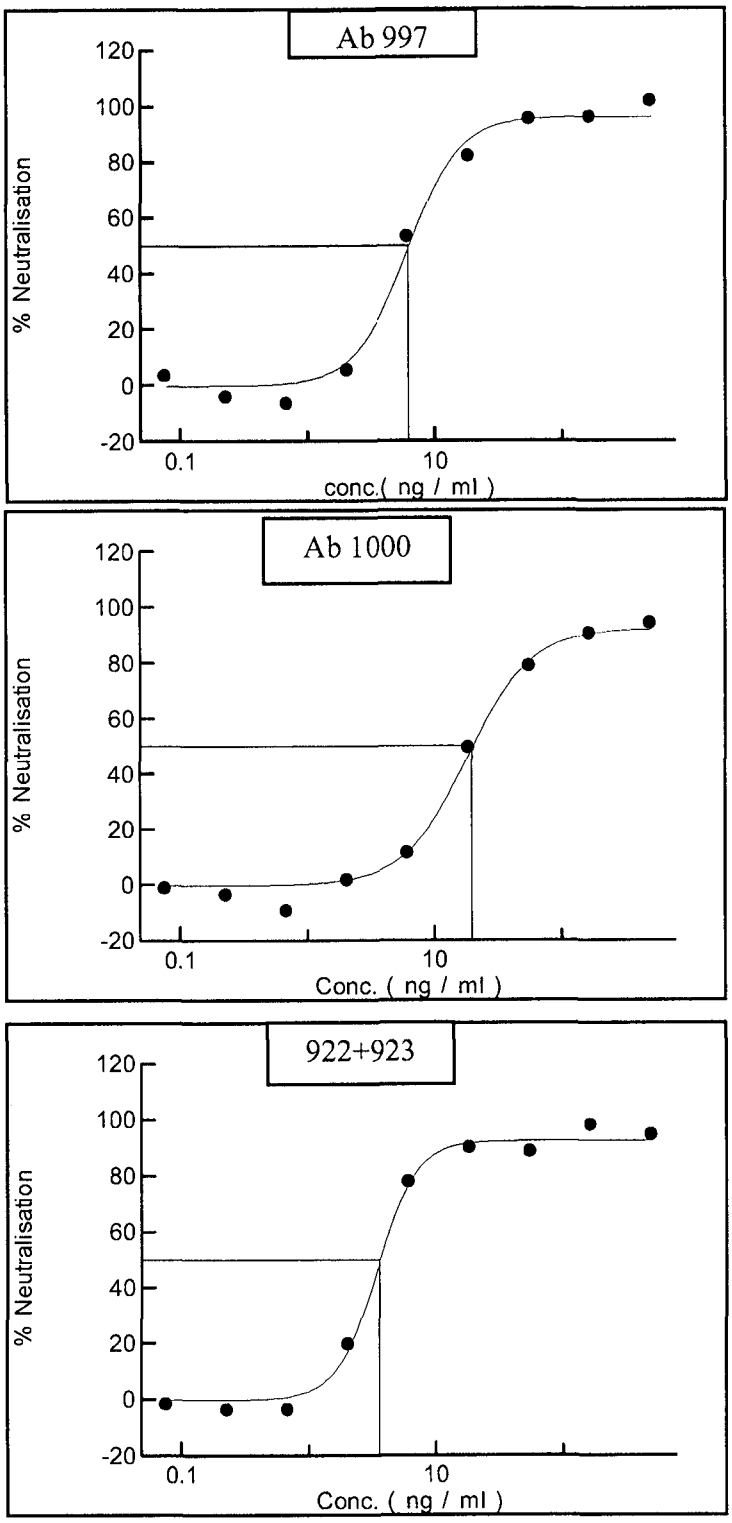


Figure 14 Anti TcdA (Ribotype 003) in-vitro neutralization data for paired Mabs (X axis conc. (ng/ml) and Y axis % Neutralization)

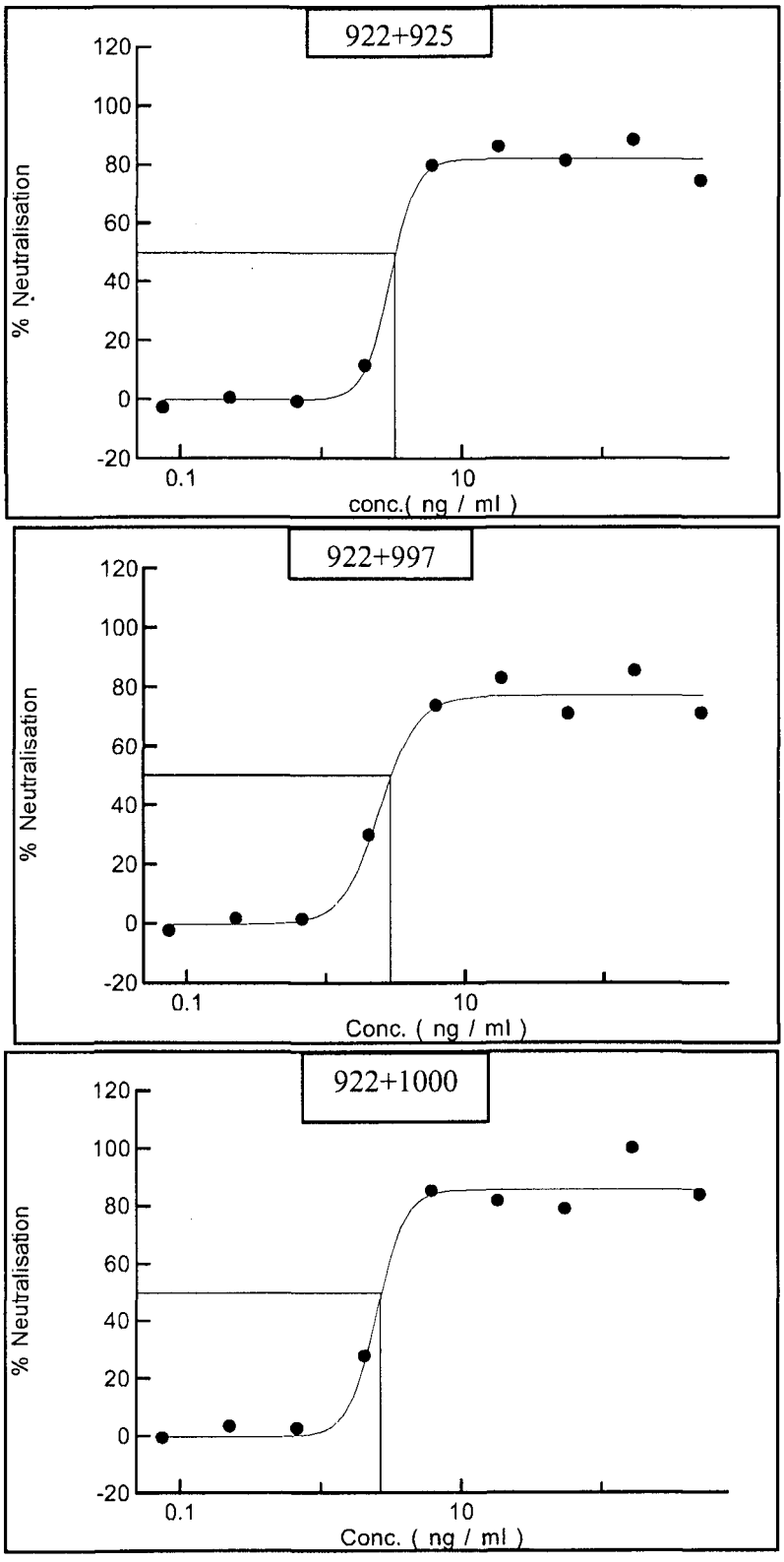


Figure 15 Anti TcdA (Ribotype 003) in-vitro neutralization data for paired Mabs (X axis conc. (ng/ml) and Y axis % Neutralization)

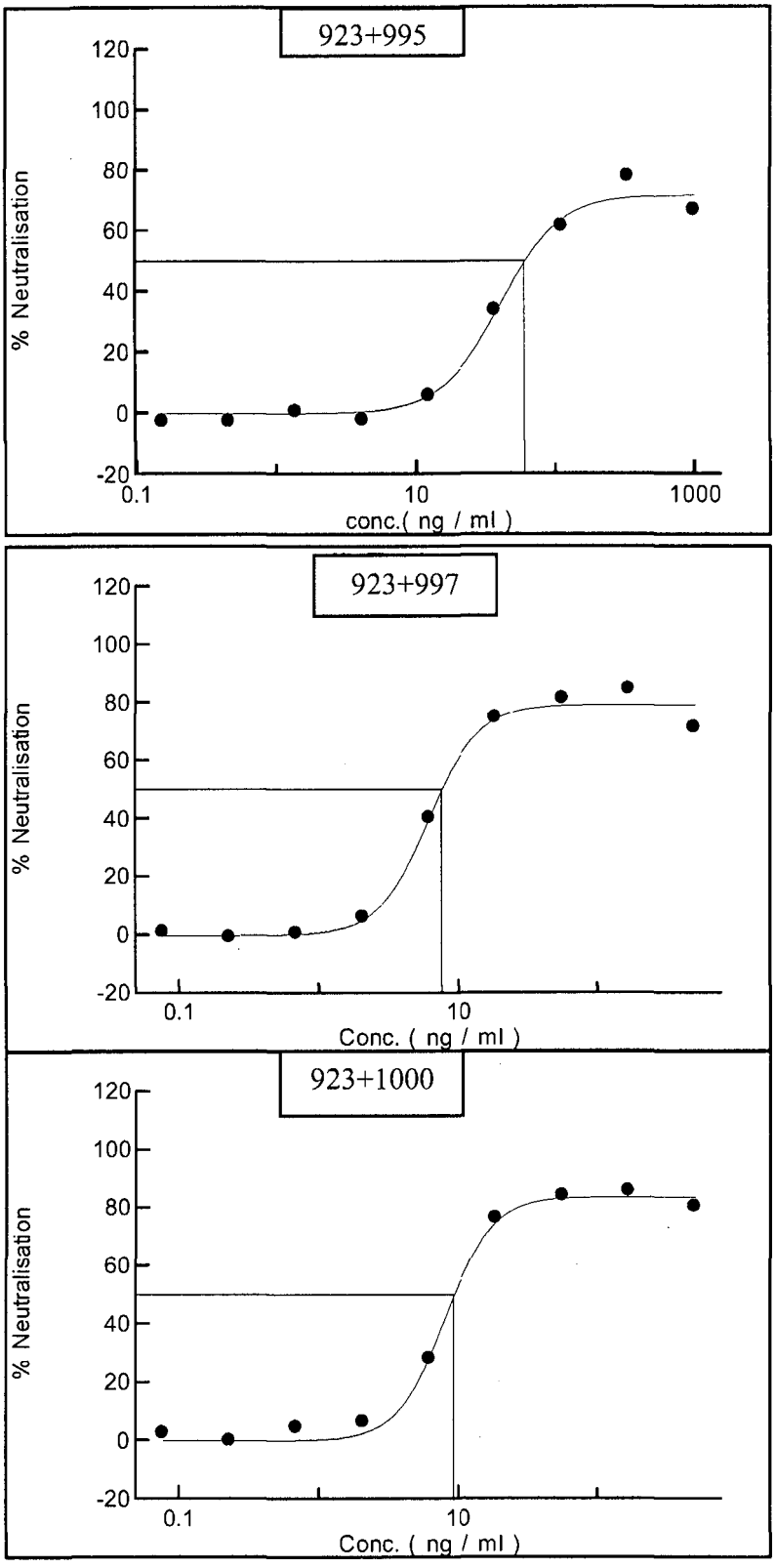


Figure 16 Anti TcdA (Ribotype 003) in-vitro neutralization data for three Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

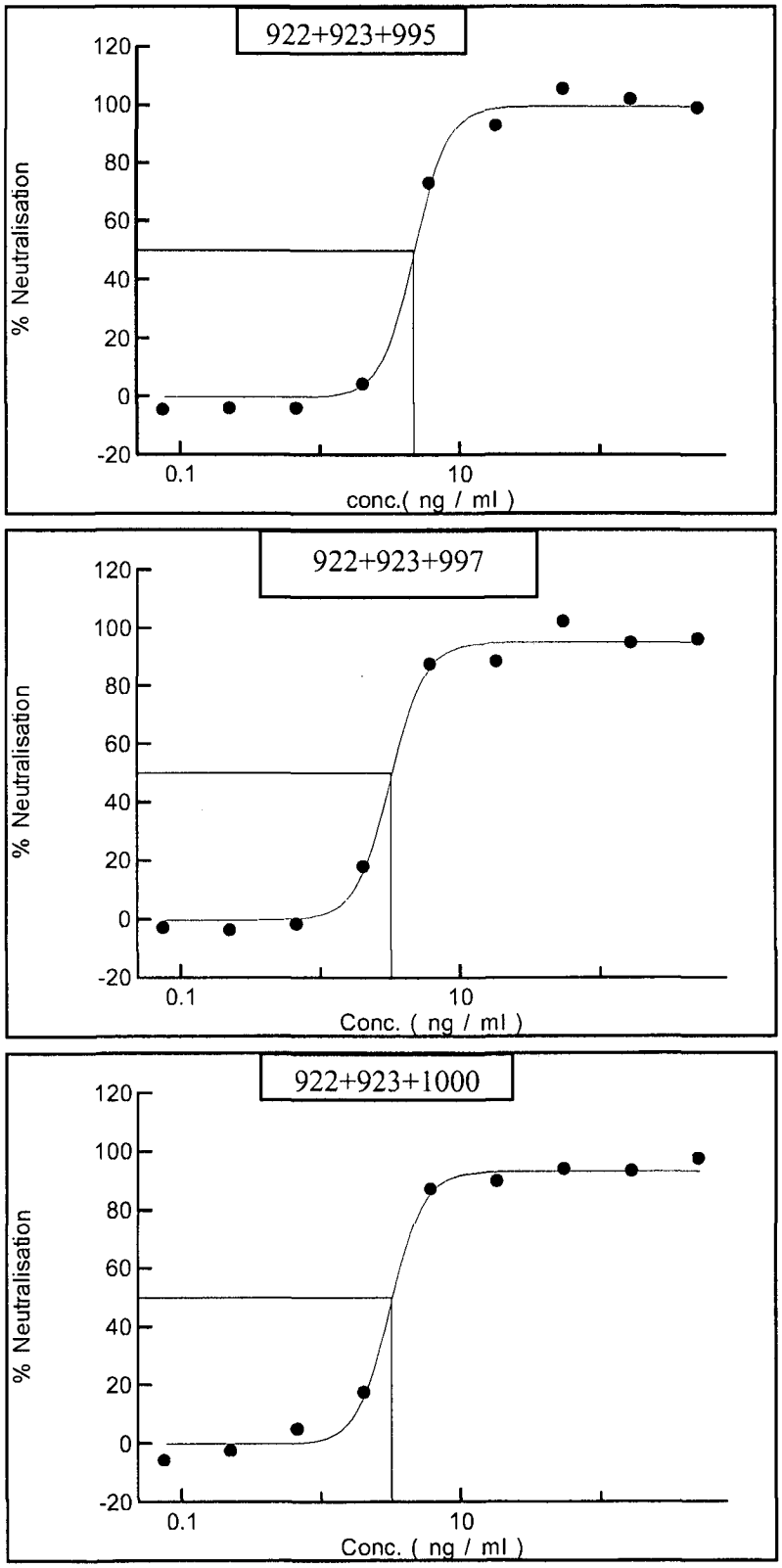


Figure 17 Anti TcdA (Ribotype 003) in-vitro neutralization data for three Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

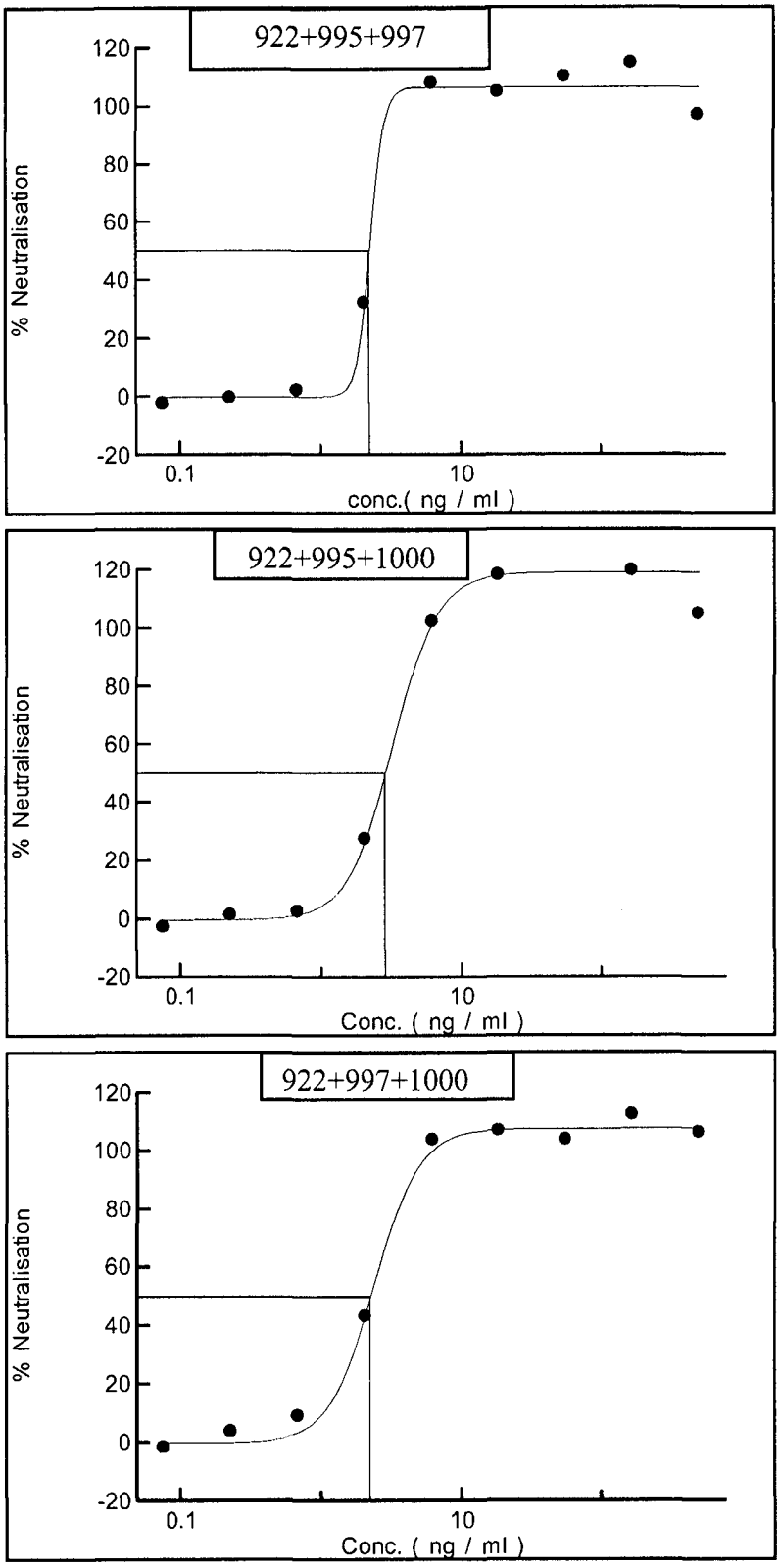


Figure 18 Anti TcdA (Ribotype 003) in-vitro neutralization data for three Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

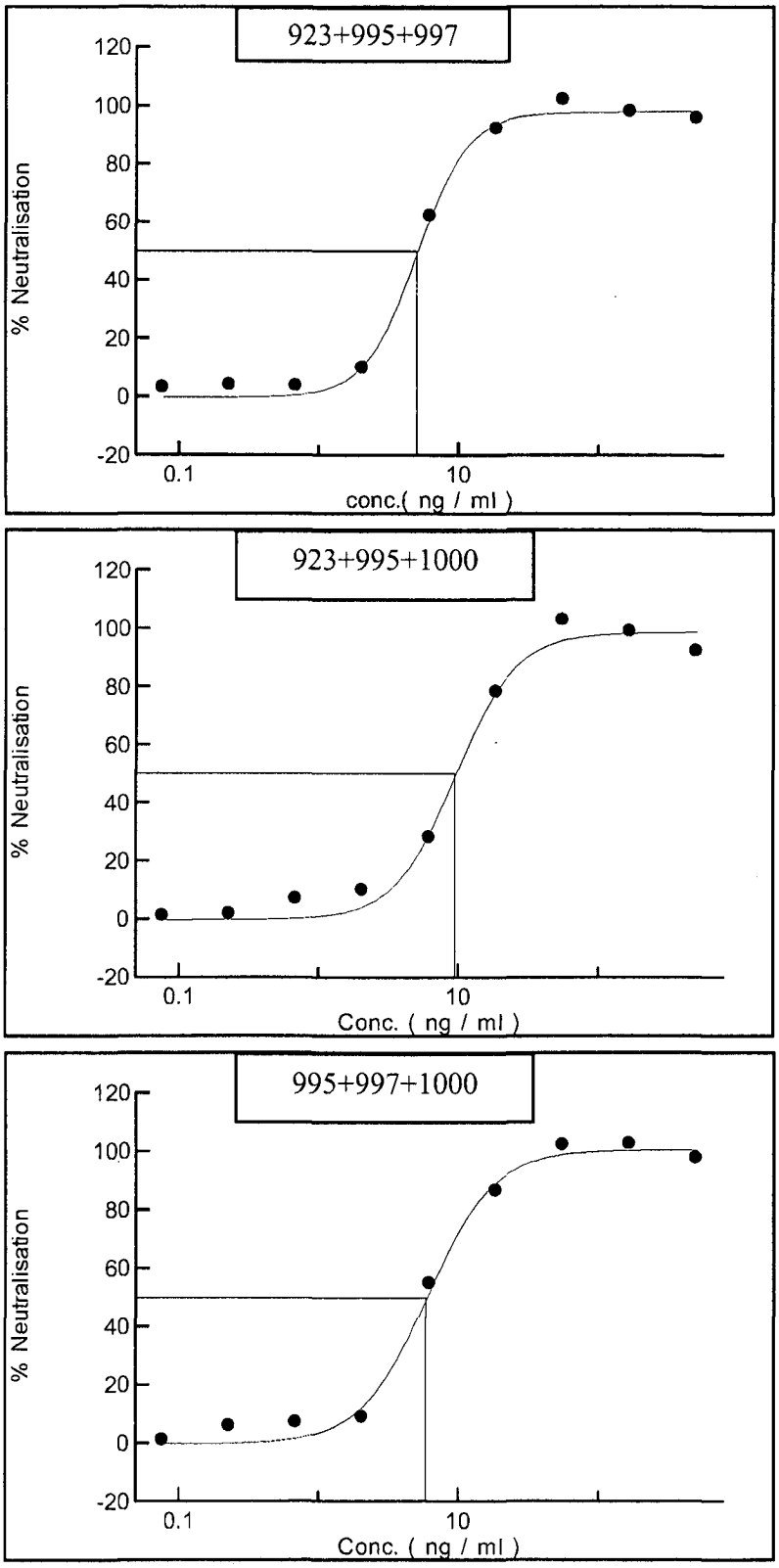


Figure 19 Anti TcdA (Ribotype 003) in-vitro neutralization data for four and five Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

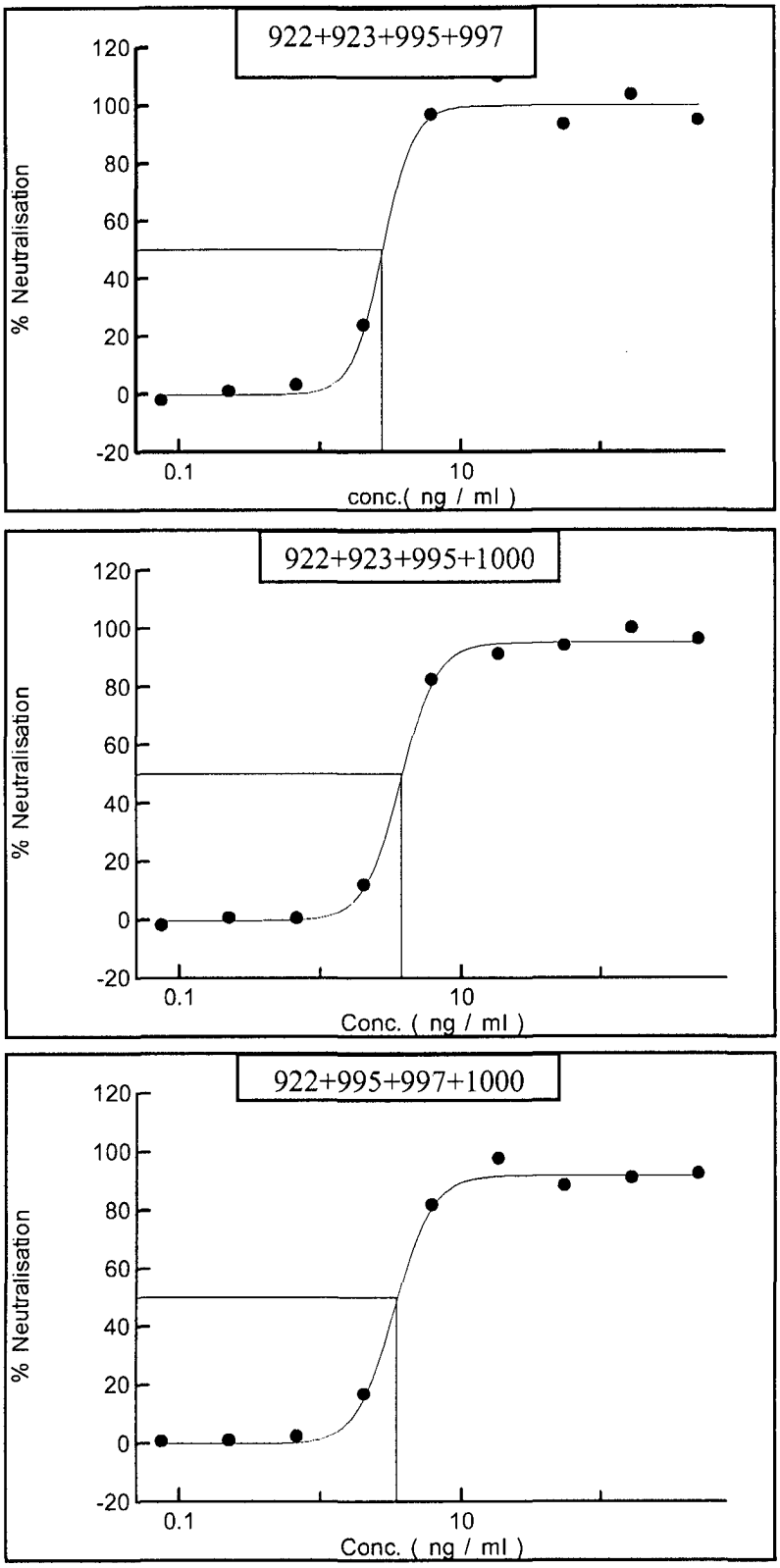


Figure 20 Anti TcdA (Ribotype 003) in-vitro neutralization data for four and five Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

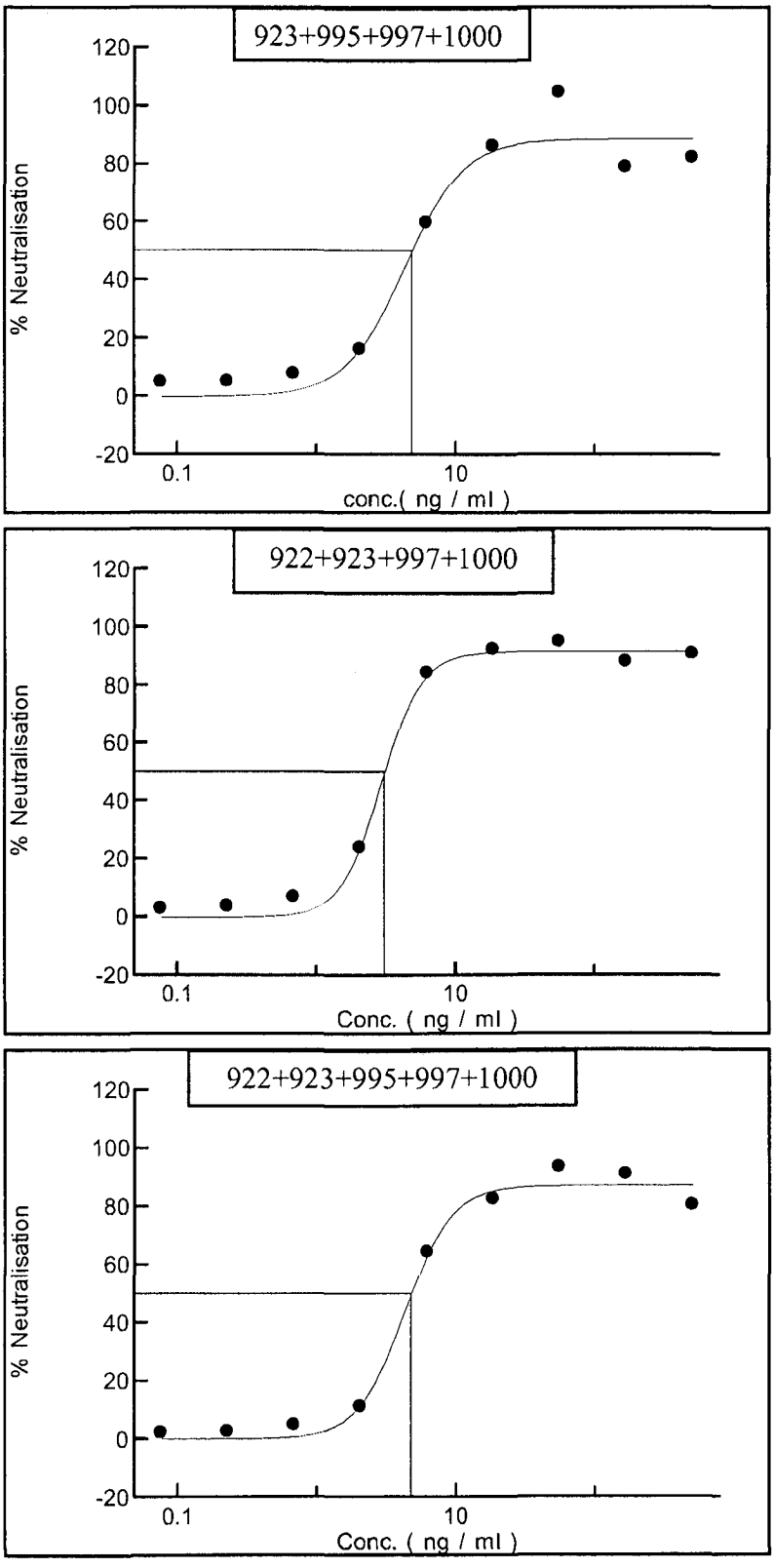
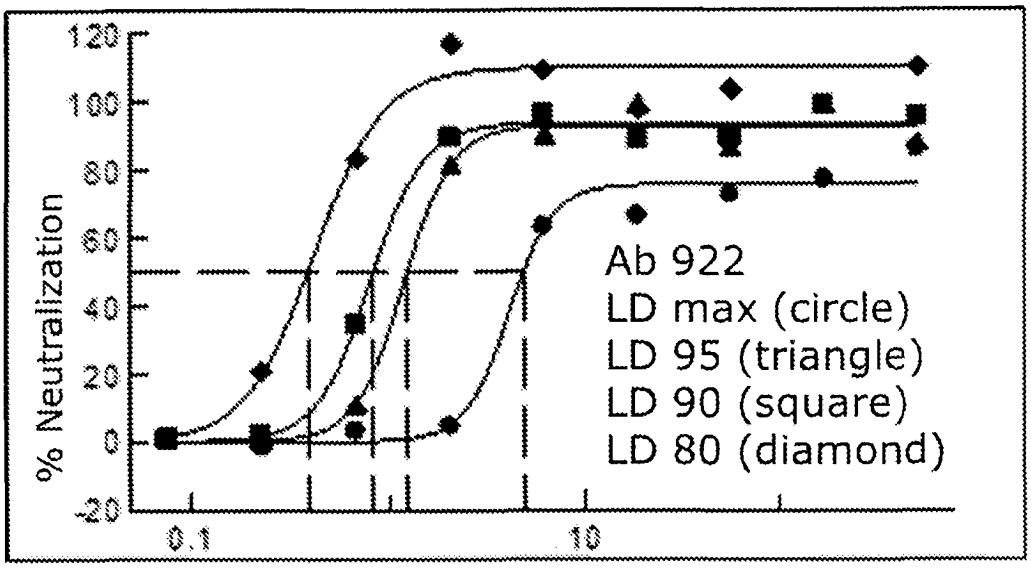
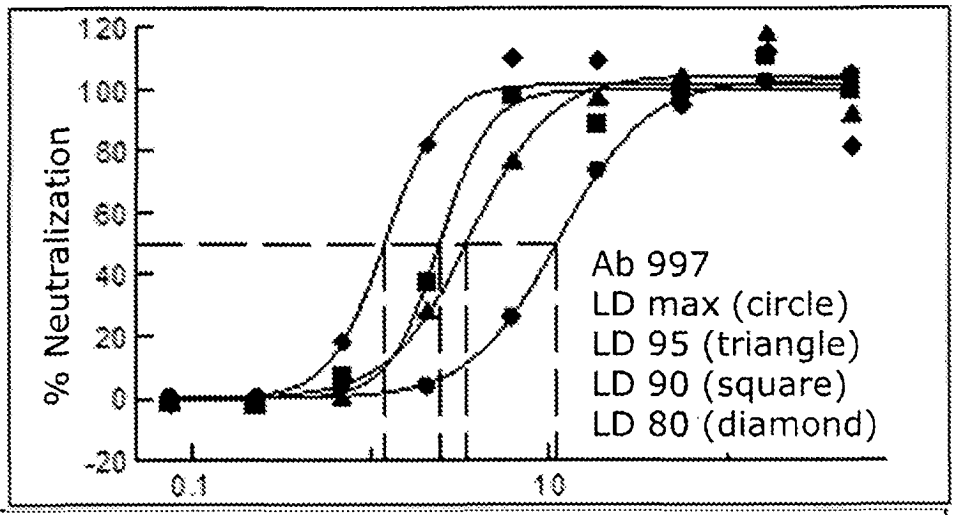


Figure 21 Anti TcdA (Ribotype 003) in-vitro neutralization data for single and paired Mabs at different TcdA concentrations (X axis is conc ng/ml)





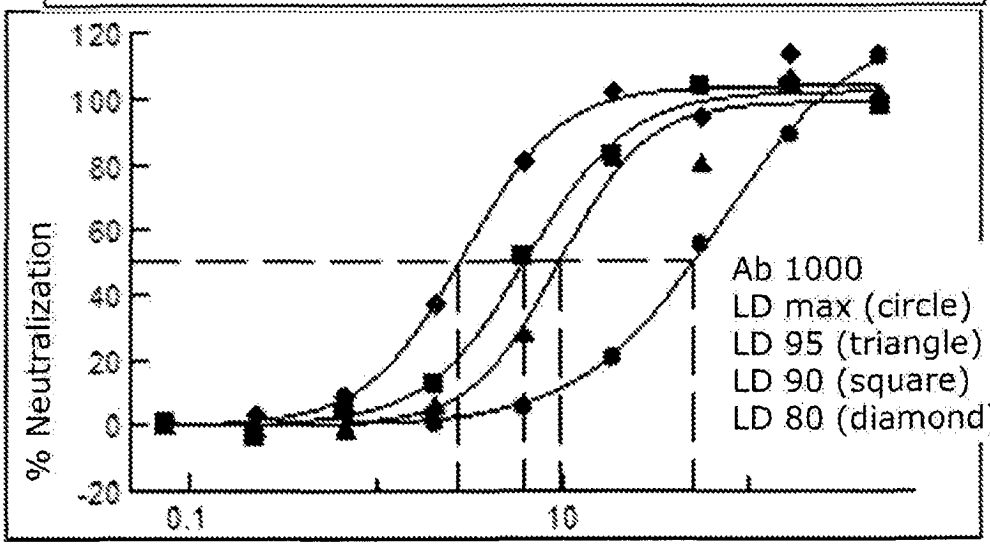
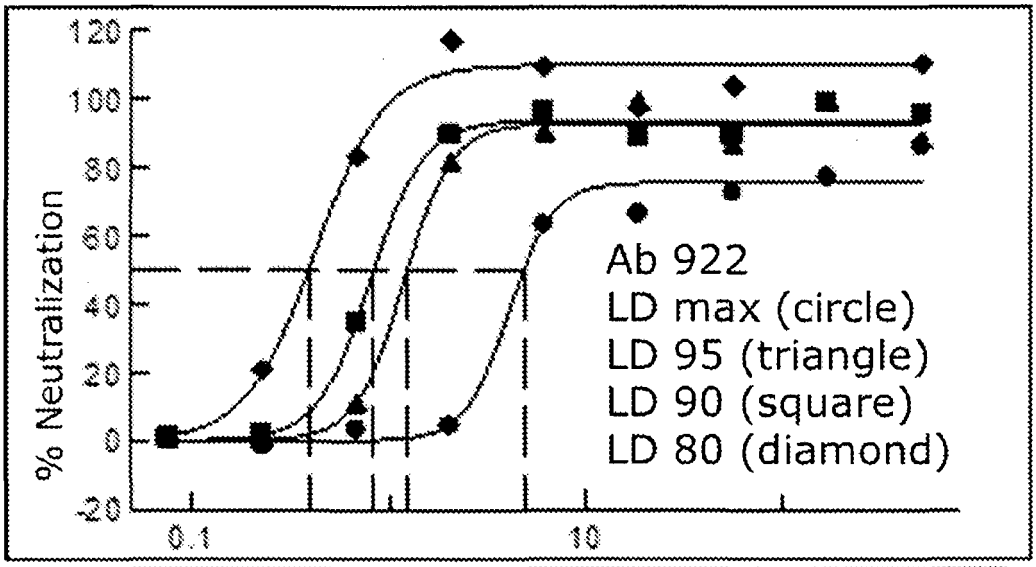
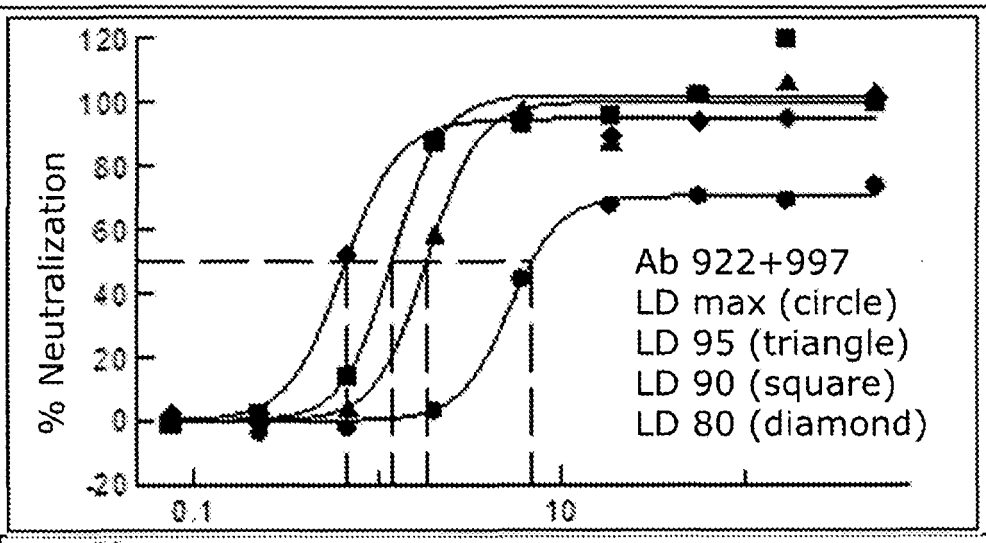


Figure 22 Anti TcdA (Ribotype 003) in-vitro neutralization data for single and paired Mabs at different TcdA concentrations (X axis is conc. ng/ml)





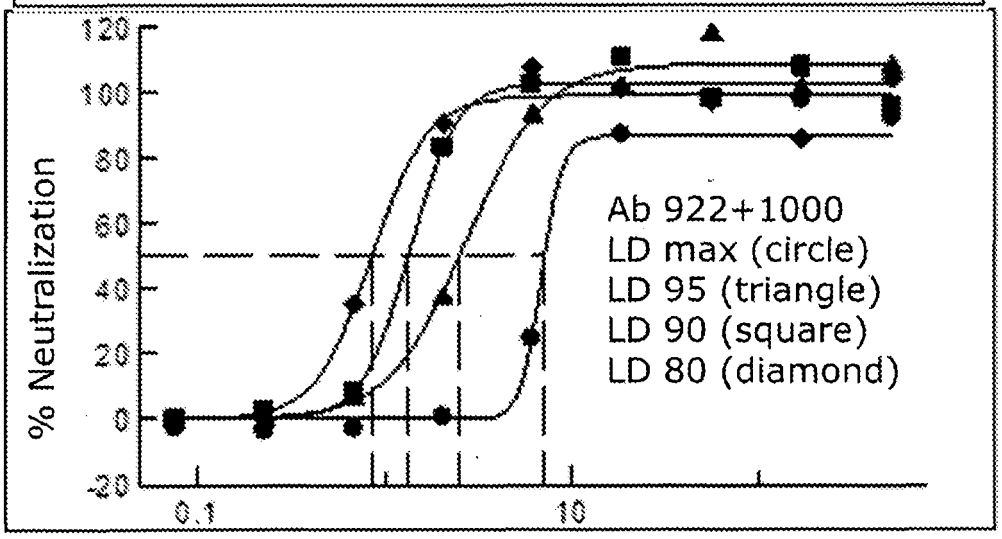


Figure 23 Anti TcdA (Ribotype 003) in-vitro neutralization data for single and to five Mab mixtures at different TcdA concentrations (X axis conc. ng/ml)

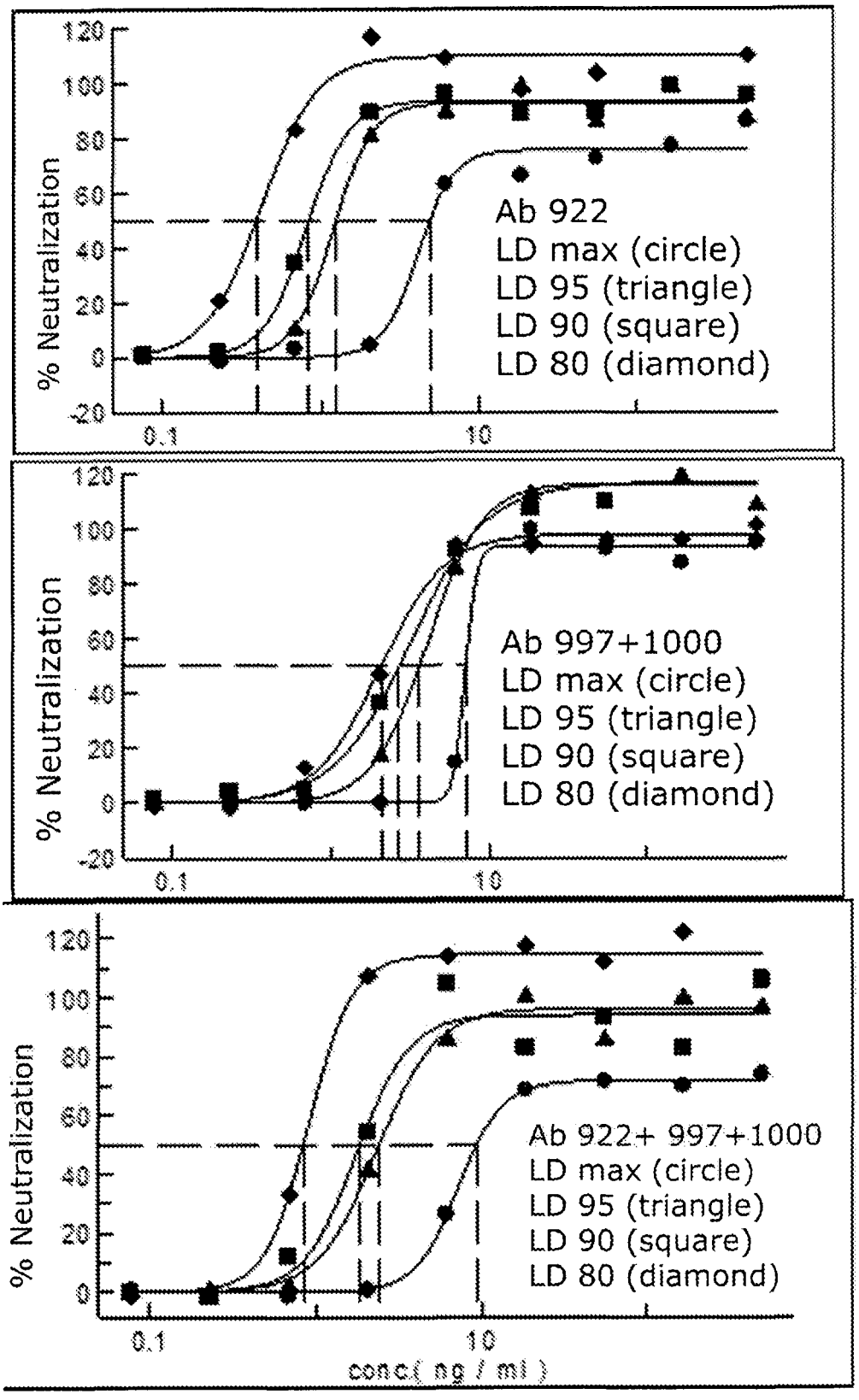
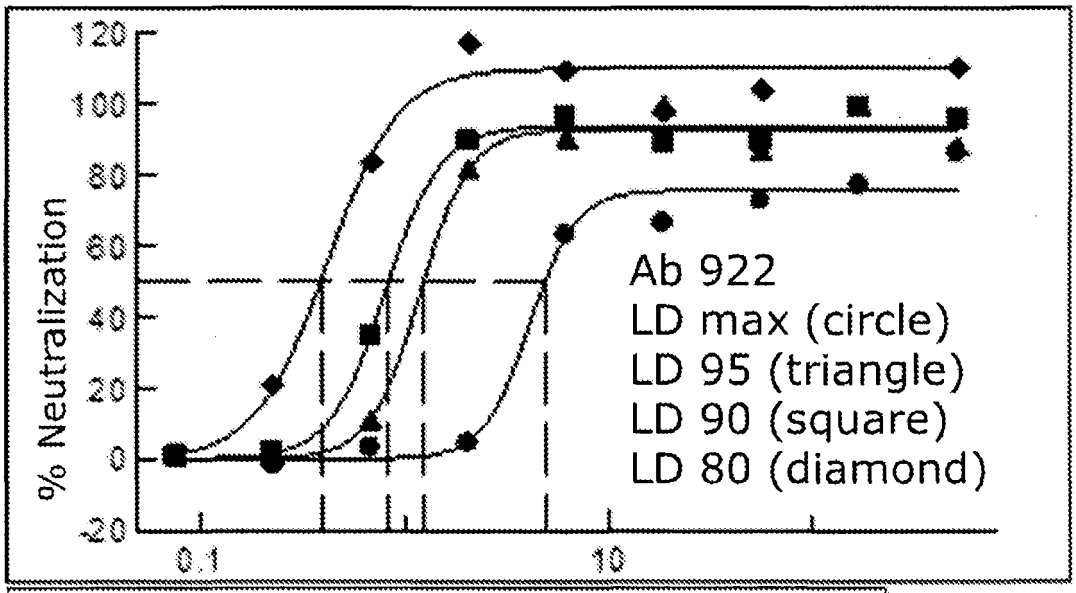
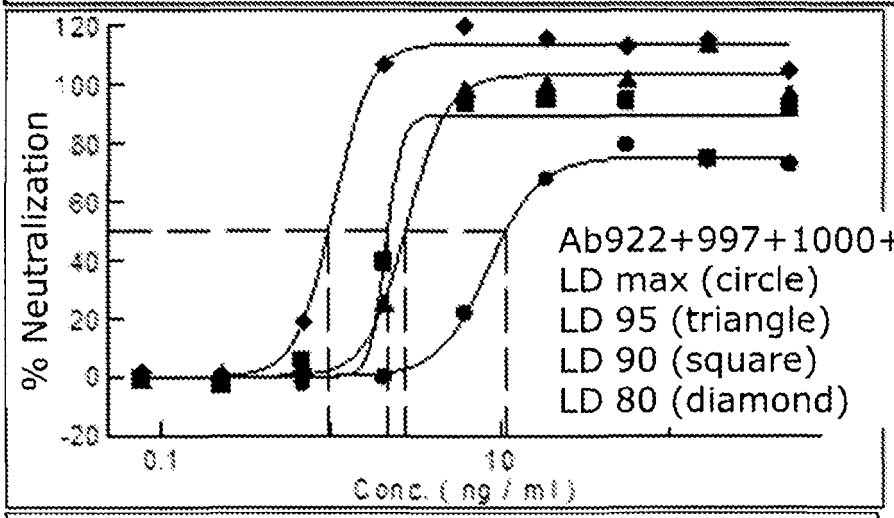


Figure 24 Anti TcdA (Ribotype 003) in-vitro neutralization data for single and to five Mab mixtures at different TcdA concentrations (X axis is conc. ng/ml





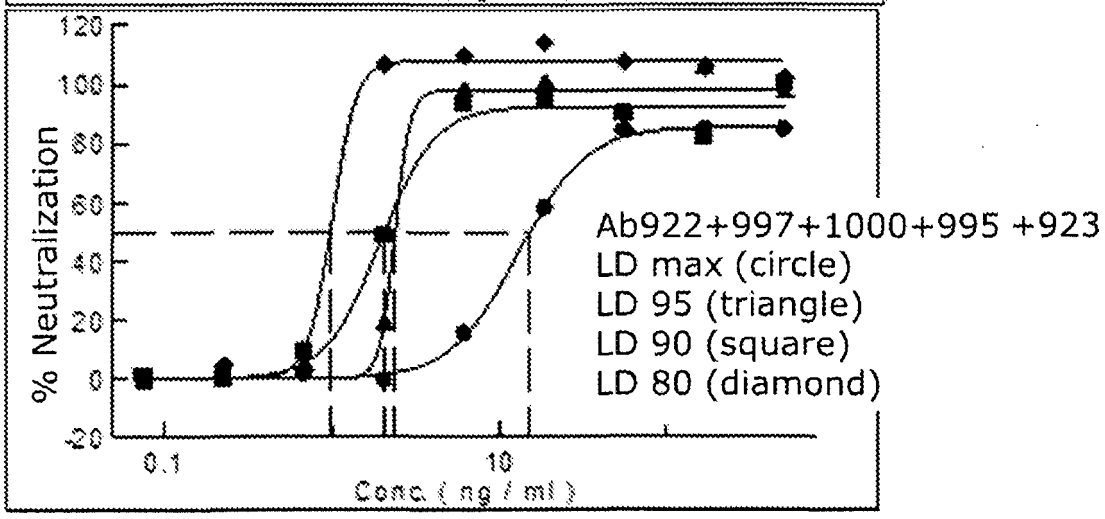


Figure 25 Anti TcdB (Ribotype 003) in-vitro neutralization data for single Mabs (Y axis neutralization X axis conc ng/ml for 1125.g2, 1134.g5 and 927.g2 respectively)

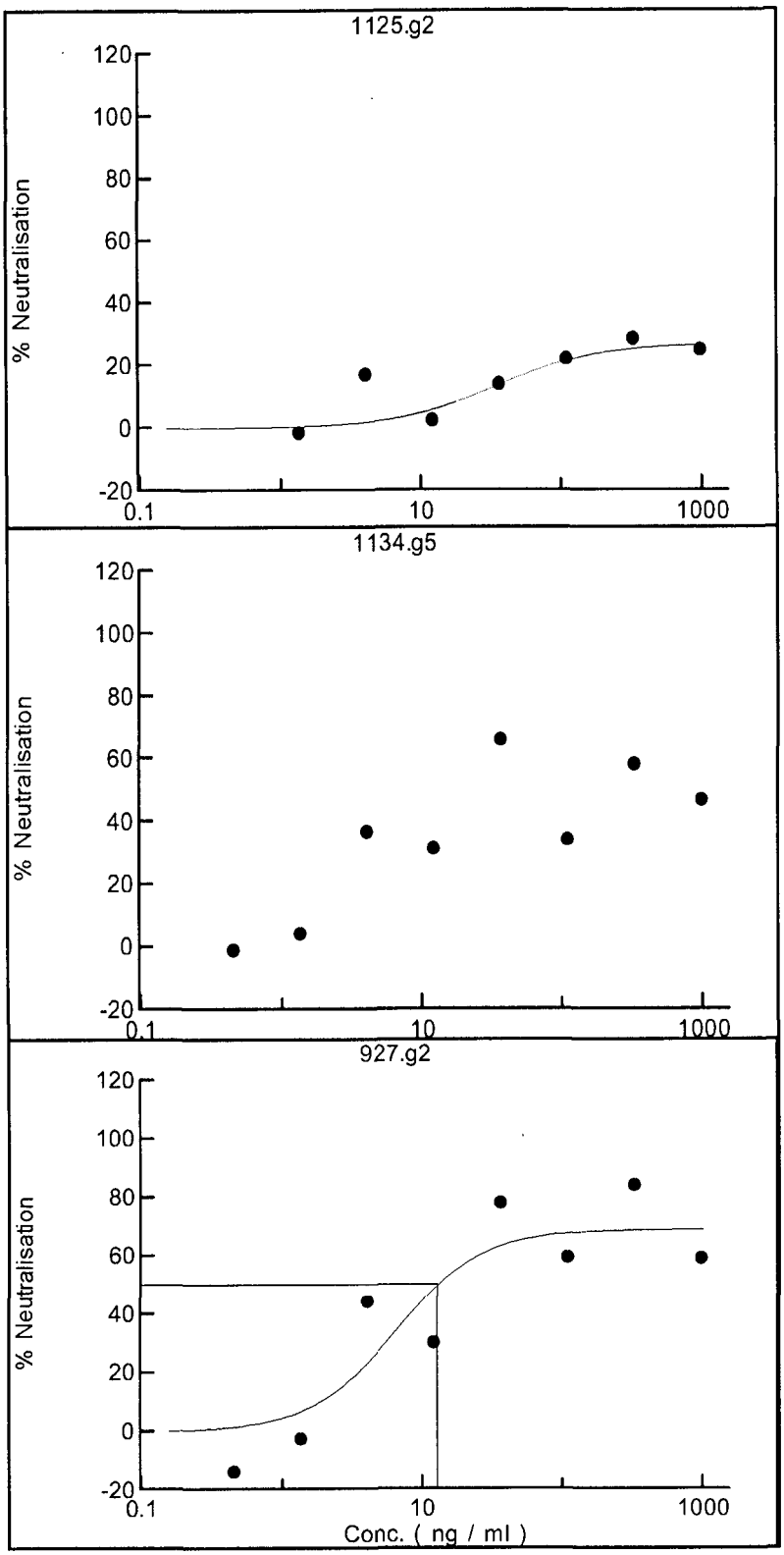


Figure 26 Anti TcdB (Ribotype 003) in-vitro neutralization data for single Mabs Y axis neutralization X axis conc ng/ml for1153.g8 and 1102.g4 respectively)

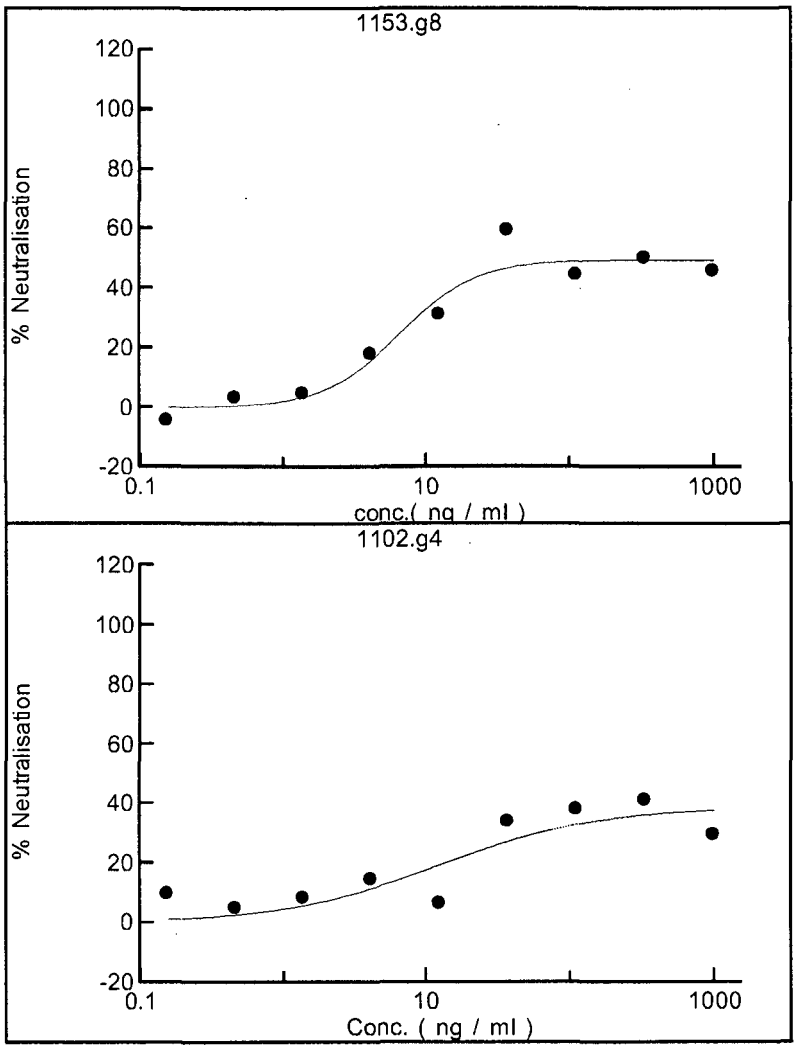


Figure 27 Anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs Y axis neutralization X axis conc ng/ml for combinations of 927+1099, 927+1102, 927+1114 respectively)

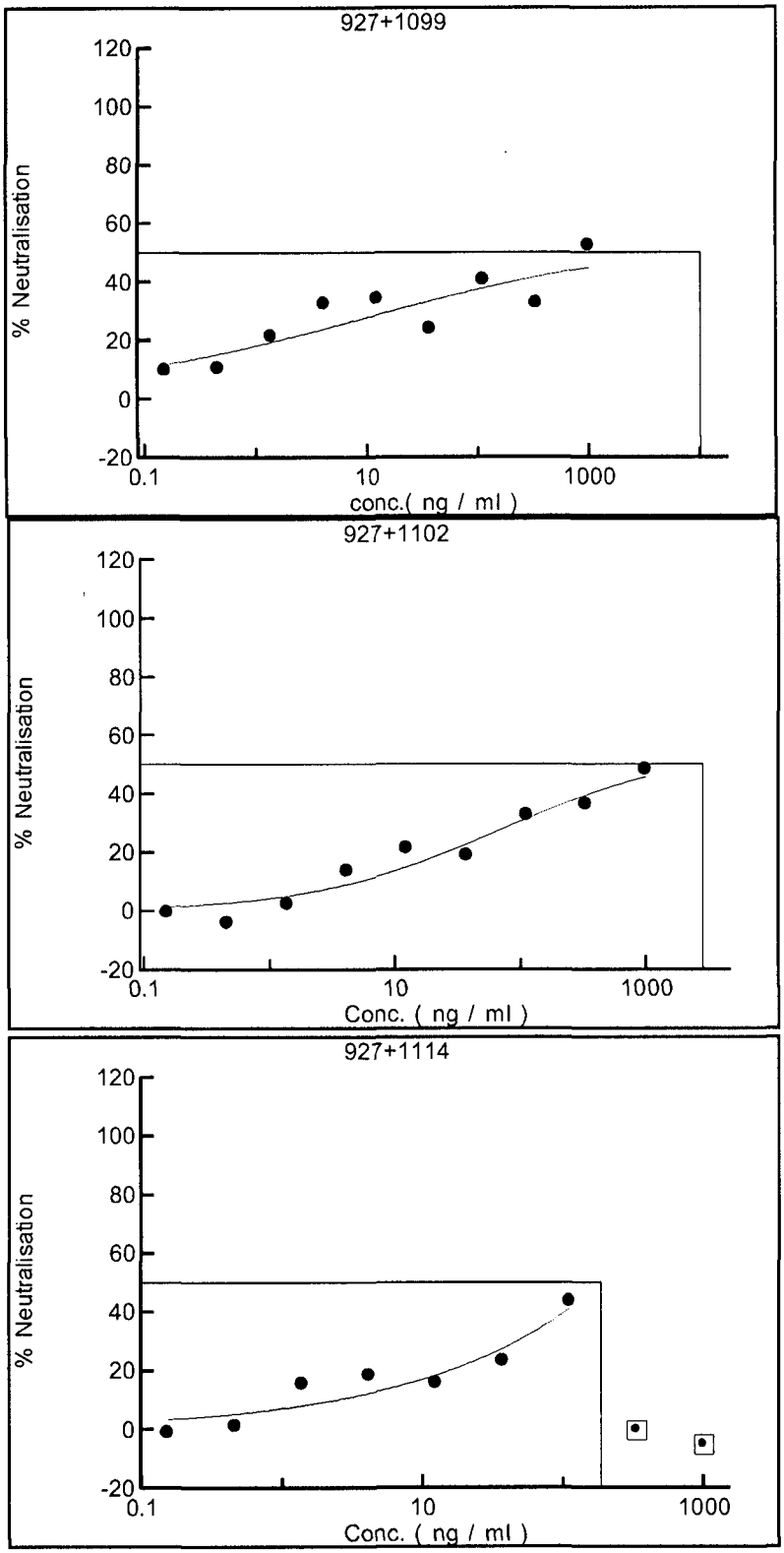


Figure 28 Anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs (Y axis neutralization X axis conc ng/ml for combinations of 927+1125, 927+1134, 1099+1114 respectively)

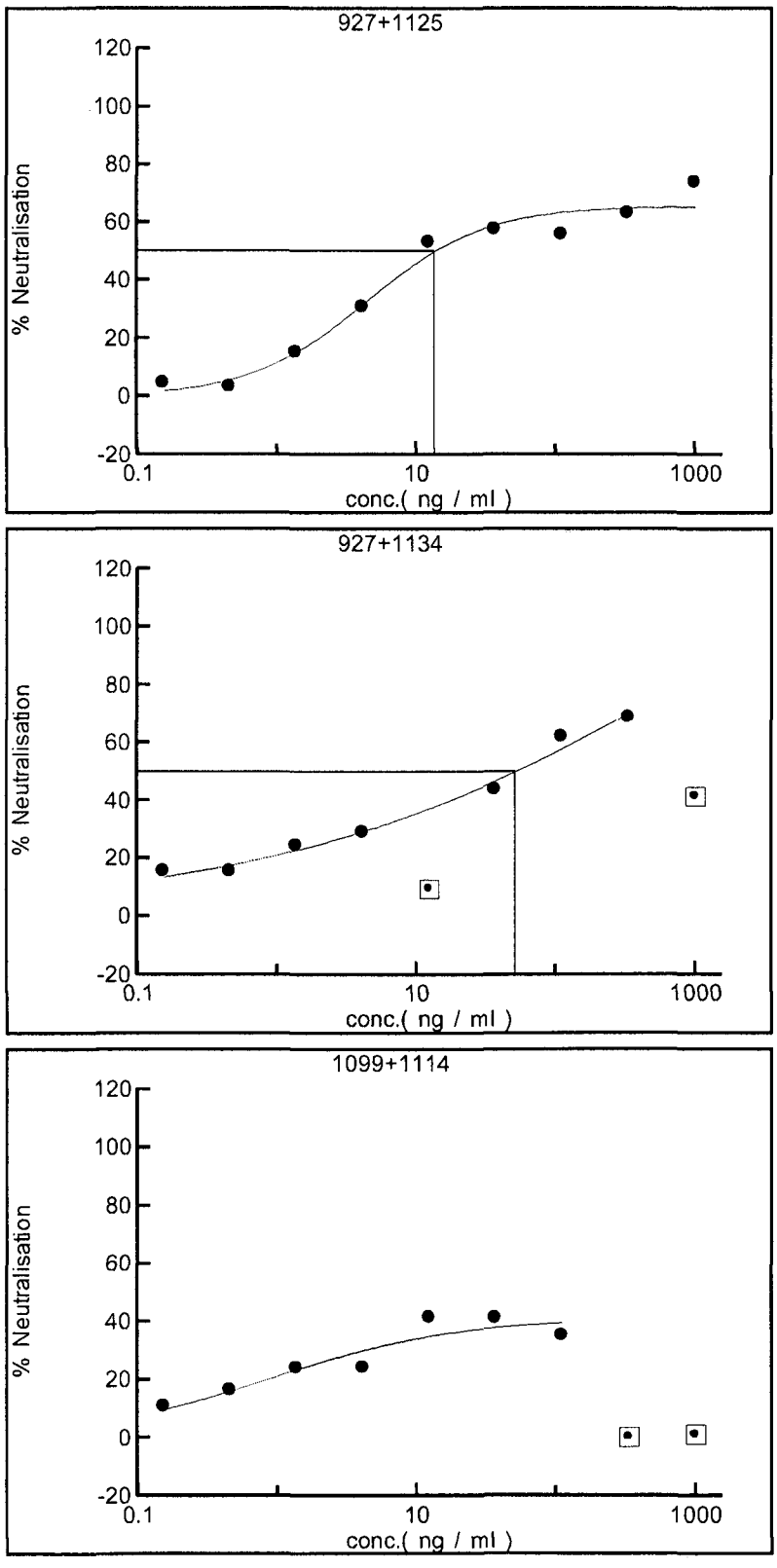


Figure 29 Anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs (Y axis neutralization X axis conc ng/ml for combinations of 1102+1114, 1102+1125, 1114+1134 respectively)

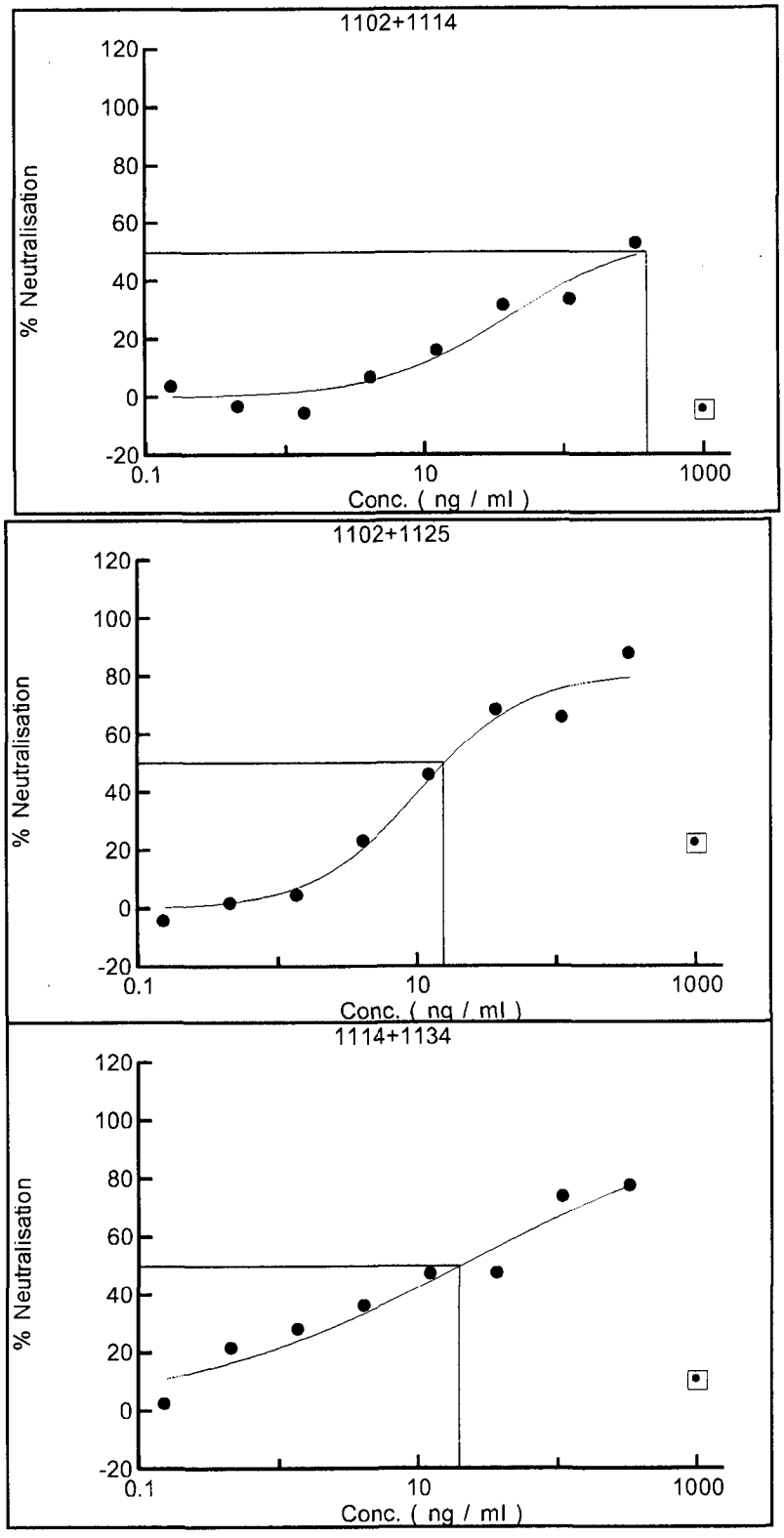


Figure 30 Anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs (Y axis neutralization X axis conc ng/ml for combinations of 1114+1151, 1114+1153, 1125+1134 respectively)

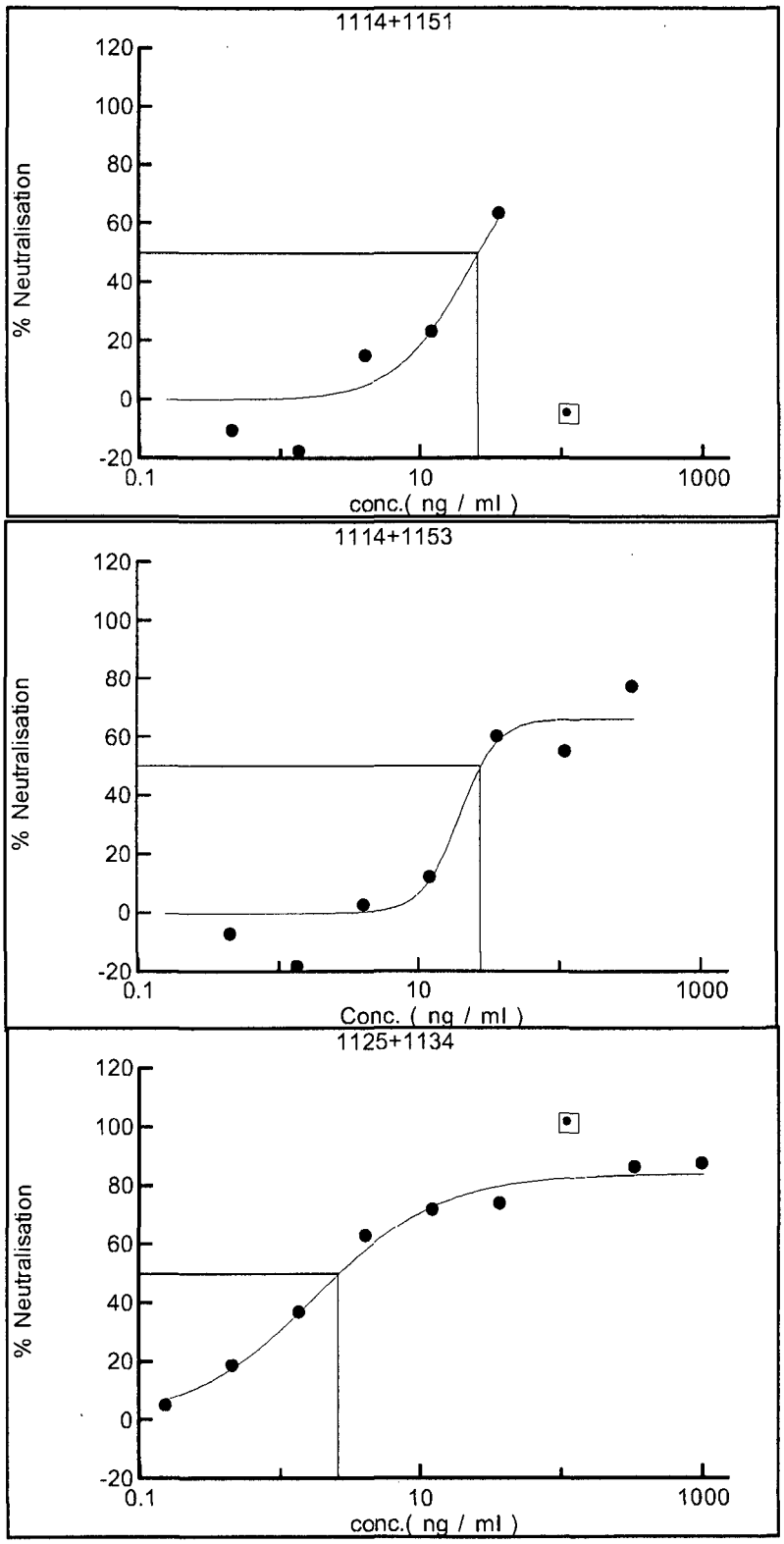


Figure 31 Anti TcdB (Ribotype 003) in-vitro neutralization data for three Mab mixtures (Y axis neutralization X axis conc ng/ml for combinations of 1125+1134+1114, 1125+1134+927, 1125+1151+*1114* respectively)

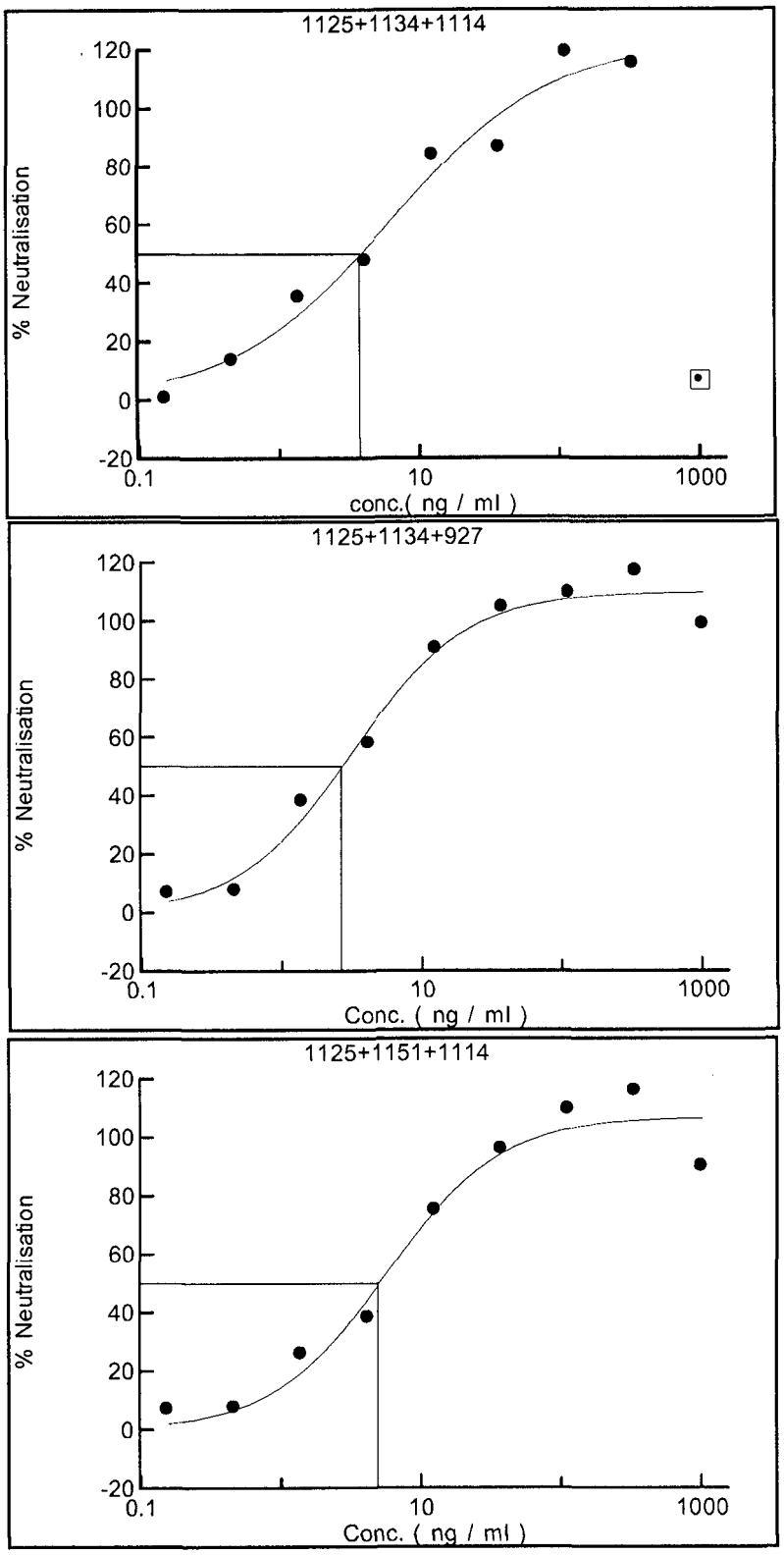


Figure 32 Anti TcdB (Ribotype 003) in-vitro neutralization data for three Mab mixtures (Y axis neutralization X axis conc ng/ml for 1125.+1151+927, 1125.g2+1134.g5+927.g2 respectively)

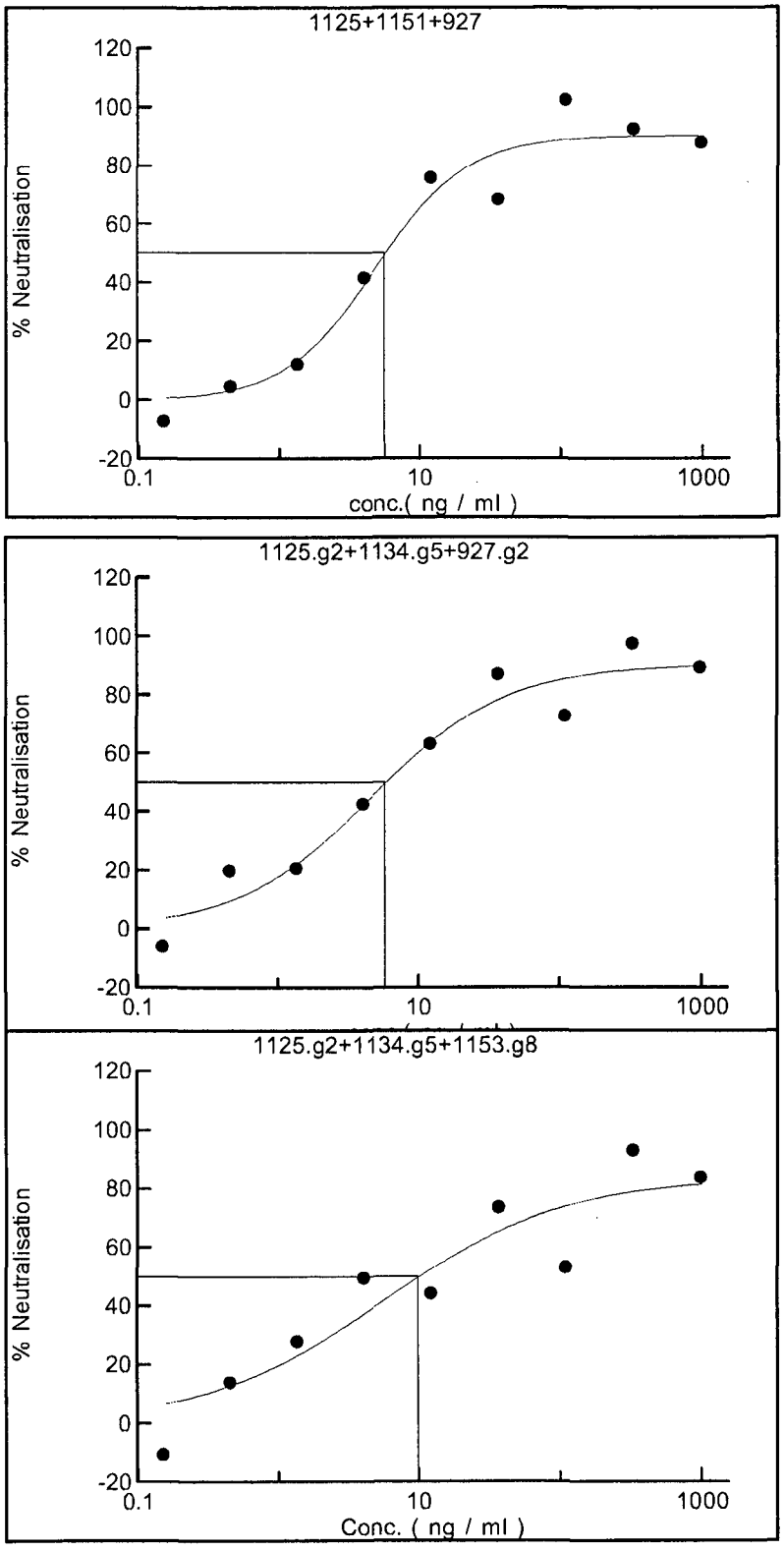


Figure 33 Anti TcdB (Ribotype 003) in-vitro neutralization data for three Mab mixtures

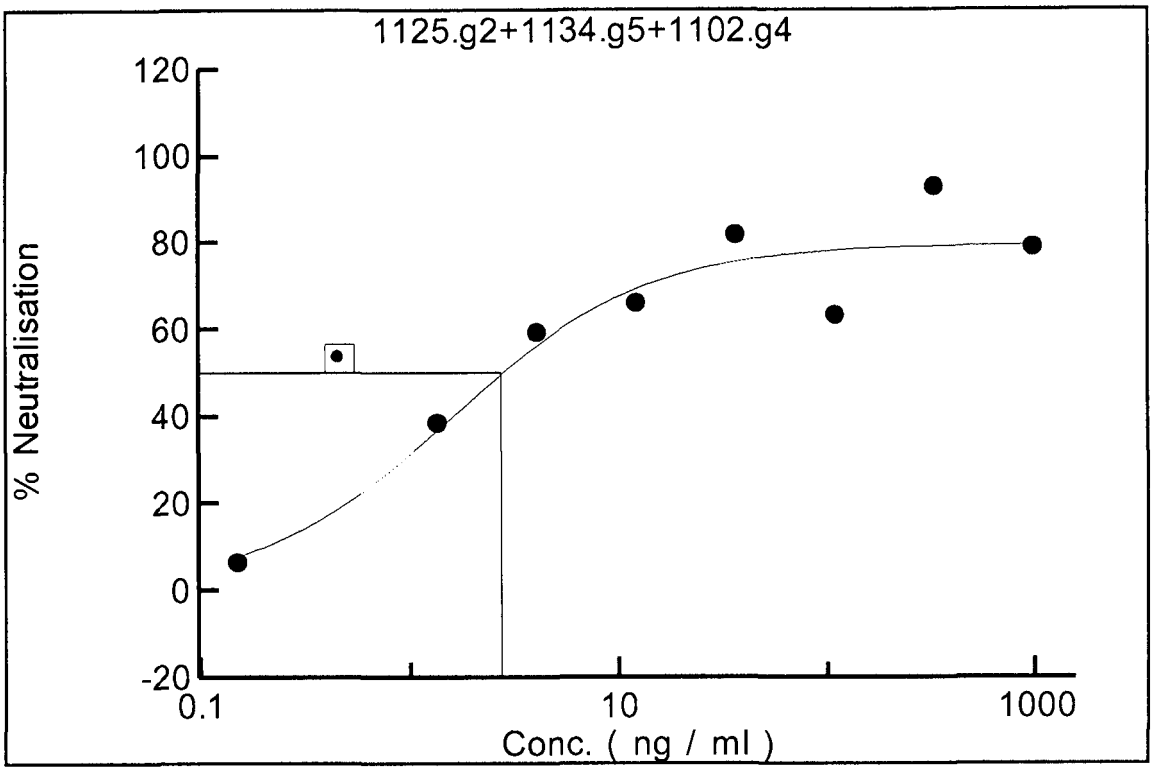


Figure 34 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

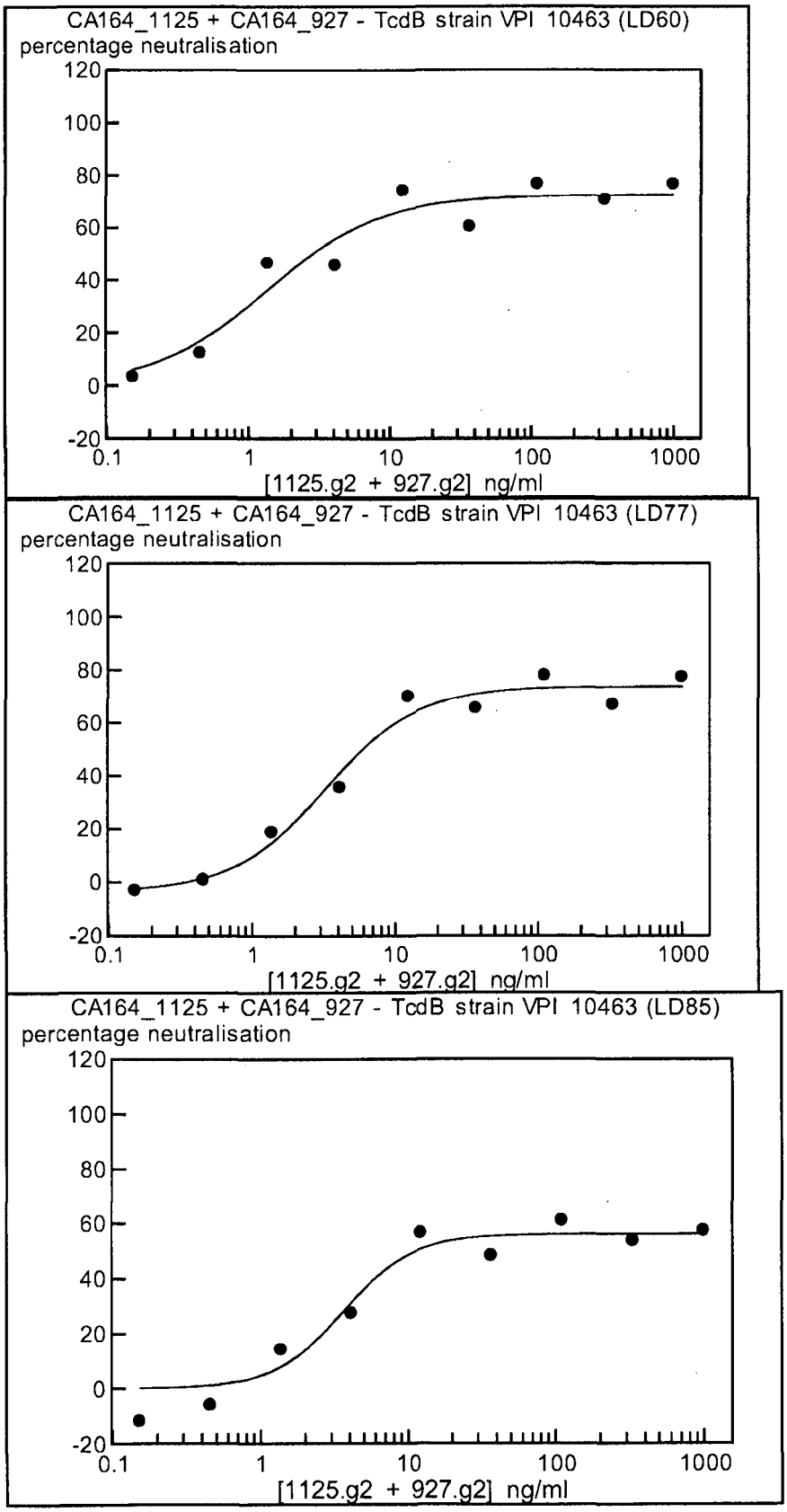


Figure 35 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

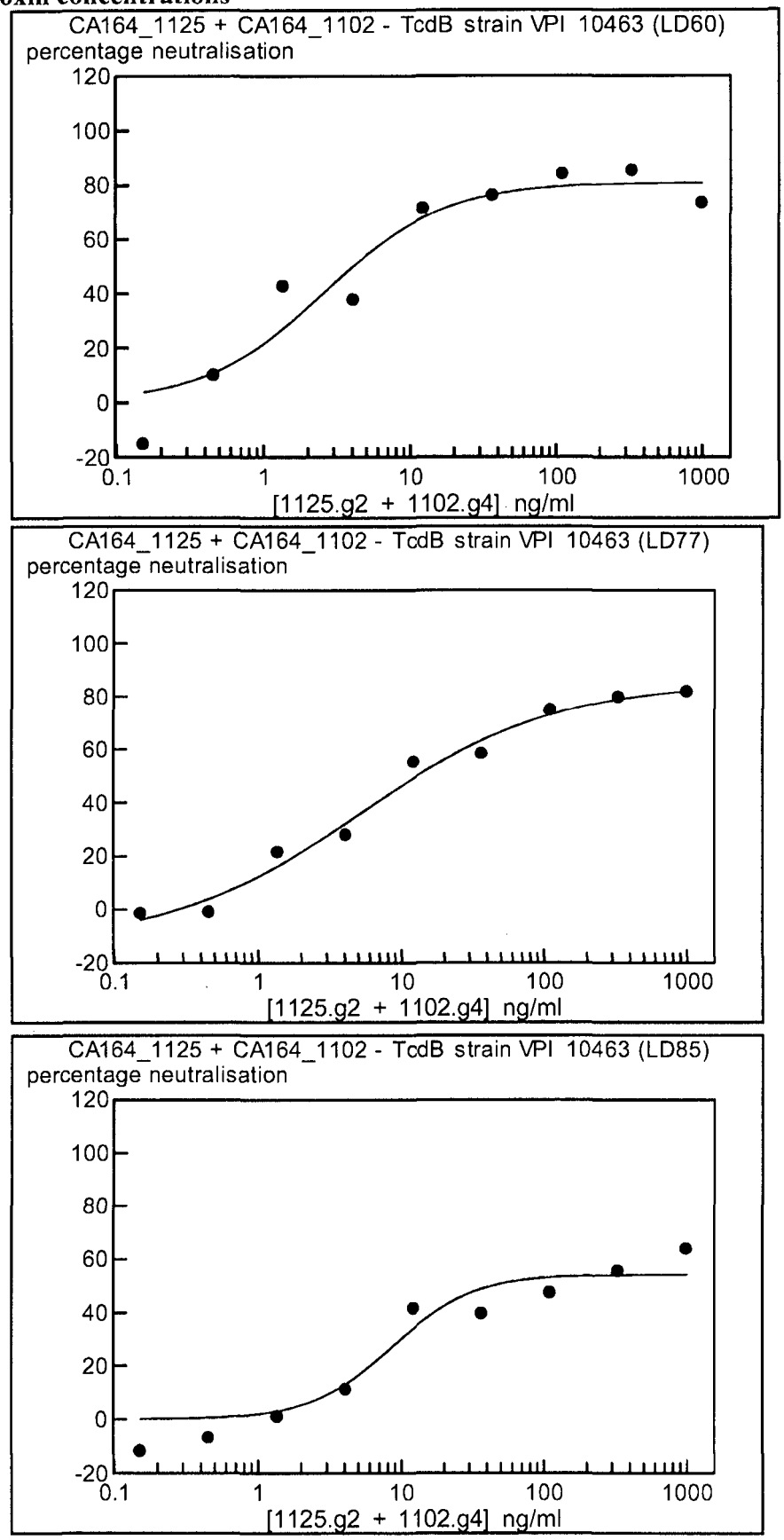
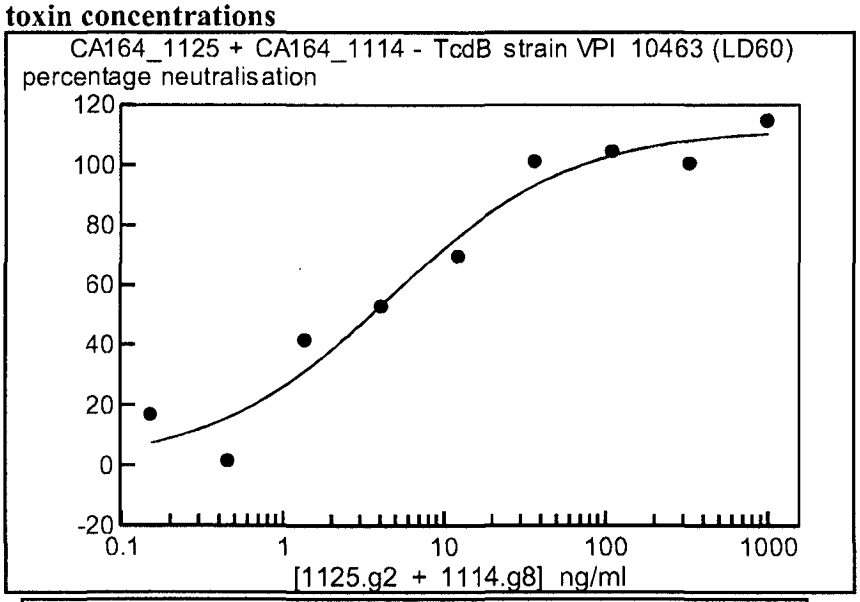
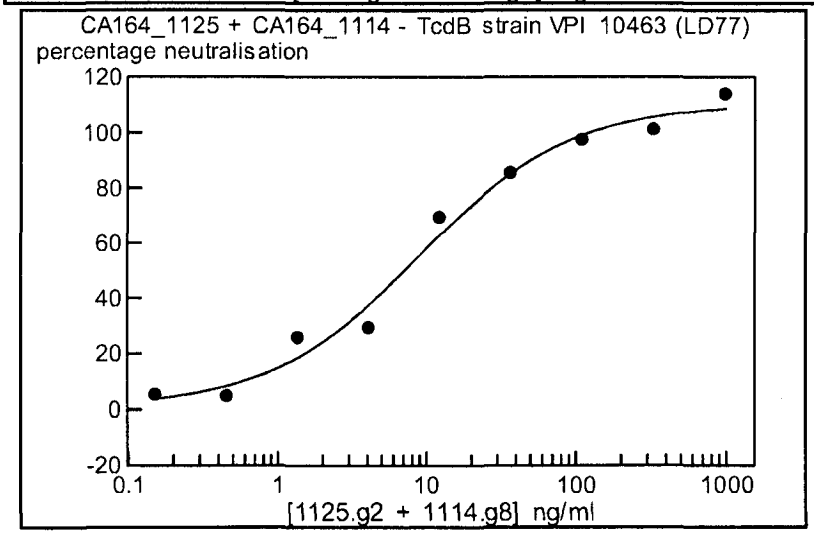


