

Fig. 1: *C. difficile* triple toxin vaccine constructs

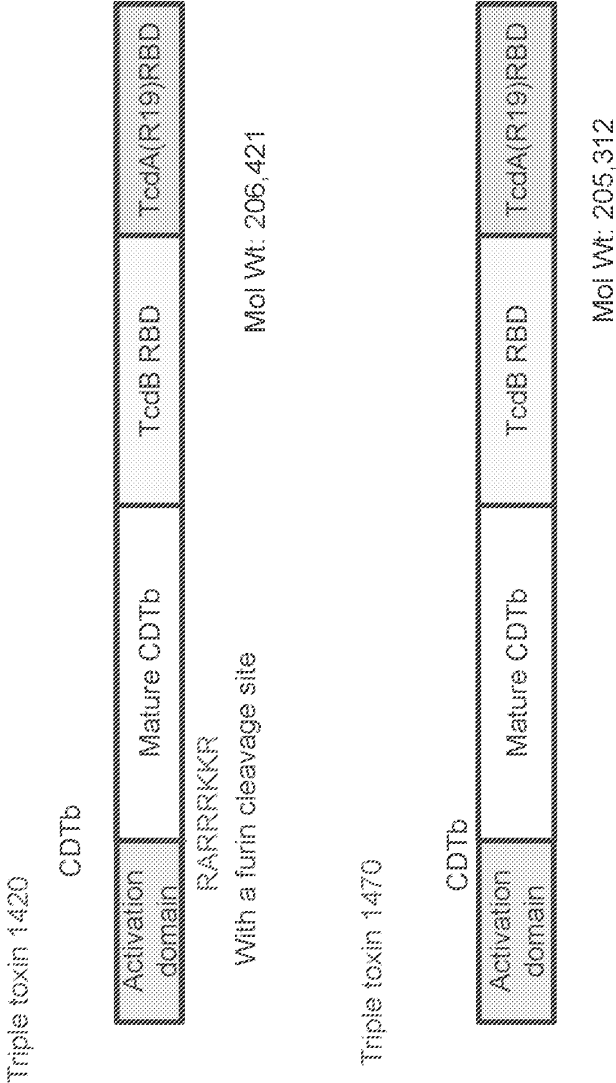


Fig. 2: Expression and solubility of triple toxin vaccine BV1470 and BV1420

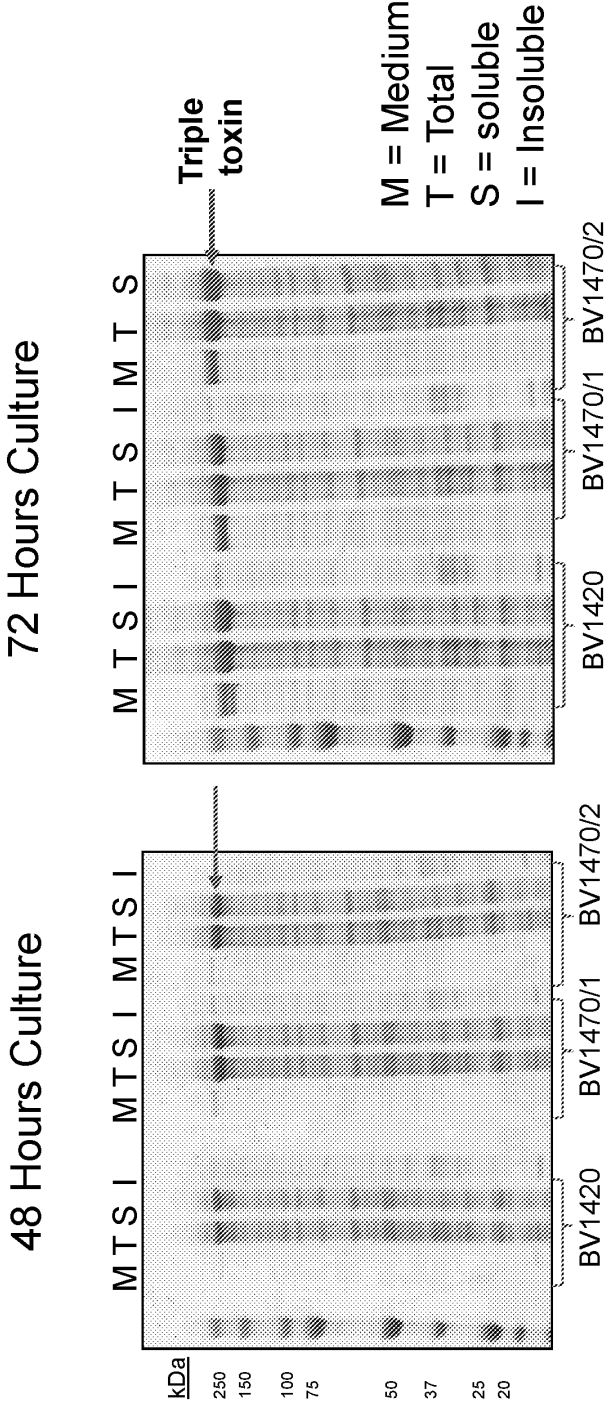


Fig. 3: Time course expression of triple toxin vaccine
BV1470 and BV1420

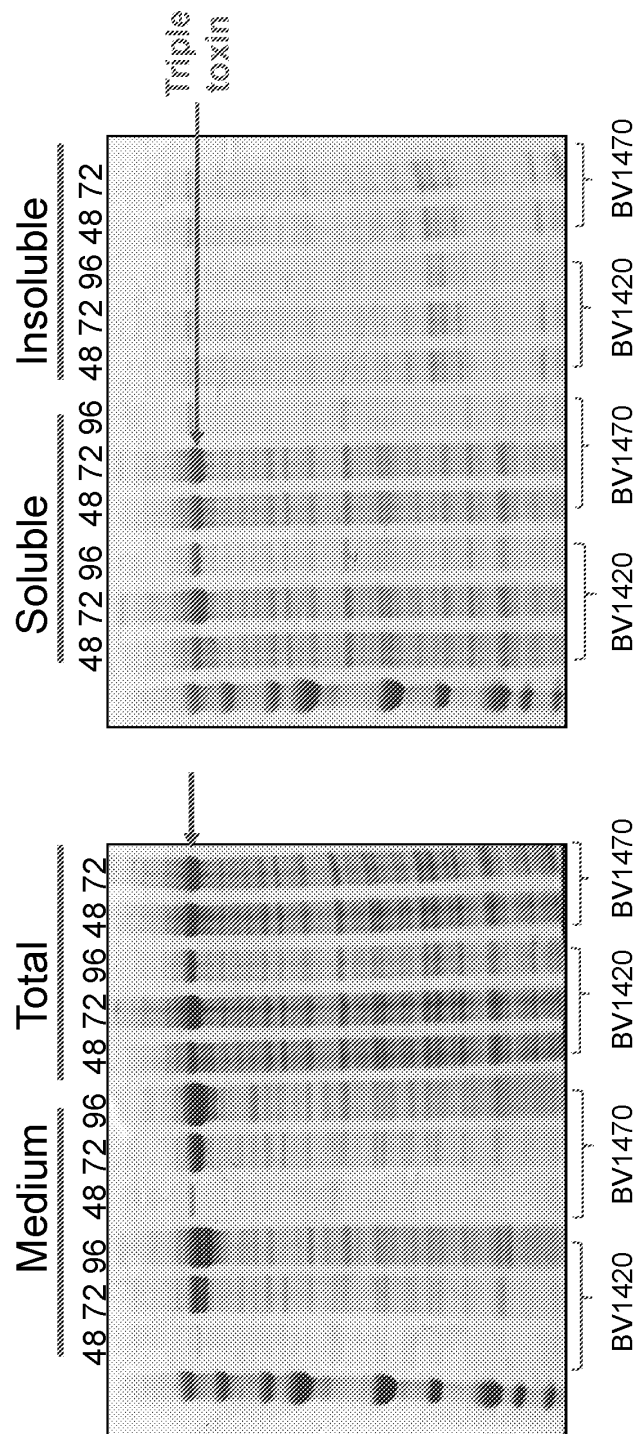


Fig. 4: Purification of triple toxin vaccine

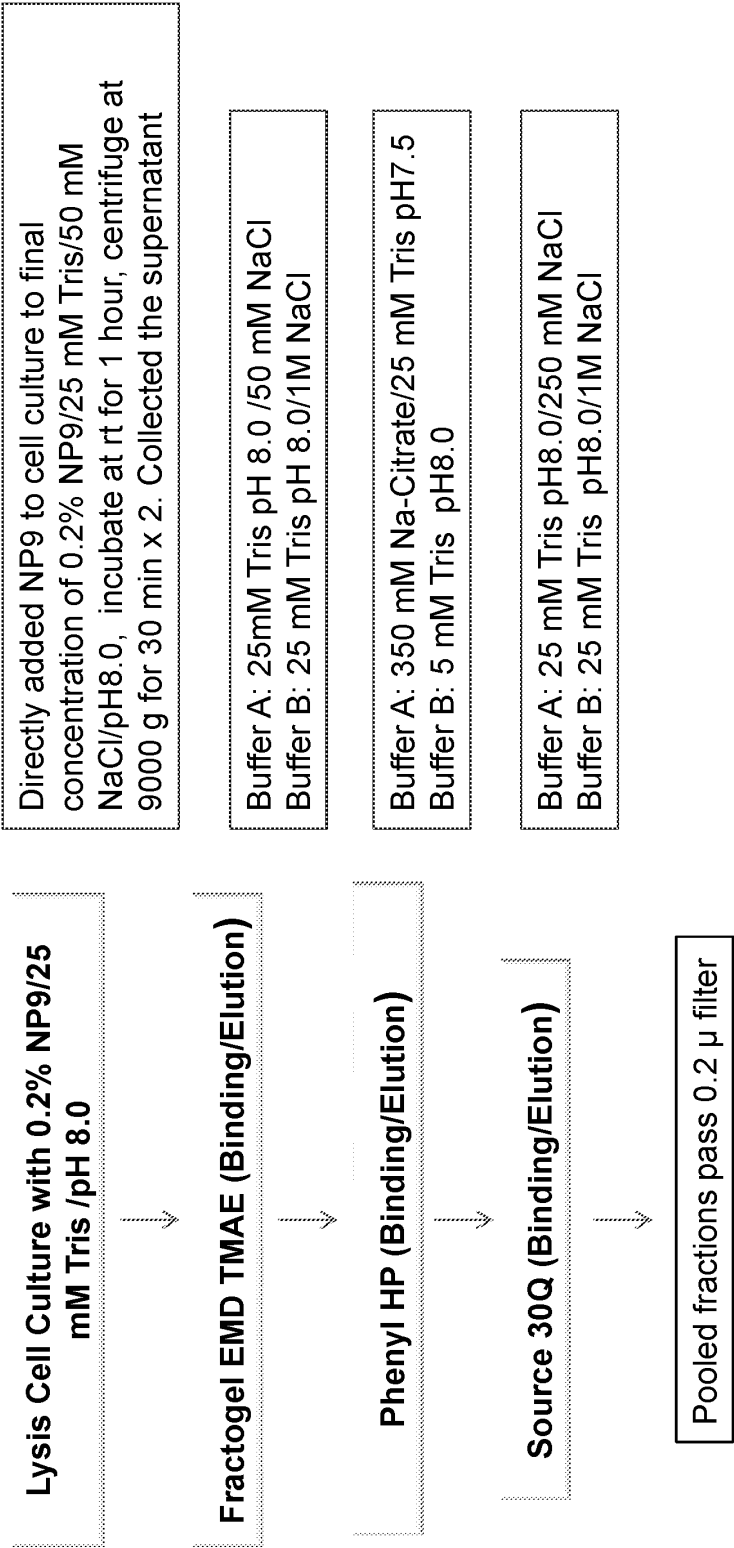
