

Overcoming Unexpected Interference in a Highly Drug-Tolerant and Ultrasensitive ADA-SPEAD Assay

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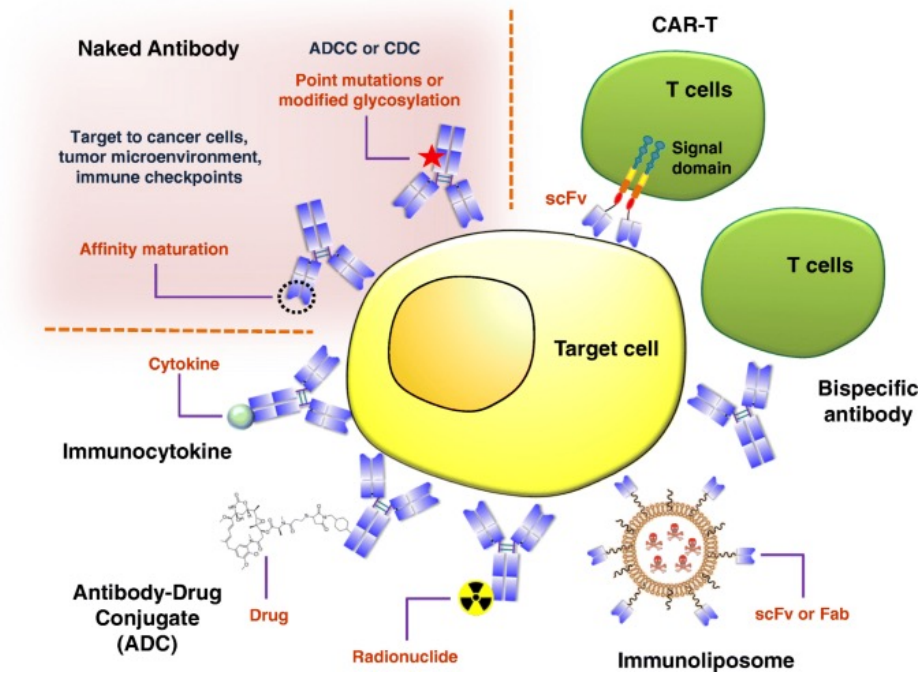
European Immunogenicity Platform (EIP) Symposium 2026



Agenda

- Introduction
- Unexpected Interference
- Characterization of Interference
- Solutions to reduce interference

Introduction



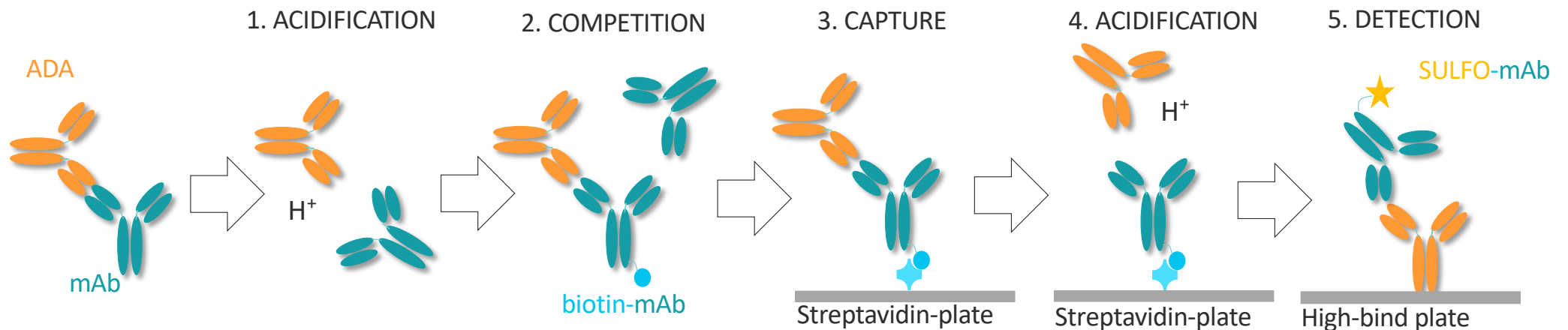
Ruei-Min Lu et al, 2020

- Monoclonal blocking antibodies often require high doses
- Affinity
 - Target
 - Tissue Distribution

