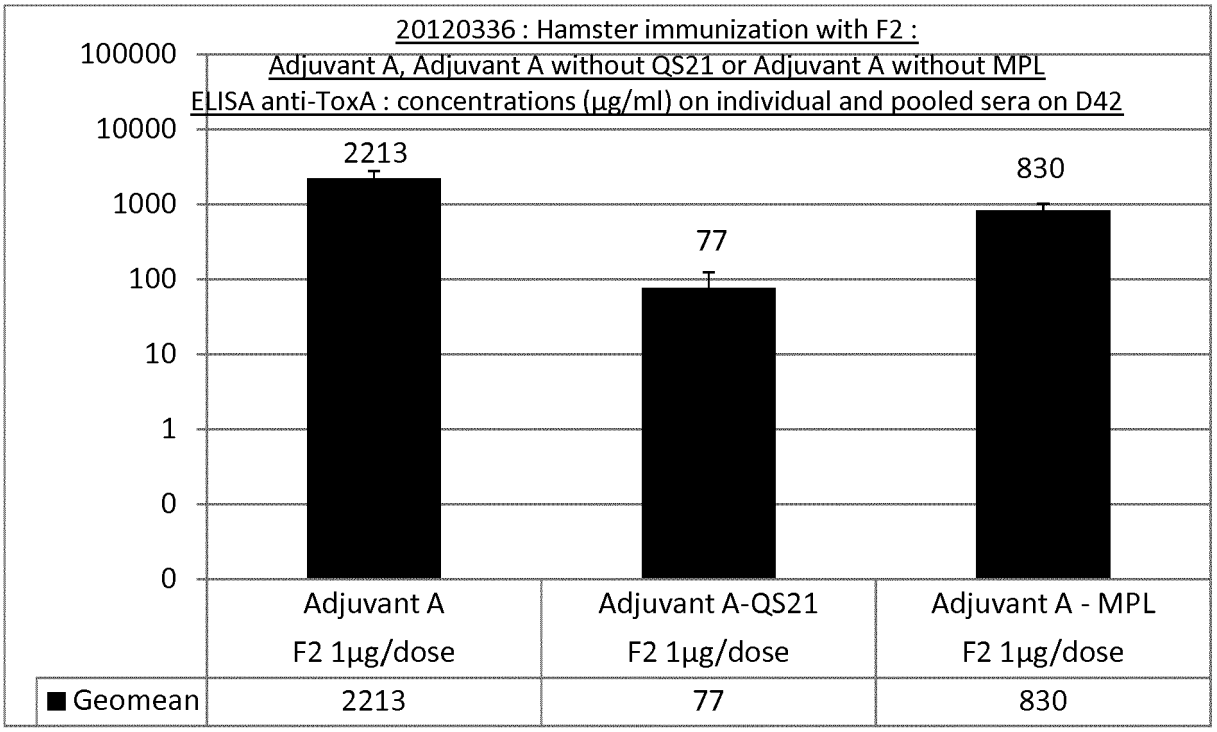
Figures

Figure 1



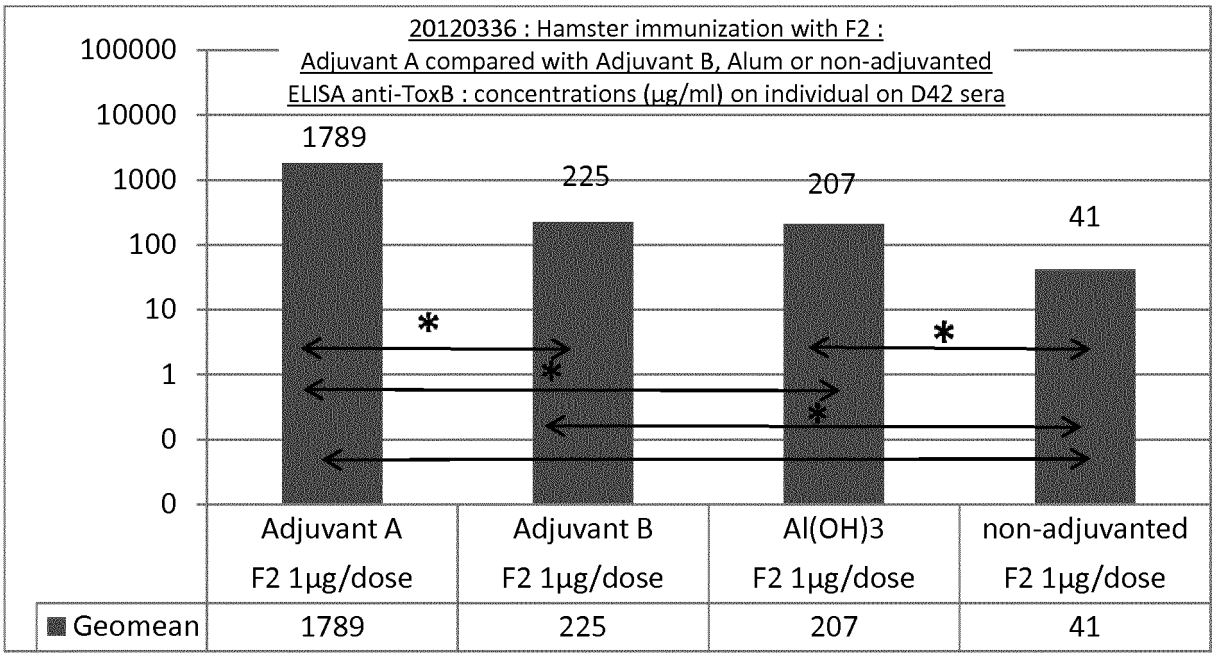
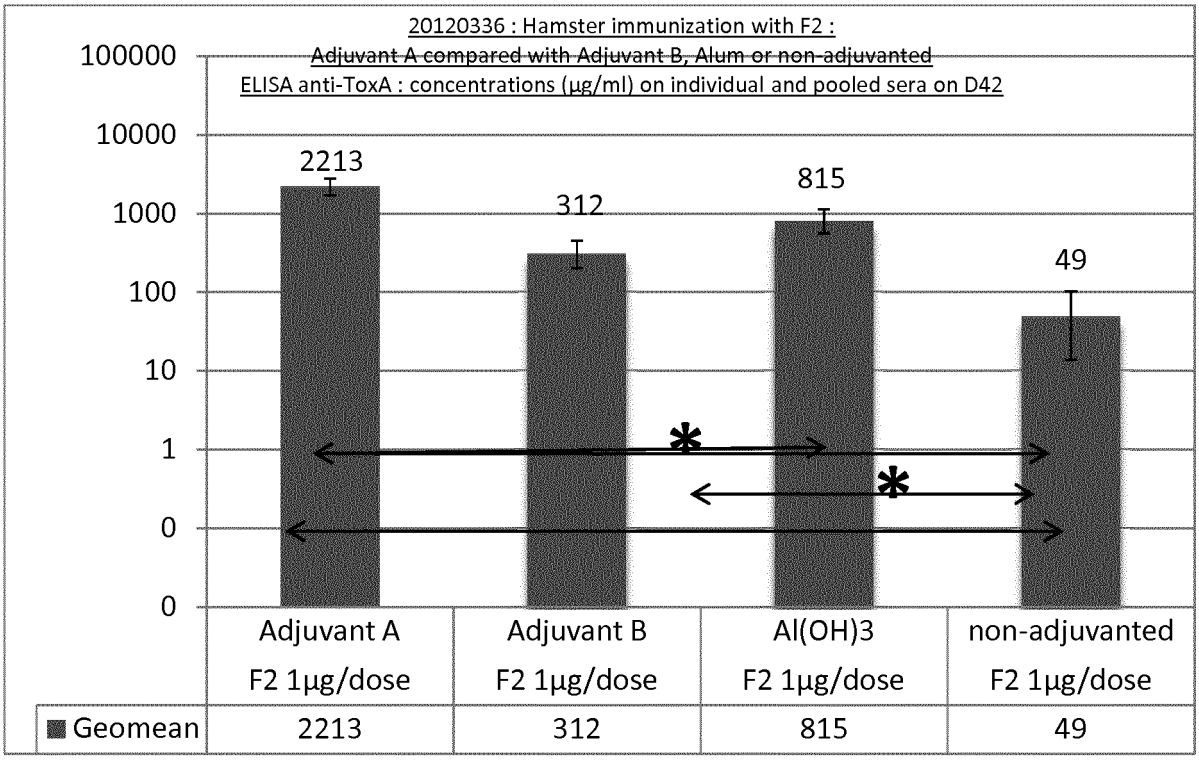


Figure 2


20120336 : Hamster immunization with F2 :
Adjuvant A compared with Adjuvant B, Alum or non-adjuvanted
ELISA anti-ToxA : concentrations (µg/ml) on individual and pooled sera on D42
100000
10000
1000
100
10
1
0
0
2213
312
815
49
*
*
Adjuvant A F2 1µg/dose
Adjuvant B F2 1µg/dose
Al(OH)3 F2 1µg/dose
non-adjuvanted F2 1µg/dose
Geomean
2213
312
815
49

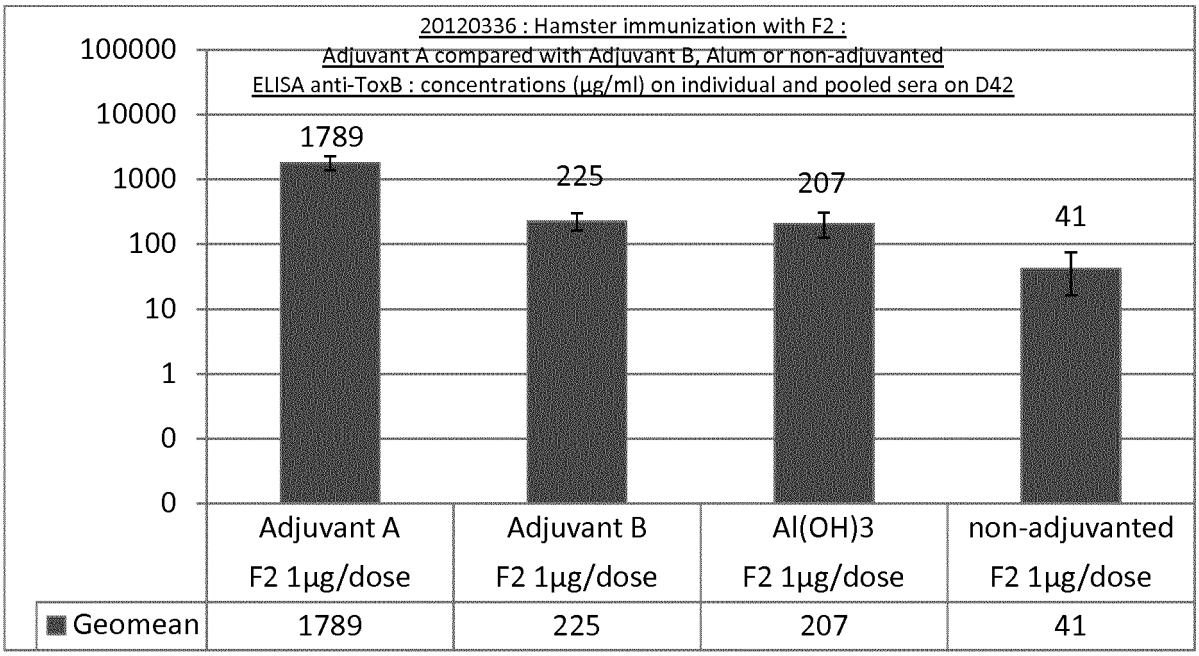
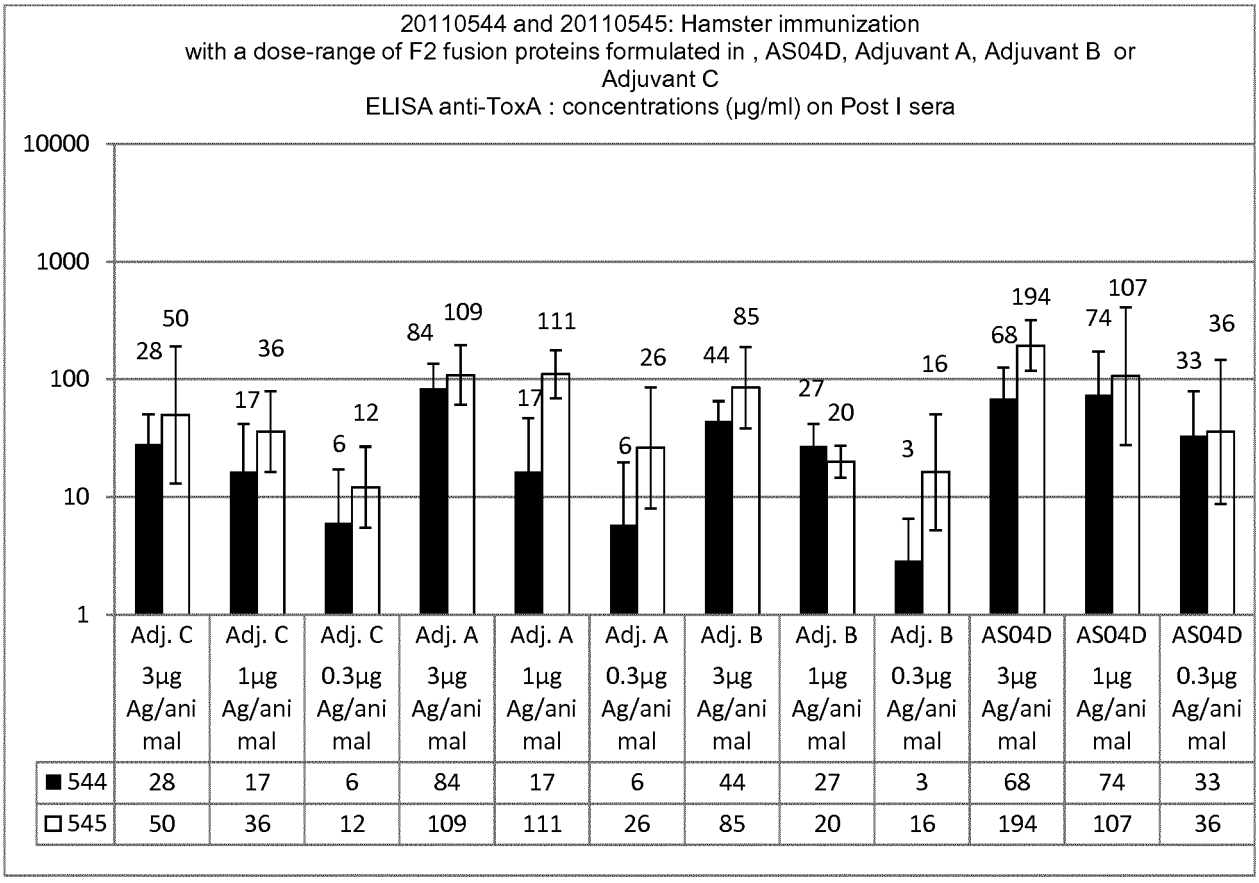

20120336 : Hamster immunization with F2 :
Adjuvant A compared with Adjuvant B, Alum or non-adjuvanted
ELISA anti-ToxB : concentrations (µg/ml) on individual and pooled sera on D42
100000
10000
1000
100
10
1
0
0
1789
225
207
41
Adjuvant A F2 1µg/dose
Adjuvant B F2 1µg/dose
Al(OH)3 F2 1µg/dose
non-adjuvanted F2 1µg/dose
Geomean
1789
225
207
41