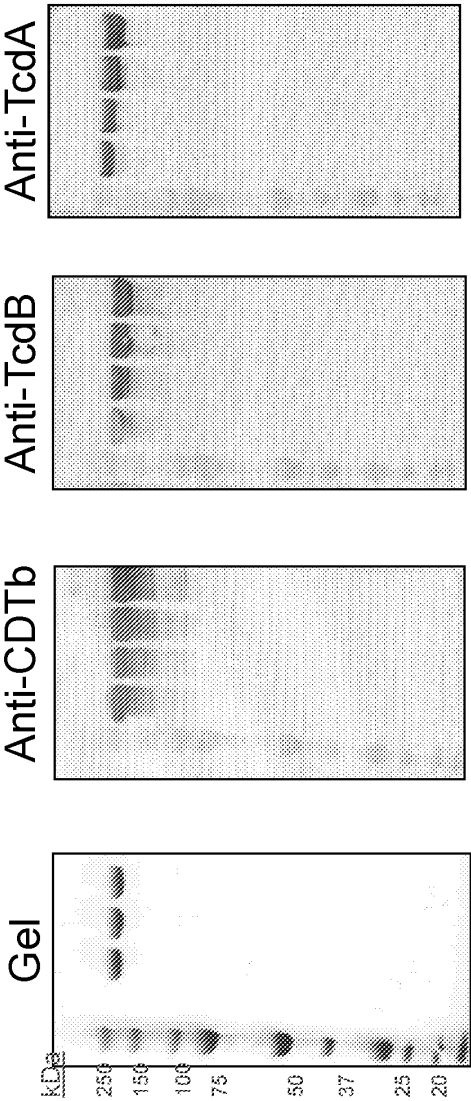
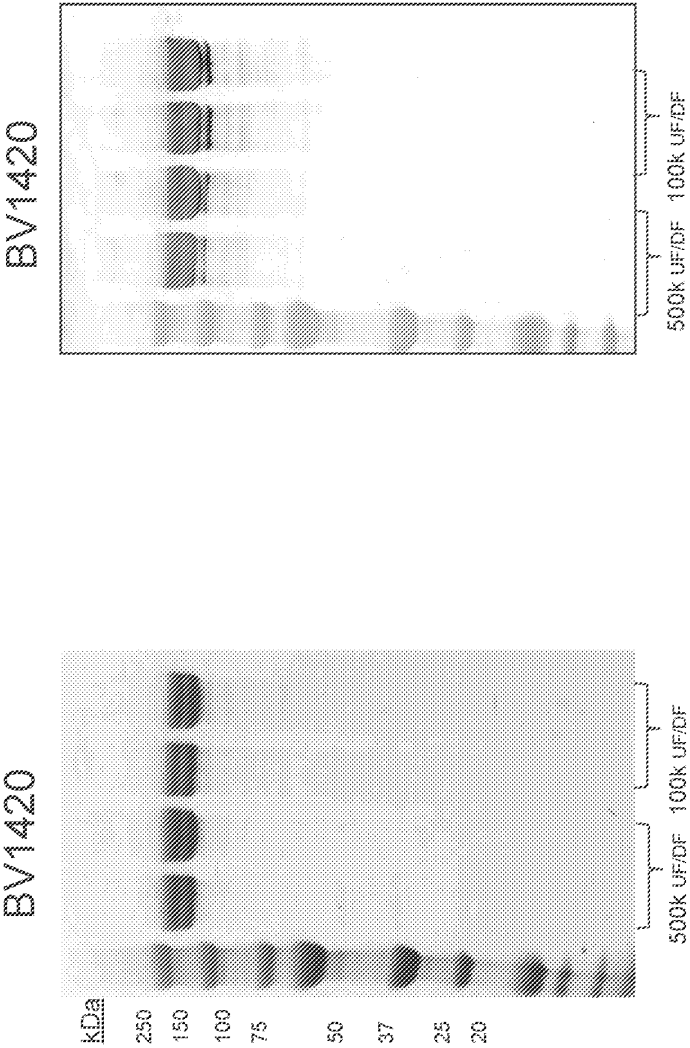


Fig. 5: Purification of triple toxin vaccines
1470 from Sf9 cells



yield: 269 mg/liter

Fig. 6: Purification of triple toxin vaccines
1420 from Sf9 cells



yield: 166 mg/liter

Fig. 7: Particle size distribution by volume graph for triple toxin BV1420

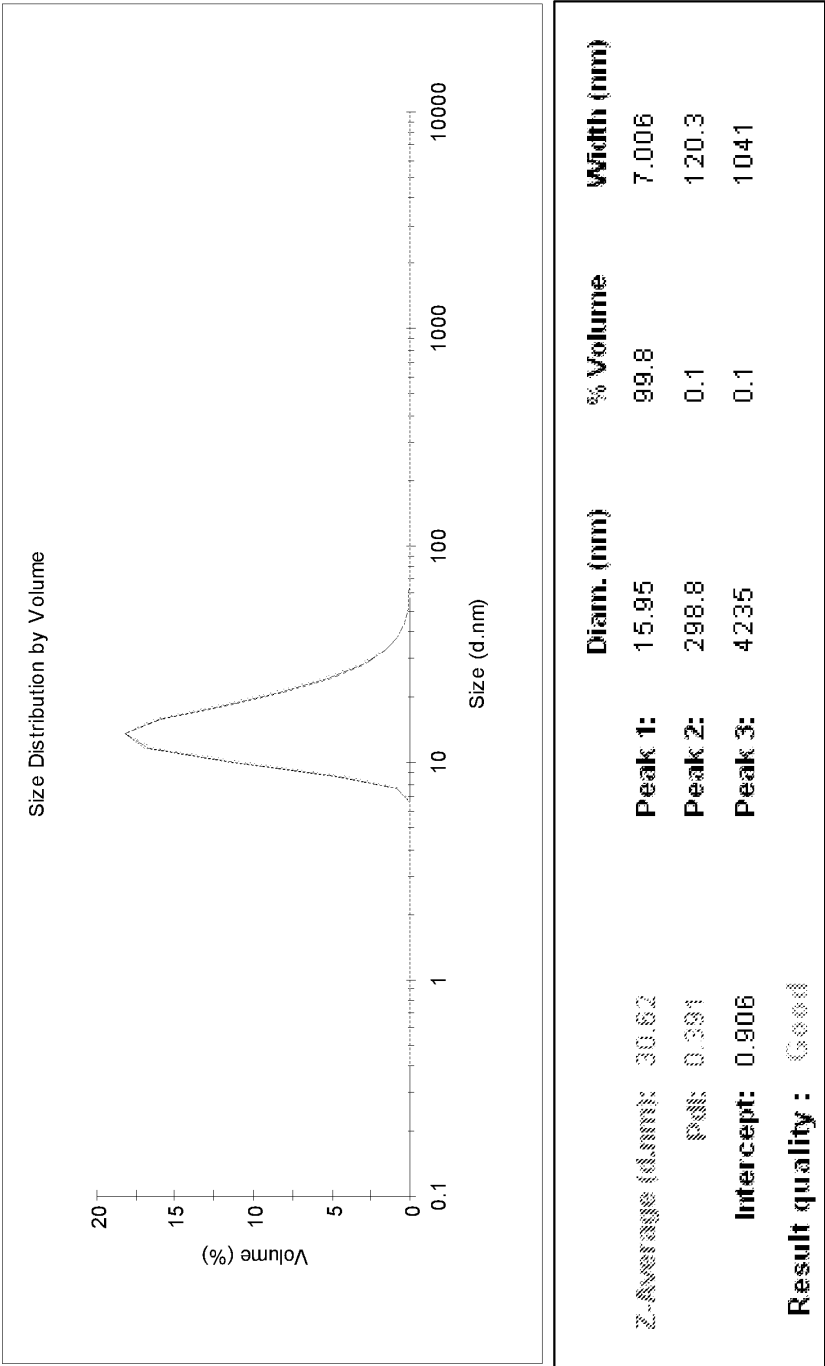
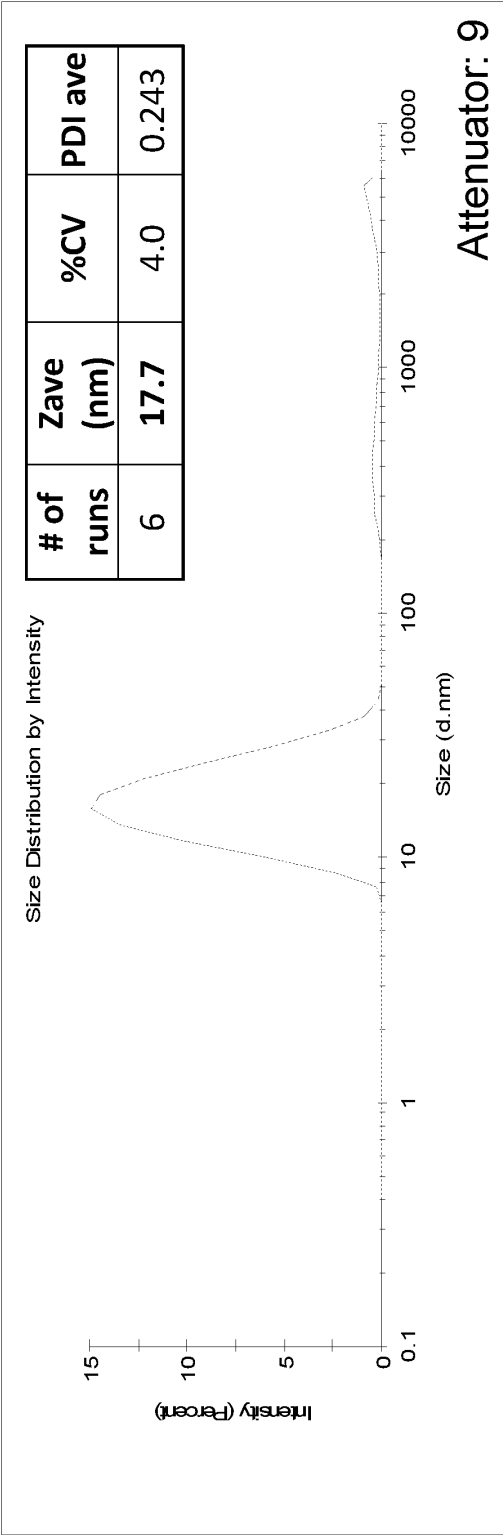


