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Translating Science to Medicine

Case Study:
Establishing a Singlicate Assay Detecting Cross-Reactive Anti-Drug Antibodies Supporting Clinical Development of a Peptide Hormone Therapeutic

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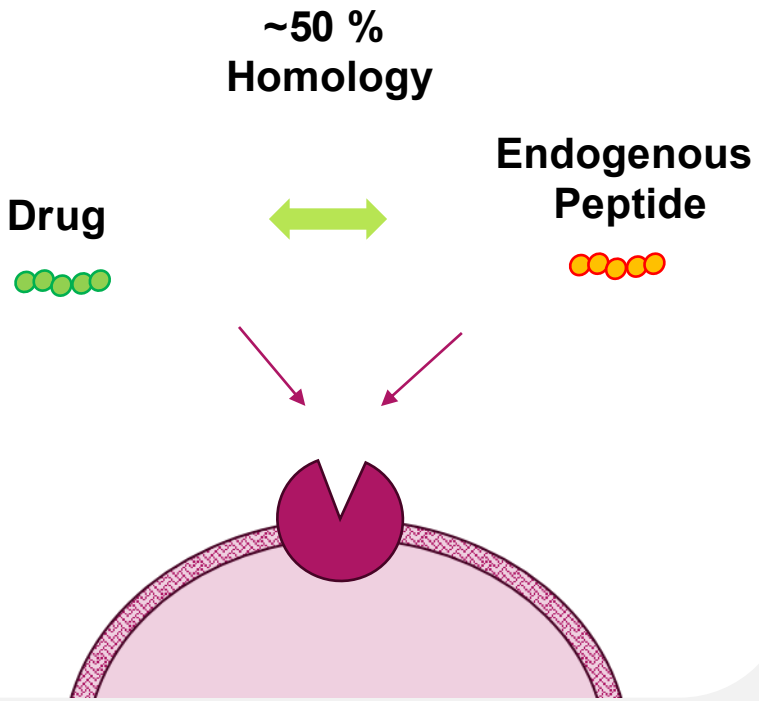
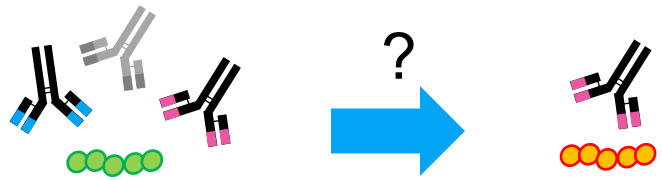
Cross-Reactivity Assessment: A Critical Component of Immunogenicity Testing Strategy

