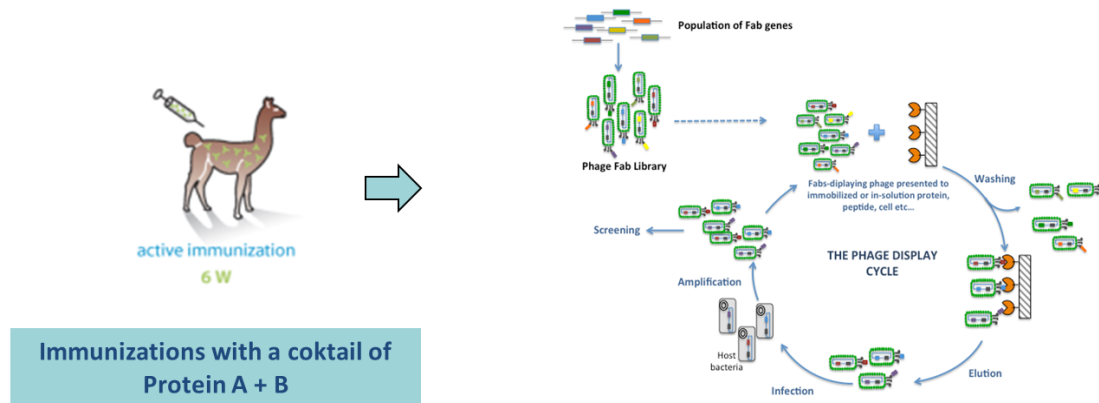


# Case study VII

## Generation of capturing and detection mAbs (sandwich ELISA) for diagnostic application

- Aim: Identify capturing and detection monoclonal antibodies (sandwich ELISA) for diagnostic application
  - Protein A (Cancer and heart failure biomarker)
  - Protein B (Cancer and nephropathy biomarker)
- Technology: Llama immunizations + phage display

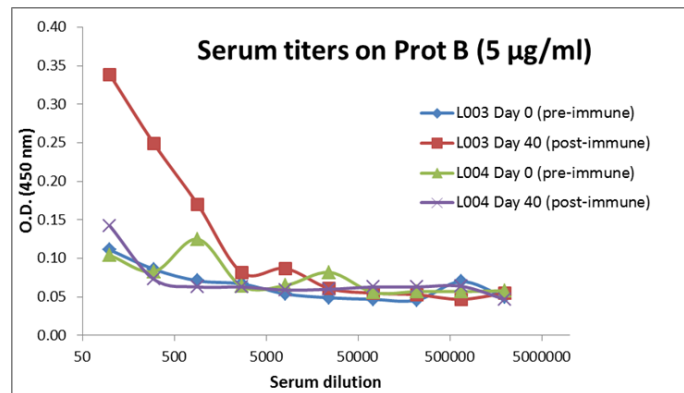
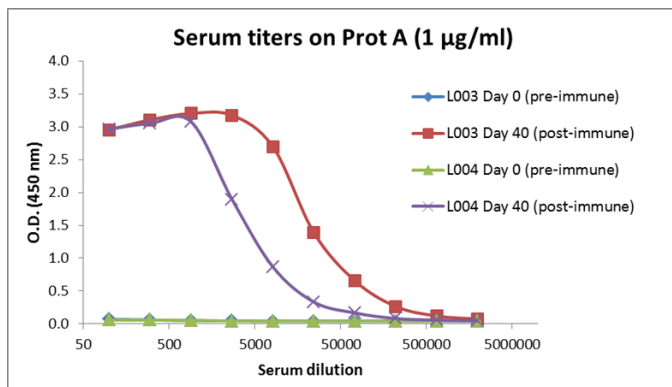


