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Translating Science to Medicine

How to Deal with the Challenges of NAb Assays: Case Studies Evaluated

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Neutralizing Antibodies: General Information

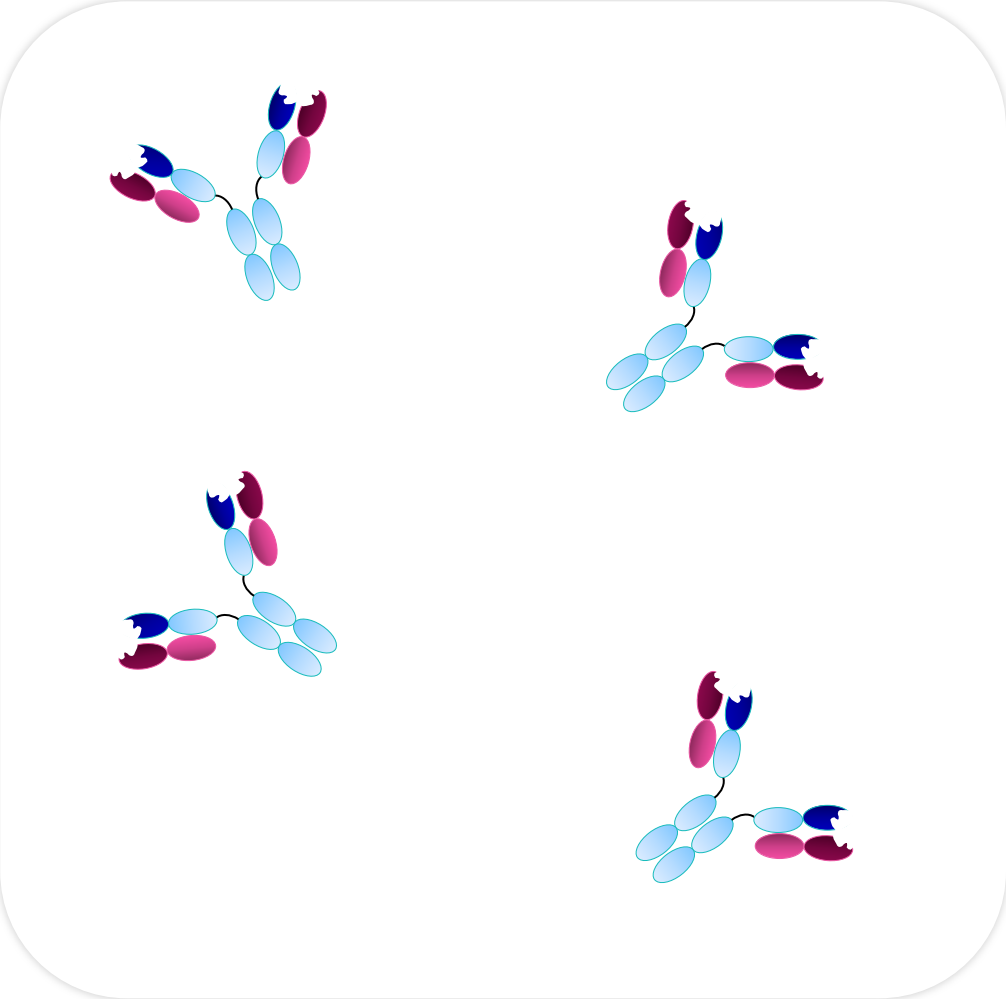
Immunogenicity assessment

- Critical step in the safety and efficacy evaluation of biopharmaceuticals

Neutralizing antibodies (NAbs) may block the biological activity of a therapeutic drug

- Altered PK and PD profiles

Worst-case scenario → cross-reaction with the endogenous counterpart of the biotherapeutic