Drug Candidate



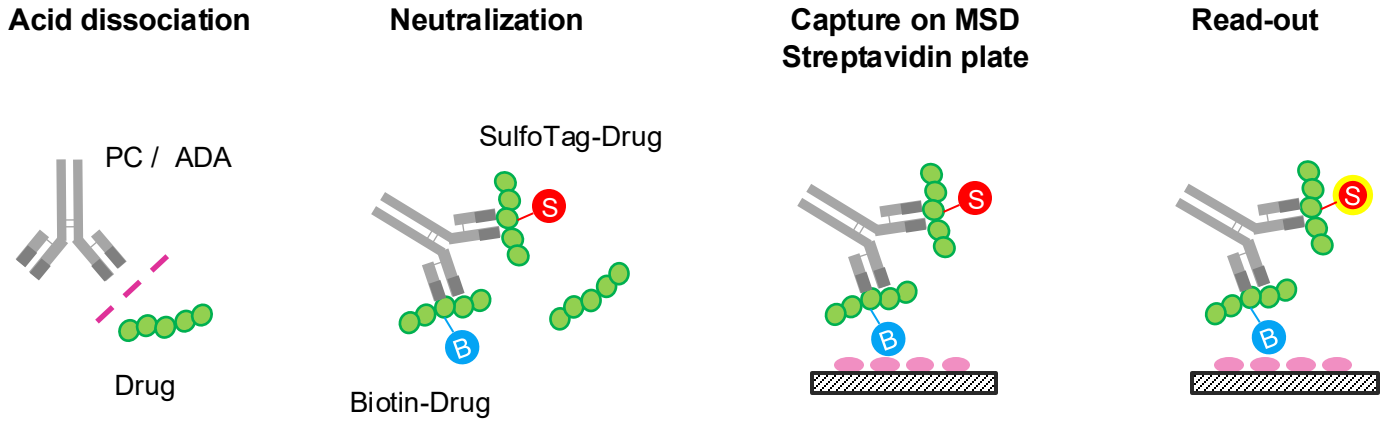
Cross-Reactivity Assessment



→ need to assess ADA cross-reactivity to endogenous peptide

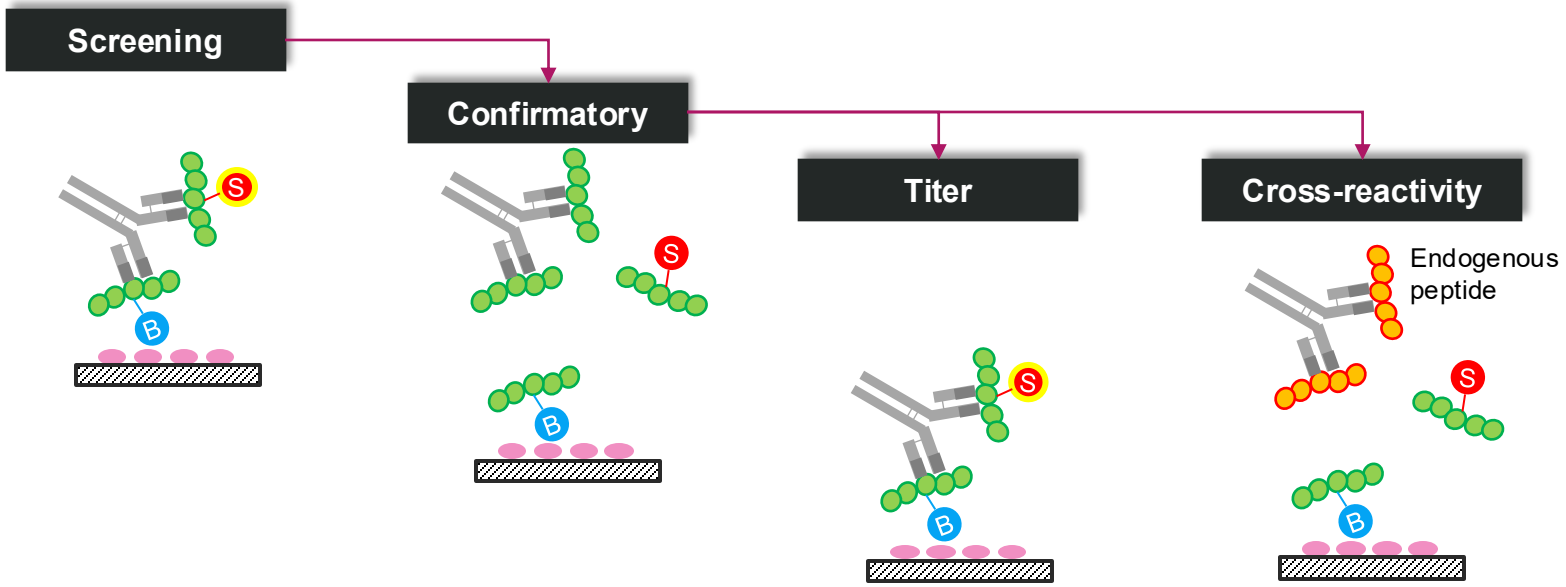
Maintaining Preclinical ADA Assay Format in Clinical Studies

Bridging ADA Assay with Acidification to Mitigate Drug Interference



Drug Tolerance	
Pre-clinical	580 nM at 1000 ng/mL pAb PC
Clinical Requirement	200 nM at 100 ng/mL PC

Program-Tailored Multi-tiered Testing Strategy



Assay	PC: Preferred Option	PC: Alternative Option
1 Screening	PC1	PC1
2 Confirmatory		
3a Titer		
3b Cross-reactivity		PC 2

