

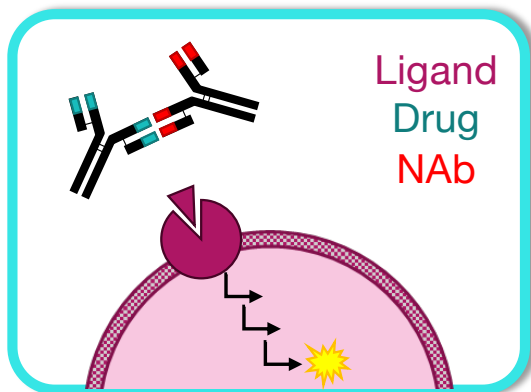
PABAD in Practice: Achieving High Drug Tolerance in NAb Assays

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March 17th 2026



MoA and NAb Testing Strategy of an Antagonist mAb Therapeutic

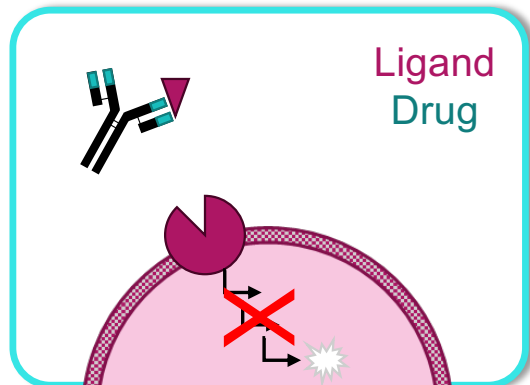


- Drug: mAb therapeutic
- Mode of action: mAb drug prevents a peptide hormone from binding to its receptor and inhibits the downstream signaling cascade
- NAb assay requirements:
 - Drug Tolerance: 200 µg/mL
 - Target Tolerance: 300 ng/mL
- Assay Reagents:
 - Matrix: human serum
 - Positive Control: Affinity-purified anti-drug polyclonal antibody from goat antiserum

NAb Assay: Choosing the Right Starting Format



Cell-Based Assay or Competitive Ligand Binding Assay?



- Drug target = soluble ligand
- Binding of the drug to its target reflects its biological activity
- Balancing drug tolerance (200 µg/mL) and sensitivity (500–1000 ng/mL) is challenging with CBA

Conclusion: **Competitive Ligand Binding Assay (CLBA)** is preferred

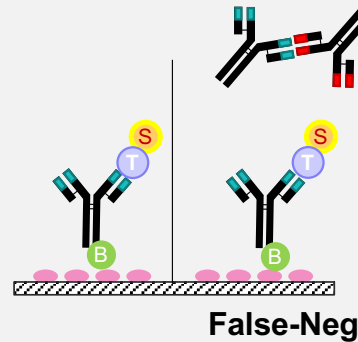
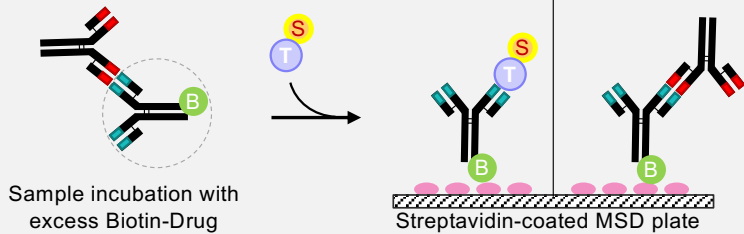
Selection of CLBA Format to Detect NAb

Drug+Target
→ Signal

Drug+Target+NAb
→ No signal

+ High Drug Levels

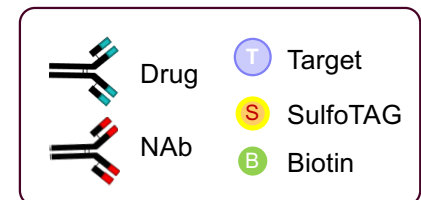
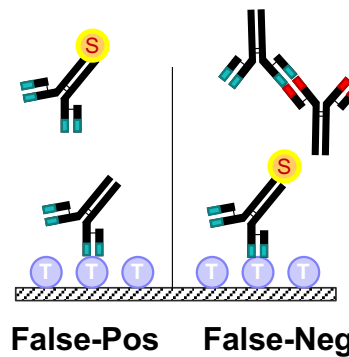
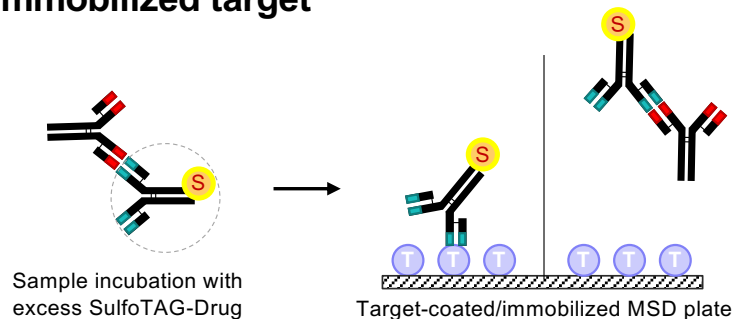
Immobilized drug



