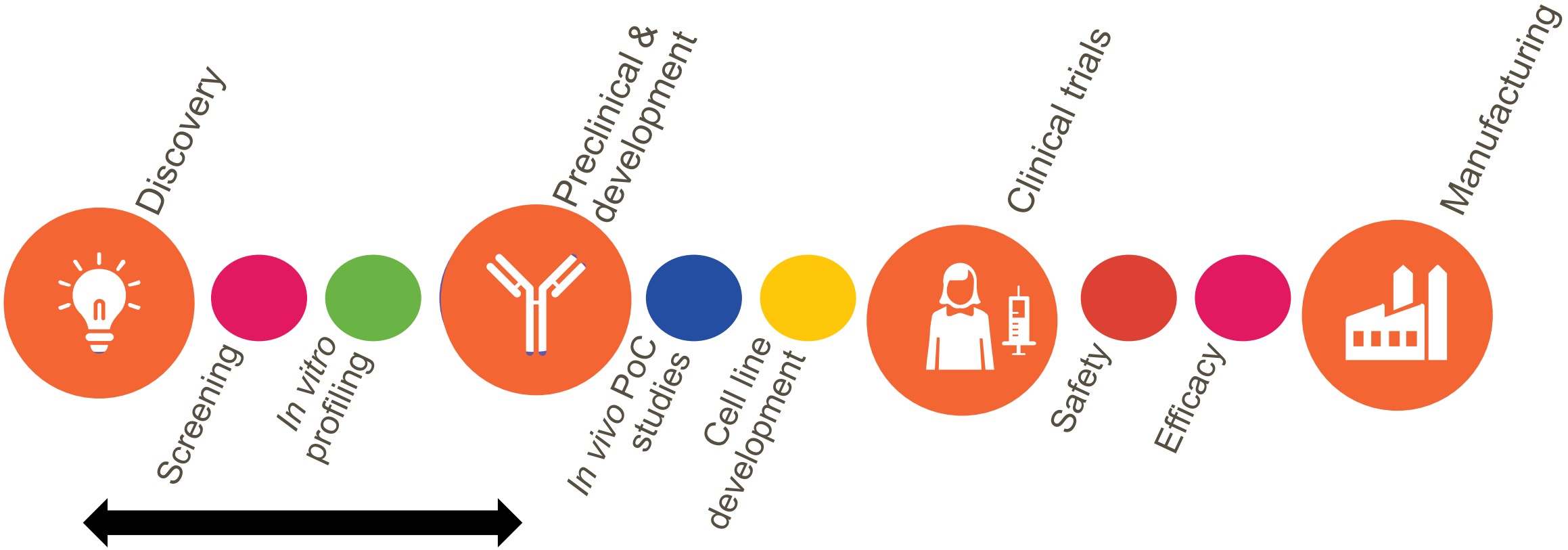
Evaluating Consistent Antibody Affinity Measurements Using Varying Capture Concentrations on Carterra LSA and LSA XT

Tomas Rodriguez

Carterra Symposium, 6th June 2025

Large Molecule Discovery



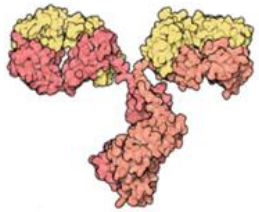
Antibody Discovery Process

In-vitro and *in-vivo* selection work flow

Antigen

Input

In vitro Antibody Selections



High Throughput Expression

Optimisation

Binding, functional and biophysical characterisation

In vivo immunisation campaign

B cell sorting, FACS and NGS

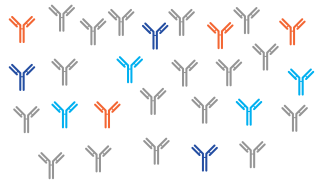
DNA generation and High Throughput Expression

Binding, functional and biophysical characterisation

GSK

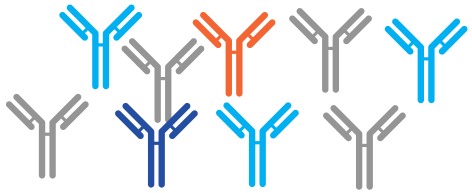
Binding and Biophysical Characterisation during Discovery

High-Throughput Characterisation



100s Abs

Lead Triaging



<100 Abs

Lead Declaration

Development

≥1 Ab

More molecules, less material, predictive assays used

Fewer molecules, more material, more in depth characterisation can be carried out

Use of Carterra LSA® and LSA^{XT} in Antibody Screening Cascade

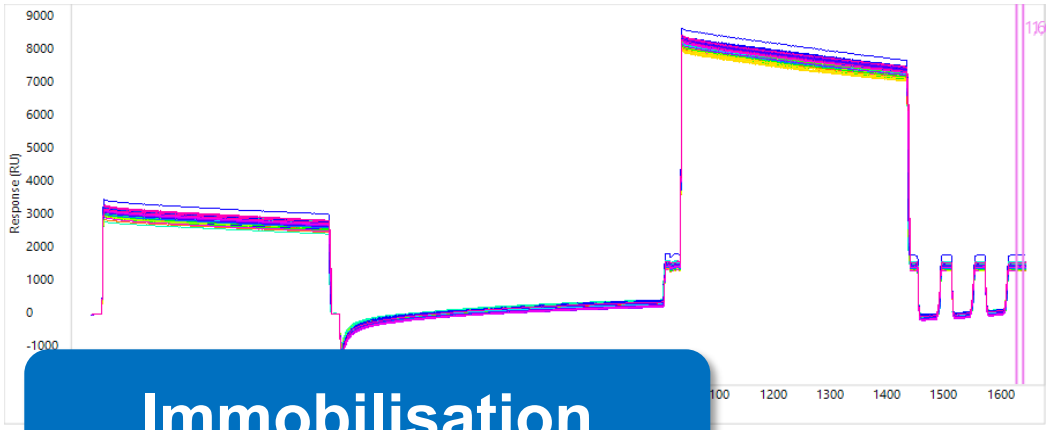
HIGH THROUGHPUT SCREENING

- Binding checks
- Kinetics and affinities
- Epitope binning

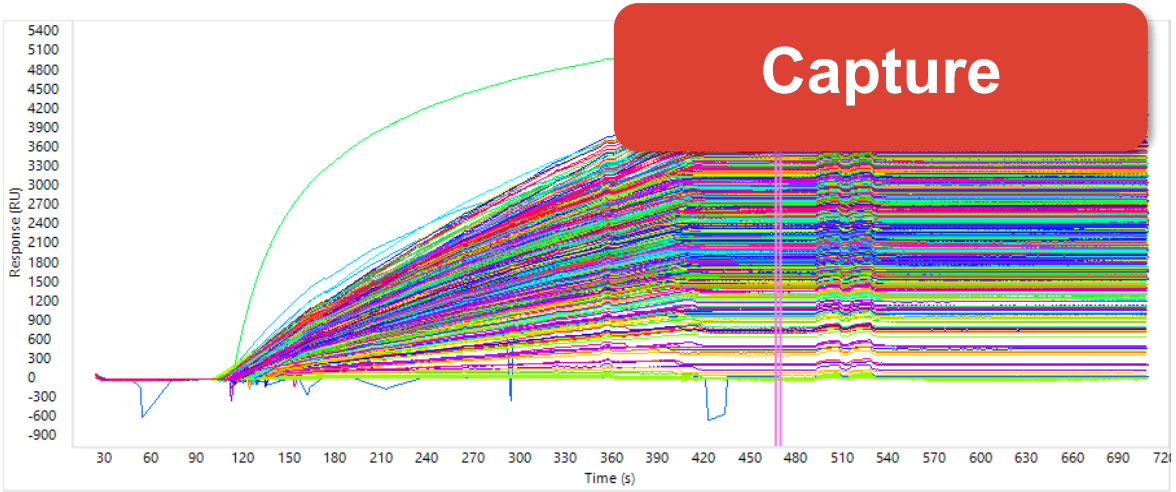
LSA®

LSA^{XT}

Obstacles in our existing Carterra workflows.