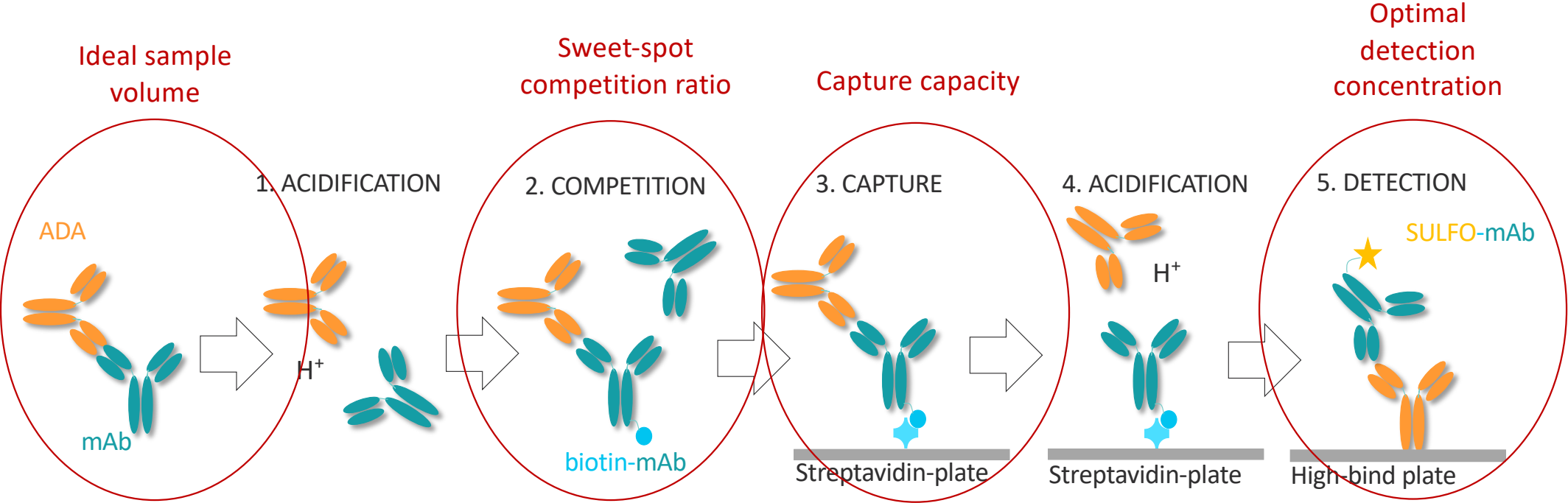
Case study

- ADA assay for a mAb therapeutic
- Up to 1000 $\mu\text{g}/\text{mL}$ drug tolerance requirement

➤ Solid Phase Extraction And Acid Dissociation (SPEAD) format



ADA SPEAD format optimization

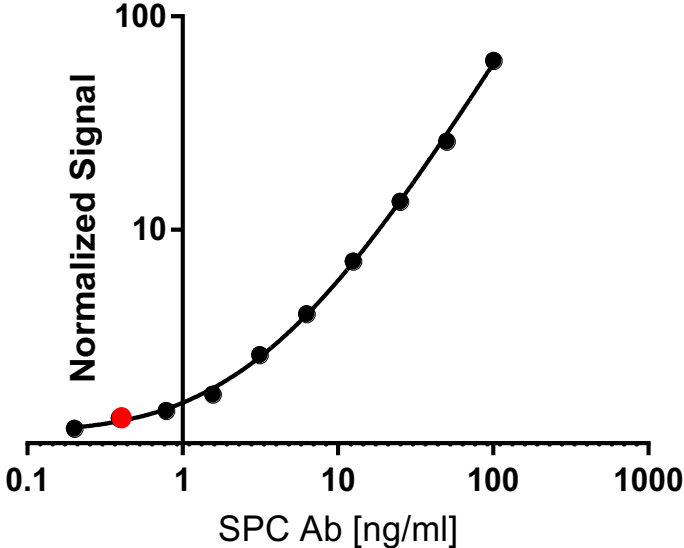


Optimization leads to highly drug tolerant and ultra-sensitive assay

Drug tolerance

SPC Ab [ng/mL]	mAb [μ g/mL]	mean RLU	NC normalized
100	1000	78	1.333
100	500	103	1.761
100	250	203	3.462
100	125	341	5.821

