FIG. 1

Amino Acid Sequences of Monoclonal Anti-Toxin A Antibodies Variable Light Chain Regions (L) and Variable Heavy Chain Regions (H)

Clone	chain		Amino Acid Sequence	SEQ ID NO: (SEQ ID NO: without leader sequence)
3D8	L	I	mdmmvpaqllglllwfpgsrcDIQMTQSPSSVSASVGDRVTITCRASQGISS WLAWYQHKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQANSFPWTFGQGTKVEIK	SEQ ID NO:43 (SEQ ID NO:4)
		II	mdmrvlaqllglllcfpgarcDIQMTQSPSSLSASVGDRVTITCRASQGISSWL AWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQYNSYPWTFGQGTKVEIK	SEQ ID NO:44 (SEQ ID NO:30)
		III	mdmrvpaqllglllwlpgarcVIWMTQSPSLLSASTGDRVTISCRMSQGISSY LAWYQQKPGKAPELLIYAASTLQSGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQYNSYPWTFGQGTKVEIK	SEQ ID NO:45 (SEQ ID NO:31)
		IV	mdmmvpaqllglllwfpgsrcDIQMTQSPSSVSASVGDRVTITCRASQGISS WLAWYQHKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQYNSYPWTFGQGTKVEIK	SEQ ID NO:46 (SEQ ID NO:32)
		V	mdmrvpaqllglllcfpgarcDIQMTQSPSSVSASVGDRVTITCRASQGISSW LAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQ PEDFATYYCQQYNSYPWTFGQGTKVEIK	SEQ ID NO:47 (SEQ ID NO:33)
		VI	mdmrvlaqllglllcfpgarcDIQMTQSLSSLSASVGDRVTITCRASQGISSWL AWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQANSFPWTFGQGTKVEIK	SEQ ID NO:48 (SEQ ID NO:34)
	H		mefglswvflvallrgvqcQVQLVESGGGVVQPGRSLRLSCAASGFSFSNYG MHWVRQAPGKGLEWVALIWYDGSNEDYTDSVKGRFTISRDNSKN TLYLQMNSLRAEDTAVYYCARWGMVRGVIDVFDIWGQGTVVTVS S	SEQ ID NO:49 (SEQ ID NO:1)
1B11	L		meapaqllflllwlpdttgEIVLTQSPATLSLSPGERATLSCRASQSVSSYLA WYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPE DFAVYYCQQRSNWSQFTFGPGTKVDIK	SEQ ID NO:50 (SEQ ID NO:5)
	H		mefglswvflvallrgvqcQMQLVESGGGVVQPGRSLRLSCEASGFSFNSYG MHWVRQAPGKGLEWVSVIWASGNKKYYIESVEGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCARANFDYWGQGTLVTVSS	SEQ ID NO:51 (SEQ ID NO:2)
33.3H2	L		mdmrvlaqllglllcfpgarcDIQMTQSPSSLSASVGDRVTITCRASQGISSWL AWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQYKSYPVTFGGGTKVEIK	SEQ ID NO:52 (SEQ ID NO:6)
	H		mefglswvflvallrgvqcQVQLVESGGGVVQPGRSLRLSCAASGFTFNKY GMHWVRQAPGKGLEWVAVIWYDGTNKYYADSMKGRFTISRDNS KNMLYLQMNSLRAEDTAVYYCARDPPTANYWGQGTLVTVSS	SEQ ID NO:53 (SEQ ID NO:3)

FIG. 2A

Anti-Toxin A 3D8 VK Sequences

V-segment: L19
J-segment: JK1

```
      D   I   Q   M   T   Q   S   P   S   S   V   S   A   S   V   G   D   R
1     GAC ATC CAG ATG ACC CAG TCT CCA TCT TCC GTG TCT GCA TCT GTA GGA GAC AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      V   T   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
55    GTC ACC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                                CDR2
                                                            ~~~~~~~~~~~~~~~~~~
      Q   H   K   P   G   K   A   P   K   L   L   I   Y   A   A   S   S   L
109   CAG CAT AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC TAT GCT GCA TCC AGT TTG

      CDR2
      ~~~~~~~~
      Q   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163   CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                    CDR3
                                                                ~~~~~~~~
      L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAC TAT TGT CAA CAG

          CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   N   S   F   P   W   T   F   G   Q   G   T   K   V   E   I   K
271   GCT AAT AGT TTC CCT TGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
                      |
                      ↳ JK1
```

Amino acid sequence = SEQ ID NO:4
Nucleic acid sequence= SEQ ID NO:35

3 / 35

FIG. 2B

Anti-Toxin A 3D8 VH Sequences

V-segment: VH3-33
D-segment: D3-10
J-segment: JH3b

```
      Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
  1   CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGC AGG TCC CTG

                                                        CDR1
                                                    ----------
      R   L   S   C   A   A   S   G   F   S   F   S   N   Y   G   M   H   W
 55   AGA CTC TCC TGT GCG GCG TCT GGA TTC AGC TTC AGT AAC TAT GGC ATG CAC TGG

                                                            CDR2
                                                    ----------
      V   R   Q   A   P   G   K   G   L   E   W   V   A   L   I   W   Y   D
109   GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA CTT ATA TGG TAT GAT

                  CDR2
      ----------------------------------------------
      G   S   N   E   D   Y   T   D   S   V   K   G   R   F   T   I   S   R
163   GGA AGT AAT GAG GAC TAT ACA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA


      D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D
217   GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                            CDR3
                                    ----------------------------------
      T   A   V   Y   Y   C   A   R   W   G   M   V   R   G   V   I   D   V
271   ACG GCT GTG TAT TAC TGT GCG AGA TGG GGG ATG GTT CGG GGA GTT ATC GAT GTT
                                    |  D3-10/DXP'1  |  |
                                    ----------------->  JH3b
        CDR3
      ----------
      F   D   I   W   G   Q   G   T   V   V   T   V   S   S
325   TTT GAT ATC TGG GGC CAA GGG ACA GTG GTC ACC GTC TCT TCA
```

amino acid sequence=SEQ ID NO:1
nucleic acid sequence=SEQ ID NO:38

FIG. 3A

Anti-Toxin A 1B11 VK Sequences

V-segment: L6
J-segment: JK3

```
      E   I   V   L   T   Q   S   P   A   T   L   S   L   S   P   G   E   R
1     GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT TTG TCT CCA GGG GAA AGA

                                  CDR1
                          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      A   T   L   S   C   R   A   S   Q   S   V   S   S   Y   L   A   W   Y
55    GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC TAC TTA GCC TGG TAC

                                                                CDR2
                                                        ~~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   D   A   S   N   R
109   CAA CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GAT GCA TCC AAC AGG

      CDR2
      ~~~~~~~~
      A   T   G   I   P   A   R   F   S   G   S   G   S   G   T   D   F   T
163   GCC ACT GGC ATC CCA GCC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACT

                                                                    CDR3
                                                                    ~~~~~~~
      L   T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTA GAG CCT GAA GAT TTT GCA GTT TAT TAC TGT CAG CAG

            CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      R   S   N   W   S   Q   F   T   F   G   P   G   T   K   V   D   I   K
271   CGT AGC AAC TGG TCT CAA TTC ACT TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA
                              └──►
                                JK3
```

Amino acid sequence = SEQ ID NO:5
Nucleic acid sequence= SEQ ID NO:36

FIG. 3B

Anti-Toxin A 1B11 VH Sequences

V-segment: VH3-33
D-segment: unknown
J-segment: JH4b

```
      Q   M   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
1     CAG ATG CAG CTG GTG GAG TCT GGG GGC GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                        CDR1
                                                    ~~~~~~~~~~~~~~~~~~~~
      R   L   S   C   E   A   S   G   F   S   F   N   S   Y   G   M   H   W
55    AGA CTC TCC TGT GAA GCG TCT GGA TTC TCC TTC AAT AGC TAT GGC ATG CAC TGG

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~~
      V   R   Q   A   P   G   K   G   L   E   W   V   S   V   I   W   A   S
109   GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG TCA GTC ATA TGG GCC AGT

                        CDR2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      G   N   K   K   Y   Y   I   E   S   V   E   G   R   F   T   I   S   R
163   GGA AAT AAG AAA TAT TAT ATA GAA TCC GTG GAG GGC CGA TTC ACC ATC TCC AGA

      D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D
217   GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC

                                        CDR3
                                    ~~~~~~~~~~~~~~~~~~~~
      T   A   V   Y   Y   C   A   R   A   N   F   D   Y   W   G   Q   G   T
271   ACG GCT GTG TAT TAC TGT GCG AGA GCC AAT TTT GAC TAC TGG GGC CAG GGA ACC
                                        |_____>
                                                JH4b
      L   V   T   V   S   S
325   CTG GTC ACC GTC TCC TCA
```

Amino acid sequence = SEQ ID NO:2
Nucleic acid sequence= SEQ ID NO:39

FIG. 4A

Anti-Toxin A 33.3H2 VK Sequences

V-segment: L15
J-segment: JK4

```
      D   I   Q   M   T   Q   S   P   S   S   L   S   A   S   V   G   D   R
1     GAC ATC CAG ATG ACC CAG TCT CCA TCC TCA CTG TCT GCA TCT GTA GGA GAC AGA

                                CDR1
                            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      V   T   I   T   C   R   A   S   Q   G   I   S   S   W   L   A   W   Y
55    GTC ACC ATC ACT TGT CGG GCG AGT CAG GGT ATT AGC AGC TGG TTA GCC TGG TAT

                                                            CDR2
                                                        ~~~~~~~~~~~~~~~~~~~
      Q   Q   K   P   E   K   A   P   K   S   L   I   Y   A   A   S   S   L
109   CAG CAG AAA CCA GAG AAA GCC CCT AAG TCC CTG ATC TAT GCT GCA TCC AGT TTG

      CDR2
      ~~~~~~~~
      Q   S   G   V   P   S   R   F   S   G   S   G   S   G   T   D   F   T
163   CAA AGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT

                                                                    CDR3
                                                                ~~~~~~~~
      L   T   I   S   S   L   Q   P   E   D   F   A   T   Y   Y   C   Q   Q
217   CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT TAT TAC TGC CAA CAG

              CDR3
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      Y   K   S   Y   P   V   T   F   G   G   G   T   K   V   E   I   K
271   TAT AAG AGT TAC CCG GTC ACT TTC GGC GGA GGG ACC AAG GTG GAG ATC AAA
                          |
                          └──► JK4
```

Amino acid sequence = SEQ ID NO:6
Nucleic acid sequence= SEQ ID NO:37

FIG. 4B

Anti-Toxin A 33.3H2 VH Sequences

V-segment: VH3-33
D-segment: Unknown
J-segment: JH4b

```
      Q   V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   R   S   L
1     CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG

                                                        CDR 1
                                                    ~~~~~~~~~~~~~~~~~~~~
      R   L   S   C   A   A   S   G   F   T   F   N   K   Y   G   M   H   W
55    AGA CTC TCC TGT GCA GCG TCT GGA TTC ACC TTC AAT AAA TAT GGC ATG CAC TGG

                                                            CDR 2
                                                        ~~~~~~~~~~~~~~~~~~~
      V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   W   Y   D
109   GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TGG TAT GAT

                  CDR 2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      G   T   N   K   Y   Y   A   D   S   M   K   G   R   F   T   I   S   R
163   GGA ACT AAT AAA TAC TAT GCA GAC TCC ATG AAG GGC CGA TTC ACC ATC TCC AGA

      D   N   S   K   N   M   L   Y   L   Q   M   N   S   L   R   A   E   D
217   GAC AAT TCC AAG AAT ATG CTG TAT CTG CAA ATG AAC AGC CTA AGA GCC GAG GAC

                                        CDR 3
                                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
      T   A   V   Y   Y   C   A   R   D   P   P   T   A   N   Y   W   G   Q
271   ACG GCT GTG TAT TAC TGT GCG AGA GAT CCC CCC ACT GCT AAC TAC TGG GGC CAG

                                                        └──► JH4b
      G   T   L   V   T   V   S   S
325   GGA ACC CTG GTC ACC GTC TCC TCA
```

