

Application of Octet for Rapid Screening of Assay Reagents for Pharmacokinetic Ligand Binding Assays for Antibody Drug Conjugates

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WORLDWIDE RESEARCH & DEVELOPMENT
Pharmacokinetics, Dynamics & Metabolism – NBE

Agenda

- PDM-NBE Bioanalytical - What we do...
- Antibody Drug Conjugates (ADC)
 - Overview
 - Regulated PK Assay Strategy
- Application of Octet® RED96 System
 - Reagent Screening
 - Reagent Characterization
 - Epitope Binning

PDM-NBE Bioanalytical

- Develop and Validate Ligand Binding Assays (LBAs) for Large Molecule Therapeutics from Discovery through Clinical
 - Pharmacokinetics/Toxicokinetics (quantitation of drug)
 - Immunogenicity (antibody response to drug)
 - Biomarkers
- Sample Analysis (in-house / outsourced to CRO)

Antibody Drug Conjugates (ADCs)

- ADC



Specific monoclonal antibody



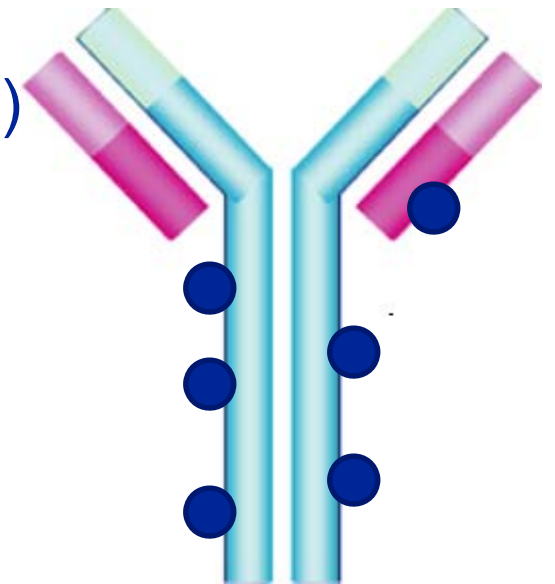
Small molecule cytotoxic drug (Payload)

- Oncology therapeutic

- Antibody binds to target, providing a selective delivery mechanism of the payload which then kills/arrests the targeted cell.

- ADCs (one size does not fit all)

- Multiple combinations of antibodies, linkers, and payloads
- Varying drug to antibody ratios (DAR) resulting from the conjugation chemistry and *in vivo* circulation



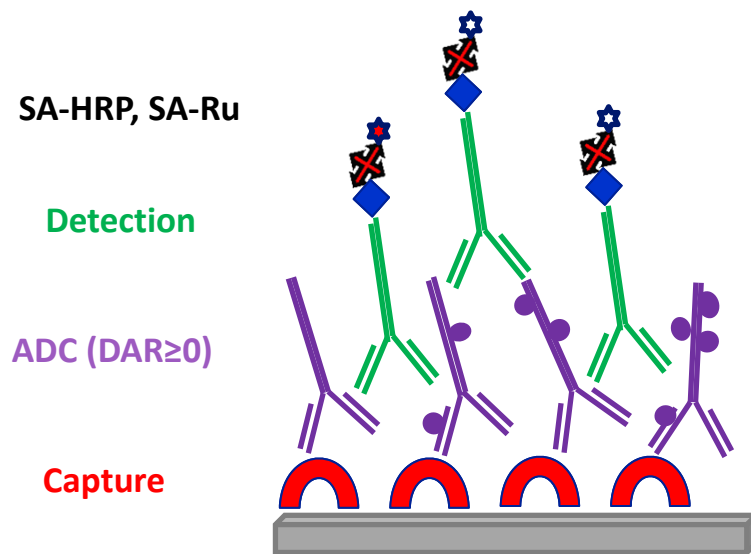
Unique LBA Challenges Posed by ADC Heterogeneity & Complex Structure

- DAR Independence
- Specificity
- Sensitivity
 - Lower dose requires more sensitive assay
- Pre-existing reactivity against ADC component
 - PK and ADA assays
- Stability of the Therapeutic