Typical Nab Assays and their Problems

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Therapeutic MoA

Assay Performance Characteristics

Risk Assessment

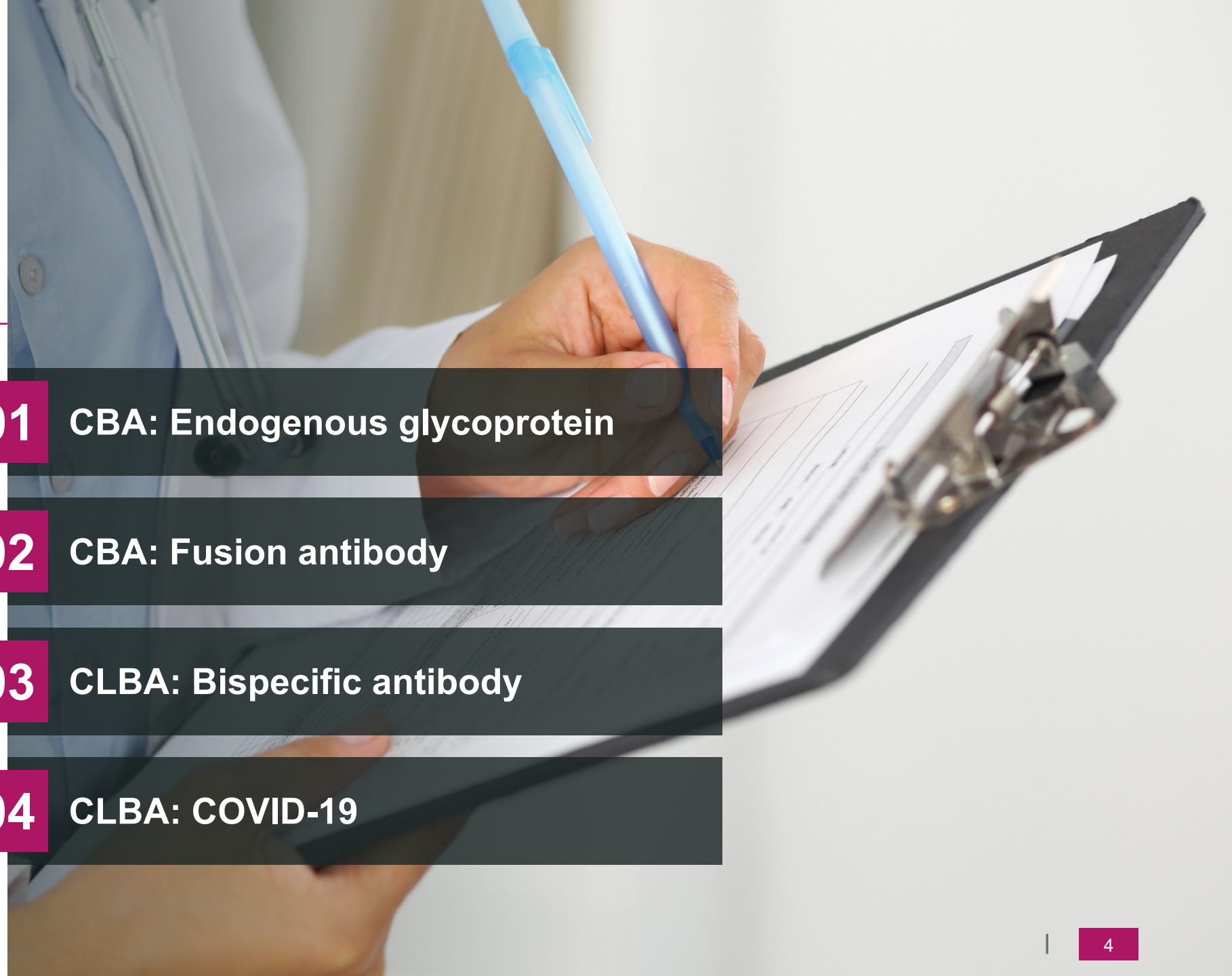
NAb assay categories:

- Cell-based assays (CBA)
- Competitive ligand-binding assay (CLBA)