Positive Control Design and Implementation Strategy

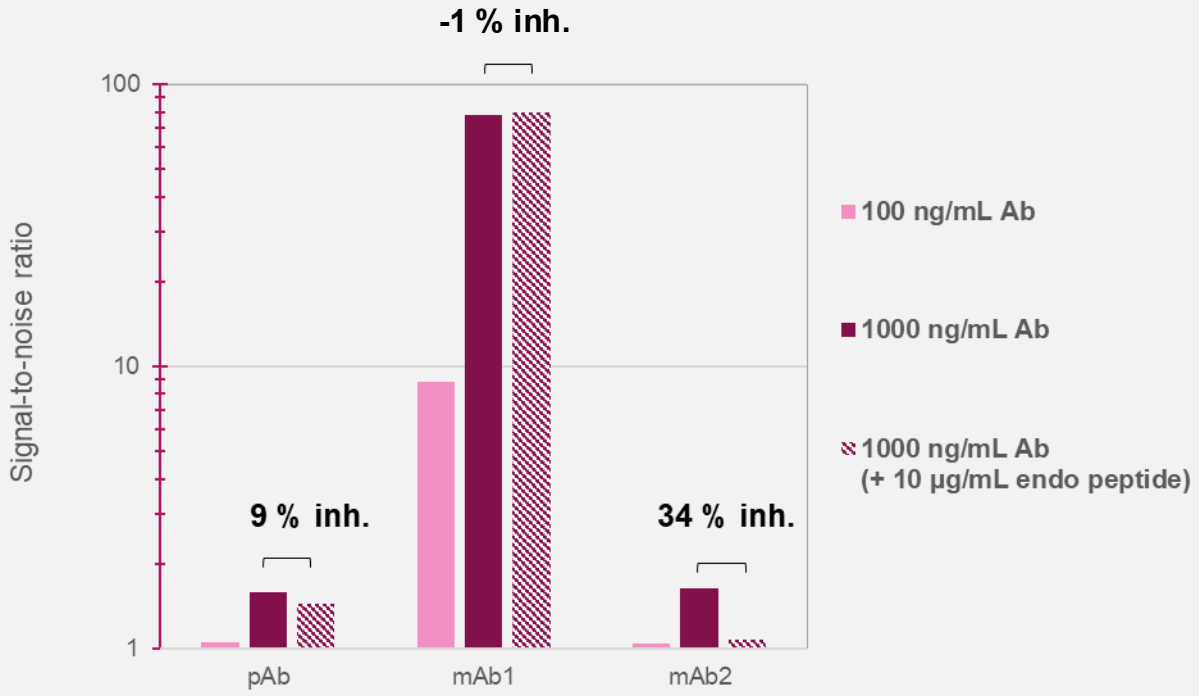
Postive Control Antibody	Description	Binding to endogenous peptide
pAb	pAb against therapeutic	Not determined
mAb1	mAb against therapeutic	Poor
mAb2	mAb against therapeutic	Good

One positive control

Two positive controls

Option 1	Option 2	Option 3	Option 4
pAb: all tiers	mAb2: all tiers	Mix of mAb1 + mAb2: all tiers	mAb1: screening / confirmatory / titer mAb2: cross-reactivity

Assessing Positive Controls: Cross-reactivity



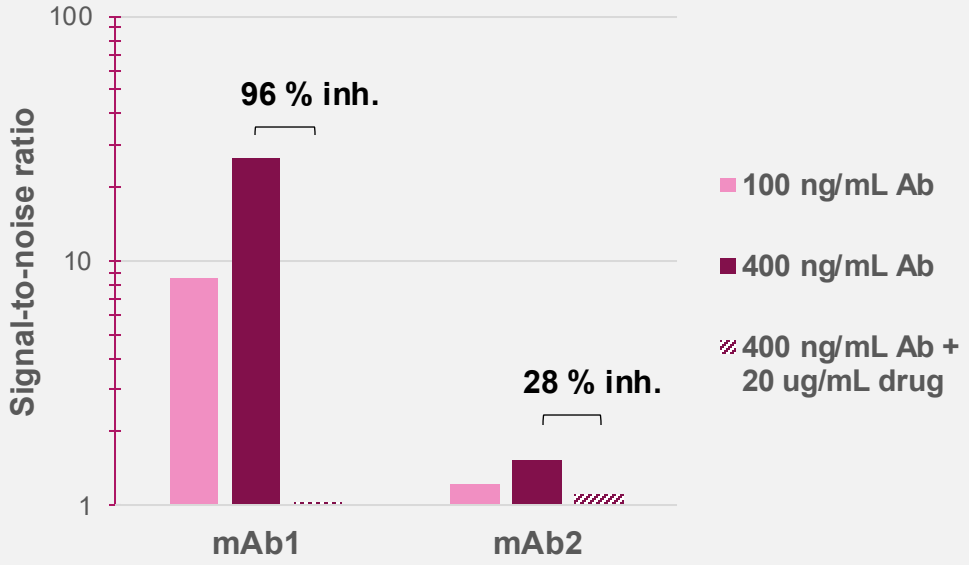
pAb as Positive Control

- Poor cross-reactivity to endogenous peptide
- Limited pAb supply

→ Excluded from further testing

Option 1	Option 2	Option 3	Option 4
pAb: all tiers	mAb2: all tiers	Mix of mAb1 + mAb2: all tiers	mAb1: screening / confirmatory / titer mAb2: cross-reactivity

Assessing Positive Controls: Drug Inhibition



mAb as Positive Control

- mAb1 and mAb2 are sensitive to drug inhibition
- mAb2 affinity to drug is low
- **Estimated sensitivity close to 100 ng/mL**