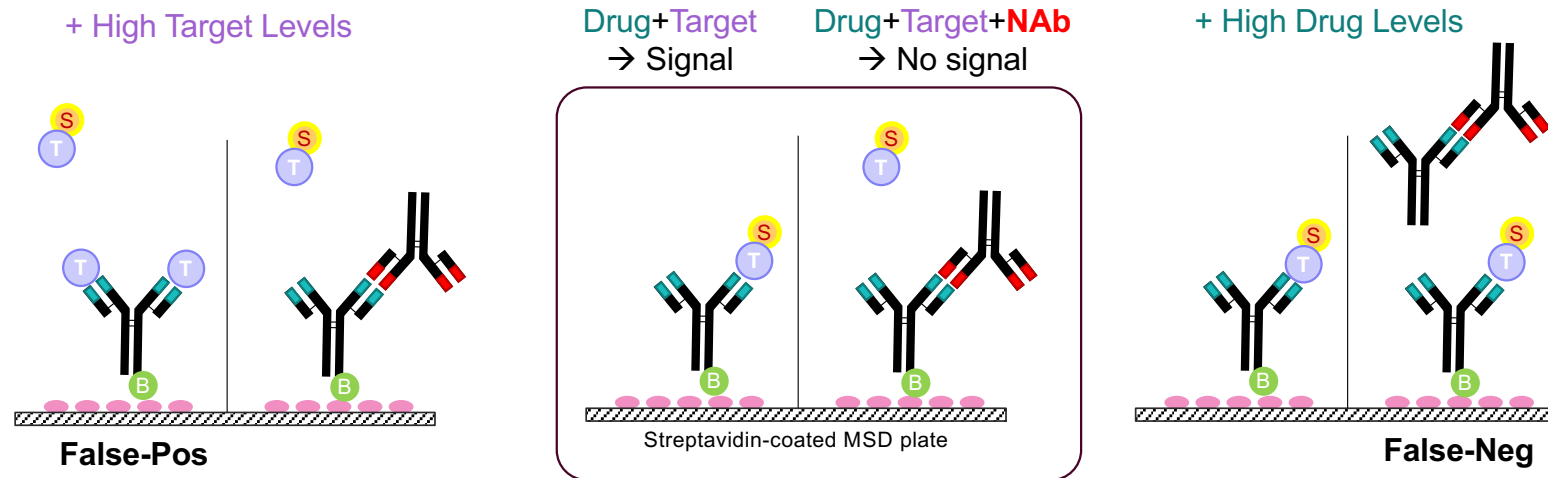
Less affected by drug interference (high drug tolerance required).
Target can be labelled without masking epitope.