Figure 3


20110544 and 20110545: Hamster immunization
with a dose-range of F2 fusion proteins formulated in , AS04D, Adjuvant A, Adjuvant B or Adjuvant C
ELISA anti-ToxA : concentrations (µg/ml) on Post I sera
10000
1000
100
10
1
Adj. C 3µg Ag/animal
Adj. C 1µg Ag/animal
Adj. C 0.3µg Ag/animal
Adj. A 3µg Ag/animal
Adj. A 1µg Ag/animal
Adj. A 0.3µg Ag/animal
Adj. B 3µg Ag/animal
Adj. B 1µg Ag/animal
Adj. B 0.3µg Ag/animal
AS04D 3µg Ag/animal
AS04D 1µg Ag/animal
AS04D 0.3µg Ag/animal
544 28 17 6 84 17 6 44 27 3 68 74 33
545 50 36 12 109 111 26 85 20 16 194 107 36

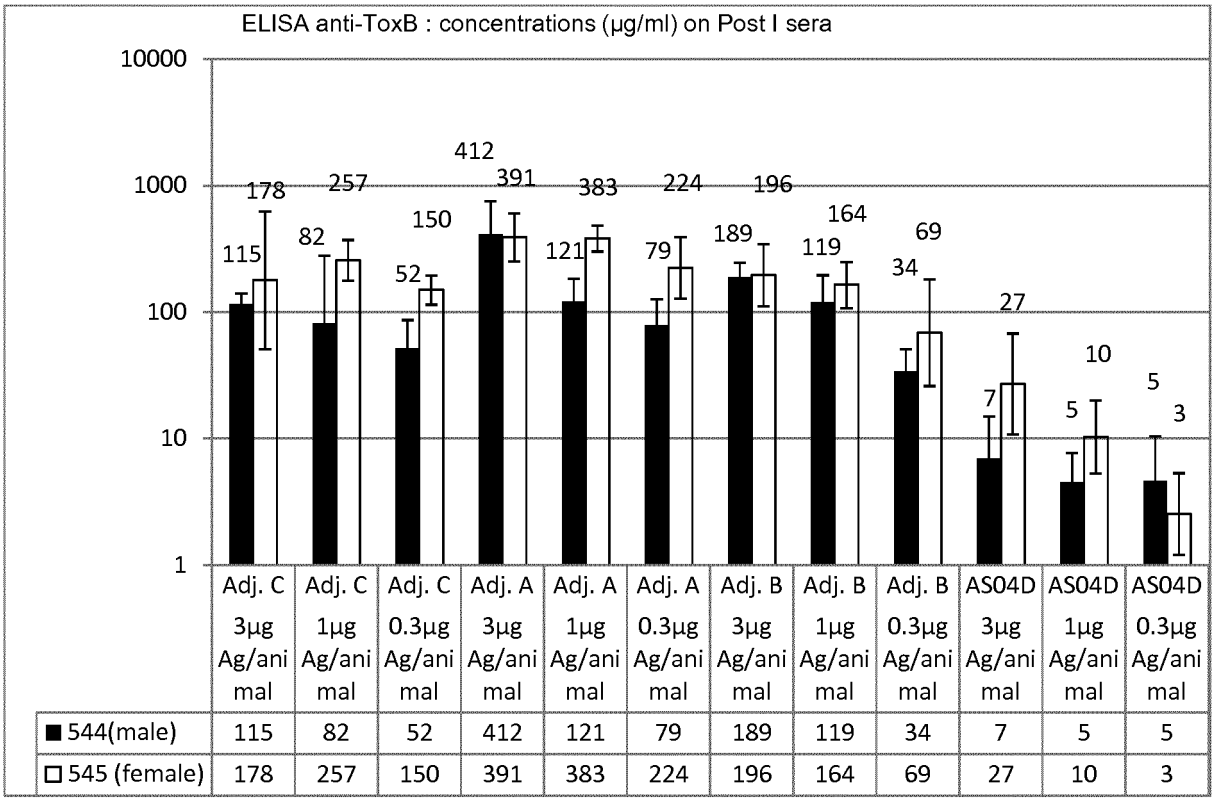
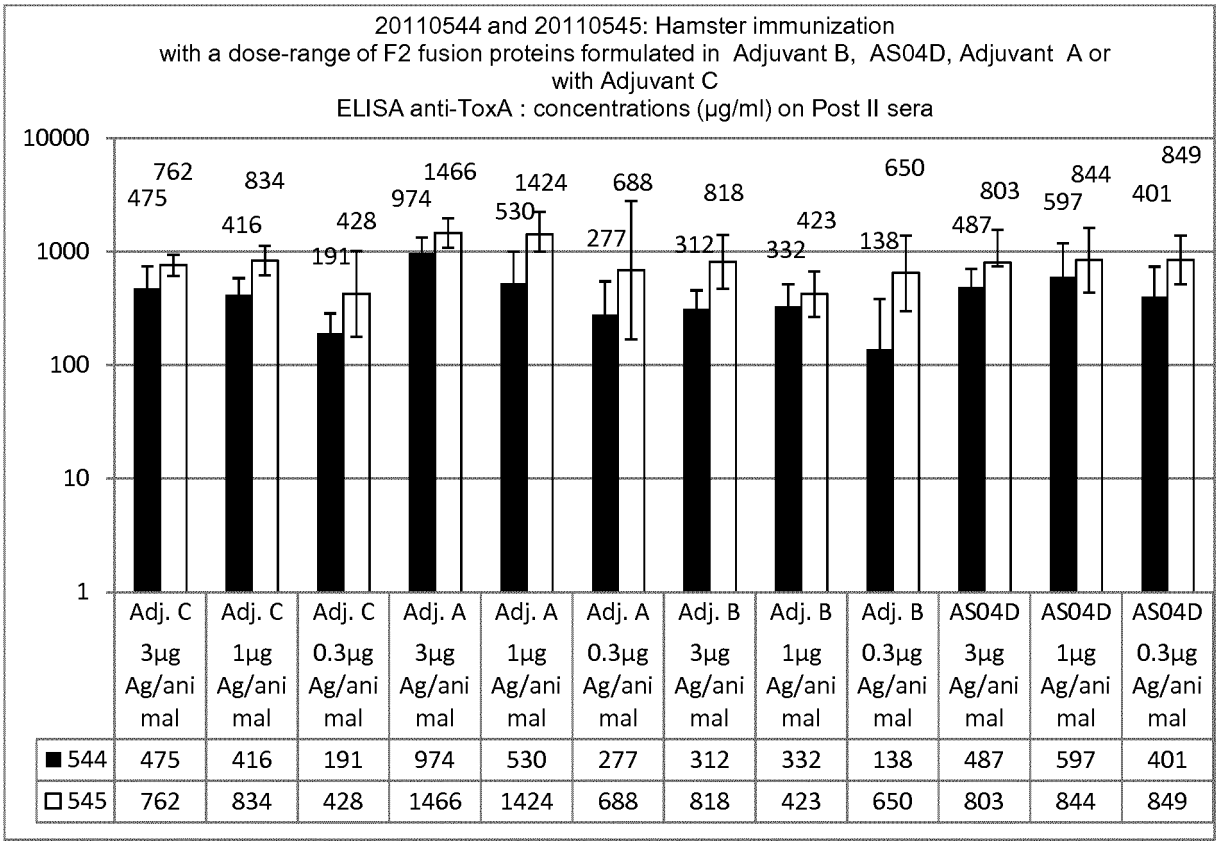
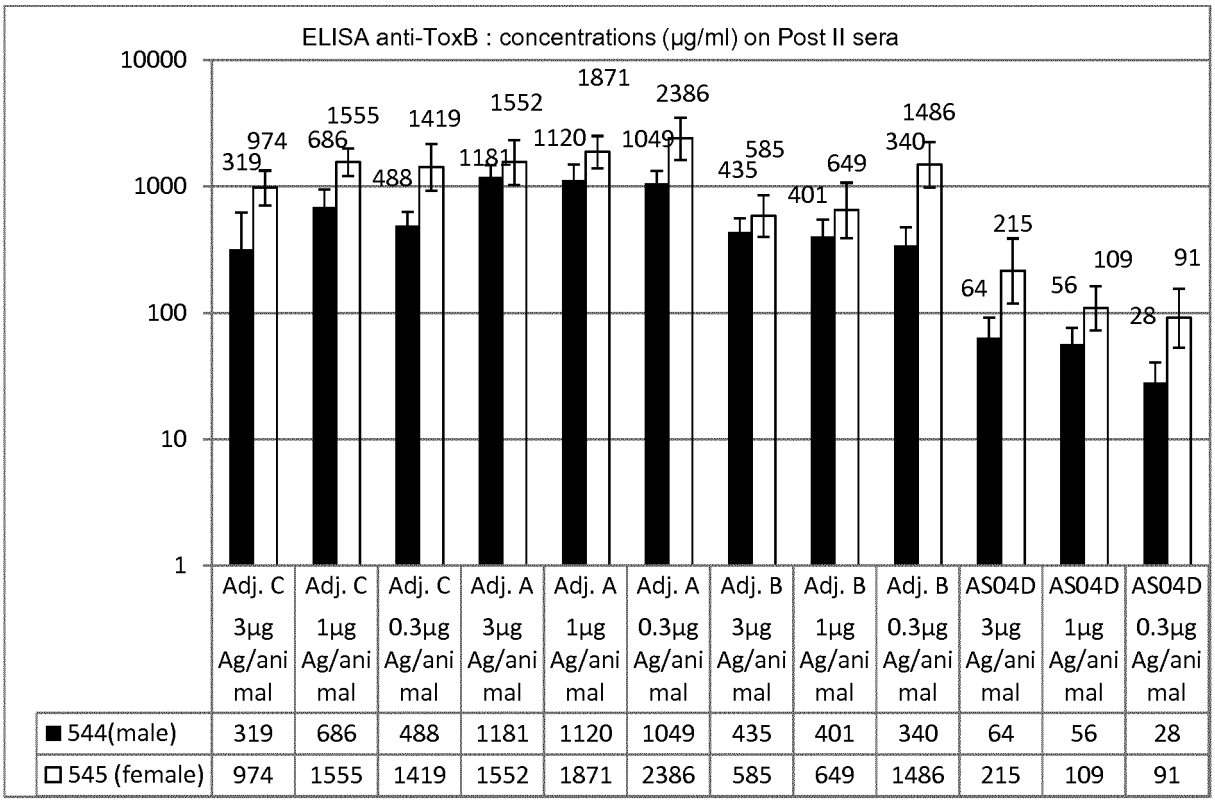

ELISA anti-ToxB : concentrations (µg/ml) on Post I sera
10000
1000
100
10
1
Adj. C 3µg Ag/animal
Adj. C 1µg Ag/animal
Adj. C 0.3µg Ag/animal
Adj. A 3µg Ag/animal
Adj. A 1µg Ag/animal
Adj. A 0.3µg Ag/animal
Adj. B 3µg Ag/animal
Adj. B 1µg Ag/animal
Adj. B 0.3µg Ag/animal
AS04D 3µg Ag/animal
AS04D 1µg Ag/animal
AS04D 0.3µg Ag/animal
544(male) 115 82 52 412 121 79 189 119 34 7 5 5
545 (female) 178 257 150 391 383 224 196 164 69 27 10 3

Figure 4

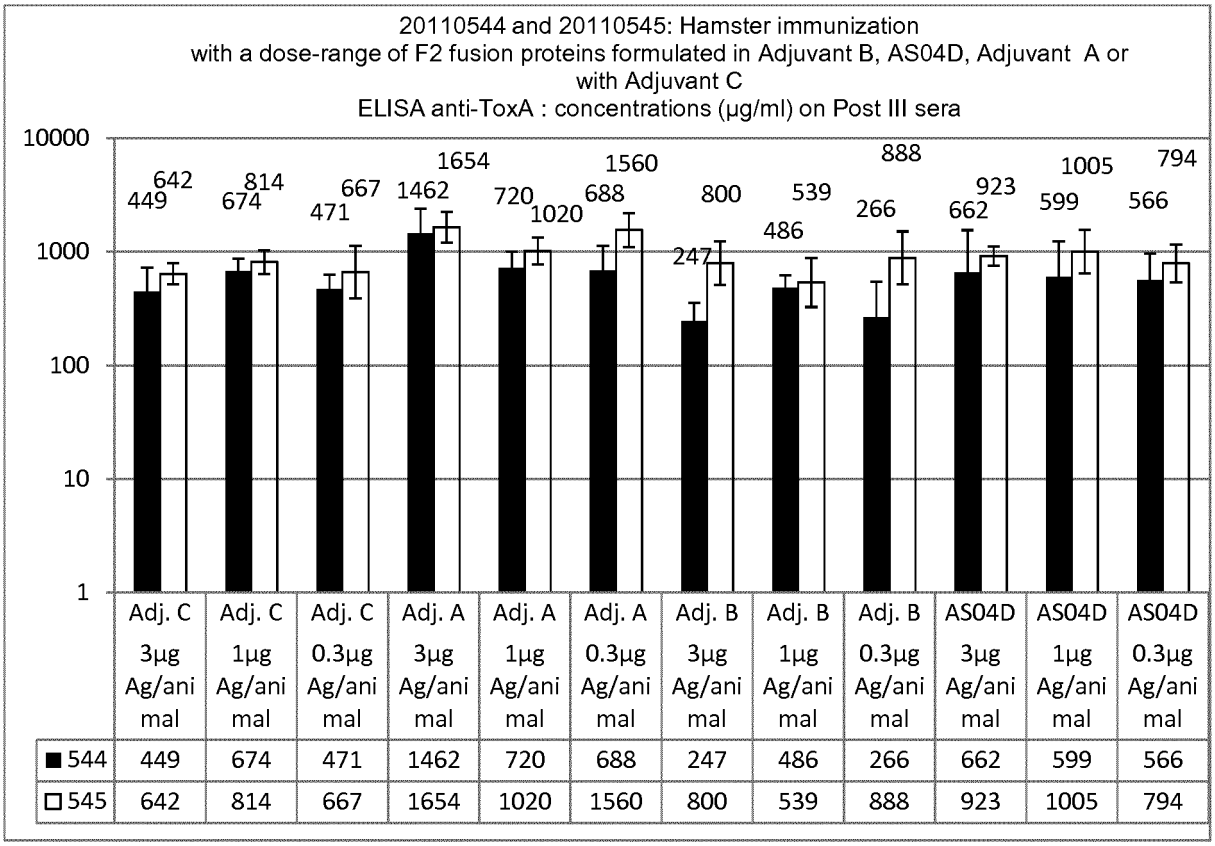


	Adj. C 3µg Ag/animal	Adj. C 1µg Ag/animal	Adj. C 0.3µg Ag/animal	Adj. A 3µg Ag/animal	Adj. A 1µg Ag/animal	Adj. A 0.3µg Ag/animal	Adj. B 3µg Ag/animal	Adj. B 1µg Ag/animal	Adj. B 0.3µg Ag/animal	AS04D 3µg Ag/animal	AS04D 1µg Ag/animal	AS04D 0.3µg Ag/animal
■ 544	475	416	191	974	530	277	312	332	138	487	597	401
□ 545	762	834	428	1466	1424	688	818	423	650	803	844	849