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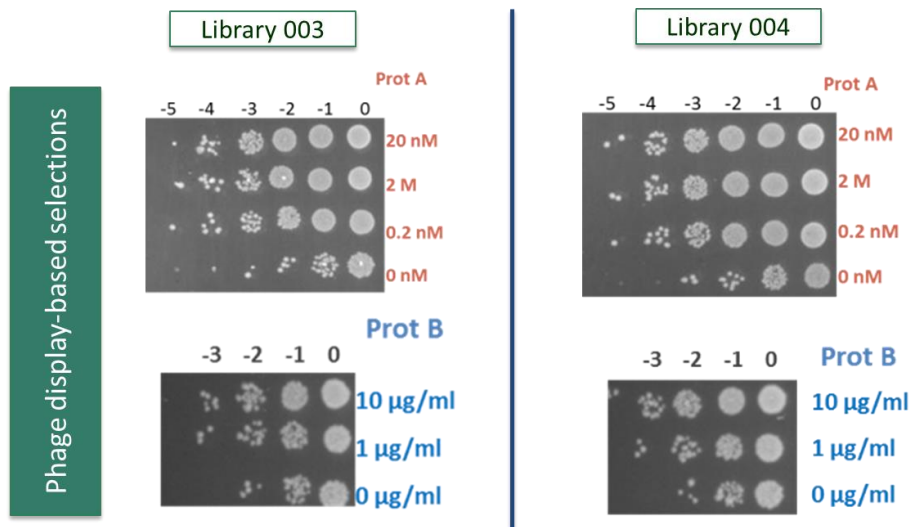
### Llama immune responses



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Titration of *E. coli* infected with phage from a Fab immune library selected after two-three rounds on protein A (Prot A) or on protein B (Prot B)

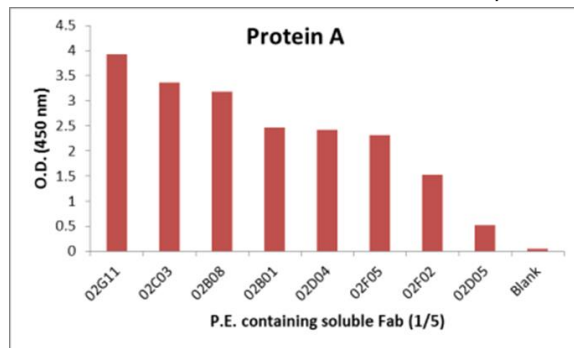
# Case study VII

## Generation of capturing and detection mAbs (sandwich ELISA) for diagnostic application

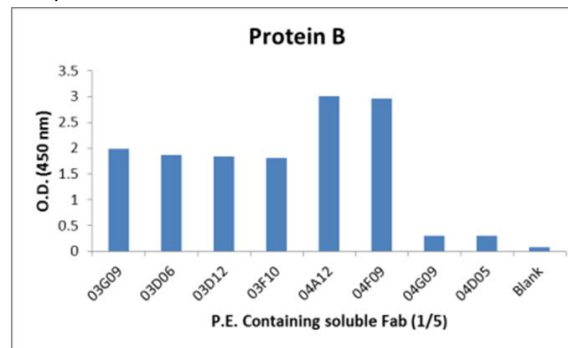
- Aim: Identify capturing and detection monoclonal antibodies (sandwich ELISA) for diagnostic application
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### Screening

- Neutravidin coated plates
- Biotinylated protein A or B
- Fab containing-Periplasmic extract (P.E)
- Anti c-myc-HRP antibody



Library	Hit-rate (OD450≥0.2) N=94
003	32.0%
004	34.0%



Library	Hit-rate (OD450≥0.3) N=94
003	55.0%
004	58.0%

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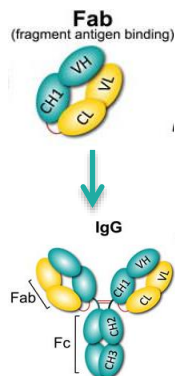
## Sequencing reveals diversity

Protein	# Sequenced clones	# Families (HCDR3)	# VH	# V $\lambda$	# V $\kappa$
A	25	14	21	17	5
B	31	5	8	10	2

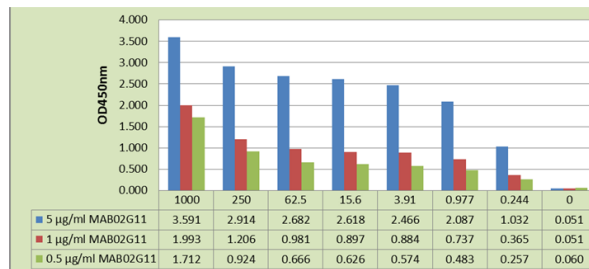
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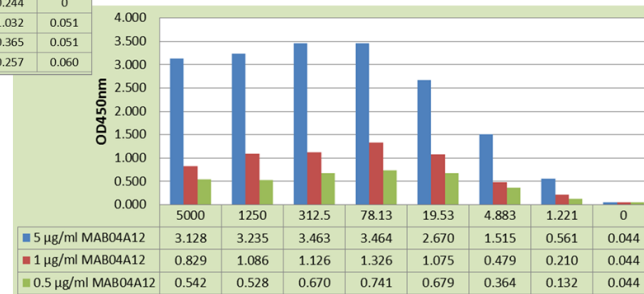