Next Steps

→ Improve sensitivity

→ Test mix of mAbs

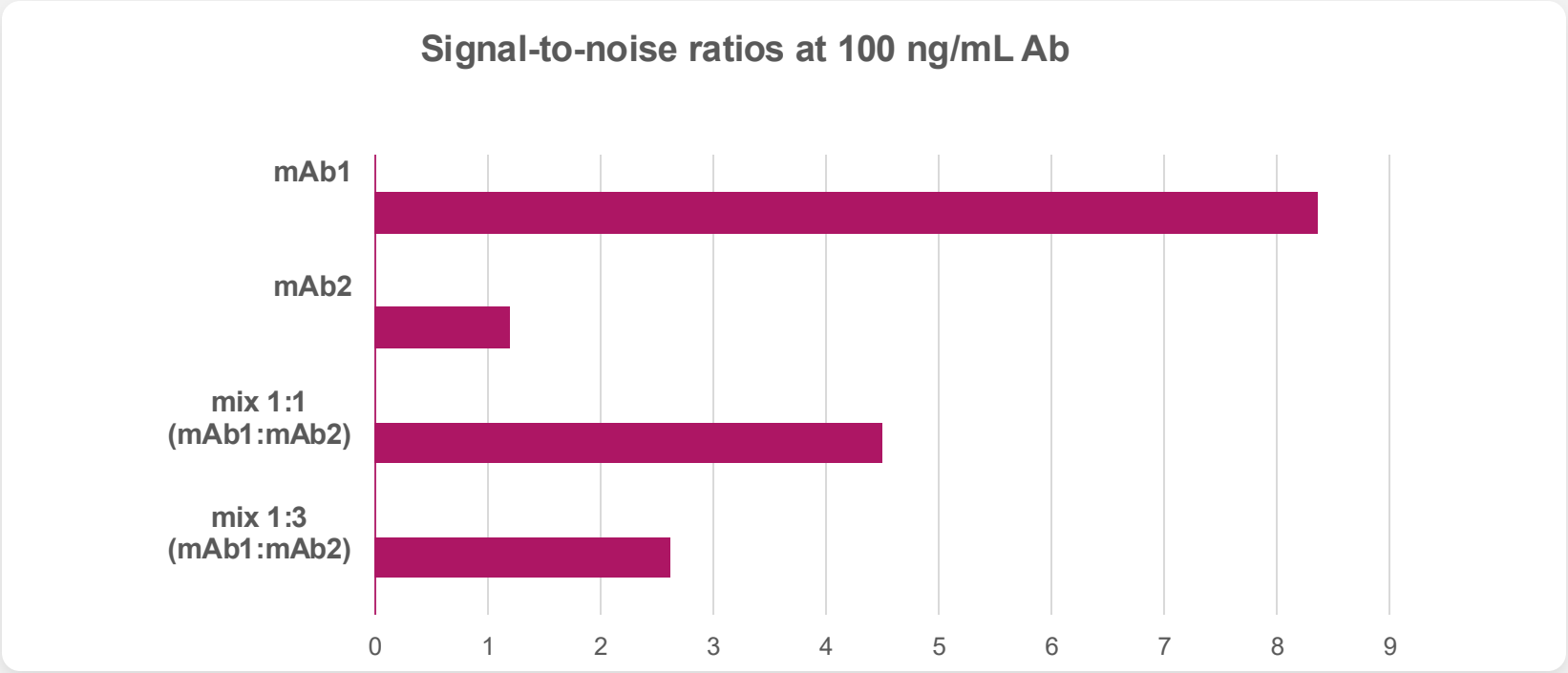
Option	Option 2	Option 3	Option 4
pAb: tiers	mAb2: all tiers	Mix of mAb1 + mAb2: all tiers	mAb1: screening / confirmatory / titer mAb2: cross-reactivity