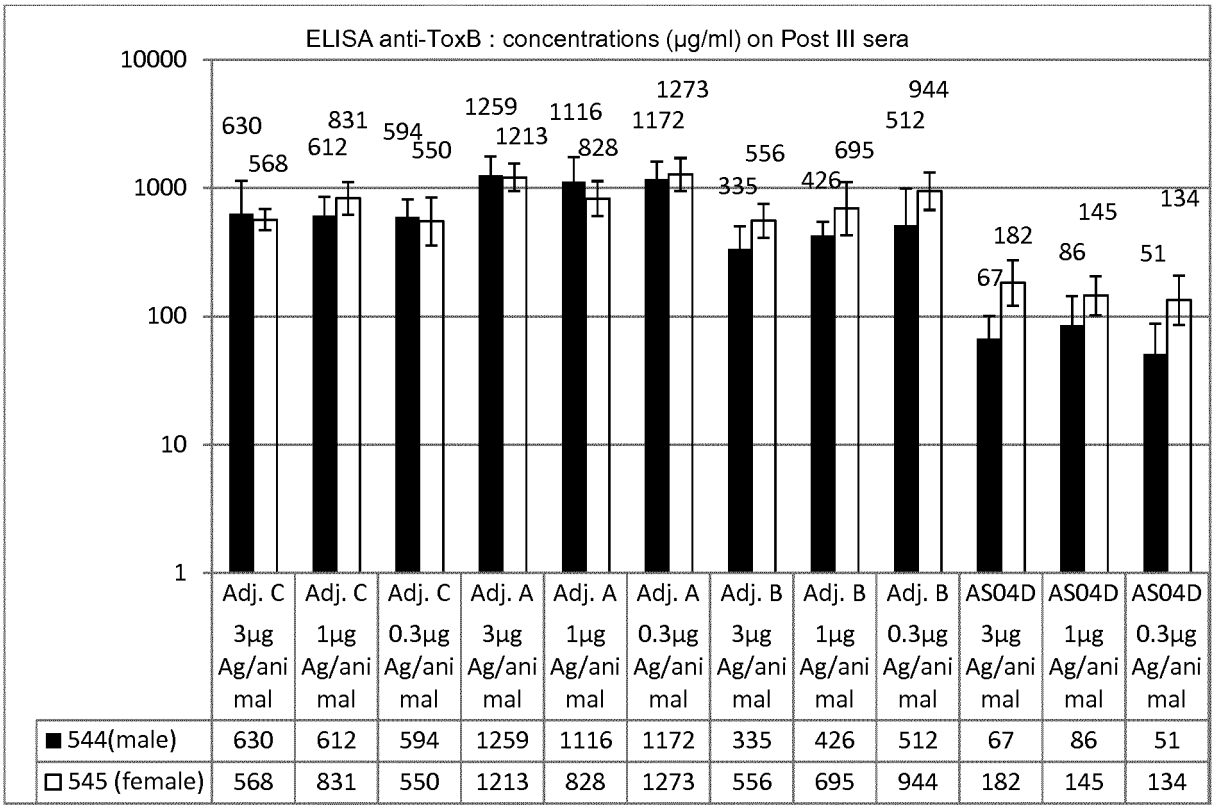


	Adj. C 3µg Ag/animal	Adj. C 1µg Ag/animal	Adj. C 0.3µg Ag/animal	Adj. A 3µg Ag/animal	Adj. A 1µg Ag/animal	Adj. A 0.3µg Ag/animal	Adj. B 3µg Ag/animal	Adj. B 1µg Ag/animal	Adj. B 0.3µg Ag/animal	AS04D 3µg Ag/animal	AS04D 1µg Ag/animal	AS04D 0.3µg Ag/animal
■ 544(male)	319	686	488	1181	1120	1049	435	401	340	64	56	28
□ 545 (female)	974	1555	1419	1552	1871	2386	585	649	1486	215	109	91

Figure 5

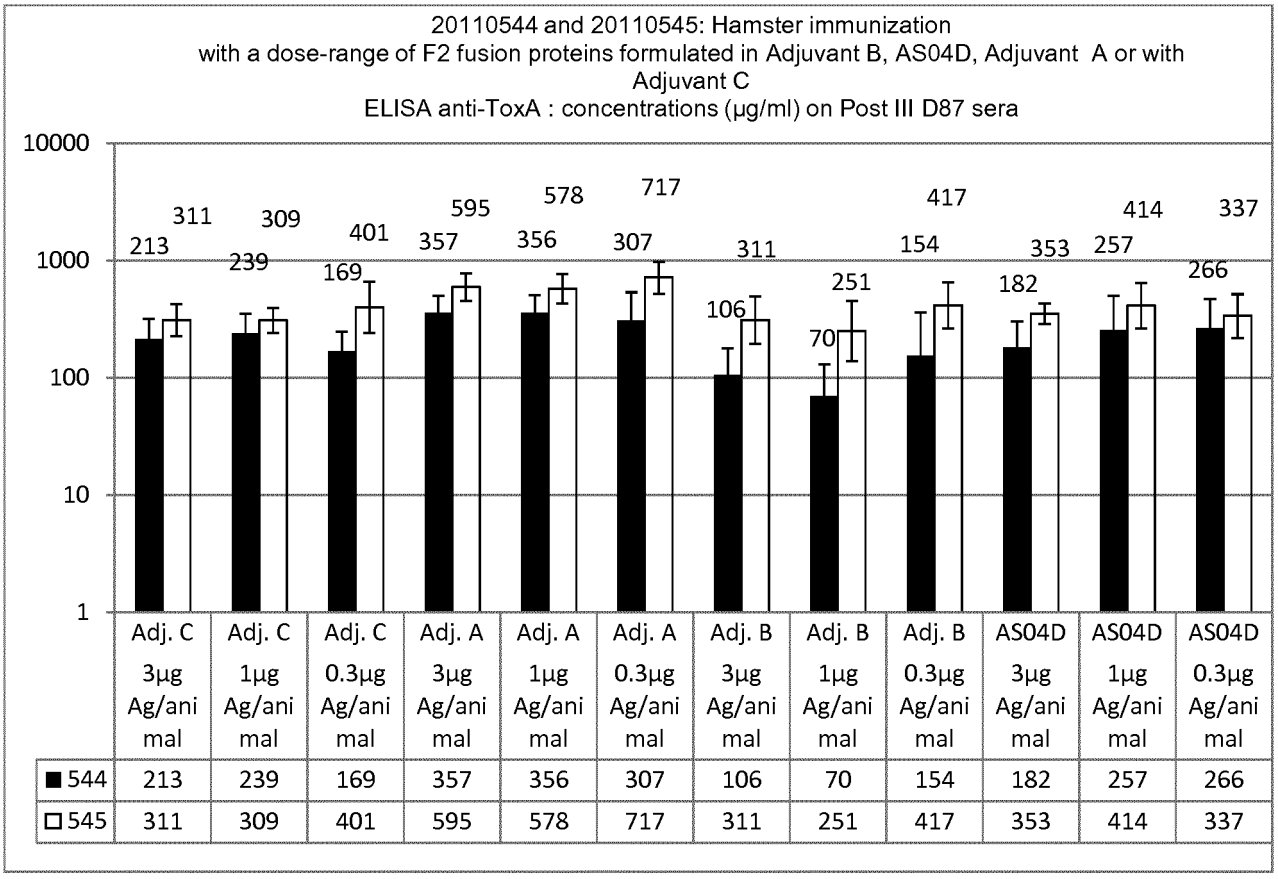


	Adj. C 3µg Ag/animal	Adj. C 1µg Ag/animal	Adj. C 0.3µg Ag/animal	Adj. A 3µg Ag/animal	Adj. A 1µg Ag/animal	Adj. A 0.3µg Ag/animal	Adj. B 3µg Ag/animal	Adj. B 1µg Ag/animal	Adj. B 0.3µg Ag/animal	AS04D 3µg Ag/animal	AS04D 1µg Ag/animal	AS04D 0.3µg Ag/animal
■ 544	449	674	471	1462	720	688	247	486	266	662	599	566
□ 545	642	814	667	1654	1020	1560	800	539	888	923	1005	794

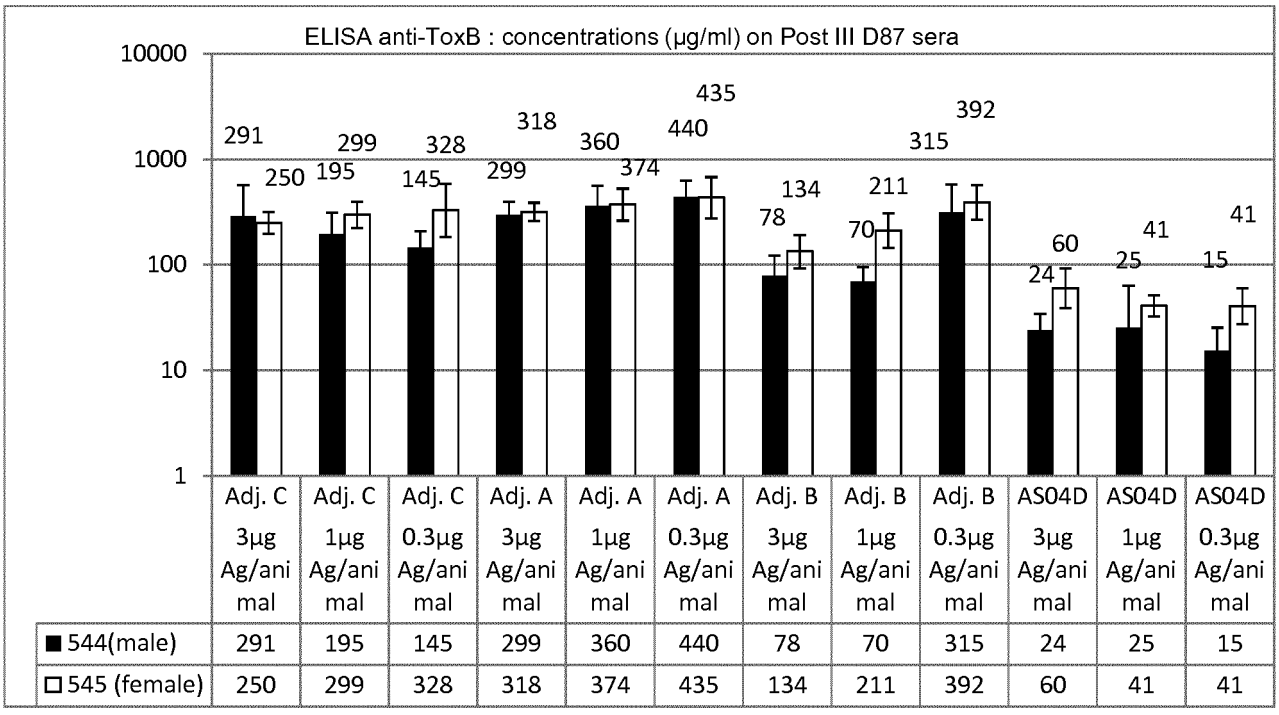


	Adj. C 3µg Ag/animal	Adj. C 1µg Ag/animal	Adj. C 0.3µg Ag/animal	Adj. A 3µg Ag/animal	Adj. A 1µg Ag/animal	Adj. A 0.3µg Ag/animal	Adj. B 3µg Ag/animal	Adj. B 1µg Ag/animal	Adj. B 0.3µg Ag/animal	AS04D 3µg Ag/animal	AS04D 1µg Ag/animal	AS04D 0.3µg Ag/animal
■ 544(male)	630	612	594	1259	1116	1172	335	426	512	67	86	51
□ 545 (female)	568	831	550	1213	828	1273	556	695	944	182	145	134

Figure 6

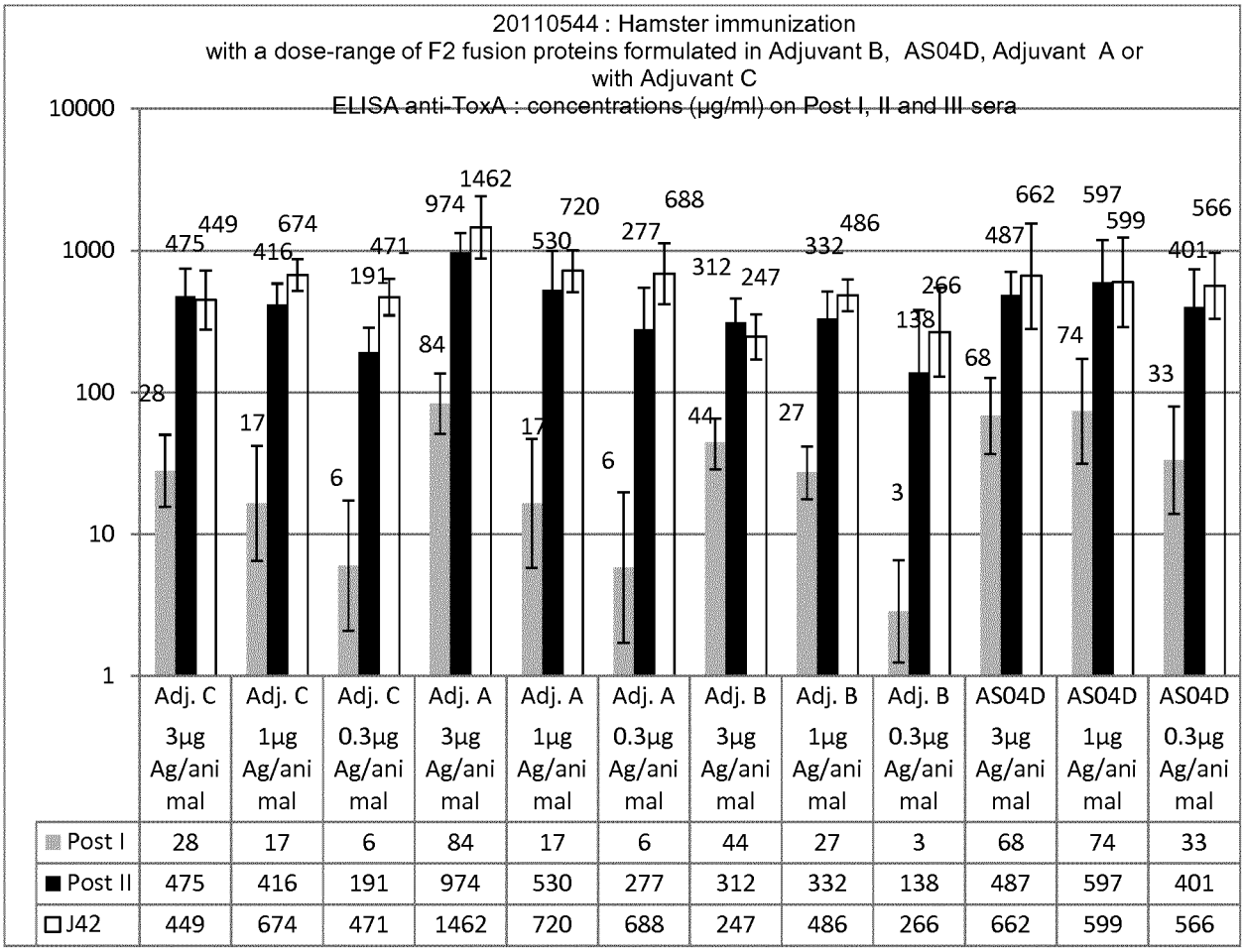


	Adj. C 3µg Ag/animal	Adj. C 1µg Ag/animal	Adj. C 0.3µg Ag/animal	Adj. A 3µg Ag/animal	Adj. A 1µg Ag/animal	Adj. A 0.3µg Ag/animal	Adj. B 3µg Ag/animal	Adj. B 1µg Ag/animal	Adj. B 0.3µg Ag/animal	AS04D 3µg Ag/animal	AS04D 1µg Ag/animal	AS04D 0.3µg Ag/animal
■ 544	213	239	169	357	356	307	106	70	154	182	257	266
□ 545	311	309	401	595	578	717	311	251	417	353	414	337