Fig. 8: Particle size distribution by intensity graph for triple toxin BV1470



Z-Average (d.nm): 17.66
PDI: 0.243
Intercept: 0.934
Result quality : Good

Size (d.nm):
Peak 1: 17.84
Peak 2: 558.7
Peak 3: 4210
% Intensity: 92.3 4.2 3.5
St Dev (d.nm): 6.148 341.0 1134

Fig. 9A: Electronmicrographs of negative stained
triple toxin BV1420

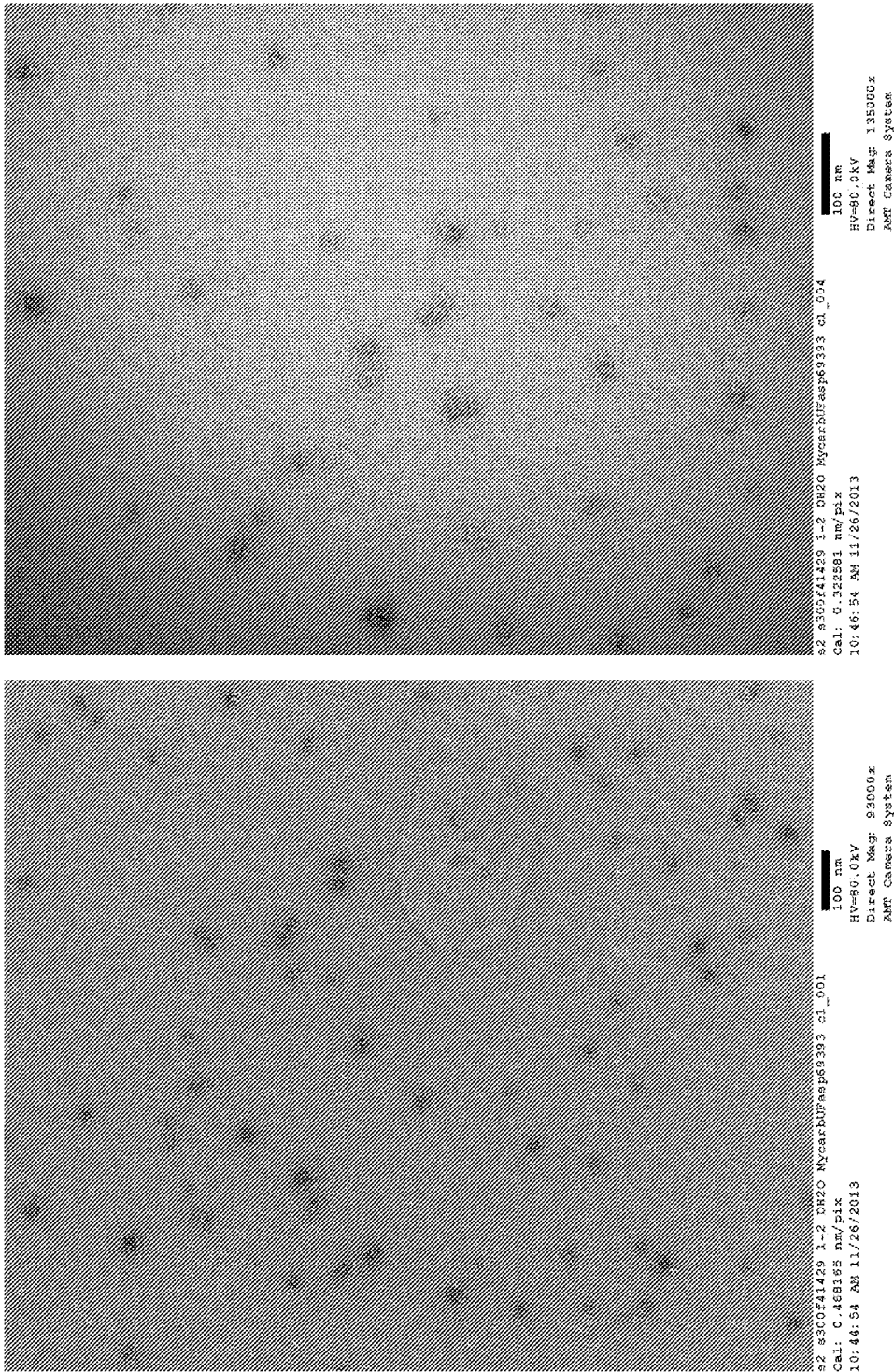


Fig. 9B

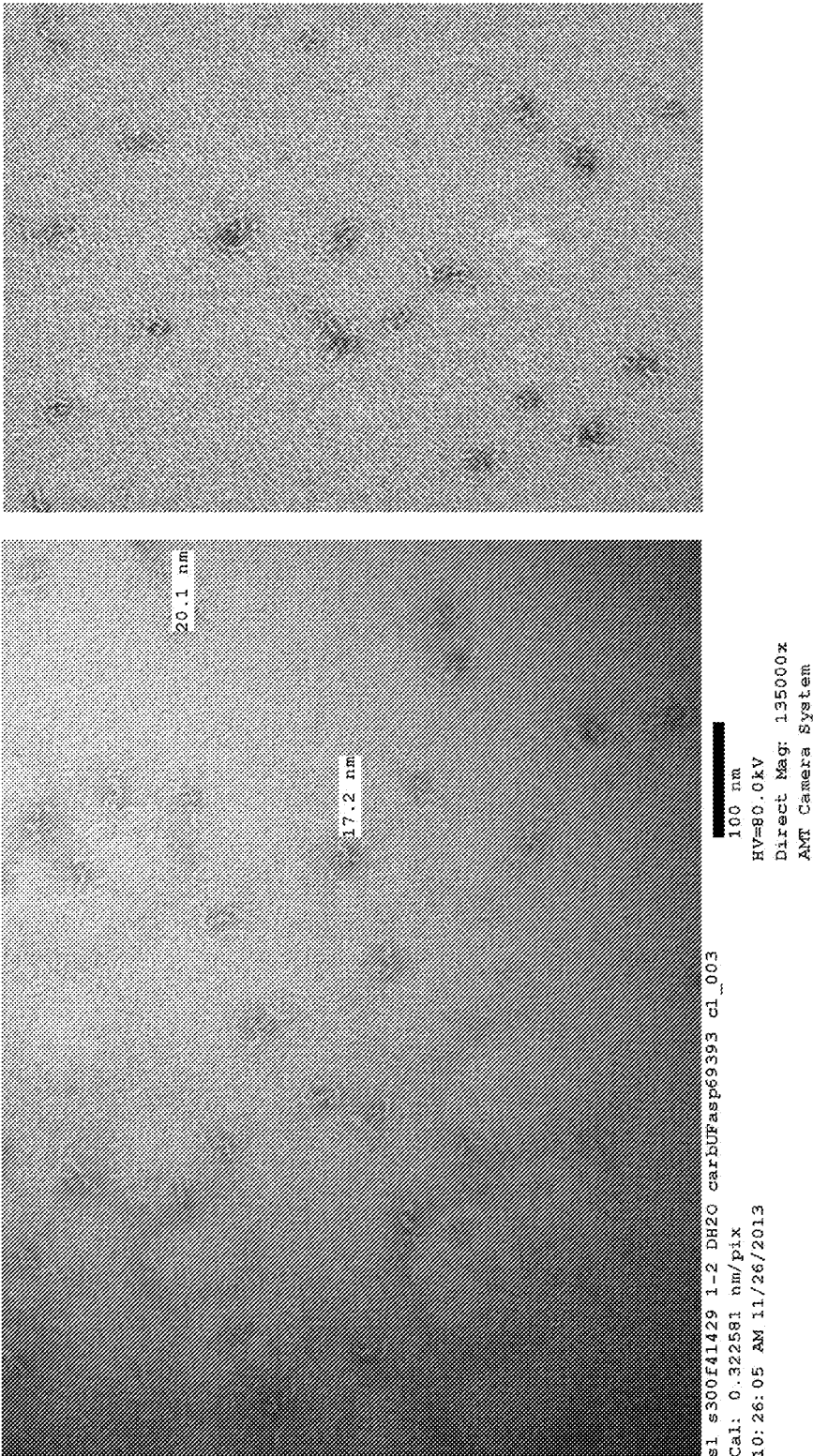


Fig. 9C

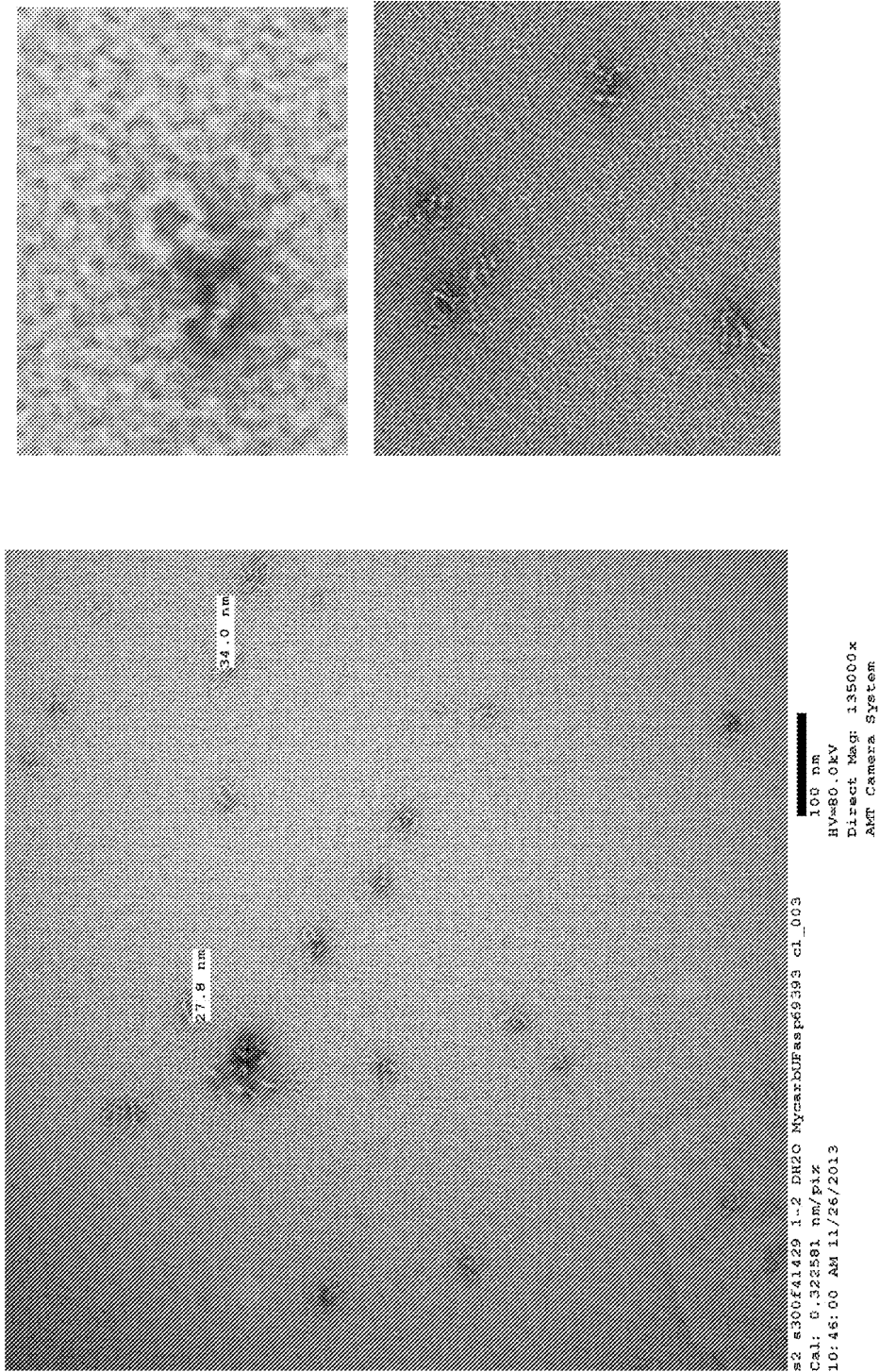
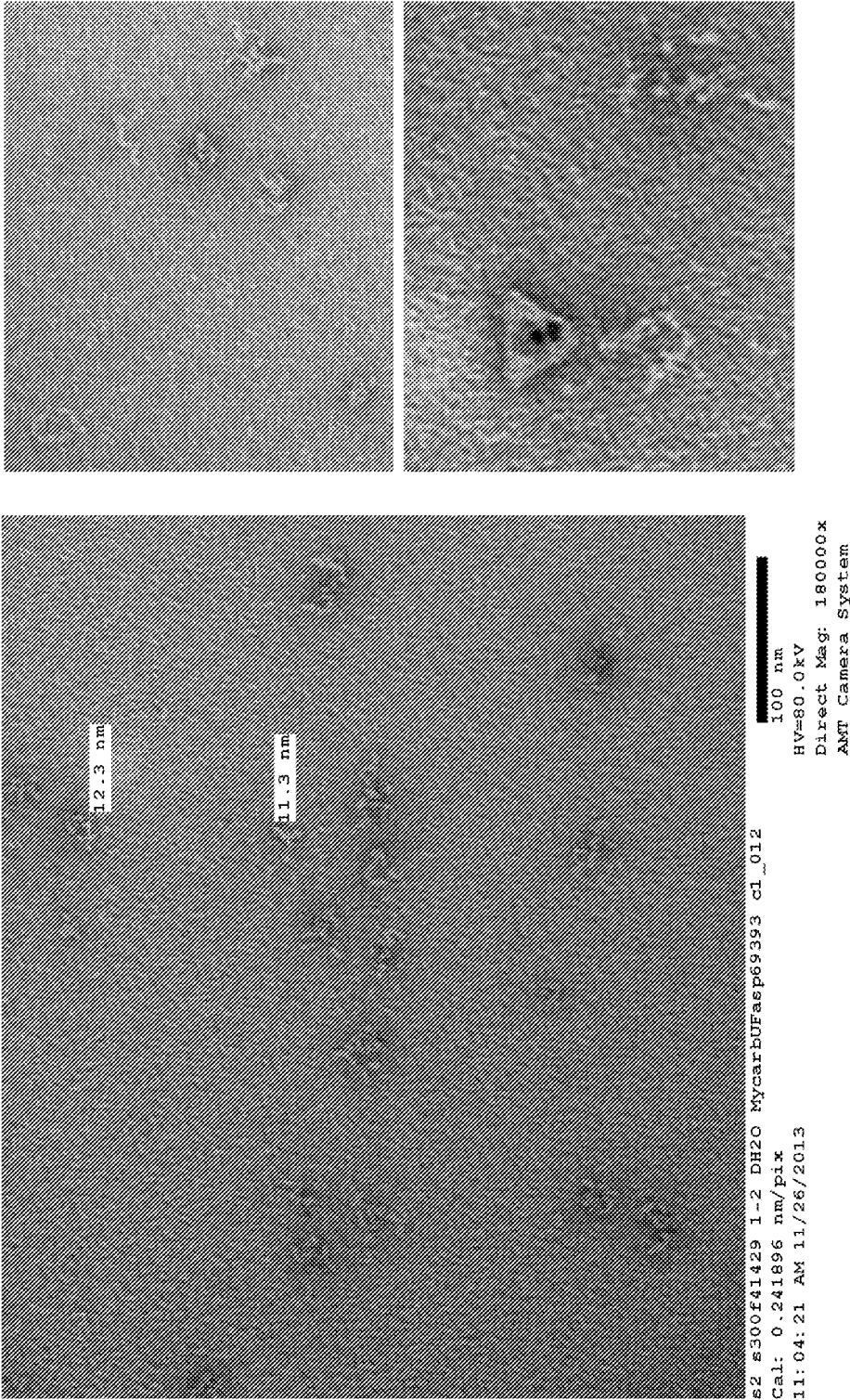


Fig. 9D



**Fig. 10: BV1420 triple toxin vaccine mouse lethal
toxin challenge study 1**