Improving Sensitivity: Optimization Efforts

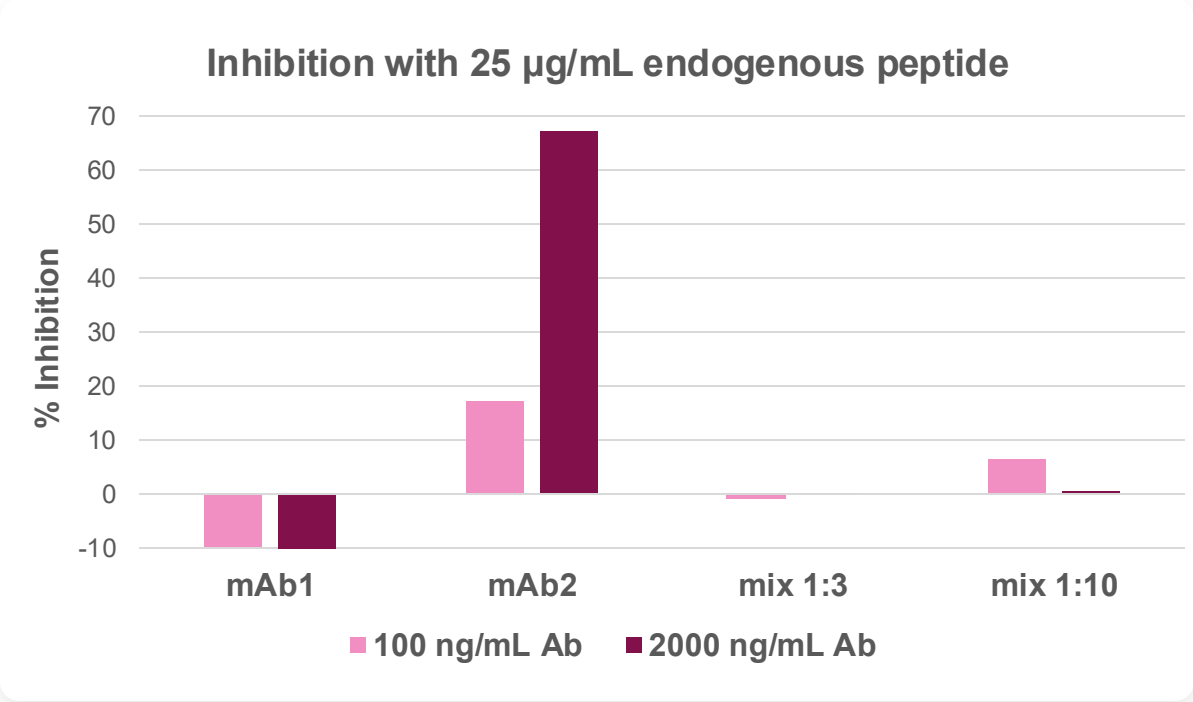
Critical Reagents	Titration of Critical Reagents	☒
Incubation Time	Increase sample incubation with master mix	☒
Acid Sensitivity	Is mAb2 sensitive to acid treatment?	☒
Buffer	Testing different block and sample diluent	☒
MRD	Testing Different MRD — Estimated sensitivity ~100 ng/mL for mAb2	☑

Evaluation of Mixed Ab Positive Control

Option 1	Option 2	Option 3	Option 4
pAb: all tiers	mAb2: all tiers	Mix of mAb1 and mAb2: all tiers	mAb1: screening / confirmatory / titer mAb2: cross-reactivity