Figure 36 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations





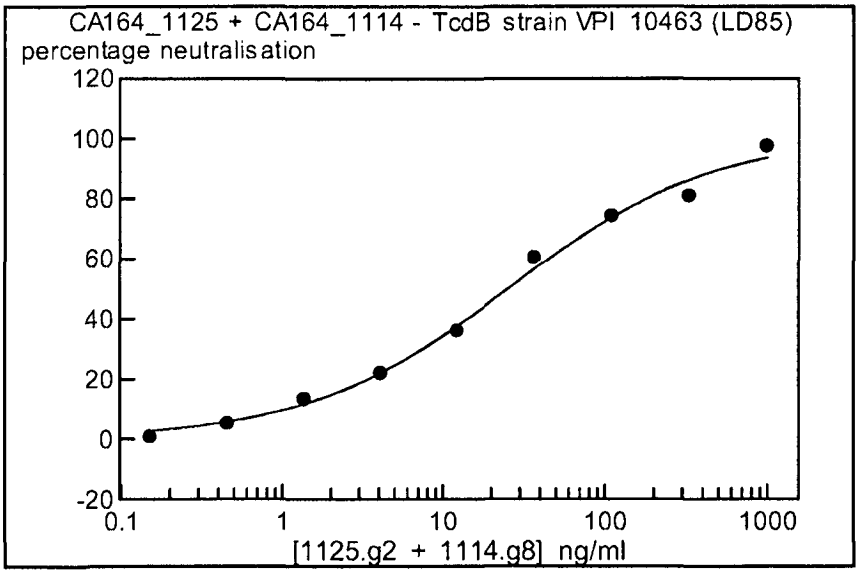


Figure 37 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

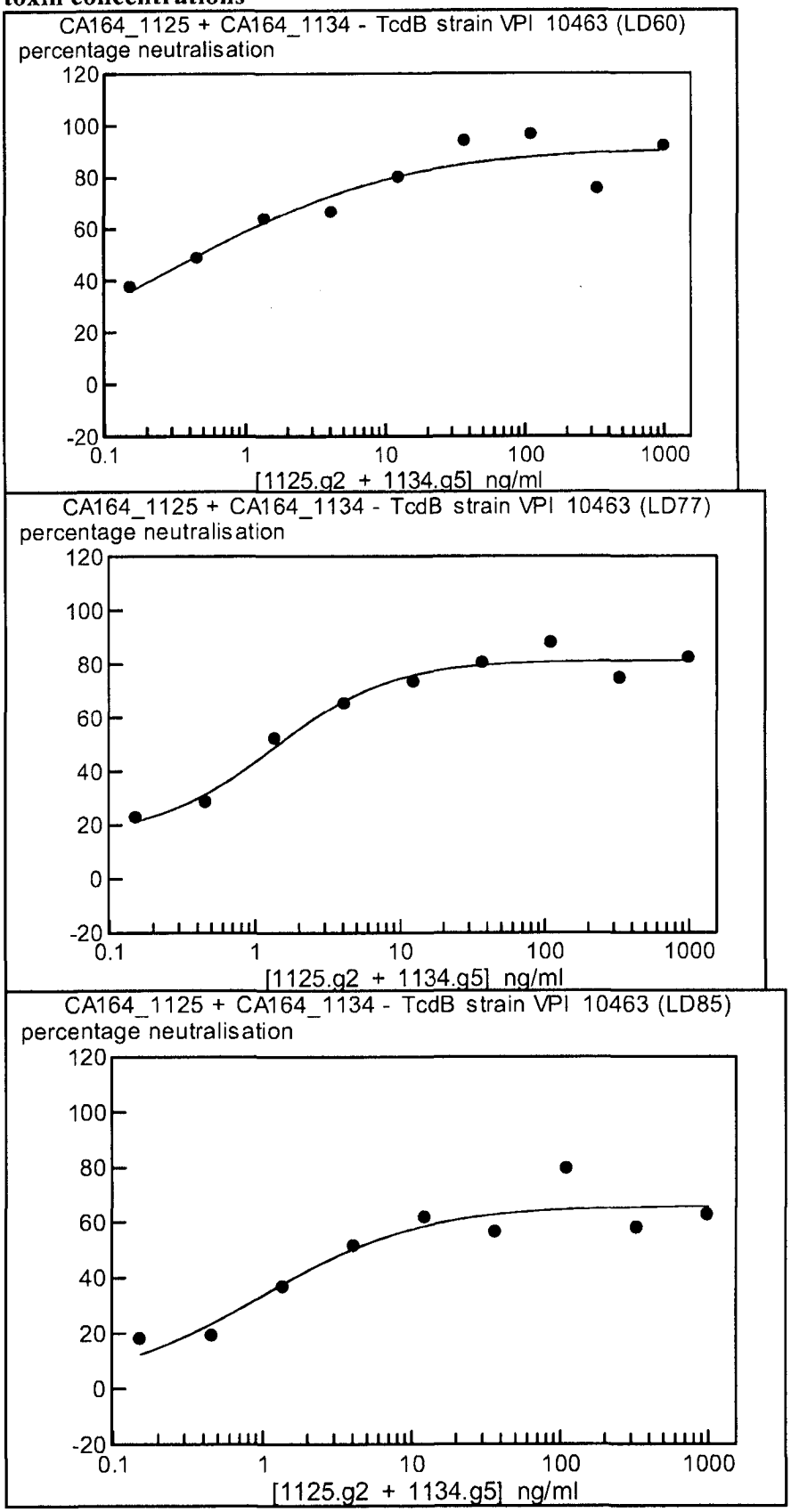


Figure 38 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

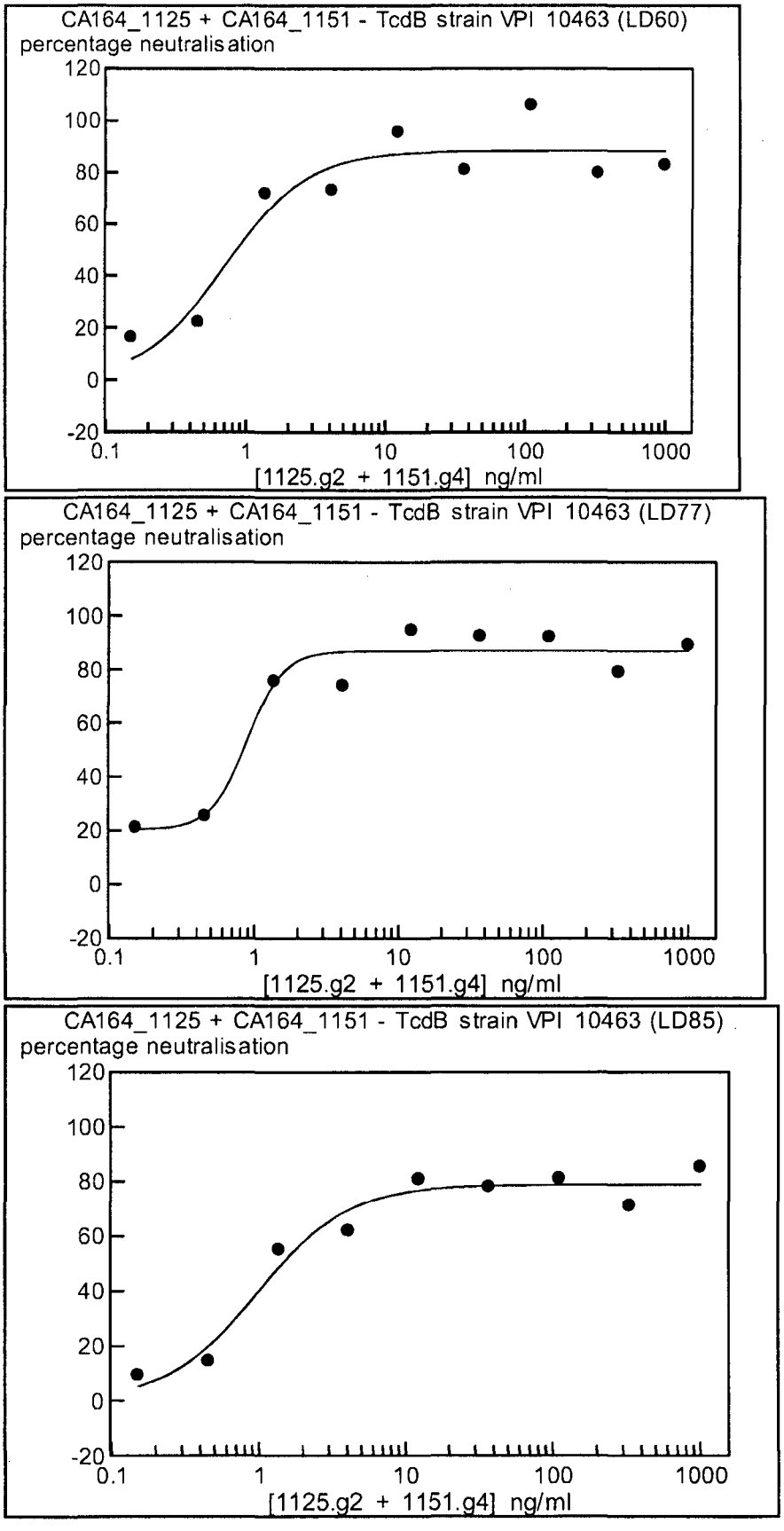


Figure 39 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

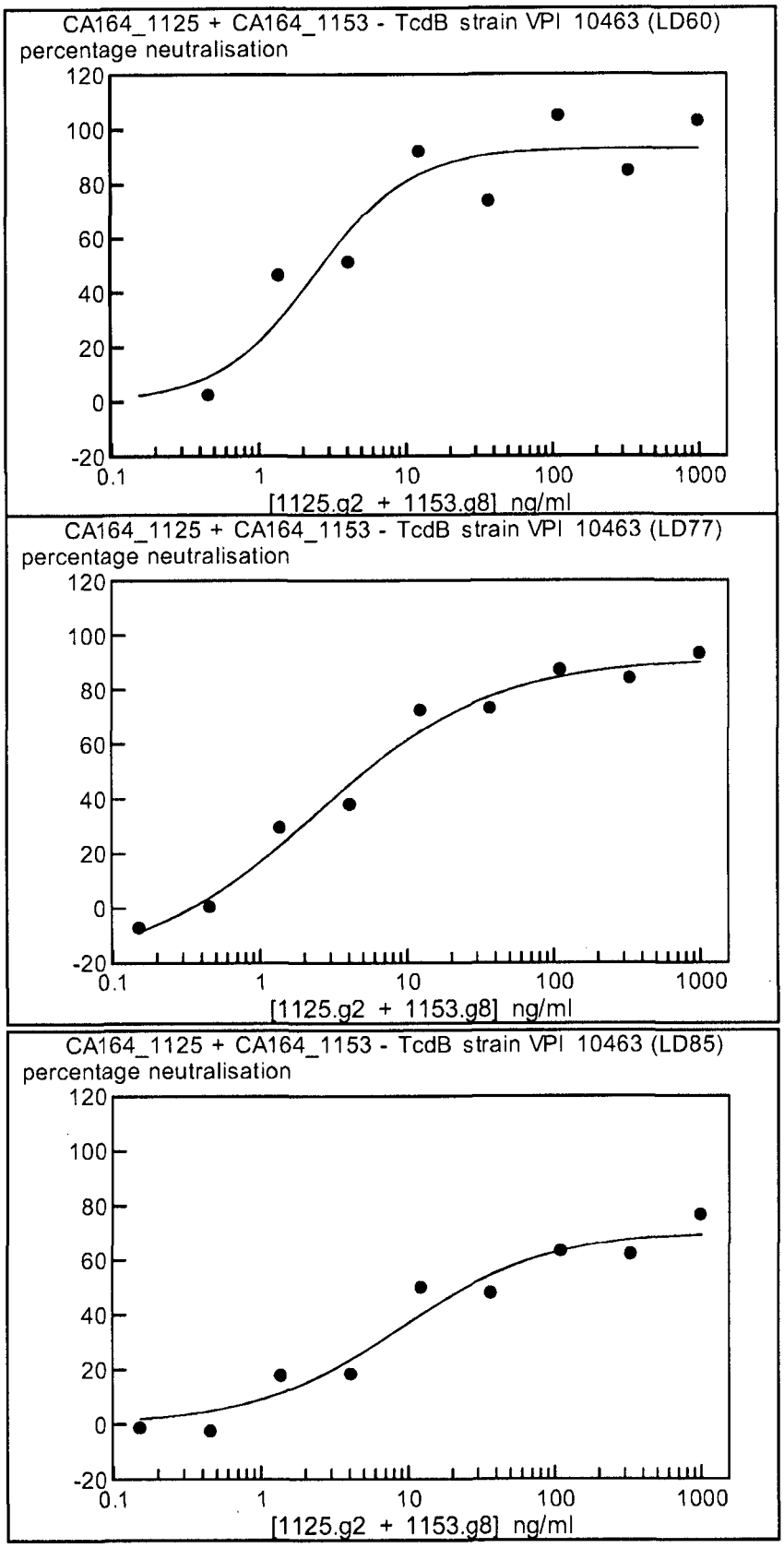


Figure 40 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

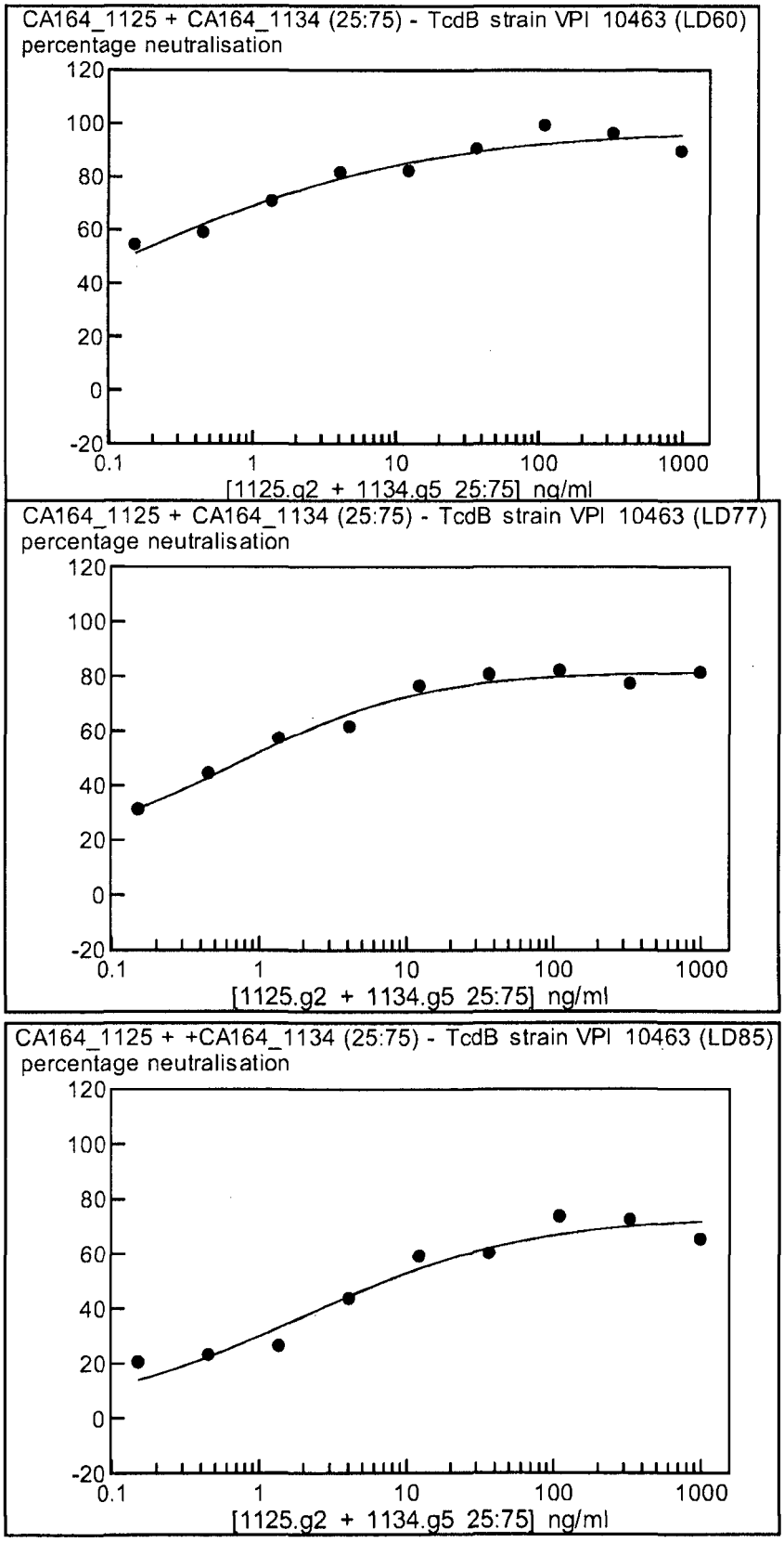


Figure 41 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations

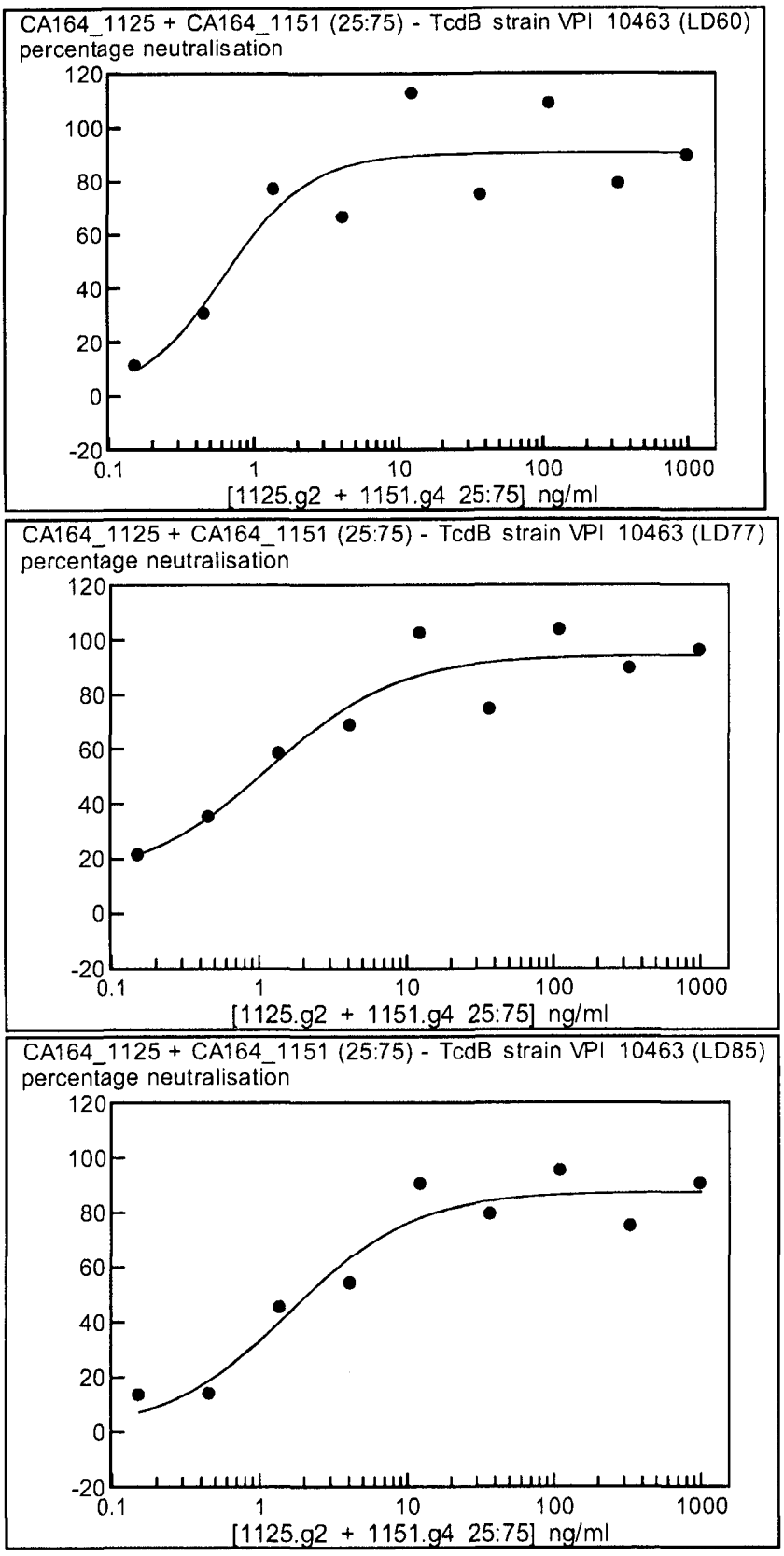


Figure 42 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations

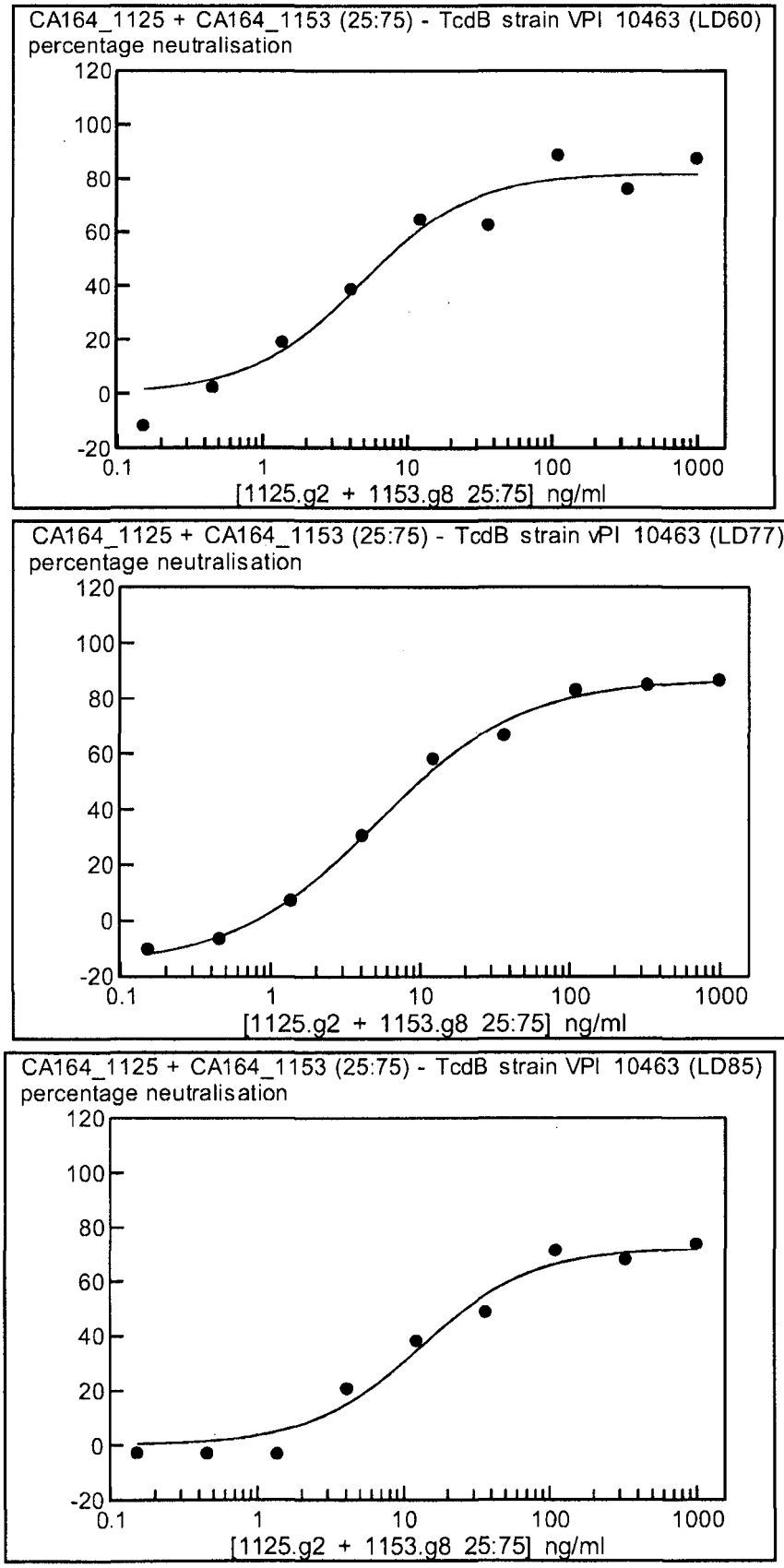


Figure 43 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations

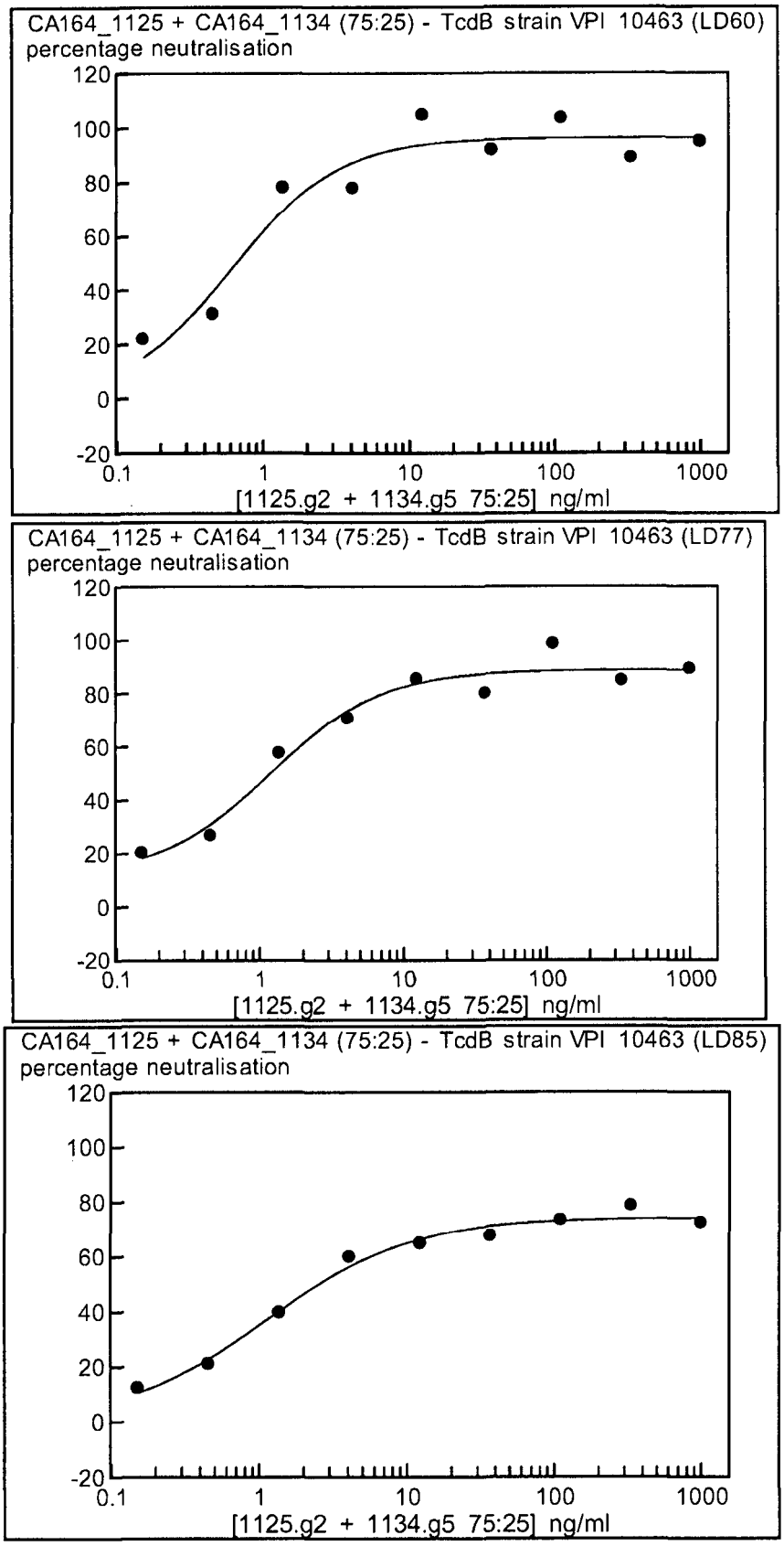
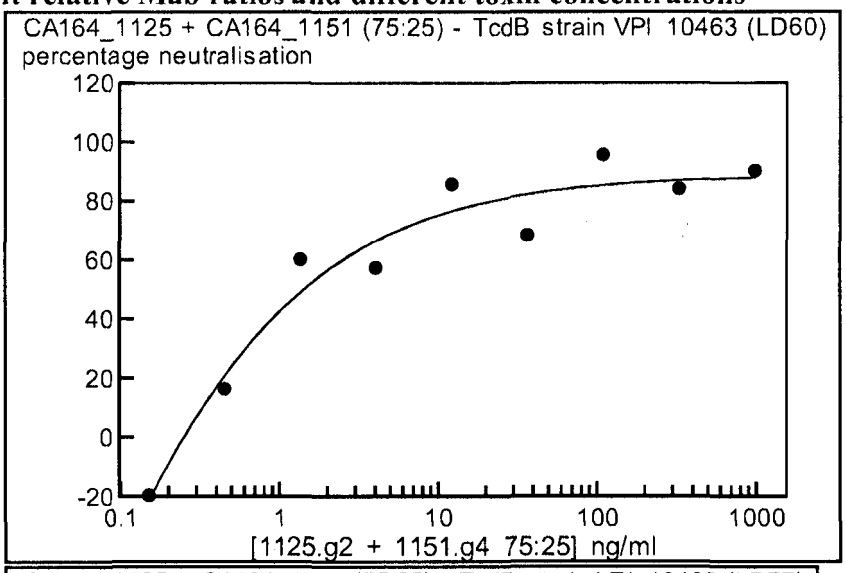
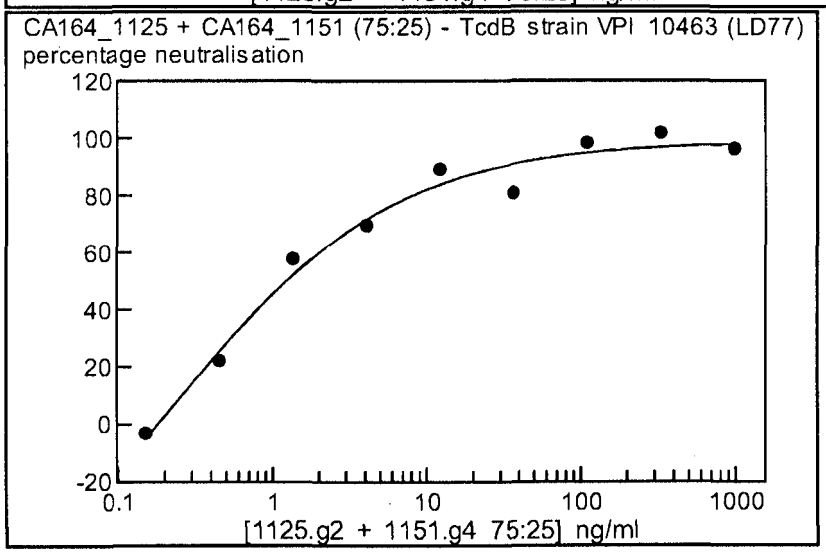


Figure 44 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations





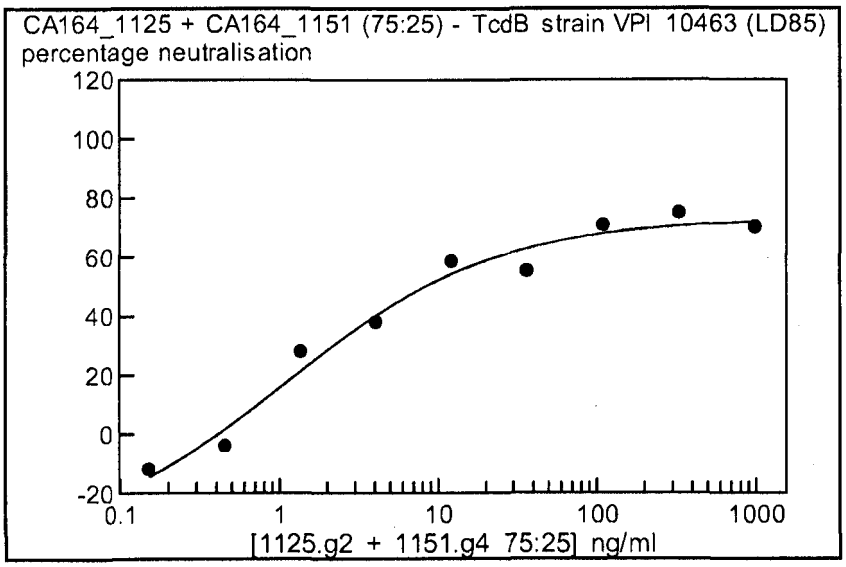


Figure 45 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations

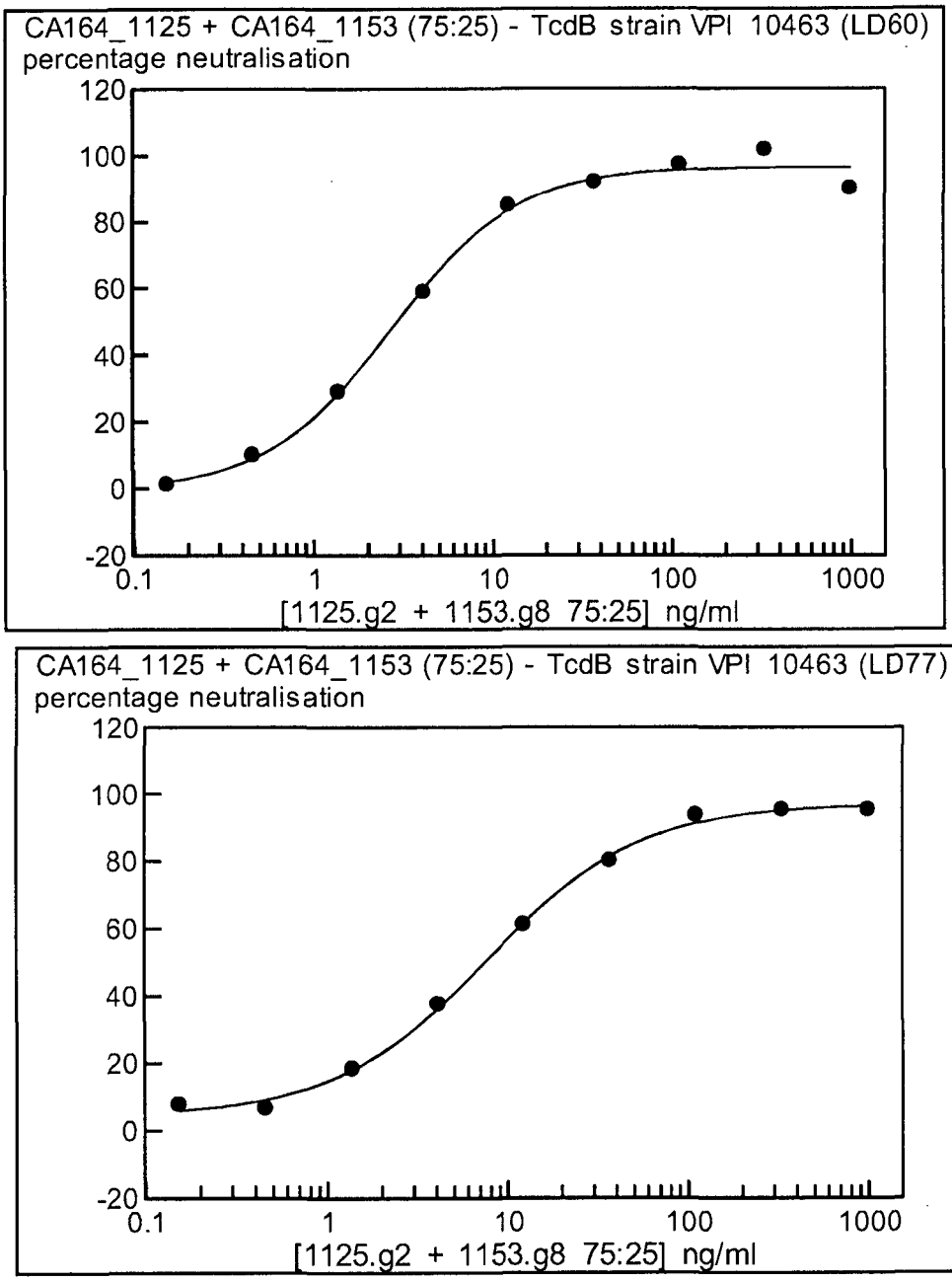


Figure 46 TcdB strain VPI 10463 neutralisation, Antibody singles and pairs, Constant toxin dose (LD80)

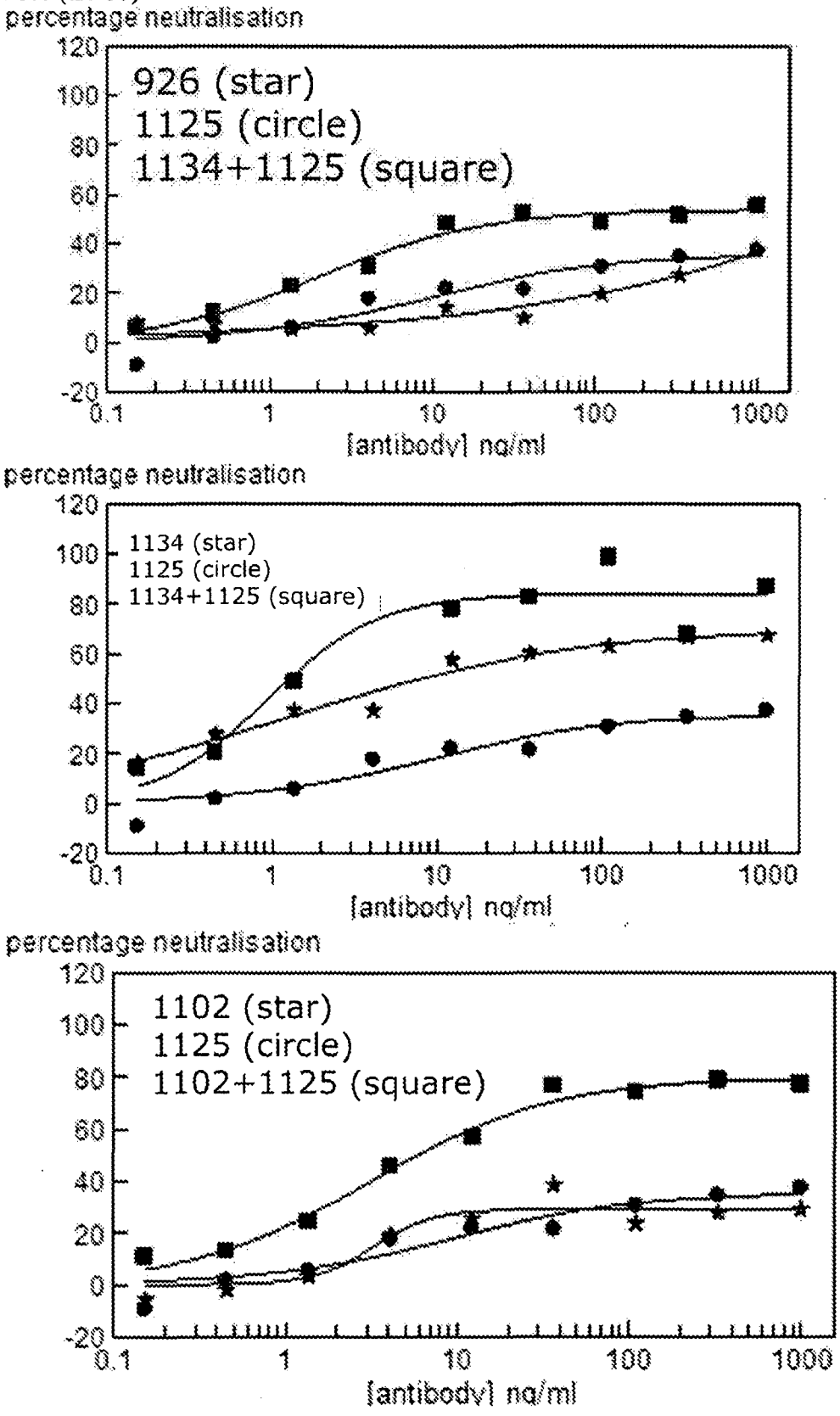


Figure 47 TcdB neutralisation, Antibody singles and pairs, Constant toxin dose (LD80)

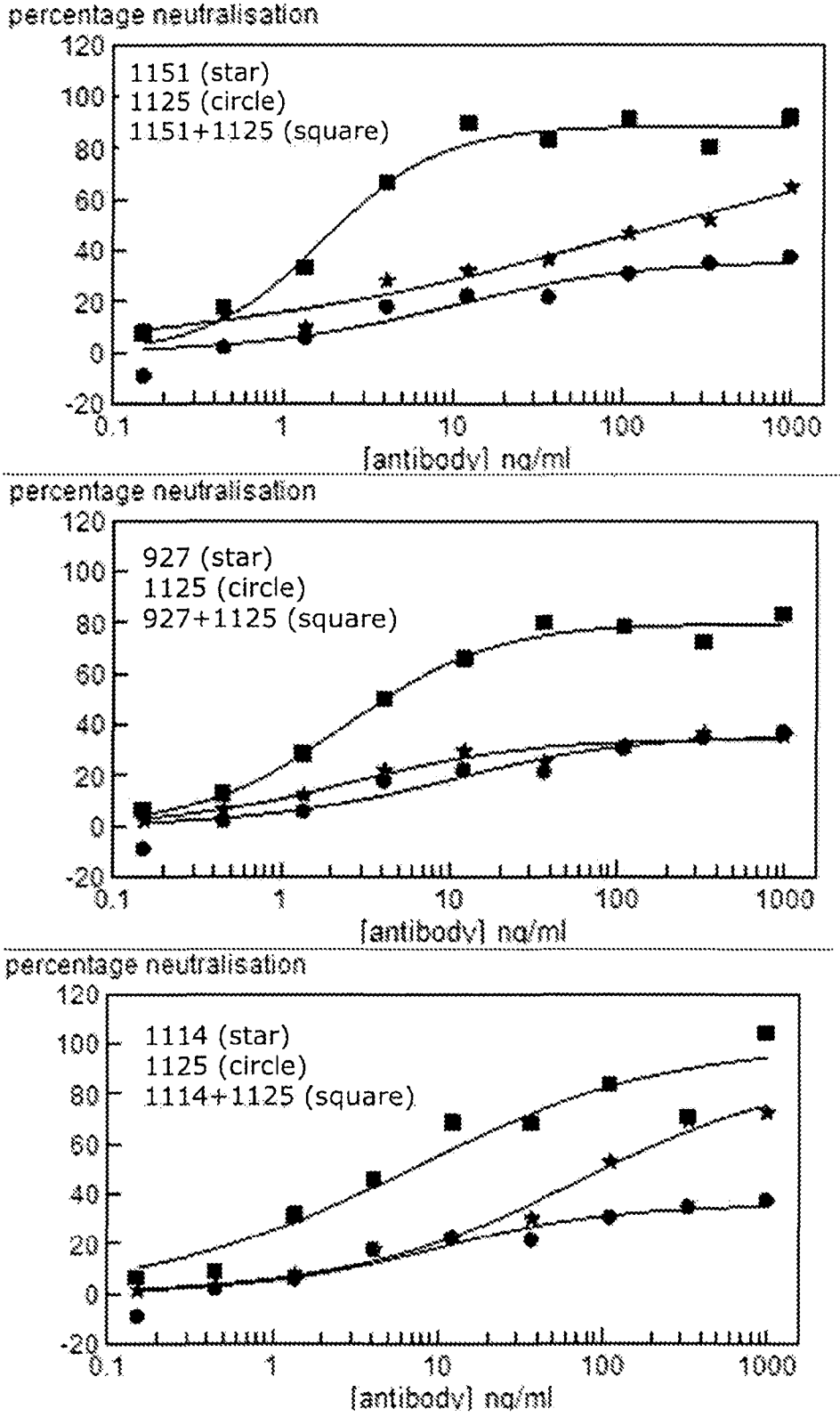


Figure 48 TcdB neutralisation, Antibody singles and pairs, Constant toxin dose (LD80)

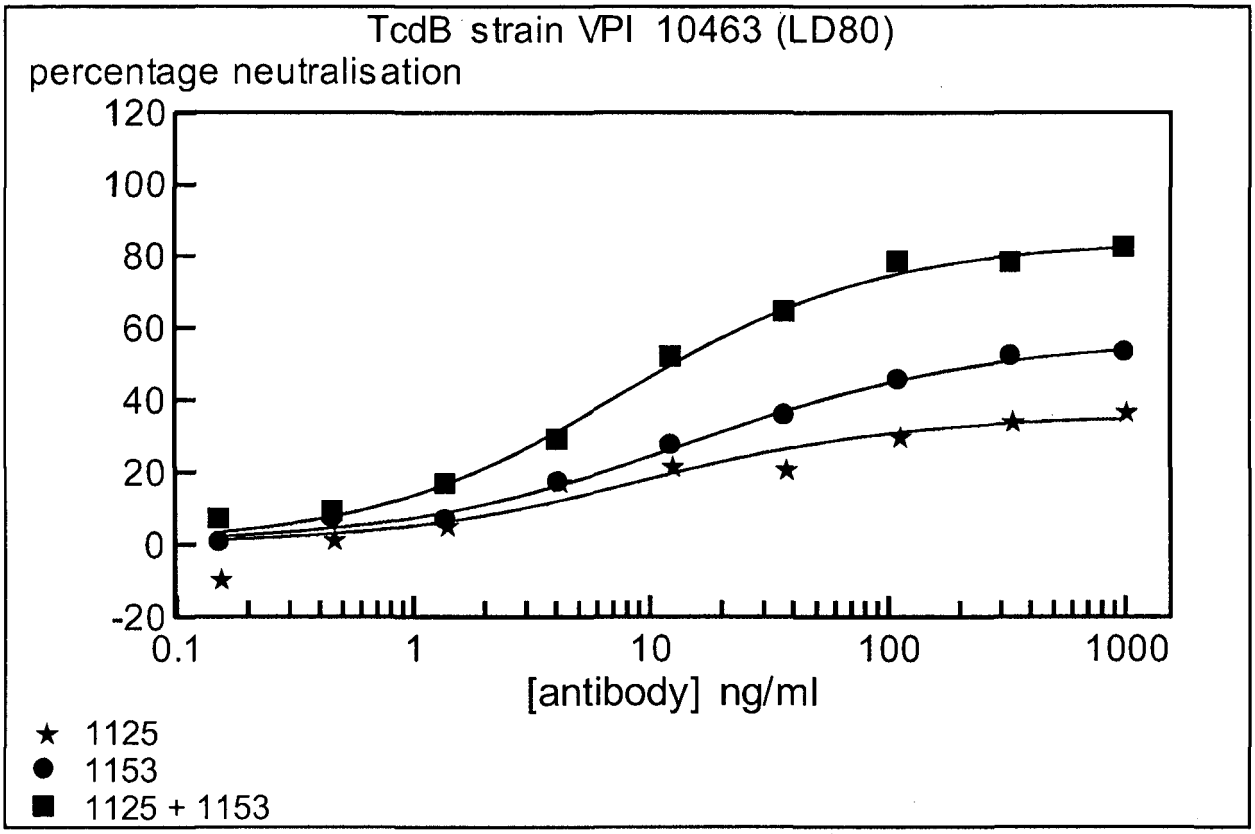


Figure 49 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

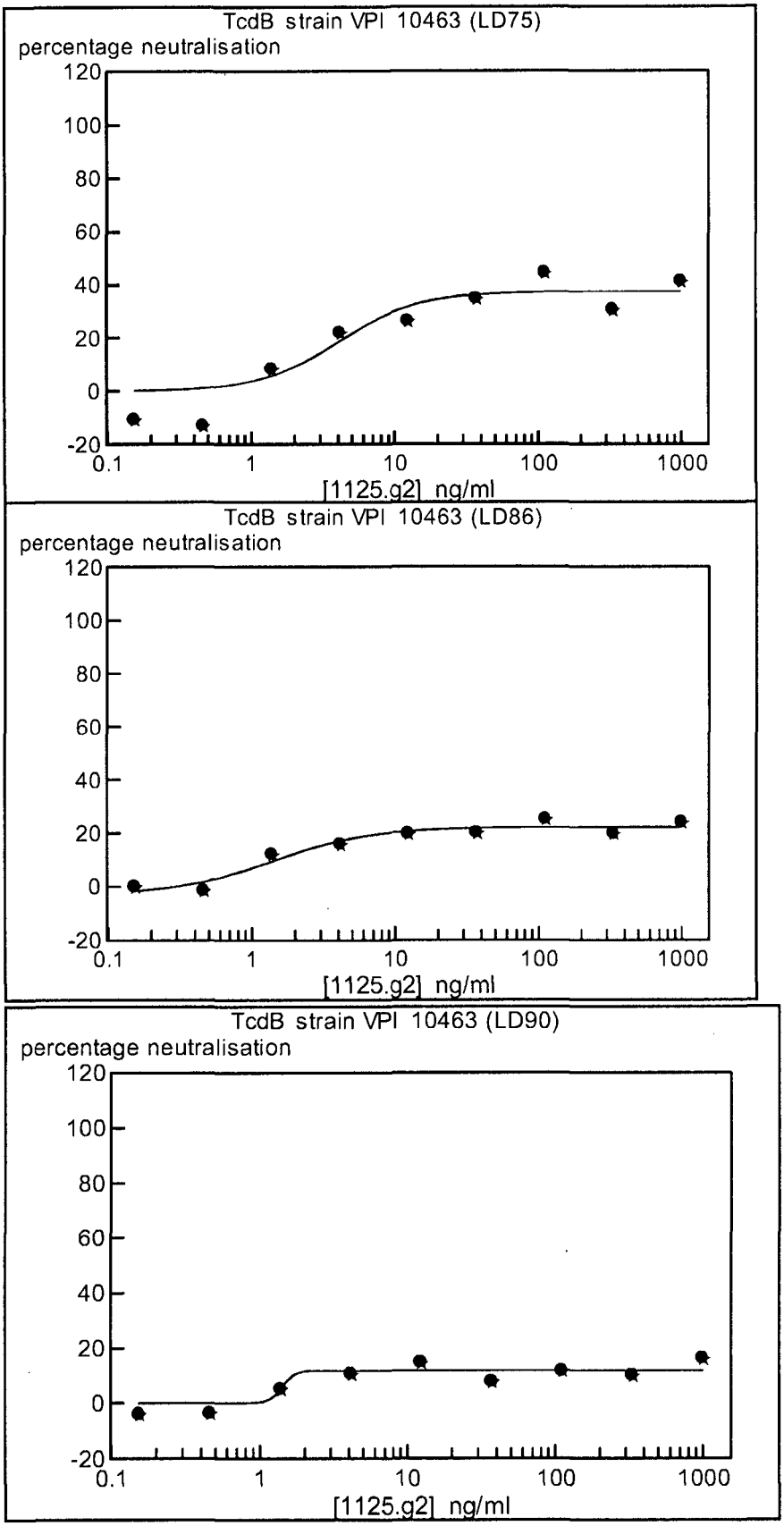


Figure 50 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

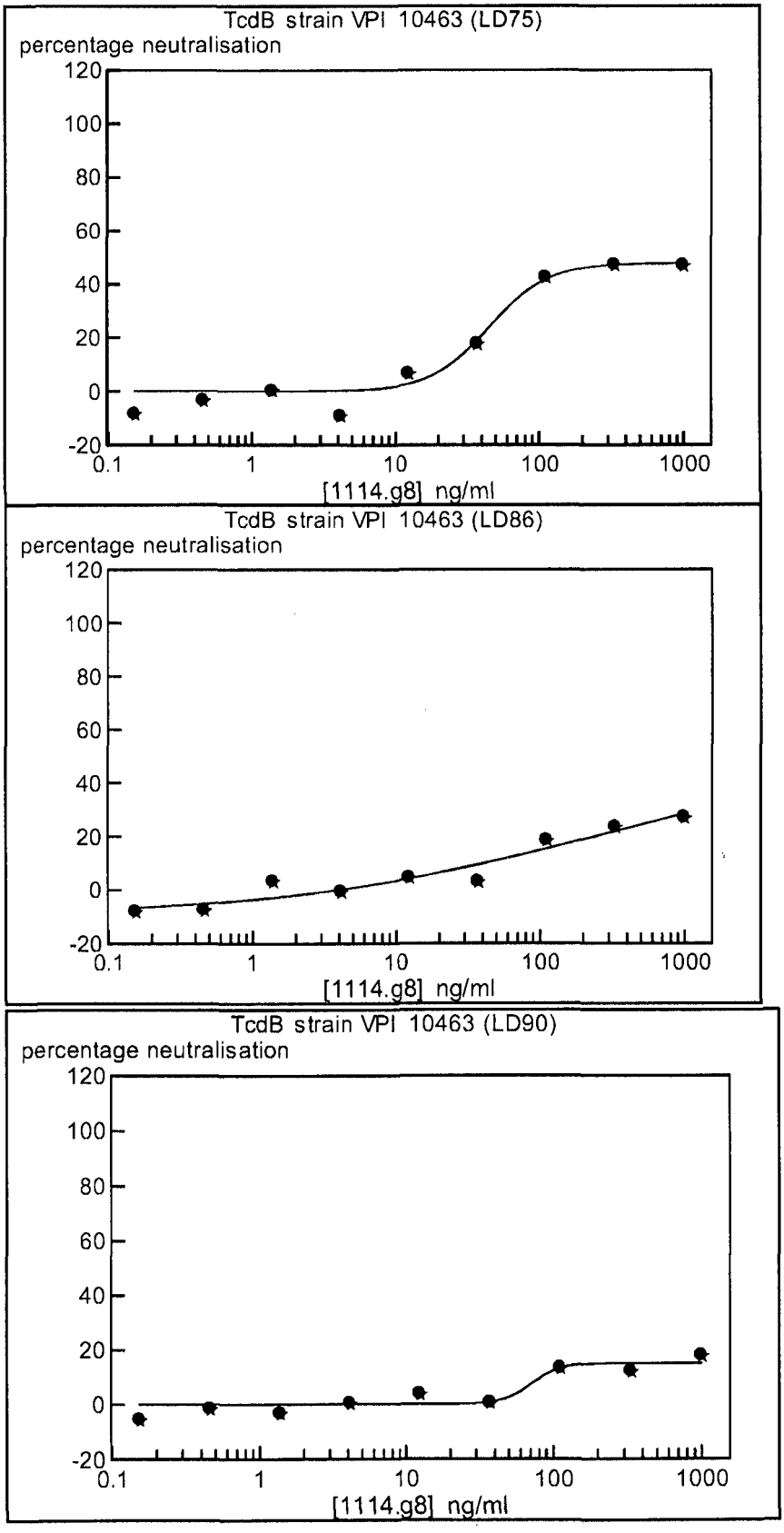


Figure 51 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

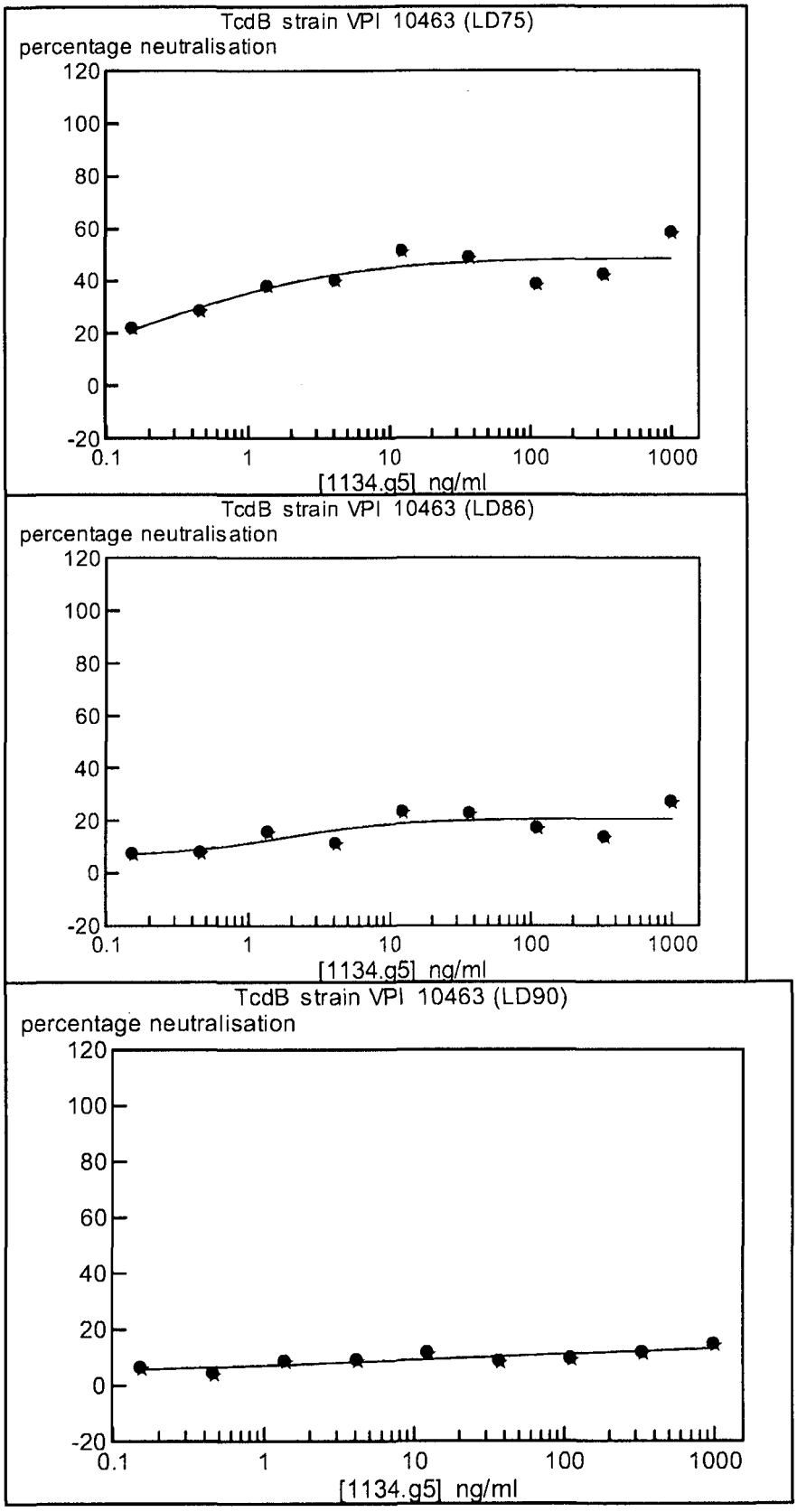


Figure 52 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

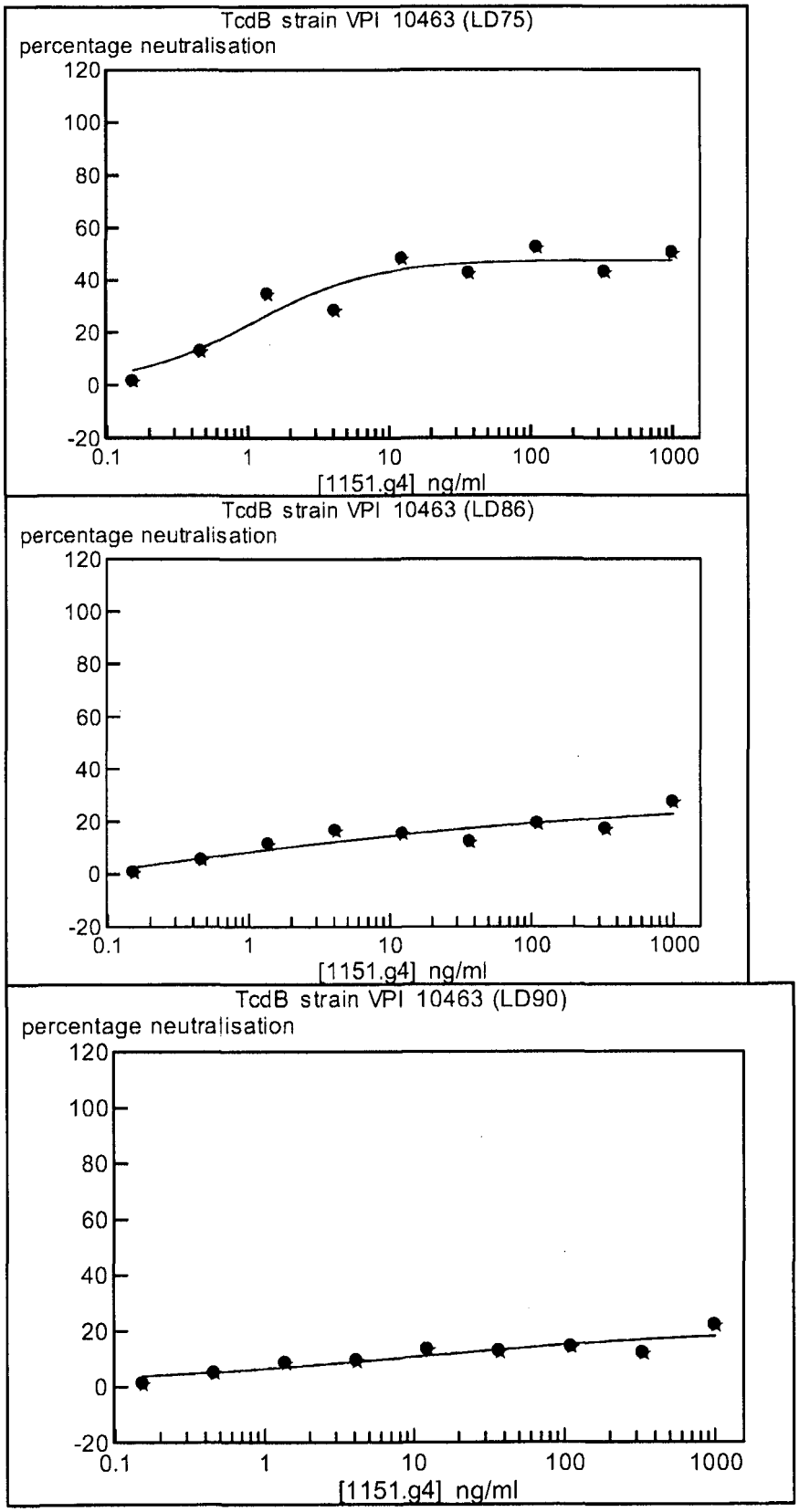


Figure 53 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

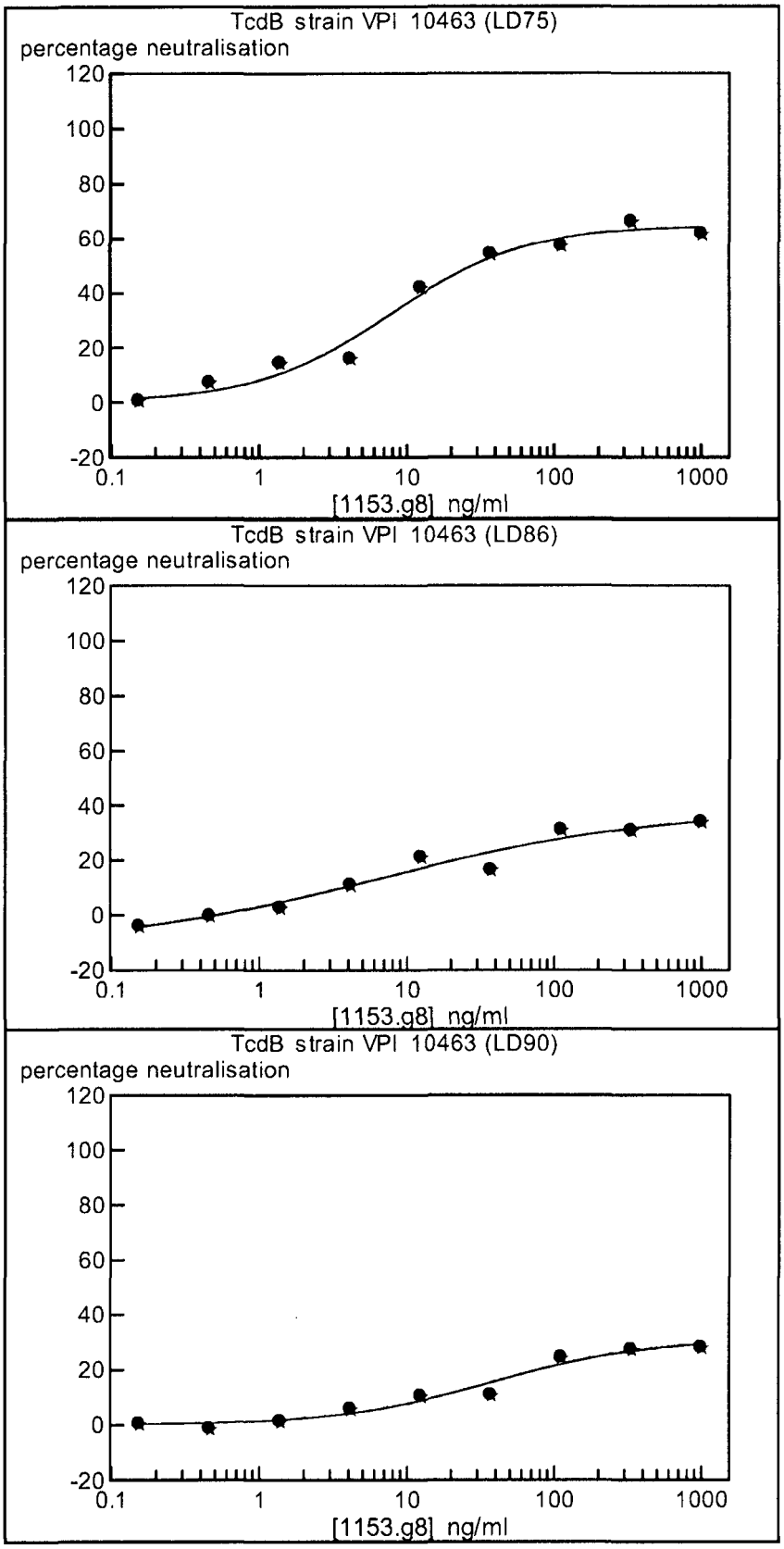


Figure 54 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

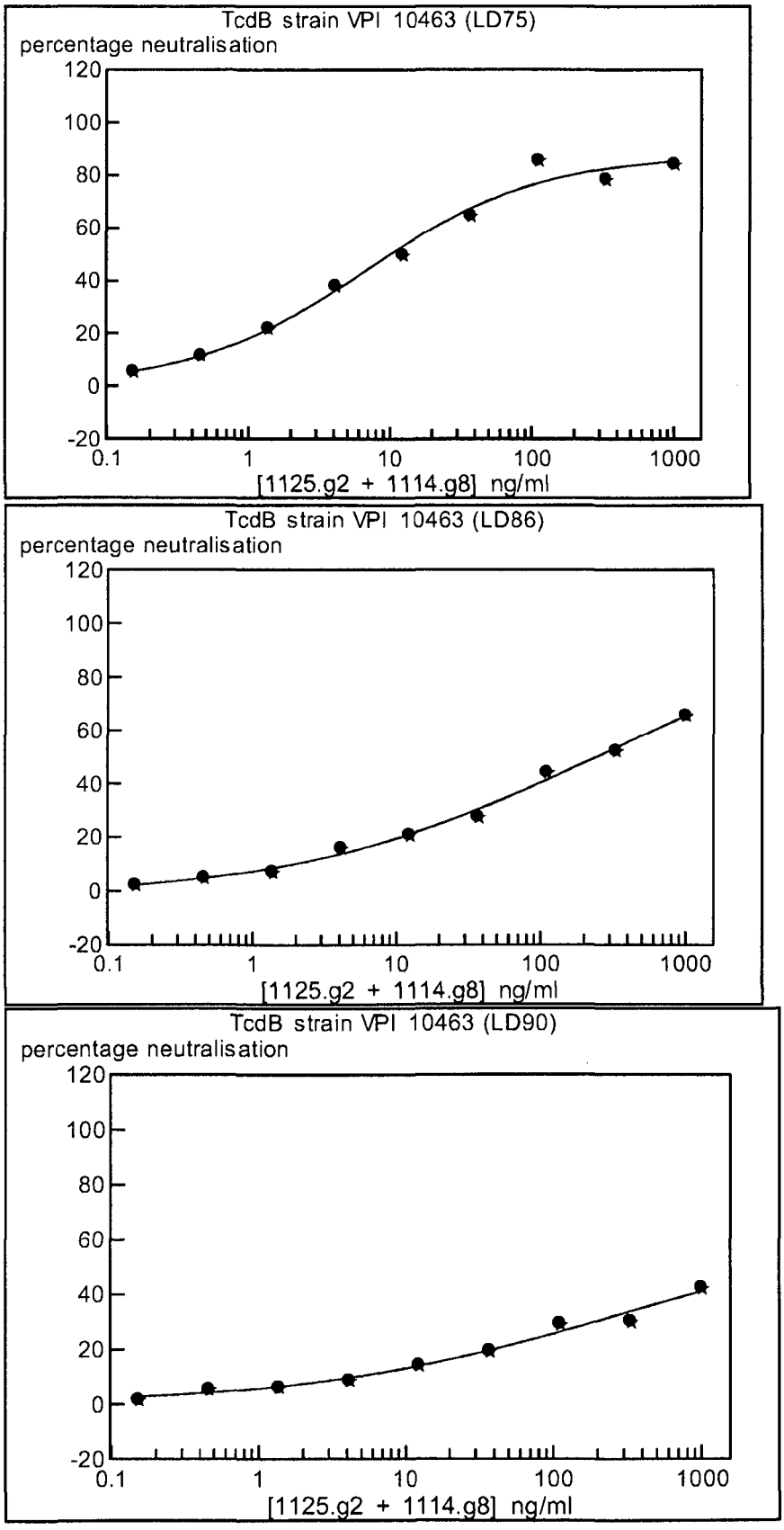


Figure 55 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

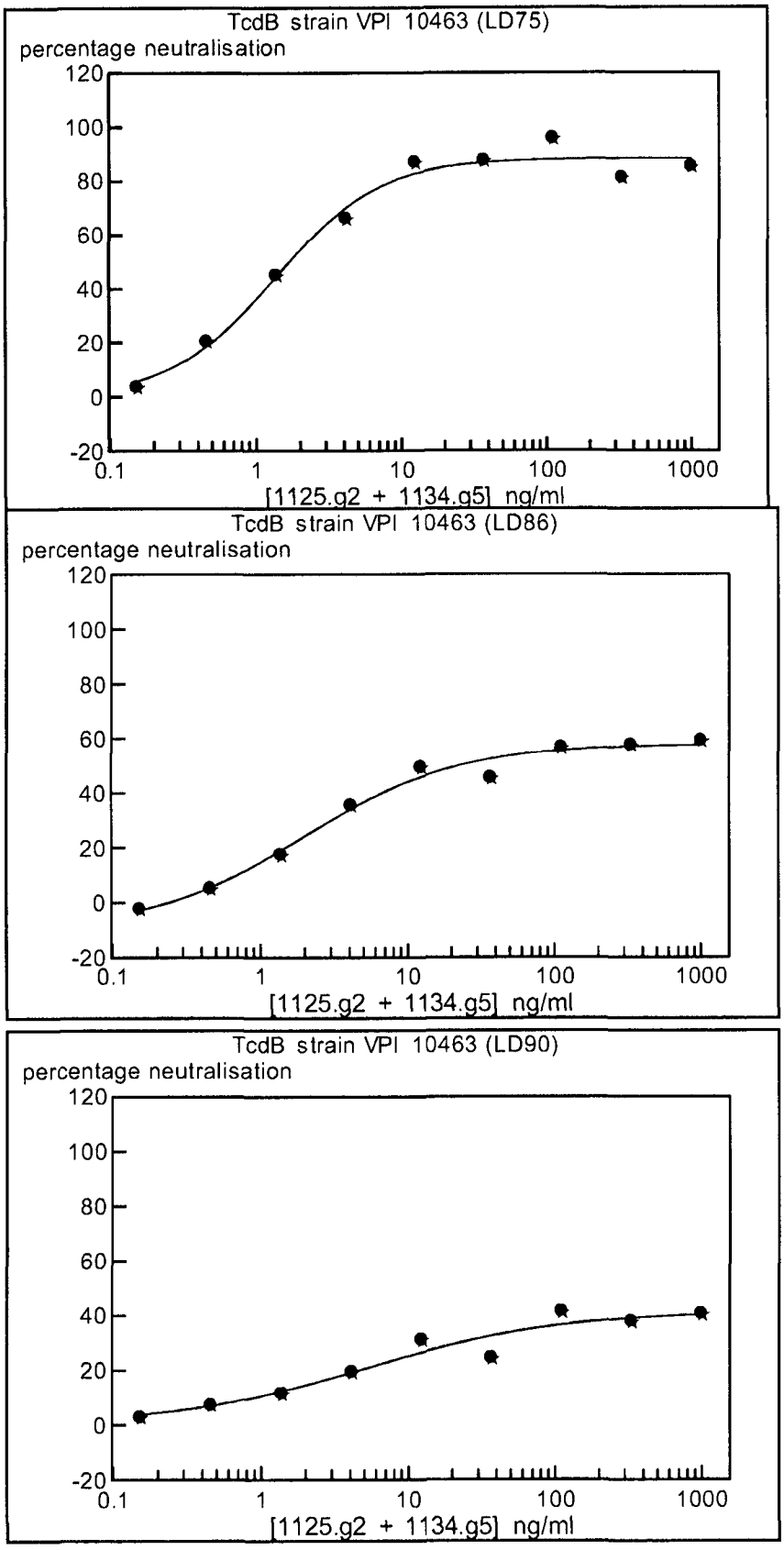
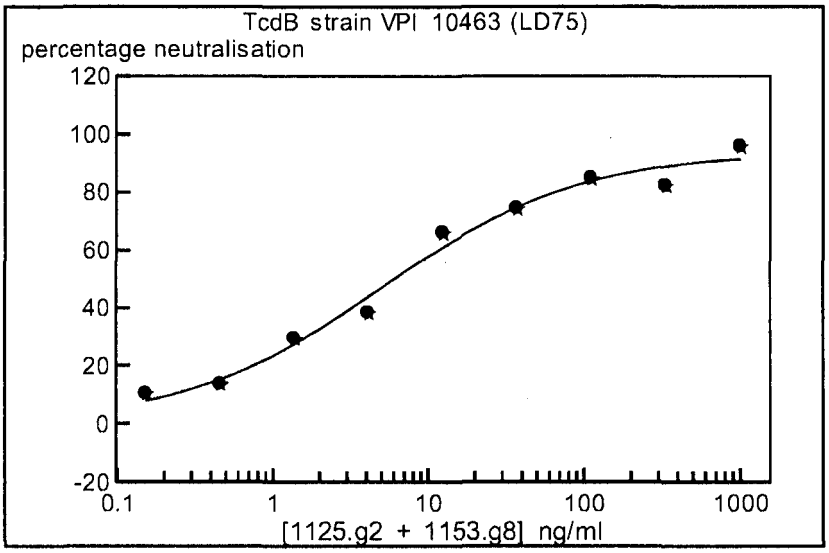
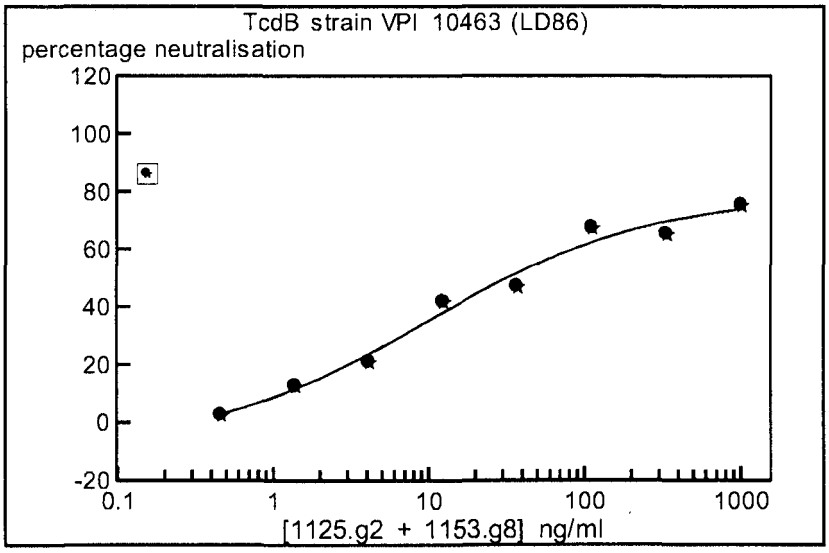


Figure 56 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)





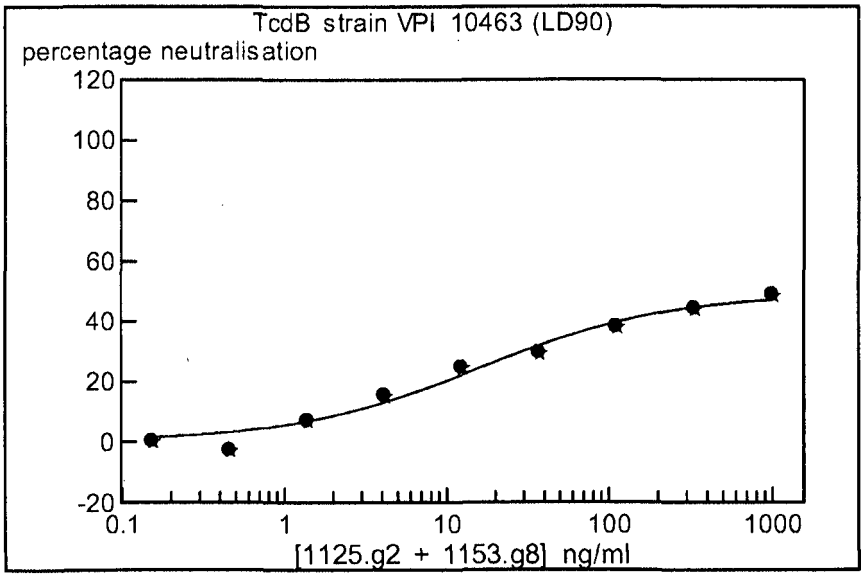


Figure 57 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

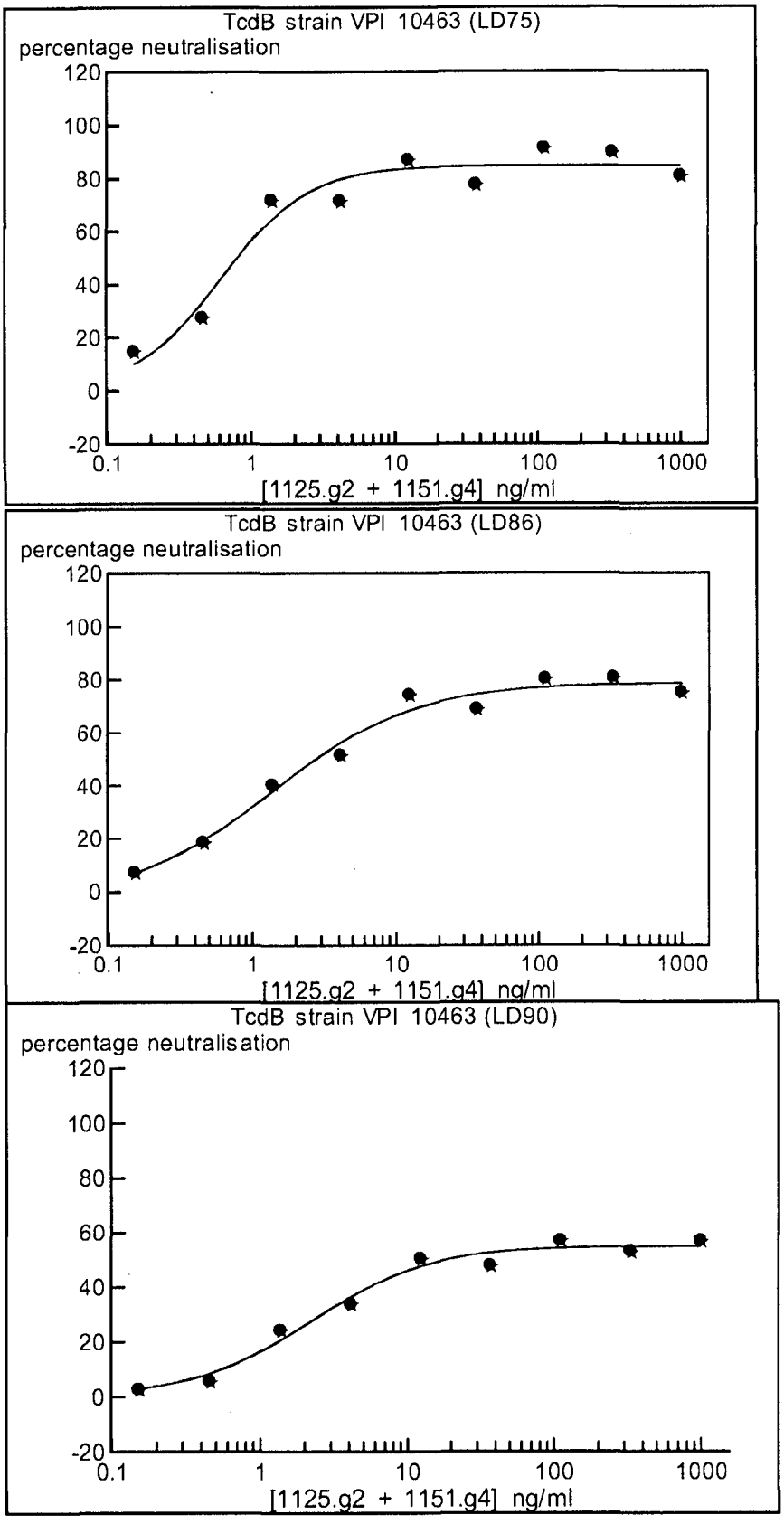


Figure 58 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

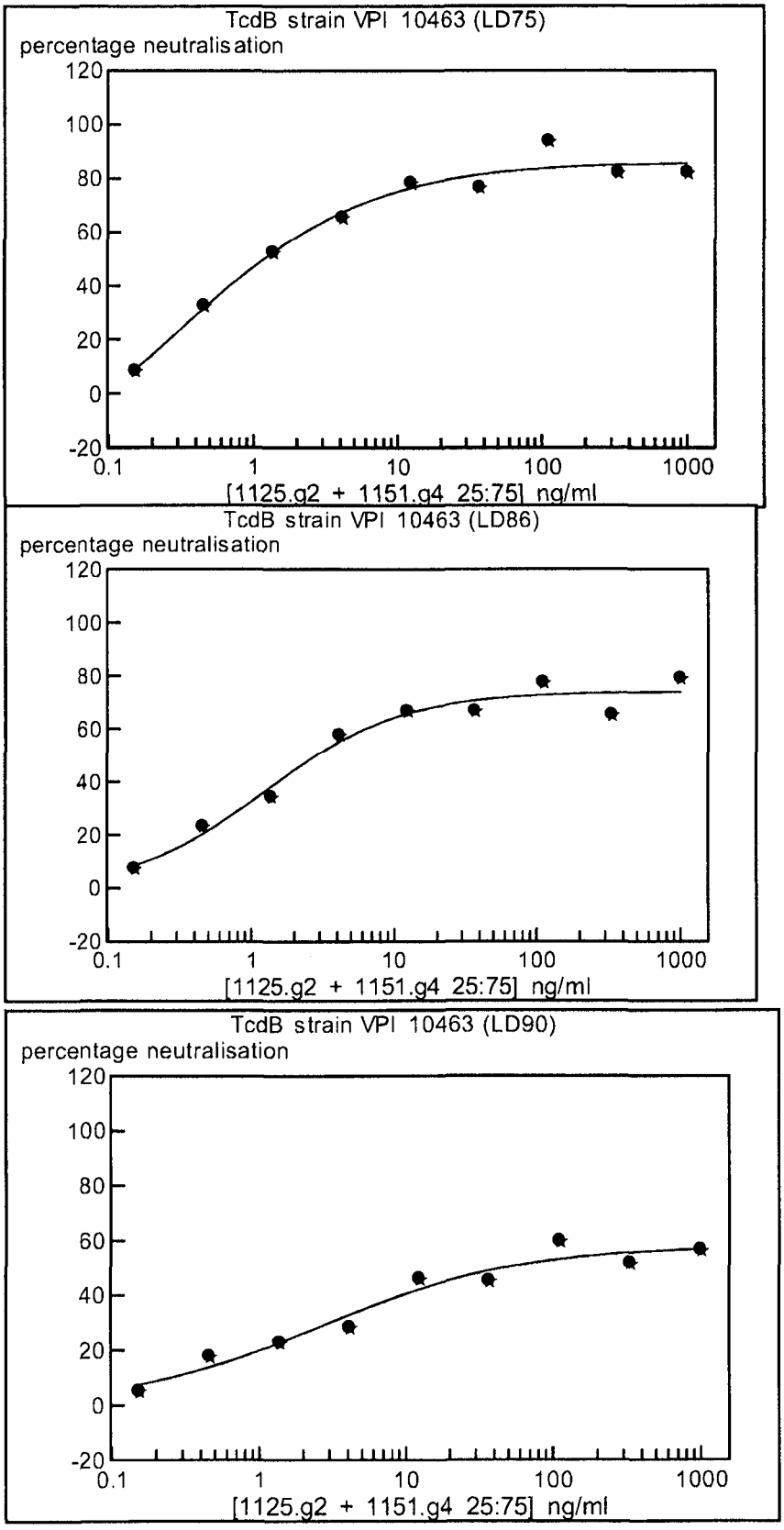


Figure 59 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

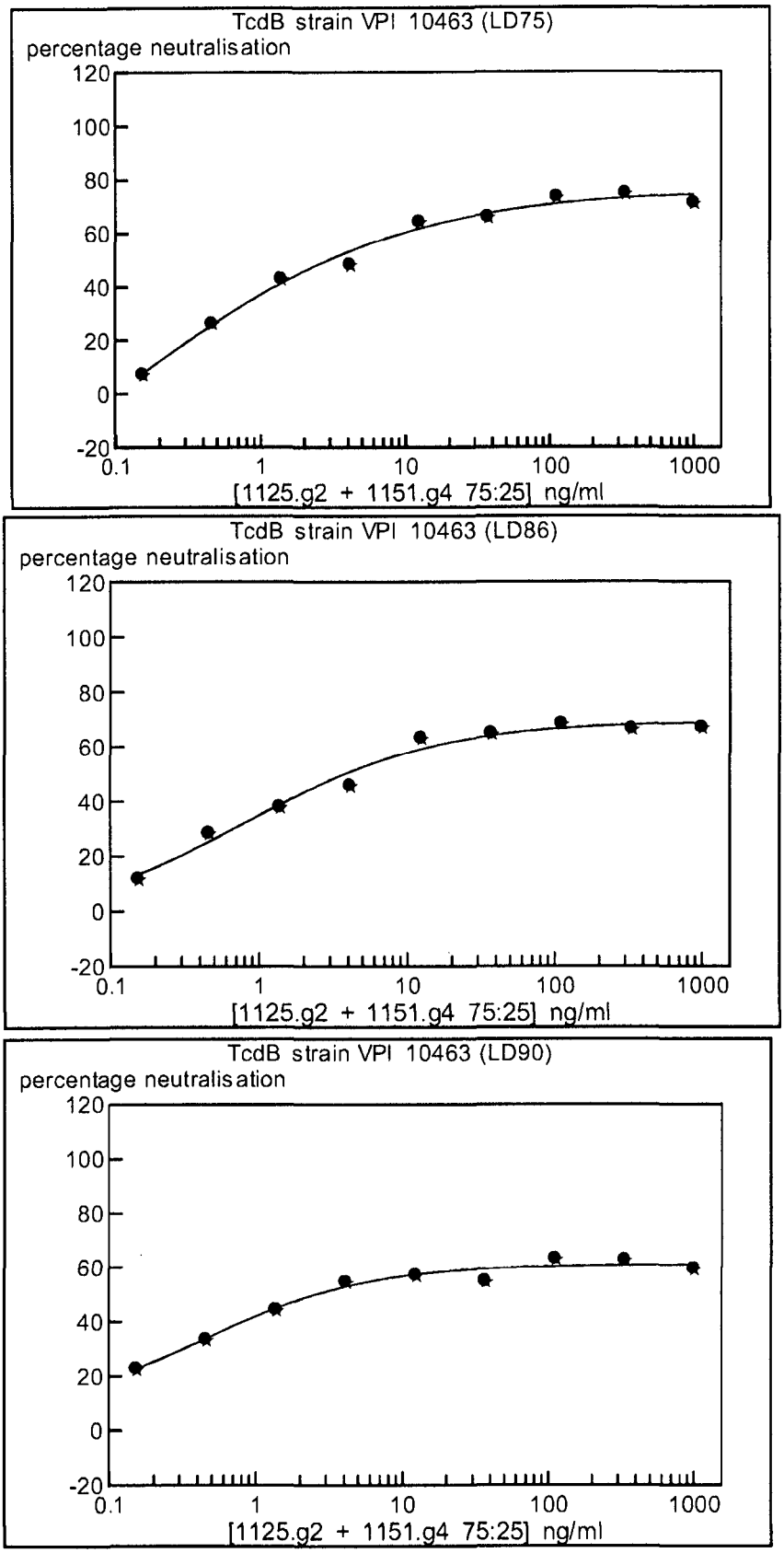


Figure 60 Amino Acid sequence for TcdA SEQ ID NO: 171

MSLISKEELI KLAYSIRPRE NEYKTILTNL DEYNKLTTNN NENKYLQLKK LNESIDVFMN
KYKTSSRNRA LSNLKKDILK EVILIKNSNT SPVEKNLHFV WIGGEVSDIA LEYIKQWADI
NAEYNIKLWY DSEAFLVNTL KKAIVESSTT EALQLLEEEI QNPQFDNMKF YKKRMEFIYD
RQKRFINYYK SQINKPTVPT IDDIIKSHLV SEYNRDETVL ESYRTNSLRK INSNHGIDIR
ANSLFTEQEL LNIYSQELLN RGNLAAASDI VRLLALKNFG GVYLDVDMLP GIHSDLFKTI
SRPSSIGLDR WEMIKLEAIM KYKKYINNYT SENFDKLDQQ LKDNFKLIIE SKSEKSEIFS
KLENLNVSDL EIKIAFALGS VINQALISKQ GSYLTNLVIE QVKNRYQFLN QHLNPAIESD
NNFTDTTKIF HDSLFNSATA ENSMFLTKIA PYLQVGFMPE ARSTISLSGP GAYASAYYDF
INLQENTIEK TLKASDLIEF KFPENNLSQL TEQEINSLWS FDQASAKYQF EKYVRDYTGG
SLSEDNGVDF NKNTALDKNY LLNNKIPSNN VEEAGSKNYV HYIIQLQGDD ISYEATCNLF
SKNPKNSIII QRNMNESAKS YFLSDDGESI LELNKYRIPE RLKNKEKVKV TFIGHGKDEF
NTSEFARLSV DSLSNEISSF LDTIKLDISP KNVEVNLLGC NMFSYDFNVE ETYPGKLLLS
IMDKITSTLP DVNKNSITIG ANQYEVRINS EGRKELLAHS GKWINKEEAI MSDLSSKEYI
FFDSIDNKLK AKSKNIPGLA SISEDIKTLL LDASVSPDTK FILNNLKLNI ESSIGDYIYY
EKLEPVKNII HNSIDDLIDE FNLLENVSDE LYELKKLNNL DEKYLISFED ISKNNSTYSV
RFINKSNGES VYVETEKEIF SKYSEHITKE ISTIKNSIIT DVNGNLLDNI QLDHTSQVNT
LNAAFFIQSL IDYSSNKDVL NDLSTSVKVQ LYAQLFSTGL NTIYDSIQLV NLISNAVNDT

INVLPTITEG IPIVSTILDG INLGAAIKEL LDEHDPLLKK ELEAKVGVLA INMSLSIAAT
VASIVGIGAE VTIFLLPIAG ISAGIPSLVN NELILHDKAT SVVNYFNHLS ESKKYGPLKT
EDDKILVPID DLVISEIDFN NNSIKLGTCN ILAMEGGSGH TVTGNIDHFF SSPSISSHIP
SLSIYSAIGI ETENLDFSKK IMMLPNAPSR VFWWETGAVP GLRSLENDGT RLLDSIRDLY
PGKFYWRFYA FFDYAITTLK PVYEDTNIKI KLDKDTRNFI MPTITTNEIR NKLSYSFDGA
GGTYSLLLSS YPISTNINLS KDDLWIFNID NEVREISIEN GTIKKGKLIK DVLSKIDINK
NKLIIGNQTI DFSGDIDNKD RYIFLTCELD DKISLIIEIN LVAKSYSLLL SGDKNYLISN
LSNTIEKINT LGLDSKNIAY NYTDESNNKY FGAISKTSQK SIIHYKKDSK NILEFYNDST
LEFNSKDFIA EDINVFMKDD INTITGKYYV DNNTDKSIDF SISLVSKNQV KVNGLYLNES
VYSSYLDFVK NSDGHHNTSN FMNLFLDNIS FWKLFGFENI NFVIDKYFTL VGKTNLGYVE
FICDNNKNID IYFGEWKTSS SKSTIFSGNG RNVVVEPIYN PDTGEDISTS LDFSYEPLYG
IDRYINKVLI APDLYTSLIN INTNYYSNEY YPEIIVLNPN TFHKKVNINL DSSSFEYKWS
TEGSDFILVR YLEESNKKIL QKIRIKGILS NTQSFNKMSI DFKDIKKLSL GYIMSNFKSF
NSENELDRDH LGFKIIDNKT YYYDEDSKLV KGLININNSL FYFDPIEFNL VTGWQTINGK
KYYFDINTGA ALTSYKIING KHFYFNNDGV MQLGVFKGPD GFEYFAPANT QNNNIEGQAI
VYQSKFLTLN GKKYYFDNNS KAVTGWRIIN NEKYYFNPNN AIAAVGLQVI DNNKYYFNPD
TAIISKGWQT VNGSRYYFDT DTAIAFNGYK TIDGKHFYFD SDCVVKIGVF STSNGFEYFA
PANTYNNNIE GQAIVYQSKF LTLNGKKYYF DNNSKAVTGL QTIDSKKYYF NTNTAEAATG

WQTIDGKKYY FNTNTAEAAT GWQTIDGKKY YFNTNTAIAS TGYTIINGKH FYFNTDGIMQ
IGVFKGPNGF EYFAPANTDA NNIEGQAILY QNEFLTLNGK KYYFGSDSKA VTGWRIINNK
KYYFNPNNAI AAIHLCTINN DKYYFSYDGI LQNGYITIER NNFYFDANNE SKMVTGVFKG
PNGFEYFAPA NTHNNNIEGQ AIVYQNKFLT LNGKKYYFDN DSKAVTGWQT IDGKKYYFNL
NTAEAATGWQ TIDGKKYYFN LNTAEAATGW QTIDGKKYYF NTNTFIASTG YTSINGKHFY
FNTDGIMQIG VFKGPNGFEY FAPANTDANN IEGQAILYQN KFLTLNGKKY YFGSDSKAVT
GLRTIDGKKY YFNTNTAVAV TGWQTINGKK YYFNTNTSIA STGYTIISGK HFYFNTDGIM
QIGVFKGPDG FEYFAPANTD ANNIEGQAIR YQNRFLYLHD NIYYFGNNSK AATGWVTIDG
NRYYFEPNTA MGANGYKTID NKNFYFRNGL PQIGVFKGSN GFEYFAPANT DANNIEGQAI

RYQNRFLHLL GKIYYFGNNS KAVTGWQTIN GKVYYFMPDT AMAAAGGLFE IDGVIYFFGV
DGVKAPGIYG

Figure 61 Amino Acid sequence for TcdB SEQ ID NO: 172

MSLVNRKQLE KMANVRFRTQ EDEYVAILDA LEEYHNMSEN TVVEKYLKLK DINSLTDIYI
DTYKKSCRNK ALKKFKEYLV TEVLELKNNN LTPVEKNLHF VWIGGQINDT AINYINQWKD
VNSDYNVNVF YDSNAFLINT LKKTVVESAI NDTLESFREN LNDPRFDYNK FFRKRMEIIY
DKQKNFINYY KAQREENPEL IIDDIVKTYL SNEYSKEIDE LNTYIEESLN KITQNSGNDV
RNFEEFKNGE SFNLYEQELV ERWNLAAASD ILRISALKEI GGMYLDVDML PGIQPDLFES
IEKPSSVTVD FWEMTKLEAI MKYKEYIPEY TSEHFDMLDE EVQSSFESVL ASKSDKSEIF
SSLGDMEASP LEVKIAFNSK GIINQGLISV KDSYCSNLIV KQIENRYKIL NNSLNPAISE
DNDFNTTTNT FIDSIMAEAN ADNGRFMMEL GKYLRVGFFP DVKTTINLSG PEAYAAAYQD
LLMFKEGSMN IHLIEADLRN FEISKTNISQ STEQEMASLW SFDDARAKAQ FEEYKRNYFE
GSLGEDDNLD FSQNIVVDKE YLLEKISSLA RSSERGYIHY IVQLQGDKIS YEAACNLFAK
TPYDSVLFQK NIEDSEIAYY YNPGDGEIQE IDKYKIPSII SDRPKIKLTF IGHGKDEFNT
DIFAGFDVDS LSTEIEAAID LAKEDISPKS IEINLLGCNM FSYSINVEET YPGKLLLKVK

DKISELMPSI SQDSIIVSAN QYEVRINSEG RRELLDHSGE WINKEESIIK DISSKEYISF
NPKENKITVK SKNLPELSTL LQEIRNNSNS SDIELEEKVM LTECEINVIS NIDTQIVEER
IEEAKNLTSD SINYIKDEFK LIESISDALC DLKQQNELED SHFISFEDIS ETDEGFSIRF
INKETGESIF VETEKTIFSE YANHITEEIS KIKGTIFDTV NGKLVKKVNL DTTHEVNTLN
AAFFIQSLIE YNSSKESLSN LSVAMKVQVY AQLFSTGLNT ITDAAKVVEL VSTALDETID
LLPTLSEGLP IIATIIDGVS LGAAIKELSE TSDPLLRQEI EAKIGIMAVN LTTATTAIIT
SSLGIASGFS ILLVPLAGIS AGIPSLVNNE LVLRDKATKV VDYFKHVSLV ETEGVFTLLD

DKIMMPQDDL VISEIDFNNN SIVLGKCEIW RMEGGSGHTV TDDIDHFFSA PSITYREPHL
SIYDVLEVQK EELDLSKDLM VLPNAPNRVF AWETGWTPGL RSLENDGTKL LDRIRDNYEG
EFYWRYFAFI ADALITTLKP RYEDTNIRIN LDSNTRSFIV PIITTEYIRE KLSYSFYGSG
GTYALSLSQY NMGINIELSE SDVWIIDVDN VVRDVTIESD KIKKGDLIEG ILSTLSIEEN
KIILNSHEIN FSGEVNGSNG FVSLTFSILE GINAIIEVDL LSKSYKLLIS GELKILMLNS

NHIQQKIDYI GFNSELQKNI PYSFVDSEGK ENGFINGSTK EGLFVSELPD VVLISKVYMD
DSKPSFGYYS NNLKDVKVIT KDNVNILTGY YLKDDIKISL SLTLQDEKTI KLNSVHLDES
GVAEILKFMN RKGNTNTSDS LMSFLESMNI KSIFVNFLQS NIKFILDANF IISGTTSIGQ
FEFICDENDN IQPYFIKFNT LETNYTLYVG NRQNMIVEPN YDLDDSGDIS STVINFSQKY
LYGIDSCVNK VVISPNIYTD EINITPVYET NNTYPEVIVL DANYINEKIN VNINDLSIRY
VWSNDGNDFI LMSTSEENKV SQVKIRFVNV FKDKTLANKL SFNFSDKQDV PVSEIILSFT
PSYYEDGLIG YDLGLVSLYN EKFYINNFGM MVSGLIYIND SLYYFKPPVN NLITGFVTVG
DDKYYFNPIN GGAASIGETI IDDKNYYFNQ SGVLQTGVFS TEDGFKYFAP ANTLDENLEG
EAIDFTGKLI IDENIYYFDD NYRGAVEWKE LDGEMHYFSP ETGKAFKGLN QIGDYKYYFN
SDGVMQKGFV SINDNKHYFD DSGVMKVGYT EIDGKHFYFA ENGEMQIGVF NTEDGFKYFA
HHNEDLGNEE GEEISYSGIL NFNNKIYYFD DSFTAVVGWK DLEDGSKYYF DEDTAEAYIG
LSLINDGQYY FNDDGIMQVG FVTINDKVFY FSDSGIIESG VQNIDDNYFY IDDNGIVQIG
VFDTSDGYKY FAPANTVNDN IYGQAVEYSG LVRVGEDVYY FGETYTIETG WIYDMENESD
KYYFNPETKK ACKGINLIDD IKYYFDEKGI MRTGLISFEN NNYYFNENGE MQFGYINIED
KMFYFGEDGV MQIGVFNTPD GFKYFAHQNT LDENFEGESI NYTGWLDLDE KRYYFTDEYI
AATGSVIIDG EEYYFDPDTA QLVISE