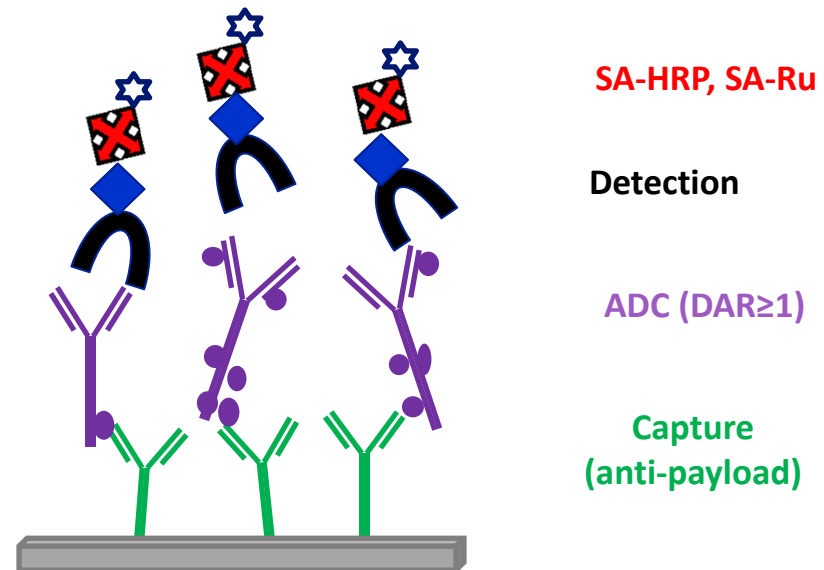
Utilized octet to accelerate assay development cycles

Recommendations for LBA PK Assays

Total Antibody Assay



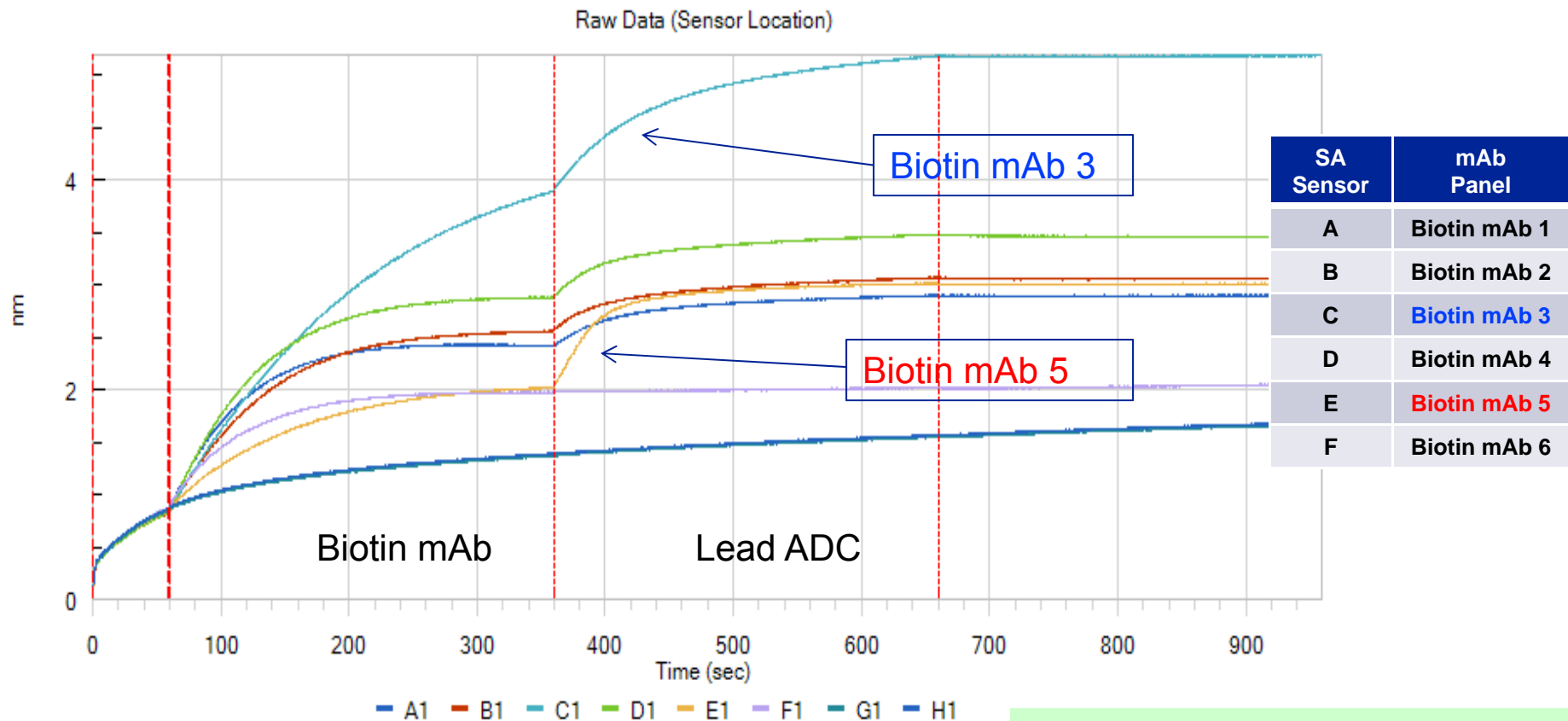
Conjugated Antibody Assay



- ✓ Analyte: ADC with DAR \geq 0
- ✓ Reference Standard & QCs: ADC

- ✓ Analyte: ADC with DAR \geq 1
- ✓ Reference Standard & QCs: ADC

Reagent Screen 1: Binding of Lead ADC to panel of mAbs against drug Ab



MABs 3 and 5 are potential capture/detector reagents for both the total Ab and conjugated Ab assays.