### Fab formatting to mAb QC and selection of capturing antibody



- Coating: MAB02G11 (5 µg/ml, 1 µg/ml, 0.5 µg/ml)
- Capture Bio-Protein A (1000 nM → 0.244 nM)
- Detection: Extravidin-HRP (1:1000)

- Coating: MAB04A12 (5 µg/ml, 1 µg/ml, 0.5 µg/ml)
- Capture Bio-Protein B (5000 nM → 1.221 nM)
- Detection: Extravidin-HRP (1:1000)

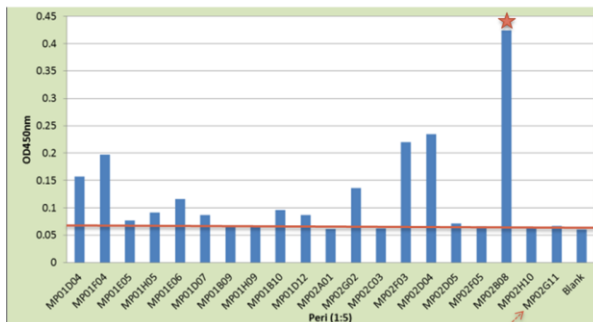


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## Generation of capturing and detection mAbs (sandwich ELISA) for diagnostic application

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  - Protein A (Cancer and heart failure biomarker)
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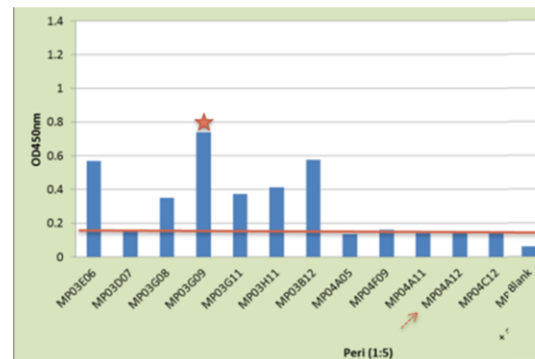
Screening of detection antibody (Protein A)



- **Coating:** MAB02G11 (5 µg/ml)
- **Capture:** Protein A (5 nM)
- **Sample:** anti protein A Fab's peri (1:5)
- **Detection:** Anti c-myc-HRP (1:5000)



Screening of detection antibody (Protein B)



- **Coating:** MAB04A12 (5 µg/ml)
- **Capture:** Protein B (25 nM)
- **Sample:** anti Protein B Fab's peri (1:5)
- **Detection:** Anti c-myc-HRP (1:5000)

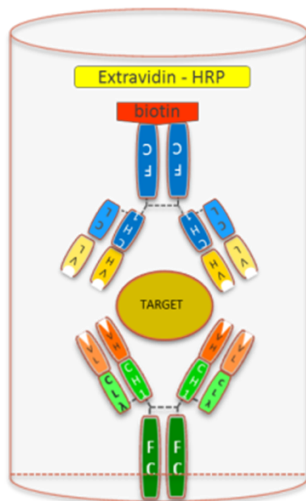


# Case study VII

## Generation of capturing and detection mAbs (sandwich ELISA) for diagnostic application

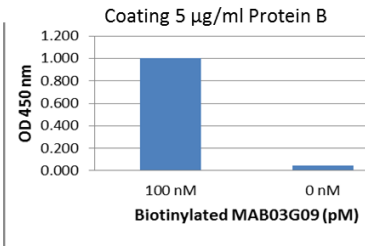
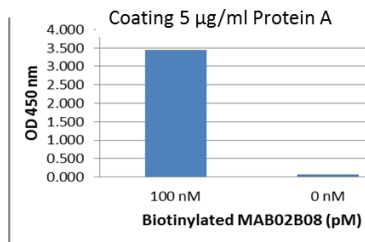
- Aim: Identify capturing and detection monoclonal antibodies (sandwich ELISA) for diagnostic application
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### Final sandwich ELISA