→ Preferred Assay Format

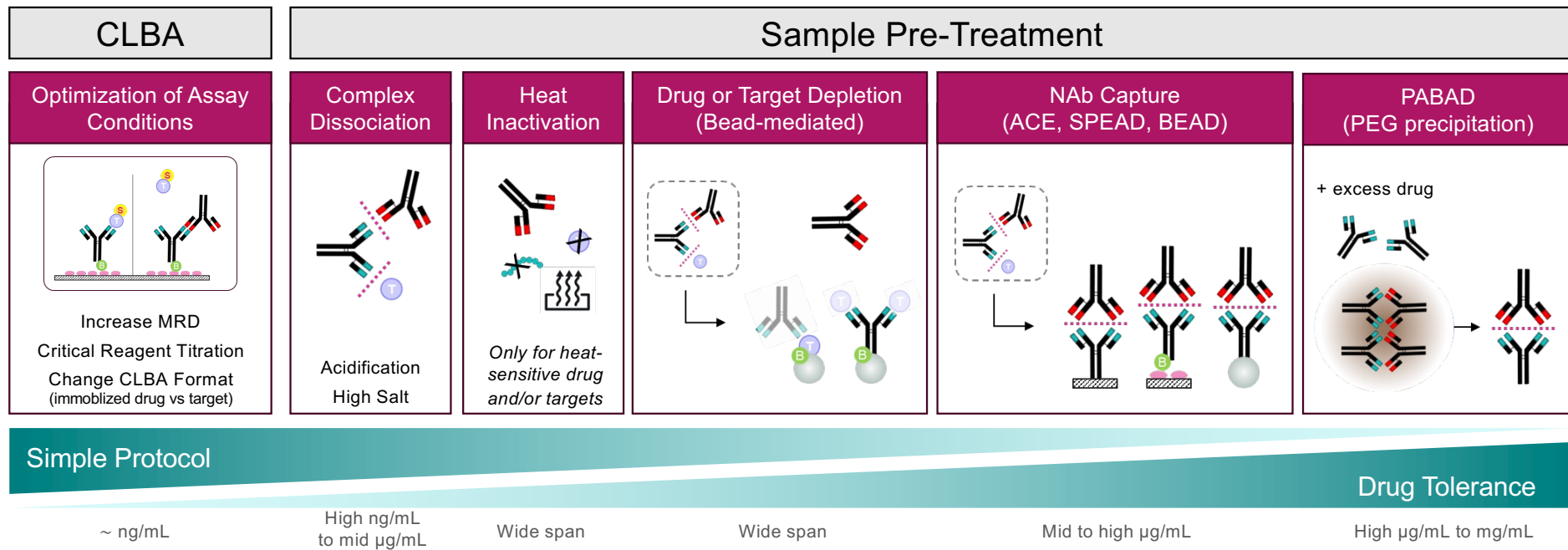
Immobilized target



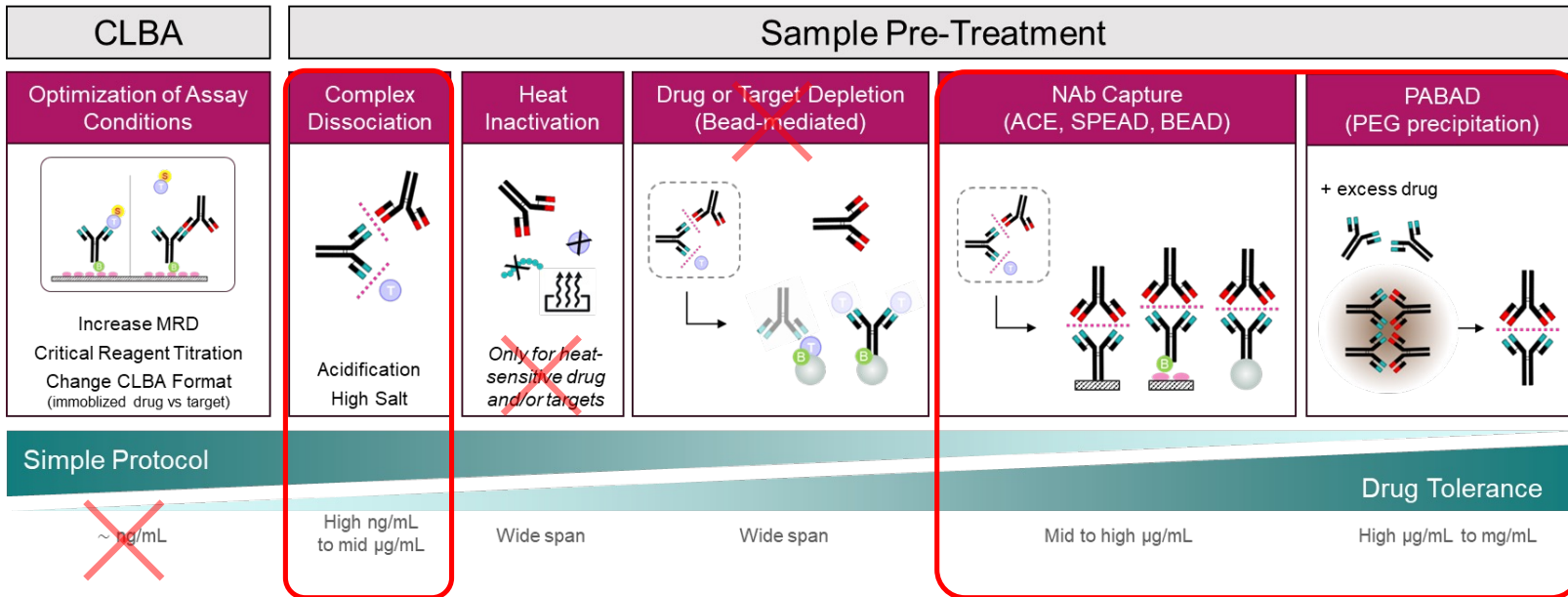
Impact of Drug and Target on NAb Assay Performance



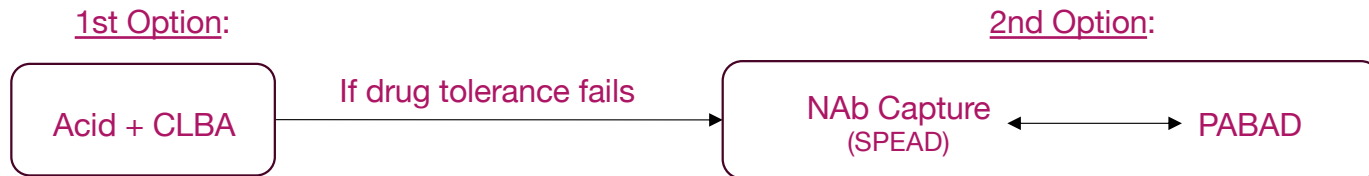
Strategies to Mitigate Drug and/or Target Interference



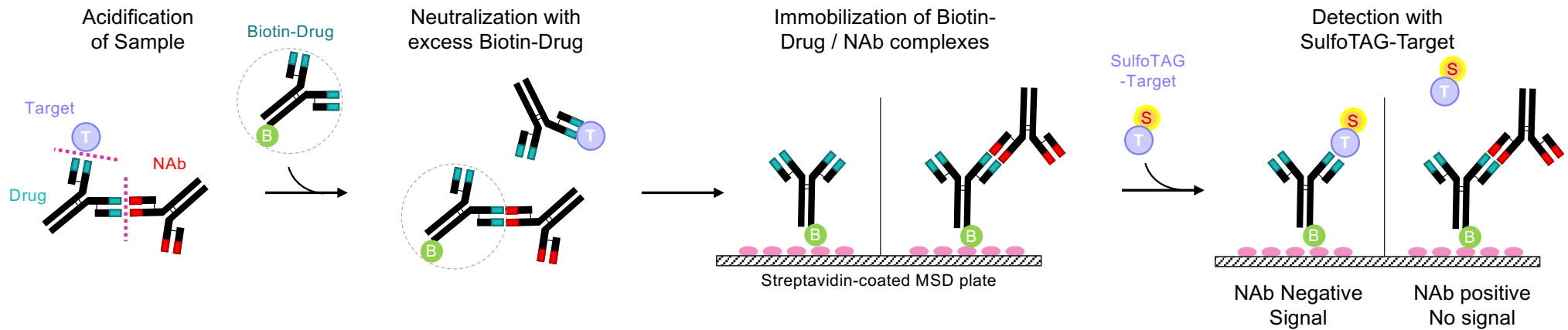
Fit-for-Program NAb Assay Development Strategy