Amino acid sequence = SEQ ID NO:3
Nucleic acid sequence= SEQ ID NO:40

FIG. 5

Comparison of *C. difficile* MAbs in the Toxin A Binding ELISA; Standard 8E6.1G12.2G2; 3D8.2A4.2A4; 33.3H2.2H8.2B8; 1B11.2A10.4A7

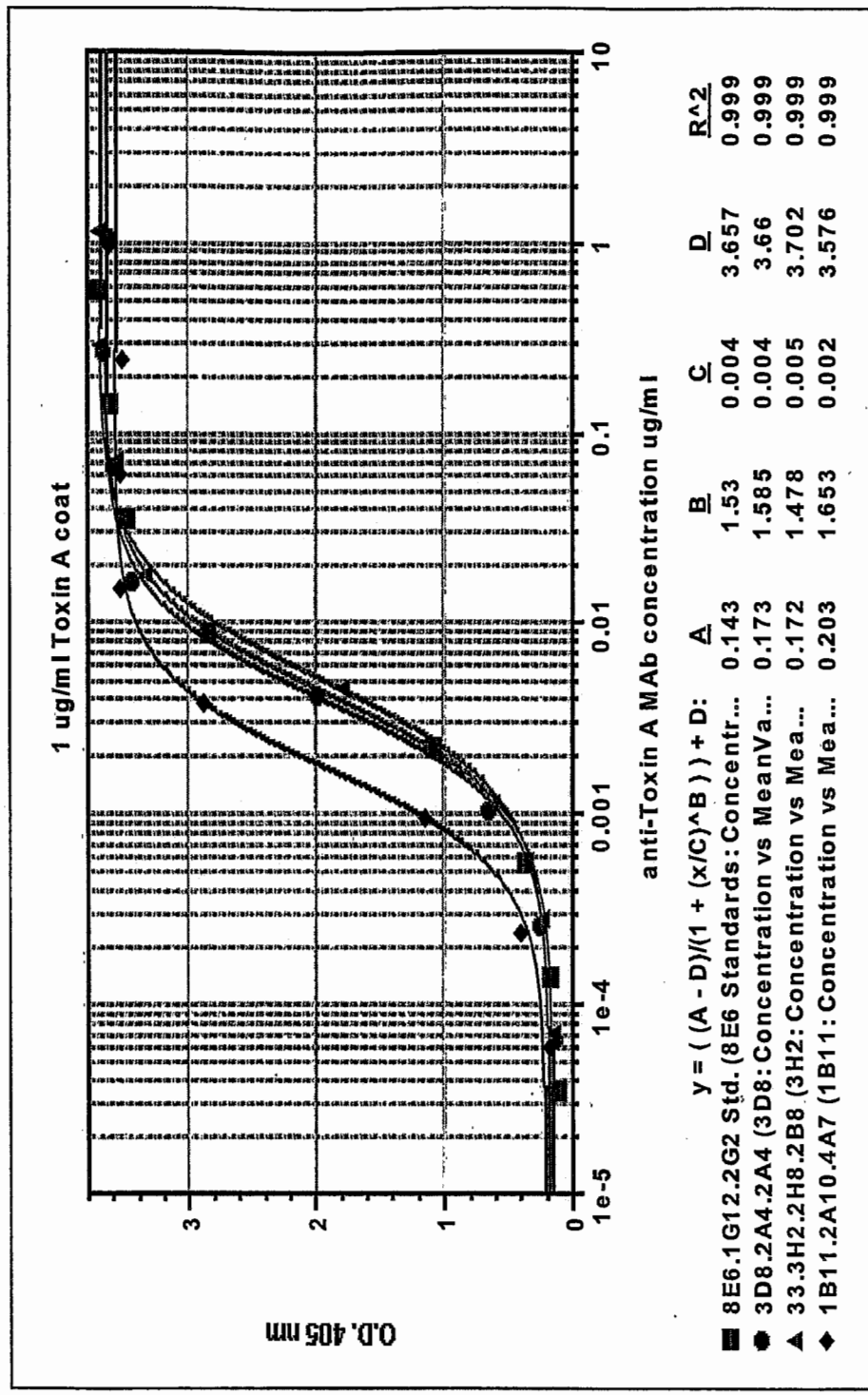


FIG. 6

FIG. 6A

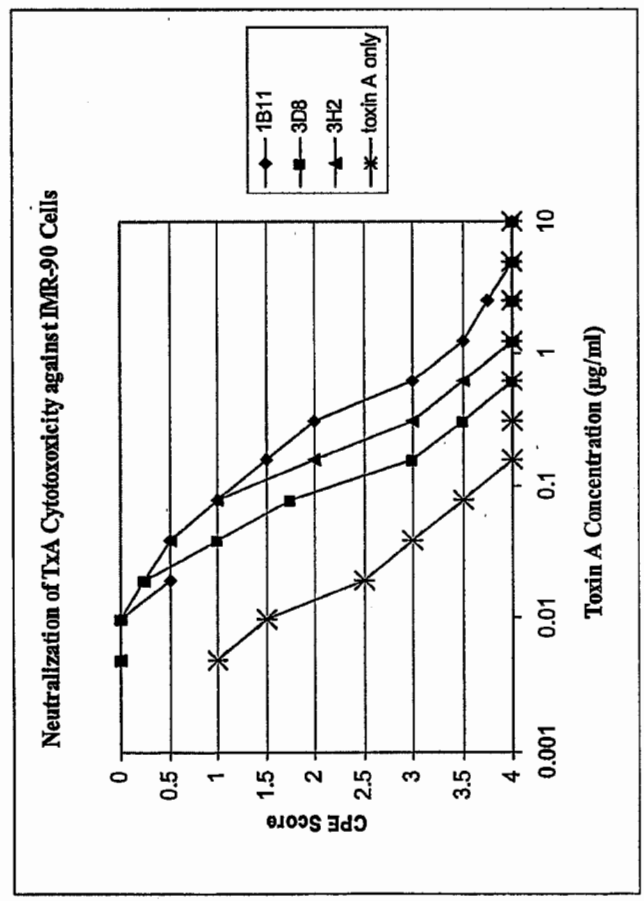
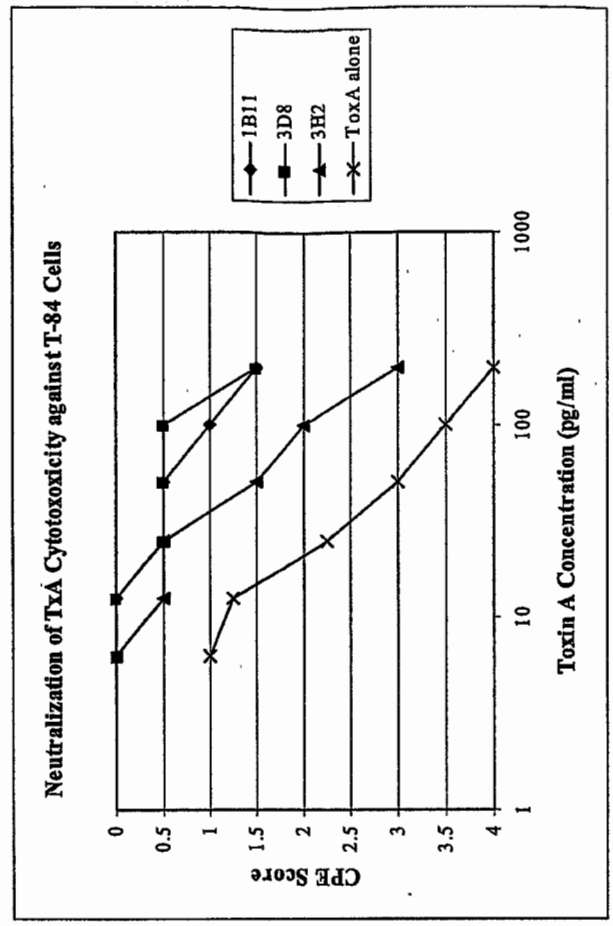


FIG. 6B



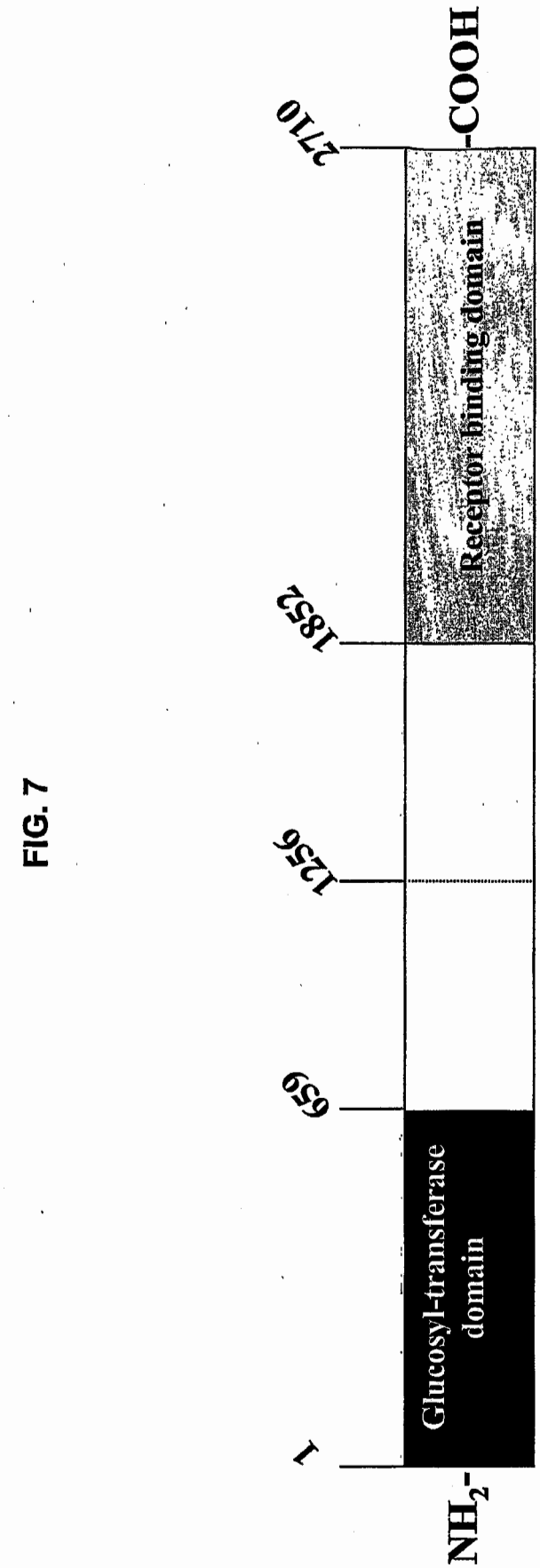
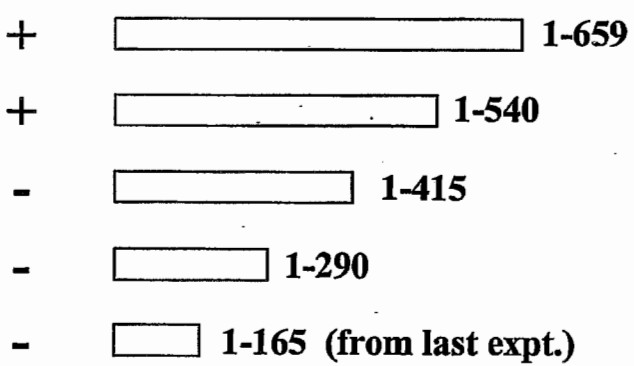

NH2-
1
659
1256
1852
2710
-COOH
Glucosyl-transferase domain
Receptor binding domain
Fragment 1
Fragment 2
Fragment 3
Fragment 4

FIG. 7

FIG. 8

FIG. 8A

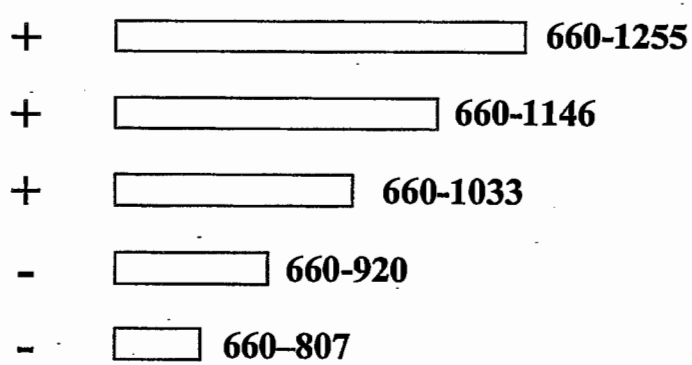
Fragment 1 - Enzymatic



1B11 epitope = AA 415-540.