Typical technical challenges:

- Low sensitivity
- High individual variability
- Interference of free drug

Case studies:



01 CBA: Endogenous glycoprotein

02 CBA: Fusion antibody

03 CLBA: Bispecific antibody

04 CLBA: COVID-19

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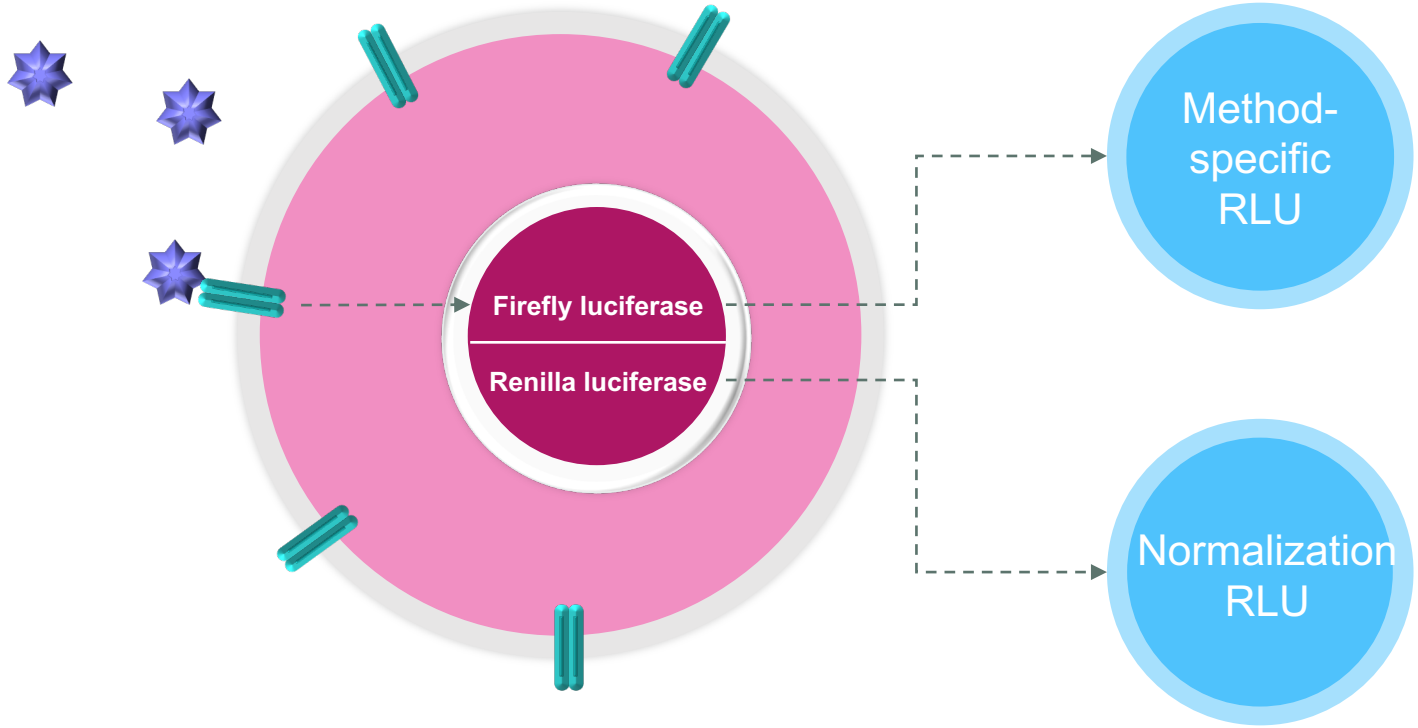
01 CBA: Endogenous glycoprotein

