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(71) Applicant(s)
UCB Pharma S.A.

(72) Inventor(s)
Humphreys, David Paul;Lightwood, Daniel John;Tyson, Kerry Louise;Knight, David Edward Ormonde;Herve, Karine Jeannine Madeleine;Compson, Joanne Elizabeth;Page, Matthew Jon Timothy;Payne, Andrew Charles;Fisher, Nicola Louise;Mackenzie, Brendon;Cox, Matthew

(74) Agent / Attorney
Griffith Hack, GPO Box 1285, Melbourne, VIC, 3001, AU

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(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).

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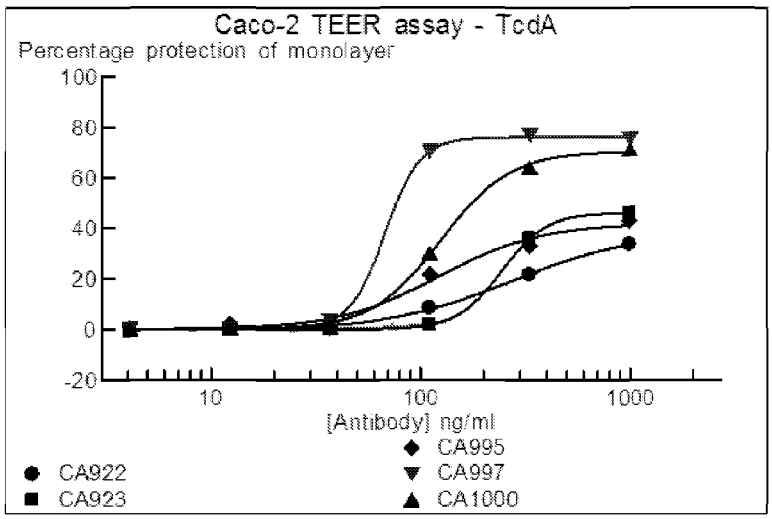
[Continued on next page]

(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

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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.

It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art in Australia or any other country.

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).

A first aspect provides a pharmaceutical composition 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, the composition comprising one or more monoclonal antibodies that specifically bind antigen TcdA123 and/or TcdA456, wherein the antibody has high affinity of 500pM or higher for the target antigen TcdA123 and said one or more monoclonal antibodies are independently selected from:

i) an antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:4 for CDR-H1, a sequence given in SEQ ID NO:5 for CDR-H2 and a sequence given in SEQ ID NO:6 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:1 for CDR-L1, a sequence given in in SEQ ID NO:2 for CDR-L2 and a sequence given in SEQ ID NO:3 for CDR-L3;

ii) an antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:34 for CDR-H1, a sequence given in SEQ ID NO:35 for CDR-H2 and a sequence given in SEQ ID NO:36 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:31 for CDR-L1, a sequence given in in SEQ ID NO:32 for CDR-L2 and a sequence given in SEQ ID NO:33 for CDR-L3;

iii) an antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:44 for CDR-H1, a sequence given in SEQ ID NO:45 for CDR-H2 and a sequence given in SEQ ID NO:46 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:41 for CDR-L1, a sequence given in in SEQ ID NO:42 for CDR-L2 and a sequence given in SEQ ID NO:43 for CDR-L3; and

iv) an antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:54 for CDR-H1, a sequence given in SEQ ID NO:55 for CDR-H2 and sequence given in SEQ ID NO:56 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:51 for CDR-L1, a sequence given in in SEQ ID NO:52 for CDR-L2 and a sequence given in SEQ ID NO:53 for CDR-L3.

A second aspect provides use of a pharmaceutical composition according to the first aspect in the manufacture of a medicament for treating or preventing *Clostridium difficile* infection or a complication therefrom.

A third aspect provides use of one or more monoclonal antibodies independently selected from:

i) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:4 for CDR-H1, a sequence given in SEQ ID NO:5 for CDR-H2 and a sequence given in SEQ ID NO:6 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:1 for CDR-L1, a sequence given in in SEQ ID NO:2 for CDR-L2 and a sequence given in SEQ ID NO:3 for CDR-L3;

ii) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:34 for CDR-H1, a sequence given in SEQ ID NO:35 for CDR-H2 and a sequence given in SEQ ID NO:36 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:31 for CDR-L1, a sequence given in in SEQ ID NO:32 for CDR-L2 and a sequence given in SEQ ID NO:33 for CDR-L3;

iii) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:44 for CDR-H1, a sequence given in SEQ ID NO:45 for CDR-H2 and a sequence given in SEQ ID NO:46 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:41 for CDR-L1, a sequence given in in SEQ ID NO:42 for CDR-L2 and a sequence given in SEQ ID NO:43 for CDR-L3; and

iv) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:54 for CDR-H1, a sequence given in SEQ ID NO:55 for CDR-H2 and sequence given in SEQ ID NO:56 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:51

for CDR-L1, a sequence given in in SEQ ID NO:52 for CDR-L2 and a sequence given in SEQ ID NO:53 for CDR-L3,

in the manufacture of a medicament for treating or preventing *Clostridium difficile* infection or a complication therefrom.

A fourth aspect provides a method for treating or preventing *Clostridium difficile* infection or a complication therefrom, the method comprising administering to a patient a pharmaceutical composition according to the first aspect.

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):7843-7848l; 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.

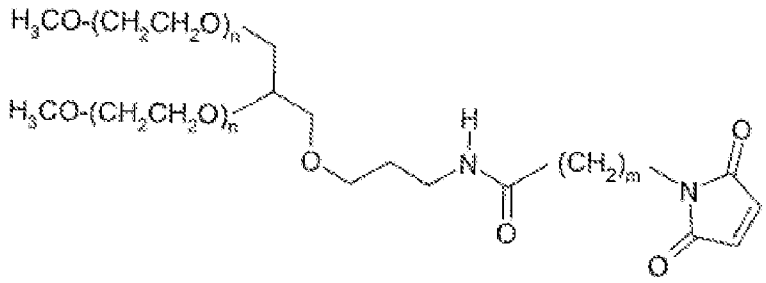
Suitably PEG molecules are covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent 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).

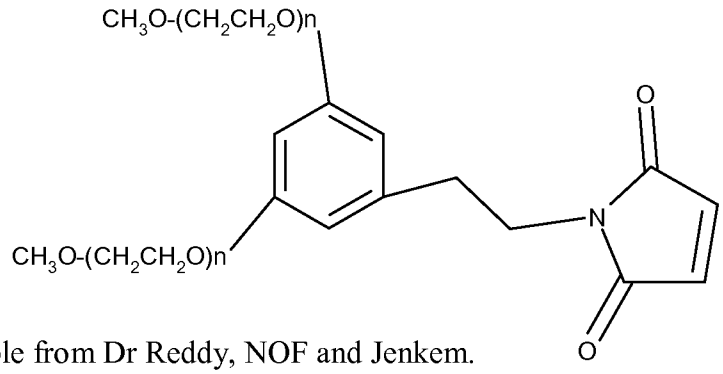
Alternative sources of PEG linkers include NOF who supply GL2-400MA2 (wherein m in the structure below is 5) and GL2-400MA (where m is 2) and n is approximately 450:



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

Anti-TcdA IgG1	**measured pI**	**Tm(Fab) in PBS**	**Tm(CH2)**
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

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CLAIMS

1. A pharmaceutical composition 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, the composition comprising one or more monoclonal antibodies that specifically bind antigen TcdA123 and/or TcdA456, wherein the monoclonal antibody has high affinity of 500pM or higher for the antigen TcdA123 and/or TcdA456 and said one or more monoclonal antibodies are independently selected from:

i) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:4 for CDR-H1, a sequence given in SEQ ID NO:5 for CDR-H2 and a sequence given in SEQ ID NO:6 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:1 for CDR-L1, a sequence given in in SEQ ID NO:2 for CDR-L2 and a sequence given in SEQ ID NO:3 for CDR-L3;

ii) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:34 for CDR-H1, a sequence given in SEQ ID NO:35 for CDR-H2 and a sequence given in SEQ ID NO:36 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:31 for CDR-L1, a sequence given in in SEQ ID NO:32 for CDR-L2 and a sequence given in SEQ ID NO:33 for CDR-L3;

iii) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:44 for CDR-H1, a sequence given in SEQ ID NO:45 for CDR-H2 and a sequence given in SEQ ID NO:46 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:41 for CDR-L1, a sequence given in in SEQ ID NO:42 for CDR-L2 and a sequence given in SEQ ID NO:43 for CDR-L3; and

iv) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:54 for CDR-H1, a sequence given in SEQ ID NO:55 for CDR-H2 and sequence given in SEQ ID NO:56 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:51 for CDR-L1, a sequence given in in SEQ ID NO:52 for CDR-L2 and a sequence given in SEQ ID NO:53 for CDR-L3.

2. A pharmaceutical composition according to claim 1 wherein the one or more monoclonal antibodies comprise a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:44 for CDR-H1, a sequence given in SEQ ID NO:45 for CDR-H2 and a sequence given in SEQ ID NO:46 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:41 for CDR-L1, a sequence given in in SEQ ID NO:42 for CDR-L2 and a sequence given in SEQ ID NO:43 for CDR-L3.

3. A pharmaceutical composition according to claim 2 wherein the one or more monoclonal antibodies comprise 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.

4. A pharmaceutical composition according to claim 1 wherein the one or more monoclonal antibodies comprise a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:54 for CDR-H1, a sequence given in SEQ ID NO:55 for CDR-H2 and a sequence given in SEQ ID NO:56 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:51 for CDR-L1, a sequence given in SEQ ID NO:52 for CDR-L2 and a sequence given in SEQ ID NO:53 for CDR-L3.

5. A pharmaceutical composition according to claim 4 wherein the one or more monoclonal antibodies comprise 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.

6. A pharmaceutical composition according to any one of claims 1 to 5, wherein at least one monoclonal antibody is a neutralizing antibody that maintains neutralizing activity at high concentrations of toxin, optionally wherein the monoclonal antibody is effective against ribotypes 003, 012, 027 and 078.

7. A pharmaceutical composition according to any one of claims 1 to 6, wherein the one or more monoclonal antibodies have an EC_{50} in a TEER assay in the range of 60 to 80ng/ml when measured at 4h after initiation of the assay.

8. A pharmaceutical composition according to any one of claims 1 to 7 further comprising a monoclonal antibody which specifically binds TcdB, the anti-TcdB monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain 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, and a light chain wherein the light chain

variable domain 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.

9. A pharmaceutical composition according to claim 8 wherein the anti-TcdB monoclonal antibody comprises 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.

10. A pharmaceutical composition according to any one of claims 1 to 9 further comprising a monoclonal antibody which specifically binds TcdB, the anti-TcdB monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain 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, and a light chain wherein the light chain variable domain 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.

11. A pharmaceutical composition according to claim 10 wherein the anti-TcdB monoclonal antibody comprises 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.

12. A pharmaceutical composition according to any one of claims 1 to 11, comprising two or more monoclonal antibodies that specifically bind TcdB.

13. A pharmaceutical composition according to any one of claims 1 to 12, comprising two or more monoclonal antibodies that specifically bind to TcdA.

14. A pharmaceutical composition according to any one of claims 1 to 13, wherein the composition comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 distinct monoclonal antibodies.

15. A pharmaceutical composition according to any one of claims 1 to 15, further comprising a pharmaceutically acceptable excipient.

16. Use of a pharmaceutical composition according to any one of claims 1 to 15 in the manufacture of a medicament for treating or preventing *Clostridium difficile* infection or a complication therefrom.

17. Use of one or more monoclonal antibodies independently selected from:

i) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:4 for CDR-H1, a sequence given in SEQ ID NO:5 for CDR-H2 and a sequence given in SEQ ID NO:6 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:1 for CDR-L1, a sequence given in in SEQ ID NO:2 for CDR-L2 and a sequence given in SEQ ID NO:3 for CDR-L3;

ii) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:34 for CDR-H1, a sequence given in SEQ ID NO:35 for CDR-H2 and a sequence given in SEQ ID NO:36 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:31 for CDR-L1, a sequence given in in SEQ ID NO:32 for CDR-L2 and a sequence given in SEQ ID NO:33 for CDR-L3;

iii) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:44 for CDR-H1, a sequence given in SEQ ID NO:45 for CDR-H2 and a sequence given in SEQ ID NO:46 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:41 for CDR-L1, a sequence given in in SEQ ID NO:42 for CDR-L2 and a sequence given in SEQ ID NO:43 for CDR-L3; and

iv) a monoclonal antibody comprising a heavy chain wherein the heavy chain variable domain comprises a sequence given in SEQ ID NO:54 for CDR-H1, a sequence given in SEQ ID NO:55 for CDR-H2 and sequence given in SEQ ID NO:56 for CDR-H3, and a light chain wherein the light chain variable domain comprises a sequence given in SEQ ID NO:51 for CDR-L1, a sequence given in in SEQ ID NO:52 for CDR-L2 and a sequence given in SEQ ID NO:53 for CDR-L3,

in the manufacture of a medicament for treating or preventing *Clostridium difficile* infection or a complication therefrom.

18. A method for treating or preventing *Clostridium difficile* infection or a complication therefrom, the method comprising administering to a patient a pharmaceutical composition according to any one of claims 1 to 15.

19. Use according to claim 16 or claim 17 or a method according to claim 18, wherein treating or preventing further comprises administering a compound selected the group comprising metronidazole, vancomycin, clindamycin, fidaxomicin and combinations thereof.

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

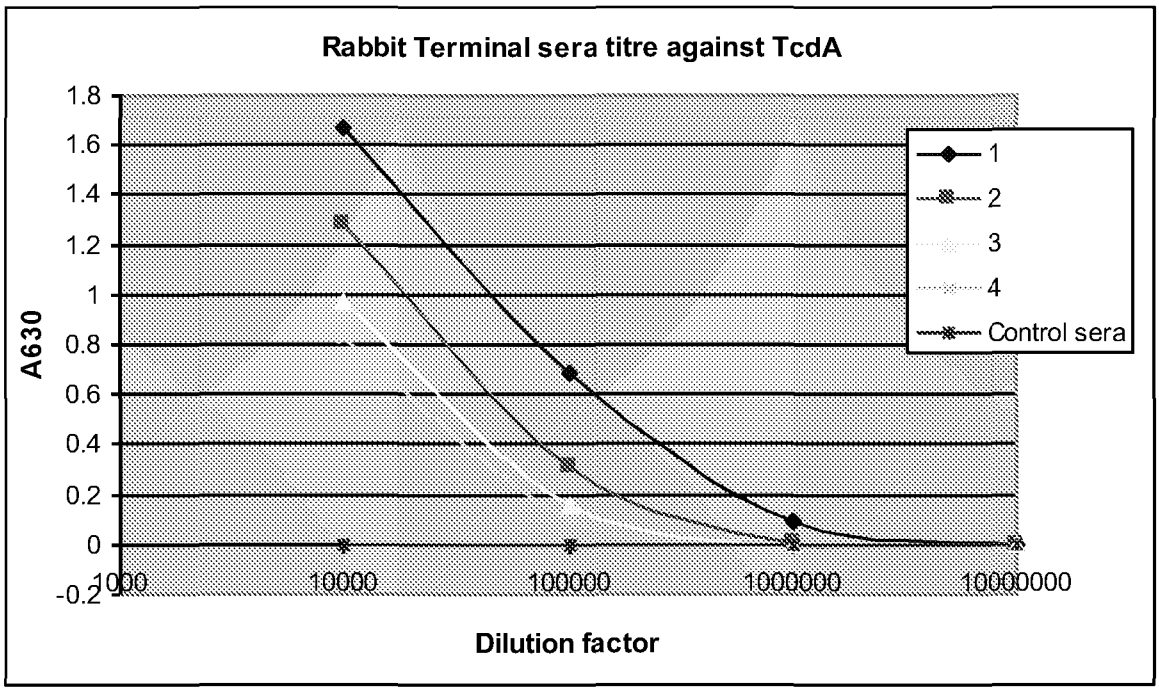
SEQ ID NO: 168 polynucleotide sequence encoding anti-toxin B antibody 1153.g8 VL region

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



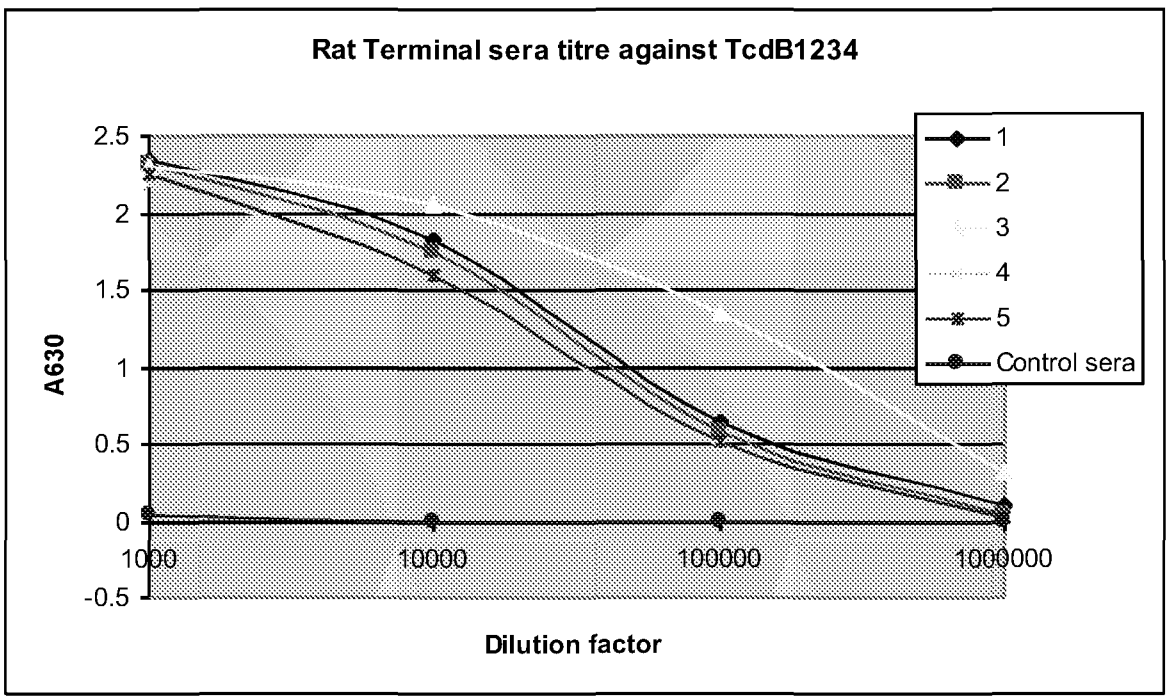


Figure 12 Anti TcdA (Ribotype 003) in-vitro neutralization data for single Mabs (X axis conc. (ng/ml) and Y axis % Neutralization)

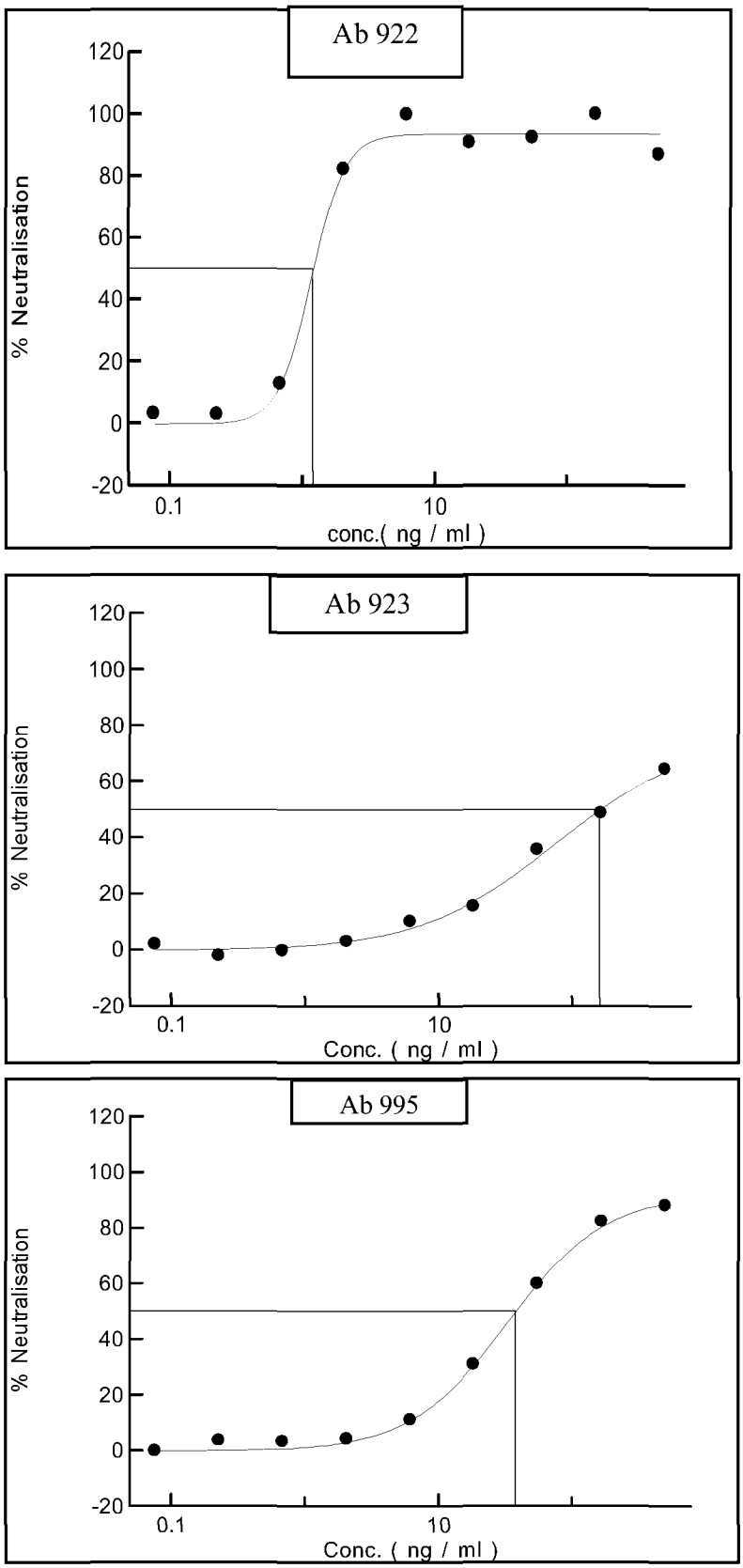


Figure 13 Anti TcdA (Ribotype 003) in-vitro neutralization data for single Mabs (X axis conc. (ng/ml) and Y axis % Neutralization)

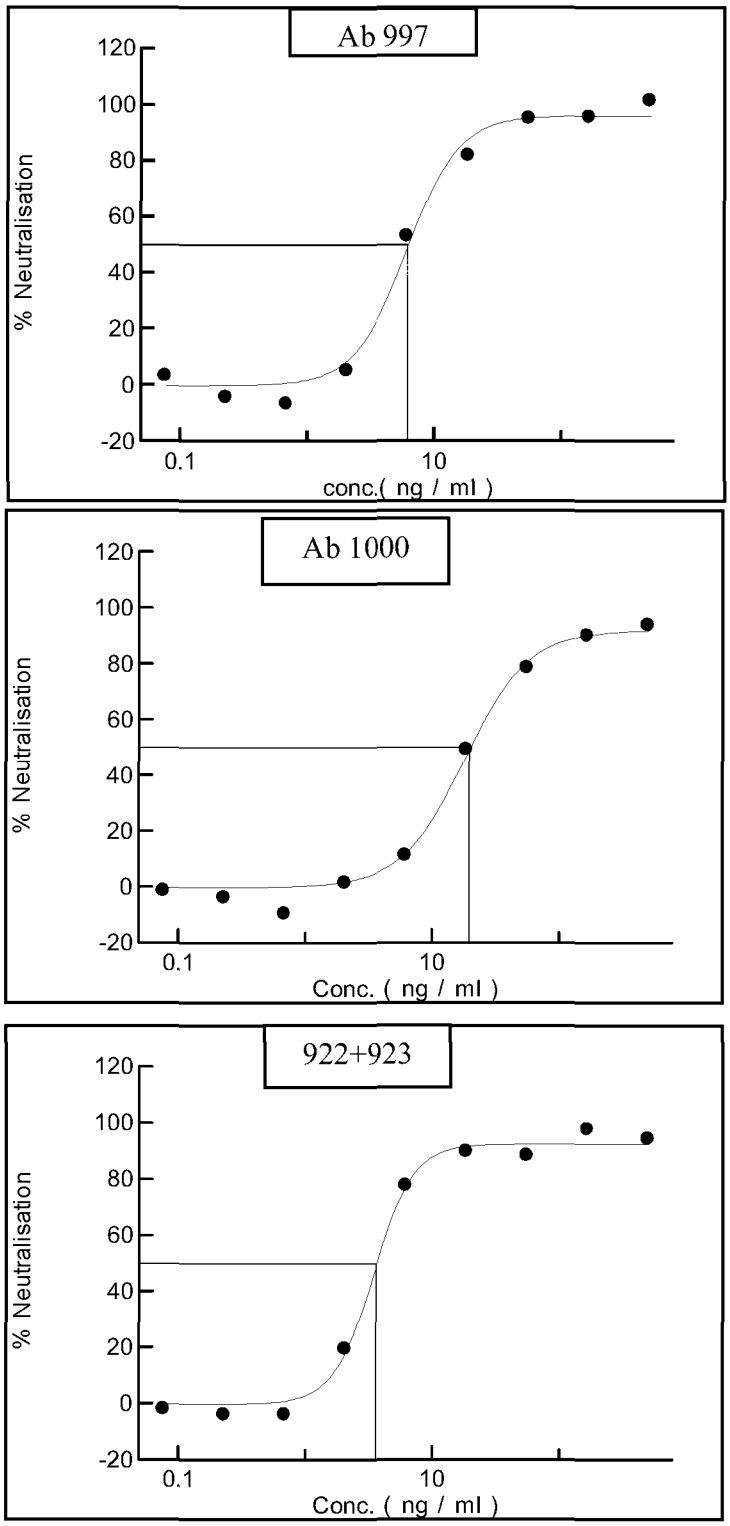


Figure 14 Anti TcdA (Ribotype 003) in-vitro neutralization data for paired Mabs (X axis conc. (ng/ml) and Y axis % Neutralization)

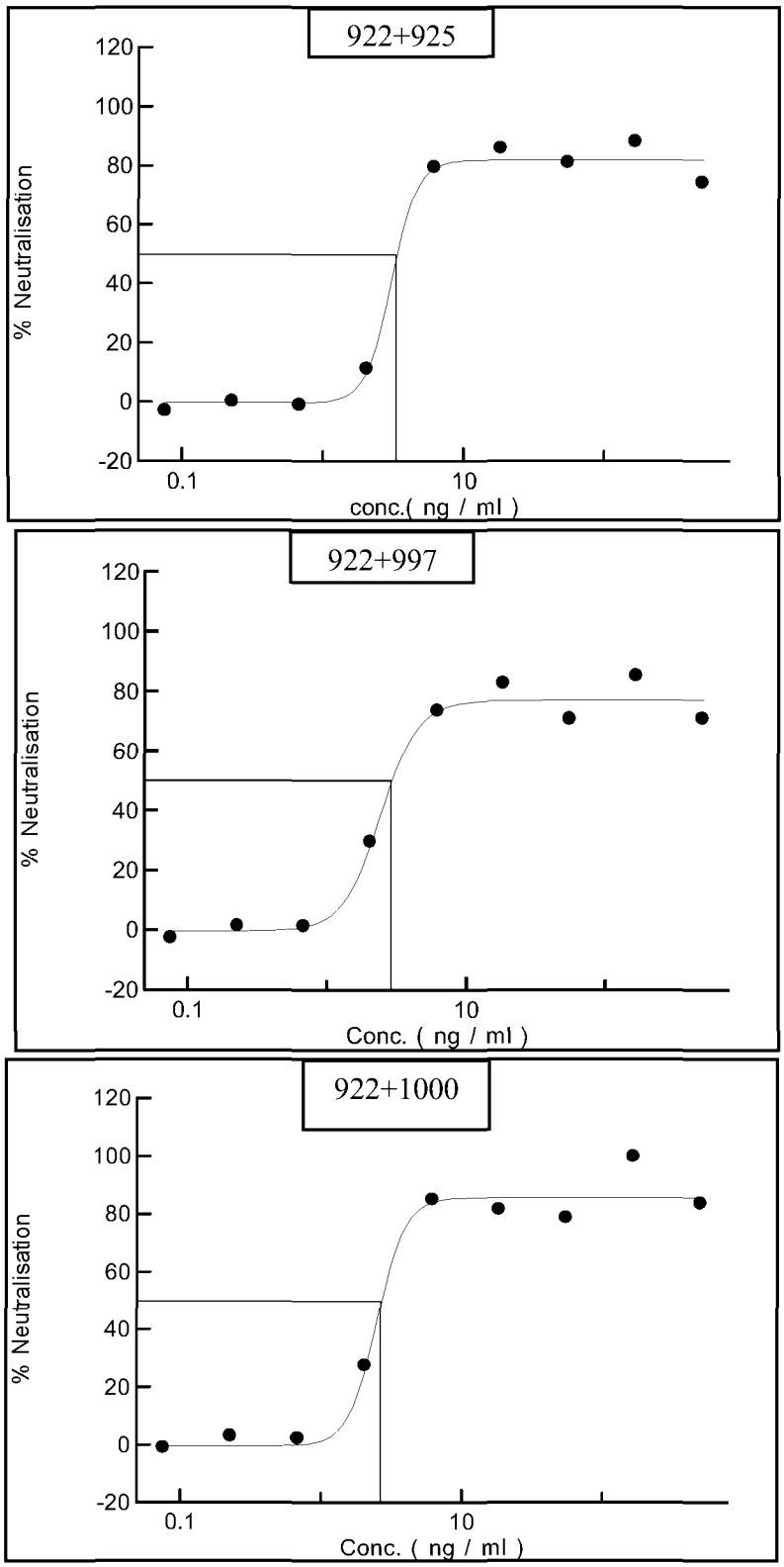


Figure 15 Anti TcdA (Ribotype 003) in-vitro neutralization data for paired Mabs (X axis conc. (ng/ml) and Y axis % Neutralization)

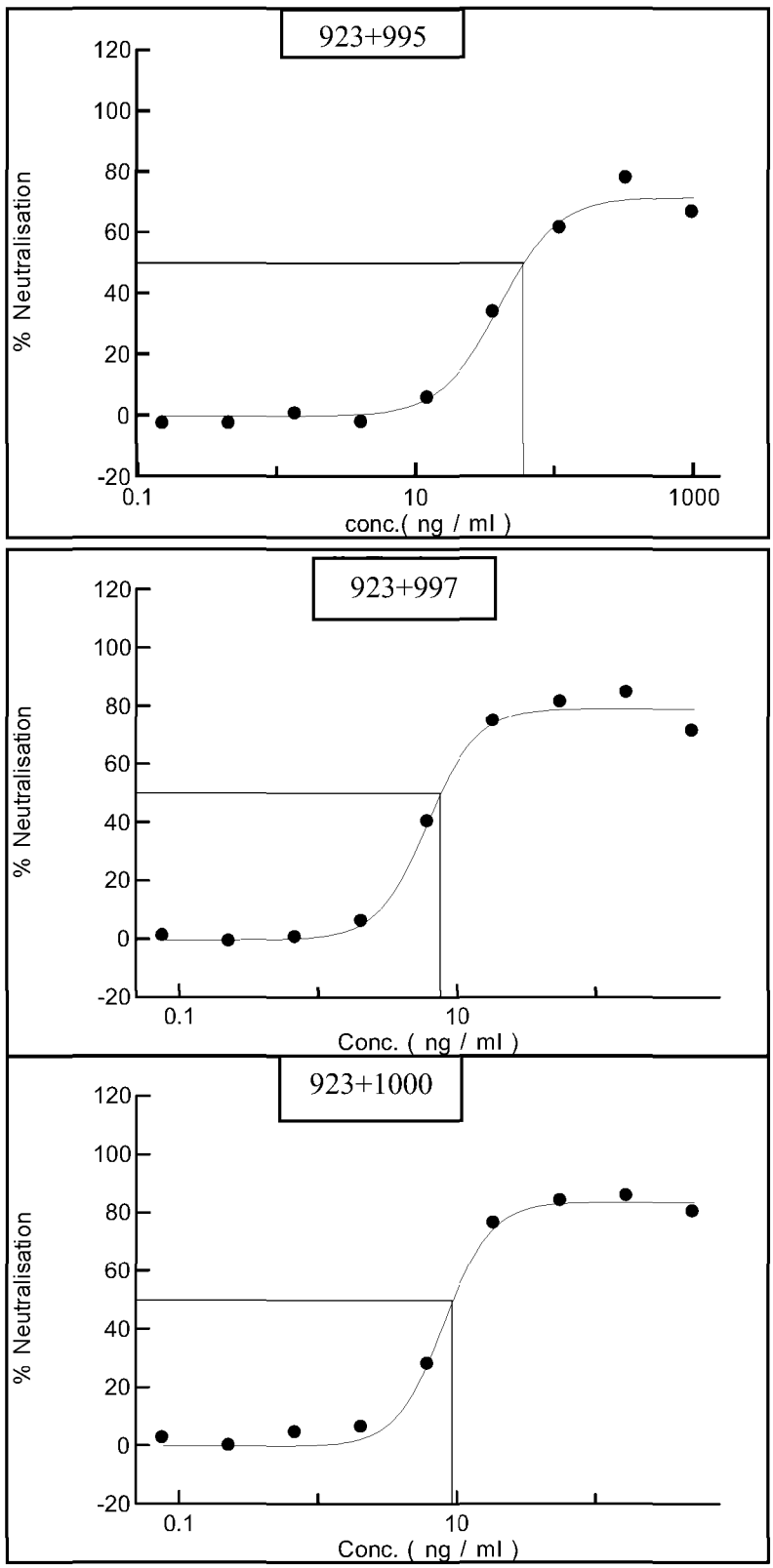


Figure 16 Anti TcdA (Ribotype 003) in-vitro neutralization data for three Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