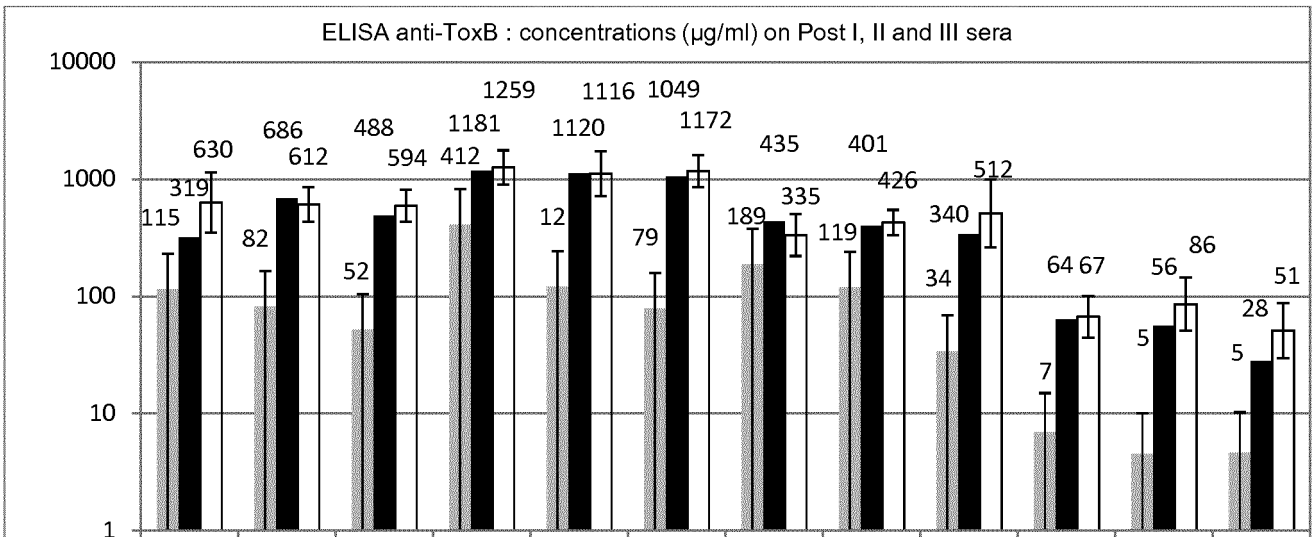


	Adj. C 3µg Ag/animal	Adj. C 1µg Ag/animal	Adj. C 0.3µg Ag/animal	Adj. A 3µg Ag/animal	Adj. A 1µg Ag/animal	Adj. A 0.3µg Ag/animal	Adj. B 3µg Ag/animal	Adj. B 1µg Ag/animal	Adj. B 0.3µg Ag/animal	AS04D 3µg Ag/animal	AS04D 1µg Ag/animal	AS04D 0.3µg Ag/animal
■ 544(male)	291	195	145	299	360	440	78	70	315	24	25	15
□ 545 (female)	250	299	328	318	374	435	134	211	392	60	41	41

Figure 7



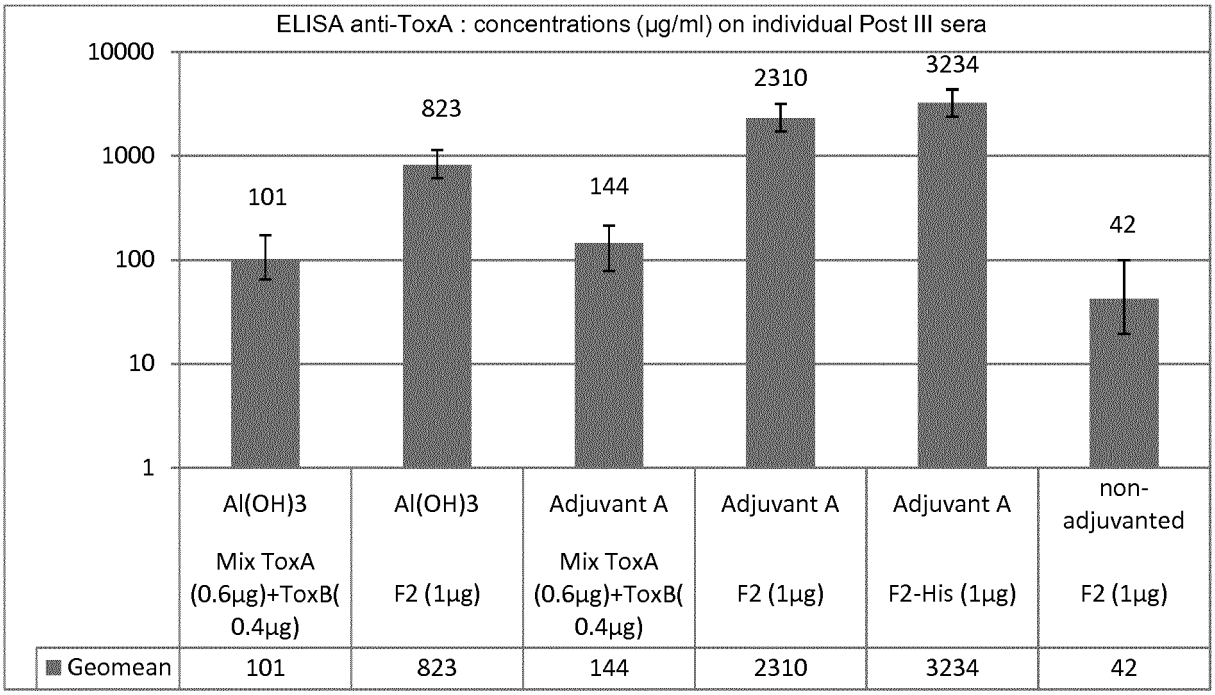
	Adj. C 3µg Ag/animal	Adj. C 1µg Ag/animal	Adj. C 0.3µg Ag/animal	Adj. A 3µg Ag/animal	Adj. A 1µg Ag/animal	Adj. A 0.3µg Ag/animal	Adj. B 3µg Ag/animal	Adj. B 1µg Ag/animal	Adj. B 0.3µg Ag/animal	AS04D 3µg Ag/animal	AS04D 1µg Ag/animal	AS04D 0.3µg Ag/animal
Post I	28	17	6	84	17	6	44	27	3	68	74	33
Post II	475	416	191	974	530	277	312	332	138	487	597	401
J42	449	674	471	1462	720	688	247	486	266	662	599	566



	Adj. C 3µg Ag/animal	Adj. C 1µg Ag/animal	Adj. C 0.3µg Ag/animal	Adj. A 3µg Ag/animal	Adj. A 1µg Ag/animal	Adj. A 0.3µg Ag/animal	Adj. B 3µg Ag/animal	Adj. B 1µg Ag/animal	Adj. B 0.3µg Ag/animal	AS04D 3µg Ag/animal	AS04D 1µg Ag/animal	AS04D 0.3µg Ag/animal
Post I	115	82	52	412	121	79	189	119	34	7	5	5
Post II	319	686	488	1181	1120	1049	435	401	340	64	56	28
J42	630	612	594	1259	1116	1172	335	426	512	67	86	51

Figure 8

20120008 and 20120011 : Female and Male hamster immunization. Comparison of the immunogenicity of a Mix (ToxA+ToxB) with the F2 fusion protein formulated in Alum or Adjuvant A



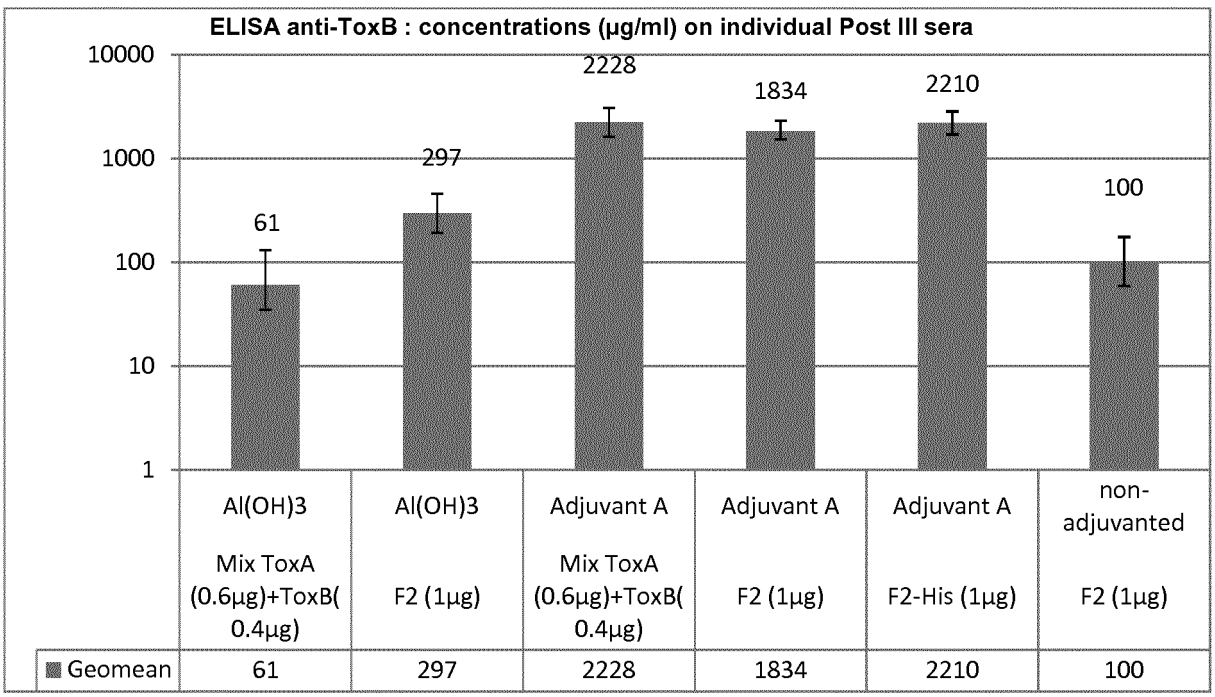


Figure 9

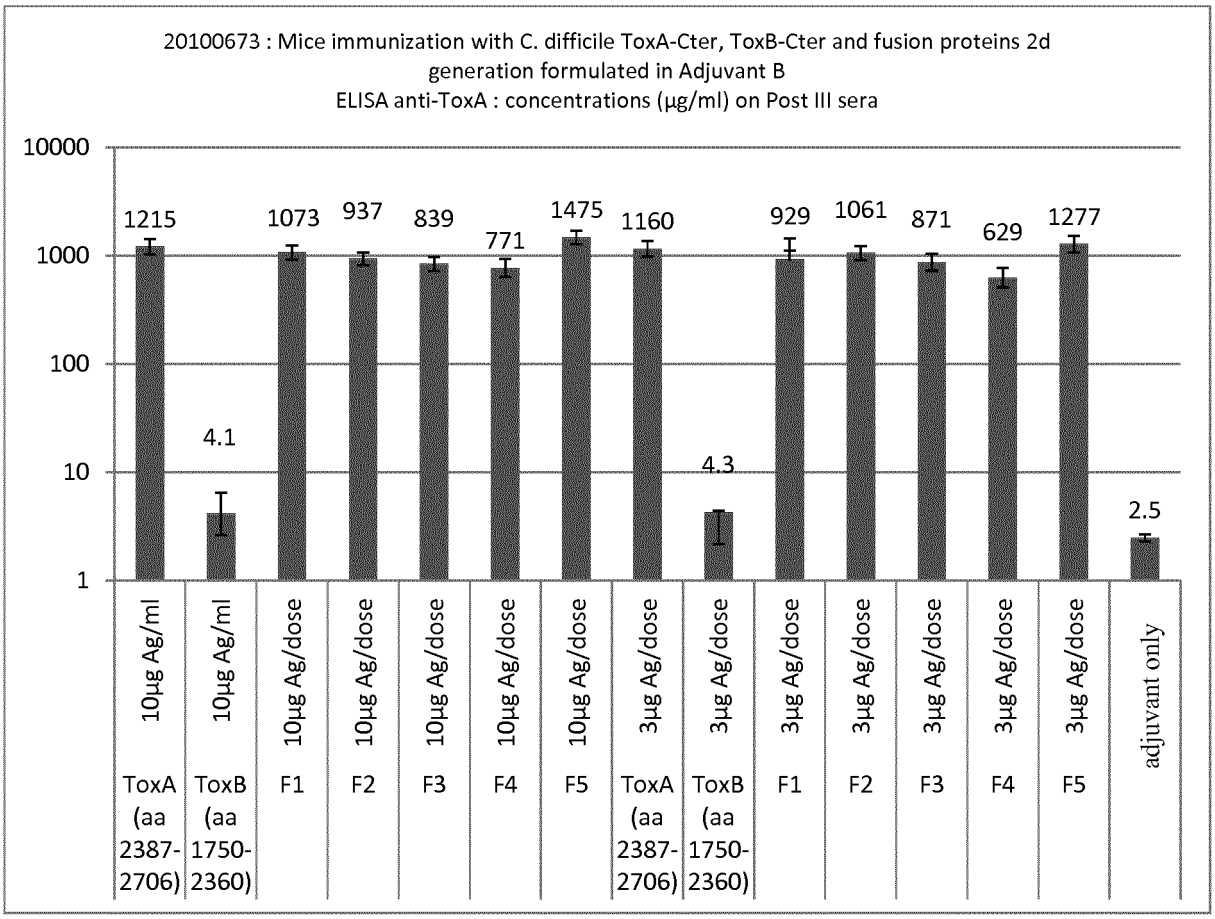

20100673 : Mice immunization with C. difficile ToxA-Cter, ToxB-Cter and fusion proteins 2d generation formulated in Adjuvant B
ELISA anti-ToxA : concentrations (µg/ml) on Post III sera
10000
1000
100
10
1
1215
4.1
1073
937
839
771
1475
1160
4.3
929
1061
871
629
1277
2.5
10µg Ag/ml ToxA (aa 2387-2706)
10µg Ag/ml ToxB (aa 1750-2360)
10µg Ag/dose F1
10µg Ag/dose F2
10µg Ag/dose F3
10µg Ag/dose F4
10µg Ag/dose F5
3µg Ag/dose ToxA (aa 2387-2706)
3µg Ag/dose ToxB (aa 1750-2360)
3µg Ag/dose F1
3µg Ag/dose F2
3µg Ag/dose F3
3µg Ag/dose F4
3µg Ag/dose F5
adjuvant only