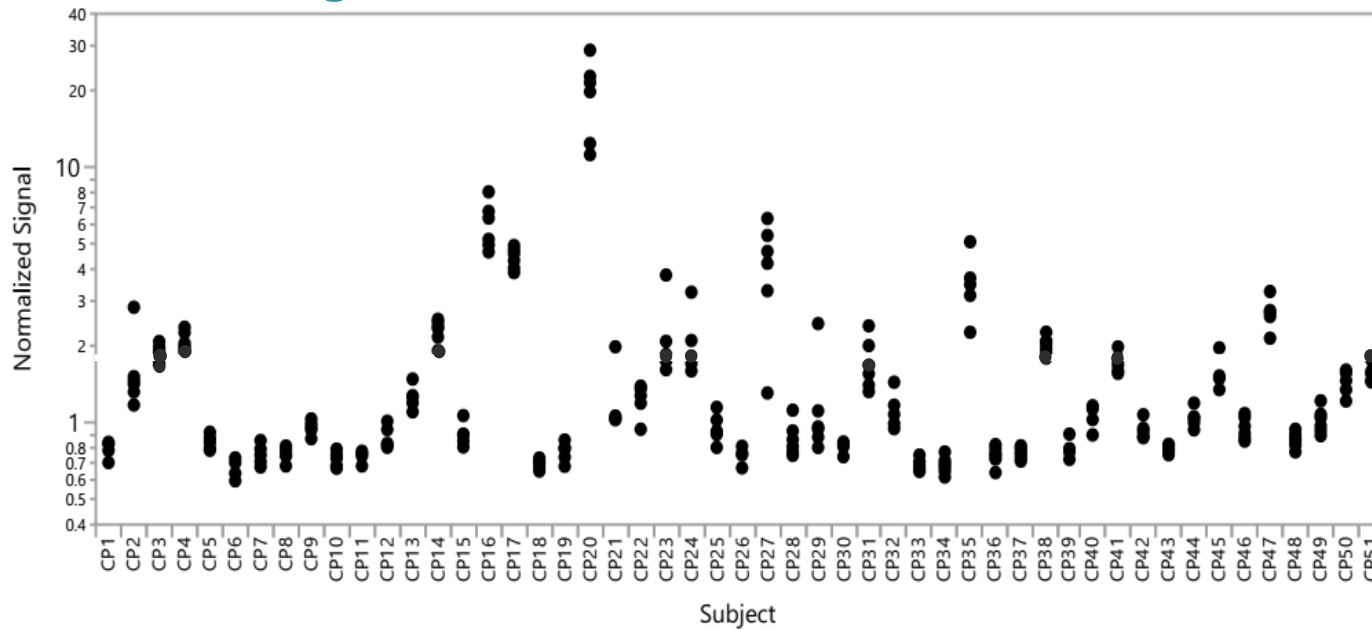
Sensitivity



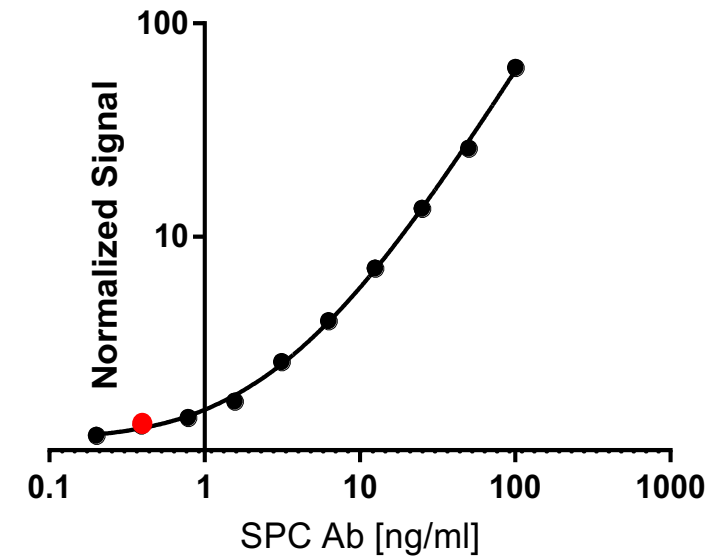
Unexpected interferences in the CP data set

(for a mAb therapeutic)

Screening CP data



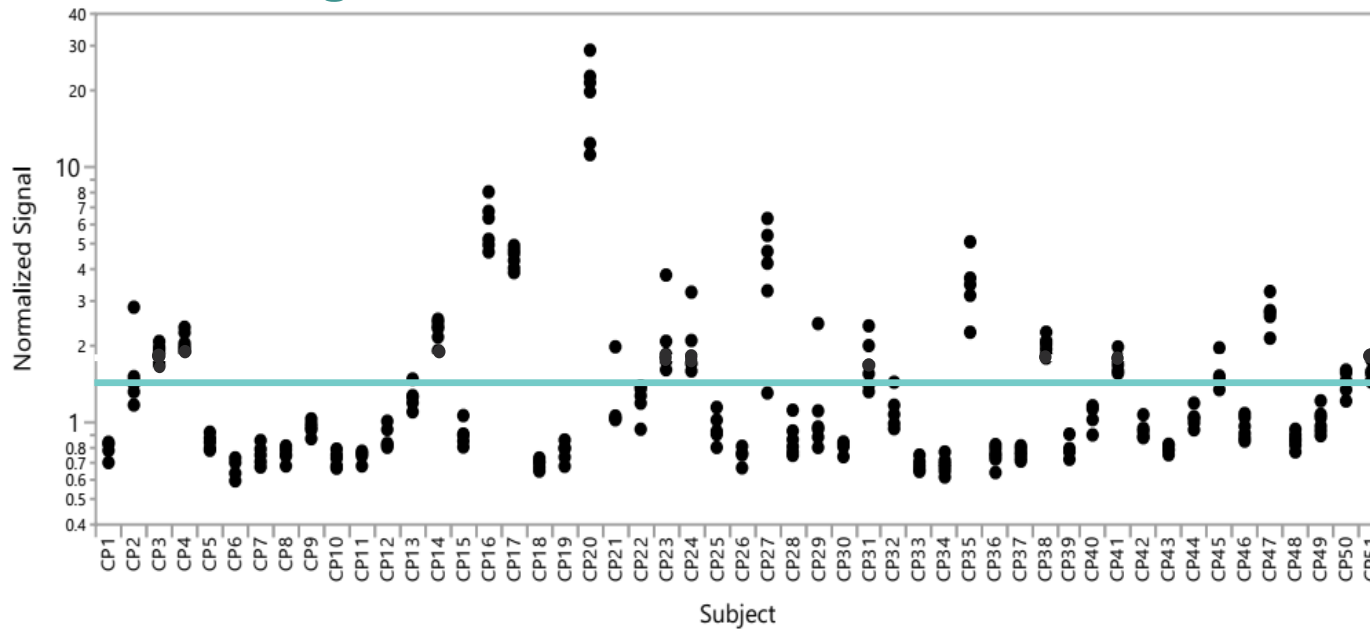
Sensitivity



Unexpected interferences in the CP data set

(for a mAb therapeutic)