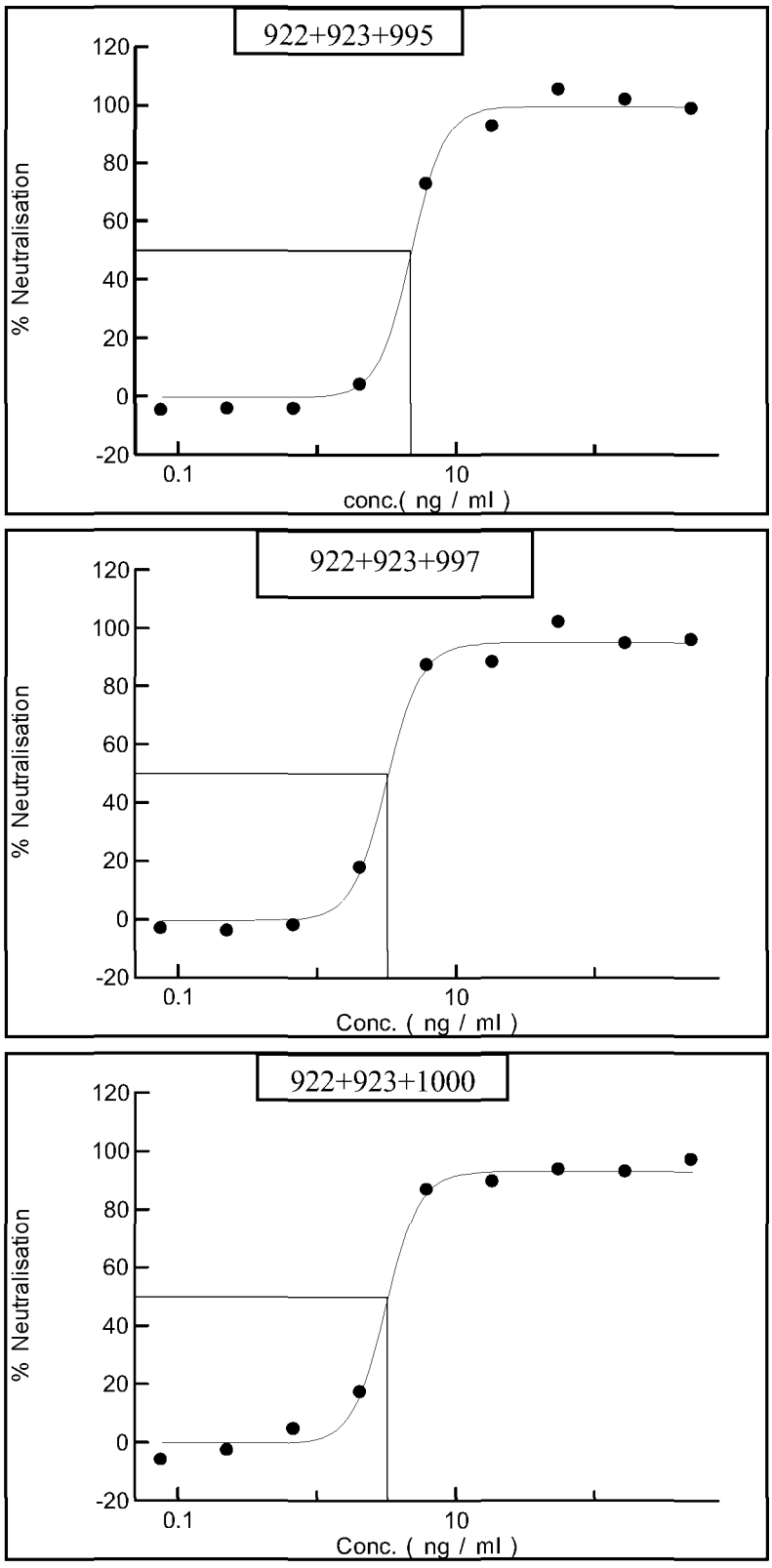


Figure 17 Anti TcdA (Ribotype 003) in-vitro neutralization data for three Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

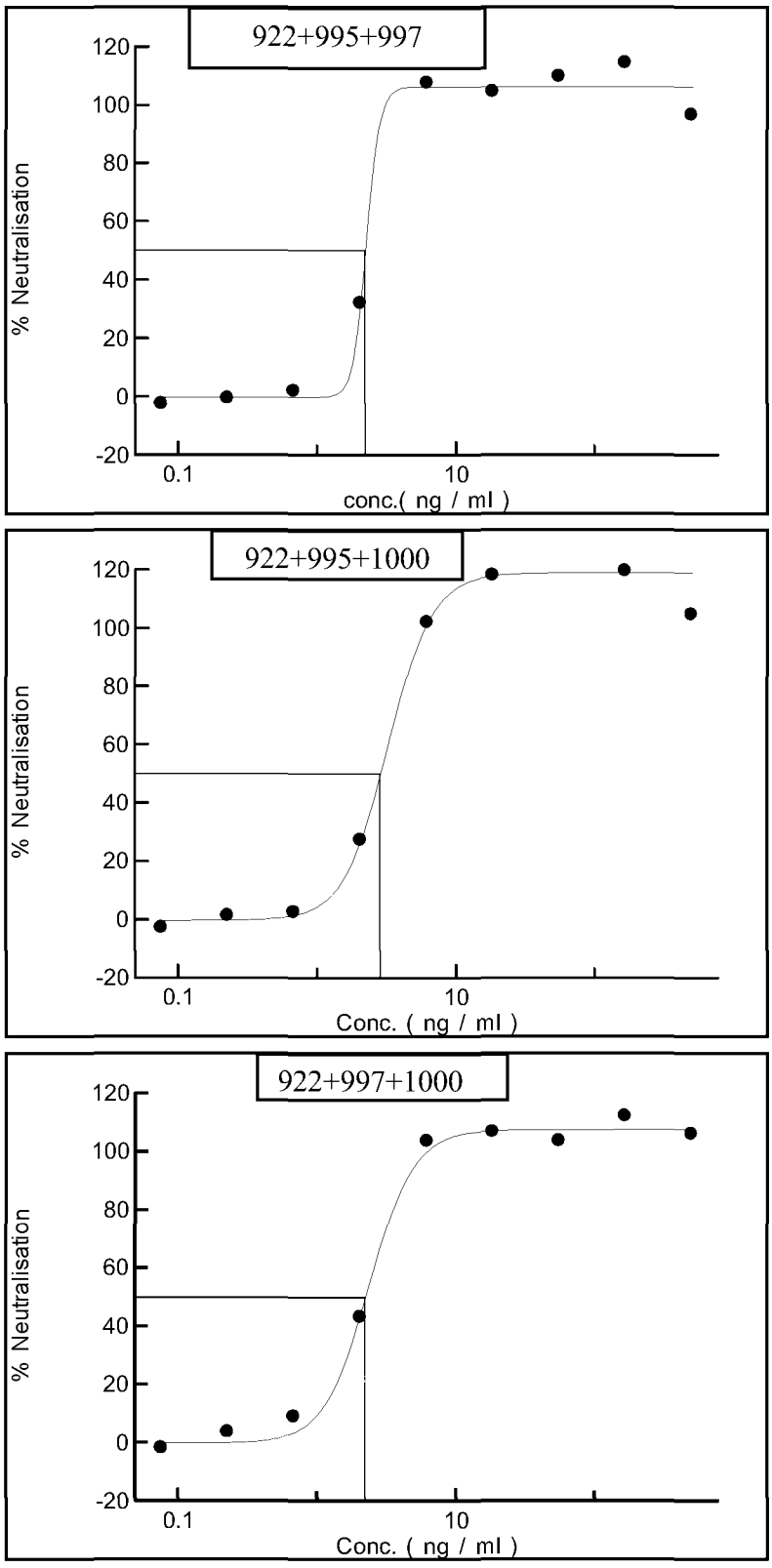


Figure 18 Anti TcdA (Ribotype 003) in-vitro neutralization data for three Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

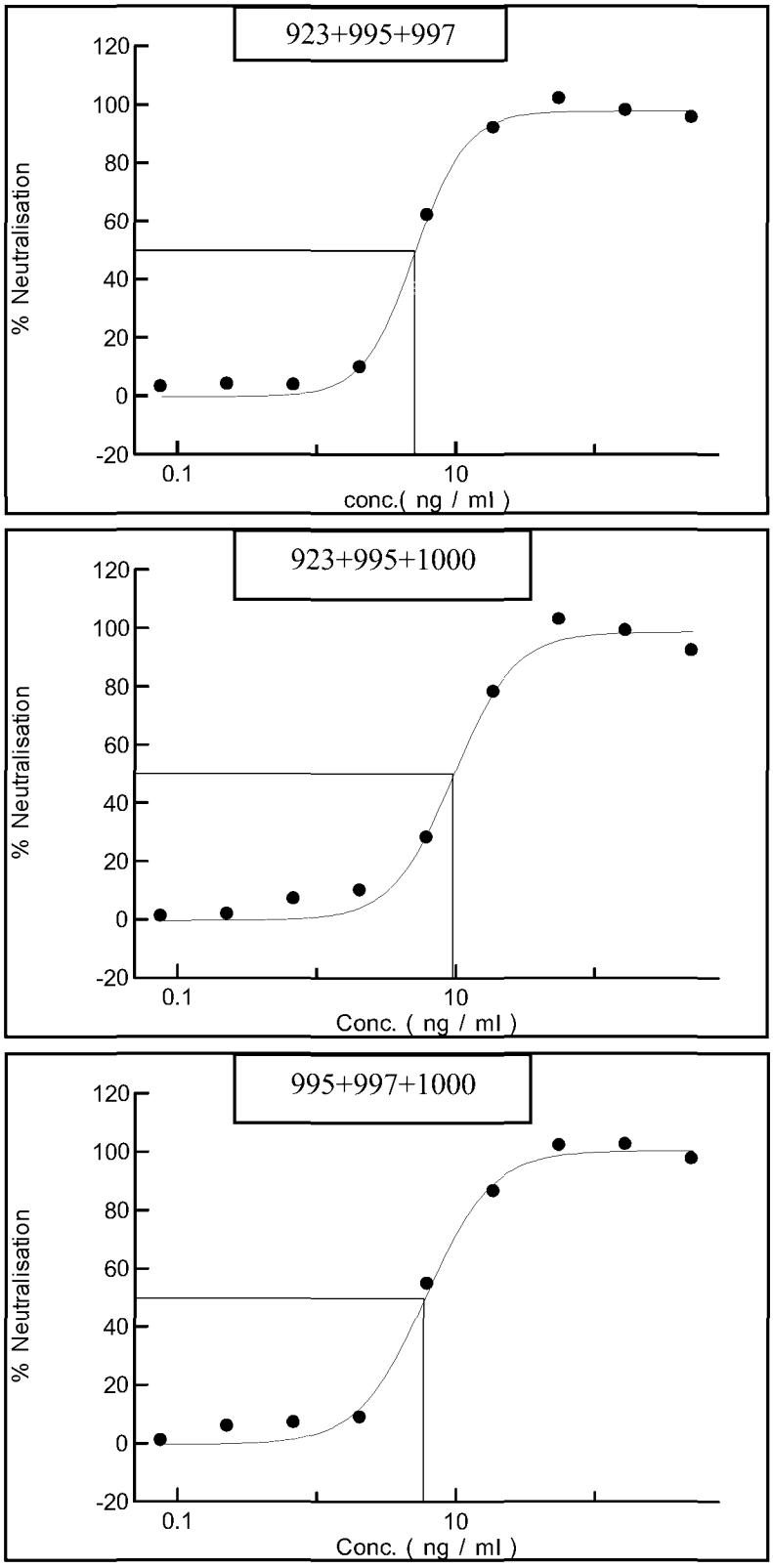


Figure 19 Anti TcdA (Ribotype 003) in-vitro neutralization data for four and five Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

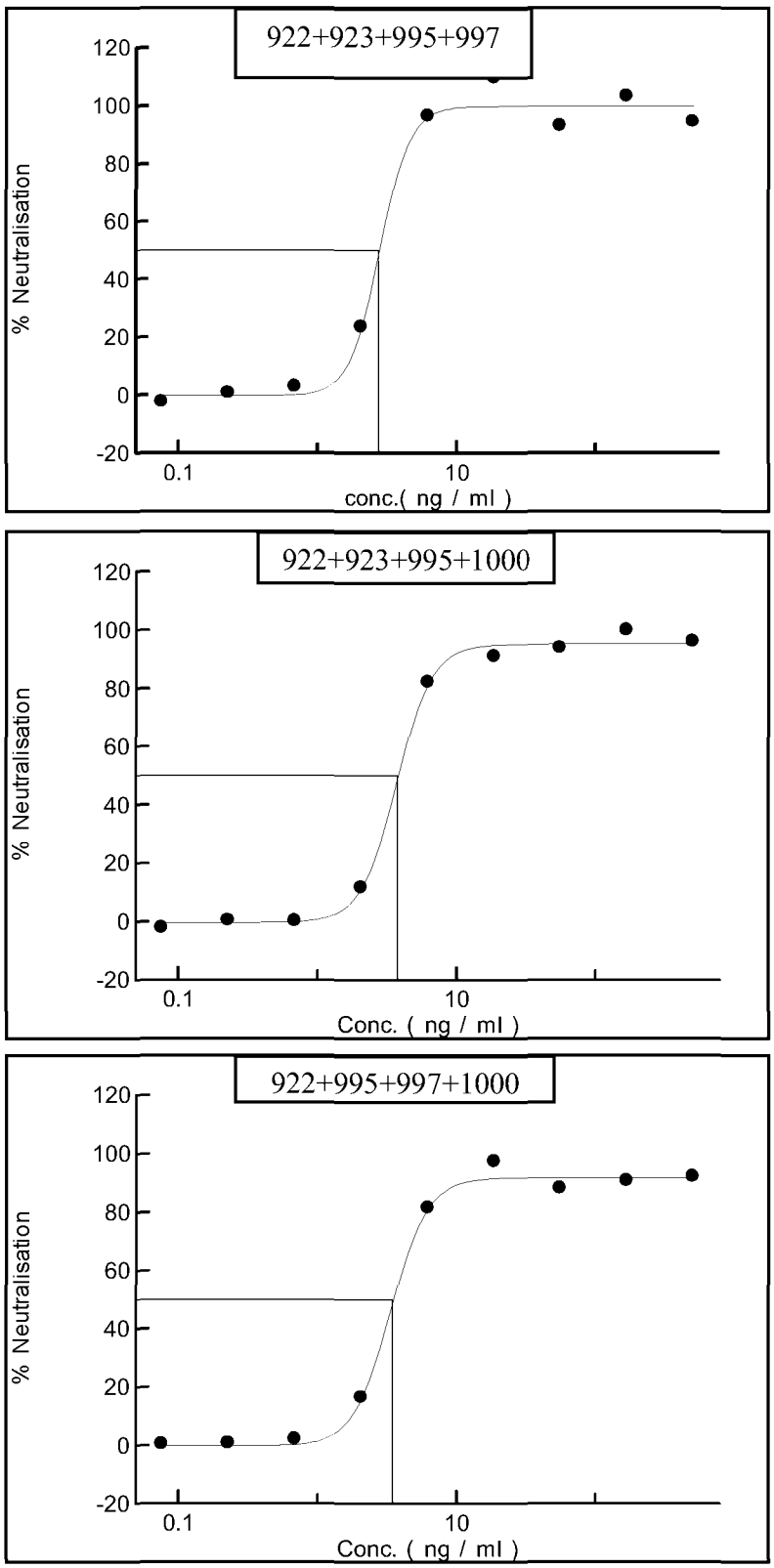


Figure 20 Anti TcdA (Ribotype 003) in-vitro neutralization data for four and five Mab mixtures (X axis conc. (ng/ml) and Y axis % Neutralization)

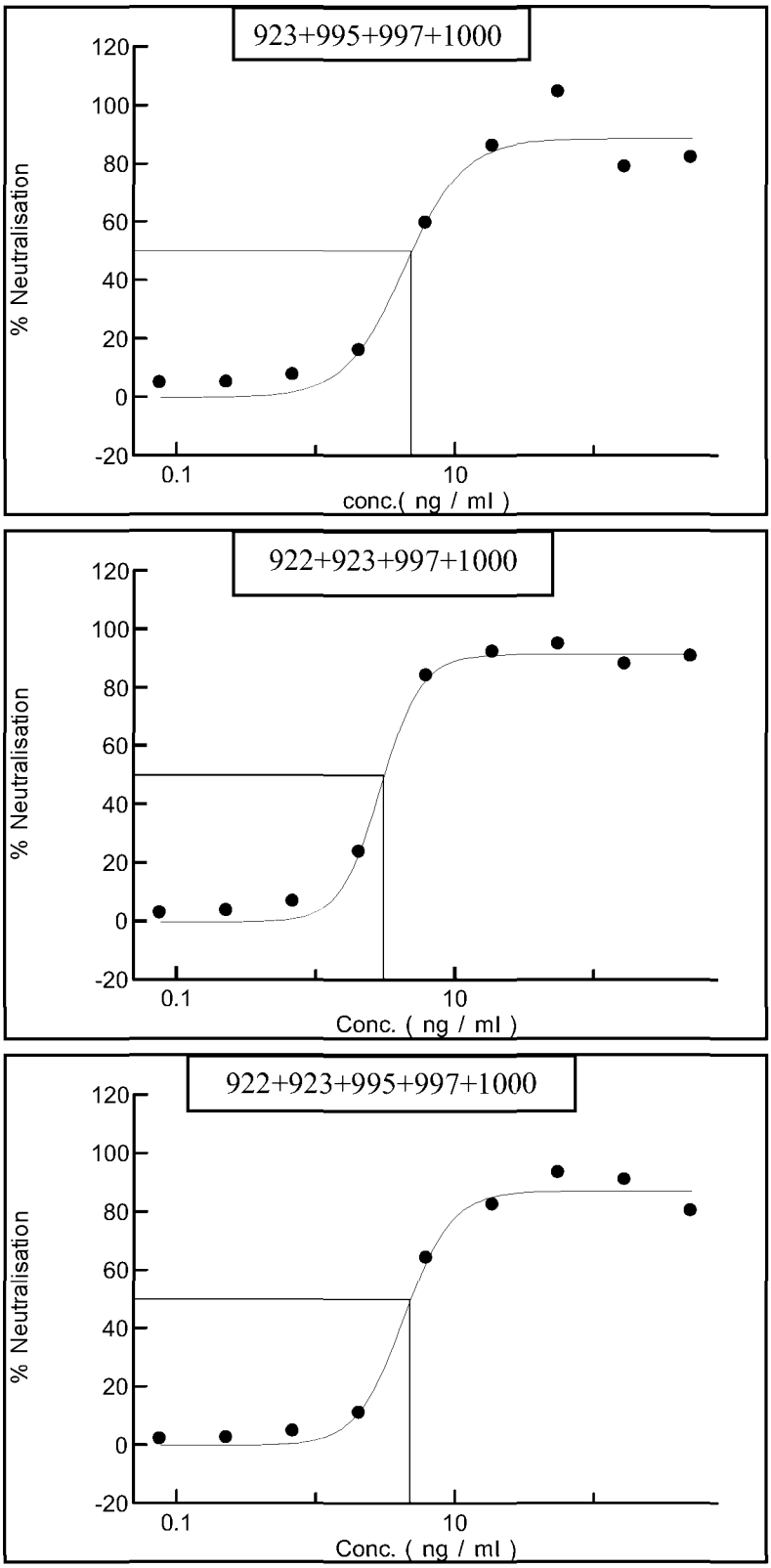
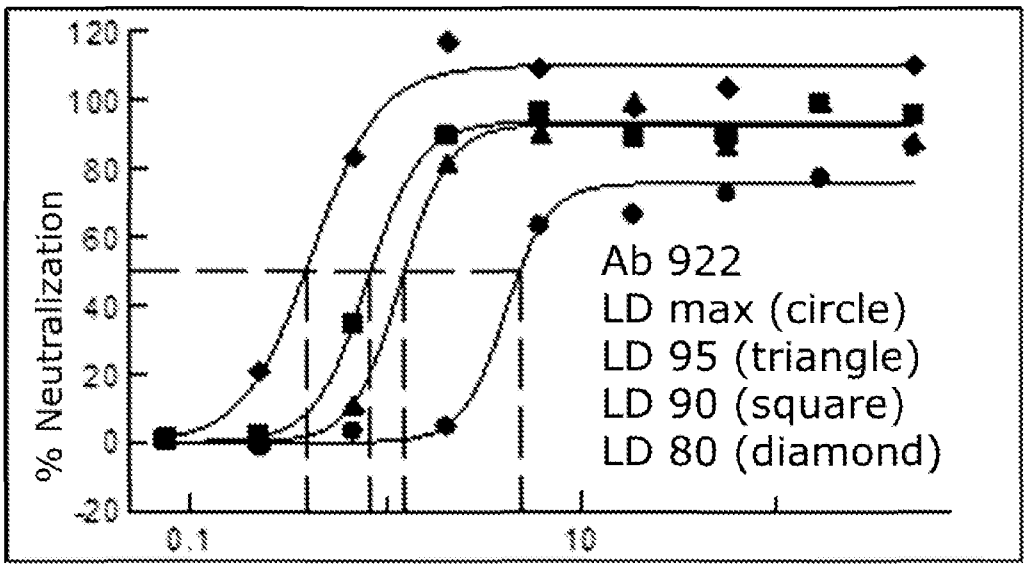
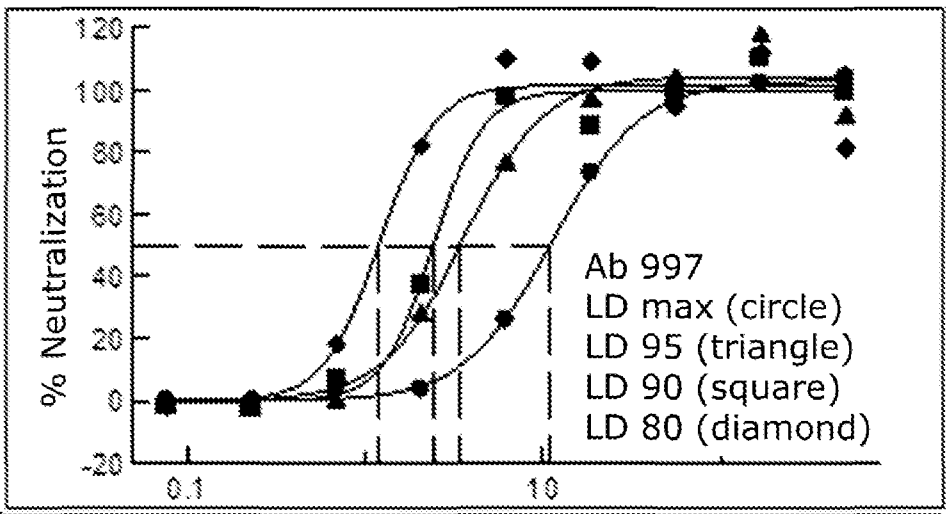


Figure 21 Anti TcdA (Ribotype 003) in-vitro neutralization data for single and paired Mabs at different TcdA concentrations (X axis is conc ng/ml)





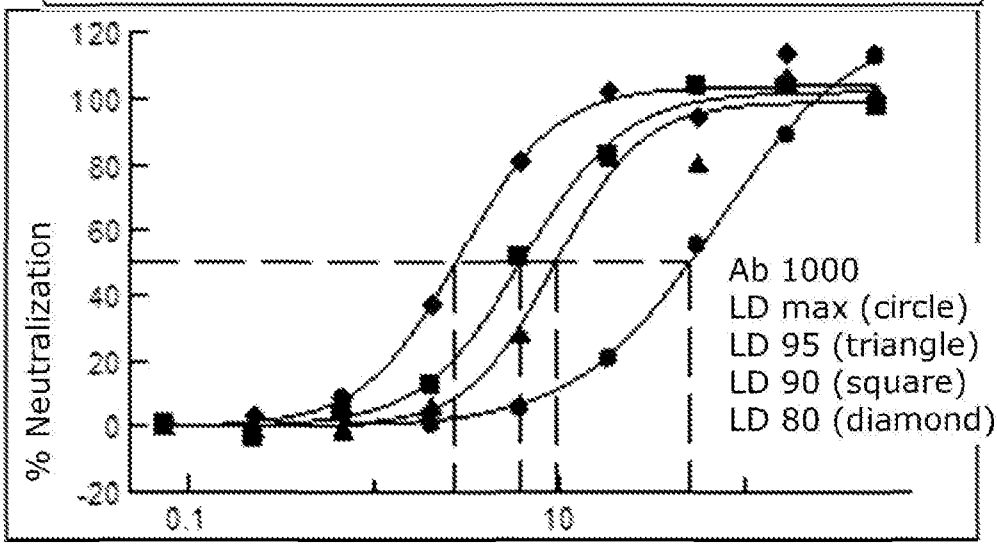


Figure 22 Anti TcdA (Ribotype 003) in-vitro neutralization data for single and paired Mabs at different TcdA concentrations (X axis is conc. ng/ml)

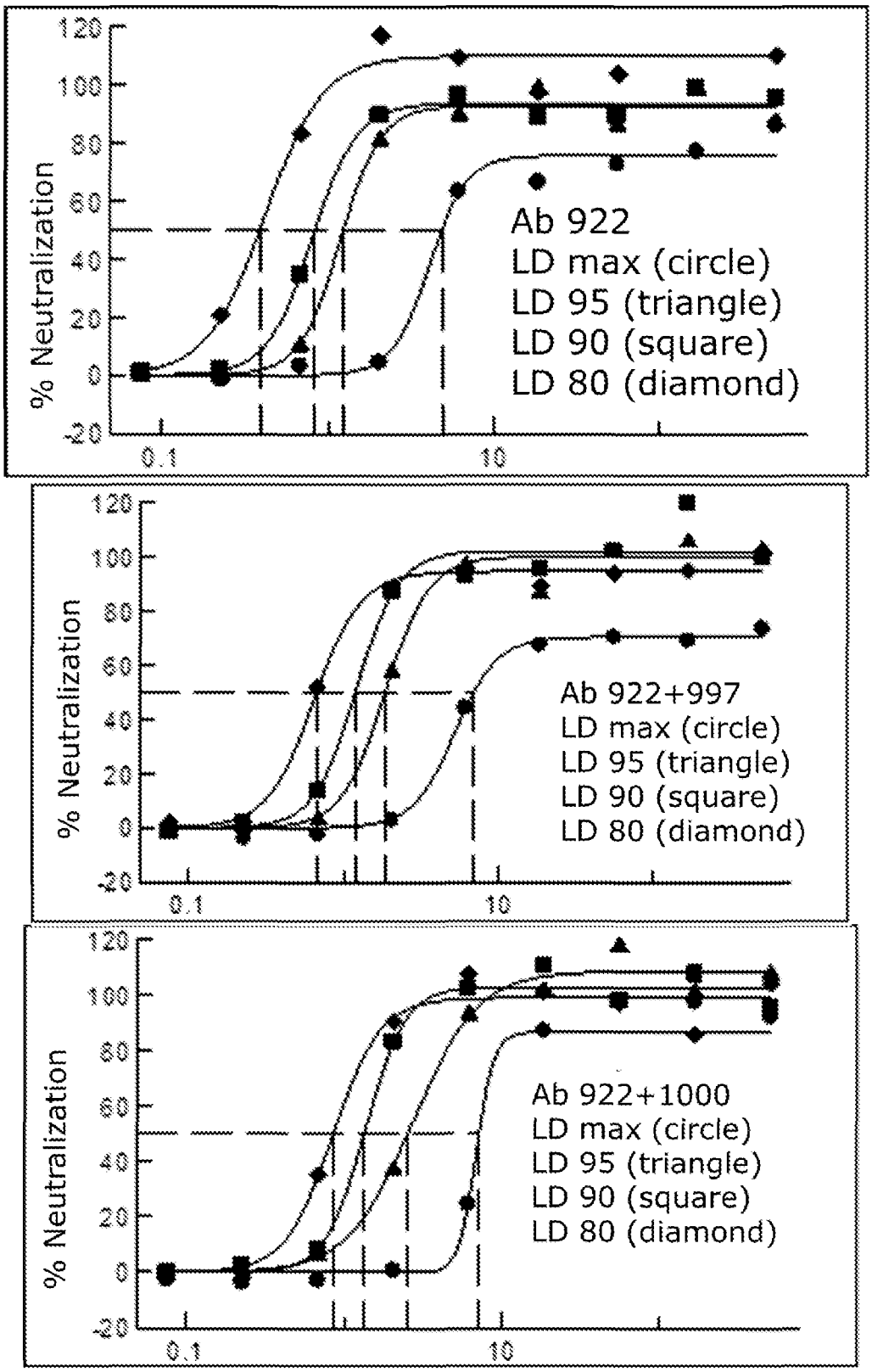


Figure 23 Anti TcdA (Ribotype 003) in-vitro neutralization data for single and to five Mab mixtures at different TcdA concentrations (X axis conc. ng/ml)

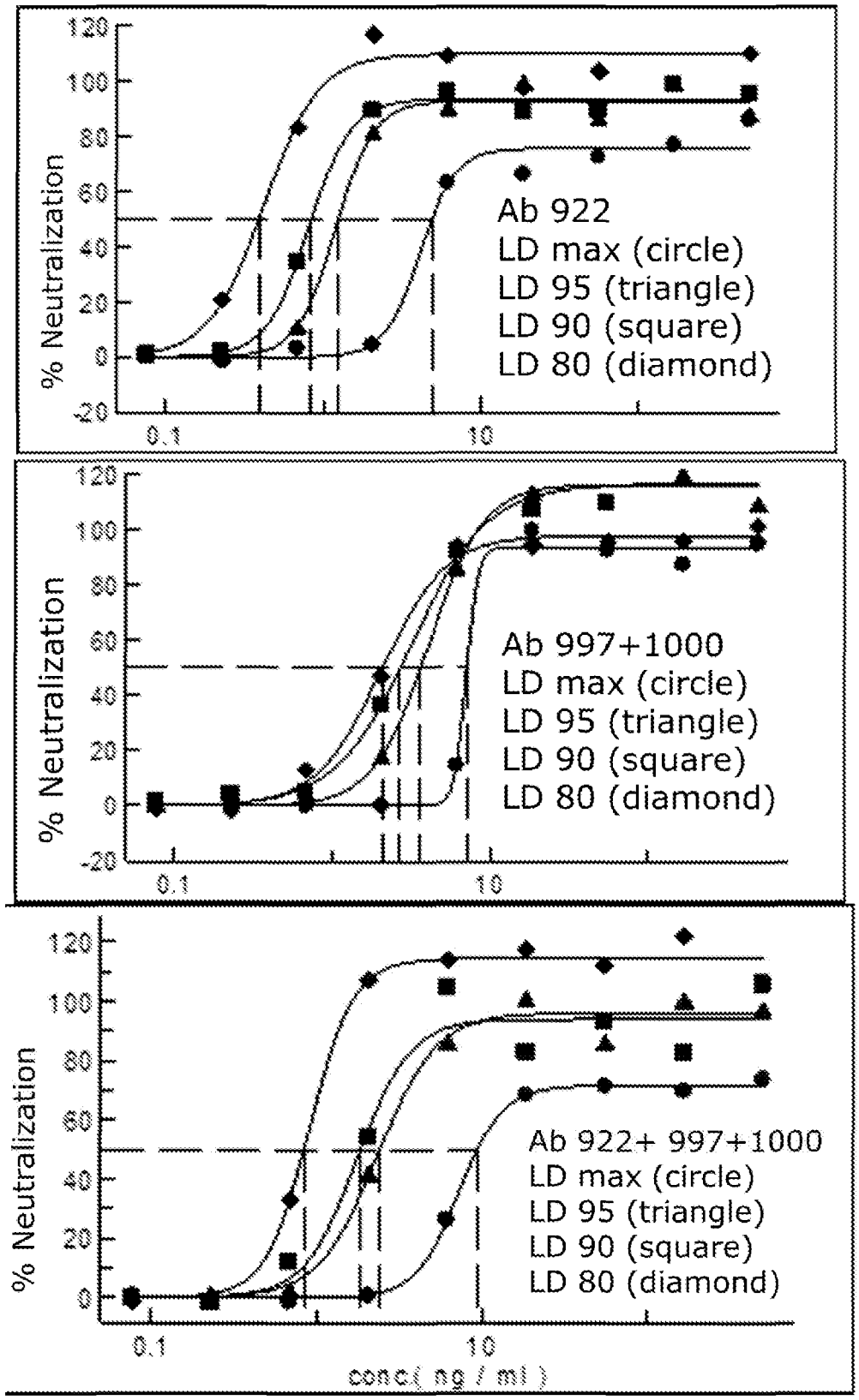
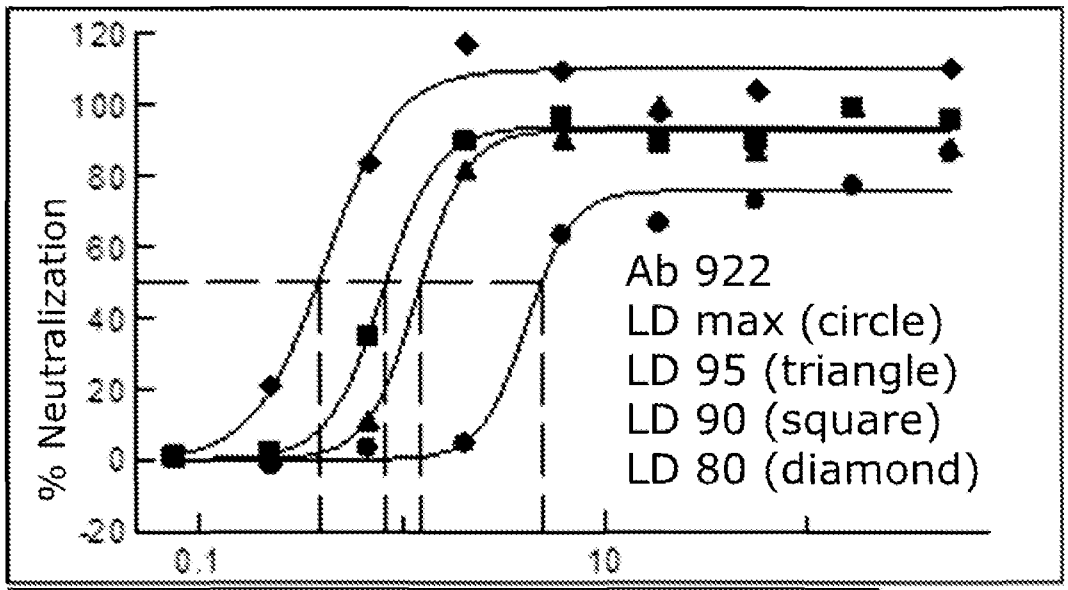
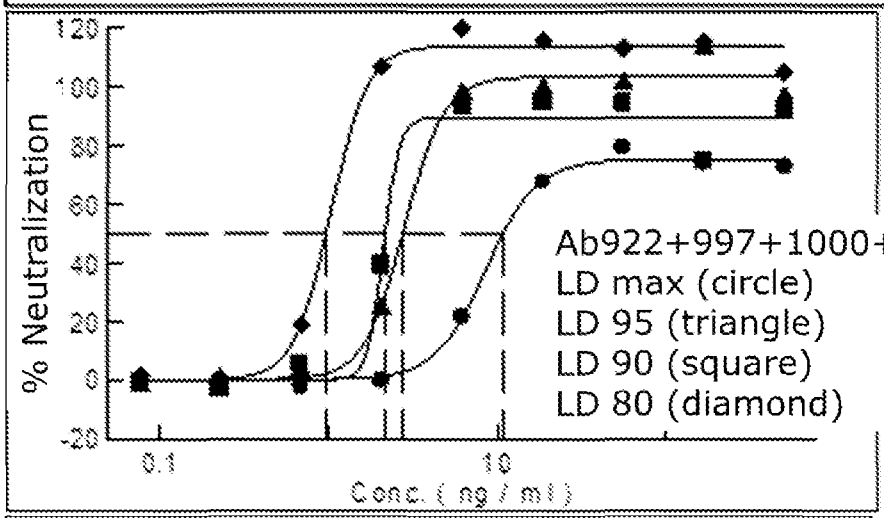