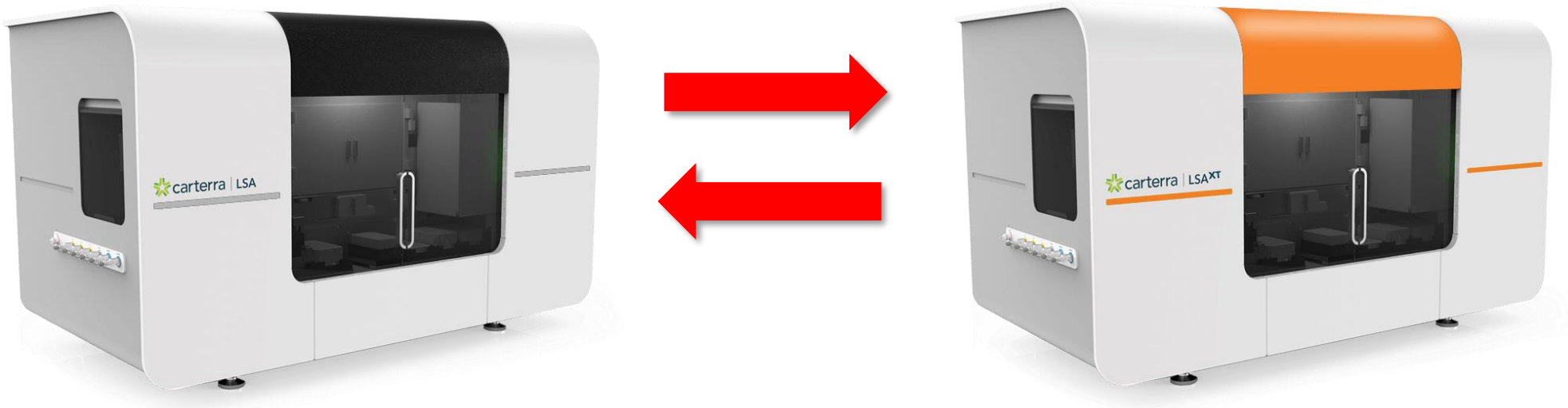
Immobilisation



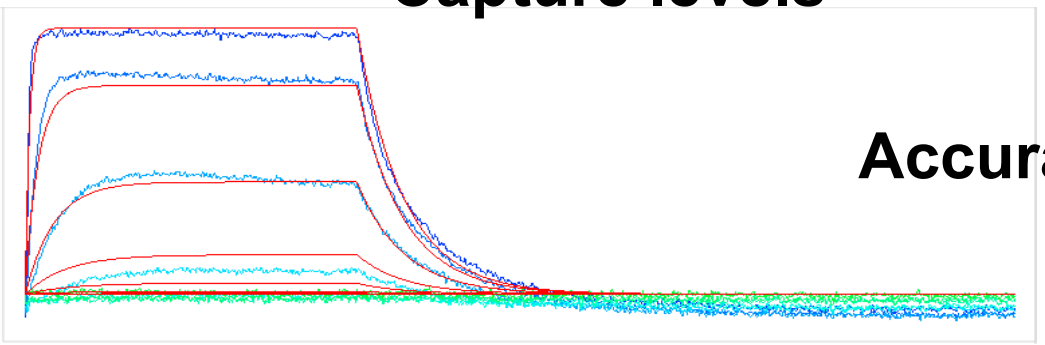
Capture

Optimisation

My Industrial Placement Project



Capture levels

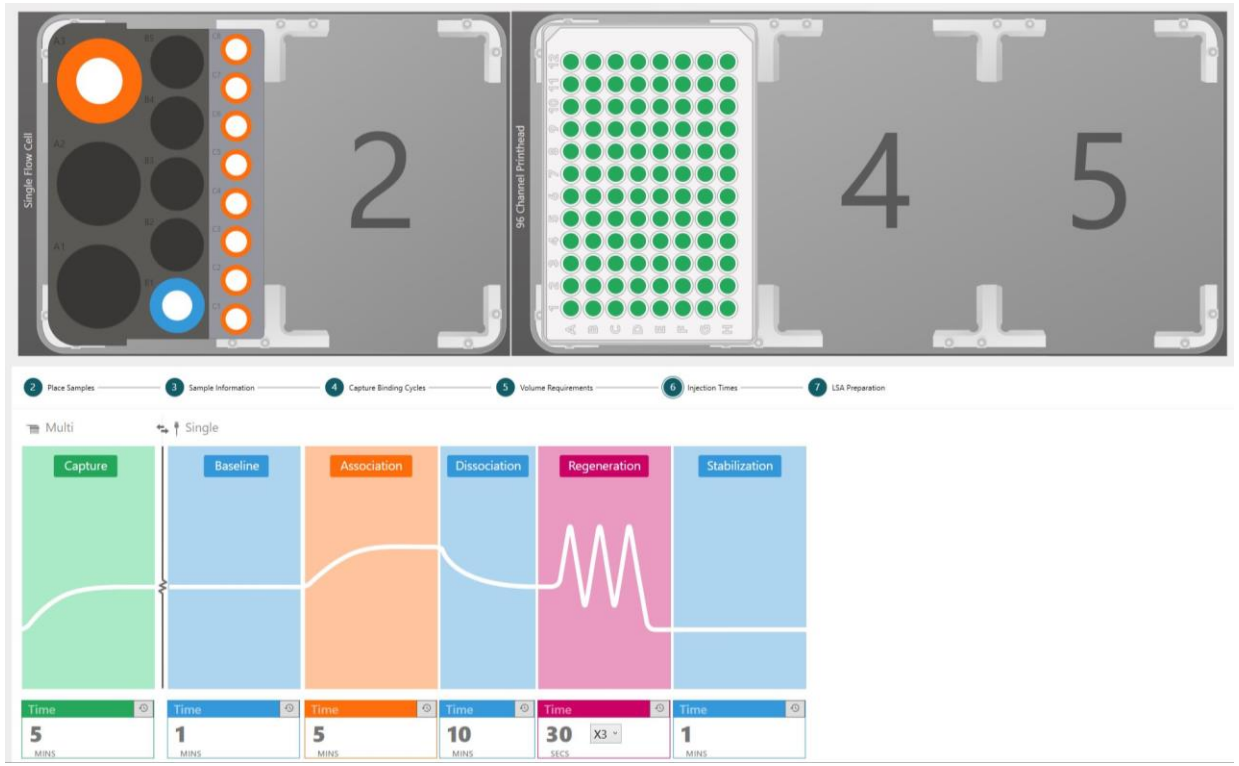


Accuracy

Reproducibility

Method

LSA® - CDMP



LSA^{XT} - CMDP

12  **x 6 concentrations**

Immobilisations and captures

75 ug/ml of anti-human Fc antibodies in acetate pH 4.5
7-minute activation, 10-minute coupling, 7-minute blocking, and three 30 second washes

LSA® - CMDP

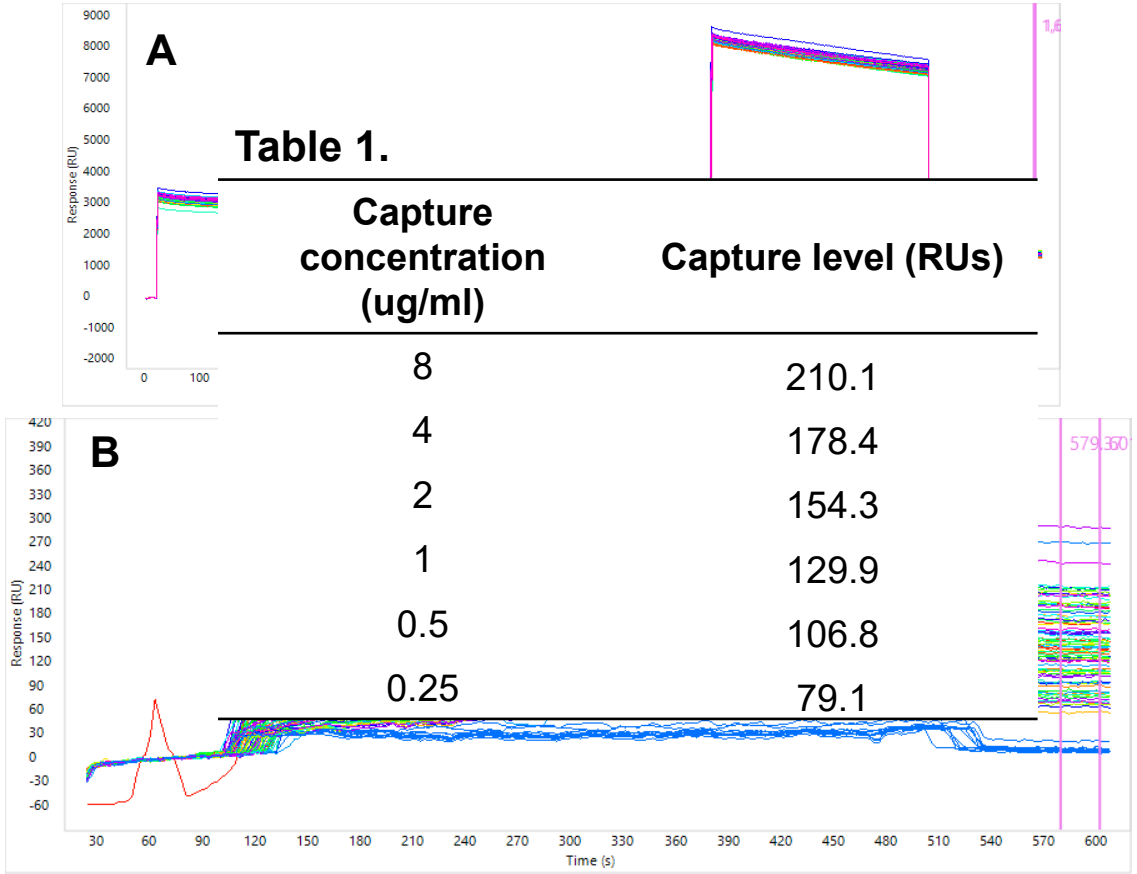


Table 1.

Capture concentration (ug/ml)	Capture level (RUs)
8	210.1
4	178.4
2	154.3
1	129.9
0.5	106.8
0.25	79.1