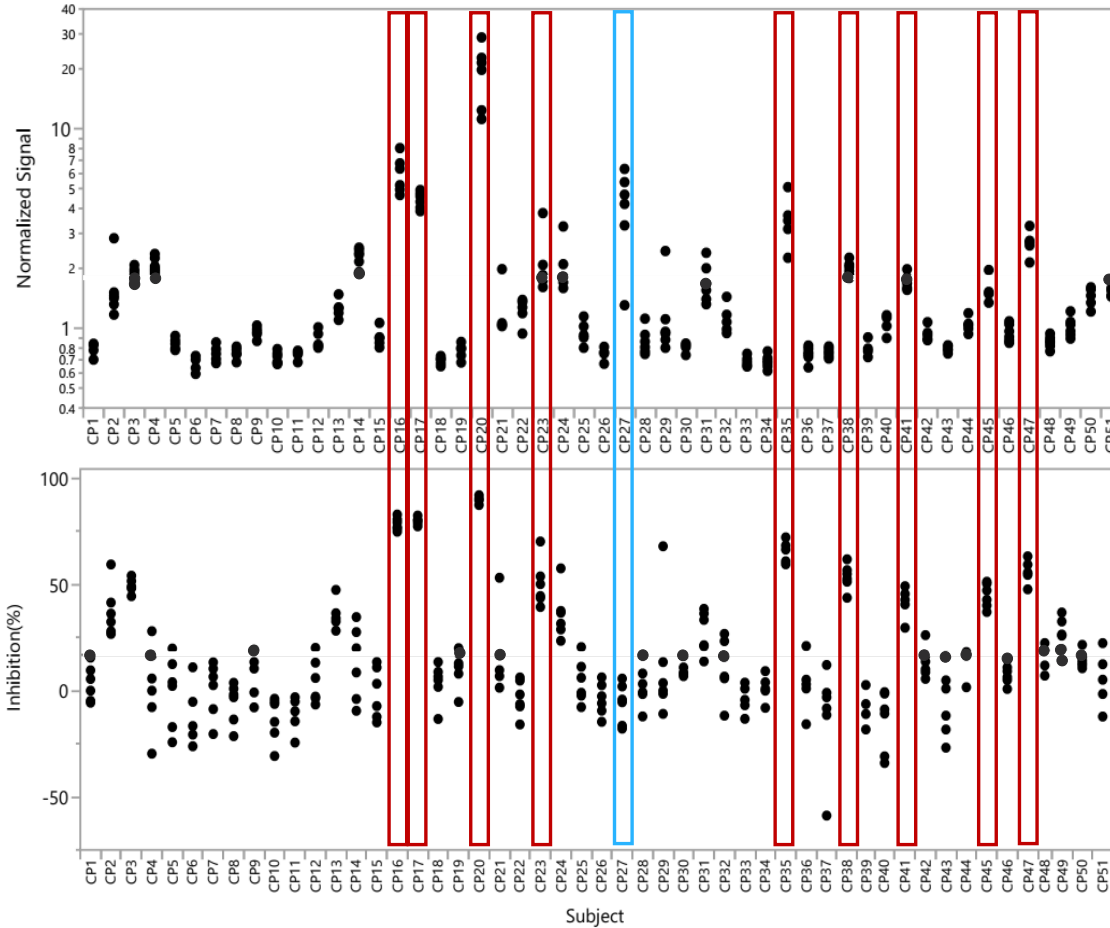
Screening CP data



SCPF = 1.36 (partitioning approach)


34.3 % positivity rate!


Unexpected interferences is heterogenous



Screening assay

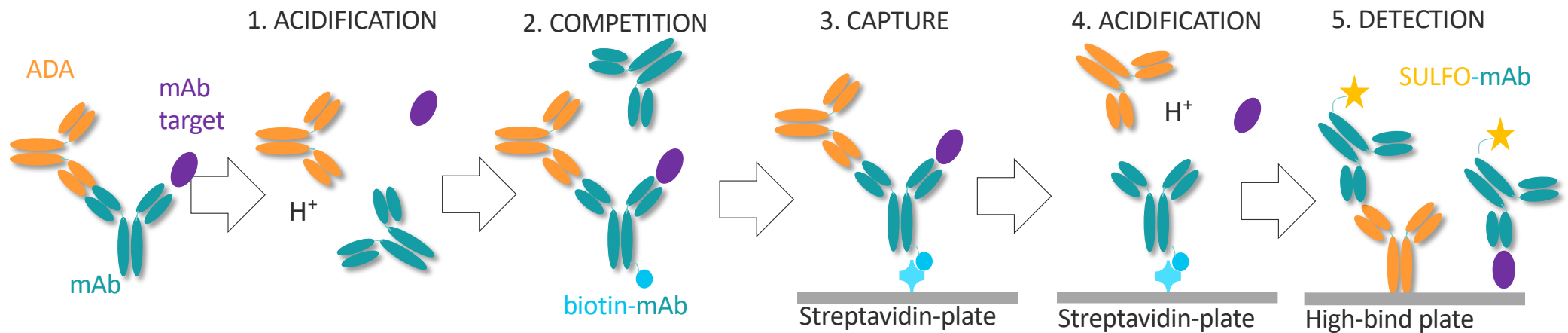
Confirmatory assay

 High signal Samples that likely confirm

 High signal Samples that likely do not confirm

Characterizing the interference: mAb target?