Figure 24 Anti TcdA (Ribotype 003) in-vitro neutralization data for single and to five Mab mixtures at different TcdA concentrations (X axis is conc. ng/ml





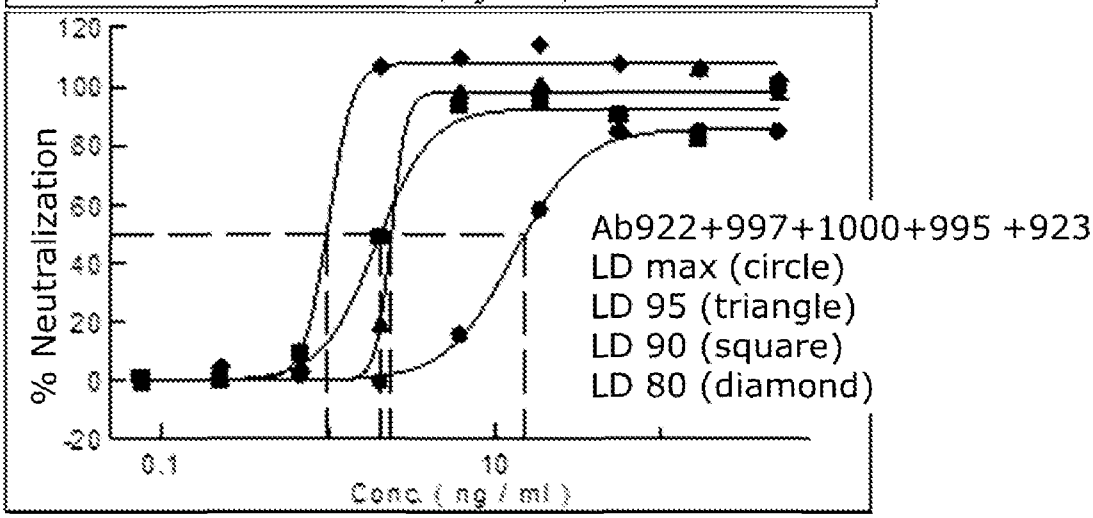


Figure 25 Anti TcdB (Ribotype 003) in-vitro neutralization data for single Mabs (Y axis neutralization X axis conc ng/ml for 1125.g2, 1134.g5 and 927.g2 respectively)

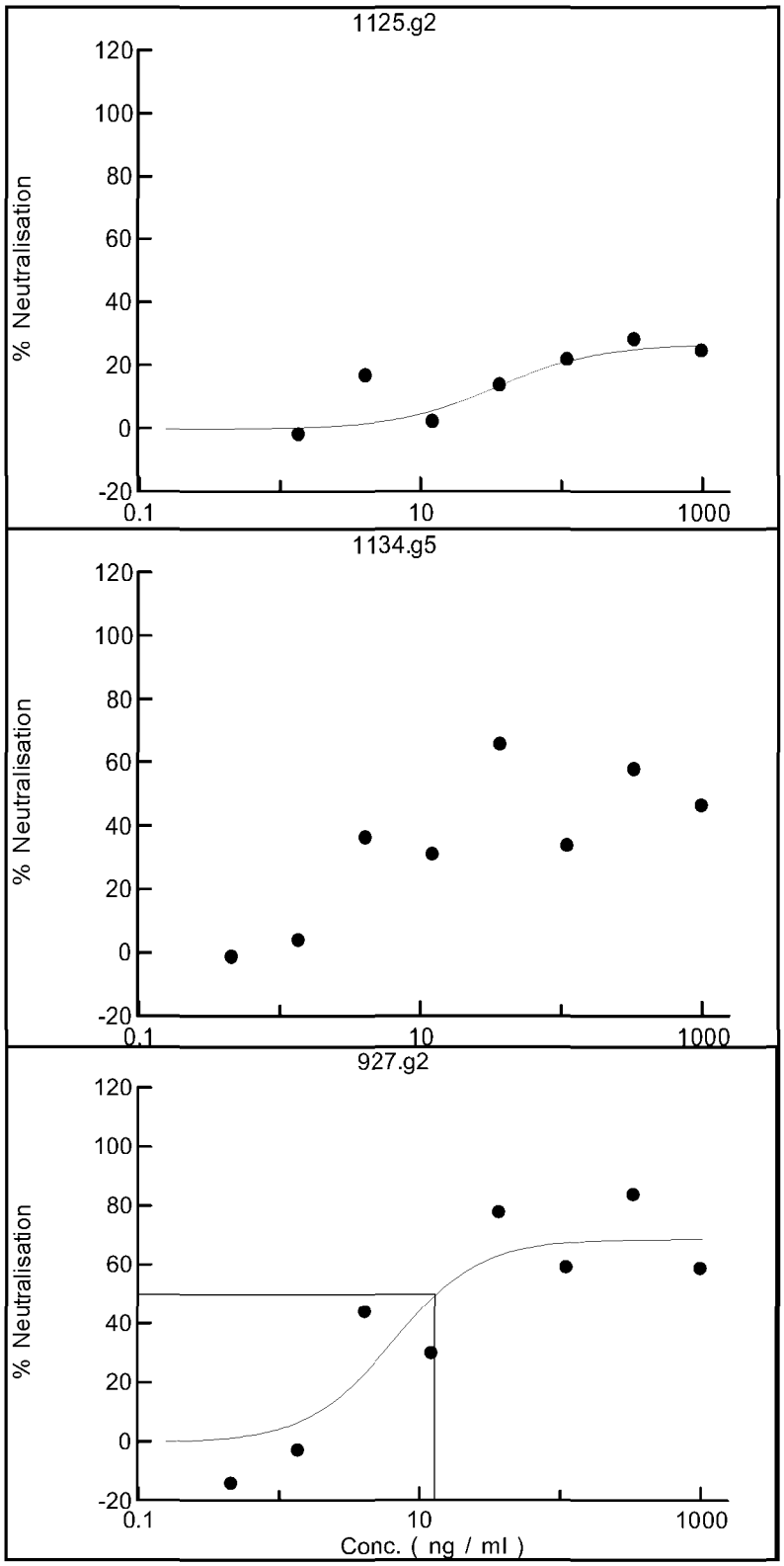


Figure 26 Anti TcdB (Ribotype 003) in-vitro neutralization data for single Mabs Y axis neutralization X axis conc ng/ml for1153.g8 and 1102.g4 respectively)

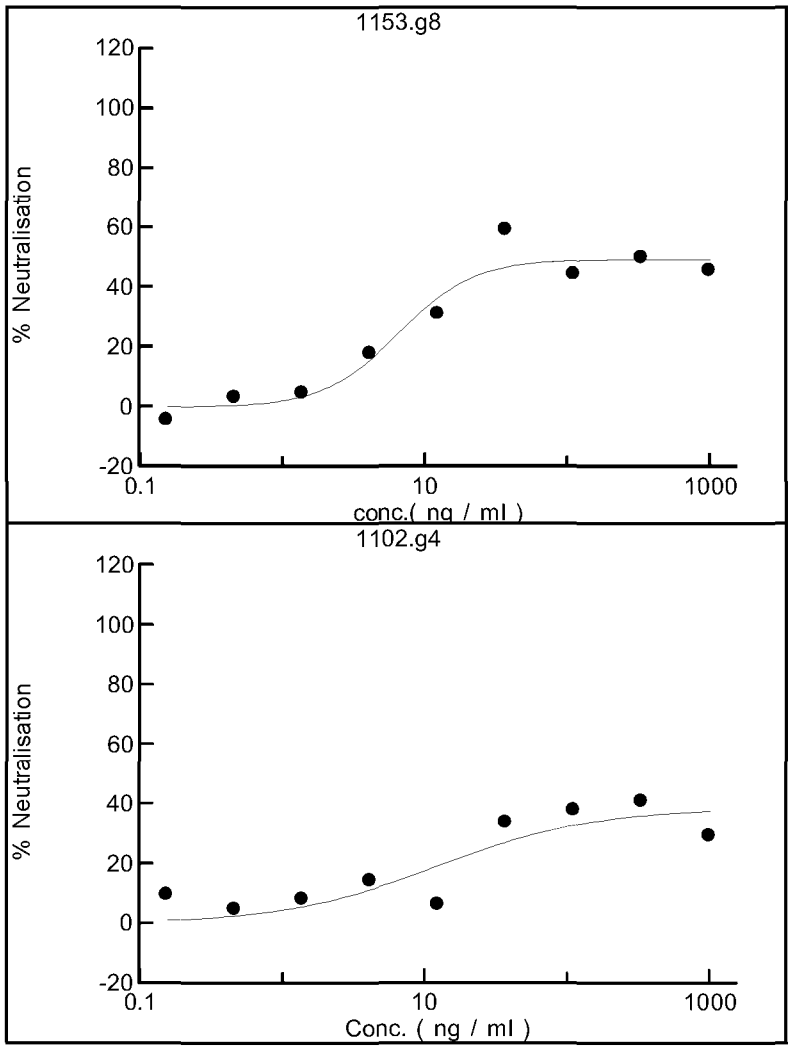


Figure 27 Anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs Y axis neutralization X axis conc ng/ml for combinations of 927+1099, 927+1102, 927+1114 respectively)

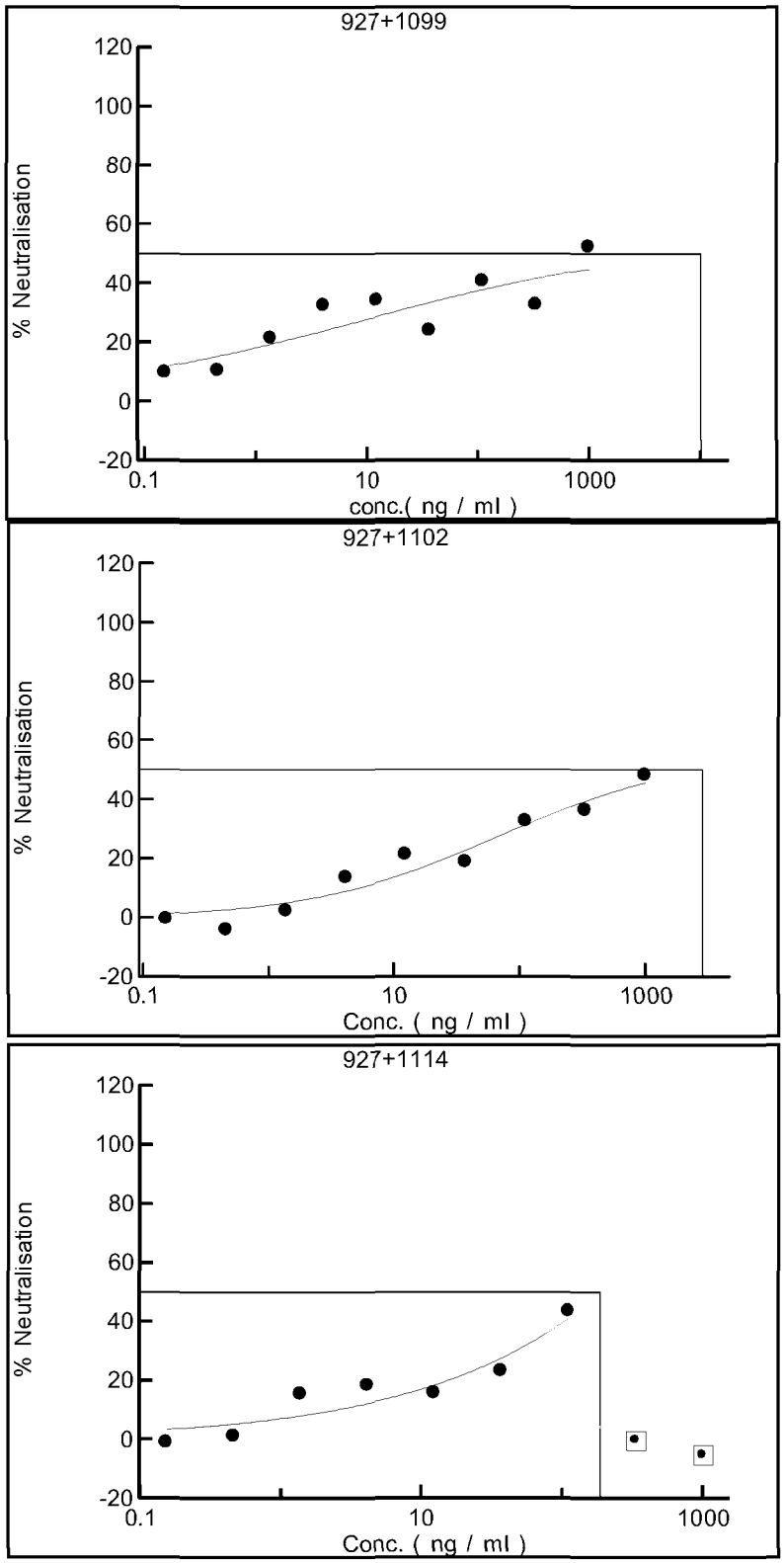
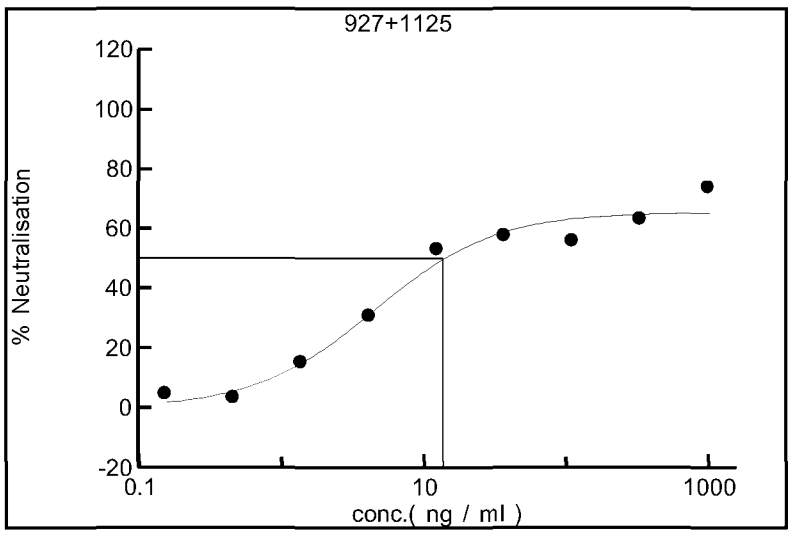
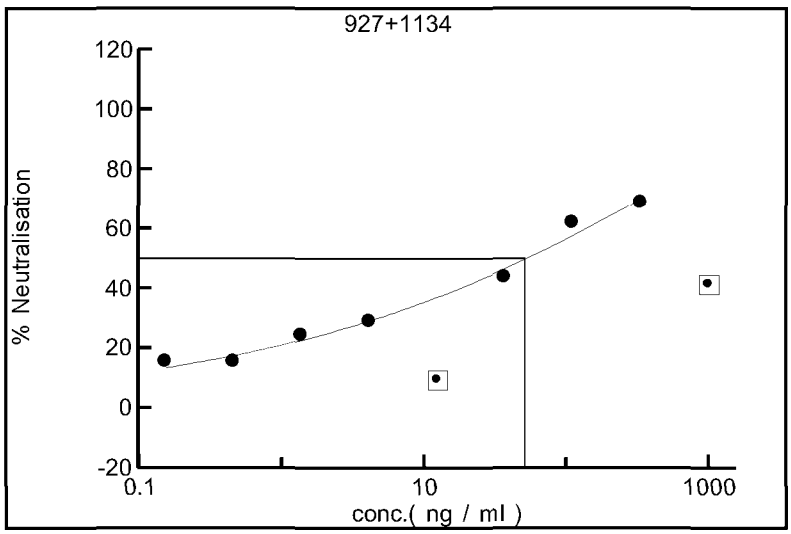


Figure 28 Anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs (Y axis neutralization X axis conc ng/ml for combinations of 927+1125, 927+1134, 1099+1114 respectively)





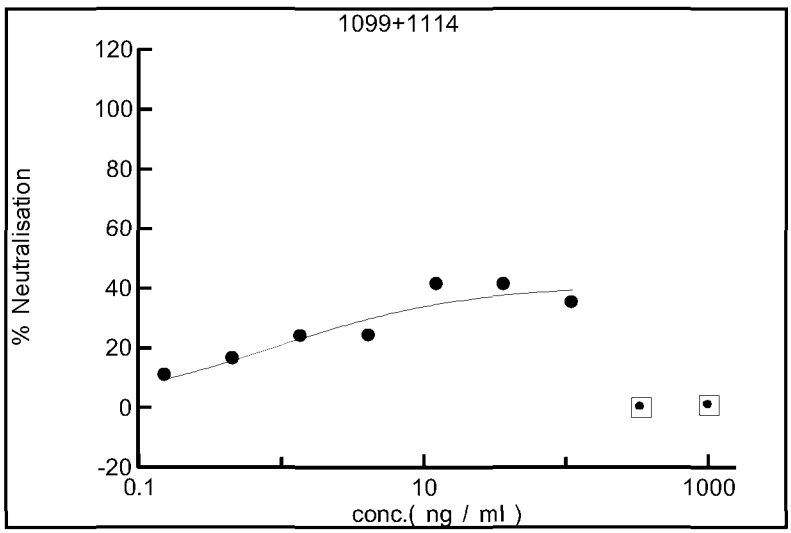


Figure 29 Anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs (Y axis neutralization X axis conc ng/ml for combinations of 1102+1114, 1102+1125, 1114+1134 respectively)

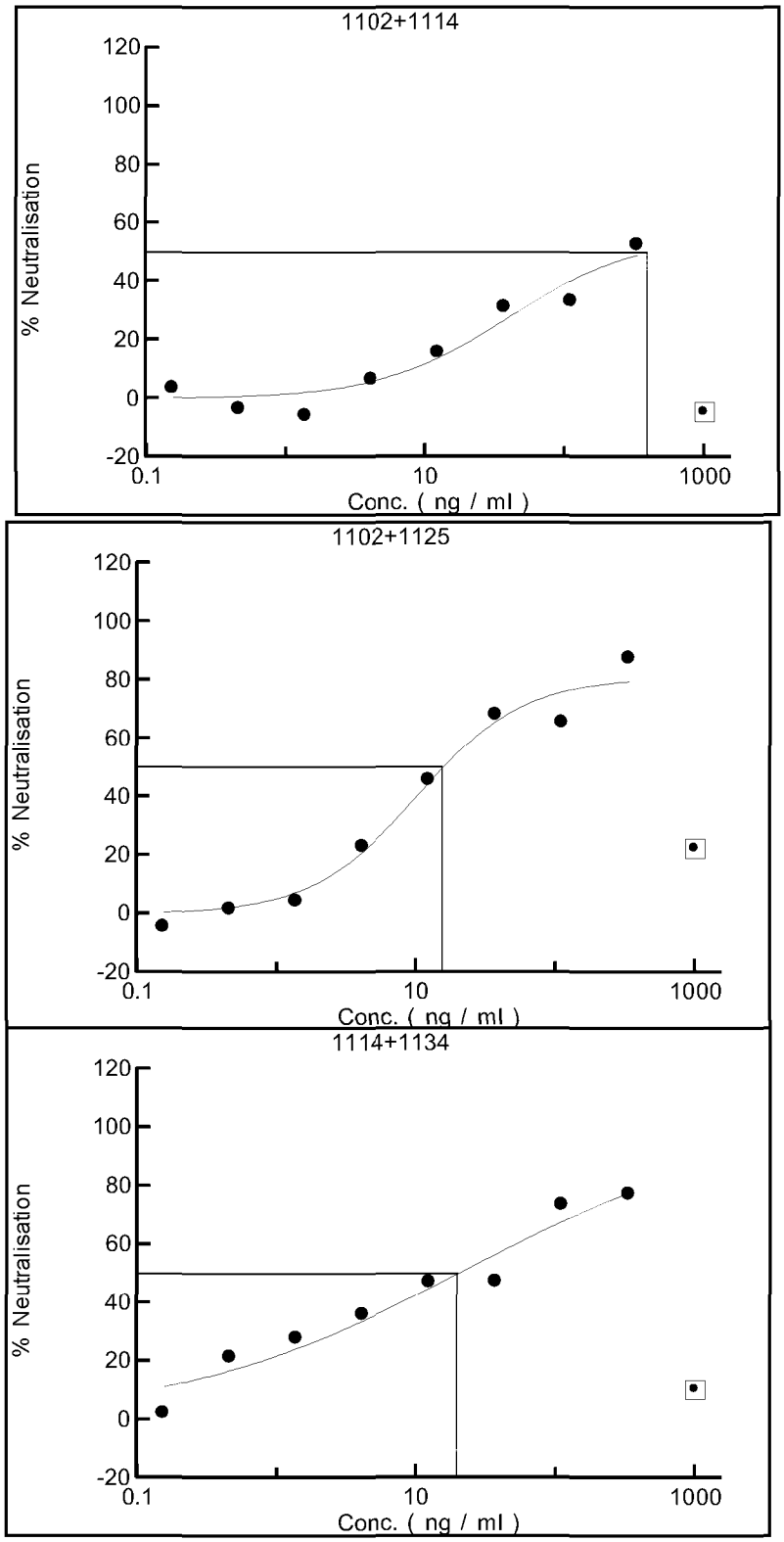


Figure 30 Anti TcdB (Ribotype 003) in-vitro neutralization data for paired Mabs (Y axis neutralization X axis conc ng/ml for combinations of 1114+1151, 1114+1153, 1125+1134 respectively)

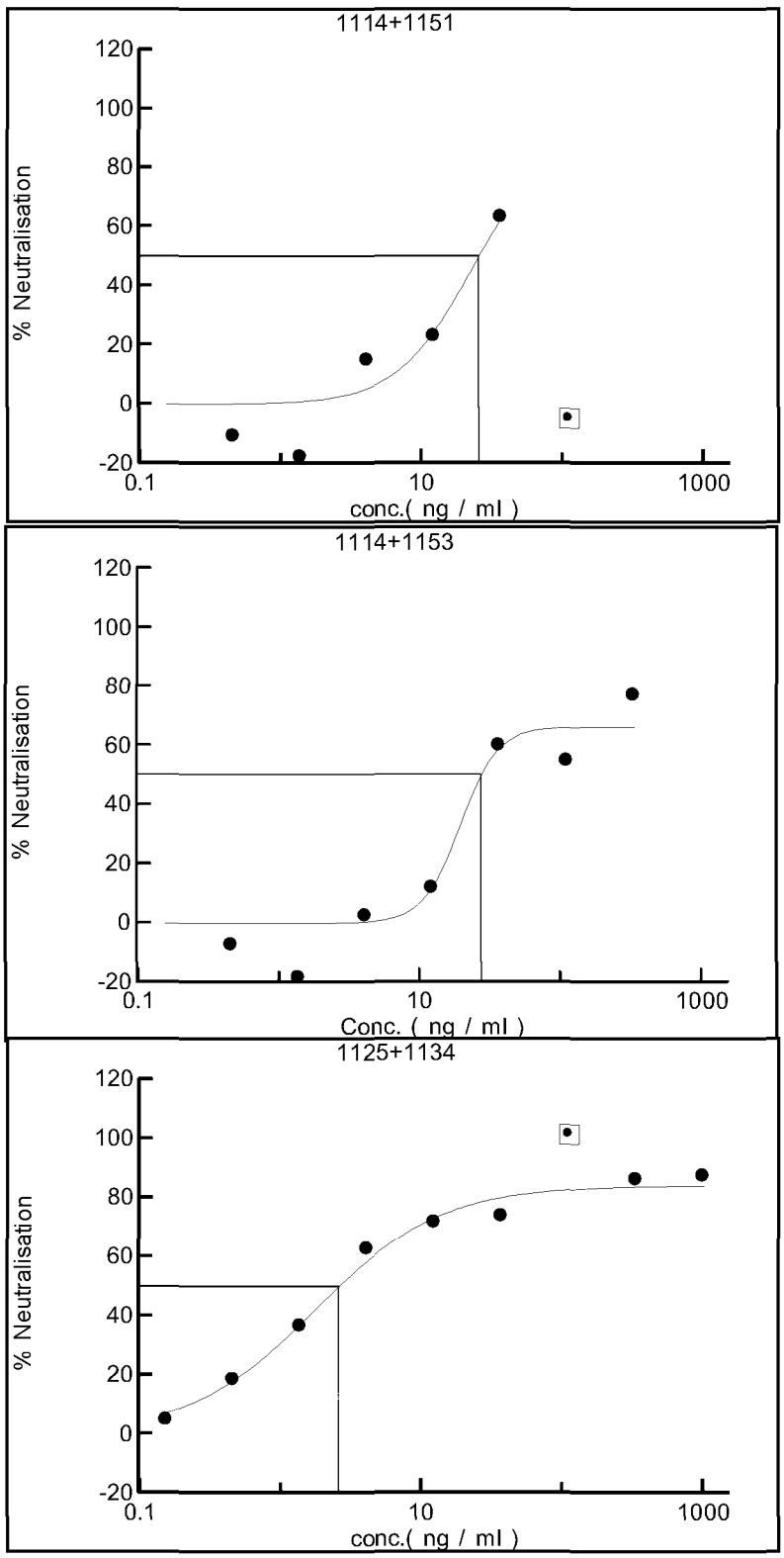


Figure 31 Anti TcdB (Ribotype 003) in-vitro neutralization data for three Mab mixtures (Y axis neutralization X axis conc ng/ml for combinations of 1125+1134+1114, 1125+1134+927, 1125+1151+*1114* respectively)

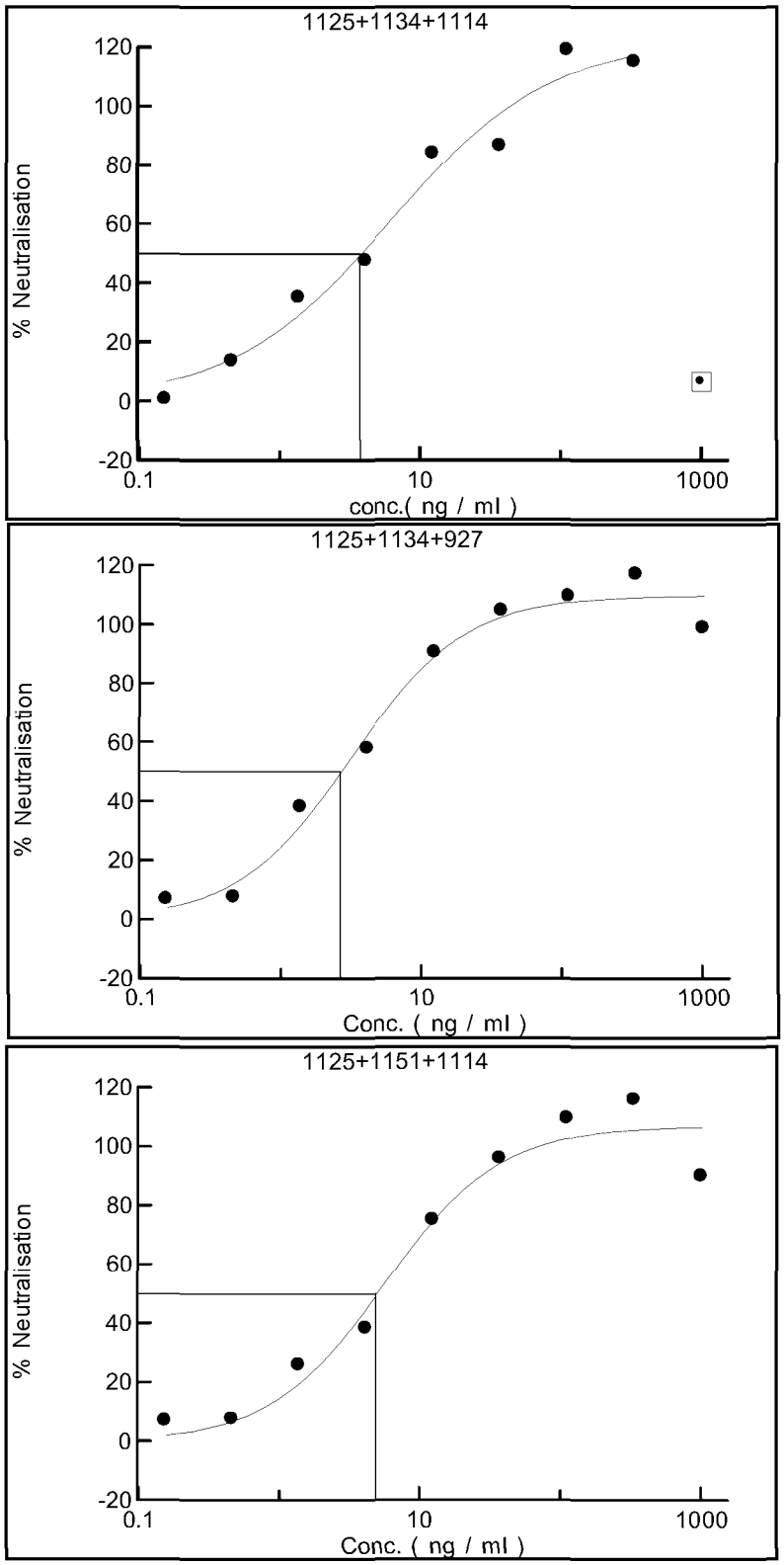


Figure 32 Anti TcdB (Ribotype 003) in-vitro neutralization data for three Mab mixtures (Y axis neutralization X axis conc ng/ml for 1125.+1151+927, 1125.g2+1134.g5+927.g2 respectively)