Figure 10

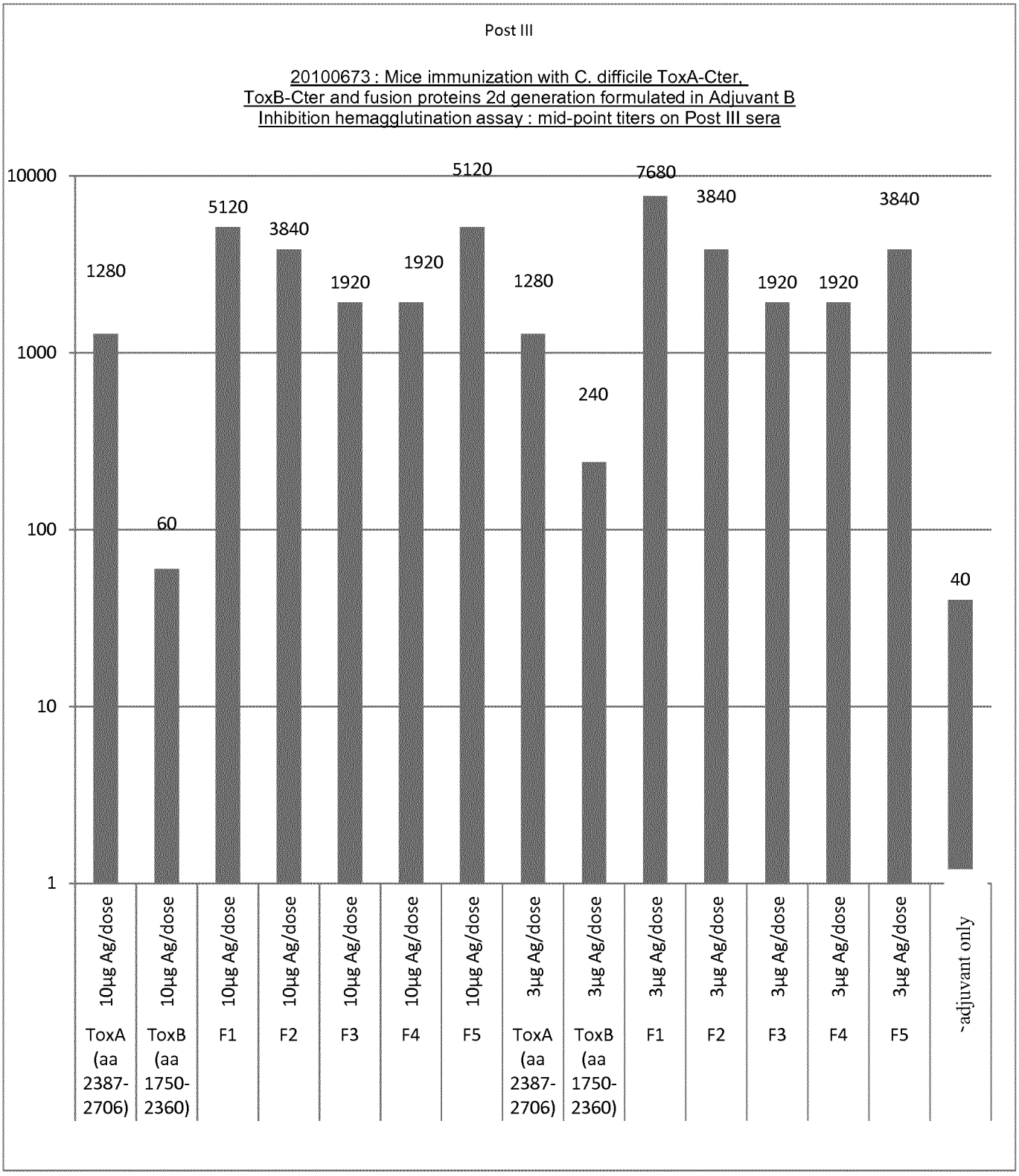

Post III
20100673 : Mice immunization with C. difficile ToxA-Cter, ToxB-Cter and fusion proteins 2d generation formulated in Adjuvant B
Inhibition hemagglutination assay : mid-point titers on Post III sera
10000
1000
100
10
1
1280
60
5120
3840
1920
1920
5120
1280
240
7680
3840
1920
1920
3840
40
10µg Ag/dose ToxA (aa 2387-2706)
10µg Ag/dose ToxB (aa 1750-2360)
10µg Ag/dose F1
10µg Ag/dose F2
10µg Ag/dose F3
10µg Ag/dose F4
10µg Ag/dose F5
3µg Ag/dose ToxA (aa 2387-2706)
3µg Ag/dose ToxB (aa 1750-2360)
3µg Ag/dose F1
3µg Ag/dose F2
3µg Ag/dose F3
3µg Ag/dose F4
3µg Ag/dose F5
adjuvant only

Figure 11

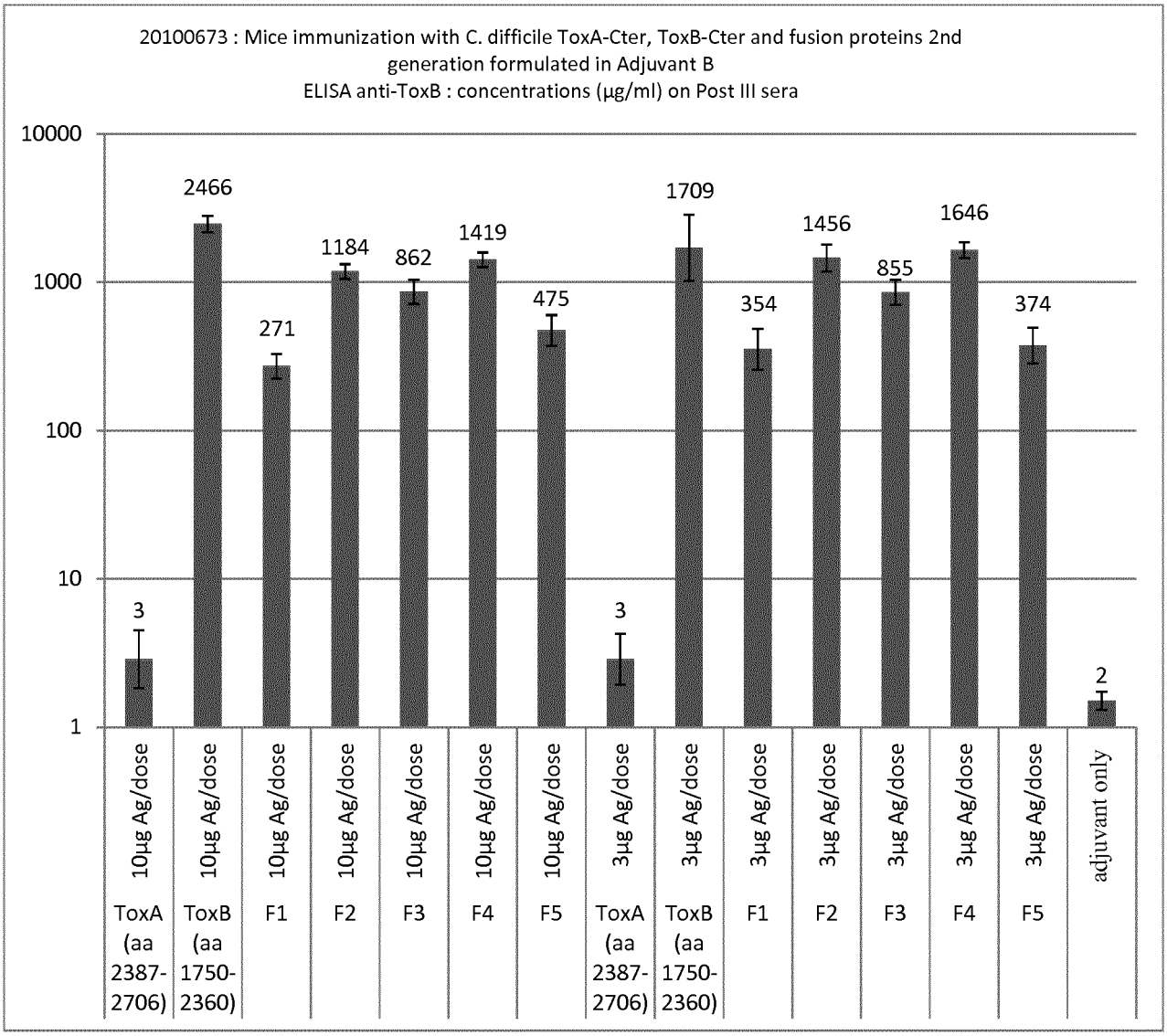

20100673 : Mice immunization with C. difficile ToxA-Cter, ToxB-Cter and fusion proteins 2nd generation formulated in Adjuvant B
ELISA anti-ToxB : concentrations (µg/ml) on Post III sera
10000
1000
100
10
1
3
2466
271
1184
862
1419
475
3
1709
354
1456
855
1646
374
2
10µg Ag/dose ToxA (aa 2387-2706)
10µg Ag/dose ToxB (aa 1750-2360)
10µg Ag/dose F1
10µg Ag/dose F2
10µg Ag/dose F3
10µg Ag/dose F4
10µg Ag/dose F5
3µg Ag/dose ToxA (aa 2387-2706)
3µg Ag/dose ToxB (aa 1750-2360)
3µg Ag/dose F1
3µg Ag/dose F2
3µg Ag/dose F3
3µg Ag/dose F4
3µg Ag/dose F5
adjuvant only

Figure 12

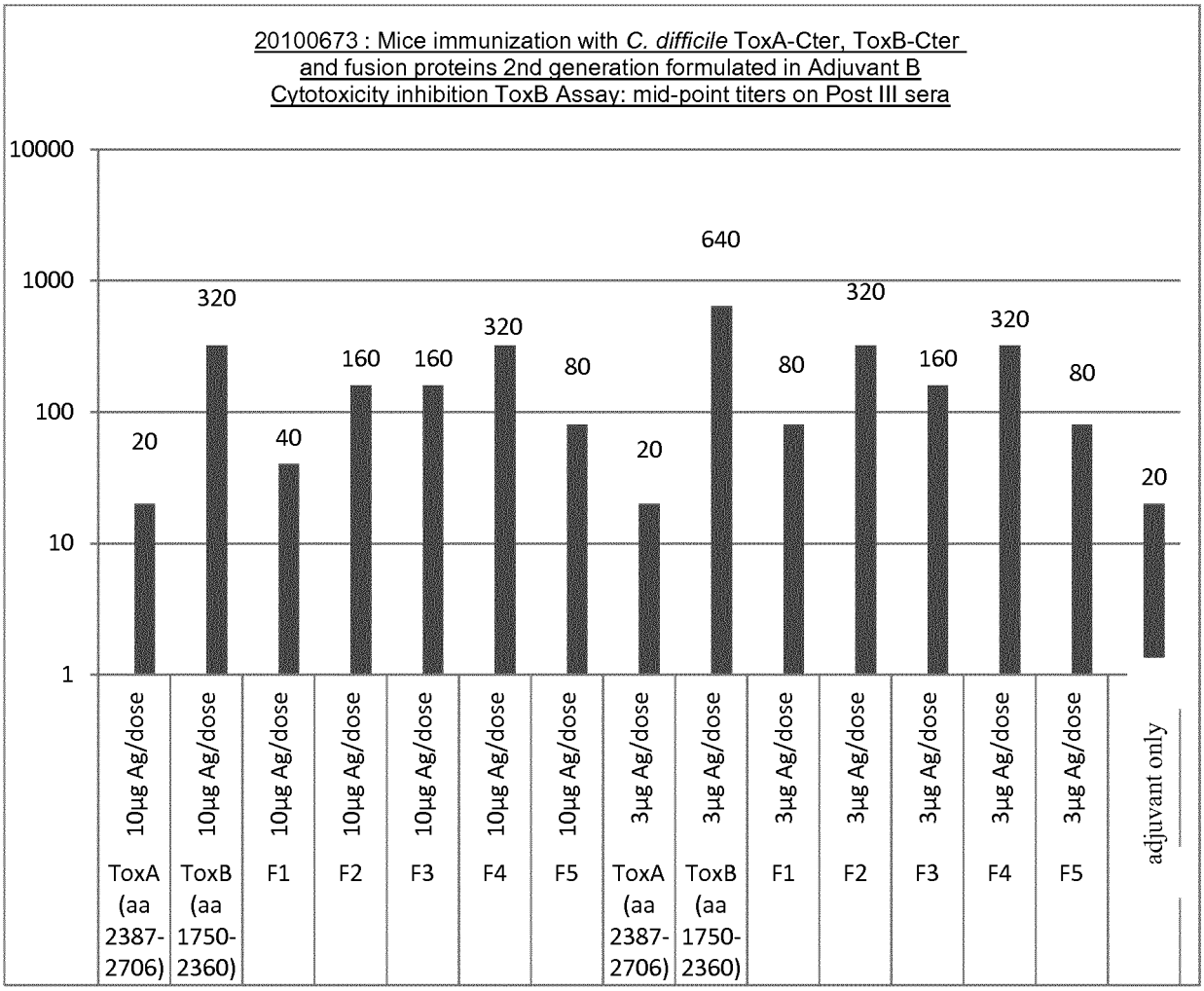

20100673 : Mice immunization with C. difficile ToxA-Cter, ToxB-Cter and fusion proteins 2nd generation formulated in Adjuvant B
Cytotoxicity inhibition ToxB Assay: mid-point titers on Post III sera
10000
1000
100
10
1
20
320
40
160
160
320
80
20
640
80
320
160
320
80
20
10µg Ag/dose ToxA (aa 2387-2706)
10µg Ag/dose ToxB (aa 1750-2360)
10µg Ag/dose F1
10µg Ag/dose F2
10µg Ag/dose F3
10µg Ag/dose F4
10µg Ag/dose F5
3µg Ag/dose ToxA (aa 2387-2706)
3µg Ag/dose ToxB (aa 1750-2360)
3µg Ag/dose F1
3µg Ag/dose F2
3µg Ag/dose F3
3µg Ag/dose F4
3µg Ag/dose F5
adjuvant only

Figure 13

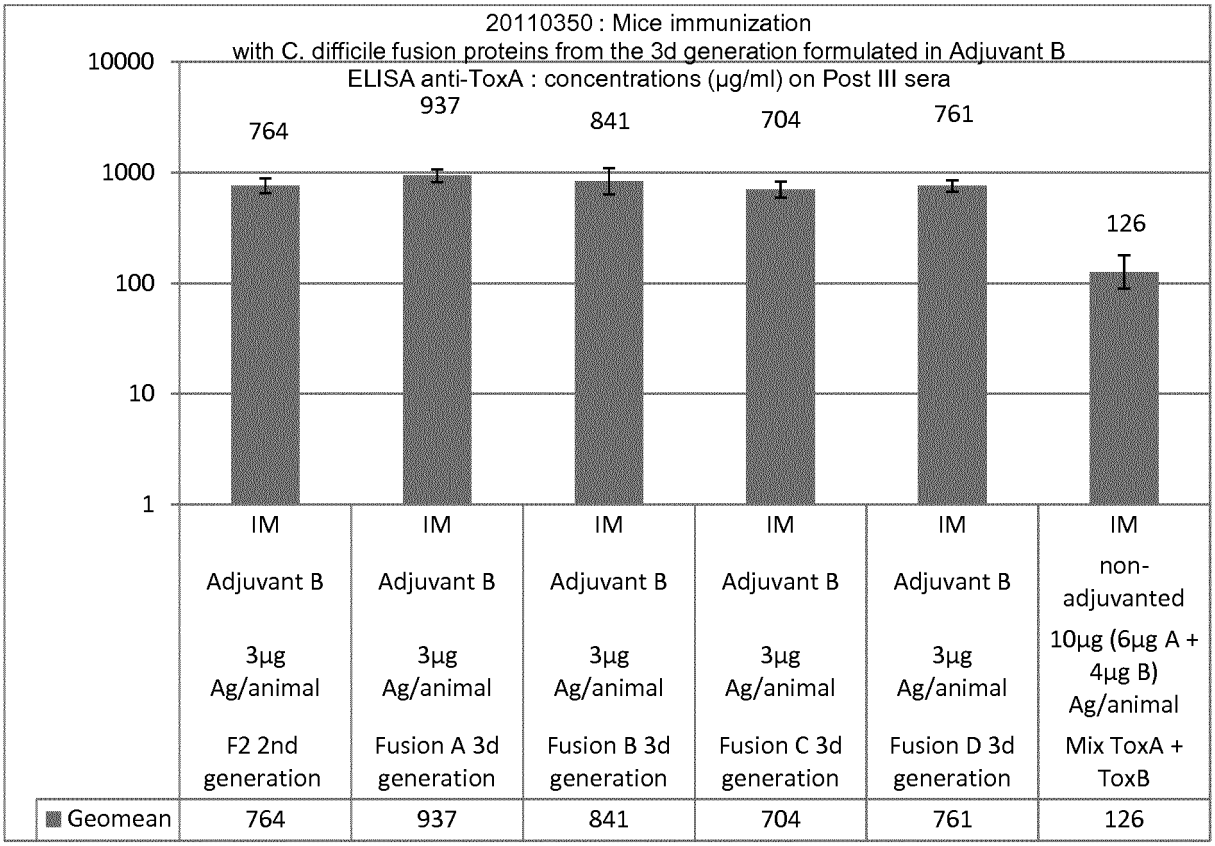

20110350 : Mice immunization
with C. difficile fusion proteins from the 3d generation formulated in Adjuvant B
ELISA anti-ToxA : concentrations (µg/ml) on Post III sera
10000
1000
100
10
1
764
937
841
704
761
126
IM
Adjuvant B
3µg Ag/animal
F2 2nd generation
IM
Adjuvant B
3µg Ag/animal
Fusion A 3d generation
IM
Adjuvant B
3µg Ag/animal
Fusion B 3d generation
IM
Adjuvant B
3µg Ag/animal
Fusion C 3d generation
IM
Adjuvant B
3µg Ag/animal
Fusion D 3d generation
IM
non-adjuvanted
10µg (6µg A + 4µg B) Ag/animal
Mix ToxA + ToxB
Geomean
764
937
841
704
761
126