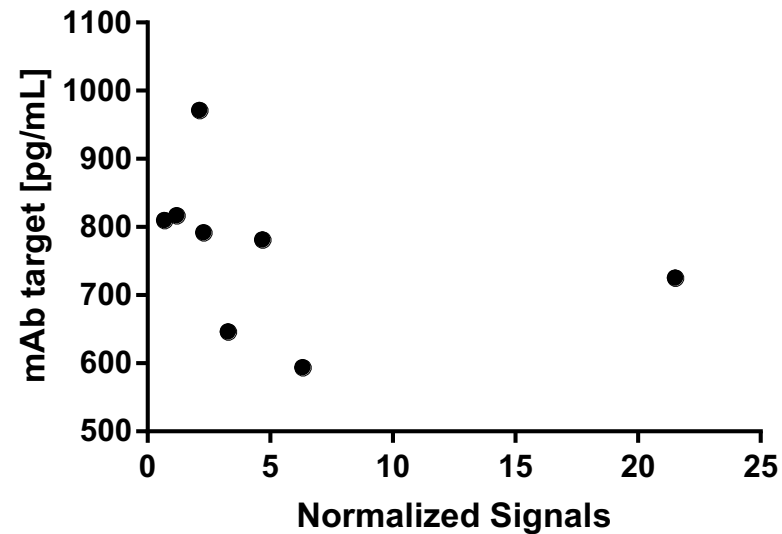
Target intolerance of SPEAD format



Characterizing the interference: correlation of mAb target concentration and ADA screening signals?

Total mAb target biomarker assay:

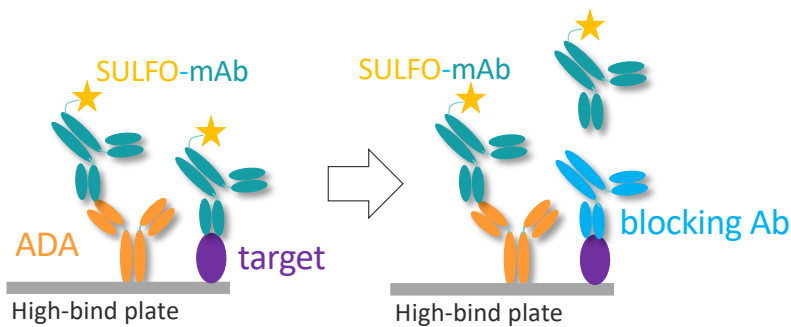
serum samples	mAb target [pg/mL] Biomarker Assay	median Normalized Signals ADA assay
CP7	810.471	0.672
CP40	817.160	1.167
CP24	971.543	2.103
CP35	792.193	2.264
CP47	646.732	3.271
CP16	781.573	4.672
CP27	594.233	6.314
CP20	725.659	21.513



No correlation!

Characterizing the interference: mAb target block and target interference

Target blocking:



sample	mean RLU	Normalized Signals	condition
CP17	214	3.267	no target block
CP27	194	2.962	
CP47	98	1.496	
CP17	212	3.593	1 µg/mL anti mAb target Ab
CP27	190	3.220	
CP47	95	1.602	

Target interference:

NC pool + recombinant mAb target

sample	mean RLU	Normalized Signals
NC + 1 ng/mL mAb target	59	0.959
NC + 100 ng/mL mAb target	61	0.984
NC + 1000 ng/mL mAb target	64	1.033

No interference of mAb target in ADA SPEAD assay!

Characterizing the interference: pre-existing ADA?

IgG depletion with Protein A/G Sepharose

sample	non-depleted		IgG depleted		% Inhibition of IgG depletion	Median % Inhibition of confirmatory
	mean RLU	Normalized Signal	mean RLU	Normalized Signal		
CP16	214	3.963	104	1.926	51.4	78.1
CP40	61	1.130	65	1.204	-6.6	-9.8
CP17	256	4.154	63	1.016	75.5	80.1
CP20	998	16.220	754	12.252	24.5	91.2
CP27	359	5.829	497	8.081	-38.6	-4.8
CP35	217	3.528	118	1.919	45.6	66.6
CP47	148	2.398	153	2.480	-3.4	55.8
CP49	65	1.049	83	1.350	-28.7	26.2
HPC	164063	2667.691	11810	192.024	92.8	>98.0
MPC	2759	44.854	226	3.667	91.8	>90.0

(Selected samples)

High screen signal but no depletion and no confirmation

High % inhibition in confirmatory but no depletion

Mixed results => specific and unspecific signals

Consequences of high prevalence of interference

For the Client

- Reporting of incorrect ADA incidence in product label
- Laborious assessment of clinical significance
- Higher costs of confirmation and titration efforts

For the CRO

- Potentially unreliable data with unforeseen in-study issues
 - In-study selectivity
 - In-study CP

Options to overcome interference

➤ Make the assay less sensitive while keeping its drug tolerance?

➤ Is there an alternative assay format with less interference?