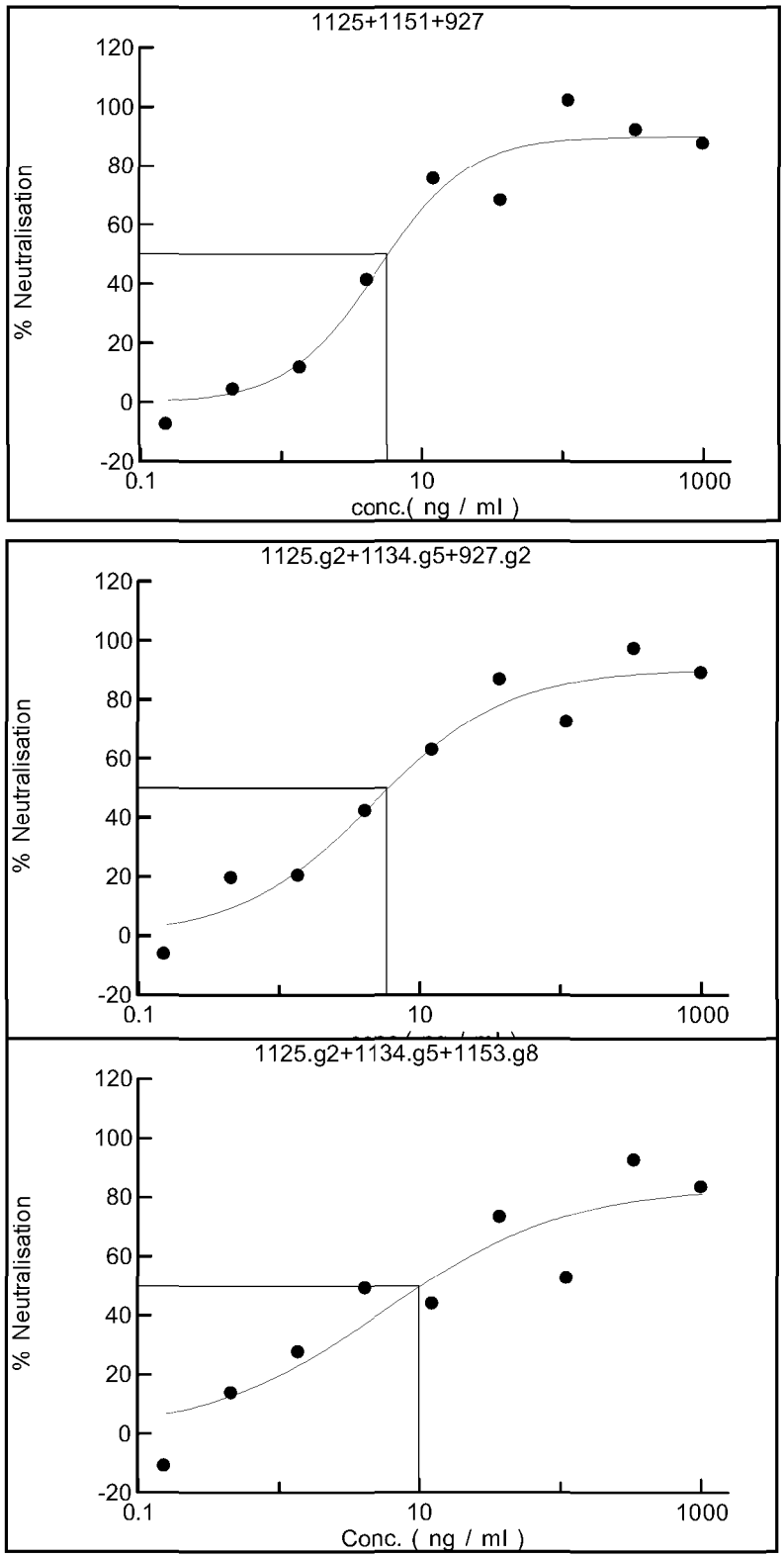


Figure 33 Anti TcdB (Ribotype 003) in-vitro neutralization data for three Mab mixtures

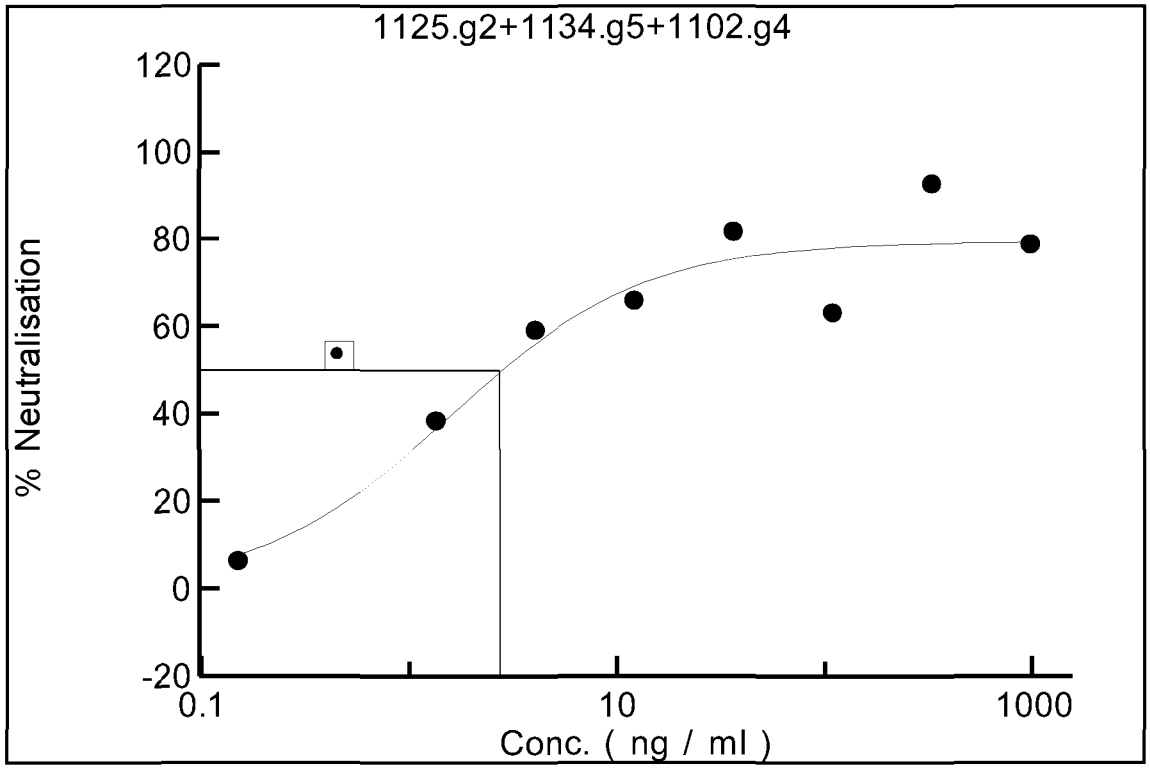


Figure 34 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

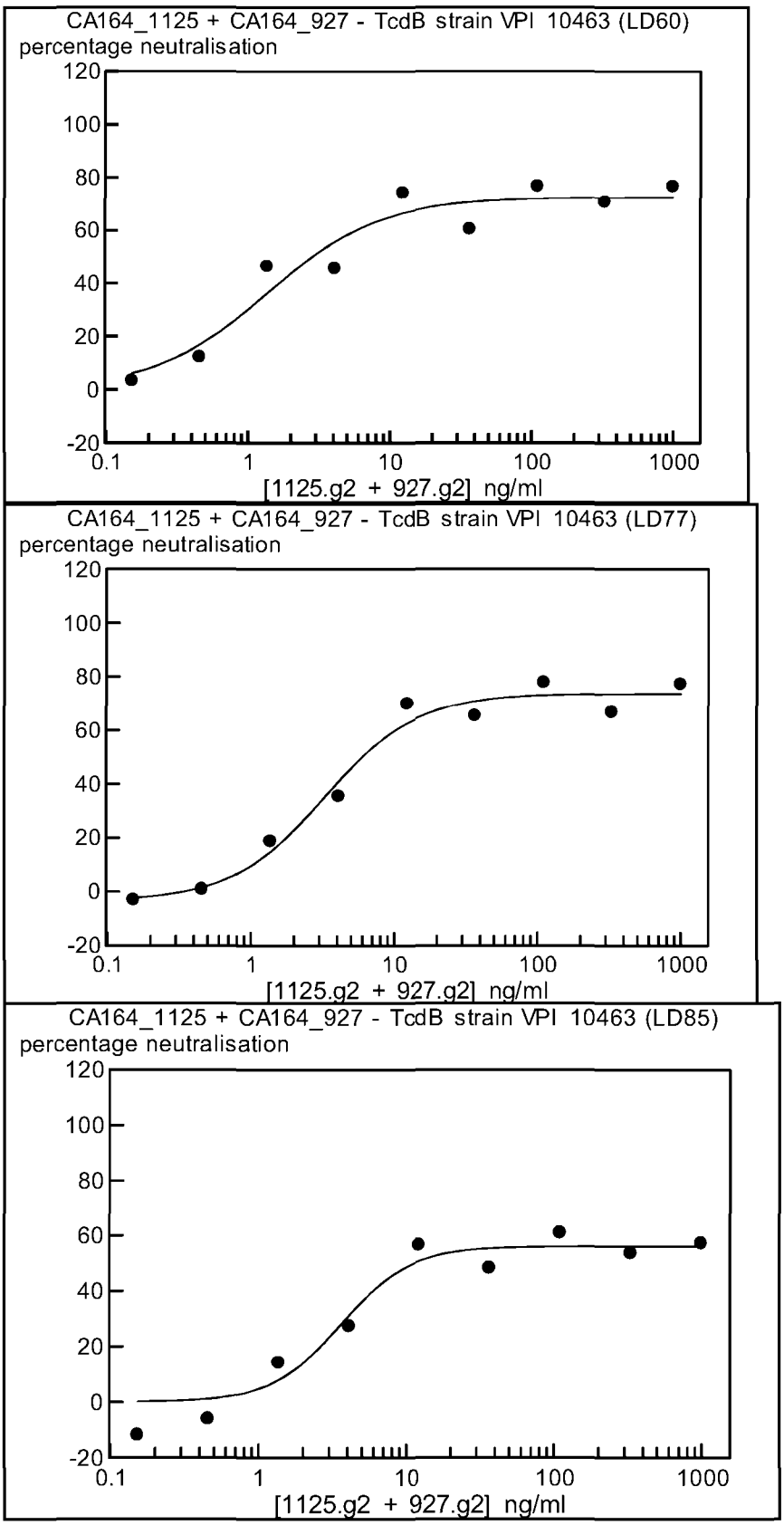


Figure 35 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

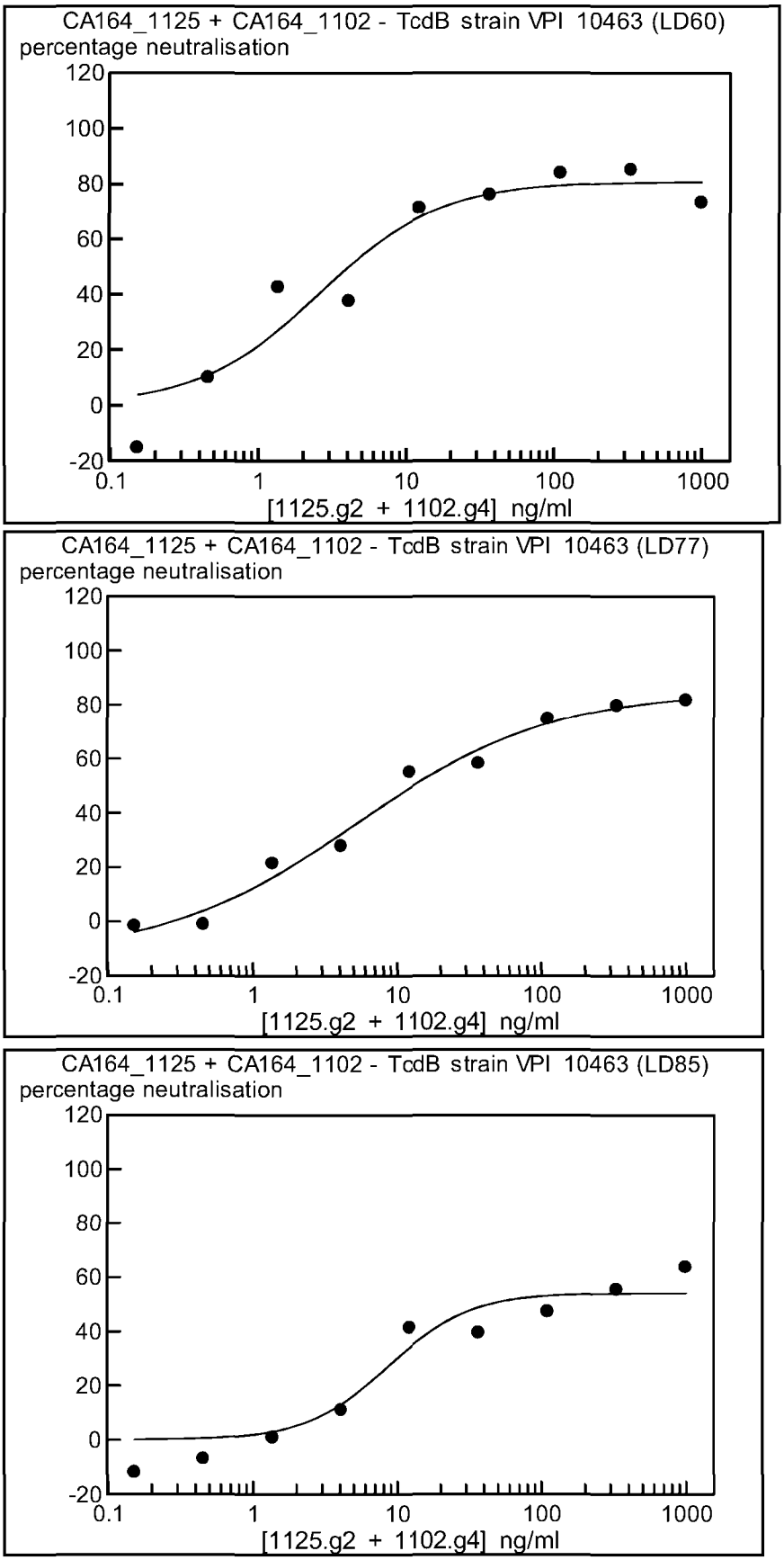
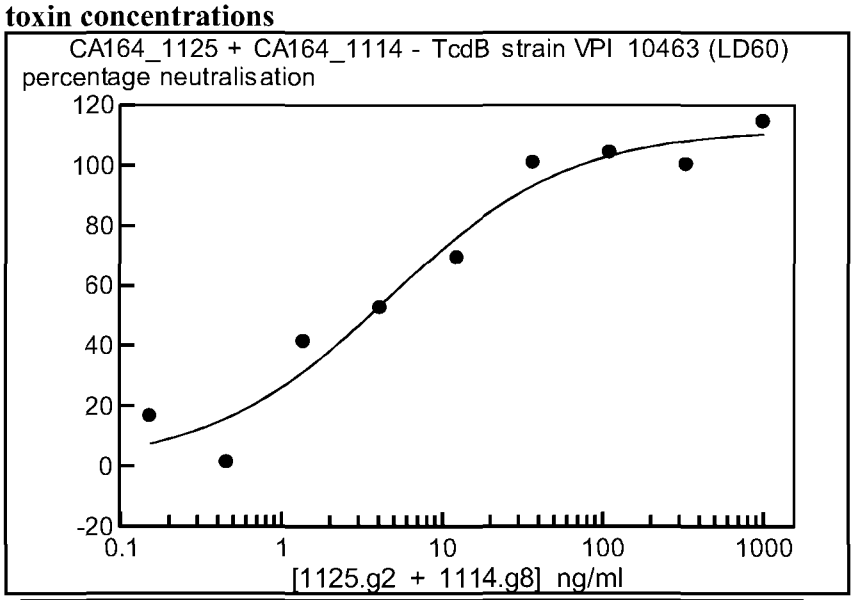
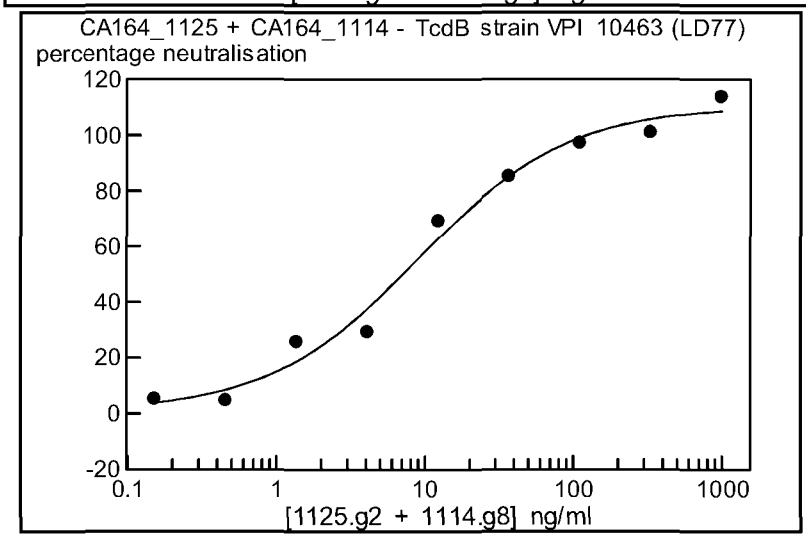


Figure 36 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations





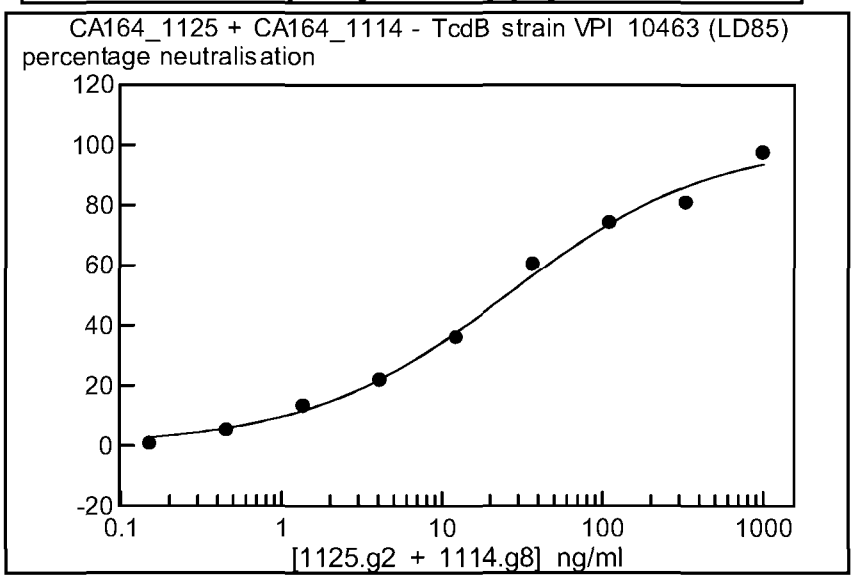


Figure 37 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

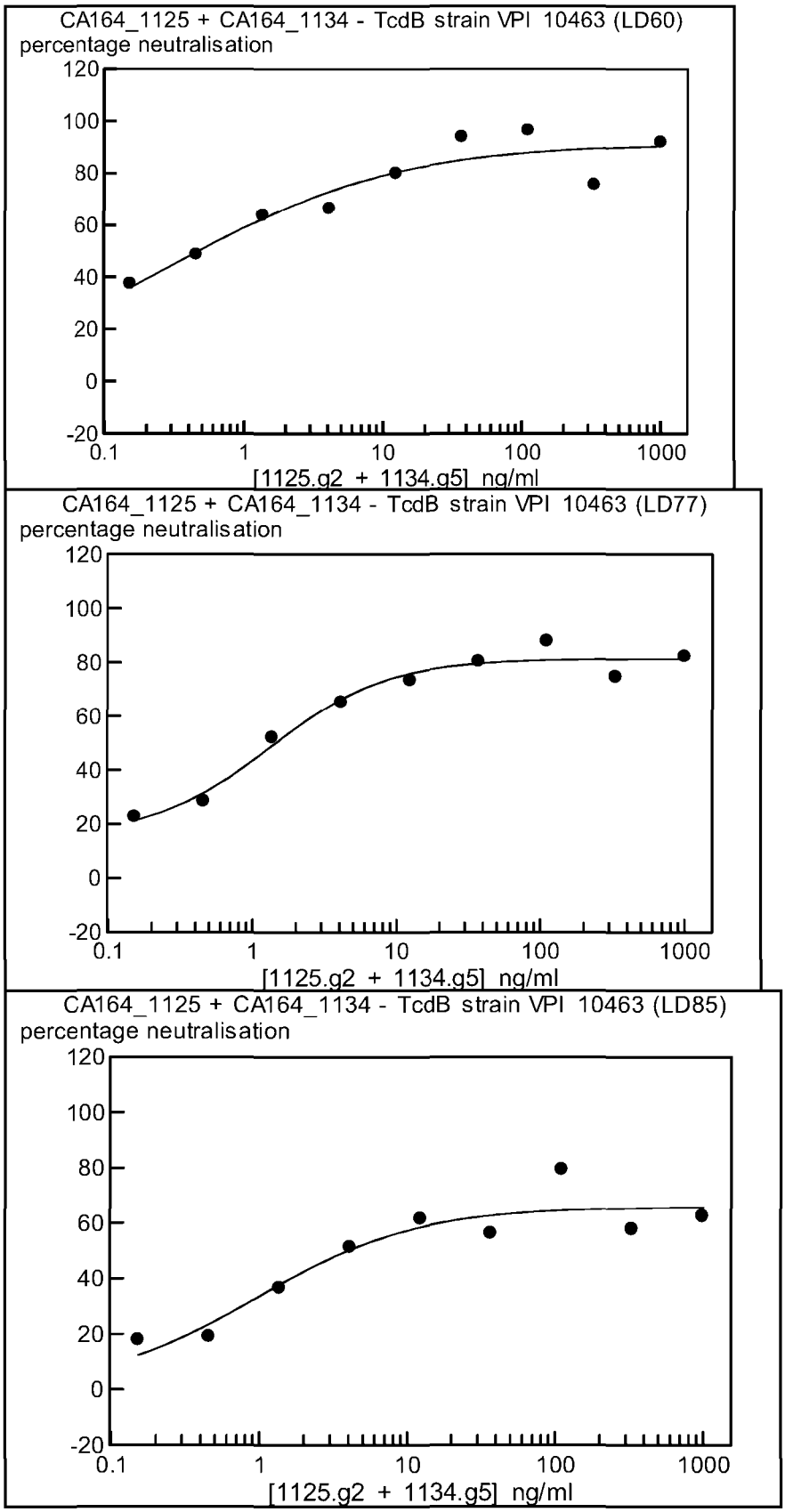
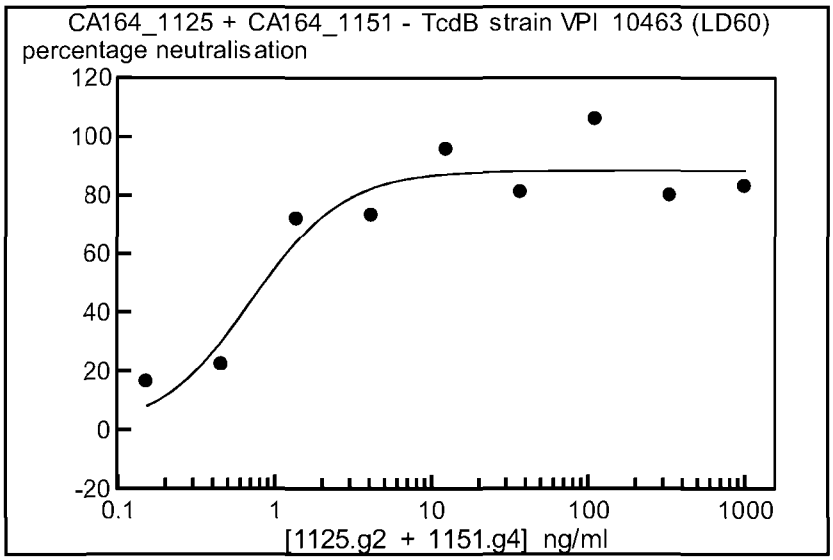
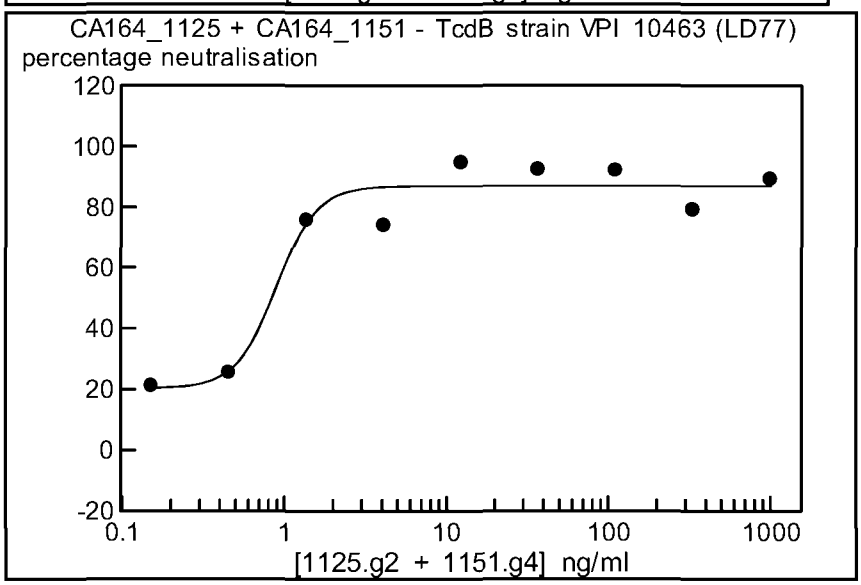


Figure 38 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations





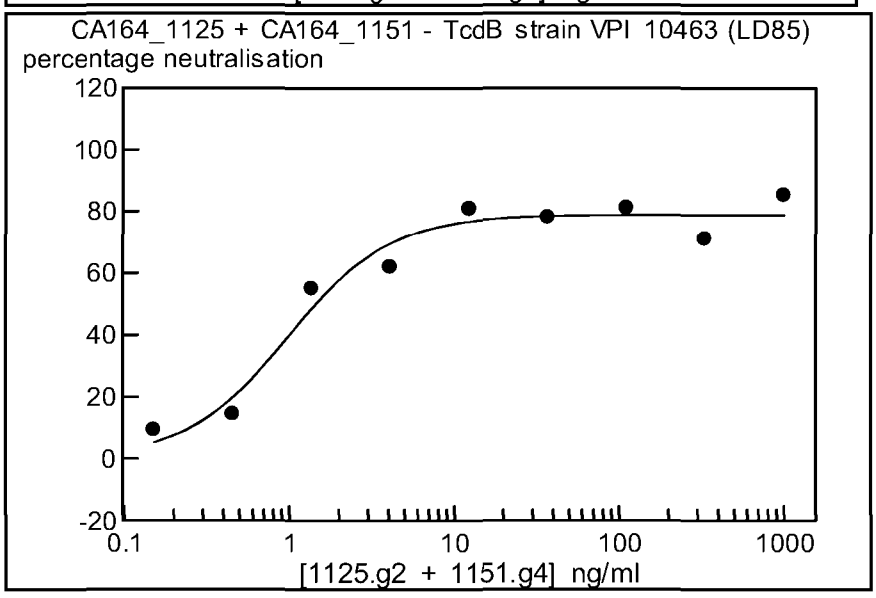


Figure 39 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

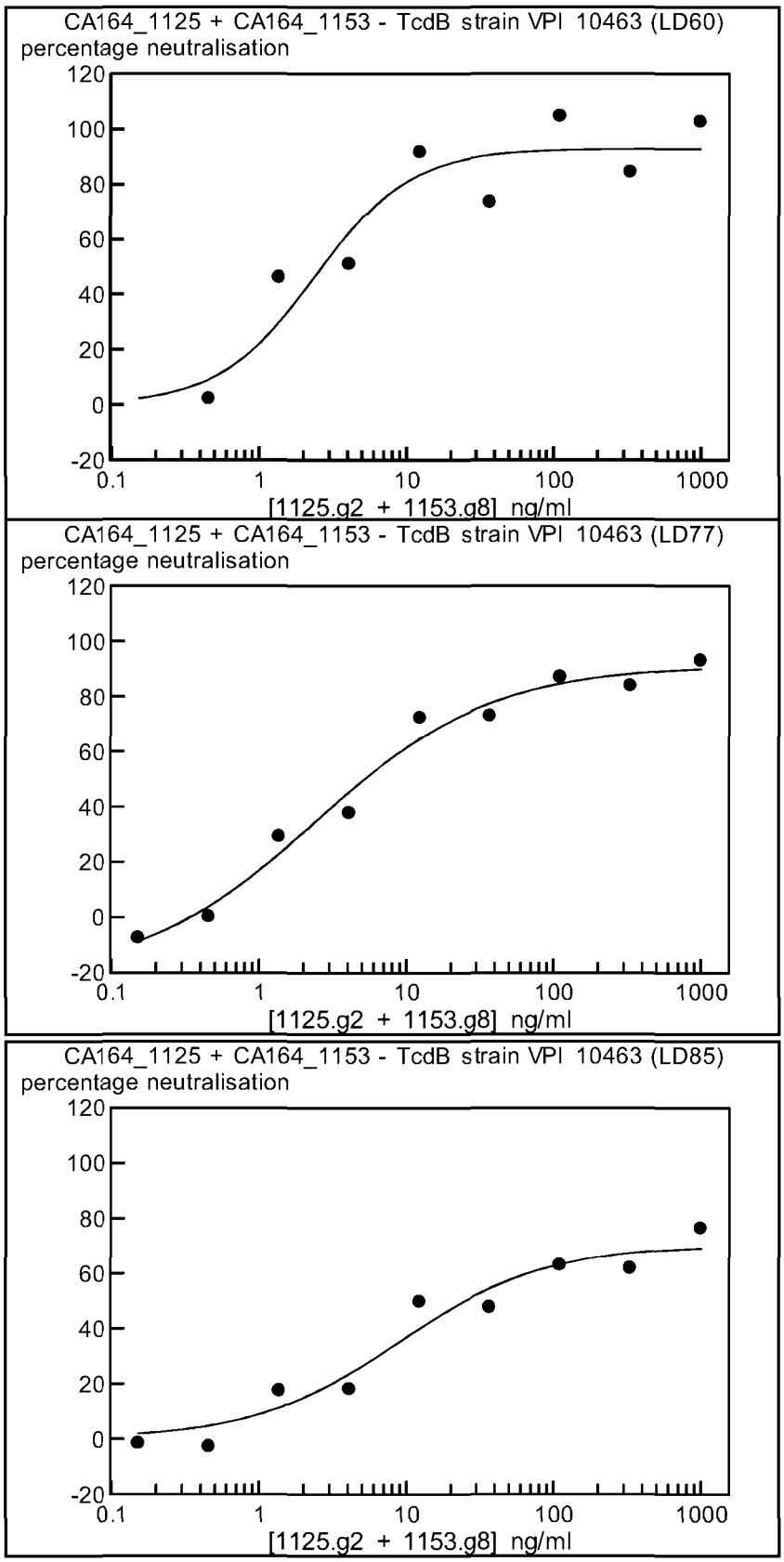


Figure 40 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different toxin concentrations