METHOD (5% matrix)

- pre-wet SA biosensor tips
- Baseline (1 min) – assay buffer
- Association (5 min) - 5 ug/mL of biotin mAbs
- Association (5 min) - 5 ug/mL of ADC
- Dissociation – assay buffer



Reagent Screen 2: Binding of Anti-Payload Abs to Lead ADC

SA Sensor	Sample ID	Response	Anti-Payload -ADC Response
A1	Biotin mAb 3	3.9223	
	ADC	5.1254	
	anti-Payload-X	7.1253	+1.9999
B1	Biotin mAb 3	3.4066	
	ADC	4.5292	
	anti-Payload-Y	4.5927	+0.0635
C1	Biotin mAb 3	3.3639	
	ADC	4.4605	
	Negative Ctrl	4.4878	+0.0273
D1	Biotin mAb 5	1.7997	
	ADC	2.5601	
	anti-Payload-X	3.9334	+1.3733
E1	Biotin mAb 5	1.7582	
	ADC	2.5276	
	anti-Payload-Y	2.587	+0.0594
F1	Biotin mAb 5	1.7909	
	ADC	2.5799	
	Negative Ctrl	2.6222	+0.0423

METHOD (5% matrix)

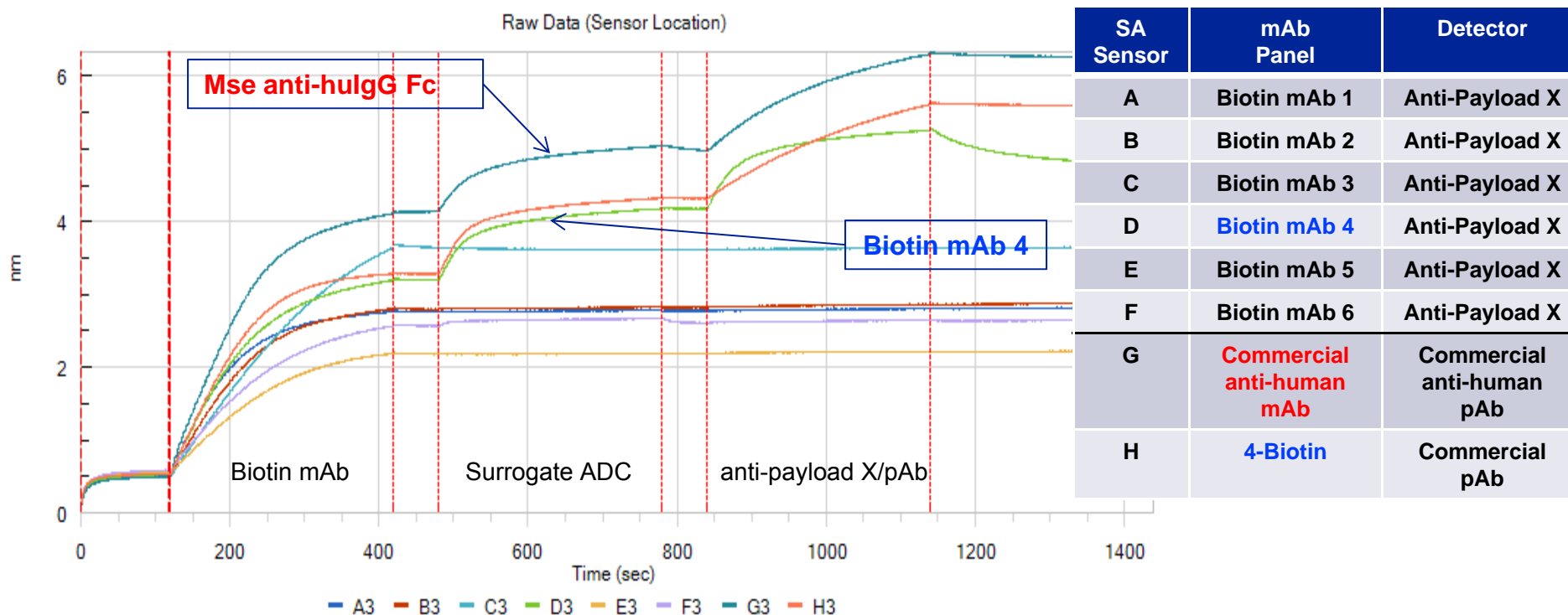
- pre-wet SA biosensor tips with PBS-CMF
- Baseline (2 min) – assay buffer
- Association (5 min) - 5 ug/mL Biotin mAbs 3 & 5
- Baseline (1 min) – assay buffer
- Association (5 min) - 5 ug/mL of ADC
- Baseline (1 min) – assay buffer
- Association (5 min) - 5 ug/mL of anti-payload Abs
- Dissociation (5 mins) – assay buffer