Figure 62 Caco-2 monolayer (Trans-Epithelial Electrical Resistance) data – TcdA

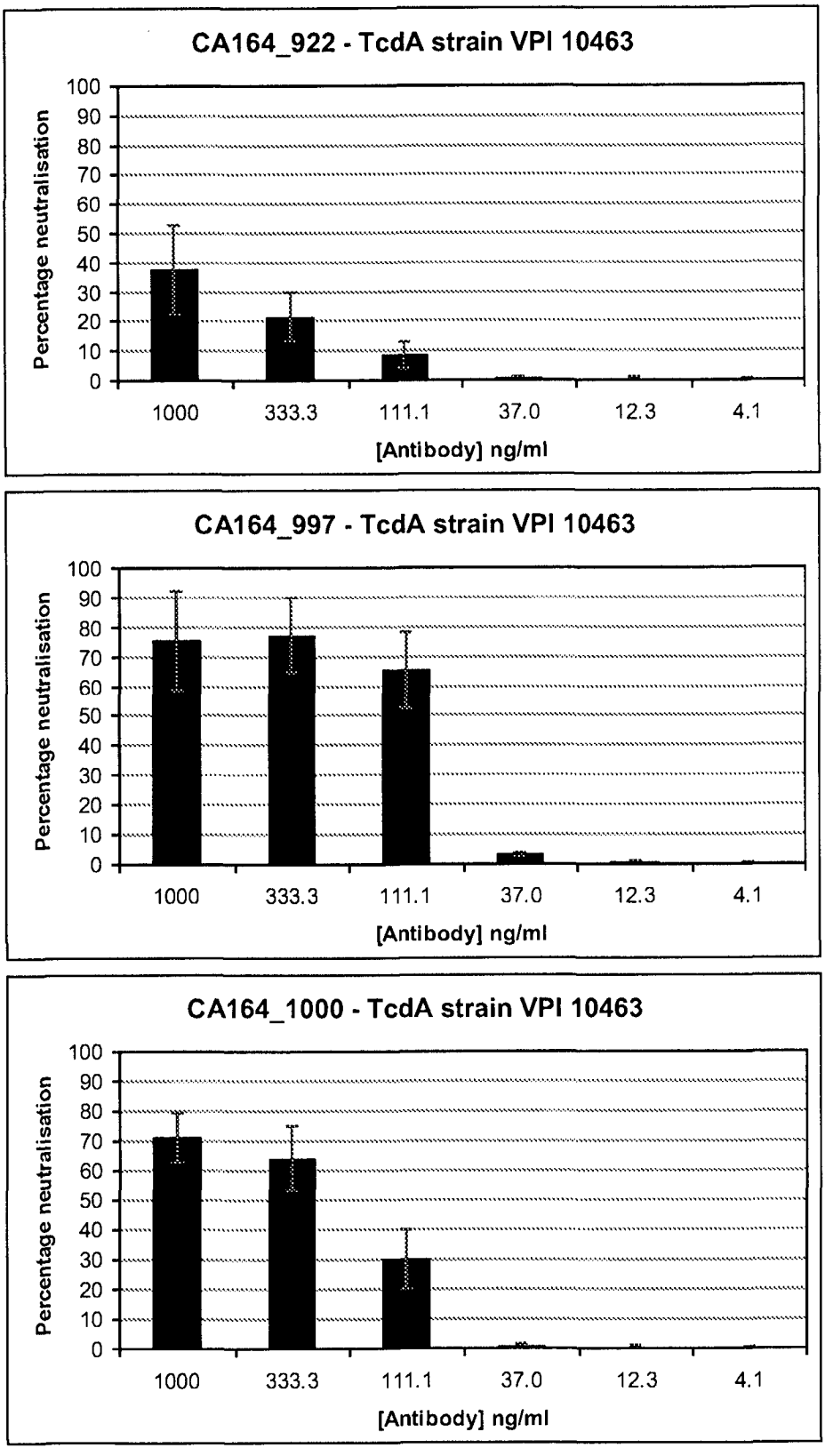


Figure 62A

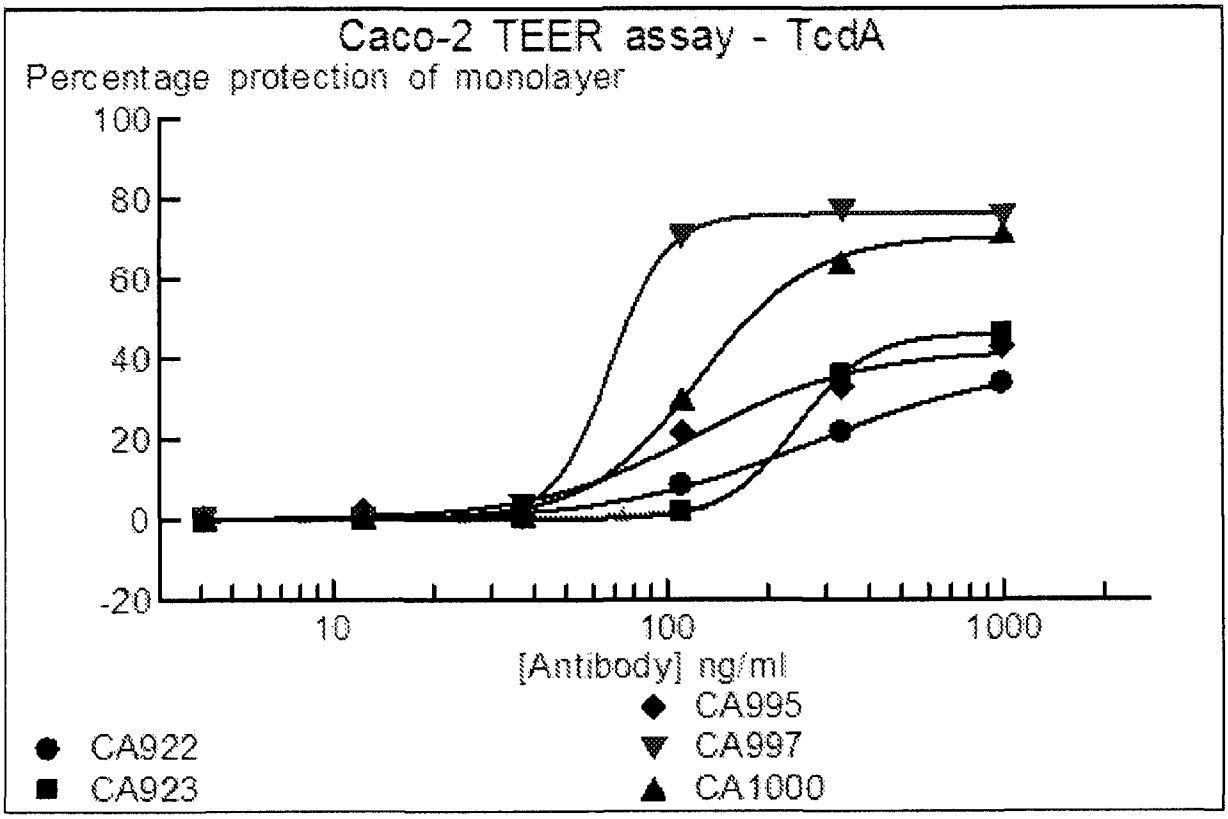

Caco-2 TEER assay - TcdA
Percentage protection of monolayer
100
80
60
40
20
0
-20
10
100
1000
[Antibody] ng/ml
CA922
CA923
CA995
CA997
CA1000

Figure 63

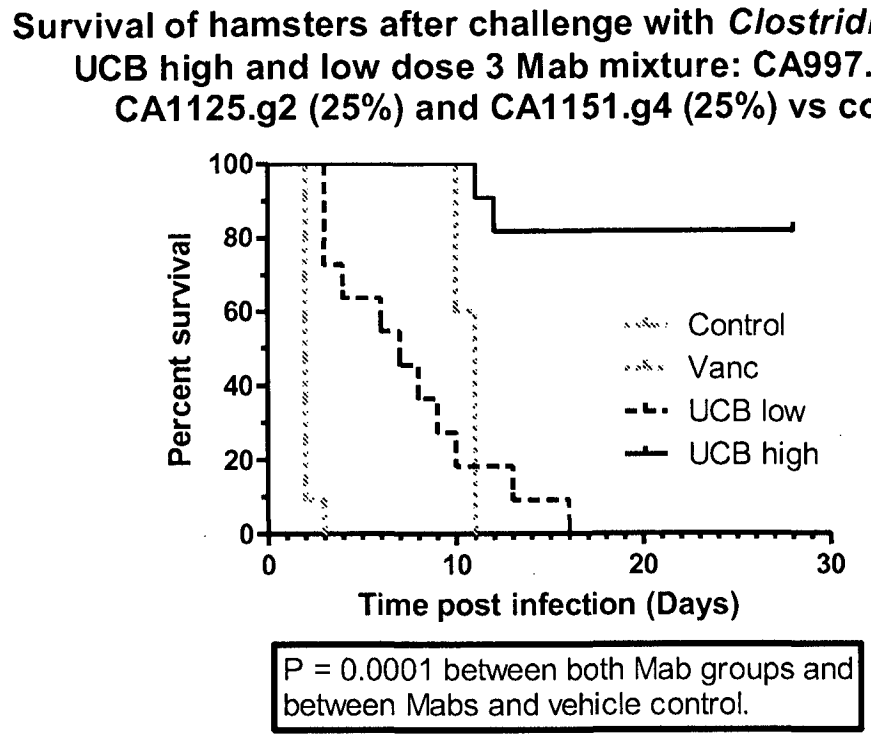


Figure 64 Hamster body weight changes

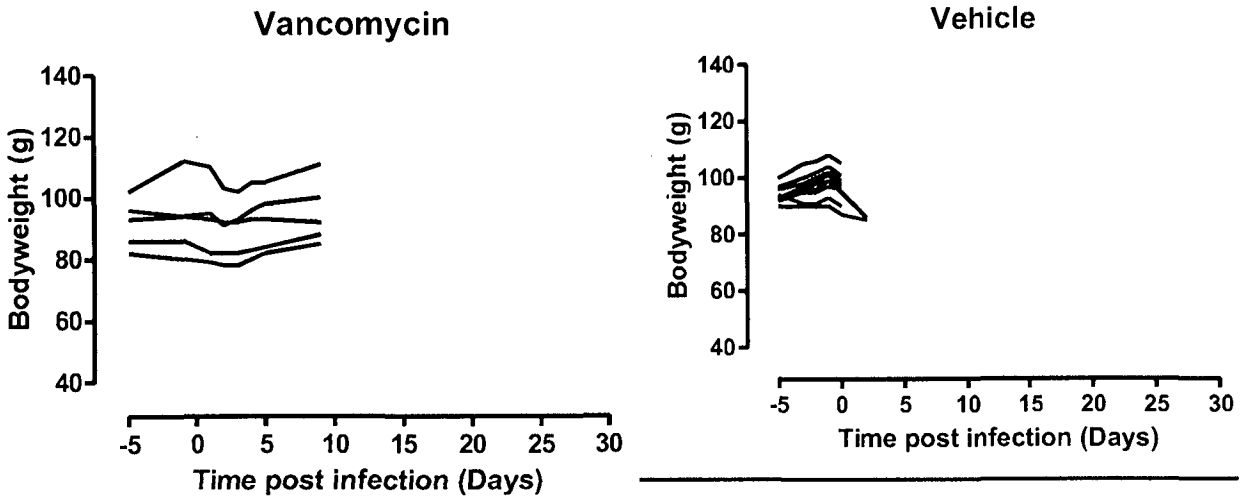


Figure 65

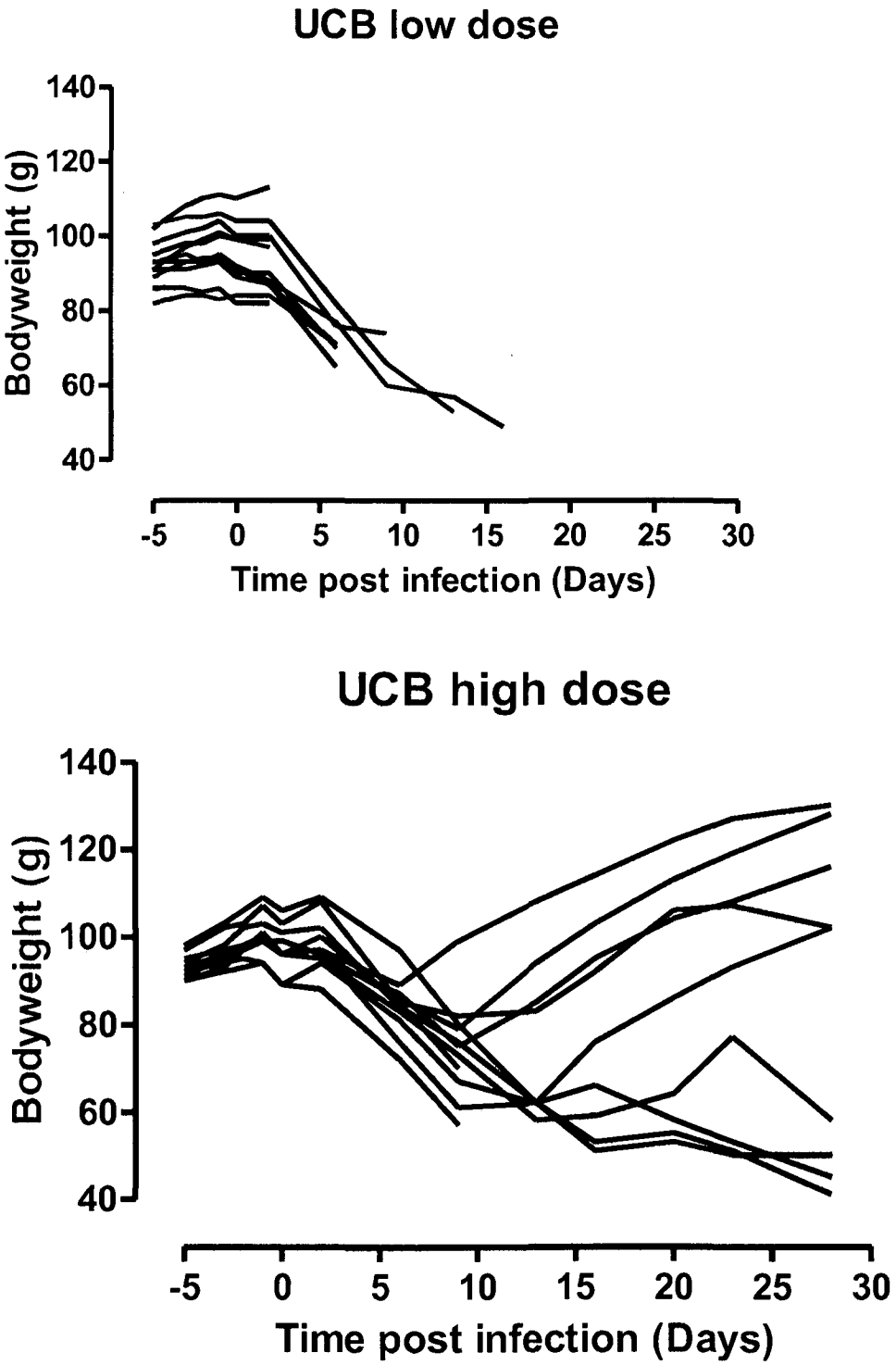
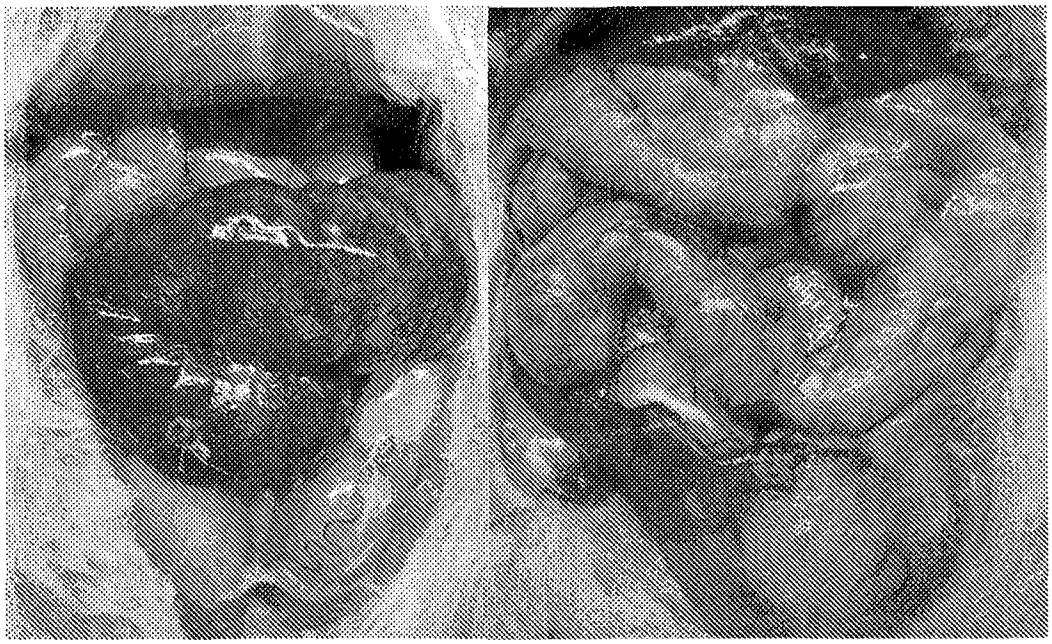

UCB low dose
Bodyweight (g)
140
120
100
80
60
40
-5
0
5
10
15
20
25
30
Time post infection (Days)
UCB high dose
Bodyweight (g)
140
120
100
80
60
40
-5
0
5
10
15
20
25
30
Time post infection (Days)

Figure 66

PBS control

UCB high dose



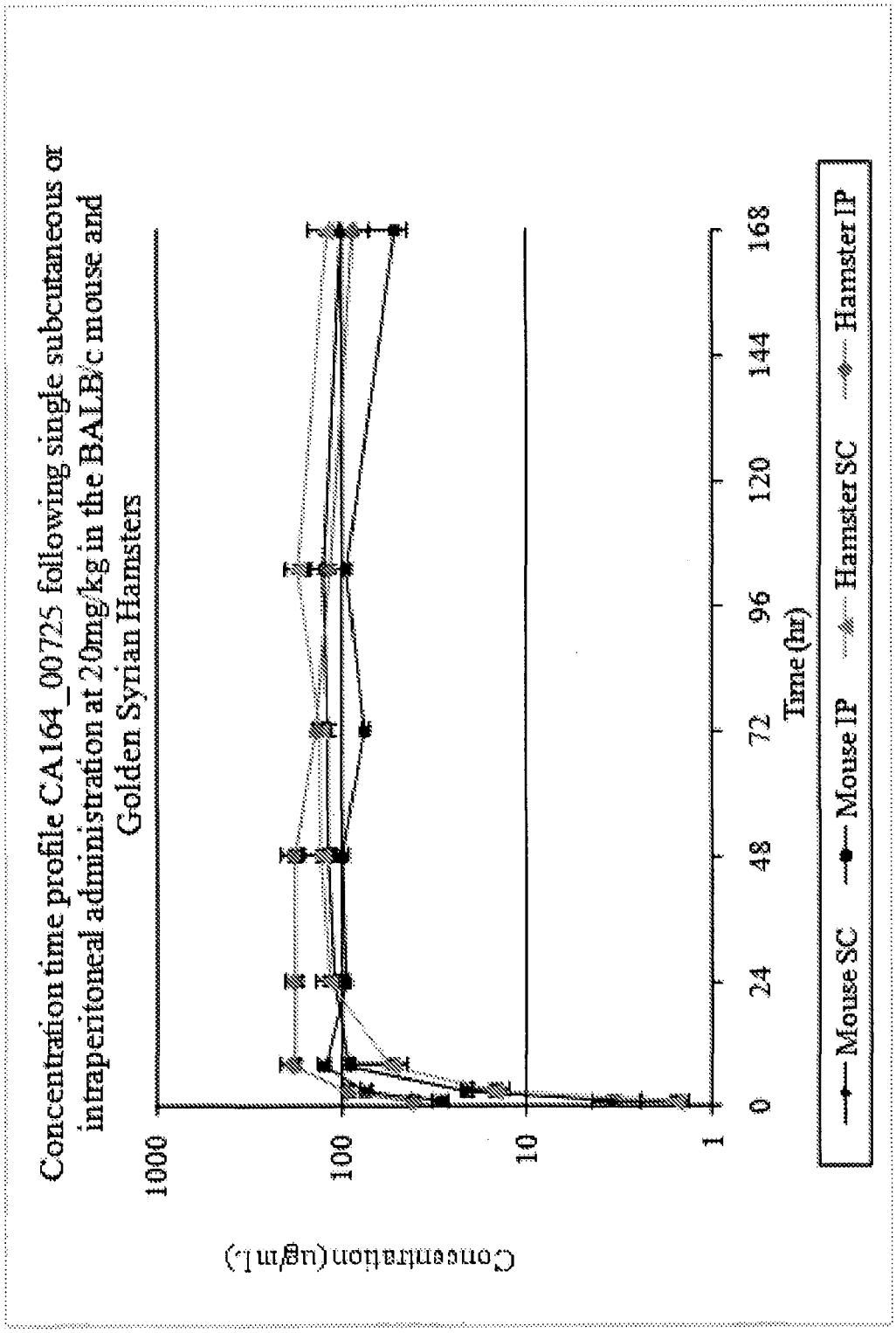


Figure 67 Serum pharmacokinetics of a human IgG1 in mice and hamsters

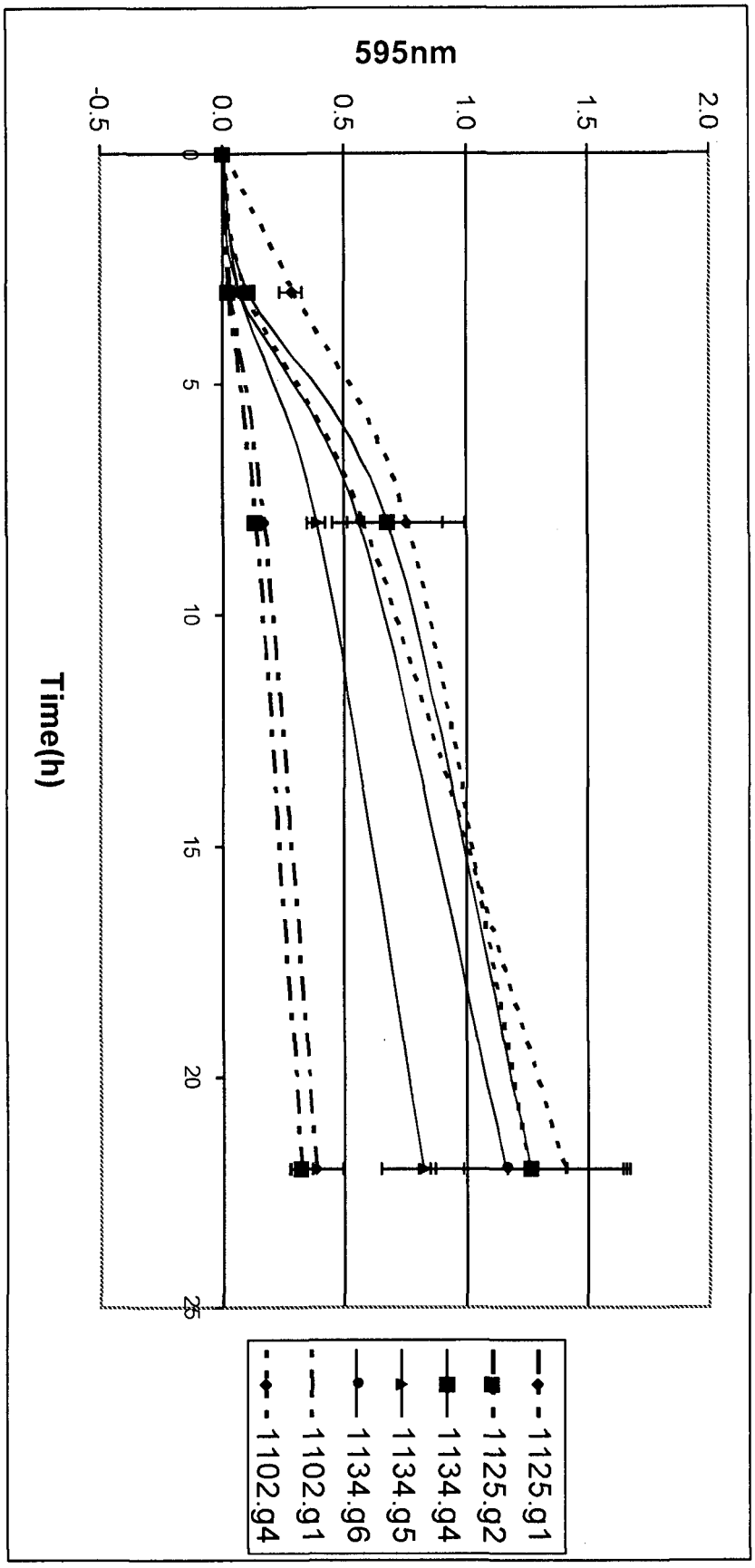


Figure 68 Effect of Agitation *via* Vortexing on anti-TcdB IgG1 Molecules in PBS, pH 7.4(n=3)

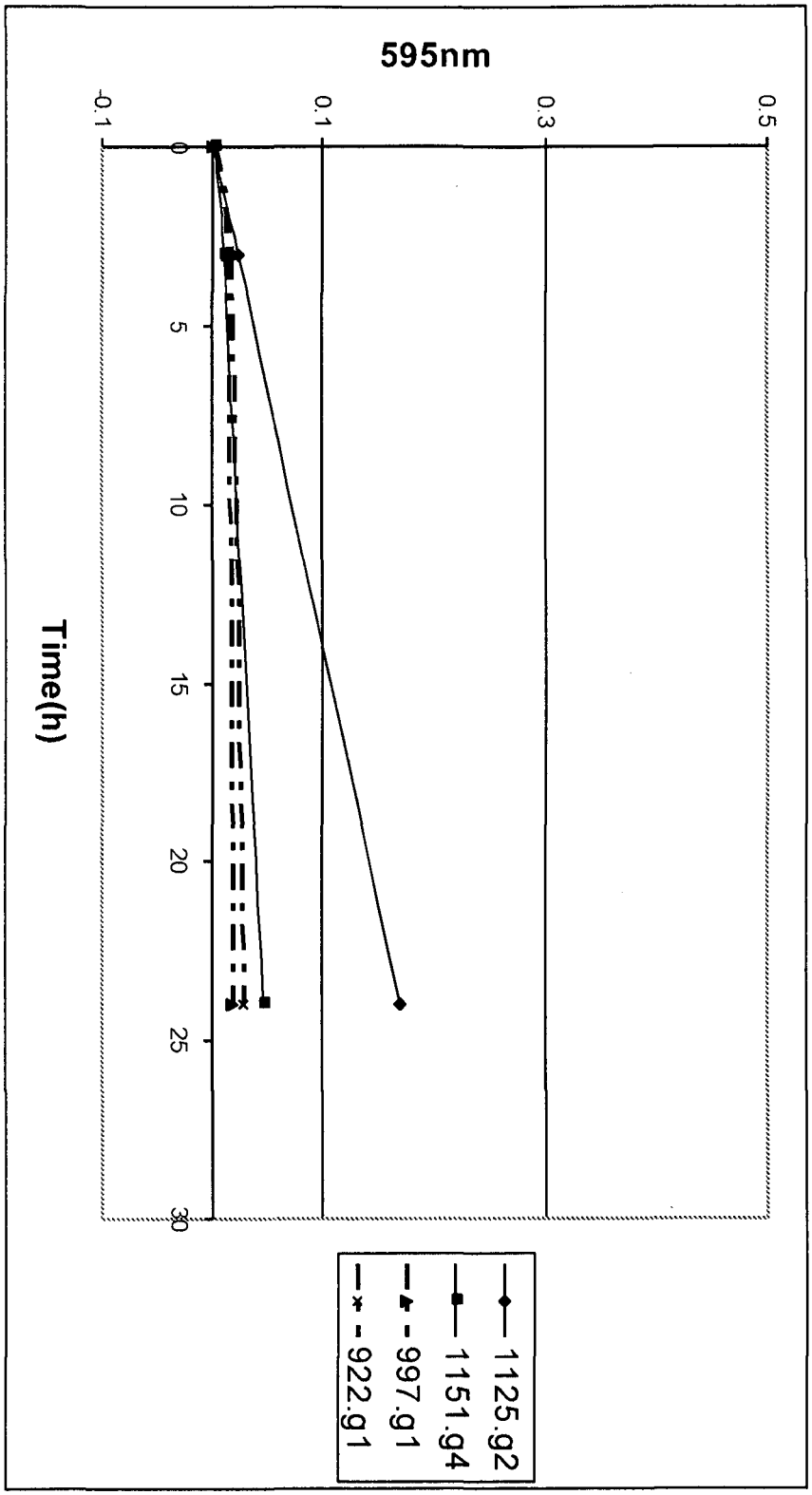


Figure 69 : Comparison of Aggregation Stability of anti-TcdA and anti-TcdB IgG1 Molecules in PBS pH 7.4

Figure 70 Neutralisation against ribotype 003

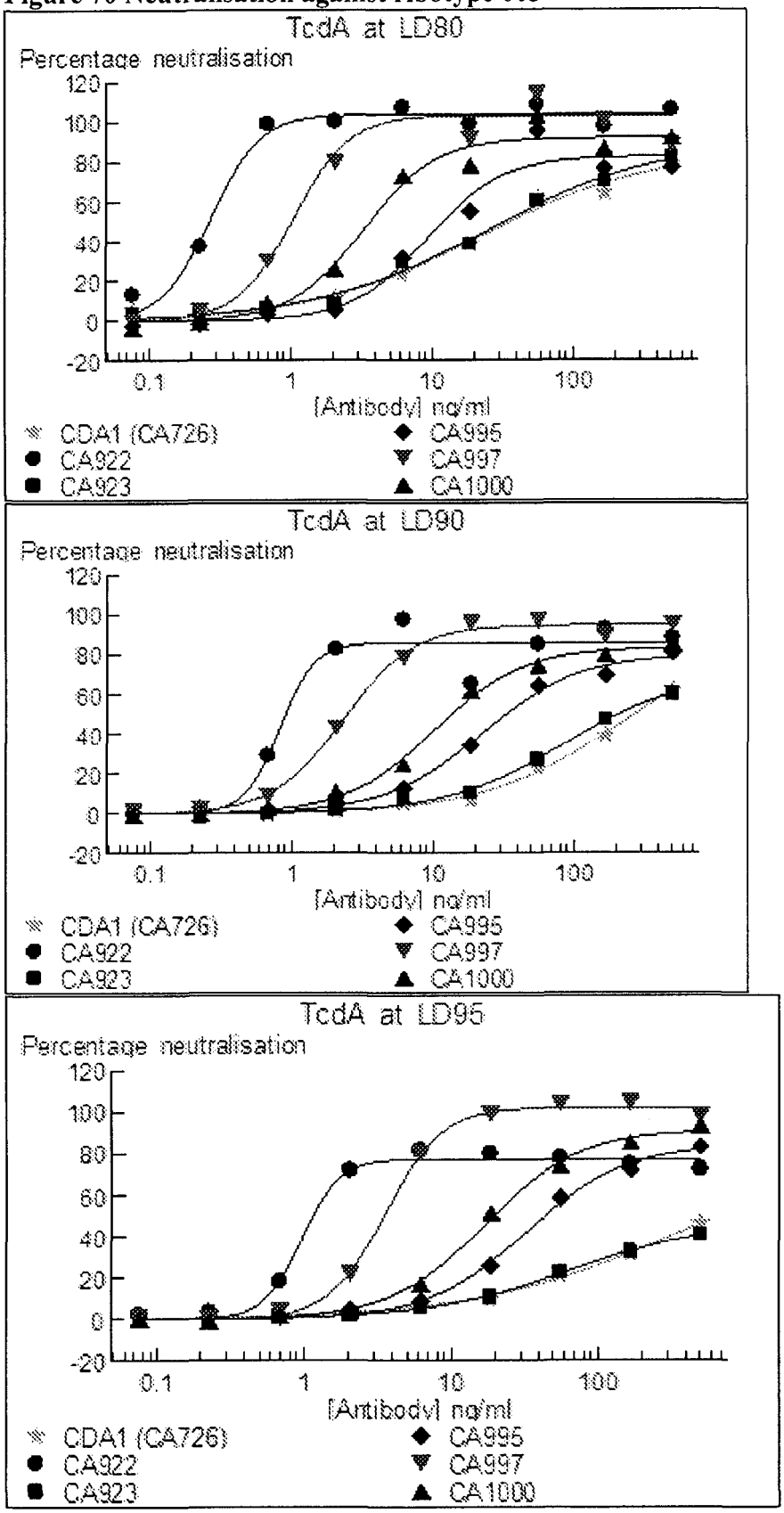


Figure 71 Neutralisation against ribotype 003

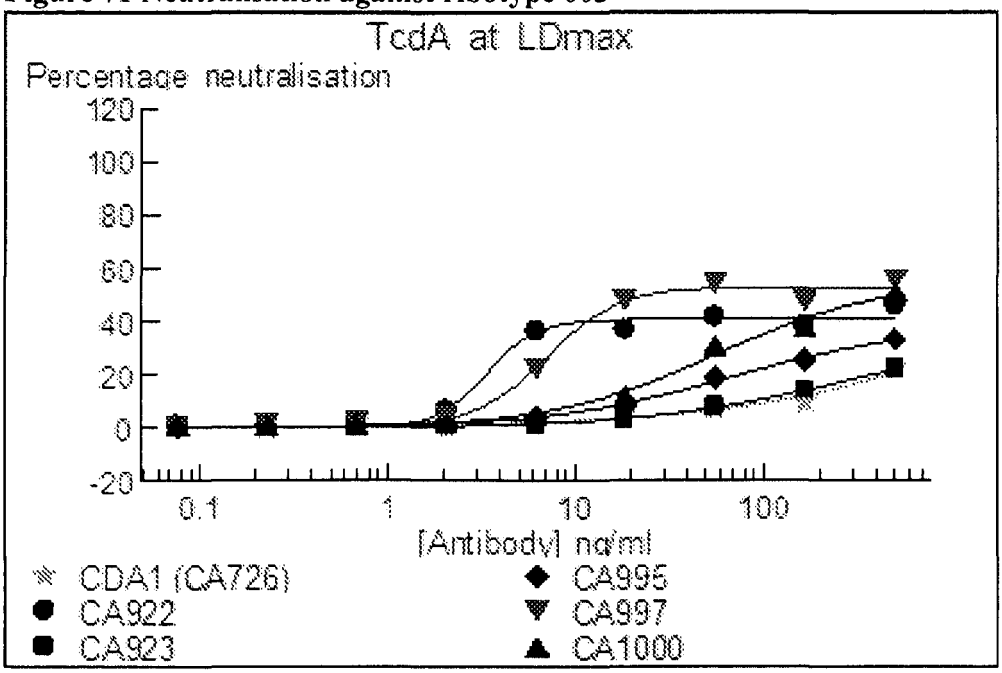


Figure 72 Neutralisation against ribotype 027

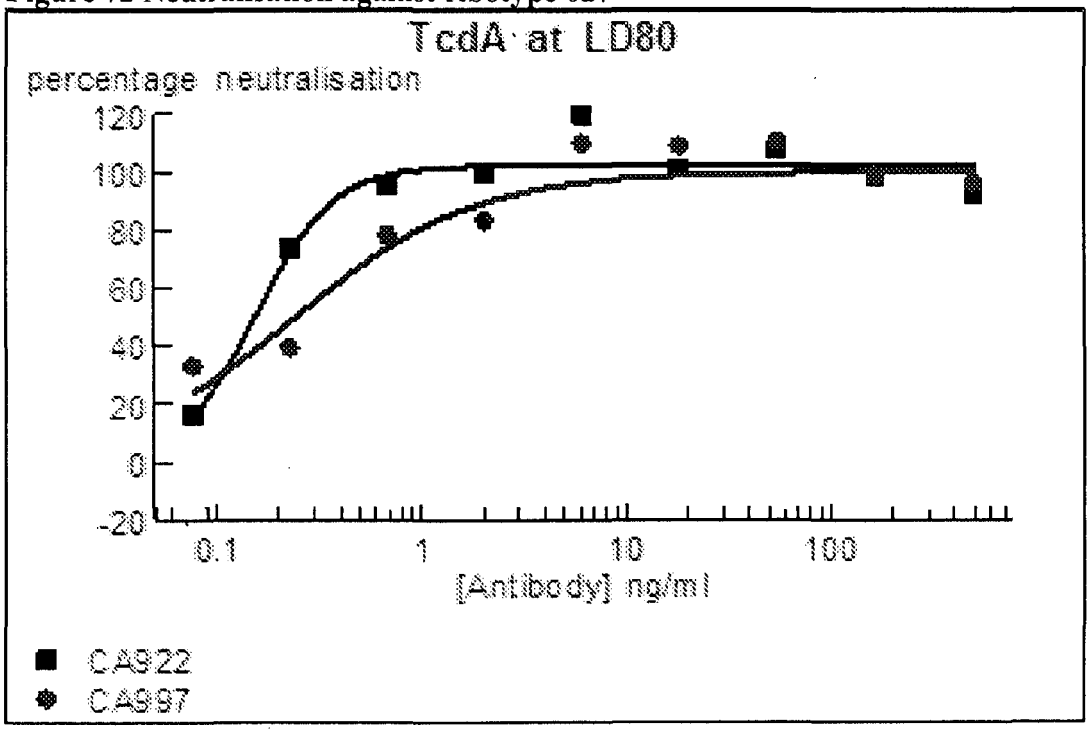
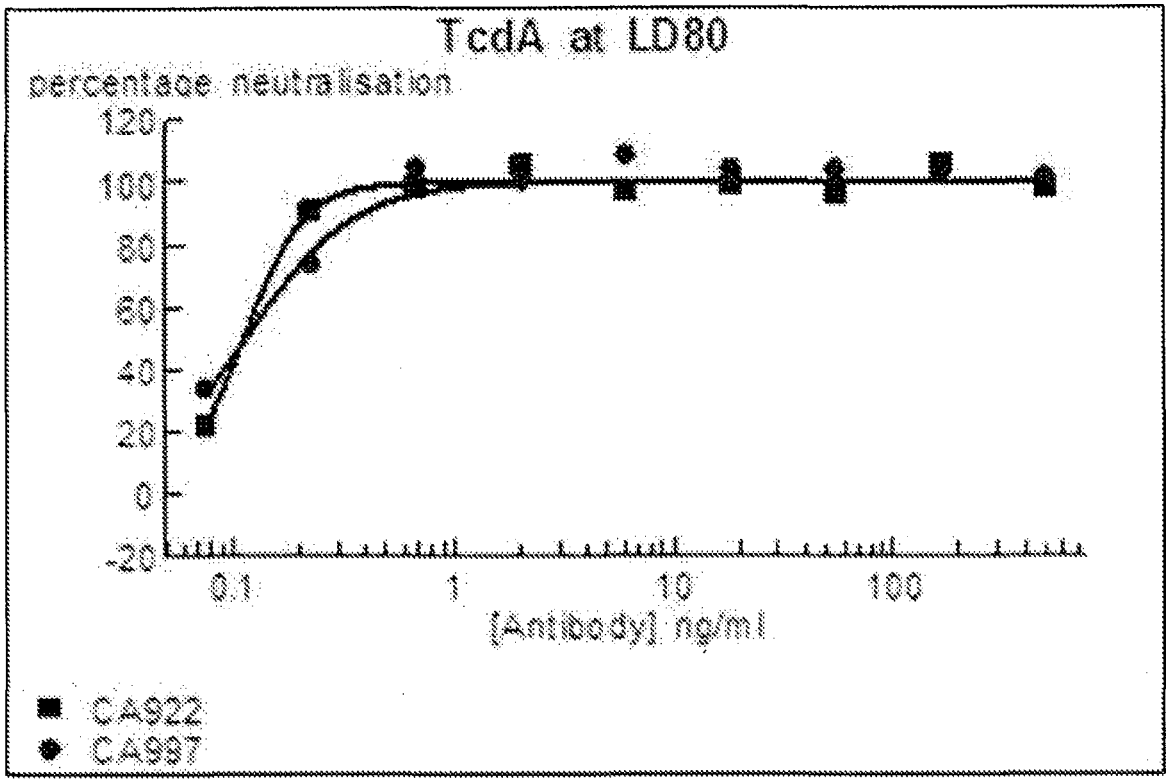


Figure 73 Neutralisation against ribotype 078



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/052222

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/12 A61K39/40 A61P31/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBASE, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/121422 A2 (UNIV MASSACHUSETTS [US]; MEDAREX INC [US]; AMBROSINO DONNA [US]; BABCO) 16 November 2006 (2006-11-16) cited in the application claims 1-7; examples 2,5,7,12,16 -----	1-14, 18-20, 26-37,41
X	BABCOCK GREGORY J ET AL: "Human monoclonal antibodies directed against toxins A and B prevent Clostridium difficile-induced mortality in hamsters", INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MACROBIOLOGY, USA, vol. 74, no. 11, 1 November 2006 (2006-11-01), pages 6339-6347, XP009109025, ISSN: 0019-9567, DOI: 10.1128/IAI.00982-06 cited in the application figure 4 ----- -/--	1-14, 18-20, 26-37,41

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 February 2013	28/02/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weikl, Martina

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2012/052222

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUSSACK GREG ET AL: "Toxin-Specific Antibodies for the Treatment of Clostridium difficile: Current Status and Future Perspectives", TOXINS, vol. 2, no. 5, May 2010 (2010-05), pages 998-1018 URL, XP002686777, ISSN: 2072-6651 the whole document -----	1-14, 18-20, 26-37,41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2012/052222

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-14, 18-20, 26-37, 41

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 12-14(completely); 1-7, 9-11, 26, 28-37, 41(partially)

Claims relating to a monoclonal antibody specific to antigen TcdA wherein the antibody is the antibody 997

---

2. claims: 15-17(completely); 1-7, 9-11, 26, 28-31, 33-37, 41(partially)

Claims relating to a monoclonal antibody specific to antigen TcdA wherein the antibody is the antibody 1000

---

3. claims: 18-20(completely); 1-6, 8-11, 26, 27, 29-37, 41(partially)

Claims relating to a monoclonal antibody specific to antigen TcdB wherein the antibody is the antibody 1125

---

4. claims: 21-23(completely); 1-6, 8-11, 26, 27, 29-37, 41(partially)

Claims relating to a monoclonal antibody specific to antigen TcdB wherein the antibody is the antibody 1151

5-8. claims: 24(completely); 1-7, 9-11, 26, 28-31, 33-37, 41(partially)

Claims relating to monoclonal antibodies specific to antigen TcdA wherein the antibodies are the antibodies 922, 923, 993 and 995

9-17. claims: 25(completely); 1-6, 8-11, 26, 27, 29-31, 33-37, 41(partially)

Claims relating to monoclonal antibodies specific to antigen TcdB wherein the antibodies are the antibodies 926, 927, 1099, 1102, 1114, 1114(grafted), 1129, 1134 and 1151

---

18. claims: 38(completely); 40(partially)

a method of selecting an antibody as defined in the application using assays to measure protection against loss of TEER

---

19. claims: 39(completely); 40(partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

a method of selecting an antibody as defined in the application using assays to measure thermal stability and resistance to shaking aggregation

---

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2012/052222

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006121422 A2	16-11-2006	AT 512986 T	15-07-2011
		AU 2005320349 A1	24-08-2006
		BR PI0507433 A	03-07-2007
		CA 2553946 A1	06-08-2005
		DK 1766093 T3	03-10-2011
		EP 1766093 A2	28-03-2007
		EP 2270045 A1	05-01-2011
		HK 1102287 A1	04-11-2011
		HR P20110599 T1	31-10-2011
		JP 4588763 B2	01-12-2010
		JP 2007533330 A	22-11-2007
		JP 2011019516 A	03-02-2011
		KR 20070050863 A	16-05-2007
		KR 20120003017 A	09-01-2012
		KR 20120083534 A	25-07-2012
		NZ 548821 A	24-12-2009
		PT 1766093 E	24-08-2011
		RS 51908 B	29-02-2012
		US 2005287150 A1	29-12-2005
		US 2010233181 A1	16-09-2010
		US 2010233182 A1	16-09-2010
		US 2012288508 A1	15-11-2012
		WO 2006121422 A2	16-11-2006

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 103958544 A
(43)申请公布日 2014.07.30

(21)申请号 201280049537.6
(22)申请日 2012.09.10
(30)优先权数据
61/535,532 2011.09.16 US
61/638,731 2012.04.26 US
(85)PCT国际申请进入国家阶段日
2014.04.09
(86)PCT国际申请的申请数据
PCT/GB2012/052222 2012.09.10
(87)PCT国际申请的公布数据
WO2013/038156 EN 2013.03.21
(71)申请人 UCB医药有限公司
地址 比利时布鲁塞尔
(72)发明人 D·P·哈姆费雷斯 D·J·莱特伍德
K·L·泰森 D·E·O·奈特
K·J·M·赫伍 J·E·康普森
M·J·T·裴知 A·C·佩内
N·L·菲舍 B·麦肯泽 M·寇克斯
(74)专利代理机构 中国国际贸易促进委员会专利商标事务所 11038
代理人 李程达
(51)Int.Cl.
C07K 16/12(2006.01)
A61K 39/40(2006.01)
A61P 31/04(2006.01)

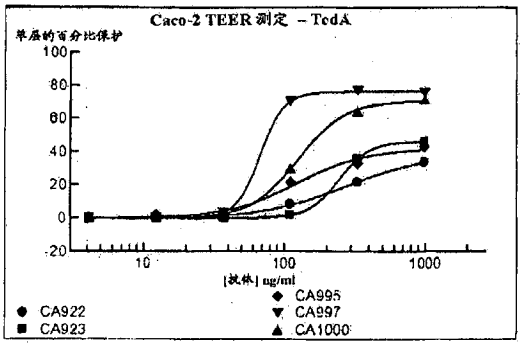
权利要求书3页 说明书68页
序列表70页 附图69页

(54)发明名称

针对艰难梭菌的主要外毒素TcdA和TcdB的中和抗体

(57)摘要

本发明描述了能够中和艰难梭菌的主要外毒素TcdA和TcdB的抗体的衍生和选择。本发明还描述了单个Mab及其混合物的新型中和和抗原结合性质。



CN 103958544 A

1. 一种特异于抗原 TcdA 或 TcdB 的单克隆抗体，其中所述抗体具有对于靶抗原的高亲和力，并且适合用于减少具有艰难梭菌 (Clostridium difficile) 感染或处于所述感染的风险中的患者的腹泻的持续时间和 / 或严重度、发病率和 / 或死亡率。

2. 权利要求 1 的单克隆抗体，其中当以等于或高于 LD_{80} 使用毒素时，所述抗体具有高效力，例如 200ng/ml 或更少，例如 150ng/ml 或更少，特别是 100ng/ml 或更少的 EC_{50}。

3. 权利要求 2 的单克隆抗体，其中当以等于或高于 LD_{80} 使用毒素时，所述抗体的 EC_{50} 为 0.1 至 10ng/ml。

4. 权利要求 2 或权利要求 3 的单克隆抗体，其中当以等于或高于 LD_{80} 使用毒素时，毒素的最大抑制为 50 至 100%。

5. 权利要求 1 至 4 的任一项的单克隆抗体，其中所述抗体结合靶抗原多次。

6. 权利要求 5 的单克隆抗体，其中所述抗体结合靶抗原 2、3、4、5、6、7、8、9、10、11、12、13、14 或 15 次或更多次。

7. 权利要求 1 至 6 的任一项的单克隆抗体，其中所述抗体特异于 TcdA。

8. 权利要求 1 至 6 的任一项的单克隆抗体，其中所述抗体特异于 TcdB。

9. 权利要求 1 至 8 的任一项的单克隆抗体，其中所述抗体具有 1nM 或更少，例如 600pM，例如 50 至 600pM 的亲和力。

10. 权利要求 1 至 9 的任一项的单克隆抗体，其中所述抗体是中和抗体，包括在高毒素浓度、特别是有效地抗核糖型 003、012、027 和 078 时是中和抗体。

11. 权利要求 1 至 10 的任一项的单克隆抗体，其中当在测定开始后 4 小时测量时，所述抗体在 TEER 测定中具有在 60 至 80ng/ml 的范围内的 EC_{50}。

12. 权利要求 1 的单克隆抗体，其特异性结合 TcdA，其包含重链，其中重链的可变结构域包含具有 SEQ ID NO:44 中所示的 CDR-H1 的序列的 CDR、具有 SEQ ID NO:45 中所示的 CDR-H2 的序列的 CDR 和具有 SEQ ID NO:46 中所示的 CDR-H3 的序列的 CDR 的至少一个。

13. 权利要求 12 的单克隆抗体，其还包含轻链，其中轻链的可变结构域包含具有 SEQ ID NO:41 中所示的 CDR-L1 的序列的 CDR、具有 SEQ ID NO:42 中所示的 CDR-L2 的序列的 CDR 和具有 SEQ ID NO:43 中所示的 CDR-L3 的序列的 CDR 的至少一个。

14. 权利要求 13 的单克隆抗体，其具有重链和轻链，所述重链包含 SEQ ID NO:49 所示的序列并且所述轻链包含 SEQ ID NO:47 所示的序列。

15. 权利要求 1 的单克隆抗体，其特异性结合 TcdA，其包含重链，其中重链的可变结构域包含具有 SEQ ID NO:54 中所示的 CDR-H1 的序列的 CDR、具有 SEQ ID NO:55 中所示的 CDR-H2 的序列的 CDR 和具有 SEQ ID NO:56 所示的 CDR-H3 的序列的 CDR 的至少一个。

16. 权利要求 15 的单克隆抗体，其还包含轻链，其中轻链的可变结构域包含具有 SEQ ID NO:51 中所示的 CDR-L1 的序列的 CDR、具有 SEQ ID NO:52 中所示的 CDR-L2 的序列的 CDR 和具有 SEQ ID NO:53 中所示的 CDR-L3 的序列的 CDR 的至少一个。

17. 权利要求 16 的单克隆抗体，其具有重链和轻链，所述重链包含 SEQ ID NO:59 中所示的序列并且所述轻链包含 SEQ ID NO:57 中所示的序列。

18. 权利要求 1 的单克隆抗体，其特异性结合 TcdB，其包含重链，其中重链的可变结构域包含具有 SEQ ID NO:124 中所示的 CDR-H1 的序列的 CDR、具有 SEQ ID NO:125 中所示的 CDR-H2 的序列的 CDR 和具有 SEQ ID NO:126 所示的 CDR-H3 的序列的 CDR 的至少一个。

19. 权利要求 18 的单克隆抗体，其还包含轻链，其中轻链的可变结构域包含具有 SEQ ID NO:121 中所示的 CDR-L1 的序列的 CDR、具有 SEQ ID NO:122 中所示的 CDR-L2 的序列的 CDR 和具有 SEQ ID NO:123 中所示的 CDR-L3 的序列的 CDR 的至少一个。

20. 权利要求 19 的单克隆抗体，其具有重链和轻链，所述重链包含 SEQ ID NO:129 中所示的序列并且所述轻链包含 SEQ ID NO:127 中所示的序列。

21. 权利要求 1 的单克隆抗体，其特异性结合 TcdB，其包含重链，其中重链的可变结构域包含具有 SEQ ID NO:154 中所示的 CDR-H1 的序列的 CDR、具有 SEQ ID NO:155 中所示的 CDR-H2 的序列的 CDR 和具有 SEQ ID NO:156 所示的 CDR-H3 的序列的 CDR 的至少一个。

22. 权利要求 21 的单克隆抗体，其还包含轻链，其中轻链的可变结构域包含具有 SEQ ID NO:151 中所示的 CDR-L1 的序列的 CDR、具有 SEQ ID NO:152 中所示的 CDR-L2 的序列的 CDR 和具有 SEQ ID NO:153 中所示的 CDR-L3 的序列的 CDR 的至少一个。

23. 权利要求 22 的单克隆抗体，其具有包含 SEQ ID NO:159 中所示的序列的重链和包含 SEQ ID NO:157 中所示的序列的轻链。

24. 权利要求 1 的单克隆抗体，其特异性结合 TcdA，其具有重链和轻链，其中重链可变区包含选自 SEQ ID NO:9、SEQ ID NO:19、SEQ ID NO:29 和 SEQ ID NO:39 的序列并且轻链可变区包含选自 SEQ ID NO:7、SEQ ID NO:17、SEQ ID NO:27 和 SEQ ID NO:37 的序列。

25. 权利要求 1 的单克隆抗体，其特异性结合 TcdB，其具有重链和轻链，其中重链可变区包含选自的SEQ ID NO:69、SEQ ID NO:79、SEQ ID NO:89、SEQ ID NO:99、SEQ ID NO:109、SEQ ID NO:119、SEQ ID NO:139、SEQ ID NO:149 和 SEQ ID NO:159 的序列，并且轻链可变区包含选自 SEQ ID NO:67、SEQ ID NO:77、SEQ ID NO:87、SEQ ID NO:97、SEQ ID NO:107、SEQ ID NO:117、SEQ ID NO:137、SEQ ID NO:147 和 SEQ ID NO:157 的序列。

26. 药物组合物，其包含一种或多种权利要求 1 至 25 的任一项中定义的抗体。

27. 权利要求 26 的药物组合物，其包含两种或更多种特异于 TcdB 的抗体。

28. 权利要求 26 的药物组合物，其包含两种或更多种特异于 TcdA 的抗体。

29. 权利要求 26 的药物组合物，其中组合物中的至少一种抗体特异于 TcdA 并且组合物中的至少一种抗体特异于 TcdB。

30. 权利要求 29 的药物组合物，其中所述组合物还包含至少一种特异于 TcdB 的第二抗体。

31. 权利要求 26 至 30 的药物组合物，其中所述组合物包含 2、3、4、5、6、7、8、9、10、11、12、13、14、15 种不同的针对靶抗原的抗体，例如 2、3、4 或 5 种抗体。

32. 包含权利要求 14, 20 和 23 的抗体的药物组合物或混合物。

33. 权利要求 26 至 32 的任一项的药物组合物，其还包含药学上可接受的赋形剂。

34. 权利要求 1 至 25 的任一项的单克隆抗体或权利要求 26 至 33 任一项的药物组合物，其用于治疗例如治疗或预防艰难梭菌感染或源自其的并发症。

35. 治疗具有艰难梭菌感染或处于源自其的风险中的患者的方法，包括施用治疗有效量的权利要求 1 至 25 的任一项的单克隆抗体或权利要求 26 至 33 的任一项的药物组合物。

36. 权利要求 35 的治疗方法，其中将所述治疗与用于艰难梭菌治疗的其它治疗、例如选自甲硝唑、万古霉素、克林霉素、非达米星及其组合组合使用。

37. 权利要求 1 至 25 的任一项中定义的抗体或权利要求 26 至 33 的任一项中定义的组

合物用于制造用于治疗或预防艰难梭菌感染或来自其的并发症的药剂的用途。

38. 使用测量抵抗 TEER(跨上皮电阻)的丢失的保护作用的测定来选择权利要求 1 至 11 的任一项中定义的抗体的方法。

39. 使用测量热稳定性 (Tm) 和对摇动聚集的抗性的测定来选择权利要求 1 至 11 的任一项中定义的抗体的方法。

40. 通过将毒素中和的测量、TEER 测量、热稳定性测量 (Tm)、摇动聚集测量和适合用于共配制的等电点 (pI) 组合来选择用于治疗艰难梭菌感染的抗体混合物的方法。

41. 与类毒素或包含所述类毒素的药物组合物组合的权利要求 1 至 26 的任一项的单克隆抗体,其例如用于接种,例如治疗或预防艰难梭菌感染或来自其的并发症。

针对艰难梭菌的主要外毒素 TcdA 和 TcdB 的中和抗体

[0001] 本发明涉及针对艰难梭菌 (Clostridium difficile) 的外毒素例如 TcdA 和 TcdB 的抗体、包含所述抗体的药物组合物、产生所述抗体和组合物的方法以及所述抗体和组合物用于治疗和 / 或预防，特别是治疗或预防艰难梭菌感染、假膜性结肠炎、暴发性结肠炎和 / 或中毒性巨结肠的用途。

[0002] 两种主要外毒素 TcdA 和 TcdB 已在许多体外和体内研究中被确定为艰难梭菌的主要致病性决定子。非产毒株对动物和人是非致病性的 (1, 2)。迄今为止仍然需要确立对二元毒素的作用的明确理解 (3)。

[0003] 两种毒素都是肠毒性的和细胞毒性的，但综合各种证据显示 TcdA 是比 TcdB 更强的肠毒素，而通常观察到 TcdB 的细胞毒性为 TcdA 的约 1000 倍 (4)。虽然两种毒素都能够诱发炎症反应，但 TcdA 似乎帮助更多炎症性 TcdB 迁移进入更深的肠黏膜 (5)。

[0004] 总体来说，30 多年产生的一大批数据支持其中两种毒素都可能在人疾病过程中具有非常重要的意义的模型。TcdA 可能通过紧密连接的丢失和绒毛尖的破坏和从而腹泻，可能地通过白蛋白驱动的液体流失来起始早期（即在 TcdB 之前）和快速（即 1-3 小时）肠损伤。对肠衬里的完整性的这种损害使得 TcdB 能够更快和更有效地（即深入更深的组织，或细胞靶并且全身性破坏可及的器官）施加其优良的摩尔效力 (TcdB 通常被引证为是 TcdA 的细胞毒性的 1000 倍）。任一毒素在体外对人或动物细胞和组织是单独有效的。取决于其它诱发因素例如机械损伤、屏障过载和宿主特异性敏感性，任一毒素单独地在动物的体内是有效的。现已清楚在仓鼠中，至少通过艰难梭菌肠感染递送的单独的 TcdA 或 TcdB 可引起死亡 (1)。已证明 A-B+ 菌株能够引起人的症状和死亡 (6, 7)。然而，绝大部分（～ 95%）临床菌株为 A+B+，从而目标在于治疗艰难梭菌感染 (CDI) 的药物必须能够有效中和两种毒素的活性和清除所述毒素。

[0005] CDI 是老年患者或具有并发合并症的那些患者的最常见的医院内感染。然而，社区获得性感染的增加已引起人们的注意。感染几乎总是与广谱抗生素的使用相关或由其诱发。仅在美国医疗保健相关花费据估计每年超过 10 亿美元。这些花费主要因具有更长的住院期的患者造成。目前的疗法包括使用杀死肠内的艰难梭菌细胞的抗生素，例如克林霉素、万古霉素或非达米星。目前的疗法解决了细菌感染但不能对付或直接防止由 TcdA 和 TcdB 引发的显著发病机制，所述 TcdA 和 TcdB 是 CDI 症状和死亡率的主要贡献者。

[0006] 人的 CDI 症状包括轻度至严重的腹泻、假膜性结肠炎 (PMC) 和暴发性结肠炎或所谓的中毒性巨结肠。死亡导致 5-15% 的接受目前最好的照护的患者。因此，目前还没有患者可获得用于防止在感染后由艰难梭菌毒素引起的损害和损伤的特异性疗法。

[0007] 已显示通过接种产生抗体反应和胃肠外施用多克隆和单克隆抗体能够保护动物免受腹泻的症状和死亡 (8-15)。仓鼠中的早期研究表明单独的抗 TcdA 抗体是获得保护作用所需的所有物质。然而，功能上缺失 TcdA 或 TcdB 的菌株的使用证明了任一毒素都能够引起仓鼠的疾病，但两种毒素一起更有效 (1)。

[0008] 对于治疗性应用，单克隆抗体 (Mab) 可提供优于血清来源的多克隆抗体或血清来源的高免疫血清的效力、安全性、制造和监管有利方面。由于这些原因，Mab 对于治疗产品

通常是优选的选择。

[0009] 已进行了许多努力来产生抗 TcdA 和 TcdB 保护性 Mab。这些 Mab 中在临床上最先进的 Mab 是 2 种 IgG1Mab 的混合物，由 MBL 和 Medarex 开发的最初称为 CDA1 和 MDX1388 的各自抗 TcdA 和 TcdB 的 Mab。已显示它们在急性或复发性感染的模型中不能完全保护仓鼠(15)。该 Mab 组合现由 Merck Inc 开发为 MK3415A。在人 II 期临床试验中，MK3415A 导致统计上显著的疾病复发的减少 (p=0.006)（也参见 Lowy 等人，NEJM(2010)362:197-205）但不影响腹泻的持续时间 / 严重度或死亡率 (16)。这可能意味着这些抗体仅对预防感染的复发有用。感染的复发导致约 25% 的患者。因此，可能存在这些抗体在其中无效的大量患者群体。

[0010] 为了能够对腹泻（例如作为因 TcdA 引起的对肠紧密连接的急性损害的结果）和死亡（例如因下列情况而引起的：延长的不良营养状况、脱水应激和炎症级联的引发、对肠衬里的广泛解剖学上的损伤和可能地因全身性毒性 TcdB（更甚于 TcdA）引起的对远距离器官的损害）产生积极影响，需要具有优良亲和力、毒素中和、对 TEER（跨上皮电阻）的丢失的优良防止、抗原修饰和抗原免疫清除的 Mab。

[0011] 发明概述

[0012] 本发明提供了具有极高水平的体外和体内效力的 Mab，其具有对患有艰难梭菌感染 (CDI) 的人的腹泻的持续和严重度以及死亡率产生影响的潜力。

[0013] 在一个实施方案中，提供了特异于抗原 TcdA 或 TcdB 的单克隆抗体，其中所述抗体对靶抗原具有高亲和力，并且适合用于减少患有艰难梭菌感染或处于发生所述感染的风险中的患者的腹泻的持续时间和 / 或严重度以及发病率。

[0014] 在一个实施方案中，提供了特异于的 TcdA 或 TcdB 的 Mab，或至少两种 Mab 的群体，所述 Mab 的至少一种特异于 TcdA 并且所述 Mab 的至少一种特异于 TcdB，其中所述抗体或每一种抗体或抗体的组合的 EC_{50} 为 200ng/ml 或更少，例如 150ng/ml 或更小，例如 100ng/ml。

[0015] 本公开内容的抗体是有用的，因为它们可能提供治疗患者的原发感染例如腹泻的症状的严重度和持续时间或防止死亡而不仅仅是预防疾病症状的复发的手段。

[0016] 在至少一些实施方案中，根据本公开内容的抗体在高浓度毒素存在的情况下不显示效力的减小。

[0017] 发明详述

[0018] 如本文中所用，特异性意指这样的抗体，所述抗体仅识别其所特异于的抗原，或这样的抗体，所述抗体对于其所特异性于的抗原的结合，相较于该抗体对于其所非特异性于的抗原的结合，具有显著更高的结合亲和力，例如为 5、6、7、8、9、10 倍的结合亲和力。

[0019] 结合亲和力可通过标准测定例如表面等离子体共振技术例如 BIAcore 来测量。

[0020] 在一个实施方案中，对于细胞培养测定和患者的艰难梭菌感染，EC_{50} 小于 75、70、60、65、55、50、45、40、35、30、25、20、15、10、9、8、7、6、5、4、3、2、1.5ng/ml。这显著低于（效力更强）已知抗体，并且被认为是关于为什么本公开内容的抗体对接受治疗的受试者的存活具有显著的和积极影响的主要因素。

[0021] 如本文中所用，效力是抗体在给定的剂量或浓度诱发适当的生物反应例如有害毒素作用的中和的能力。效力的实例包括毒素活性的最大中和百分比（保护的程度）、Mab 对抗原的最低相对浓度（例如 EC_{50}）、中和活性的速度和耐久性。

[0022] 在细胞培养测定中，中和可能被观察为下列方面的一个或多个方面：毒素结合至细胞的防止、毒素从溶液的免疫沉淀、细胞形式和形状丢失的防止、细胞骨架结构的丢失的防止、细胞单层紧密连接和跨上皮电阻的丢失的防止、细胞死亡、细胞凋亡的防止以及促炎细胞因子例如 TNFα、IL-1β、IL-6 和 MIP1α 的产生。

[0023] 在组织切片和外植体测定中，中和可以例如被观察为坏死和/或水肿液积累的防止。

[0024] 在体外测定中，中和可被观察为如下方面的一个或多个方面：液体在结扎的回肠袢中的积累的防止以及肠组织坏死、腹泻、动物死亡的假膜形成的防止，

[0025] 因此，在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗-毒素 A 抗体），所述 CDR 选自：

[0026]

QASQSISNALA	SEQ ID NO: 1
SASSLAS	SEQ ID NO: 2
QYTHYSHTSKNP	SEQ ID NO: 3
GFTISSYYMS	SEQ ID NO: 4
IISSGGHFTWYANWAKG	SEQ ID NO: 5
AYVSGSSFNGYAL	SEQ ID NO: 6

[0027] 在一个实施方案中，序列 1 至 3 存在于抗体的轻链中。

[0028] 在一个实施方案中，序列 4 至 6 存在于抗体的重链中。

[0029] 在一个实施方案中，SEQ ID NO:1 为 CDR L1，SEQ ID NO:2 为 CDR L2 以及 SEQ ID NO:3 为 CDR L3。

[0030] 在一个实施方案中，SEQ ID NO:4 为 CDR H1，SEQ ID NO:5 为 CDR H2 以及 SEQ ID NO:6 为 CDR H3。

[0031] 在一个实施方案中，SEQ ID NO:1 为 CDR L1，SEQ ID NO:2 为 CDR L2，SEQ ID NO:3 为 CDR L3，SEQ ID NO:4 为 CDR H1，SEQ ID NO:5 为 CDR H2 以及 SEQ ID NO:6 为 CDR H3。

[0032] 在一个实施方案中，提供了可变区，例如具有下列序列的轻链可变区（抗体 922 抗-毒素 A 抗体；轻链可变区序列）SEQ ID NO:7:

[0033] DPVMTQSPSTLSASVGDRVTITCQASQSISNALAWYQQKPGKAPKLLIYSASSLASGVPSRFKGSGSGTEFTLTISSLQPDDFATYYCQYTHYSHTSKNPFGGGTKVEIK

[0034] 其中将 CDR 加以下划线并且构建体在本文中被称为 922.g1VK(gL1)。

[0035] 编码 SEQ ID NO:7 的多核苷酸序列示于图 1 中和 SEQ ID NO:8。

[0036] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:9 的重链可变区（抗体 922 抗-毒素 A 抗体重链可变区序列）：

[0037] EVQLVESGGGLVQPGGSLRLSCAASGFTISSYYMSWVRQAPGKGLEWIGIISSGGHFTWYANWAKGRFTISSDSTTVYLQMNSLRDEDTATYFCARAYVSGSSFNGYALWGQGTLVTVS

[0038] 其中将 CDR 加以下划线并且构建体在本文中被称为 922.g1VH(gH1)

[0039] 编码 SEQ ID NO:9 的多核苷酸序列示于图 1 中和 SEQ ID NO:10。

[0040] 在一个实施方案中，抗体包含 SEQ ID NO:7 和 9 中显示的可变区。

[0041] 因此，在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 A 抗体），所述 CDR 选自：

[0042]

QASQSISNYLA	SEQ ID NO: 11
SASTLAS	SEQ ID NO: 12
QYSHYGTGVFGA	SEQ ID NO: 13
AFSLSNYYMS	SEQ ID NO: 14
IISSGSNALKWYASWPKG	SEQ ID NO: 15
NYVGSGSYYGMDL	SEQ ID NO: 16

[0043] 在一个实施方案中，序列 11 至 13 存在于抗体的轻链中。

[0044] 在一个实施方案中，序列 14 至 16 存在于抗体的重链中。

[0045] 在一个实施方案中，SEQ ID NO:11 为 CDR L1，SEQ ID NO:12 为 CDR L2 以及 SEQ ID NO:13 为 CDR L3。

[0046] 在一个实施方案中，SEQ ID NO:14 为 CDR H1，SEQ ID NO:15 为 CDR H2 以及 SEQ ID NO:16 为 CDR H3。

[0047] 在一个实施方案中，SEQ ID NO:11 为 CDR L1，SEQ ID NO:12 为 CDR L2，SEQ ID NO:13 为 CDR L3，SEQ ID NO:14 为 CDR H1，SEQ ID NO:15 为 CDR H2 以及 SEQ ID NO:16 为 CDR H3。

[0048] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:17 的轻链可变区（抗体 923 抗 - 毒素 A 抗体；轻链可变区序列）：

[0049] DVVMTQSPSSLSASVGDRVTITCQASQSISNYLAWYQQKPGKVPKLLIYSASTLASGVPSRFKGSGSGTQFTLTISSLQPEDVATYYCQYSHYGTGVFGAFGGGTKVEIK

[0050] 其中将 CDR 加以下划线并且构建体在本文中被称为 CA923.g1gL1

[0051] 编码 SEQ ID NO:17 的多核苷酸序列示于图 1 中和 SEQ ID NO:18。

[0052] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:19 的重链可变区（抗体 923 抗 - 毒素 A 抗体重链可变区序列）：

[0053] EVQLVESGGGLVQPGGSLRLSCAASAFSLSNYYMSWVRQAPGKGLEWIGIISSGSNALKWYASWPKGRFTISKDSTTVYLQMNSLRAEDTATYFCARNYVGSGSYYGMDLWGQGTLVTVS

[0054] 其中将 CDR 加以下划线并且构建体在本文中被称为 CA923.g1gH1

[0055] 编码 SEQ ID NO:19 的多核苷酸序列示于图 2 中和 SEQ ID NO:20。

[0056] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:17 和 SEQ ID NO:19 中所示的可变区。

[0057] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 A 抗体），所述 CDR 选自：

[0058]

QASQSISSYFS	SEQ ID NO: 21
GASTLAS	SEQ ID NO: 22
QCTDYSGIYFGG	SEQ ID NO: 23
GFSLSSYYMS	SEQ ID NO: 24
IISSGSSTTFTWYASWAKG	SEQ ID NO: 25
AYVGSSSYYGFDP	SEQ ID NO: 26

[0059] 在一个实施方案中，序列 21 至 23 存在于抗体的轻链中。

[0060] 在一个实施方案中，序列 24 至 26 存在于抗体的重链中。

[0061] 在一个实施方案中，SEQ ID NO:21 为 CDR L1，SEQ ID NO:22 为 CDR L2 以及 SEQ ID NO:23 为 CDR L3。

[0062] 在一个实施方案中，SEQ ID NO:24 为 CDR H1，SEQ ID NO:25 为 CDR H2 以及 SEQ ID NO:26 为 CDR H3。

[0063] 在一个实施方案中，SEQ ID NO:21 为 CDR L1，SEQ ID NO:22 为 CDR L2，SEQ ID NO:23 为 CDR L3，SEQ ID NO:24 为 CDR H1，SEQ ID NO:25 为 CDR H2 以及 SEQ ID NO:26 为 CDR H3。

[0064] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:27 的轻链可变区（抗体 993 抗－毒素 A 抗体；轻链可变区序列）：

[0065] DVVMTQSPSTLSASVGDRVTITCQASQSISSYFSWYQQKPGKAPQLLIYGASTLASGVPSRFKGSGSGTELTLTISSLQPDDFATYYCQCTDYSGIYFGGFGGGTKVEIK

[0066] 其中将 CDR 加以下划线并且构建体在本文中被称为 CA993.g1gL1

[0067] 编码 SEQ ID NO:27 的多核苷酸序列示于图 2 中和 SEQ ID NO:28。

[0068] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:29 的重链可变区（抗体 993 抗－毒素 A 抗体重链可变区序列）：

[0069] EVQLVESGGGLVQPGGSLKLSCTASGFSLSSYYMSWVRQAPGKGLEWIGIISSGSSTTFTWYASWAKGRFTISKTSTTVYLQMNSLKTEDTATYFCARAYVGSSSYYGFDPWGQGTLVTVS

[0070] 其中将 CDR 加以下划线并且构建体在本文中被称为 CA993.g1gH1

[0071] 编码 SEQ ID NO:29 的多核苷酸序列示于图 2 中和 SEQ ID NO:30。

[0072] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:27 和 SEQ ID NO:29 中显示的可变区。

[0073] 在一个实施方案中，提供了包含 CDR 例如 1，2，3，4，5 或 6 个 CDR 的抗体（例如抗－毒素 A 抗体），所述 CD 选自：

[0074]

QASQSINNYFS	SEQ ID NO: 31
GAANLAS	SEQ ID NO: 32
QNNYGVHIYGAA	SEQ ID NO: 33
GFSLSNYDMI	SEQ ID NO: 34
FINTGGITYYASWAKG	SEQ ID NO: 35
VDDYIGAWGAGL	SEQ ID NO: 36

[0075] 在一个实施方案中，序列 31 至 33 存在于抗体的轻链中。

[0076] 在一个实施方案中，序列 34 至 36 存在于抗体的重链中。

[0077] 在一个实施方案中，SEQ ID NO:31 为 CDR L1，SEQ ID NO:32 为 CDR L2 以及 SEQ ID NO:33 为 CDR L3。

[0078] 在一个实施方案中，SEQ ID NO:34 为 CDR H1，SEQ ID NO:35 为 CDR H2 以及 SEQ ID NO:36 为 CDR H3。

[0079] 在一个实施方案中，SEQ ID NO:31 为 CDR L1，SEQ ID NO:32 为 CDR L2，SEQ ID NO:33 为 CDR L3，SEQ ID NO:34 为 CDR H1，SEQ ID NO:35 为 CDR H2 以及 SEQ ID NO:36 为 CDR H3。

[0080] 在一个实施方案中，提供了可变区，例如具有下列序列的轻链可变区（抗体 995 抗 - 毒素 A 抗体；轻链可变区序列）SEQ ID NO:37:

[0081] DVVMTQSPSTLSASVGDRVTITCQASQSINNYFSWYQQKPGKAPKLLIYGAANLASGVPSRFKGSGSGTEYTLTISSLQPDDFATYSCQNNYGVHIYGAAFGGGTKVEIK

[0082] 其中将 CDR 加以下划线

[0083] 编码 SEQ ID NO:37 的多核苷酸序列示于图 3 中和 SEQ ID NO:38。

[0084] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:39 的重链可变区（抗体 995 抗 - 毒素 A 抗体重链可变区序列）：

[0085] EVQLVESGGGLVQPGGSLRLSCTASGFSLSNYDMIWVRQAPGKGLEYIGFINTGGITYYASWAKGRFTISRDSSTVYLQMNSLRAEDTATYFCARVDDYIGAWGAGLWGQGTLVTVS

[0086] 其中将 CDR 加以下划线

[0087] 编码 SEQ ID NO:39 的多核苷酸序列示于图 3 中和 SEQ ID NO:40。

[0088] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:37 和 SEQ ID NO:39 中显示的可变区。

[0089] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 A 抗体），所述 CDR 选自：

[0090]

QASQSISSYLS	SEQ ID NO: 41
RASTLAS	SEQ ID NO: 42
LGVYGYSNDDGIA	SEQ ID NO: 43
GIDLSSHHMC	SEQ ID NO: 44
VIYHFGSTYYANWATG	SEQ ID NO: 45
ASIAGYSAFDP	SEQ ID NO: 46

[0091] 在一个实施方案中，序列 41 至 43 存在于抗体的轻链中。

[0092] 在一个实施方案中，序列 44 至 46 存在于抗体的重链中。

[0093] 在一个实施方案中，SEQ ID NO:41 为 CDR L1，SEQ ID NO:42 为 CDR L2 以及 SEQ ID NO:43 为 CDR L3。

[0094] 在一个实施方案中，SEQ ID NO:44 为 CDR H1，SEQ ID NO:45 为 CDR H2 以及 SEQ ID NO:46 为 CDR H3。

[0095] 在一个实施方案中，SEQ ID NO:41 为 CDR L1，SEQ ID NO:42 为 CDR L2，SEQ ID NO:43 为 CDR L3，SEQ ID NO:44 为 CDR H1，SEQ ID NO:45 为 CDR H2 以及 SEQ ID NO:46 为 CDR H3。

[0096] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:47 的轻链可变区（抗体 997 抗 - 毒素 A 抗体；轻链可变区序列）：

[0097] ALVMTQSPSSFSASTGDRVTITCQASQSISSYLSWYQQKPGKAPKLLIYRASTLASGVPSRFSGSGSGTEYTLTISCLQSEDFATYYCLGVYGYSNDDGIAFGGGTKVEIK

[0098] 其中将 CDR 加以下划线

[0099] 编码 SEQ ID NO: 的多核苷酸序列 47 示于图 3 中和 SEQ ID NO:48。

[0100] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:49 的重链可变区（抗体 997 抗 - 毒素 A 抗体重链可变区序列）：

[0101] EVQLVESGGGLVQPGGSLRLSCTVSGIDLSSHHMCWVRQAPGKGLEYIGVIYHFGSTYYANWATGRFTISKDSTTVYLQMNSLRAEDTATYFCARASIAGYSAFDPWGQGTLVTVS

[0102] 其中将 CDR 加以下划线

[0103] 编码 SEQ ID NO:49 的多核苷酸序列示于图 4 中和 SEQ ID NO:50。

[0104] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:47 和 SEQ ID NO:49 中显示的可变区。

[0105] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 A 抗体），所述 CDR 选自：

[0106]

QASQSIYSYLA	SEQ ID NO: 51
DASTLAS	SEQ ID NO: 52
QGNAYTSNSHDNA	SEQ ID NO: 53
GIDLSSDAVG	SEQ ID NO: 54
IIATFDSTYYASWAKG	SEQ ID NO: 55
TGSWYYISGWGSYYYGMDL	SEQ ID NO: 56

[0107] 在一个实施方案中，序列 51 至 53 存在于抗体的轻链中。

[0108] 在一个实施方案中，序列 54 至 56 存在于抗体的重链中。

[0109] 在一个实施方案中，SEQ ID NO:51 为 CDR L1，SEQ ID NO:52 为 CDR L2 以及 SEQ ID NO:53 为 CDR L3。

[0110] 在一个实施方案中，SEQ ID NO:54 为 CDR H1，SEQ ID NO:55 为 CDR H2 以及 SEQ ID NO:56 为 CDR H3。

[0111] 在一个实施方案中，SEQ ID NO:51 为 CDR L1，SEQ ID NO:52 为 CDR L2，SEQ ID NO:53 为 CDR L3，SEQ ID NO:54 为 CDR H1，SEQ ID NO:55 为 CDR H2 以及 SEQ ID NO:56 为 CDR H3。

[0112] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:57 的轻链可变区（抗体 1000 抗 - 毒素 A 抗体；轻链可变区序列）：

[0113] EIVMTQSPSTLSASVGDRVTITCQASQSIYSYLAWYQQKPGKAPKLLIYDASTLASGVPSRFKGSGSGTEFTLTISSLQPDDFATYYCQGNAYTSNSHDNAFGGGTKVEIK

[0114] 其中将 CDR 加以下划线。

[0115] 编码 SEQ ID NO:57 的多核苷酸序列示于图 4 中和 SEQ ID NO:58。

[0116] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:59 的重链可变区（抗体 1000 抗 - 毒素 A 抗体重链可变区序列）：

[0117] EVQLVESGGGLIQPGGSLRLSCTVSGIDLSSDAVGWVRQAPGKGLEYIGIIATFDSTYYASWAKGRFTISKASSTTVYLQMNSLRAEDTATYFCARTGSWYYISGWGSYYYGMDLWGQGTLVTVS

[0118] 其中将 CDR 加以下划线。

[0119] 编码 SEQ ID NO:59 的多核苷酸序列示于图 4 中和 SEQ ID NO:60。

[0120] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:57 和 SEQ ID NO:59 中显示的可变区。

[0121] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 B 抗体），所述 CDR 选自：

[0122]

RASKSVSTLMH	SEQ ID NO: 61
LASNLES	SEQ ID NO: 62
QQTWNDPWT	SEQ ID NO: 63
GFTFSNYGMA	SEQ ID NO: 64
SISSSGGSTYYRDSVKG	SEQ ID NO: 65
VIRGYVMDA	SEQ ID NO: 66

[0123] 在一个实施方案中，序列 61 至 63 存在于抗体的轻链中。

[0124] 在一个实施方案中，序列 64 至 66 存在于抗体的重链中。

[0125] 在一个实施方案中，SEQ ID NO:61 为 CDR L1，SEQ ID NO:62 为 CDR L2 以及 SEQ ID NO:63 为 CDR L3。

[0126] 在一个实施方案中，SEQ ID NO:64 为 CDR H1，SEQ ID NO:65 为 CDR H2 以及 SEQ ID NO:66 为 CDR H3。

[0127] 在一个实施方案中，SEQ ID NO:61 为 CDR L1，SEQ ID NO:62 为 CDR L2，SEQ ID NO:63 为 CDR L3，SEQ ID NO:64 为 CDR H1，SEQ ID NO:65 为 CDR H2 以及 SEQ ID NO:66 为 CDR H3。

[0128] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:67 的轻链可变区（抗体 926 抗－毒素 B 抗体；轻链可变区序列）：

[0129] DTVLTQSPATLSLSPGERATLSCRASKSVSTLMHWFQQKPGQAPKLLIYLASNLESGVPARFSGSGSGT DFTLTISSLEPEDFAVYYCQQTWNDPWTFGGGTKVEIK

[0130] 其中将 CDR 加以下划线。

[0131] 编码 SEQ ID NO:67 的多核苷酸序列示于图 5 中和 SEQ ID NO:68。

[0132] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:69 的重链可变区（抗体 926 抗－毒素 B 抗体重链可变区序列）：

[0133] EVELLESGGGLVQPGGSLRLSCEASGFTFSNYGMAWVRQAPTKGLEWVTSISSSGGSTYYRDSVKGRFT ISRDNAKSSLYLQMNSLRAEDTATYYCTTVIRGYVMDAWGQGTLVTVS

[0134] 其中将 CDR 加以下划线

[0135] 编码 SEQ ID NO:69 的多核苷酸序列示于图 5 中和 SEQ ID NO:70。

[0136] 在一个实施方案中，提供了包含 CDR 例如 1，2，3，4，5 或 6 个 CDR 的抗体（例如抗－毒素 B 抗体），所述 CDR 选自：

[0137]

RASGSVSTLMH	SEQ ID NO: 71
KASNLAS	SEQ ID NO: 72
HQSWNSDT	SEQ ID NO: 73
GFTFSNYGMA	SEQ ID NO: 74
TINYDGRTTHYRDSVKG	SEQ ID NO: 75
ISRSHYFDC	SEQ ID NO: 76

[0138] 在一个实施方案中，序列 71 至 73 存在于抗体的轻链中。

[0139] 在一个实施方案中，序列 74 至 76 存在于抗体的重链中。

[0140] 在一个实施方案中，SEQ ID NO:71 为 CDR L1，SEQ ID NO:72 为 CDR L2 以及 SEQ ID NO:73 为 CDR L3。

[0141] 在一个实施方案中，SEQ ID NO:74 为 CDR H1，SEQ ID NO:75 为 CDR H2 以及 SEQ ID NO:76 为 CDR H3。

[0142] 在一个实施方案中，SEQ ID NO:71 为 CDR L1，SEQ ID NO:72 为 CDR L2，SEQ ID NO:73 为 CDR L3，SEQ ID NO:74 为 CDR H1，SEQ ID NO:75 为 CDR H2 以及 SEQ ID NO:76 为 CDR H3。

[0143] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:77 的轻链可变区（抗体 927 抗 - 毒素 B 抗体；轻链可变区序列）：

[0144] DTQMTQSPSTLSASVGDRVTITCRASGSVSTLMHWYQQKPGKAPKLLIYKASNLASGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCHQSWNSDTFGQGTRLEIK

[0145] 其中将 CDR 加以下划线

[0146] 编码 SEQ ID NO:77 的多核苷酸序列示于图 5 中和 SEQ ID NO:78。

[0147] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:79 的重链可变区（抗体 927 抗 - 毒素 B 抗体重链可变区序列）：

[0148] EVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMAWVRQAPGKGLEWVATINYDGRTTHYRDSVKGRFTISRDNSKSTLYLQMNSLRAEDTAVYYCTSISRSHYFDCWGQGTLVTVS

[0149] 其中将 CDR 加以下划线

[0150] 编码 SEQ ID NO:79 的多核苷酸序列示于图 5 中和 SEQ ID NO:80。

[0151] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:77 和 SEQ ID NO:79 中显示的可变区。

[0152] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 B 抗体），所述 CDR 选自：

[0153]

KASKSISNHLA	SEQ ID NO: 81
SGSTLQS	SEQ ID NO: 82
QQYDEYPYT	SEQ ID NO: 83
GFSLQSYTIS	SEQ ID NO: 84
AISGGGSTYYNLPLKS	SEQ ID NO: 85
PRWYPRSYFDY	SEQ ID NO: 86

[0154] 在一个实施方案中，序列 81 至 83 存在于抗体的轻链中。

[0155] 在一个实施方案中，序列 84 至 86 存在于抗体的重链中。

[0156] 在一个实施方案中，SEQ ID NO:81 为 CDR L1，SEQ ID NO:82 为 CDR L2 以及 SEQ ID NO:83 为 CDR L3。

[0157] 在一个实施方案中，SEQ ID NO:84 为 CDR H1，SEQ ID NO:85 为 CDR H2 以及 SEQ ID NO:86 为 CDR H3。

[0158] 在一个实施方案中，SEQ ID NO:81 为 CDR L1，SEQ ID NO:82 为 CDR L2，SEQ ID NO:83 为 CDR L3，SEQ ID NO:84 为 CDR H1，SEQ ID NO:85 为 CDR H2 以及 SEQ ID NO:86 为 CDR H3。

[0159] 在一个实施方案中，提供了可变区，例如具有下列序列的轻链可变区（抗体 1099 抗－毒素 B 抗体；轻链可变区序列）SEQ ID NO:87:

[0160] DVQLTQSPSFLSASVGDRVTITCKASKSISNHLAWYQEKPGKANKLLIHSGSTLQSGTPSRFSGSGSGT EFTLTISSLQPEDFATYYCQQYDEYPYTFGQGTRLEIKRT

[0161] 其中将 CDR 加以下划线

[0162] 在一个实施方案中，SEQ ID NO:87 的最后两个氨基酸（RT）被省略。

[0163] 编码 SEQ ID NO:87 的多核苷酸序列示于图 6 中和 SEQ ID NO:88。在一个实施方案中，编码最后两个氨基酸（RT）的密码子被省略。

[0164] 在一个实施方案中，提供了可变区，例如具有下列序列的重链可变区（抗体 1099 抗－毒素 B 抗体重链可变区序列）SEQ ID NO:89:

[0165] EVQLQESGPGLVKPSETLSLTCTVSGFSLQSYTISWVRQPPGKGLEWIAAISGGGSTYYNLPLKSRVTI SRDTSKSQVSLKLSSVTAADTAVYYCTRPRWYPRSYFDYWGRGTLVTVS

[0166] 其中将 CDR 加以下划线

[0167] 编码 SEQ ID NO:89 的多核苷酸序列示于图 6 中和 SEQ ID NO:90。

[0168] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:87 和 SEQ ID NO:89 中所示的可变区。

[0169] 在一个实施方案中，提供了包含 CDR 例如 1，2，3，4，5 或 6 个 CDR 的抗体（例如抗－毒素 B 抗体），所述 CDR 选自：

[0170]

RASQRISTSIH	**SEQ ID NO: 91**
YASQSIS	**SEQ ID NO: 92**
QQSYSSLYT	**SEQ ID NO: 93**
GFTFSDSYMA	**SEQ ID NO: 94**
SISYGGTIIQYGDSVKG	**SEQ ID NO: 95**
RQGTYARYLDF	**SEQ ID NO: 96**

[0171] 在一个实施方案中，序列 91 至 93 存在于抗体的轻链中。

[0172] 在一个实施方案中，序列 94 至 96 存在于抗体的重链中。

[0173] 在一个实施方案中，SEQ ID NO:91 为 CDR L1，SEQ ID NO:92 为 CDR L2 以及 SEQ ID NO:93 为 CDR L3。

[0174] 在一个实施方案中，SEQ ID NO:94 为 CDR H1，SEQ ID NO:95 为 CDR H2 以及 SEQ ID NO:96 为 CDR H3。

[0175] 在一个实施方案中，SEQ ID NO:91 为 CDR L1，SEQ ID NO:92 为 CDR L2，SEQ ID NO:93 为 CDR L3，SEQ ID NO:94 为 CDR H1，SEQ ID NO:95 为 CDR H2 以及 SEQ ID NO:96 为 CDR H3。

[0176] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:97 的轻链可变

区（抗体 1102 抗 - 毒素 B 抗体；轻链可变区序列）：

[0177] NIVLTQSPATLSLSPGERATLSCRASQRISTSIHWYQQKPGQAPRLLIKYASQSISGIPARFSGSGSGT DFTLTISSLEPEDFAVYYCQQSYSSLYTFGQGTKLEIK

[0178] 其中将 CDR 加以下划线

[0179] 编码 SEQ ID NO:97 的多核苷酸序列示于图 6 中和 SEQ ID NO:98。

[0180] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:99 的重链可变区（抗体 1102 抗 - 毒素 B 抗体重链可变区序列）：

[0181] EVQLVESGGGLVQPGGSLRLSCAVSGFTFSDSYMAWVRQAPGKGLEWIASISYGGTIIQYGDSVKGRFT ISRDNAKSSLYLQMNSLRAEDTAVYYCARRQGTYARYLDFWGQGTLVTVS

[0182] 其中将 CDR 加以下划线

[0183] 编码 SEQ ID NO:99 的多核苷酸序列示于图 7 中和 SEQ ID NO:100。

[0184] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:97 和 SEQ ID NO:99 中所示的可变区。

[0185] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 B 抗体），所述 CDR 选自：

[0186]

RASESVSTLLH	SEQ ID NO: 101
KASNLAS	SEQ ID NO: 102
HQSWNSPPT	SEQ ID NO: 103
GFTFSNYGMA	SEQ ID NO: 104
IINYDASTTHYRDSVKG	SEQ ID NO: 105
YGRSHYFDY	SEQ ID NO: 106

[0187] 在一个实施方案中，序列 101 至 103 存在于抗体的轻链中。

[0188] 在一个实施方案中，序列 104 至 106 存在于抗体的重链中。

[0189] 在一个实施方案中，SEQ ID NO:101 为 CDR L1，SEQ ID NO:102 为 CDR L2 以及 SEQ ID NO:103 为 CDR L3。

[0190] 在一个实施方案中，SEQ ID NO:104 为 CDR H1，SEQ ID NO:105 为 CDR H2 以及 SEQ ID NO:106 为 CDR H3。

[0191] 在一个实施方案中，SEQ ID NO:101 为 CDR L1，SEQ ID NO:102 为 CDR L2，SEQ ID NO:103 为 CDR L3，SEQ ID NO:104 为 CDR H1，SEQ ID NO:105 为 CDR H2 以及 SEQ ID NO:106 为 CDRH3。

[0192] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:107 的轻链可变区（抗体 1114 抗 - 毒素 B 抗体；轻链可变区序列）：

[0193] ATQMTQSPSSLSASVGDRVTITCRASESVSTLLHWYQQKPGKAPKLLIYKASNLASGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCHQSWNSPPTFGQGTKLEIK

[0194] 其中将 CDR 加以下划线

[0195] 编码 SEQ ID NO:107 的多核苷酸序列示于图 7 中和 SEQ ID NO:108。

[0196] 在一个实施方案中，提供了可变区，例如具有下列序列SEQ ID NO:109的重链可变区（抗体1114抗-毒素B抗体重链可变区序列）：

[0197] EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYGMAWVRQAPGKGLEWVAIINYDASTTHYRDSVKGRFTISRDNAKSSLYLQMNSLRAEDTAVYYCTRYGRSHYFDYWGQGTLVTVS

[0198] 其中将CDR加以下划线

[0199] 编码SEQ ID NO:109的多核苷酸序列示于图7中和SEQ ID NO:110。

[0200] 在一个实施方案中，根据本发明的抗体包含SEQ ID NO:107和SEQ ID NO:109中显示的可变区。

[0201] 在一个实施方案中，提供了包含CDR例如1,2,3,4,5或6个CDR的抗体（例如抗-毒素B抗体），所述CDR选自：

[0202]

RASESVSTLLH	SEQ ID NO: 111
KASNLAS	SEQ ID NO: 112
HQSWNSPPT	SEQ ID NO: 113
GFTFSNYGMA	SEQ ID NO: 114
IINYDASTTHYRDSVK	SEQ ID NO: 115
YGRSHYFDY	SEQ ID NO: 116

[0203] 在一个实施方案中，序列111至113存在于抗体的轻链中。

[0204] 在一个实施方案中，序列114至116存在于抗体的重链中。

[0205] 在一个实施方案中，SEQ ID NO:111为CDR L1，SEQ ID NO:112为CDR L2以及SEQ ID NO:113为CDR L3。

[0206] 在一个实施方案中，SEQ ID NO:114为CDR H1，SEQ ID NO:115为CDR H2以及SEQ ID NO:116为CDR H3。

[0207] 在一个实施方案中，SEQ ID NO:111为CDR L1，SEQ ID NO:112为CDR L2，SEQ ID NO:113为CDR L3，SEQ ID NO:114为CDR H1，SEQ ID NO:115为CDR H2以及SEQ ID NO:116为CDRH3。

[0208] 在一个实施方案中，提供了可变区，例如具有下列序列SEQ ID NO:117的轻链可变区（抗体1114graft8抗-毒素B抗体；轻链可变区序列）：

[0209] DTVLTQSPSSLSASVGDRVTITCRASESVSTLLHWYQQKPGKAPKLLIYKASNLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHQSWNSPPTFGQGTKLEIK

[0210] 其中将CDR加以下划线。

[0211] 编码SEQ ID NO:117的多核苷酸序列示于图8中和SEQ ID NO:118。

[0212] 在一个实施方案中，提供了可变区，例如具有下列序列SEQ ID NO:119的重链可变区（抗体1114graft8抗-毒素B抗体重链可变区序列）：

[0213] EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYGMAWVRQAPGKGLEWVAIINYDASTTHYRDSVKGRFTISRDNAKSSLYLQMNSLRAEDTAVYYCTRYGRSHYFDYWGQGTLVTVS

[0214] 其中将CDR加以下划线

[0215] 编码 SEQ ID NO:119 的多核苷酸序列示于图 8 中和 SEQ ID NO:120。

[0216] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:117 和 SEQ ID NO:119 中显示的可变区。

[0217] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 B 抗体），所述 CDR 选自：

[0218]

KASQNIYMYLN	SEQ ID NO: 121
NTNKLHT	SEQ ID NO: 122
LQHKSFPYT	SEQ ID NO: 123
GFTFRDSFMA	SEQ ID NO: 124
SISYEGDKTYYGDSVKG	SEQ ID NO: 125
LTITTSGDS	SEQ ID NO: 126

[0219] 在一个实施方案中，序列 121 至 123 存在于抗体的轻链中。

[0220] 在一个实施方案中，序列 124 至 126 存在于抗体的重链中。

[0221] 在一个实施方案中，SEQ ID NO:121 为 CDR L1，SEQ ID NO:122 为 CDR L2 以及 SEQ ID NO:123 为 CDR L3。

[0222] 在一个实施方案中，SEQ ID NO:124 为 CDR H1，SEQ ID NO:125 为 CDR H2 以及 SEQ ID NO:126 为 CDR H3。

[0223] 在一个实施方案中，SEQ ID NO:121 为 CDR L1，SEQ ID NO:122 为 CDR L2，SEQ ID NO:123 为 CDR L3，SEQ ID NO:124 为 CDR H1，SEQ ID NO:125 为 CDR H2 以及 SEQ ID NO:126 为 CDRH3。

[0224] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:127 的轻链可变区（抗体 1125 抗 - 毒素 B 抗体；轻链可变区序列）：

[0225] DIQMTQSPSSLSASVGDRVTITCKASQNIYMYLNWYQQKPGKAPKRLIYNTNKLHTGVPSRFSGSGSGTEYTLTISSLQPEDFATYYCLQHKSFPYTFGQGTKLEIK

[0226] 其中将 CDR 加以下划线

[0227] 编码 SEQ ID NO:127 的多核苷酸序列示于图 8 中和 SEQ ID NO:128。

[0228] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:129 的重链可变区（抗体 1125 抗 - 毒素 B 抗体重链可变区序列）：

[0229] EVQLVESGGGLVQPGGSLRLSCAASGFTFRDSFMAWVRQAPGKGLEWVASISYEGDKTYYGDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARLTITTSGDSWGQGTMVTVSS

[0230] 其中将 CDR 加以下划线

[0231] 在一个实施方案中，SEQ ID NO:129 的最后一个氨基酸 (S) 被省略。

[0232] 编码 SEQ ID NO:129 的多核苷酸序列示于图 9 中和 SEQ ID NO:130。在一个实施方案中，编码最后 1 个氨基酸 S 的密码子 AGC 被省略。

[0233] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:127 和 SEQ ID NO:129 中显示的可变区。

[0234] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 B 抗体），所述 CDR 选自：

[0235]

KASQHVGTNVD	SEQ ID NO: 131
GASIRYT	SEQ ID NO: 132
LQYNYNPYT	SEQ ID NO: 133
GFIFSNFGMS	SEQ ID NO: 134
SISPSGGNAYYRDSVKG	SEQ ID NO: 135
RAYSSPFAF	SEQ ID NO: 136

[0236] 在一个实施方案中，序列 131 至 133 存在于抗体的轻链中。

[0237] 在一个实施方案中，序列 134 至 136 存在于抗体的重链中。

[0238] 在一个实施方案中，SEQ ID NO:131 为 CDR L1，SEQ ID NO:132 为 CDR L2 以及 SEQ ID NO:133 为 CDR L3。

[0239] 在一个实施方案中，SEQ ID NO:134 为 CDR H1，SEQ ID NO:135 为 CDR H2 以及 SEQ ID NO:136 为 CDR H3。

[0240] 在一个实施方案中，SEQ ID NO:131 为 CDR L1，SEQ ID NO:132 为 CDR L2，SEQ ID NO:133 为 CDR L3，SEQ ID NO:134 为 CDR H1，SEQ ID NO:135 为 CDR H2 以及 SEQ ID NO:136 为 CDR H3。