FIG. 8B

Fragment 2 - Unknown



3H2 epitope = AA 920-1033

FIG. 9

Summary of mouse protection from Toxin A lethality by 1B11, 3D8, and 3H2.

TxA lot # (dose)	Control (Synagis)	3D8 % survivial (dose*)	3H2 % survival (dose*)	1B11, % survivial (dose*)
0100005 (100 ng)	10	80		50
0100005 (100 ng)	40	100		
0100005 (100 ng)	0		100	
0100005 (100 ng)	50		100	
0100005 (100 ng)	10	10-50	60	0-60
		(250, 50, 10)	(50)	(250, 50, 10)
1002047 (500 ng)	30	10-80	20-40	0-30
		(250, 25, 2.5)	(250, 25, 2.5)	(250, 25, 2.5)
1002047 (300 ng)	20	40, 30	90, 30	40, 20
		(500, 50)	(150, 15)	(500, 50)
1002047 (100 ng)	30	80, 70	90, 80	
		(250, 25)	(250, 25)	
1002047 (100 ng)	30	30, 40	100, 80	
		(250, 25)	(250, 25)	
1002047 (100 ng)	25	50, 10, 10	90, 40, 10	10, 10, 30
		(100, 10, 1)	(100, 10, 1)	(100, 10, 1)
1002047 (100 ng)	15	50, 50, 20	90, 60, 60	
		(100, 10, 1)	(100, 10, 1)	
1002047 (100 ng)	15	60, 60, 40***		
		(100, 10, 1)		
*HuMAb dose was 250 μg unless otherwize noted				
** HuMAbs were injected 24 hours prior to challenge				
*** expressed from CHO cells				

FIG. 10

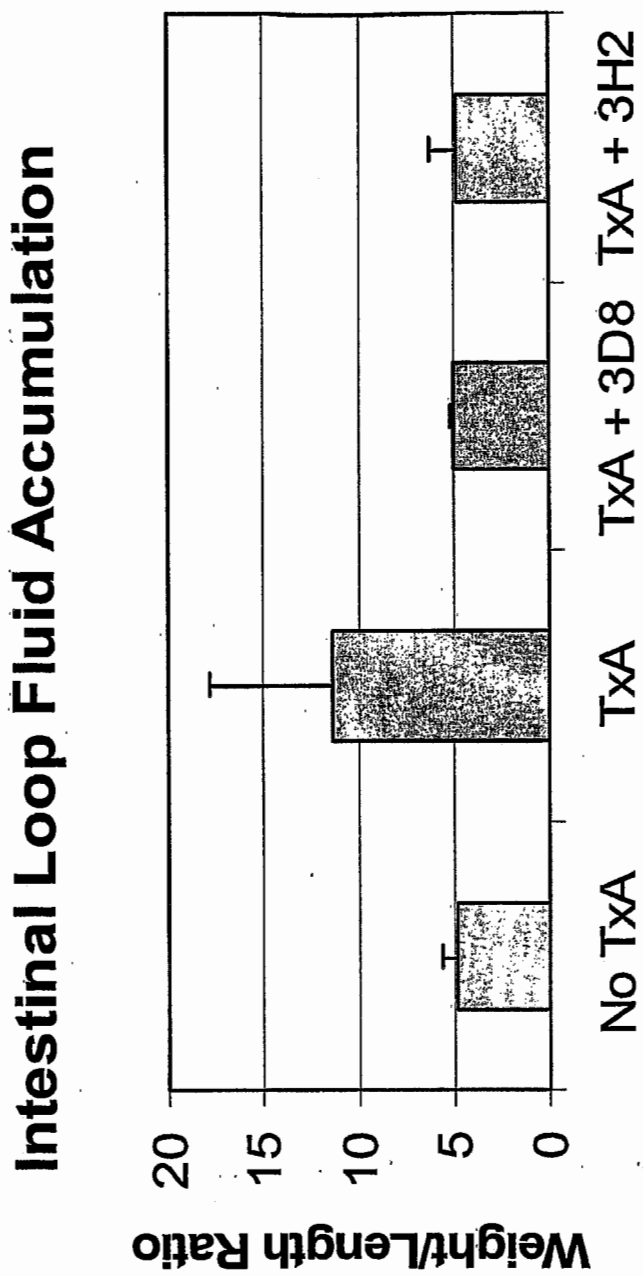

Intestinal Loop Fluid Accumulation
Weight/Length Ratio
20
15
10
5
0
No TxA
TxA
TxA + 3D8
TxA + 3H2
Treatment