LBA Feasibility: anti-payload-X capture & Biotin mAb 3 detector

Concentration (ng/mL)	Mean RLU	Signal to Noise
500	608999	5296
167	498492	4335
55.6	274264	2385
18.5	99879	869
6.17	34015	296
2.06	11905	104
0.686	4193	36
0	115	



Reagent Screen 3: Surrogate ADC pairing of mAbs against drug Ab and anti-payload Ab



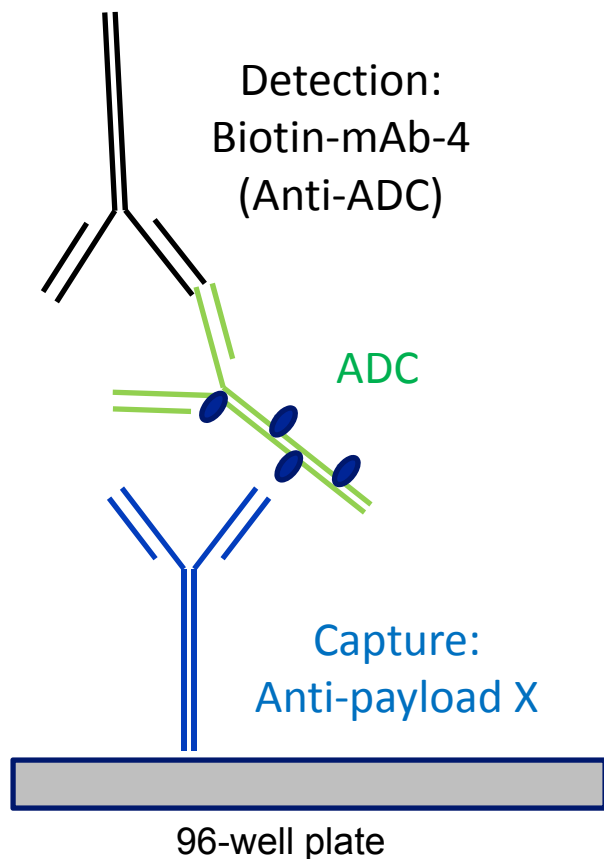
- Getting smarter... combined screening experiments 1 and 2 into a single experiment
- Surrogate ADC did not bind to biotinylated mAbs 3 and 5
- Biotinylated mAb 4 is a potential rgt for conjugated Ab assay
- Biotin mAb 4, as well as commercial mAb and pAb are potential rgts for total Ab assay.

METHOD (5% matrix)

- pre-wet SA biosensor tips
- Baseline (1 min) – assay buffer
- Association (5 min) - 10 ug/mL of biotinylated mAbs
- Baseline (1 min) – assay buffer
- Association (5 min) - 10 ug/mL of Surrogate ADC
- Baseline (1 min) – assay buffer
- Association (5 min) - 10 ug/mL of anti-payload X / Commercial pAb
- Dissociation – assay buffer



Surrogate ADC: DAR Evaluation with Anti-Payload Capture



Sample ID	Octet %RE	LBA* %RE
ADC (~4DAR)	REF	6
Ab (0 DAR)	-97	Not Tested
2 DAR	-39	-70
4 DAR	-10	55
6 DAR	-3	42
ADC		
+ 1 mg/mL unconjugated Ab	Not Tested	3