Fig. 1

LSA^{XT} - CMDP

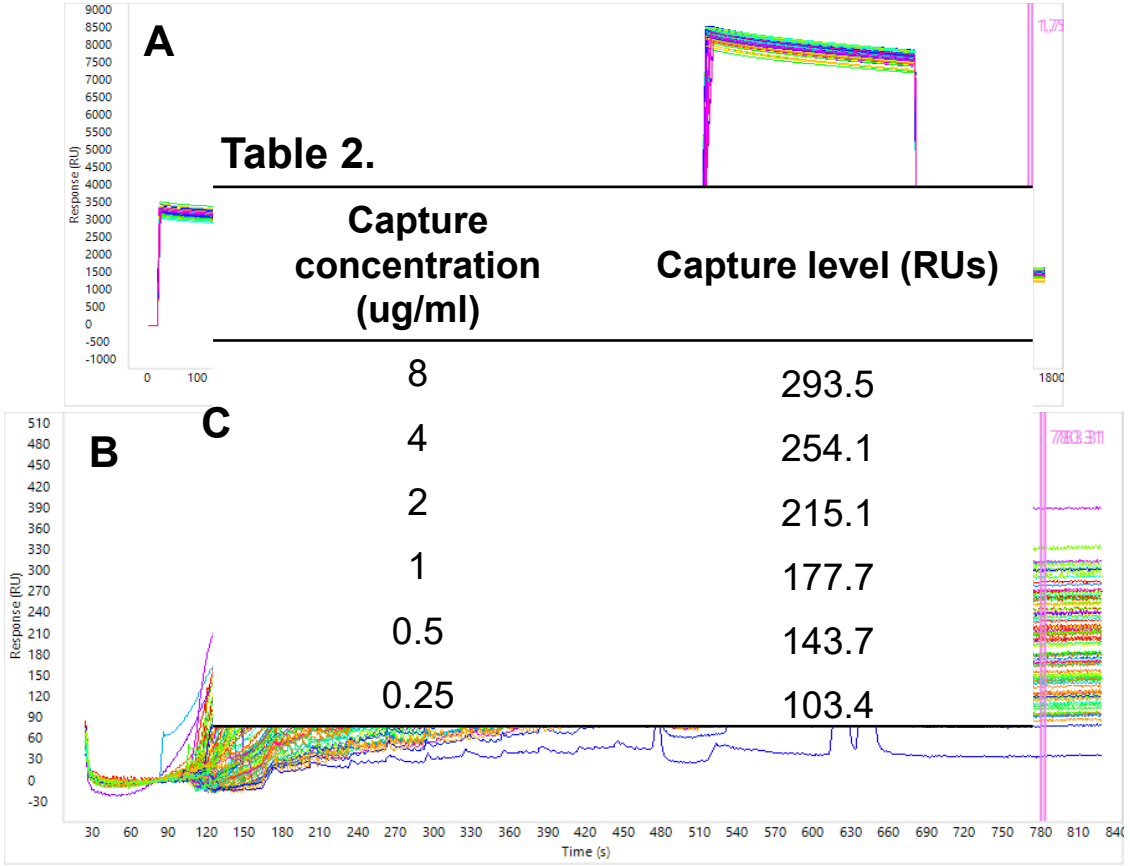
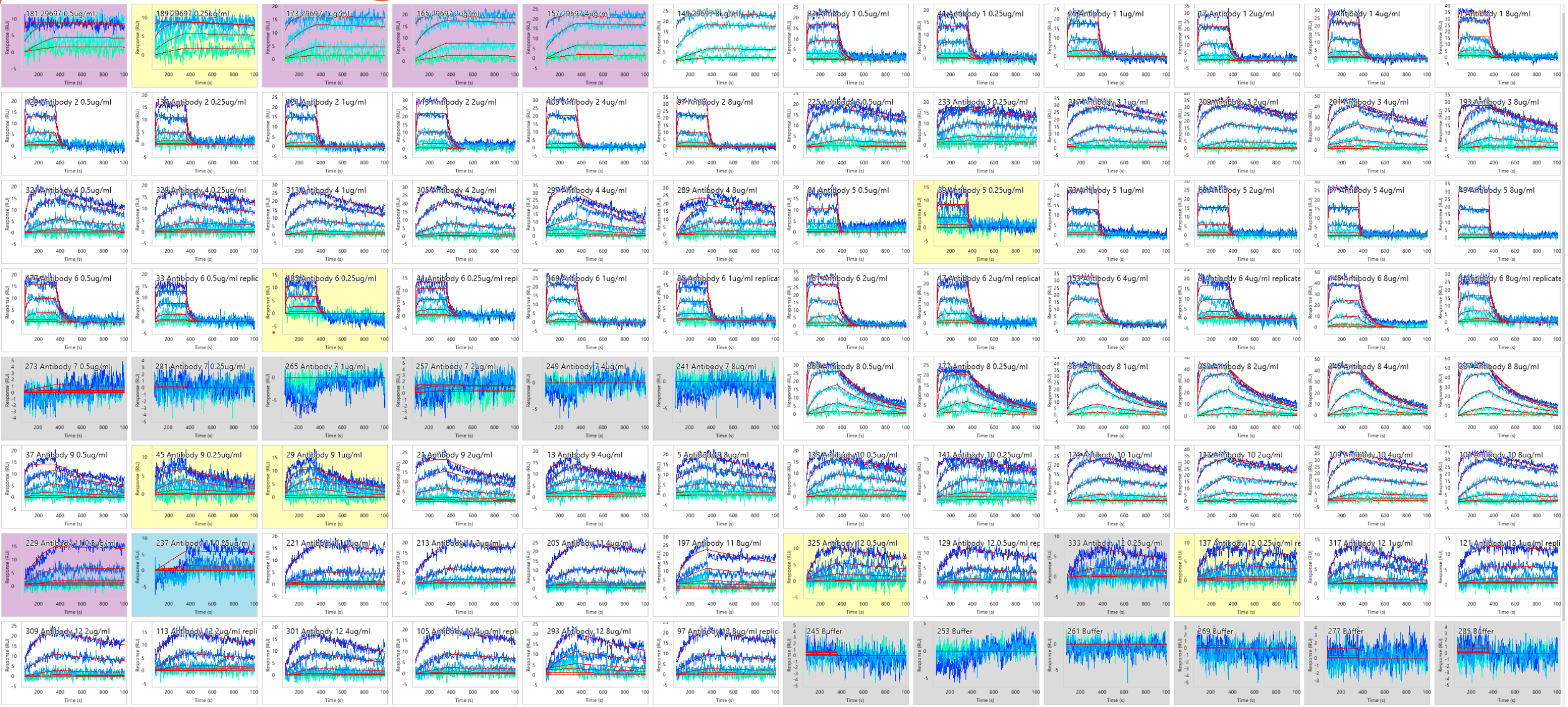


Table 2.

Capture concentration (ug/ml)	Capture level (RUs)
8	293.5
4	254.1
2	215.1
1	177.7
0.5	143.7
0.25	103.4