Tolerance Requirements:
 200 µg/mL Drug;
 300 ng/mL Target



CLBA with Acid Dissociation: Assay Design Overview



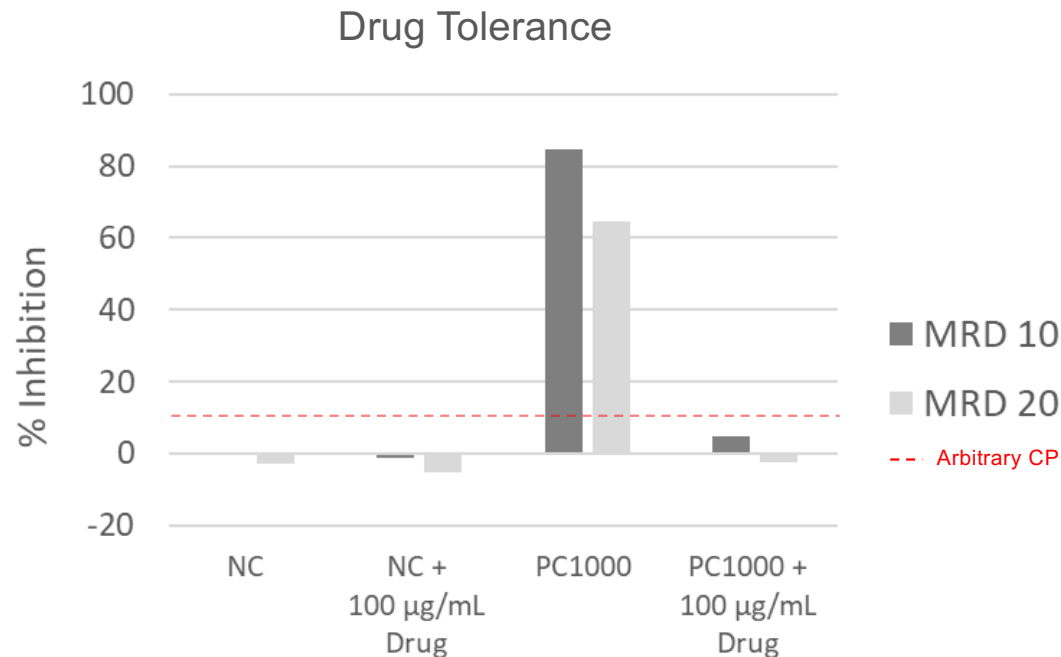
Pros

- Simple
- Fast (1-day assay)

Cons

- DT: Acid dissociation may not be sufficient to reach the targeted drug tolerance

No Drug Tolerance Using CLBA With Acid Dissociation

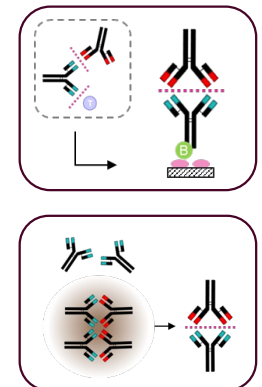


- 100 µg/mL free drug (half of the targeted DT) completely inhibits assay sensitivity:
- Further assay optimization (e.g., higher MRD or alternative acids) may not sufficiently improve drug tolerance but will markedly reduce sensitivity.

Conclusion: CLBA using acid dissociation is not appropriate
 → Highly effective sample pre-treatment is required to improve DT

NAb Assay Development Strategy: SPEAD or PABAD?

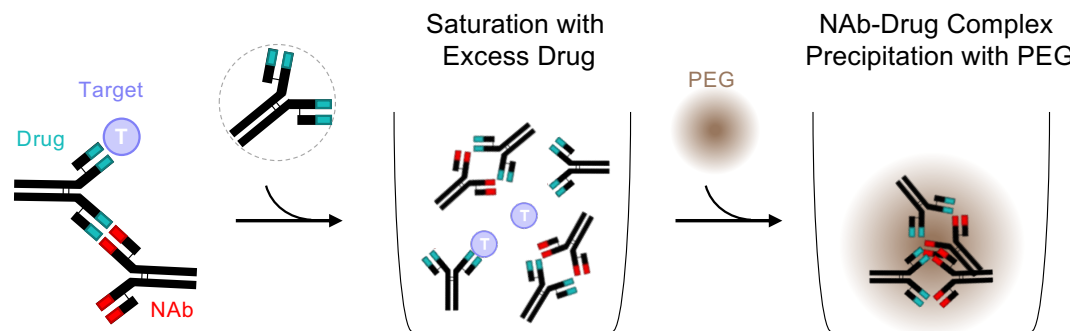
	Drug Tolerance	Limitations
SPEAD	Effective improvement in drug tolerance up to 50 µg/mL (Xiang et al., 2018; Butterfield et al., 2010; Smith et al., 2007)	<ul style="list-style-type: none"> ▪ Labor-intensive ▪ Stringent - often using two acidification steps (risk for acid-sensitive antibodies) ▪ Or long – if using o/n incubation instead of first acidification steps (2-days protocol)
PABAD	PEG precipitation-based methods are highly effective at reducing drug and target interference up to high µg/mL to mg/mL levels (Pham et al., 2026; Wickramarachchi et al., 2023; Zoghbi et al., 2015)	<ul style="list-style-type: none"> ▪ Labor-intensive ▪ Controlled pellet washing required ▪ Long: 2-days protocol
Summary	→ PABAD is more effective in overcoming drug interference	→ Both methods are complex and have limitations