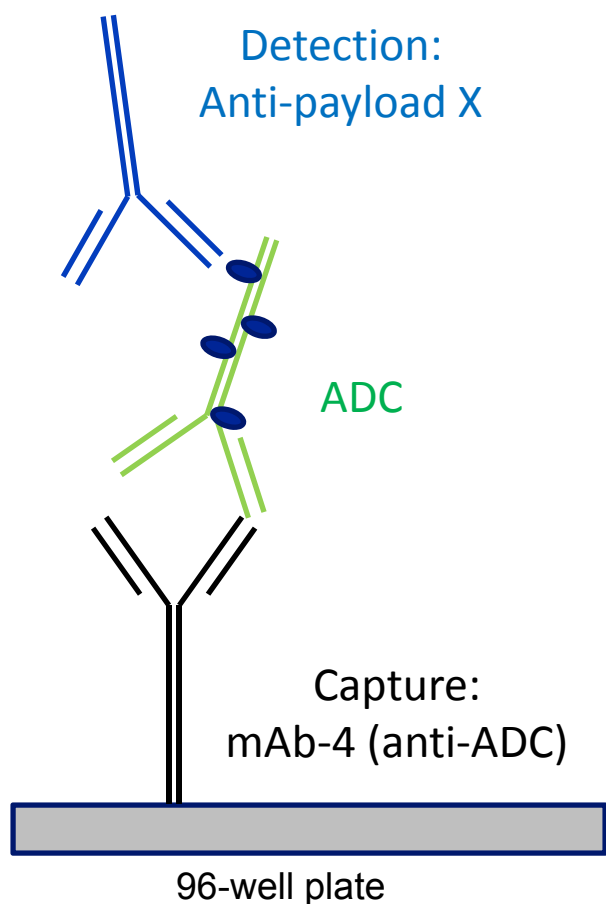
*Mean of %RE at upper and lower limits of ROQ; target %RE \pm 25%.

RESULTS

- DAR trends are similar between Octet and LBA
 - 2 DAR under-recovers
 - 4 DAR and 6 DAR have comparable recoveries
- Although qualitative, Octet results were predictive of LBA
- Assay is specific for quantitation of conjugated Ab



Surrogate ADC: DAR Evaluation with Anti-Payload Detector



Sample ID	Octet %RE	LBA* %RE
ADC (~4DAR)	REF	6
Ab (0 DAR)	-104	Not Tested
2 DAR	-67	-83
4 DAR	2	20
6 DAR	67	100
ADC		
+ 1 mg/mL unconjugated Ab	Not Tested	-98

*Mean of %RE at upper and lower limits of ROQ; target %RE \pm 25%.

RESULTS

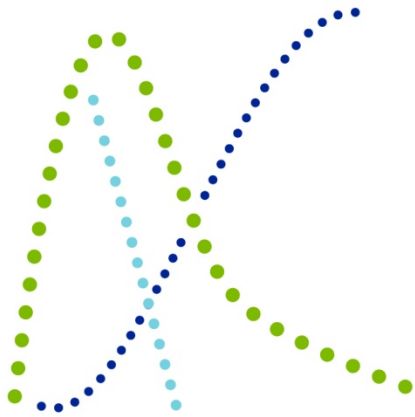
- DAR trends are similar between Octet and LBA
 - 2 DAR under-recovers
 - 4 DAR demonstrates acceptable recovery
 - 6 DAR over-recovers
- Although qualitative, Octet results were predictive of LBA
- Assay is NOT specific for quantitation of conjugated Ab



Reagent Screening & Characterization Summary

- Octet is a quick and efficient, user-friendly method to screen reagents
 - Can conduct multiple experiments in a day (~30 minutes/screening), whereas ELISA may take several days to screen all pairs
- Screening performed in matrix
- Identified capture/detector reagents in both the total Ab and conjugated Ab assays
 - The lead and surrogate ADCs demonstrated optimal binding to different mAbs (anti-drug)
 - Anti-payload mAb was identified as a capture/detector reagent for the conjugated Ab assay
- For DAR evaluation in conjugated Ab assay, the Octet was predictive of LBA results





Characterization for Total mAb Assay



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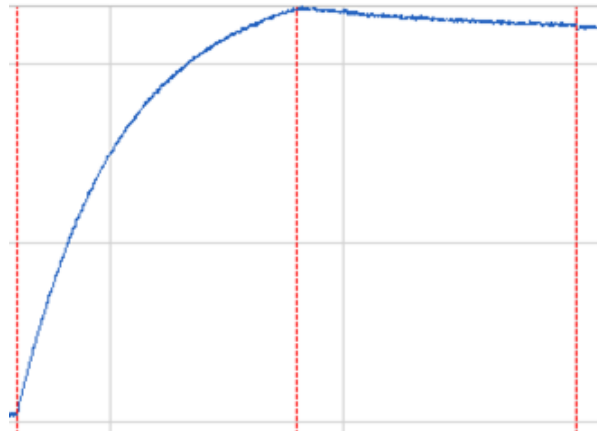
OBJECTIVE: Total Ab Plate Based LBA for Quantitation of Lead Candidate in Plasma

- Assay for total antibody measurement must be DAR insensitive
- Further characterize binding of available reagents to ADC
- Choose antibody for use in LBA

	Source	Antigen
mAb-3:	In-house	Unconjugated Ab
mAb-5:	In-house	Unconjugated Ab
mAb-7:	Commercial	Human Fc
pAb 1:	Commercial	Human IgG (H+L)
pAb 2:	Commercial	Human IgG (H+L)