Fig. 2

LSA® – Sensorgrams



Res sd 10% Rmax

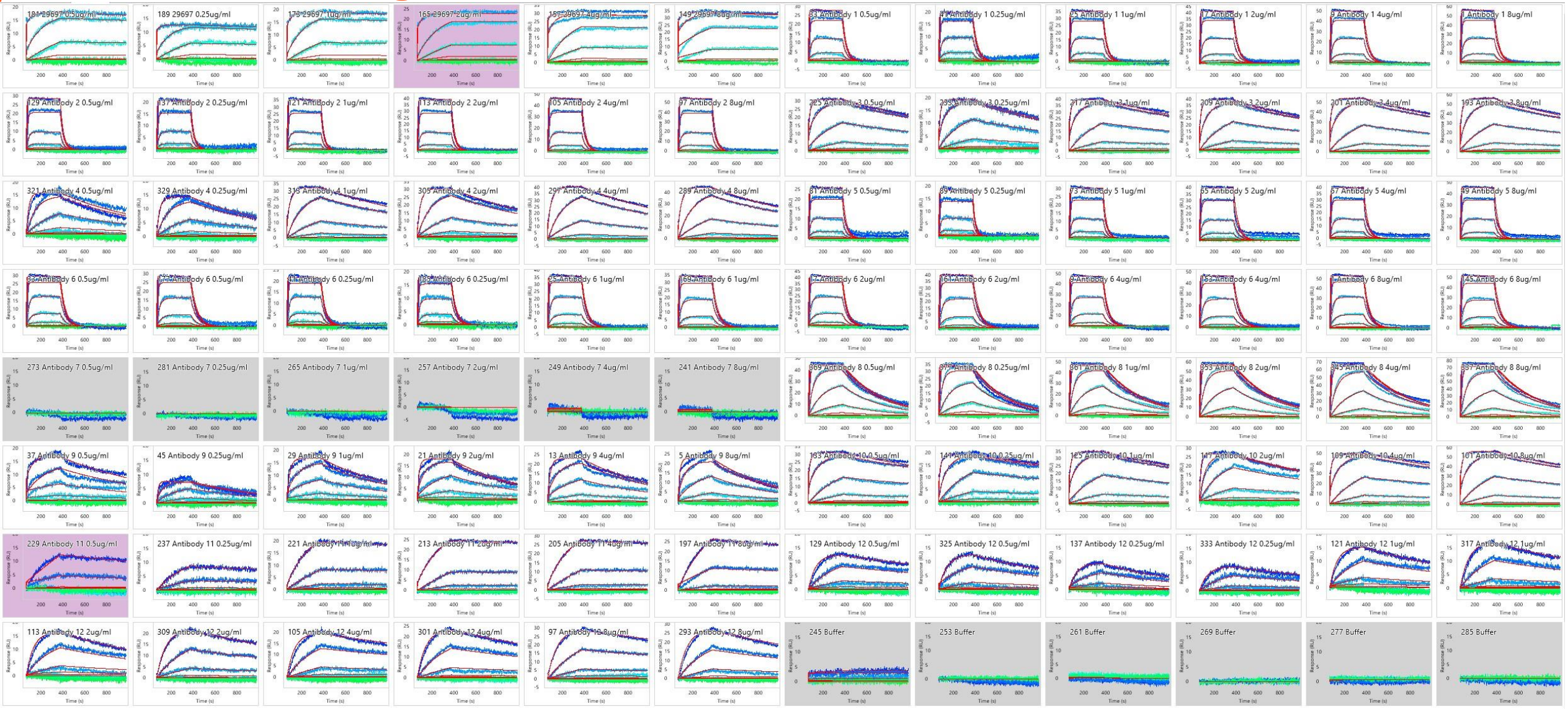
Kd below limit

Data < 50% Rmax

Non-binders

Fig. 3

LSA^{XT} – Sensorgrams



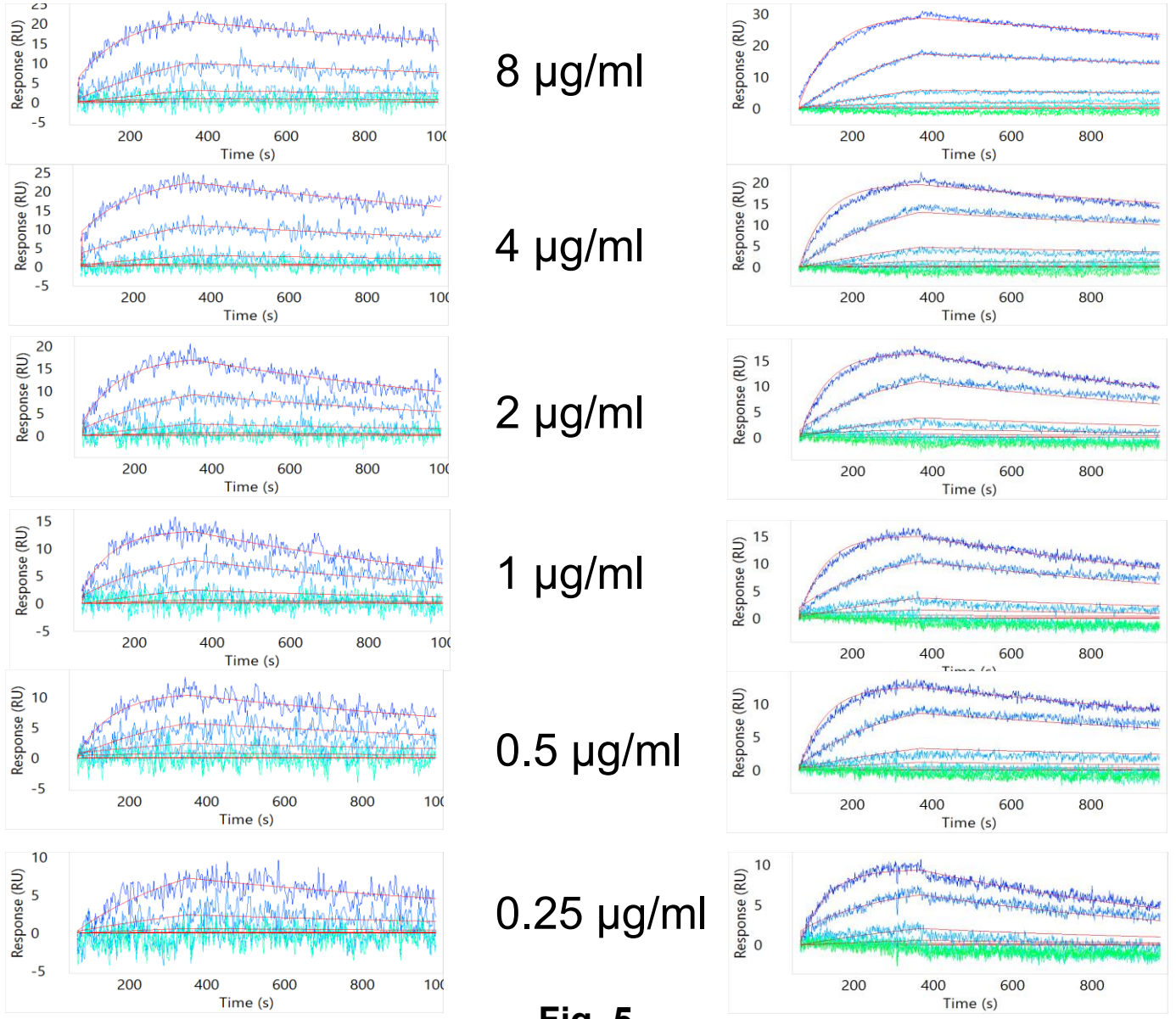
Kd below limit

Non-binders

Fig. 4

Select sensorgrams – Antibody 12

LSA®

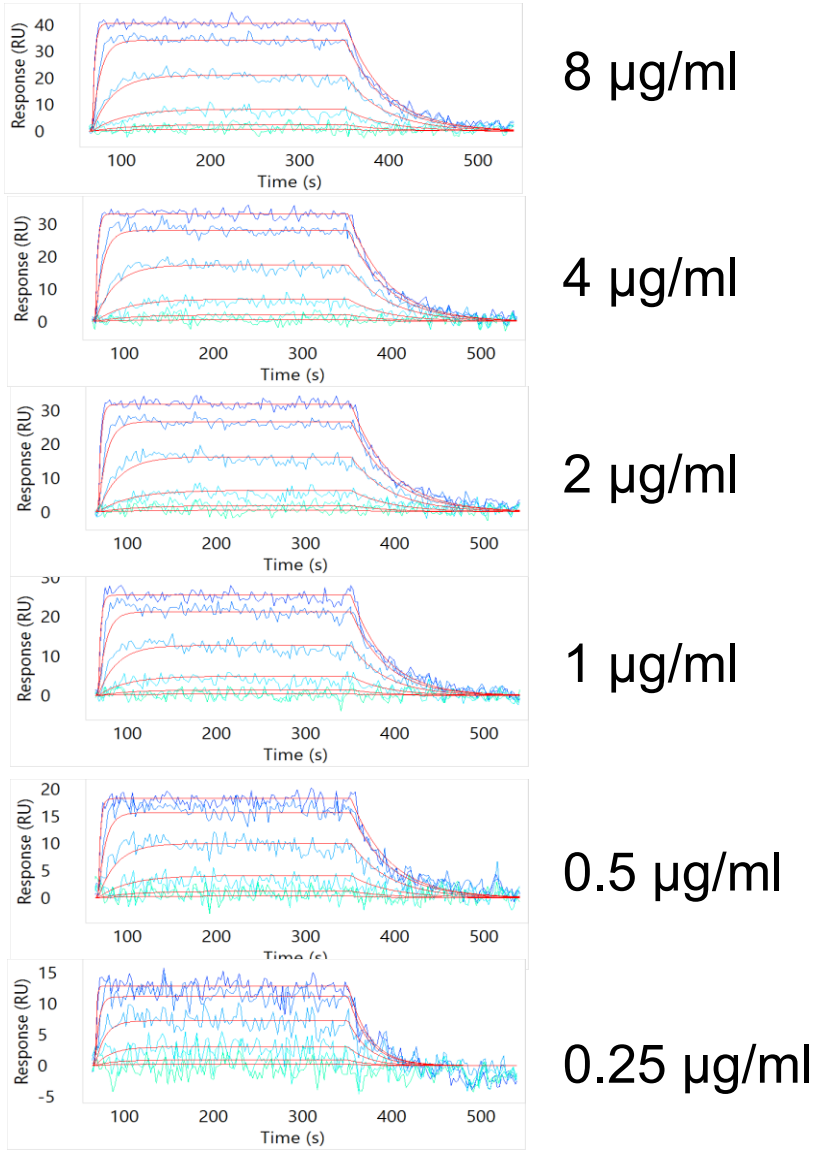


LSA^{XT}

Fig. 5

Select sensorgrams – Antibody 6

LSA®



LSA^{XT}

Fig. 6

Affinities across capture concentrations – LSA® repeats

Affinities across capture concentrations (LSA - First replicate)

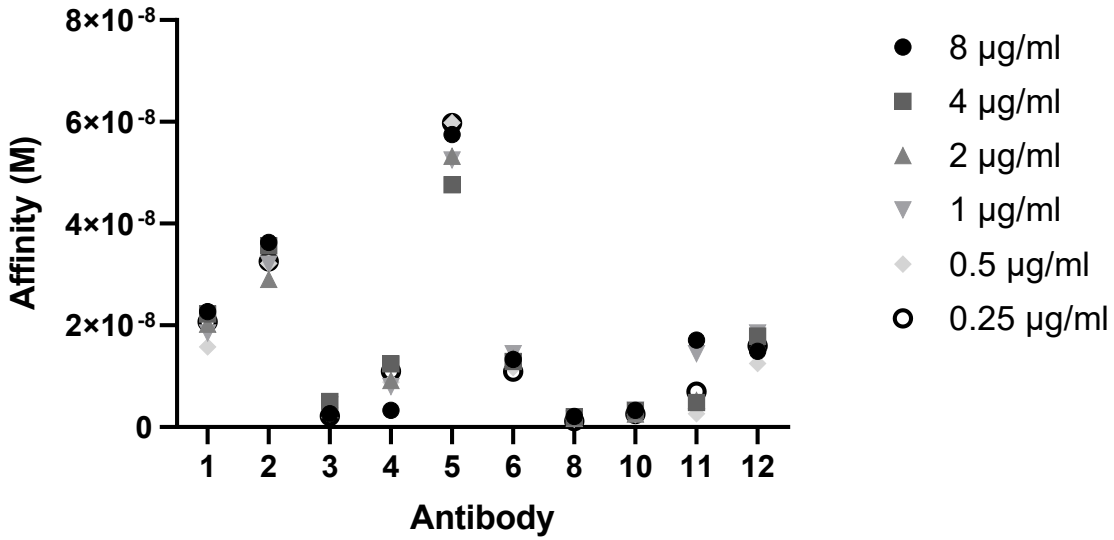


Fig. 7

Affinities across capture concentrations (LSA - Second replicate)

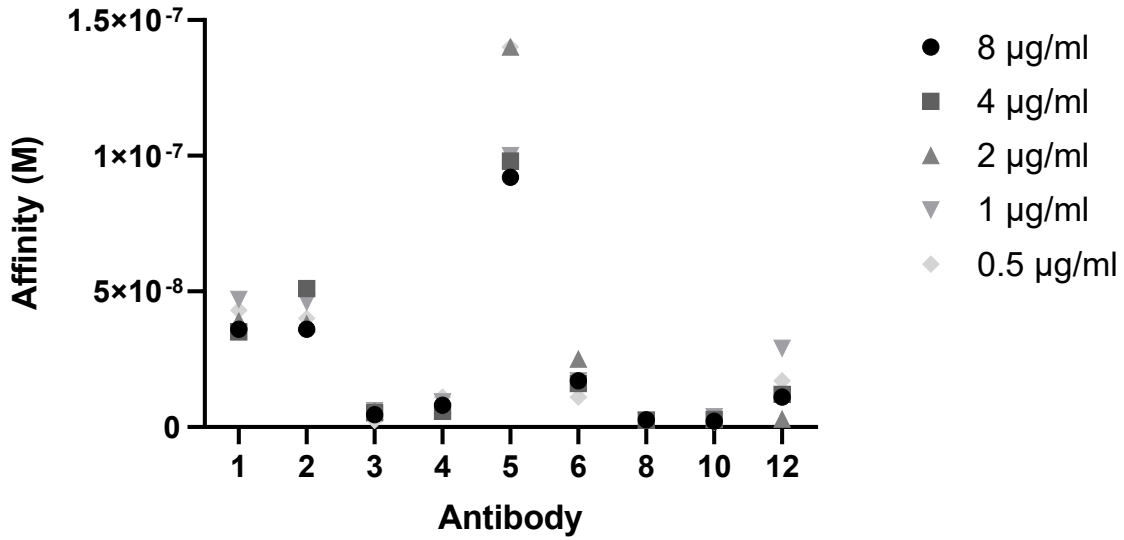


Fig. 8

No significant differences across capture concentrations (Friedman test; $p > 0.05$)