FIG. 11A

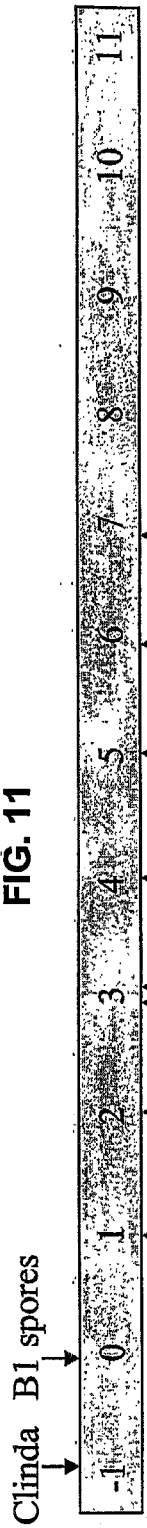


FIG. 11

FIG. 11B

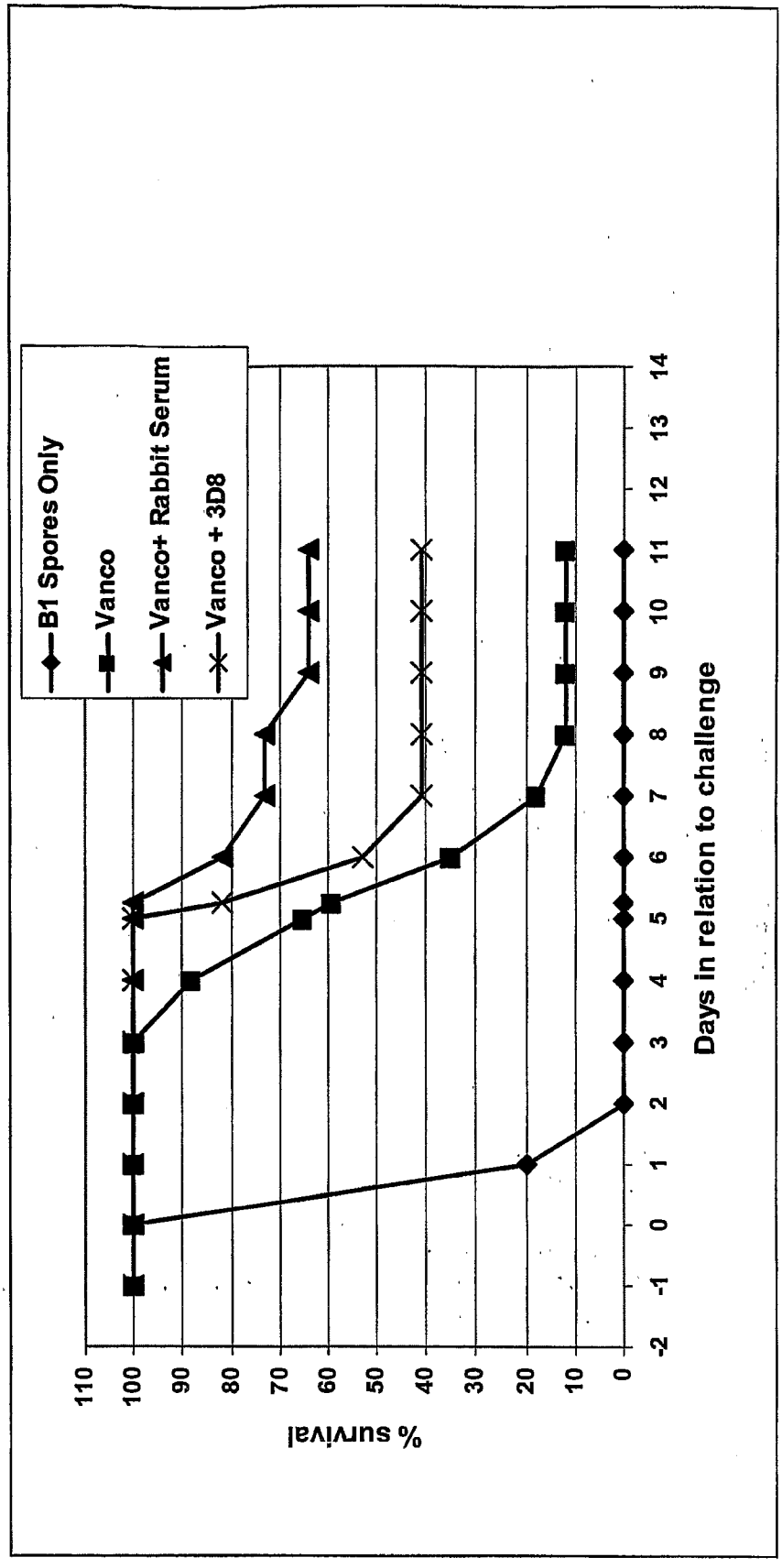


FIG. 12

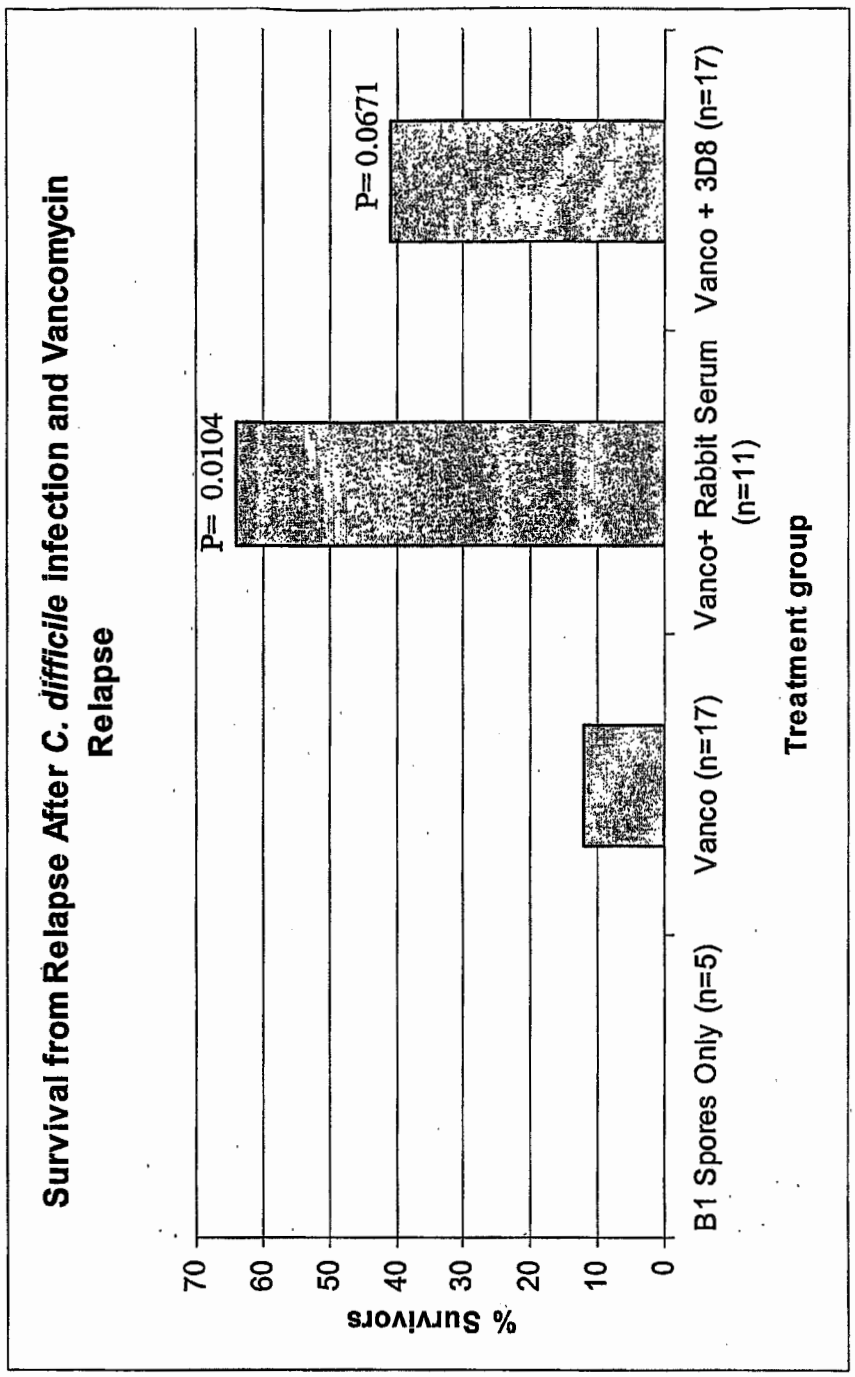

Survival from Relapse After C. difficile Infection and Vancomycin
Relapse
% Survivors
0
10
20
30
40
50
60
70
P= 0.0104
P= 0.0671
B1 Spores Only (n=5)
Vanco (n=17)
Vanco+ Rabbit Serum (n=11)
Vanco + 3D8 (n=17)
Treatment group

FIG. 13

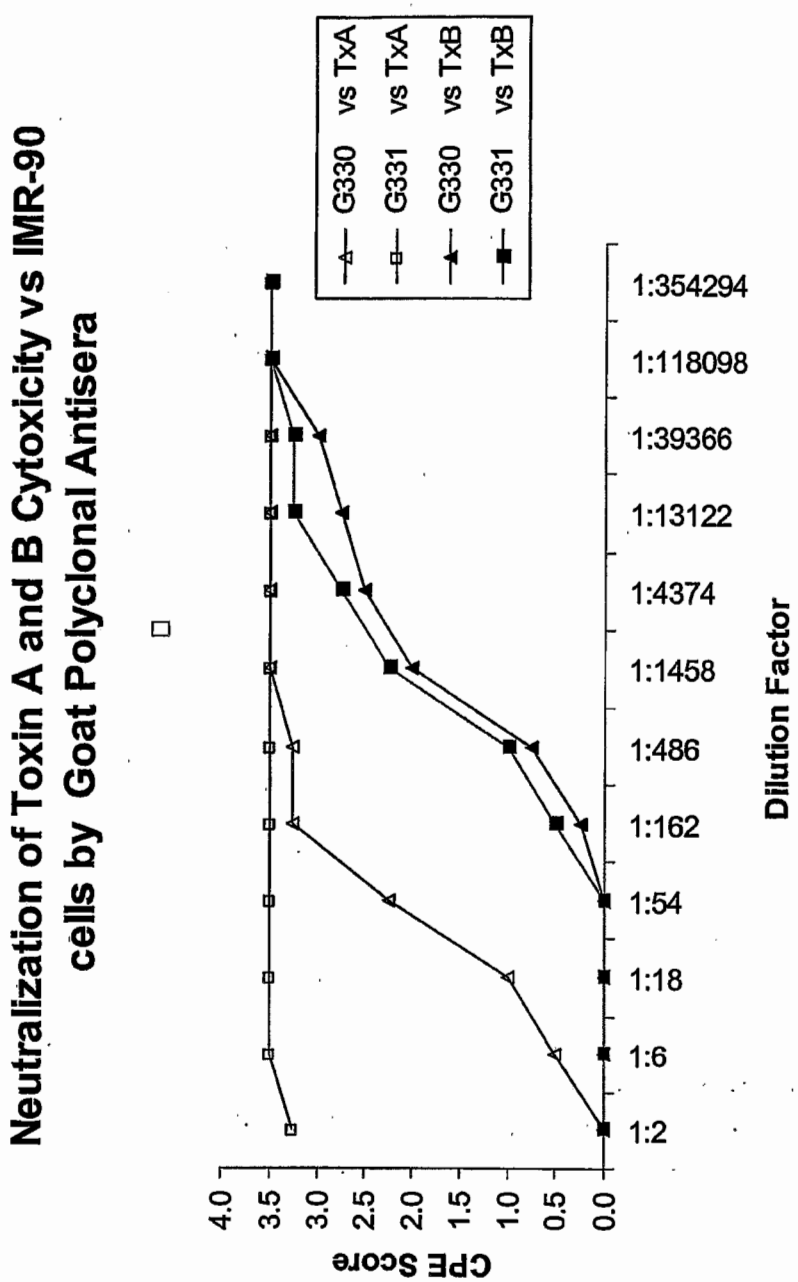

Neutralization of Toxin A and B Cytoxicity vs IMR-90 cells by Goat Polyclonal Antisera
G330 vs TxA
G331 vs TxA
G330 vs TxB
G331 vs TxB
CPE Score
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1:354294
1:118098
1:39366
1:13122
1:4374
1:1458
1:486
1:162
1:54
1:18
1:6
1:2
Dilution Factor

FIG. 14

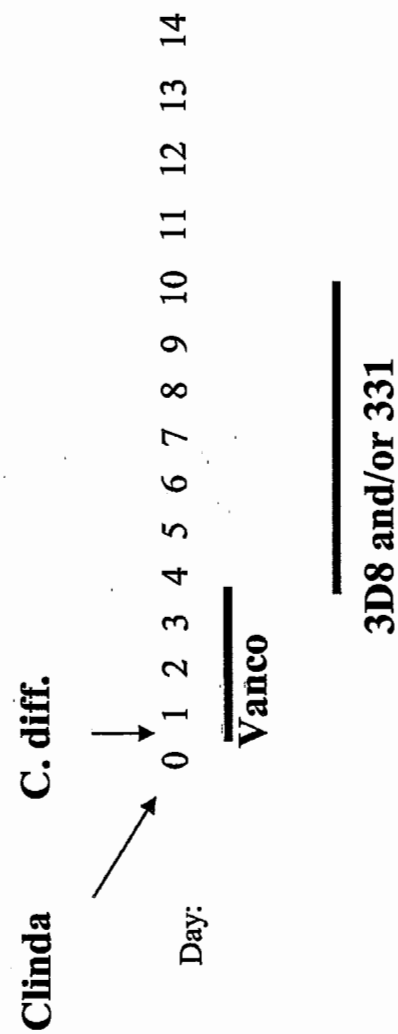

Clinda
C. diff.
Day:
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Vanco
3D8 and/or 331

FIG. 15

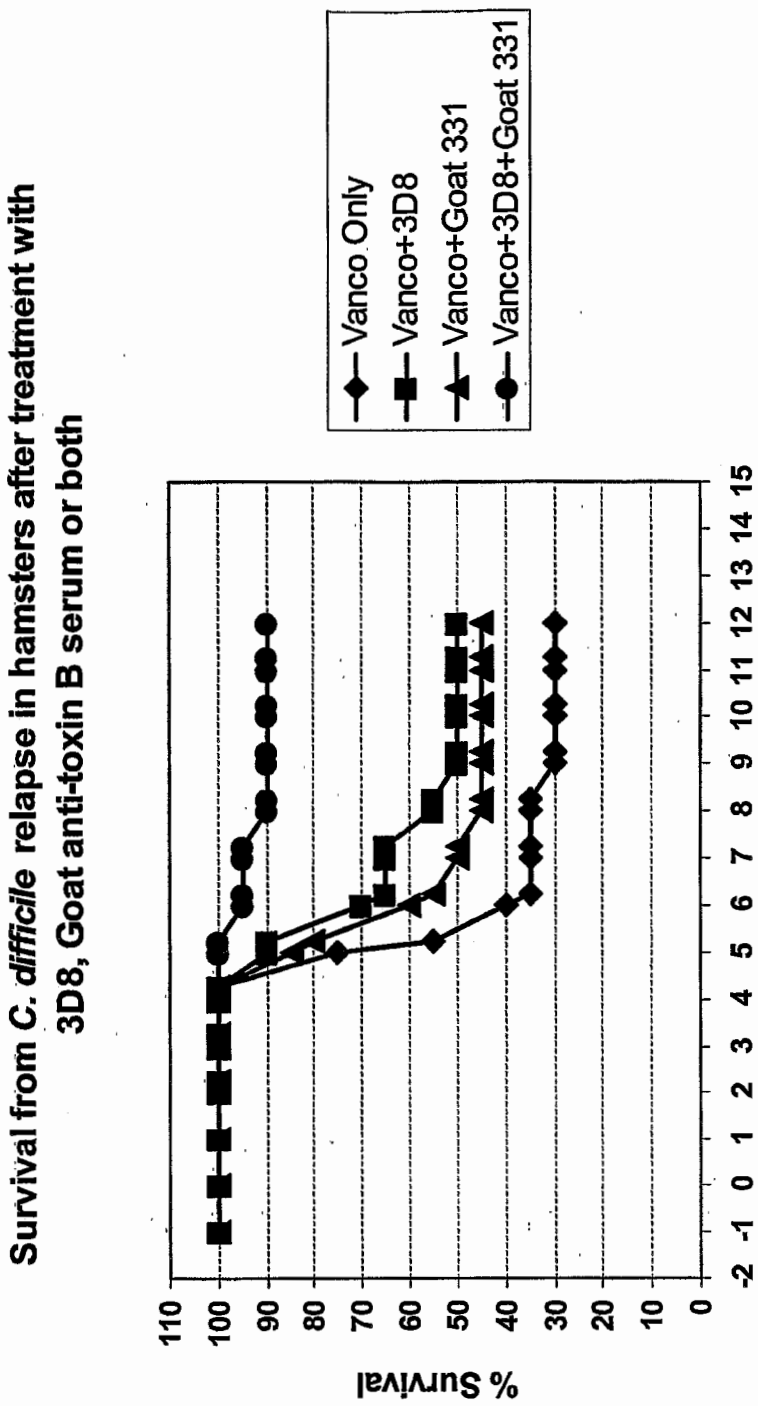

Survival from C. difficile relapse in hamsters after treatment with 3D8, Goat anti-toxin B serum or both
Vanco Only
Vanco+3D8
Vanco+Goat 331
Vanco+3D8+Goat 331
% Survival
110
100
90
80
70
60
50
40
30
20
10
0
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days in Relation to Challenge