Screening In-house mAbs for Binding to FC



Loading SA Tip: Biotin-mAb
Association: Human Fc

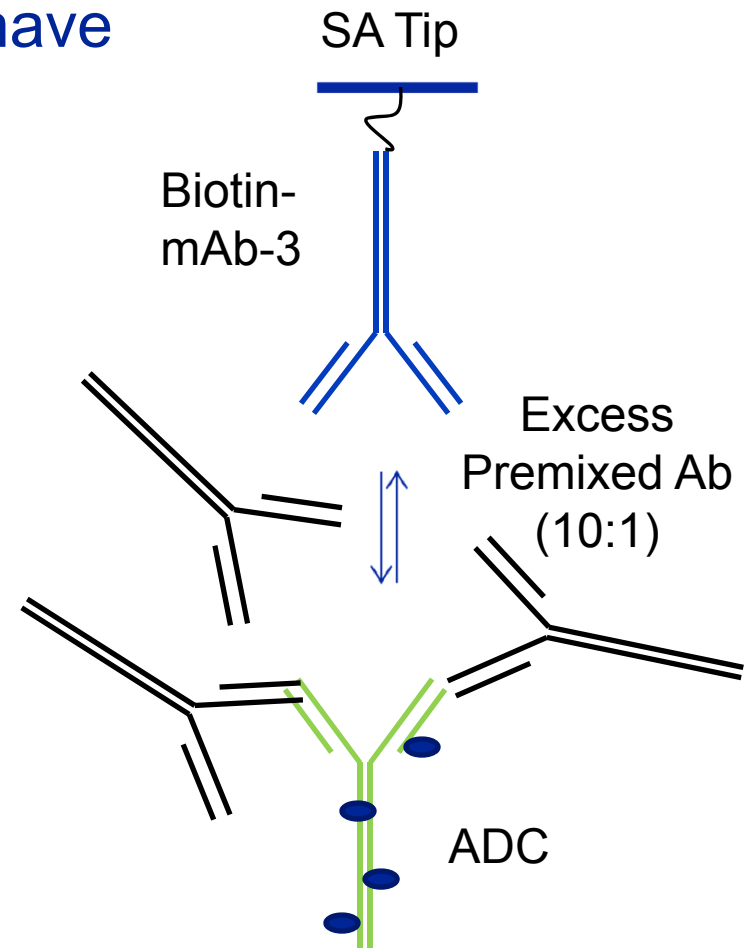
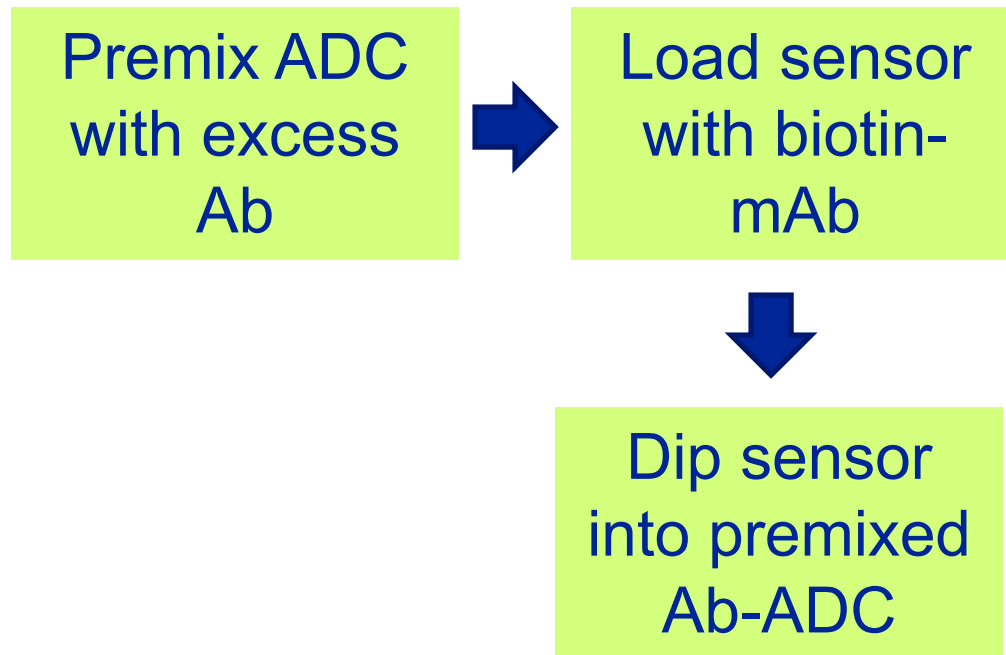
Loading Protein	Conc (ug/mL)	Sample ID	Sensor	Conc. (ug/mL)	Response
Biotin-mAb-3	10	Human FC	A1	10	-0.02802
Biotin-mAb-3	10	Human FC	B1	2.5	-0.01924
Biotin-mAb-3	10	Human FC	C1	0.625	-0.01761
Biotin-mAb-3	10	Human FC	D1	0	-0.01887
Biotin-mAb-5	10	Human FC	E1	0.4	-0.00406
Biotin-mAb-5	10	Human FC	F1	0.1	-0.00136
Biotin-mAb-5	10	Human FC	G1	0.025	-0.00201
Biotin-mAb-5	10	Human FC	H1	0	-0.00319