Affinities across capture concentrations – LSA^{XT} repeats

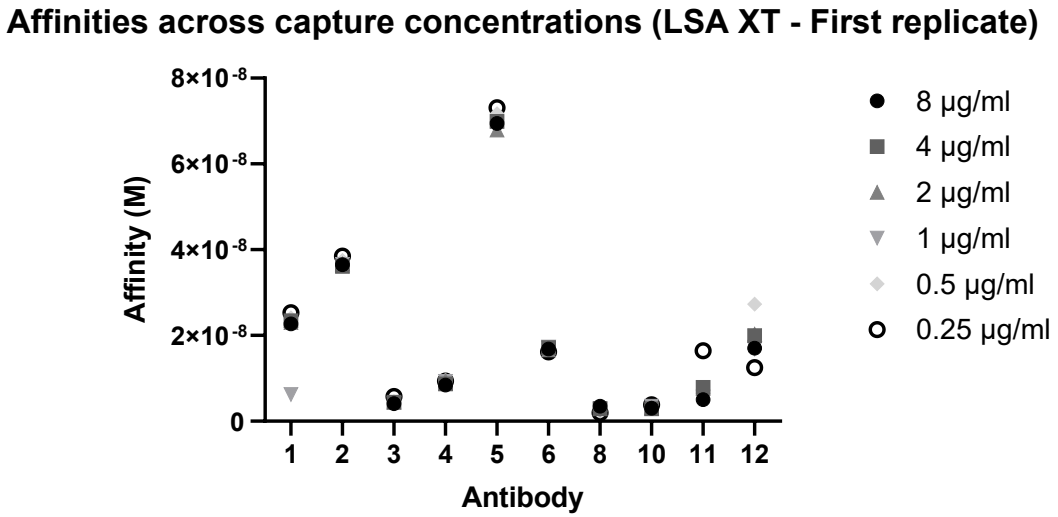


Fig. 9

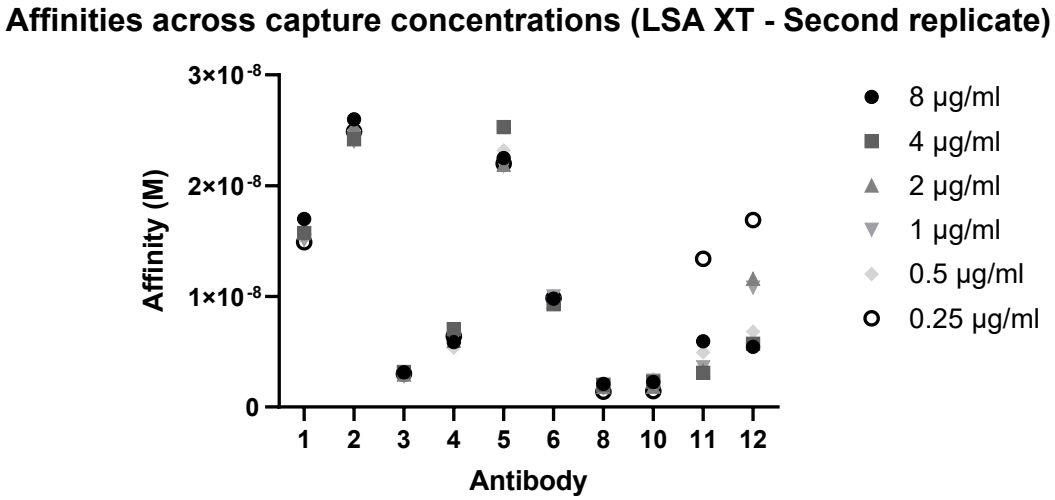


Fig. 10

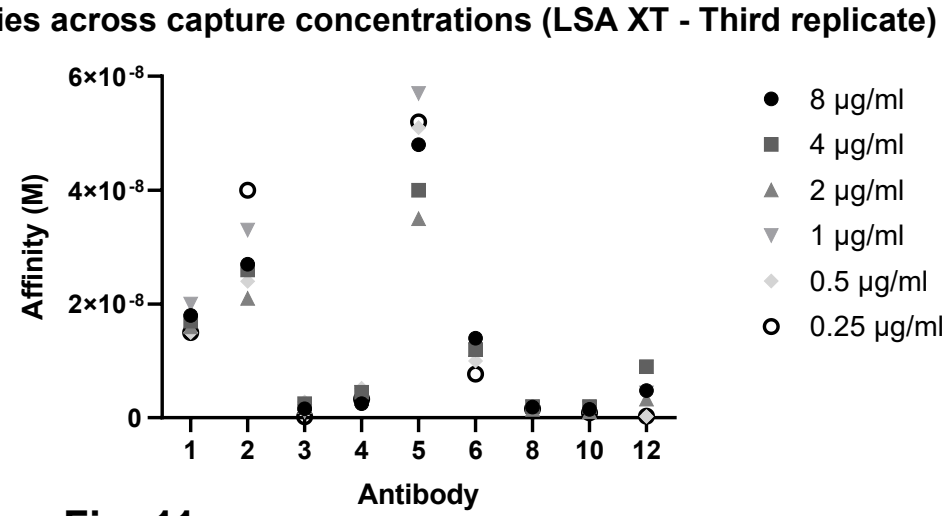


Fig. 11

No significant differences across capture concentrations (Friedman test; $p > 0.05$)

Average affinities compared – LSA® repeats

Table 3.	Average affinity in first repeat (nM)	Average affinity in second repeat (nM)
Antibody 1	19.9	39.8
Antibody 2	32.9	41.7
Antibody 3	3.44	4.64
Antibody 4	8.15	8.22
Antibody 5	54.9	113
Antibody 6	12.7	16.6
Antibody 8	1.65	2.57
Antibody 10	2.90	2.60
Antibody 11	6.98	N/A
Antibody 12	15.9	11.3

- Affinities within 2-3-fold for all clones.
- Affinity measurements consistent in the LSA®.

Average affinities compared – LSA^{XT} repeats

Table 4.	Average affinity in first repeat (nM)	Average affinity in second repeat (nM)	Average affinity in third repeat (nM)
Antibody 1	19.0E	15.7	16.8
Antibody 2	37.0	24.9	28.0
Antibody 3	4.46	3.02	1.28
Antibody 4	9.14	6.23	3.79
Antibody 5	70.0	22.7	46.8
Antibody 6	16.8	9.73	11.1
Antibody 8	2.67	1.77	1.79
Antibody 10	3.22	2.09	1.45
Antibody 11	7.52	4.93	N/A
Antibody 12	18.5	8.74	1.83

- Affinities within 2-3-fold for all clones.
- Affinity measurements consistent in the LSA^{XT}.

Comparability by capture level

- Assumed comparability across capture concentrations (levels!) is the same.
- Relative p values can illustrate how closely comparable each capture concentration is across repeats.
- The larger the p value the more comparable.

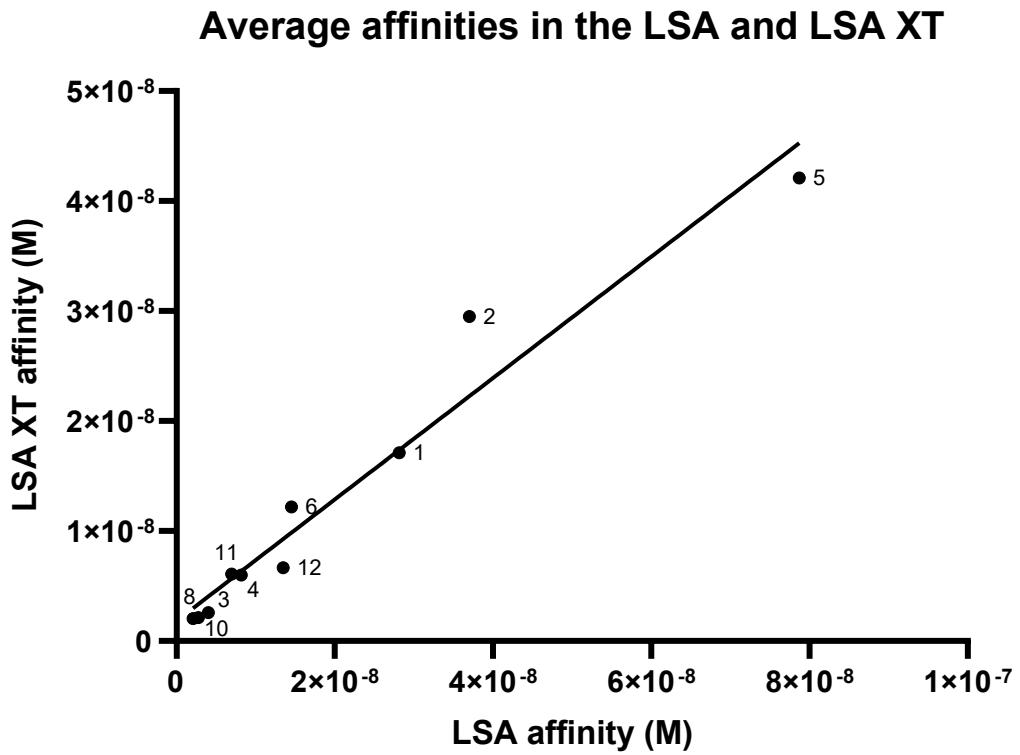
Table 5.

		8µg/ml	4µg/ml	2µg/ml	1µg/ml	0.5µg/ml	0.25µg/ml
p value	**LSA®**	0.1641	0.3008	0.1641	0.0039	0.0742	-
	LSA^{XT}	0.0003	0.0003	<0.0001	0.0158	0.0002	0.0158

- For the **LSA®**, lower capture concentrations tend to be **less comparable**, corresponding to capture levels ranging between **~ 70 – 120 RUs**. Capture levels between **~ 140 – 200 RUs** seem **reliable**.
- For the **LSA^{XT}** higher capture concentrations, translating to capture levels between **~ 200 – 270 RUs**, result in seemingly less comparable results than capture levels between **~ 100 – 180 RUs**.

LSA – Wilcoxon test (2 replicates only)
LSA XT – Friedman test (3 replicates)

Comparing average affinities between the LSA® and LSA^{XT}



r = 0.9879; Spearman's p < 0.001 ***

Fig. 12

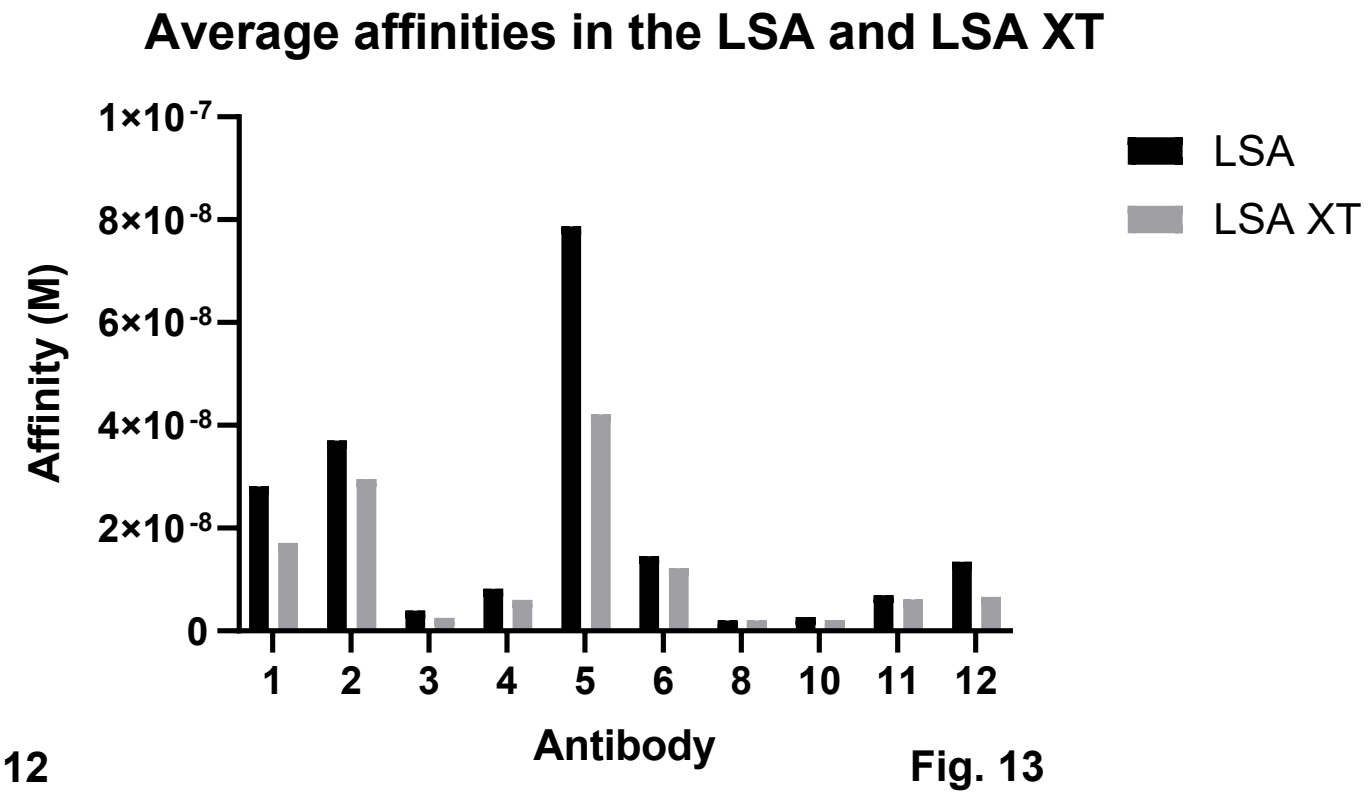


Fig. 13

- Very high agreement between instruments, affinities ranked similarly.
- Average affinities in instruments within 2-3-fold range.
- Slight differences between the LSA® and LSA^{XT}.