[0241] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:137 的轻链可变区（抗体 1129 抗 - 毒素 B 抗体；轻链可变区序列）：

[0242] DTQMTQSPSSLSASVGDRVTITCKASQHVGTNVDWYQQKPGKVPKLLIYGASIRYTGVPDRFTGSGSGTDFTLTISSLQPEDVATYYCLQYNYNPYTFGQGTKLEIK

[0243] 其中将 CDR 加以下划线

[0244] 编码 SEQ ID NO:137 的多核苷酸序列示于图 8 中和 SEQ ID NO:138。

[0245] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:139 的重链可变区（抗体 1129 抗 - 毒素 B 抗体重链可变区序列）：

[0246] EVQLVESGGGVVQPGRSLRLSCATSGFIFSNFGMSWVRQAPGKGLEWVASISPSGGNAYYRDSVKGRFTISRDNSKTTLYLQMNSLRAEDTAVYYCTRRAYSSPFAFWGQGTLVTVSS

[0247] 其中将 CDR 加以下划线

[0248] 在一个实施方案中，SEQ ID NO:139 的最后一个氨基酸 (S) 被省略。

[0249] 编码 SEQ ID NO:139 的多核苷酸序列示于图 8 中和 SEQ ID NO:140。在一个实施方案中，编码最后一个氨基酸 S 的密码子 AGC 被省略。

[0250] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:137 和 SEQ ID NO:139 中显示的可变区。

[0251] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 B 抗体），所述 CDR 选自：

[0252]

KASKSISNHLA	SEQ ID NO: 141
SGSTLQP	SEQ ID NO: 142
QQYDEYPYT	SEQ ID NO: 143
GFSLNSYTIT	SEQ ID NO: 144
AISGGGSTYFNSALKS	SEQ ID NO: 145
PRWYPRSYFDY	SEQ ID NO: 146

[0253] 在一个实施方案中，序列 141 至 143 存在于抗体的轻链中。

[0254] 在一个实施方案中，序列 144 至 146 存在于抗体的重链中。

[0255] 在一个实施方案中，SEQ ID NO:141 为 CDR L1；SEQ ID NO:142 为 CDR L2 以及 SEQ ID NO:143 为 CDR L3。

[0256] 在一个实施方案中，SEQ ID NO:144 为 CDR H1，SEQ ID NO:145 为 CDR H2 以及 SEQ ID NO:146 为 CDR H3。

[0257] 在一个实施方案中，SEQ ID NO:141 为 CDR L1，SEQ ID NO:142 为 CDR L2，SEQ ID NO:143 为 CDR L3，SEQ ID NO:144 为 CDR H1，SEQ ID NO:145 为 CDR H2 以及 SEQ ID NO:146 为 CDRH3。

[0258] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:147 的轻链可变区（抗体 1134 抗 - 毒素 B 抗体；轻链可变区序列）：

[0259] DVQLTQSPSFLSASVGDRVTITCKASKSISNHLAWYQEKPGKANKLLIHSGSTLQPGTPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYDEYPYTFGQGTRLEIK

[0260] SEQ ID NO:147

[0261] 其中将 CDR 加以下划线。

[0262] 编码 SEQ ID NO:147 的多核苷酸序列示于图 9 中和 SEQ ID NO:148。

[0263] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:149 的重链可变区（抗体 1134 抗 - 毒素 B 抗体重链可变区序列）：

[0264] EVQLQESGPGLVKPSETLSLTCTVSGFSLNSYTITWVRQPPGKGLEWIAAISGGGSTYFNSALKSRVTISRDTSKSQVSLKLSSVTAADTAVYYCTRPRWYPRSYFDYWGRGTLVTVS

[0265] 其中将 CDR 加以下划线

[0266] 编码 SEQ ID NO:149 的多核苷酸序列示于图 9 中和 SEQ ID NO:150。

[0267] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:147 和 SEQ ID NO:149 中所示的可变区。

[0268] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 B 抗体），所述 CDR 选自：

[0269]

KASQNVGNNVA	SEQ ID NO: 151
YASNRFT	SEQ ID NO: 152
QRVYQSTWT	SEQ ID NO: 153
GFSLTSYYVH	SEQ ID NO: 154
CIRTGGNTEYQSEFKS	SEQ ID NO: 155
GNYGFAY	SEQ ID NO: 156

[0270] 在一个实施方案中，序列 151 至 153 存在于抗体的轻链中。

[0271] 在一个实施方案中，序列 154 至 156 存在于抗体的重链中。

[0272] 在一个实施方案中，SEQ ID NO:151 为 CDR L1，SEQ ID NO:152 为 CDR L2 以及 SEQ ID NO:153 为 CDR L3。

[0273] 在一个实施方案中，SEQ ID NO:154 为 CDR H1，SEQ ID NO:155 为 CDR H2 以及 SEQ ID NO:156 为 CDR H3。

[0274] 在一个实施方案中，SEQ ID NO:151 为 CDR L1，SEQ ID NO:152 为 CDR L2，SEQ ID NO:153 为 CDR L3，SEQ ID NO:154 为 CDR H1，SEQ ID NO:155 为 CDR H2 以及 SEQ ID NO:156 为 CDR H3。

[0275] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:157 的轻链可变区（抗体 1151 抗 - 毒素 B 抗体；轻链可变区序列）：

[0276] AIQMTQSPSSLSASVGDRVTITCKASQNVGNNVAWYQHKPGKAPKLLIYYASNRFTGVPSRFTGGGYGT DFTLTISSLQPEDFATYYCQRVYQSTWTFGQGTKVEIK

[0277] 其中将 CDR 加以下划线

[0278] 编码 SEQ ID NO:157 的多核苷酸序列示于图 9 中和 SEQ ID NO:158。

[0279] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:159 的重链可变区（抗体 1151 抗 - 毒素 B 抗体重链可变区序列）：

[0280] EVQLQESGPGLVKPSETLSLTCTVSGFSLTSYYVHWVRQPPGKGLEWMGCIRTGGNTEYQSEFKSRVTI SRDTSKNQVSLKLSSVTAADTAVYYCARGNYGFAYWGQGTLVTVS

[0281] 其中将 CDR 加以下划线

[0282] 编码 SEQ ID NO:159 的多核苷酸序列示于图 9 中和 SEQ ID NO:160。

[0283] 在一个实施方案中，根据本发明的抗体包含 SEQ ID NO:157 和 SEQ ID NO:159 中显示的可变区。

[0284] 在一个实施方案中，提供了包含 CDR 例如 1, 2, 3, 4, 5 或 6 个 CDR 的抗体（例如抗 - 毒素 B 抗体），所述 CDR 选自：

[0285]

KASQNINKYLD	SEQ ID NO: 161
NIQSLHT	SEQ ID NO: 162
FQHNSGW	SEQ ID NO: 163
GFTFTQAAMF	SEQ ID NO: 164
RISTKSNNFATYYPDSVKG	SEQ ID NO: 165
PAYYYDGTVPFAY	SEQ ID NO: 166

[0286] 在一个实施方案中，序列 161 至 163 存在于抗体的轻链中。

[0287] 在一个实施方案中，序列 164 至 166 存在于抗体的重链中。

[0288] 在一个实施方案中，SEQ ID NO:161 为 CDR L1，SEQ ID NO:162 为 CDR L2 以及 SEQ ID NO:163 为 CDR L3。

[0289] 在一个实施方案中，SEQ ID NO:164 为 CDR H1，SEQ ID NO:165 为 CDR H2 以及 SEQ ID NO:166 为 CDR H3。

[0290] 在一个实施方案中，SEQ ID NO:161 为 CDR L1，SEQ ID NO:162 为 CDR L2，SEQ ID NO:163 为 CDR L3，SEQ ID NO:164 为 CDR H1，SEQ ID NO:165 为 CDR H2 以及 SEQ ID NO:166 为 CDR H3。

[0291] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:167 的轻链可变区（抗体 1153 抗 - 毒素 B 抗体；轻链可变区序列）：

[0292] DIQMTQSPSSLSASVGDRVTITCKASQNINKYLDWYQQKPGKVPKLLIYNIQSLHTGIPSRFSGSGSGT DFTLTISSLQPEDVATYYCFQHNSGWTFGQGTRLEIK

[0293] 其中将 CDR 加以下划线

[0294] 编码 SEQ ID NO:167 的多核苷酸序列示于图 10 中和 SEQ ID NO:168。

[0295] 在一个实施方案中，提供了可变区，例如具有下列序列 SEQ ID NO:169 的重链可变区（抗体 1153 抗 - 毒素 B 抗体重链可变区序列）：

[0296] EVQLVESGGGLVQPGGSLKLSCAASGFTFTQAAMFWVRQASGKGLEGIARISTKSNNFATYYPDSVKGR FTISRDDSKNTVYLQMNSLKTEDTAVYYCTAPAYYYDGTVPFAYWGQGTLVTVS

[0297] 其中将 CDR 加以下划线

[0298] 编码 SEQ ID NO:169 的多核苷酸序列示于图 10 中和 SEQ ID NO:170。

[0299] 在一个实施方案中，根据本发明的抗体包含中 SEQ ID NO:167 和 SEQ ID NO:169 显示的可变区。

[0300] 在一个实施方案中，提供了包含 6 个 CDR 的抗体，所述 CDR 独立地选自 SEQ ID NO1、2、3、4、5、6、11、12、13、14、15、16、21、22、23、24、25、26、31、32、33、34、35、36、41、42、43、44、45、46、51、52、53、54、55、56、61、62、63、64、65、66、71、72、73、74、75、76、81、82、83、84、85、86、91、92、93、94、95、96、101、102、103、104、105、106、111、112、113、114、115、116、121, 122、123、124、125、126、131、132、133、134、135、136、141、142、143、144、145、146、151、152、153、154、155、156、161、162、163、164、165 和 166。

[0301] 在一个实施方案中，提供了包含 6 个 CDR 的抗 -TcdA 抗体，所述 CDR 独立地选自 SEQ ID NO1、2、3、4、5、6、11、12、13、14、15、16、21、22、23、24、25、26、31、32、33、34、35、36、41、42、43、44、45、46、51、52、53、54、55 和 56。

[0302] 在一个实施方案中，提供了包含6个CDR的抗-TcdB抗体，所述CDR独立地选自SEQ ID NO61、62、63、64、65、66、71、72、73、74、75、76、81、82、83、84、85、86、91、92、93、94、95、96、101、102、103、104、105、106、111、112、113、114、115、116、121, 122、123、124、125、126、131、132、133、134、135、136、141、142、143、144、145、146、151、152、153、154、155、156、161、162、163、164、165 和 166。

[0303] 在一个实施方案中，提供了包含两个可变区的抗体，所述可变区独立地选自 SEQ ID NO:7、9、17、19、27、29、37、39、47、49、57、59、67、69、77、79、87、89、97、99、107、109、117、119、127、129、137、139、147、149、157 和 159。

[0304] 在一个实施方案中，提供了包含两个可变区的抗体，所述可变区独立地选自 SEQ ID NO:7、9、17、19、27、29、37、39、47、49、57 和 59。

[0305] 在一个实施方案中，提供了包含两个可变区的抗体，所述可变区独立地选自 SEQ ID NO:67、69、77、79、87、89、97、99、107、109、117、119、127、129、137、139、147、149、157 和 159。

[0306] 在一个实施方案中，根据本发明的抗体是人源化的。

[0307] 在一个实施方案中，抗体是针对 TcdA 和 / 或 TcdB 毒素的 C 末端“细胞结合”部分的。

[0308] 在一个实施方案中，根据本发明的抗体适合用于中和毒素 A 或毒素 B。

[0309] 如本文中所用，中和意指消除或减小目标毒素的有害 / 有毒作用，例如至少 50% 的相关有害作用的减小。

[0310] 本发明人通过使用本申请中发现的抗体之间的内部比较和通过与本领域中良好描述的抗体 (Babcock 等人 2006;Lowy 等人，2010) 比较，已确定一些抗体即使在高毒素浓度下也具有期望的维持有效中和的特征（例如低 EC_{50} 和高百分比保护作用）。包括本领域中描述的那些抗体的其它抗体在高毒素浓度下不维持有效毒素中和。

[0311] 有效毒素浓度在滴定研究中可被定义为在中和抗体不存在的情况下的‘致死剂量’(LD)。中和测定通常在 50% 的完全细胞杀死的 LD（即 LD_{50}）下进行，但也可在 LD_{80} 下更严苛地进行。

[0312] 测定还可在更具挑战性的条件例如 LD_{90}、LD_{95} 和 / 或 LD_{max}（LD_{max} 是可包括在测定中的受测定体积和最大毒素浓度 / 溶解度限制的最大毒素量）下进行。这样的测定目的在于模拟当肠中生长的艰难梭菌蔓延时并且腹泻和其它症状导致人们假设毒素浓度处于它们的最高值时人的感染的早期阶段。在高毒素浓度条件下有效地中和有害毒素活性的抗体被本发明人认为具有针对人感染的症状的控制的特殊临床值。在一个实施方案中，本公开内容的抗体具有有用的例如低 EC_{50} 值和 / 或对于 LD_{80}、LD_{90}、LD_{95} 和 / 或 LD_{max} 中的一个或多个使细胞免于死亡的高百分比保护作用。在一个实施方案中，后一种情况的一个或多个中的 EC_{50} 为 15ng/ml 或更少，例如 10ng/ml 或更少，例如 5ng/ml 或更少，特别地 1ng/ml 或更少。在一个实施方案中，使细胞免于死亡的百分比保护作用大于 90%，或大于 75% 或大于 50%。

[0313] 因此，在一个实施方案中，本公开内容提供了即使在高水平毒素存在的情况下仍然维持毒素中和作用（例如如本文中提供的测定中测量的）的抗体或抗体组合。

[0314] 可以例如在适当的体外测定中测量毒素的有害作用。在一个实施方案中，在下面实施例 1 中给出的测定中测量中和作用。还提供了在中和测定中鉴定的抗体，例如其中抗

体的效力在高水平毒素存在的情况下得到维持。

[0315] 毒素 A 可与 TcdA 互换使用。

[0316] 毒素 B 可与 TcdB 互换使用。

[0317] 在一个实施方案中，根据本发明的抗体为单克隆抗体或其结合片段。

[0318] 在一个实施方案中，根据本发明的单克隆抗体能够以极高的效力和亲和力中和 TcdA。

[0319] 在一个实施方案中，根据本发明的单克隆抗体能够以极高的效力和亲和力以及高亲合性 (avidity) 中和 TcdA。

[0320] 如本文中所用，亲合性是指多个结合亲和力的组合强度。

[0321] 在一个实施方案中，根据本发明的单克隆抗体能够以极高的效力和亲和力以及高亲合性和高结合效价中和 TcdA。

[0322] 如本文中所用，结合效价是指单克隆抗体多次结合抗原的能力。高结合效价因而导致高水平的抗体对抗原的修饰和 / 或高水平的毒素分子的交联，这被认为是有利的。

[0323] 根据本公开内容的抗 -TcdA Mab 可适合于中和 TcdA 的早期作用，例如细胞上的例如紧密连接的丢失。

[0324] 如本文中所用，紧密连接意欲指单层或解剖组织结构内的细胞之间的不能渗透的连接区域。当紧密连接保持它们的结构和功能完整性时，液体丢失不发生。紧密连接的丢失是细胞已被毒素损害的指征，并且被良好地记录为 TcdA 和 TcdB 的毒性作用的早期步骤 (25)，并且导致包含血清、免疫球蛋白和离子的流体的丢失 (26, 3)。紧密连接的丢失被认为是人的腹泻发作的第一步骤。

[0325] TEER 测定系统可用于体外测量紧密连接的丢失。TEER 为跨上皮电阻测定的首字母缩略词并且其通常用于测量代表肠内皮衬里的分化细胞层的通透性。然而，在抗体筛选的背景中，TEER 丢失可用于鉴定减缓或防止对紧密连接的损害的抗体，从而为抗导致腹泻的组织损害的保护作用的替代者。

[0326] 通常地使用 Caco-2 细胞，因为它们来源于人结肠细胞并且已知形成分化的单层，细胞通过紧密连接进行连接。试剂盒可从 Becton-Dickinson 命名的 Caco-2BioCoat HTS 平板系统 (BDBiosciences/354802) 商购获得。试剂盒中的说明书适合用于本说明书中的测试。当膜已受损后，膜的电阻改变。

[0327] 通常地，在添加至 TEER 系统以确定抗体是否可防止或减缓对由毒素引起的对膜的损害之前，用毒素预温育抗体。可在一段适当的时间例如 24 小时内通过在某些时间点上进行测量来进行测定。本发明人已确定 4 小时的时间点特别适合鉴别治疗上有用的抗体。

[0328] TEER 测定中使用的毒素的浓度通常在 100-200ng/ml 的范围内，最优选为 125ng/ml。

[0329] TEER 测定中使用的抗体 (例如 IgG1) 的浓度通常在 4 至 2000ng/ml，例如 50 至 1000ng/ml，例如 100 至 500ng/ml 的范围内。

[0330] 在一个实施方案中，在所述条件中使用的 TEER 测定中的抗体的 EC_{50} 为至少 200ng/ml，例如少于 100ng/ml，例如约 60-80ng/ml。

[0331] 在一个实施方案中，提供了在艰难梭菌感染的治疗或预防中适合用作治疗剂的抗 -TcdA 抗体或抗 -TcdB 抗体，其中使用 TEER 测定筛选和选择所述抗体。

[0332] 在一个方面，提供了就减缓或防止紧密连接丢失的能力在 TEER 测定中筛选抗体的方法。在一个实施方案中，筛选的抗体是抗 -TcdA 抗体。在一个实施方案中，筛选的抗体是抗 -TcdB 抗体。在一个实施方案中，筛选的抗体是抗 -TcdA 与抗 -TcdB 抗体的组合。在一个实施方案中，方法包括鉴定适当的抗体和表达适当量的所述抗体的步骤。在一个实施方案中，方法还包括在药物制剂中配制所述抗体的步骤。在一个实施方案中，方法还包括将所述抗体或所述制剂给有此需要的患者施用的步骤。

[0333] 在一个实施方案中，本公开内容的多个抗体可以能够结合目标毒素 (TcdA 或 TcdB)，所述抗体可帮助毒素的免疫清除。

[0334] 如本文中所用，多个抗体意欲指多个拷贝的具有相同序列的抗体或具有相同氨基酸序列的抗体，或特异于相同靶抗原但具有不同氨基酸序列的抗体。

[0335] 在一个实施方案中，根据本发明的抗体特异于靶抗原，例如特异于靶抗原中的表位。

[0336] 在一个实施方案中，本发明的抗体能够在两个或更多个位置，例如两个或 3 个位置，例如 4、5、6、7、8、9、10 或更多个位置上结合靶抗原，例如毒素可包含重复结构域，从而抗体对于表位可以是特异性的并且事实上表位可数次存在于抗原中，即存在于超过一个位置中。因此，给定的抗体可在抗原的不同位置结合相同表位数次。

[0337] 在一个实施方案中，抗体结合给定的抗原数次，例如 2 至 20 次例如 3、4、5、6、7、8、9、10、11、12、13、14、15 或 16 次。在一个实施方案中，抗体结合给定的抗原至少 3 次。该多次结合被认为在毒素的中和和 / 或清除中是非常重要的。不希望受理论束缚，认为多次结合例如 3 次或更多次结合，即被 3 个或更多个 Fc 片段修饰，在触发主要通过肝和脾 (27, 28) 进行的毒素快速清除 (24) 中是非常重要的。

[0338] 在一个实施方案中，抗 -TcdA 抗体结合 3 次或更多次，例如 3 至 16 次。

[0339] 在一个实施方案中，抗 -TcdA 抗体结合 12 次。

[0340] 在一个实施方案中，抗 -TcdA 抗体结合 2 次。

[0341] 在一个实施方案中，抗 -TcdA 抗体在 TcdA 的催化末端细胞结合结构域中结合。

[0342] 在一个实施方案中，抗 -Tcd B 抗体结合 2 次或更多次，例如 2 次。

[0343] 在一个实施方案中，抗 -TcdB 抗体在 TcdB 的催化末端细胞结合结构域中结合。

[0344] 在一个实施方案中，根据本公开内容的抗体能够交联毒素分子，例如抗体分子的一个臂结合一个毒素分子，抗体的另一个臂结合不同毒素分子中的表位，从而形成一种免疫复合物。后者的形成还可促进免疫系统的活化，以清除相关毒素，从而使所述毒素的有害体内作用减小至最小。

[0345] 在一个实施方案中，先天性免疫反应，例如补体被活化。

[0346] 在一个实施方案中，本公开内容的抗体具有高的抗来源于不同核糖型例如 003、027、078 的菌株的毒素的效力。

[0347] 在一个实施方案中，抗 TcdA 抗体，例如针对自核糖型 003、027 和 078 的菌株的毒素，可具有在 0.1 - 100ng/ml，例如 1 至 10ng/ml 范围内的 EC_{50} 以及在 LD_{80-95} 的毒素浓度具有在 50-100% 的范围内的最大抑制。

[0348] 在一个实施方案中，抗 TcdA 抗体，例如针对自核糖型 003、027 和 078 的菌株的毒素，可具有在 0.1 - 100ng/ml，例如 1 至 10ng/ml 范围内的 EC_{50} 以及在 LD_{80-95} 的毒素浓度具

有在 60-100%、70-100%、80-100% 或 90-100% 的范围内的最大抑制。

[0349] 在一个实施方案中，抗 TcdB 抗体，例如针对自核糖型 003 的菌株的毒素，可具有在 0.1 - 100ng/ml，例如 1 至 10ng/ml 范围内的 EC_{50} 以及在 LD_{80-95} 的毒素浓度具有在 50-100% 的范围内的最大抑制。

[0350] 在一个实施方案中，抗 TcdB 抗体，例如针对自核糖型 003 的菌株的毒素，可具有在 0.1 - 100ng/ml，例如 1 至 10ng/ml 范围内的 EC_{50} 以及在 LD_{80-95} 的毒素浓度具有在 60-100%、70-100%、80-100% 或 90-100% 的范围内的最大抑制。

[0351] 在一个实施方案中，提供了根据本发明的抗体的组合，例如特异于 TcdA 的抗体的组合，特异于 TcdB 的抗体的组合或特异于 TcdA 的抗体与特异于 TcdB 的抗体的组合。

[0352] 特异于 TcdA 的抗体的组合通常是指针对靶抗原 TcdA 上的不同表位的抗体的组合，或至少具有两个不同结合性质的抗体的组合。

[0353] 特异于 TcdB 的抗体的组合通常是指针对靶抗原 TcdB 上的不同表位的抗体的组合，或至少具有两个不同结合性质的抗体的组合。

[0354] 组合可包括 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 或 15 种不同抗体，例如 2, 3, 4 或 5 种抗体。

[0355] 在一个实施方案中，提供了一种抗 -TcdA 抗体与两种抗 -TcdB 抗体的组合，例如其中抗 -TcdA 抗体为 997 并且其中抗 -TcdB 抗体为 1125 和 1151。

[0356] 具体地，提供了一种抗 -TcdA 抗体（其包含具有 SEQ ID NO:49 中显示的序列的重链可变区和具有 SEQ ID NO:47 中显示的序列的轻链可变区）与两种抗 -TcdB 抗体（第一种抗 -TcdB 抗体具有 SEQ ID NO:129 中显示的重链可变区和 SEQ ID NO:127 中显示的轻链可变区，第二种抗 -TcdB 抗体具有 SEQ ID NO:159 中显示的重链可变区和 SEQ ID NO:157 中显示的轻链可变区）的组合。

[0357] 如本文中所用，不同的抗体意欲指具有不同的氨基酸序列的抗体，其可结合靶抗原上的相同表位或不同表位。

[0358] 本发明还提供的是被本发明提供的抗体（特别是包含 SEQ ID NO:49 中显示的重链序列和 SEQ ID NO:47 中显示的轻链序列的抗体）结合的 TcdA 的特定区域或表位。

[0359] 本发明还提供的是被本发明提供的抗体（特别地包含 SEQ ID NO:129 中显示的重链序列和 SEQ ID NO:127 中显示的轻链序列的抗体，或包含 SEQ ID NO:159 中显示的重链序列和 SEQ ID NO:157 中显示的轻链序列的抗体）结合的 TcdB 的特定区域或表位。

[0360] TcdA 或 TcdB 毒素的该特定区域或表位可通过本领域内已知的任何适当的表位作图法与本发明提供的抗体的任一种相组合来鉴定。这样的方法的实例包括就与本发明的抗体的结合筛选来源于毒素的具有不同长度的肽，可特异性结合抗体的最小片段包含被抗体识别的表位的序列。可合成产生或通过毒素多肽的蛋白水解消化产生肽。结合抗体的肽可以通过例如质谱分析来鉴定。在另一个实例中，NMR 光谱学或 X 射线晶体学可用于鉴定被本发明的抗体结合的表位。鉴定后，必要时，可将结合本发明的抗体的表位片段用作获得结合相同表位的其它拮抗性抗体的免疫原。

[0361] 交叉阻断根据本发明的抗体的结合的抗体可类似地用于中和毒素活性。因此，本发明还提供了具有对于 TcdA 或 TcdB 的特异性的中和抗体，所述中和抗体交叉阻断上述抗体的任一种对 TcdA 或 TcdB 的结合和 / 或被那些抗体的任一种交叉阻断对这些毒素的结

合。在一个实施方案中，这样的抗体结合与本文中上述抗体相同的表位。在另一个实施方案中，交叉阻断中和抗体结合与被本文中上述抗体结合的表位相邻和 / 或重叠的表位。在另一个实施方案中，本发明的该方面的交叉阻断中和抗体不结合与本发明的抗体结合的表位相同的表位或与所述表位相邻和 / 或重叠的表位。

[0362] 交叉阻断抗体可使用本领域中的任何适当方法来鉴定，例如通过使用竞争性的 ELISA 或 BIAcore 测定来进行，其中交叉阻断抗体对 TcdA 或 TcdB 的结合阻止本发明的抗体的结合或反之亦然。

[0363] 在一个实施方案中，提供了产生抗 -TcdA 或抗 -TcdB 抗体，特别是交叉阻断本文中描述的抗体的结合的中和抗体的方法，所述方法包括步骤：用适当的抗原例如 SEQ ID NO173 至 194 的任一个所示的抗原或其组合免疫宿主。所述方法还包括一个或多个下列步骤，例如鉴定目标抗体（特别是使用功能测定例如 TEER 测定），表达目标抗体，和任选地将抗体配制为药学上可接受的组合物。

[0364] 因此在一个方面，本公开内容提供了利用 SEQ ID NO173 至 194 中所示的氨基酸序列或其组合免疫宿主的方法。

[0365] 在一个实施方案中，根据本发明的抗体具有 10nM 或更少，例如 1nM 或更少例如 900pM，特别是 800pM、700pM、600pM 或 500pM，例如 60pM 的对靶抗原的亲和力。

[0366] 在一个实施方案中，亲和力是针对 TcdA 或 TcdB 或其片段的。在一个实例中，片段为对应于 TcdA 的 S1827-D2249 残基的 TcdA123。在一个实例中，片段为对应于残基 G2205-R2608 的 TcdA456。在一个实施方案中，片段为对应于 TcdB 的残基 S1833-E2366 的 TcdB1234。

[0367] 在一个实施方案中，根据本发明的抗体或其组合具有 200ng/ml 或更少，例如 150ng/ml 或更少例如 100ng/ml 或更少，例如在 0.1 至 10ng/ml 的范围内的 EC_{50}。

[0368] 混合物的单个组分抗体不必具有在所述范围内的 EC_{50}，只要当它们与一种或多种抗体组合使用时组合具有在所述范围内的 EC_{50} 即可。

[0369] 有利地，本发明的抗体是稳定的，例如在高于 50℃例如 60 或 70℃的温度是热稳定的。

[0370] 根据本发明的抗体和组合还具有下列有利性质的一个或多个性质：慢的解离速率 (off rate)、高亲和力、高效力、多次结合靶抗原的能力、利用减少可测量的 TEER 活性的损失的机制中和毒素、刺激或帮助宿主天然免疫反应、催化或帮助病原体（或毒素）的免疫清除和 / 或训练免疫系统对病原体（或毒素）作出适当反应的能力。

[0371] 按照由 Kabat 等人设计的系统常规地给抗体可变结构域中的残基进行编号。该系统示于 Kabat 等人，1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA（在下文中“Kabat 等人（同上）”）中。除其中另有所指的地方外，该编码系统用于本说明书中。

[0372] Kabat 残基命名不总是直接对应于氨基酸残基的线性编号。实际的线性氨基酸序列可包含比严格 Kabat 编号中的氨基酸更少或更多的氨基酸，对应于基本可变结构域结构的结构组分（无论是构架区还是互补决定区 (CDR)）的缩短或插入。可通过将抗体序列中具有同源性的残基与“标准”Kabat 编号的序列比对来确定给定的抗体的正确残基 Kabat 编号。

[0373] 重链可变结构域的CDR位于根据Kabat编号系统的残基31-35(CDR-H1)、残基50-65(CDR-H2)和残基95-102(CDR-H3)上。然而根据Chothia(Chothia,C.和Lesk,A.M.J.Mol.Biol.,196,901-917(1987)),等同于CDR-H1的环从残基26延伸至残基32。因此除非另外指出,否则如本文中所用,'CDR-H1'意指残基26至35,如通过Kabat编号系统和Chothia's拓扑学环定义的组合描述的。

[0374] 轻链可变结构域的CDR位于根据Kabat编号系统的残基24-34(CDR-L1)、残基50-56(CDR-L2)和残基89-97(CDR-L3)上。

[0375] 本发明中使用的抗体可使用本领域已知的任何适当的方法来获得。可使用毒素A和/或毒素B多肽/蛋白质,包括融合蛋白例如毒素-Fc融合蛋白或表达(重组地或天然)多肽的细胞(例如活化的T细胞)来产生特异性识别目标毒素的抗体。毒素多肽可以是全长多肽或其生物活性片段或衍生物。

[0376] 多肽可通过本领域公知的方法从包含表达系统的遗传工程化宿主细胞制备,或可从天然生物来源回收它们。在本申请中,术语"多肽"包括肽、多肽和蛋白质。除非另外明确地指出,否则这些术语可互换使用。来自核糖型027的TcdA的序列示于SEQ ID NO:171(Uniprot登录号C9YJ37),来自核糖型027的TcdB的序列示于SEQ ID NO:172(Uniprot登录号C9YJ35)。

[0377] 抗原多肽可在一些情况下为更大的蛋白质例如融合蛋白(例如融合至亲和标记物)的一部分。

[0378] 可通过使用公知的和常规方案给动物,优选非人动物施用多肽来获得针对抗原多肽产生的抗体,其中动物的免疫是必需的,参见,例如Handbook of Experimental Immunology,D.M.Weir(ed.),第4卷,Blackwell Scientific Publishers,Oxford,England,1986)。可免疫许多温血动物例如兔、小鼠、大鼠、绵羊、牛、骆驼或猪。然而,小鼠、兔、猪和大鼠通常是最适当的。

[0379] 单克隆抗体可通过本领域已知的任何方法例如杂交瘤技术(Kohler&Milstein,1975,Nature,256:495-497)、三源杂交瘤技术、人B细胞杂交瘤技术(Kozbor等人,1983,Immunology Today,4:72)和EBV-杂交瘤技术(Cole等人,Monoclonal Antibody and Cancer Therapy,pp77-96,Alan R Liss,Inc.,1985)来制备。

[0380] 本发明中使用的抗体还可使用单淋巴细胞抗体法,通过克隆和表达从通过例如Babcook,J.等人,1996,Proc.Natl.Acad.Sci.USA93(15):7843-78481;WO92/02551;WO2004/051268和国际专利申请号WO2004/106377中描述的方法选择的产生特定抗体的单个淋巴细胞产生的免疫球蛋白可变区cDNA来产生。

[0381] 人源化抗体(其包含CDR移植的抗体)是具有来自非人物种的一个或多个互补决定区(CDR)和来自人免疫球蛋白分子的构架区的抗体分子(参见,例如US5,585,089;WO91/09967)。应理解,可能仅必需转移CDR的特异性决定残基而非整个CDR(参见例如,Kashmiri等人,2005,Methods,36,25-34)。人源化抗体可任选地还包含来源于CDR所源自的非人物种的一个或多个构架残基。

[0382] 如本文中所用,术语'人源化抗体分子'是这样的抗体分子,在所述抗体分子中,重链和/或轻链包含来自移植至受体抗体(例如,人抗体)的重链和/或轻链可变构架内的供体抗体(例如鼠单克隆抗体)的一个或多个CDR(包括,必要时,一个或多个修饰的CDR)。

关于综述，参见 Vaughan 等人，Nature Biotechnology, 16, 535-539, 1998。在一个实施方案中，不是整个 CDR 被移植，而是将来自本文中上述 CDR 的任一个的特异性决定残基的仅一个或多个转移至人抗体构架（参见例如，Kashmiri 等人，2005, Methods, 36, 25-34）。在一个实施方案中，仅将本文中上述 CDR 的一个或多个的特异性决定残基转移至人抗体构架。在另一个实施方案中，仅将本文中上述 CDR 的每一个的特异性决定残基转移至人抗体构架。

[0383] 当移植 CDR 或特异性决定残基时，通过考虑 CDR 所源自的供体抗体的种类 / 类型，可使用任何适当的受体可变区构架序列，包括小鼠、灵长类动物和人构架区。适当地，根据本发明的人源化抗体具有包含人受体构架区以及本文中提供的 CDR 的一个或多个的可变结构域。

[0384] 因此，在一个实施方案中提供的是结合毒素 A 或毒素 B 的人源化抗体，其中可变结构域包含人受体构架区和非人供体 CDR。

[0385] 可用于本发明的人构架的实例是 KOL、NEWM、REI、EU、TUR、TEI、LAY 和 POM(Kabat 等人，同上）。例如，KOL 和 NEWM 可用于重链，REI 可用于轻链，EU、LAY 和 POM 可用于重链和轻链。或者，可使用人种系序列；这些序列可在 :http://vbase.mrc-cpe.cam.ac.uk/ 上获得。

[0386] 在本发明的人源化抗体中，受体重链和轻链不必来源于相同抗体，并且必要时，可包含具有来源于不同链的构架区的混合链。

[0387] 同样地，在本发明的人源化抗体中，构架区不必具有与受体抗体的构架区完全相同的序列。例如，可将罕见残基改变成对于该受体链种类或类型是更加频繁存在的残基。或者，可改变受体构架区中选择的残基，以便它们对应于在供体抗体中相同位置上发现的残基（参见 Reichmann 等人，1998, Nature, 332, 323-324）。应当使这样的变化保持至恢复供体抗体的亲和力所必需的最低程度。用于选择可能需要被改变的受体构架区中的残基的方案示于 WO91/09967 中。

[0388] 一般而言，本说明书中公开的抗体序列被人源化。

[0389] 本发明还提供了与本文中公开的序列或抗体具有 80%、90%、91%、92%、93%94%、95%96%、97%、98% 或 99% 的相似性的序列。

[0390] 如本文中所用，"同一性"表示在对齐的序列中的任意特定位置上，氨基酸残基在序列之间是相同的。如本文中所用，"相似性"意指，在对齐的序列的任意特定位置上，氨基酸残基在序列之间具有相似类型。例如，亮氨酸可置换异亮氨酸或缬氨酸。通常可彼此置换的其它氨基酸包括但不限于：

[0391] - 苯丙氨酸、酪氨酸和色氨酸（具有芳香族侧链的氨基酸）；

[0392] - 赖氨酸、精氨酸和组氨酸（具有碱性侧链的氨基酸）；

[0393] - 天冬氨酸和谷氨酸（具有酸性侧链的氨基酸）；

[0394] - 天冬酰胺和谷氨酰胺（具有酰胺侧链的氨基酸）；和

[0395] - 半胱氨酸和甲硫氨酸（具有含硫侧链的氨基酸）。

[0396] 同一性和相似性的程度可容易地计算 (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, Part1, Griffin, A.M., 和 Griffin, H.

G.,eds.,Humana Press,New Jersey,1994;Sequence Analysis in Molecular Biology,von Heinje,G.,Academic Press,1987,Sequence Analysis Primer,Gribskov,M. 和 Devereux,J.,eds.,M Stockton Press,New York,1991, 可从 NCBI 获得的 BLAST™ 软件 (Altschul,S.F. 等人,1990,J.Mol.Biol.215:403-410;Gish,W.&States,D.J.1993,Nature Genet.3:266-272.Madden,T.L. 等 人 ,1996,Meth.Enzymol.266:131-141;Altschul,S.F. 等 人 ,1997,Nucleic Acids Res.25:3389-3402;Zhang,J.&Madden,T.L.1997;Genome Res.7:649-656,)。

[0397] 本发明的抗体分子包括具有全长重链和轻链或其片段的完全抗体分子,其可以是但不限于 Fab、修饰的 Fab、Fab'、修饰的 Fab'、F(ab')2、Fv、Fab-Fv、Fab-dsFv、单结构域抗体 (例如 VH 或 VL 或 VHH)、scFv、二价、三价或四价抗体、双 -scFv、双链抗体、三链抗体、四链抗体以及上述任一个的表位结合片段 (参见例如,Holliger 和 Hudson,2005,Nature Biotech.23(9):1126-1136;Adair 和 Lawson,2005,Drug Design Reviews-Online2(3),209-217)。用于产生和制造这类抗体片段的方法在本领域是公知的 (参见例如 Verma 等人,1998,Journal of Immunological Methods,216,165-181)。用于本发明的其它抗体片段包括国际专利申请 WO2005/003169、WO2005/003170 和 WO2005/003171 中描述的 Fab 和 Fab'片段。多价抗体可包括多特异性,例如双特异性或可以是单特异性的 (参见例如 WO92/22853 和 WO05/113605)。在本实例中特别考虑了双特异性和多特异性抗体变体,因为目的是中和两种独立的目标蛋白 :TcdA 和 TcdB。来自本文中公开的抗体的可变区可以以这样的方式构造来产生能够结合并中和 TcdA 和 TcdB 的单一抗体变体。

[0398] 在一个实施方案中,根据本公开内容的抗体被提供为 TcdA 或 TcdB 结合抗体融合蛋白,其包含免疫球蛋白部分,例如 Fab 或 Fab'片段,和一个或两个直接或间接与其连接的单结构域抗体 (dAb),例如如 WO2009/040562 中描述的。

[0399] 在一个实施方案中,融合蛋白包含两个结构域抗体,例如,任选地通过二硫键连接的可变重链 (VH) 和可变轻链 (VL) 配对,例如如 WO2010/035012 中描述的。

[0400] 在一个实施方案中,融合蛋白的 Fab 或 Fab'元件具有相同的或相似的对于单结构域抗体的特异性。在一个实施方案中,Fab 或 Fab' 具有不同的对单结构域抗体的特异性,也就是说融合蛋白是多价的。在一个实施方案中,根据本发明的多价融合蛋白具有白蛋白结合位点,例如本文中的 VH/VL 对提供了白蛋白结合位点。

[0401] 在一个实施方案中,根据本发明的多价融合蛋白结合 TcdA 和 TcdB。

[0402] 在一个实施方案中,根据本发明的多价融合蛋白在多个位置结合 TcdB,例如具有特异于两个不同表位的不同的结合区域。

[0403] 可以就预期的抗体分子的功能,特别是可能需要的效应子功能,来选择本发明的抗体分子的恒定区结构域 (如果存在的话)。例如,恒定区结构域可以是人 IgA,IgD,IgE,IgG 或 IgM 结构域。具体地,当意欲将抗体分子用于治疗用途并且需要抗体效应功能时,可使用人 IgG 恒定区结构域,特别是 IgG1 和 IgG3 同种型的恒定区结构域。或者,当意欲将抗体分子用于治疗目的并且不需要抗体效应子功能 (例如仅用于中和或激动抗原) 时,可使用 IgG2 和 IgG4 同种型。应理解,还可使用这些恒定区结构域的序列变体。例如可使用其中位置 241 上的丝氨酸已被改变成脯氨酸的 IgG4 分子 (如 Angal 等人,Molecular Immunology,1993,30(1),105-108 中描述的)。本领域技术人员还应理解,抗体可经历多种

翻译后修饰。这些修饰的类型和程度通常取决于用于表达抗体的宿主细胞系以及培养条件。这样的修饰可包括糖基化、甲硫氨酸氧化、哌嗪二酮形成、天冬氨酸异构化和天冬酰胺脱酰胺作用的变化。常见修饰是因羧肽酶的作用而导致的羧基末端碱性残基（例如赖氨酸或精氨酸）的丢失（如 Harris, RJ. Journal of Chromatography705:129-134, 1995 中描述的）。

[0404] 在一个实施方案中，抗体重链包含 CH1 结构域并且抗体轻链包含 CL 结构域（κ 或 λ）。

[0405] 生物分子例如抗体或片段包含酸性和 / 或碱性官能团，从而赋予分子净正电荷或负电荷。总的"观察到的"电荷的量将取决于物质的绝对氨基酸序列，3D 结构中的带电荷的局部环境和分子的环境条件。等电点 (pI) 是特定分子或其溶剂可及表面不携带净电荷时的 pH。在一个实例中，本发明的抗体和片段可被工程化来具有适当的等电点。这可导致具有更加强劲的性质，特别是适当的溶解度和 / 或稳定性特征和 / 或改善的纯化特征的抗体和 / 或片段。

[0406] 因此，在本发明的一个方面，提供了经工程化从而具有与其所源自的原始鉴定的抗体的等电点不同的等电点的人源化抗体。抗体可以例如通过替代氨基酸残基，例如用一个或多个碱性氨基酸残基替代酸性氨基酸残基来进行工程化。或者，可引入碱性氨基酸残或可除去酸性氨基酸残基。或者，如果分子具有不可接受的高 pI 值，则可视需要引入酸性残基来降低 pI。重要的是当操纵 pI 时，必须小心以保持抗体或片段的期望的活性。因此，在一个实施方案中，工程化抗体或片段具有与"未修饰"抗体或片段相同或大体上相同的活性。

[0407] 程序例如 **ExPASY http://www.expasy.ch/tools/pi_tool.html, and http://www.iut-arles.up.univ-mrs.fr/w3bb/d_abim/compo-p.html，可用于预测抗体或片段的等电点。

[0408] 应理解，可使用本领域已知的任何适当方法来改变本发明提供的抗体的亲和力。抗体或其变体的亲和力可使用本领域已知的任何适当方法，包括 BIAcore，使用适当的分离的天然的或重组蛋白或适当的融合蛋白 / 多肽来测量。

[0409] 本发明从而还涉及视情况而定具有改善的对于 TcdA 或 TcdB 的亲和力的本发明的抗体分子的变体。这样的变体可通过许多亲和力成熟方案来获得，包括突变 CDR(Yang 等人，J.Mol.Biol., 254, 392-403, 1995)、链改组 (Marks 等人，Bio/Technology, 10, 779-783, 1992)、大肠杆菌的增变株的使用 (Low 等人，J.Mol.Biol., 250, 359-368, 1996)、DNA 改组 (Patten 等人，Curr. Opin.Biotechnol., 8, 724-733, 1997)、噬菌体展示 (Thompson 等人，J.Mol. Biol., 256, 77-88, 1996) 和有性 PCR(Crameri 等人，Nature, 391, 288-291, 1998)。Vaughan 等人（同上）论述了这些亲和力成熟的方法。

[0410] 本文中使用的改善的亲和力在本说明书中是指高于起始分子的改善。

[0411] 必要时，可将本发明中使用的抗体缀合至一个或多个效应分子。应理解，效应分子可包括单个效应分子，或连接以形成可连接至本发明的抗体的单个部分的两个或更多个这样的分子。当期望获得连接至效应分子的抗体片段时，这可通过标准化学或重组 DNA 法来制备，其中将抗体片段直接地或通过偶联剂连接至效应分子。用于将这样的效应分子缀合

至抗体的技术在本领域是公知的（参见，Hellstrom 等人，Controlled Drug Delivery，第 2 版，Robinson 等人，eds.，1987，pp. 623-53；Thorpe 等人，1982，Immunol. Rev.，62:119-58 和 Dubowchik 等人，1999，Pharmacology and Therapeutics，83，67-123）。特定的化学方法包括例如 WO93/06231、WO92/22583、WO89/00195、WO89/01476 和 WO03031581 中描述的方法。或者，当效应分子是蛋白质或多肽时，可使用重组 DNA 法，例如 WO86/01533 和 EP0392745 中描述的方法来实现连接。

[0412] 如本文中所用，术语效应分子包括例如生物活性蛋白，例如酶、其它抗体或抗体片段、合成或天然存在的聚合物、核酸及其片段例如 DNA、RNA 及其片段、放射性核素，特别是放射性碘、放射性同位素、螯合的金属、纳米颗粒和受体基团例如荧光化合物或可通过 NMR 或 ESR 光谱学检测的化合物。

[0413] 其它效应分子可包括螯合的放射性核素例如 111In 和 90Y、Lu177、铋 213、锕 252、铱 192 和钨 188/ 铼 188；或药物例如但不限于烷基磷酸胆碱、DNA 拓扑异构酶 I 抑制剂、紫杉烷和舒拉明。

[0414] 其它效应分子包括蛋白质、肽和酶。目标酶包括但不限于蛋白水解酶、水解酶、裂解酶、异构酶、转移酶。目标蛋白质、多肽和肽包括但不限于免疫球蛋白、毒素例如相思豆毒蛋白、蓖麻蛋白 A、假单胞菌外毒素或白喉毒素、蛋白质例如胰岛素、肿瘤坏死因子、α－干扰素、β－干扰素、神经生长因子、血小板衍生的生长因子或组织型纤溶酶原激活物、血栓形成剂或抗－血管生成剂例如血管他丁或内皮他丁，或生物应答调节剂例如淋巴因子、白细胞介素 -1（IL-1）、白细胞介素 -2（IL-2）、粒细胞巨噬细胞集落刺激因子（GM-CSF）、粒细胞集落刺激因子（G-CSF）、神经生长因子（NGF）或其它生长因子和免疫球蛋白。

[0415] 其它效应分子可包括用于例如诊断的可检测物质。可检测物质的实例包括各种酶、辅基、荧光材料、发光材料、生物发光材料、放射性核素、正电子发射金属（用于正电子发射断层摄影术）和非放射性顺磁性金属离子。关于可被缀合至抗体以用作诊断剂的金属离子，通常参见美国专利号 4,741,900。适当的酶包括辣根过氧化物酶、碱性磷酸酶、β半乳糖苷酶或乙酰胆碱酯酶；适当的辅基包括链霉抗生物素蛋白、抗生物素蛋白和生物素；适当的荧光材料包括伞形酮、荧光素、异硫氰酸荧光素、罗丹明、二氯三嗪胺荧光素、丹磺酰氯和藻红蛋白；适当的发光材料包括鲁米诺；适当的生物发光材料包括萤光素酶、萤光素和水母发光蛋白；以及适当的放射性核素包括 125I、131I、111In 和 99Tc。

[0416] 在另一个实例中，效应分子可增加抗体的体内半衰期和／或减小抗体的免疫原性和／或增强抗体穿过上皮屏障至免疫系统的递送。该类型的适当的效应分子的实例包括聚合物、白蛋白、白蛋白结合蛋白或白蛋白结合化合物例如 WO05/117984 中描述的那些化合物。

[0417] 当效应分子为聚合物时，其通常可以是合成的或天然存在的聚合物，例如任选地取代的直链或支链聚亚烷基、聚亚链烯基或聚氧化烯基聚合物或分支或未分枝多糖例如同多糖或异多糖。

[0418] 可存在于上述合成聚合物上的具体的任选的取代基包括一个或多个羟基、甲基或甲氧基。

[0419] 合成聚合物的具体实例包括任选地取代的直链或支链聚（乙二醇）、聚（丙二醇）聚（乙烯醇）或其衍生物，特别是任选地取代的聚（乙二醇）例如甲氧基聚（乙二醇）或

其衍生物。

[0420] 具体的天然存在的聚合物包括乳糖、直链淀粉、葡聚糖、糖原或其衍生物。

[0421] 如本文中所用，“衍生物”意欲包括反应性衍生物，例如巯基选择性反应性基团例如马来酰亚胺等。可将反应性基团直接或通过接头区段连接至聚合物。应理解，这样的基团的残基在一些情况下形成作为抗体片段与聚合物之间的连接基团的产物的部分。

[0422] 聚合物的大小可按需变化，但通常在 500Da 至 50000Da，例如 5000 至 40000Da，例如 20000 至 40000Da 的平均分子量范围内。可以特别地基于产物的期望用途例如定位至某些组织例如肿瘤或延长循环半衰期的能力选择聚合物大小（关于综述，参见 Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545）。因此，例如，当期望产物离开循环并渗入组织，例如用于治疗肿瘤时，可有利地使用小分子量例如具有约 5000Da 的分子量的聚合物。关于其中产物保留在循环中的应用，可有利地使用更高分子量例如具有在 20000Da 至 40000Da 的范围内的分子量的聚合物。

[0423] 适当的聚合物包括聚烷基聚合物，例如聚（乙二醇）或，特别是甲氧基聚（乙二醇）或其衍生物，特别是具有在约 15000Da 至约 40000Da 的范围内的分子量。

[0424] 在一个实例中，可将用于本发明的抗体连接至聚（乙二醇）(PEG) 部分。在一个具体实例中，抗体是抗体片段，并且可通过位于抗体片段中的任何可获得的氨基酸侧链或末端氨基酸官能团，例如任何游离氨基、亚氨基、巯基、羟基或羧基连接 PEG 分子。这样的氨基酸可天然地存在于抗体片段中，或可使用重组 DNA 法将其工程化入片段（参见例如，US5, 219, 996; US5, 667, 425; WO98/25971, WO2008/038024）。在一个实例中，本发明的抗体分子是修饰的 Fab 片段，其中将修饰添加至其重链的 C 末端上的一个或多个氨基酸以允许效应分子的连接。适当地，另外的氨基酸形成包含一个或多个可将效应分子与其连接的半胱氨酸残基的修饰的铰链区。多个位点可用于连接两个或更多个 PEG 分子。

[0425] 适当地可通过位于抗体片段中的至少一个半胱氨酸残基的硫基共价地连接 PEG 分子。可将连接至修饰的抗体片段的每一个聚合物分子共价地连接至位于片段中的半胱氨酸残基的硫原子。共价连接通常是二硫键，或具体地，硫 - 碳键。当巯基用作适当地活化的效应分子的连接点时，可使用例如巯基选择性衍生物例如马来酰亚胺和半胱氨酸衍生物。活化的聚合物可在上述聚合物修饰的抗体片段的制备中用作起始材料。活化的聚合物可以是任意聚合物，所述聚合物包含巯基反应性基团例如 α - 卤代羧酸或酯，例如碘乙酰胺、酰亚胺例如马来酰亚胺、乙烯基砜或二硫化物。这样的起始材料可商购获得（例如来自 Nektar, 以前称为 Shearwater Polymers Inc., Huntsville, AL, USA）或可使用常规化学方法从商购可得的起始材料制备而来。具体的 PEG 分子包括 20K 甲氧基 -PEG- 胺（可从 Nektar, formerly Shearwater; Rapp Polymere; 和 SunBio 获得）和 M-PEG-SPA（可从 Nektar, 以前称为 Shearwater 获得）。

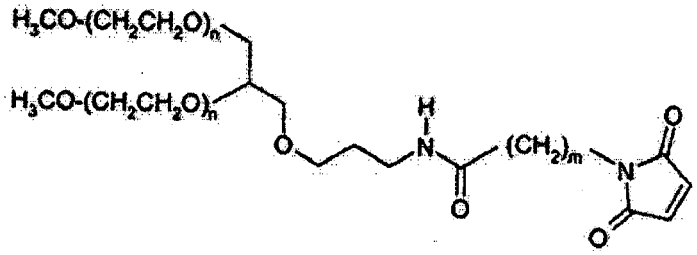
[0426] 在一个实施方案中，抗体是被 PEG 化的，即具有例如按照 EP0948544 或 EP1090037 中公开的方法共价地连接至其的 PEG（聚（乙二醇））的修饰的 Fab 片段或 diFab[也参见 "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris 和 S. Zalipsky (eds), American Chemical

Society, Washington DC and"Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam 和 A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews2002, 54:531-545]。在一个实例中，PEG 被连接至铰链区中的半胱氨酸。在一个实例中，PEG 修饰的 Fab 片段具有共价地连接至修饰的铰链区中的单个巯基的马来酰亚胺基。可将赖氨酸残基共价地连接至马来酰亚胺基和将赖氨酸残基上的每一个胺基连接至具有约 20,000Da 的分子量的甲氧基聚（乙二醇）聚合物。连接至 Fab 片段的 PEG 的总分子量从而可以为约 40,000Da。

[0427] 具体的 PEG 分子包括 N,N'-双（甲氧基丙基（乙二醇）MW20,000）的 2-[3-(N-马来酰亚胺基）丙酰胺基]乙基酰胺修饰的赖氨酸，也称为 PEG2MAL40K（可获自 Nektar，先前称为 Shearwater）。

[0428] PEG 接头的可选择来源包括 NOF，其提供 GL2-400MA2（其中结构中的 m 小于 5）和 GL2-400MA（其中 m 是 2）并且 n 为约 450；

[0429]

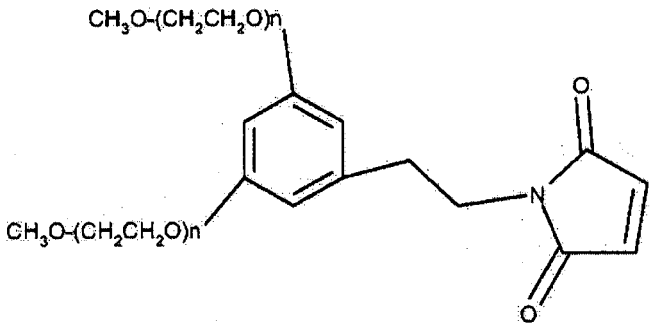


m是2或5

[0430] 即每一个 PEG 为约 20,000Da。

[0431] 下列类型的其它可选择的 PEG 效应分子：

[0432]



[0433] 可获自 Dr Reddy, NOF 和 Jenkem。

[0434] 在一个实施方案中，提供了通过链中的氨基酸 226（例如重链的氨基酸 226（按照序列编号））上或其附近的半胱氨酸残基连接的被 PEG 化（例如利用本文中描述的 PEG）的抗体。

[0435] 在一个实施方案中，根据本发明的一个特定抗体具有下列性质：

[0436]

抗体	亲和力(pM)		结合的效价	EC_{50}(ng/ml)
	$TcdA_{123}$	$TcdA_{456}$	TcdA, est.	
CA922	4.06	2.59	16	1.21
CA923	64.7	312	12	160.42
CA995	0	119	1	37.64
CA997	132	66.8	12	6.25
CA1000	73.3	84.1	2	19.73

[0437] 本发明还提供了组合物，例如本文中定义的抗体或抗体的组合的药物组合物。

[0438] 本发明还提供了包含至少两种根据本发明的抗体的组合物，例如其中其中的至少一种抗体特异于 TcdA 并且其中的至少一种抗体特异于 TcdB，或可选择地至少两种抗体特异于 TcdA 或至少两种抗体特异于 TcdB。

[0439] 在一个实施方案中，提供了包含多种特异于 TcdA 的抗体和任选地一种或多种特异于 TcdB 的抗体的组合物。

[0440] 在一个实施方案中，提供了包含多种特异于 TcdB 的抗体和任选地一种或多种特异于 TcdA 的抗体的组合物。

[0441] 因此，在一个实施方案中，提供了包含 2、3、4、5、6、7、8、9、10、11、12、13、14 或 15 种根据本发明的抗体，即不同的抗体的组合物。

[0442] 本发明描述了一种包含 3 种 Mab 的具体混合物，所述 Mab 的一种特异于 TcdA 并且所述 Mab 的两种特异于 TcdB。该混合物显示极高水平的使仓鼠免受致死感染性口服剂量的艰难梭菌引起的死亡和肠炎症的保护作用。

[0443] 具体地，提供了包含一种抗 -TcdA 抗体（包含具有 SEQ ID NO:49 中显示的序列的重链可变区和具有 SEQ ID NO:47 中显示的轻链可变区）和两种抗 -TcdB（第一种具有 SEQ ID NO:129 显示的重链可变区和 SEQ ID NO:127 中显示的轻链可变区，第二种具有 SEQ ID NO:159 中显示的重链可变区和 SEQ ID NO:157 中显示的轻链可变区）的组合的组合物。

[0444] 在一个实施方案中，其中组合物包含 3 种抗体，例如一种抗 -TcdA 和两种抗 -TcdB 抗体，所述抗体以分别为其总抗体含量的 50%、25% 和 25% 的比率存在。

[0445] 在一个实施方案中，提供了包含特异于 2、3、4 或 5 个特异于 TcdA 的抗体和任选地 1、2、3、4 或 5 种特异于 TcdB 的抗体的组合物。

[0446] 在一个实施方案中，根据本发明提供的组合物已被良好地定义，例如为单克隆抗体的混合物而不只是来源于免疫的的宿主或具有免疫活性的宿主的多克隆组合物。

[0447] 在一个实施方案中，抗体的组合物具有 200ng/ml 或更少，例如 150ng/ml 或更少，例如 100ng/ml 或更少，例如 0.1 至 10ng/ml 的 EC_{50}。

[0448] 有利地，本文中描述的抗体具有极高水平的生物物理稳定性，从而适合包含在抗

体的混合物中。

[0449] 在一个方面，根据本发明的药物制剂或组合物还包含药学上可接受的赋形剂。

[0450] 治疗性组合物中的药学上可接受的载体可额外地包含液体例如水、盐水、甘油和乙醇。此外，辅助物质例如湿润剂或乳化剂或 pH 缓冲物质可存在于这样的组合物中。这样的载体使得药物组合物能够被配制为片剂、丸剂、锭剂、胶囊剂、液体、凝胶、糖浆剂、膏剂和栓剂，以被患者摄入。

[0451] 用于施用的适当形式包括适合用于例如通过注射或输注（例如通过单次快速静脉注射或连续输注）胃肠外施用的形式。当产品用于注射或输注时，其可采用油性或含水媒介物中的悬浮液、溶液或乳剂的形式，并且其可包含配制剂，例如悬浮剂、防腐剂、稳定剂和 / 或分散剂。或者，抗体分子可以以无水形式存在，以在使用前利用适当的无菌液体重建。

[0452] 配制后，可将本发明的组合物直接施用至受试者。待治疗的受试者可以是动物。然而，在一个或多个实施方案中，组合物可被改造来适合给人受试者施用。

[0453] 适当地在根据本公开内容的制剂中，最终制剂的 pH 与抗体或片段的等电点的值不相似，例如如果制剂的 pH 为 7，则为 8-9 或更高的 pI 可以是适当的。虽然不希望受理论束缚，但认为这可最终提供具有改善的稳定性的最终制剂，例如抗体或片段保持在溶液中。

[0454] 在一个实施方案中，本公开内容的组合物或制剂包含 1-200mg/mL 的抗体，这就是说组合的抗体含量，例如 150mg/mL 或更少，例如 100mg/mL 或更少，具体地 90、80、70、60、50、40、30、20、10mg/mL 或更少。

[0455] 在一个实施方案中，本公开内容的组合物或制剂包含其中各 20mg/mL 的每一种抗体。

[0456] 在一个实施方案中，提供了制剂，所述制剂包含：

[0457] 33mg/mL 或更少的一种包含具有 SEQ ID NO:49 中所示序列的重链可变区和具有 SEQ ID NO:47 所示的序列的轻链可变区的抗 -TcdA 抗体，和

[0458] 28mg/mL 或更少的具有 SEQ ID NO:129 显示的重链可变区和 SEQ ID NO:127 显示的轻链可变区的第一抗 -TcdB 抗体，和

[0459] 25mg/mL 的具有 SEQ ID NO:159 显示的重链可变区和 SEQ ID NO:157 显示的轻链可变区的第二抗 -TcdB 抗体。

[0460] 在一个实施方案中，pH 在 4.0 至 7.0 的范围内的药物制剂包含：1 至 200mg/mL 的根据本公开内容的抗体、1 至 100mM 的缓冲剂、0.001 至 1% 的表面活性剂，

[0461] a) 10 至 500mM 的稳定剂，

[0462] b) 5 至 500mM 的张度剂，或

[0463] c) 10 至 500mM 的稳定剂和 5 至 500mM 的张度剂。

[0464] 在一个实施方案中，根据本公开内容的组合物或制剂包含缓冲剂液磷酸盐缓冲盐水。

[0465] 例如，具有约 pH6 的制剂可包含 1 至 50mg/mL 的抗体、20mM L- 盐酸组氨酸、240mM 海藻糖和 0.02% 的聚山梨醇 20。或者，具有约 pH5.5 的制剂可包含 1 至 50mg/mL 的抗体、20mM 柠檬酸盐缓冲液、240mM 蔗糖、20mM 精氨酸和 0.02% 的聚山梨醇 20。

[0466] 本发明的药物组合物可通过许多途径来施用，包括但不限于口服、静脉内、肌内、

动脉内、髓内、硬膜内、心室内、经真皮、经皮（例如，参见WO98/20734）、皮下、腹膜内、鼻内、肠内、局部、舌下、阴道内或直肠途径。皮下注射器也可用于施用本发明的药物组合物。通常地，可将治疗性组合物配制为可注射液体溶液或悬浮液。还可制备在注射之前适合用于在液体媒介物中配制成溶液或悬浮液的固体形式。

[0467] 组合物的直接递送通常可通过皮下、腹膜内、静脉内或肌内注射来实现，或被递送至组织的细胞间隙。

[0468] 还可通过例如用于吞咽的封装的口服剂量，通过鼻胃管至胃或回肠，通过直肠管或灌肠溶液或通过直肠胶囊将组合物施用至伤口或直接施用至胃肠道中。剂量处理可以是单剂量方案或多剂量方案。

[0469] 应理解，组合物中的活性成分可为抗体分子。这样，其易于在胃肠道中降解。因此，如果将通过使用胃肠道的途径施用组合物，则组合物需要包含试剂，所述试剂保护抗体免受降解，但一旦其已从胃肠道吸收就释放抗体。

[0470] 药学上可接受的载体的详尽论述可在《雷氏药学大全》(MackPublishing Company, N. J. 1991) 中获得。

[0471] 本发明还提供了如本文中描述的抗体或抗体组合或包含所述抗体或抗体组合的组合物，其用于治疗，例如用于治疗或预防艰难梭菌感染或与所述感染相关的并发症例如腹泻、结肠炎特别是假膜性结肠炎、胃气胀、腹痛和中毒性巨结肠。

[0472] 预防还可通过施用预形成的灭活的毒素抗原（类毒素）与抗体的复合物（以产生疫苗）来实现。

[0473] 在一个实施方案中，根据本发明的抗体、其组合和包含所述抗体的组合物适合用于治疗艰难梭菌的所谓超级菌株（即超毒力菌株例如核糖型027）的感染。

[0474] 根据本发明的抗体和组合物适合用于治疗或预防相关艰难梭菌毒素在原发感染过程中的急性和/或慢性作用。

[0475] 根据本发明的抗体和组合物适合用于治疗或预防相关艰难梭菌毒素在继发感染或再感染过程中的作用。国际指南(International guidelines)确立原发感染后的时间间隔，这从而将继发（或回归）感染定义为与现有症状的持续不同（有时描述为复发）(29)。研究已显示继发感染可以是与原发感染相同的菌株或核糖型的结果。在这样的情况下，回归感染而非复发依赖于达成的时间约束。然而，研究还显示继发感染还可以是与原发感染的菌株或核糖型不同的菌株或核糖型的感染的结果。在一个研究中，48%的疾病回归是与已引起第一感染的菌株不同的第二菌株的结果 (30)。在另一个研究中，超过56%的疾病回归是与引起第一感染的菌株不同的第二菌株的结果 (31)。

[0476] 在一个实施方案中，根据本发明的抗体、其组合和包含所述抗体的组合物适合用于防止损伤，例如对结肠上皮的长期结构损伤。

[0477] 在一个实施方案中，抗体、组合和组合物适合用于防止艰难梭菌感染，包括感染特别是医院内感染的回归。

[0478] 在一个实施方案中，根据本发明的抗体、其组合和包含所述抗体的组合物适合用于减小艰难梭菌感染回归的风险。

[0479] 有利地，可预防性施用本公开内容的抗体以防止感染或再感染，因为在抗体所特异于毒素不存在的情况下，抗体只是被从身体清除而不引起不利的与受试者身体组织的相

互作用。

[0480] 有利地，本公开内容的抗体似乎在施用后引发快速反应，例如在 1 或 2 天的施用内，激起目标毒素的快速清除，这可阻止重要器官例如肺、心脏和肾被损伤。这是第一次可获得可用于在急性艰难梭菌感染期防止毒素 A 和 / 或 B 对患者的损害或损伤的试剂。

[0481] 因此，在一个实施方案中，根据本发明的抗体、其组合和包含所述抗体的组合物适合用于防止对重要器官的损害。

[0482] 在一个实施方案中，本文中描述的抗体、组合或制剂适合用于防止被感染的患者死亡，如果在毒素已产生不可挽回的损伤之前在适当的时间施用的话。

[0483] 本公开内容的抗体具有快速的结合速率，其促进体内快速作用。

[0484] 在一个实施方案中，患者群体超过 60 岁，例如超过 65 岁。

[0485] 在一个实施方案中，患者群体为 5 岁或更小。

[0486] 可将根据本发明的抗体与抗生素治疗例如甲硝唑、万古霉素或非达米星组合使用。

[0487] 一系列体外数据证明了 Mab 和 Mab 混合物的性质。我们显示 3 种 Mab 的一种混合物（50% 摩尔当量的抗 -TcdA 和 50% 摩尔当量的抗 -TcdB 组分）能够保护仓鼠免受致死性 CDI。

[0488] 在一个实施方案中，提供了通过例如在艰难梭菌感染或与所述感染相关的并发症例如腹泻、结肠炎特别是假膜性结肠炎、胃气胀、腹痛和中毒性巨结肠的治疗或预防中，施用治疗有效量的本文中描述的抗体或抗体组合或包含所述抗体的组合物来治疗有此需要的患者的方法。

[0489] 在一个实施方案中，通过常规途径例如皮下、腹膜内、静脉内或肌内施用抗体、组合或制剂。仓鼠中产生的实施例的数据表明通过该途径施用的剂量到达肠，从而能够产生治疗作用。

[0490] 在一个实施方案中，通过口服（例如肠溶衣制剂）施用抗体、组合或制剂。

[0491] 在一个实施方案中，提供了本文中描述的抗体、组合或制剂用于制造用于治疗或预防艰难梭菌感染的药物的用途。

[0492] 在一个实施方案中，施用的剂量在 1 至 1000mg/Kg，例如 10 至 75mg/Kg 例如 20 至 50mg/Kg 的范围内。

[0493] 在一个实施方案中，抗体在小鼠和仓鼠中的体内半衰期在健康（未感染的）动物中在 6 至 8 天的范围内，从而预期具有在 14-28 天的范围内的人中的半衰期。

[0494] 在一个实施方案中，仅以一个剂量提供抗体。

[0495] 在一个实施方案中，以每周一个剂量或每两周一个剂量提供抗体。

[0496] 在一个实施方案中，以每天一次的剂量提供抗体。

[0497] 在一个实施方案中，提供了包含与一种或多种本文中定义的抗 -TcdA 抗体复合的 TcdA 或其免疫原性片段的复合物。复合物可在疫苗制剂中用作抗原，例如适合用于在给人施用后体内产生针对毒素 A 的保护性抗体。

[0498] 在一个实施方案中，提供了包含与一种或多种本文中定义的抗 -TcdB 抗体复合的 TcdB 或其免疫原性片段的复合物。复合物可在疫苗制剂中用作抗原，例如适合用于在给人施用后体内产生针对毒素 B 的保护性抗体。

[0499] 可被配制来产生适合本发明中使用的佐剂的 Th1 型免疫刺激物包括并且不限于下列物质。

[0500] 在一个实施方案中，提供了包含 TcdA 或其免疫原性片段和 TcdB 或其免疫原性片段的复合物，其中将每一种毒素或片段与一种或多种特异于其的抗体复合，其中复合物适合用于作为疫苗制剂施用。

[0501] 已知抗体：抗原复合物在 Fc 受体介导的过程中被免疫系统吸收 (27, 28)，并且抗体：抗原复合物的预形成的复合物已在人临床试验中被成功地用作疫苗 (22)。

[0502] 在一个或多个实施方案中，疫苗制剂还包含佐剂作为免疫刺激物。

[0503] 单磷酰脂质 A，特别是 3- 脱 -O- 酰化单磷酰脂质 A(3D-MPL) 是用于本发明的优选 Th1 型免疫刺激物。3D-MPL 是由 Ribi Immunochem, Montana 生产的公知佐剂。在化学上，其通常以 3- 脱 -O- 酰化单磷酰脂质 A 与 4, 5 或 6 个酰化链的混合物提供。可通过 GB2122204B 中教导的方法纯化和制备，所述参考资料还公开了二磷酰脂质 A 及其 3-O- 脱酰基变体的制备。已描述了其它纯化和合成的脂多糖 (US6, 005, 099 和 EP0729473B1; Hilgers 等人，1986, Int. Arch. Allergy. Immunol., 79(4) :392-6; Hilgers 等人，1987, Immunology, 60(1) :141-6; 和 EP0549 074 B1)。3D-MPL 的优选形式以具有直径小于 0.2mm 的小的颗粒尺寸的颗粒制剂的形式存在，其制造方法公开于 EP0 689 454 中。

[0504] 根据本发明，皂苷也是优选的 Th1 免疫刺激物。皂苷是公知的佐剂并且教导于：Lacaille-Dubois, M 和 Wagner H. (1996. A review of the biological and pharmacological activities of saponins. Phytomedicine 第 2 卷，第 363-386 页）中。例如 Quil A（来源于南美的树石碱木 (Quillaja Saponaria Molina) 的树皮）及其级分描述于 US5, 057, 540 和“Saponins as vaccine adjuvants”, Kensil, C. R., Crit Rev Ther Drug Carrier Syst, 1996, 12(1-2) :1-55; 和 EP0 362 279B1 中。溶血性皂苷 QS21 和 QS17(Quil A 的 HPLC 纯化的级分）已被描述为有效的系统性佐剂，其生产方法描述于美国专利号 5, 057, 540 和 EP0 362 279B1。这些文献中还描述了 QS7(Quil-A 的非溶血性级分）的使用，其用作系统性疫苗的有效佐剂。QS21 的使用进一步描述于 Kensil 等人 (1991. J. Immunology 第 146 卷，431-437) 中。QS21 与聚山梨酯或环糊精的组合也是已知的 (WO99/10008)。包含 QuilA 级分、例如 QS21 和 QS7 的颗粒佐剂系统描述于 WO96/33739 和 WO96/11711 中。一个这样的系统称为 Iscorn 并且可包含一种或多种皂苷。

[0505] 另一种优选免疫刺激物是包含未甲基化的 CpG 二核苷酸（“CpG”）的免疫刺激性寡核苷酸。CpG 是存在于 DNA 中的胞苷 - 鸟苷二核苷酸基序的缩写。当通过系统性和粘膜途径施用时，CpG 在本领域已知为佐剂 (WO96/02555, EP468520, Davis 等人，J. Immunol, 1998, 160(2) :870-876; McCluskie 和 Davis, J. Immunol., 1998, 161(9) :4463-6)。历史上，观察到 BCG 的 DNA 级分可产生抗肿瘤作用。在其它研究中，已显示来源于 BCG 基因序列的合成寡核苷酸能够诱导免疫刺激性作用（在体外和体内）。这些研究的研究人员得出某些回文序列 (包括中央 CG 基序) 具有该活性。CG 基序在免疫刺激中的作用后来在 Krieg 的出版物，Nature374, p5461995 中进行了阐明。详细分析已显示 CG 基序必须存在于某些序列背景中，并且这样的序列在细菌 DNA 中是常见的但在脊椎动物 DNA 中是稀有的。免疫刺激性序列通常是：嘌呤、嘌呤、C、G、嘧啶、嘧啶；其中 CG 基序未被甲基化，但已知其它未甲基化的 CpG 序列是免疫刺激性的并且可用于本发明。

[0506] 在6个核苷酸的某些组合中，存在回文序列。这些基序中的几个，作为一个基序的重复或不同基序的组合，可存在于相同的寡核苷酸中。这些含免疫刺激性序列的寡核苷酸的一个或多个的存在可活化各种免疫亚组，包括天然杀伤细胞（其产生干扰素g并且具有溶细胞活性）和巨噬细胞(Wooldrige等人第89卷(no.8),1977)。其它不具有该共有序列的含未甲基化CpG的序列现也显示是免疫刺激性的。

[0507] 当配制成疫苗时，通常将CpG与游离抗原一起在自由溶液中施用(WO96/02555;McCluskie和Davis，同上）或共价地缀合至抗原(WO98/16247)，或利用载体例如氢氧化铝来配制((肝炎表面抗原)，Davis等人（同上）;Brazolot-Millan等人，Proc. Natl. Acad. Sci.，USA,1998,95(26),15553-8)。

[0508] 可将上述这样的免疫刺激物与载体例如脂质体、水包油乳剂和/或金属性盐包括铝盐（例如氢氧化铝）一起配制。例如，3D-MPL可用氢氧化铝(EP0 689 454)或水包油乳剂(WO95/17210)来配制；QS21可以有利地利用含胆固醇脂质体(WO96/33739)、水包油乳剂(WO95/17210)或明矾(WO98/15287)来配制；CpG可以利用明矾(Davis等人（同上）;Brazolot-Millan（同上））或利用其它阳离子载体来配制。

[0509] 免疫刺激物的组合也是优选的，特别是单磷酰脂质A与皂苷衍生物的组合(WO94/00153;WO95/17210;WO96/33739;WO98/56414;WO99/12565;WO99/11241)，更特别是WO94/00153中公开的QS21与3D-MPL的组合。或者，CpG+皂苷例如QS21的组合也形成用于本发明的有效佐剂。或者，可将皂苷配制在脂质体中或Iscorn中，以及与免疫刺激性寡核苷酸组合。

[0510] 因此，适当的佐剂系统包括例如单磷酰脂质A，优选3D-MPL与铝盐的组合。

[0511] 因此在一个实施方案中，佐剂是QS21与3D-MPL在水包油或脂质体制剂中的组合。

[0512] 增强的系统包括单磷酰脂质A与皂苷衍生物的组合，特别是QS21和3D-MPL的组合（如WO94/00153中公开的），或其中QS21在含胆固醇脂质体(DQ)中被淬灭的不太具有反应原性的组合物，如WO96/33739中公开的。该组合可额外地包含免疫刺激性寡核苷酸。

[0513] 在水包油乳剂中包含QS21、3D-MPL和生育酚的特别有效的佐剂制剂描述于WO95/17210中，并且是用于本发明的另一种优选制剂。

[0514] 另一种优选制剂单独地或与铝盐一起地包含CpG寡核苷酸。

[0515] 在本发明的其它方面，提供了制造本文中描述的疫苗制剂的方法，其中所述方法包括将根据本发明的多肽与适当的佐剂混合。

[0516] 用于根据本发明的制剂的特别适合的佐剂组合如下：

[0517] i)3D-MPL+QS21，在脂质体中

[0518] ii)铝+3D-MPL

[0519] iii)铝+脂质体中的QS21+3D-MPL

[0520] iv)铝+CpG

[0521] v)3D-MPL+QS21+水包油乳剂

[0522] vi)CpG

[0523] 如本文中所用，本说明书的上下文中的术语“包含”应当被解释为“包括”。

[0524] 当技术上恰当时，可组合实施方案与参数选择。

[0525] 本文中的公开内容描述了包含某些整体的实施方案。本公开内容还扩展至由所述

整体组成或基本上由所述整体组成的相同实施方案。

[0526] 图

[0527] 图 1-10 显示各种抗体和片段序列。

[0528] 图 11 显示 TcdA 和 TcdB 的血清滴度。

[0529] 图 12 显示单个 Mab 的抗 TcdA(核糖型 003)体外中和数据。

[0530] 图 13 显示单一和成对 Mab 的抗 TcdA(核糖型 003)体外中和数据。

[0531] 图 14-15 显示成对的 Mab 的抗 TcdA(核糖型 003)体外中和数据。

[0532] 图 16-18 显示 3 种 Mab 的混合物的抗 TcdA(核糖型 003)体外中和数据。

[0533] 图 19-20 显示 4 和 5 种 Mab 的混合物的抗 TcdA(核糖型 003)体外中和数据。

[0534] 图 21-22 显示在不同 TcdA 浓度单一和成对 Mab 的抗 TcdA(核糖型 003)体外中和数据。

[0535] 图 23-24 显示在不同 TcdA 浓度单一 Mab 和达到 5 种 Mab 的混合物的抗 TcdA(核糖型 003)体外中和数据。

[0536] 图 25-26 显示单一 Mab 的抗 TcdB(核糖型 003)体外中和数据。

[0537] 图 27-30 显示成对 Mab 的抗 TcdB(核糖型 003)体外中和数据。

[0538] 图 31-33 显示 3 种 Mab 的混合物的抗 TcdB(核糖型 003)体外中和数据。

[0539] 图 34-40 显示在不同毒素浓度两种 Mab 的混合物的抗 TcdB(核糖型 003)体外中和数据。

[0540] 图 41-45 显示在不同毒素浓度不同相对 Mab 比率的两种 Mab 混合物的抗 TcdB(核糖型 003)体外中和数据。

[0541] 图 46-59 显示单一抗体和成对抗体的 TcdB 中和数据。

[0542] 图 60 显示 TcdA 的氨基酸序列。

[0543] 图 61 显示 TcdB 的氨基酸序列。

[0544] 图 62 显示以直方图形式表示的 TcdA 的 TEER 测定数据。

[0545] 图 62A 显示以线图形式表示的 TcdA 的 TEER 测定数据。

[0546] 图 63 显示抗体 997、1125 和 1151 的组合的 meier-kaplan 曲线，高浓度为 50mg/Kg，低浓度为 5mg/Kg。

[0547] 50mg/kg 的剂量至第 11 天产生 100% 的保护作用，至第 28 天产生～ 82% 的保护作用。

[0548] 5mg/kg 的剂量导致非持久的且不完全保护作用。

[0549] 图 64 显示万古霉素和媒介物处理的仓鼠的体重变化。

[0550] 图 65 显示低剂量抗体 5mg/Kg 和高剂量抗体 50mg/Kg 的体重。

[0551] 图 66 显示其中接受利用根据本公开内容的抗体的处理的动物相对于对照的结肠的照片。

[0552] 图 67-68 显示涡旋对抗体稳定性的作用。

[0553] 图 69 显示不同抗体的聚集稳定性的比较。

[0554] 图 70-73 显示不同核糖型的 TcdA 的中和。

实施例

[0555] 抗体的产生

[0556] 一系列不同的免疫原和筛选试剂购自或产生自常规大肠杆菌表达技术，以提供多种多样的广泛的免疫反应和帮助单克隆抗体（表 1 中列出的）的鉴定和表征。在其中产生重组蛋白或肽的情况下，序列基于核糖型 027。来自核糖型 027 的 TcdA 的序列示于 SEQ ID NO:171(Uniprot 登录号 C9YJ37) 中，给出的核糖型 027 的 TcdB 的序列为 SEQ ID NO:172(Uniprot 登录 C9YJ35)。

[0557] 利用定位至对于 TcdA 和 TdcdB 全长毒素是共同的区域的合成肽、甲醛灭活的类毒素 A、毒素 A 的结合结构域片段 (CROPs1、2、3 或 CROPs4、5、6) 或毒素 B 的结合结构域 (CROPs1、2、3、4) 或在一些情况下上述毒素的组合来免疫斯普拉 - 道来大鼠和半垂耳兔。在 2 至 6 次免疫后，杀死动物，收集 PBMC、脾和骨髓。通过 ELISA 监控血清对毒素 A 结构域、毒素 B 结构域、毒素或类毒素的结合。2 个这样的免疫的血清滴度示于图 11 中。

[0558] UCB SLAM 用作产生单克隆抗体的工具。直接从免疫的动物培养 B 细胞 (Zubler 等人，1985)。该步骤使得能够采集大百分比的 B 细胞库。通过整合选择的淋巴细胞抗体法 (SLAM) (Babcook 等人，1996)，可能使阳性培养孔讯号去卷积 (deconvolute) 并且鉴定抗原特异性抗体分泌性细胞。此处我们使用 SLAM(UCB SLAM(Tickle 等人 2009)) 的改进形式，该形式利用基于荧光的方法来从培养孔鉴定抗原特异性 B 细胞。建立 B 细胞培养物，首先在基于珠粒的测定中，使用 Applied Biosystem8200 细胞检测系统筛选上清液的结合相关纯化的毒素结构域的能力（结合、转运或催化）。这是使用包含 IgG 的 B 细胞培养上清液、涂覆至链霉抗生物素珠粒上的生物素化毒素结构域和山羊抗大鼠 / 兔 Fc-Cy5 缀合物进行的均相测定。来自该测定的对于对 TcdA 或 TcdB 组分的结合是阳性的细胞培养物被选择用于基于细胞的功能测定来鉴定毒素诱导的细胞毒性的中和剂。在初步结合筛选中从总共 40x50- 板实验鉴定了约 12,000 个毒素特特异性阳性物。这等同于大约 5 亿个 B 细胞的筛选。在逆转录 (RT)-PCR 后，从通过显微操作从约 100 个毒素中和孔收获的单细胞分离重链和轻链可变区基因对。随后将这些 V 区基因克隆为用于大鼠可变区的小鼠 IgG1/κ 全长抗体和用于兔可变区的兔 IgG/κ 全长抗体。在 HEK-293 瞬时表达系统中再表达抗体。随后再测试这些重组抗体的在基于细胞的测定中中和毒素的能力。重组抗体也通过 BIAcore 筛选来测定对于给定的毒素结构域的亲和力，以及还测定抗体对毒素的特异性和结合事件的大致数目。基于细胞的测定中的体外活性，选择先导候选物 (lead candidate) 来进行人源化。除非另外声明，否则本文中的所有数据都使用人源化抗体来产生。

[0559] 产生了一组重组大肠杆菌产生的毒素片段 (TcdA)、艰难梭菌来源的毒素或类毒素 (A) 和合成肽 (B)，或购自商业来源。

[0560] 表 1. 用于筛选和免疫的毒素 A(TcdA) 序列相关试剂。

[0561]

片段	残基编号	来源
TcdA 催化	M1-E659	UCB 大肠杆菌表达
TcdA 迁移	K577-D1350	UCB 大肠杆菌表达

TcdA $CROPS_{123}$(TcdA123)	S1827-D2249	UCB 大肠杆菌表达
TcdA $CROPS_{456}$(TcdA456)	G2205-R2608	UCB 大肠杆菌表达
TcdA $CROP_1$	S1827-N1978	UCB 大肠杆菌表达
TcdA $CROP_2$	G1966-N2133	UCB 大肠杆菌表达
TcdA $CROP_3$	G2100-D2249	UCB 大肠杆菌表达
TcdA $CROP_4$	G2213-N2381	UCB 大肠杆菌表达
TcdA $CROP_5$	G2328-N2494	UCB 大肠杆菌表达
TcdA $CROP_6$	G2462-N2609	UCB 大肠杆菌表达
TcdA $CROP_7$	R2554-D2701	UCB 大肠杆菌表达
TcdB 催化	M1-A593	UCB 大肠杆菌表达
TcdB 迁移	R576-D1349	UCB 大肠杆菌表达
TcdB 结合 (TcdB1234)	S1833-E2366	UCB 大肠杆菌表达
TcdB $CROP_1$	S1833-S1981	UCB 大肠杆菌表达
TcdB $CROP_2$	G1968-D2113	UCB 大肠杆菌表达
TcdB $CROP_3$	G2100-E2247	UCB 大肠杆菌表达
TcdB $CROP_4$	G2234-E2366	UCB 大肠杆菌表达
毒素 A	全长	购买的
毒素 B	全长	购买的
类毒素 A	全长	购买的

[0562]

[0563] 表 2. 用于筛选和免疫的毒素 B(TcdB) 序列相关试剂。

[0564]

毒素结构域	氨基酸序列	
催化	SPVEKNLHFVWIGGEVSD	SEQ ID NO: 173
催化	NLAAASDIVRL	SEQ ID NO: 174
催化	CGGVYLDVDMLPGIH	SEQ ID NO: 175
催化	CGGVYLDVDMLPGIHSDLFK	SEQ ID NO: 176
催化	CWEMIKLEAIMKYK	SEQ ID NO: 177
催化	CTNLVIEQVKNR	SEQ ID NO: 178
催化	PEARSTISLSGP	SEQ ID NO: 179
催化	CSNLIVKQIENR	SEQ ID NO: 180
催化	TEQEINSLWSFDQA	SEQ ID NO: 181
催化	TEQEINSLWSFDPEARSTISLSGPC	SEQ ID NO: 182
转运	NVEETYPGKLLLC	SEQ ID NO: 183
转运	Acetyl-CANQYEVRINSEGR	SEQ ID NO: 184
转运	VNTLNAAFFIQSLIC	SEQ ID NO: 185
转运	YAQLFSTGLNTIC	SEQ ID NO: 186
转运	CAGISAGIPSLVNNEL	SEQ ID NO: 187
转运	DDLVISEIDFNNNSIC	SEQ ID NO: 188
转运	MEGGSGHTVT	SEQ ID NO: 189
转运	AVNDTINVLPTITEGIPIVSTILDGI	SEQ ID NO: 190
	NLGAAIKEL	
结合	CGFEYFAPANTDANNIEGQA	SEQ ID NO: 191
结合	CGYKYFAPANTVNDNIYGQA	SEQ ID NO: 192
结合	CKYYFNTNTAEA	SEQ ID NO: 193
结合	CKYYFDEDTAEA	SEQ ID NO: 194

[0565]

[0566] 艰难梭菌抗-毒素 Mab 的表达和纯化

[0567] 将单独的轻链和重链哺乳动物表达质粒以等摩尔组合，用于转染 HEK-293 或 CHO-S 细胞。对于小规模表达研究，使用 lipofectamine 和 HEK-293 细胞，而对于更大批的 IgG 的产生，至 CHO-S 内的电穿孔是优选的。

[0568] 将培养上清液加载至 MabSelect SuRe 柱（于 PBS pH7.4 中）上。利用 100%0.1M 的柠檬酸钠 pH3.4 缓冲液洗脱抗体。利用 Tris.ClpH8.0 将样品中和至 pH7.4。通过 PBS pH7.4 中的 Superdex200 凝胶过滤柱除去聚集体。

[0569] 表 3

[0570]

抗体	细胞类型	SN(L)的体积	表达类型	纯化的量(mg)
CA164_00997.g1_P3	CHO	10	瞬时	755.93
CA164_00922.g1_P3	CHO	0.5	瞬时	129.36
CA164_01125.g2_P3	CHO	10	瞬时	498.96
CA164_01151.g4_P3	CHO	5	瞬时	262.43

[0571] 实施例 1. 纯化的 Mab 对 TcdA 活性的体外中和

[0572] 在 96 孔聚苯乙烯板中运行所有中和筛选测定。测定使用在 MEM+20%FCS, 2mM Q 和 NEAA 中生长和筛选的 CACO-2 细胞。除非另外声明，否则任何抗体组合以等摩尔比率存在。第 1 天：将细胞以每孔 3000 个于 50μl 培养基中涂板，温育 24hr；第 2 天：将人源化 Mab 的纯化样品添加至 96 孔圆底聚苯乙烯无菌板；将毒素 A 以足以产生适当的致死剂量的浓度（即 LD_{80} 或以上）掺入 PP 板，在 37℃温育 1hr；将 50μl 的该混合物添加至细胞板，温育 96hr；在第 5 天：添加亚甲蓝（0.5% 亚甲蓝，50% 乙醇）；在室温温育 1hr；利用 1%N- 十二烷基肌氨酸裂解细胞，在 BIOTEK Synergy2 板读数器上于 405nm 进行读数。结果示于图 12 至 24 中。显示的 EC_{50} 和 TcdA 活性的最大中和百分比确认选择的抗体具有作为单一试剂的极高效力。这些抗体的 2 至 5 种的组合不提高最佳 EC_{50} 或最大中和百分比。当与 MabCA922、923、995、997 和 1000 组合时任何协同作用的不存在是非常重要的观察，并且可归因于每一种抗体单独地具有特别高水平的亲和力和效力的事实。实施例 5 中的支持数据也显示一些 Mab（例如 CA997）能够结合 TcdA 亚结构域许多次。因此，这 5 种 Mab 似乎可能代表当靶向 TcdA 的 C 末端细胞结合结构域时可获得的最大亲和力、效力和效价。抗体在中和范围从 LD80 变化至大于 LD_{95}（LD_{max}）的极高毒素浓度时是有效的，但对于高水平的 [TcdA] 观察到 EC_{50} 的一些适度的增加（即效力的减小）。这些数据也是令人惊讶的，因为其它数据已显示当测试升高的 TcdA 浓度时效力显著下降 (20)。

[0573] 此处描述的 Mab（例如对于 CA997）的高效力和亲和力并不仅仅归因于它们的高结合效价。其它研究 (20 和 WO06/071877) 描述了能够结合达到 14 次的抗 -TcdA Mab。这些 Mab 仅具有在 0.3 至 100nM 的范围内的亲和力，并且显示不完全的抗 TcdA 介导的细胞杀伤的保护作用：单独地 (26-63% 的保护作用）或成对地 (31-73% 的保护作用）。因此已显示对 TcdA 的结合的高效价不一定激起对 TcdA 的结合的高亲和力或中和。未描述 Mab CDA-1 的对 TcdA 的结合的亲和力和效价 (18 和 US7625559)。因此，本文中描述的 Mab 具有令人惊讶的亲和力、效力和效价。

[0574] 表 4 单一 TcdA 浓度 (LD_{80}) 时的抗 TcdA1、2 和 3Mab 组合

[0575]

抗体	最终(最高)Mab 浓度 ng/ml	EC_{50}(ng/ml)
922	500	1.21
923	500	160.42
995	500	37.64
997	500	6.25
1000	500	19.73
922+923	500	3.58
922+925	500	3.326
922+997	500	2.88
922+1000	500	2.64
923+995	500	60.23
923+997	500	7.54
923+1000	500	9.24
995+997	500	7.29
995+1000	500	19.63
997+1000	500	4.46
922+923+995	500	4.72
922+923+997	500	3.23
922+923+1000	500	3.21
922+995+997	500	2.22
922+995+1000	500	2.85
922+997+1000	500	2.22
923+995+997	500	5.04
923+995+1000	500	9.49
995+997+1000	500	5.84
922+923+995+997	500	2.75
922+923+995+1000	500	3.75
922+995+997+1000	500	3.46
923+995+997+1000	500	4.81
922+923+997+1000	500	3.06
922+923+995+997+1000	500	4.72

[0576] 表 5. 不同 TcdA 浓度时的抗 TcdA 单一、成对和三个一组的 Mab 组合，其中 TcdA 在其 LD_{80}, LD_{90}, LD_{95} 和 LD_{max}。

[0577]

毒素 TcdA	样品	最终 Mab 浓度 ng/ml	EC_{50}(ng/ml)
@3000 pg/ml(LD_{MAX})	922	500	4.89
	997	500	10.99
	1000	500	50.17
	922+997	500	7.18
	922+1000	500	6.99
	997+1000	500	9.437
	922+997+1000	500	10.80
	922+997+1000+995	500	15.03
	922+997+1000+995+923	500	7.16
@ 1000 pg/ml(LD_{95})	922	500	1.24
	997	500	3.42
	1000	500	9.60
	922+997	500	1.85
	922+1000	500	2.51
	997+1000	500	3.61
	922+997+1000	500	2.40
	922+997+1000+995	500	2.74
	922+997+1000+995+923	500	2.38
@ 700 pg/ml(LD_{90})	922	500	0.84
	997	500	2.40
	1000	500	6.23
	922+997	500	1.19
	922+1000	500	1.33
	997+1000	500	2.68
	922+997+1000	500	1.84

[0578]

	922+997+1000+995	500	2.17
	922+997+1000+995+923	500	2.06
@ 350 pg/ml(LD_{80})	922	500	0.39
	997	500	1.18
	1000	500	2.76
	922+997	500	0.67
	922+1000	500	0.85
	997+1000	500	2.06
	922+997+1000	500	0.83
	922+997+1000+995	500	0.97
	922+997+1000+995+923	500	0.98

[0579] 实施例 2. 纯化的 Mab 的抗 TcdB 体外中和作用

[0580] 测定法描述：

[0581] 在 96 孔聚苯乙烯板中进行所有中和筛选测定。

[0582] 测定使用在 MEM+20%FCS, 2mM Q 和 NEAA 中生长和筛选的 CACO-2 细胞。除非另外声明，否则所有 Ab 组合以等比率存在。

[0583] • 第 1 天：以每孔 3000 将细胞于 50 μ l 培养基中涂板，温育 24hr

[0584] • 第 2 天：将人源化 Mab 的纯化样品添加至 96 孔圆底聚苯乙烯无菌板中

[0585] • 将毒素 B 批次 #031 掺入 PP 板，在 37℃温育 1hr

[0586] • 将 50 μ l 的该混合物添加至细胞板中

[0587] • 温育 96hr

[0588] • 第 5 天：添加亚甲蓝 (0.5% 亚甲蓝，50% 乙醇)

[0589] • 在室温温育 1hr

[0590] • 用 1%N- 十二烷基肌氨酸裂解细胞

[0591] • 在 BIOTEK Synergy2 板计数器上于 405nm 进行读数

[0592] 图 25 至 33 中的数据显示：就百分比最大中和作用和活性 (EC_{50}) 而言，单独的单一 Mab 在中和 TcdB 上是相对无效的。然而，当将 2 种和 3 种抗体组合时，在百分比最大中和作用和活性 (EC_{50}) 上观察到相当大的改善。1125 和 1151 被选择作为最佳配对，尽管观察到其它良好配对：1125+1153、1125+1134。

[0593] 最有效的 Mab 配对凭经验地选择，并且当考虑每一种 Mab 的单个效力时，并回顾地发现产生预料之外的令人惊讶的组合。例如，在表 6 中，仅 CA927 具有可导致确定的 EC_{50} 的 TcdB 中和效力，然而 CA1125 和 CA1151 的 TcdB 中和效力在这些测定条件下都不足以导致确定的 EC_{50}。然而，发现 CA927 不是组合内使用的最有效的 Mab。最佳的含 CA927 的组合具有 13.5ng/ml 的 EC_{50}，然而其它两种 Mab 组合具有低至 2.59 和 4.71ng/ml 的 EC_{50}。在另一个实

例中，在表 8 中，CA1099 在使用的测定条件下具有最低 TcdB 中和 EC_{50}。然而，发现 CA1099 不是组合中使用的最有效 Mab。最佳的含 CA1099 组合具有 6ng/ml 的 EC_{50}，然而其它两种 Mab 组合具有低至 2 和 1ng/ml 的 EC_{50}。我们可推测最有效的 Mab 配对通过它们的协同结合方式来确定，特别是如通过具有非重叠表位来确定。

[0594] 表 6. 抗 -TcdB Mab 组合和在恒定毒素浓度时的相对 Mab 比率

[0595]

样品	最终 Mab 浓度 ng/ml	EC_{50}(ng/ml)
1125.g2	1000	>1000
1134.g5	1000	>1000
927.g2	1000	12.89
1153.g8	1000	>1000
1102.g4	1000	>1000
927+1099	1000	>1000
927+1102	1000	>1000
927+1114	1000	>111.111
927+1125	1000	13.55
927+1134	1000	51.58
1099+1114	1000	>1000
1102+1114	1000	>333.333
1102+1125	1000	15.51
1114+1134	1000	19.70
1114+1151	1000	25.69
1114+1153	1000	27.48
1125+1134	1000	2.59
1125+1151	1000	4.71
1125+1153	1000	21.23

1125+1134+1114	1000	3.77
1125+1134+927	1000	2.63
1125+1151+1114	1000	4.90
1125+1151+927	1000	5.69
1125.g2+1134.g5+927.g2	1000	5.83
1125.g2+1134.g5+1153.g8	1000	9.89
1125.g2+1134.g5+1102.g4	1000	2.72

[0596]

[0597] 实施例 3. 纯化的 Mab 的组合对 TcdB 的中和作用

[0598] 在 96 孔聚苯乙烯板中进行所有中和筛选测定。

[0599] 测定使用在 MEM+20%FCS, 2mM Q 和 NEAA 中生长和筛选的 CACO-2 细胞。

[0600] • 第 1 天 :以每孔 3000 将细胞于 50μl 培养基中涂板,温育 24hr

[0601] • 第 2 天 :将人源化 Mab 的纯化样品添加至 96 孔圆底聚苯乙烯无菌板中

[0602] • 将毒素 B(VPI10463) 掺入 PP 板,在 37℃温育 1hr

[0603] • 将 50μl 的该混合物添加至细胞板中

[0604] • 温育 72hr

[0605] • 第 5 天 :添加亚甲蓝 (0.5% 亚甲蓝,50%ETOH)

[0606] • 在室温温育 1hr

[0607] • 用 1%N- 十二烷基肌氨酸裂解细胞

[0608] • 在 BIOTEK Synergy2 板计数器上于 405nm 进行读数

[0609] 结果示于图 34 至 45。

[0610] 这些数据显示在一系列毒素浓度时中和 TcdB 的最佳 Mab 配对是 CA1125 和 CA1151。此外,1125+1151 组合大体上不受相对摩尔比率的变化影响,这与 1125+1153 相反。

[0611] 表 7. 抗 -TcdB Mab 组合和在 3 个不同的毒素浓度时的相对 Mab 比率

[0612]

	EC50 值(ng/ml)		
抗体组合	TcdB LD60	TcdB LD77	TcdB LD85
1125.g2 + 927.g2 (50:50)	2.8	6	11.3
1125.g2 + 1102.g4 (50:50)	4	13	44
1125.g2 + 1114.g8 (50:50)	3.5	7.1	25.4
1125.g2 + 1134.g5 (50:50)	0.48	1.4	4
1125.g2 + 1151.g4 (50:50)	0.85	0.85	1.5
1125.g2 + 1153.g8 (50:50)	2.7	5.2	25.2
1125.g2 + 1134.g5 (25:75)	<0.15	0.84	7.2
1125.g2 + 1151.g4 (25:75)	0.73	1	2.1
1125.g2 + 1153.g8 (25:75)	7	10	27
1125.g2 + 1134.g5 (75:25)	0.66	1.2	2.5
1125.g2 + 1151.g4 (75:25)	1.4	1.2	8.3
1125.g2 + 1153.g8 (75:25)	2.9	7.5	30