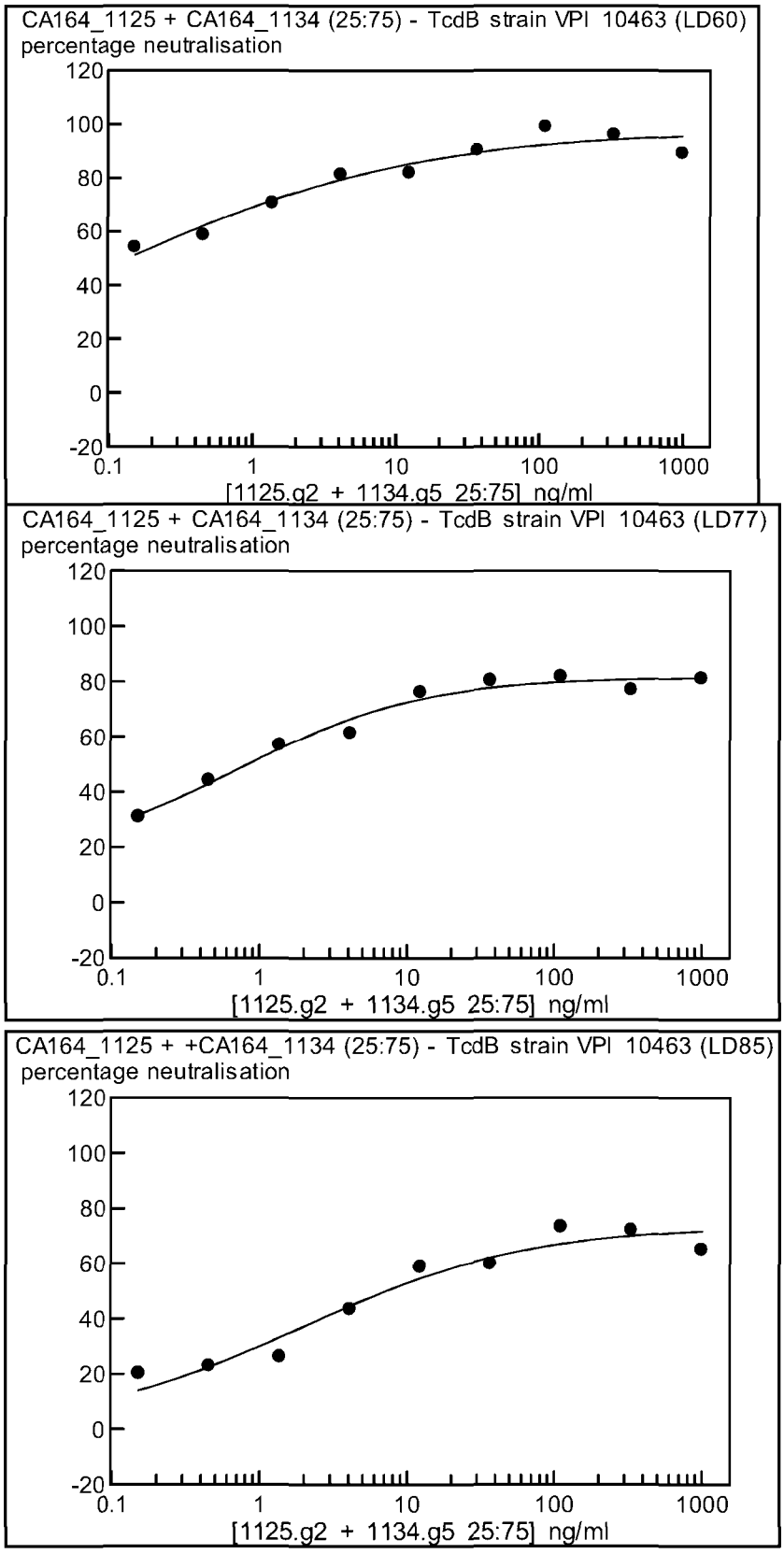


Figure 41 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations

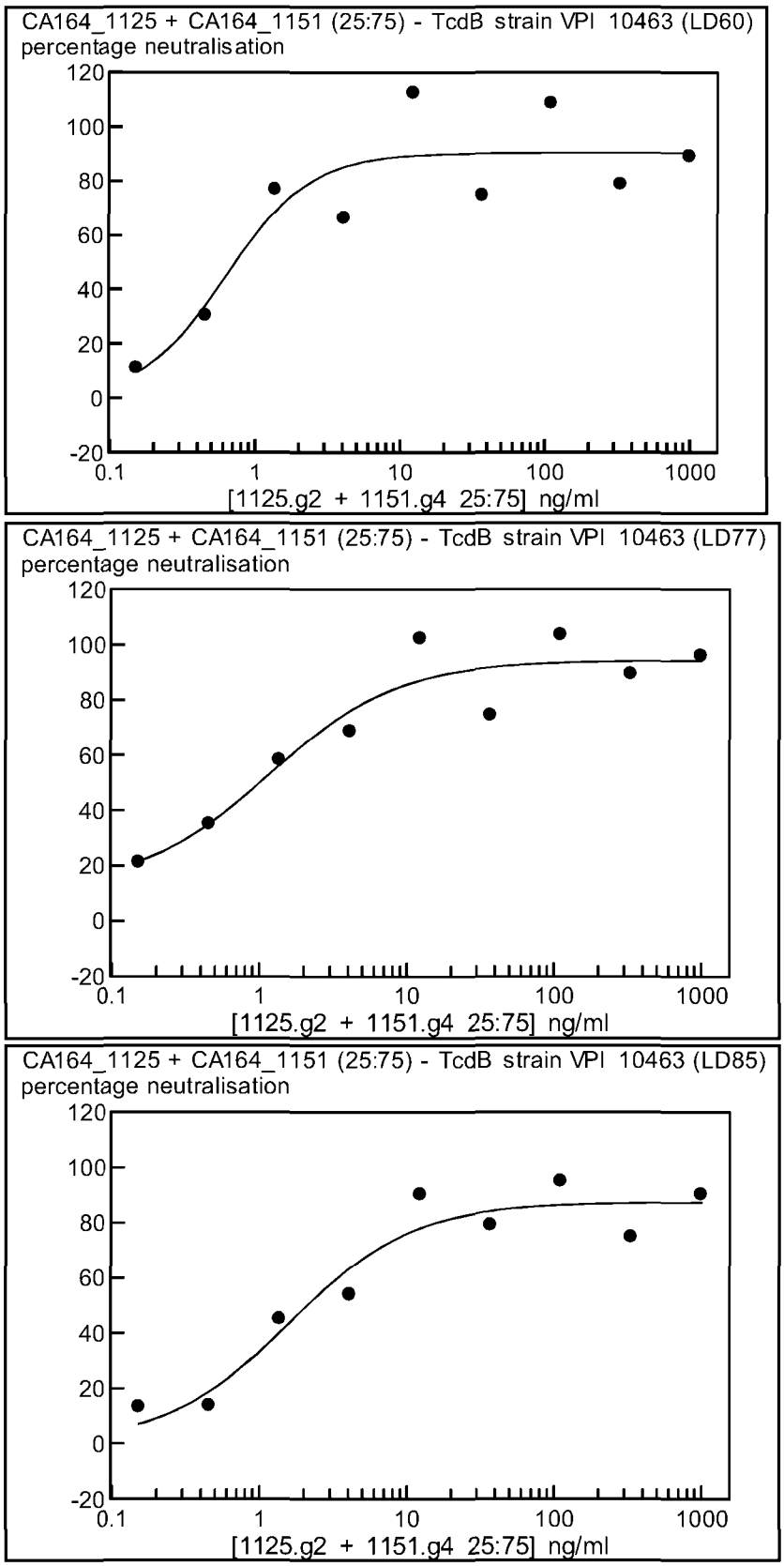
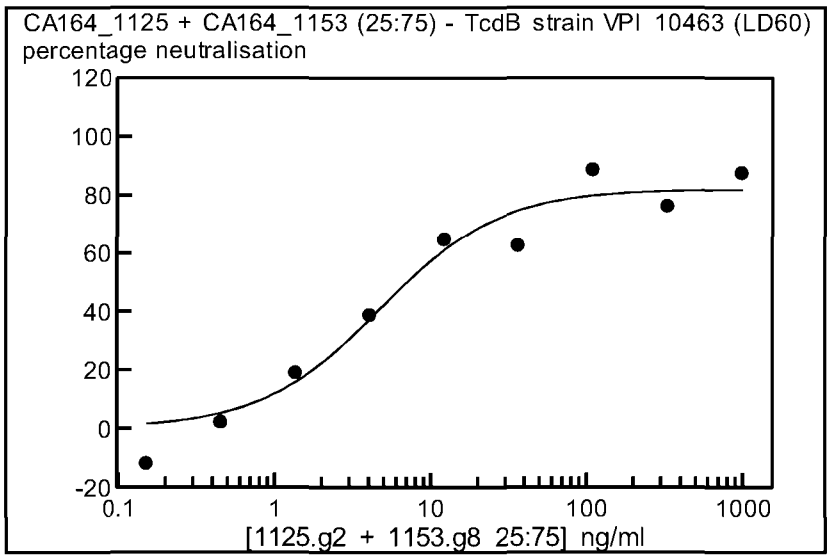
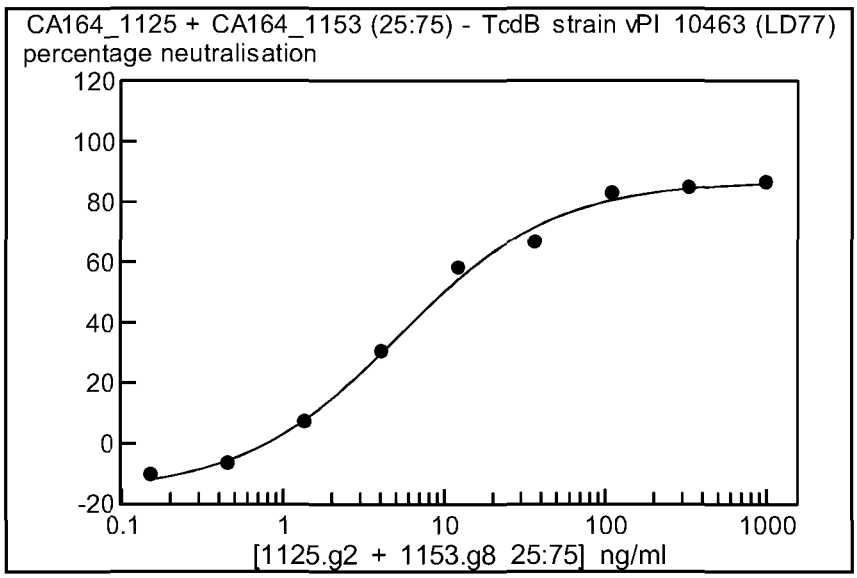


Figure 42 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations





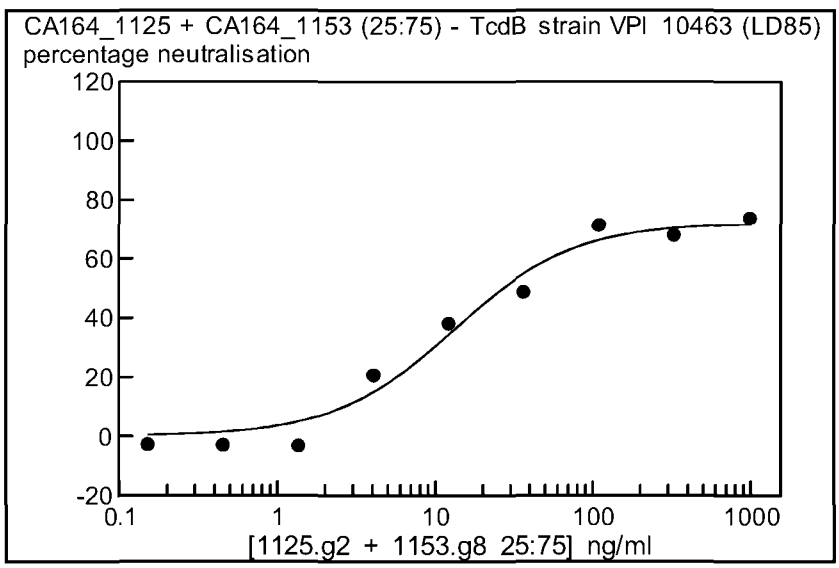


Figure 43 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations

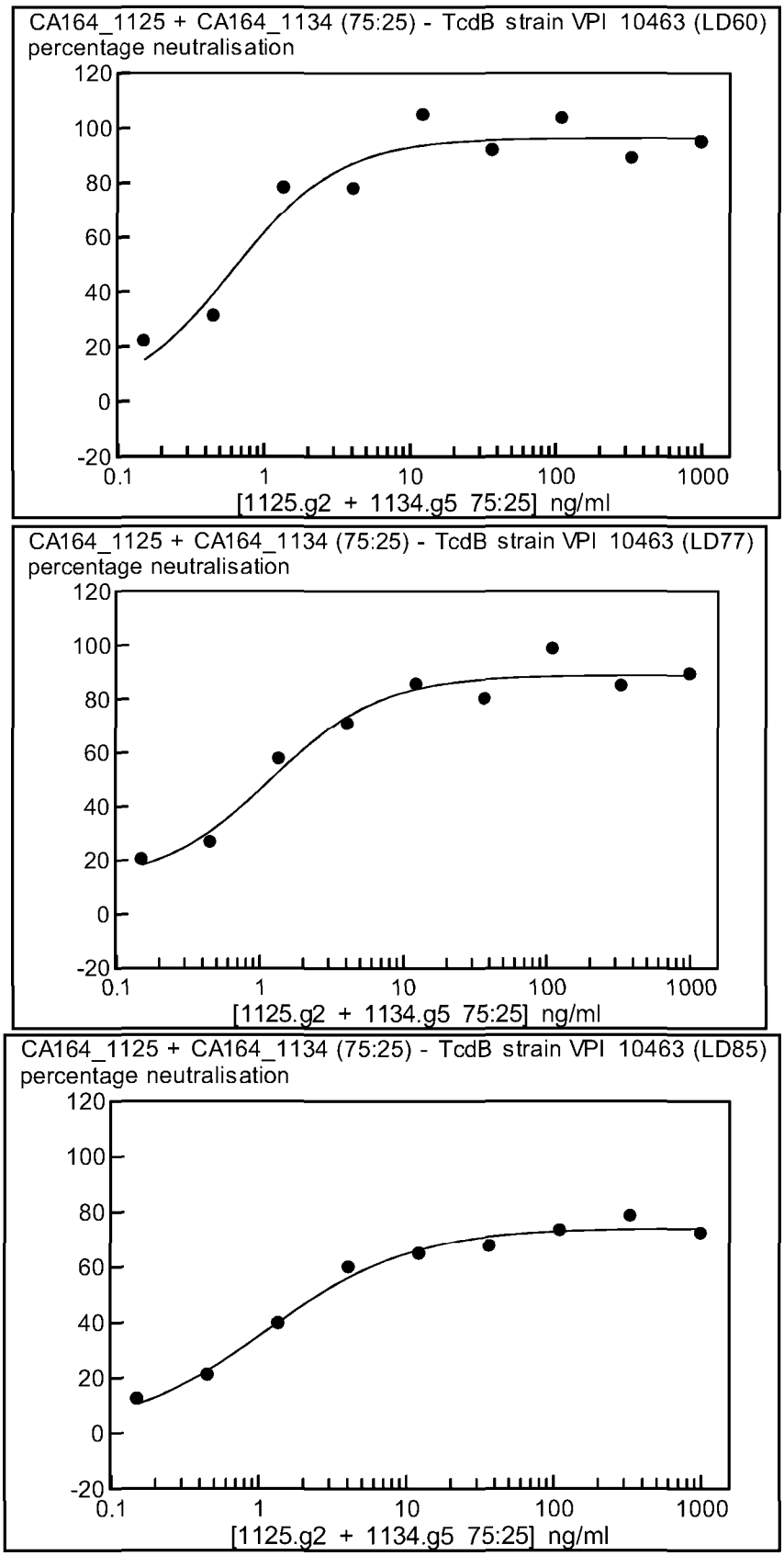
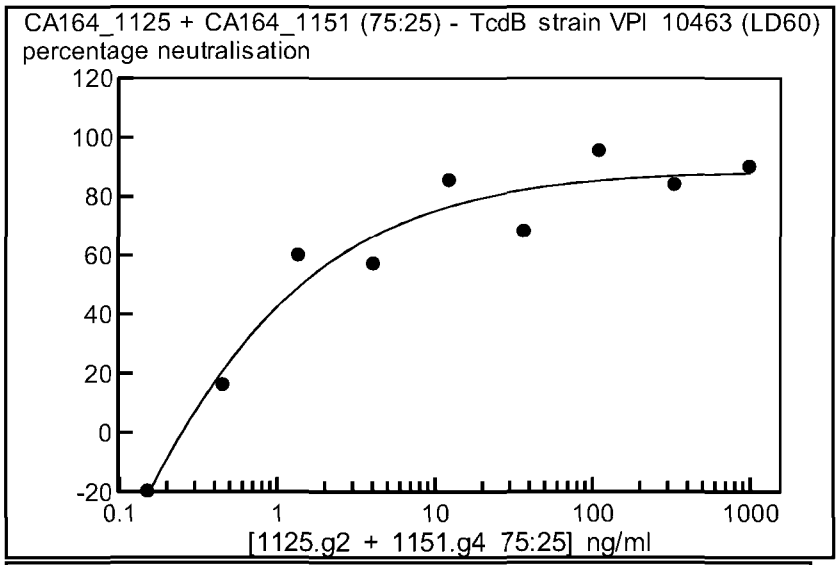
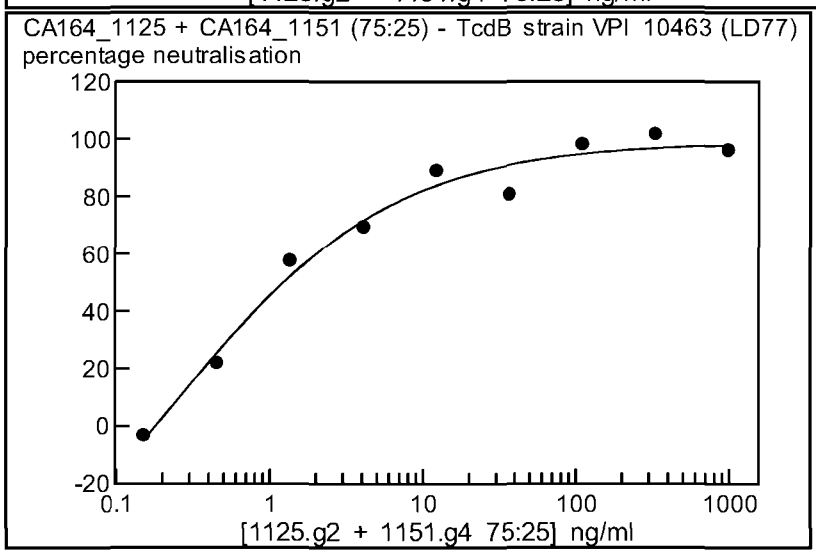


Figure 44 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations





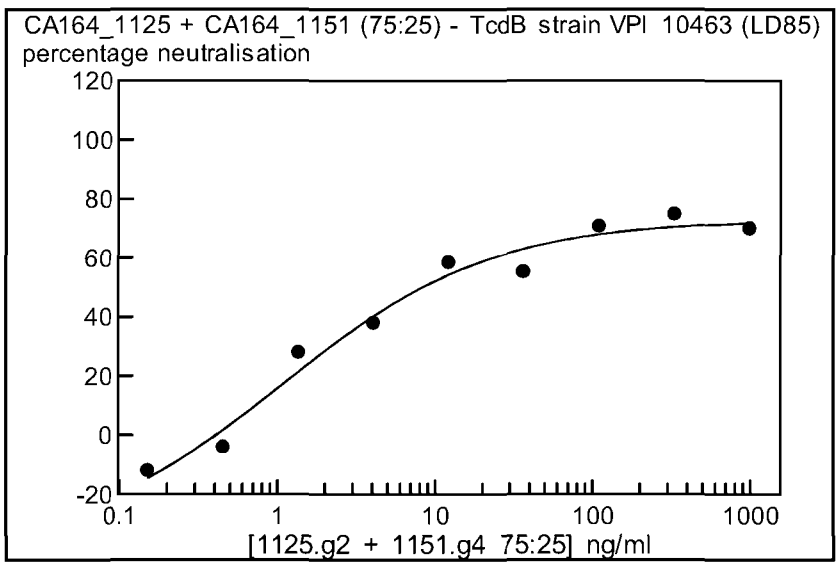


Figure 45 Anti TcdB (Ribotype 003) in-vitro neutralization data for two Mab mixtures at different relative Mab ratios and different toxin concentrations

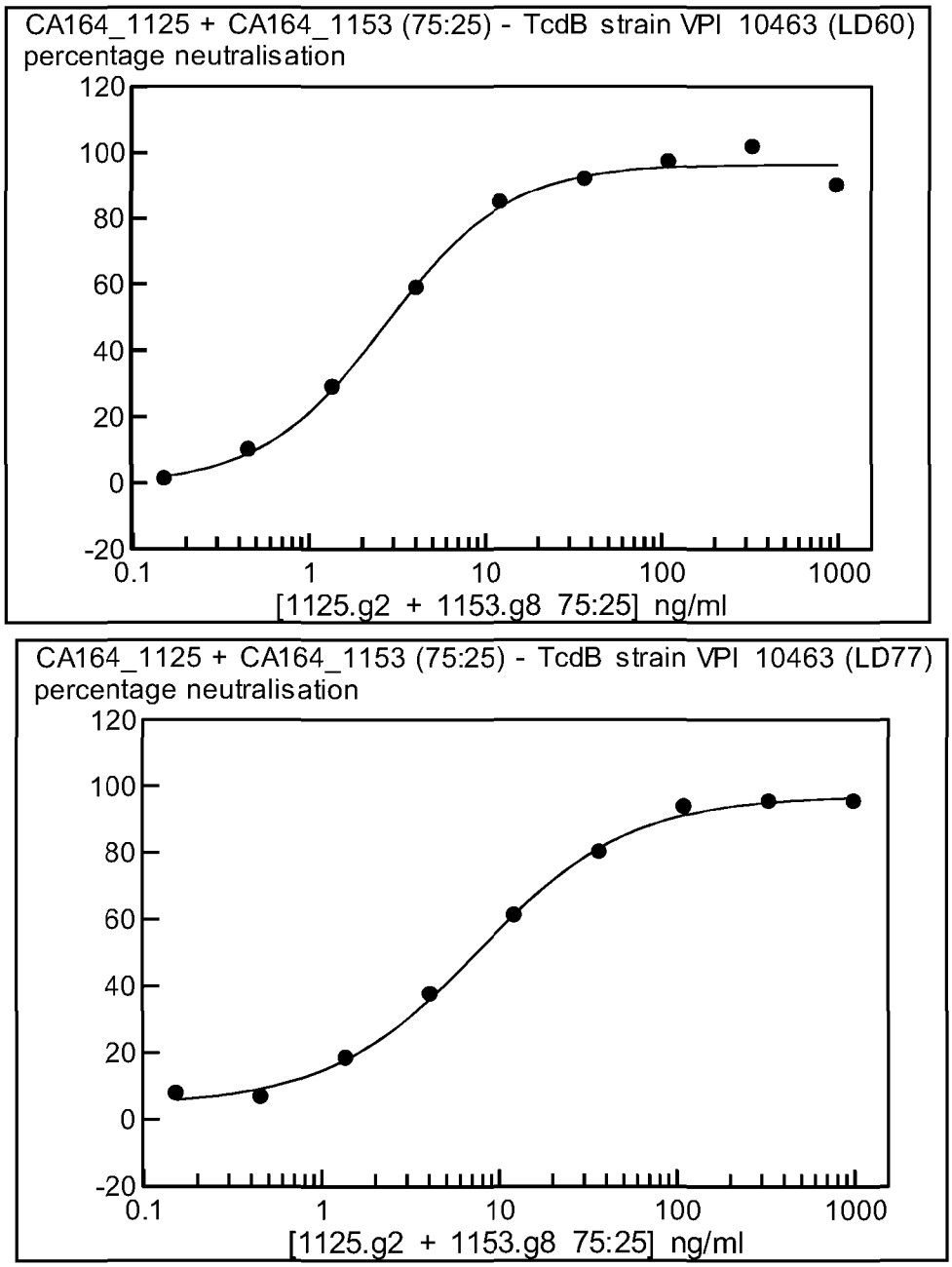


Figure 46 TcdB strain VPI 10463 neutralisation, Antibody singles and pairs, Constant toxin dose (LD80)

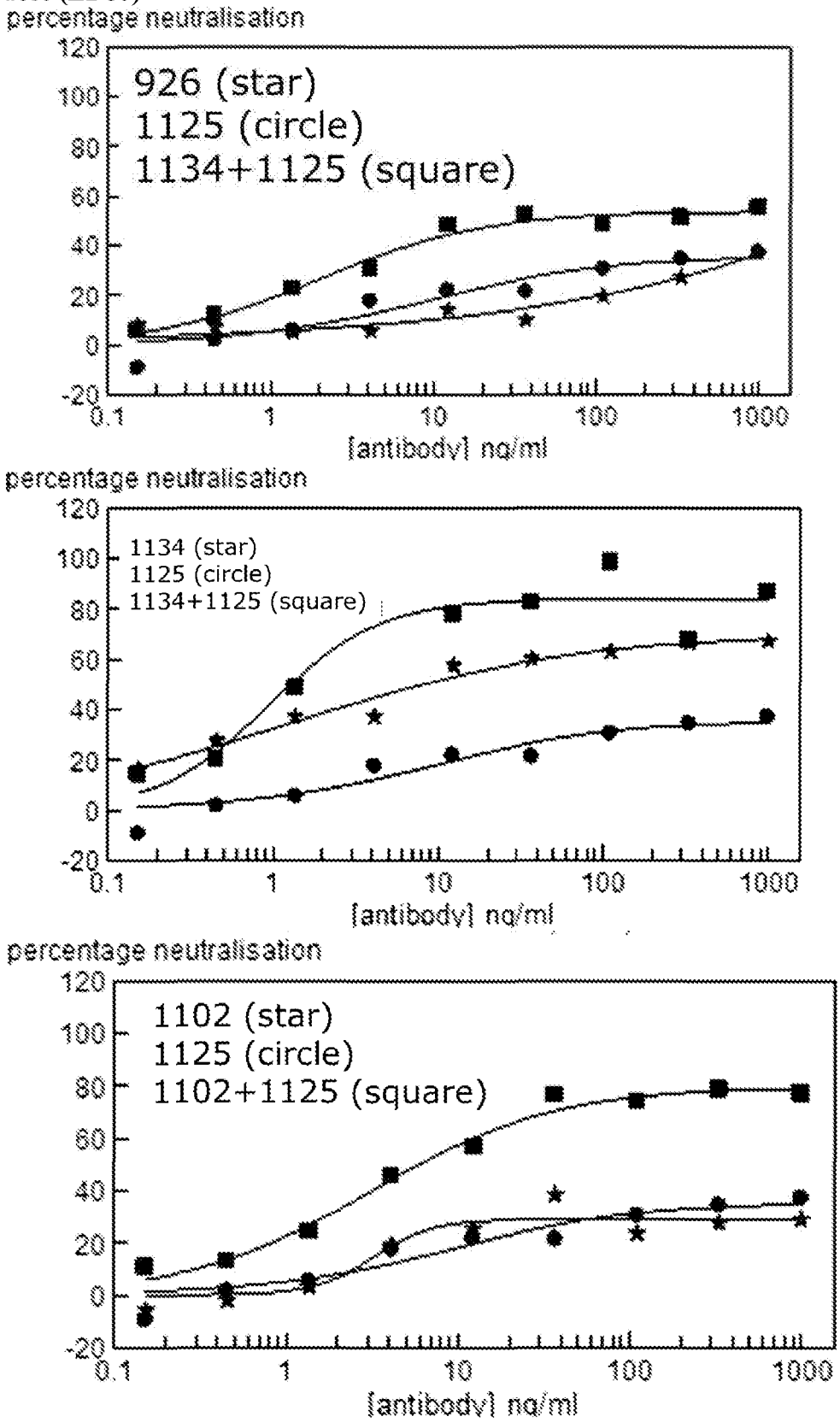


Figure 47 TcdB neutralisation, Antibody singles and pairs, Constant toxin dose (LD80)

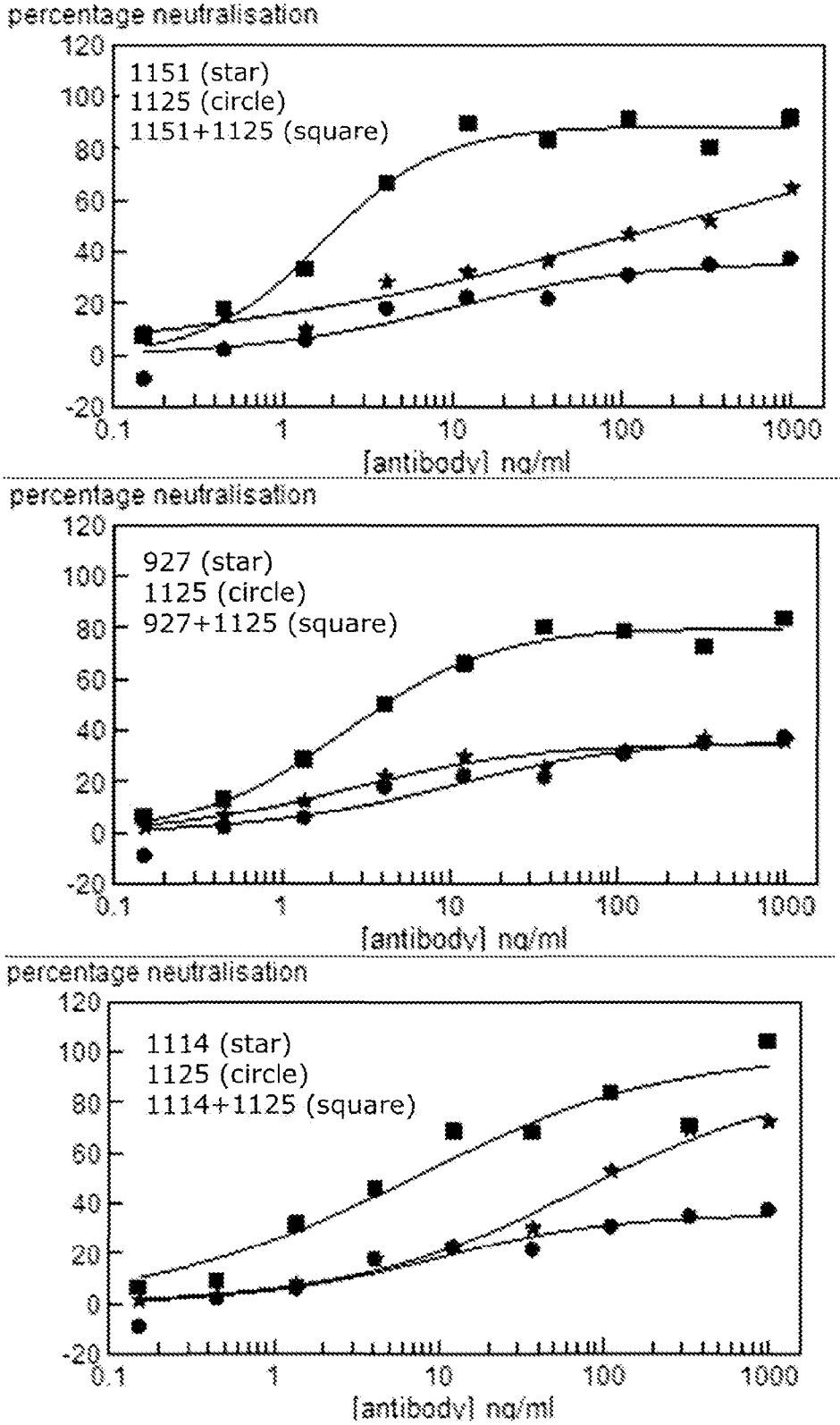


Figure 48 TcdB neutralisation, Antibody singles and pairs, Constant toxin dose (LD80)