Conclusions

- ✓ Affinity **measurements** are **consistent** across a range of capture concentrations (levels) in both Carterras.
- ✓ Replicate experiments show affinities are within the acceptable 2-3-fold range in both instruments.
- ✓ The most reliable capture levels range from at least ~ **140 – 200 RUs** in the LSA® and ~ **100 – 180 RUs** in the LSA^{XT}.
- ✓ Overall, **less noise** observed using the LSA^{XT}, especially at **lower** capture levels.

Conclusions

- ✓ Very **strong correlation** in affinity ranking by instruments.
- ✓ Within the comparable 2-3-fold, **LSA XT** measured **stronger** affinities for **all** clones.
- ✓ **GSK methods** appear reliable **across** instruments.
- ✓ **CMDP** only!

Immobilisations conditions

LSA® - HC30M

- 75 ug/ml SB anti-human Fc antibodies.
- Acetate pH 4.5
- 5-minute activation, 7-minute coupling, 5-minute blocking, and three 30 second washes.

LSA^{XT} - CMDP

- 75 ug/ml SB anti-human Fc antibodies.
- Acetate pH 4.5
- 7-minute activation, 10-minute coupling, 7-minute blocking, and three 30 second washes.

Immobilisations and captures

LSA® - HC30M

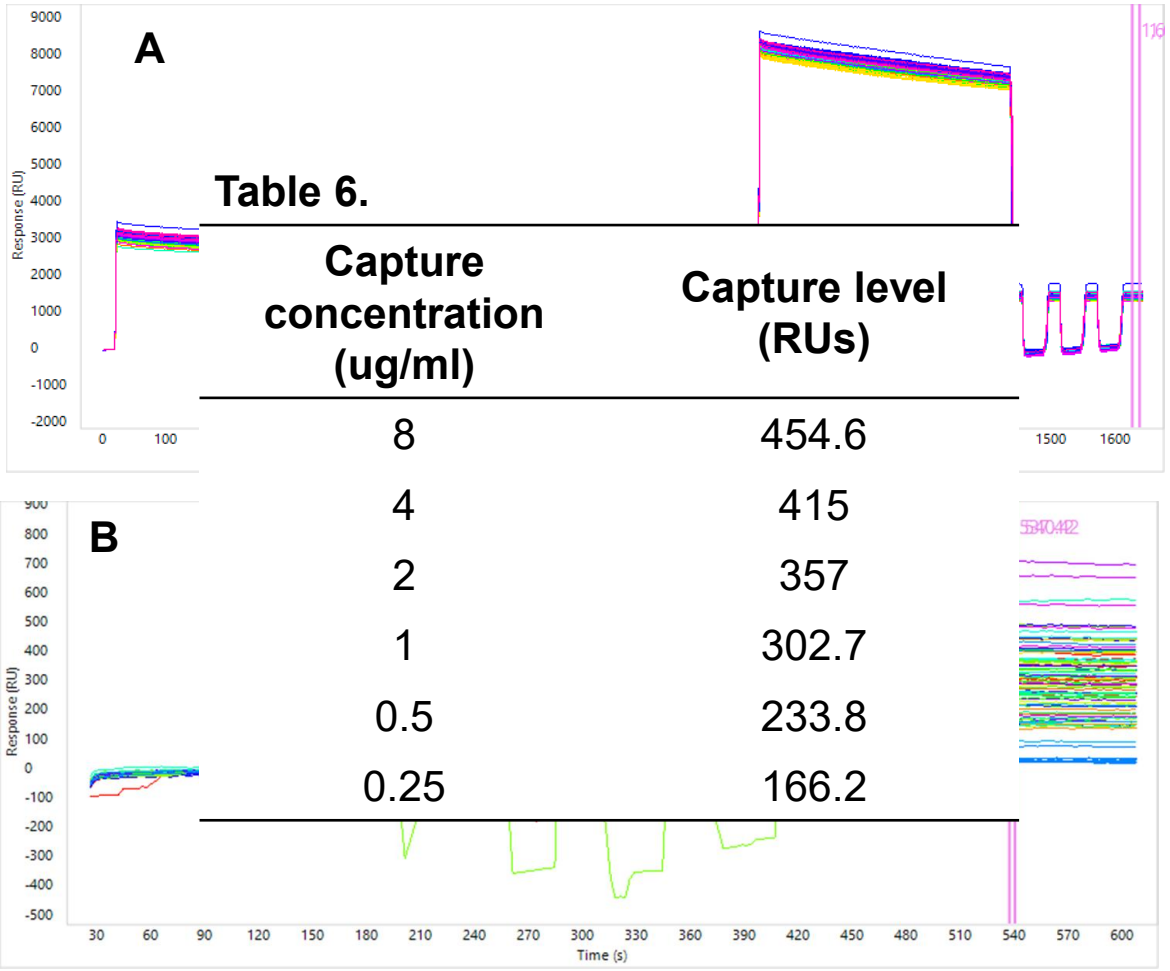


Table 6.

Capture concentration (ug/ml)	Capture level (RUs)
8	454.6
4	415
2	357
1	302.7
0.5	233.8
0.25	166.2

Fig. 14

LSA^{XT} - CMDP

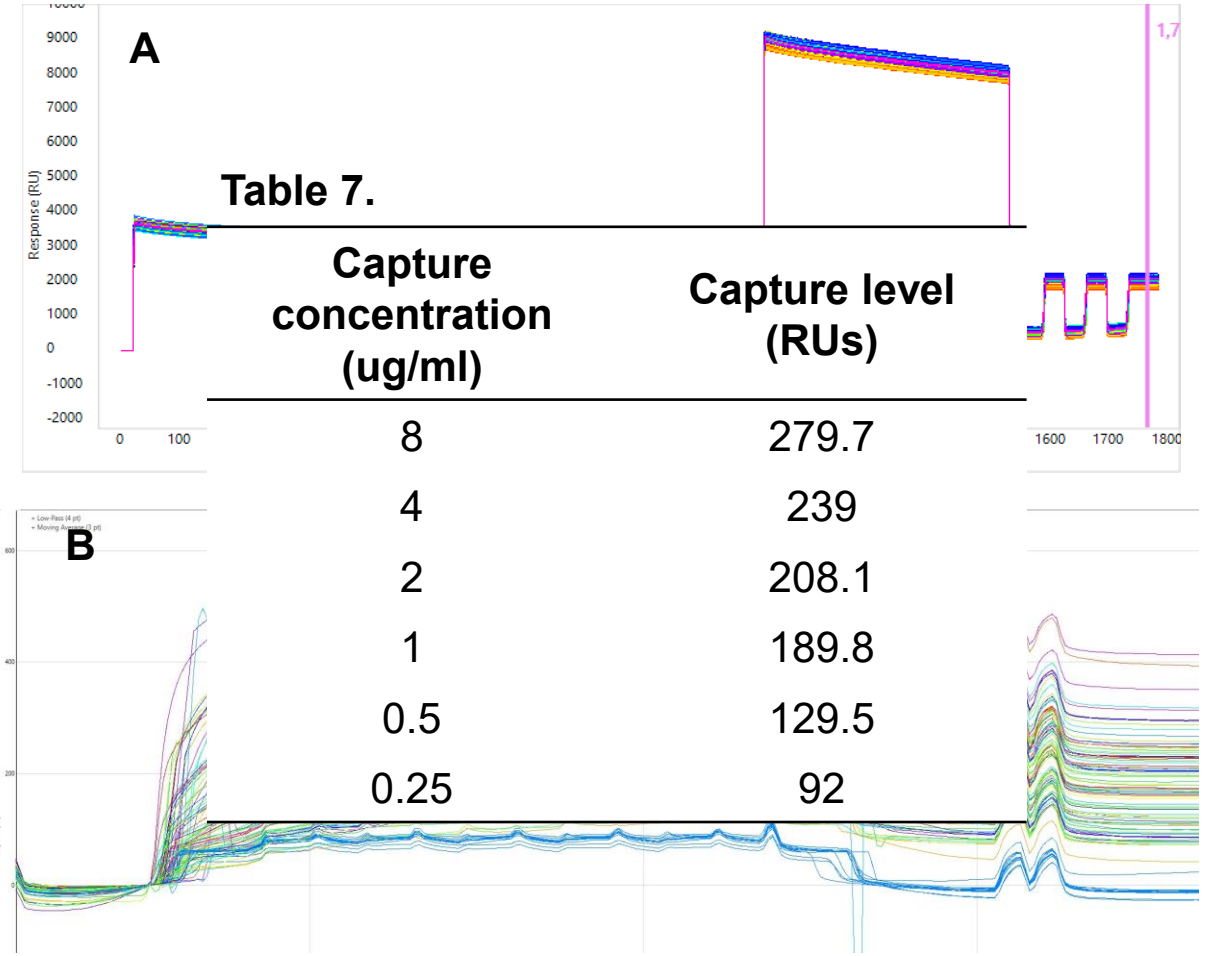
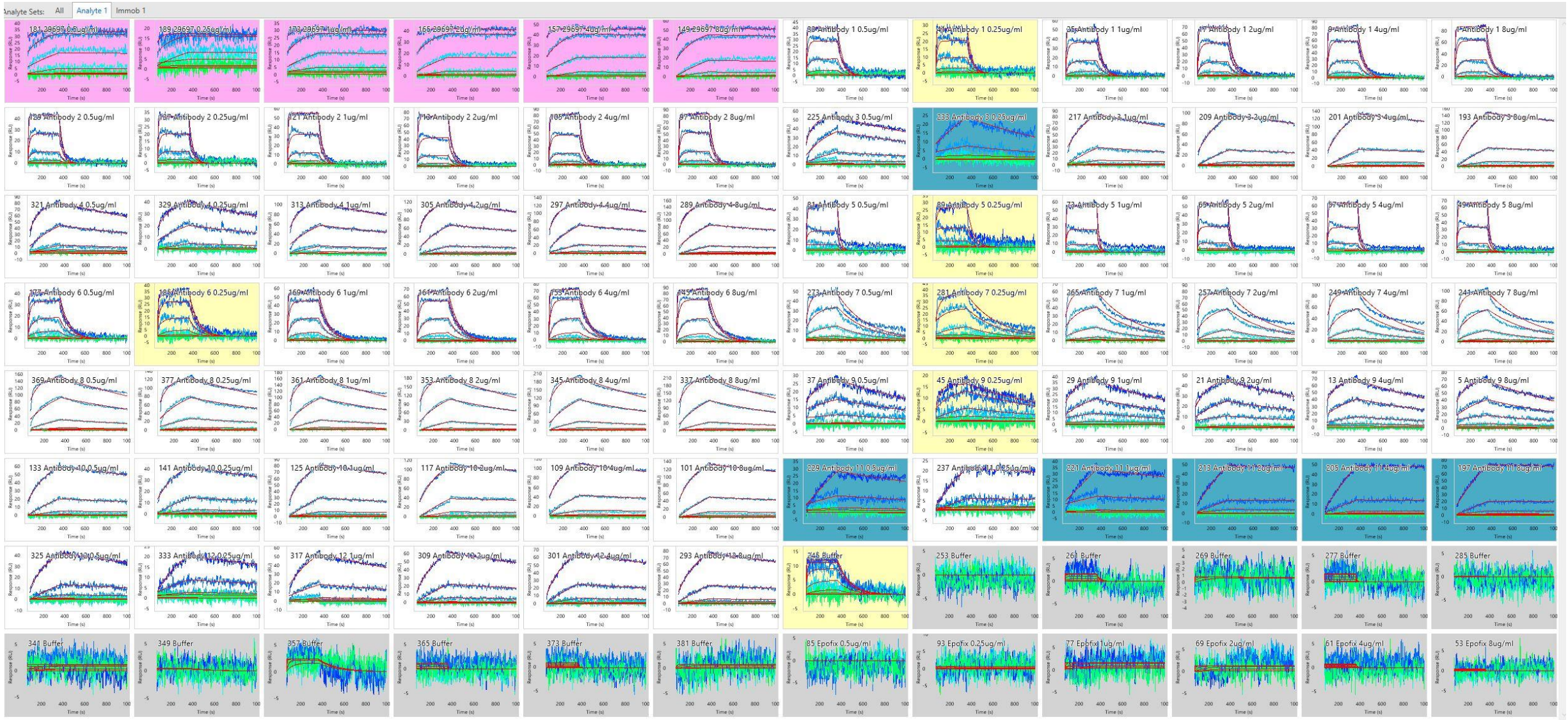