Gp	Antigen	Adjuvant (μ g)	Dose (μ g)	N	Day Immun.	Bleed	Challenge day 35	Terminal bleed
1	BV1420	Alum OH 50.0	30.0	8	0, 14	0, 14, 32	Toxin A	42
2	BV1420	Alum OH 50.0/ Matrix M 5.0	30.0	8	0, 14	0, 14, 32	Toxin A	42
3	PBS			8	0, 14	0, 14, 32	Toxin A	42
4	BV1420	Alum OH 50.0	30.0	8	0, 14	0, 14, 32	Binary Toxin	42
5	BV1420	Alum OH 50.0/ Matrix M 5.0	30.0	8	0, 14	0, 14, 32	Binary Toxin	42
6	PBS			8	0, 14	0, 14, 32	Binary Toxin	42

Fig. 11: BV1420 triple toxin vaccine mouse lethal toxin challenge study 1
Serum IgG responses

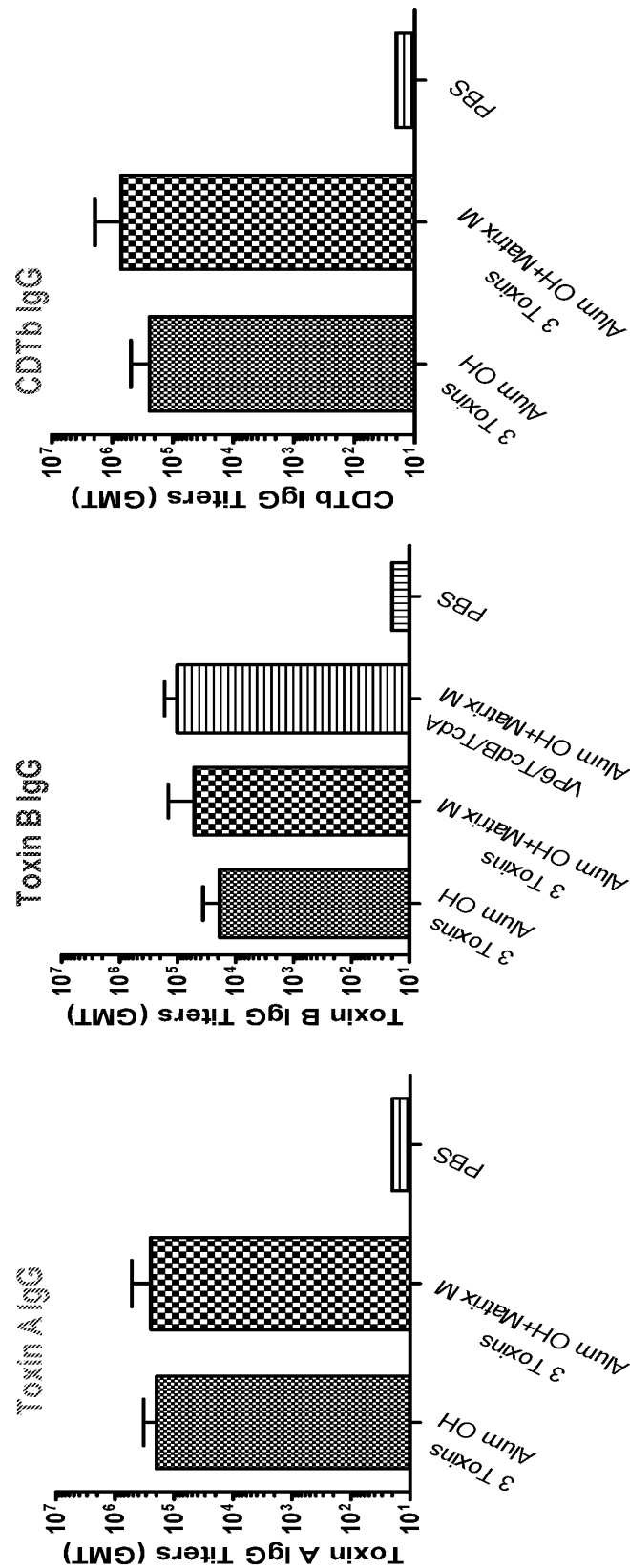
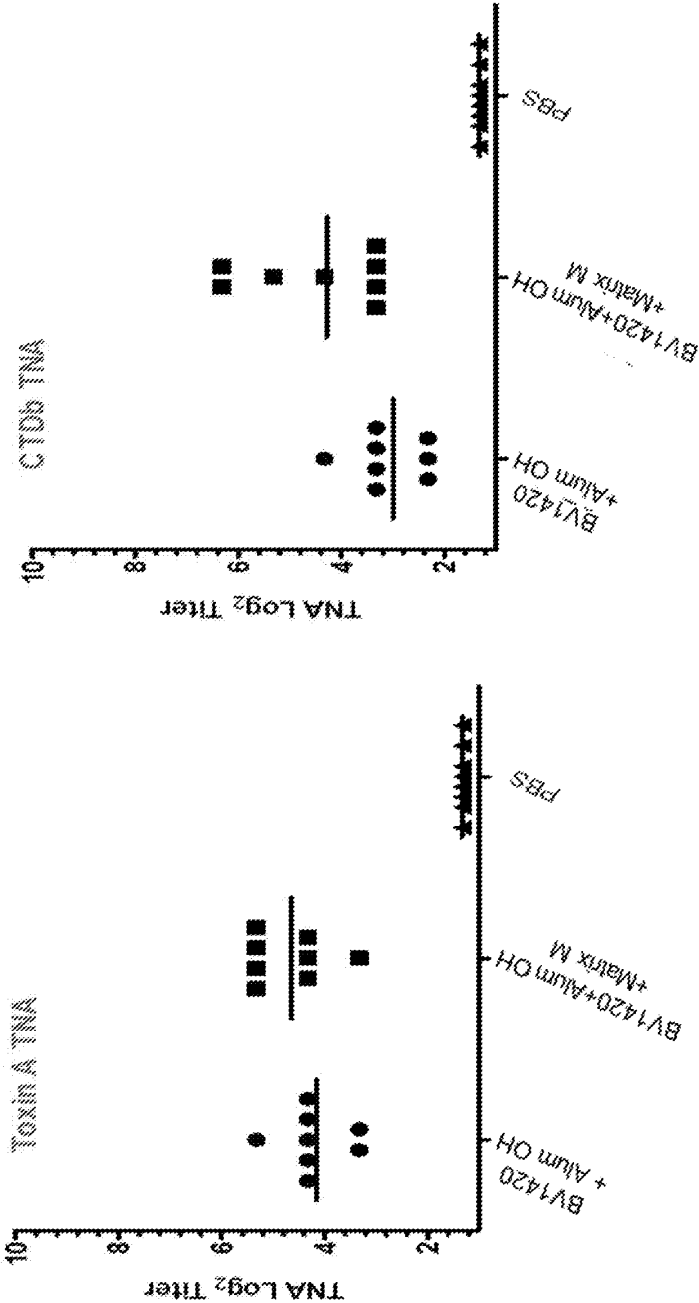