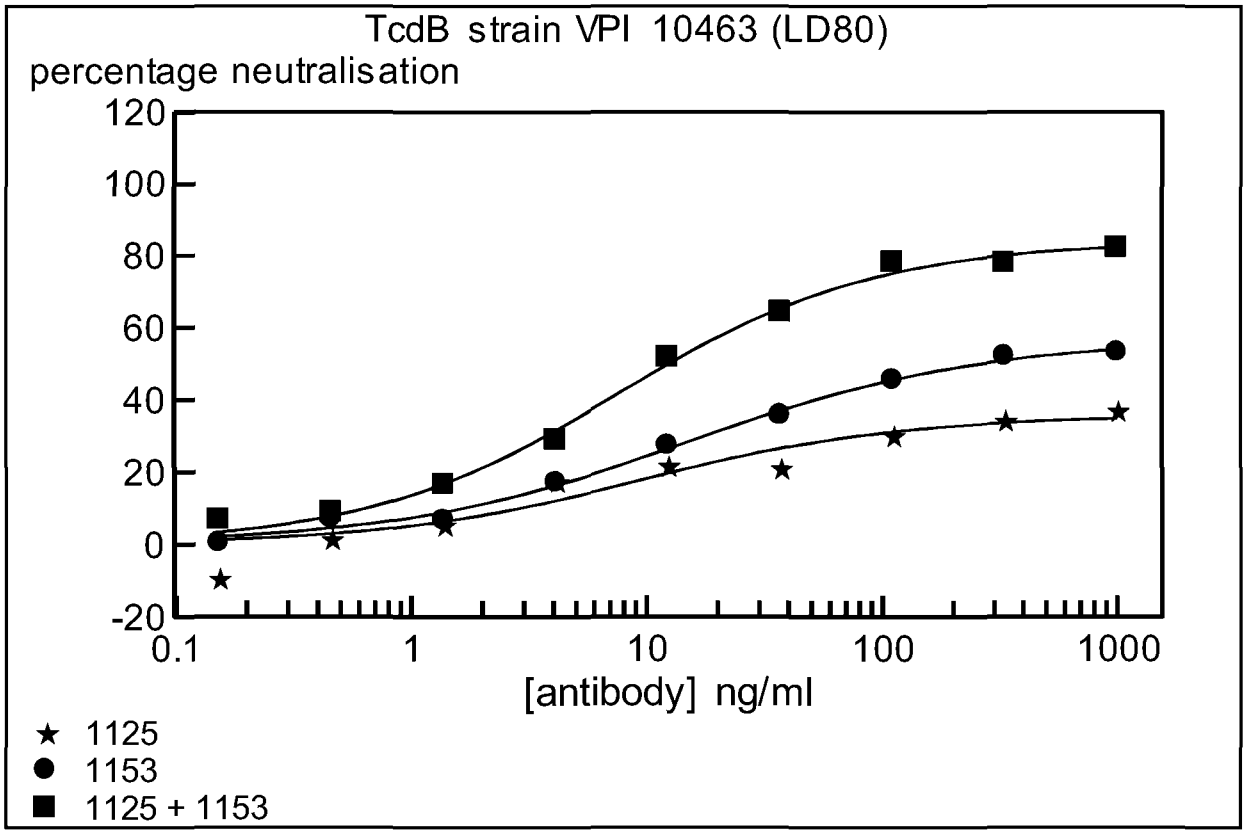


Figure 49 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

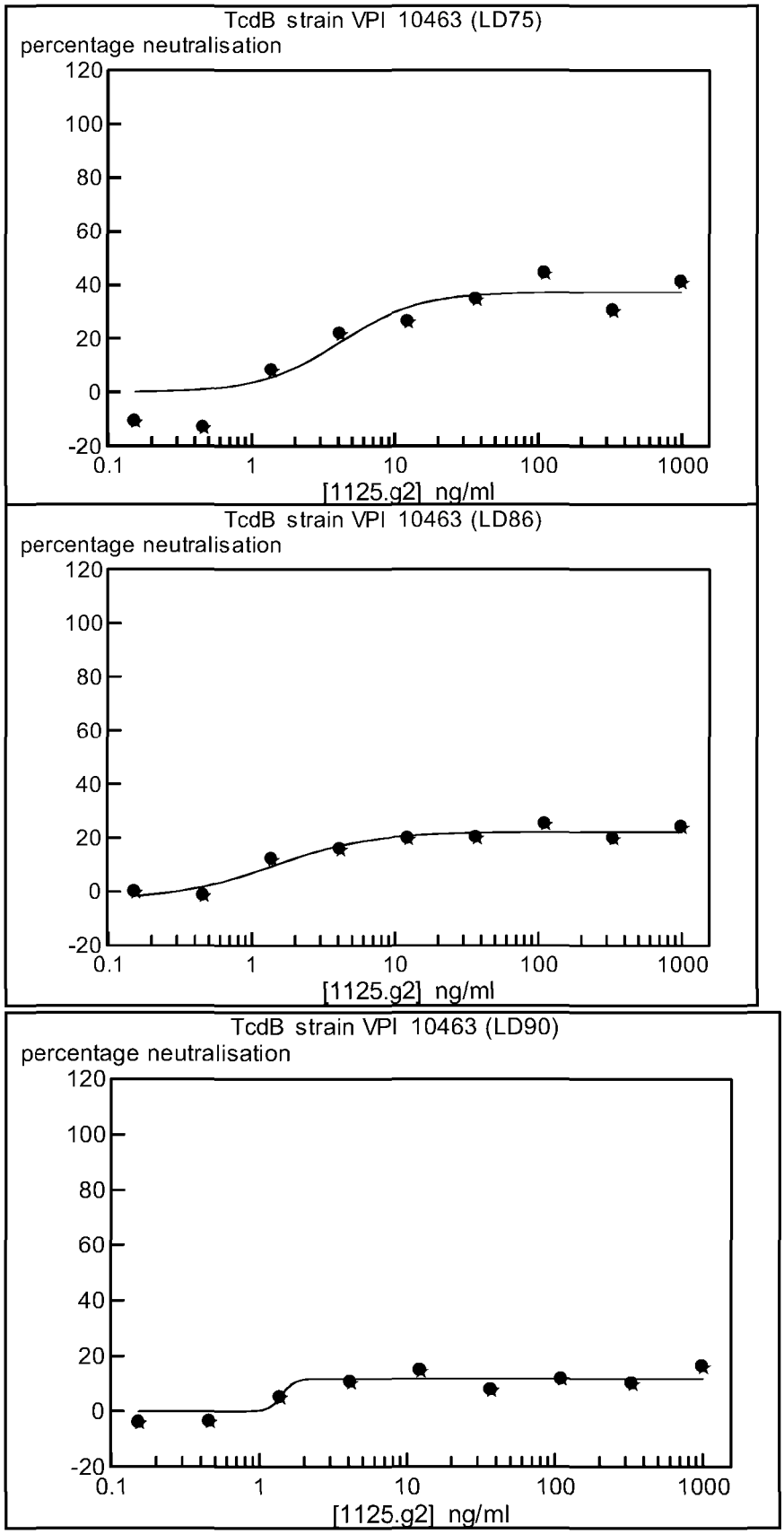


Figure 50 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

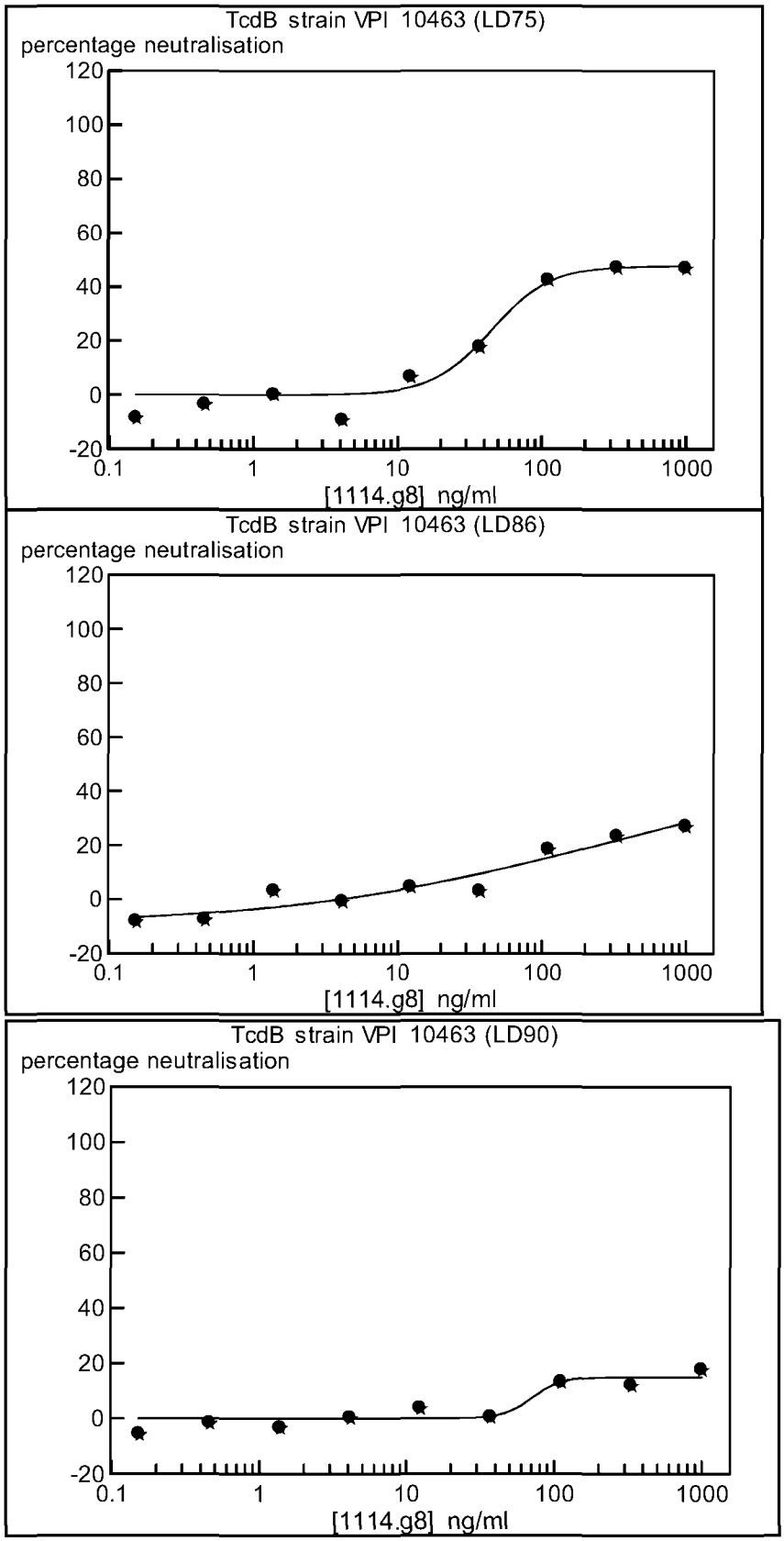


Figure 51 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

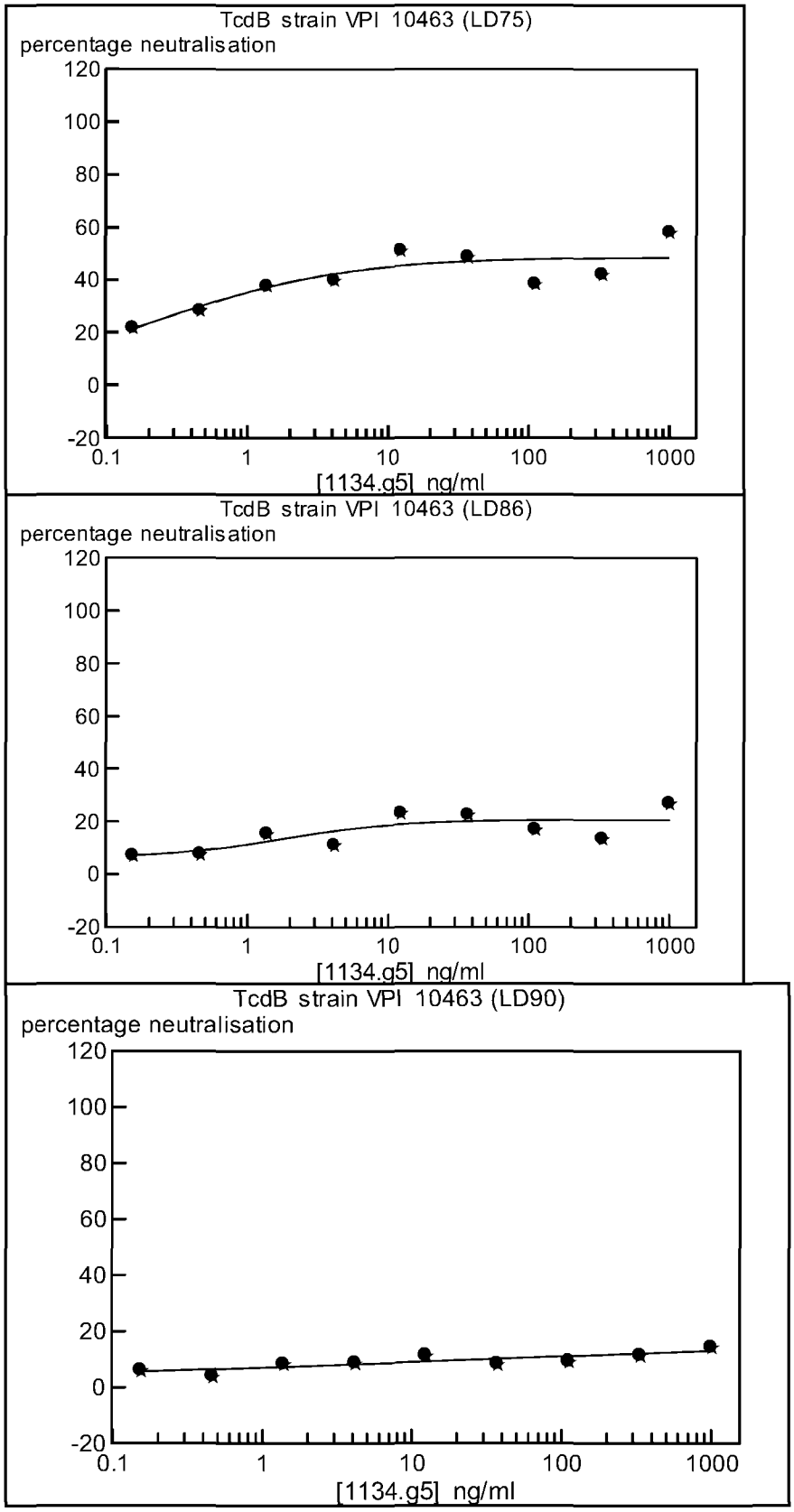


Figure 52 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

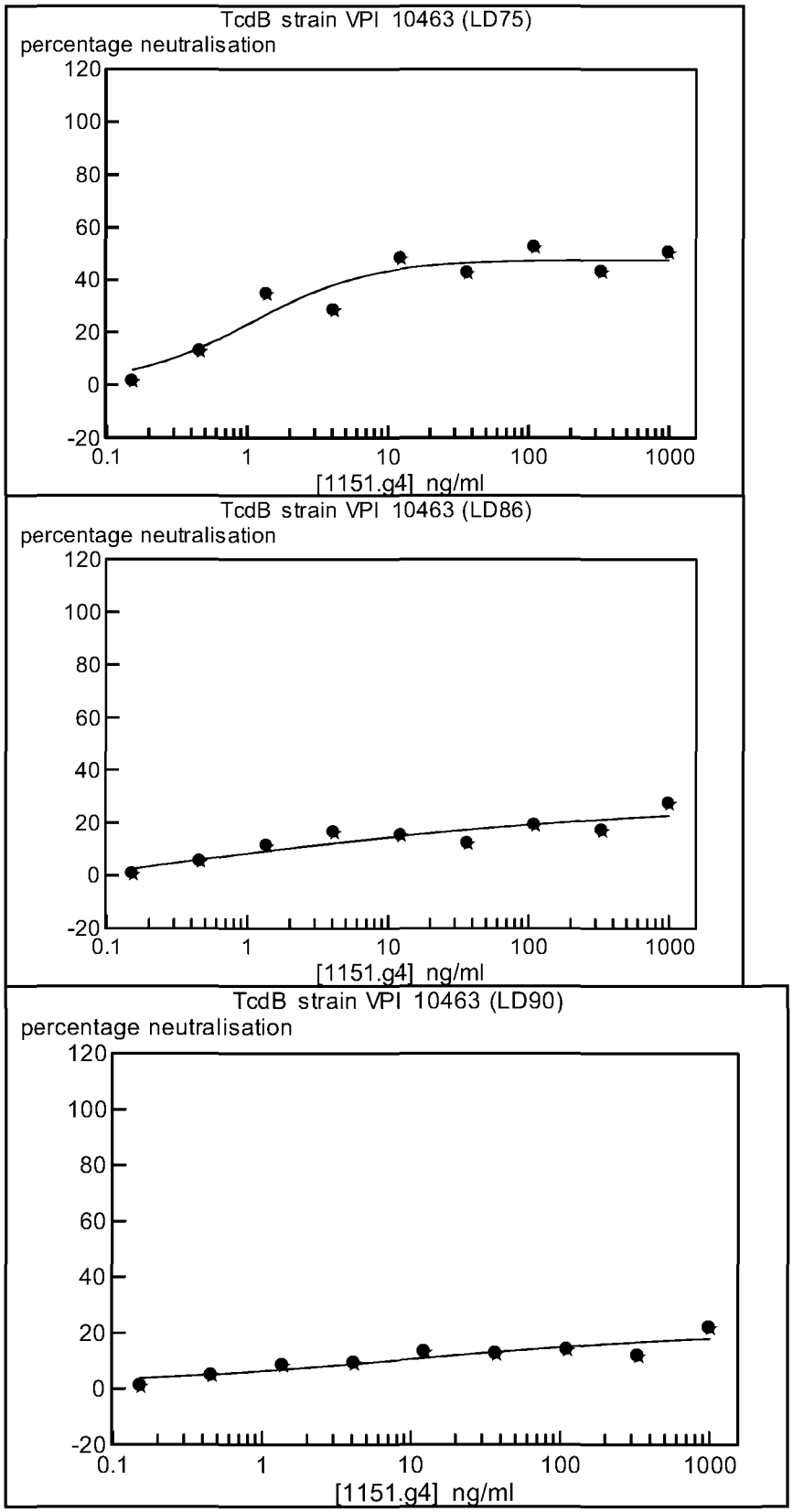


Figure 53 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

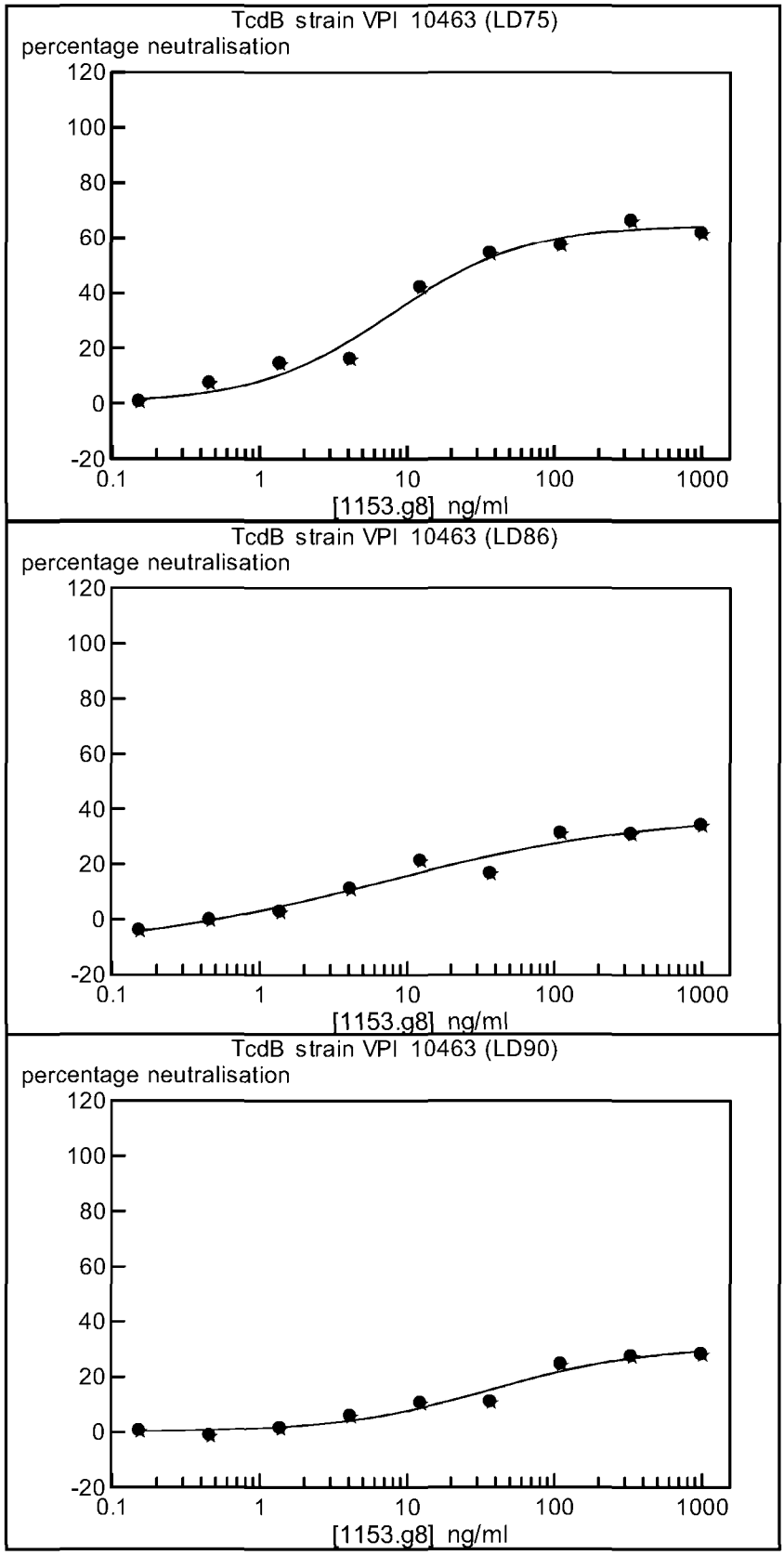


Figure 54 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

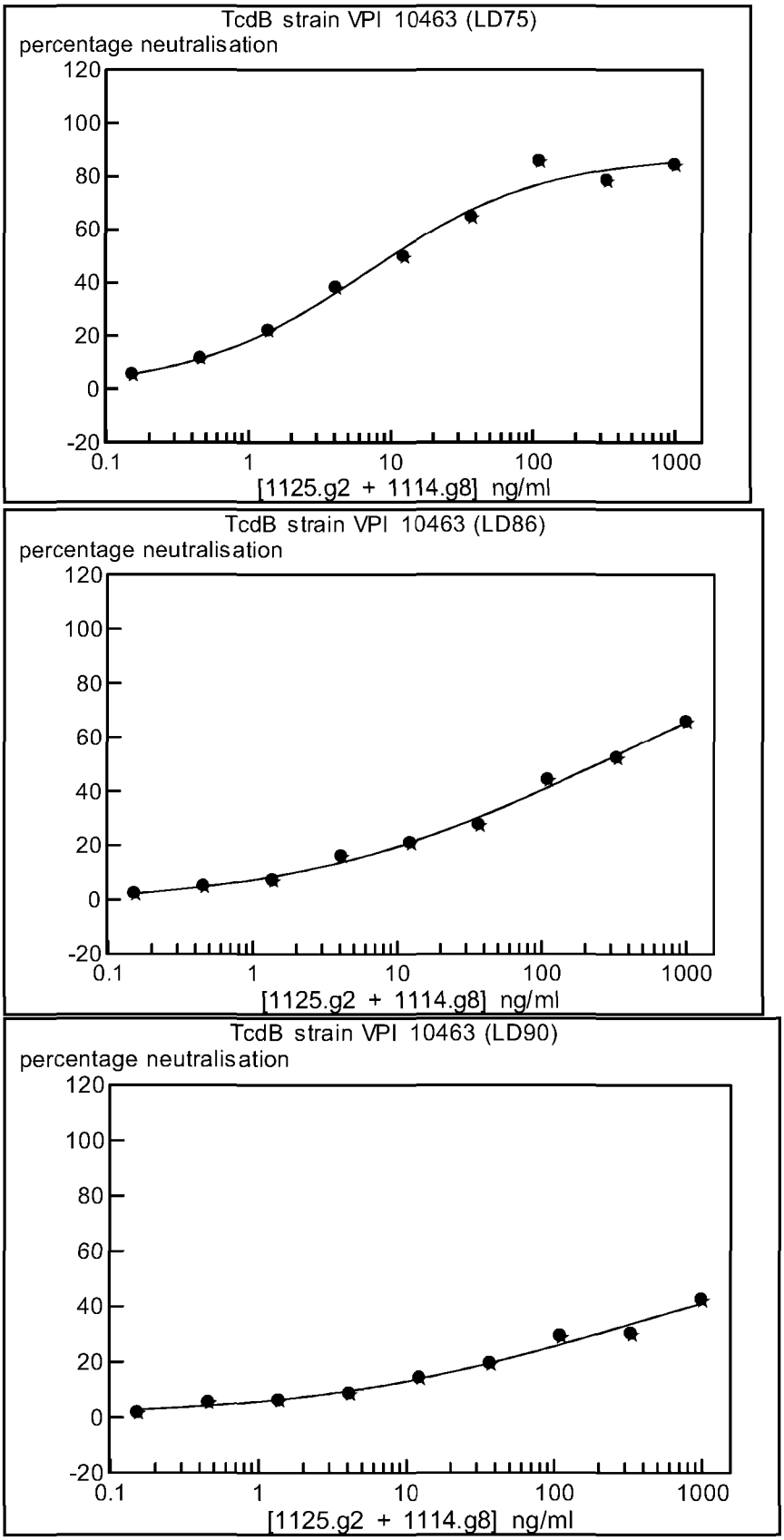


Figure 55 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

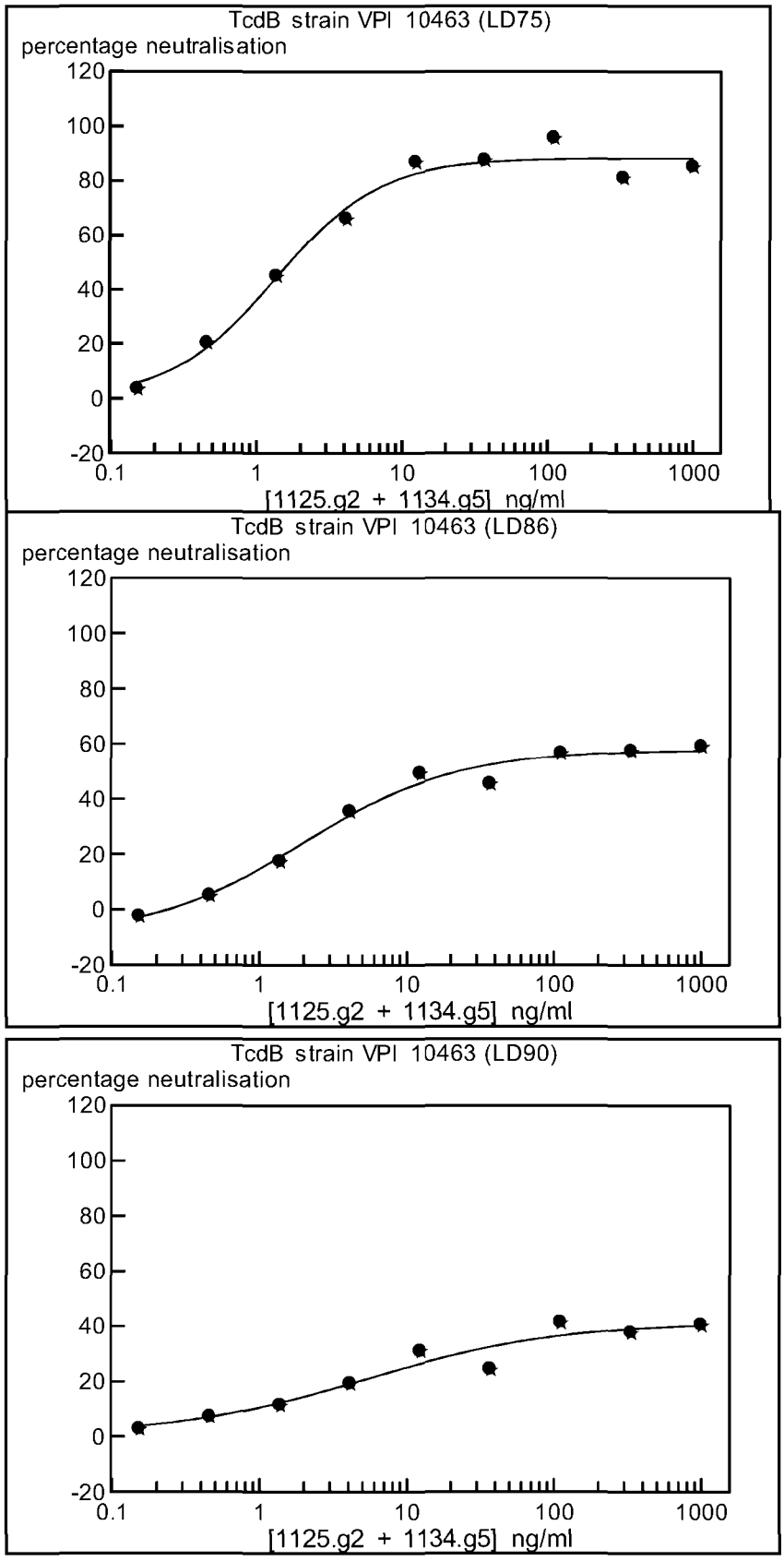


Figure 56 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

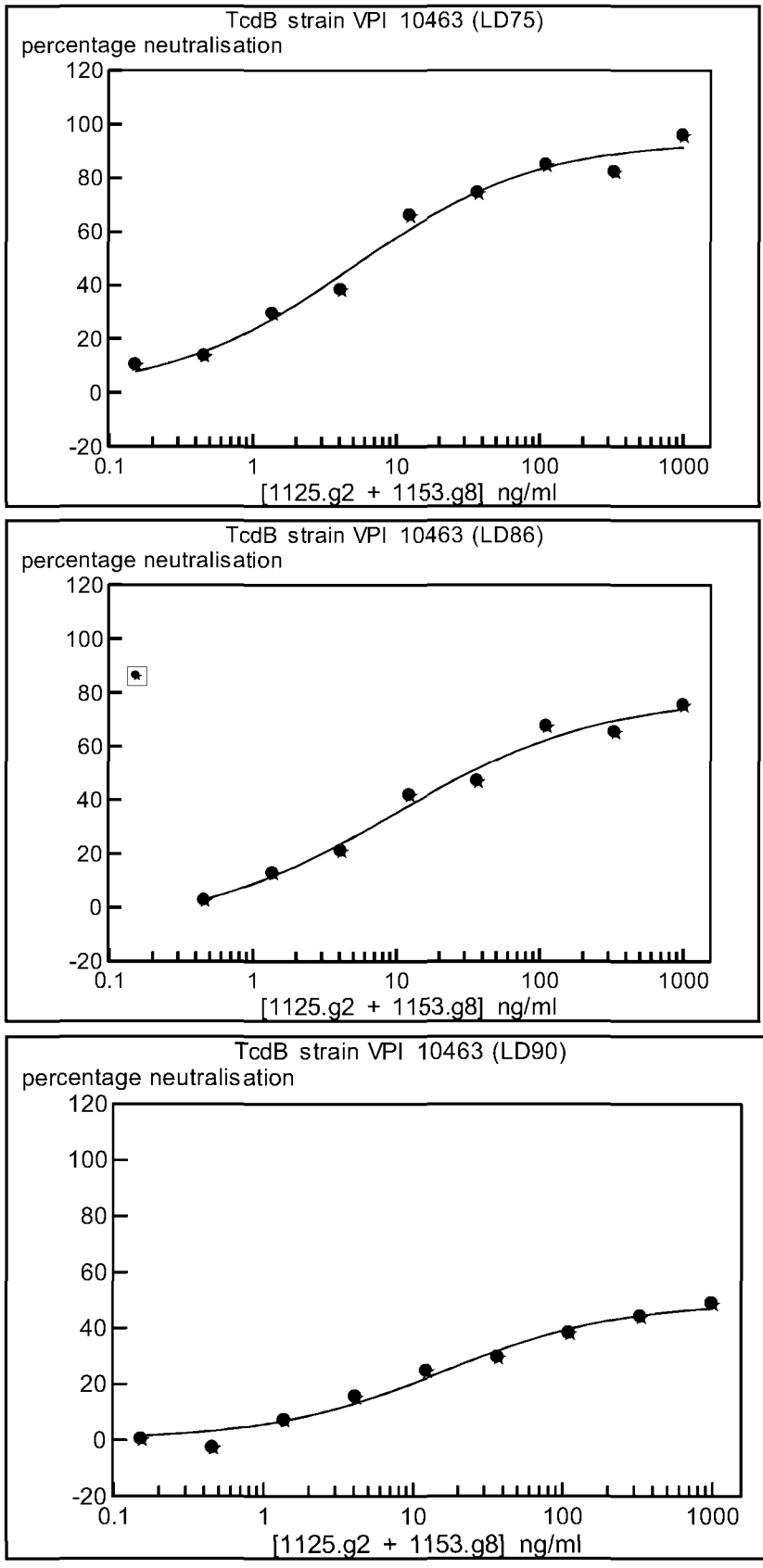


Figure 57 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

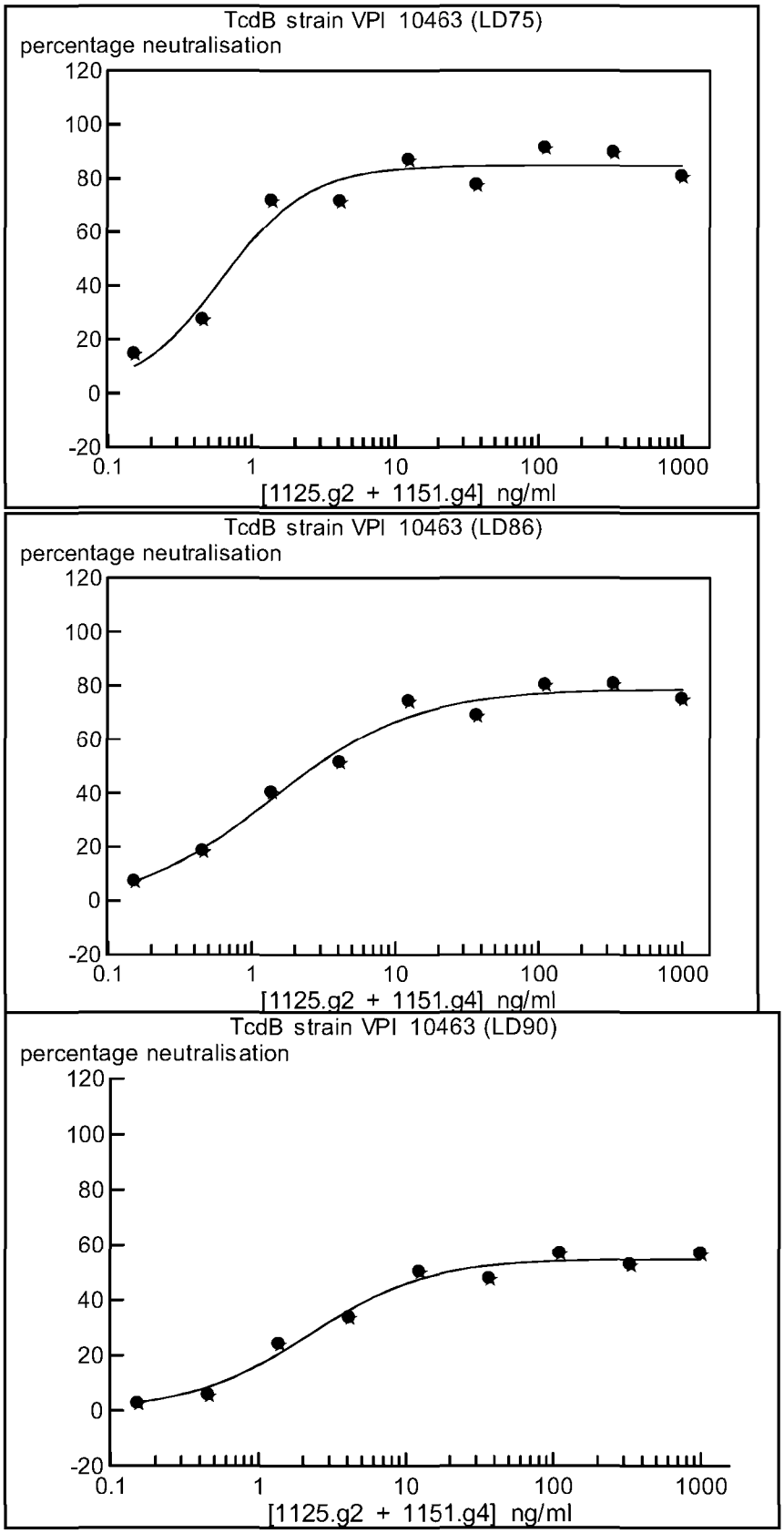


Figure 58 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

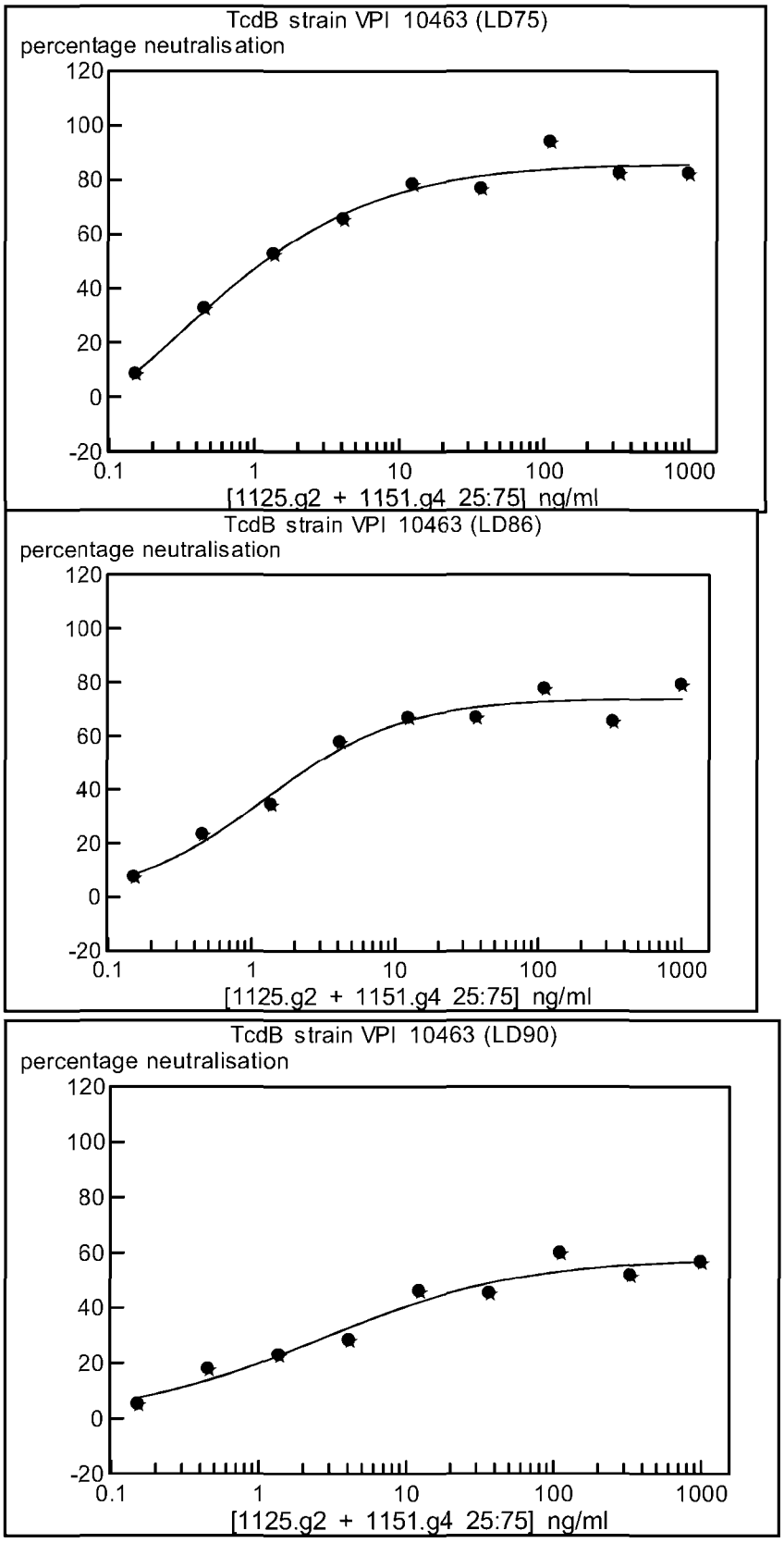


Figure 59 TcdB neutralisation, Antibody singles and pairs, Varying toxin dose (straddling)