FIG. 16

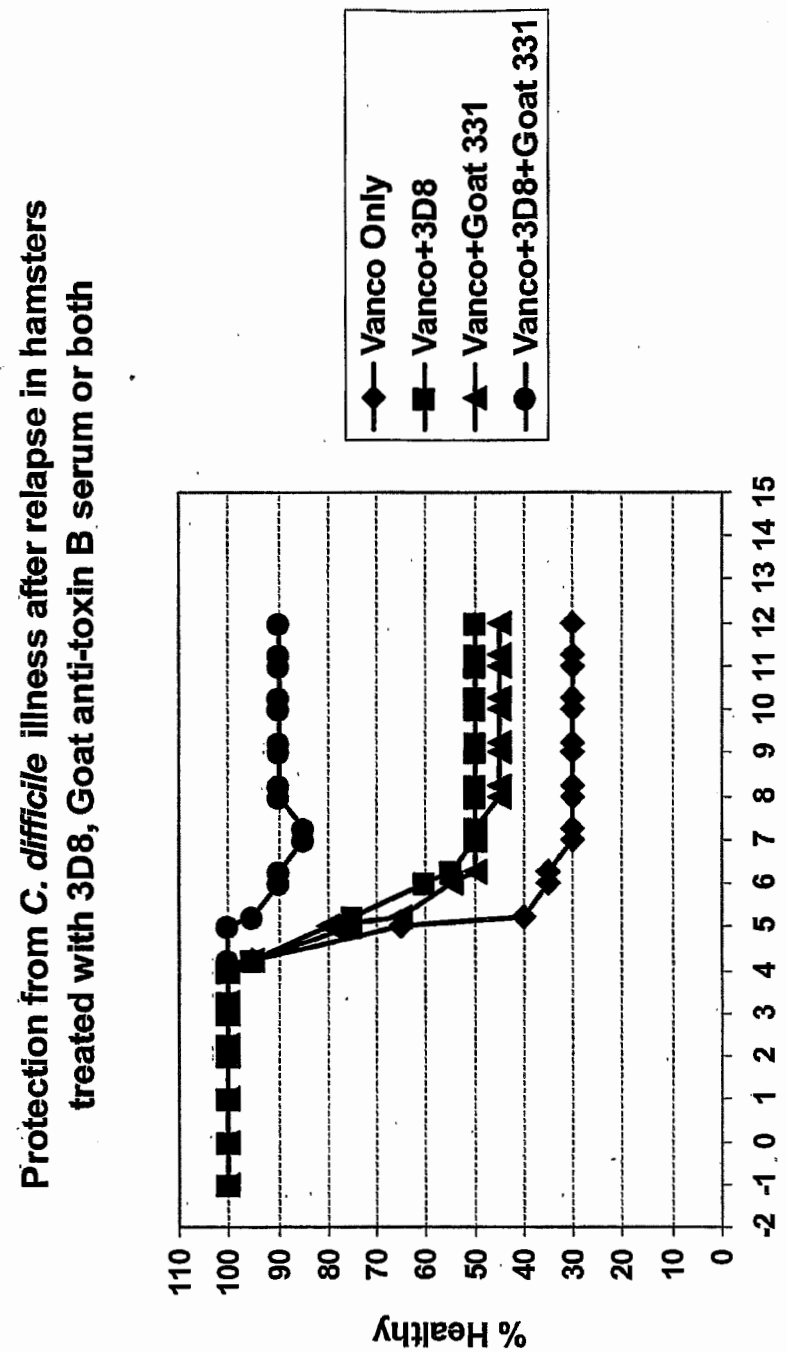

Protection from C. difficile illness after relapse in hamsters
treated with 3D8, Goat anti-toxin B serum or both
Vanco Only
Vanco+3D8
Vanco+Goat 331
Vanco+3D8+Goat 331
% Healthy
110
100
90
80
70
60
50
40
30
20
10
0
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days in Relation to Challenge

FIG. 17

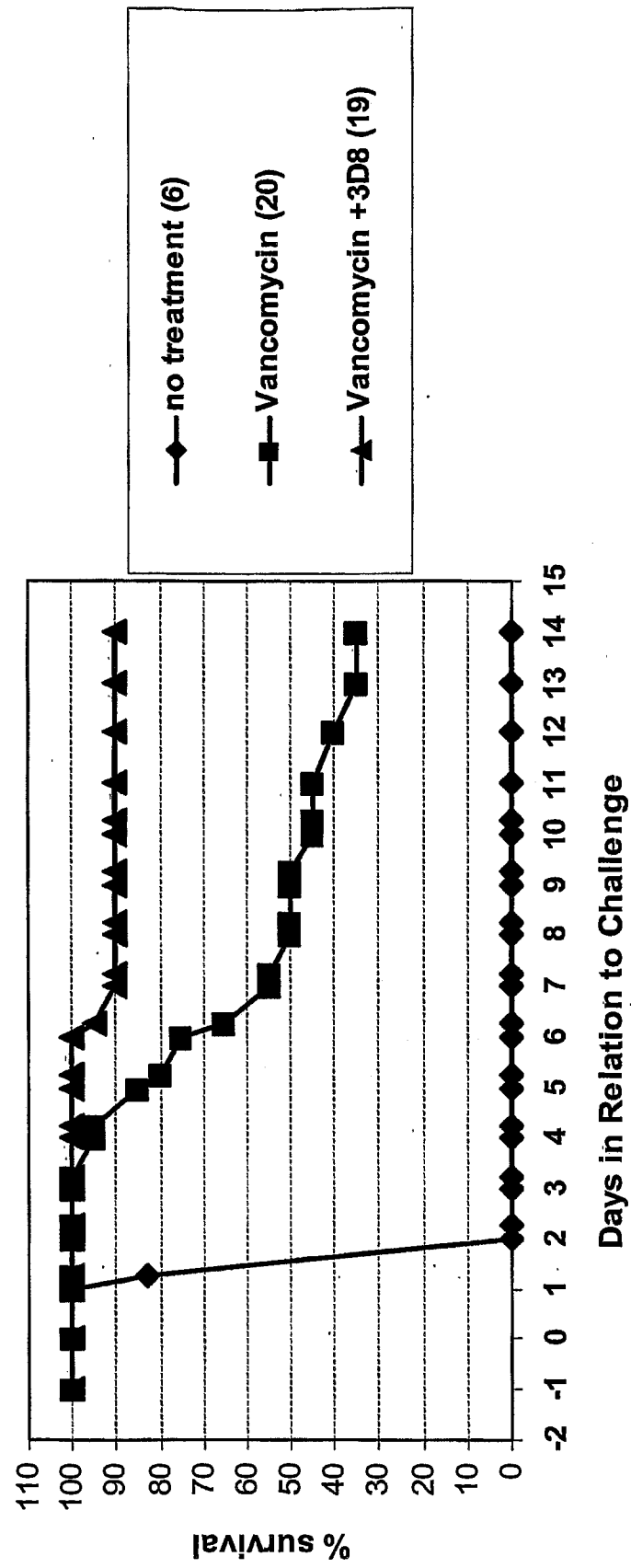

Hamster Immunization with Toxin B fragment 4 / Relapse
Protection (Survival) Experiment
no treatment (6)
Vancomycin (20)
Vancomycin +3D8 (19)
% survival
110
100
90
80
70
60
50
40
30
20
10
0
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days in Relation to Challenge

FIG. 18

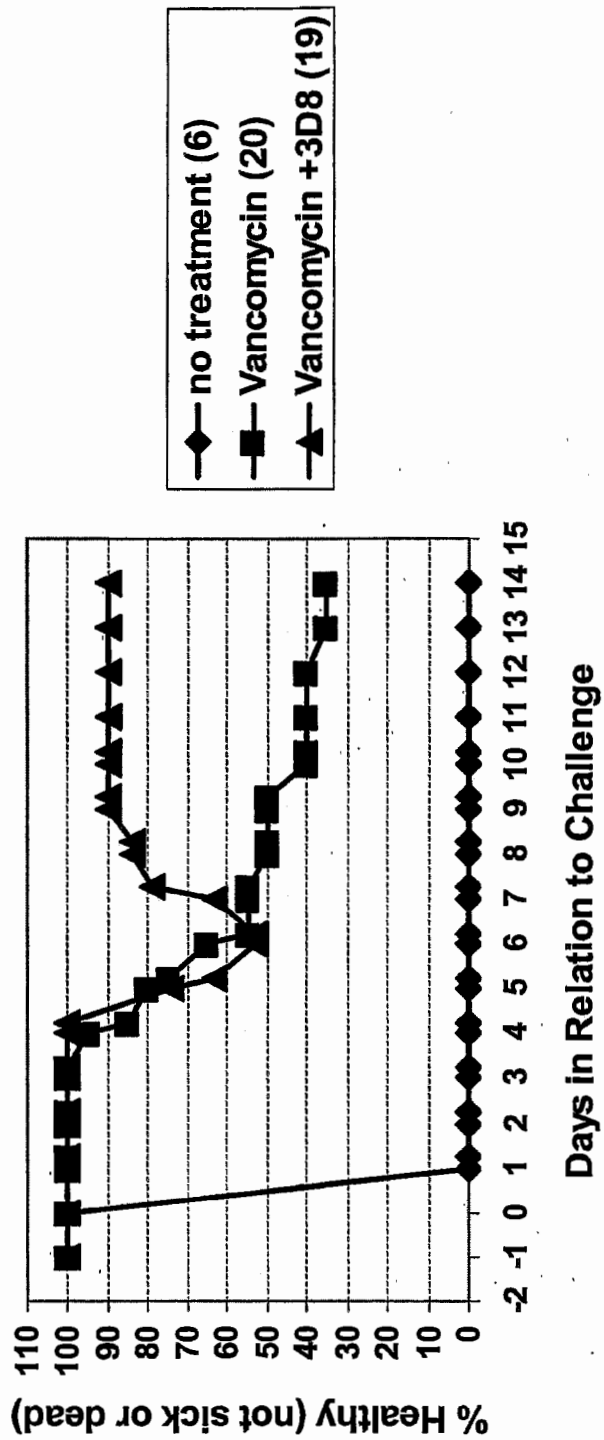

Hamster Immunization with Toxin B fragment 4 / Relapse
Protection Experiment (illness)
no treatment (6)
Vancomycin (20)
Vancomycin +3D8 (19)
% Healthy (not sick or dead)
110
100
90
80
70
60
50
40
30
20
10
0
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Days in Relation to Challenge

