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



Carterra Symposium 2025

Binding and Biophysical Characterisation during Discovery



GSK

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

HIGH THROUGHPUT SCREENING

- Binding checks
- Kinetics and affinities
- Epitope binning









• Obstacles in our existing Carterra workflows.







My Industrial Placement Project









LSA[®] - CDMP LSAXT - CMDP -2 Place San 8 Summary 3 Sample I Capture Binding Cycles 5 Volume Require 6 Injection Tim 7 LSA Preparation Regeneration Setu G Capture Binding Cycles Tsc 🔩 🕴 Single Hulti Regeneration Time Time 30 30 X3 * 5 1 5 10 30 X3 * 1 5 5 10 1 MINS MINS

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



GSK

420

390

360

330

300

270

240

€ 210

<u>ल</u> 180

ຂຶ້ 120

90

60

30

0

-30

-60

150

LSA[®] – Sensorgrams

Rmax



Rmax

Non-binders



LSA^{XT} – Sensorgrams

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GSK

Kd below limit

Non-binders

Select sensorgrams – Antibody 12



LSAXT

LSA[®]

Select sensorgrams – Antibody 6



LSAXT

GSK

LSA[®]

13

Affinities across capture concentrations – LSA[®] repeats



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



Affinities across capture concentrations – LSA^{XT} repeats



No significant differences across capture concentrations (Friedman test; p > 0.05) Carterra Symposium 2025

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.

GSK

• 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.

Table 5.

• The larger the p value the more comparable.

		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.

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



- 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.



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.

- 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.

LSA^{XT} - CMDP

Immobilisations and captures



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LSA[®] - HC30M



Res sd 10% Rmax

Kd below limit

Data < 50% Rmax

Non-binders

Fig. 16 Carterra Symposium 2025

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LSA^{XT}- CMDP



Res sd 10% Rmax

GSK

Data < 50% Rmax

Non-binders

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



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

Comparability by capture level – HC30M

55K

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



Average affinity by capture concentration

- 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



Average affinity by capture concentration

- 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



- 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?
- \checkmark 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

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