Evaluation of Mixed Ab Positive Control – Cross-reactivity



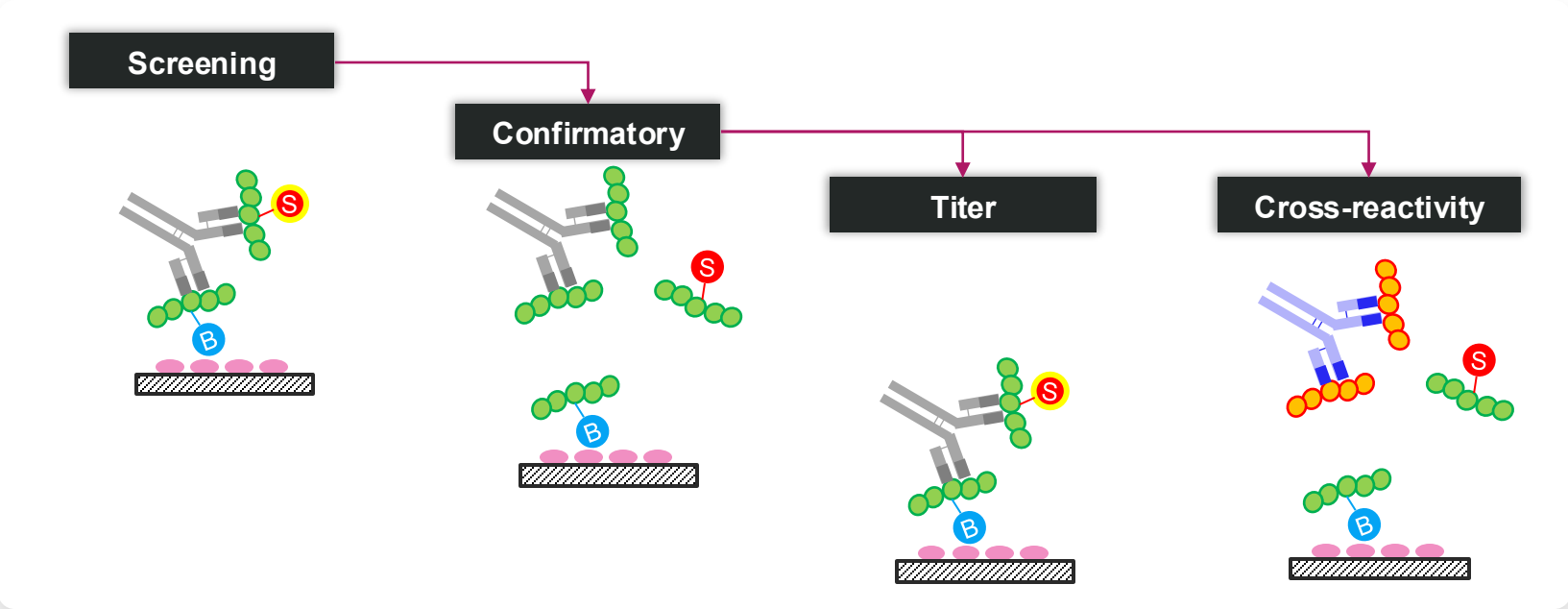
mAb mix

- Mix of mAb1 + mAb2 is not sensitive to inhibition

→ mAb1 + mAb2 is not a viable option

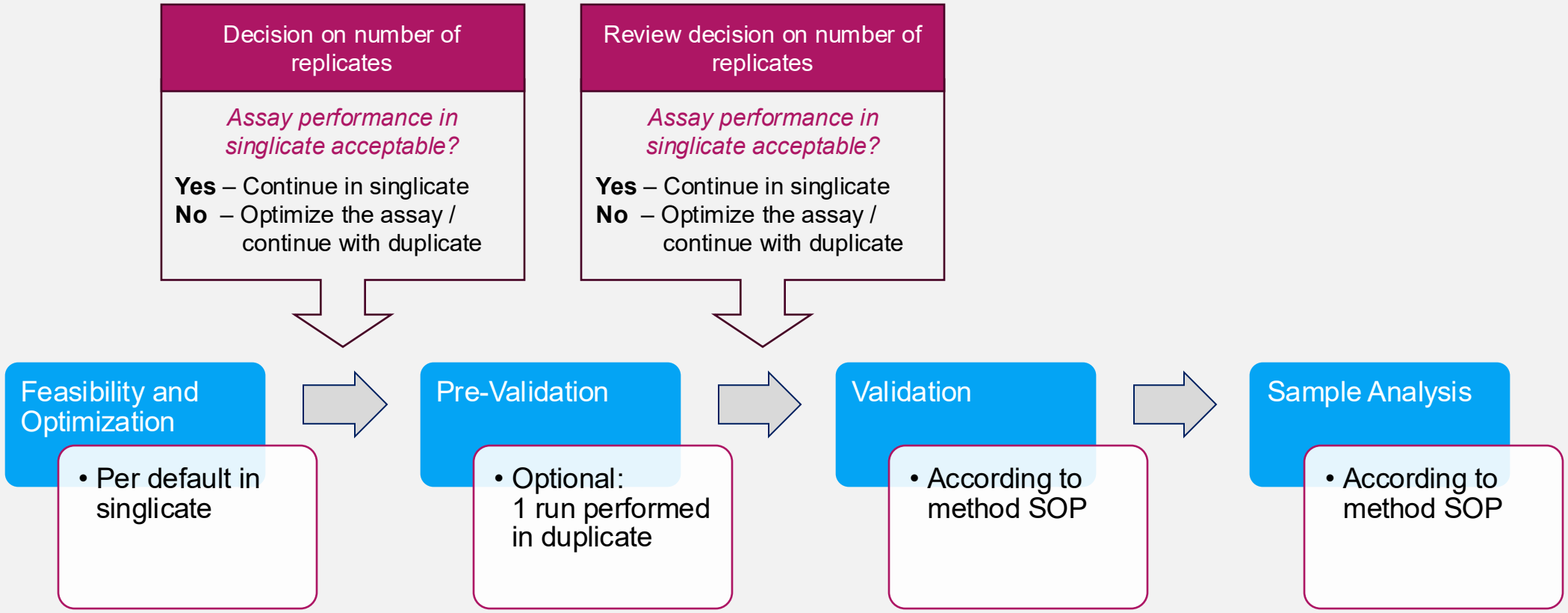
Option 1	Option 2	Option 3	Option 4
pAb: all titers	mAb2: all titers	Mix of mAb1 and mAb2: all titers	mAb1: screening / confirmatory / titer mAb2: cross-reactivity

Program-Tailored Multi-tiered Testing Strategy



- ✓ Assay format could be maintained for all tiers
- ✓ Suitable positive controls selected
- ✓ Confirmatory assay with excess drug (10 µg/mL)
- ✓ Cross-reactivity assay with excess of endogenous peptide (25 µg/mL) and a separate positive control

Standard ADA Testing Strategy: Singlicate?