Figure 14

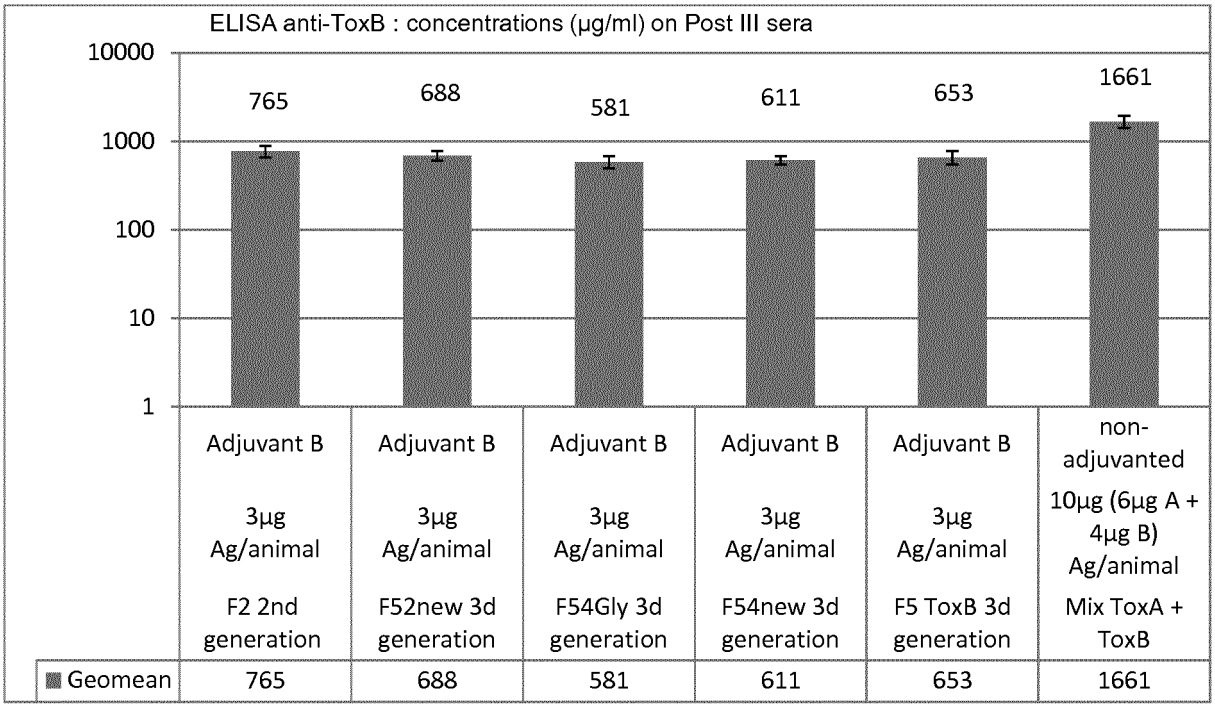

ELISA anti-ToxB : concentrations (µg/ml) on Post III sera
10000
1000
100
10
1
765
688
581
611
653
1661
Adjuvant B 3µg Ag/animal F2 2nd generation
Adjuvant B 3µg Ag/animal F52new 3d generation
Adjuvant B 3µg Ag/animal F54Gly 3d generation
Adjuvant B 3µg Ag/animal F54new 3d generation
Adjuvant B 3µg Ag/animal F5 ToxB 3d generation
non-adjuvanted 10µg (6µg A + 4µg B) Ag/animal Mix ToxA + ToxB
Geomean 765 688 581 611 653 1661

Figure 15

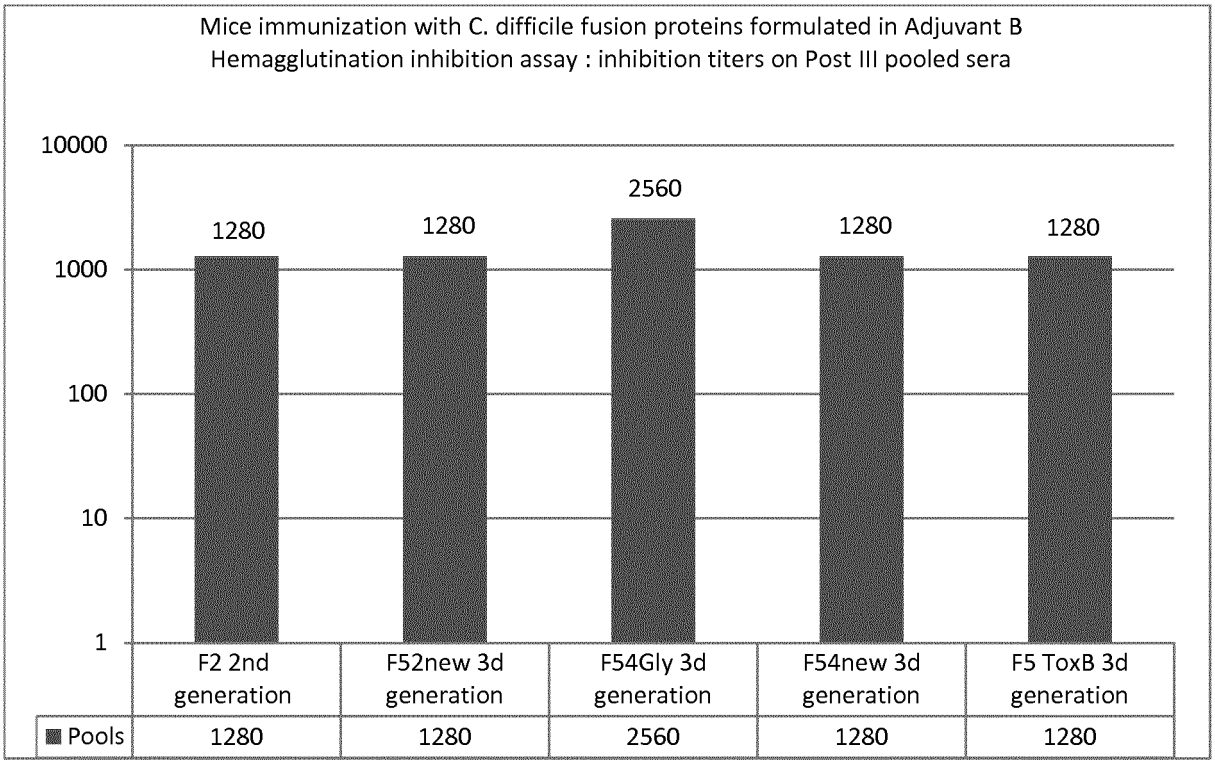

Mice immunization with C. difficile fusion proteins formulated in Adjuvant B
Hemagglutination inhibition assay : inhibition titers on Post III pooled sera
10000
1000
100
10
1
1280
1280
2560
1280
1280
F2 2nd generation
F52new 3d generation
F54Gly 3d generation
F54new 3d generation
F5 ToxB 3d generation
Pools
1280
1280
2560
1280
1280

Figure 16

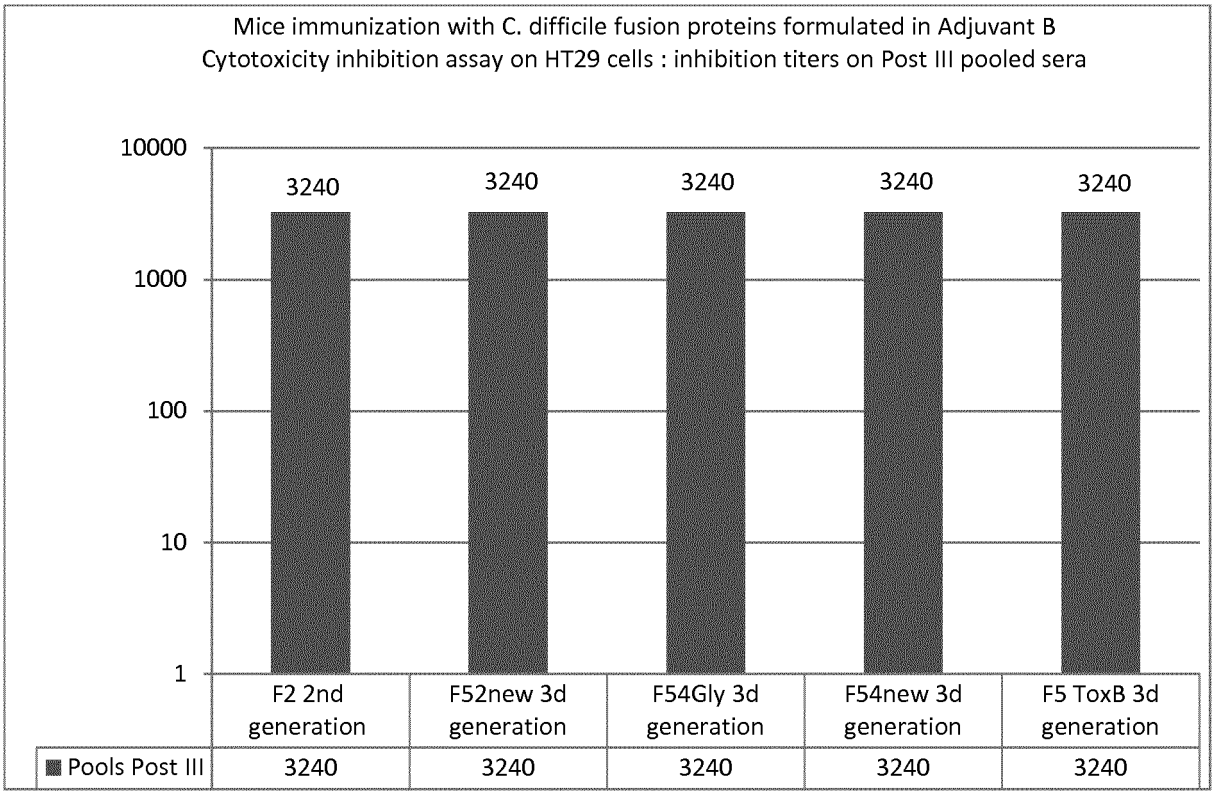

Mice immunization with C. difficile fusion proteins formulated in Adjuvant B
Cytotoxicity inhibition assay on HT29 cells : inhibition titers on Post III pooled sera
10000
1000
100
10
1
3240
3240
3240
3240
3240
F2 2nd generation
F52new 3d generation
F54Gly 3d generation
F54new 3d generation
F5 ToxB 3d generation
Pools Post III
3240
3240
3240
3240
3240

Figure 17

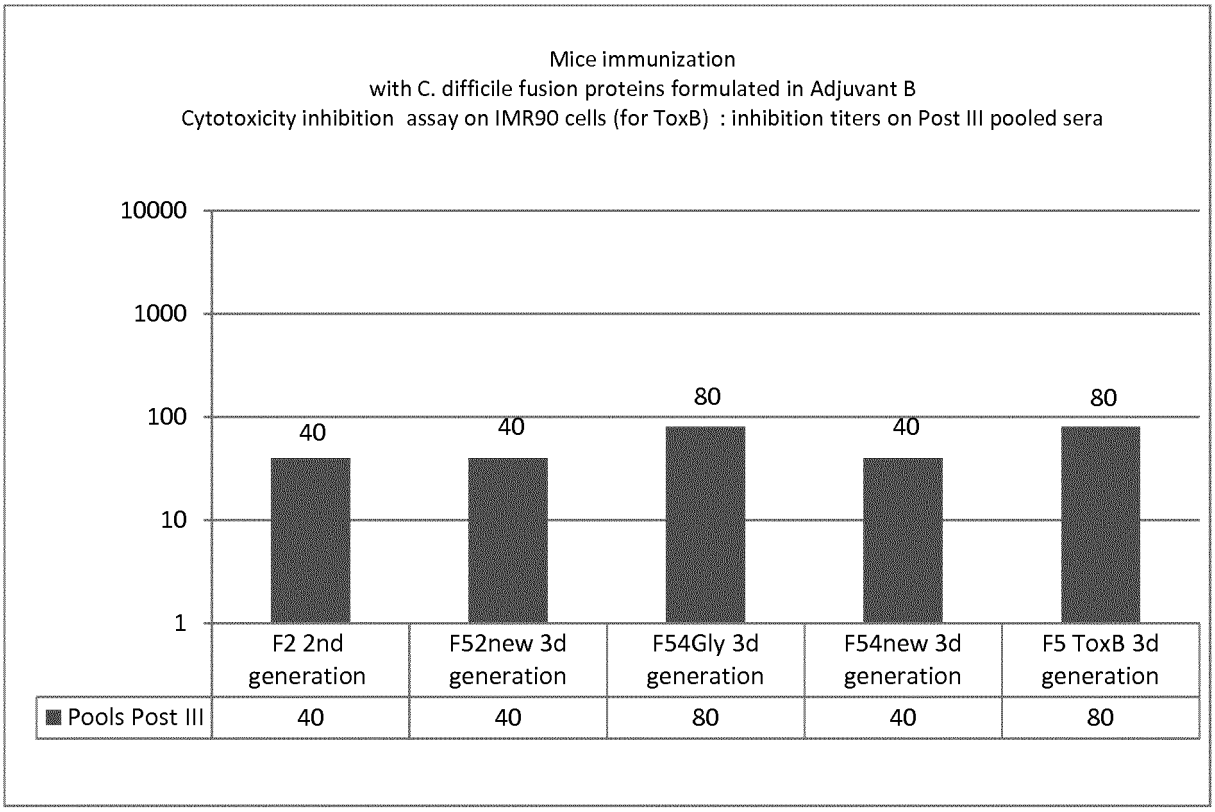

Mice immunization
with C. difficile fusion proteins formulated in Adjuvant B
Cytotoxicity inhibition assay on IMR90 cells (for ToxB) : inhibition titers on Post III pooled sera
10000
1000
100
10
1
40
40
80
40
80
F2 2nd generation
F52new 3d generation
F54Gly 3d generation
F54new 3d generation
F5 ToxB 3d generation
Pools Post III
40
40
80
40
80