Fig. 12: BV1420 triple toxin vaccine mouse lethal toxin challenge study 1
toxin neutralizing antibody (TNA) titers



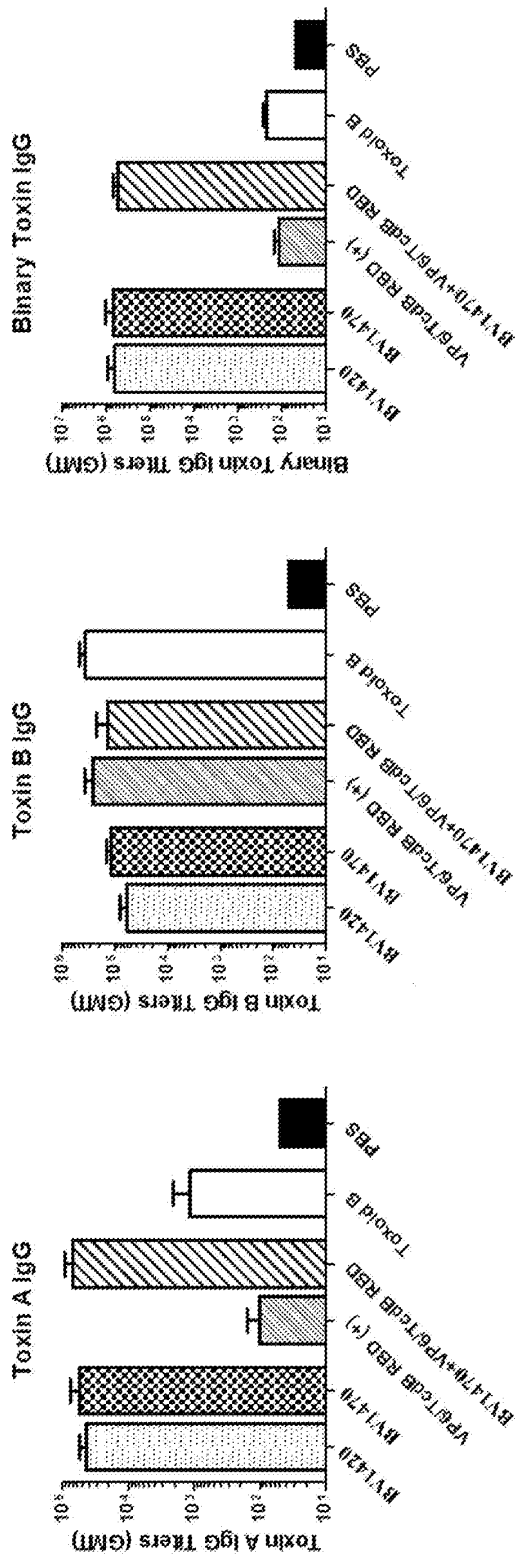
**Fig. 13: BV1420 triple toxin vaccine mouse lethal toxin challenge study 1
animal survival**

Gps	Antigen	Adjuvant (ug)	Toxin	1/6/14 Survivor	1/7/14 Survivor	1/8/14 Survivor	1/9/14 Survivor	1/10/14 Survivor	1/11/14 Survivor	1/12/14 Survivor	1/13/14 Survivor	Survival %
1	BV1420	Alum OH 50.0	Toxin A	8	8	8	8	8	8	8	8	100%
2	BV1420	Alum OH 50.0/ Matrix M 5.0	Toxin A	8	8	8	8	8	8	8	8	100%
3	PBS	NA	Toxin A	8	7	0	0	0	0	0	0	0%
4	BV1420	Alum OH 50.0	Binary Toxin	8	8	8	8	8	8	8	8	100%
5	BV1420	Alum OH 50.0/ Matrix M 5.0	Binary Toxin	8	8	8	8	8	8	8	8	100%
6	PBS	NA	Binary Toxin	8	3	2	2	2	2	2	2	25%

Fig. 14: BV1420 triple toxin vaccine mouse lethal toxin challenge study 2

Gp	N	Antigen	Adjuvant (μ g)	Dose (μ g)	Day Immun.	Bleed	Challenge day 35	Terminal bleed
1	20	BV1420	Alum OH	30.0	0, 14	0, 14, 32	Toxin B	42
2	20	BV1470	Alum OH	30.0	0, 14	0, 14, 32	Toxin B	42
3	20	VP6/TcdB RBD	Alum OH	10.0	0, 14	0, 14, 32	Toxin B	42
4	20	BV1470 + VP6/TcdB RBD	Alum OH	30+10	0, 14	0, 14, 32	Toxin B	42
5	20	Toxoid B	Alum OH	10	0, 14	0, 14, 32	Toxin B	42
6	20	PBS	-	-	0, 14	0, 14, 32	Toxin B	42

Fig. 15: BV1420 triple toxin vaccine mouse lethal toxin challenge study 2
Anti-toxin IgG levels



All vaccine groups adjuvanted with Alum

Fig. 16: BV1420 triple toxin vaccine mouse lethal toxin challenge study 2
Toxin B TNA titers

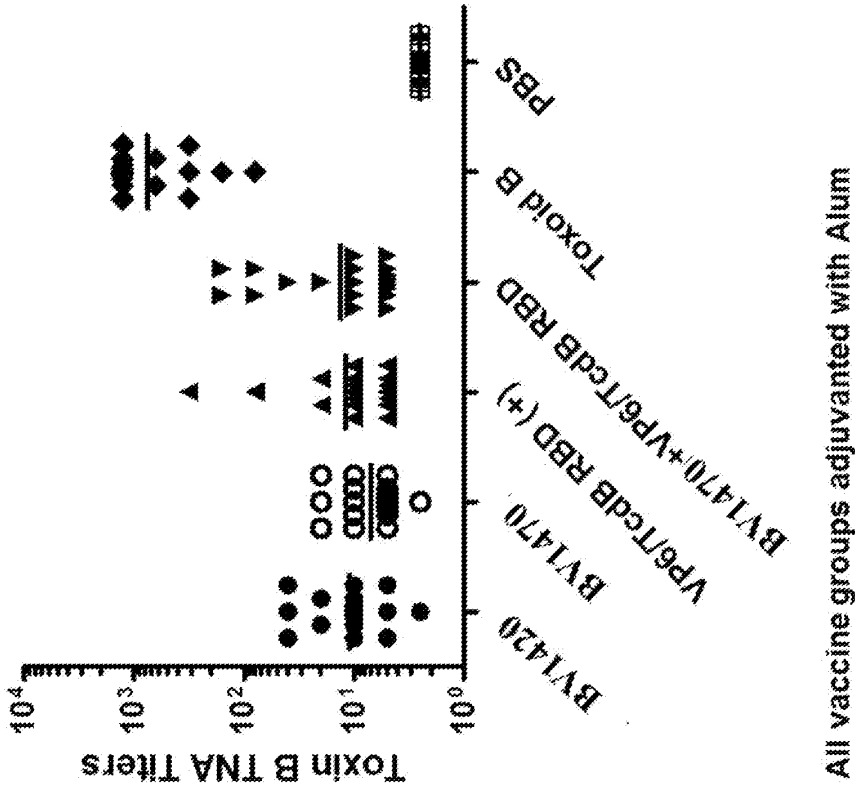


Fig. 17: BV1420 triple toxin vaccine mouse lethal toxin challenge study 2
Toxin B survival

Gps	Antigen	Adjuvant (ug)	Toxin	Day 0 Survivor	Day 1 Survivor	Day 2 Survivor	Day 3 Survivor	Day 4 Survivor	Day 5 Survivor	Day 6 Survivor	Day 7 Survivor	Survival %
1	BV1420	Alum OH 50.0	Toxin B	6	6	6	5	5	4	4	4	67%
2	BV1470	Alum OH 50.0	Toxin B	6	6	6	6	6	6	5	5	83%
3	VP6/TcdB RBD	Alum OH 50.0	Toxin B	6	6	6	5	5	5	5	5	83%
4	BV1470 + VP6/TcdB RBD	Alum OH 50.0	Toxin B	6	5	5	5	5	5	5	5	83%
5	Toxoid B	Alum OH 50.0	Toxin B	6	6	6	6	6	6	6	6	100%
6	PBS	NA	Toxin B	6	1	1	0	0	0	0	0	0%