FIG. 19

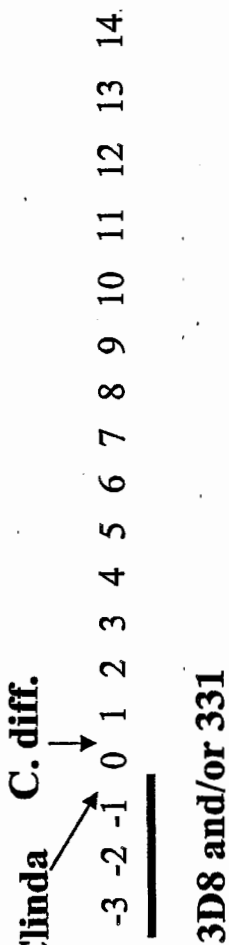


FIG. 20

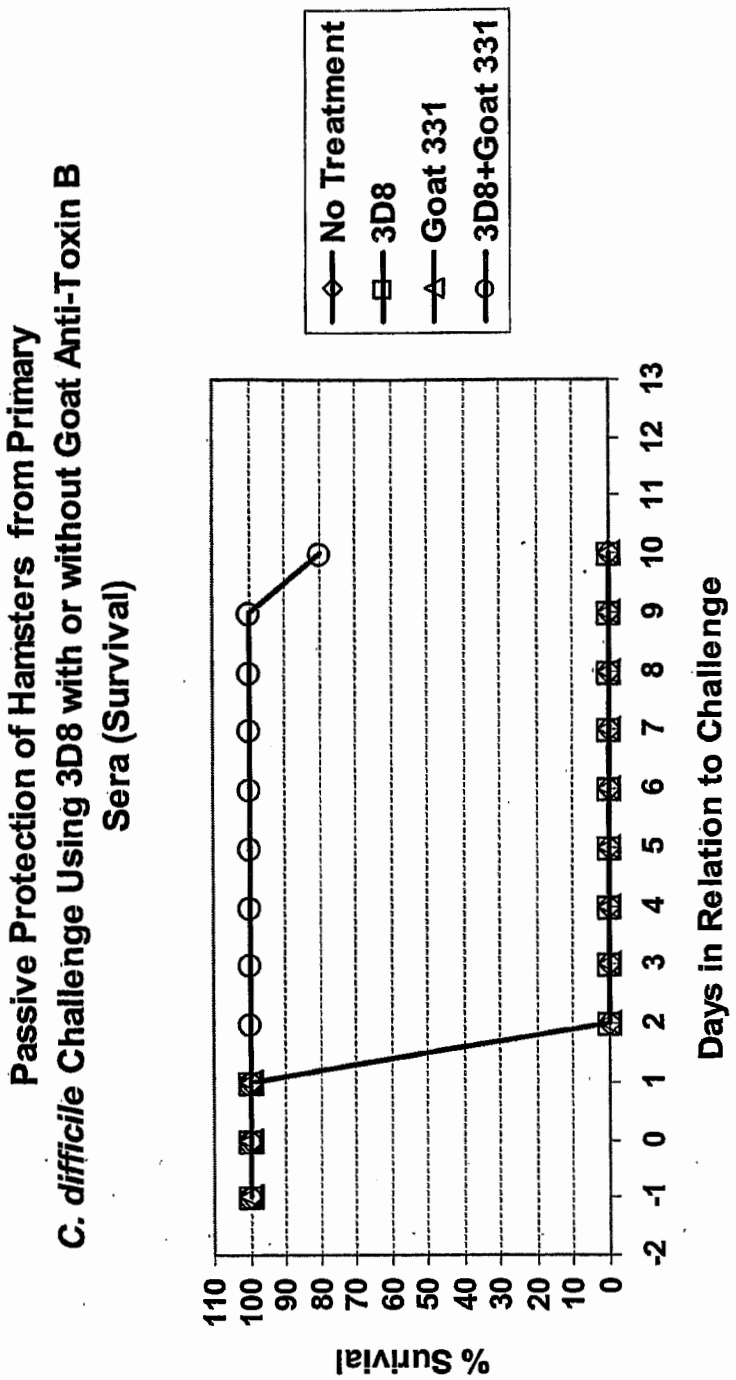
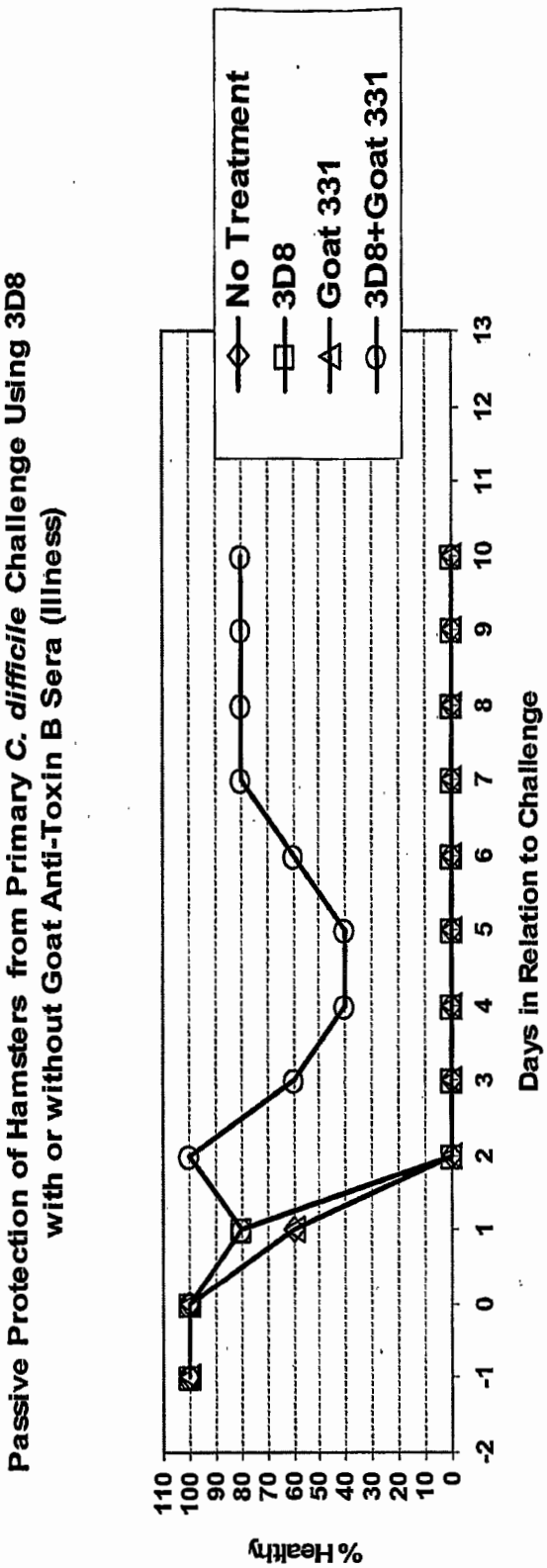

Passive Protection of Hamsters from Primary
C. difficile Challenge Using 3D8 with or without Goat Anti-Toxin B
Sera (Survival)
No Treatment
3D8
Goat 331
3D8+Goat 331
% Survival
110
100
90
80
70
60
50
40
30
20
10
0
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13
Days in Relation to Challenge

FIG. 21


Passive Protection of Hamsters from Primary C. difficile Challenge Using 3D8
with or without Goat Anti-Toxin B Sera (Illness)
No Treatment
3D8
Goat 331
3D8+Goat 331
% Healthy
110
100
90
80
70
60
50
40
30
20
10
0
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Days in Relation to Challenge

C. difficile Toxin A Amino Acid Sequence
(See also GenBank® GI No:98593, Acc. No:A37052)

MSLISKEELIKLAYSIRPRENEYKTILTNLDEYNKLTTNNNENKYLQLKKLNESIDVFMNKY
KTSSRNRALSNLKKDILKEVILIKNSNTSPVEKNLHFVWIGGEVSDIALEYIKQWADINAEY
NIKLWYDSEAFLVNTLKKAIVESSTTEALQLLEEEIQNPQFDNMKFYKKRMEFIYDRQKRFI
NYYKSQINKPTVPTIDDIIKSHLVSEYNRDETVLESYRTNSLRKINSNHGIDIRANSLFTEQ
ELLNIYSQELLNRGNLAAASDIVRLLALKNFGGVYLDVDMLPGIHSDLFKTISRPSSIGLDR
WEMIKLEAIMKYKKYINNYTSENFDKLDQQLKDNFKLIIESKSEKSEIFSKLENLNVSDLEI
KIAFALGSVINQALISKQGSYLTNLVIEQVKNRYQFLNQHLNPAIESDNNFTDTTKIFHDSL
FNSATAENSMFLTKIAPYLQVGFMPEARSTISLSGPGAYASAYYDFINLQENTIEKTLKASD
LIEFKFPENNLSQLTEQEINSLWSFDQASAKYQFEKYVRDYTGGSLSEDNGVDFNKNTALDK
NYLLNNKIPSNNVEEAGSKNYVHYIIQLQGDDISYEATCNLFSKNPKNSIIIQRNMNESAKS
YFLSDDGESILELNKYRIPERLKNKEKVKVTFIGHGKDEFNTSEFARLSVDSLSNEISSFLD
TIKLDISPKNVEVNLLGCNMFSYDFNVEETYPGKLLLSIMDKITSTLPDVNKNSITIGANQY
EVRINSEGRKELLAHSGKWINKEEAIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDI
KTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVS
DELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKE
ISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLY
AQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEH
DPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILH
DKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGS
GHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVP
GLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMP
TITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIK
KGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSY
SLLLSGDKNYLISNLSNTIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKD
SKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQV
KVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVG
KTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFS
YEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFE
YKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFK
SFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGK
KYYFDINTGAALTSYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVY
QSKFLTLNGKKYYFDNNSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAII
SKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYN
NNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKK
YYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGF
EYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAA
IHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHN
NNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKK
YYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGF
EYFAPANTDANNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAV
TGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDAN
NIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNF
YFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGW
QTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG (SEQ ID NO:41)

C. difficile Toxin B Amino Acid Sequence
(See also GenBank® GI No: 7476000, Acc. No: S70172)

MSLVNRKQLEKMANVRFRVQEDEYVAILDALEEYHNMSENTVVEKYLKLKDINSLTDTYIDT
YKKSGRNKALKKFKEYLVIEILELKNSNLTPVEKNLHFIWIGGQINDTAINYINQWKDVNSD
YNVNVFYDSNAFLINTLKKTIIESASNDTLESFRENLNDPEFNHTAFFRKRMQIIYDKQQNF
INYYKAQKEENPDLIIDDIVKTYLSNEYSKDIDELNAYIEESLNKVTENSGNDVRNFEEFKT
GEVFNLYEQESVERWNLAGASDILRVAILKNIGGVYLDVDMLPGIHPDLFKDINKPDSVKTA
VDWEEMQLEAIMKHKEYIPEYTSKHFDTLDEEVQSSFESVLASKSDKSEIFLPLGDIEVSPL
EVKIAFAKGSIINQALISAKDSYCSDLLIKQIQNRYKILNDTLGPIISQGNDFNTTMNNFGE
SLGAIANEENISFIAKIGSYLRVGFYPEANTTITLSGPTIYAGAYKDLLTFKEMSIDTSILS
SELRNFEFPKVNISQATEQEKNSLWQFNEERAKIQFEEYKKNYFEGALGEDDNLDFSQNTVT
DKEYLLEKISSSTKSSEGGYVHYIVQLQGDKISYEAACNLFAKNPYDSILFQRNIEDSEVAY
YYNPTDSEIQEIDKYRIPDRISDRPKIKLTFIGHGKAEFNTDIFAGLDVDSLSSEIETAIGL
AKEDISPKSIEINLLGCNMFSYSVNVEETYPGKLLLRVKDKVSELMPSMSQDSIIVSANQYE
VRINSEGRRELLDHSGEWINKEESIIKDISSKEYISFNPKENKIIVKSKNLPELSTLLQEIR
NNSNSSDIELEEKVMLAECEINVISNIETQVVEERIEEAKSLTSDSINYIKNEFKLIESISE
ALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEI
SKIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYA
QLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELSETSD
PLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELVLRD
KATKVVDYFKHVSLVETEGVFTLLDDKVMMQQDDLVISEIDFNNNSIVLGKCEIWRMEGGSG
HTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPG
LRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVP
IITTEYIREKLSYSFYGSGGTYALPLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIK
KGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSY
KLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSE
LPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKT
IKLNSVHLDESGVAEILKFMNRKGSTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFI
ISGTTSIGQFEFICDENNNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTV
INFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNIND
LSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIIL
SFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTV
GDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGE
AIDFTGKLIIDENIYYFEDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDDKYYFNSDG
VMQKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNED
LGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDG
QYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYK
YFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFVPETKKA
CKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEIQFGYINIEDKMFYFGEDGVMQI
GVFNTPDGFKYFAHQNTLDENFEGESINYTGWLGLDEKRYYFTDEYIAATGSVIIDGEEYYF
DPDTAQLVISE