I. ADA bridging format with Acid Treatment?

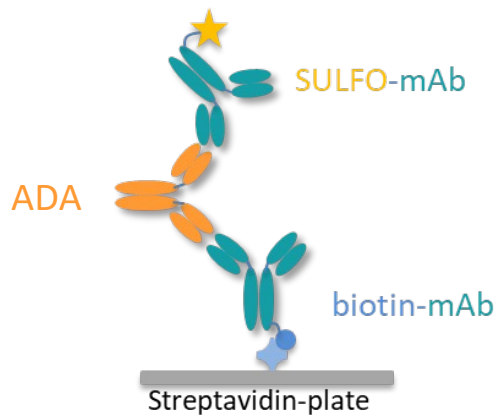
➤ Make the SPEAD assay less prone to interference?

II. Re-optimizing blocking condition

BSA/Casein vs

- 1) Bovine Gamma Globulin
- 2) Horse Serum

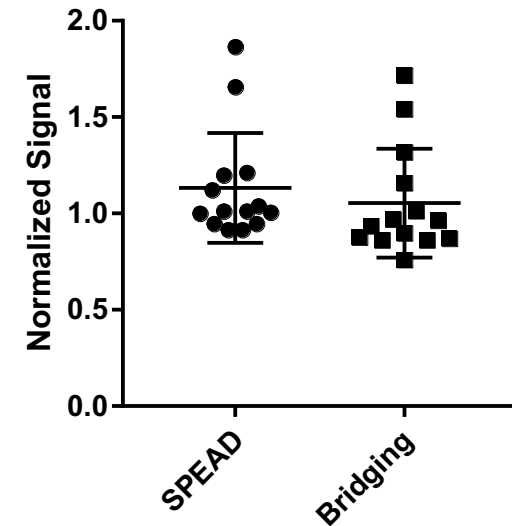
ADA bridging format: an option to reduce interference?



Drug Tolerance:

	SPC Ab [ng/mL]	mAb [μ g/mL]	mean RLU	NC normalized
Bridging	100	100	136	1.964
SPEAD	100	125	341	5.821

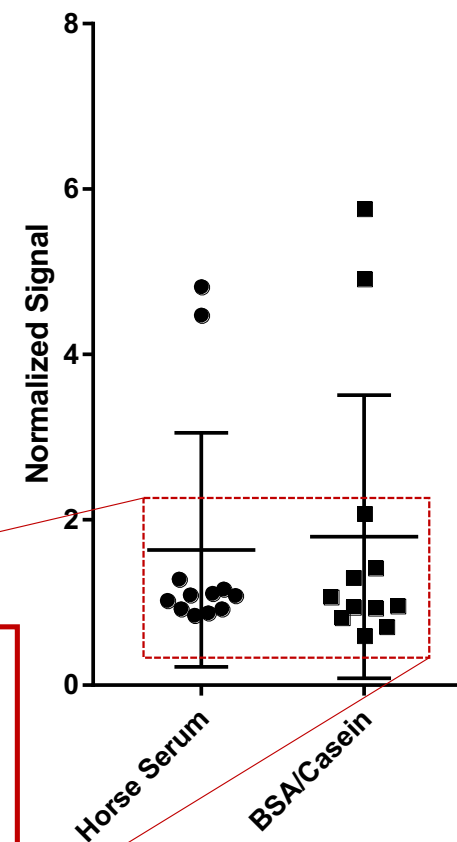
Variability:



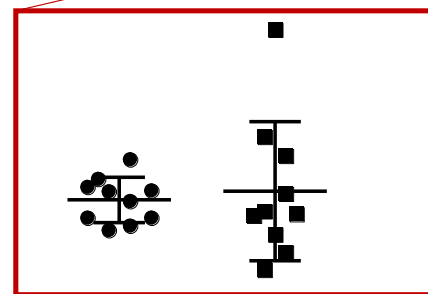
Significantly poorer drug tolerance and same variability

Optimizing blocking condition to reduce interference

	Casein/BSA				Horse Serum			
	screening		confirmatory		screening		confirmatory	
	mean RLU	Ratio	mean RLU	%Inhibition	mean RLU	Ratio	mean RLU	%Inhibition
CP1	54	1.597	47	12.1	95	1.027	82	13.2
CP2	79	2.358	48	39.2	101	1.092	136	-34.8
CP5	46	1.358	52	-13.2	85	0.924	80	6.5
CP6	34	1.000	40	-17.9	81	0.875	78	3.1
CP9	60	1.776	48	20.2	100	1.087	84	16.0
CP13	73	2.164	49	32.4	107	1.163	83	22.4
CP16	320	9.552	62	80.6	412	4.478	103	75.1
CP27	273	8.149	245	10.3	444	4.821	292	34.3
CP31	116	3.448	89	22.9	118	1.283	117	0.8
CP32	53	1.567	64	-21.0	103	1.114	90	12.7
CP33	40	1.179	36	8.9	78	0.848	87	-10.9
CP44	52	1.552	57	-8.7	85	0.924	88	-3.5