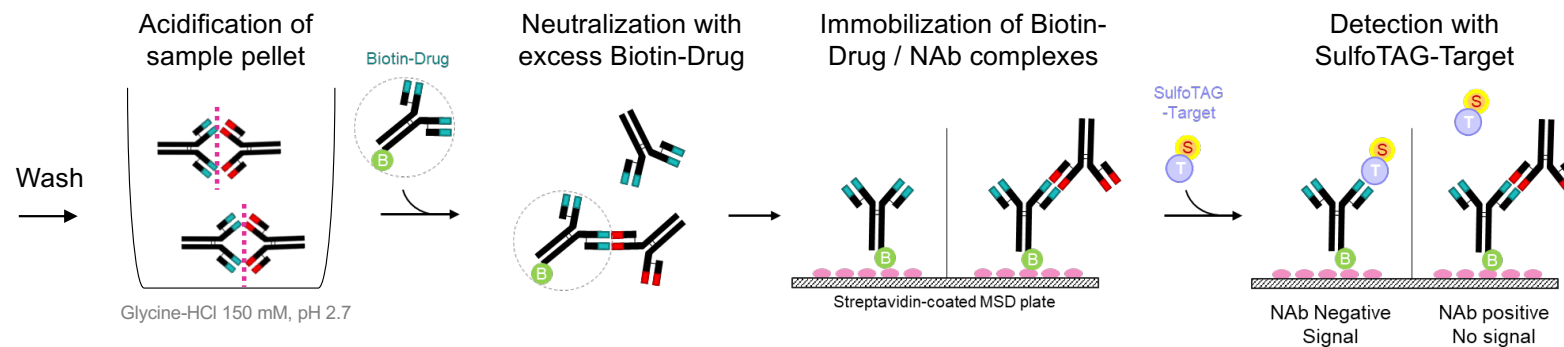
Conclusion: PABAD selected for further evaluation

PABAD Assay Format

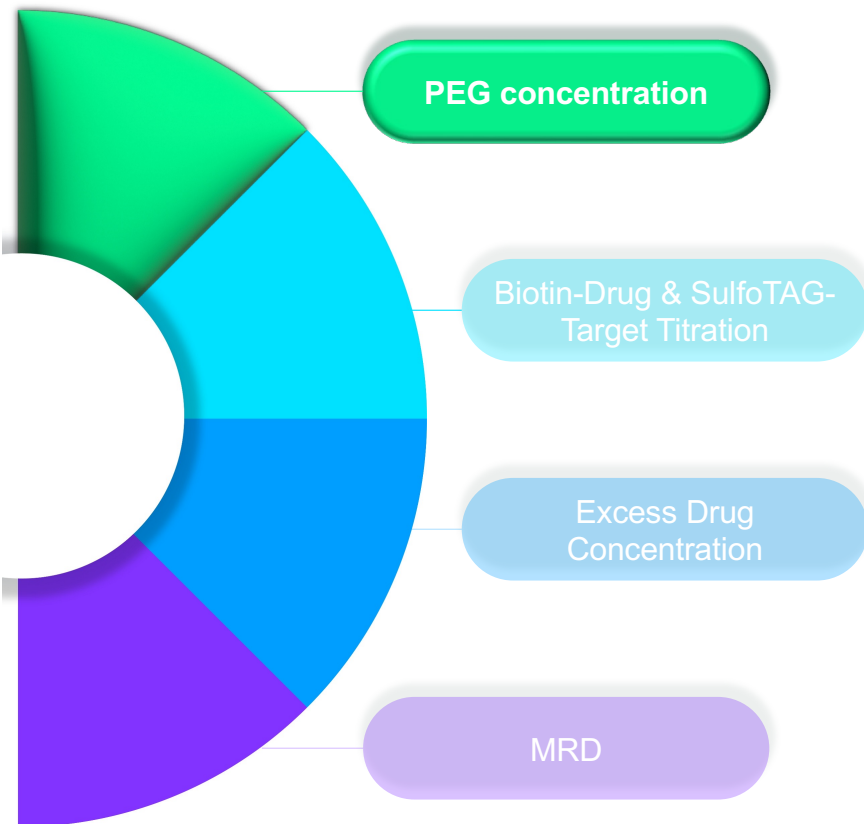
DAY 1: Drug Saturation and PEG precipitation of Nab-Drug complexes



DAY 2: CLBA with Acid Dissociation (using immobilized Drug)