Fig. 24

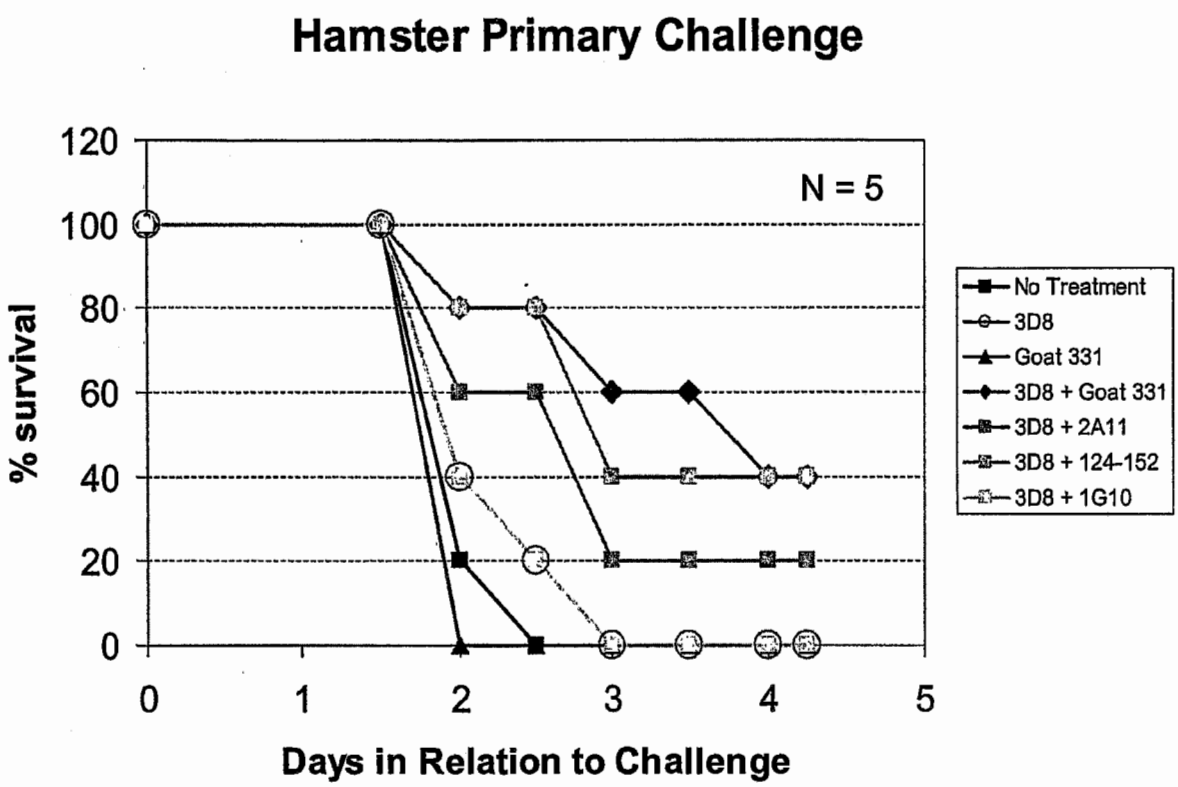

Hamster Primary Challenge
N = 5
% survival
120
100
80
60
40
20
0
0
1
2
3
4
5
Days in Relation to Challenge
No Treatment
3D8
Goat 331
3D8 + Goat 331
3D8 + 2A11
3D8 + 124-152
3D8 + 1G10

Fig. 25

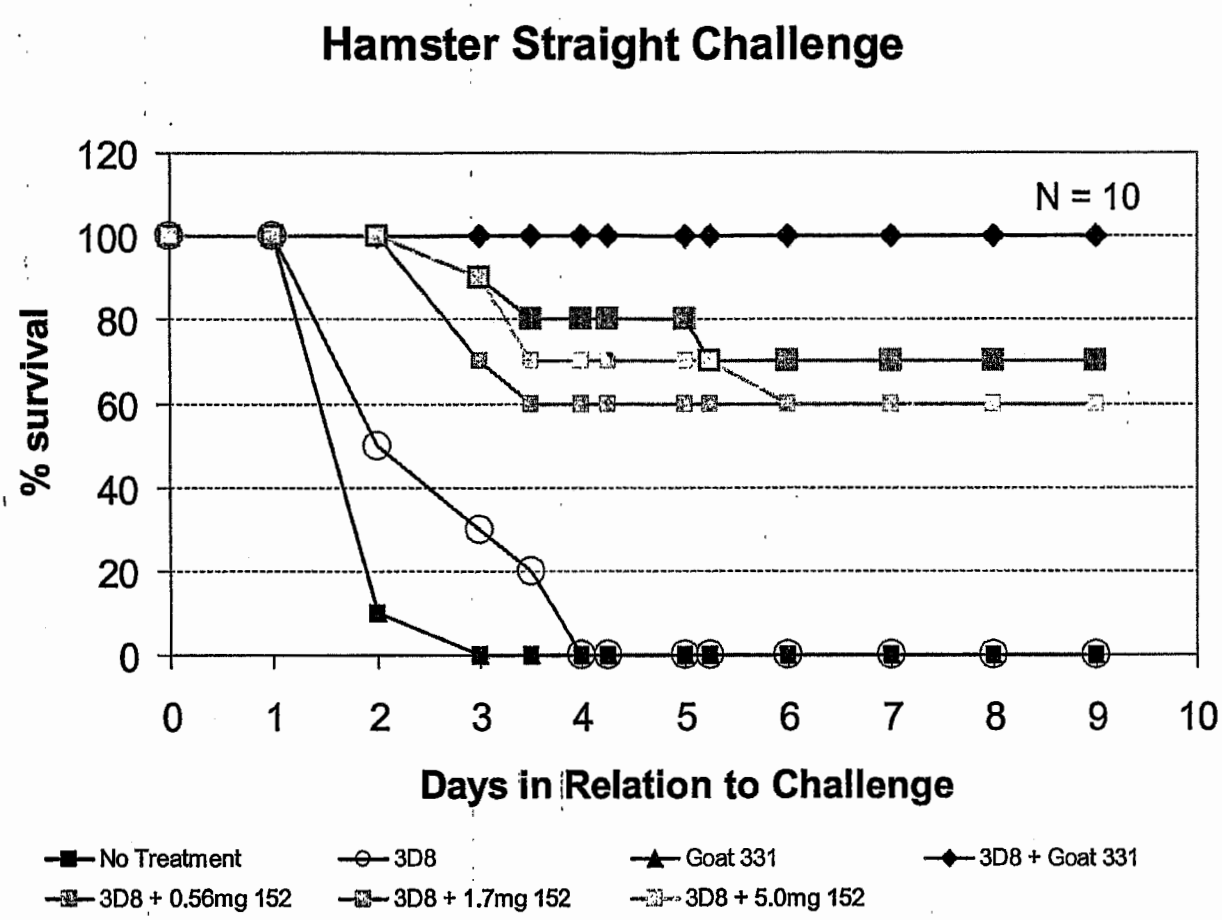

Hamster Straight Challenge
N = 10
% survival
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
Days in Relation to Challenge
No Treatment
3D8
Goat 331
3D8 + Goat 331
3D8 + 0.56mg 152
3D8 + 1.7mg 152
3D8 + 5.0mg 152

Fig. 26

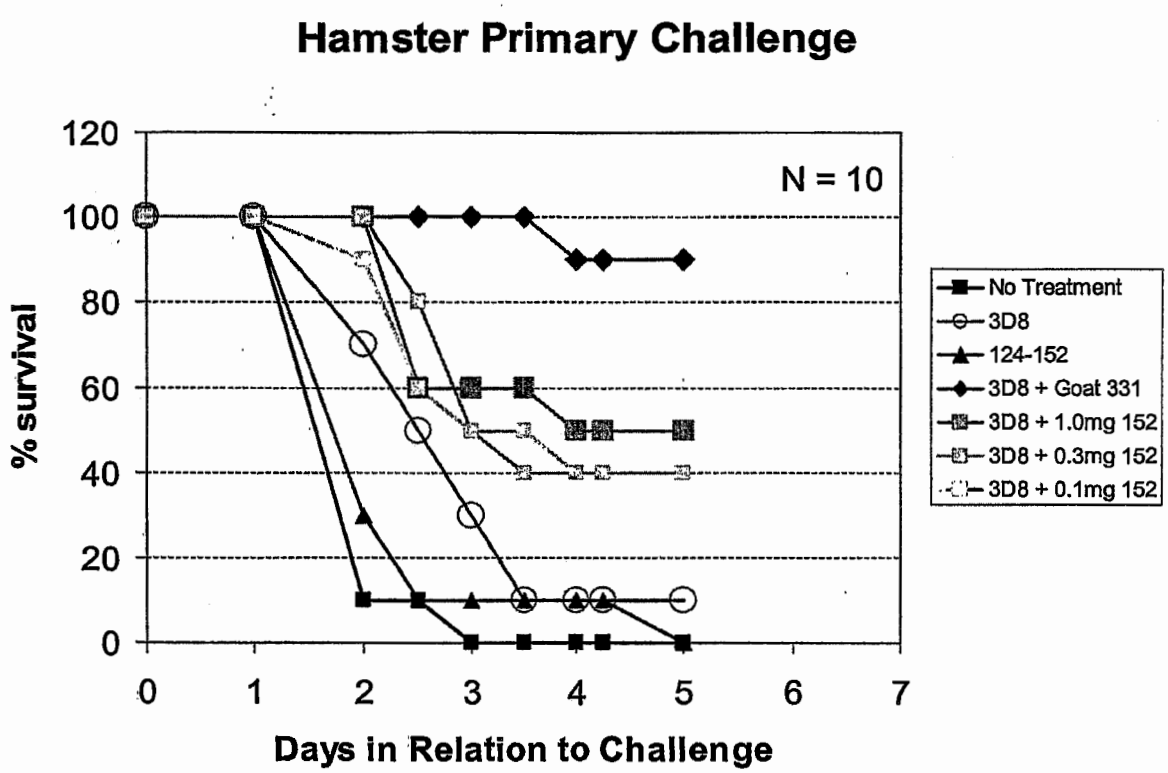
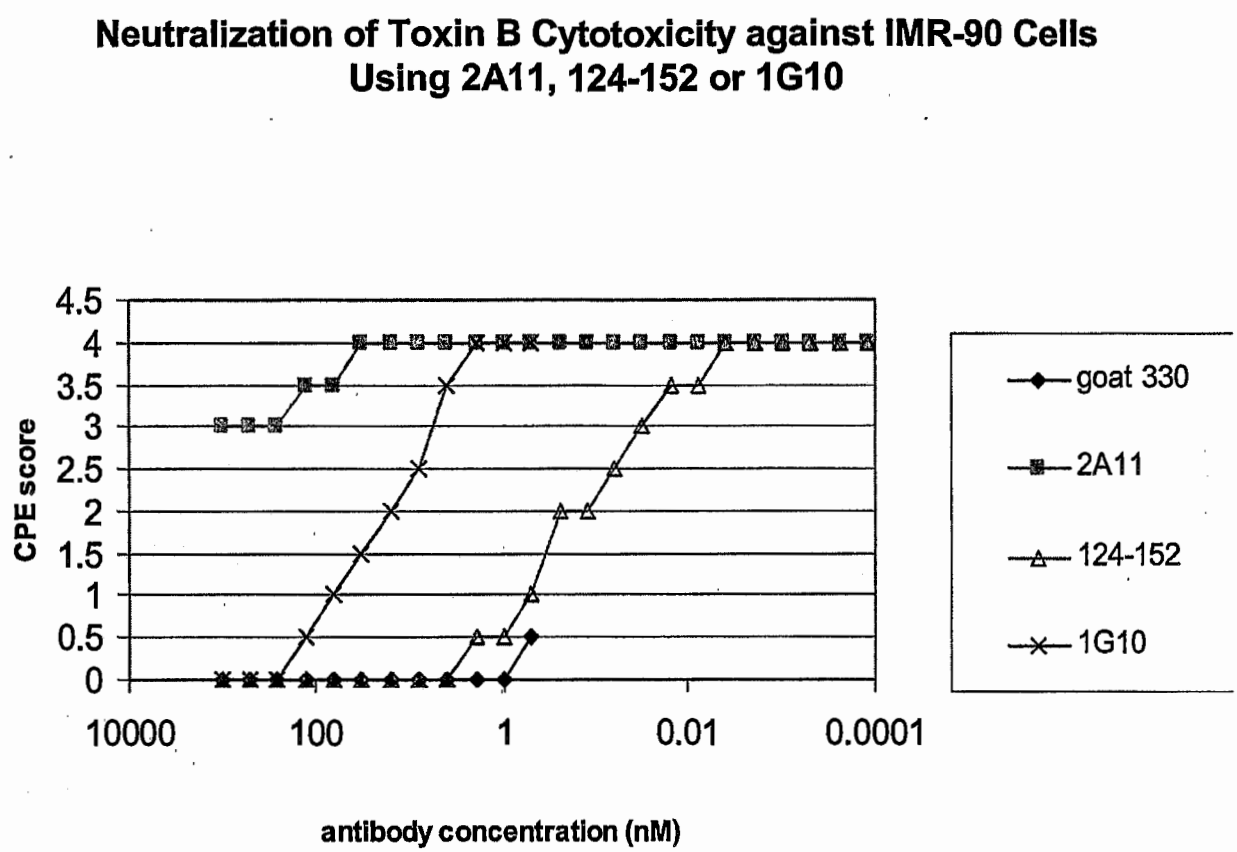