Fig. 18: Alternative triple toxin vaccines

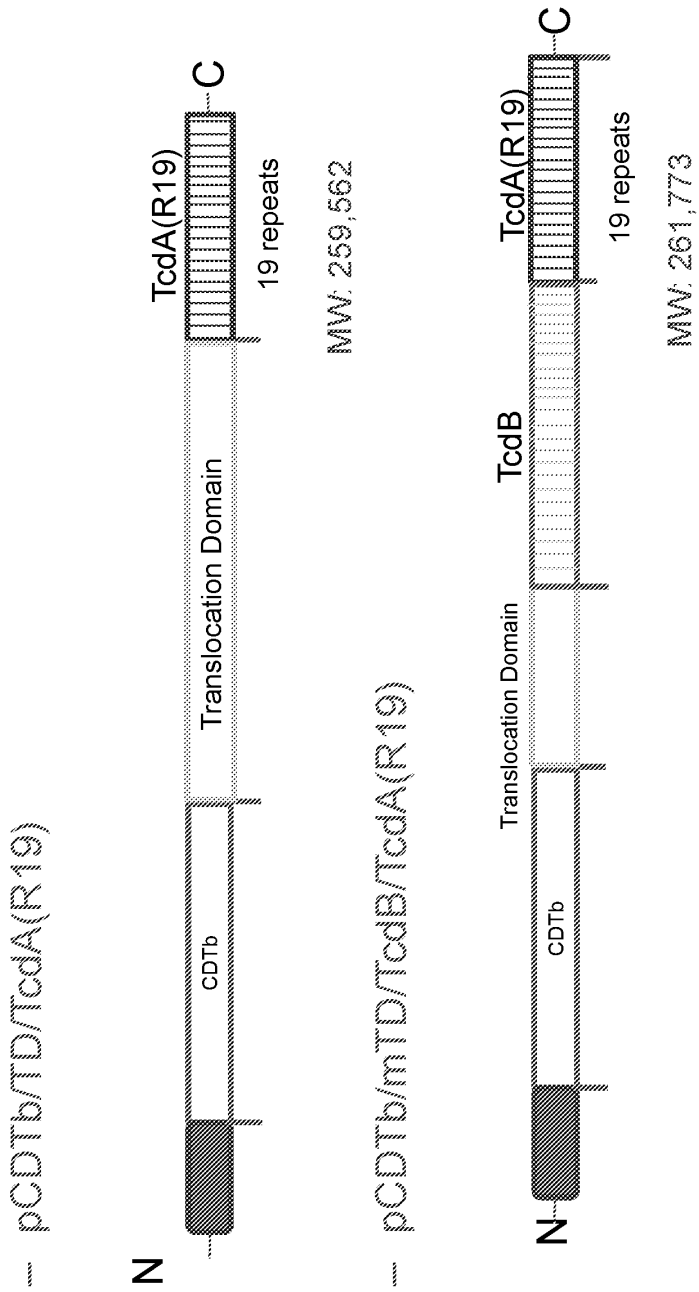


Fig. 19: Alternative triple toxin vaccine – BV1512
purified pCDTb/TD/TcdAR19

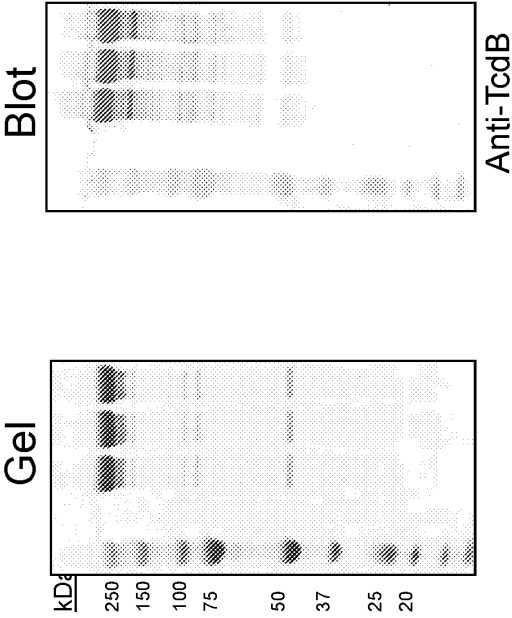


Fig. 20: Quadrivalent toxin vaccine

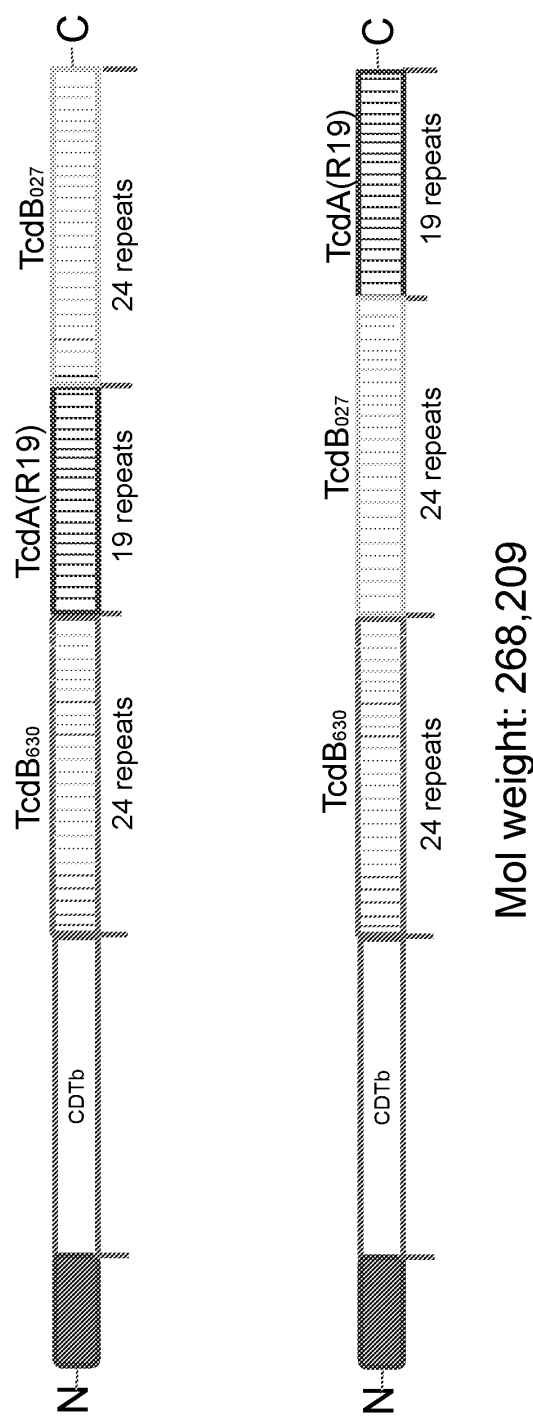
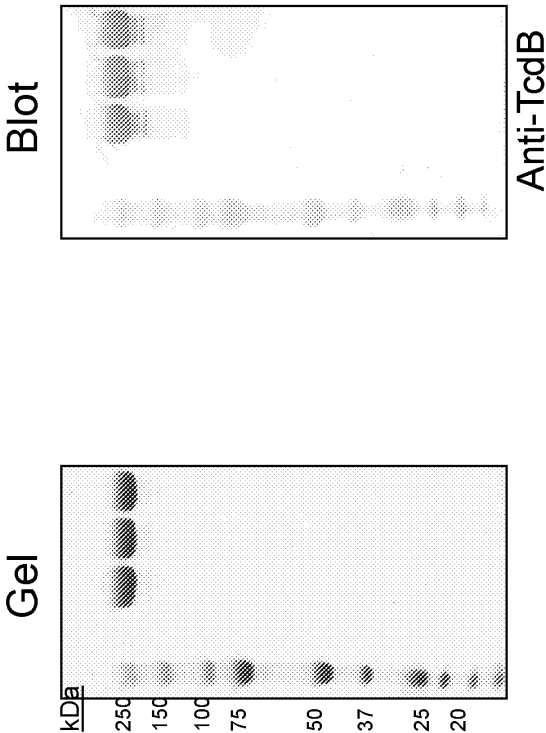
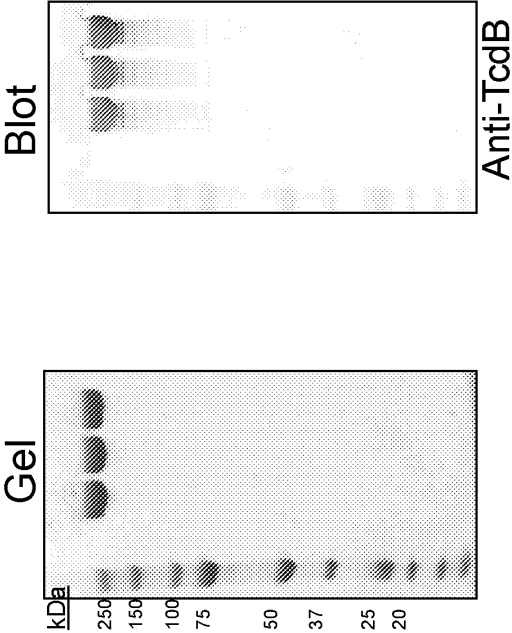