Protein	Capturing mAb
A	MAB02G11
B	MAB 04A12

Protein	Detection mAb
A	MAB02B08
B	MAB03G09



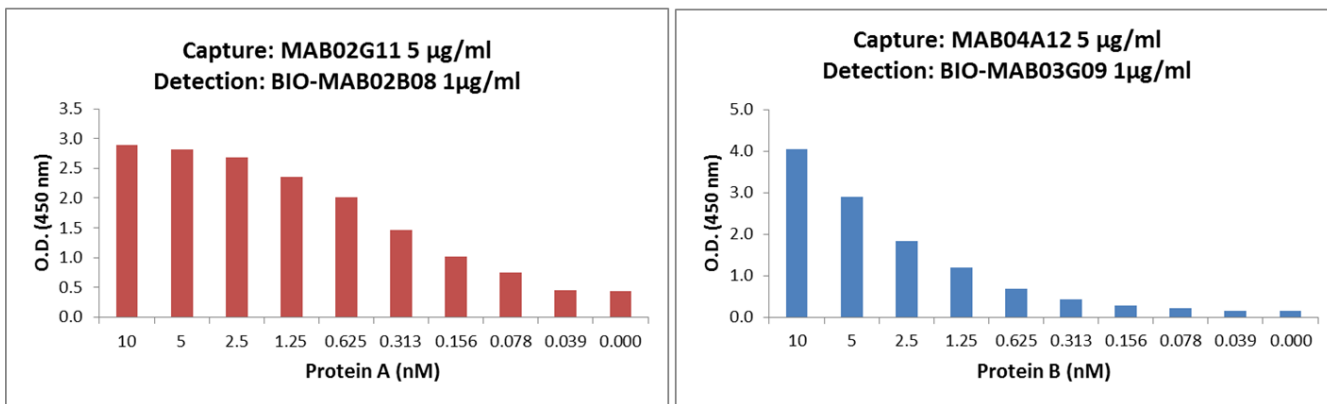


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## Sandwich ELISA sensitivity

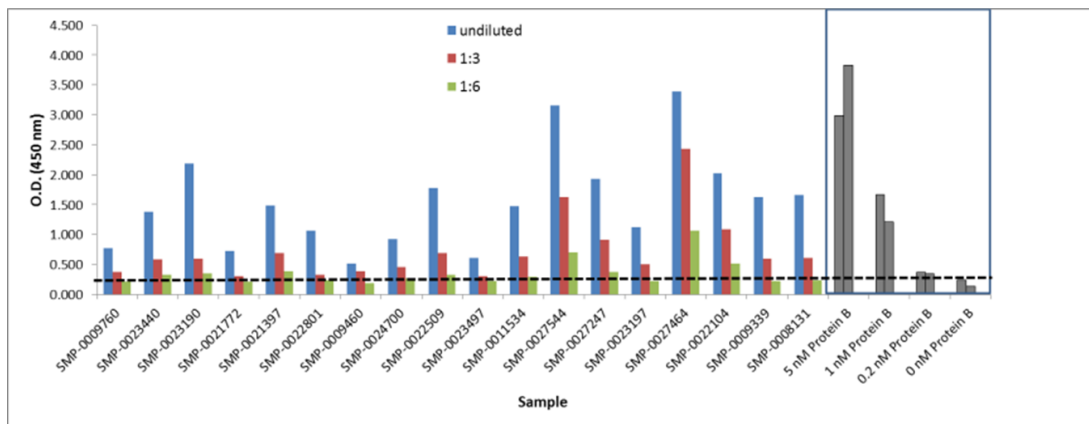


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### Detection of protein B in Human plasma samples in Sandwich ELISA



# Case study VII

## Generation of capturing and detection mAbs (sandwich ELISA) for diagnostic application

- 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.

- Antibodies were isolated even in the case of target specific weak immune responses
- For each target a diverse panel of antibodies recognizing different epitopes were identified
- Affinities of the selected antibodies allowed the capturing and detection of proteins A and B when they are present in concentrations as low as 0.5 pmol/ml