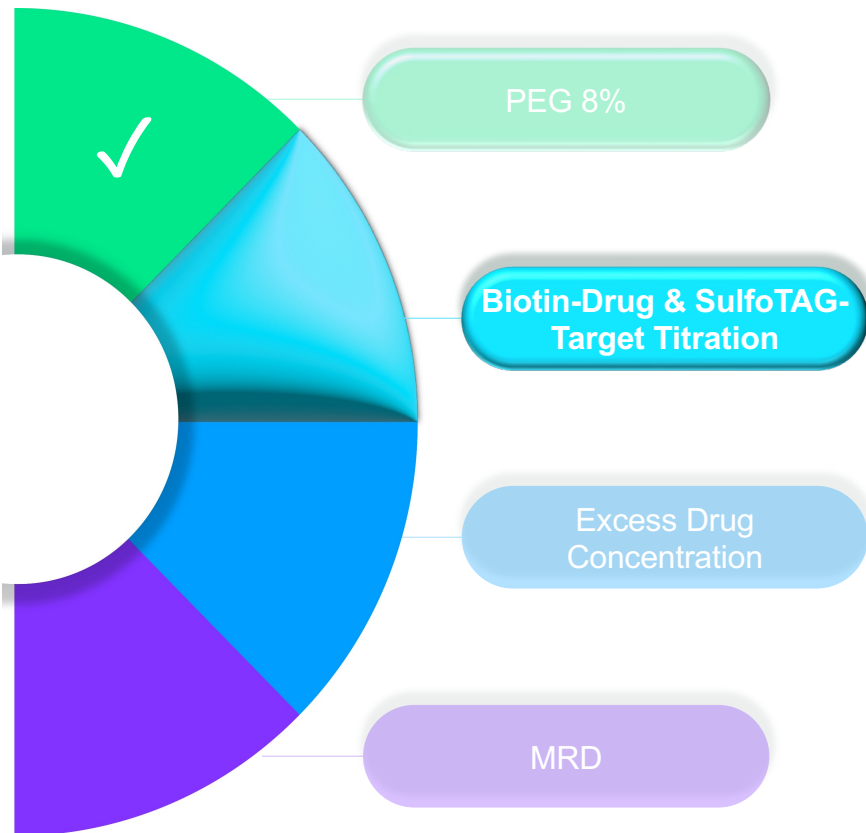
PABAD Assay Optimization Steps



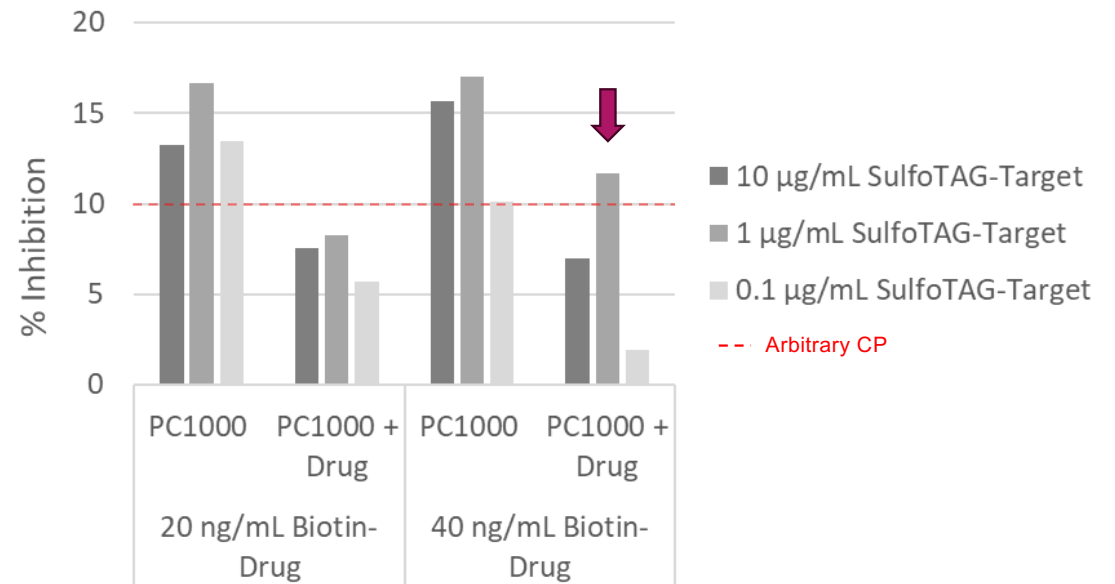
Highest NAb recovery using 8% PEG

% Inhibition (1000 ng/mL PC)		20 ng/mL Biotin-Drug		40 ng/mL Biotin-Drug	
		PEG 4%	PEG 8%	PEG 4%	PEG 8%
SulfoTAG -Target	10 µg/mL	10	21	9	20
	1 µg/mL	17	24	10	21
	0.1 µg/mL	19	23	13	19