Figure 18

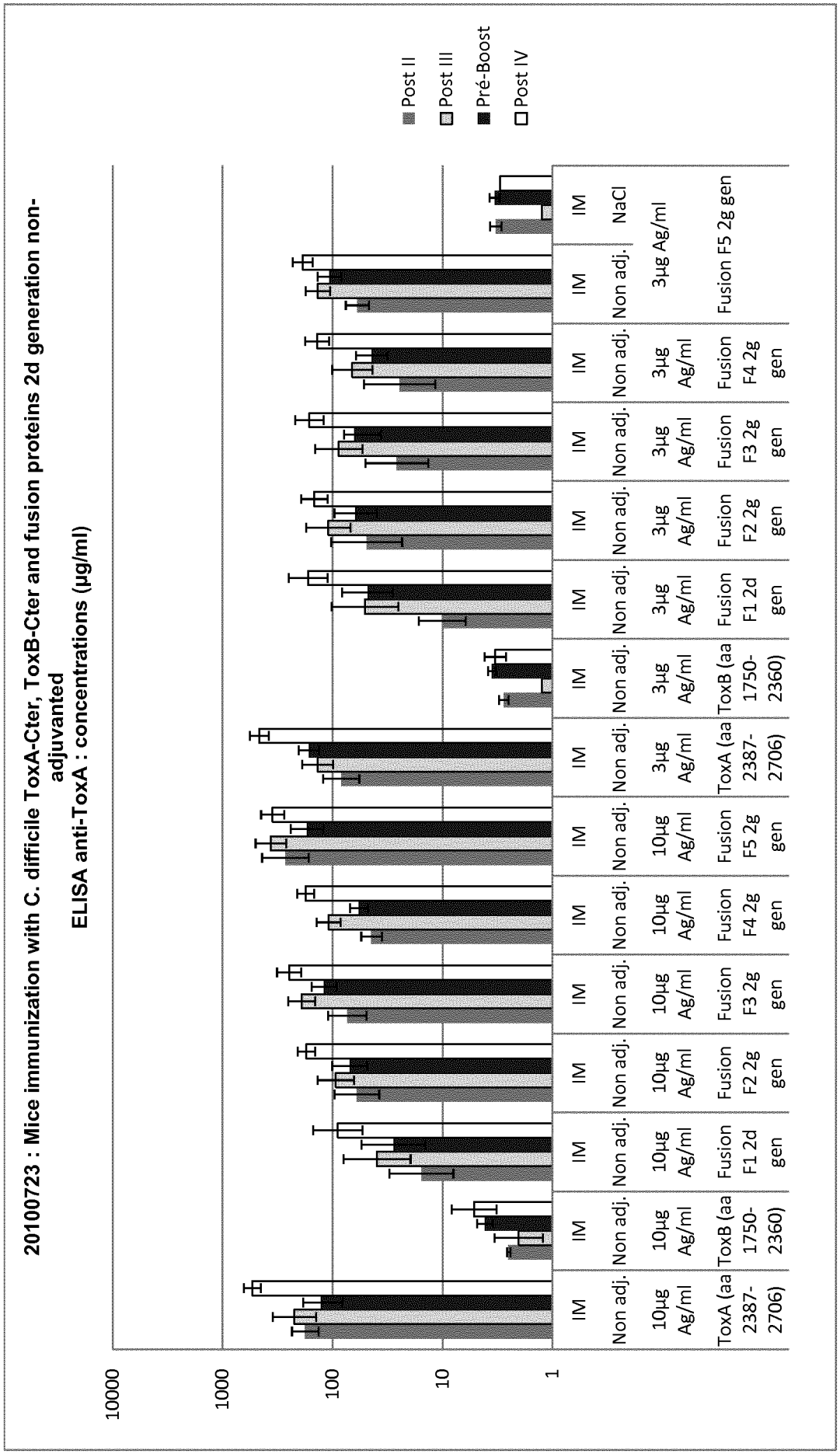

20100723 : Mice immunization with C. difficile ToxA-Cter, ToxB-Cter and fusion proteins 2d generation non-adjuvanted
ELISA anti-ToxA : concentrations (µg/ml)
Post II
Post III
Pré-Boost
Post IV
10000
1000
100
10
1
ToxA (aa 2387-2706) 10µg Ag/ml IM
Non adj. 10µg Ag/ml IM
ToxB (aa 1750-2360) 10µg Ag/ml Non adj. IM
Fusion F1 2d gen 10µg Ag/ml Non adj. IM
Fusion F2 2g gen 10µg Ag/ml Non adj. IM
Fusion F3 2g gen 10µg Ag/ml Non adj. IM
Fusion F4 2g gen 10µg Ag/ml Non adj. IM
Fusion F5 2g gen 10µg Ag/ml Non adj. IM
ToxA (aa 2387-2706) 3µg Ag/ml Non adj. IM
ToxB (aa 1750-2360) 3µg Ag/ml Non adj. IM
Fusion F1 2d gen 3µg Ag/ml Non adj. IM
Fusion F2 2g gen 3µg Ag/ml Non adj. IM
Fusion F3 2g gen 3µg Ag/ml Non adj. IM
Fusion F4 2g gen 3µg Ag/ml Non adj. IM
Fusion F5 2g gen 3µg Ag/ml Non adj. IM
NaCl IM

Figure 19

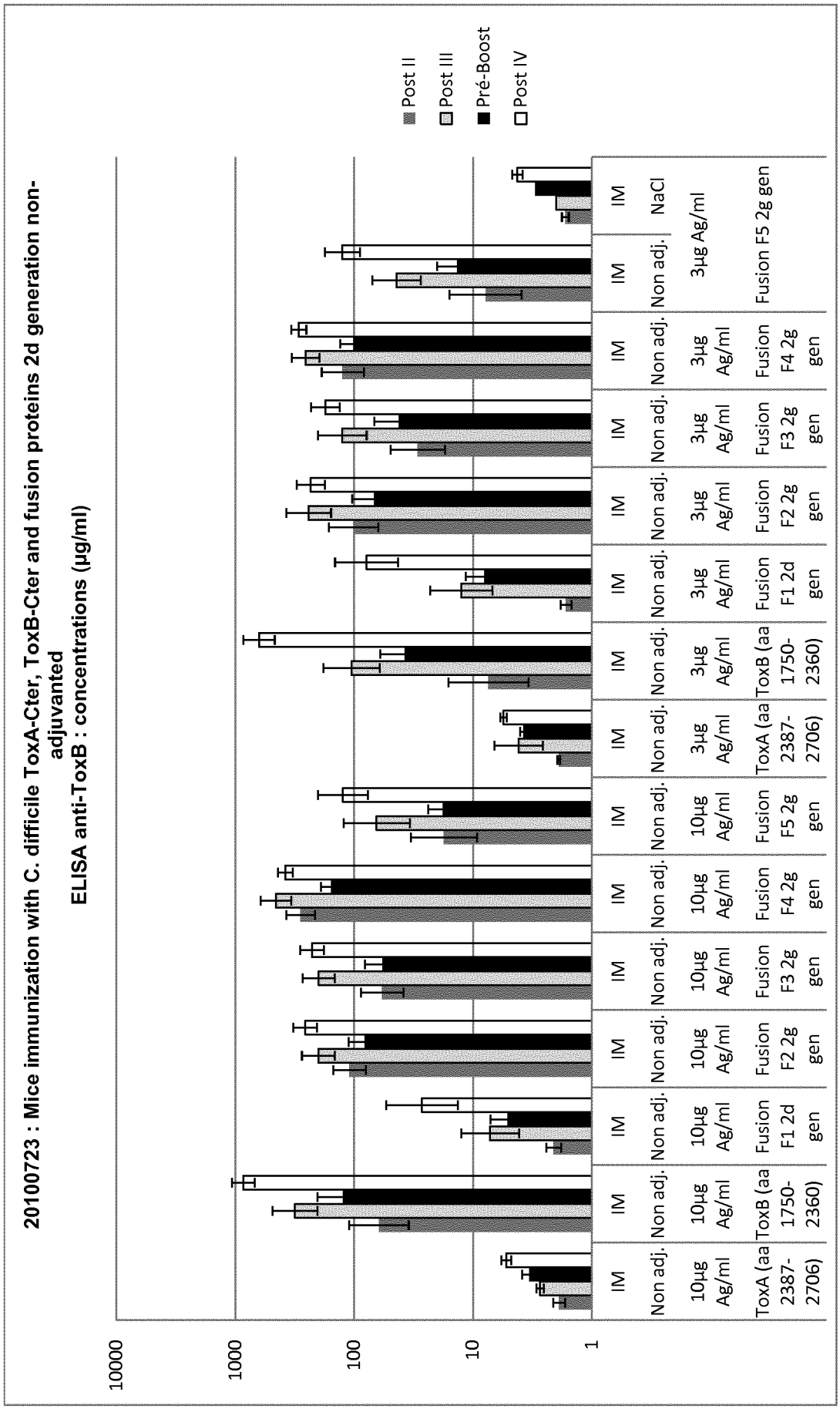

20100723 : Mice immunization with C. difficile ToxA-Cter, ToxB-Cter and fusion proteins 2d generation non-adjuvanted
ELISA anti-ToxB : concentrations (µg/ml)
Post II
Post III
Pré-Boost
Post IV
10000
1000
100
10
1
IM
Non adj.
10µg Ag/ml
ToxA (aa 2387-2706)
ToxB (aa 1750-2360)
Fusion F1 2d gen
Fusion F2 2g gen
Fusion F3 2g gen
Fusion F4 2g gen
Fusion F5 2g gen
3µg Ag/ml
NaCl

Figure 20

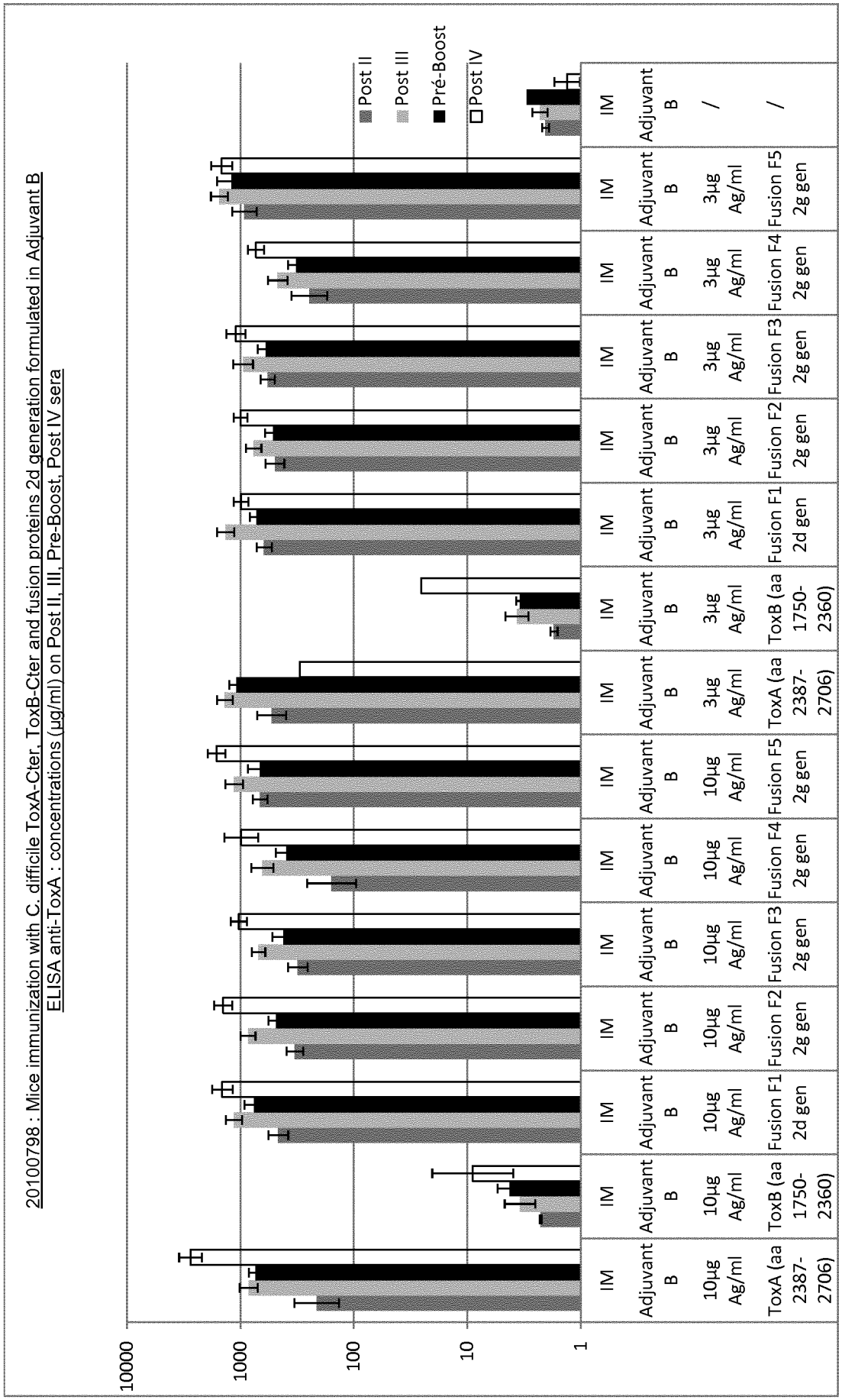

20100798 : Mice immunization with C. difficile ToxA-Cter, ToxB-Cter and fusion proteins 2d generation formulated in Adjuvant B
ELISA anti-ToxA : concentrations (µg/ml) on Post II, III, Pre-Boost, Post IV sera
Post II
Post III
Pré-Boost
Post IV
10000
1000
100
10
1
ToxA (aa 2387-2706)
10µg Ag/ml
Adjuvant B
IM
ToxB (aa 1750-2360)
Fusion F1 2d gen
Fusion F2 2g gen
Fusion F3 2g gen
Fusion F4 2g gen
Fusion F5 2g gen
3µg Ag/ml
/

Figure 21

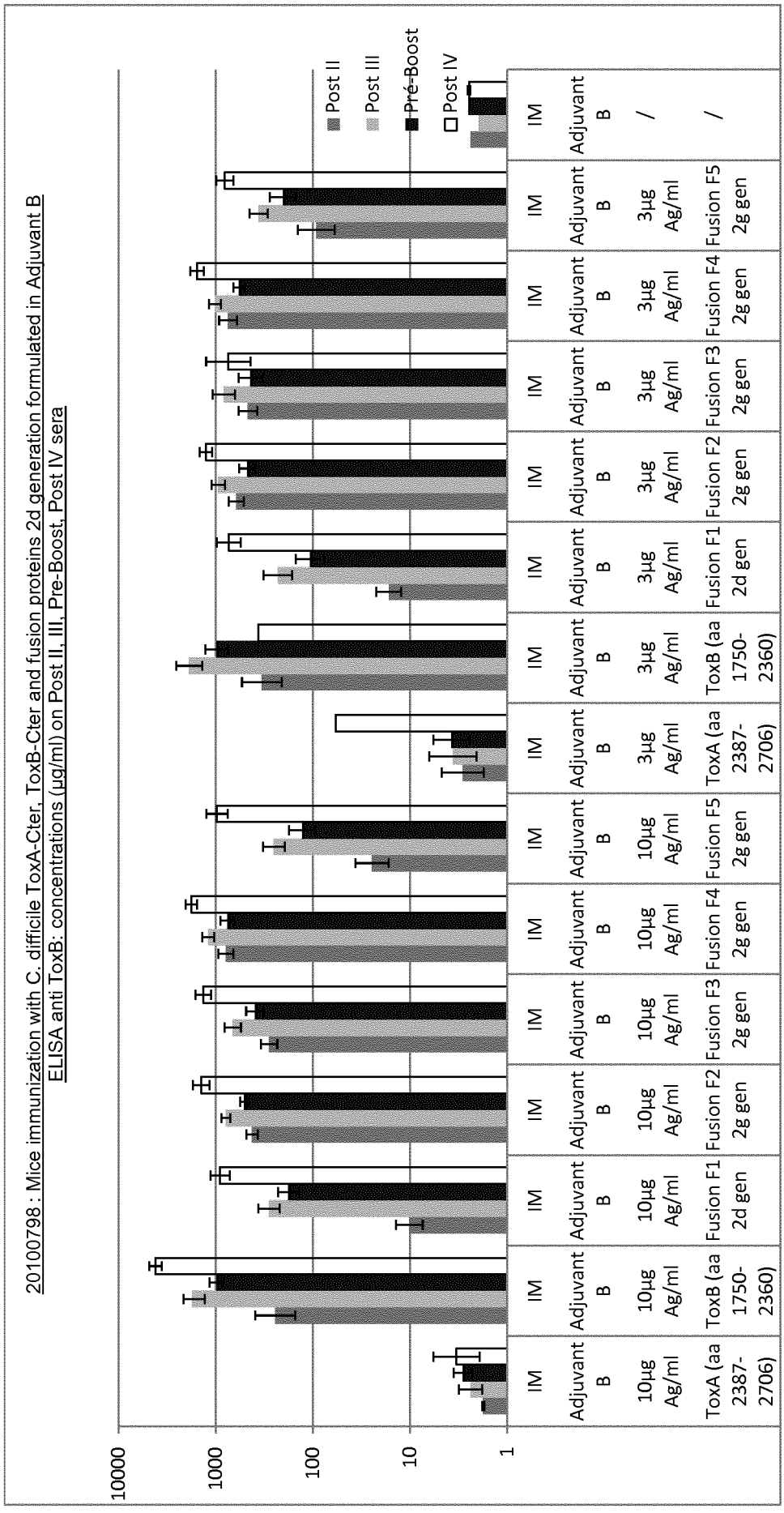

20100798 : Mice immunization with C. difficile ToxA-Cter, ToxB-Cter and fusion proteins 2d generation formulated in Adjuvant B
ELISA anti ToxB: concentrations (µg/ml) on Post II, III, Pre-Boost, Post IV sera
Post II
Post III
Pré-Boost
Post IV
10000
1000
100
10
1
IM
Adjuvant B
10µg Ag/ml
ToxA (aa 2387-2706)
ToxB (aa 1750-2360)
Fusion F1 2d gen
Fusion F2 2g gen
Fusion F3 2g gen
Fusion F4 2g gen
Fusion F5 2g gen
3µg Ag/ml
/