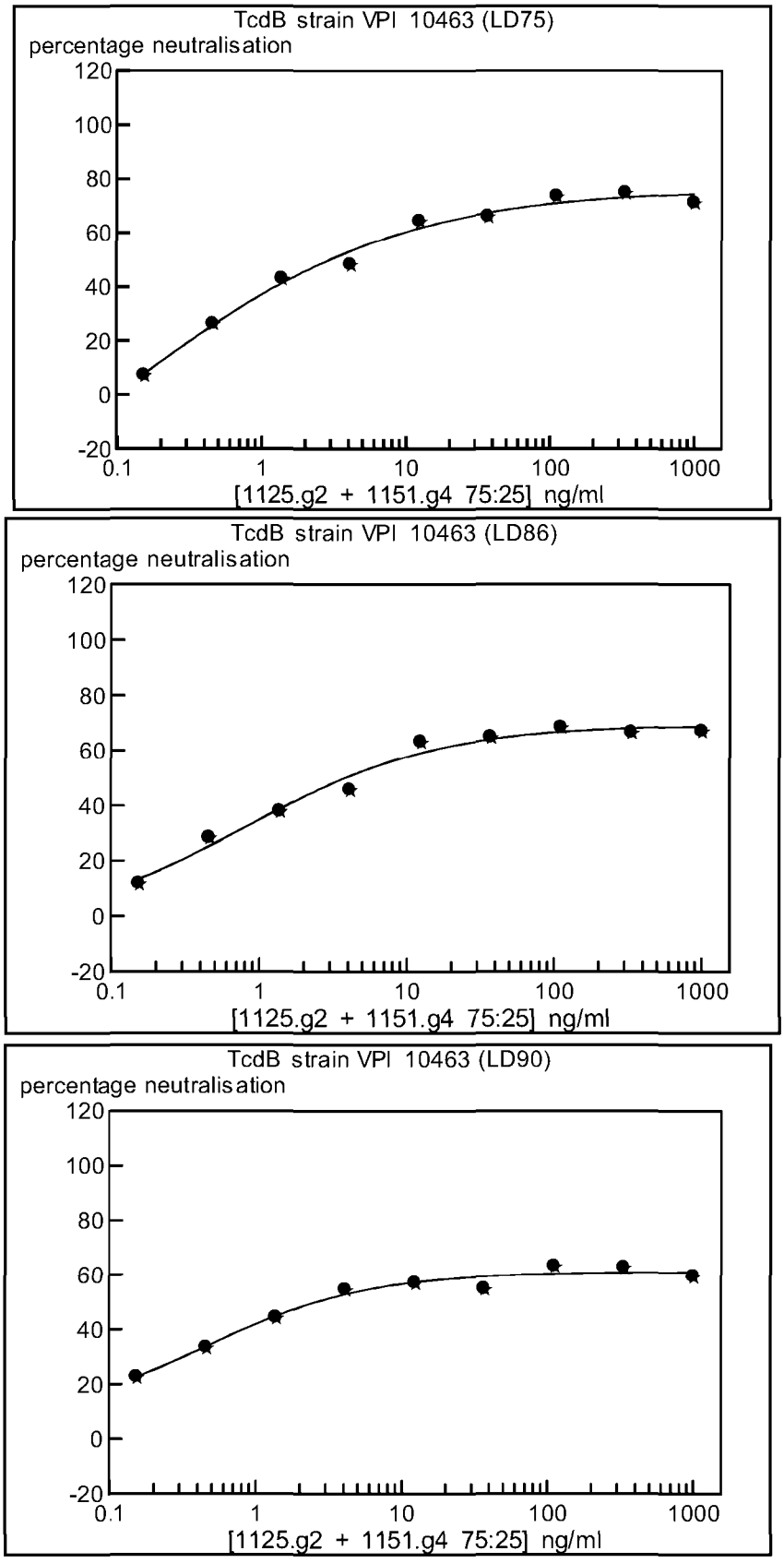


Figure 60 Amino Acid sequence for TcdA SEQ ID NO: 171

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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

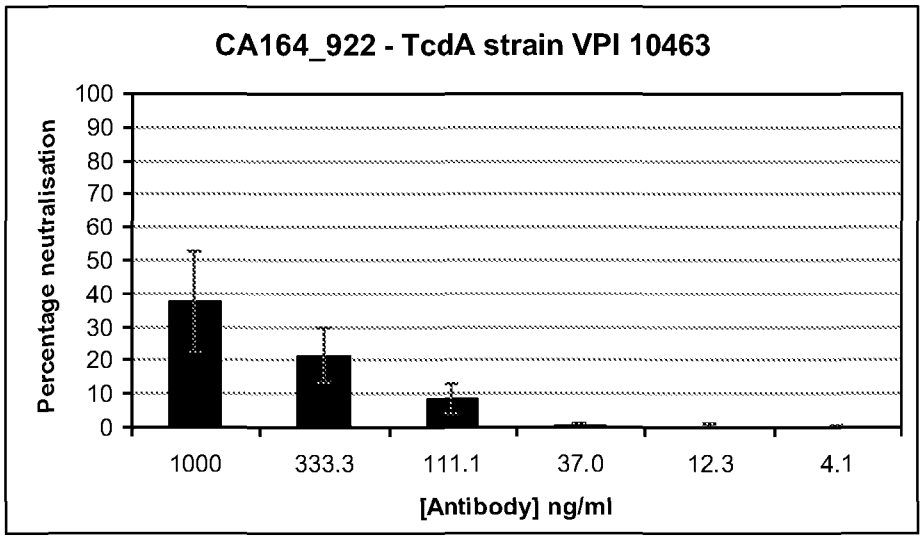
MSLVNRKQLE KMANVRFRTQ EDEYVAILDA LEEYHNMSEN TVVEKYLKLK DINSLTDIYI
DTYKKSCRNK ALKKFKEYLV TEVLELKNNN LTPVEKNLHF VWIGGQINDT AINYINQWKD
VNSDYNVNVF YDSNAFLINT LKKTVVESAI NDTLESFREN LNDPRFDYNK FFRKRMEIIY
DKQKNFINYY KAQREENPEL IIDDIVKTYL SNEYSKEIDE LNTYIEESLN KITQNSGNDV
RNFEEFKNGE SFNLYEQELV ERWNLAAASD ILRISALKEI 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

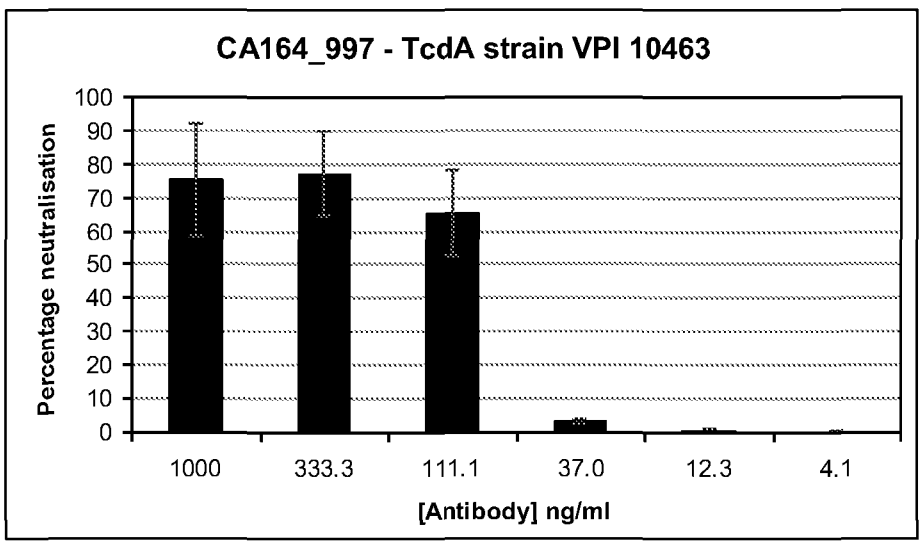
DKISELMPSI SQDSIIVSAN QYEVRINSEG RRELLDHSGE WINKEESIIK DISSKEYISF
NPKENKITVK SKNLPELSTL LQEIRNNSNS SDIELEEKVM LTECEINVIS NIDTQIVEER
IEEAKNLTSD SINYIKDEFK LIESISDALC DLKQQNELED SHFISFEDIS ETDEGFSIRF
INKETGESIF VETEKTIFSE YANHITEEIS KIKGTIFDTV NGKLVKKVNL DTTHEVNTLN
AAFFIQSLIE YNSSKESLSN LSVAMKVQVY AQLFSTGLNT 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





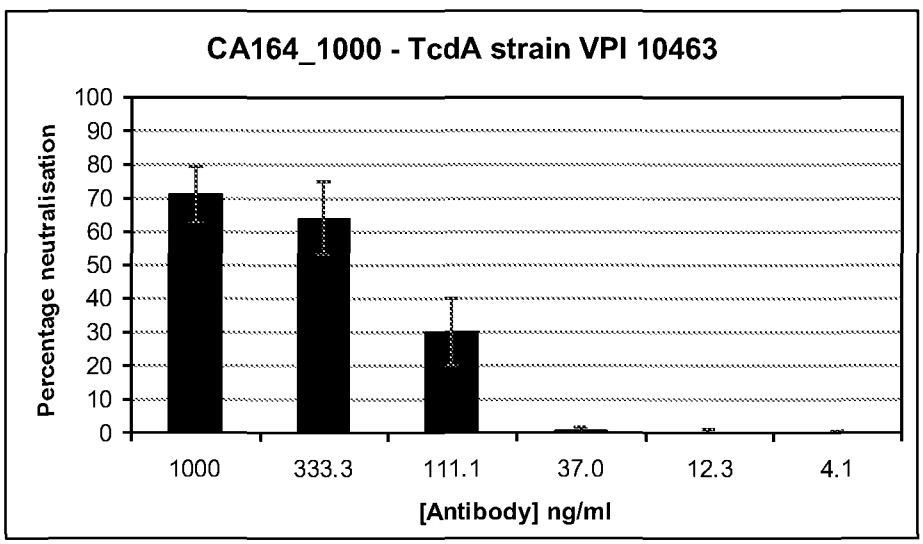


Figure 62A

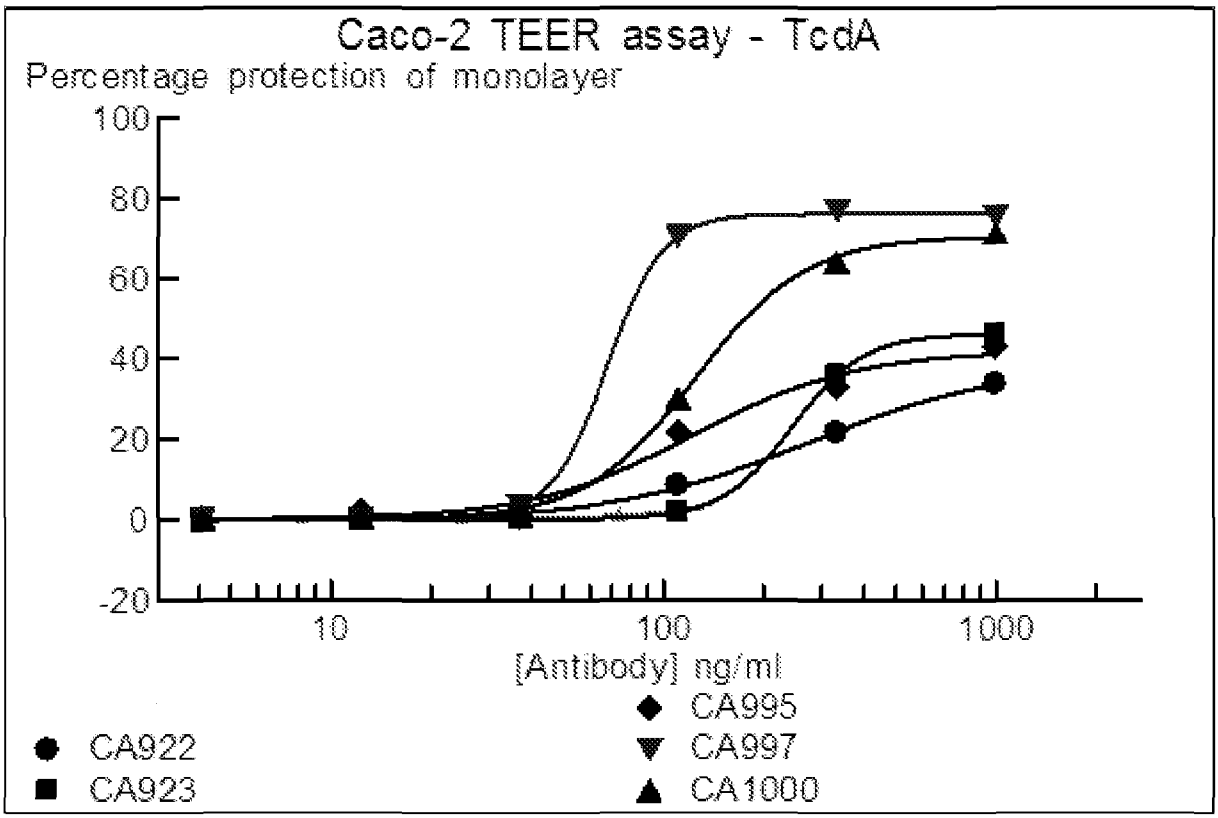

Caco-2 TEER assay - TcdA
Percentage protection of monolayer
100
80
60
40
20
0
-20
10
100
1000
[Antibody] ng/ml
CA922
CA923
CA995
CA997
CA1000

Figure 63

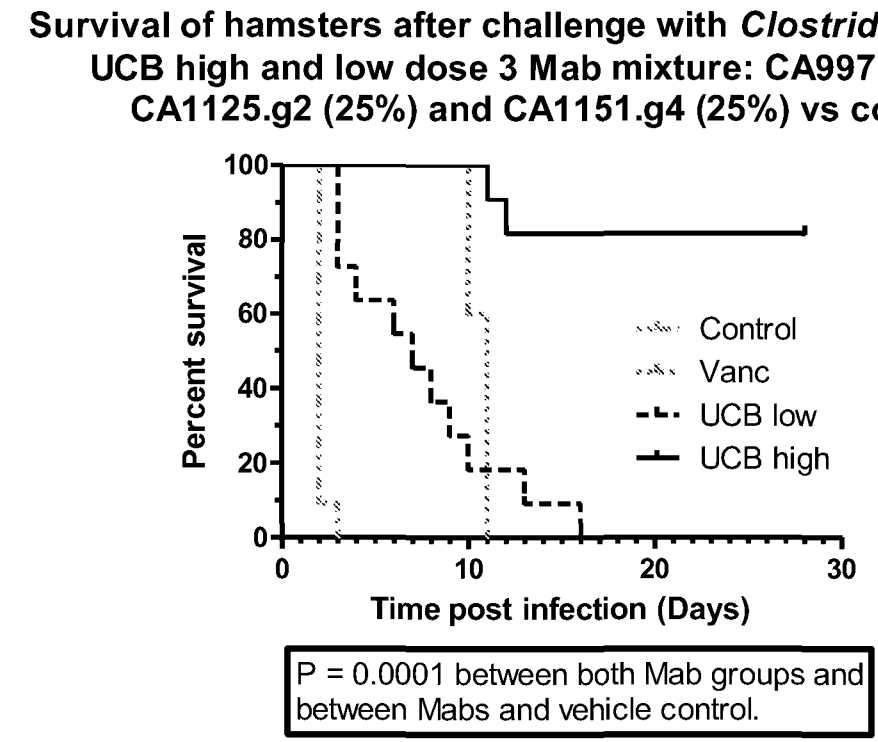


Figure 64 Hamster body weight changes

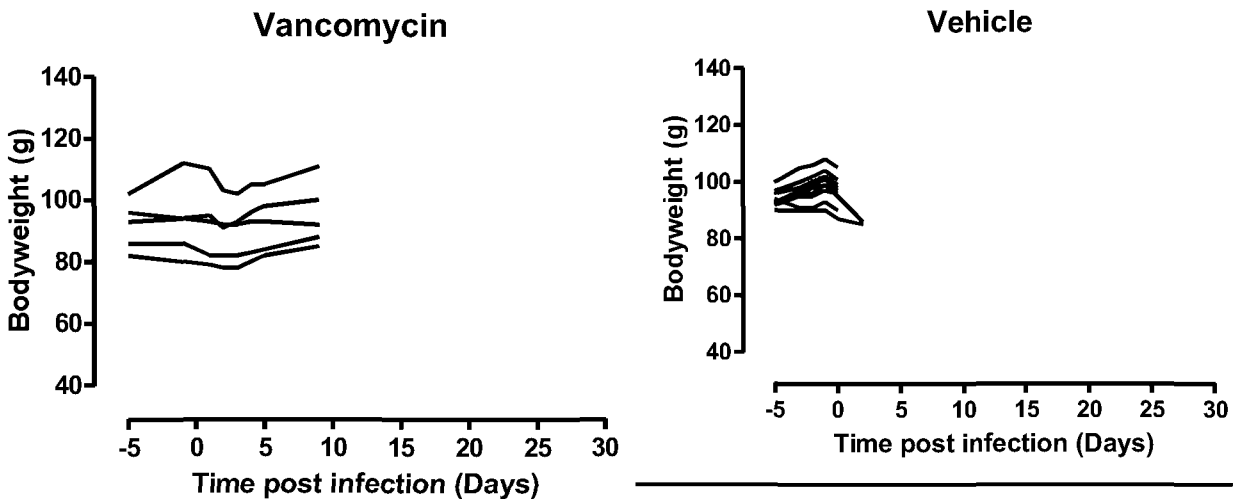


Figure 65

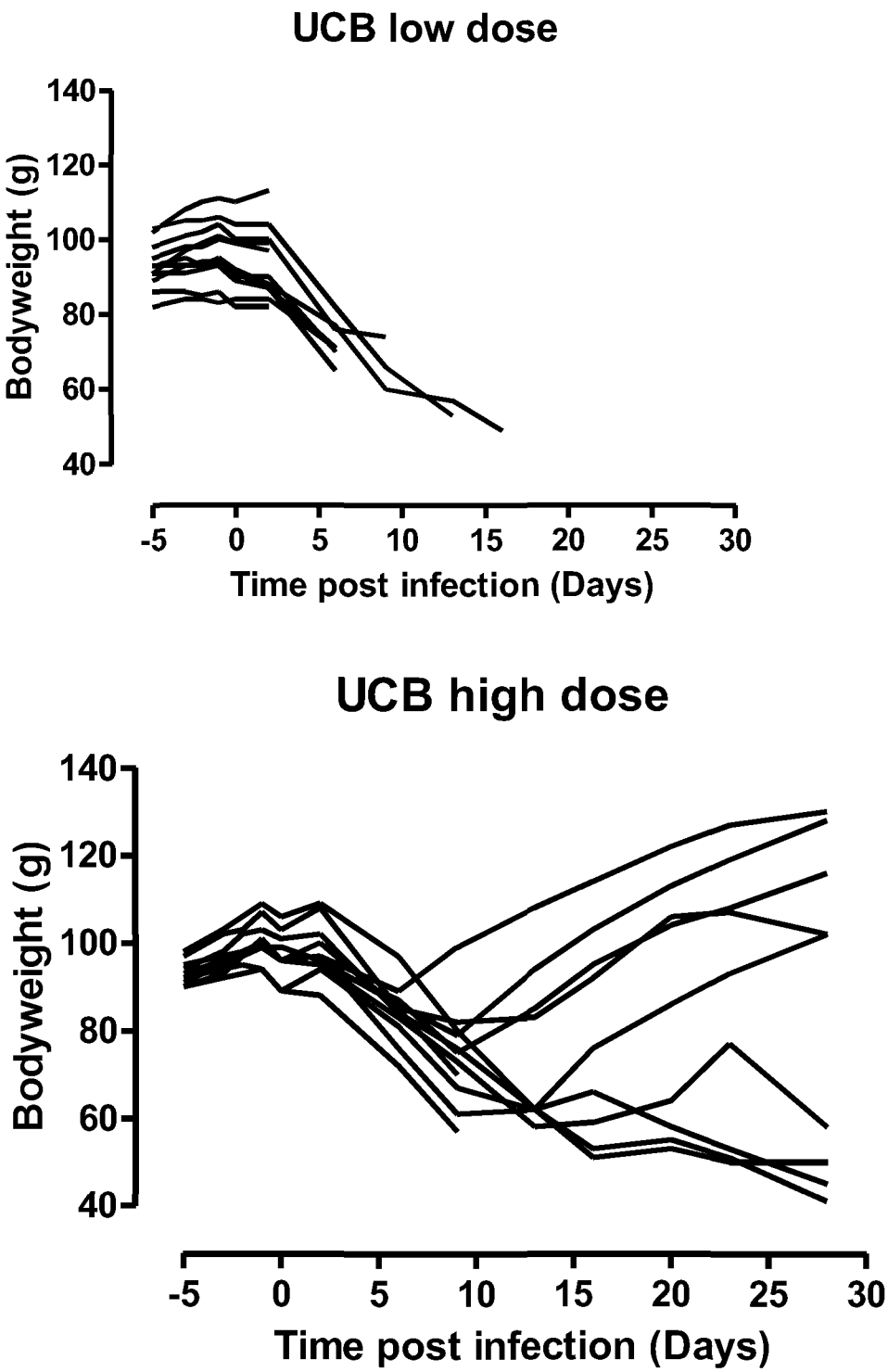
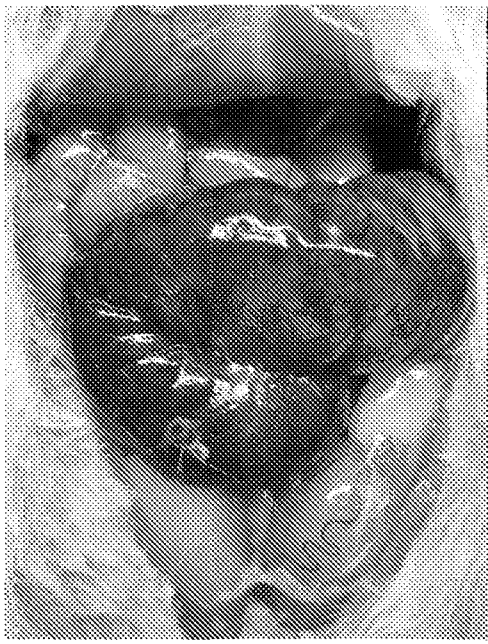

UCB low dose
Bodyweight (g)
140
120
100
80
60
40
-5
0
5
10
15
20
25
30
Time post infection (Days)
UCB high dose
Bodyweight (g)
140
120
100
80
60
40
-5
0
5
10
15
20
25
30
Time post infection (Days)

Figure 66

PBS control



UCB high dose

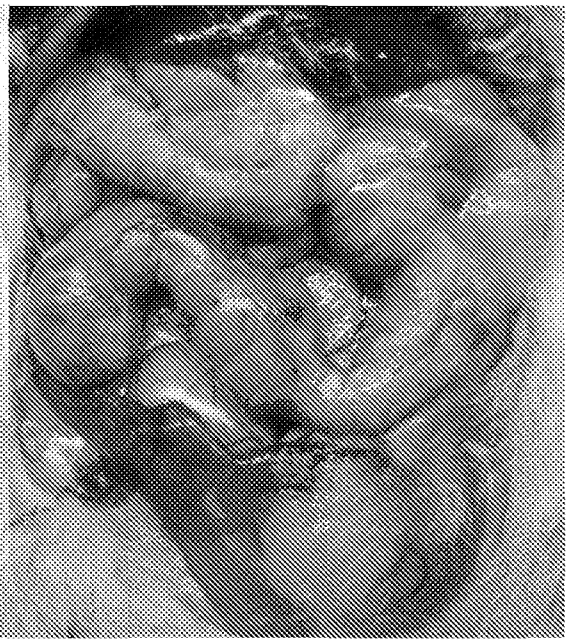


Figure 67 Serum pharmacokinetics of a human IgG1 in mice and hamsters

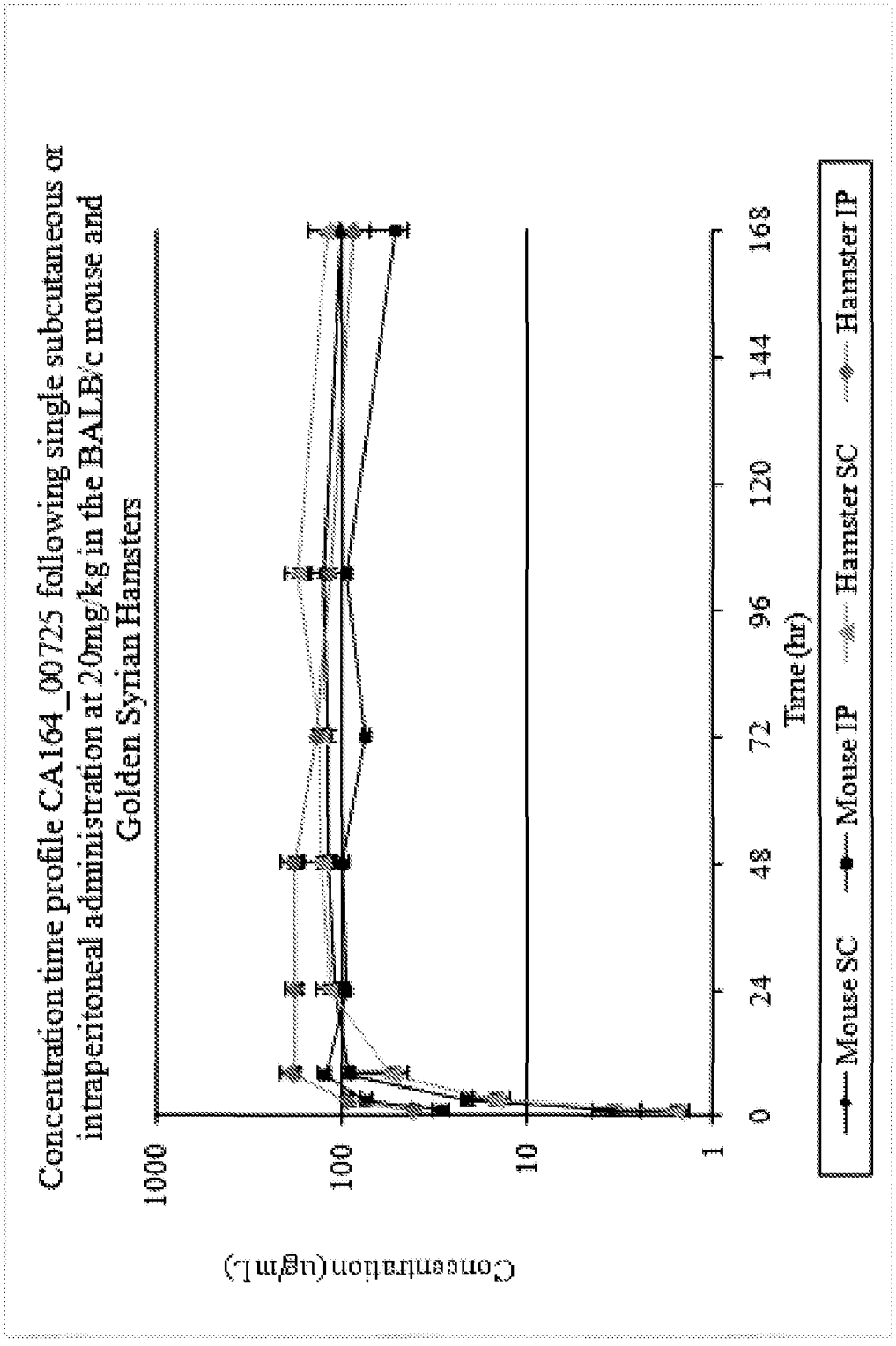
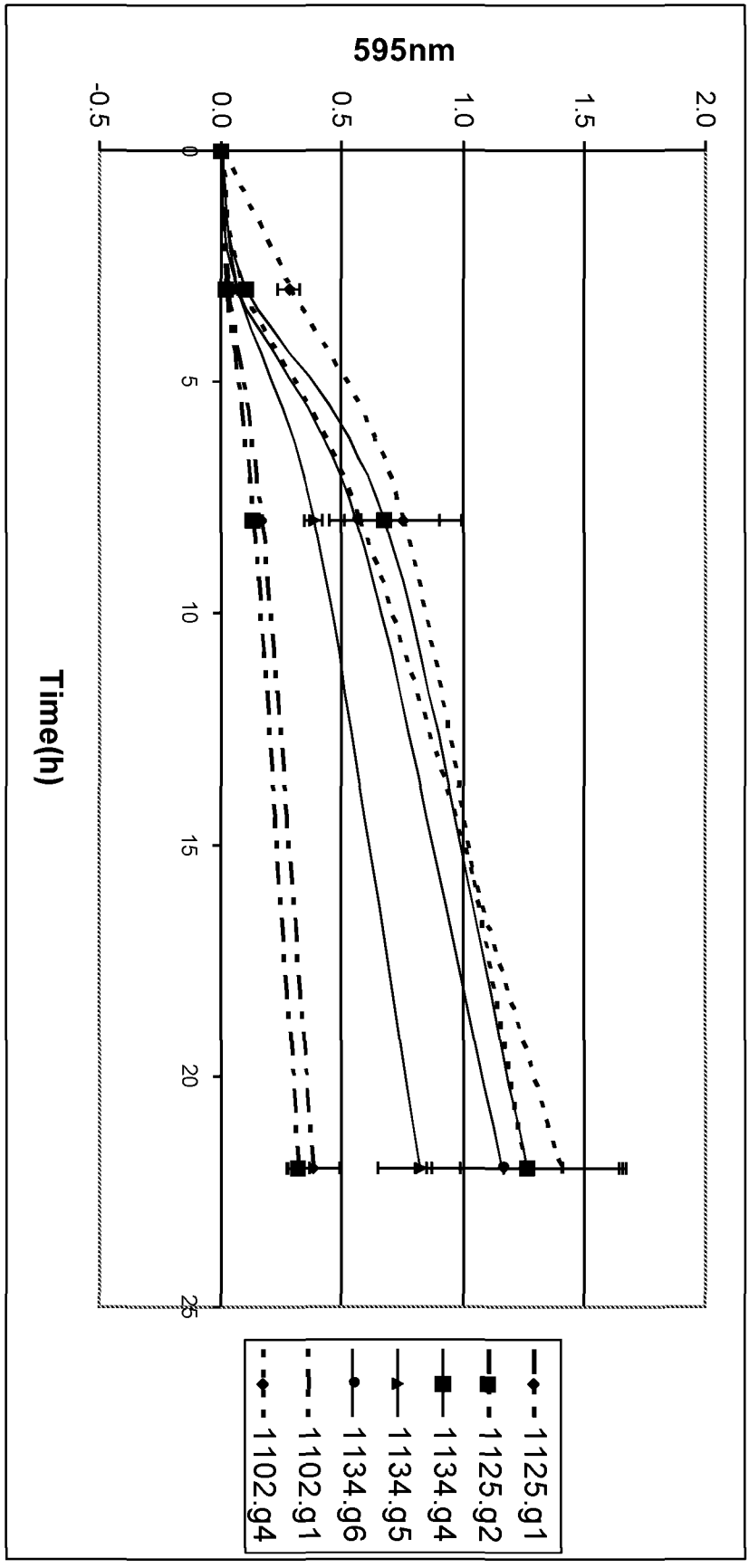


Figure 68 Effect of Agitation *via* Vortexing on anti-TcdB IgG1 Molecules in PBS, pH 7.4(n=3)