Horse serum reduces baseline variability

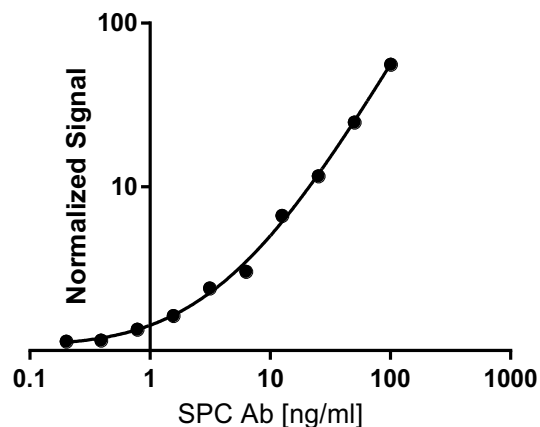
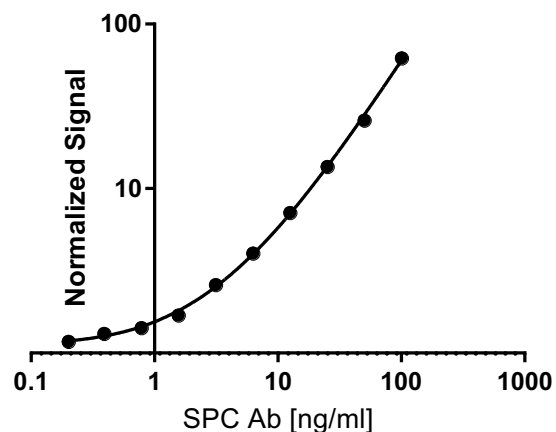


Optimizing blocking condition to reduce interference

Comparison: Casein/BSA

Horse Serum blocking

Sensitivity:



➤ Similar sensitivity

Drug Tolerance:

SPC Ab [ng/mL]	mAb [μ g/mL]	mean RLU	NC normalized
100	1000	78	1.333
100	500	103	1.761
100	250	203	3.462
100	125	341	5.821

SPC Ab [ng/mL]	mAb [μ g/mL]	mean RLU	NC normalized
100	1000	NA	NA
100	500	140	1.150
100	250	174	1.440
100	100	304	2.510