Figure 22

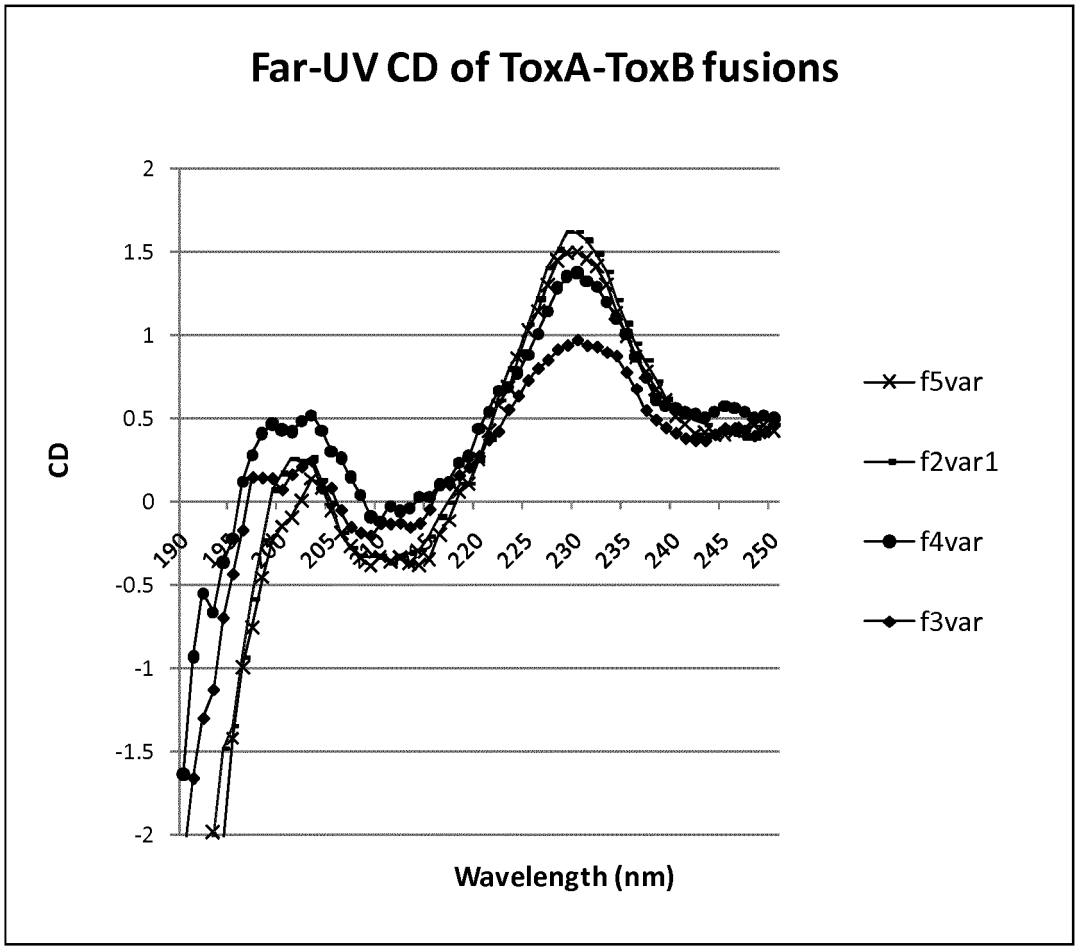

Far-UV CD of ToxA-ToxB fusions
CD
Wavelength (nm)
f5var
f2var1
f4var
f3var

Figure 23

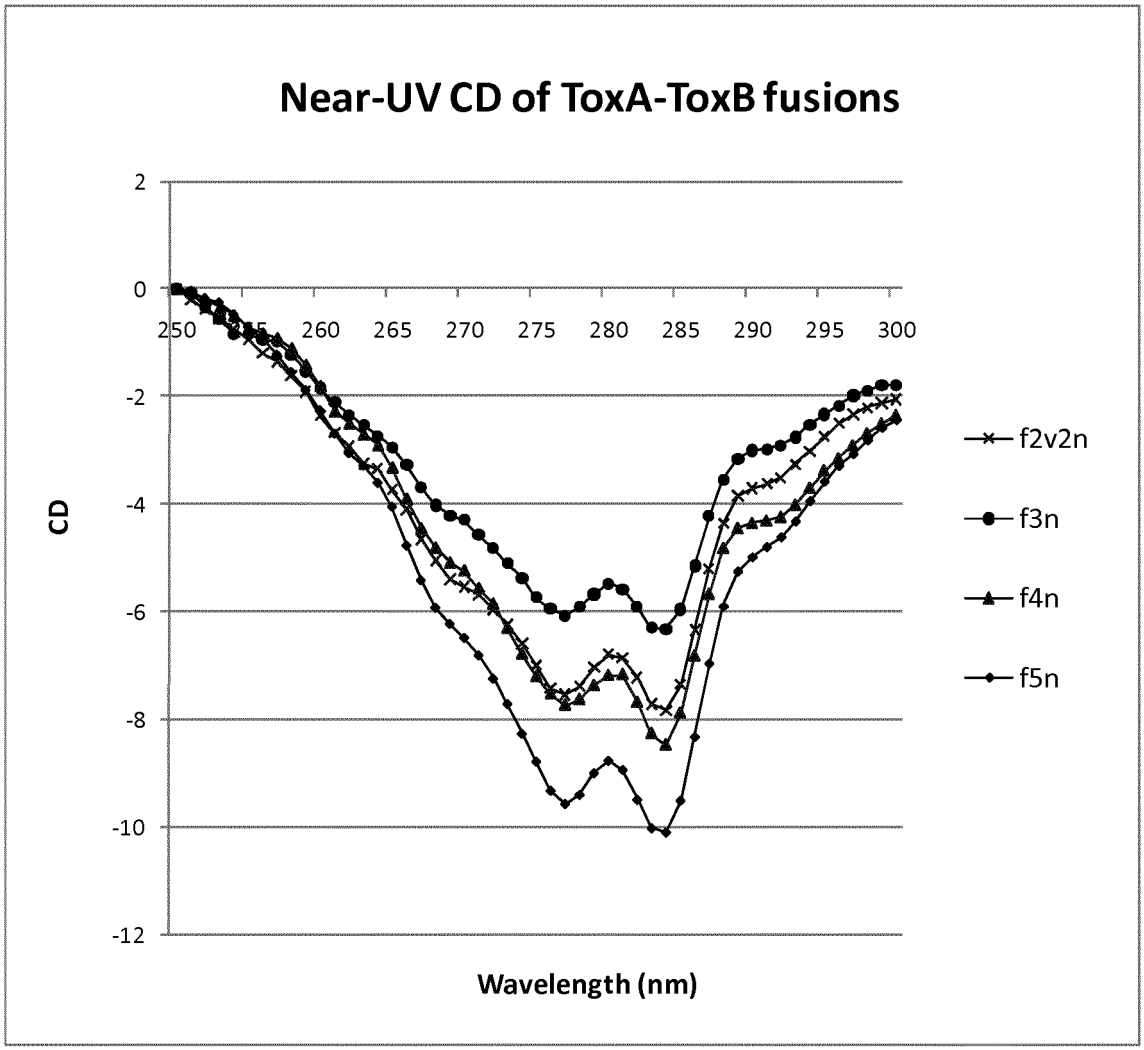

Near-UV CD of ToxA-ToxB fusions
2
0
-2
-4
-6
-8
-10
-12
250
260
265
270
275
280
285
290
295
300
CD
Wavelength (nm)
f2v2n
f3n
f4n
f5n

Figure 24

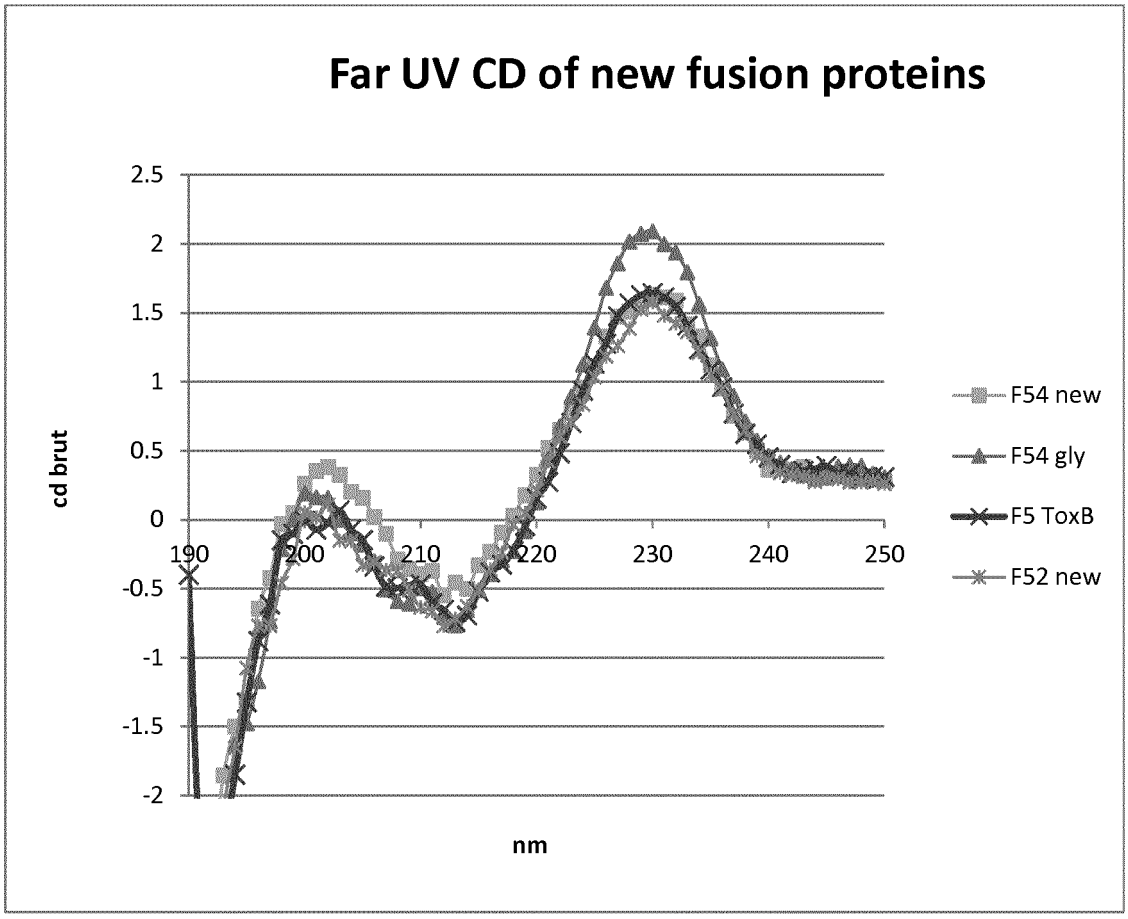
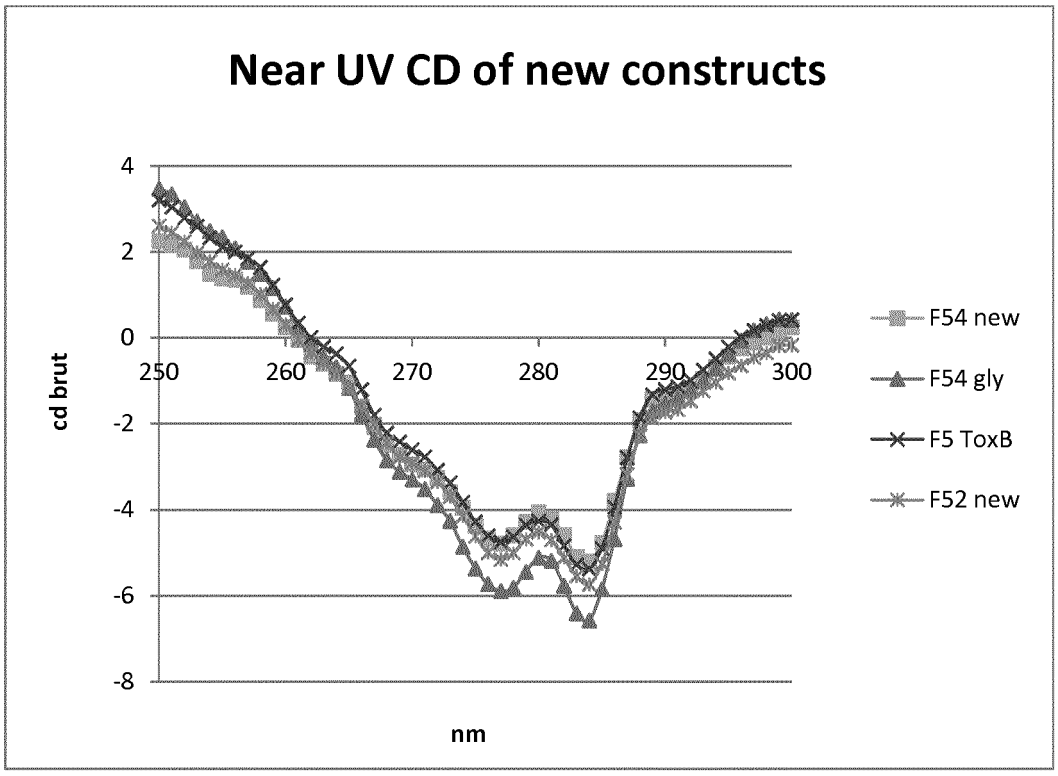

Far UV CD of new fusion proteins
cd brut
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
190
200
210
220
230
240
250
nm
F54 new
F54 gly
F5 ToxB
F52 new

Figure 25


Near UV CD of new constructs
4
2
0
-2
-4
-6
-8
250
260
270
280
290
300
cd brut
nm
F54 new
F54 gly
F5 ToxB
F52 new