Table 7.

Capture concentration (ug/ml)	Capture level (RUs)
8	279.7
4	239
2	208.1
1	189.8
0.5	129.5
0.25	92

Fig. 15

LSA® - HC30M



Res sd 10% Rmax

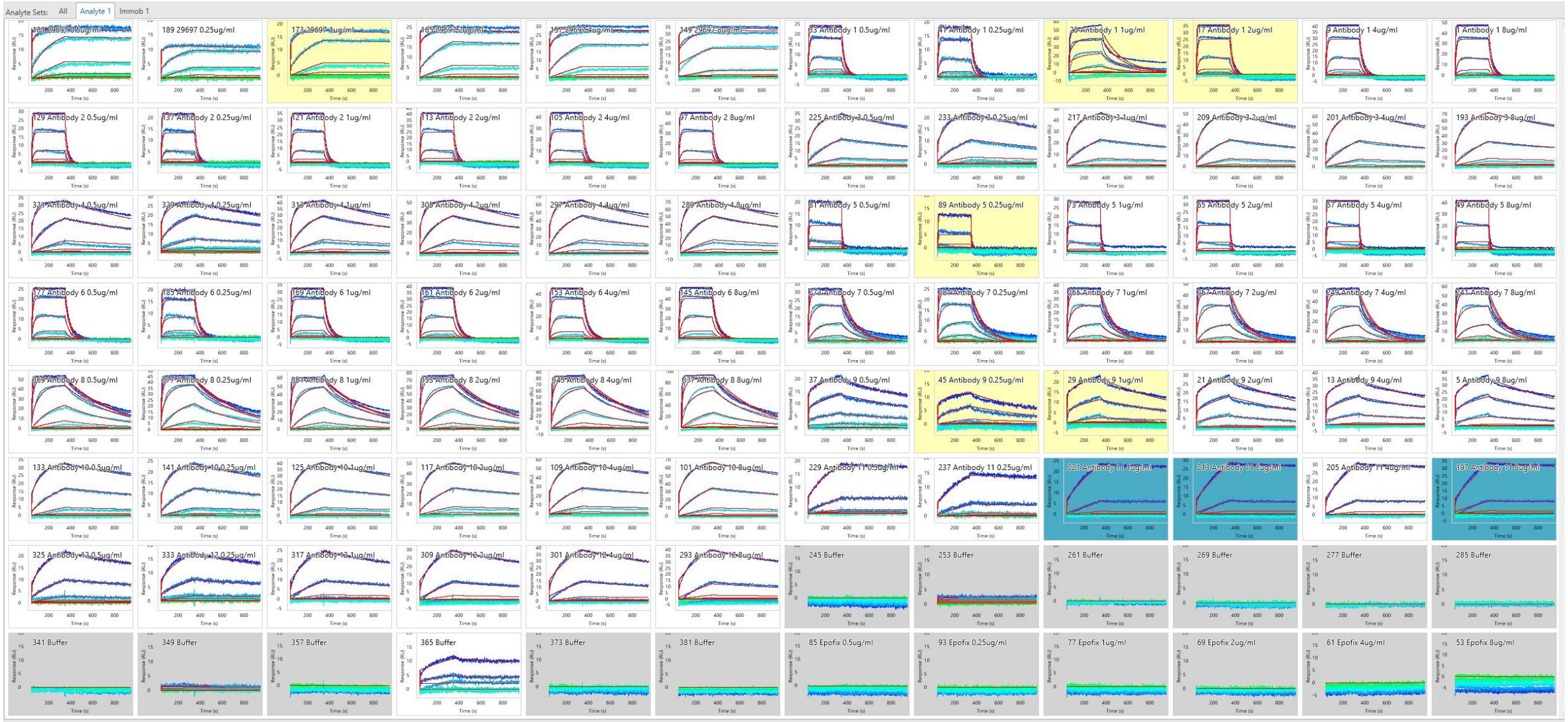
Kd below limit

Data < 50% Rmax

Non-binders

Fig. 16

LSA^{XT}- CMDP



Res sd 10% Rmax

Data < 50% Rmax

Non-binders

Fig. 17

Affinities across capture concentrations – LSA® and LSA^{XT}

Affinities across capture concentrations (LSA - HC30M)

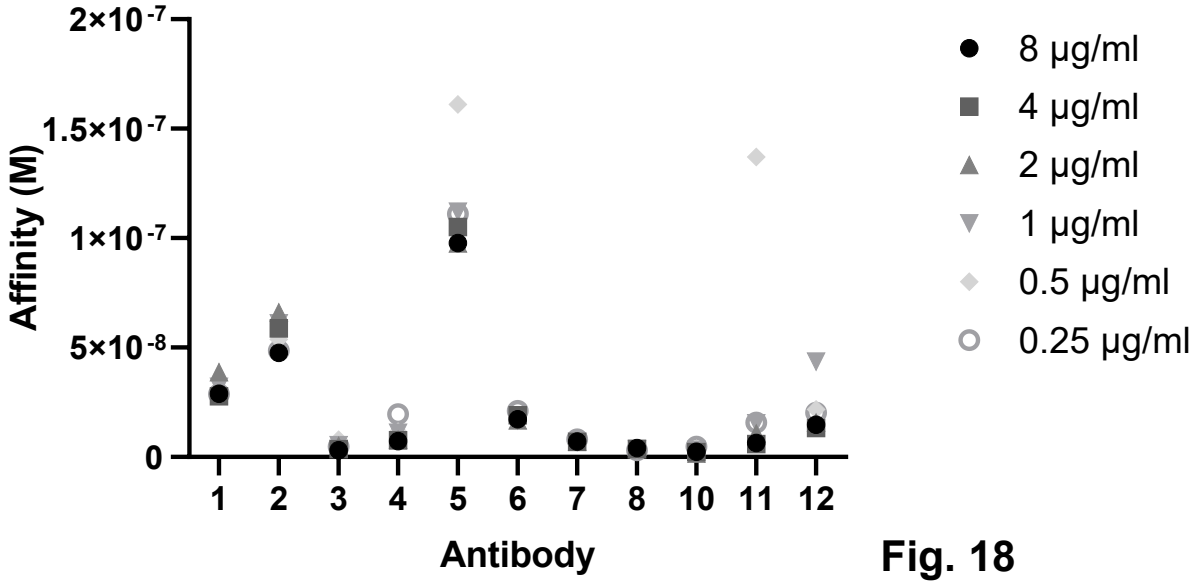


Fig. 18

Affinities across capture concentrations (LSA XT - CMDP)

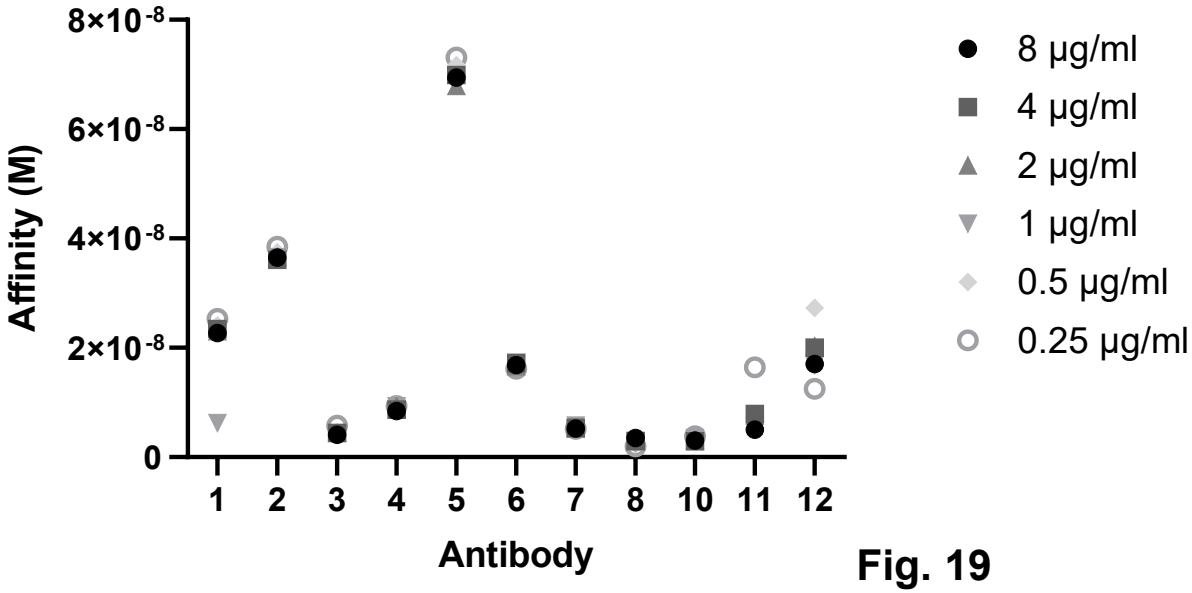


Fig. 19

No significant differences across capture concentrations (Friedman test; $p > 0.05$)

Comparability by capture level – HC30M

- Scatter plots to visualise variance by capture concentration (replicates needed).