[0613] 数据显示甚至最具有活性的特异性配对的组合具有令人惊讶地和不可预测地彼此相对不同的性质。等摩尔比率的 CA1125 与 CA1151 的优选组合的 EC_{50} 大体上不受递增的 [TcdB] 影响。测试的 Mab 的 3 个相对摩尔比率（即 25:75 相对于 50:50 相对于 75:25）具有彼此极其相似的 EC_{50}，从而表明 CA1125 和 CA1151 特别地具有互补作用模式。这与 CA1125 与 CA1134 的组合相反，在所述 CA1125 与 CA1134 的组合中，对于更高 [TcdB]，EC_{50} 的增加（即效力的减少）更显著，并且其中 3 个 Mab 摩尔比率效力不同 :25:75 的 CA1125:CA1134 比率的效力明显低于 50:50 和 75:25。这表明 CA1125+CA1134 的组合效力更加依赖于 CA1125 组分。CA1125 与 CA1153 的所有 3 个摩尔组合的 EC_{50} 基本上不受递增的 [TcdB] 影响，从而表明 CA1153 是不太适合的与 CA1125 的组合的伴侣。总体上，这些数据显示 CA1125 和 CA1151 是特别有利的组合，因为最高效力在一系列 Mab 与 TcdB 的摩尔比率上得到维持。

[0614] 表 8. TcdB 中和 - 在恒定毒素剂量 (LD_{80}) 时的 1 种或 2 种抗 -TcdB

[0615]

抗体	IC50(ng/ml)
1099	2
1102	N/A
1114	103
1125	N/A
1134	8
1151	182
1153	260
926	N/A
927	N/A
1099+1125	6
1114+1125	7
1151+1125	2
1134+1125	1
1102+1125	6
1125+1153	12
926+1125	42
927+1125	4

[0616] 表 9. TcdB 中和 - 在不同 TcdB 剂量时的 1 种或 2 种抗 -TcdB Mab

[0617]

	EC50 值(ng/ml)			最大中和		
抗体组合	TcdB LD75	TcdB LD86	TcdB LD90	TcdB LD75	TcdB LD86	TcdB LD90
1125.g2	n/a	n/a	n/a	40%	25%	15%
1114.g8	n/a	n/a	n/a	45%	25%	15%
1134.g5	n/a	n/a	n/a	45%	25%	15%
1151.g4	n/a	n/a	n/a	45%	25%	20%
1153.g8	28.3	n/a	n/a	65%	35%	28%
1125.g2+1114.g8(50:50)	10.1	243.8	n/a	85%	65%	40%
1125.g2+1134.g5(50:50)	1.7	22.6	n/a	87%	60%	40%
1125.g2+1153.g8(50:50)	6.1	32.2	n/a	95%	75%	48%
1125.g2+1151.g4(50:50)	0.8	2.8	19.1	85%	80%	55%
1125.g2+1151.g4(25:75)	1.2	2.8	47.2	85%	75%	60%
1125.g2+1151.g4(75:25)	2.9	3.8	2.6	75%	70%	60%

[0618] 这些数据显示 Mab 的组合，特别是 CA1125 与 CA1151 的组合，改善了效力（如通过 EC_{50} 测量的，还有如通过百分比最大保护作用测量的）。百分比最大保护作用在本测定法中具有特别的相关性，因为将 Mab:TcdB 混合物与细胞一起温育了很长时间 (72h)。因为 TcdB 在 2-4 小时内在 pg/ml 的范围内对于 Caco-2 细胞是有毒的，该测量可被认为是非常难的 Mab 中和能力的测试，并且可在它们的结合动力学或模式方面反映 Mab 混合物的能力。继而，当大量 TcdB 可在组织内存在许多小时时，这可反映 Mab 混合物在建立的感染过程中保护免受 TcdB 的作用的能力。

[0619] 在图 46-59 中进一步显示了来自表 6-9 的选择的数据。

[0620] 实施例 4. Mab 对 TcdB 亚结构域的结合的效价

[0621] 可在 Biacore3000 (GE Healthcare) 上通过表面等离子体共振 (SPR) 来测定抗-艰难梭菌 TcdB 抗体对 $TcdB_{1234}$ 的结合事件的摩尔数。通过胺偶联将链霉抗生物素蛋白固定在 CM5 传感器芯片 (GE Healthcare) 上至～4000RU 的水平，生物素化的 $TcdB_{1234}$ 在 500-600RU 被结合。以 10μl/min 在该表面上方进行 2 次 20μl 的相同抗-TcdB 抗体混合物（每一种抗体的终浓度为 500nM) 的注射，记录饱和结合反应。在每一轮后使用 HCl 再生表面。使用对仅有链霉抗生物素蛋白的参照流动池的反应来针对本底结合校正所有数据。

[0622] 表 10. $TcdB_{1234}$ 上的 IgG 结合位点的数目的表面等离子体共振分析

[0623]

抗体组合	结合循环重复的次数	结合反应(RU)	相对于 CA927 平均反应的结合
CA1125.g2	10	750	0.9
CA1151.g4	10	1232	1.6
CA1125_CA1151	4	1941	2.5
CA1125_CA927	3	1570	2.0
CA1151_CA927	3	1959	2.5
CA927	8	791	1.0

[0624] 相对于多个 CA927 平均反应来表达所有反应（表 10 的最后一列），因为 CA927 似乎代表仅结合 $TcdB_{1234}$ 一次的 Mab。

[0625] 当结合至 $TcdB_{1234}$ 时，固定的 CA1125 不允许 CA1125 进一步结合，从而支持 CA1125 在 $TcdB_{1234}$ 上具有一个结合位点并且在该位点已被饱和后未能发现 CA1125 的其它结合位点的想法。然而，当 $TcdB_{1234}$ 已被 CA1125 饱和后，CA1151 仍然可结合。这表明 CA1151 在作为被 CA1125 占据的位点的替代位点上结合。这些数据一起显示了 CA1125 是 $TcdB_{1234}$ 的单结合剂，然而 1151IgG 结合 $TcdB_{1234}$ 超过一次，最可能地 2 次。因此，CA1125 与 CA1151 的混合物可结合 $TcdB_{1234}$ 大致 3 次。

[0626] 所有抗体组合具有额外的结合反应，显示了在 $TcdB_{1234}$ 上存在 2 个或更多个被这些组合结合的非竞争性位点。

[0627] 实施例 5. Mab 对 TcdA 亚结构域的结合的效价

[0628] 在 Biacore3000(GE Healthcare) 上通过表面等离子体共振 (SPR) 测定抗 - 艰难梭菌 TcdA 抗体对 $TcdA_{123}$ 和 A_{456} 的结合事件的摩尔数。通过胺偶联将链霉抗生物素蛋白固定在 CM5 传感器芯片 (GEHealthcare) 上至～ 4000RU 的水平，以及将生物素化的 $TcdA_{123}$ 结合至一个流动池，将 $TcdA_{456}$ 结合至不同流动池至～ 500RU 的反应。以 10μl/min 在两个流动池上方进行 2 次 30μl 的 1μM 的相同抗 -TcdA 抗体的注射，记录饱和结合反应。在每一轮后，使用 HCl 再生表面。使用对仅有链霉抗生物素蛋白的参照流动池的反应来针对本底结合校正所有数据。

[0629] 表 11. IgG 对固定的 $TcdA_{123}$ 和 $TcdA_{456}$ 的结合反应的 SPR 分析

[0630]

	CA997	CA1000	CA997/CA1000 的比率
TcdA123	1069	166	6
TcdA456	1285	407	3

[0631] 抗体 CA997 和 CA1000 以 6 个 CA997 对 1 个 CA1000 的比率结合 TcdA123，然而它们以 3 个 CA997 对 1 个 CA1000 的比率结合 TcdA456（表 2）。

[0632] 就分子量和固定的毒素水平进行了修正的 CA997 针对 TcdA123 与针对 TcdA456 的

最大抗体反应相似。这表明 CA997 结合 TcdA4566 次并且 CA1000 结合 TcdA4562 次。因此抗体 CA997 可能结合完整 TcdA 毒素 (TcdA) 约 12 次。

[0633] 总体上 CA997 结合 A1236 次或更多次，结合 A4566 次或更多次，然而 CA1000 结合 A123 至少 1 次，结合 A4562 次。

[0634] 增加的对 TcdA 和 TcdB 结合的效价可具有两个重要的体内作用。第一个作用是能够结合 TcdB 超过一次的任何 Mab 或 Mab 混合物将具有增加的形成毒素间结合事件和从而免疫沉淀的潜能。免疫沉淀可通过降低毒素的溶解性和形成非常大的大分子复合物(这从而降低毒素的有效工作浓度)来提供效力。这样的大的蛋白质复合物可被驻留在组织中的巨噬细胞和单核细胞吸收并且可能促成增强的宿主免疫应答。具有 Fc 片段的抗原：抗体复合物已明确地显示能够引发抗肠病原体的宿主免疫反应 (21)。同样地，可溶性抗原：抗体复合物已在人临床试验中被成功地用作抗病原体疫苗 (22)。此外，利用具有 Fc 的 IgG 对毒素的免疫修饰可促成通过肝和脾使用正常机制进行的免疫清除。一般而言，更高水平的抗原的 Fc 修饰导致更快更完全水平的消除 (23)。极其重要地，每毒素 2 个或更多个 Mab Fc 结构域的存在，特别是每毒素 3 个 Fc 结构域的存在，可能可以代表毒素的极快速的和大量清除所需的 Fc 的临界数目 (24)。抗 -TcdA Mab CA997 可能能够结合 TcdA 多至 12 次，CA1125 与 CA1151 的组合可能能够结合 TcdB3 次。因此 3 种 Mab 的混合物极可能能够在体内提供这些类型的额外的效力机制。

[0635] 实施例 6. 由 TcdA 引起的 TEER 的丢失的 Mab 中和

[0636] 使用 Becton-Dickinson(BD) Caco-2BioCoat HTS 平板系统进行艰难梭菌单层完整性测定。

[0637] 第 1 天 - 将 Caco-2 细胞以每孔 2x105/ml 于 500μl 基础接种培养基 (由 BD 提供) 中接种在插入板中。将 35ml 的基础接种培养基添加至进料盘。将细胞在 37℃温育 24 小时。第 2 天 - 从插入板除去基础接种培养基，替换为 Entero-STIM 分化培养基 (由 BD 提供)。每孔添加 500μl，向进料盘中添加 35ml。将细胞在 37℃再温育 72 小时。第 5 天 - 以相对于待用于聚丙烯板中的测定孔的浓度的 2 倍的浓度制备的抗体和毒素 A。将毒素 A 以 125ng/ml 的浓度添加至抗体，将板在 37℃温育 1hr。将 1ml 的 Caco-2 生长培养基 (MEM+20%FCS, 2mM Q, NEAA) 添加至标准 24 孔 TC 板的每一个孔。将 BioCoat 插入板转移至 24 孔 TC 板。从插入板除去 Entero-STIM 培养基，替换为 400μl 的毒素 :Ab 混合物。

[0638] 肠细胞之间的紧密连接的丧失是 TcdA 对细胞单层和肠组织切片的重要早期作用，并且是腹泻的主要原因。白蛋白和其它血清蛋白质随同伴随的血清液一起流失至肠腔中。已形成单层的分化的培养细胞的跨上皮电阻的丢失是抗 TcdA 的急性作用的保护作用的有用替代。显示的 3 种抗体具有良好水平的抗 TEER 丢失的保护作用，图 62。令人瞩目和惊讶的是这些 Mab 在 TEER 测定中的能力不反映在毒素中和中看到的能力，如在细胞增殖测定中测量的。CA922 在细胞增殖测定中具有最佳性能 (EC50=1.21ng/ml)，然而这被在细胞增殖测定中具有小于 10 倍的效力的抗体 (CA1000) 远远超过 (EC50=19.73ng/ml)。CA997 在 TEER 测定中具有最佳性能，因为其具有高水平的保护作用并且在更低的 Mab 浓度上维持该保护作用。CA997 具有很大的可能在第 4 小时时以达到 80% 的最大抑制和约 80ng/ml 的 EC50 中和 TEER 丢失。这些观察是预料之外的，因为所述 Mab 全都具有对于 TcdA 结构域的高亲和力 (CA922 ～ 4pM、CA997 ～ 132pM、CA1000 ～ 73pM)。这些数据表明 CA997 和 CA1000

识别在 TEER 丢失中是重要的表位或通过与对其它 Mab 不同的机制中和 TcdA。此外，由于 CA1000 据估计结合完全毒素 2 次（一次在 TcdA123 中以及一次在 TcdA456 中），因此 CA1000 可确定 TcdA 细胞结合区域内的 'TEER 决定性' 表位，这可能对确定疫苗免疫原具有特殊价值。结果示于图 62 中。

[0639] 实施例 7. 抗 - 艰难梭菌毒素抗体对于 TcdA 和 TcdB 的亚结构域 :$TcdA_{123}$、$TcdA_{456}$ 和 $TcdB_{1234}$ 的亲和力

[0640] 通过在 BIAcore3000 上使用 CM5 传感器芯片进行的表面等离子体共振测定抗 - 艰难梭菌 TcdA 和 TcdB 抗体的相互作用的动力学常数。

[0641] 所有实验在 25oC 进行。通过胺偶联化学将特异性 Affinipure $F(ab')_2$ 片段山羊抗 - 人 IgG、Fc 片段 (Jackson ImmunoResearch) 固定在 CM5 传感器芯片 (GE) 上至～ 7000 反应单位 (RU) 的捕获水平。以 10μL/min 的流速将 HBS-EP 缓冲液 (10mM HEPES pH7.4, 0.15MNaCl, 3mM EDTA, 0.005% 表面活性剂 P20, Biacore AB) 用作运行缓冲液。将 10μL 的 1ug/ml 或更低的每一种抗体通过注射用于利用固定的抗 - 人 IgG、Fc 进行的捕获。以 30μL/min 的流速，从 12.5nM 开始以二倍稀释在捕获的纯化的抗体上滴定 TcdA123、TcdA456 或 TcdB1234。对于存在于培养上清液中的抗体，以 30ul/min 在抗体上通过单个浓度的 12.5nM 的 TcdA123 或 TcdA456 和 50nM 的 TcdB1234。在 n=2 时计算动力学。以 10uL/min 的流速通过两次 10μL 的 40mM HCl 的注射和 5μL 的 5mM NaOH 的注射再生表面。

[0642] 按照标准方法使用 BIAevaluation 软件 (3.2 版) 分析扣除两倍参照本底的结合曲线。根据拟合算法测定动力学参数。

[0643] 表 12. 抗 -TcdA Mab 亲和力和结合动力学

[0644]

	抗体 ID	ka(1/Ms)	kd(1/s)	KD(M)	KD(pM)	材料/测定
TcdA123	CA164_00922.g1	1.09E+06	4.43E-06	4.06E-12	4.06	纯化的 Mab 5 点滴定
	CA164_00923.g1	5.36E+05	3.47E-05	6.47E-11	64.7	
	CA164_00995.g1	无结合			无结合	
	CA164_00997.g1	7.84E+05	1.03E-04	1.32E-10	132	
	CA164_01000.g1	1.33E+05	9.78E-06	7.33E-11	73.3	
	CA164_00993.g1	9.00E+05	5.00E-06	5.56E-12	5.56	上清液 2x 1 点滴定
TcdA456	CA164_00922.g1	1.29E+06	3.33E-06	2.59E-12	2.59	纯化的 Mab 5 点滴定
	CA164_00923.g1	6.16E+05	1.92E-04	3.12E-10	312	
	CA164_00995.g1	2.87E+05	3.42E-05	1.19E-10	119	
	CA164_00997.g1	9.21E+05	6.15E-05	6.68E-11	66.8	
	CA164_01000.g1	3.55E+05	2.98E-05	8.41E-11	84.1	
	CA164_00993.g1	1.25E+06	5.00E-06	4.00E-12	4.00	上清液 2x 1 点滴定

[0645] 表 13. 抗 -TcdB Mab 亲和力和结合动力学

[0646]

	抗体 ID	ka(1/Ms)	kd(1/s)	KD(M)	KD(pM)	材料/测定
TcdB1234	CA164_1125.g2	2.64E+05	3.23E-05	1.22E-10	122	纯化的 Mab 3 点滴定
	CA164_1151.g4	7.49E+05	4.13E-04	5.51E-10	551	纯化的 Mab 3 点滴定
	CA164_926.g1	1.38E+05	7.12E-05	5.16E-10	516	上清液 2x 1 点滴定
	CA164_927.g2	3.97E+05	3.61E-05	9.11E-11	91	纯化的 Mab 3 点滴定
	CA164_1099.g2	5.24E+05	1.63E-05	3.10E-11	31	纯化的 Mab 3 点滴定
	CA164_1102.g4	1.17E+05	3.78E-04	3.25E-09	3250	上清液 2x 1 点滴定
	CA164_1114.g2	2.87E+05	1.97E-03	6.87E-09	6870	上清液 2x 1 点滴定
	CA164_1114.g8	2.55E+05	1.85E-03	7.25E-09	7250	上清液 2x 1 点滴定
	CA164_1129.g1	1.89E+05	2.30E-04	1.22E-09	1220	上清液 2x 1 点滴定
	CA164_1134.g5	5.09E+05	2.45E-05	4.81E-11	48	纯化的 Mab 3 点滴定
	CA164_1153.g8	1.43E+05	4.48E-05	3.14E-10	314	纯化的 Mab 3 点滴定

[0647] 抗 -TcdA 亲和力相较于公开的其它 Mab 的亲和力特别高。我们证明低至 4pM 的亲和力是可实现的。优选的 CA997 具有 132pM 的亲和力，CA1125 具有 122pM 的亲和力，CA115 具有 551pM 的亲和力。CA995 明确地显示其不结合 CROPs A_{123}，从而以令人惊讶和意外的方式显示此处显示的 Mab 具有彼此不同的性质。CA922、923、997 和 1000 结合 CROPs A123 和 A456 至少一次。因此这 4 种 Mab 确认了每一种必须结合完全毒素至少 2 次。我们因技术限制而不能得出这些 Mab 对完全毒素的结合的亲和力。然而，鉴于针对抗 -TcdA Mab 显示的高亲和力和效价，可能推测抗完全毒素的功能性亲和力可能甚至比针对对毒素亚结构域的结合证明的所述功能性亲和力更强。抗 -TcdBMab 还显示达到低至 31pM 的强亲和力。具体地，CA1125、1151、927、1099、1134 和 1153 显示超过由其它抗体显示的亲和力的亲和力。

[0648] 实施例 8. 艰难梭菌抗 - 毒素人源化 IgG1 分子的生物物理学表征

[0649] 分析的分子

[0650] 抗 -TcdA IgG1:

[0651] CA164_00922.g1

[0652] CA164_0923.g1

[0653] CA164_0995.g1

[0654] CA164_0997.g1

[0655] CA164_01000.g1

[0656] 抗 -TcdB IgG1

[0657] CA164_01125.g1

[0658] CA164_01125.g2

[0659] CA164_01134.g4

[0660] CA164_01134.g5

[0661] CA164_01134.g6

[0662] CA164_01102.g1

[0663] CA164_01102.g4

[0664] CA164_01151.g4

[0665] 抗体组合需要由具有高水平稳定性的 Mab 组成以在长期贮存中减小聚集的潜在风险。热稳定性 (Tm) 用作一个量度。对于 Mab 混合物特别有价值的是测量它们对因物理应激例如搅拌或摇动而聚集的倾向性。聚集体是药物组合物的不想要的组分，因为它们可减少贮存期限，并且可在某些水平上对患者产生安全性风险。Tm 数据显示所有 5 种抗 -TcdA Mab 具有高 Tm 稳定性，而 3 种 (CA922、923 和 997) 具有在 79-81℃的范围内的极高 Tm。在测试的抗 -TcdB Mab 中，除两个外全都具有极高的 Tm。注意在仓鼠感染研究 (实施例 9) 中测试的 CA997、CA1125 和 CA1151 具有极高的 Tm(分别地 79.2℃、79.3℃和 80.8℃)，这使得它们适合用于 Mab 混合物。

[0666] 在摇动聚集测定中，CA997 和 922 具有最低的对 5 种抗 -TcdA Mab 的聚集的倾向性。类似地，CA115 和 1151 具有最低的抗 -TcdB Mab 的聚集倾向性。因此，CA997、1125 和 1151 作为 Mab 混合物的用途可具有特别的价值，因为它们更可能在共配制后保持活性，以及以高蛋白质浓度贮存。

[0667] 利用毛细管 IEF 估计等电点 (pI)

[0668] 通过混合下列组分来制备样品：30ul 的 2mg/ml 的蛋白质样品、0.35% 的甲基纤维素、4% 的 pH3-10 两性电解质 (Pharmalyte)、合成的 pI 标准产品 (4.65 和 9.77)、各 1ul 的原液和加 HPLC 级水至 200ul 的终体积。随后使用 iCE280IEF 分析仪（在 1500V 预聚焦 1min，随后在 3000V 聚焦 6min）分析混合物。随后使用 Empower 软件（来自 Waters）整合校准的电泳图。

[0669] 通过 Thermofluor 测定法测量的热稳定性 (Tm)

[0670] 本方法使用 Sypro 橙荧光染料监控蛋白质结构域的解折叠过程。染料结合作为解折叠的结果而变得暴露的暴露的疏水区域，这导致发射光谱的改变。

[0671] 将样品 (5ul，1mg/ml) 与 5ul 的 Sypro 橙的原液 (30x) 混合，利用 pH7.40 的 PBS 使体积达到 50ul。

[0672] 将 10ul 的该溶液的等份添加至 384 孔板的孔中 (n=4)。

[0673] 将板置于包含用于精确温度控制的加热装置的 7900HT 快速实时 PCR 系统中。使温度从 20℃升高至 99℃ (1.1℃ /min 的升降温速度)。CCD 装置同时监控孔中的荧光变化。将算法用于处理强度数据并且将多重转变 (multiple transitions) 加以考虑。

[0674] 通过搅拌对样品进行应激

[0675] 在制备过程中，将抗体样品经历通过方法例如抽吸和过滤产生的机械应激。这可引起由于蛋白质对气 - 液界面和剪切力的暴露而引起的变性和随后聚集，从而导致生物活性的最终丢失。通过涡旋产生的应激是筛选抗体样品鲁棒性以用于预测聚集稳定性的方法。

[0676] 将抗 -TcdA 和抗 -TcdB IgG1 分子经历通过搅拌，通过涡旋（在 25℃，以 1400rpm 使用 Eppendorf Thermomixer Comfort）产生的应激。样品大小为 250uL(x3/ 样品），在 1.5ml 圆锥形 Eppendorf- 式加帽的管（塑料）中，于 PBS pH7.4 中进行。使每一种样品达到 1mg/ml 的浓度（使用从序列计算的消光系数），通过使用 Varian Cary50-Bio 分光光度计，在一定间隔 (总共达到 24 小时) 测量的 340nm 和 / 或 595nm 处的吸光度监控聚集。

[0677] 结果：表 14 提供了针对抗 -TcdA 和抗 -TcdB IgG1 分子测量的 pI 和 Tm 数据的总结

[0678] 表 14. pI 和 Tm 数据的汇编

[0679]

抗-TcdA IgG1	测量的 pI	PBS 中的 Tm(Fab)	Tm(CH2)
CA164_00922.g1	8.8	81	69.2
CA164_0923.g1	9.2	79	69.3
CA164_0995.g1	8.5	71	无数据*
CA164_0997.g1	8.3	79.2	68.4
CA164_01000.g1	7.74	70.5	无数据*
抗-TcdB IgG1			
CA164_01125.g1	9.2	79.3	69.4
CA164_01125.g2	9.2	79.5	69.3
CA164_01134.g4	9.3	78.4	69.4
CA164_01134.g5	9.2	76.4	69.2
CA164_01134.g6	9.2	76.6	69.6
CA164_01102.g1	9.1	69	无数据*
CA164_01102.g4	9.1	69.1	无数据*
CA164_01151.g4	9.2	80.8	69.8

[0680] *表示不可能辨别 Fab 和 CH2 结构域。

[0681] 测量的 pI

[0682] 分子的测量的 pI 是高的（除 CA164_01000.g1_P3 外）并且远离配制缓冲液例如 PBS,pH7.4 和 50m 醋酸钠 /125mM 氯化钠,pH5 的 pH。这可表示可选择具有适合于两种或更多种 Mab 的共配制的 pH 的缓冲液。

[0683] 通过 Thermofluor 测定法测量的热稳定性 (Tm)

[0684] 由于所有分子是 IgG1,因此 Fc 结构域的 Tm(Tm(CH2)) 相同。分子之间热稳定性的差异可通过 Fab' 结构域的 Tm(Tm(Fab)) 来测定。

[0685] 对于抗 -TcdA 分子,等级次序（首先是最稳定的）为 CA922 ≥ 997>923>995>1000,对于抗 -TcdB 分子（首先是最稳定的）是 CA1151.g4>1125.g1,g4>1134.g4>1134.g5 ≥ 1134.g6>1102.g1=1102.g4。

[0686] 通过搅拌进行的样品的应激反应

[0687] 测定不同抗体之间的不同聚集稳定性是可能的,图 67 显示通过涡旋对 PH7.4 的 PBS 中的不同抗 -TcdA IgG1 分子的搅拌的作用。

[0688] 确定等级次序（首先最稳定聚合的）是可能的 :CA922 ≥ 997>923 ≥ 995>1000。

[0689] 图 68 显示通过涡旋对不同抗 -TcdB 分子的搅拌的作用。

[0690] 确定聚集稳定性的等级顺序是可能的,以便 CA1125 移植物表现比 CA1134 分子更

稳定，所述 CA1134 分子比 CA1102 分子更稳定。

[0691] 进行了进一步研究以直接比较抗 -TcdB 分子 (CA1151.g4) 与更稳定的分子 CA1125.g2（参见图 2）和更加聚集稳定的抗 -TcdA 分子 (CA922.g1 和 CA997.g1) 的聚集稳定性。结果可见于图 69 中。

[0692] 这 4 种 Mab 的其它结果也示于图 67 和 68 中。

[0693] 对于抗 -TcdA 分子 CA922.g1 和 CA977.g1，基于上述分析，CA922 是优选的，尽管除 CA1000 外，所有分子可被当作用作治疗性 IgG1 的适当候选者。

[0694] 对于抗 -TcdB 分子，可基于聚集稳定性和 Tm 将生物物理学特征归类在移植物的家族内，以便 CA1125 移植物潜在地证明更加稳定。CA1102 移植物显示最差的 Tm 数据并且还显示最大的对通过应激（通过搅拌产生的）产生的聚集的倾向性。

[0695] 使用 CA1151.g4 的研究显示该分子相较于 CA11125.g2 显示略微增加的聚集稳定性并且似乎等于 TcdA 分子 (CA922.g1 和 CA997.g1)。所有 4 种分子都显示相等的 Tm 值。CA997、CA1125 和 CA1151 显示极高水平的热稳定性和在搅拌后极低水平的聚集体形成。

[0696] 实施例 9. 抗 - 艰难梭菌毒素 Mab 仓鼠感染研究

[0697] 仓鼠感染研究由 Ricerca Biosciences LLC, Cleveland, Ohio, USA 来进行。研究方案由 Ricerca IACUC 委员会批准。在完成计划的 28 天的研究期之前，活性组分和对照组分（组成和剂量）对于 Ricerca 员工来说是未知的。

[0698] 将 Golden Syrian 雄性仓鼠（体重 82-103g，54 日龄）单个地圈养在 HEPA 过滤的一次性笼子中，并且随意地喂食 Teklad Global Diet2016 和水。适应后，利用 Mab 混合物或 PBS（媒介物对照）给仓鼠预给药 (i.p.)，每天一次，进行 4 天：第 -3、-2、-1 和 0 天。研究了两个剂量的 Mab：高剂量 = 各 50mg/kg 的抗 -TcdA 和抗 -TcdB 组分；低剂量 = 各 5mg/kg 的抗 -TcdA 和抗 -TcdB 组分。

[0699] 测试的药物组合由下列组成：一种构成 50% 的注射蛋白的抗 -TcdA 抗体 (CA997.g1) 和共同构成 50% 的注射蛋白（但单独地构成 25% 的注射蛋白）的两种抗 -TcdB 抗体 (CA1125.g2 和 CA1151.g4)。在刺激之前，利用 50mg/kg 的 PBS 中的克林霉素磷酸酯使仓鼠致敏 (s.c.)（第 -1 天），1 天后（第 0 天）用 $3.4x10^6$c.f.u. 的来自菌株 ATCC43596 的营养细胞进行刺激。在第 1、2、3、4、5 天每天两次以 5mg/kg 施用 (p.o.) 万古霉素即进行 5 天。

[0700] 每两天一次对动物进行存活性检查，将被发现濒死的动物无痛致死，并将其计数为死亡。在给药的每一天测定体重，随后每周 2 次测定体重直至对存活者者实施无痛致死术。对所有动物进行肉眼尸检。利用 Kaplan 和 Meier 的方法产生存活曲线。使用相较于 P=0.005 的 Bonferroni 修正的阈值的来自时序检验的 P 值分析存活曲线。分析不包括万古霉素处理的组。利用 Prism v5.04 进行所有统计检验。所有组包含 11 只动物，除包含 5 只动物的万古霉素对照组外。

[0701] 存活曲线可见于图 63 中。接受 PBS（对照）的仓鼠在第 +2 和 +3 天都死亡，然而接受 5 天万古霉素处理的组在第 +10 和 +11 天都死亡。接受高剂量的 UCB Mab 混合物的仓鼠直至第 +11 天都存活，此后直至 28 天的研究结束仅两只动物死亡。接受低剂量的 UCB Mab 混合物的仓鼠直至第 +3 天都存活，此后动物相当稳定地失去，直至第 +16 天，此时所有动物都死亡。当与在仓鼠中使用抗 - 毒素 Mab 的公开的数据 (18) 相比较时，本数据显示异常水平和持续时间的保护作用。这些体内数据支持中和和稳定性的极高水平的性能的体外

观察。

[0702] 在感染的急性期（第 1-5 天）期间死亡与体重之间不存在明显的关联性，图 64-65。因此，可猜测仓鼠死于 TcdA 和 TcdB 的压倒性直接和间接作用。因中和 Mab 的部分保护作用（UCB 低剂量）而活过急性期的仓鼠重量减轻，假定归因于肠损伤和改变的营养状况。值得注意的是许多因 UCB 高剂量 Mab 的保护作用而持续活过 28 天的研究期的仓鼠从体重减轻恢复并且事实上甚至体重增加。这可被当作 UCB Mab 使得肠正常发挥作用的优良保护作用的证据

[0703] 表 15. 总体病理学评分

[0704]

组	黑色盲肠	暗红色盲肠	红色盲肠	粉红色盲肠	正常盲肠	肛门生殖器染色'湿尾症'	红色小肠
PBS 对照	1	9	1	0	0	1	1
UCB 低	0	4	5	2	0	4	1
UCB 高	0	0	1	1	9	3	0

[0705] 很清楚，UCB Mab 能够保护大肠和小肠免受由 TcdA 和 TcdB 引起的血液渗出。

[0706] 结果示于图 63 至 66。

[0707] 图 66 中的照片显示了由 TcdA 和 TcdB 引起的盲肠的膨胀和血液渗出（左图，PBS 对照，动物在第 2 天死亡）的典型肉眼检测病理学和在利用 UCB 高剂量 Mab 的保护后充满粪便的正常盲肠（右图，UCB 高剂量，动物存活至第 28 天）。这些数据显示在利用高剂量的 UCB Mab 保护后，大肠可恢复至正常形态和功能。

[0708] 实施例 10. 纯化的 Mab 对来自不同核糖体分型的菌株的 TcdA 的中和作用

[0709] 临床感染由多种不同的菌株引起。使用许多方法（其中核糖体分型是至关重要的）表征菌株差异。观察到不同的核糖型菌株具有不同的致病性、感染和芽孢形成性质。所有上文中显示的 TcdA 中和使用从称为 VPI10463 的菌株纯化的 TcdA。然而，与暴发相关的优势侵袭性致病性菌株称为核糖型 027。其它重要的核糖型包括 078、001、106。已在由不同核糖型产生的毒素之间观察到氨基酸序列差异，因此重要的是 Mab 能够中和来自不同组的临床分离株的毒素。测试 CA922、997 和 1000 中和来自菌株 027 和 078 的 TcdA 的能力，将其与它们抗来自 VPI10463 的 TcdA 的能力相比较。在 4 个 [TcdA] 测试 Mab，发现其能够中和所有毒素而在 LD_{80}、LD_{90} 和 LD_{95} 无显著差异。

[0710] 表 16

[0711]

	EC50 值(ng/ml) – TcdA 菌株 VPI 10463			
抗体	LD80	LD90	LD95	LDmax
CA164_922	0.27	0.9	1.2	>500
CA16_997	1	2.5	3.5	25.4
CA164_1000	3.6	13.5	19.3	>500

[0712] 表 17

[0713]

	EC50 值(ng/ml) – TcdA 核糖型 027			
抗体	LD80	LD90	LD95	LDmax
CA164_922	0.19	0.25	0.41	1.46
CA16_997	0.92	1.27	1.75	7.19
CA164_1000	2.25	2.49	3.52	16.32

[0714] 表 18

[0715]

	EC50 值(ng/ml) – TcdA 核糖型 078			
抗体	LD80	LD90	LD95	LDmax
CA164_922	0.11	0.12	0.25	0.68
CA16_997	0.33	0.64	1.11	2.57
CA164_1000	2.04	2.41	5.03	14.16

[0716] 实施例 11. PK 数据

[0717] 在健康仓鼠中进行的人 IgG1(20mg/kg) 的 PK 研究。发现仓鼠 PK 具有与小鼠或大鼠中的 Mab 相似的半衰期 (t1/2 6-8 天)。i.p. 和 s.c. 给药基本上相同。

[0718] 在'正常'(非感染的)golden Syrian 仓鼠中研究了 hIgG1Mab 的药代动力学和至肠的分配。通过 CARE Research LLC, Fort Collins, Colorado, USA 将纯化的 Mab 施用至雄性仓鼠 (120-135g),利用 UCBPharma 测定样品。研究获得 CARE IACUC 委员会批准。8 只动物各自接受单一剂量的 20mg/kg 的 IgG1,4 只 i.p. 给药,4 只 s.c. 给药。在给药后 1、3、8、24、48、72、103 和 168 小时收集血液,分离血清,随后在 -80℃贮存。还从两只未处理的仓鼠采集血液以提供测定对照。在无痛致死后,从每一只仓鼠的盲肠联接处向前切取 2cm 长的结肠。用洗涤缓冲液 (50%(v/v) 含 50%(v/v)Sigma 蛋白酶抑制剂混合物 (P2714) 的 PBS) 冲洗结肠片段,随后打开、分离并从下面的肌肉取出粘膜。将粘膜样品置于 0.5ml 的洗涤缓冲液中进行匀浆,直至目视均匀,在于湿冰上立即装运之前将其在 4℃贮存。对于抗 - 人 IgG1,利用山羊 $F(ab')_2$ 抗 - 人 IgG-Fc γ 片段 (Jackson109-006-098) 于 0.1M $NaHCO_3$pH8.3 中涂

覆 ELISA Nunc maxisorp96 孔板，过夜，用 PBS-Tween(PBS/0.1%(v/v)Tween20) 洗涤板，随后用 1.0%(w/v) 的 PBS 中的 BSA&0.1%(v/v)Tween 封闭板。将血清样品在样品－缀合物缓冲液 (1%(w/v) 的 PBS 中的 BSA,0.2%Tween) 中进行稀释，洗涤后，利用样品－缀合物缓冲液中的山羊抗－人 κ -HRP(Cambridge Bioscience2060-05) 和 2.5M H_2SO_4 终止溶液中的 TMB 显色。

[0719] 肠、粘膜和血清水平：

[0720] 此后取出从在第 168 小时时间点上采集的血液收集的血清样品和结肠样品。

[0721] 第 168 小时的 20mg/kg IP

[0722]

样品	ng/mL/cm 粘膜	血清 μg/mL
1001	23.2	75.0
1002	13.7	90.8
1003	21.8	70.5
1004	53.8	119.4

[0723] 第 168 小时的 20mg/kg SC

[0724]

样品	ng/mL/cm 粘膜	血清 μg/mL
2001	41.4	108.7
2002	62.1	76.6
2003	35.6	163.7
2004	37.3	153.3

[0725] 血清数据

[0726]

		仓鼠 *i.p.*		仓鼠 *s.c.*	
		平均值	平均值的 SE	平均值	平均值的 SE
C_{max}:	μg/mL	202	12	186	21
T_{max}:	hr	36	7	76	16
$AUC_{(last)}$:	hr·μg/mL	22626	1378	22371	2258
$AUC_{(inf)}$:	hr·μg/mL	43287	7169	61290	17637
百分比外推:		43.7	9.2	54	11.7
CL/F	mL/hr/kg	0.50	0.07	0.43	0.13
MRT_{inf}	h	223	53	310	88
$t_{½,z}$:	h	149.2	36.9	188.5	61.9

[0727] 数据也示于图 70 和 71 中。

[0728]

仓鼠 ID		平均值	SE
IP 血清动力学			
C_{max}:	μg/mL	202	12
T_{max}:	hr	36	7
$AUC_{(last)}$:	hr·μg/mL	22626	1378
$AUC_{(inf)}$:	hr·μg/mL	43287	7169
百分比外推:		43.7	9.2
CL/F	mL/hr/kg	0.50	0.07
MRT_{inf}	h	223	53
$t_{½,z}$:	h	149.2	36.9
SC 血清动力学			
仓鼠 ID		平均值	SE

[0729]

C_{max}:	μg/mL	186	21
T_{max}:	hr	76	16
$AUC_{(last)}$:	hr·μg/mL	22371	2258
$AUC_{(inf)}$:	hr·μg/mL	61290	17637
百分比外推:		54	11.7
CL/F	mL/hr/kg	0.43	0.13
MRT_{inf}	h	310	88
$t_{½}$:	h	188.5	61.9

[0730] 还显示hIgG1可见于肠的'削刮的碎屑',即hIgG1进入健康肠的血管系统-从而在'预防性给药'中可具有保护性。该作用在人中甚至更重大,因为它们具有同源hFcRn。

[0731] 实施例12.具有艰难梭菌感染的仓鼠的血清水平

[0732] 本研究是测定i.p.施用(下面详述的不同剂量)后Golden Syrian仓鼠中的CA725.0、CA726.0、CA997.g1CA1125.g2和CA01151.g4的血清浓度。

[0733] 在胰蛋白酶消化后使用液相色谱串联质谱(LC-MS/MS)分析定量人源化Mab。通过将以已知浓度掺入空白基质的真正的标准材料与用作内部标准的掺入的马肌红蛋白相比较来实现定量。

[0734] 选择对于所有研究的人源化Mab是共同的独特("裂解蛋白质(proteotypic)")肽(DTLMISR,CH2区肽),如所概述的使用胰蛋白酶消化样品和校准样品。在利用乙腈/Tris(2-羧乙基)膦变性/还原和利用碘乙酰(Sigma-Aldrich,Poole,UK)进行胺氨甲酰-甲基化之后,使用测序级的修饰的胰蛋白酶对5μl血清样品进行胰蛋白酶消化过夜。

[0735] LC-MS/MS系统由CTC HTS-x自动采样器(CTC Analytics,Zwingen,Switzerland)、Agilent1290LC系统(Agilent Technologies,Stockport,UK)和配备有以电喷雾模式操作的Turbo V离子源的Sciex5500QTrap MS系统(AB Sciex,Warrington,UK)组成。使用Onyx Monolithic C18柱(100x4.6mm,Phenomenex,Macclesfield,UK),通过以1.5mL/min递送的2至95%(v/v)的水/乙腈(0.1%的甲酸)的梯度分离分析物,进行6分钟。注射体积为10μL;将所有洗脱剂引入质谱仪源。将质谱仪的源温度维持在600℃,并且将其它源参数(例如碰撞能、去簇电压、帘式气压等)最优化以获得对目标肽的最大敏感性。监控每一个目标裂解蛋白质的选择跃迁(selective transition)。

[0736] 对于所有目标分析物,选择独特的("裂解蛋白质")肽;在胰蛋白酶消化后分析样品。

[0737] 基于监控的肽计算的血浆浓度概述于下文中。

[0738] 对于 CA164_00997 和 CA164_01151，在 MRM 描迹 (MRM trace) 中观察到干扰峰。为此，不能定量样品中的这两种分析物。

[0739] 使用对于所有目标分析物来说是共同的肽来定量所有样品中的总 h-IgG。这使用所有 5 种分析物的组合标准曲线来进行。该方法的有效性通过这样的事实来证明：对于 CA164_00725 和 CA164_00726 观察到的浓度之和与对于总的 h-IgG 观察到的浓度充分一致（在实验误差内）。

[0740] 通过使用该方法，测定利用 CA164_00997、CA164_01125 和 CA164_01151 给药的动物的样品中的 h-IgG 的总浓度。

[0741] 总体上，获得的数据表明全部 5 个目标分析物的暴露对于给定的剂量是相似的。

[0742] 研究组

[0743]

不知情 标记				处理组分		
组	处理	实际处理	剂量天数	抗毒素 A	抗毒素 B	
4	处理 3	媒介物 PBS 5mL/kg i/p/	3,-2,-1,0			
2	万古霉素	万古霉素 5mg/kgb.i.d.p.o.	1,2,3,4,5			
1	处理 1	UCB LD* 5mg/kg A 5mg/kg i.p.	3,-2,-1,0	CA997.g1_P3 5mg/kg	CA1125.g2_p3 2.5mg/kg	CA1151.g4_P3 2.5mg/kg
5	处理 4	UCB HD* 50mg/kg A 50mg/kg i.p.	3,-2,-1,0	CA997.g1_P3 50mg/kg	CA1125.g2_P3 25mg/kg	CA1151.g4_P3 25mg/kg
6	处理 5	竞争剂 LD* 5mg/kg A 5mg/kg i.p.	3,-2,-1,0	CA726_P3 5mg/kg	CA725_P3 25mg/kg	
3	处理 2	竞争剂 HD* 50mg/kg A 50mg/kg i.p.	3,-2,-1,0	CA726_P3 50mg/kg	CA725_P3 50mg/kg	

[0744] 表 19

[0745]

组/时间	天	动物编号	剂量	血清浓度 μg/mL 总的 h-IgG
1	1	44	5 mg/kg 997, 2.5 mg/kg 1125, 2.5 mg/kg 1151	280
	1	45		302
	1	46		182
	6	45		61
	6	47		71
	6	49		45

[0746]

3	1	60	50 mg/kg 725, 50 mg/kg 726	3040
	1	61		3330
	1	62		2990
	6	62		583
	6	63		913
	6	64		1240
	28	64		199
	28	65		36
4	1	71	媒介物	nd
	1	72		nd
	1	73		nd
5	1	82	50 mg/kg 997, 25 mg/kg 1125, 25 mg/kg 1151	3050
	1	83		2790
	1	84		2370
	6	82		838
	6	83		645
	6	84		855
	28	82		116
	28	83		65
	28	84		66
	28	85		44
	28	86		101
	28	87		89
	28	88		27
	28	89		31
	28	90		66
6	1	93	5 mg/kg 725, 5 mg/kg 726	335
	1	94		322
	1	95		260

[0747]

	6	200		103
	6	202		62
	6	203		79
	28	203		nd

[0748] nd - 未检测到（对于所有分析 LOQ=2.5μg/mL）

[0749] na - 未分析；对于 997 和 1151 观察到样品中的干扰

[0750] 表 20. 抗体 CA725 是现有技术抗体 MDX1388。抗体 CA726 是如描述的现有技术抗体 CDA1(15)。该数据的概述示于图 72 中。

[0751]

組	盲肠病状					小肠病状	
	黑色	暗红色	红色	粉红色	正常	暗红色	红色
PBS 对照	1	9	1	0	0	0	1
高 MDX 50mg/Kg x4	0	1	4	4	2	1	0
高 UCB 50mg/Kg x4	0	0	1	1	9	0	0

[0752] 参考资料

[0753] 1. Kuehne, S et al., "The role of toxin A and toxin B in Clostridium difficile Infection" Nature (2010) 467:711-713.

[0754] 2. Davies AH et al., "Super toxins from a super bug: structure and function of Clostridium difficile toxins" Biochem. J (2011) 436:517-526.

[0755] 3. Rothman, S et al., "Differential Cytotoxic Effects of Toxins A and B Isolated from Clostridium difficile" Infect. Imm. (1984) 46:324-331.

[0756] 4. Du, T and Alfa, MJ "Translocation of Clostridium difficile toxin B across polarized Caco-2cell monolayers is enhanced by toxin A" Can J Infect Dis. (2004) 15:83-88.

[0757] 5. Kim, Iaconis and Rolfe. "Immunization of Adult Hamsters against Clostridium diffcile-Associated Ileocecitis and Transfer of Protection to Infant Hamsters" Infect. Imm. (1987) 55:2984-2992

[0758] 6. Rupnik JCM (2003) 41:1118-1125

[0759] 7. Chaves-Olarte JBC (1999) 274:11046-11052.

[0760] 8. Lylerly, DM et al., "Passive Immunization of Hamsters against Disease Caused by Clostridium difficile by Use of Bovine Immunoglobulin G

Concentrate" Infection and Immunity (1991) 59:2215-2218.

[0761] 9. Lylerly, DM et al., "Vaccination against Lethal Clostridium difficile Enterocolitis with a Nontoxic Recombinant Peptide of Toxin A" Current Microbiology (1990) 21:29-32.

[0762] 10. Lylerly, DM et al., "Characterization of Toxins A and B of Clostridium difficile with Monoclonal Antibodies" Infect. Imm. (1986) 54:70-76.

[0763] 11. Corthier et al., "Protection against Experimental Pseudomembranous Colitis in Gnotobiotic Mice by Use of Monoclonal Antibodies against Clostridium difficile Toxin A" Infect. Imm. (1991) 59:1192-1195.

[0764] 12. Kink JA and Williams JA, "Antibodies to Recombinant Clostridium difficile Toxins A and B Are an Effective Treatment and Prevent Relapse of C. difficile-Associated Disease in a Hamster Model of Infection" Infect. Imm. (1998) 66:2018-2025.

[0765] 13. Ma D, et al., Progenics inc. ASM Poster27th May2010

[0766] 14. Hansen, G and Demarest, SJ. WO2006/0718877A2

[0767] 15. Babcock GJ, et al., "Human monoclonal antibodies directed against toxins A and B prevent Clostridium difficile-induced mortality in hamster" Infect. Imm. (2006) 74:6339-6347.

[0768] 16. Lowy I et al., "Treatment with Monoclonal Antibodies against Clostridium difficile Toxins" NEJM (2010) 362:197-205.

[0769] 17. Zubler, R. H., Erard, F., Lees, R. K., Van, L. M., Mingari, C., Moretta, L. &Mac Donald, H. R. (1985). Mutant EL-4thymoma cells polyclonally activate murine and human B cells via direct cell interaction. J. Immunol. 134, 3662-3668

[0770] 18. Babcook, J. S., Leslie, K. B., Olsen, O. A., Salmon, R. A. &Schrader, J. W. (1996). A novel strategy for generating monoclonal antibodies from single, isolated lymphocytes producing antibodies of defined specificities. Proc. Natl. Acad. Sci. U. S. A93, 7843-7848

[0771] 19. Tickle, S., Adams, R., Brown, D., Griffiths, M., Lightwood, D. &Lawson, A. (2010). High-Throughput Screening for High Affinity Antibodies., pp. 303-307.

[0772] 20. Demarest et al., mAbs (2010) 2:190-198

[0773] 21. Yoshida et al., J. Clin. Invest. (2006) 116:2142-2151

[0774] 22. Xu et al., Vaccine (2005) 23:2658-2664.

[0775] 23. Yousaf et al., Clin. Exp. Immunol. (1986) 66:654-660

[0776] 24. Mannik et al., J. Exp. Med. (1971) 133:713-739

[0777] 25. Nusrat et al., Infection and Immunity (2001) 69:1329-1336

[0778] 26. Lima et al., Infect Immun (1988) 56:582-588

[0779] 27. Ravichandran et al J of Pharmacology and Experimental Therapeutics. (2006) 318:1343-1351

[0780] 28. Takahashi et al., (2009) Vaccine27:2616-2619

[0781] 29. Cohen et al., Infect. Cont. and Hosp. Epidem. (2010)31:431-455

[0782] 30. Barbut et al., J. Clin. Microbiol. (2000)38:2386-2388

[0783] 31. Wilcox et al., J. Hospital Infection(1998)38:93-100

[0001]

序列表

<110> UCB Pharma S.A.

<120> 针对艰难梭菌的主要外毒素TcdA和TcdB的中和抗体

<130> G0159-WO

<150> US 61/535,532
<151> 2011-09-16

<150> US 61/638,731
<151> 2012-04-26

<160> 194

<170> PatentIn version 3.5

<210> 1
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 1

Gln Ala Ser Gln Ser Ile Ser Asn Ala Leu Ala
1 5 10

<210> 2
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 2

Ser Ala Ser Ser Leu Ala Ser
1 5

<210> 3
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 3

Gln Tyr Thr His Tyr Ser His Thr Ser Lys Asn Pro
1 5 10

<210> 4
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 4

Gly Phe Thr Ile Ser Ser Tyr Tyr Met Ser
1 5 10

<210> 5
<211> 17

[0002]

<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 5

Ile Ile Ser Ser Gly Gly His Phe Thr Trp Tyr Ala Asn Trp Ala Lys
1 5 10 15

Gly

<210> 6
<211> 13
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 6

Ala Tyr Val Ser Gly Ser Ser Phe Asn Gly Tyr Ala Leu
1 5 10

<210> 7
<211> 110
<212> PRT
<213> 人工的

<220>
<223> 抗体可变区

<400> 7

Asp Pro Val Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Ser Asn Ala
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Ser Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Tyr Thr His Tyr Ser His Thr
85 90 95

Ser Lys Asn Pro Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 8
<211> 330
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体922.g1的可变区的多核苷酸

[0003]

<400> 8
gaccctgtga tgacccagag tccgagcact ctttctgcct ccgtgggaga ccgcgtgacc 60

attacatgtc aggcttcaca aagtatctcc aatgctctgg cctggtatca gcagaaaccc 120

ggcaaagccc ctaagctgct catctactct gcatcaagcc tggctagcgg cgtgccaagc 180

cgattcaagg ggagcggttc tggcactgag tttacgctga ccatcagtag cttgcagcct 240

gacgattttg caacctatta ctgccagtac acacactact cccatacatc taaaaaccca 300

ttcggagggg gtactaaggt cgaaataaag 330

<210> 9
<211> 119
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体922的抗体可变区

<400> 9

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Ile Ser Ser Tyr
20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Ile Ile Ser Ser Gly Gly His Phe Thr Trp Tyr Ala Asn Trp Ala
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ser Asp Ser Thr Thr Val Tyr Leu Gln
65 70 75 80

Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg
85 90 95

Ala Tyr Val Ser Gly Ser Ser Phe Asn Gly Tyr Ala Leu Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser
115

<210> 10
<211> 357
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体922的可变区(重链可变区)的多核苷酸

<400> 10
gaagtgcaat tggtggaaag tggcggagga ctggtgcaac ccgggggtag tctgcgactg 60

agctgtgctg cctccggctt taccattagc tcctactata tgagctgggt tcgacaggcc 120

cctggaaaag gactcgaatg gatcggcatc atatcttccg gtgggcattt cacctggtac 180

gcaaactggg ctaaggggag attcacgatt agcagcgact ccacaaccgt gtacctgcaa 240

atgaacagcc tgagggatga ggacactgcc acatatttct gcgcacgcgc ttacgtgagc 300

ggaagctcat ttaatggcta tgcactgtgg gggcaaggaa cactcgtgac tgtctcg 357

[0004]

<210> 11
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 11

Gln Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala
1 5 10

<210> 12
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 12

Ser Ala Ser Thr Leu Ala Ser
1 5

<210> 13
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 13

Gln Tyr Ser His Tyr Gly Thr Gly Val Phe Gly Ala
1 5 10

<210> 14
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 14

Ala Phe Ser Leu Ser Asn Tyr Tyr Met Ser
1 5 10

<210> 15
<211> 18
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 15

Ile Ile Ser Ser Gly Ser Asn Ala Leu Lys Trp Tyr Ala Ser Trp Pro
1 5 10 15

Lys Gly

[0005]

<210> 16
<211> 13
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 16

Asn Tyr Val Gly Ser Gly Ser Tyr Tyr Gly Met Asp Leu
1 5 10

<210> 17
<211> 110
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体923的抗体可变区

<400> 17

Asp Val Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Ser Asn Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Gln Tyr Ser His Tyr Gly Thr Gly
85 90 95

Val Phe Gly Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 18
<211> 330
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体923.g1的抗体可变区的多核苷酸

<400> 18
gacgtcgtga tgactcagag cccatctagt ctgagcgcta gcgtcggaga ccgagtcaca 60

attacctgtc aagcctccca gagcatctcc aactacctgg cctggtacca acagaaacct 120

ggcaaggtgc ccaagctgct gatctatagt gcttccacac tcgcaagcgg cgttccgtca 180

cgctttaagg gatctggctc tggcactcag ttcaccttga cgatctcaag cctgcagcca 240

gaagatgtgg ccacctatta ctgccagtat tcccactacg ggactggggt gttcggtgcc 300

tttggaggtg ggaccaaagt ggagataaag 330

<210> 19
<211> 120

[0006]

<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体923的抗体可变区

<400> 19

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Ala Phe Ser Leu Ser Asn Tyr
20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Ile Ile Ser Ser Gly Ser Asn Ala Leu Lys Trp Tyr Ala Ser Trp
50 55 60

Pro Lys Gly Arg Phe Thr Ile Ser Lys Asp Ser Thr Thr Val Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Thr Tyr Phe Cys Ala
85 90 95

Arg Asn Tyr Val Gly Ser Gly Ser Tyr Tyr Gly Met Asp Leu Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser
115 120

<210> 20
<211> 360
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体923.g1的抗体可变区的多核苷酸

<400> 20
gaagttcaac ttgtggaatc tggaggcggg ctcgtgcagc ctggtggaag ccttagactg 60

agctgcgctg catccgcatt ttccctgtcc aactactaca tgagctgggt gcgacaagca 120

ccaggcaagg gactggaatg gattggcatc ataagctccg gttccaatgc cctgaaatgg 180

tacgcatcat ggccgaaagg ccgctttacc ataagcaagg actccaccac cgtctatctg 240

cagatgaact cattgcgtgc cgaggacact gcaacgtact tctgtgctcg caactacgtg 300

ggaagcggat cttattatgg catggatctg tggggacaag gtacactcgt gaccgtctcg 360

<210> 21
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 21

Gln Ala Ser Gln Ser Ile Ser Ser Tyr Phe Ser
1 5 10

[0007]

<210> 22
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 22

Gly Ala Ser Thr Leu Ala Ser
1 5

<210> 23
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 23

Gln Cys Thr Asp Tyr Ser Gly Ile Tyr Phe Gly Gly
1 5 10

<210> 24
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 24

Gly Phe Ser Leu Ser Ser Tyr Tyr Met Ser
1 5 10

<210> 25
<211> 19
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 25

Ile Ile Ser Ser Gly Ser Ser Thr Thr Phe Thr Trp Tyr Ala Ser Trp
1 5 10 15

Ala Lys Gly

<210> 26
<211> 13
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 26

Ala Tyr Val Gly Ser Ser Ser Tyr Tyr Gly Phe Asp Pro
1 5 10

<210> 27
<211> 110

[0008]

<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体993的抗体可变区

<400> 27

Asp Val Val Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Phe Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Gln Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly
50 55 60

Ser Gly Ser Gly Thr Glu Leu Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Cys Thr Asp Tyr Ser Gly Ile
85 90 95

Tyr Phe Gly Gly Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 28
<211> 330
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体933.g1的抗体可变区的多核苷酸

<400> 28
gatgtcgtga tgactcagtc ccctctaca ttgagtgcct ctgtcggtga tcgagttacc 60

atcacctgtc aagcaagcca gagcatcagc tcctacttct cttggtacca gcaaaagccg 120

ggaaaagccc ctcaactgct gatttatggg gcctcaacac tggcttctgg cgtgccatca 180

agattcaagg gatctggctc cggcactgag cttacactga ccattagctc cctgcaacct 240

gacgattttg ctacctacta ctgccagtgc accgactata gtgggatata tttcggcgga 300

tttgggggag ggacgaaagt ggaaatcaag 330

<210> 29
<211> 121
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体993的抗体可变区（重链）

<400> 29

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Thr Ala Ser Gly Phe Ser Leu Ser Ser Tyr
20 25 30

[0009]

Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Ile Ile Ser Ser Gly Ser Ser Thr Thr Phe Thr Trp Tyr Ala Ser
50 55 60

Trp Ala Lys Gly Arg Phe Thr Ile Ser Lys Thr Ser Thr Thr Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Arg Ala Tyr Val Gly Ser Ser Ser Tyr Tyr Gly Phe Asp Pro Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser
115 120

<210> 30
<211> 363
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体993.g1的抗体可变区的多核苷酸

<400> 30
gaagttcagc tggtcgagag cggaggcgga ctggtgcaac ctggtggtag cctgaaactc 60

tcttgtactg cctccgggtt ttccctgagc tcttactata tgtcatgggt gagacaggct 120

cccgggaaag gattggaatg gatcgggatt atctcctccg gctcttccac cactttcaca 180

tggtacgcct catgggcaaa ggggaggttt accataagca agacaagcac gaccgtgtat 240

cttcagatga actccctgaa gacggaggat actgccacct acttttgcgc tcgggcctat 300

gtgggctcaa gctcttacta tggcttcgac ccatggggac agggcacact tgtgaccgtc 360

tcg 363

<210> 31
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 31

Gln Ala Ser Gln Ser Ile Asn Asn Tyr Phe Ser
1 5 10

<210> 32
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 32

Gly Ala Ala Asn Leu Ala Ser
1 5

[0010]

<210> 33
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 33

Gln Asn Asn Tyr Gly Val His Ile Tyr Gly Ala Ala
1 5 10

<210> 34
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 34

Gly Phe Ser Leu Ser Asn Tyr Asp Met Ile
1 5 10

<210> 35
<211> 16
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 35

Phe Ile Asn Thr Gly Gly Ile Thr Tyr Tyr Ala Ser Trp Ala Lys Gly
1 5 10 15

<210> 36
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 36

Val Asp Asp Tyr Ile Gly Ala Trp Gly Ala Gly Leu
1 5 10

<210> 37
<211> 110
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体995的抗体可变区

<400> 37

Asp Val Val Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Asn Asn Tyr
20 25 30

Phe Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

[0011]

Tyr Gly Ala Ala Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly
50 55 60

Ser Gly Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Ser Cys Gln Asn Asn Tyr Gly Val His Ile
85 90 95

Tyr Gly Ala Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 38
<211> 330
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体995.gl的抗体可变区的多核苷酸

<400> 38
gacgtcgtga tgacacagag cccttcaaca ctgtctgcaa gcgtgggcga tagggtcacc 60

ataacgtgcc aggcctctca atccatcaac aactatttta gctggtacca gcagaagcca 120

ggcaaggctc cgaaacttct gatctacgga gctgccaacc tggcaagtgg cgtgccatca 180

cggttcaagg gatccgggag cggtactgag tataccctga ccatttcatc tctccaaccc 240

gacgatttcg ccacctactc ctgccagaat aattacggcg tgcacatcta tggagctgcc 300

tttggcggtg ggacaaaagt ggaaattaag 330

<210> 39
<211> 117
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体995 的抗体可变区(重链)

<400> 39

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Phe Ser Leu Ser Asn Tyr
20 25 30

Asp Met Ile Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Ile
35 40 45

Gly Phe Ile Asn Thr Gly Gly Ile Thr Tyr Tyr Ala Ser Trp Ala Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Ser Ser Thr Val Tyr Leu Gln Met
65 70 75 80

Asn Ser Leu Arg Ala Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Val
85 90 95

Asp Asp Tyr Ile Gly Ala Trp Gly Ala Gly Leu Trp Gly Gln Gly Thr
100 105 110

[0012]

Leu Val Thr Val Ser
115

<210> 40
<211> 351
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体的抗体可变区的多核苷酸

<400> 40
gaagttcagc tggtcgagag tgggggaggg cttgtgcaac ctggtggctc cctccgtctg 60

agctgtactg cttctggatt ctcactgagc aattacgaca tgatctgggt gcgacaggca 120

cccggcaaag gactggagta cattggcttc atcaacaccg ggggtataac gtactatgcc 180

tcatgggcta aggggcgctt tacaattagt agggattcct ctaccgtgta cctgcagatg 240

aactcactga gagccgagga cactgccaca tatttctgcg ctcgggtgga tgactatatc 300

ggggcctggg gcgccggatt gtggggccaa ggaacactgg tcaccgtctc g 351

<210> 41
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 41

Gln Ala Ser Gln Ser Ile Ser Ser Tyr Leu Ser
1 5 10

<210> 42
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 42

Arg Ala Ser Thr Leu Ala Ser
1 5

<210> 43
<211> 13
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 43

Leu Gly Val Tyr Gly Tyr Ser Asn Asp Asp Gly Ile Ala
1 5 10

<210> 44
<211> 10
<212> PRT
<213> 人工的

<220>

[0013]

<223> 抗体CDR

<400> 44

Gly Ile Asp Leu Ser Ser His His Met Cys
1 5 10

<210> 45
<211> 16
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 45

Val Ile Tyr His Phe Gly Ser Thr Tyr Tyr Ala Asn Trp Ala Thr Gly
1 5 10 15

<210> 46
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 46

Ala Ser Ile Ala Gly Tyr Ser Ala Phe Asp Pro
1 5 10

<210> 47
<211> 111
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体997的抗体可变区

<400> 47

Ala Leu Val Met Thr Gln Ser Pro Ser Ser Phe Ser Ala Ser Thr Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30

Leu Ser Trp Tyr Gln Glu Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Arg Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Cys Leu Gln Ser
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gly Val Tyr Gly Tyr Ser Asn
85 90 95

Asp Asp Gly Ile Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 48
<211> 333

[0014]

<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体997.g1的抗体可变区的多核苷酸

<400> 48
gcactcgtga tgacacagag cccgagtagc tttagtgctt caaccggtga tagggtcact 60

attacttgcc aagcctctca gagtatatct agctatctga gctggtacca gcaaaagccc 120

gggaaggctc ctaaactgct gatctaccgg gcttccacat tggcctccgg cgttccctca 180

cgctttagcg gctccggatc cggaaccgag tacaccctga ctatctcttg cctgcaatct 240

gaggacttcg caacctacta ttgtctgggc gtctacggat atagcaacga tgacgggatc 300

gccttcggcg gcggtaccaa agtggaaatt aag 333

<210> 49
<211> 116
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体997的抗体可变区(重链)

<400> 49

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Val Ser Gly Ile Asp Leu Ser Ser His
20 25 30

His Met Cys Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Ile
35 40 45

Gly Val Ile Tyr His Phe Gly Ser Thr Tyr Tyr Ala Asn Trp Ala Thr
50 55 60

Gly Arg Phe Thr Ile Ser Lys Asp Ser Thr Thr Val Tyr Leu Gln Met
65 70 75 80

Asn Ser Leu Arg Ala Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Ala
85 90 95

Ser Ile Ala Gly Tyr Ser Ala Phe Asp Pro Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser
115

<210> 50
<211> 348
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体997.g1的抗体可变区(重链)的多核苷酸

<400> 50
gaggtgcaac ttgtggaaag cgggggagga ctggtgcagc ctgggggctc attgagactg 60

agctgcaccg tttctggtat tgacctgagc tcccatcata tgtgctgggt gcgccaggca 120

cccggaaaag gactggaata catcggcgtc atataccact ttggctctac atactatgcc 180

[0015]

aactgggcaa ctgggcgatt cacaattagc aaggactcaa ctaccgttta cctgcaaatg 240

aatagcctga gggctgagga tactgccacc tatttctgtg cccgggcttc aatcgccggc 300

tattctgcct ttgatccatg ggggcaagga acactcgtga ccgtctcg 348

<210> 51
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 51

Gln Ala Ser Gln Ser Ile Tyr Ser Tyr Leu Ala
1 5 10

<210> 52
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 52

Asp Ala Ser Thr Leu Ala Ser
1 5

<210> 53
<211> 13
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 53

Gln Gly Asn Ala Tyr Thr Ser Asn Ser His Asp Asn Ala
1 5 10

<210> 54
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 54

Gly Ile Asp Leu Ser Ser Asp Ala Val Gly
1 5 10

<210> 55
<211> 16
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 55

Ile Ile Ala Thr Phe Asp Ser Thr Tyr Tyr Ala Ser Trp Ala Lys Gly
1 5 10 15

[0016]

<210> 56
<211> 19
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 56

Thr Gly Ser Trp Tyr Tyr Ile Ser Gly Trp Gly Ser Tyr Tyr Tyr Gly
1 5 10 15

Met Asp Leu

<210> 57
<211> 111
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体1000的抗体可变区

<400> 57

Glu Ile Val Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Ser Ile Tyr Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gly Asn Ala Tyr Thr Ser Asn
85 90 95

Ser His Asp Asn Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 58
<211> 333
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体1000.g1的抗体可变区的多核苷酸

<400> 58
gaaatcgtga tgacgcagtc accaagcaca ctgagcgctt ctgtgggaga tcgggtcaca 60

ataacctgtc aggcctccca gagcatctac tcttatctgg catggtacca gcagaagcca 120

gggaaagctc ccaagctgct gatttatgac gccagcactt tggcttccgg tgttcctagt 180

aggttcaaag gctccggaag cggtaccgag tttaccctga ccatctcatc tctgcaaccc 240

gatgactttg ccacatacta ttgccagggg aatgcctaca cttccaactc acacgacaac 300

[0017]

gcattcgggg gaggcaccaa agtcgaaatt aag 333

<210> 59
<211> 125
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdA抗体1000的抗体可变区(重链)

<400> 59

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Ile Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Thr Val Ser Gly Ile Asp Leu Ser Ser Asp
20 25 30

Ala Val Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Ile
35 40 45

Gly Ile Ile Ala Thr Phe Asp Ser Thr Tyr Tyr Ala Ser Trp Ala Lys
50 55 60

Gly Arg Phe Thr Ile Ser Lys Ala Ser Ser Thr Thr Val Tyr Leu Gln
65 70 75 80

Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg
85 90 95

Thr Gly Ser Trp Tyr Tyr Ile Ser Gly Trp Gly Ser Tyr Tyr Tyr Gly
100 105 110

Met Asp Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120 125

<210> 60
<211> 375
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdA抗体1000.g1的抗体可变区(重链)的多核苷酸

<400> 60
gaagttcagc tggtcgagag cggaggggat ttgattcagc ccggtggctc acttagattg 60

agctgcaccg tgtccggaat cgatctgtca tctgatgccg tgggctgggt gcgacaggca 120

cctgggaaag gactggagta tatagggatc atcgccacct tcgactccac atactacgct 180

agctgggcaa aagggcgctt tacgattagc aaggcctcct ctactacggt gtacctccaa 240

atgaactcac tgagggccga ggacactgcc acttatttct gtgctcggac cggtagctgg 300

tactacatct ctggctgggg ctcctactat tatggcatgg acctgtgggg acagggacca 360

ctcgtgaccg tctcg 375

<210> 61
<211> 11
<212> PRT
<213> 人工的

<220>

[0018]

<223> 抗体CDR

<400> 61

Arg Ala Ser Lys Ser Val Ser Thr Leu Met His
1 5 10

<210> 62
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 62

Leu Ala Ser Asn Leu Glu Ser
1 5

<210> 63
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 63

Gln Gln Thr Trp Asn Asp Pro Trp Thr
1 5

<210> 64
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 64

Gly Phe Thr Phe Ser Asn Tyr Gly Met Ala
1 5 10

<210> 65
<211> 17
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 65

Ser Ile Ser Ser Ser Gly Gly Ser Thr Tyr Tyr Arg Asp Ser Val Lys
1 5 10 15

Gly

<210> 66
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

[0019]

<400> 66

Val Ile Arg Gly Tyr Val Met Asp Ala
1 5

<210> 67
<211> 107
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体926的抗体可变区

<400> 67

Asp Thr Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Lys Ser Val Ser Thr Leu
20 25 30

Met His Trp Phe Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Leu Ala Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Thr Trp Asn Asp Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 68
<211> 321
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体926.g1的抗体可变区的多核苷酸

<400> 68
gataccgtgc tgacccagag ccctgctaca ttgtcactga gccccgggga gagggccaca 60

ttgagctgcc gggcttcaaa atccgtgtcc accctcatgc actggtttca gcaaaagccc 120

gggcaggccc caaaactgct gatctacctc gcatctaacc ttgaatctgg cgtgccggcc 180

cgctttagtg gctccggaag cggaaccgac ttcacactga cgattagctc cctggagcct 240

gaggatttcg ccgtgtacta ttgccagcaa acttggaatg acccttggac tttcgggggc 300

ggtactaagg tcgaaataaa g 321

<210> 69
<211> 117
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体92 6的抗体可变区(重链)

<400> 69

[0020]

Glu Val Glu Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Glu Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Gly Met Ala Trp Val Arg Gln Ala Pro Thr Lys Gly Leu Glu Trp Val
35 40 45

Thr Ser Ile Ser Ser Ser Gly Gly Ser Thr Tyr Tyr Arg Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Ser Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Thr Tyr Tyr Cys
85 90 95

Thr Thr Val Ile Arg Gly Tyr Val Met Asp Ala Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser
115

<210> 70
<211> 351
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体926.g1的抗体可变区(重链)的多核苷酸

<400> 70
gaggtggaac tgctcgaatc tggtggtggg ctggtgcagc ccggtggatc tctgagattg 60

tcatgcgagg catccggctt tacctttcc aactacggaa tggcctgggt gagacaggcc 120

ccaacgaagg ggctcgaatg ggttacaagc atcagctctt ctgggggatc tacttactat 180

cgcgatagcg tcaaaggccg gtttaccatt agccgagata atgccaaatc aagcctgtat 240

ctgcaaatga acagcctgag ggctgaggac accgccacat actattgtac aaccgtgata 300

aggggctacg tgatggacgc atggggacag gggacattgg ttaccgtctc g 351

<210> 71
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 71

Arg Ala Ser Gly Ser Val Ser Thr Leu Met His
1 5 10

<210> 72
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

[0021]

<400> 72

Lys Ala Ser Asn Leu Ala Ser
1 5

<210> 73
<211> 8
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 73

His Gln Ser Trp Asn Ser Asp Thr
1 5

<210> 74
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 74

Gly Phe Thr Phe Ser Asn Tyr Gly Met Ala
1 5 10

<210> 75
<211> 17
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 75

Thr Ile Asn Tyr Asp Gly Arg Thr Thr His Tyr Arg Asp Ser Val Lys
1 5 10 15

Gly

<210> 76
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 76

Ile Ser Arg Ser His Tyr Phe Asp Cys
1 5

<210> 77
<211> 106
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体927的抗体可变区

<400> 77

[0022]

Asp Thr Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gly Ser Val Ser Thr Leu
20 25 30

Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys His Gln Ser Trp Asn Ser Asp Thr
85 90 95

Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105

<210> 78
<211> 318
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体927.g2的抗体可变区的多核苷酸

<400> 78
gacacacaga tgacccagag cccatccact ttgtctgcat ccgtgggcga ccgagtgacc 60

atcacctgta gagcaagcgg ttccgtgagc acactgatgc attggtacca gcagaagcct 120

gggaaggctc ccaagctgct gatctacaaa gccagcaacc ttgcctccgg cgttccaagc 180

cggtttagcg gttccggatc tggaaccgag ttcaccctga ccatatcaag cctgcaaccc 240

gacgacttcg ccacctacta ttgccaccag agctggaata gcgacacgtt cgggcaaggc 300

acaaggctgg aaatcaaa 318

<210> 79
<211> 117
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体927的抗体可变区(重链)

<400> 79

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Gly Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Thr Ile Asn Tyr Asp Gly Arg Thr Thr His Tyr Arg Asp Ser Val
50 55 60

[0023]

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Ser Ile Ser Arg Ser His Tyr Phe Asp Cys Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser
115

<210> 80
<211> 351
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体927.g2的抗体可变区(重链)的多核苷酸

<400> 80
gaggtgcaac ttgtggaaag cggaggggc gtggtccaac ccggaagaag tctccgtctt 60

tcttgcgccg caagtggctt caccttttcc aactacggaa tggcctgggt tcgacaagct 120

cctgggaaag gattggagtg ggtggccact atcaactatg acggacgcac gacacactac 180

cgagactctg ttaaggggcg ctttacgatt tcccgcgaca atagcaagag caccctctac 240

ctgcaaatga atagcctccg ggccgaggat actgctgtgt actattgtac ctccatctca 300

cggagccact acttcgattg ctggggacaa ggcacactcg tgactgtctc g 351

<210> 81
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 81

Lys Ala Ser Lys Ser Ile Ser Asn His Leu Ala
1 5 10

<210> 82
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 82

Ser Gly Ser Thr Leu Gln Ser
1 5

<210> 83
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 83

[0024]

Gln Gln Tyr Asp Glu Tyr Pro Tyr Thr
1 5

<210> 84
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 84

Gly Phe Ser Leu Gln Ser Tyr Thr Ile Ser
1 5 10

<210> 85
<211> 16
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 85

Ala Ile Ser Gly Gly Gly Ser Thr Tyr Tyr Asn Leu Pro Leu Lys Ser
1 5 10 15

<210> 86
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 86

Pro Arg Trp Tyr Pro Arg Ser Tyr Phe Asp Tyr
1 5 10

<210> 87
<211> 109
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1099的抗体可变区

<400> 87

Asp Val Gln Leu Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Lys Ser Ile Ser Asn His
20 25 30

Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Ala Asn Lys Leu Leu Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Thr Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

[0025]

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Glu Tyr Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr
100 105

<210> 88
<211> 327
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1099.g2的抗体可变区的多核苷酸

<400> 88
gacgtccagc tcactcaatc tccctccttt ctgtctgctt ctgtgggcga tcgcgtgaca 60

ataacctgca aggcctccaa atcaattagc aaccatctgg catggtatca ggagaagcct 120

ggcaaagcca ataagctgct gatccactcc ggctcaactc tgcaatccgg taccccaagc 180

cgatttagcg gatctgggag cggaaccgag ttcacactta ccattagctc cctgcaaccg 240

gaggacttcg ccacctatta ctgccagcaa tacgacgaat acccctatac gttcggccaa 300

gggacaagat tggaaatcaa gcgtacg 327

<210> 89
<211> 118
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1099的抗体可变区(重链)

<400> 89

Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Gln Ser Tyr
20 25 30

Thr Ile Ser Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Ala Ala Ile Ser Gly Gly Gly Ser Thr Tyr Tyr Asn Leu Pro Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Arg Asp Thr Ser Lys Ser Gln Val Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Thr
85 90 95

Arg Pro Arg Trp Tyr Pro Arg Ser Tyr Phe Asp Tyr Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser
115

<210> 90
<211> 354
<212> DNA
<213> 人工的

[0026]

<220>
<223> 编码抗-TcdB抗体1099.g2的抗体可变区(重链)的多核苷酸

<400> 90
gaagttcagc tgcaggaatc tggacctggc ttggtgaaac caagcgagac acttagtctc 60

acttgcaccg tttccggctt ctcccttcaa tcctacacga tctcttgggt gcggcaacca 120

cccgggaaag gactggaatg gatcgcagcc attagcgggg gagggagcac ctattacaac 180

ttgcctctca agagccgcgt gaccatatcc cgtgacacaa gcaagagcca ggtttccctg 240

aagctgagct ccgtgactgc tgccgatacg gctgtttact attgcacccg acctcgctgg 300

tatccccgtt cctatttcga ctactgggga agaggcacac tggttaccgt ctcg 354

<210> 91
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 91

Arg Ala Ser Gln Arg Ile Ser Thr Ser Ile His
1 5 10

<210> 92
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 92

Tyr Ala Ser Gln Ser Ile Ser
1 5

<210> 93
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 93

Gln Gln Ser Tyr Ser Ser Leu Tyr Thr
1 5

<210> 94
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDRs

<400> 94

Gly Phe Thr Phe Ser Asp Ser Tyr Met Ala
1 5 10

<210> 95
<211> 17

[0027]

<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 95

Ser Ile Ser Tyr Gly Gly Thr Ile Ile Gln Tyr Gly Asp Ser Val Lys
1 5 10 15

Gly

<210> 96
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 96

Arg Gln Gly Thr Tyr Ala Arg Tyr Leu Asp Phe
1 5 10

<210> 97
<211> 107
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1102的抗体可变区

<400> 97

Asn Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Ile Ser Thr Ser
20 25 30

Ile His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Lys Tyr Ala Ser Gln Ser Ile Ser Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Ser Leu Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 98
<211> 321
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1102.g4的抗体可变区的多核苷酸

[0028]

<400> 98
aacatcgtgc tgacacagtc tcctgcaacc ctttcactgt ctccaggtga acgagcaacc 60

ctgagttgta gagccagtca gaggatctcc acgagcattc actggtatca gcaaaagcct 120

gggcaagctc ccagactctt gatcaagtac gcctctcaga gcataagtgg cattccagct 180

aggtttagcg gctcaggctc aggaacagac ttcactctga ccatcagctc cctggaaccg 240

gaggactttg ccgtctatta ctgccagcaa tcctactcca gtctgtacac cttcgggcag 300

ggtactaaac tggagataaa g 321

<210> 99
<211> 119
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1102的抗体可变区(重链)

<400> 99

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Thr Phe Ser Asp Ser
20 25 30

Tyr Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Ala Ser Ile Ser Tyr Gly Gly Thr Ile Ile Gln Tyr Gly Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Ser Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Arg Gln Gly Thr Tyr Ala Arg Tyr Leu Asp Phe Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser
115

<210> 100
<211> 357
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1002.g4的抗体可变区(重链)的多核苷酸

<400> 100
gaagtgcagc tggtcgaatc cgggggaggt ttggtgcaac caggtggctc actgagactg 60

agctgtgccg tttccggctt tacgttctca gacagttata tggcctgggt gcgtcaagca 120

cctggaaaag ggctggagtg gattgccagt atcagctatg gtgggaccat aatccagtac 180

ggcgatagcg tcaagggcag gtttactatc tccagggaca acgccaagtc aagcctttac 240

ctgcagatga attctctccg cgcagaggat accgctgtgt attactgcgc tagacggcag 300

[0029]

ggaacctacg ctcgatacct ggacttctgg ggtcagggaa cactcgttac agtctcg 357

<210> 101
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 101

Arg Ala Ser Glu Ser Val Ser Thr Leu Leu His
1 5 10

<210> 102
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 102

Lys Ala Ser Asn Leu Ala Ser
1 5

<210> 103
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 103

His Gln Ser Trp Asn Ser Pro Pro Thr
1 5

<210> 104
<211> 10
<212> PRT
<213> 人工的

<220>
<223> Anitbody CDR

<400> 104

Gly Phe Thr Phe Ser Asn Tyr Gly Met Ala
1 5 10

<210> 105
<211> 17
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 105

Ile Ile Asn Tyr Asp Ala Ser Thr Thr His Tyr Arg Asp Ser Val Lys
1 5 10 15

Gly

[0030]

<210> 106
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 106

Tyr Gly Arg Ser His Tyr Phe Asp Tyr
1 5

<210> 107
<211> 107
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1114的抗体可变区

<400> 107

Ala Thr Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Ser Thr Leu
20 25 30

Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Ser Trp Asn Ser Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 108
<211> 321
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1114.g2的抗体可变区的多核苷酸

<400> 108
gcgacgcaaa tgactcagtc gccctcatcg cttagcgcgt ccgtcggaga tagagtgacg 60

atcacctgcc gcgcatcaga gtcggtgtcc acactcctcc actggtatca gcagaaaccg 120

gggaaggcac caaaactctt gatctacaaa gccagcaacc ttgcgtccgg tgtcccgtca 180

aggttctccg ggagcggttc ggggacagac tttactttga ccatttcgtc gcttcagccg 240

gaggacttcg ccacctatta ctgtcatcag tcatggaact cacctcccac atttggccag 300

ggaacgaaac tcgaaatcaa g 321

<210> 109

[0031]

<211> 117
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1114的抗体可变区(重链)

<400> 109

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Gly Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ile Ile Asn Tyr Asp Ala Ser Thr Thr His Tyr Arg Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Ser Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Arg Tyr Gly Arg Ser His Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser
115

<210> 110
<211> 351
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1114.g2的抗体可变区(重链)的多核苷酸

<400> 110
gaagtacaac tcgtagagtc aggggtggg ctggtccaac ctggcggctc ccttcggctt 60

tcgtgtgccg cctcgggatt cacgtttagc aattacggta tggcctgggt gaggcaggca 120

ccagggaagg gtcttgagtg ggtagcgatc atcaactatg atgcaagcac cacccactac 180

agggatagcg tcaagggacg ctttactatc agccgggata atgcgaaatc ctcgctctat 240

ctgcagatga actccctcag agccgaggac accgcagtgt actattgcac acgatacgga 300

cgctcgcact atttcgacta ttggggacag gggacgctcg taactgtctc g 351

<210> 111
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 111

Arg Ala Ser Glu Ser Val Ser Thr Leu Leu His
1 5 10

[0032]

<210> 112
<211> 7
<212> PRT
<213> 人工的

<220>
<223> Anitbody CDR

<400> 112

Lys Ala Ser Asn Leu Ala Ser
1 5

<210> 113
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 113

His Gln Ser Trp Asn Ser Pro Pro Thr
1 5

<210> 114
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 114

Gly Phe Thr Phe Ser Asn Tyr Gly Met Ala
1 5 10

<210> 115
<211> 16
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 115

Ile Ile Asn Tyr Asp Ala Ser Thr Thr His Tyr Arg Asp Ser Val Lys
1 5 10 15

<210> 116
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 116

Tyr Gly Arg Ser His Tyr Phe Asp Tyr
1 5

<210> 117
<211> 107
<212> PRT
<213> 人工的

[0033]

<220>
<223> 抗-TcdB抗体1114 graft 8的抗体可变区

<400> 117

Asp Thr Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ser Val Ser Thr Leu
20 25 30

Leu His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Lys Ala Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys His Gln Ser Trp Asn Ser Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 118
<211> 321
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1114.g8的抗体可变区的多核苷酸

<400> 118
gacacggtcc tgactcagtc gccctcatcg cttagcgcgt ccgtcggaga tagagtgacg 60

atcacctgcc gcgcatcaga gtcggtgtcc acactcctcc actggtatca gcagaaaccg 120

gggaaggcac caaaactctt gatctacaaa gccagcaacc ttgcgtccgg tgtcccgtca 180

aggttctccg ggagcggttc ggggacagac tttactttga ccatttcgtc gcttcagccg 240

gaggacttcg ccacctatta ctgtcatcag tcatggaact cacctcccac atttggccag 300

ggnacgnaac tcgnaatcaa g 321

<210> 119
<211> 117
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1114 graft 8的抗体可变区(重链)

<400> 119

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

Gly Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

[0034]

Ala Ile Ile Asn Tyr Asp Ala Ser Thr Thr His Tyr Arg Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Ser Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Arg Tyr Gly Arg Ser His Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser
115

<210> 120
<211> 351
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1114.g8的抗体可变区的多核苷酸

<400> 120
gaagtacaac tcgtagagtc aggggtgggg ctggtccaac ctggcggctc ccttcggctt 60

tcgtgtgccg cctcgggatt cacgtttagc aattacggta tggcctgggt gaggcaggca 120

ccagggaagg gtcttgagtg ggtagcgatc atcaactatg atgcaagcac cacccactac 180

agggatagcg tcaagggacg ctttactatc agccgggata atgcgaaatc ctcgctctat 240

ctgcagatga actccctcag agccgaggac accgcagtgt actattgcac acgatacgga 300

cgctcgcact atttcgacta ttggggacag gggacgctcg taactgtctc g 351

<210> 121
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 121

Lys Ala Ser Gln Asn Ile Tyr Met Tyr Leu Asn
1 5 10

<210> 122
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 122

Asn Thr Asn Lys Leu His Thr
1 5

<210> 123
<211> 9
<212> PRT
<213> 人工的

[0035]

<220>
<223> 抗体CDR

<400> 123

Leu Gln His Lys Ser Phe Pro Tyr Thr
1 5

<210> 124
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 124

Gly Phe Thr Phe Arg Asp Ser Phe Met Ala
1 5 10

<210> 125
<211> 17
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 125

Ser Ile Ser Tyr Glu Gly Asp Lys Thr Tyr Tyr Gly Asp Ser Val Lys
1 5 10 15

Gly

<210> 126
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 126

Leu Thr Ile Thr Thr Ser Gly Asp Ser
1 5

<210> 127
<211> 107
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1125的抗体可变区

<400> 127

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Ile Tyr Met Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

[0036]

Tyr Asn Thr Asn Lys Leu His Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Lys Ser Phe Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 128
<211> 321
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1125.g2的抗体可变区的多核苷酸

<400> 128
gatatacaaa tgactcagag ccctagctca ctgagcgctt ctgtgggcga tcgtgtgaca 60

atcacttgca aagcaagcca gaacatctat atgtacctga attggtacca gcaaaaaccg 120

ggaaaagctc ccaagcgcct gatttacaac accaataagc tgcataccgg cgtgccaagc 180

cgttttagcg gatctggctc tggaaccgaa tatacactga ccataagctc cctgcaaccg 240

gaagactttg caacttacta ttgcctccag cacaaatcct tccctatac gttcggacaa 300

gggaccaaac tggaaatcaa a 321

<210> 129
<211> 118
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1125的抗体可变区(重链)

<400> 129

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asp Ser
20 25 30

Phe Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Tyr Glu Gly Asp Lys Thr Tyr Tyr Gly Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Leu Thr Ile Thr Thr Ser Gly Asp Ser Trp Gly Gln Gly Thr
100 105 110

[0037]

Met Val Thr Val Ser Ser
115

<210> 130
<211> 354
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1125.g2的抗体可变区(重链)的多核苷酸

<400> 130
gaagtgcagc tggtcgaaag cggcggagga ttggtgcaac ctggtggctc tcttcgcctg 60

tcttgcgctg caagcggctt tacgttccgc gatagcttta tggcttgggt gcgacaagct 120

cctgggaaag ggctggaatg ggtcgctagc ataagctacg aaggcgacaa gacttactat 180

ggggactctg tgaaaggccg attcaccatt agccgagaca acgcaaagaa ctccctgtac 240

ctgcagatga actccctgcg tgccgaagat accgccgtgt actattgcgc taggctgacg 300

atcactacaa gcggagatag ctggggacaa gggacaatgg tgaccgtctc gagc 354

<210> 131
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 131

Lys Ala Ser Gln His Val Gly Thr Asn Val Asp
1 5 10

<210> 132
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 132

Gly Ala Ser Ile Arg Tyr Thr
1 5

<210> 133
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 133

Leu Gln Tyr Asn Tyr Asn Pro Tyr Thr
1 5

<210> 134
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

[0038]

<400> 134

Gly Phe Ile Phe Ser Asn Phe Gly Met Ser
1 5 10

<210> 135
<211> 17
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 135

Ser Ile Ser Pro Ser Gly Gly Asn Ala Tyr Tyr Arg Asp Ser Val Lys
1 5 10 15

Gly

<210> 136
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 136

Arg Ala Tyr Ser Ser Pro Phe Ala Phe
1 5

<210> 137
<211> 107
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1129的抗体可变区

<400> 137

Asp Thr Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln His Val Gly Thr Asn
20 25 30

Val Asp Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Ile Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Leu Gln Tyr Asn Tyr Asn Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

[0039]

<210> 138
<211> 321
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1129.g1的抗体可变区的多核苷酸

<400> 138
gacacccaga tgactcagtc tccgtcaagc ctttctgcct ctgttggaga tcgagtcaca 60

attacgtgca aggcaagcca acacgtgggt accaacgtgg actggtatca acagaagcca 120

gggaaggtcc ccaaactgct gatctacggt gccagtattc gctataccgg cgtgcctgat 180

cgcttcaccg gaagcgggtc agggaccgat ttcacactga caatcagctc cctgcaacct 240

gaagacgtgg ctacttacta ctgcctgcag tacaactata atccctacac ctttggccag 300

ggcaccaaac tggagataaa g 321

<210> 139
<211> 118
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1129的抗体可变区(重链)

<400> 139

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Ile Phe Ser Asn Phe
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Ser Ile Ser Pro Ser Gly Gly Asn Ala Tyr Tyr Arg Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Thr Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Arg Arg Ala Tyr Ser Ser Pro Phe Ala Phe Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 140
<211> 354
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1129.g1的抗体可变区(重链)的多核苷酸

<400> 140
gaggtgcaac ttgtggaatc aggaggtggc gtggttcagc ccggtagatc acttcgtctg 60

[0040]

agttgtgcaa caagcggctt tatcttctcc aacttcggga tgtcttgggt tagacaggct 120

cctggtaagg gcctcgaatg ggtggctagt attagcccaa gcgggggaaa cgcctactat 180

agggacagcg tgaaaggacg cttcactatc agccgagata actccaagac cacgctgtat 240

ctgcagatga atagtctgag ggccgaggat accgcagtgt actactgcac tcgacgggcc 300

tattcttccc ctttttgcctt ttggggacag gggactctgg tgacagtctc gagc 354

<210> 141
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 141

Lys Ala Ser Lys Ser Ile Ser Asn His Leu Ala
1 5 10

<210> 142
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 142

Ser Gly Ser Thr Leu Gln Pro
1 5

<210> 143
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 143

Gln Gln Tyr Asp Glu Tyr Pro Tyr Thr
1 5

<210> 144
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 144

Gly Phe Ser Leu Asn Ser Tyr Thr Ile Thr
1 5 10

<210> 145
<211> 16
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 145

[0041]

Ala Ile Ser Gly Gly Gly Ser Thr Tyr Phe Asn Ser Ala Leu Lys Ser
1 5 10 15

<210> 146
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 146

Pro Arg Trp Tyr Pro Arg Ser Tyr Phe Asp Tyr
1 5 10

<210> 147
<211> 107
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1134的抗体可变区

<400> 147

Asp Val Gln Leu Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Lys Ser Ile Ser Asn His
20 25 30

Leu Ala Trp Tyr Gln Glu Lys Pro Gly Lys Ala Asn Lys Leu Leu Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Pro Gly Thr Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Glu Tyr Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105

<210> 148
<211> 321
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1134.g5的抗体可变区的多核苷酸

<400> 148
gacgtccagc tcactcaatc tccctccttt ctgtctgctt ctgtgggcga tcgcgtgaca 60

ataacctgca aggcctccaa atcaattagc aaccatctgg catggtatca ggagaagcct 120

ggcaaagcca ataagctgct gatccactcc ggctcaactc tgcaacccgg taccccaagc 180

cgatttagcg gatctgggag cggaaccgag ttcacactta ccattagctc cctgcaaccg 240

gaggacttcg ccacctatta ctgccagcaa tacgacgaat accctatac gttcggccaa 300

[0042]

gggacaagat tggaaatcaa g 321

<210> 149
<211> 118
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1134的抗体可变区(重链)

<400> 149

Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Asn Ser Tyr
20 25 30

Thr Ile Thr Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Ala Ala Ile Ser Gly Gly Gly Ser Thr Tyr Phe Asn Ser Ala Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Arg Asp Thr Ser Lys Ser Gln Val Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Thr
85 90 95

Arg Pro Arg Trp Tyr Pro Arg Ser Tyr Phe Asp Tyr Trp Gly Arg Gly
100 105 110

Thr Leu Val Thr Val Ser
115

<210> 150
<211> 354
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1134.g5的抗体可变区(重链)的多核苷酸
<400> 150
gaagttcagc tgcaggaatc tggacctggc ttggtgaaac caagcgagac acttagtctc 60

acttgcaccg tttccggctt ctcccttaat tcctacacga tcacttgggt gcggcaacca 120

cccgggaaag gactggaatg gatcgcagcc attagcgggg gagggagcac ctatttcaac 180

tcggctctca agagccgcgt gaccatatcc cgtgacacaa gcaagagcca ggtttccctg 240

aagctgagct ccgtgactgc tgccgatacg gctgtttact attgcacccg acctcgctgg 300

tatccccgtt cctatttcga ctactgggga agaggcacac tggttacgct ctcg 354

<210> 151
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 151

[0043]

Lys Ala Ser Gln Asn Val Gly Asn Asn Val Ala
1 5 10

<210> 152
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 152

Tyr Ala Ser Asn Arg Phe Thr
1 5

<210> 153
<211> 9
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 153

Gln Arg Val Tyr Gln Ser Thr Trp Thr
1 5

<210> 154
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 154

Gly Phe Ser Leu Thr Ser Tyr Tyr Val His
1 5 10

<210> 155
<211> 16
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 155

Cys Ile Arg Thr Gly Gly Asn Thr Glu Tyr Gln Ser Glu Phe Lys Ser
1 5 10 15

<210> 156
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 156

Gly Asn Tyr Gly Phe Ala Tyr
1 5

<210> 157
<211> 107

[0044]

<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1151的抗体可变区

<400> 157

Ala Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Gly Asn Asn
20 25 30

Val Ala Trp Tyr Gln His Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Tyr Ala Ser Asn Arg Phe Thr Gly Val Pro Ser Arg Phe Thr Gly
50 55 60

Gly Gly Tyr Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Arg Val Tyr Gln Ser Thr Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 158
<211> 321
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1151.g1的抗体可变区的多核苷酸

<400> 158
gcgattcaaa tgactcagtc gccctcatcg cttagcgcgt ccgtcggaga tagagtgacg 60

atcacgtgca aagcatcaca aaatgtcggg aacaatgtgg catggtatca gcataaaccg 120

gggaaggcac caaaactctt gatctactac gccagcaaca ggtttactgg tgtcccgtca 180

aggttcacgg gaggggtta cgggacagac tttactttga ccatttcgtc gcttcagccg 240

gaggacttcg ccacctatta ctgtcagagg gtctaccagt caacgtggac atttggccag 300

ggaacgaaag tggaaatcaa g 321

<210> 159
<211> 114
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1151的抗体可变区(重链)

<400> 159

Glu Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Ser Tyr
20 25 30

[0045]

Tyr Val His Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Cys Ile Arg Thr Gly Gly Asn Thr Glu Tyr Gln Ser Glu Phe Lys
50 55 60

Ser Arg Val Thr Ile Ser Arg Asp Thr Ser Lys Asn Gln Val Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Gly Asn Tyr Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser

<210> 160
<211> 342
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1151.g4的抗体可变区(重链)的多核苷酸

<400> 160
gaagtacaac tccaagagtc ggggcctggt ctggtcaagc cgtccgaaac actttcgctg 60

acgtgtacgg tatcaggatt ctcacttaca tcatactacg tccactgggt gaggcagcca 120

cccgggaagg gtcttgagtg gatgggctgc attagaaccg gagggaatac cgagtaccag 180

agcgaattta agagccgcgt cactatcagc cgggatacgt ccaaaaacca ggtgtcgctc 240

aaattgtcct ccgtgacggc cgctgacacc gcagtgtact attgcgcgcg aggaaactat 300

ggctttgcgt attggggaca ggggacgctc gtaactgtct cg 342

<210> 161
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 161

Lys Ala Ser Gln Asn Ile Asn Lys Tyr Leu Asp
1 5 10

<210> 162
<211> 7
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 162

Asn Ile Gln Ser Leu His Thr
1 5

<210> 163
<211> 7

[0046]

<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 163

Phe Gln His Asn Ser Gly Trp
1 5

<210> 164
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 164

Gly Phe Thr Phe Thr Gln Ala Ala Met Phe
1 5 10

<210> 165
<211> 19
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 165

Arg Ile Ser Thr Lys Ser Asn Asn Phe Ala Thr Tyr Tyr Pro Asp Ser
1 5 10 15

Val Lys Gly

<210> 166
<211> 13
<212> PRT
<213> 人工的

<220>
<223> 抗体CDR

<400> 166

Pro Ala Tyr Tyr Tyr Asp Gly Thr Val Pro Phe Ala Tyr
1 5 10

<210> 167
<211> 106
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1153.g8的抗体可变区

<400> 167

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Ile Asn Lys Tyr
20 25 30

[0047]

Leu Asp Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile
35 40 45

Tyr Asn Ile Gln Ser Leu His Thr Gly Ile Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Val Ala Thr Tyr Tyr Cys Phe Gln His Asn Ser Gly Trp Thr
85 90 95

Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
100 105

<210> 168
<211> 318
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1153.g8的抗体可变区的多核苷酸

<400> 168
gatatacaga tgactcagtc ccctctagc ctttcagctt ccgtgggcga tagagtgact 60

atcacgtgta aggctagtca gaacattaac aagtatctgg actggtacca gcagaaaccc 120

gggaaggttc ccaagctgct gatctacaac atccagtccc tgcatacagg cattcctagc 180

cggtttagcg gatctggttc agggaccgac ttcaccctga caatcagctc tctgcaacca 240

gaagacgtgg ccacctatta ctgcttccag cacaatagtg gctggacttt tggacaaggt 300

accaggctgg agatcaaa 318

<210> 169
<211> 123
<212> PRT
<213> 人工的

<220>
<223> 抗-TcdB抗体1153的抗体可变区(graft 8
重链)

<400> 169

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Gln Ala
20 25 30

Ala Met Phe Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Gly Ile
35 40 45

Ala Arg Ile Ser Thr Lys Ser Asn Asn Phe Ala Thr Tyr Tyr Pro Asp
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80

Val Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

[0048]