PABAD Assay Optimization Steps

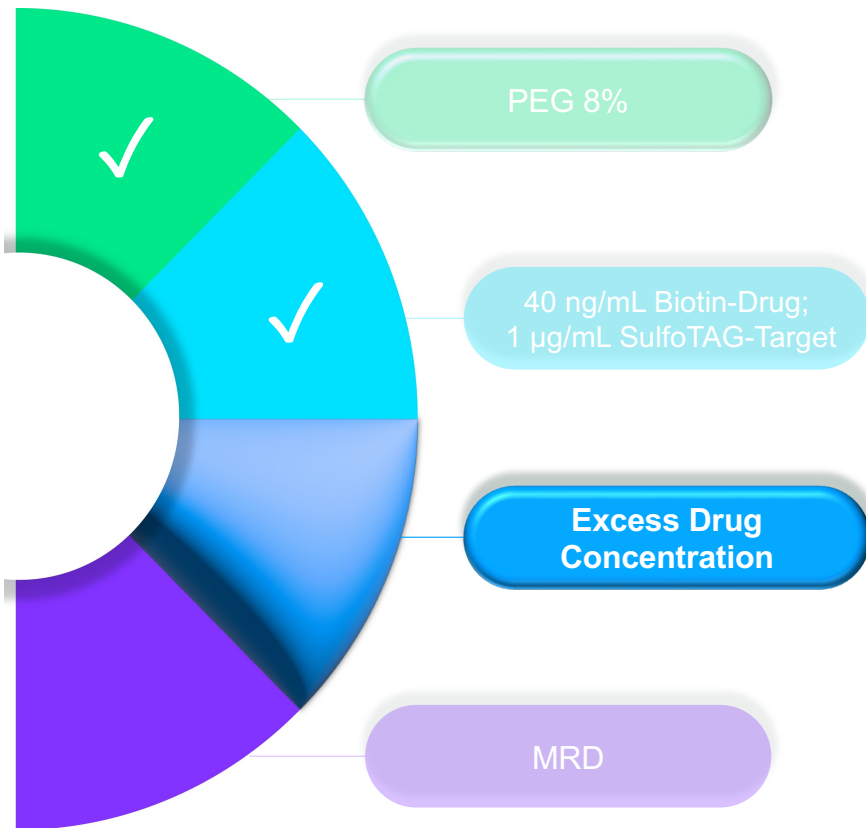


Drug Tolerance achieved with 40 ng/mL Biotin-Drug and 1 µg/mL SulfoTAG-Target



PC: 1000 ng/mL; Drug: 200 µg/mL

PABAD Assay Optimization Steps

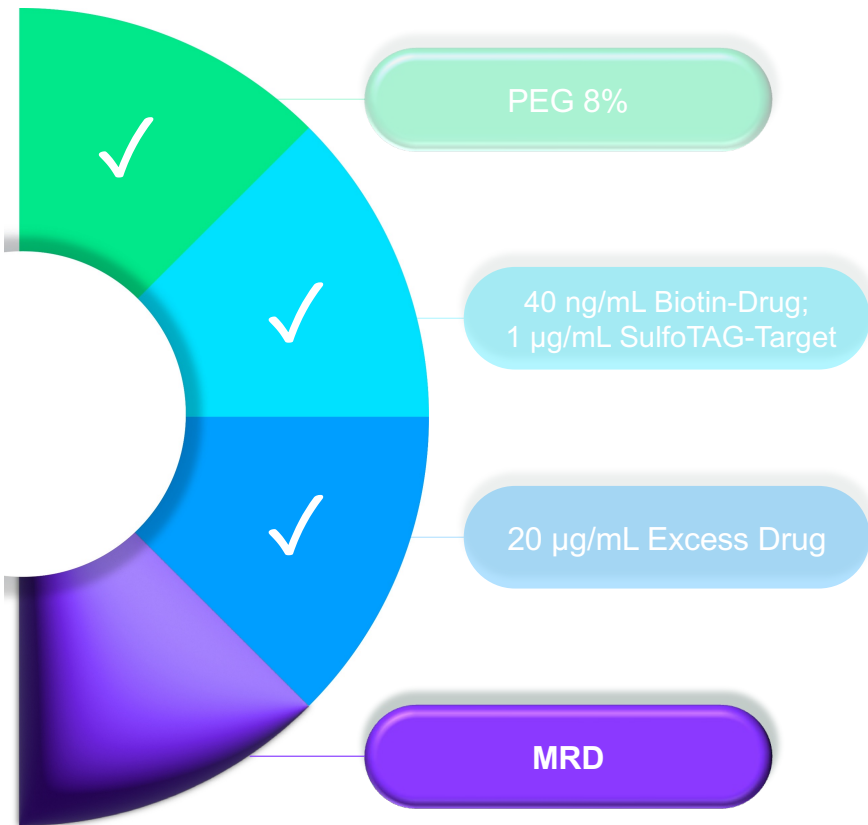


Improved sensitivity using 20 µg/mL Excess Drug

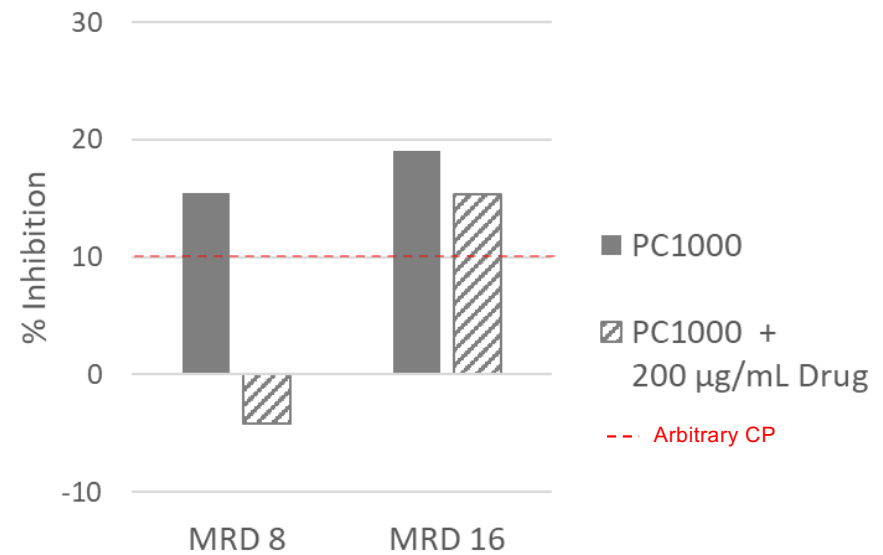
% Inhibition		Excess Drug		
		20 µg/mL	50 µg/mL	100 µg/mL
PC (ng/mL)	10000	54	39	30
	2000	27	15	10
	1000	19	13	9
	500	16	9	6
	250	9	6	-1

Arbitrary CP: 10%

PABAD Assay Optimization Steps



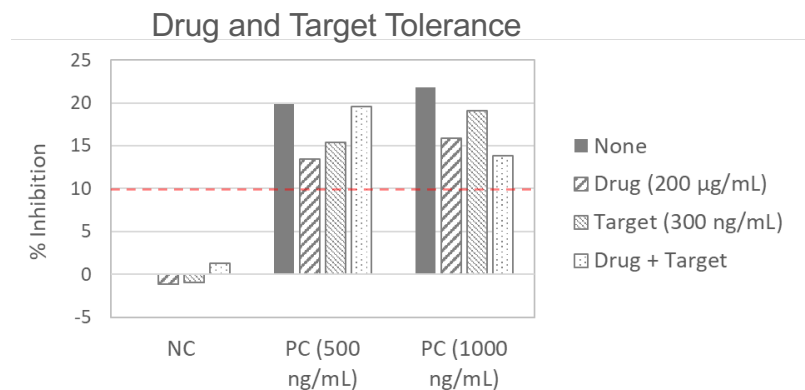
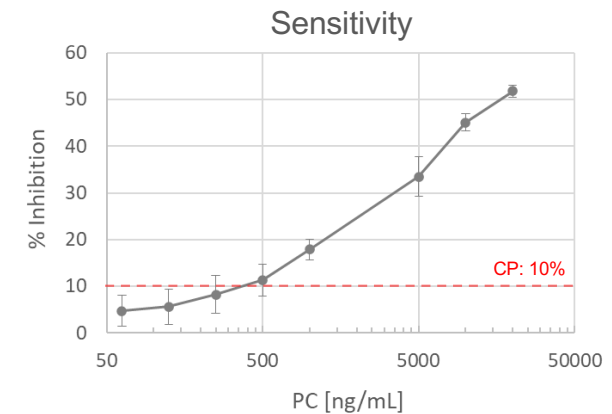
Highest Drug Tolerance at MRD 16



Pre-validation Confirms a Drug and Target Tolerant Assay

Assay Parameter	Results
Cut point (2x 30 data points)	0.8991 (approx. 10% Inhibition) Robust parametric approach; no outliers
Sensitivity	1000 ng/mL
Provisory LPC	1000 ng/mL (confirmed by selectivity – 9/10 individuals passed)
Drug tolerance	Up to 200 µg/mL (± 300 ng/mL Target) → requirement met
Target tolerance	Up to 300 ng/mL (± 200 µg/mL Drug) → requirement met
Precision (intra and inter-run)	< 5 % CV (two operators) Controlled pellet washing protocol documented in SOP
Robustness	2 lots of Biotin-Drug and SulfoTAG-target evaluated

NAb assay performance confirms the suitability of singlicate analysis



NAb Success: Pick the Winning Development Strategy

Step	Options						Criteria
CLBA vs CBA	<div data-bbox="898 596 1205 764" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">CLBA</div>						<p>Drug MoA</p> <p>Assay requirements</p>
CLBA Format	<div data-bbox="703 828 1010 995" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">Immobilized Drug</div>		<div data-bbox="1081 828 1388 995" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">Immobilized Target</div>				<p>Drug-target binding.</p> <p>Drug/target structural stability during passive coating or labeling.</p>
Sample Pre-Treatment	<div data-bbox="450 1062 589 1230" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">None</div>	<div data-bbox="611 1062 842 1230" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">Complex Dissociation (e.g. acid)</div>	<div data-bbox="864 1062 1050 1230" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">Heat Denaturation</div>	<div data-bbox="1072 1062 1258 1230" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">Drug or Target Depletion</div>	<div data-bbox="1281 1062 1467 1230" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">NAb Capture (with drug)</div>	<div data-bbox="1489 1062 1697 1230" style="background-color: #800040; color: white; padding: 10px; display: inline-block; border-radius: 5px;">PABAD (PEG Precipitation)</div>	<p>Assay requirements</p> <p>Drug/target properties</p>

Acknowledgments:
A. D'Alessandro
W. Lembke
P. Struwe

Thank You