No Binding to Fc: epitope for MAbs 3 and 5 are in Fab region

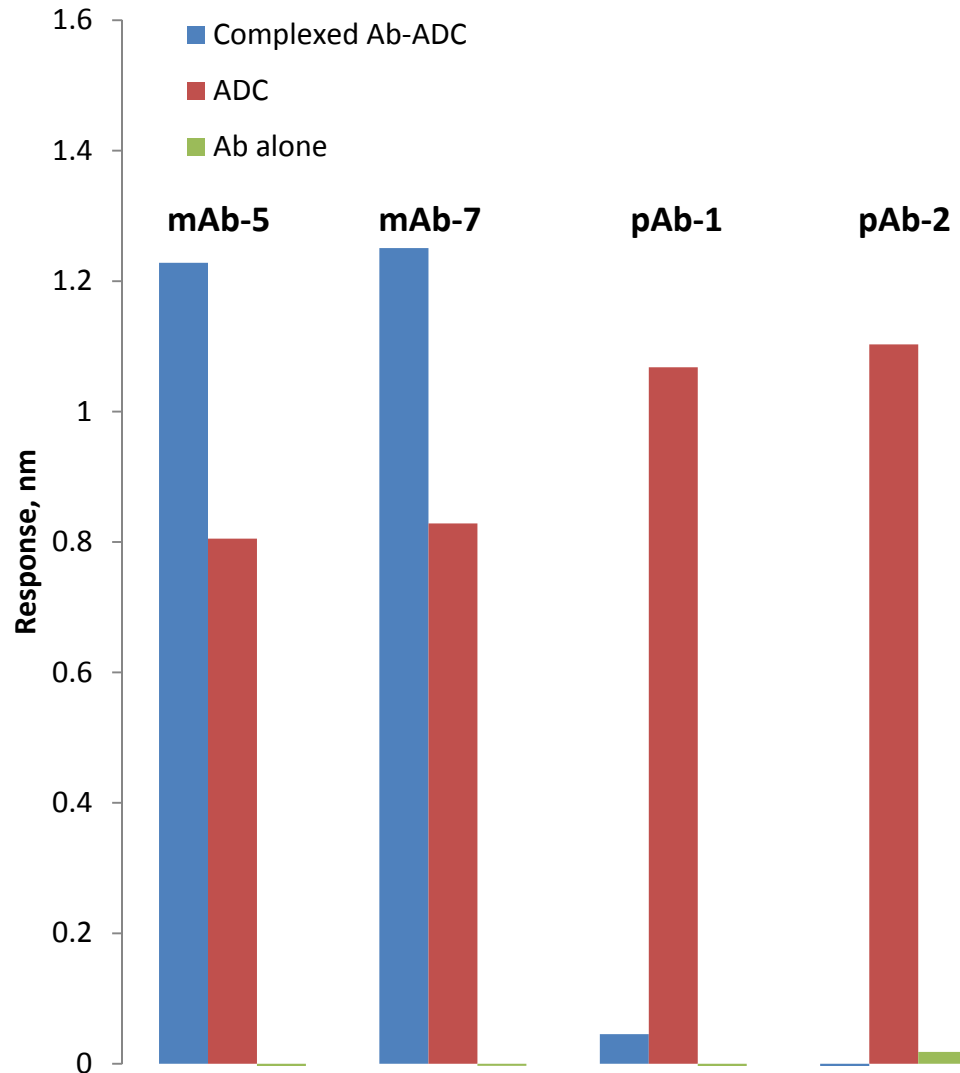


In Solution Epitope Binning

Identify Ab pairs that do not share or have overlapping epitopes



EPITOPE BINNING: Reagents Towards ADC

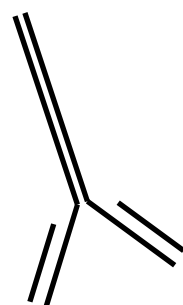


- mAb-5 & mAb-7 have distinct epitopes from mAb-3

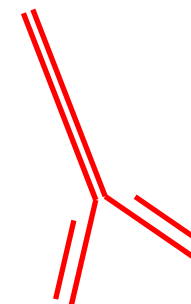
- pAb-1 and pAb-2 block binding of mAb-3