Tyr Cys Thr Ala Pro Ala Tyr Tyr Tyr Asp Gly Thr Val Pro Phe Ala
100 105 110

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
115 120

<210> 170
<211> 369
<212> DNA
<213> 人工的

<220>
<223> 编码抗-TcdB抗体1163.g8的抗体可变区(重链)的多核苷酸

<400> 170
gaggttcagc tggtggaatc aggaggggt ctggtgcaac caggaggctc cctgaaactg 60

tcttgcgccg caagcggctt tacgtttacc caggccgcta tgttctgggt taggcaggcc 120

agtgggaagg gtcttgaagg catcgcaaga atcagcacca agagcaacaa tttcgctacg 180

tactatccgg actccgtgaa aggccggttt accatttctc gcgatgacag caagaacacc 240

gtgtacctgc agatgaacag tctcaagacc gaggacacag ccgtgtacta ttgtactgct 300

cccgcctatt attacgatgg cacagtgcct ttcgcatact ggggacaggg tactttggtg 360

actgtctcg 369

<210> 171
<211> 2710
<212> PRT
<213> 梭状芽胞杆菌

<400> 171

Met Ser Leu Ile Ser Lys Glu Glu Leu Ile Lys Leu Ala Tyr Ser Ile
1 5 10 15

Arg Pro Arg Glu Asn Glu Tyr Lys Thr Ile Leu Thr Asn Leu Asp Glu
20 25 30

Tyr Asn Lys Leu Thr Thr Asn Asn Asn Glu Asn Lys Tyr Leu Gln Leu
35 40 45

Lys Lys Leu Asn Glu Ser Ile Asp Val Phe Met Asn Lys Tyr Lys Thr
50 55 60

Ser Ser Arg Asn Arg Ala Leu Ser Asn Leu Lys Lys Asp Ile Leu Lys
65 70 75 80

Glu Val Ile Leu Ile Lys Asn Ser Asn Thr Ser Pro Val Glu Lys Asn
85 90 95

Leu His Phe Val Trp Ile Gly Gly Glu Val Ser Asp Ile Ala Leu Glu
100 105 110

Tyr Ile Lys Gln Trp Ala Asp Ile Asn Ala Glu Tyr Asn Ile Lys Leu
115 120 125

Trp Tyr Asp Ser Glu Ala Phe Leu Val Asn Thr Leu Lys Lys Ala Ile
130 135 140

[0049]

Val Glu Ser Ser Thr Thr Glu Ala Leu Gln Leu Leu Glu Glu Glu Ile
145 150 155 160

Gln Asn Pro Gln Phe Asp Asn Met Lys Phe Tyr Lys Lys Arg Met Glu
165 170 175

Phe Ile Tyr Asp Arg Gln Lys Arg Phe Ile Asn Tyr Tyr Lys Ser Gln
180 185 190

Ile Asn Lys Pro Thr Val Pro Thr Ile Asp Asp Ile Ile Lys Ser His
195 200 205

Leu Val Ser Glu Tyr Asn Arg Asp Glu Thr Val Leu Glu Ser Tyr Arg
210 215 220

Thr Asn Ser Leu Arg Lys Ile Asn Ser Asn His Gly Ile Asp Ile Arg
225 230 235 240

Ala Asn Ser Leu Phe Thr Glu Gln Glu Leu Leu Asn Ile Tyr Ser Gln
245 250 255

Glu Leu Leu Asn Arg Gly Asn Leu Ala Ala Ala Ser Asp Ile Val Arg
260 265 270

Leu Leu Ala Leu Lys Asn Phe Gly Gly Val Tyr Leu Asp Val Asp Met
275 280 285

Leu Pro Gly Ile His Ser Asp Leu Phe Lys Thr Ile Ser Arg Pro Ser
290 295 300

Ser Ile Gly Leu Asp Arg Trp Glu Met Ile Lys Leu Glu Ala Ile Met
305 310 315 320

Lys Tyr Lys Lys Tyr Ile Asn Asn Tyr Thr Ser Glu Asn Phe Asp Lys
325 330 335

Leu Asp Gln Gln Leu Lys Asp Asn Phe Lys Leu Ile Ile Glu Ser Lys
340 345 350

Ser Glu Lys Ser Glu Ile Phe Ser Lys Leu Glu Asn Leu Asn Val Ser
355 360 365

Asp Leu Glu Ile Lys Ile Ala Phe Ala Leu Gly Ser Val Ile Asn Gln
370 375 380

Ala Leu Ile Ser Lys Gln Gly Ser Tyr Leu Thr Asn Leu Val Ile Glu
385 390 395 400

Gln Val Lys Asn Arg Tyr Gln Phe Leu Asn Gln His Leu Asn Pro Ala
405 410 415

Ile Glu Ser Asp Asn Asn Phe Thr Asp Thr Thr Lys Ile Phe His Asp
420 425 430

Ser Leu Phe Asn Ser Ala Thr Ala Glu Asn Ser Met Phe Leu Thr Lys
435 440 445

[0050]

Ile Ala Pro Tyr Leu Gln Val Gly Phe Met Pro Glu Ala Arg Ser Thr
450 455 460

Ile Ser Leu Ser Gly Pro Gly Ala Tyr Ala Ser Ala Tyr Tyr Asp Phe
465 470 475 480

Ile Asn Leu Gln Glu Asn Thr Ile Glu Lys Thr Leu Lys Ala Ser Asp
485 490 495

Leu Ile Glu Phe Lys Phe Pro Glu Asn Asn Leu Ser Gln Leu Thr Glu
500 505 510

Gln Glu Ile Asn Ser Leu Trp Ser Phe Asp Gln Ala Ser Ala Lys Tyr
515 520 525

Gln Phe Glu Lys Tyr Val Arg Asp Tyr Thr Gly Gly Ser Leu Ser Glu
530 535 540

Asp Asn Gly Val Asp Phe Asn Lys Asn Thr Ala Leu Asp Lys Asn Tyr
545 550 555 560

Leu Leu Asn Asn Lys Ile Pro Ser Asn Asn Val Glu Glu Ala Gly Ser
565 570 575

Lys Asn Tyr Val His Tyr Ile Ile Gln Leu Gln Gly Asp Asp Ile Ser
580 585 590

Tyr Glu Ala Thr Cys Asn Leu Phe Ser Lys Asn Pro Lys Asn Ser Ile
595 600 605

Ile Ile Gln Arg Asn Met Asn Glu Ser Ala Lys Ser Tyr Phe Leu Ser
610 615 620

Asp Asp Gly Glu Ser Ile Leu Glu Leu Asn Lys Tyr Arg Ile Pro Glu
625 630 635 640

Arg Leu Lys Asn Lys Glu Lys Val Lys Val Thr Phe Ile Gly His Gly
645 650 655

Lys Asp Glu Phe Asn Thr Ser Glu Phe Ala Arg Leu Ser Val Asp Ser
660 665 670

Leu Ser Asn Glu Ile Ser Ser Phe Leu Asp Thr Ile Lys Leu Asp Ile
675 680 685

Ser Pro Lys Asn Val Glu Val Asn Leu Leu Gly Cys Asn Met Phe Ser
690 695 700

Tyr Asp Phe Asn Val Glu Glu Thr Tyr Pro Gly Lys Leu Leu Leu Ser
705 710 715 720

Ile Met Asp Lys Ile Thr Ser Thr Leu Pro Asp Val Asn Lys Asn Ser
725 730 735

Ile Thr Ile Gly Ala Asn Gln Tyr Glu Val Arg Ile Asn Ser Glu Gly
740 745 750

[0051]

Arg Lys Glu Leu Leu Ala His Ser Gly Lys Trp Ile Asn Lys Glu Glu
755 760 765

Ala Ile Met Ser Asp Leu Ser Ser Lys Glu Tyr Ile Phe Phe Asp Ser
770 775 780

Ile Asp Asn Lys Leu Lys Ala Lys Ser Lys Asn Ile Pro Gly Leu Ala
785 790 795 800

Ser Ile Ser Glu Asp Ile Lys Thr Leu Leu Leu Asp Ala Ser Val Ser
805 810 815

Pro Asp Thr Lys Phe Ile Leu Asn Asn Leu Lys Leu Asn Ile Glu Ser
820 825 830

Ser Ile Gly Asp Tyr Ile Tyr Tyr Glu Lys Leu Glu Pro Val Lys Asn
835 840 845

Ile Ile His Asn Ser Ile Asp Asp Leu Ile Asp Glu Phe Asn Leu Leu
850 855 860

Glu Asn Val Ser Asp Glu Leu Tyr Glu Leu Lys Lys Leu Asn Asn Leu
865 870 875 880

Asp Glu Lys Tyr Leu Ile Ser Phe Glu Asp Ile Ser Lys Asn Asn Ser
885 890 895

Thr Tyr Ser Val Arg Phe Ile Asn Lys Ser Asn Gly Glu Ser Val Tyr
900 905 910

Val Glu Thr Glu Lys Glu Ile Phe Ser Lys Tyr Ser Glu His Ile Thr
915 920 925

Lys Glu Ile Ser Thr Ile Lys Asn Ser Ile Ile Thr Asp Val Asn Gly
930 935 940

Asn Leu Leu Asp Asn Ile Gln Leu Asp His Thr Ser Gln Val Asn Thr
945 950 955 960

Leu Asn Ala Ala Phe Phe Ile Gln Ser Leu Ile Asp Tyr Ser Ser Asn
965 970 975

Lys Asp Val Leu Asn Asp Leu Ser Thr Ser Val Lys Val Gln Leu Tyr
980 985 990

Ala Gln Leu Phe Ser Thr Gly Leu Asn Thr Ile Tyr Asp Ser Ile Gln
995 1000 1005

Leu Val Asn Leu Ile Ser Asn Ala Val Asn Asp Thr Ile Asn Val
1010 1015 1020

Leu Pro Thr Ile Thr Glu Gly Ile Pro Ile Val Ser Thr Ile Leu
1025 1030 1035

Asp Gly Ile Asn Leu Gly Ala Ala Ile Lys Glu Leu Leu Asp Glu
1040 1045 1050

[0052]

His Asp Pro Leu Leu Lys Lys Glu Leu Glu Ala Lys Val Gly Val
1055 1060 1065

Leu Ala Ile Asn Met Ser Leu Ser Ile Ala Ala Thr Val Ala Ser
1070 1075 1080

Ile Val Gly Ile Gly Ala Glu Val Thr Ile Phe Leu Leu Pro Ile
1085 1090 1095

Ala Gly Ile Ser Ala Gly Ile Pro Ser Leu Val Asn Asn Glu Leu
1100 1105 1110

Ile Leu His Asp Lys Ala Thr Ser Val Val Asn Tyr Phe Asn His
1115 1120 1125

Leu Ser Glu Ser Lys Lys Tyr Gly Pro Leu Lys Thr Glu Asp Asp
1130 1135 1140

Lys Ile Leu Val Pro Ile Asp Asp Leu Val Ile Ser Glu Ile Asp
1145 1150 1155

Phe Asn Asn Asn Ser Ile Lys Leu Gly Thr Cys Asn Ile Leu Ala
1160 1165 1170

Met Glu Gly Gly Ser Gly His Thr Val Thr Gly Asn Ile Asp His
1175 1180 1185

Phe Phe Ser Ser Pro Ser Ile Ser Ser His Ile Pro Ser Leu Ser
1190 1195 1200

Ile Tyr Ser Ala Ile Gly Ile Glu Thr Glu Asn Leu Asp Phe Ser
1205 1210 1215

Lys Lys Ile Met Met Leu Pro Asn Ala Pro Ser Arg Val Phe Trp
1220 1225 1230

Trp Glu Thr Gly Ala Val Pro Gly Leu Arg Ser Leu Glu Asn Asp
1235 1240 1245

Gly Thr Arg Leu Leu Asp Ser Ile Arg Asp Leu Tyr Pro Gly Lys
1250 1255 1260

Phe Tyr Trp Arg Phe Tyr Ala Phe Phe Asp Tyr Ala Ile Thr Thr
1265 1270 1275

Leu Lys Pro Val Tyr Glu Asp Thr Asn Ile Lys Ile Lys Leu Asp
1280 1285 1290

Lys Asp Thr Arg Asn Phe Ile Met Pro Thr Ile Thr Thr Asn Glu
1295 1300 1305

Ile Arg Asn Lys Leu Ser Tyr Ser Phe Asp Gly Ala Gly Gly Thr
1310 1315 1320

Tyr Ser Leu Leu Leu Ser Ser Tyr Pro Ile Ser Thr Asn Ile Asn
1325 1330 1335

[0053]

Leu Ser Lys Asp Asp Leu Trp Ile Phe Asn Ile Asp Asn Glu Val
1340 1345 1350

Arg Glu Ile Ser Ile Glu Asn Gly Thr Ile Lys Lys Gly Lys Leu
1355 1360 1365

Ile Lys Asp Val Leu Ser Lys Ile Asp Ile Asn Lys Asn Lys Leu
1370 1375 1380

Ile Ile Gly Asn Gln Thr Ile Asp Phe Ser Gly Asp Ile Asp Asn
1385 1390 1395

Lys Asp Arg Tyr Ile Phe Leu Thr Cys Glu Leu Asp Asp Lys Ile
1400 1405 1410

Ser Leu Ile Ile Glu Ile Asn Leu Val Ala Lys Ser Tyr Ser Leu
1415 1420 1425

Leu Leu Ser Gly Asp Lys Asn Tyr Leu Ile Ser Asn Leu Ser Asn
1430 1435 1440

Thr Ile Glu Lys Ile Asn Thr Leu Gly Leu Asp Ser Lys Asn Ile
1445 1450 1455

Ala Tyr Asn Tyr Thr Asp Glu Ser Asn Asn Lys Tyr Phe Gly Ala
1460 1465 1470

Ile Ser Lys Thr Ser Gln Lys Ser Ile Ile His Tyr Lys Lys Asp
1475 1480 1485

Ser Lys Asn Ile Leu Glu Phe Tyr Asn Asp Ser Thr Leu Glu Phe
1490 1495 1500

Asn Ser Lys Asp Phe Ile Ala Glu Asp Ile Asn Val Phe Met Lys
1505 1510 1515

Asp Asp Ile Asn Thr Ile Thr Gly Lys Tyr Tyr Val Asp Asn Asn
1520 1525 1530

Thr Asp Lys Ser Ile Asp Phe Ser Ile Ser Leu Val Ser Lys Asn
1535 1540 1545

Gln Val Lys Val Asn Gly Leu Tyr Leu Asn Glu Ser Val Tyr Ser
1550 1555 1560

Ser Tyr Leu Asp Phe Val Lys Asn Ser Asp Gly His His Asn Thr
1565 1570 1575

Ser Asn Phe Met Asn Leu Phe Leu Asp Asn Ile Ser Phe Trp Lys
1580 1585 1590

Leu Phe Gly Phe Glu Asn Ile Asn Phe Val Ile Asp Lys Tyr Phe
1595 1600 1605

Thr Leu Val Gly Lys Thr Asn Leu Gly Tyr Val Glu Phe Ile Cys
1610 1615 1620

[0054]

Asp Asn Asn Lys Asn Ile Asp Ile Tyr Phe Gly Glu Trp Lys Thr
1625 1630 1635

Ser Ser Ser Lys Ser Thr Ile Phe Ser Gly Asn Gly Arg Asn Val
1640 1645 1650

Val Val Glu Pro Ile Tyr Asn Pro Asp Thr Gly Glu Asp Ile Ser
1655 1660 1665

Thr Ser Leu Asp Phe Ser Tyr Glu Pro Leu Tyr Gly Ile Asp Arg
1670 1675 1680

Tyr Ile Asn Lys Val Leu Ile Ala Pro Asp Leu Tyr Thr Ser Leu
1685 1690 1695

Ile Asn Ile Asn Thr Asn Tyr Tyr Ser Asn Glu Tyr Tyr Pro Glu
1700 1705 1710

Ile Ile Val Leu Asn Pro Asn Thr Phe His Lys Lys Val Asn Ile
1715 1720 1725

Asn Leu Asp Ser Ser Ser Phe Glu Tyr Lys Trp Ser Thr Glu Gly
1730 1735 1740

Ser Asp Phe Ile Leu Val Arg Tyr Leu Glu Glu Ser Asn Lys Lys
1745 1750 1755

Ile Leu Gln Lys Ile Arg Ile Lys Gly Ile Leu Ser Asn Thr Gln
1760 1765 1770

Ser Phe Asn Lys Met Ser Ile Asp Phe Lys Asp Ile Lys Lys Leu
1775 1780 1785

Ser Leu Gly Tyr Ile Met Ser Asn Phe Lys Ser Phe Asn Ser Glu
1790 1795 1800

Asn Glu Leu Asp Arg Asp His Leu Gly Phe Lys Ile Ile Asp Asn
1805 1810 1815

Lys Thr Tyr Tyr Tyr Asp Glu Asp Ser Lys Leu Val Lys Gly Leu
1820 1825 1830

Ile Asn Ile Asn Asn Ser Leu Phe Tyr Phe Asp Pro Ile Glu Phe
1835 1840 1845

Asn Leu Val Thr Gly Trp Gln Thr Ile Asn Gly Lys Lys Tyr Tyr
1850 1855 1860

Phe Asp Ile Asn Thr Gly Ala Ala Leu Thr Ser Tyr Lys Ile Ile
1865 1870 1875

Asn Gly Lys His Phe Tyr Phe Asn Asn Asp Gly Val Met Gln Leu
1880 1885 1890

Gly Val Phe Lys Gly Pro Asp Gly Phe Glu Tyr Phe Ala Pro Ala
1895 1900 1905

[0055]

Asn Thr Gln Asn Asn Asn Ile Glu Gly Gln Ala Ile Val Tyr Gln
1910 1915 1920

Ser Lys Phe Leu Thr Leu Asn Gly Lys Lys Tyr Tyr Phe Asp Asn
1925 1930 1935

Asn Ser Lys Ala Val Thr Gly Trp Arg Ile Ile Asn Asn Glu Lys
1940 1945 1950

Tyr Tyr Phe Asn Pro Asn Asn Ala Ile Ala Ala Val Gly Leu Gln
1955 1960 1965

Val Ile Asp Asn Asn Lys Tyr Tyr Phe Asn Pro Asp Thr Ala Ile
1970 1975 1980

Ile Ser Lys Gly Trp Gln Thr Val Asn Gly Ser Arg Tyr Tyr Phe
1985 1990 1995

Asp Thr Asp Thr Ala Ile Ala Phe Asn Gly Tyr Lys Thr Ile Asp
2000 2005 2010

Gly Lys His Phe Tyr Phe Asp Ser Asp Cys Val Val Lys Ile Gly
2015 2020 2025

Val Phe Ser Thr Ser Asn Gly Phe Glu Tyr Phe Ala Pro Ala Asn
2030 2035 2040

Thr Tyr Asn Asn Asn Ile Glu Gly Gln Ala Ile Val Tyr Gln Ser
2045 2050 2055

Lys Phe Leu Thr Leu Asn Gly Lys Lys Tyr Tyr Phe Asp Asn Asn
2060 2065 2070

Ser Lys Ala Val Thr Gly Leu Gln Thr Ile Asp Ser Lys Lys Tyr
2075 2080 2085

Tyr Phe Asn Thr Asn Thr Ala Glu Ala Ala Thr Gly Trp Gln Thr
2090 2095 2100

Ile Asp Gly Lys Lys Tyr Tyr Phe Asn Thr Asn Thr Ala Glu Ala
2105 2110 2115

Ala Thr Gly Trp Gln Thr Ile Asp Gly Lys Lys Tyr Tyr Phe Asn
2120 2125 2130

Thr Asn Thr Ala Ile Ala Ser Thr Gly Tyr Thr Ile Ile Asn Gly
2135 2140 2145

Lys His Phe Tyr Phe Asn Thr Asp Gly Ile Met Gln Ile Gly Val
2150 2155 2160

Phe Lys Gly Pro Asn Gly Phe Glu Tyr Phe Ala Pro Ala Asn Thr
2165 2170 2175

Asp Ala Asn Asn Ile Glu Gly Gln Ala Ile Leu Tyr Gln Asn Glu
2180 2185 2190

[0056]

Phe Leu Thr Leu Asn Gly Lys Lys Tyr Tyr Phe Gly Ser Asp Ser
2195 2200 2205

Lys Ala Val Thr Gly Trp Arg Ile Ile Asn Asn Lys Lys Tyr Tyr
2210 2215 2220

Phe Asn Pro Asn Asn Ala Ile Ala Ala Ile His Leu Cys Thr Ile
2225 2230 2235

Asn Asn Asp Lys Tyr Tyr Phe Ser Tyr Asp Gly Ile Leu Gln Asn
2240 2245 2250

Gly Tyr Ile Thr Ile Glu Arg Asn Asn Phe Tyr Phe Asp Ala Asn
2255 2260 2265

Asn Glu Ser Lys Met Val Thr Gly Val Phe Lys Gly Pro Asn Gly
2270 2275 2280

Phe Glu Tyr Phe Ala Pro Ala Asn Thr His Asn Asn Asn Ile Glu
2285 2290 2295

Gly Gln Ala Ile Val Tyr Gln Asn Lys Phe Leu Thr Leu Asn Gly
2300 2305 2310

Lys Lys Tyr Tyr Phe Asp Asn Asp Ser Lys Ala Val Thr Gly Trp
2315 2320 2325

Gln Thr Ile Asp Gly Lys Lys Tyr Tyr Phe Asn Leu Asn Thr Ala
2330 2335 2340

Glu Ala Ala Thr Gly Trp Gln Thr Ile Asp Gly Lys Lys Tyr Tyr
2345 2350 2355

Phe Asn Leu Asn Thr Ala Glu Ala Ala Thr Gly Trp Gln Thr Ile
2360 2365 2370

Asp Gly Lys Lys Tyr Tyr Phe Asn Thr Asn Thr Phe Ile Ala Ser
2375 2380 2385

Thr Gly Tyr Thr Ser Ile Asn Gly Lys His Phe Tyr Phe Asn Thr
2390 2395 2400

Asp Gly Ile Met Gln Ile Gly Val Phe Lys Gly Pro Asn Gly Phe
2405 2410 2415

Glu Tyr Phe Ala Pro Ala Asn Thr Asp Ala Asn Asn Ile Glu Gly
2420 2425 2430

Gln Ala Ile Leu Tyr Gln Asn Lys Phe Leu Thr Leu Asn Gly Lys
2435 2440 2445

Lys Tyr Tyr Phe Gly Ser Asp Ser Lys Ala Val Thr Gly Leu Arg
2450 2455 2460

Thr Ile Asp Gly Lys Lys Tyr Tyr Phe Asn Thr Asn Thr Ala Val
2465 2470 2475

[0057]

Ala Val Thr Gly Trp Gln Thr Ile Asn Gly Lys Lys Tyr Tyr Phe
2480 2485 2490

Asn Thr Asn Thr Ser Ile Ala Ser Thr Gly Tyr Thr Ile Ile Ser
2495 2500 2505

Gly Lys His Phe Tyr Phe Asn Thr Asp Gly Ile Met Gln Ile Gly
2510 2515 2520

Val Phe Lys Gly Pro Asp Gly Phe Glu Tyr Phe Ala Pro Ala Asn
2525 2530 2535

Thr Asp Ala Asn Asn Ile Glu Gly Gln Ala Ile Arg Tyr Gln Asn
2540 2545 2550

Arg Phe Leu Tyr Leu His Asp Asn Ile Tyr Tyr Phe Gly Asn Asn
2555 2560 2565

Ser Lys Ala Ala Thr Gly Trp Val Thr Ile Asp Gly Asn Arg Tyr
2570 2575 2580

Tyr Phe Glu Pro Asn Thr Ala Met Gly Ala Asn Gly Tyr Lys Thr
2585 2590 2595

Ile Asp Asn Lys Asn Phe Tyr Phe Arg Asn Gly Leu Pro Gln Ile
2600 2605 2610

Gly Val Phe Lys Gly Ser Asn Gly Phe Glu Tyr Phe Ala Pro Ala
2615 2620 2625

Asn Thr Asp Ala Asn Asn Ile Glu Gly Gln Ala Ile Arg Tyr Gln
2630 2635 2640

Asn Arg Phe Leu His Leu Leu Gly Lys Ile Tyr Tyr Phe Gly Asn
2645 2650 2655

Asn Ser Lys Ala Val Thr Gly Trp Gln Thr Ile Asn Gly Lys Val
2660 2665 2670

Tyr Tyr Phe Met Pro Asp Thr Ala Met Ala Ala Ala Gly Gly Leu
2675 2680 2685

Phe Glu Ile Asp Gly Val Ile Tyr Phe Phe Gly Val Asp Gly Val
2690 2695 2700

Lys Ala Pro Gly Ile Tyr Gly
2705 2710

<210> 172
<211> 2366
<212> PRT
<213> 梭状芽胞杆菌

<400> 172

Met Ser Leu Val Asn Arg Lys Gln Leu Glu Lys Met Ala Asn Val Arg
1 5 10 15

Phe Arg Thr Gln Glu Asp Glu Tyr Val Ala Ile Leu Asp Ala Leu Glu

[0058]

20 25 30

Glu Tyr His Asn Met Ser Glu Asn Thr Val Val Glu Lys Tyr Leu Lys
35 40 45

Leu Lys Asp Ile Asn Ser Leu Thr Asp Ile Tyr Ile Asp Thr Tyr Lys
50 55 60

Lys Ser Gly Arg Asn Lys Ala Leu Lys Lys Phe Lys Glu Tyr Leu Val
65 70 75 80

Thr Glu Val Leu Glu Leu Lys Asn Asn Asn Leu Thr Pro Val Glu Lys
85 90 95

Asn Leu His Phe Val Trp Ile Gly Gly Gln Ile Asn Asp Thr Ala Ile
100 105 110

Asn Tyr Ile Asn Gln Trp Lys Asp Val Asn Ser Asp Tyr Asn Val Asn
115 120 125

Val Phe Tyr Asp Ser Asn Ala Phe Leu Ile Asn Thr Leu Lys Lys Thr
130 135 140

Val Val Glu Ser Ala Ile Asn Asp Thr Leu Glu Ser Phe Arg Glu Asn
145 150 155 160

Leu Asn Asp Pro Arg Phe Asp Tyr Asn Lys Phe Phe Arg Lys Arg Met
165 170 175

Glu Ile Ile Tyr Asp Lys Gln Lys Asn Phe Ile Asn Tyr Tyr Lys Ala
180 185 190

Gln Arg Glu Glu Asn Pro Glu Leu Ile Ile Asp Asp Ile Val Lys Thr
195 200 205

Tyr Leu Ser Asn Glu Tyr Ser Lys Glu Ile Asp Glu Leu Asn Thr Tyr
210 215 220

Ile Glu Glu Ser Leu Asn Lys Ile Thr Gln Asn Ser Gly Asn Asp Val
225 230 235 240

Arg Asn Phe Glu Glu Phe Lys Asn Gly Glu Ser Phe Asn Leu Tyr Glu
245 250 255

Gln Glu Leu Val Glu Arg Trp Asn Leu Ala Ala Ala Ser Asp Ile Leu
260 265 270

Arg Ile Ser Ala Leu Lys Glu Ile Gly Gly Met Tyr Leu Asp Val Asp
275 280 285

Met Leu Pro Gly Ile Gln Pro Asp Leu Phe Glu Ser Ile Glu Lys Pro
290 295 300

Ser Ser Val Thr Val Asp Phe Trp Glu Met Thr Lys Leu Glu Ala Ile
305 310 315 320

Met Lys Tyr Lys Glu Tyr Ile Pro Glu Tyr Thr Ser Glu His Phe Asp

[0059]

325 330 335

Met Leu Asp Glu Glu Val Gln Ser Ser Phe Glu Ser Val Leu Ala Ser
340 345 350

Lys Ser Asp Lys Ser Glu Ile Phe Ser Ser Leu Gly Asp Met Glu Ala
355 360 365

Ser Pro Leu Glu Val Lys Ile Ala Phe Asn Ser Lys Gly Ile Ile Asn
370 375 380

Gln Gly Leu Ile Ser Val Lys Asp Ser Tyr Cys Ser Asn Leu Ile Val
385 390 395 400

Lys Gln Ile Glu Asn Arg Tyr Lys Ile Leu Asn Asn Ser Leu Asn Pro
405 410 415

Ala Ile Ser Glu Asp Asn Asp Phe Asn Thr Thr Thr Asn Thr Phe Ile
420 425 430

Asp Ser Ile Met Ala Glu Ala Asn Ala Asp Asn Gly Arg Phe Met Met
435 440 445

Glu Leu Gly Lys Tyr Leu Arg Val Gly Phe Phe Pro Asp Val Lys Thr
450 455 460

Thr Ile Asn Leu Ser Gly Pro Glu Ala Tyr Ala Ala Ala Tyr Gln Asp
465 470 475 480

Leu Leu Met Phe Lys Glu Gly Ser Met Asn Ile His Leu Ile Glu Ala
485 490 495

Asp Leu Arg Asn Phe Glu Ile Ser Lys Thr Asn Ile Ser Gln Ser Thr
500 505 510

Glu Gln Glu Met Ala Ser Leu Trp Ser Phe Asp Asp Ala Arg Ala Lys
515 520 525

Ala Gln Phe Glu Glu Tyr Lys Arg Asn Tyr Phe Glu Gly Ser Leu Gly
530 535 540

Glu Asp Asp Asn Leu Asp Phe Ser Gln Asn Ile Val Val Asp Lys Glu
545 550 555 560

Tyr Leu Leu Glu Lys Ile Ser Ser Leu Ala Arg Ser Ser Glu Arg Gly
565 570 575

Tyr Ile His Tyr Ile Val Gln Leu Gln Gly Asp Lys Ile Ser Tyr Glu
580 585 590

Ala Ala Cys Asn Leu Phe Ala Lys Thr Pro Tyr Asp Ser Val Leu Phe
595 600 605

Gln Lys Asn Ile Glu Asp Ser Glu Ile Ala Tyr Tyr Tyr Asn Pro Gly
610 615 620

Asp Gly Glu Ile Gln Glu Ile Asp Lys Tyr Lys Ile Pro Ser Ile Ile

[0060]

625 630 635 640

Ser Asp Arg Pro Lys Ile Lys Leu Thr Phe Ile Gly His Gly Lys Asp
645 650 655

Glu Phe Asn Thr Asp Ile Phe Ala Gly Phe Asp Val Asp Ser Leu Ser
660 665 670

Thr Glu Ile Glu Ala Ala Ile Asp Leu Ala Lys Glu Asp Ile Ser Pro
675 680 685

Lys Ser Ile Glu Ile Asn Leu Leu Gly Cys Asn Met Phe Ser Tyr Ser
690 695 700

Ile Asn Val Glu Glu Thr Tyr Pro Gly Lys Leu Leu Leu Lys Val Lys
705 710 715 720

Asp Lys Ile Ser Glu Leu Met Pro Ser Ile Ser Gln Asp Ser Ile Ile
725 730 735

Val Ser Ala Asn Gln Tyr Glu Val Arg Ile Asn Ser Glu Gly Arg Arg
740 745 750

Glu Leu Leu Asp His Ser Gly Glu Trp Ile Asn Lys Glu Glu Ser Ile
755 760 765

Ile Lys Asp Ile Ser Ser Lys Glu Tyr Ile Ser Phe Asn Pro Lys Glu
770 775 780

Asn Lys Ile Thr Val Lys Ser Lys Asn Leu Pro Glu Leu Ser Thr Leu
785 790 795 800

Leu Gln Glu Ile Arg Asn Asn Ser Asn Ser Ser Asp Ile Glu Leu Glu
805 810 815

Glu Lys Val Met Leu Thr Glu Cys Glu Ile Asn Val Ile Ser Asn Ile
820 825 830

Asp Thr Gln Ile Val Glu Glu Arg Ile Glu Glu Ala Lys Asn Leu Thr
835 840 845

Ser Asp Ser Ile Asn Tyr Ile Lys Asp Glu Phe Lys Leu Ile Glu Ser
850 855 860

Ile Ser Asp Ala Leu Cys Asp Leu Lys Gln Gln Asn Glu Leu Glu Asp
865 870 875 880

Ser His Phe Ile Ser Phe Glu Asp Ile Ser Glu Thr Asp Glu Gly Phe
885 890 895

Ser Ile Arg Phe Ile Asn Lys Glu Thr Gly Glu Ser Ile Phe Val Glu
900 905 910

Thr Glu Lys Thr Ile Phe Ser Glu Tyr Ala Asn His Ile Thr Glu Glu
915 920 925

Ile Ser Lys Ile Lys Gly Thr Ile Phe Asp Thr Val Asn Gly Lys Leu

[0061]

930 935 940

Val Lys Lys Val Asn Leu Asp Thr Thr His Glu Val Asn Thr Leu Asn
945 950 955 960

Ala Ala Phe Phe Ile Gln Ser Leu Ile Glu Tyr Asn Ser Ser Lys Glu
965 970 975

Ser Leu Ser Asn Leu Ser Val Ala Met Lys Val Gln Val Tyr Ala Gln
980 985 990

Leu Phe Ser Thr Gly Leu Asn Thr Ile Thr Asp Ala Ala Lys Val Val
995 1000 1005

Glu Leu Val Ser Thr Ala Leu Asp Glu Thr Ile Asp Leu Leu Pro
1010 1015 1020

Thr Leu Ser Glu Gly Leu Pro Ile Ile Ala Thr Ile Ile Asp Gly
1025 1030 1035

Val Ser Leu Gly Ala Ala Ile Lys Glu Leu Ser Glu Thr Ser Asp
1040 1045 1050

Pro Leu Leu Arg Gln Glu Ile Glu Ala Lys Ile Gly Ile Met Ala
1055 1060 1065

Val Asn Leu Thr Thr Ala Thr Thr Ala Ile Ile Thr Ser Ser Leu
1070 1075 1080

Gly Ile Ala Ser Gly Phe Ser Ile Leu Leu Val Pro Leu Ala Gly
1085 1090 1095

Ile Ser Ala Gly Ile Pro Ser Leu Val Asn Asn Glu Leu Val Leu
1100 1105 1110

Arg Asp Lys Ala Thr Lys Val Val Asp Tyr Phe Lys His Val Ser
1115 1120 1125

Leu Val Glu Thr Glu Gly Val Phe Thr Leu Leu Asp Asp Lys Ile
1130 1135 1140

Met Met Pro Gln Asp Asp Leu Val Ile Ser Glu Ile Asp Phe Asn
1145 1150 1155

Asn Asn Ser Ile Val Leu Gly Lys Cys Glu Ile Trp Arg Met Glu
1160 1165 1170

Gly Gly Ser Gly His Thr Val Thr Asp Asp Ile Asp His Phe Phe
1175 1180 1185

Ser Ala Pro Ser Ile Thr Tyr Arg Glu Pro His Leu Ser Ile Tyr
1190 1195 1200

Asp Val Leu Glu Val Gln Lys Glu Glu Leu Asp Leu Ser Lys Asp
1205 1210 1215

Leu Met Val Leu Pro Asn Ala Pro Asn Arg Val Phe Ala Trp Glu

[0062]

1220 1225 1230

Thr Gly Trp Thr Pro Gly Leu Arg Ser Leu Glu Asn Asp Gly Thr
1235 1240 1245

Lys Leu Leu Asp Arg Ile Arg Asp Asn Tyr Glu Gly Glu Phe Tyr
1250 1255 1260

Trp Arg Tyr Phe Ala Phe Ile Ala Asp Ala Leu Ile Thr Thr Leu
1265 1270 1275

Lys Pro Arg Tyr Glu Asp Thr Asn Ile Arg Ile Asn Leu Asp Ser
1280 1285 1290

Asn Thr Arg Ser Phe Ile Val Pro Ile Ile Thr Thr Glu Tyr Ile
1295 1300 1305

Arg Glu Lys Leu Ser Tyr Ser Phe Tyr Gly Ser Gly Gly Thr Tyr
1310 1315 1320

Ala Leu Ser Leu Ser Gln Tyr Asn Met Gly Ile Asn Ile Glu Leu
1325 1330 1335

Ser Glu Ser Asp Val Trp Ile Ile Asp Val Asp Asn Val Val Arg
1340 1345 1350

Asp Val Thr Ile Glu Ser Asp Lys Ile Lys Lys Gly Asp Leu Ile
1355 1360 1365

Glu Gly Ile Leu Ser Thr Leu Ser Ile Glu Glu Asn Lys Ile Ile
1370 1375 1380

Leu Asn Ser His Glu Ile Asn Phe Ser Gly Glu Val Asn Gly Ser
1385 1390 1395

Asn Gly Phe Val Ser Leu Thr Phe Ser Ile Leu Glu Gly Ile Asn
1400 1405 1410

Ala Ile Ile Glu Val Asp Leu Leu Ser Lys Ser Tyr Lys Leu Leu
1415 1420 1425

Ile Ser Gly Glu Leu Lys Ile Leu Met Leu Asn Ser Asn His Ile
1430 1435 1440

Gln Gln Lys Ile Asp Tyr Ile Gly Phe Asn Ser Glu Leu Gln Lys
1445 1450 1455

Asn Ile Pro Tyr Ser Phe Val Asp Ser Glu Gly Lys Glu Asn Gly
1460 1465 1470

Phe Ile Asn Gly Ser Thr Lys Glu Gly Leu Phe Val Ser Glu Leu
1475 1480 1485

Pro Asp Val Val Leu Ile Ser Lys Val Tyr Met Asp Asp Ser Lys
1490 1495 1500

Pro Ser Phe Gly Tyr Tyr Ser Asn Asn Leu Lys Asp Val Lys Val

[0063]

1505 1510 1515

Ile Thr Lys Asp Asn Val Asn Ile Leu Thr Gly Tyr Tyr Leu Lys
1520 1525 1530

Asp Asp Ile Lys Ile Ser Leu Ser Leu Thr Leu Gln Asp Glu Lys
1535 1540 1545

Thr Ile Lys Leu Asn Ser Val His Leu Asp Glu Ser Gly Val Ala
1550 1555 1560

Glu Ile Leu Lys Phe Met Asn Arg Lys Gly Asn Thr Asn Thr Ser
1565 1570 1575

Asp Ser Leu Met Ser Phe Leu Glu Ser Met Asn Ile Lys Ser Ile
1580 1585 1590

Phe Val Asn Phe Leu Gln Ser Asn Ile Lys Phe Ile Leu Asp Ala
1595 1600 1605

Asn Phe Ile Ile Ser Gly Thr Thr Ser Ile Gly Gln Phe Glu Phe
1610 1615 1620

Ile Cys Asp Glu Asn Asp Asn Ile Gln Pro Tyr Phe Ile Lys Phe
1625 1630 1635

Asn Thr Leu Glu Thr Asn Tyr Thr Leu Tyr Val Gly Asn Arg Gln
1640 1645 1650

Asn Met Ile Val Glu Pro Asn Tyr Asp Leu Asp Asp Ser Gly Asp
1655 1660 1665

Ile Ser Ser Thr Val Ile Asn Phe Ser Gln Lys Tyr Leu Tyr Gly
1670 1675 1680

Ile Asp Ser Cys Val Asn Lys Val Val Ile Ser Pro Asn Ile Tyr
1685 1690 1695

Thr Asp Glu Ile Asn Ile Thr Pro Val Tyr Glu Thr Asn Asn Thr
1700 1705 1710

Tyr Pro Glu Val Ile Val Leu Asp Ala Asn Tyr Ile Asn Glu Lys
1715 1720 1725

Ile Asn Val Asn Ile Asn Asp Leu Ser Ile Arg Tyr Val Trp Ser
1730 1735 1740

Asn Asp Gly Asn Asp Phe Ile Leu Met Ser Thr Ser Glu Glu Asn
1745 1750 1755

Lys Val Ser Gln Val Lys Ile Arg Phe Val Asn Val Phe Lys Asp
1760 1765 1770

Lys Thr Leu Ala Asn Lys Leu Ser Phe Asn Phe Ser Asp Lys Gln
1775 1780 1785

Asp Val Pro Val Ser Glu Ile Ile Leu Ser Phe Thr Pro Ser Tyr

[0064]

1790 1795 1800

Tyr Glu Asp Gly Leu Ile Gly Tyr Asp Leu Gly Leu Val Ser Leu
1805 1810 1815

Tyr Asn Glu Lys Phe Tyr Ile Asn Asn Phe Gly Met Met Val Ser
1820 1825 1830

Gly Leu Ile Tyr Ile Asn Asp Ser Leu Tyr Tyr Phe Lys Pro Pro
1835 1840 1845

Val Asn Asn Leu Ile Thr Gly Phe Val Thr Val Gly Asp Asp Lys
1850 1855 1860

Tyr Tyr Phe Asn Pro Ile Asn Gly Gly Ala Ala Ser Ile Gly Glu
1865 1870 1875

Thr Ile Ile Asp Asp Lys Asn Tyr Tyr Phe Asn Gln Ser Gly Val
1880 1885 1890

Leu Gln Thr Gly Val Phe Ser Thr Glu Asp Gly Phe Lys Tyr Phe
1895 1900 1905

Ala Pro Ala Asn Thr Leu Asp Glu Asn Leu Glu Gly Glu Ala Ile
1910 1915 1920

Asp Phe Thr Gly Lys Leu Ile Ile Asp Glu Asn Ile Tyr Tyr Phe
1925 1930 1935

Asp Asp Asn Tyr Arg Gly Ala Val Glu Trp Lys Glu Leu Asp Gly
1940 1945 1950

Glu Met His Tyr Phe Ser Pro Glu Thr Gly Lys Ala Phe Lys Gly
1955 1960 1965

Leu Asn Gln Ile Gly Asp Tyr Lys Tyr Tyr Phe Asn Ser Asp Gly
1970 1975 1980

Val Met Gln Lys Gly Phe Val Ser Ile Asn Asp Asn Lys His Tyr
1985 1990 1995

Phe Asp Asp Ser Gly Val Met Lys Val Gly Tyr Thr Glu Ile Asp
2000 2005 2010

Gly Lys His Phe Tyr Phe Ala Glu Asn Gly Glu Met Gln Ile Gly
2015 2020 2025

Val Phe Asn Thr Glu Asp Gly Phe Lys Tyr Phe Ala His His Asn
2030 2035 2040

Glu Asp Leu Gly Asn Glu Glu Gly Glu Glu Ile Ser Tyr Ser Gly
2045 2050 2055

Ile Leu Asn Phe Asn Asn Lys Ile Tyr Tyr Phe Asp Asp Ser Phe
2060 2065 2070

Thr Ala Val Val Gly Trp Lys Asp Leu Glu Asp Gly Ser Lys Tyr

[0065]

2075 2080 2085

Tyr Phe Asp Glu Asp Thr Ala Glu Ala Tyr Ile Gly Leu Ser Leu
2090 2095 2100

Ile Asn Asp Gly Gln Tyr Tyr Phe Asn Asp Asp Gly Ile Met Gln
2105 2110 2115

Val Gly Phe Val Thr Ile Asn Asp Lys Val Phe Tyr Phe Ser Asp
2120 2125 2130

Ser Gly Ile Ile Glu Ser Gly Val Gln Asn Ile Asp Asp Asn Tyr
2135 2140 2145

Phe Tyr Ile Asp Asp Asn Gly Ile Val Gln Ile Gly Val Phe Asp
2150 2155 2160

Thr Ser Asp Gly Tyr Lys Tyr Phe Ala Pro Ala Asn Thr Val Asn
2165 2170 2175

Asp Asn Ile Tyr Gly Gln Ala Val Glu Tyr Ser Gly Leu Val Arg
2180 2185 2190

Val Gly Glu Asp Val Tyr Tyr Phe Gly Glu Thr Tyr Thr Ile Glu
2195 2200 2205

Thr Gly Trp Ile Tyr Asp Met Glu Asn Glu Ser Asp Lys Tyr Tyr
2210 2215 2220

Phe Asn Pro Glu Thr Lys Lys Ala Cys Lys Gly Ile Asn Leu Ile
2225 2230 2235

Asp Asp Ile Lys Tyr Tyr Phe Asp Glu Lys Gly Ile Met Arg Thr
2240 2245 2250

Gly Leu Ile Ser Phe Glu Asn Asn Asn Tyr Tyr Phe Asn Glu Asn
2255 2260 2265

Gly Glu Met Gln Phe Gly Tyr Ile Asn Ile Glu Asp Lys Met Phe
2270 2275 2280

Tyr Phe Gly Glu Asp Gly Val Met Gln Ile Gly Val Phe Asn Thr
2285 2290 2295

Pro Asp Gly Phe Lys Tyr Phe Ala His Gln Asn Thr Leu Asp Glu
2300 2305 2310

Asn Phe Glu Gly Glu Ser Ile Asn Tyr Thr Gly Trp Leu Asp Leu
2315 2320 2325

Asp Glu Lys Arg Tyr Tyr Phe Thr Asp Glu Tyr Ile Ala Ala Thr
2330 2335 2340

Gly Ser Val Ile Ile Asp Gly Glu Glu Tyr Tyr Phe Asp Pro Asp
2345 2350 2355

Thr Ala Gln Leu Val Ile Ser Glu

[0066]

2360 2365

<210> 173
<211> 18
<212> PRT
<213> 人工的

<220>
<223> 来自艰难梭菌毒素TcdB的片段

<400> 173

Ser Pro Val Glu Lys Asn Leu His Phe Val Trp Ile Gly Gly Glu Val
1 5 10 15

Ser Asp

<210> 174
<211> 11
<212> PRT
<213> 人工的

<220>
<223> 来自艰难梭菌毒素TcdB的片段

<400> 174

Asn Leu Ala Ala Ala Ser Asp Ile Val Arg Leu
1 5 10

<210> 175
<211> 15
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 175

Cys Gly Gly Val Tyr Leu Asp Val Asp Met Leu Pro Gly Ile His
1 5 10 15

<210> 176
<211> 20
<212> PRT
<213> 人工的

<220>
<223> 来自艰难梭菌毒素TcdB的片段

<400> 176

Cys Gly Gly Val Tyr Leu Asp Val Asp Met Leu Pro Gly Ile His Ser
1 5 10 15

Asp Leu Phe Lys
20

<210> 177
<211> 14
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 177

[0067]

Cys Trp Glu Met Ile Lys Leu Glu Ala Ile Met Lys Tyr Lys
1 5 10

<210> 178
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 178

Cys Thr Asn Leu Val Ile Glu Gln Val Lys Asn Arg
1 5 10

<210> 179
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 179

Pro Glu Ala Arg Ser Thr Ile Ser Leu Ser Gly Pro
1 5 10

<210> 180
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 180

Cys Ser Asn Leu Ile Val Lys Gln Ile Glu Asn Arg
1 5 10

<210> 181
<211> 14
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 181

Thr Glu Gln Glu Ile Asn Ser Leu Trp Ser Phe Asp Gln Ala
1 5 10

<210> 182
<211> 25
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 182

Thr Glu Gln Glu Ile Asn Ser Leu Trp Ser Phe Asp Pro Glu Ala Arg
1 5 10 15

Ser Thr Ile Ser Leu Ser Gly Pro Cys

[0068]

20 25

<210> 183
<211> 13
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 183

Asn Val Glu Glu Thr Tyr Pro Gly Lys Leu Leu Leu Cys
1 5 10

<210> 184
<211> 14
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 184

Cys Ala Asn Gln Tyr Glu Val Arg Ile Asn Ser Glu Gly Arg
1 5 10

<210> 185
<211> 15
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 185

Val Asn Thr Leu Asn Ala Ala Phe Phe Ile Gln Ser Leu Ile Cys
1 5 10 15

<210> 186
<211> 13
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 186

Tyr Ala Gln Leu Phe Ser Thr Gly Leu Asn Thr Ile Cys
1 5 10

<210> 187
<211> 16
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 187

Cys Ala Gly Ile Ser Ala Gly Ile Pro Ser Leu Val Asn Asn Glu Leu
1 5 10 15

<210> 188
<211> 16
<212> PRT

[0069]

<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 188

Asp Asp Leu Val Ile Ser Glu Ile Asp Phe Asn Asn Asn Ser Ile Cys
1 5 10 15

<210> 189
<211> 10
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 189

Met Glu Gly Gly Ser Gly His Thr Val Thr
1 5 10

<210> 190
<211> 35
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 190

Ala Val Asn Asp Thr Ile Asn Val Leu Pro Thr Ile Thr Glu Gly Ile
1 5 10 15

Pro Ile Val Ser Thr Ile Leu Asp Gly Ile Asn Leu Gly Ala Ala Ile
20 25 30

Lys Glu Leu
35

<210> 191
<211> 20
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 191

Cys Gly Phe Glu Tyr Phe Ala Pro Ala Asn Thr Asp Ala Asn Asn Ile
1 5 10 15

Glu Gly Gln Ala
20

<210> 192
<211> 20
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 192

Cys Gly Tyr Lys Tyr Phe Ala Pro Ala Asn Thr Val Asn Asp Asn Ile

[0070]

1 5 10 15

Tyr Gly Gln Ala
20

<210> 193
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 193

Cys Lys Tyr Tyr Phe Asn Thr Asn Thr Ala Glu Ala
1 5 10

<210> 194
<211> 12
<212> PRT
<213> 人工的

<220>
<223> 艰难梭菌毒素TcdB的片段

<400> 194

Cys Lys Tyr Tyr Phe Asp Glu Asp Thr Ala Glu Ala
1 5 10

SEQ IN NO:8 编码抗毒素 A 抗体 922.g1 VK(gL1)的多核苷酸序列

GACCCTGTGA TGACCCAGAG TCCGAGCACT CTTTCTGCCT CCGTGGGAGA CCGCGTGACC
ATTACATGTC AGGCTTCACA AAGTATCTCC AATGCTCTGG CCTGGTATCA GCAGAAACCC
GGCAAAGCCC CTAAGCTGCT CATCTACTCT GCATCAAGCC TGGCTAGCGG CGTGCCAAGC
CGATTCAAGG GGAGCGGTTC TGGCACTGAG TTTACGCTGA CCATCAGTAG CTTGCAGCCT
GACGATTTTG CAACCTATTA CTGCCAGTAC ACACACTACT CCCATACATC TAAAAACCCA
TTCGGAGGGG GTACTAAGGT CGAAATAAAG

SEQ IN NO:10 编码抗毒素 A 抗体 922.g1 VH(gH1)的多核苷酸序列

GAAGTGCAAT TGGTGGAAAG TGGCGGAGGA CTGGTGCAAC CCGGGGGTAG TCTGCGACTG
AGCTGTGCTG CCTCCGGCTT TACCATTAGC TCCTACTATA TGAGCTGGGT TCGACAGGCC
CCTGGAAAAG GACTCGAATG GATCGGCATC ATATCTTCCG GTGGGCATTT CACCTGGTAC
GCAAACTGGG CTAAGGGGAG ATTCACGATT AGCAGCGACT CCACAACCGT GTACCTGCAA
ATGAACAGCC TGAGGGATGA GGACACTGCC ACATATTTCT GCGCACGCGC TTACGTGAGC
GGAAGCTCAT TTAATGGCTA TGCACTGTGG GGGCAAGGAA CACTCGTGAC TGTCTCG

SEQ IN NO:18 编码抗毒素 A 抗体 CA923.g1 gL1 的多核苷酸序列

GACGTCGTGATGACTCAGAGCCCATCTAGTCTGAGCGCTAGCGTCGGAGACCGAGTCACAATTACC
TGTCAAGCCTCCCAGAGCATCTCCAACTACCTGGCCTGGTACCAACAGAAACCTGGCAAGGTGCCC
AAGCTGCTGATCTATAGTGCTTCCACACTCGCAAGCGGCGTTCCGTCACGCTTTAAGGGATCTGGC
TCTGGCACTCAGTTCACCTTGACGATCTCAAGCCTGCAGCCAGAAGATGTGGCCACCTATTACTGC
CAGTATTCCCACTACGGGACTGGGGTGTTCGGTGCCTTTGGAGGTGGGACCAAAGTGGAGATAAAG

图 1

SEQ IN NO:20 编码抗毒素 A 抗体的多核苷酸序列 CA923.g1 gH1

GAAGTTCAACTTGTGGAATCTGGAGGCGGGCTCGTGCAGCCTGGTGGAAGCCTTAGACTGAGCTGC
GCTGCATCCGCATTTTCCCTGTCCAACTACTACATGAGCTGGGTGCGACAAGCACCAGGCAAGGGA
CTGGAATGGATTGGCATCATAAGCTCCGGTTCCAATGCCCTGAAATGGTACGCATCATGGCCGAAA
GGCCGCTTTACCATAAGCAAGGACTCCACCACCGTCTATCTGCAGATGAACTCATTGCGTGCCGAG
GACACTGCAACGTACTTCTGTGCTCGCAACTACGTGGGAAGCGGATCTTATTATGGCATGGATCTG
TGGGGACAAGGTACACTCGTGACCGTCTCG

SEQ IN NO:28 编码抗毒素 A 抗体 CA993.g1 gL1 的多核苷酸序列

GATGTCGTGA TGACTCAGTC CCCCTCTACA TTGAGTGCCT CTGTCGGTGA TCGAGTTACC
ATCACCTGTC AAGCAAGCCA GAGCATCAGC TCCTACTTCT CTTGGTACCA GCAAAAGCCG
GGAAAAGCCC CTCAACTGCT GATTTATGGG GCCTCAACAC TGGCTTCTGG CGTGCCATCA
AGATTCAAGG GATCTGGCTC CGGCACTGAG CTTACACTGA CCATTAGCTC CCTGCAACCT
GACGATTTTG CTACCTACTA CTGCCAGTGC ACCGACTATA GTGGGATATA TTTCGGCGGA
TTTGGGGGAG GGACGAAAGT GGAAATCAAG

SEQ IN NO:30 编码抗毒素 A 抗体 CA993.g1 gH1 的多核苷酸序列

GAAGTTCAGC TGGTCGAGAG CGGAGGCGGA CTGGTGCAAC CTGGTGGTAG CCTGAAACTC
TCTTGTACTG CCTCCGGGTT TTCCCTGAGC TCTTACTATA TGTCATGGGT GAGACAGGCT
CCCGGGAAAG GATTGGAATG GATCGGGATT ATCTCCTCCG GCTCTTCCAC CACTTTCACA
TGGTACGCCT CATGGGCAAA GGGGAGGTTT ACCATAAGCA AGACAAGCAC GACCGTGTAT
CTTCAGATGA ACTCCCTGAA GACGGAGGAT ACTGCCACCT ACTTTTGCGC TCGGGCCTAT
GTGGGCTCAA GCTCTTACTA TGGCTTCGAC CCATGGGGAC AGGGCACACT TGTGACCGTC
TCG

图 2

SEQ IN NO:38 编码抗毒素 A 抗体 995.g1 VL 区域的多核苷酸序列

GACGTCGTGA TGACACAGAG CCCTTCAACA CTGTCTGCAA GCGTGGGCGA TAGGGTCACC
ATAACGTGCC AGGCCTCTCA ATCCATCAAC AACTATTTTA GCTGGTACCA GCAGAAGCCA
GGCAAGGCTC CGAAACTTCT GATCTACGGA GCTGCCAACC TGGCAAGTGG CGTGCCATCA
CGGTTCAAGG GATCCGGGAG CGGTACTGAG TATACCCTGA CCATTTCATC TCTCCAACCC
GACGATTTCG CCACCTACTC CTGCCAGAAT AATTACGGCG TGCACATCTA TGGAGCTGCC
TTTGGCGGTG GGACAAAAGT GGAAATTAAG

SEQ IN NO:40 编码抗毒素 A 抗体 995.g1 VH 区域的多核苷酸序列

GAAGTTCAGC TGGTCGAGAG TGGGGGAGGG CTTGTGCAAC CTGGTGGCTC CCTCCGTCTG
AGCTGTACTG CTTCTGGATT CTCACTGAGC AATTACGACA TGATCTGGGT GCGACAGGCA
CCCGGCAAAG GACTGGAGTA CATTGGCTTC ATCAACACCG GGGGTATAAC GTACTATGCC
TCATGGGCTA AGGGGCGCTT TACAATTAGT AGGGATTCCT CTACCGTGTA CCTGCAGATG
AACTCACTGA GAGCCGAGGA CACTGCCACA TATTTCTGCG CTCGGGTGGA TGACTATATC
GGGGCCTGGG GCGCCGGATT GTGGGGCCAA GGAACACTGG TCACCGTCTC G

SEQ IN NO:48 编码抗毒素 A 抗体 997.g1 VL 区域的多核苷酸序列

GCACTCGTGATGACACAGAGCCCGAGTAGCTTTAGTGCTTCAACCGGTGATAGGGTCACTATTACT
TGCCAAGCCTCTCAGAGTATATCTAGCTATCTGAGCTGGTACCAGCAAAAGCCCGGGAAGGCTCCT
AAACTGCTGATCTACCGGGCTTCCACATTGGCCTCCGGCGTTCCCTCACGCTTTAGCGGCTCCGGA
TCCGGAACCGAGTACACCCTGACTATCTCTTGCCTGCAATCTGAGGACTTCGCAACCTACTATTGT
CTGGGCGTCTACGGATATAGCAACGATGACGGGATCGCCTTCGGCGGCGGTACCAAAGTGGAAATT
AAG

图 3

SEQ IN NO:50 编码抗毒素 A 抗体 997.g1 VH 区域的多核苷酸序列

GAGGTGCAACTTGTGGAAAGCGGGGGAGGACTGGTGCAGCCTGGGGGCTCATTGAGACTGAGCTGC
ACCGTTTCTGGTATTGACCTGAGCTCCCATCATATGTGCTGGGTGCGCCAGGCACCCGGAAAAGGA
CTGGAATACATCGGCGTCATATACCACTTTGGCTCTACATACTATGCCAACTGGGCAACTGGGCGA
TTCACAATTAGCAAGGACTCAACTACCGTTTACCTGCAAATGAATAGCCTGAGGGCTGAGGATACT
GCCACCTATTTCTGTGCCCGGGCTTCAATCGCCGGCTATTCTGCCTTTGATCCATGGGGGCAAGGA
ACACTCGTGACCGTCTCG

SEQ IN NO:58 编码抗毒素 A 抗体 1000.g1 VL 区域的多核苷酸序列

GAAATCGTGA TGACGCAGTC ACCAAGCACA CTGAGCGCTT CTGTGGGAGA TCGGGTCACA
ATAACCTGTC AGGCCTCCCA GAGCATCTAC TCTTATCTGG CATGGTACCA GCAGAAGCCA
GGGAAAGCTC CCAAGCTGCT GATTTATGAC GCCAGCACTT TGGCTTCCGG TGTTCCTAGT
AGGTTCAAAG GCTCCGGAAG CGGTACCGAG TTTACCCTGA CCATCTCATC TCTGCAACCC
GATGACTTTG CCACATACTA TTGCCAGGGG AATGCCTACA CTTCCAACTC ACACGACAAC
GCATTCGGGG GAGGCACCAA AGTCGAAATT AAG

SEQ IN NO:60 编码抗毒素 A 抗体 1000.g1 VH 区域的多核苷酸序列

GAAGTTCAGC TGGTCGAGAG CGGAGGGGGT TTGATTCAGC CCGGTGGCTC ACTTAGATTG
AGCTGCACCG TGTCCGGAAT CGATCTGTCA TCTGATGCCG TGGGCTGGGT GCGACAGGCA
CCTGGGAAAG GACTGGAGTA TATAGGGATC ATCGCCACCT TCGACTCCAC ATACTACGCT
AGCTGGGCAA AAGGGCGCTT TACGATTAGC AAGGCCTCCT CTACTACCGT GTACCTCCAA
ATGAACTCAC TGAGGGCCGA GGACACTGCC ACTTATTTCT GTGCTCGGAC CGGTAGCTGG
TACTACATCT CTGGCTGGGG CTCCTACTAT TATGGCATGG ACCTGTGGGG ACAGGGGACA
CTCGTGACCG TCTCG

图 4

SEQ IN NO:68 编码抗毒素 B 抗体 926.g1 VL 区域的多核苷酸序列

GATACCGTGCTGACCCAGAGCCCTGCTACATTGTCACTGAGCCCCGGGGAGAGGGCCACATTGAGC
TGCCGGGCTTCAAAATCCGTGTCCACCCTCATGCACTGGTTTCAGCAAAAGCCCGGGCAGGCCCCA
AAACTGCTGATCTACCTCGCATCTAACCTTGAATCTGGCGTGCCGGCCCGCTTTAGTGGCTCCGGA
AGCGGAACCGACTTCACACTGACGATTAGCTCCCTGGAGCCTGAGGATTTCGCCGTGTACTATTGC
CAGCAAACTTGGAATGACCCTTGGACTTTCGGGGGCGGTACTAAGGTCGAAATAAAG

SEQ IN NO:70 编码抗毒素 B 抗体 926.g1 VH 区域的多核苷酸序列

GAGGTGGAACTGCTCGAATCTGGTGGTGGGCTGGTGCAGCCCGGTGGATCTCTGAGATTGTCATGC
GAGGCATCCGGCTTTACCTTTTCCAACTACGGAATGGCCTGGGTGAGACAGGCCCCAACGAAGGGG
CTCGAATGGGTTACAAGCATCAGCTCTTCTGGGGGATCTACTTACTATCGCGATAGCGTCAAAGGC
CGGTTTACCATTAGCCGAGATAATGCCAAATCAAGCCTGTATCTGCAAATGAACAGCCTGAGGGCT
GAGGACACCGCCACATACTATTGTACAACCGTGATAAGGGGCTACGTGATGGACGCATGGGGACAG
GGGACATTGGTTACCGTCTCG

SEQ IN NO:78 编码抗毒素 B 抗体 927.g2 VL 区域的多核苷酸序列

GACACACAGA TGACCCAGAG CCCATCCACT TTGTCTGCAT CCGTGGGCGA CCGAGTGACA
ATCACCTGTA GAGCAAGCGG TTCCGTGAGC ACACTGATGC ATTGGTACCA GCAGAAGCCT
GGGAAGGCTC CCAAGCTGCT GATCTACAAA GCCAGCAACC TTGCCTCCGG CGTTCCAAGC
CGGTTTAGCG GTTCCGGATC TGGAACCGAG TTCACCCTGA CCATATCAAG CCTGCAACCC
GACGACTTCG CCACCTACTA TTGCCACCAG AGCTGGAATA GCGACACGTT CGGGCAAGGC
ACAAGGCTGG AAATCAAA

SEQ IN NO:80 编码抗毒素 B 抗体 927.g2 VH 区域的多核苷酸序列

GAGGTGCAAC TTGTGGAAAG CGGAGGGGGC GTGGTCCAAC CCGGAAGAAG TCTCCGTCTT
TCTTGCGCCG CAAGTGGCTT CACCTTTTCC AACTACGGAA TGGCCTGGGT TCGACAAGCT
CCTGGGAAAG GATTGGAGTG GGTGGCCACT ATCAACTATG ACGGACGCAC GACACACTAC
CGAGACTCTG TTAAGGGGCG CTTTACGATT TCCCGCGACA ATAGCAAGAG CACCCTCTAC
CTGCAAATGA ATAGCCTCCG GGCCGAGGAT ACTGCTGTGT ACTATTGTAC CTCCATCTCA
CGGAGCCACT ACTTCGATTG CTGGGGACAA GGCACACTCG TGACTGTCTCG

图 5

SEQ IN NO:88 编码抗毒素 B 抗体 1099.g2 VL 区域的多核苷酸序列

GACGTCCAGC TCACTCAATC TCCCTCCTTT CTGTCTGCTT CTGTGGGCGA TCGCGTGACA
ATAACCTGCA AGGCCTCCAA ATCAATTAGC AACCATCTGG CATGGTATCA GGAGAAGCCT
GGCAAAGCCA ATAAGCTGCT GATCCACTCC GGCTCAACTC TGCAATCCGG TACCCCAAGC
CGATTTAGCG GATCTGGGAG CGGAACCGAG TTCACACTTA CCATTAGCTC CCTGCAACCG
GAGGACTTCG CCACCTATTA CTGCCAGCAA TACGACGAAT ACCCCTATAC GTTCGGCCAA
GGGACAAGAT TGGAAATCAA GCGTACG

SEQ IN NO:90 编码抗毒素 B 抗体 1099.g2 VH 区域的多核苷酸序列

GAAGTTCAGC TGCAGGAATC TGGACCTGGC TTGGTGAAAC CAAGCGAGAC ACTTAGTCTC
ACTTGCACCG TTTCCGGCTT CTCCCTTCAA TCCTACACGA TCTCTTGGGT GCGGCAACCA
CCCGGGAAAG GACTGGAATG GATCGCAGCC ATTAGCGGGG GAGGGAGCAC CTATTACAAC
TTGCCTCTCA AGAGCCGCGT GACCATATCC CGTGACACAA GCAAGAGCCA GGTTTCCCTG
AAGCTGAGCT CCGTGACTGC TGCCGATACG GCTGTTTACT ATTGCACCCG ACCTCGCTGG
TATCCCCGTT CCTATTTCGA CTACTGGGGA AGAGGCACAC TGGTTACCGT CTCG

SEQ IN NO:98 编码抗毒素 B 抗体 1102.g4 VL 区域的多核苷酸序列

AACATCGTGC TGACACAGTC TCCTGCAACC CTTTCACTGT CTCCAGGTGA ACGAGCAACC
CTGAGTTGTA GAGCCAGTCA GAGGATCTCC ACGAGCATTC ACTGGTATCA GCAAAAGCCT
GGGCAAGCTC CCAGACTCTT GATCAAGTAC GCCTCTCAGA GCATAAGTGG CATTCCAGCT
AGGTTTAGCG GCTCAGGCTC AGGAACAGAC TTCACTCTGA CCATCAGCTC CCTGGAACCG
GAGGACTTTG CCGTCTATTA CTGCCAGCAA TCCTACTCCA GTCTGTACAC CTTCGGGCAG
GGTACTAAAC TGGAGATAAA G

图 6

SEQ IN NO:100 编码抗毒素 B 抗体 1102.g4 VH 区域的多核苷酸序列

GAAGTGCAGC TGGTCGAATC CGGGGGAGGT TTGGTGCAAC CAGGTGGCTC ACTGAGACTG
AGCTGTGCCG TTTCCGGCTT TACGTTCTCA GACAGTTATA TGGCCTGGGT GCGTCAAGCA
CCTGGAAAAG GGCTGGAGTG GATTGCCAGT ATCAGCTATG GTGGGACCAT AATCCAGTAC
GGCGATAGCG TCAAGGGCAG GTTTACTATC TCCAGGGACA ACGCCAAGTC AAGCCTTTAC
CTGCAGATGA ATTCTCTCCG CGCAGAGGAT ACCGCTGTGT ATTACTGCGC TAGACGGCAG
GGAACCTACG CTCGATACCT GGACTTCTGG GGTCAGGGAA CACTCGTTAC AGTCTCG

SEQ IN NO:108 编码抗毒素 B 抗体 1114.g2 VL 区域的多核苷酸序列

GCGACGCAAA TGACTCAGTC GCCCTCATCG CTTAGCGCGT CCGTCGGAGA TAGAGTGACG
ATCACCTGCC GCGCATCAGA GTCGGTGTCC ACACTCCTCC ACTGGTATCA GCAGAAACCG
GGGAAGGCAC CAAAACTCTT GATCTACAAA GCCAGCAACC TTGCGTCCGG TGTCCCGTCA
AGGTTCTCCG GGAGCGGTTC GGGGACAGAC TTTACTTTGA CCATTTCGTC GCTTCAGCCG
GAGGACTTCG CCACCTATTA CTGTCATCAG TCATGGAACT CACCTCCCAC ATTTGGCCAG
GGAACGAAAC TCGAAATCAA G

SEQ IN NO:110 编码抗毒素 B 抗体 1114.g2 VH 区域的多核苷酸序列

GAAGTACAAC TCGTAGAGTC AGGGGGTGGG CTGGTCCAAC CTGGCGGCTC CCTTCGGCTT
TCGTGTGCCG CCTCGGGATT CACGTTTAGC AATTACGGTA TGGCCTGGGT GAGGCAGGCA
CCAGGGAAGG GTCTTGAGTG GGTAGCGATC ATCAACTATG ATGCAAGCAC CACCCACTAC
AGGGATAGCG TCAAGGGACG CTTTACTATC AGCCGGGATA ATGCGAAATC CTCGCTCTAT
CTGCAGATGA ACTCCCTCAG AGCCGAGGAC ACCGCAGTGT ACTATTGCAC ACGATACGGA
CGCTCGCACT ATTTCGACTA TTGGGGACAG GGGACGCTCG TAACTGTCTC G

图 7

SEQ IN NO:118 编码抗毒素 B 抗体 1114.g8 VL 区域的多核苷酸序列

GACACGGTCC TGACTCAGTC GCCCTCATCG CTTAGCGCGT CCGTCGGAGA TAGAGTGACG
ATCACCTGCC GCGCATCAGA GTCGGTGTCC ACACTCCTCC ACTGGTATCA GCAGAAACCG
GGGAAGGCAC CAAAACTCTT GATCTACAAA GCCAGCAACC TTGCGTCCGG TGTCCCGTCA
AGGTTCTCCG GGAGCGGTTC GGGGACAGAC TTTACTTTGA CCATTTCGTC GCTTCAGCCG
GAGGACTTCG CCACCTATTA CTGTCATCAG TCATGGAACT CACCTCCCAC ATTTGGCCAG
GGAACGAAAC TCGAAATCAA G

SEQ IN NO:120 编码抗毒素 B 抗体 1114.g8 VH 区域的多核苷酸序列

GAAGTACAAC TCGTAGAGTC AGGGGGTGGG CTGGTCCAAC CTGGCGGCTC CCTTCGGCTT
TCGTGTGCCG CCTCGGGATT CACGTTTAGC AATTACGGTA TGGCCTGGGT GAGGCAGGCA
CCAGGGAAGG GTCTTGAGTG GGTAGCGATC ATCAACTATG ATGCAAGCAC CACCCACTAC
AGGGATAGCG TCAAGGGACG CTTTACTATC AGCCGGGATA ATGCGAAATC CTCGCTCTAT
CTGCAGATGA ACTCCCTCAG AGCCGAGGAC ACCGCAGTGT ACTATTGCAC ACGATACGGA
CGCTCGCACT ATTTCGACTA TTGGGGACAG GGGACGCTCG TAACTGTCTC G

SEQ IN NO:128 编码抗毒素 B 抗体 1125.g2 VL 区域的多核苷酸序列

GATATACAAA TGACTCAGAG CCCTAGCTCA CTGAGCGCTT CTGTGGGCGA TCGTGTGACA
ATCACTTGCA AAGCAAGCCA GAACATCTAT ATGTACCTGA ATTGGTACCA GCAAAAACCG
GGAAAAGCTC CCAAGCGCCT GATTTACAAC ACCAATAAGC TGCATACCGG CGTGCCAAGC
CGTTTTAGCG GATCTGGCTC TGGAACCGAA TATACACTGA CCATAAGCTC CCTGCAACCG
GAAGACTTTG CAACTTACTA TTGCCTCCAG CACAAATCCT TCCCCTATAC GTTCGGACAA
GGGACCAAAC TGGAAATCAA A

SEQ IN NO:130 编码抗毒素 B 抗体 1125.g2 VH 区域的多核苷酸序列

GAAGTGCAGC TGGTCGAAAG CGGCGGAGGA TTGGTGCAAC CTGGTGGCTC TCTTCGCCTG
TCTTGCGCTG CAAGCGGCTT TACGTTCCGC GATAGCTTTA TGGCTTGGGT GCGACAAGCT
CCTGGGAAAG GGCTGGAATG GGTCGCTAGC ATAAGCTACG AAGGCGACAA GACTTACTAT
GGGGACTCTG TGAAAGGCCG ATTCACCATT AGCCGAGACA ACGCAAAGAA CTCCCTGTAC
CTGCAGATGA ACTCCCTGCG TGCCGAAGAT ACCGCCGTGT ACTATTGCGC TAGGCTGACG
ATCACTACAA GCGGAGATAG CTGGGGACAA GGGACAATGG TGACCGTCTC GAGC

SEQ IN NO:138 编码抗毒素 B 抗体 1129.g1 VL 区域的多核苷酸序列

GACACCCAGA TGACTCAGTC TCCGTCAAGC CTTTCTGCCT CTGTTGGAGA TCGAGTCACA
ATTACGTGCA AGGCAAGCCA ACACGTGGGT ACCAACGTGG ACTGGTATCA ACAGAAGCCA
GGGAAGGTCC CCAAACTGCT GATCTACGGT GCCAGTATTC GCTATACCGG CGTGCCTGAT
CGCTTCACCG GAAGCGGGTC AGGGACCGAT TTCACACTGA CAATCAGCTC CCTGCAACCT
GAAGACGTGG CTACTTACTA CTGCCTGCAG TACAACTATA ATCCCTACAC CTTTGGCCAG
GGCACCAAAC TGGAGATAAA G

SEQ IN NO:140 编码抗毒素 B 抗体 1129.g1 VH 区域的多核苷酸序列

GAGGTGCAAC TTGTGGAATC AGGAGGTGGC GTGGTTCAGC CCGGTAGATC ACTTCGTCTG
AGTTGTGCAA CAAGCGGCTT TATCTTCTCC AACTTCGGGA TGTCTTGGGT TAGACAGGCT
CCTGGTAAGG GCCTCGAATG GGTGGCTAGT ATTAGCCCAA GCGGGGGAAA CGCCTACTAT
AGGGACAGCG TGAAAGGACG CTTCACTATC AGCCGAGATA ACTCCAAGAC CACGCTGTAT
CTGCAGATGA ATAGTCTGAG GGCCGAGGAT ACCGCAGTGT ACTACTGCAC TCGACGGGCC
TATTCTTCCC CTTTTGCCTT TTGGGGACAG GGGACTCTGG TGACAGTCTC GAGC

图 8

SEQ IN NO:148 编码抗毒素 B 抗体 1134.g5 VL 区域的多核苷酸序列

GACGTCCAGC TCACTCAATC TCCCTCCTTT CTGTCTGCTT CTGTGGGCGA TCGCGTGACA
ATAACCTGCA AGGCCTCCAA ATCAATTAGC AACCATCTGG CATGGTATCA GGAGAAGCCT
GGCAAAGCCA ATAAGCTGCT GATCCACTCC GGCTCAACTC TGCAACCCGG TACCCCAAGC
CGATTTAGCG GATCTGGGAG CGGAACCGAG TTCACACTTA CCATTAGCTC CCTGCAACCG
GAGGACTTCG CCACCTATTA CTGCCAGCAA TACGACGAAT ACCCCTATAC GTTCGGCCAA
GGGACAAGAT TGGAAATCAA G

SEQ IN NO:150 编码抗毒素 B 抗体 1134.g5 VH 区域的多核苷酸序列

GAAGTTCAGC TGCAGGAATC TGGACCTGGC TTGGTGAAAC CAAGCGAGAC ACTTAGTCTC
ACTTGCACCG TTTCCGGCTT CTCCCTTAAT TCCTACACGA TCACTTGGGT GCGGCAACCA
CCCGGGAAAG GACTGGAATG GATCGCAGCC ATTAGCGGGG GAGGGAGCAC CTATTTCAAC
TCGGCTCTCA AGAGCCGCGT GACCATATCC CGTGACACAA GCAAGAGCCA GGTTTCCCTG
AAGCTGAGCT CCGTGACTGC TGCCGATACG GCTGTTTACT ATTGCACCCG ACCTCGCTGG
TATCCCCGTT CCTATTTCGA CTACTGGGGA AGAGGCACAC TGGTTACCGT CTCG

SEQ IN NO:158 编码抗毒素 B 抗体 1151.g4 VL 区域的多核苷酸序列

GCGATTCAAA TGACTCAGTC GCCCTCATCG CTTAGCGCGT CCGTCGGAGA TAGAGTGACG
ATCACGTGCA AAGCATCACA AAATGTCGGG AACAATGTGG CATGGTATCA GCATAAACCG
GGGAAGGCAC CAAAACTCTT GATCTACTAC GCCAGCAACA GGTTTACTGG TGTCCCGTCA
AGGTTCACGG GAGGGGGTTA CGGGACAGAC TTTACTTTGA CCATTTCGTC GCTTCAGCCG
GAGGACTTCG CCACCTATTA CTGTCAGAGG GTCTACCAGT CAACGTGGAC ATTTGGCCAG
GGAACGAAAG TGGAAATCAA G

图 9

SEQ IN NO:160 编码抗毒素 B 抗体 1151.g4 VH 区域的多核苷酸序列

GAAGTACAAC TCCAAGAGTC GGGGCCTGGT CTGGTCAAGC CGTCCGAAAC ACTTTCGCTG
ACGTGTACGG TATCAGGATT CTCACTTACA TCATACTACG TCCACTGGGT GAGGCAGCCA
CCCGGGAAGG GTCTTGAGTG GATGGGCTGC ATTAGAACCG GAGGGAATAC CGAGTACCAG
AGCGAATTTA AGAGCCGCGT CACTATCAGC CGGGATACGT CCAAAAACCA GGTGTCGCTC
AAATTGTCCT CCGTGACGGC CGCTGACACC GCAGTGTACT ATTGCGCGCG AGGAAACTAT
GGCTTTGCGT ATTGGGGACA GGGGACGCTC GTAACTGTCT CG

SEQ IN NO:168 编码抗毒素 B 抗体 1153.g8 VL 区域的多核苷酸序列

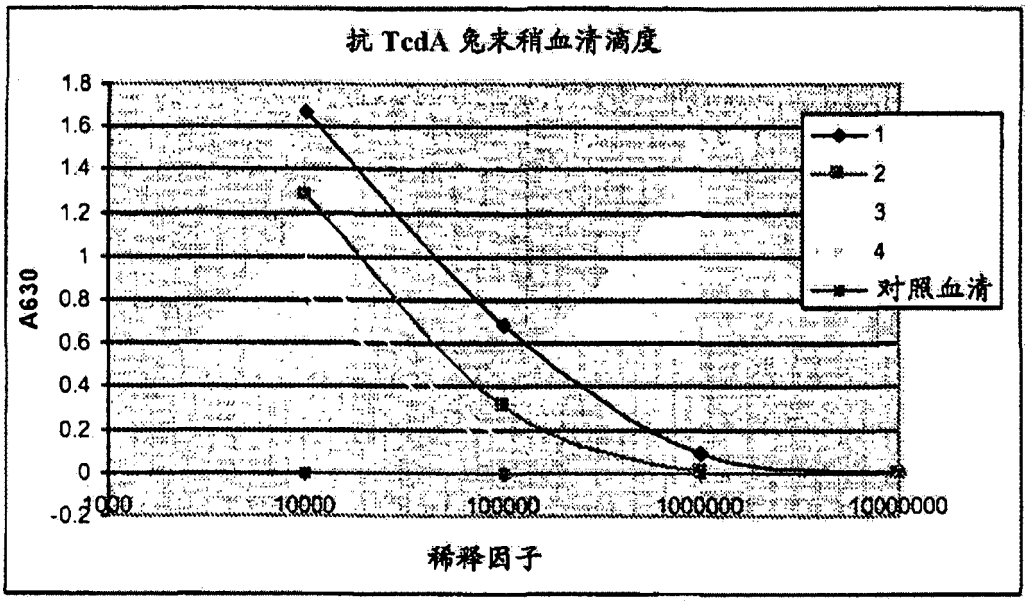
GATATACAGA TGACTCAGTC CCCTTCTAGC CTTTCAGCTT CCGTGGGCGA TAGAGTGACT
ATCACGTGTA AGGCTAGTCA GAACATTAAC AAGTATCTGG ACTGGTACCA GCAGAAACCC
GGGAAGGTTC CCAAGCTGCT GATCTACAAC ATCCAGTCCC TGCATACAGG CATTCCTAGC
CGGTTTAGCG GATCTGGTTC AGGGACCGAC TTCACCCTGA CAATCAGCTC TCTGCAACCA
GAAGACGTGG CCACCTATTA CTGCTTCCAG CACAATAGTG GCTGGACTTT TGGACAAGGT
ACCAGGCTGG AGATCAAA

SEQ IN NO:170 编码抗毒素 B 抗体 1153.g8 VH 区域的多核苷酸序列

GAGGTTCAGC TGGTGGAATC AGGAGGGGGT CTGGTGCAAC CAGGAGGCTC CCTGAAACTG
TCTTGCGCCG CAAGCGGCTT TACGTTTACC CAGGCCGCTA TGTTCTGGGT TAGGCAGGCC
AGTGGGAAGG GTCTTGAAGG CATCGCAAGA ATCAGCACCA AGAGCAACAA TTTCGCTACG
TACTATCCGG ACTCCGTGAA AGGCCGGTTT ACCATTTCTC GCGATGACAG CAAGAACACC
GTGTACCTGC AGATGAACAG TCTCAAGACC GAGGACACAG CCGTGTACTA TTGTACTGCT
CCCGCCTATT ATTACGATGG CACAGTGCCT TTCGCATACT GGGGACAGGG
TACTTTGGTG ACTGTCTCG

图 10

来自以毒素TcdA免疫的4只兔子和以TcdB结合结构（TcdB1234）免疫的5只大鼠的血清滴度。使用涂覆于ELISA板上的TcdA毒素或TcdB结合结构域产生的ELLSA数据



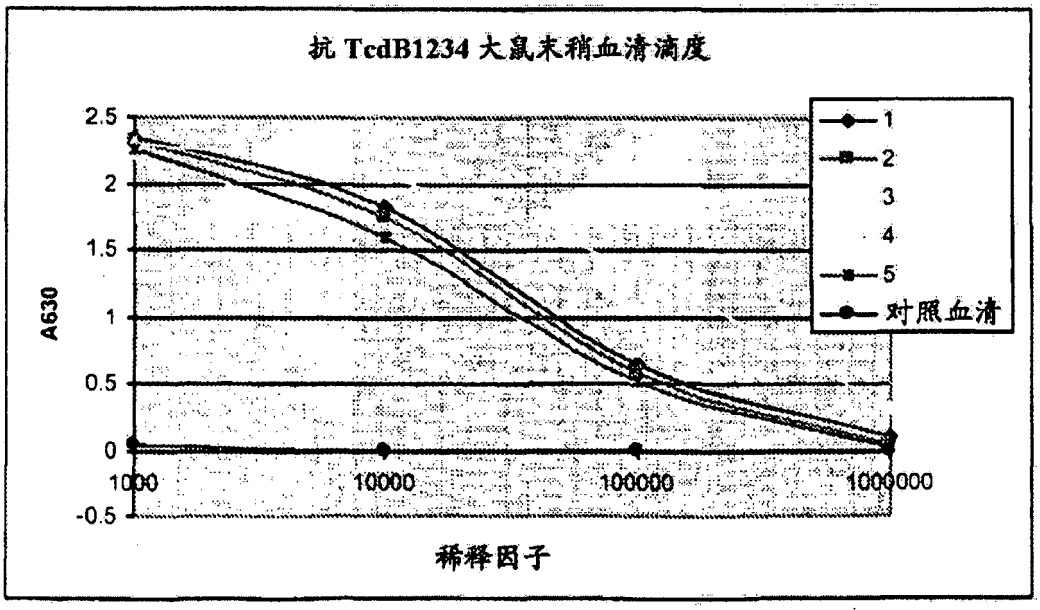


图 11

单一Mab的抗TcdA(核糖型 003)体外中和数据(X 轴浓度(ng/ml)和 Y 轴中和%)

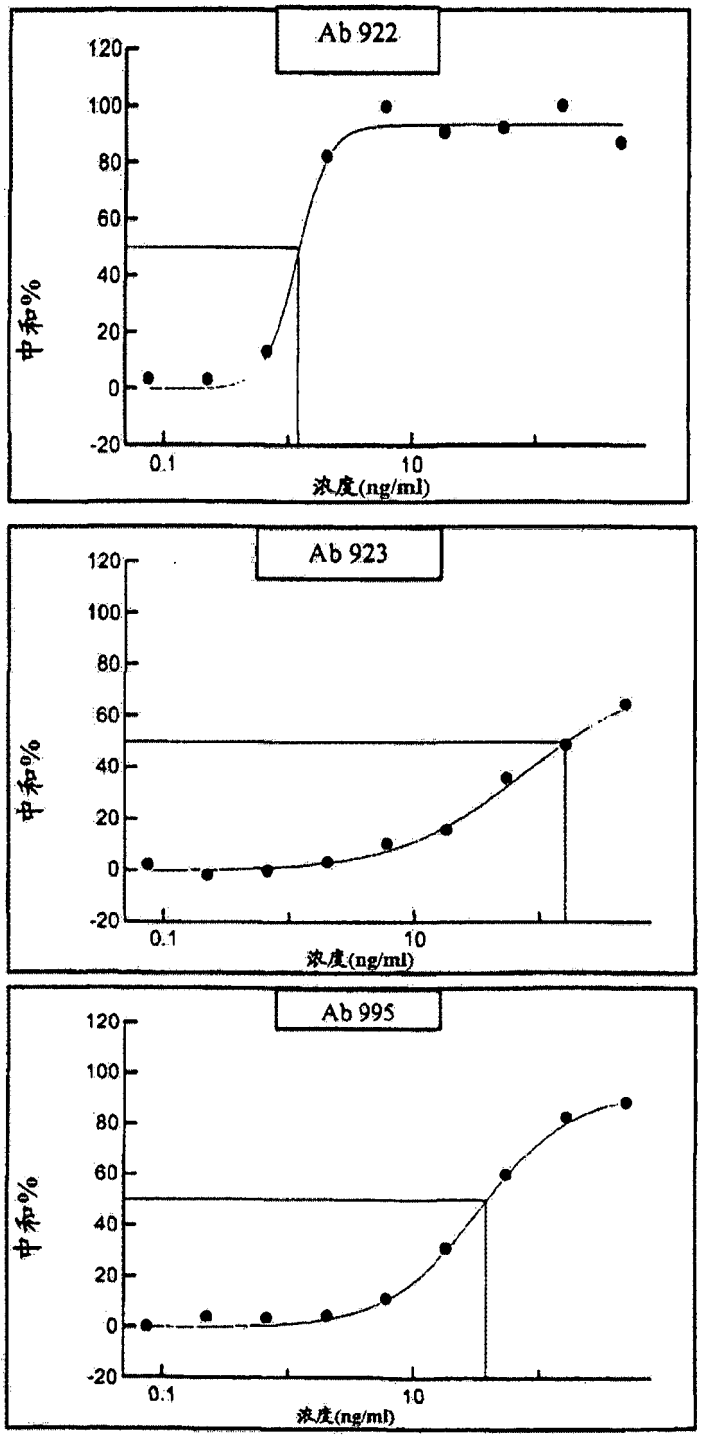


图 12

单一 Mab 的抗 TcdA(核糖型 003)体外中和数据(X 轴浓度(ng/ml)和 Y 轴中和%)

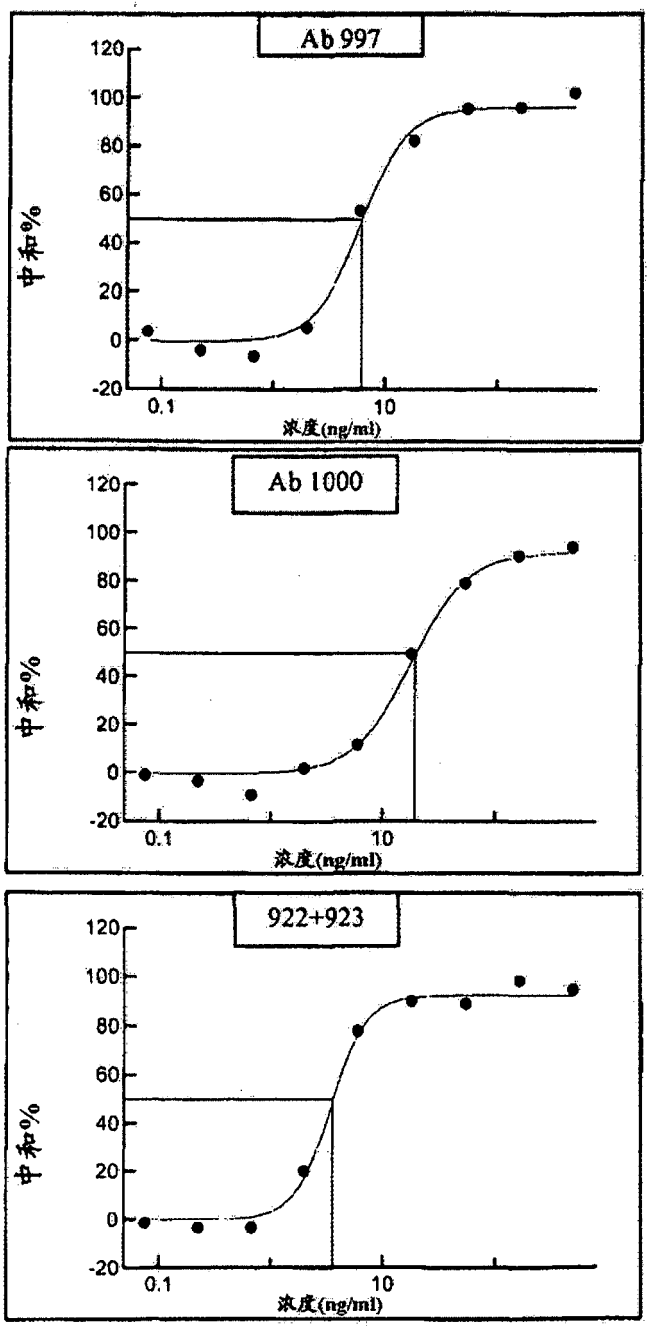


图 13

配对Mab 抗 TcdA(核糖型 003)体外中和数据(X 轴浓度(ng/ml)和 Y 轴中和%)

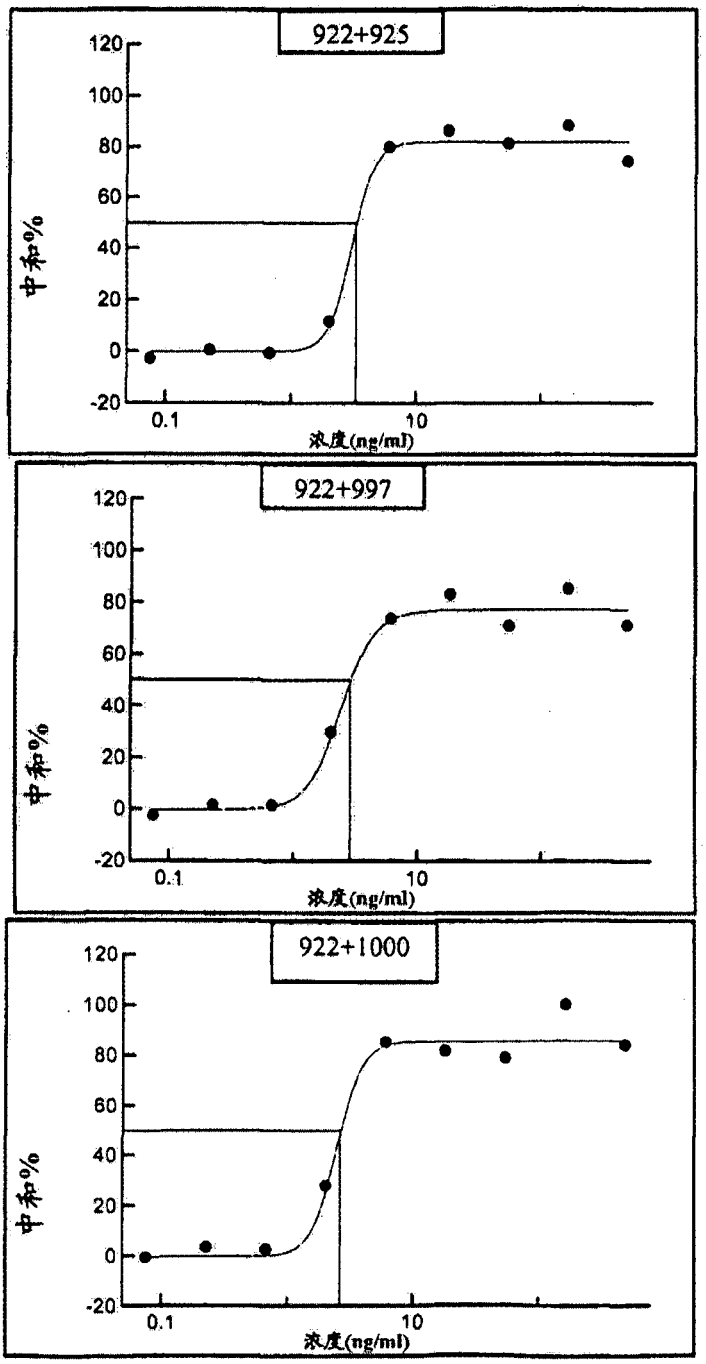


图 14

配对Mab 的抗 TcdA(核糖型 003)体外中和数据 (X 轴浓度(ng/ml)和 Y 轴中和%)

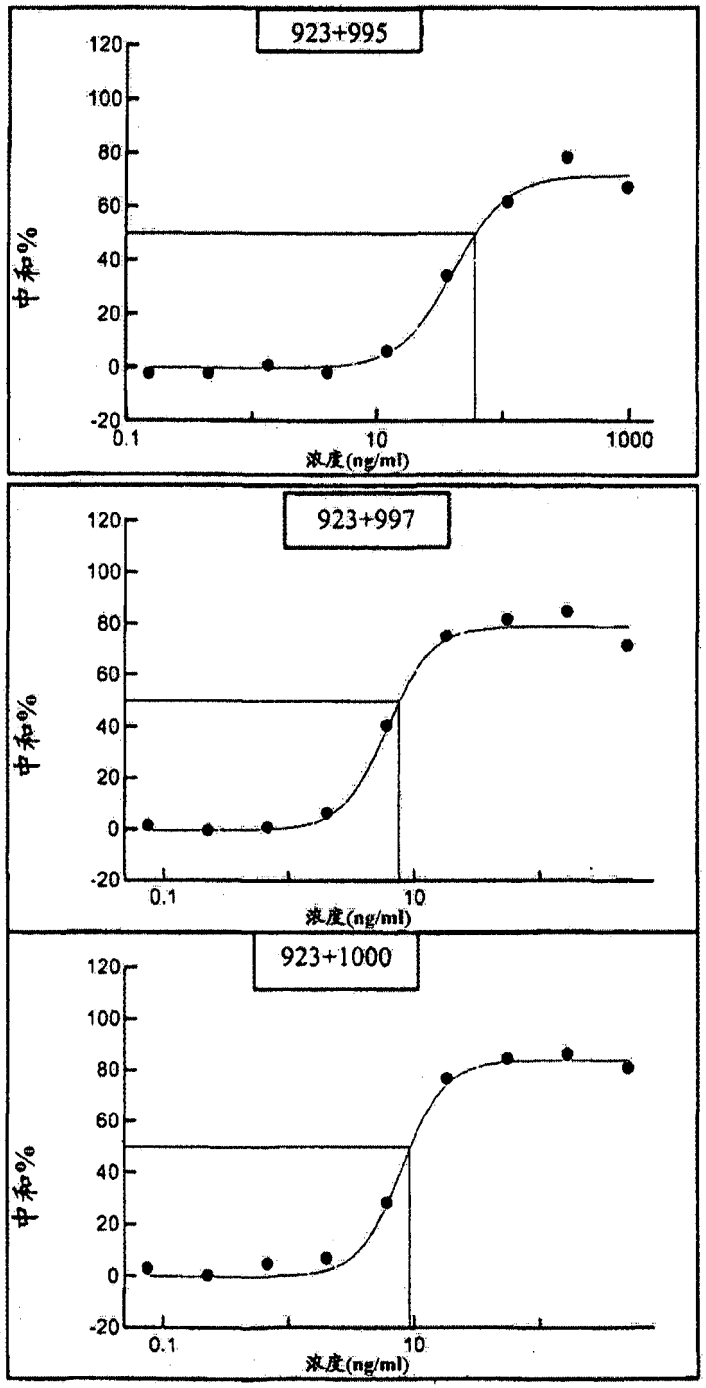


图 15

三种Mab混合物的抗 TcdA(核糖型 003)体外中和数据(X 轴浓度(ng/ml)和 Y 轴中和%)

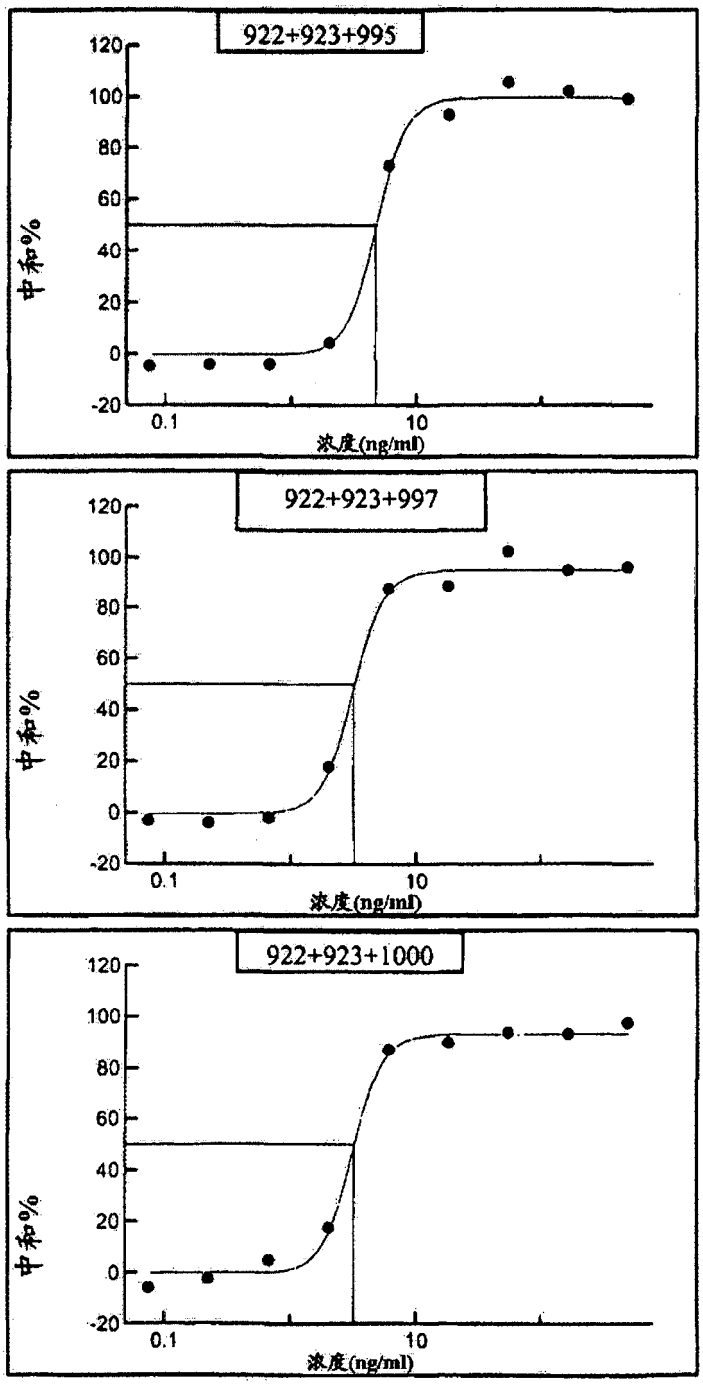


图 16

三种Mab混合物的抗 TcdA(核糖型 003)体外中和数据(X 轴浓度(ng/ml)和 Y 轴中和%)