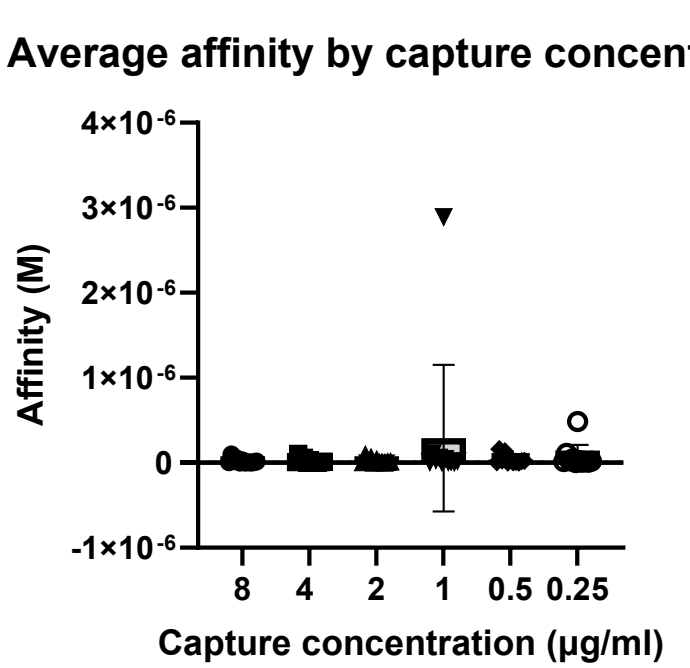


Fig. 20

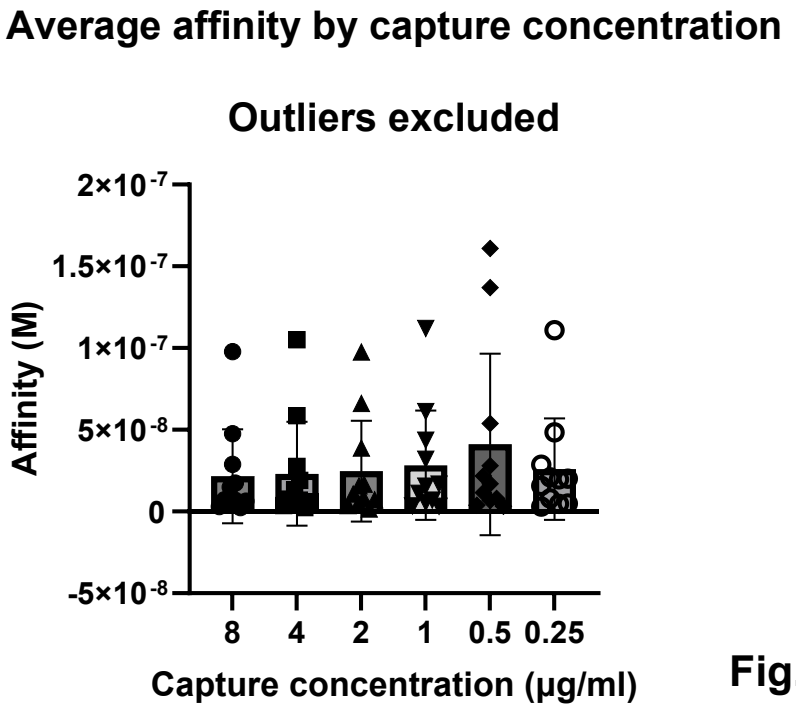


Fig. 21

- More variation in average affinities across the lower three capture concentrations (which also include the outliers).
- Consistent average affinity corresponds to **~ 350 – 460 RUs** capture in HC30M.

Comparability by capture level – CMDP

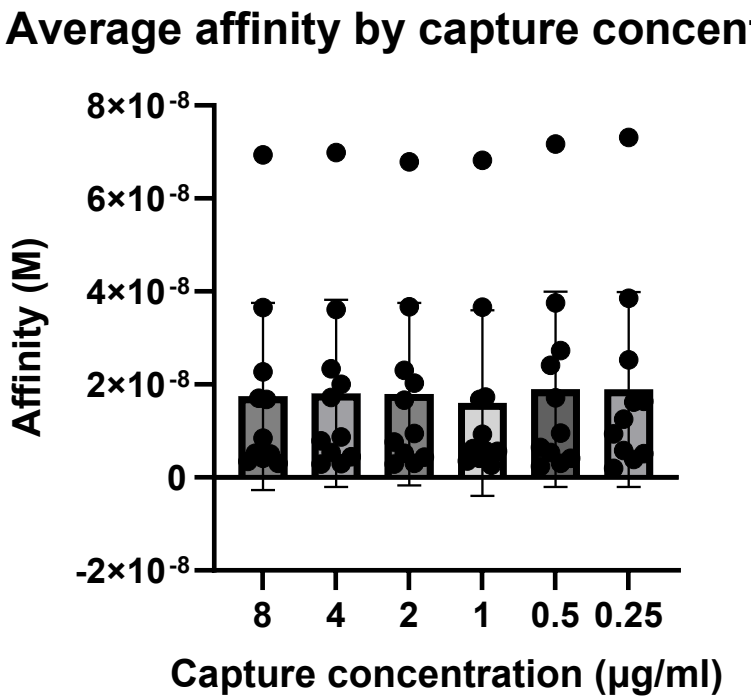


Fig. 22

- Generally consistent across capture concentrations.
- Slightly more variability in bottom three capture concentrations (SD and mean).
- Capture levels appear acceptable throughout capture range.
- Highlights importance of repeats and limitations to HC30M conclusions.

HC30M and CMDP comparison

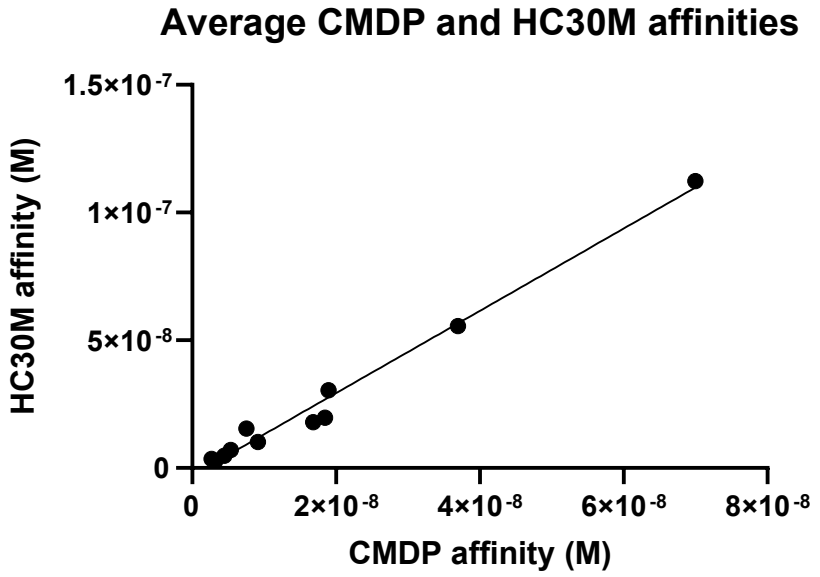


Fig. 23

r = 0.9818; Spearman's p < 0.001 ***

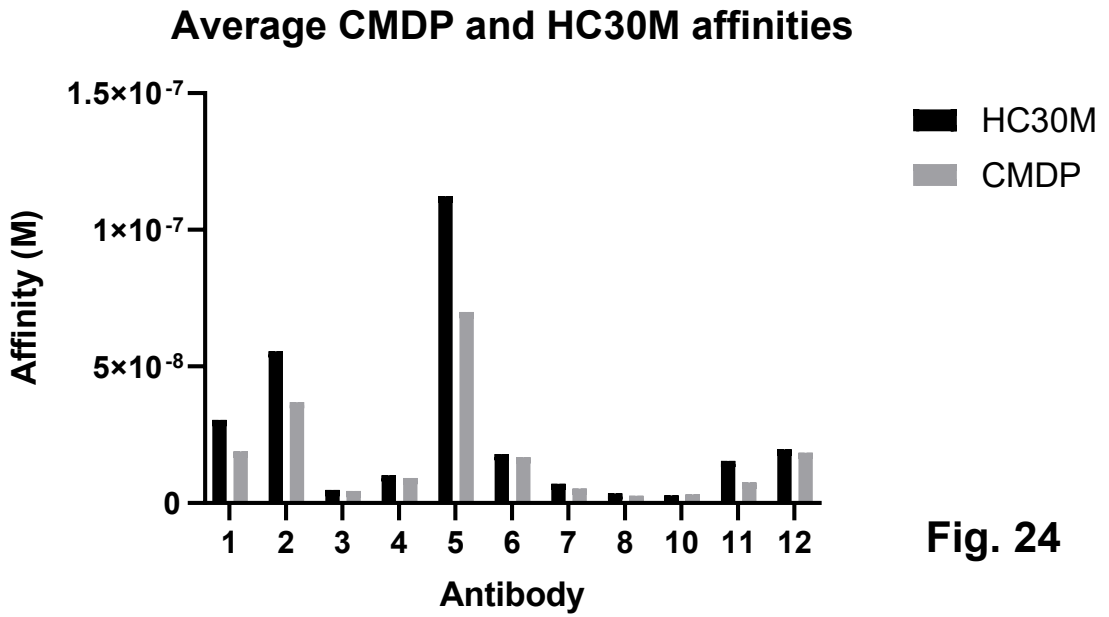


Fig. 24

- Very high agreement between instruments, affinities ranked similarly.
- Average affinities in instruments within 2-3-fold range.
- Slight differences between the HC30M and CMDP.

Conclusions – HC30M and CMDP

- ✓ Affinities are **ranked consistently** between instruments from single to triple digit nanomolar affinities.
- ✓ Affinities measured are **within 2-3-fold** range.
- ✓ Capture level range limitations **clearer on the HC30M**. Preliminary data pointing to capture ranges being even more important in HC30M?
- ✓ Data points to **increased sensitivity** in the **LSA XT.**
- ✓ GSK methods appear **acceptable** on both HC30M and CMDP chips.

Next steps

- ✓ Investigating **lower capture levels** on CMDP in **LSA XT** to test limits.
- ✓ Investigating **higher capture levels** on CMDP in **LSA®** to test limits.
- ✓ Producing **replicate experiments for the HC30M** – establish accurate capture level ranges with more confidence.
- ✓ Testing **weaker affinity** panels.
- ✓ Testing **another antibody – analyte** interaction.

Thank you for listening!
Any questions?

Acknowledgements

Gemma Burton

Sarah Stuart

Katie Smith

Rosalind Booth