Hamster Primary Challenge
N = 10
% survival
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
Days in Relation to Challenge
No Treatment
3D8
124-152
3D8 + Goat 331
3D8 + 1.0mg 152
3D8 + 0.3mg 152
3D8 + 0.1mg 152

Fig. 27


Neutralization of Toxin B Cytotoxicity against IMR-90 Cells
Using 2A11, 124-152 or 1G10
CPE score
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
10000
100
1
0.01
0.0001
antibody concentration (nM)
goat 330
2A11
124-152
1G10

Anti-CDTox B 124-152 VH

Fig. 28

V segment: 5-51
D segment: 7-27
J segment: JH3b

```
   E   V   Q   L   V   Q   S   G   A   E   V   K   K   S   G   E   S   L
 1 GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG TCC GGG GAG TCT CTG

                                                    CDR1 SEQ ID NO:62 (aa) 63 (nt)
                                                    ~~~~~~~~~~~~~~~~~~
   K   I   S   C   K   G   S   G   Y   S   F   T   S   Y   W   I   G   W
55 AAG ATC TCC TGT AAG GGT TCT GGA TAC AGC TTT ACC AGC TAC TGG ATC GGC TGG

                                                        CDR2
                                                    ~~~~~~~~~~~~~~~~~~
   V   R   Q   M   P   G   K   G   L   E   W   M   G   I   F   Y   P   G
109 GTG CGC CAG ATG CCC GGG AAG GGC CTG GAG TGG ATG GGG ATC TTC TAT CCT GGT

                    CDR2    SEQ ID NO:64 (aa) 65 (nt)
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   D   S   S   T   R   Y   S   P   S   F   Q   G   Q   V   T   I   S   A
163 GAC TCT AGT ACC AGA TAC AGC CCG TCC TTC CAA GGC CAG GTC ACC ATC TCA GCC

   D   K   S   V   N   T   A   Y   L   Q   W   S   S   L   K   A   S   D
217 GAC AAG TCC GTC AAC ACC GCC TAC CTG CAG TGG AGC AGC CTG AAG GCC TCG GAC

                                            CDR3  SEQ ID NO:66 (aa) 67 (nt)
                                        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   T   A   M   Y   Y   C   A   R   R   R   N   W   G   N   A   F   D   I
271 ACC GCC ATG TAT TAC TGT GCG AGA CGT CGA AAC TGG GGA AAT GCT TTT GAT ATC

   W   G   Q   G   T   M   V   T   V   S   S    SEQ ID NO:54
325 TGG GGC CAA GGG ACA ATG GTC ACC GTC TCT TCA  SEQ ID NO:55
```

>CDTox B, 124-152, VH-NT with leader SEQ ID NO:57
ATGGGGTCAACCGCCATCCTCGCCCTCCTCCTGGCTGTTCTCCAAGGAGTCTGTGCCGAGGTGCA
GCTGGTGCAGTCTGGAGCAGAGGTGAAAAAGTCCGGGGAGTCTCTGAAGATCTCCTGTAAGGGTT
CTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTGCGCCAGATGCCCGGGAAGGGCCTGGAG
TGGATGGGGATCTTCTATCCTGGTGACTCTAGTACCAGATACAGCCCGTCCTTCCAAGGCCAGGT
CACCATCTCAGCCGACAAGTCCGTCAACACCGCCTACCTGCAGTGGAGCAGCCTGAAGGCCTCGG
ACACCGCCATGTATTACTGTGCGAGACGTCGAAACTGGGGAAATGCTTTTGATATCTGGGGCCAA
GGGACAATGGTCACCGTCTCTTCA

>CDTox B, 124-152, VH-AA with leader SEQ ID NO:56
MGSTAILALLLAVLQGVCAEVQLVQSGAEVKKSGESLKISCKGSGYSFTSYWIGWVRQMPGKGLE
WMGIFYPGDSSTRYSPSFQGQVTISADKSVNTAYLQWSSLKASDTAMYYCARRRNWGNAFDIWGQ
GTMVTVSS

Fig. 29

Anti-CDTox B 124-152 VK

V segment: A27
J segment: JK1

SEQ ID NO:58

```
      E   I   V   L   T   Q   S   P   G   T   L   S   L   S   P   G   E   R
    1 GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT TTG TCT CCA GGG GAA AGA
```

SEQ ID NO:59

CDR1 SEQ ID NO:68 (aa) 69 (nt)

```
      A   T   L   S   C   R   A   S   Q   S   V   S   S   S   Y   L   A   W
   55 GCC ACC CTC TCC TGC AGG GCC AGT CAG AGT GTT AGC AGC AGC TAC TTA GCC TGG
```

CDR2

```
      Y   Q   Q   K   P   G   Q   A   P   R   L   L   I   Y   G   A   S   S
  109 TAC CAG CAG AAA CCT GGC CAG GCT CCC AGG CTC CTC ATC TAT GGT GCA TCC AGC
```

CDR2 SEQ ID NO:70 (aa) 71 (nt)

```
      R   A   T   G   I   P   D   R   F   S   G   S   G   S   G   T   D   F
  163 AGG GCC ACT GGC ATC CCA GAC AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC
```

CDR3

```
      T   L   T   I   S   R   L   E   P   E   D   F   A   V   Y   Y   C   Q
  217 ACT CTC ACC ATC AGC AGA CTG GAG CCT GAA GAT TTT GCA GTG TAT TAC TGT CAG
```

CDR3 SEQ ID NO:72 (aa) 73 (nt)

```
      Q   Y   G   S   S   T   W   T   F   G   Q   G   T   K   V   E   I   K
  271 CAG TAT GGT AGC TCA ACG TGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
```

>CDTox B, 124-152, VK-NT with leader SEQ ID NO:61

ATGGAAACCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGATACCACCGGAGAAAT
TGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCA
GGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCC
AGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGG
GTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACT
GTCAGCAGTATGGTAGCTCAACGTGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA

>CDTox B, 124-152, VK-AA with leader SEQ ID NO:60

METPAQLLFLLLLWLPDTTGEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQA
PRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSTWTFGQGTKVEIK

Fig. 30

Anti-CDTox B 124-152 VH region

SEQ ID NO:75

CDR1

```
5-51 germline  E V Q L V Q S G A E V K K P G E S L K I S C K G S G Y S F T S Y W I G W
124-152 VH     - - - - - - - - - - - - - S - - - - - - - - - - - - - - - - - - - - -
```

SEQ ID NO:76

CDR2

```
5-51 germline  V R Q M P G K G L E W M G I I Y P G D S D T R Y S P S F Q G Q V T I S A
124-152 VH     - - - - - - - - - - - - - F - - - - - S - - - - - - - - - - - - - - -
```

SEQ ID NO:77

CDR3

```
5-51 germline  D K S I S T A Y L Q W S S L K A S D T A M Y Y C A R
JH3b germline                                                   A F D I
124-152 VH     - - - V N - - - - - - - - - - - - - - - - - - - R R N W G N - - - -
```

SEQ ID NO:74

```
JH3b germline  W G Q G T M V T V S S
124-152 VH     - - - - - - - - - - (JH3b)
```

Fig. 31

Anti-CDTox B 124-152 VK region

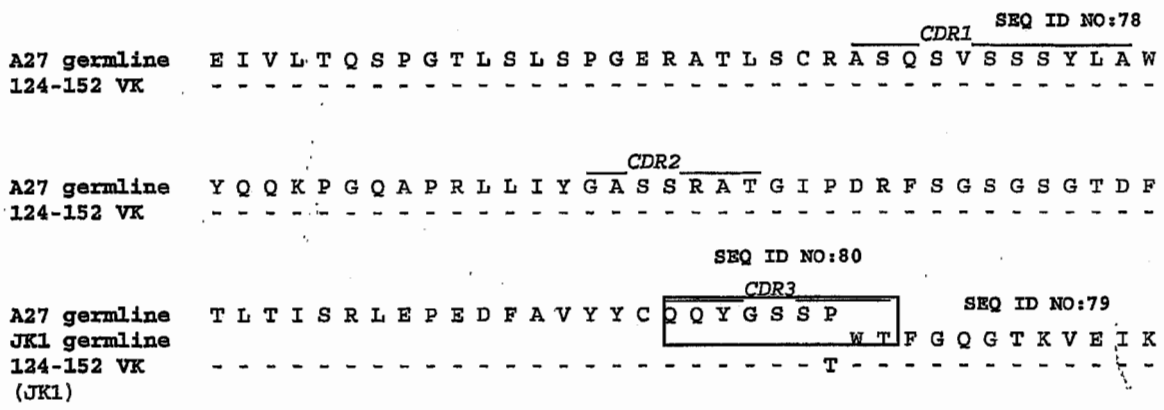
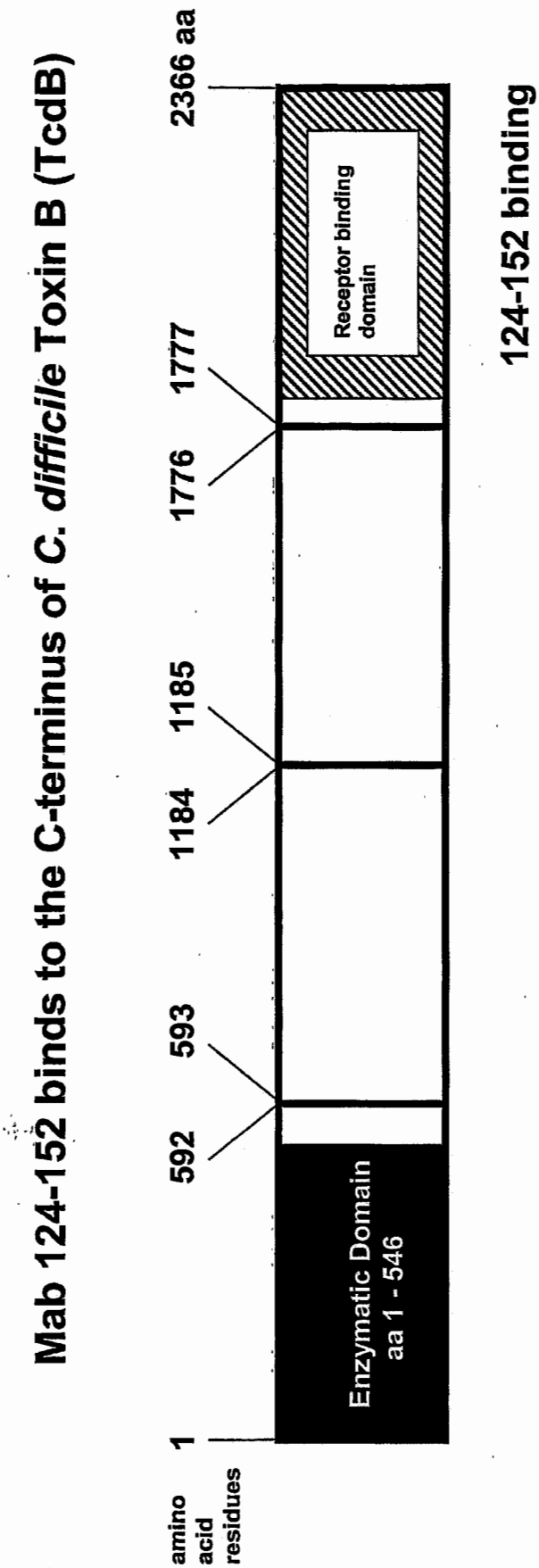


FIG. 32


Mab 124-152 binds to the C-terminus of C. difficile Toxin B (TcdB)
amino acid residues
1
592
593
1184
1185
1776
1777
2366 aa
Enzymatic Domain aa 1 - 546
Receptor binding domain
124-152 binding