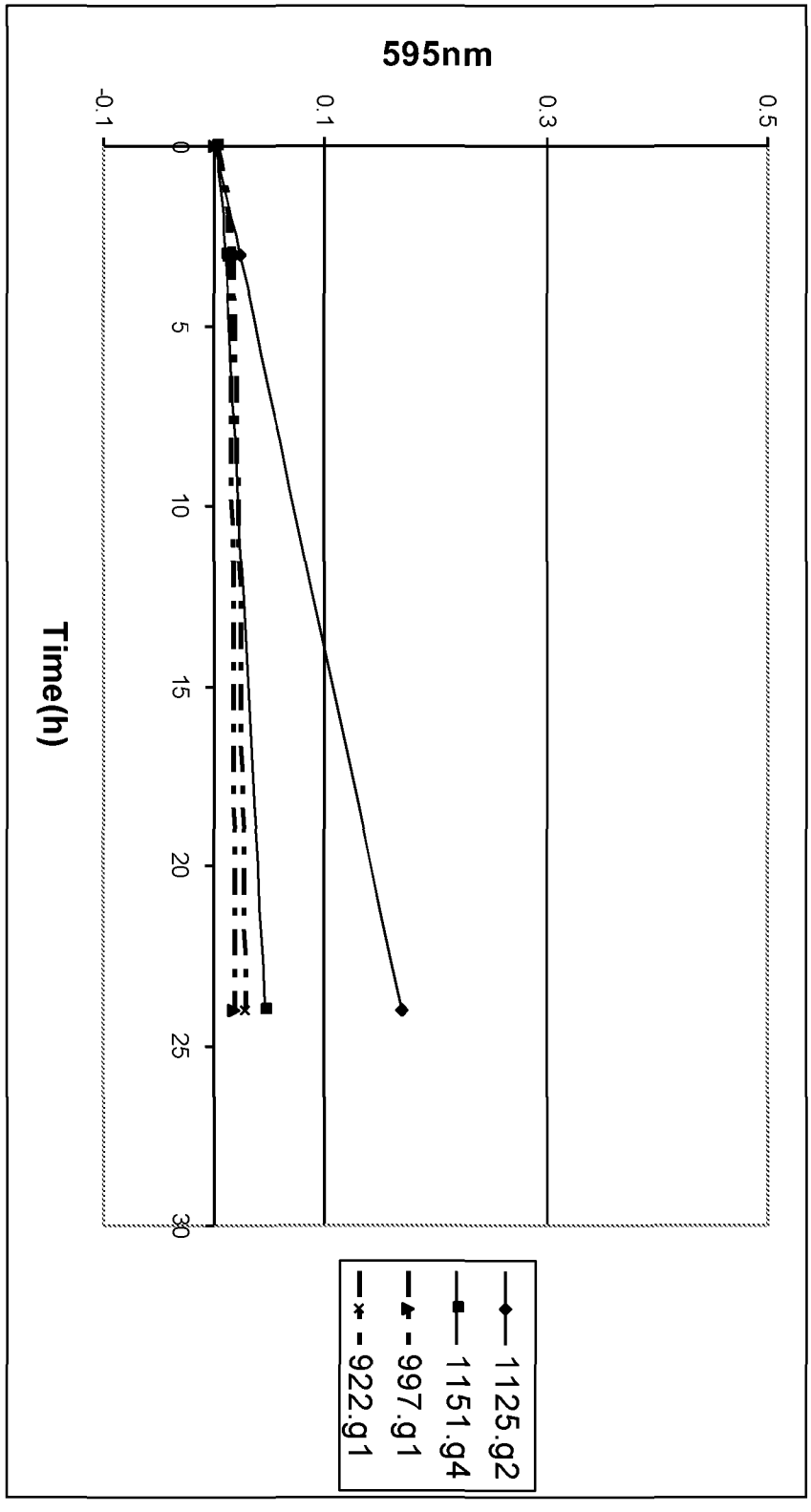


Figure 69 : Comparison of Aggregation Stability of anti-TcdA and anti-TcdB IgG1 Molecules in PBS pH 7.4

Figure 70 Neutralisation against ribotype 003

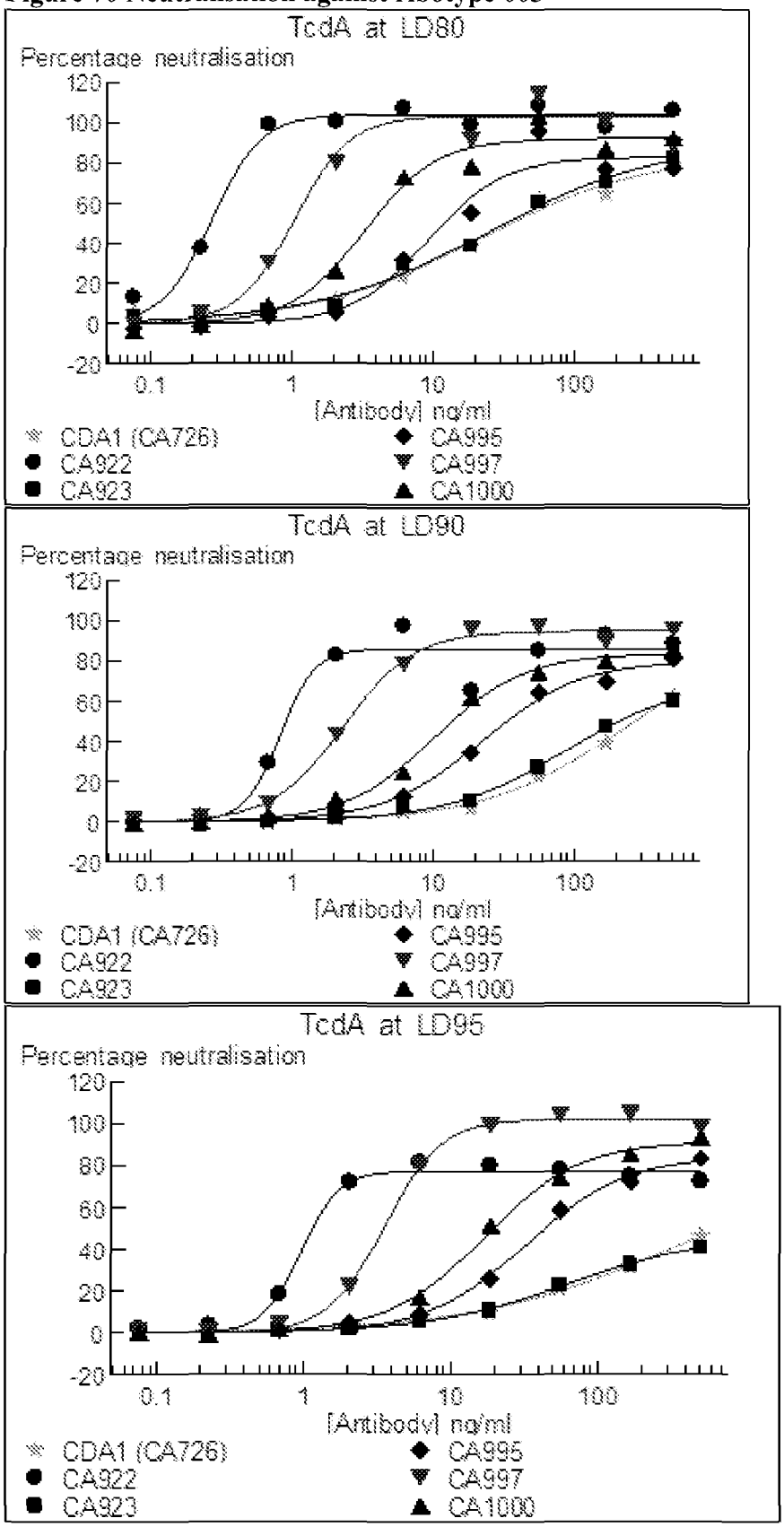


Figure 71 Neutralisation against ribotype 003

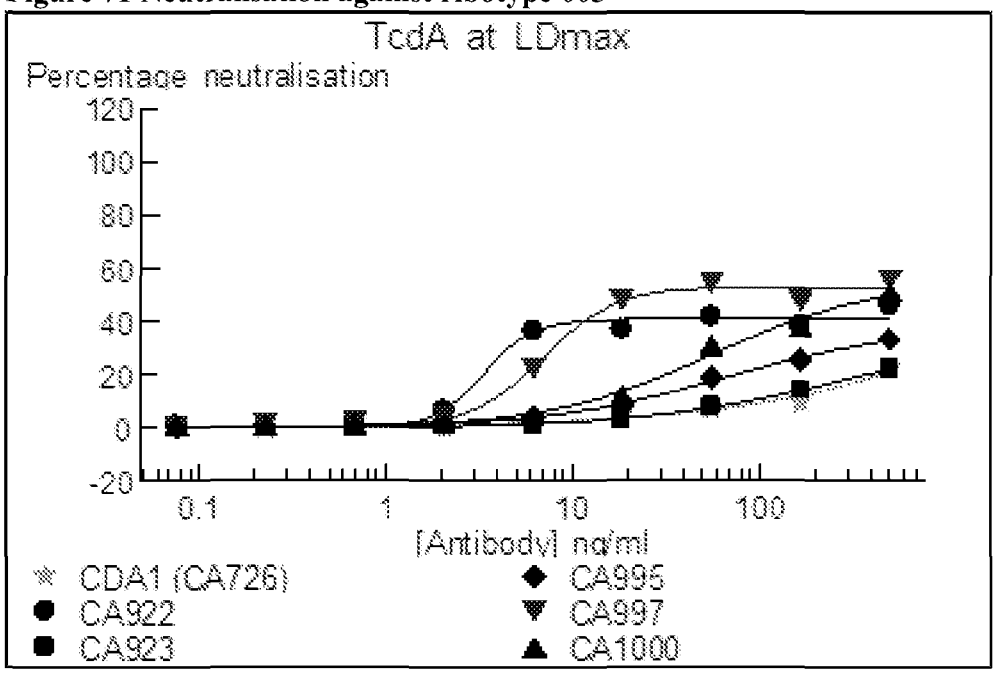


Figure 72 Neutralisation against ribotype 027

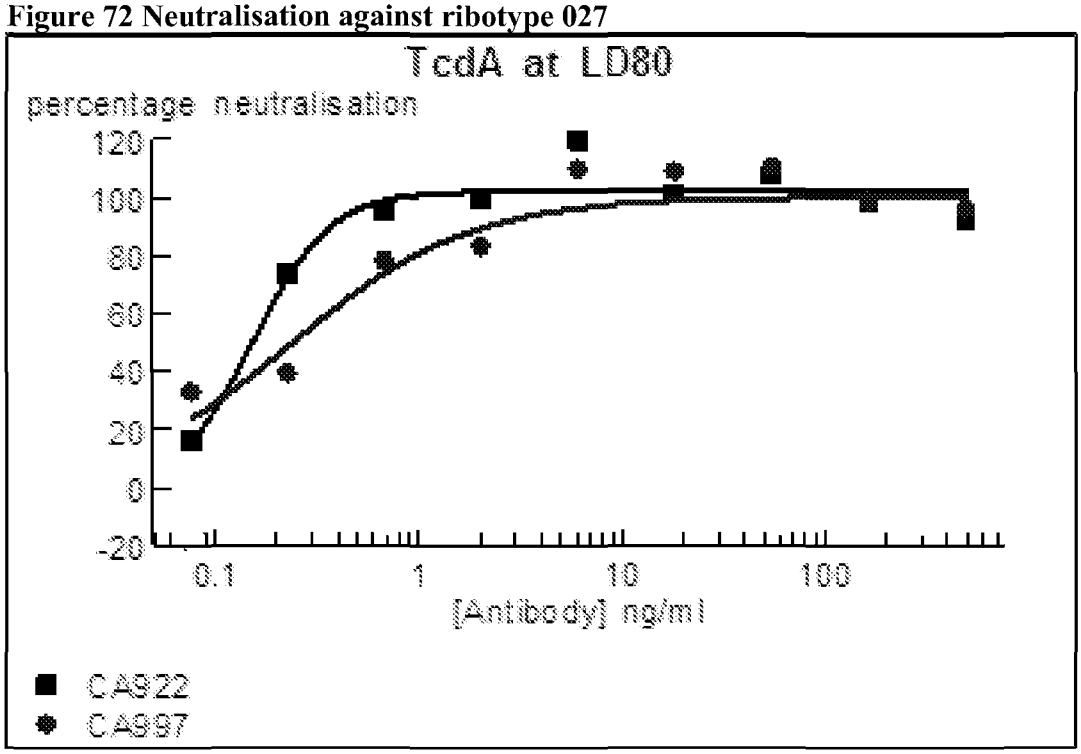
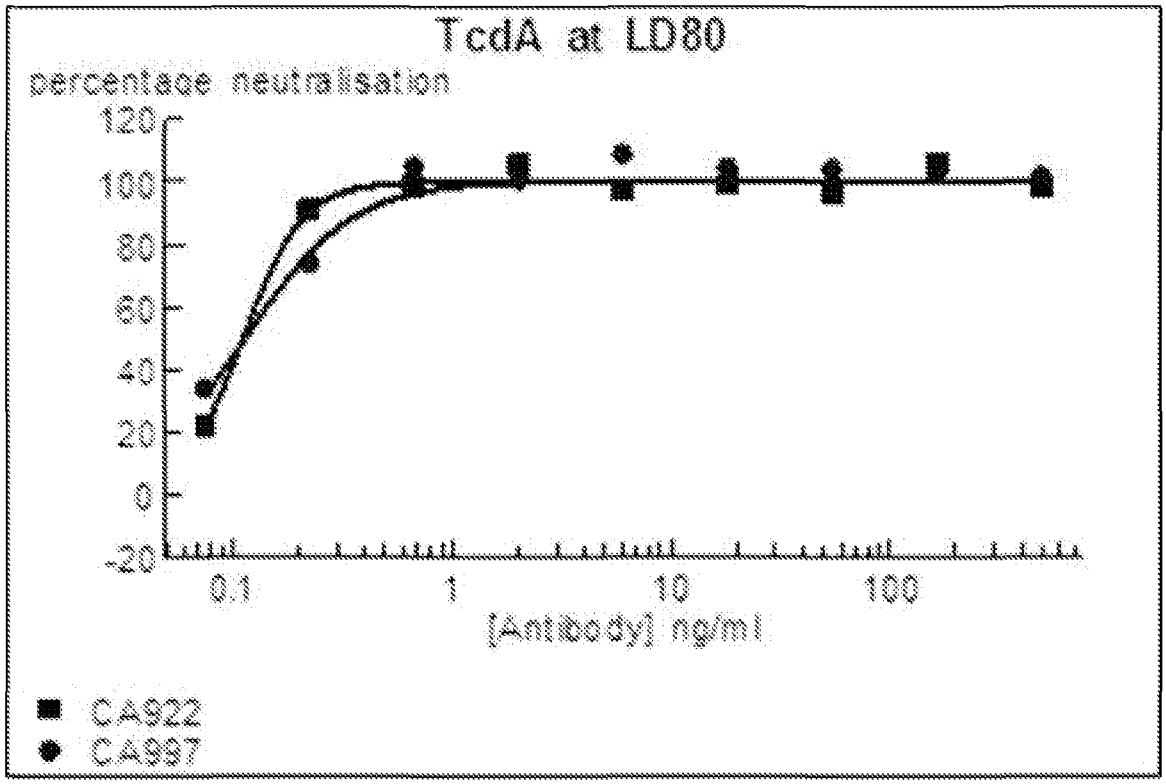


Figure 73 Neutralisation against ribotype 078



SEQUENCE LISTING

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<130> G0159-WO

<150> US 61/535,532
<151> 2011-09-16

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<151> 2012-04-26

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Ser Ala Ser Ser Leu Ala Ser
1 5

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Gln Tyr Thr His Tyr Ser His Thr Ser Lys Asn Pro
1 5 10

<210> 4
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Gly Phe Thr Ile Ser Ser Tyr Tyr Met Ser
1 5 10

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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> Artificial

<220>
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<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> Artificial

<220>
<223> Antibody variable region

<400> 7

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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> Artificial

<220>
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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> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 922

<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> Artificial

<220>
<223> Polynucleotide encoding variable region of anti-TcdA antibody 922 (heavy chain variable region)

<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

<210> 11
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 11

Gln Ala Ser Gln Ser Ile Ser Asn Tyr Leu Ala
1 5 10

<210> 12
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 12

Ser Ala Ser Thr Leu Ala Ser
1 5

<210> 13
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 14

Ala Phe Ser Leu Ser Asn Tyr Tyr Met Ser
1 5 10

<210> 15
<211> 18
<212> PRT
<213> Artificial

<220>
<223> Antibody 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

<210> 16
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Anitbody ariable region of anti-TcdA antibody 923

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdA antibody 923.g1

<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
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 923

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdA antibody 923.g1

<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> Artificial

<220>
<223> Antibody CDR

<400> 21

Gln Ala Ser Gln Ser Ile Ser Ser Tyr Phe Ser
1 5 10

<210> 22
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 22

Gly Ala Ser Thr Leu Ala Ser
1 5

<210> 23

<211> 12
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 24

Gly Phe Ser Leu Ser Ser Tyr Tyr Met Ser
1 5 10

<210> 25
<211> 19
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 26

Ala Tyr Val Gly Ser Ser Ser Tyr Tyr Gly Phe Asp Pro
1 5 10

<210> 27
<211> 110
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 993

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdA antibody 933.g1

<400> 28
gatgtcgtga tgactcagtc cccctctaca 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> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 993 (heavy chain)

<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

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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdA antibody 993.g1

<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> Artificial

<220>
<223> Antibody CDR

<400> 31

Gln Ala Ser Gln Ser Ile Asn Asn Tyr Phe Ser
1 5 10

<210> 32
<211> 7
<212> PRT

<213> Artificial

<220>
<223> Antibody CDR

<400> 32

Gly Ala Ala Asn Leu Ala Ser
1 5

<210> 33
<211> 12
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 34

Gly Phe Ser Leu Ser Asn Tyr Asp Met Ile
1 5 10

<210> 35
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 995

<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

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> Artificial

<220>
<223> Poly nucleotide encoding antibody variable region for anti-TcdA antibody 995. g1

<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> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 995 (heavy chain)

<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

Leu Val Thr Val Ser
115

<210> 40
<211> 351
<212> DNA
<213> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdA antibody

<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> Artificial

<220>
<223> Antibody CDR

<400> 41

Gln Ala Ser Gln Ser Ile Ser Ser Tyr Leu Ser

1 5 10

<210> 42
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 42

Arg Ala Ser Thr Leu Ala Ser
1 5

<210> 43
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 44

Gly Ile Asp Leu Ser Ser His His Met Cys
1 5 10

<210> 45
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 46

Ala Ser Ile Ala Gly Tyr Ser Ala Phe Asp Pro
1 5 10

<210> 47
<211> 111
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 997

<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 Gln 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
<212> DNA
<213> Artificial

<220>
<223> Polynucleotide sequence encoding antibody variable region for anti-TcdA antibody 997.g1

<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> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 997 (heavy chain)

<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> Artificial

<220>
<223> Polynucleotide sequence encoding antibody variable region for anti-TcdA anitbody 997.g1 (heavy chain)

<400> 50
gaggtgcaac ttgtggaaag cgggggagga ctggtgcagc ctgggggctc attgagactg 60

agctgcaccg tttctggtat tgacctgagc tcccatcata tgtgctgggt gcgccaggca 120

cccggaaaag gactggaata catcggcgtc atataccact ttggctctac atactatgcc 180

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> Artificial

<220>
<223> Antibody CDR

<400> 51

Gln Ala Ser Gln Ser Ile Tyr Ser Tyr Leu Ala
1 5 10

<210> 52
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 52

Asp Ala Ser Thr Leu Ala Ser
1 5

<210> 53
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 54

Gly Ile Asp Leu Ser Ser Asp Ala Val Gly
1 5 10

<210> 55
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 55

Ile Ile Ala Thr Phe Asp Ser Thr Tyr Tyr Ala Ser Trp Ala Lys Gly
1 5 10 15

<210> 56
<211> 19
<212> PRT

<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 1000

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdA antibody 1000.g1

<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

gcattcgggg gaggcaccaa agtcgaaatt aag 333

<210> 59
<211> 125
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdA antibody 1000 (heavy chain)

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdA antibody 1000.g1 (heavy chain)

<400> 60
gaagttcagc tggtcgagag cggaggggt ttgattcagc ccggtggctc acttagattg 60

agctgcaccg tgtccggaat cgatctgtca tctgatgccg tgggctgggt gcgacaggca 120

cctgggaaag gactggagta tatagggatc atcgccacct tcgactccac atactacgct 180

agctgggcaa aagggcgctt tacgattagc aaggcctcct ctactaccgt gtacctccaa 240

atgaactcac tgagggccga ggacactgcc acttatttct gtgctcggac cggtagctgg 300

tactacatct ctggctgggg ctcctactat tatggcatgg acctgtgggg acaggggaca 360

ctcgtgaccg tctcg 375

<210> 61
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 61

Arg Ala Ser Lys Ser Val Ser Thr Leu Met His
1 5 10

<210> 62
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 62

Leu Ala Ser Asn Leu Glu Ser
1 5

<210> 63
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 63

Gln Gln Thr Trp Asn Asp Pro Trp Thr
1 5

<210> 64
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 64

Gly Phe Thr Phe Ser Asn Tyr Gly Met Ala
1 5 10

<210> 65
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 66

Val Ile Arg Gly Tyr Val Met Asp Ala
1 5

<210> 67
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 926

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 926.g1

<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> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 926 (heavy chain)

<400> 69

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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdBantibody 926.g1 (heavy chain)

<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> Artificial

<220>
<223> Antibody CDR

<400> 71

Arg Ala Ser Gly Ser Val Ser Thr Leu Met His
1 5 10

<210> 72
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 72

Lys Ala Ser Asn Leu Ala Ser
1 5

<210> 73
<211> 8
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 73

His Gln Ser Trp Asn Ser Asp Thr
1 5

<210> 74
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 74

Gly Phe Thr Phe Ser Asn Tyr Gly Met Ala
1 5 10

<210> 75
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 76

Ile Ser Arg Ser His Tyr Phe Asp Cys
1 5

<210> 77
<211> 106
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 927

<400> 77

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> Artificial

<220>
<223> Polynucleotide sequence encoding antbody variable region for anti-TcdB antibody 927.g2

<400> 78
gacacacaga tgacccagag cccatccact ttgtctgcat ccgtgggcga ccgagtgaca 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> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 927 (heavy chain)

<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

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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 927.g2 (heavy chain)

<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> Artificial

<220>
<223> Antibody CDR

<400> 81

Lys Ala Ser Lys Ser Ile Ser Asn His Leu Ala
1 5 10

<210> 82
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 82

Ser Gly Ser Thr Leu Gln Ser
1 5

<210> 83
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 83

Gln Gln Tyr Asp Glu Tyr Pro Tyr Thr
1 5

<210> 84
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 84

Gly Phe Ser Leu Gln Ser Tyr Thr Ile Ser
1 5 10

<210> 85
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 86

Pro Arg Trp Tyr Pro Arg Ser Tyr Phe Asp Tyr
1 5 10

<210> 87
<211> 109
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1099

<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

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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1099.g2

<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> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1099 (heavy chain)

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1099.g2 (heavh chain)

<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> Artificial

<220>
<223> Antibody CDR

<400> 91

Arg Ala Ser Gln Arg Ile Ser Thr Ser Ile His
1 5 10

<210> 92
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 92

Tyr Ala Ser Gln Ser Ile Ser
1 5

<210> 93
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 93

Gln Gln Ser Tyr Ser Ser Leu Tyr Thr
1 5

<210> 94
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDRs

<400> 94

Gly Phe Thr Phe Ser Asp Ser Tyr Met Ala
1 5 10

<210> 95
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 96

Arg Gln Gly Thr Tyr Ala Arg Tyr Leu Asp Phe
1 5 10

<210> 97
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1102

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1102.g4

<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> Artificial

<220>
<223> Antibody variabl region for anti-TcdB antibody 1102 (heavy chain)

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1002.g4 (heavy chain)

<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

ggaacctacg ctcgatacct ggacttctgg ggtcagggaa cactcgttac agtctcg 357

<210> 101
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 101

Arg Ala Ser Glu Ser Val Ser Thr Leu Leu His
1 5 10

<210> 102
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 102

Lys Ala Ser Asn Leu Ala Ser
1 5

<210> 103
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 103

His Gln Ser Trp Asn Ser Pro Pro Thr
1 5

<210> 104
<211> 10
<212> PRT
<213> Artificial

<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> Artificial

<220>
<223> Antibody CDR

<400> 105

Ile Ile Asn Tyr Asp Ala Ser Thr Thr His Tyr Arg Asp Ser Val Lys
1 5 10 15

Gly

<210> 106
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 106

Tyr Gly Arg Ser His Tyr Phe Asp Tyr
1 5

<210> 107
<211> 107
<212> PRT

<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB anitbody 1114

<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> Artificial

<220>
<223> Polynucleotide sequence encoding antibody variable region for anti-TcdB antibody 1114.g2

<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
<211> 117
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1114 (heavy chain)

<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> Artificial

<220>
<223> Polynucleotide encoding anitbody variable region for anti-TcdB antibody 1114.g2 (heavy chain)

<400> 110
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> 111
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 111

Arg Ala Ser Glu Ser Val Ser Thr Leu Leu His
1 5 10

<210> 112
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Anitbody CDR

<400> 112

Lys Ala Ser Asn Leu Ala Ser
1 5

<210> 113
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 113

His Gln Ser Trp Asn Ser Pro Pro Thr
1 5

<210> 114
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 114

Gly Phe Thr Phe Ser Asn Tyr Gly Met Ala
1 5 10

<210> 115
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 116

Tyr Gly Arg Ser His Tyr Phe Asp Tyr
1 5

<210> 117
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1114 graft 8

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1114.g8

<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

ggaacgaaac tcgaaatcaa g 321

<210> 119
<211> 117
<212> PRT

<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1114 graft 8 (heavy chain)

<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

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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1114.g8

<400> 120
gaagtacaac tcgtagagtc agggggtggg 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> Artificial

<220>
<223> Antibody CDR

<400> 121

Lys Ala Ser Gln Asn Ile Tyr Met Tyr Leu Asn
1 5 10

<210> 122
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 122

Asn Thr Asn Lys Leu His Thr
1 5

<210> 123
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 123

Leu Gln His Lys Ser Phe Pro Tyr Thr
1 5

<210> 124
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 124

Gly Phe Thr Phe Arg Asp Ser Phe Met Ala
1 5 10

<210> 125
<211> 17
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 126

Leu Thr Ile Thr Thr Ser Gly Asp Ser
1 5

<210> 127
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1125

<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

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> Artificial

<220>
<223> Polynucleotide sequence encoding antibody variable region for anti-TcdB antibody 1125.g2

<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 tcccctatac gttcggacaa 300

gggaccaaac tggaaatcaa a 321

<210> 129
<211> 118
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1125 (heavy chain)

<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

Met Val Thr Val Ser Ser
115

<210> 130
<211> 354
<212> DNA
<213> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1125.g2 (heavy chain)

<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> Artificial

<220>
<223> Antibody CDR

<400> 131

Lys Ala Ser Gln His Val Gly Thr Asn Val Asp
1 5 10

<210> 132
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 132

Gly Ala Ser Ile Arg Tyr Thr
1 5

<210> 133
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 133

Leu Gln Tyr Asn Tyr Asn Pro Tyr Thr
1 5

<210> 134
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 134

Gly Phe Ile Phe Ser Asn Phe Gly Met Ser
1 5 10

<210> 135
<211> 17
<212> PRT
<213> Artificial

<220>

<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 136

Arg Ala Tyr Ser Ser Pro Phe Ala Phe
1 5

<210> 137
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1129

<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

<210> 138
<211> 321
<212> DNA
<213> Artificial

<220>
<223> Polynucleotide sequence encoding antibody variable region for anti-TcdB antibody 1129.g1

<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> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1129 (heavy chain)

<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> Artificial

<220>

<223> Polynucleotide sequence encoding antibody variable region for anti-TcdB antibody 1129.g1(heavy chain)

<400> 140

gaggtgcaac ttgtggaatc aggaggtggc gtggttcagc ccggtagatc acttcgtctg 60

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 cttttgcctt ttggggacag gggactctgg tgacagtctc gagc 354

<210> 141
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 141

Lys Ala Ser Lys Ser Ile Ser Asn His Leu Ala
1 5 10

<210> 142
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 142

Ser Gly Ser Thr Leu Gln Pro
1 5

<210> 143
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 143

Gln Gln Tyr Asp Glu Tyr Pro Tyr Thr
1 5

<210> 144
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 144

Gly Phe Ser Leu Asn Ser Tyr Thr Ile Thr
1 5 10

<210> 145
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 145

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> Artificial

<220>
<223> Antibody CDR

<400> 146

Pro Arg Trp Tyr Pro Arg Ser Tyr Phe Asp Tyr
1 5 10

<210> 147
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1134

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1134.g5

<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 acccctatac gttcggccaa 300

gggacaagat tggaaatcaa g 321

<210> 149
<211> 118
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1134 (heavy chain)

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1134.g5 (heavy chain)

<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 tggttaccgt ctcg 354

<210> 151
<211> 11
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 151

Lys Ala Ser Gln Asn Val Gly Asn Asn Val Ala
1 5 10

<210> 152
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 152

Tyr Ala Ser Asn Arg Phe Thr
1 5

<210> 153
<211> 9
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 153

Gln Arg Val Tyr Gln Ser Thr Trp Thr
1 5

<210> 154
<211> 10

<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 154

Gly Phe Ser Leu Thr Ser Tyr Tyr Val His
1 5 10

<210> 155
<211> 16
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody CDR

<400> 156

Gly Asn Tyr Gly Phe Ala Tyr
1 5

<210> 157
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1151

<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> Artificial

<220>
<223> Polynucleotide encoding antibody variable region for anti-TcdB antibody 1151.g1

<400> 158
gcgattcaaa tgactcagtc gccctcatcg cttagcgcgt ccgtcggaga tagagtgacg 60

atcacgtgca aagcatcaca aaatgtcggg aacaatgtgg catggtatca gcataaaccg 120

gggaaggcac caaaactctt gatctactac gccagcaaca ggtttactgg tgtcccgtca 180

aggttcacgg gaggggggtta cgggacagac tttactttga ccatttcgtc gcttcagccg 240

gaggacttcg ccacctatta ctgtcagagg gtctaccagt caacgtggac atttggccag 300

ggaacgaaag tggaaatcaa g 321

<210> 159
<211> 114
<212> PRT
<213> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1151 (heavy chain)

<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

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> Artificial

<220>
<223> Polynucleotide sequence encoding antibody variable region for anti-TcdB antibody 1151.g4 (heavy chain)

<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> Artificial

<220>
<223> Antibody CDR

<400> 161

Lys Ala Ser Gln Asn Ile Asn Lys Tyr Leu Asp
1 5 10

<210> 162
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 162

Asn Ile Gln Ser Leu His Thr
1 5

<210> 163
<211> 7
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 163

Phe Gln His Asn Ser Gly Trp
1 5

<210> 164
<211> 10
<212> PRT
<213> Artificial

<220>
<223> Antibody CDR

<400> 164

Gly Phe Thr Phe Thr Gln Ala Ala Met Phe
1 5 10

<210> 165
<211> 19
<212> PRT
<213> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody 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> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1153.g8

<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

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> Artificial

<220>
<223> Polynucleotide sequence encoding antibody variable region for anti-TcdB antibody 1153.g8

<400> 168
gatatacaga tgactcagtc cccttctagc 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> Artificial

<220>
<223> Antibody variable region for anti-TcdB antibody 1153 (graft 8 heavy chain)

<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

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> Artificial

<220>
<223> Polynucleotide sequence encoding antibody variable region for anti-TcdB antibody 1153.g8 (heavy chain)

<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> Clostridia

<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

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

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

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

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

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

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

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

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

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

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<212> PRT
<213> Clostridia

<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
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

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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
                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
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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
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
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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
    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
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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
    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
```

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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
    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
    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
    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
    2360                2365
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<210> 173
<211> 18
<212> PRT
<213> Artificial

<220>
<223> Fragment from C. difficile toxin TcdB

<400> 173

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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> Artificial

<220>
<223> Fragment from C. difficile toxin TcdB

<400> 174

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Asn Leu Ala Ala Ala Ser Asp Ile Val Arg Leu
1               5                   10
```

<210> 175
<211> 15
<212> PRT
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<220>
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<400> 175

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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> Artificial

<220>
<223> Fragment from C. difficile toxin TcdB

<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> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<400> 177

Cys Trp Glu Met Ile Lys Leu Glu Ala Ile Met Lys Tyr Lys
1 5 10

<210> 178
<211> 12
<212> PRT
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<220>
<223> Fragment of C. difficile toxin TcdB

<400> 178

Cys Thr Asn Leu Val Ile Glu Gln Val Lys Asn Arg
1 5 10

<210> 179
<211> 12
<212> PRT
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<220>
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<400> 179

Pro Glu Ala Arg Ser Thr Ile Ser Leu Ser Gly Pro
1 5 10

<210> 180
<211> 12
<212> PRT
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<220>
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<400> 180

Cys Ser Asn Leu Ile Val Lys Gln Ile Glu Asn Arg
1 5 10

<210> 181
<211> 14
<212> PRT
<213> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<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> Artificial

<220>
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<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
20 25

<210> 183
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<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> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<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> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<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> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<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> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<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
<213> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<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> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<400> 189

Met Glu Gly Gly Ser Gly His Thr Val Thr
1 5 10

<210> 190
<211> 35
<212> PRT
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<220>
<223> Fragment of C. difficile toxin TcdB

<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> Artificial

<220>
<223> Fragment of C. difficile toxin TcdB

<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> Artificial

<220>
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<400> 192

Cys Gly Tyr Lys Tyr Phe Ala Pro Ala Asn Thr Val Asn Asp Asn Ile
1 5 10 15

Tyr Gly Gln Ala
20

<210> 193
<211> 12
<212> PRT
<213> Artificial

<220>
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<400> 193

Cys Lys Tyr Tyr Phe Asn Thr Asn Thr Ala Glu Ala
1 5 10

<210> 194
<211> 12
<212> PRT
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<220>
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<400> 194

Cys Lys Tyr Tyr Phe Asp Glu Asp Thr Ala Glu Ala
1 5 10