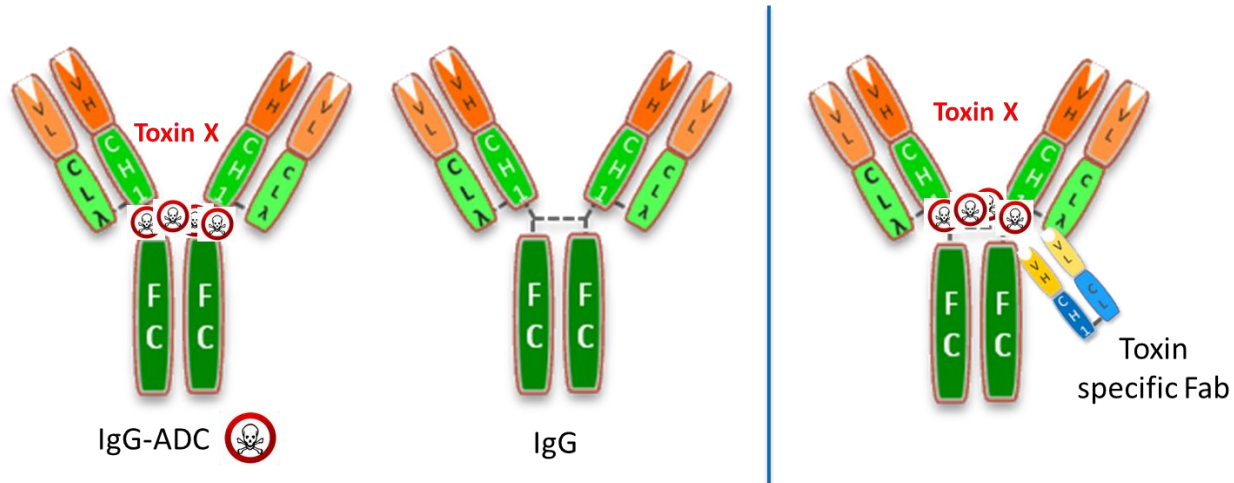


Case study VIII

Discovery of llama Fabs that binds specifically to Toxin X when bound to human IgG (ADC)

- Aim: Find Fab from immunized llamas that binds specifically to Toxin X when bound to Human antibody
 - Optional: To select Fabs that recognize both Toxin X and Y (>90% homology)



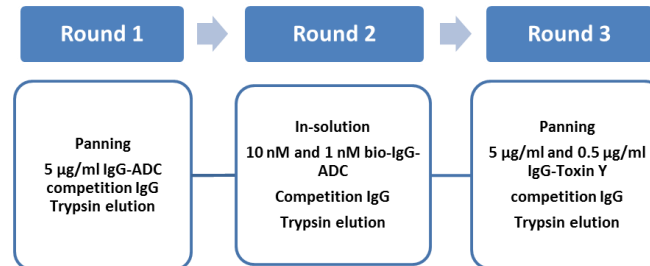
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Llama Fab phage display libraries and selections

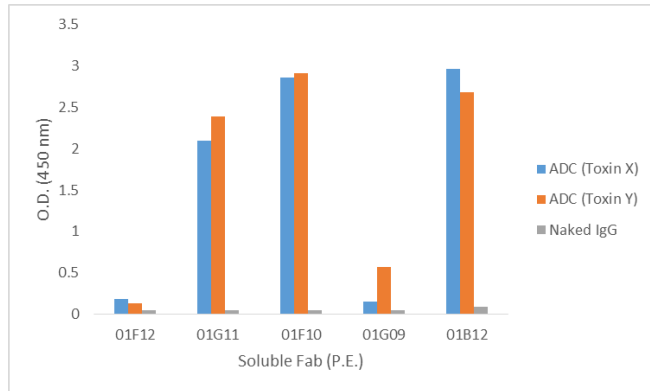
Library	Library size	Fab Insert %
L001k	3.1x10 ⁸	88 %
L001λ	3.8x10 ⁸	88 %
L002k	3.6x10 ⁸	92 %
L002λ	5.7x10 ⁸	82 %



Case study VIII

Discovery of llama Fabs that binds specifically to Toxin X when bound to human IgG (ADC)

- Aim: Find Fab from immunized llamas that binds specifically to Toxin X when bound to Human antibody
 - ELISA & sequencing and SPR reveal specificity and diversity



Clone name	sequence # VH	sequence # VL	family # HCDR3	Kd (s ⁻¹)		
				ADC (Toxin X)	ADC (Toxin Y)	Naked IgG
01F12	1	1	1	2.65x 10 ⁻³	1.45x 10 ⁻³	-
01G11	2	2	1	9.32x 10 ⁻⁴	9.90x 10 ⁻⁴	-
01F10	3	3	2	1.50x 10 ⁻³	8.40x 10 ⁻⁴	-
01G09	4	4	3	6.70x 10 ⁻⁴	9.28x 10 ⁻⁴	-
01B12	5	5	4	9.50x 10 ⁻⁴	5.8x 10 ⁻⁴	3.05x 10 ⁻³

Case study VIII



Discovery of llama Fabs that binds specifically to Toxin X when bound to human IgG (ADC)

- Conclusions

Combination of llama immunizations and phage display-based selections led to the identification of specific mAbs for 2 different proteins, allowing the establishment of sandwich ELISA for diagnostic application in 8 months.

- Although llamas were immunized with toxin conjugate antibodies (ADC) it was possible to identify toxin specific Fabs by applying phage display based counter selections in 4 months.
- At least 3 different anti toxin specific Fabs (1F10, 1G11 and 1F12) were selected that recognize specifically the X and Y toxin conjugated antibodies.
- The identified toxin specific Fabs showed off-rates for ADC varying from low 10^{-3} and high 10^{-4} sec^{-1} .
- A clone binding both the conjugate and the naked antibody was selected (1B12). However, ELISA and SPR results indicated that it binds much better to the conjugates IgGs (Toxin X and Y) than to the naked antibody.