➤ Poorer DT

Optimizing blocking condition to reduce interference

Screening assay CP data

Horse Serum Blocking

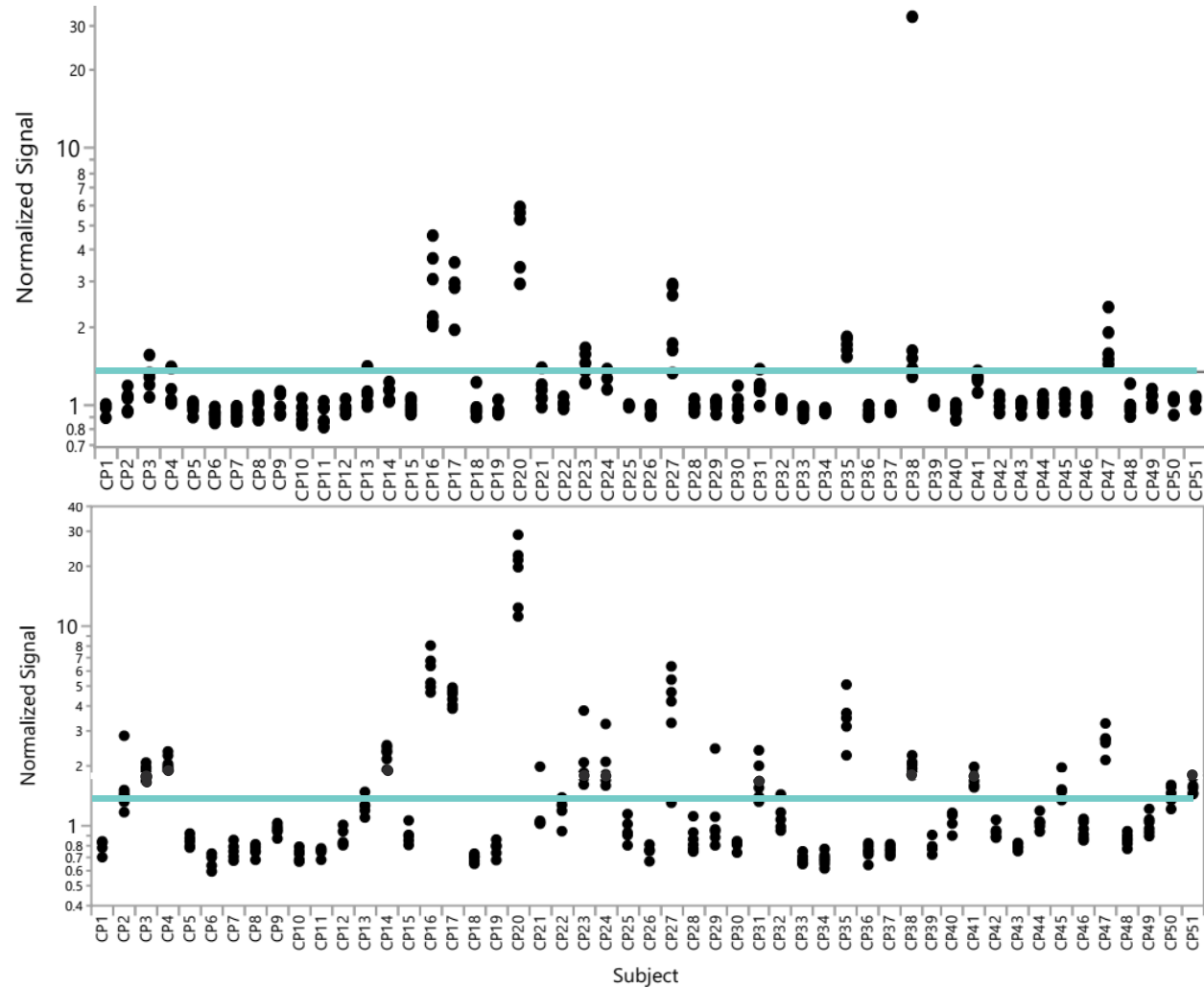
SCPF = 1.20

Tukey box plot (1.5xIQR)

Casein/BSA Blocking

SCPF = 1.36

Partitioning



Optimizing blocking condition to reduce interference

Horse Serum Blocking

Screening Assay

SCPF = 1.20

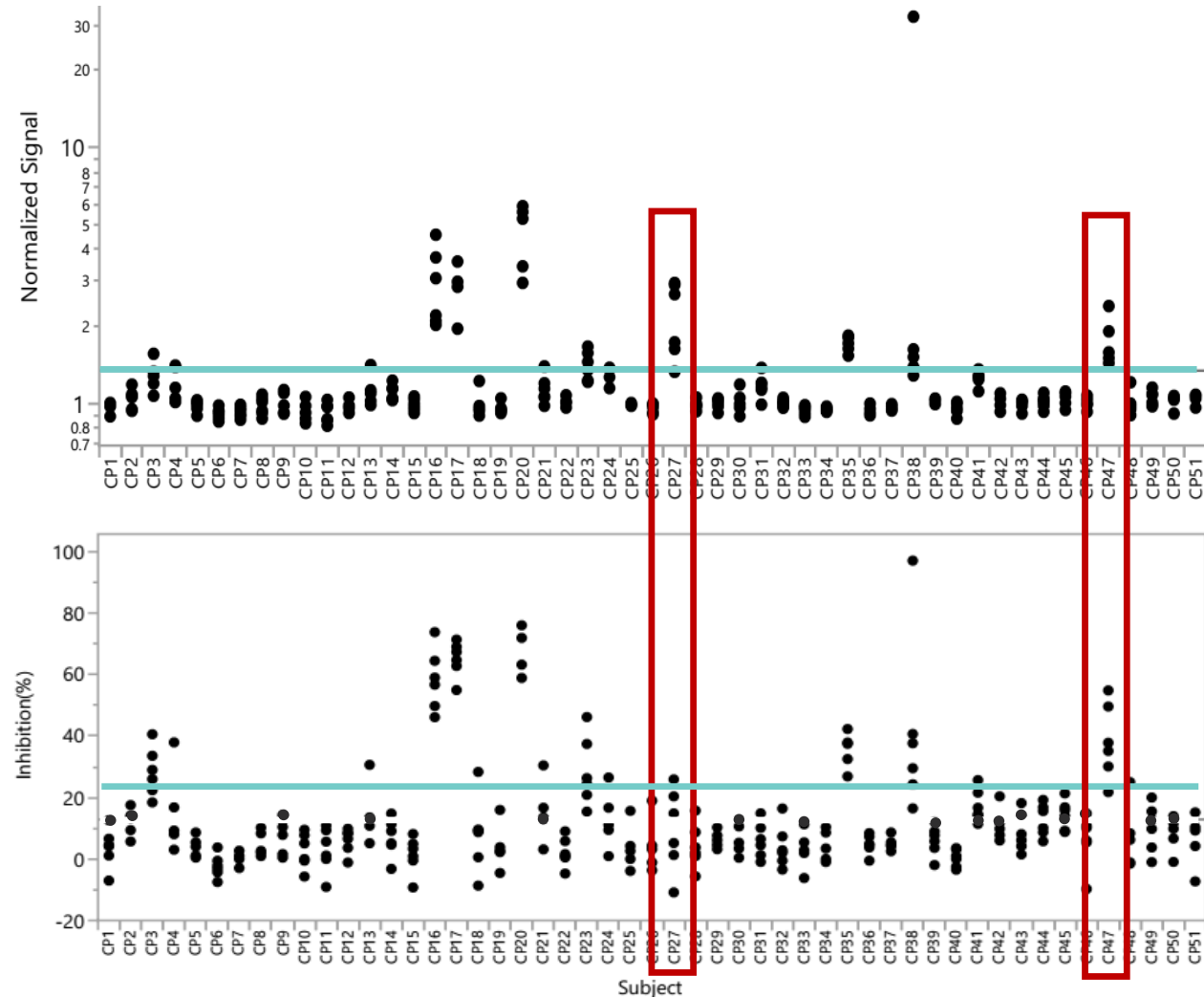
Confirmatory Assay

CCP = 22 %

25 % positives



15 % positives



Summary

- Ultrasensitive ADA assays may be prone to unspecific interference
- Stronger blocking conditions help to reduce unspecific interference, but at the consequence of poorer drug tolerance

Thank You

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