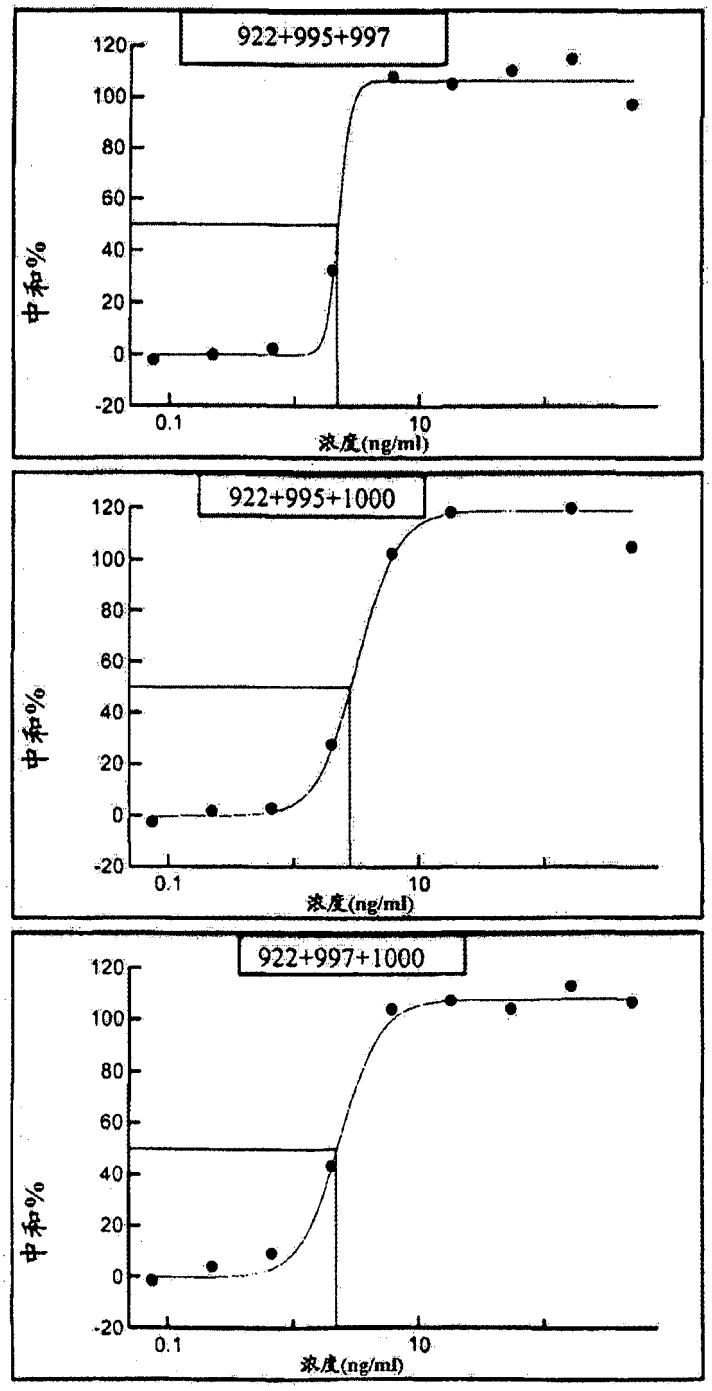


图 17

三种Mab混合物的抗 TcdA(核糖型 003)体外中和数据(X 轴浓度(ng/ml)和 Y 轴中和%)

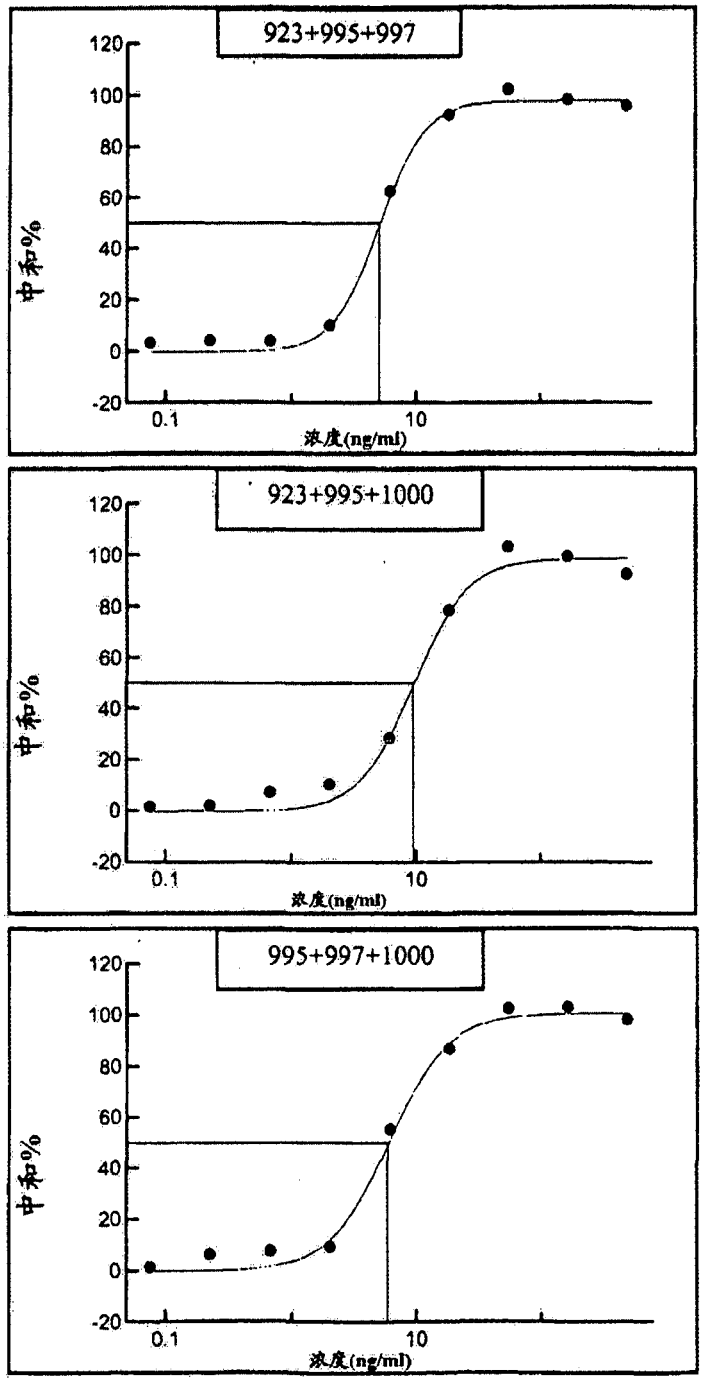


图 18

四种和五种Mab混合物的抗 TcdA(核糖型 003)体外中和数据(X 轴浓度(ng/ml)和 Y 轴中和%)

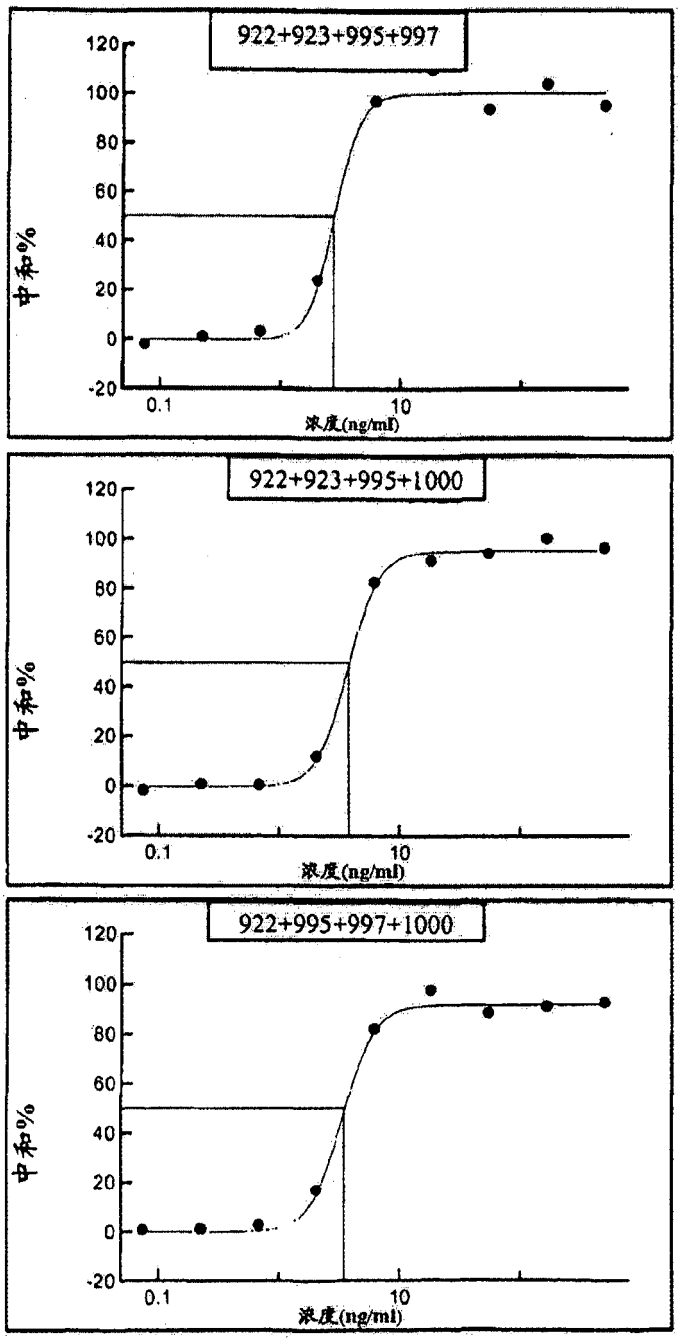


图 19

四种和五种Mab的混合物的抗TcdA(核糖型003)体外中和数据(X轴为浓度ng/ml和Y轴中和%)

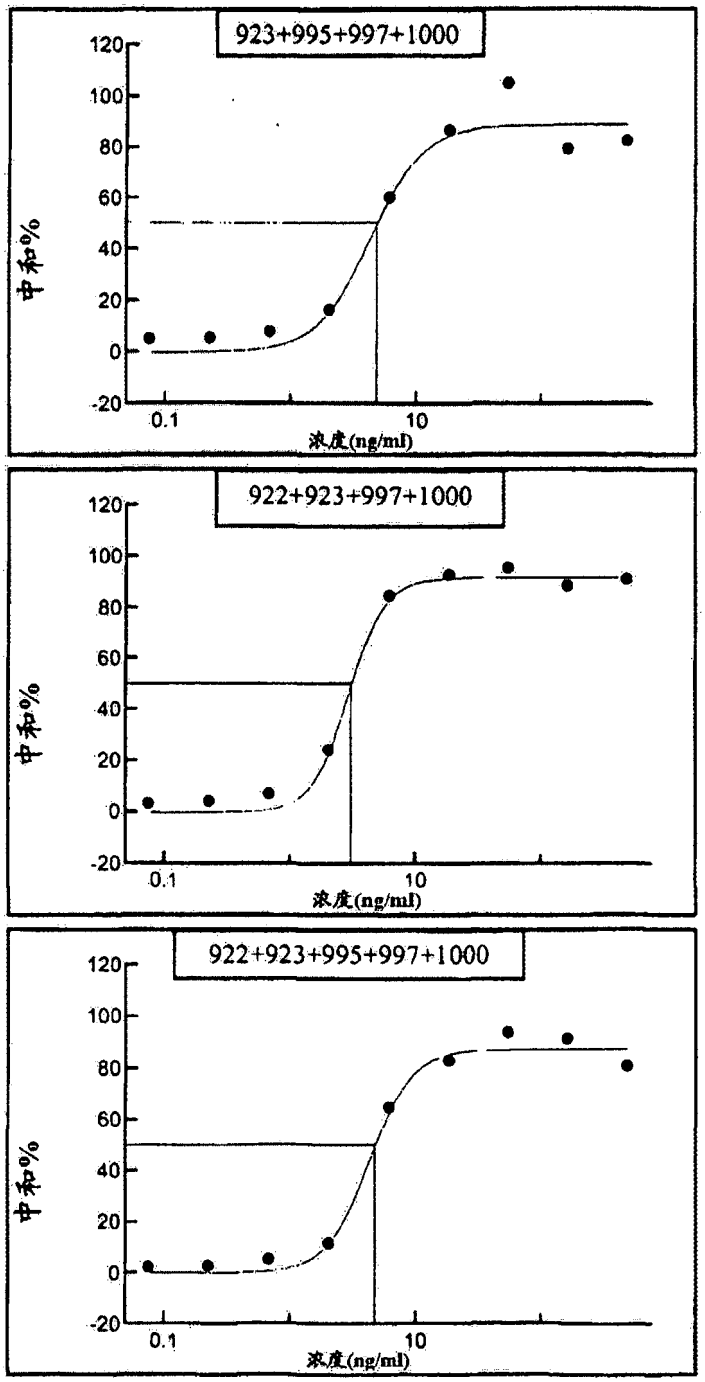


图 20

单一和成对 Mab 在不同 TcdA 浓度上的抗 TcdA(核糖型 003)体外中和数据(X 轴为浓度 ng/ml)

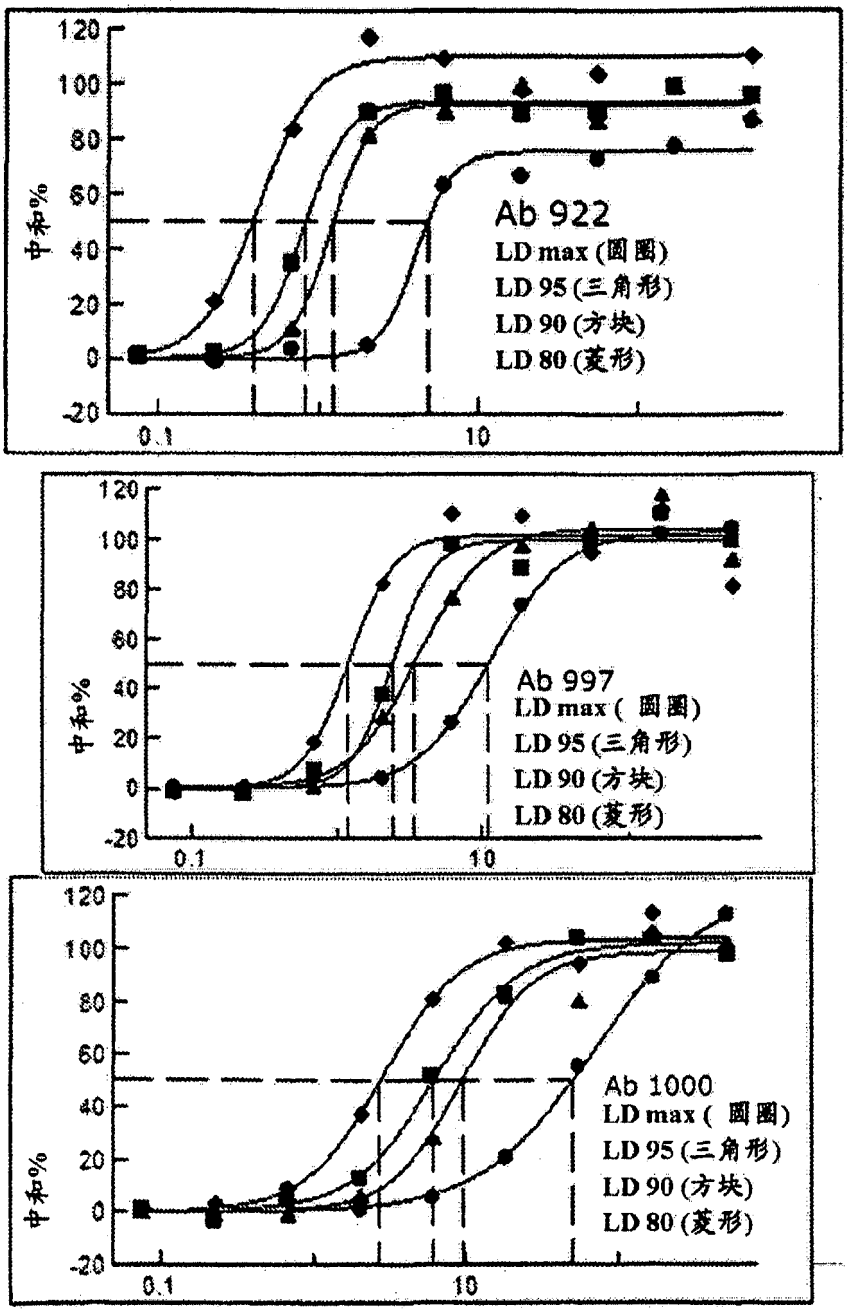


图 21

单一和成对 Mab 在不同 TcdA 浓度上的抗 TcdA(核糖型 003)体外中和数据(X 轴为浓度 ng/ml)

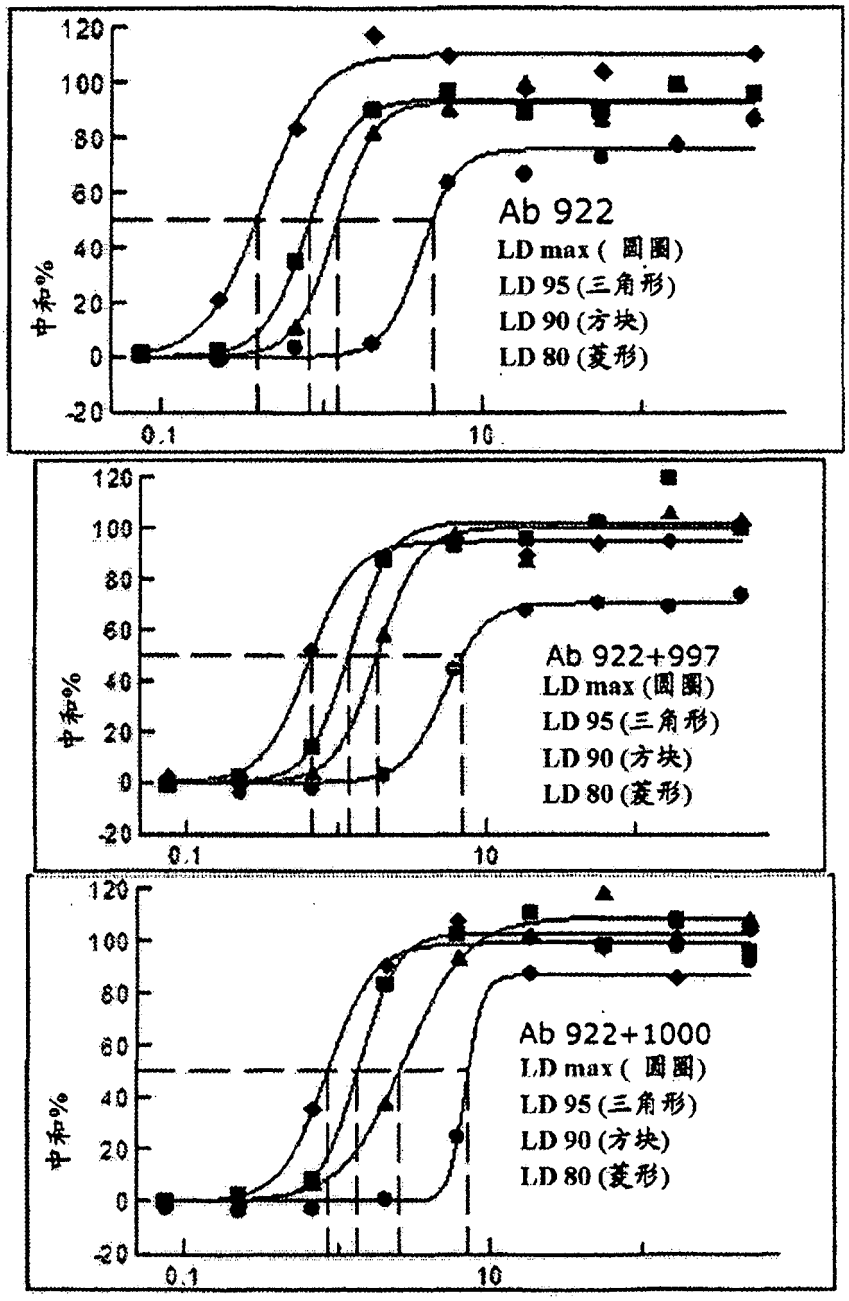


图 22

单一和五种Mab的混合物在不同TcdA浓度上的抗TcdA（核糖型003）体外中和数据(X轴为浓度ng/ml)

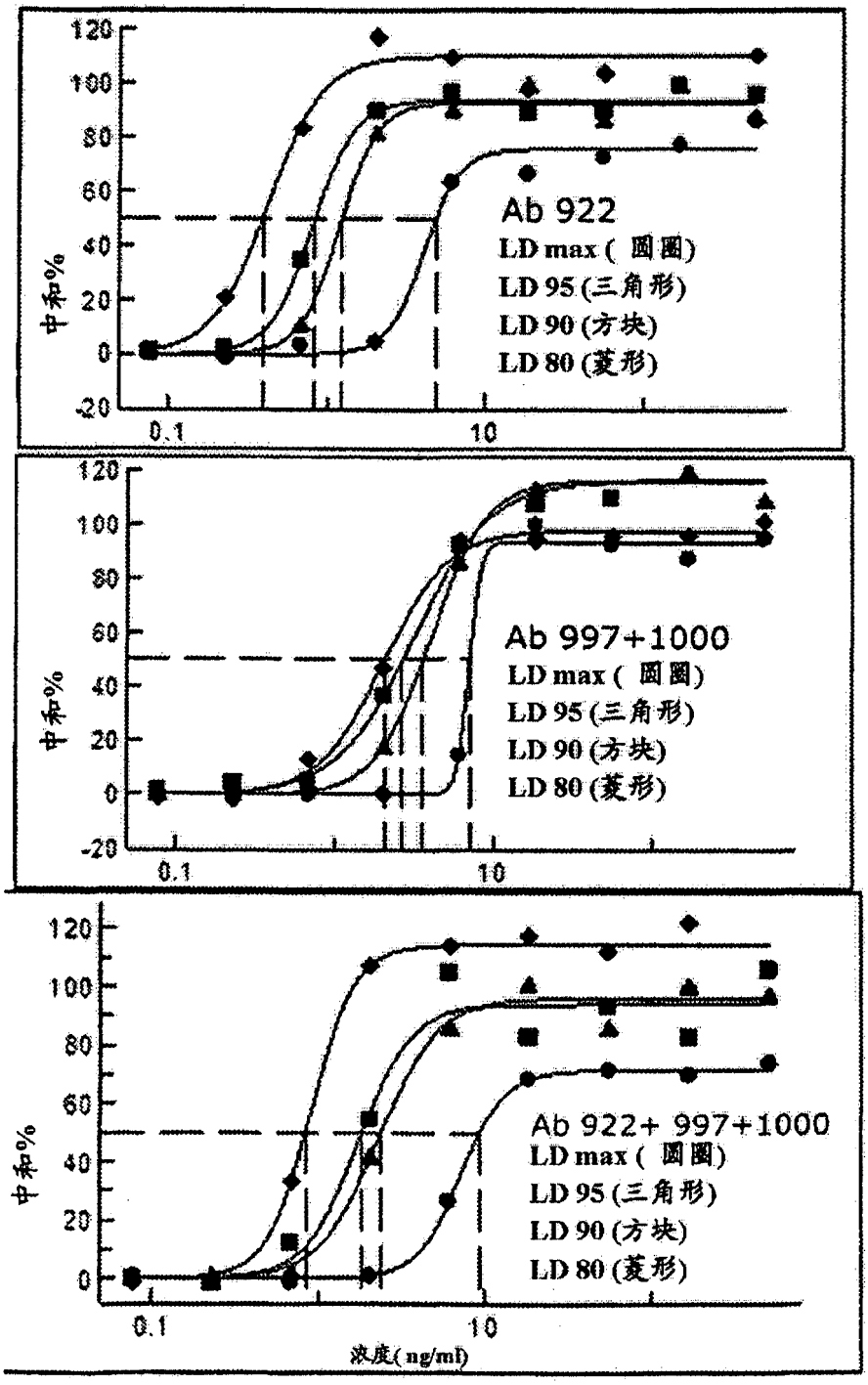


图 23

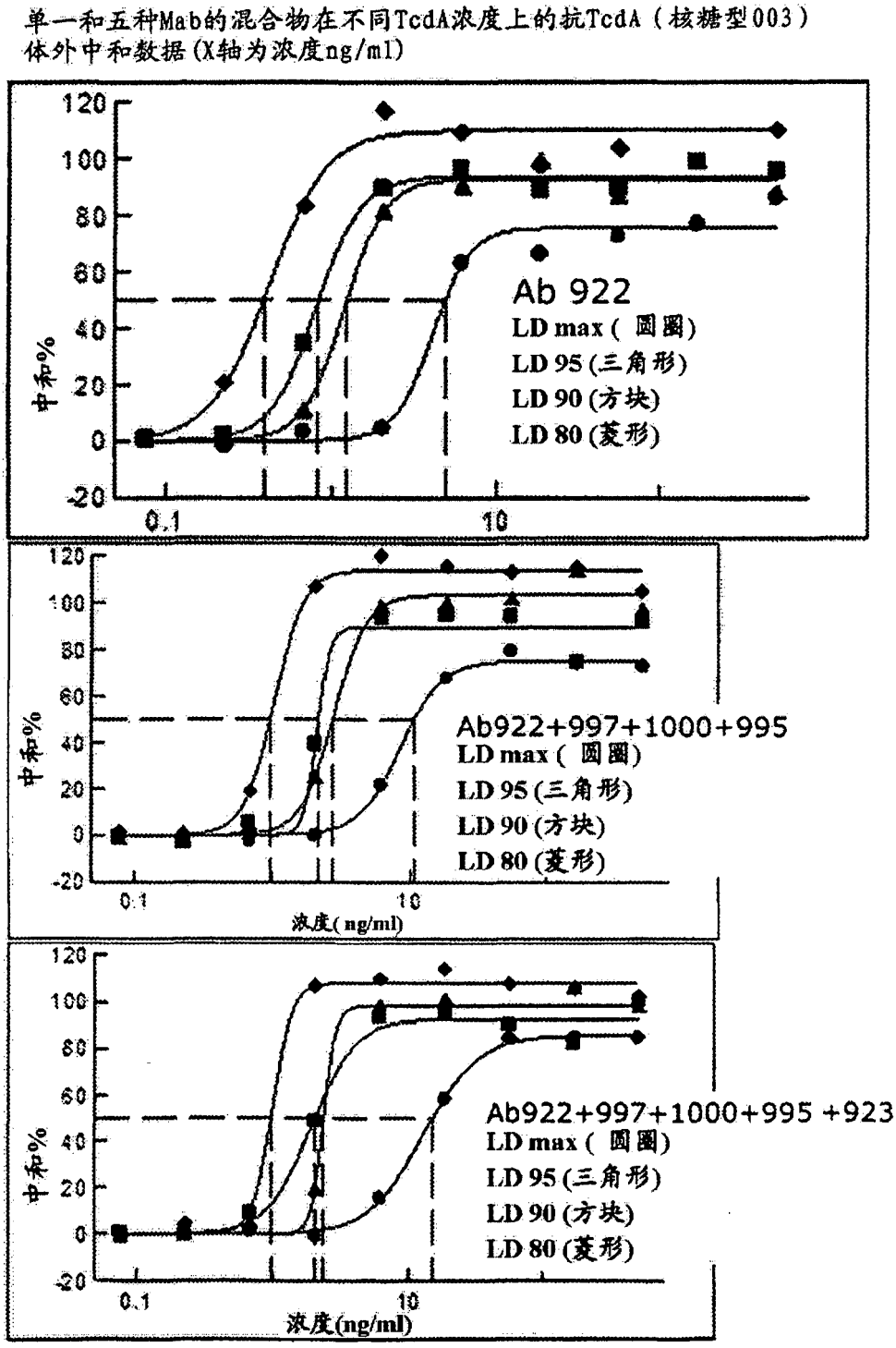

单一和五种Mab的混合物在不同TcdA浓度上的抗TcdA（核糖型003）体外中和数据(X轴为浓度ng/ml)
中和%
120
100
80
60
40
20
0
-20
0.1
10
Ab 922
LD max (圆圈)
LD 95 (三角形)
LD 90 (方块)
LD 80 (菱形)
Ab922+997+1000+995
LD max (圆圈)
LD 95 (三角形)
LD 90 (方块)
LD 80 (菱形)
浓度(ng/ml)
Ab922+997+1000+995 +923
LD max (圆圈)
LD 95 (三角形)
LD 90 (方块)
LD 80 (菱形)
浓度(ng/ml)

图 24

单一 Mab 的抗 TcdB (核糖型 003)体外中和数据(分别地对于 1125.g2, 1134.g5 和 927.g2 的 Y 轴中和 X 轴浓度 ng/ml)

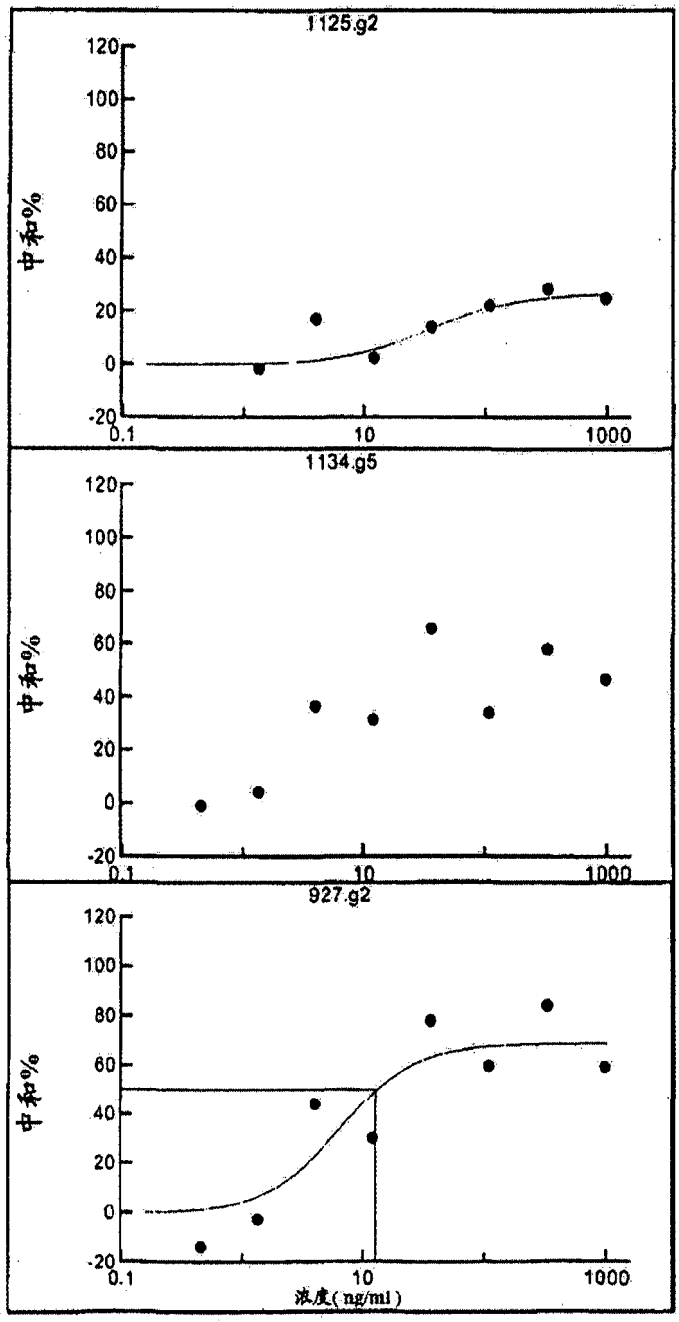


图 25

单一 Mab 的抗 TcdB (核糖型 003)体外中和数据(分别地对于 1153.g8 和 1102.g4 的 Y 轴中和 X 轴浓度 ng/ml)

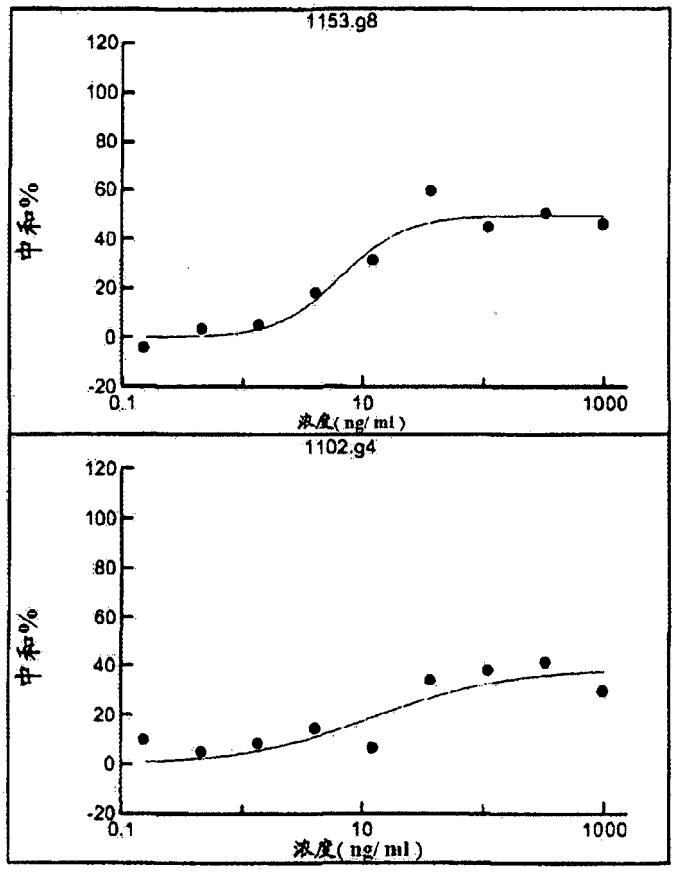


图 26

成对 Mab 的抗 TcdB (核糖型 003)体外中和数据(分别地对于 927+1099、927+1102、927+1114 的组合的 Y 轴中和 X 轴浓度 ng/ml)

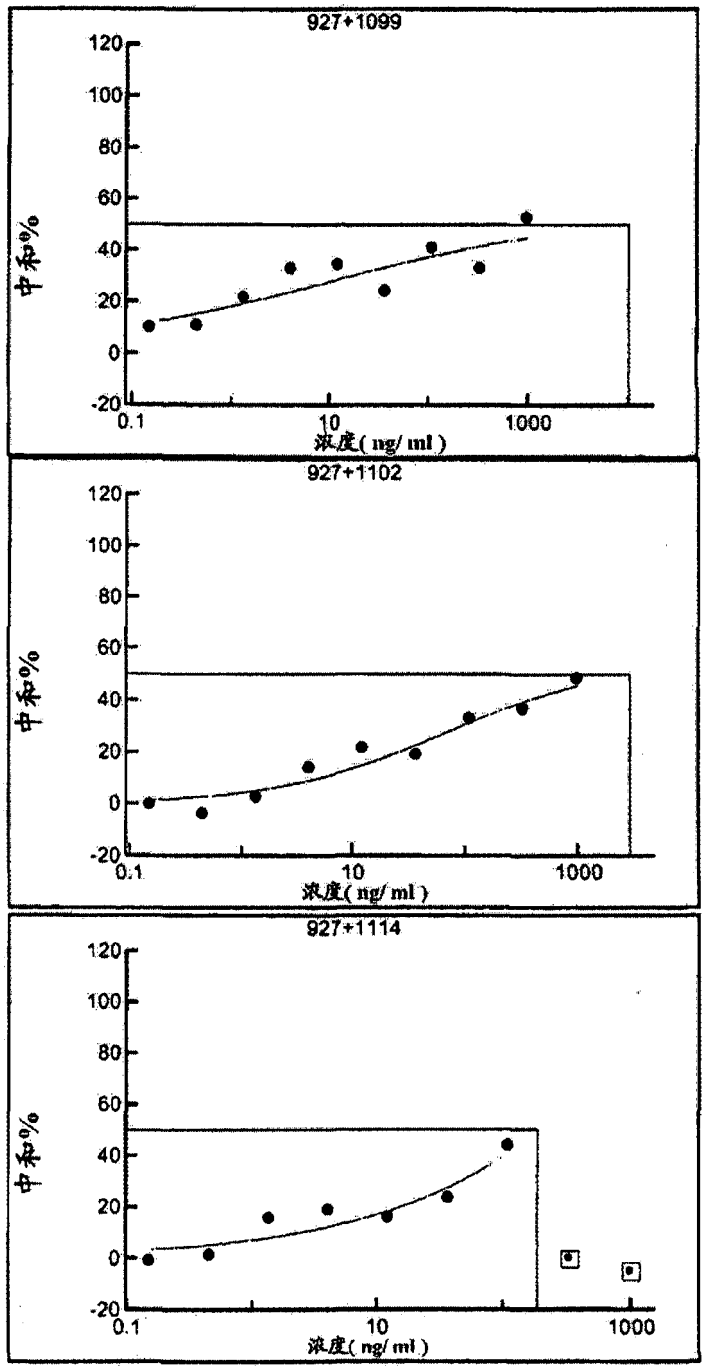


图 27

成对 Mab 的抗 TcdB (核糖型 003)体外中和数据(分别地对于 927+1125、927+1134、1099+1114 的组合的 Y 轴中和 X 轴浓度 ng/ml)

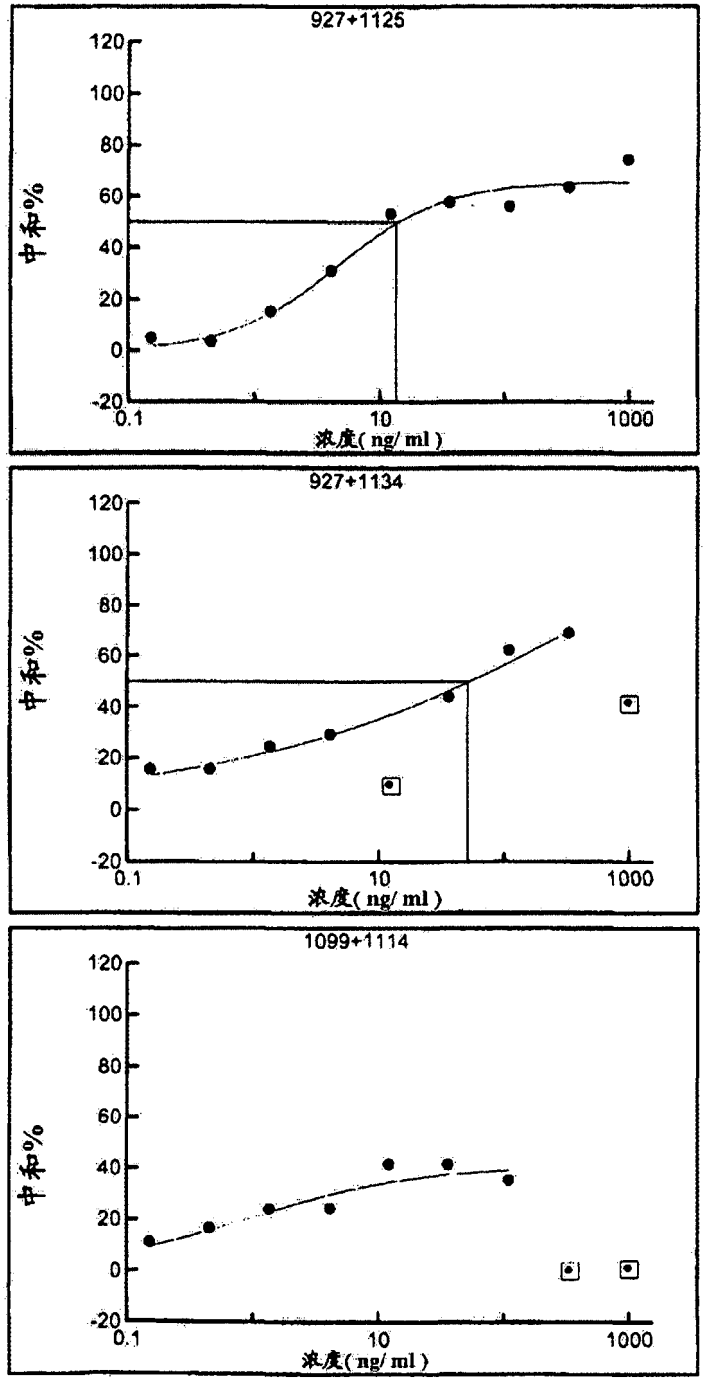


图 28

成对 Mab 的抗 TcdB (核糖型 003)体外中和数据(分别地对于 1102+1114, 1102+1125, 1114+1134 的组合的 Y 轴中和 X 轴浓度 ng/ml)

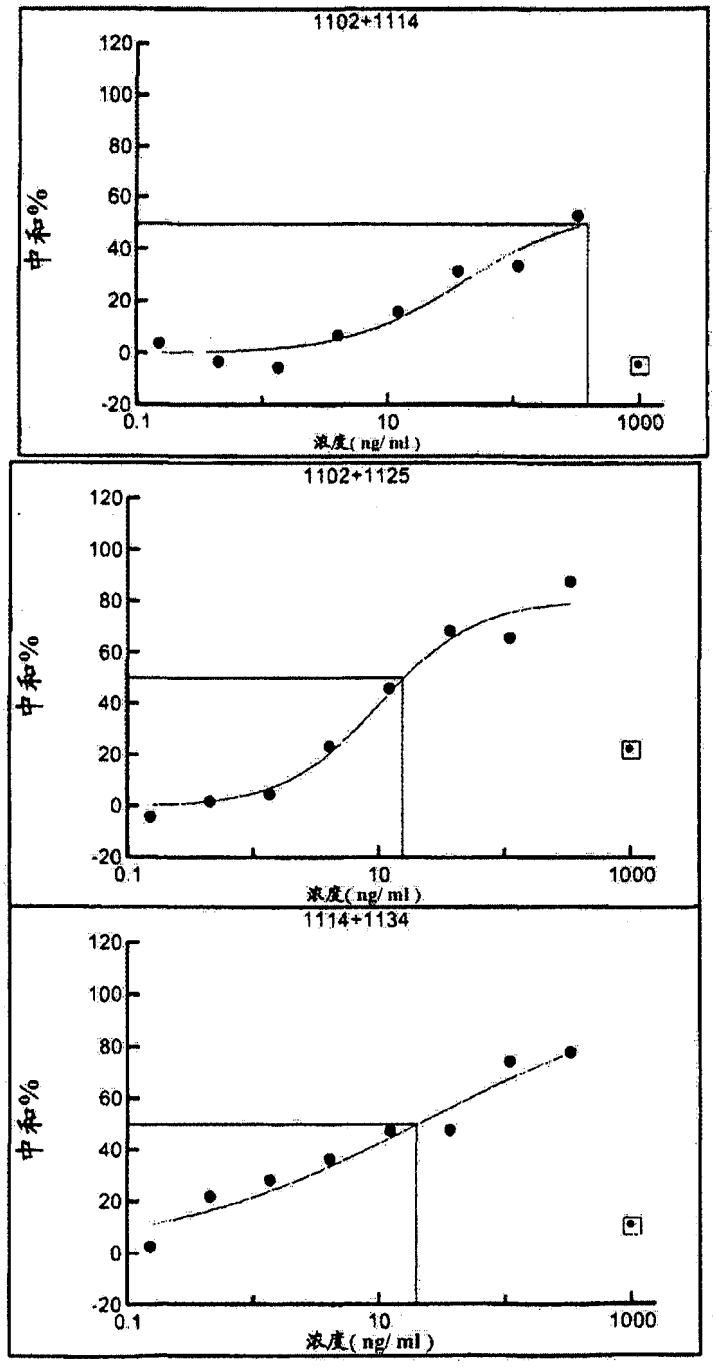


图 29

成对 Mab 的抗 TcdB (核糖型 003)体外中和数据(分别地对于 1114+1151, 1114+1153, 1125+1134 的组合的 Y 轴中和 X 轴浓度 ng/ml)

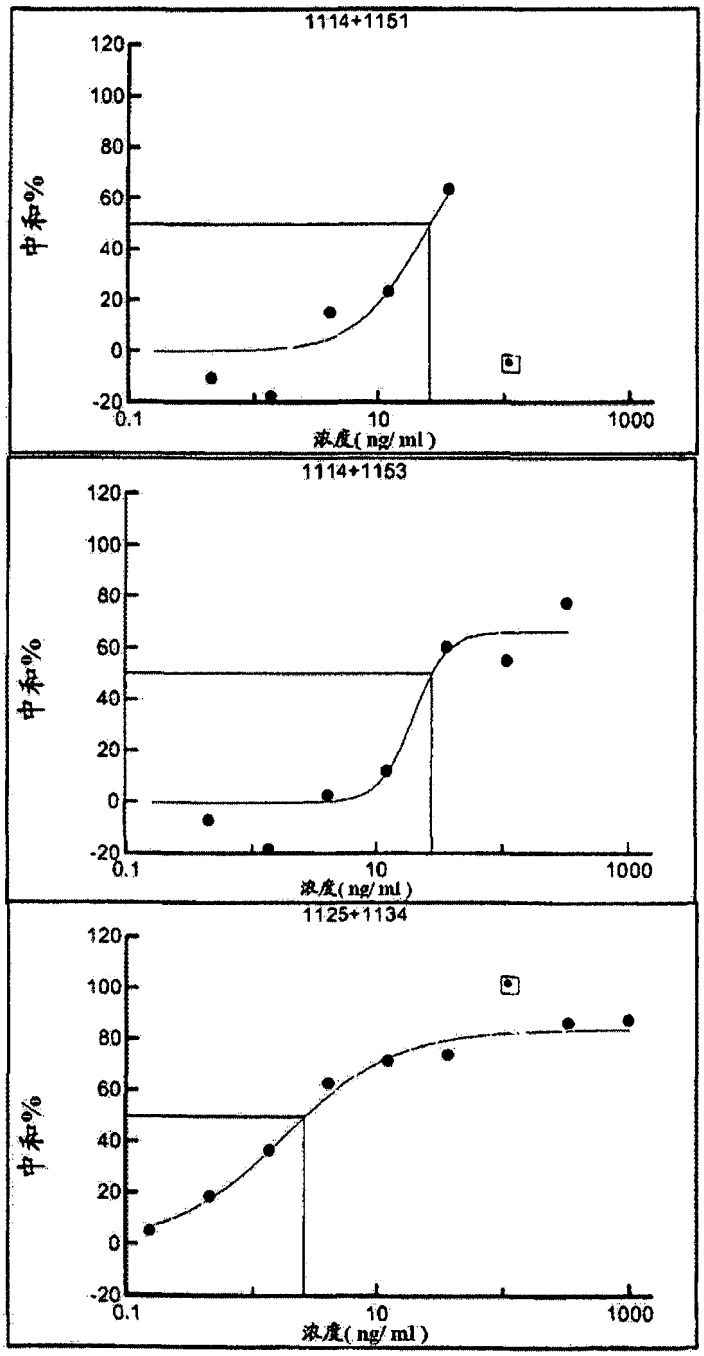


图 30

三种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据(分别地对于 1125+1134+1114, 1125+1134+927, 1125+1151+1114 的组合的 Y 轴中和 X 轴浓度 ng/ml)

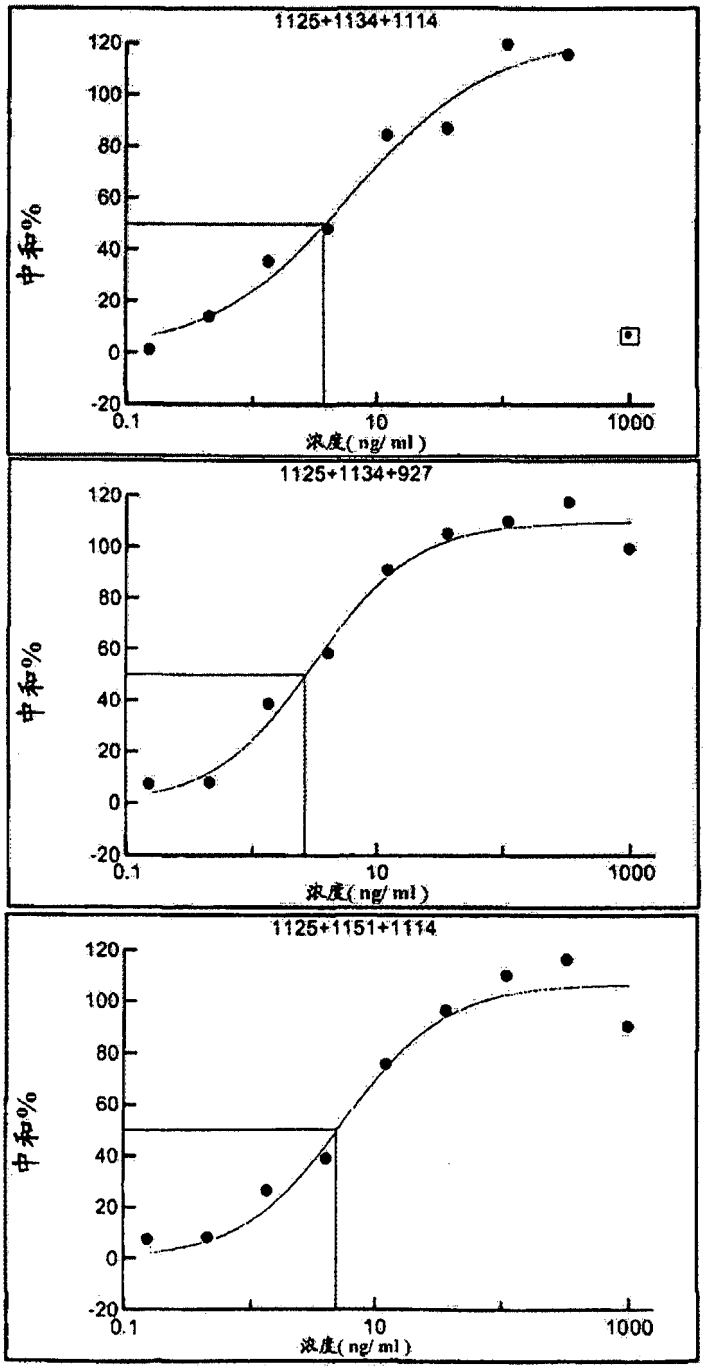


图 31

三种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据(分别地对于 1125+1151+927, 1125.g2+1134.g2+927.g2 的组合的 Y 轴中和 X 轴浓度 ng/ml)

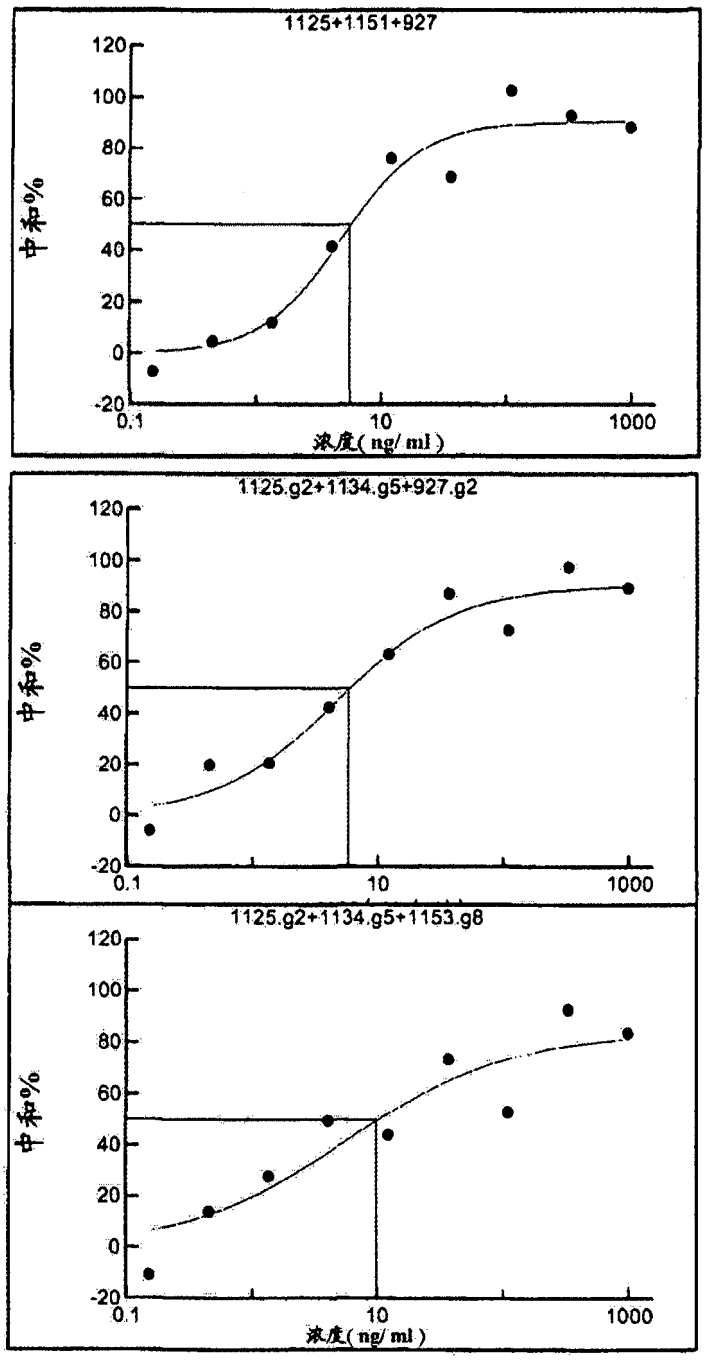


图 32

3 种 Mab 的混合物的抗 TcdB (核糖型 003)体外中和数据

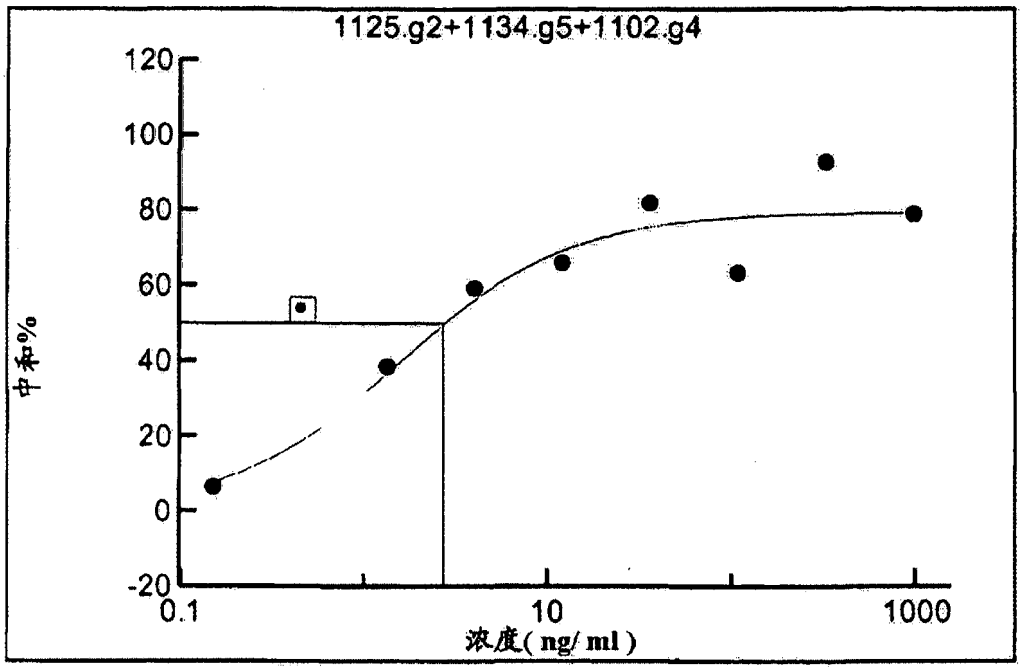


图 33

在不同毒素浓度上的两种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据

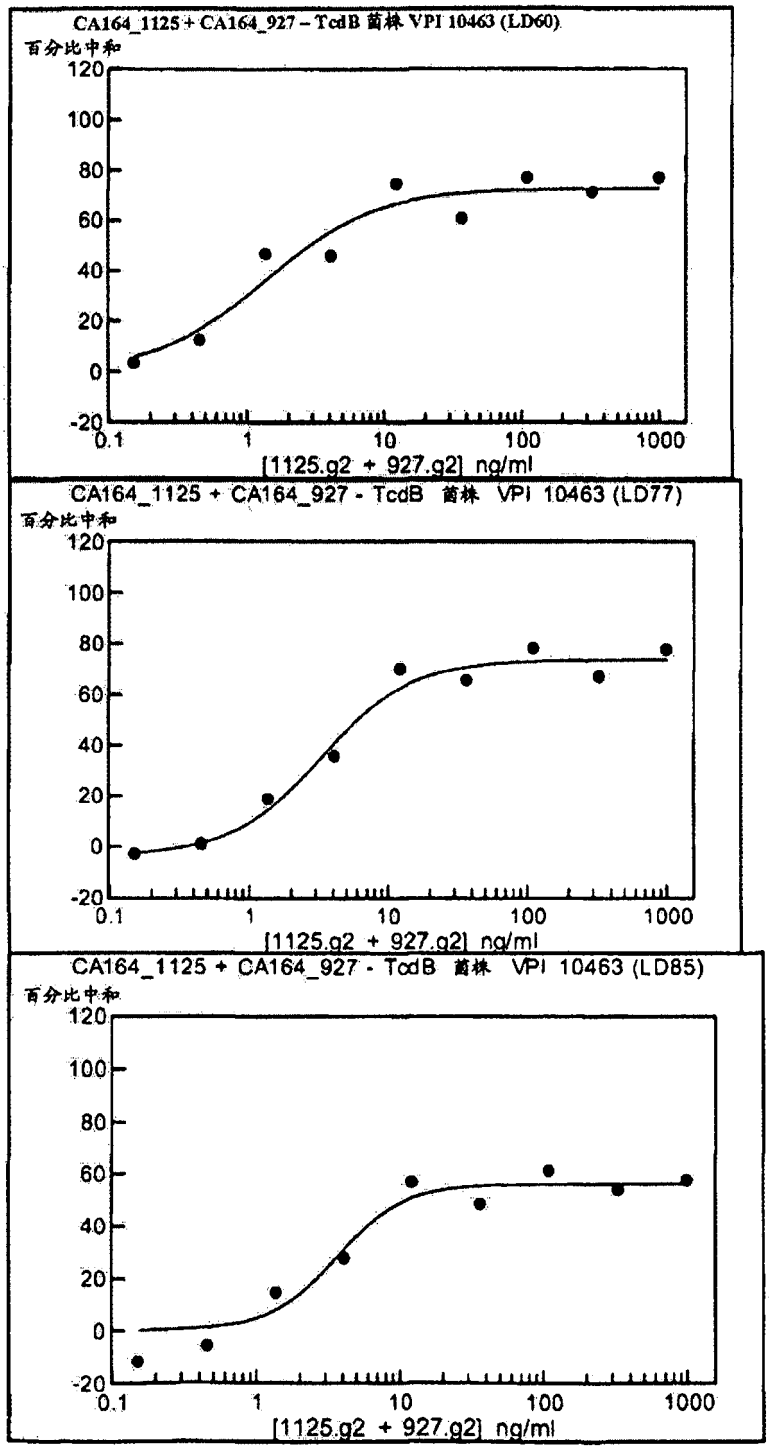


图 34

在不同毒素浓度上的两种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据

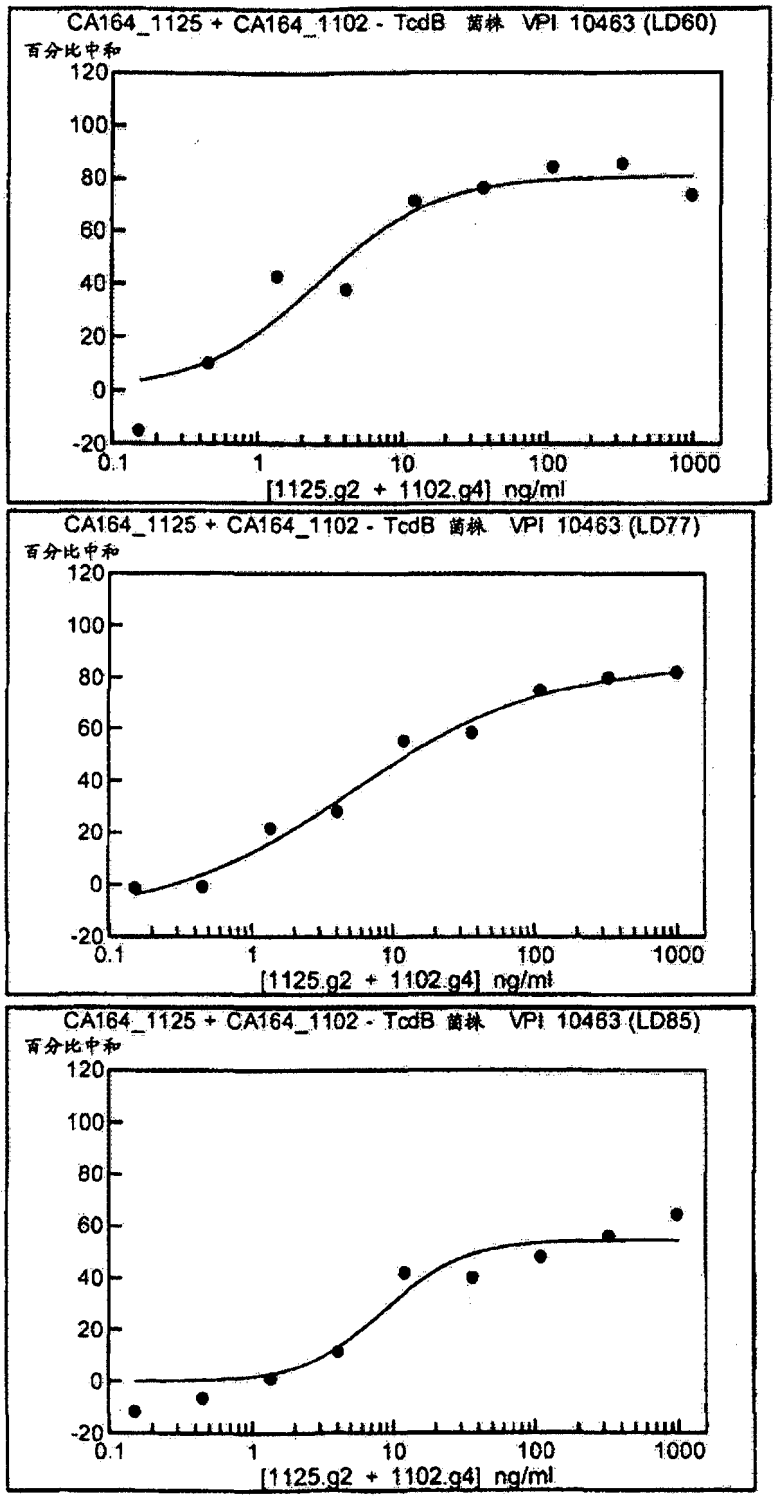


图 35

在不同毒素浓度上的两种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据

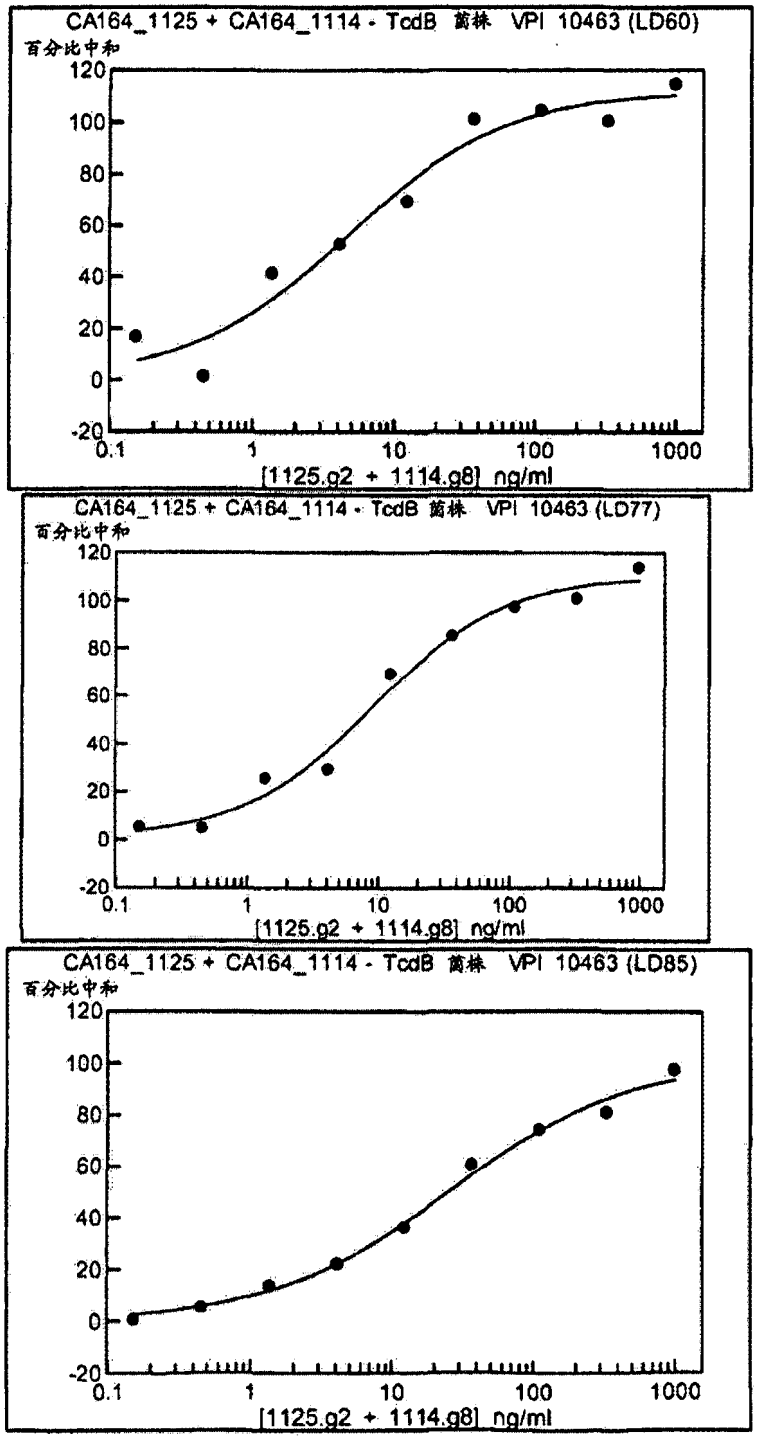


图 36

在不同毒素浓度上的两种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据

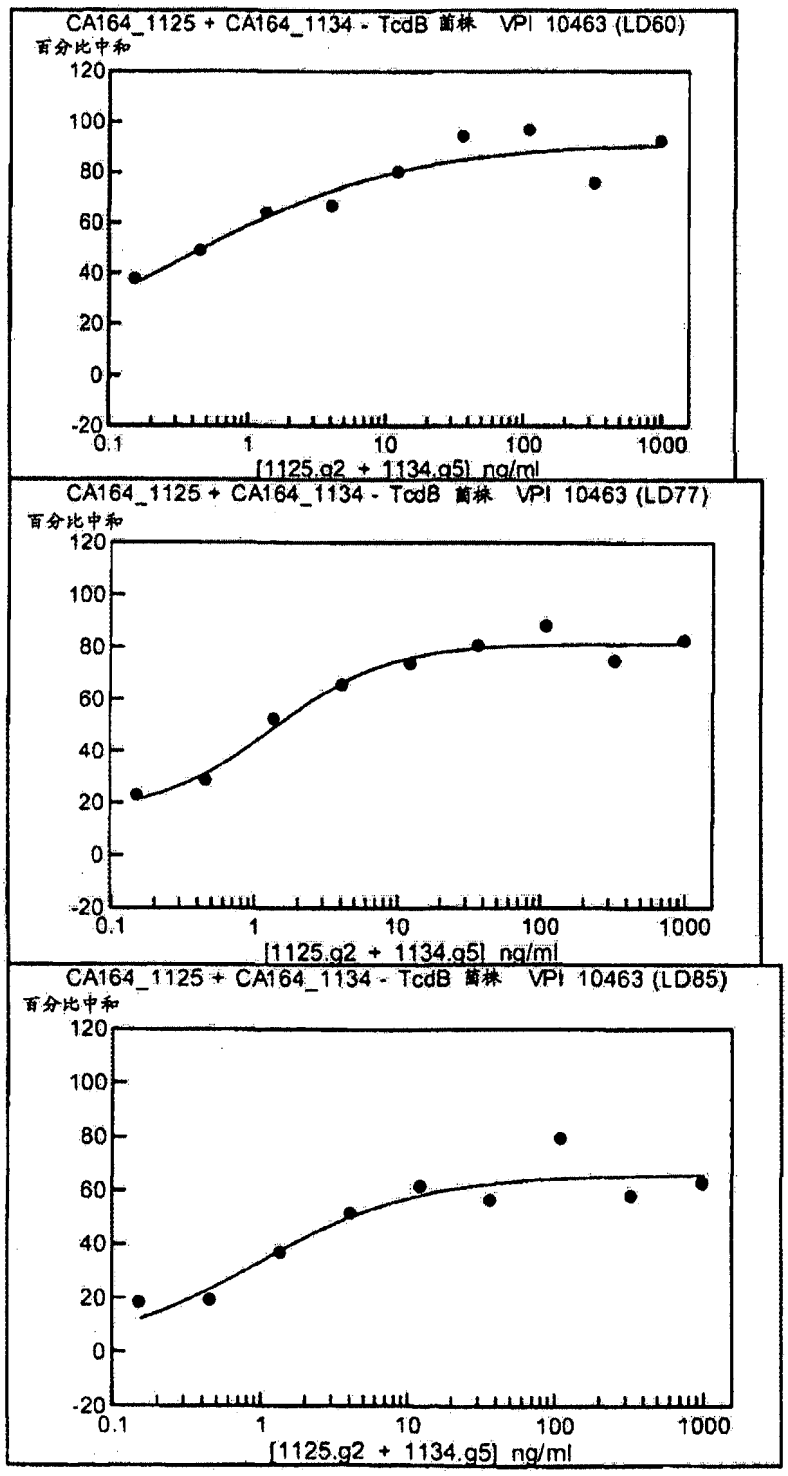


图 37

在不同毒素浓度上的两种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据

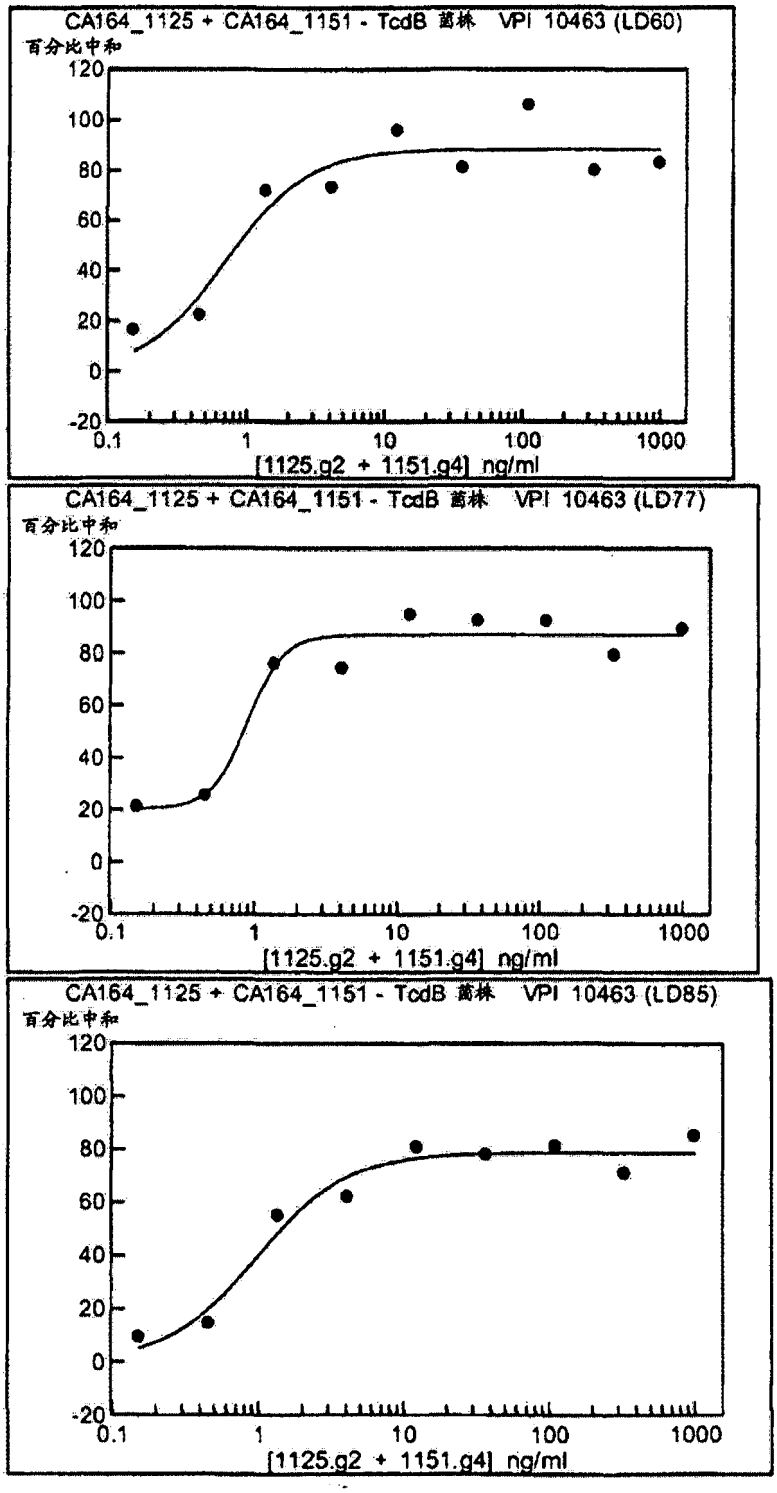


图 38

在不同毒素浓度上的两种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据

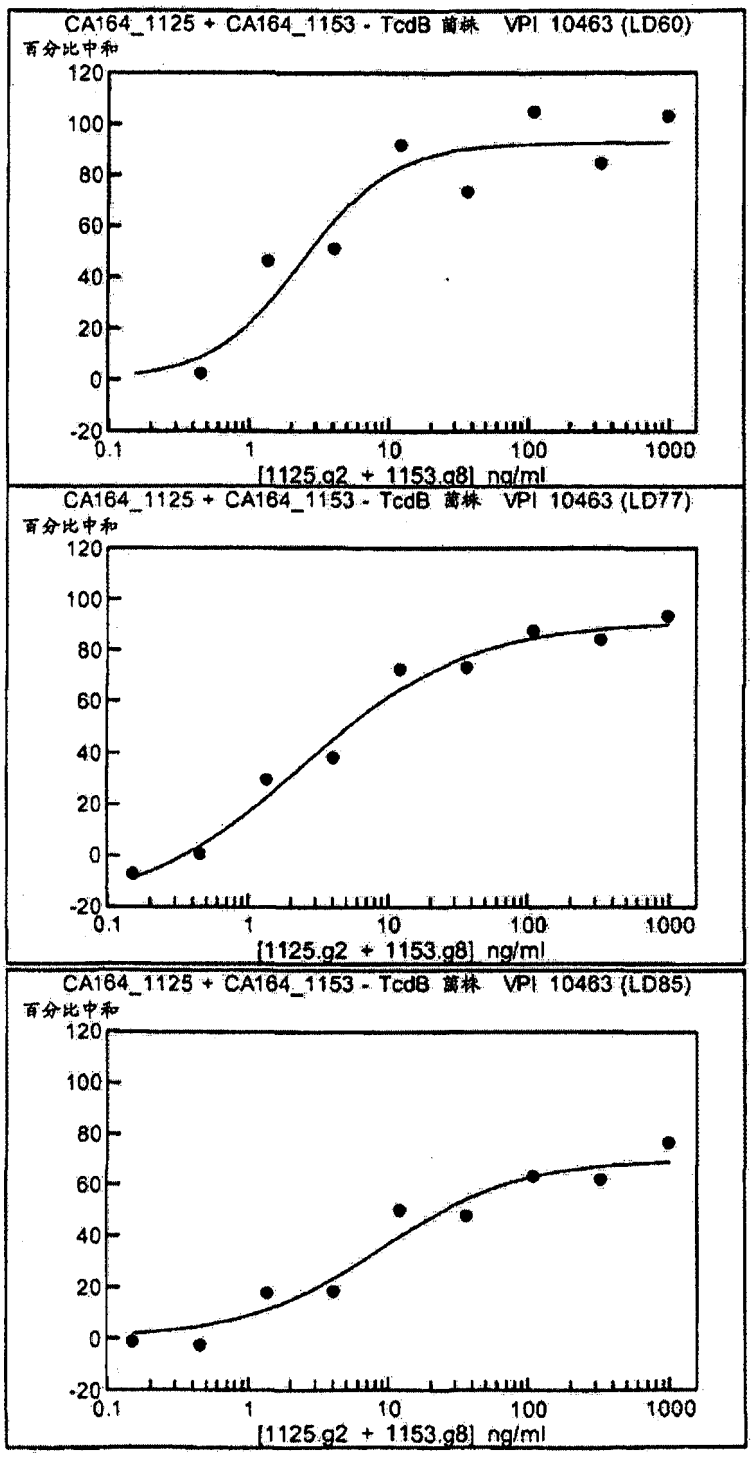


图 39

在不同毒素浓度上的两种 Mab 混合物的抗 TcdB (核糖型 003)体外中和数据

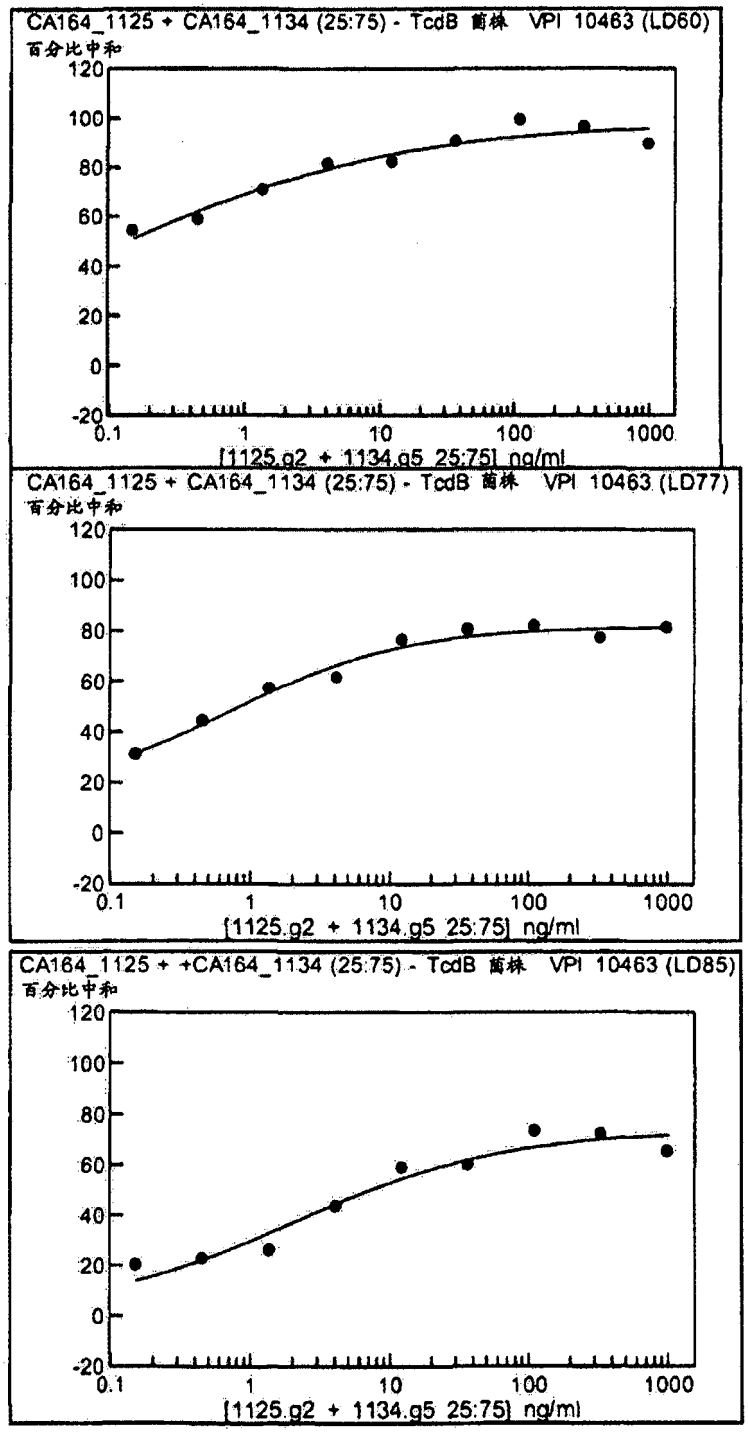


图 40

在不同相对 Mab 比率和不同毒素浓度下两种 Mab 的混合物的抗 TcdB (核糖型 003)体外中和数据

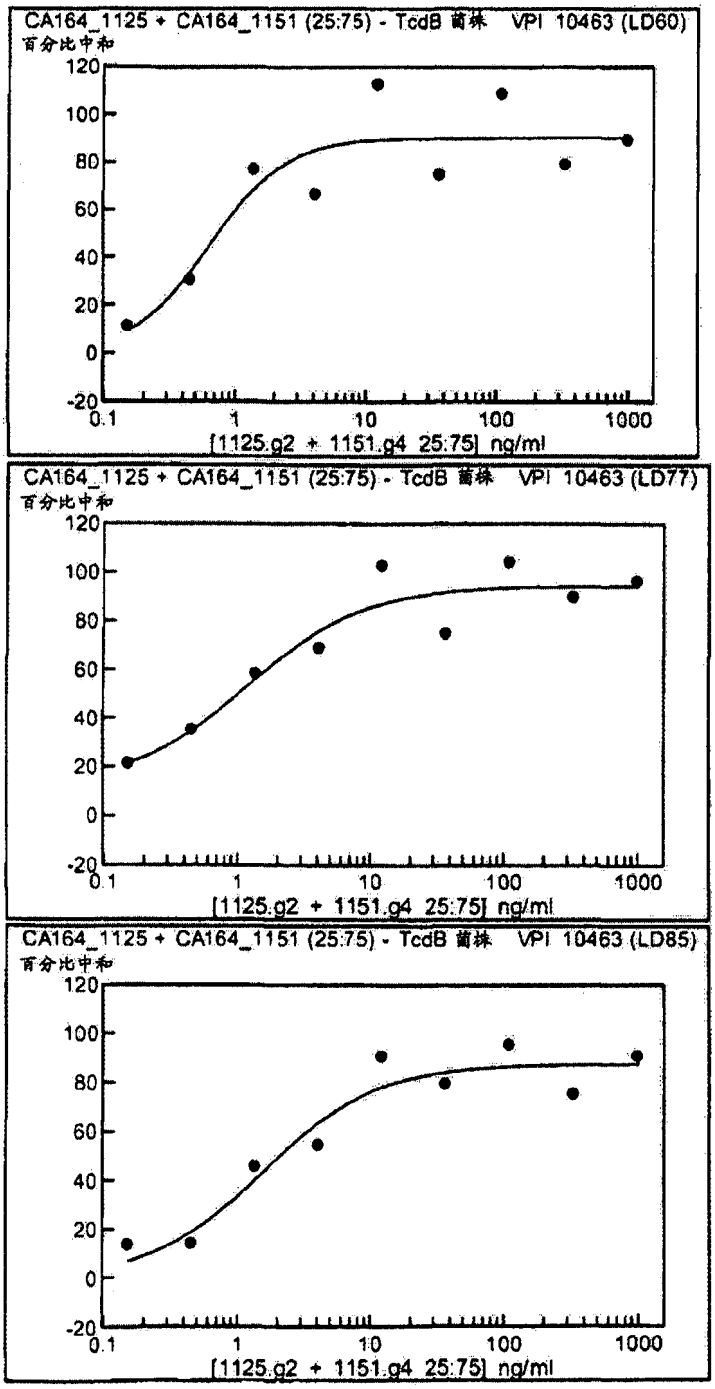


图 41

在不同相对 Mab 比率和不同毒素浓度下两种 Mab 的混合物的抗 TcdB (核糖型 003)体外中和数据

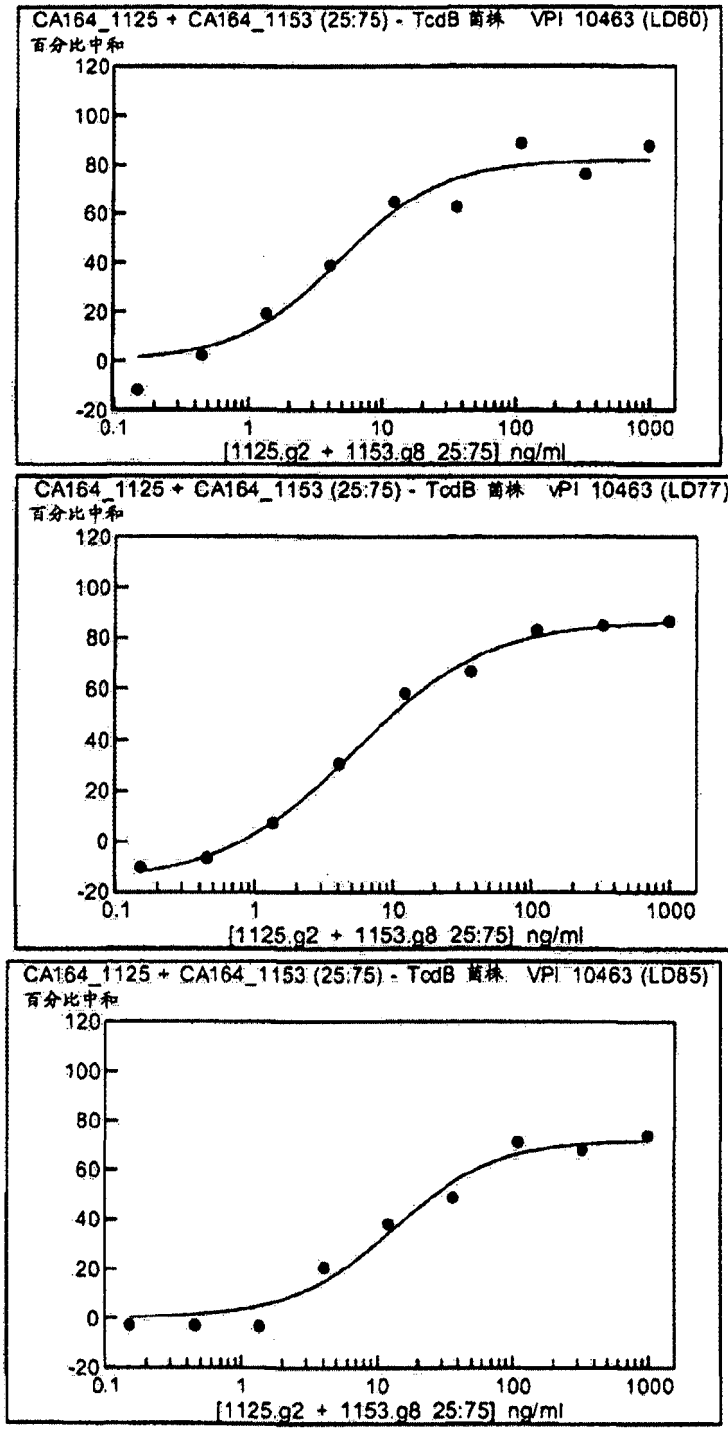


图 42

在不同相对 Mab 比率和不同毒素浓度下两种 Mab 的混合物的抗 TcdB (核糖型 003)体外中和数据

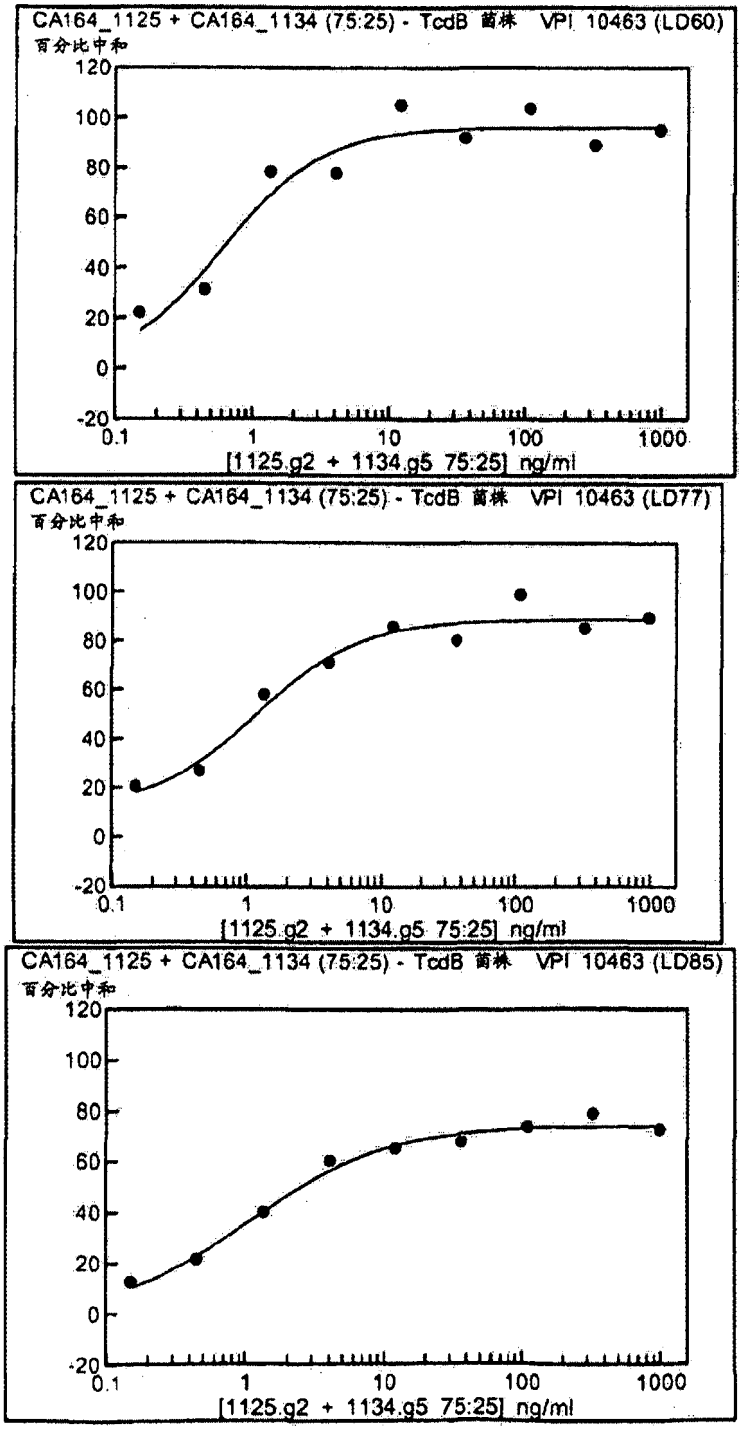


图 43

在不同相对 Mab 比率和不同毒素浓度下两种 Mab 的混合物的抗 TcdB (核糖型 003)体外中和数据

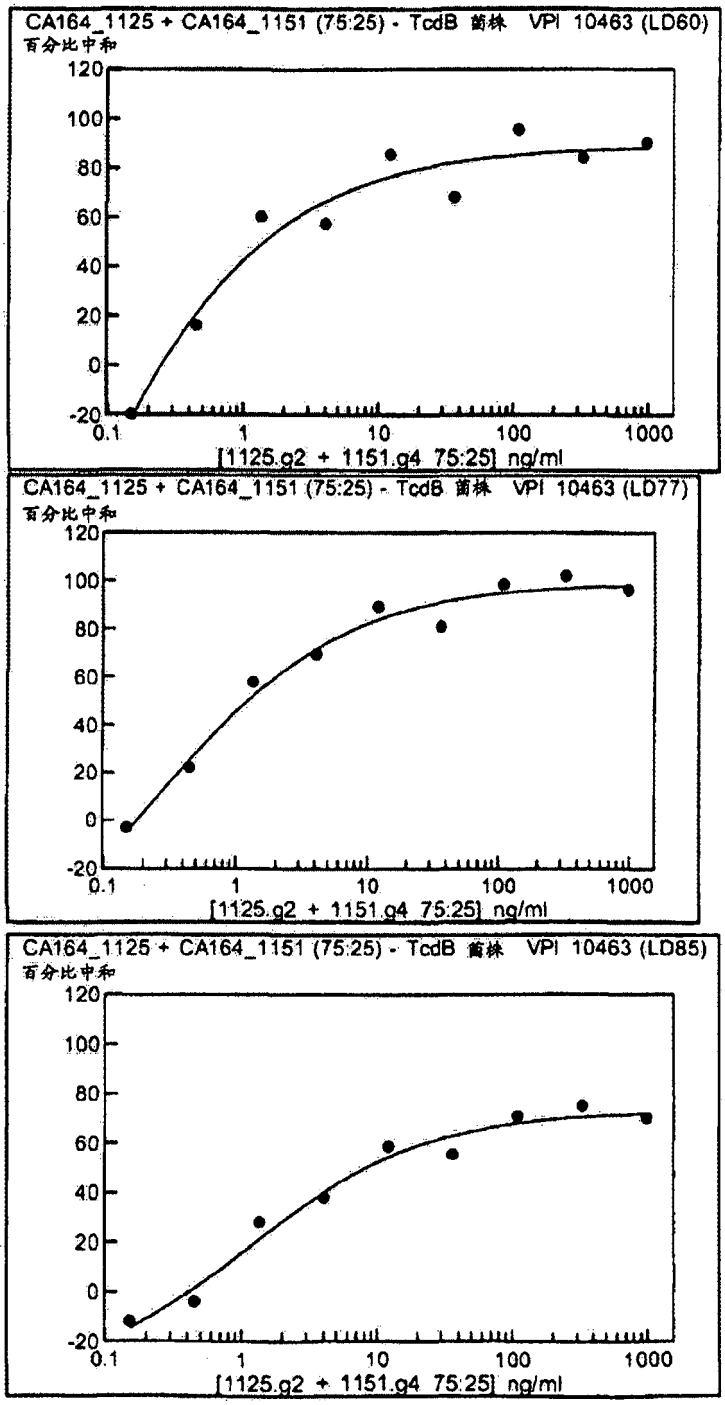


图 44

在不同相对 Mab 比率和不同毒素浓度下两种 Mab 的混合物的抗 TcdB (核糖型 003)体外中和数据

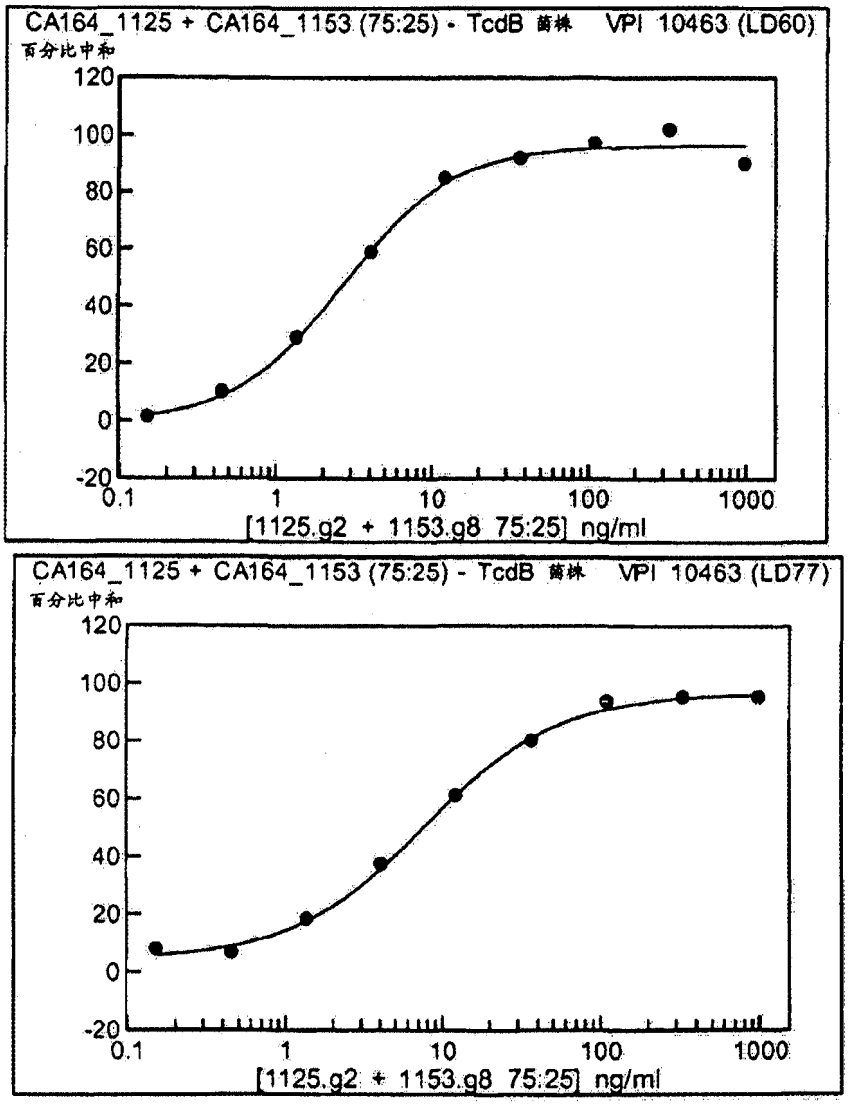


图 45

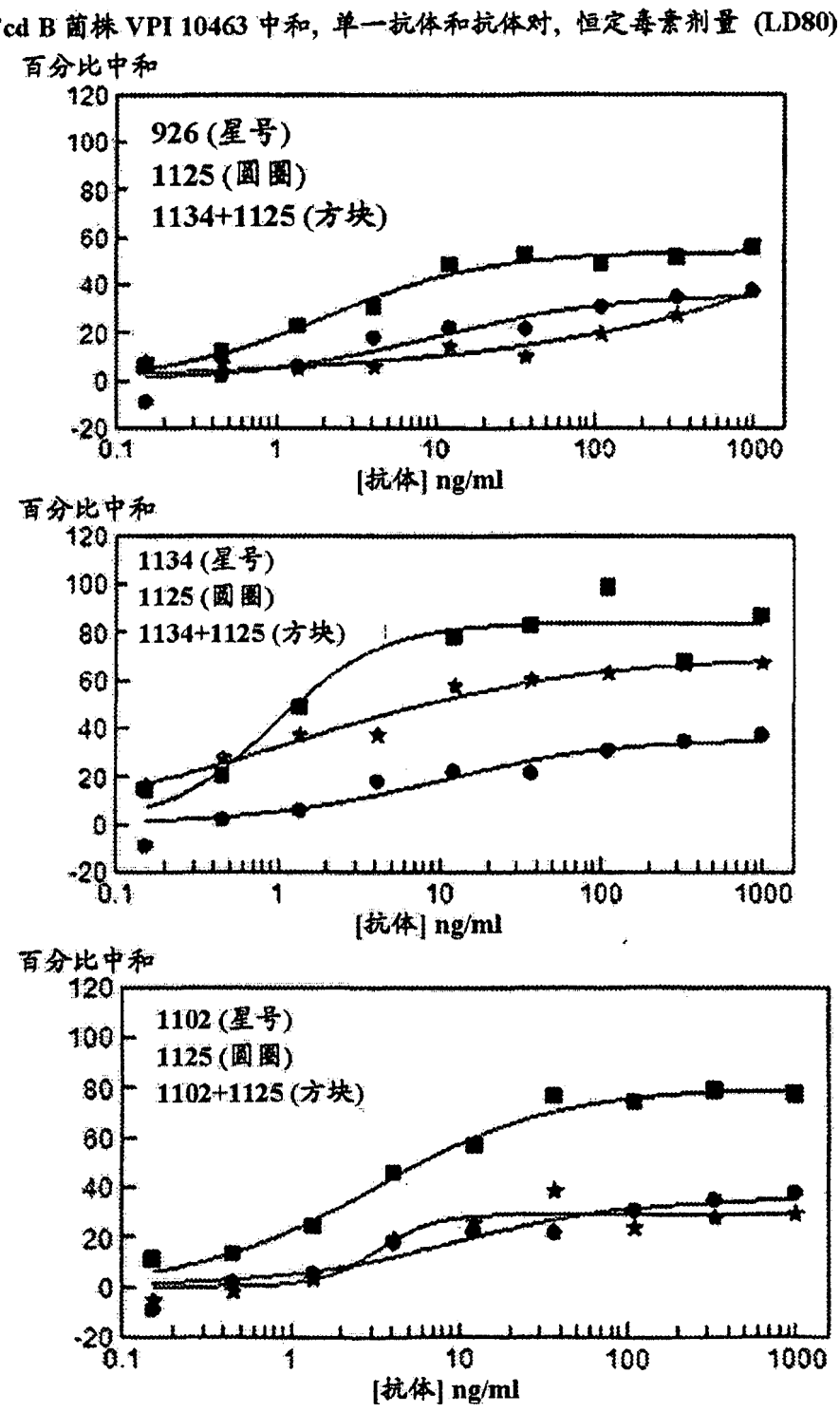

Tcd B 菌株 VPI 10463 中和，单一抗体和抗体对，恒定毒素剂量 (LD80)
百分比中和
926 (星号)
1125 (圆圈)
1134+1125 (方块)
[抗体] ng/ml
百分比中和
1134 (星号)
1125 (圆圈)
1134+1125 (方块)
[抗体] ng/ml
百分比中和
1102 (星号)
1125 (圆圈)
1102+1125 (方块)
[抗体] ng/ml