Pre-Validation Results Support Singlicate Use

Selectivity in diseased population

pLPC mAb1: 5 ng/mL
pLPC mAb2: 75 ng/mL
Blue: RLU > rCP

	Duplicate Result (RLU)			First Well Result (RLU)			Second Well Result (RLU)			%CV Between Wells		
	blank	pLPC mAb1	pLPC mAb2	blank	pLPC mAb1	pLPC mAb2	blank	pLPC mAb1	pLPC mAb2	blank	pLPC mAb1	pLPC mAb2
Buffer	56	110	70	54	111	66	57	108	73	3.82	1.94	**7.12**
Pool	57	108	66	58	110	67	56	106	65	2.48	2.62	2.14
Ind 1	56	103	63	55	102	63	56	103	63	1.27	0.69	0.00
Ind 2	59	110	92	59	108	91	59	111	93	0.00	1.94	1.54
Ind 3	63	113	69	63	112	68	63	113	69	0.00	0.63	1.03
Ind 4	59	113	68	59	113	68	58	113	68	1.21	0.00	0.00
Ind 5	56	108	65	55	107	65	56	108	64	1.27	0.66	1.10
Ind 6	67	117	75	66	118	75	67	115	74	1.06	1.82	0.95
Ind 7	55	104	64	53	104	62	56	104	66	3.89	0.00	4.42
Ind 8	57	105	64	58	105	62	56	105	66	2.48	0.00	4.42
Ind 9	56	103	63	55	101	63	56	104	63	1.27	2.07	0.00
Ind 10	56	105	64	57	106	64	55	103	64	2.53	2.03	0.00

Plate average % CV	1.66
Max % CV	7.12

→ Good precision between wells

→ Singlicate analysis is suitable

Assay Pre-Validation Summary

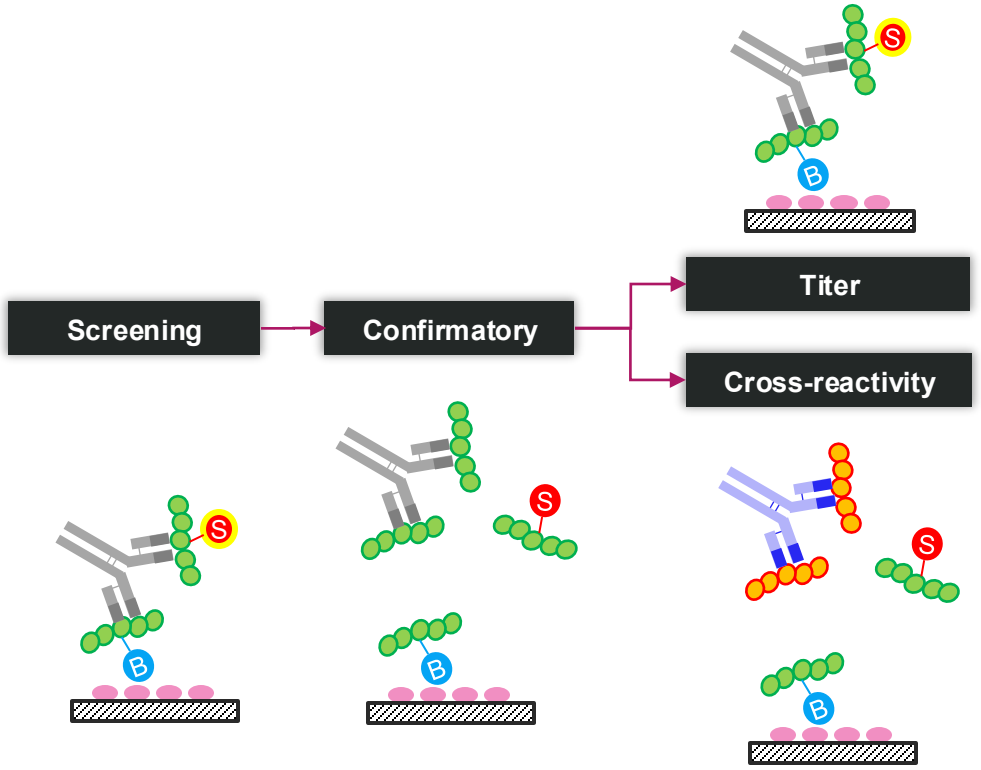
Assay Parameters	Screening, Confirmatory, Titer Assays		Cross-Reactivity Assay
PC	mAb1: pLPC: 5.00 ng/mL; MPC: 100 ng/mL; HPC: 1000 ng/mL		mAb2: pLPC: 75 ng/mL; HPC: 2000 ng/mL
Cut Point	Screening: Confirmatory:	1.07 (Robust parametric approach) 11.9 %	6.3 %
Sensitivity	Screening: Confirmatory:	1.04 ng/mL 1.28 ng/mL	184 ng/mL
Precision	Intra- and Inter-assay precision: < 20 % CV Intra-assay precision: max. 3.8 % CV		
Hook Effect	No hook effect (tested up to 42 µg/mL mAb1)		No hook effect (tested up to 6.6 µg/mL mAb2)
Drug Tolerance	> 200 nM at 100 ng/mL mAb1		> 200 nM at 100 ng/mL mAb2
Selectivity, healthy	10/10 negative 10/10 positive at pLPC and HPC		10/10 positive at pLPC and HPC
Selectivity, diseased	8/10 negative 10/10 positive at pLPC		10/10 positive at pLPC