Fig. 21: Purified quadrivalent vaccine - CBAB
pCDTb/TcdB630/TcdAR19/TcdB027



yield from culture medium: 42 mg/liter

Fig. 22: Purified quadrivalent vaccine – CBBA pCDTb/TcdB630/TcdB027/TcdAR19



yield from culture medium: 40 mg/liter

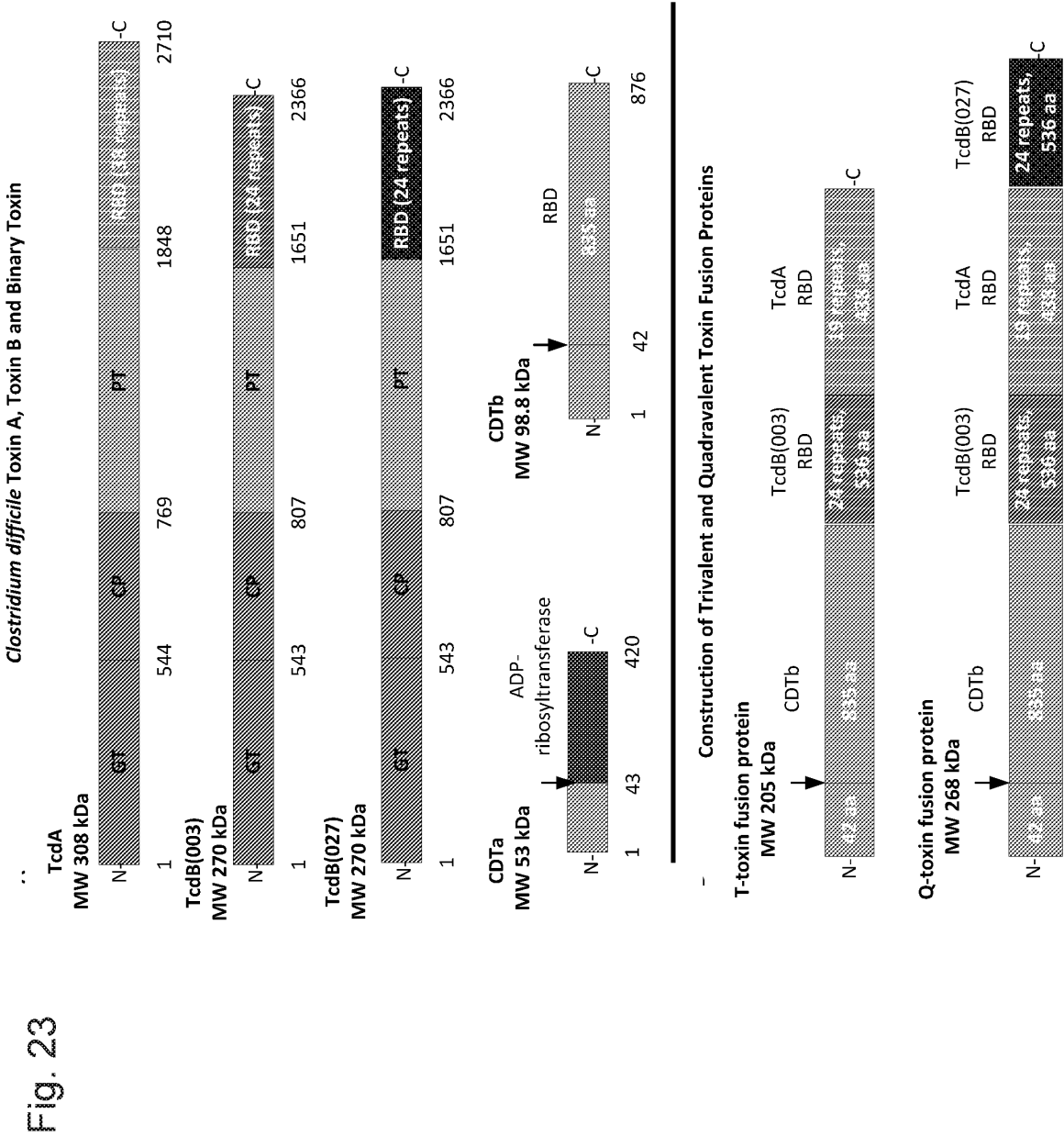


Fig. 24. Expression and purification of T-toxin and Q-toxin fusion proteins.

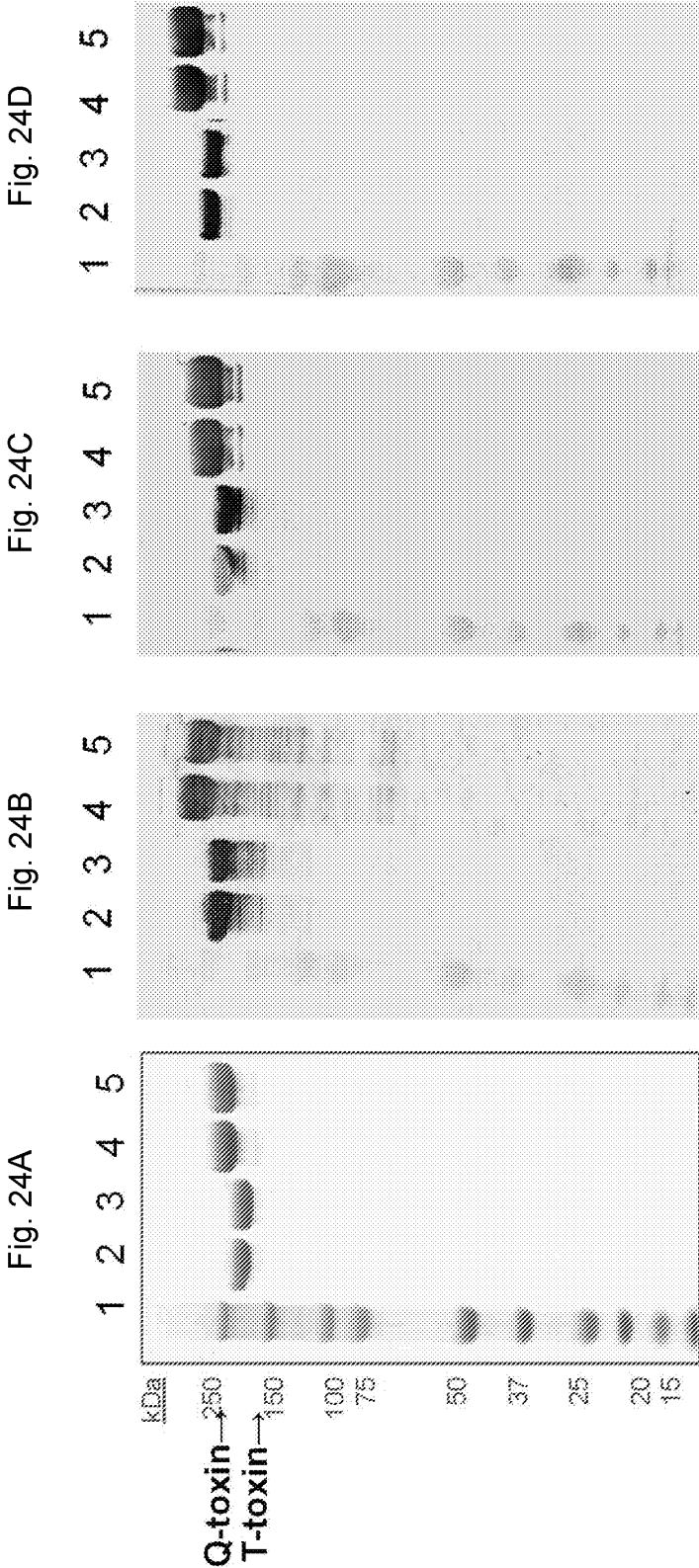


Fig. 25A: Immunogenicity of T-toxin and Q-toxin fusion proteins in mice: Serum IgG titers to TcdA, TcdB(003), and CDTb determined by ELISA

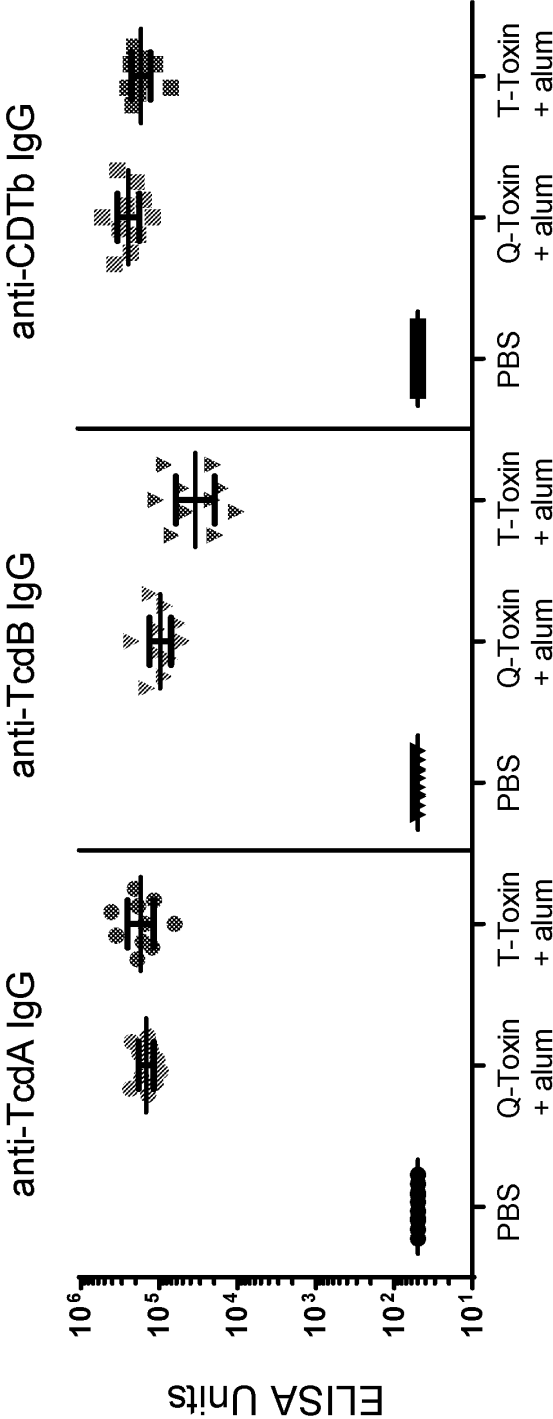


Fig. 25B: Immunogenicity of T-toxin and Q-toxin fusion proteins in mice: toxin-neutralizing antibody (TNA) titers using pooled serum

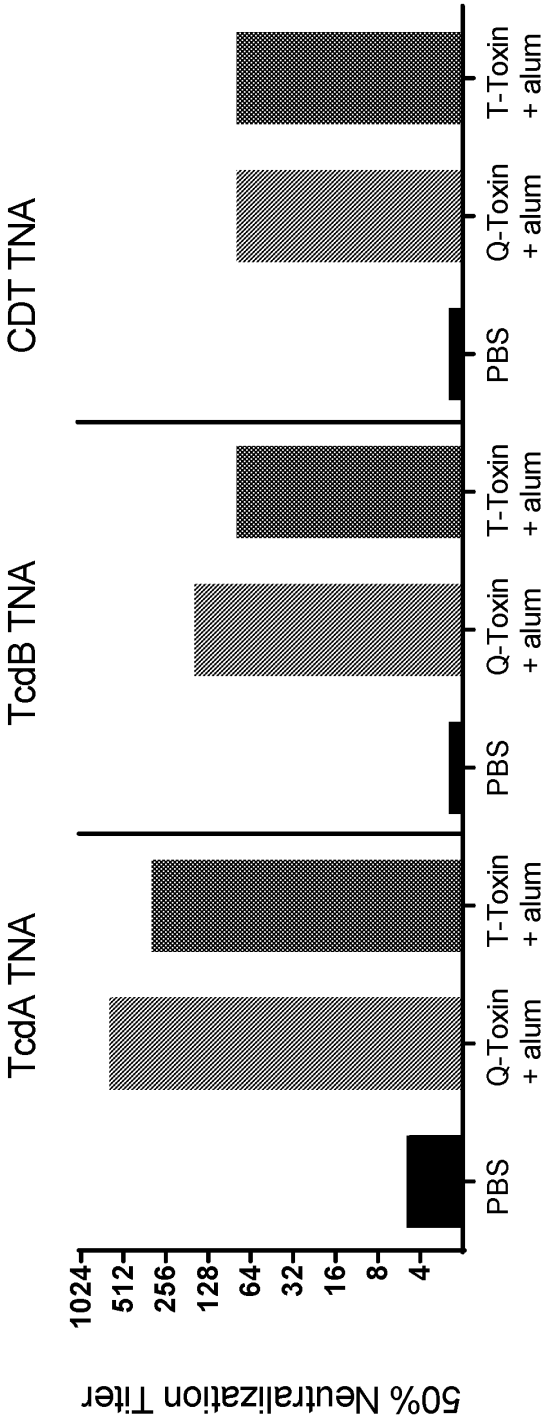


Fig. 25C: Immunogenicity of T-toxin and Q-toxin fusion proteins in mice: Protection against lethal challenge