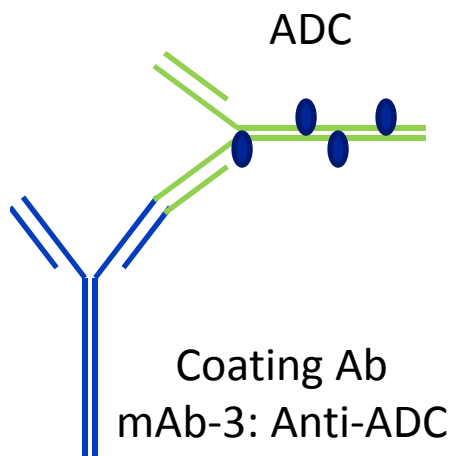
Comparing Antibody Pairs for DAR Independence in ECL-LBA



Detection Ab
Biotin-mAb-5:
Anti-ADC



Detection Ab
Biotin-mAb-7:
Anti-Hu FC



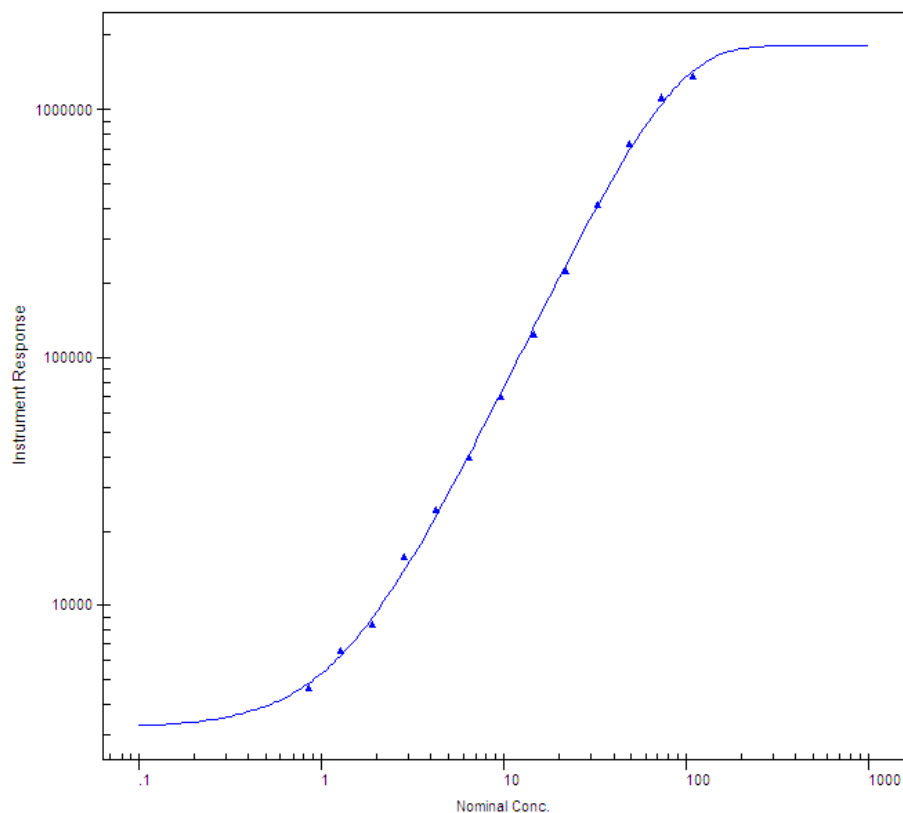
QC Concentration (ng/mL)	ADC QC (ng/mL)	Unconjugated Ab QC (ng/mL)	% Variability (ADC v Unconj)	ADC QC (ng/mL)	Unconjugated Ab QC (ng/mL)	% Variability (ADC v Unconj)
5500	4361	5971	31	4856	5488	12
1650	1398	1928	32	1404	1580	12
550	432	624	36	440	519	17

mAb-3 paired with mAb-7 provided most DAR independent format



ASSAY PERFORMANCE: Coat mAb-3, Detection mAb-7

Screened 9 antibodies, 72 distinct pairs for plate LBA



n = 10	ADC		Unconjugated Ab	
	%Bias	CV	%Bias	CV
ULOQ	-9.9	13	11	14
HQC	-14	6.9	6.8	9.5
MQC	-12	12	8.2	12
LQC	-14	10	4.8	10
LLOQ	-7.1	8.5	4.0	8.1



SUMMARY

- ADC pose unique challenge for plate based LBA
- Bio-Layer Interferometry can be utilized to screen reagents for plate based LBAs
 - Very fast compared to plate based LBA
 - Screening performed in matrix
 - Tips and plates can be discarded as hazardous waste, closed system would need to be decontaminated
- In solution epitope binning experiments can identify Ab pairs which recognize distinct epitopes

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