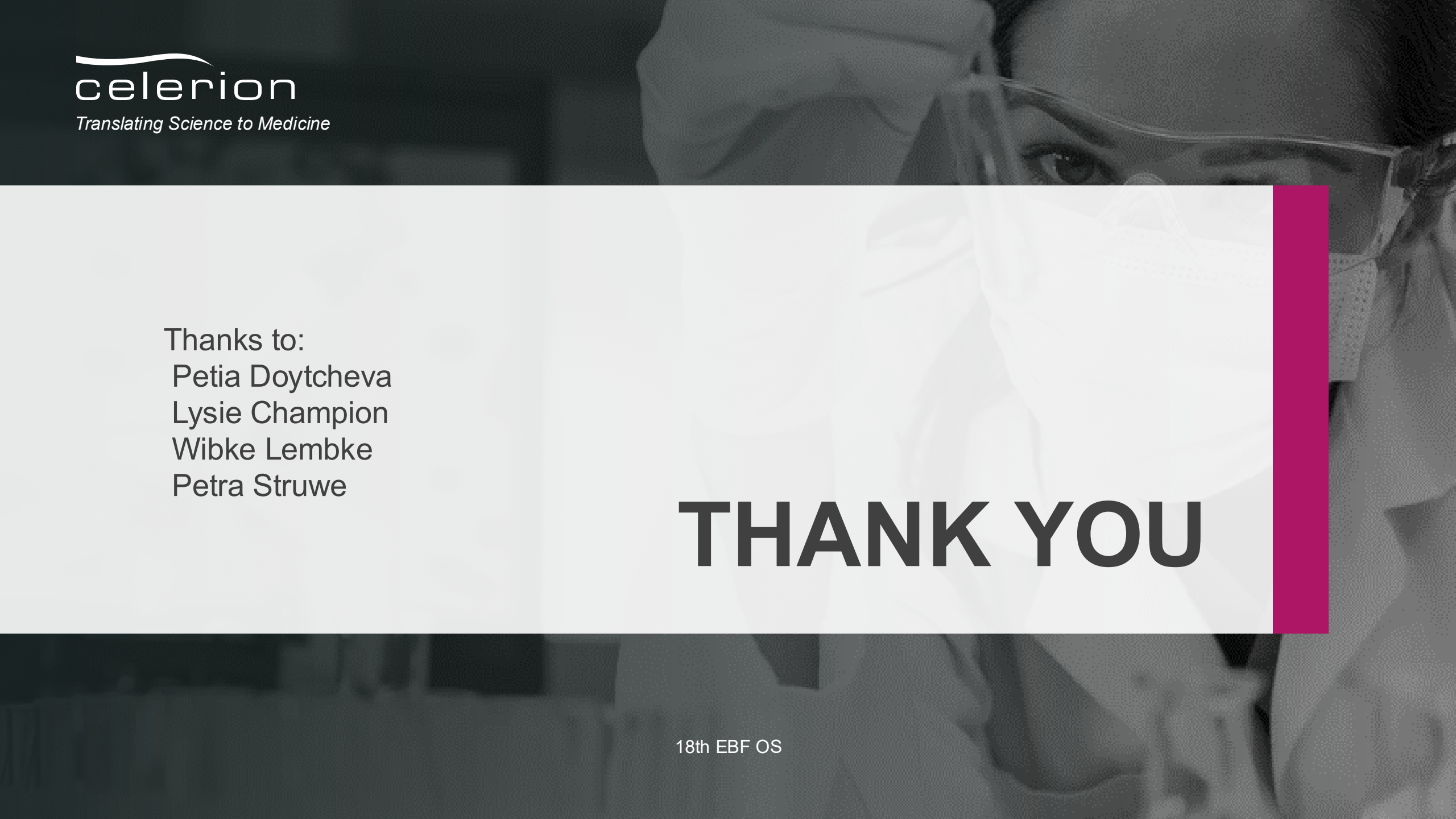
Grey: only tested in screening assay (non-inhibited)

Conclusion

- ✓ Robust, program-tailored, multi-tiered ADA testing strategy meeting both clinical and regulatory requirements
- ✓ Careful selection and evaluation of positive controls for reliable detection of ADAs including cross-reactive assessment
- ✓ Sustained supply for positive controls
- ✓ Demonstrated suitability of singlicate analysis enabling high throughput:

	Singlicate	Duplicate
Screening	88	42
Confirmatory / Cross-reactivity	40	16
Titer	16	8





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THANK YOU