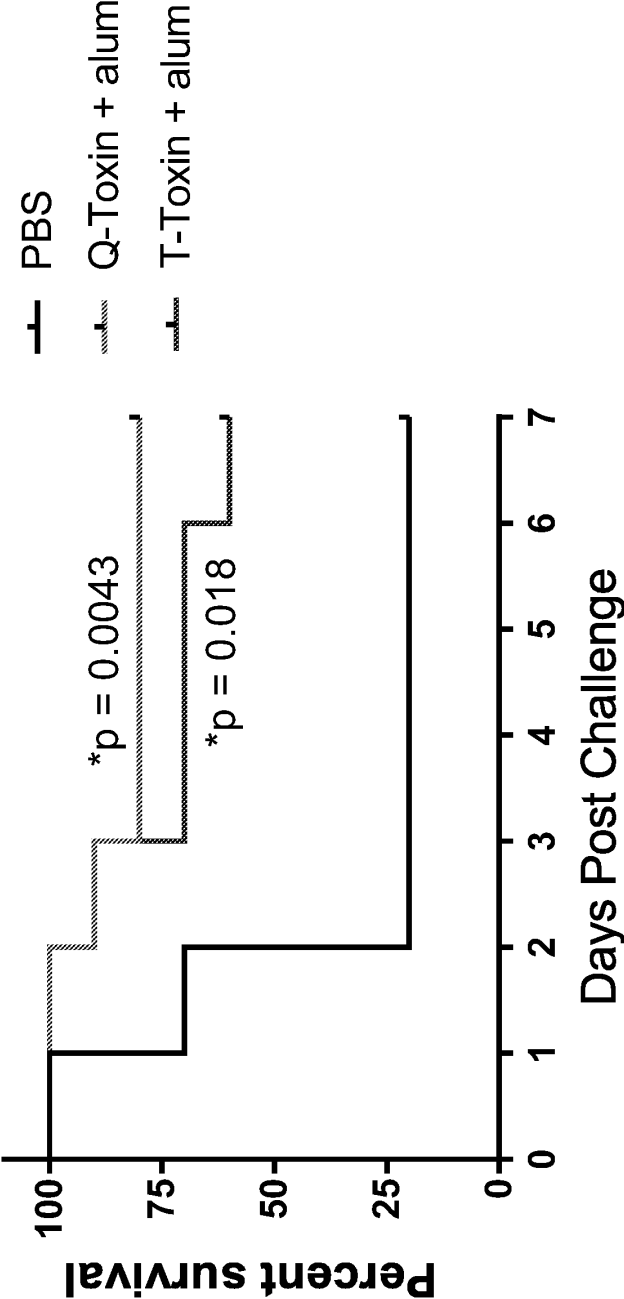


Fig. 26A: Immunogenicity of T-toxin and Q-toxin fusion proteins in hamsters:
Serum IgG titers to TcdA, TcdB(003), and CDTb determined by ELISA

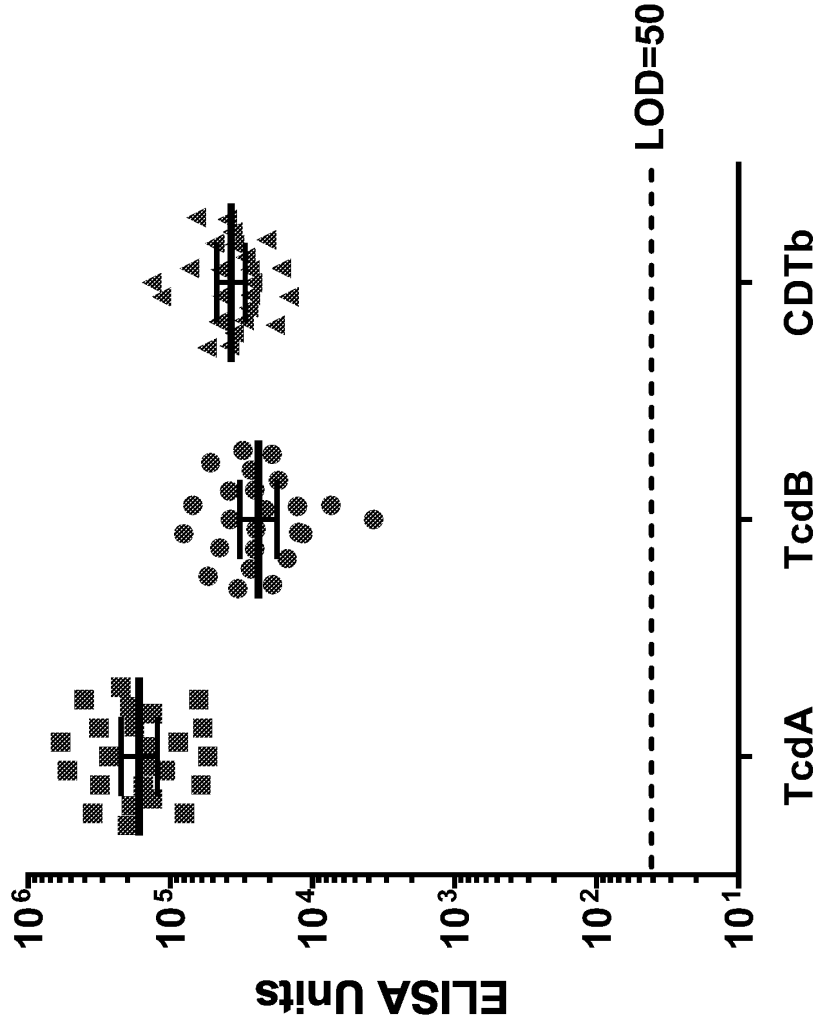


Fig. 26B: Immunogenicity of T-toxin and Q-toxin fusion proteins in hamsters :
Toxin-neutralizing antibody (TNA) titers using pooled serum

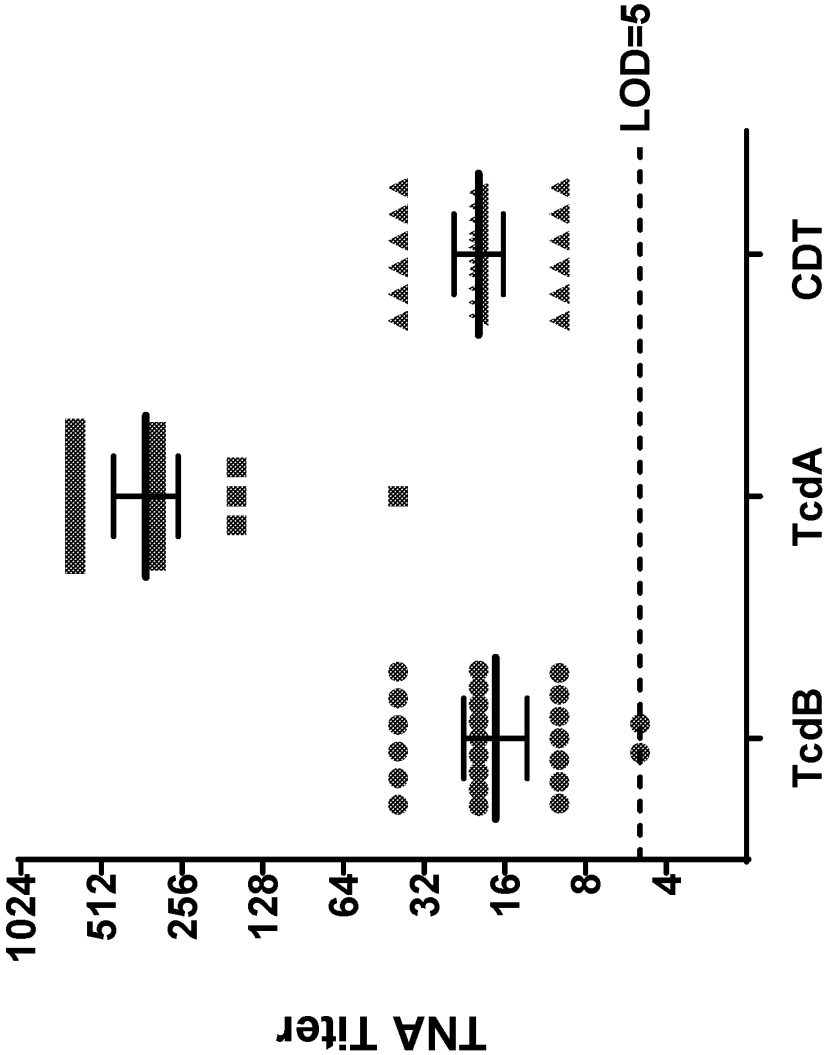
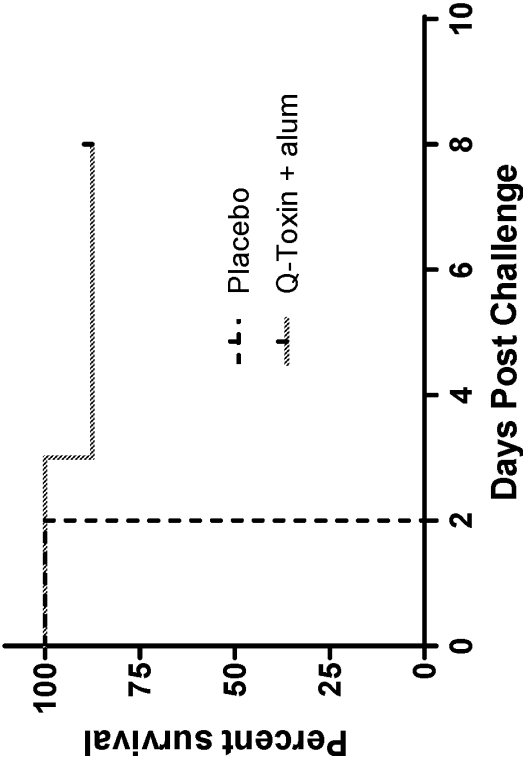
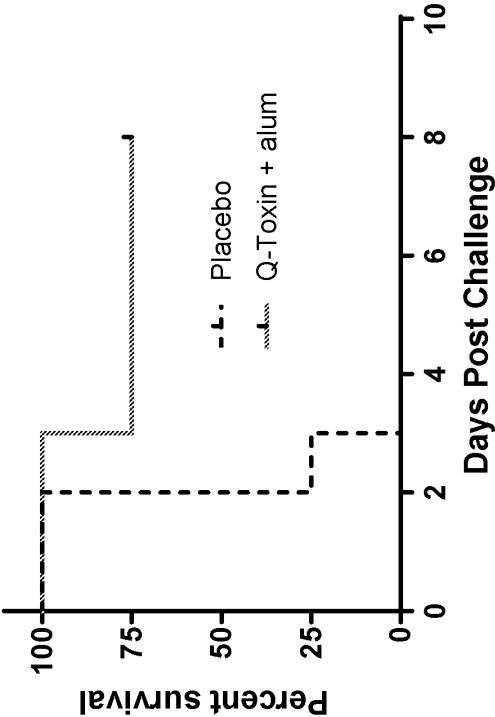


Fig. 26C



C. difficile strain 630

Fig. 26D



C. difficile B1/NAP1/027