图 46

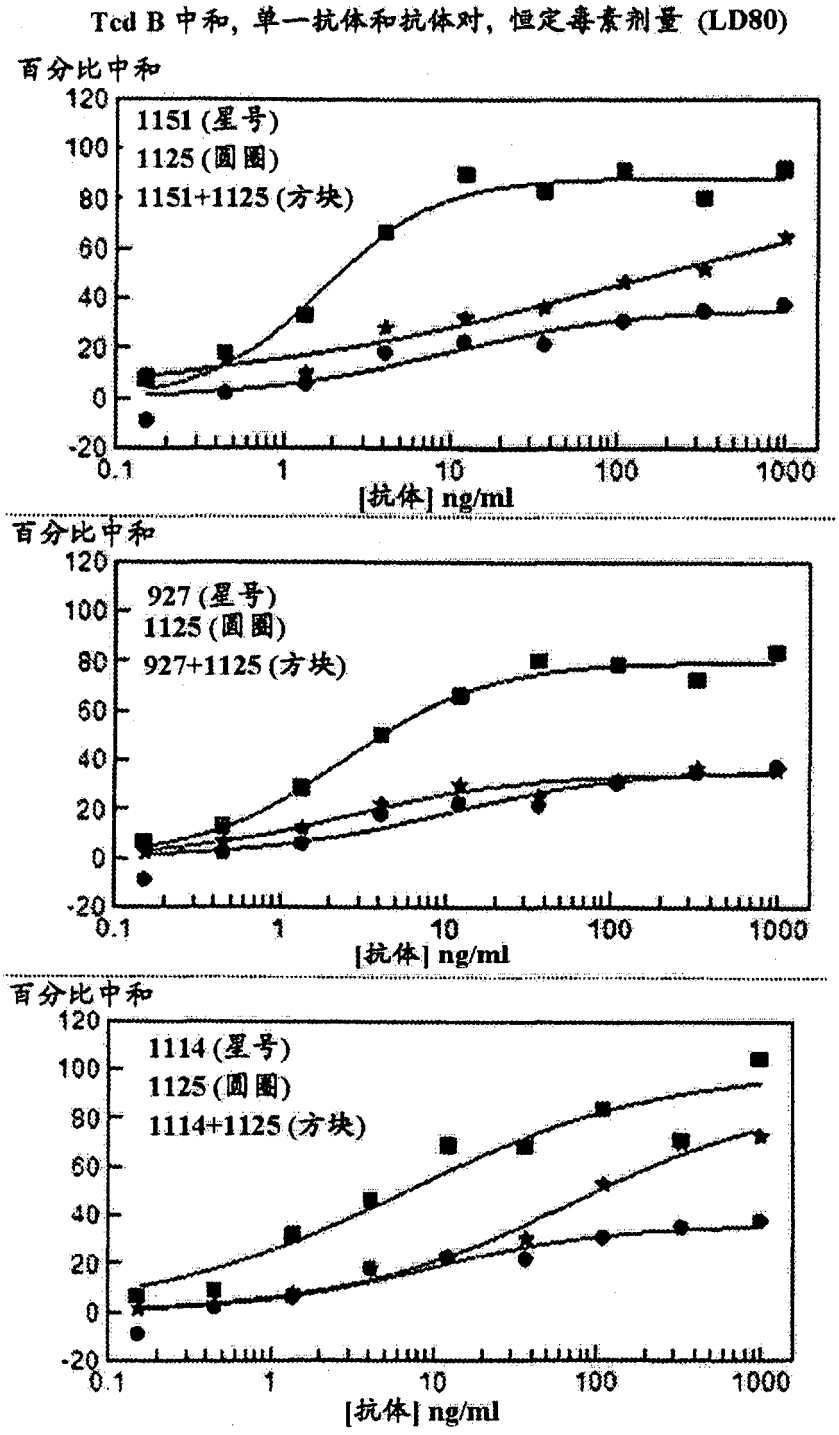

Tcd B 中和，单一抗体和抗体对，恒定毒素剂量 (LD80)
百分比中和
1151 (星号)
1125 (圆圈)
1151+1125 (方块)
[抗体] ng/ml
百分比中和
927 (星号)
1125 (圆圈)
927+1125 (方块)
[抗体] ng/ml
百分比中和
1114 (星号)
1125 (圆圈)
1114+1125 (方块)
[抗体] ng/ml

图 47

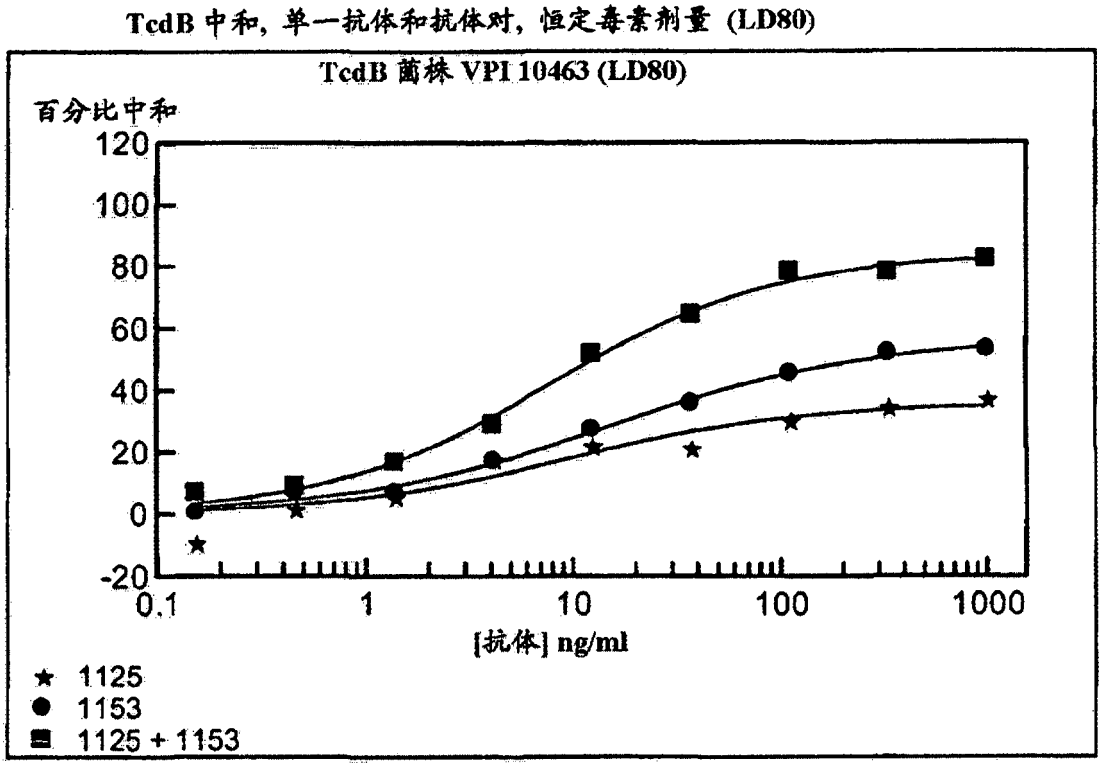

TcdB 中和，单一抗体和抗体对，恒定毒素剂量 (LD80)
TcdB 菌株 VPI 10463 (LD80)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[抗体] ng/ml
1125
1153
1125 + 1153

图 48

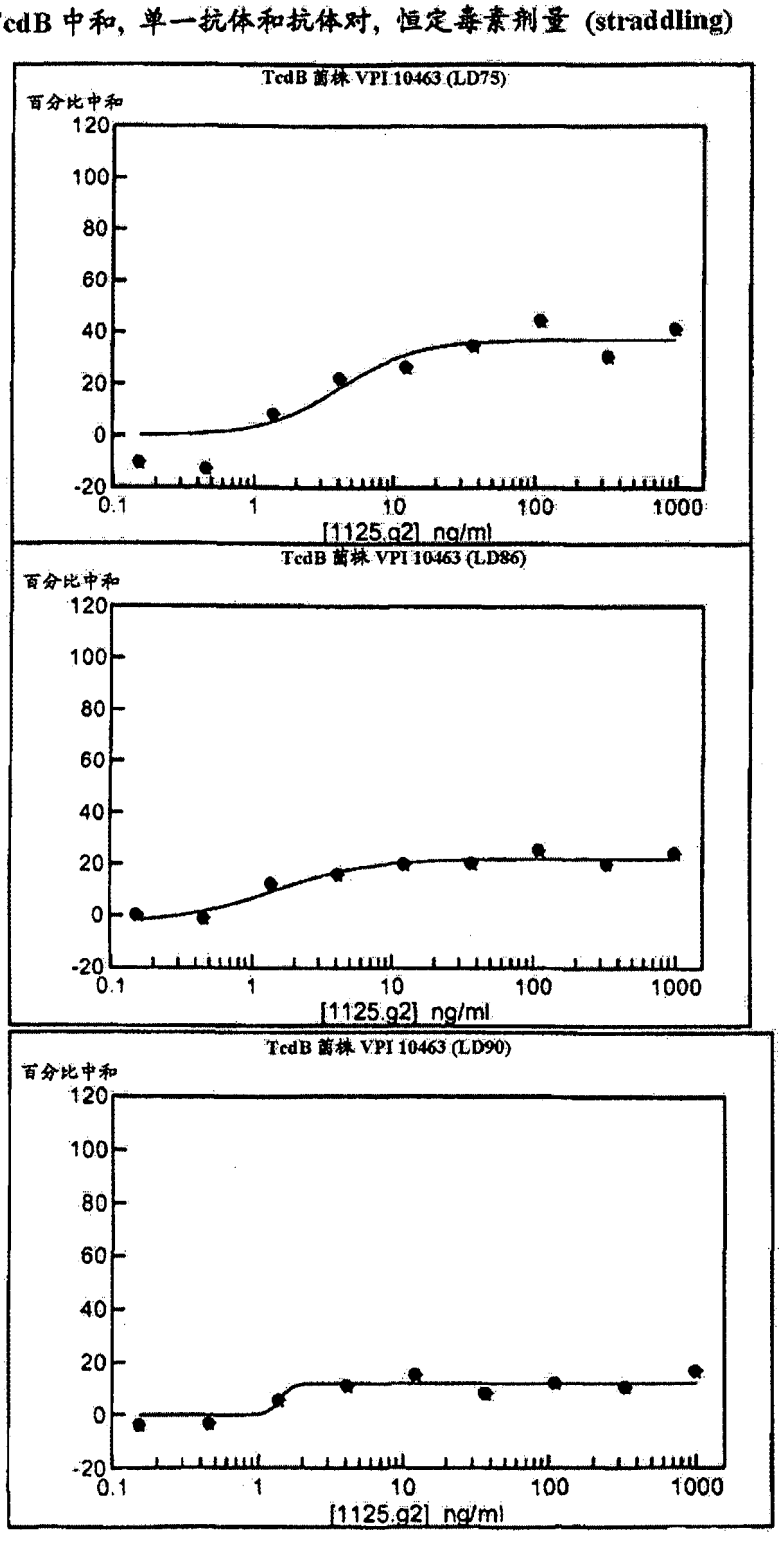

TcdB 中和，单一抗体和抗体对，恒定毒素剂量 (straddling)
TcdB 菌株 VPI 10463 (LD75)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2] ng/ml
TcdB 菌株 VPI 10463 (LD86)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2] ng/ml
TcdB 菌株 VPI 10463 (LD90)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2] ng/ml

图 49

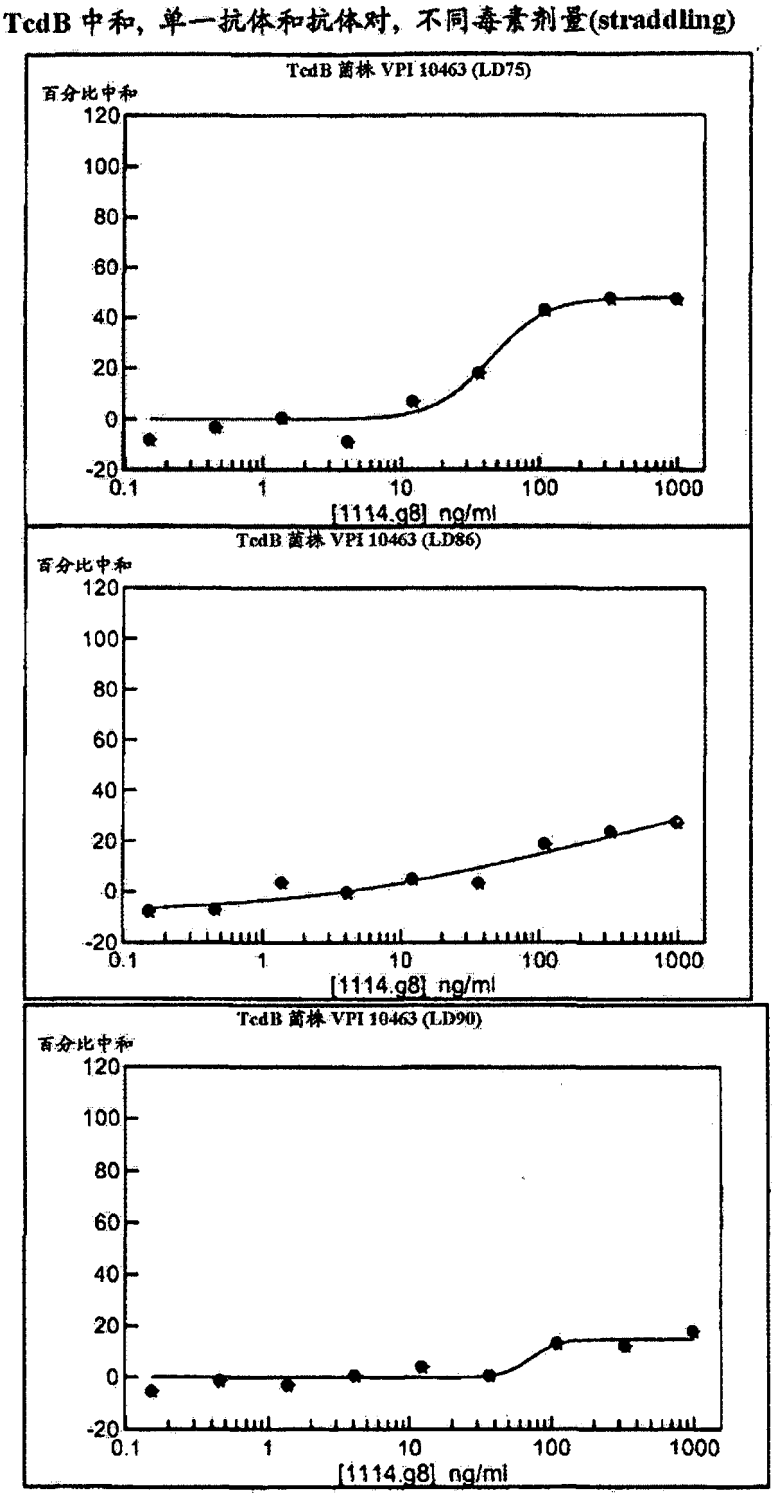

TcdB 中和，单一抗体和抗体对，不同毒素剂量(straddling)
TcdB 菌株 VPI 10463 (LD75)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1114.g8] ng/ml
TcdB 菌株 VPI 10463 (LD86)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1114.g8] ng/ml
TcdB 菌株 VPI 10463 (LD90)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1114.g8] ng/ml

图 50

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）

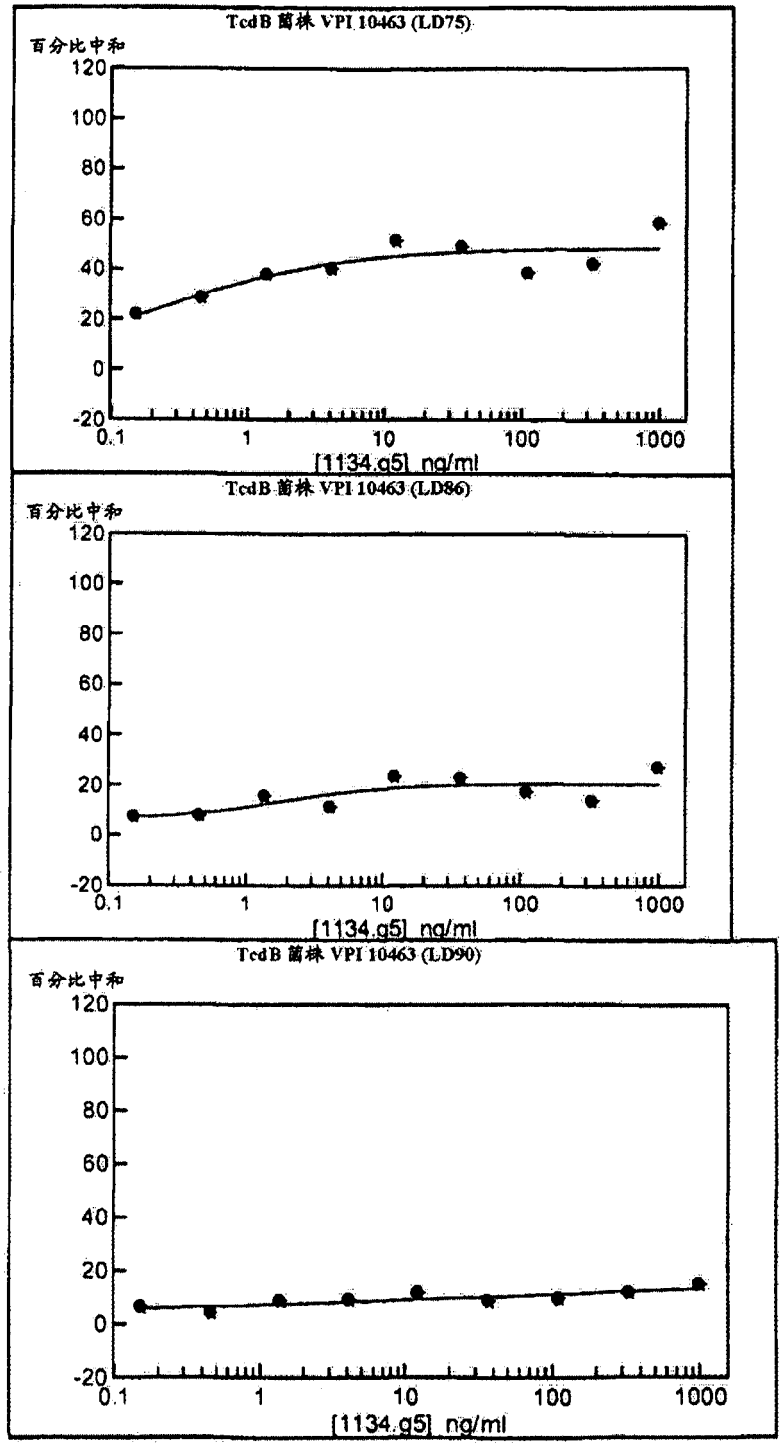


图 51

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）

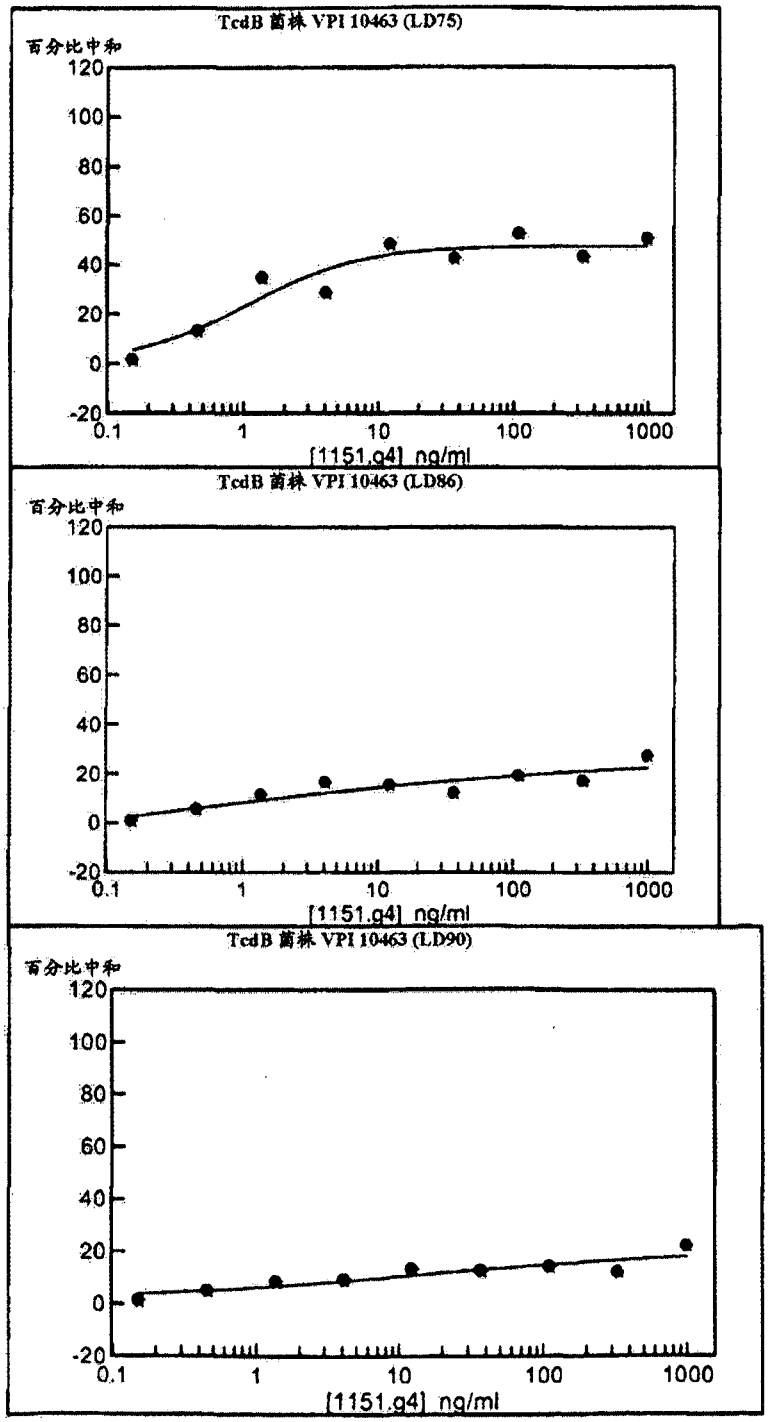


图 52

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）

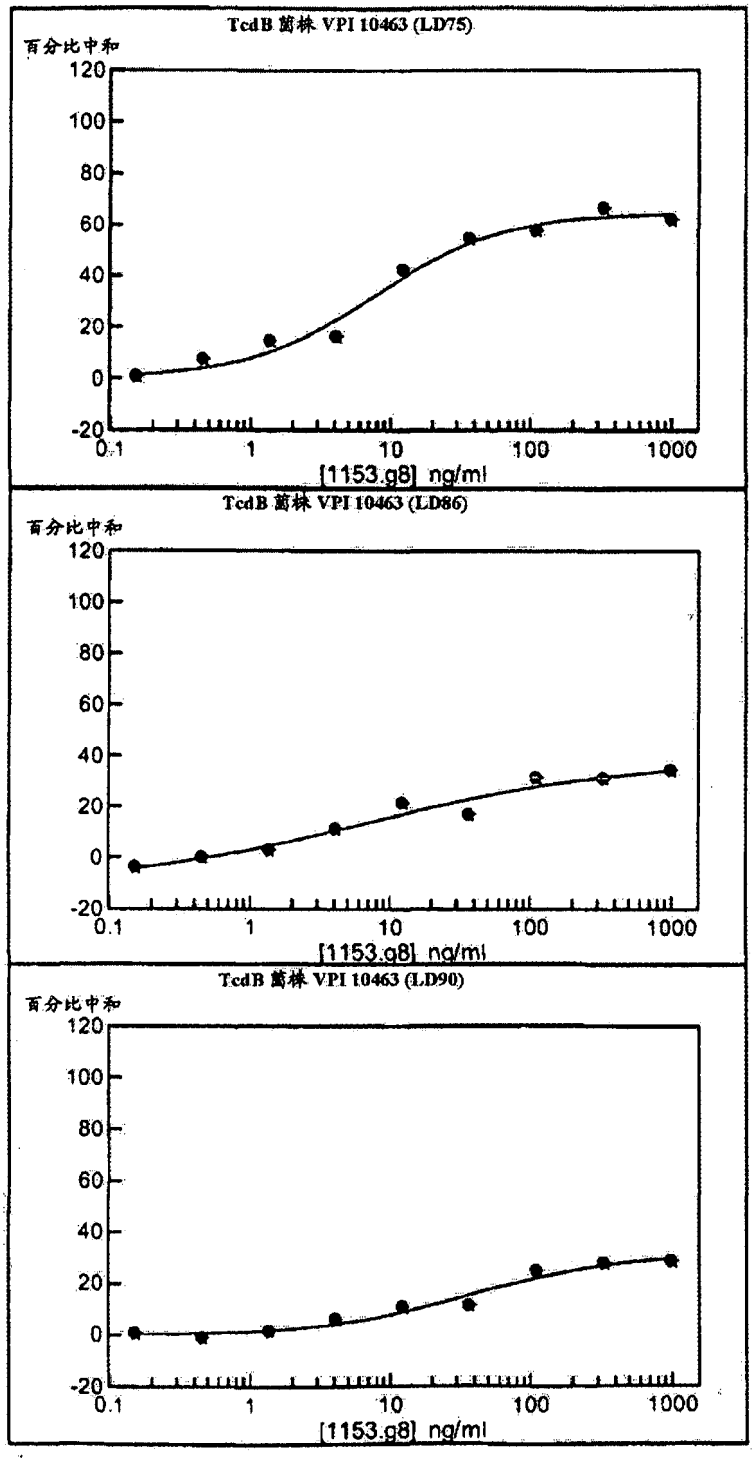


图 53

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）

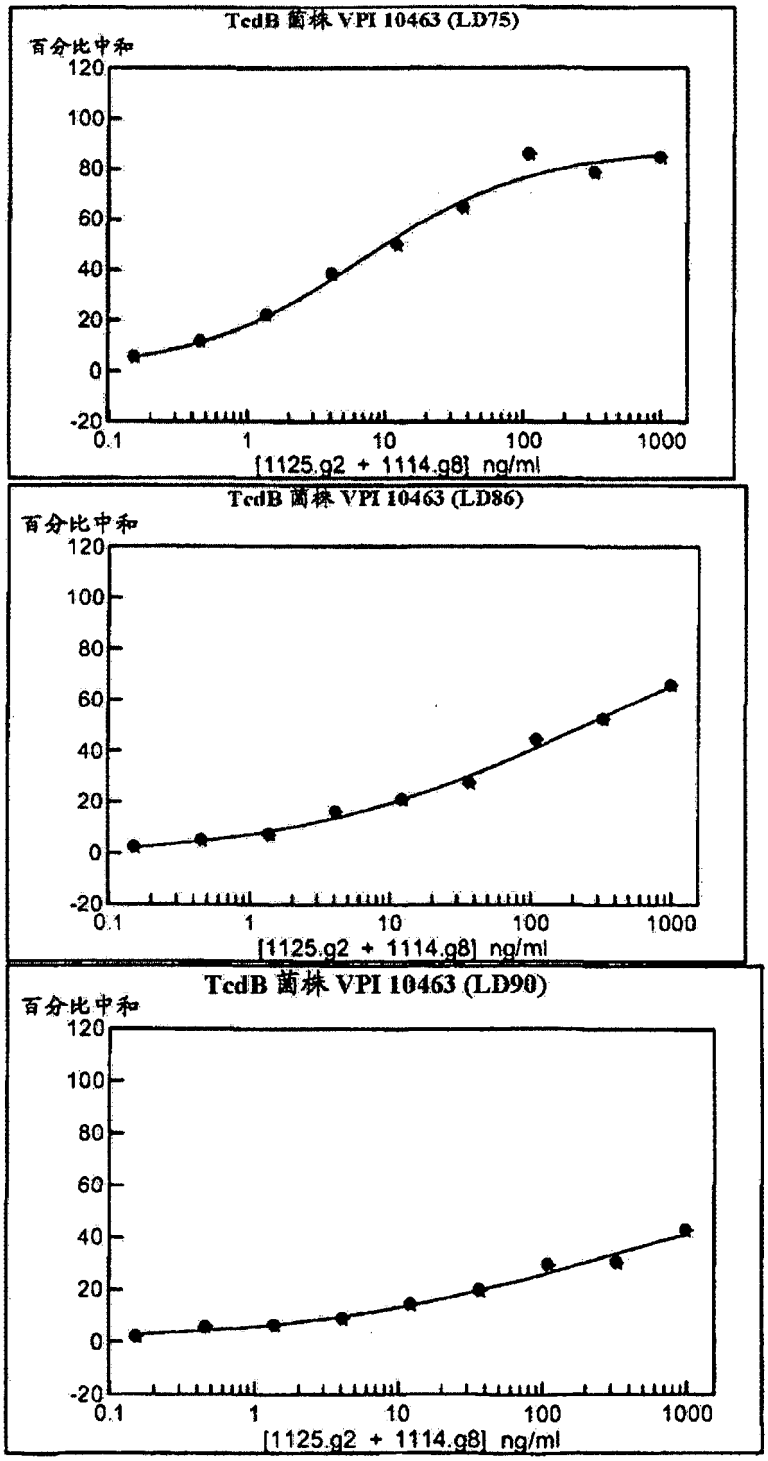


图 54

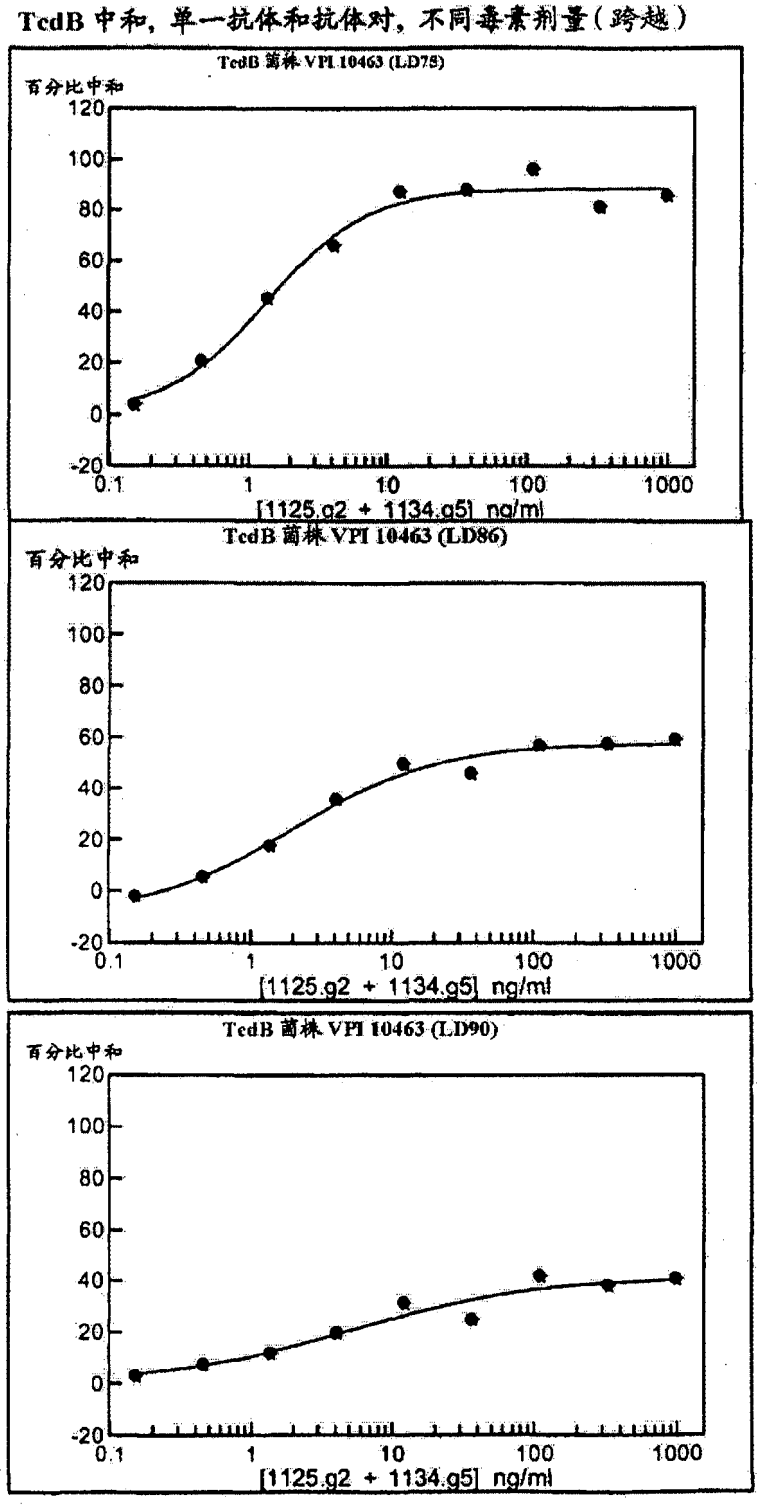

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）
TcdB 菌株 VPI 10463 (LD75)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1134.g5] ng/ml
TcdB 菌株 VPI 10463 (LD86)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1134.g5] ng/ml
TcdB 菌株 VPI 10463 (LD90)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1134.g5] ng/ml

图 55

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）

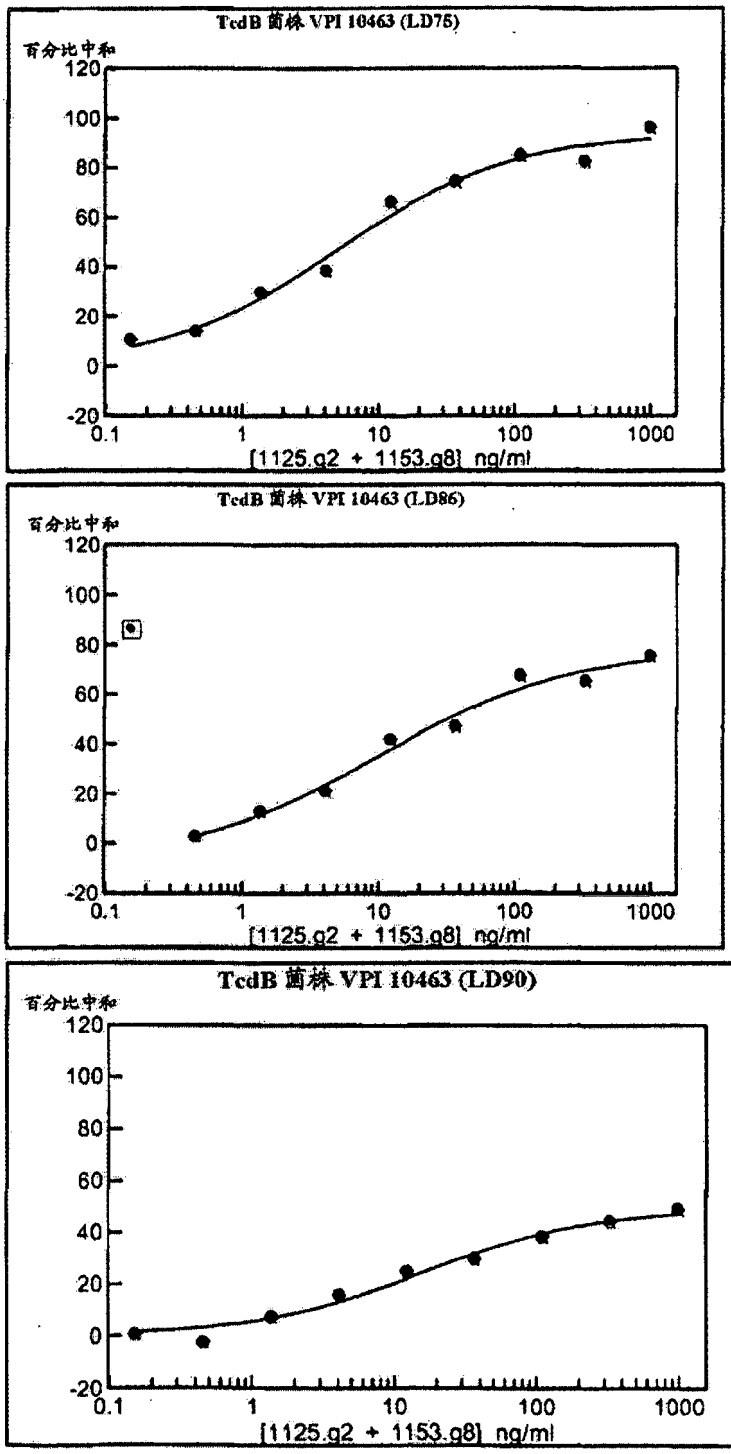


图 56

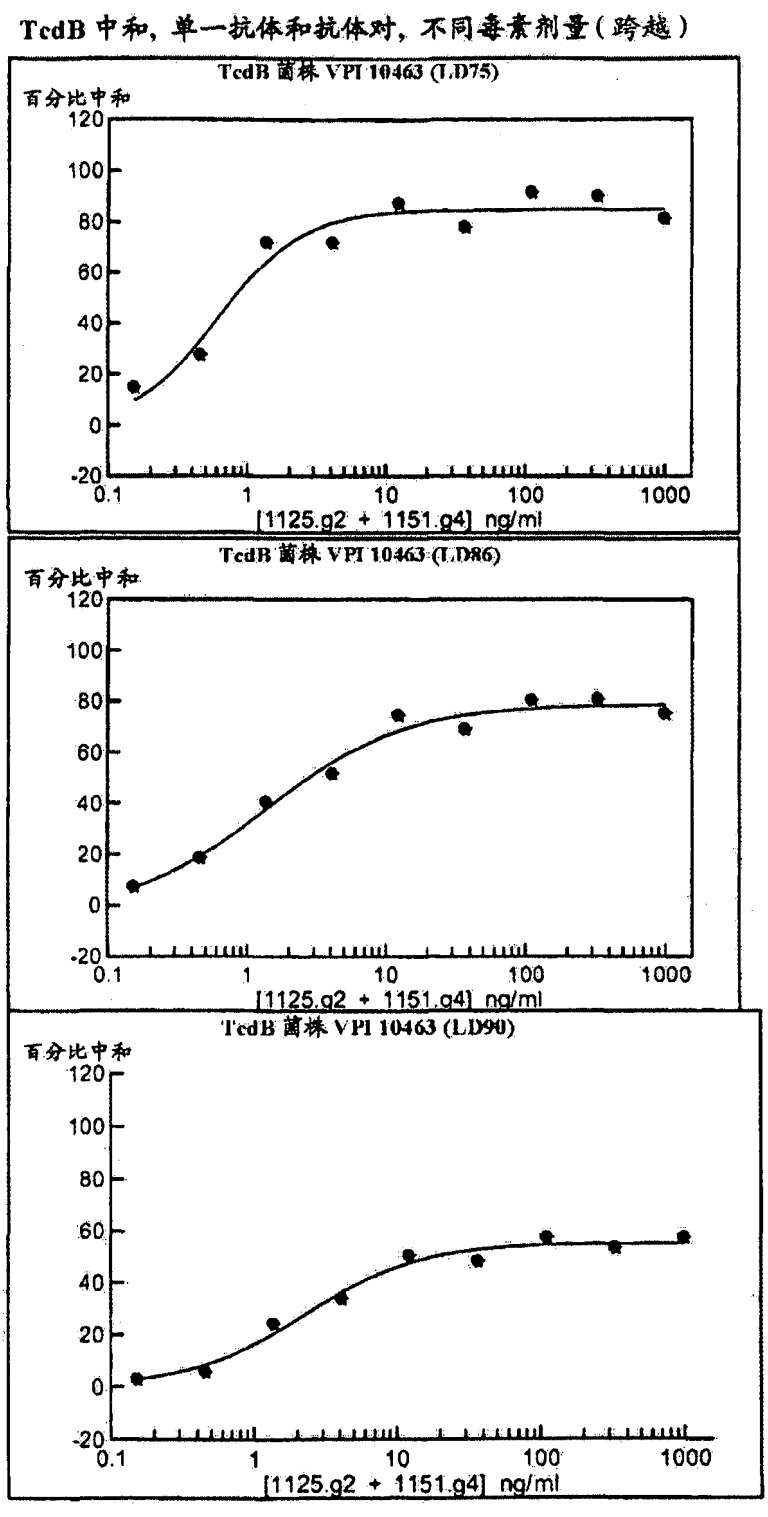

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）
TcdB 菌株 VPI 10463 (LD75)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1151.g4] ng/ml
TcdB 菌株 VPI 10463 (LD86)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1151.g4] ng/ml
TcdB 菌株 VPI 10463 (LD90)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1151.g4] ng/ml

图 57

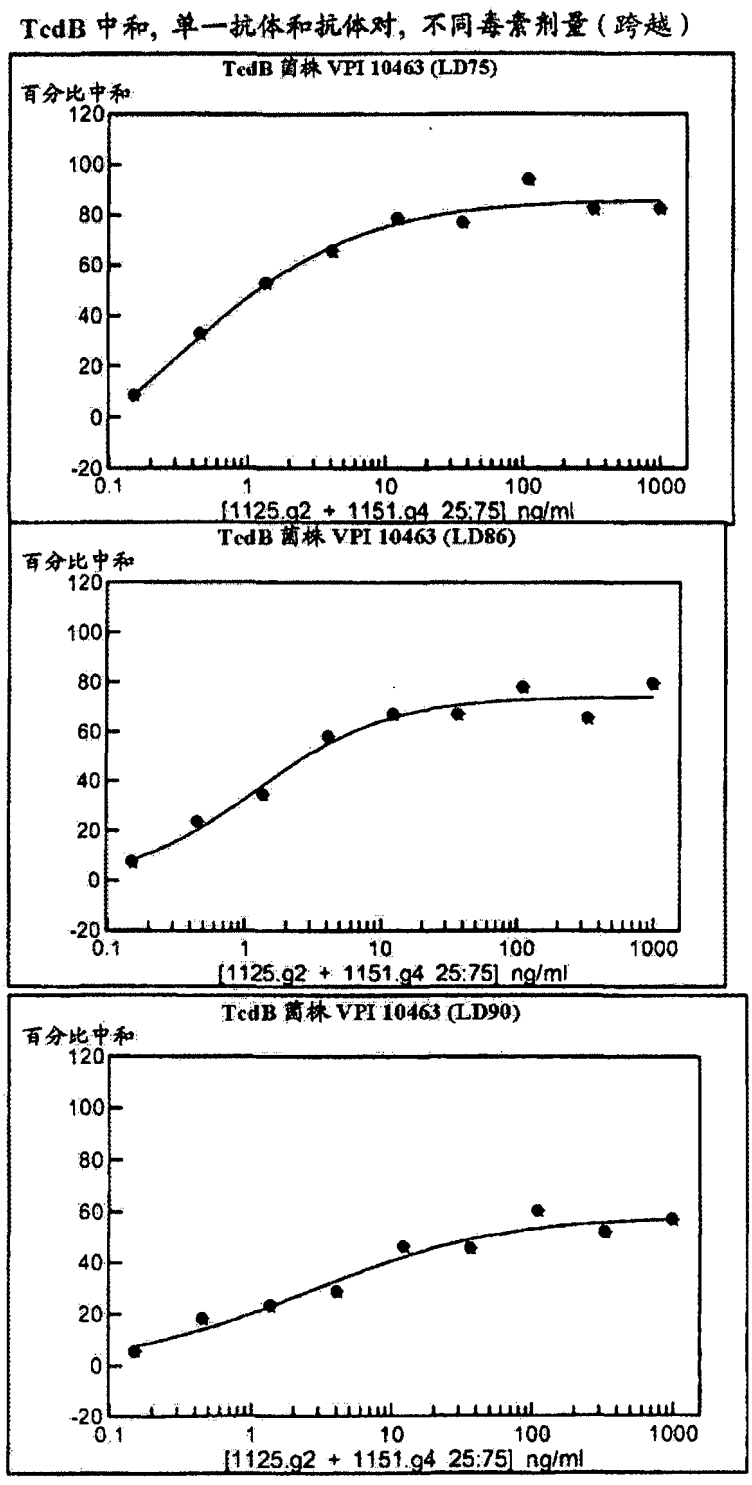

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）
TcdB 菌株 VPI 10463 (LD75)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1151.g4 25:75] ng/ml
TcdB 菌株 VPI 10463 (LD86)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1151.g4 25:75] ng/ml
TcdB 菌株 VPI 10463 (LD90)
百分比中和
120
100
80
60
40
20
0
-20
0.1
1
10
100
1000
[1125.g2 + 1151.g4 25:75] ng/ml

图 58

TcdB 中和，单一抗体和抗体对，不同毒素剂量（跨越）

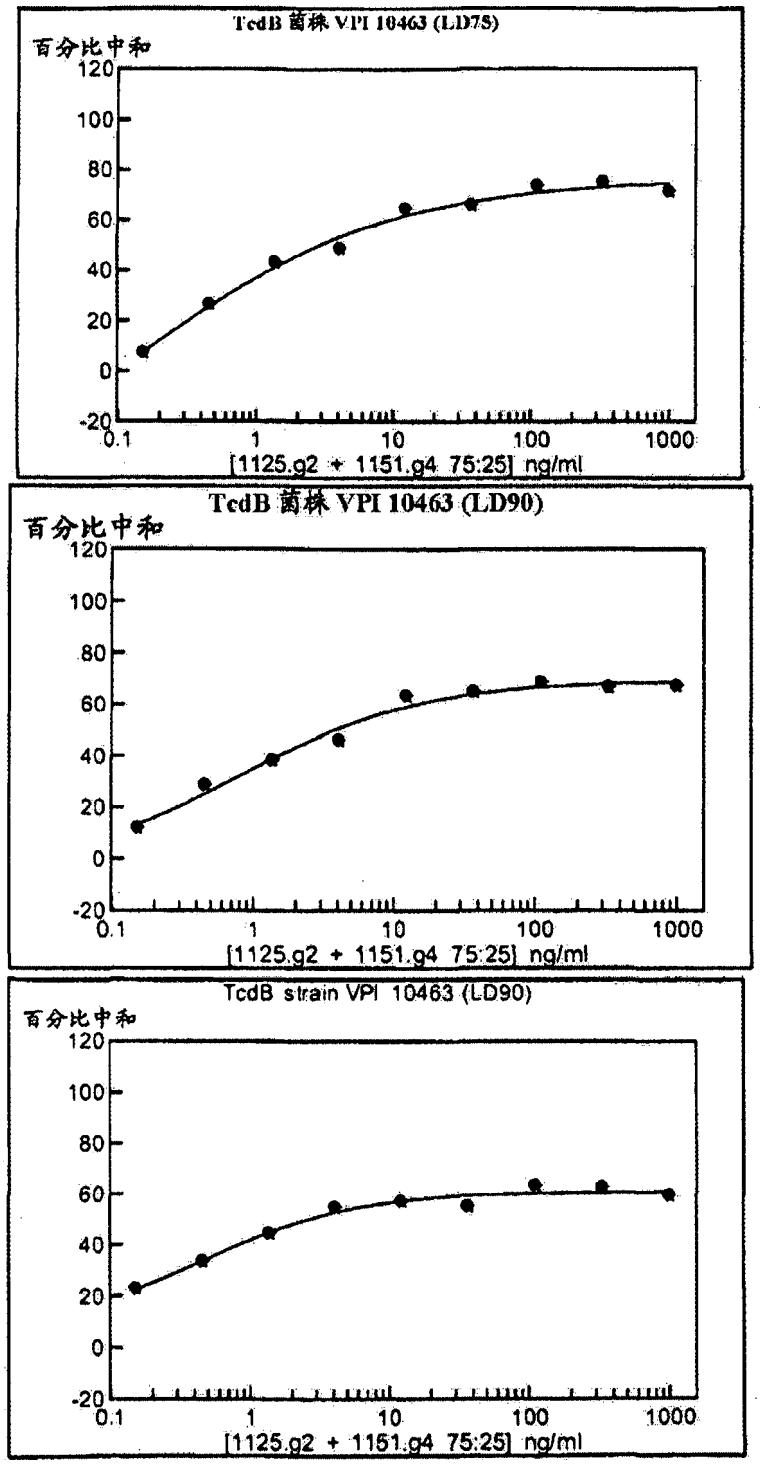


图 59

TcdA 的氨基酸序列 SEQ ID NO: 171

MSLISKEELI KLAYSIRPRE NEYKTILTNL DEYNKLTTNN NENKYLQLKK LNESIDVFMN
XYKTSSRNRA LSNLKKDILK EVILIKNSNT SPVEKNLHFV WIGGEVSDIA LEYIKQWADI
NAEYNIKLWY DSEAFLVNTL KKAIVESSTT EALQLLEEEI QNPQFDNMKF YKKRMEFIYD
RQKRFINYYK SQINKPTVPT IDDIIKSHLV SEYNRDETVL ESYRTNSLRK INSNHGIDIR
ANSLFTEQEL LNIYSQELLN RGNLAAASDI VRLLALKNFG GVYLDVDMLP GIHSDLFKTI
SRPSSIGLDR WEMIKLEAIM KYKKYINNYT SENFDKLDQQ LKDNFKLIIE SKSEKSEIFS
KLENLNVSQL EIKIAFALGS VINQALISKQ GSYLTNLVIE QVKNRYQFLN QHLNPAIESD
NNFTDTTKIP HDSLFNSATA ENSMFLTKIA PYLQVGFMPE ARSTISLSGP GAYASAYYDF
INLQENTIEK TLKASDLIET KFPENNLSQL TEQEINSLWS FDQASAKYQF EKYVRDYTGG
SLSEDNGVDF NKNTALDKNY LLNNKIPSNN VEEAGSKNYV HYIIQLQGDD ISYEATCNLF
SKNPKNSIII QRNMNESAKS YFLSDDGESI LELNKYRIPE RLKNKEKVKV TFIGHGKDEF
NTSEFARLSV DSLSNEISSF LDTIKLDISP KNVEVNLLGC NMFSYDFNVE ETYPGKLLLS
IMDKITSTLP DVNKNSITIG ANQYEVRINS EGRKELLAHS GKWINKEEAI MSDLSSKEYI
FFDSIDNKLK AKSKNIPGLA SISEDIKTLL LDASVSPDTK FILNNLKLNI ESSIGDYIYY
EKLEPVKNII HNSIDDLIDE FNLLENVSDE LYELKKLNNL DEKYLISFED ISKNNSTYSV
RFINKSNGES VYVETEKEIF SKYSEHITKE ISTIKNSIIT DVNGNLLDNI QLDHTSQVNT
LNAAFFIQSL IDYSSNKDVL NDLSTSVKVQ LYAQLFSTGL NTIYDSIQLV NLISNAVNDT

INVLPTITEG IPIVSTILDG INLGAAIKEL LDEHDPLLKK ELEAKVGVLA INMSLSIAAT
VASIVGIGAE VTIFLLPIAG ISAGIPSLVN NELILHDKAT SVVNYFNHLS ESKKYGPLKT
EDDKILVPID DLVISEIDFN NNSIKLGTCN ILAMEGGSGH TVTGNIDHFF SSPSISSHIP
SLSIYSAIGI ETENLDFSKK IMMLPNAPSR VFWWETGAVP GLRSLENDGT RLLDSIRDLY
PGKFYWRFYA FFDYAITTLK PVYEDTNIKI KLDKDTRNFI MPTITTNEIR NKLSYSFDGA
GGTYSLLLSS YPISTNINLS KDDLWIFNID NEVREISIEN GTIKKGKLIK DVLSKIDINK
NKLIIGNQTI DFSGDIDNKD RYIFLTCELD DKISLIIEIN LVAKSYSLLL SGDKNYLISN
LSNTIEKINT LGLDSKNIAY NYTDEFNNKY FGAISKTSQK SIIHYKKDSK NILEFYNDST
LEFNSKDFIA EDINVFMKDD INTITGKYYV DNNTDKSIDF SISLVSKNQV KVNGLYLNES
VYSSYLDFVK NSDGHHNTSN FMNLFLDNIS FWKLFGFENI NFVIDKYFTL VGKTNLGYVE
FICDNNKNID IYFGEWKTSS SKSTIFSGNQ RNVVVEPIYN PDTGEDISTS LDFSYEPLYG
IDRYINKVLI APDLYTSLIN INTNYYSNEY YPEIIVLNPN TFHKKVNINL DSSSFEYKWS
TEGSDFILVR YLEESNKKIL QKIRIKGILS NTQSFNKMSI DFKDIKKLSL GYIMSNFKSF
NSENELDRDH LGFKIIDNKT YYYDEDSKLV KGLININNSL FYFDPIEFNL VTGWQTINGK
KYYFDINTGA ALTSYKIING KHFYFNNDGV MQLGVFKGPD GFEYFAPANT QNNNIEGQAI
VYQSKFLTLN GKKYYFDNNS KAVTGWRIIN NEKYYFNPNN AIAAVGLQVI DNNKYYFNPD
TAIISKGWQT VNGSRYYFDT DTAIAFNGYK TIDGKHFYFD SDCVVKIGVF STSNGFEYFA
PANTYNNNIE GQAIVYQSKF LTLNGKKYYF DNNSKAVTGL QTIDSKKYYF NTNTAEAATG

WQTIDGKKYY FNTNTAEAAT GWQTIDGKKY YFNTNTAIAS TGYTIINGKH FYFNTDGIMQ
IGVFKGPNGF EYFAPANTDA NNIEGQAILY QNEFLTLNGK KYYFGSDSKA VTGWRIINNK
KYYFNPNNAI AAIHLGTINN DKYYFSYDGI LQNGYITIER NNFYFDANNE SKMVTGVFKG
PNGFEYFAPA NTHNNNIEGQ AIVYQNKFLT LNGKKYYFDN DSKAVTGWQT IDGKKYYFNL
NTAEAATGWQ TIDGKKYYFN LNTAEAATGW QTIDGKKYYF NTNTFIASTG YTSINGKHFY
FNTDGIMQIG VFKGPNGFEY FAPANTDANN IEGQAILYQN KFLTLNGKKY YEGSDSKAVT
GLRTIDGKKY YFNTNTAVAV TGWQTINGKK YYFNTNTGIA STGYTIISGK HFYFNTDGIM
QIGVFKGPDG FEYFAPANTD ANNIEGQAIR YQNRFLYLHD NIYYFGNNSK AATGWVTIDG
NRYYFEPNTA MGANGYKTID NKNFYFRNGL PQIGVFKGSN GFEYFAPANT DANNIEGQAI

RYQNRFLHLL GKIYYFGNNS KAVTGWQTIN GKVYYFMPDT AMAAAGGLFE IDGVIYFFGV
DGVKAPGIYG

图 60

TcdB 的氨基酸序列 SEQ ID NO: 172

MSLVNRKQLE KMANVRFRTQ EDEYVAILDA LEEYHNMSEN TVVEKYLKLK DINSLTDIYI
DTYKKSGRNK ALKKFKEYLV TEVLELKNNN LTPVEKNLHF VWIGGQINDT AINYINQWKD
VNSDYNVNVF YDSNAFLINT LKKTVVESAI NDTLESFREN LNDPRFDYNK FFKKRMEIIY
DKQKNFINYY KAQREENPEL IIDDIVKTYL SNEYSKEIDE LNTYIEESLN KITQNSGNDV
RNFEEFKNGE SFNLYEQELV ERWNLAAASD ILRISALKEI GGMYLDVDML PGIQPDLFES
IEKPSSVTVD FWEMTKLEAI MKYKEYIPEY TSEHFDMLDE EVQSSFESVL ASKSDKSEIF
SSLGDMEASP LEVKIAFNSK GIINQGLISV KDSYCSNLIV KQIENRYKIL NNSLNPAISE
DNDFNTTTNT FIDSIMAEAN AQNGREMMEL GKYLRVGFFP DVKTTINLSG PEAYAAAYQD
LLMFKEGSMN IHLIEADLRN FEISKTNISQ STEQEMASLW SFDDARAKAQ FEEYKRNYFE
GSLGEDDNLD FSQNIVVDKE YLLEKISSLA RSSERGYIHY IVQLQGDKIS YEAACNLFAK
TPYDSVLFQK NIEDSEIAYY YNPGDGEIQE IDKYKIPSII SDRPKIKLTF IGHGKDEFNT
DIFAGFDVDS LSTEIEAAID LAKEDISPKS IEINLLGCNM FSYSINVEET YPGKLLLKVK

DKISELMPSI SQDSIIVSAN QYEVRINSEC RRELLDHSGE WINKEDSIIK DISSKEYISF
NPKENKITVK SKNLPELSTL LQEIRNNSNS SDIELEEKVM LTECEINVIS NIDTQIVEER
IEEAKNLTSD SINYIKDEFK LIESISDALC DLKQQNELED SNFISFEDIS EIDEGFSIRF
INKETGESIF VETSKTIFSE YANHITEEIS KIKGTIFDTV NGKLVKKVNL DTTHEVNTLN
AAFFIQSLIE YNSSKESLSN LSVAMKVQVY AQLFSTGLNT ITDAAKVVEL VSTALDETID
LLPTLSEGLP IIATIIDGVS LGAAIKELSE TSDPLLRQEI EAKIGIMAVN LTTATTAIIT
SSLGIASGFS ILLVPLAGIS AGIPSLVNNE LVLRDKATKV VDYFKHVSLV ETEGVFTLLD

DKIMMPQDDL VISEIDFNNN SIVLGKCEIW RMEGGSGHTV TDDIDHFFSA PSITYREPHL
SIYDVLEVQK EELDLSKDLM VLPNAPNRVF AMETGWTPGL RSLENDGTKL LDRIRDNYEG
EFYWRYFAFI ADALITTLKP RYEDTNIRIN LDSNTRSFIV PIITTEYIRE KLSYSFYGSG
GTYALSLSQY NMGINIELSE SDVWIIDVDN VVRDVTIESD KIKKGDLIEG ILSTLSIEEN
KIILNSHEIN FSGEVNGSNG FVSLTFSILE GINAIIEVDL LSKSYKLLIS GELKILMLNS

NHIQQKIDYI GFNSELQKNI PYSFVDSEGK ENGFINGSTK EGLFVSELPD VVLISKVYMD
DSKPSFGYYS NNLKDVKVIT KDNVNILTGY YLKDDIKISL SLTLQDEKTI KLNSVHLDES
GVAEILKFMN RKGNTNTSDS LMSFLESMNI KSIFVNFLQS NIKFILDANF IISGTTSIGQ
FEFICDENDN IQPYFIKFNT LETNYTLYVG NRQNMIVEPN YDLDDSGDIS STVINFSQKY
LYGIDSCVNK VVISPNIYTD EINITPVYET NNTYPEVIVL DANYINEKIN VNINDLSIRY
VWSNDGNDFI LMSTSEENKV SQVKIRFVNV FKDKTLANKL SFNFSDKQDV PVSEIILSFT
PSYYEDGLIG YDLGLVSLYN EKFYINNPGM MVSGLIYIND SLYYFKPPVN NLITGFVTVG
DDKYYFNPIN GGAASIGETI IDDKNYYFNQ SGVLQTGVFS TEDGFKYFAP ANTLDENLEG
EAIDFTGKLI IDENIYYFDD NYRGAVEWKE LDGEMHYFSP ETGKAFKGLN QIGDYKYYFN
SDGVMQKGFV SINDNKHYFD DSGVMKVGYT EIDGKHFYFA ENGEMQIGVF NTEDGFKYFA
HHNEDLGNEE GEEISYSGIL NFNNKIYYFD DSFTAVVGWK DLEDGSKYYF DEDTAEAYIG
LSLINDGQYY FNDDGIMQVG FVTINDKVFY FSDSGIIESG VQNIDDNYFY IDDNGIVQIG
VFDTSDGYKY FAPANTVNDN IYGQAVEYSG LVRVGEDVYY FGETYTIETG WIYDMENESD
KYYFNPETKK ACKGINLIDD IKYYFDEKGI MRTGLISFEN NNYYFNENGE MQFGYINIED
KMFYFGEDGV MQIGVFNTPD GFKYFAHQNT LDENFEGESI NYTGWLDLDE KRYYFTDEYI
AATGSVIIDG EEYYFDPDTA QLVISE

图 61

Caco-2 单层(跨上皮电阻)数据 – TcdA

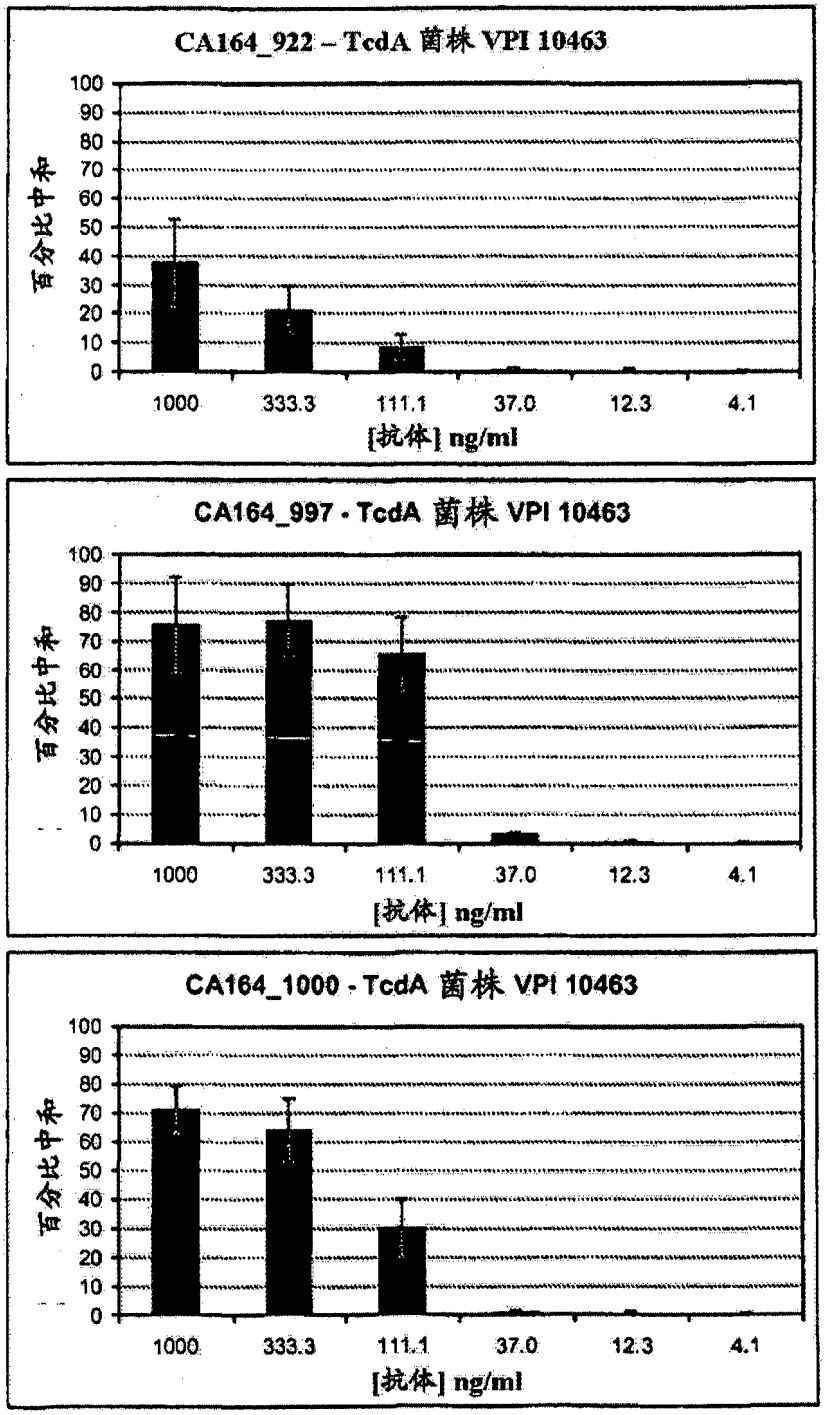


图 62

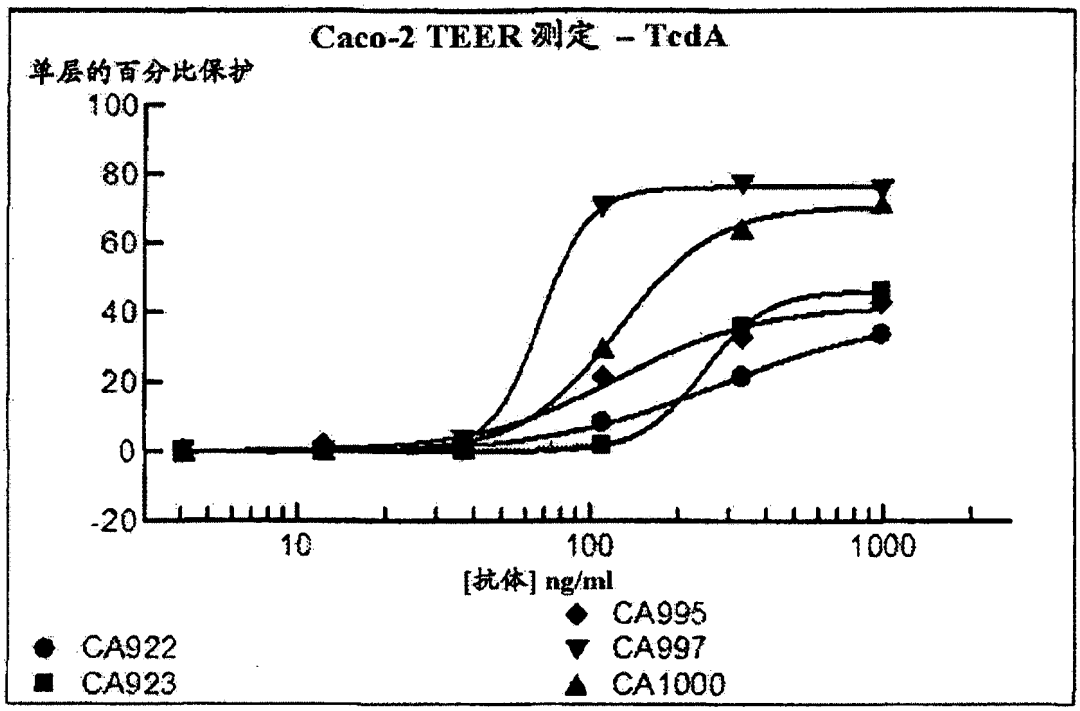


图 62A

利用艰难梭菌攻击后仓鼠的存活率。UCB 高和低剂量 3 种 Mab 的混合物：CA997.g1 (50%), CA1125.g2 (25%)和 CA1151.g4 (25%)相对于对照

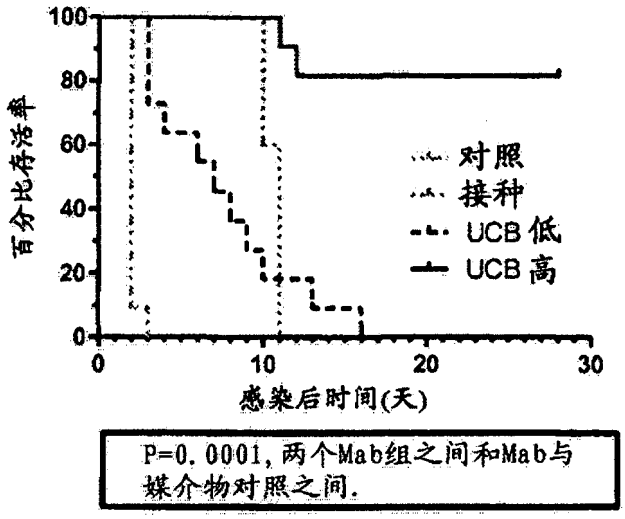


图 63

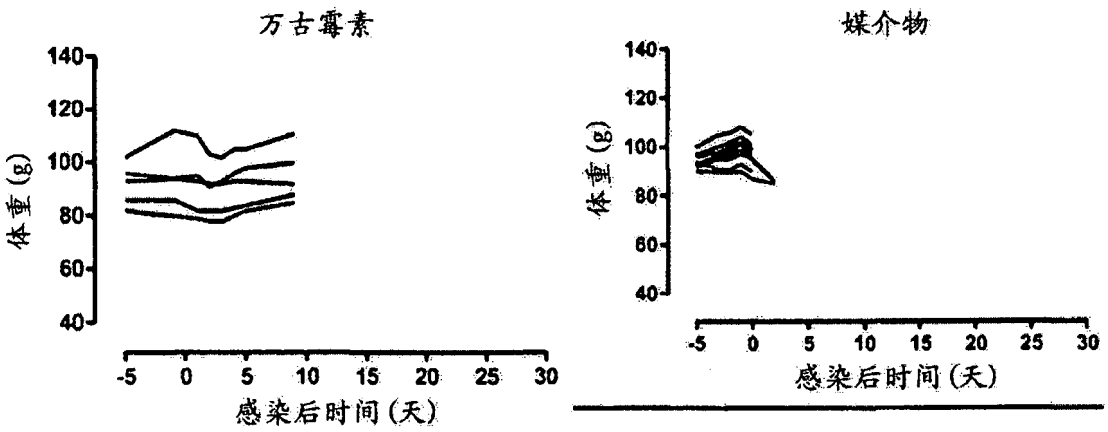

仓鼠体重变化
万古霉素
体重(g)
140
120
100
80
60
40
-5 0 5 10 15 20 25 30
感染后时间(天)
媒介物
体重(g)
140
120
100
80
60
40
-5 0 5 10 15 20 25 30
感染后时间(天)

图 64

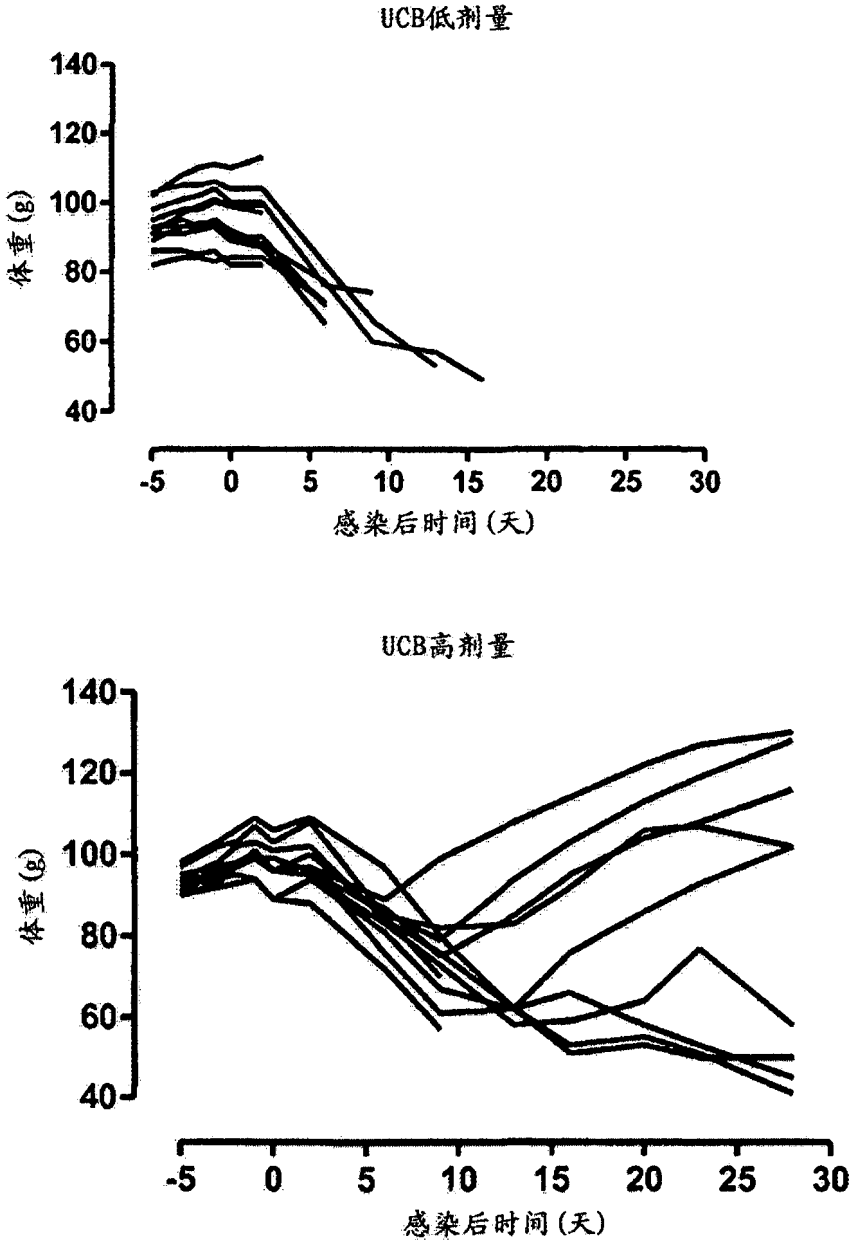

UCB低剂量
140
120
100
80
60
40
体重(g)
-5
0
5
10
15
20
25
30
感染后时间(天)
UCB高剂量
140
120
100
80
60
40
体重(g)
-5
0
5
10
15
20
25
30
感染后时间(天)

图 65

PBS 对照 UCB 高剂量

图 66

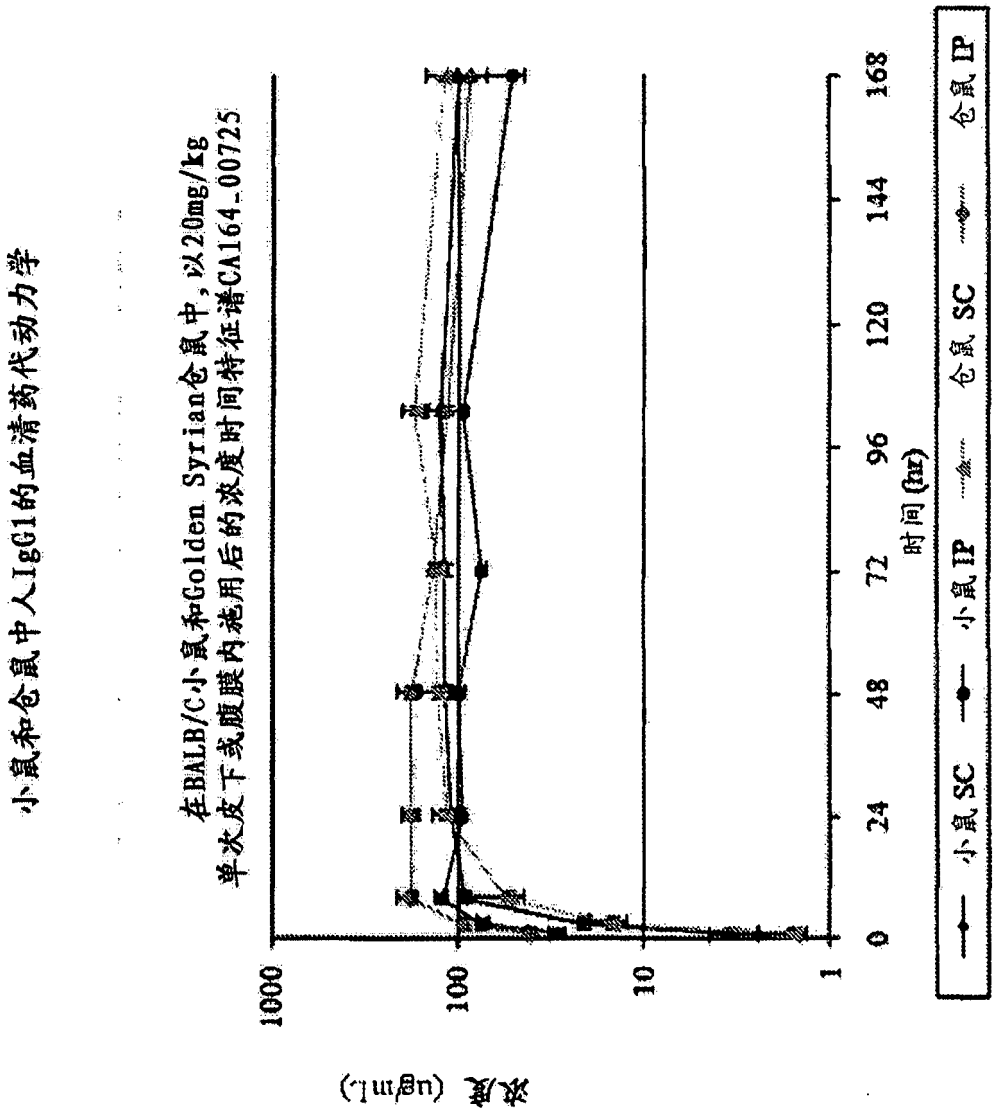

小鼠和仓鼠中人IgG1的血清药代动力学
在BALB/C小鼠和Golden Syrian仓鼠中，以20mg/kg
单次皮下或腹膜内施用后的浓度时间特征谱CA164_00725
浓度 (ug/mL)
1000
100
10
1
0
24
48
72
96
120
144
168
时间 (hr)
小鼠 SC
小鼠 IP
仓鼠 SC
仓鼠 IP

图 67

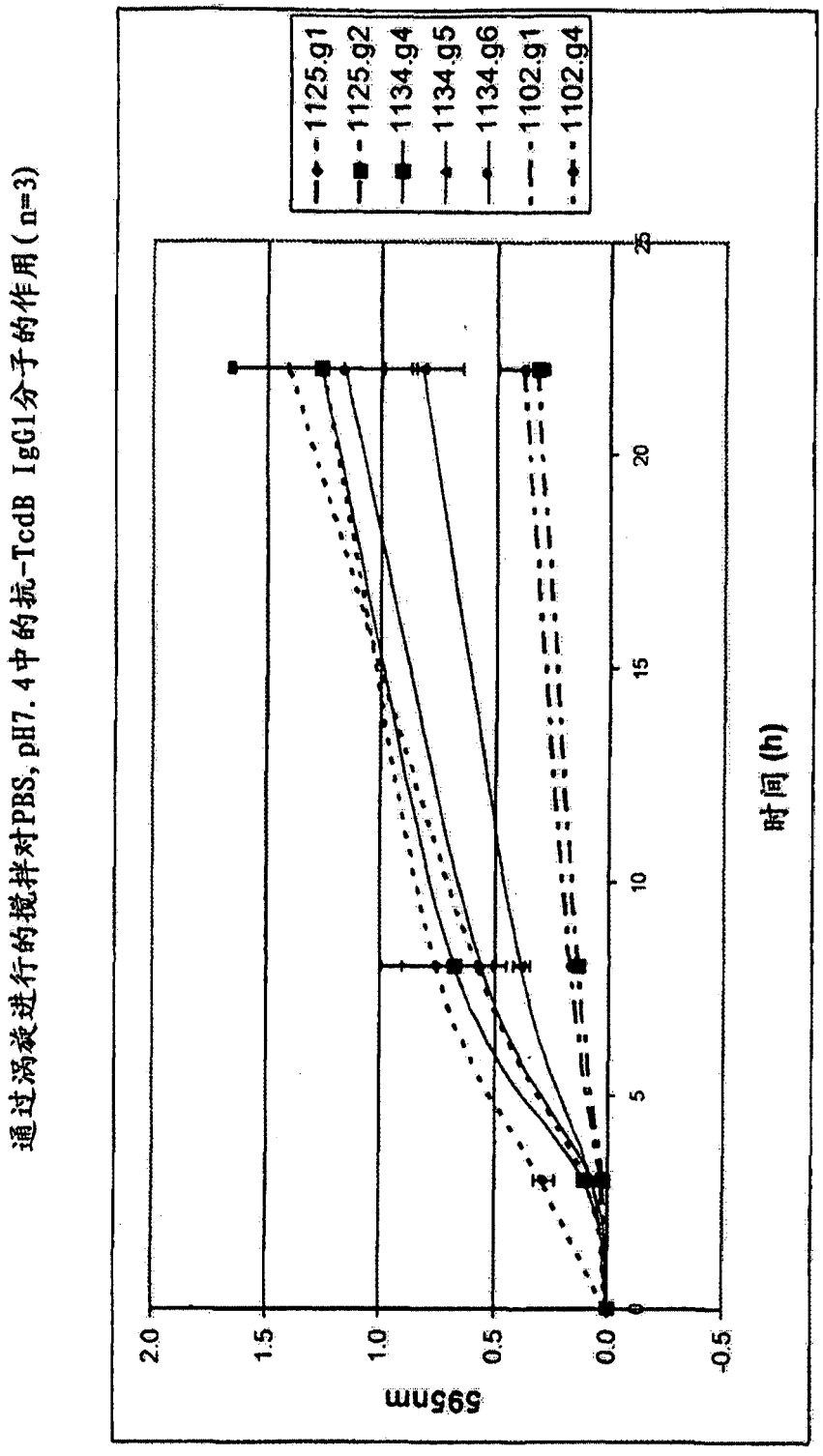

通过涡旋进行的搅拌对PBS, pH7.4中的抗-TcdB IgG1分子的作用(n=3)
1125.g1
1125.g2
1134.g4
1134.g5
1134.g6
1102.g1
1102.g4
595nm
2.0
1.5
1.0
0.5
0.0
-0.5
0
5
10
15
20
25
时间 (h)

图 68

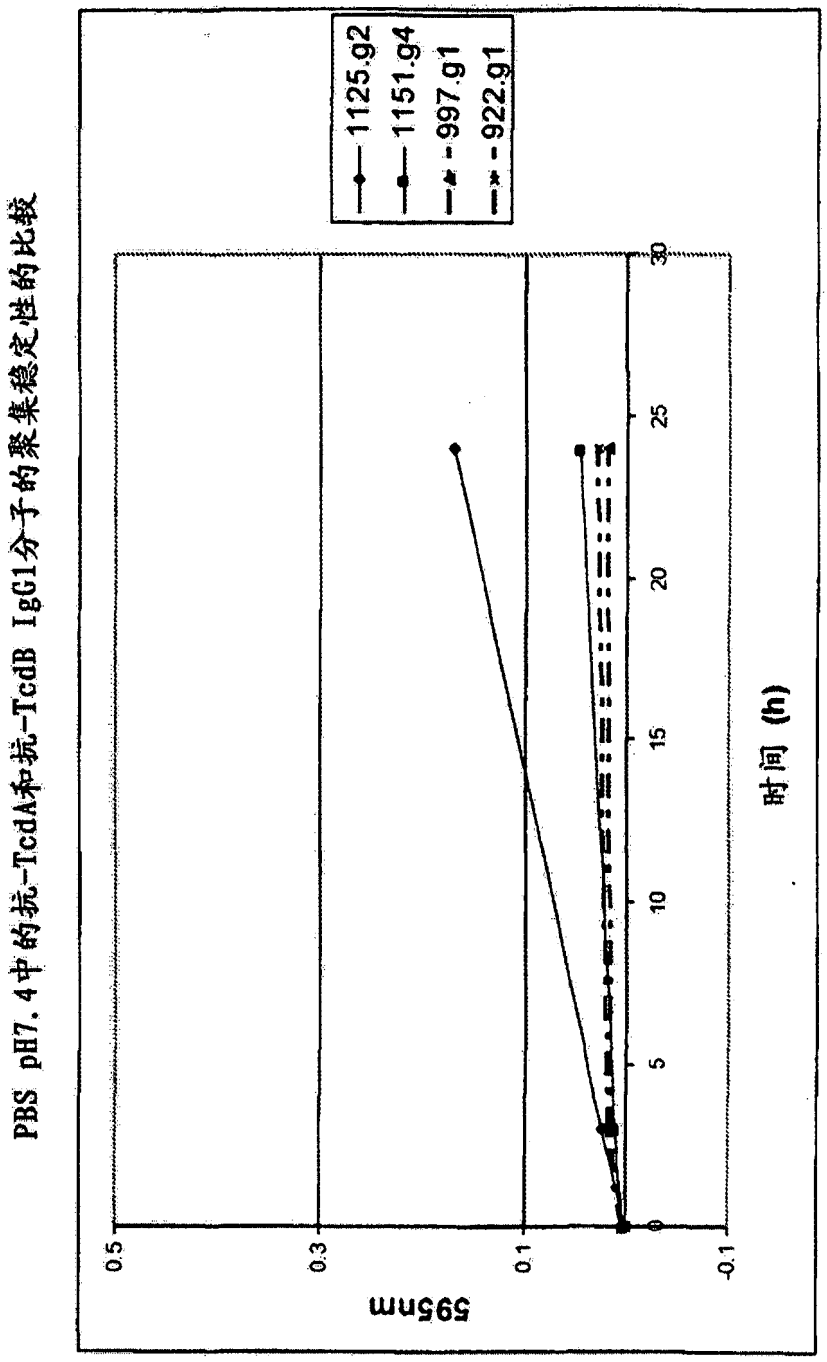

PBS pH7.4中的抗-TcdA和抗-TcdB IgG1分子的聚集稳定性的比较
1125.g2
1151.g4
997.g1
922.g1
595nm
0.5
0.3
0.1
-0.1
0
5
10
15
20
25
30
时间 (h)

图 69

抗核糖型003中和

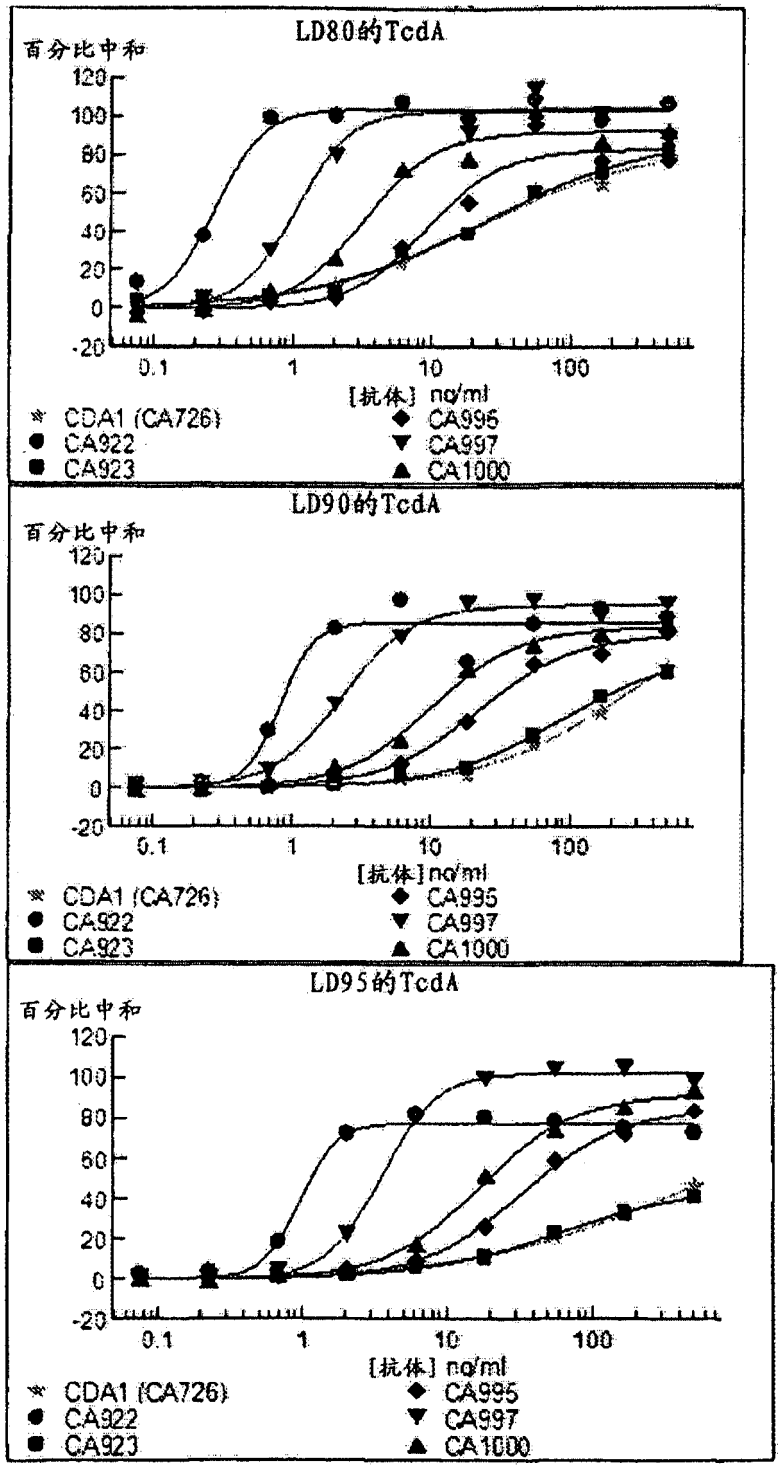


图 70

抗核糖型003中和

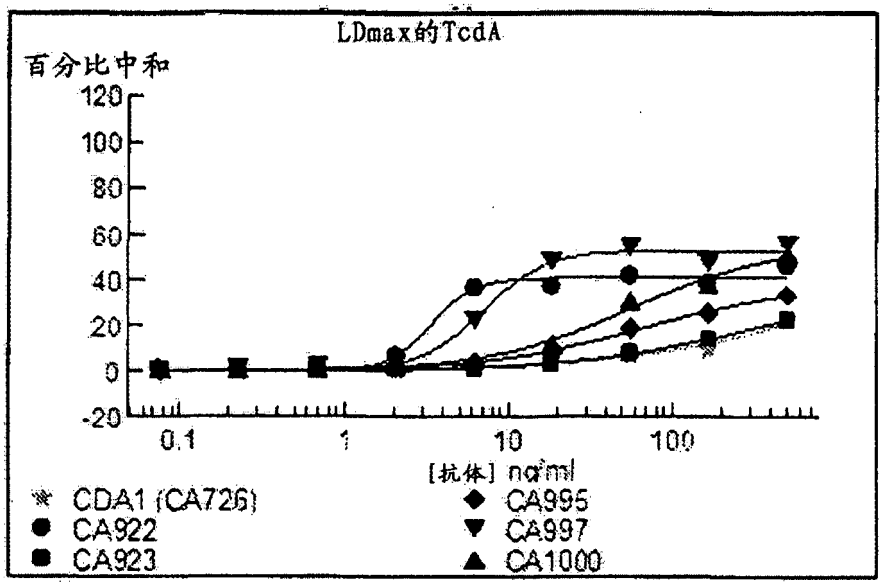


图 71

抗核糖型027中和

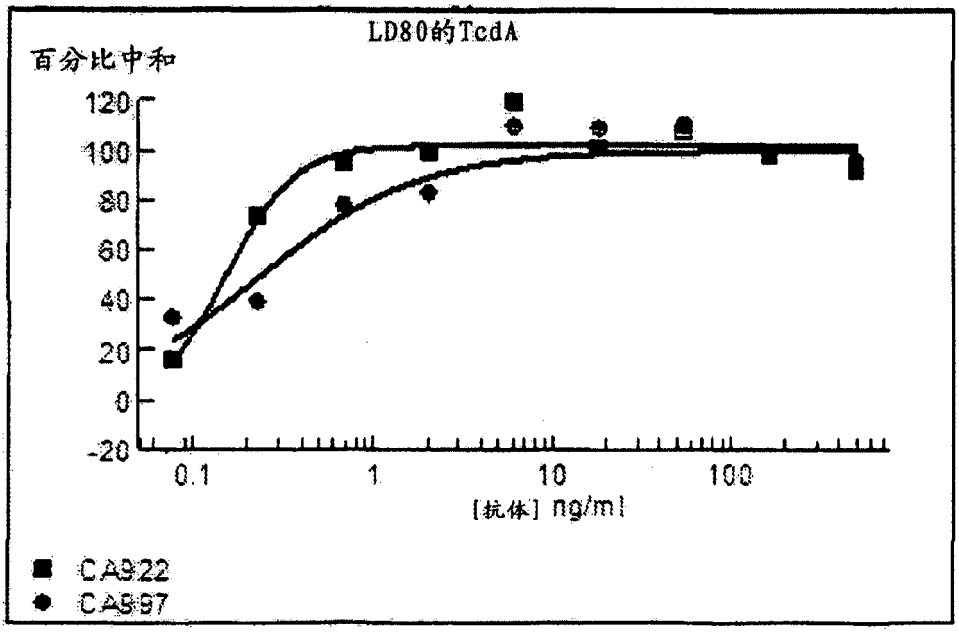


图 72

抗核糖型078中和

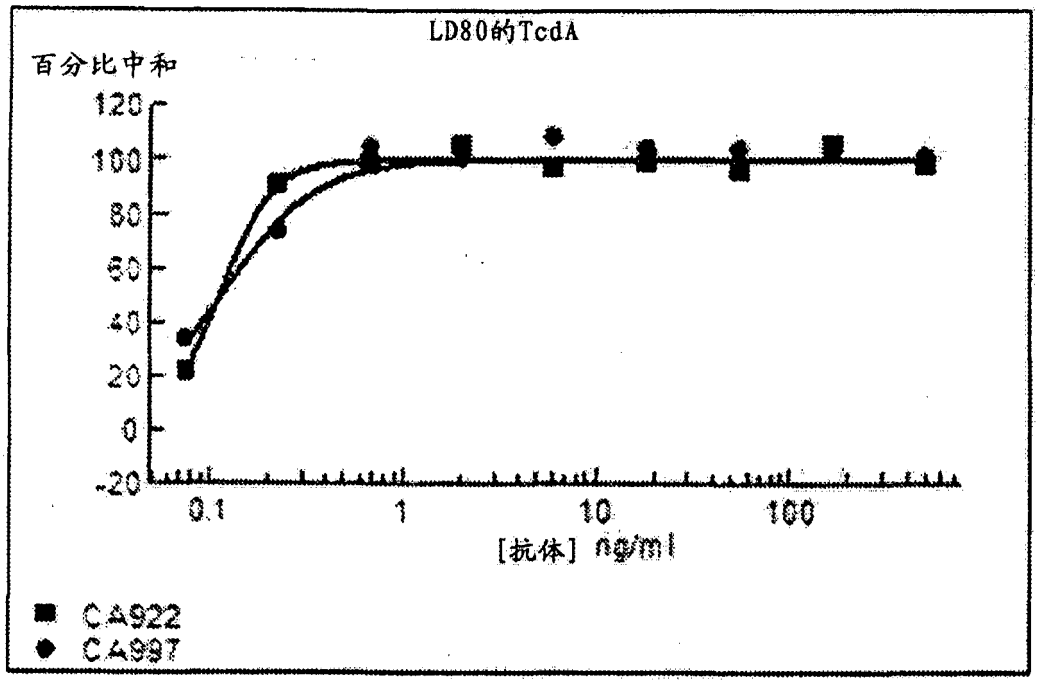


图 73