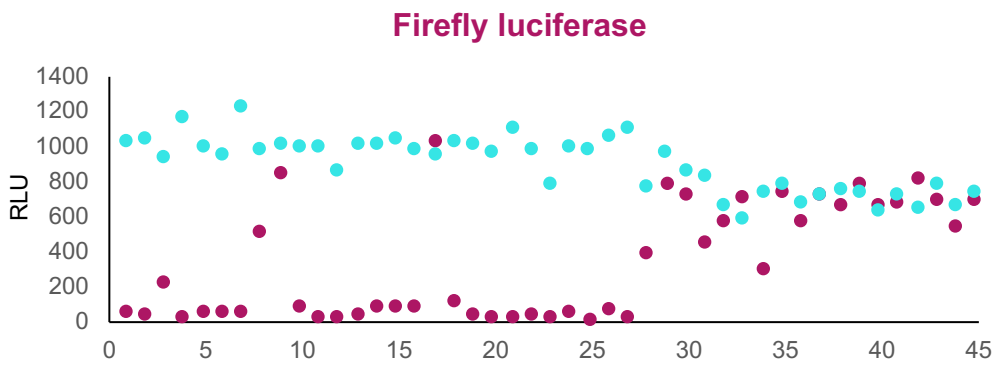
1. CBA: Endogenous Glycoprotein

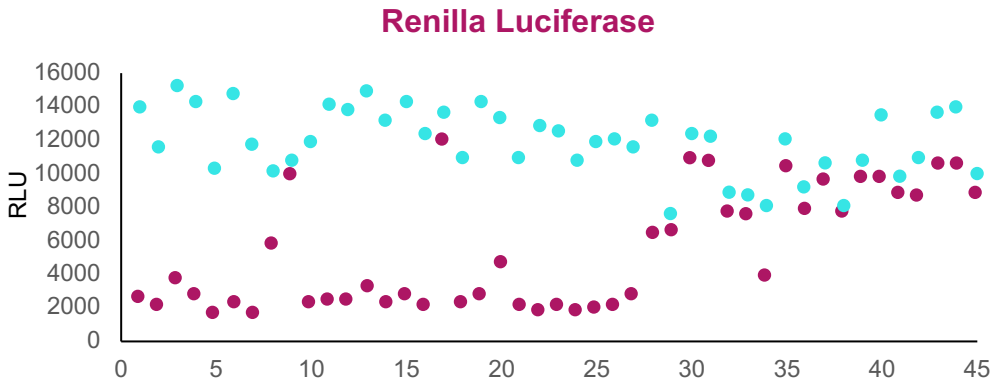


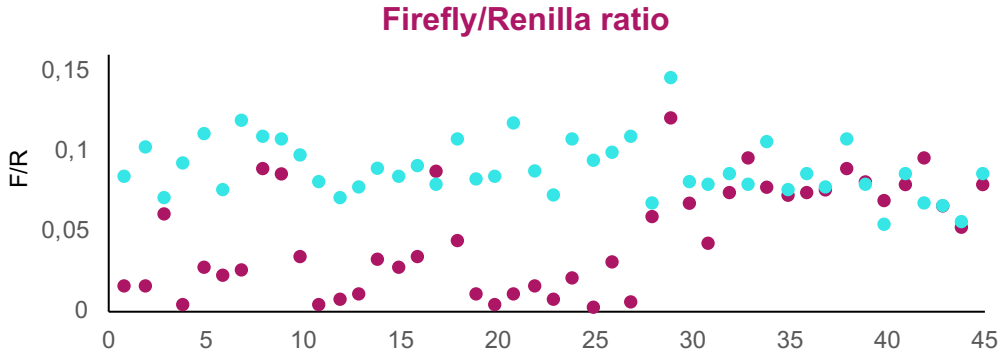
Assay challenges:

Individual variability | Precision | False positives

1. CBA: Endogenous Glycoprotein



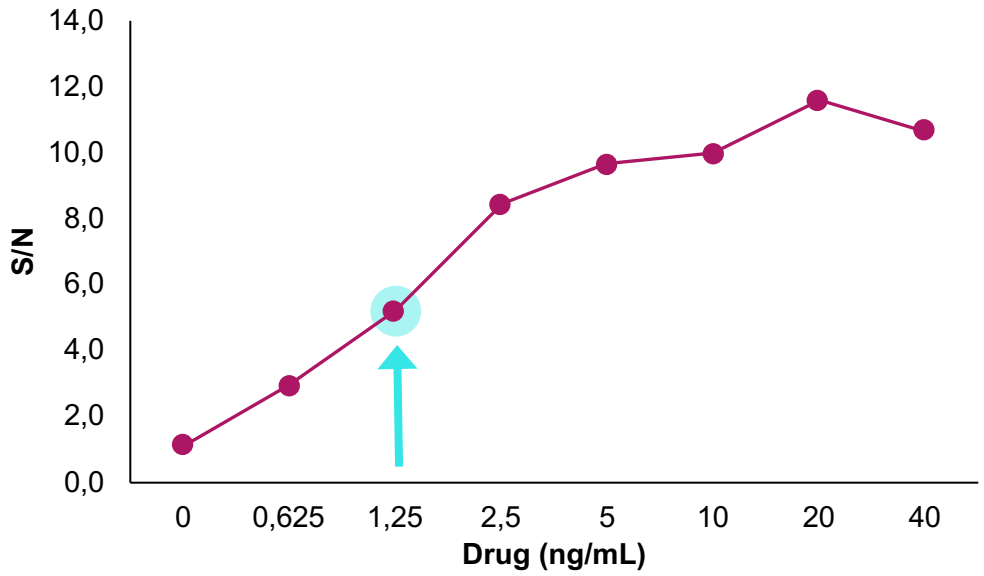




Non-treated Heat-inactivated

Assay challenge: **Individual variability**

1. CBA: Endogenous Glycoprotein

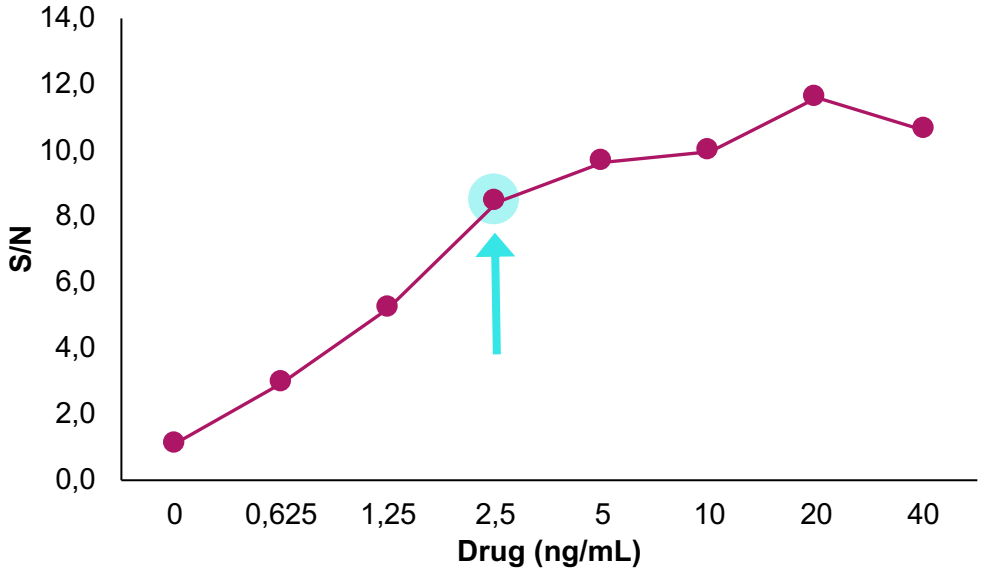


Sample	Average (S/NC)	%CV
NC	1.0	6.0
pLPC	0.6	34
MPC	0.4	16
HPC	0.3	28

rCP
0.75

Assay challenge: Precision

1. CBA: Endogenous Glycoprotein



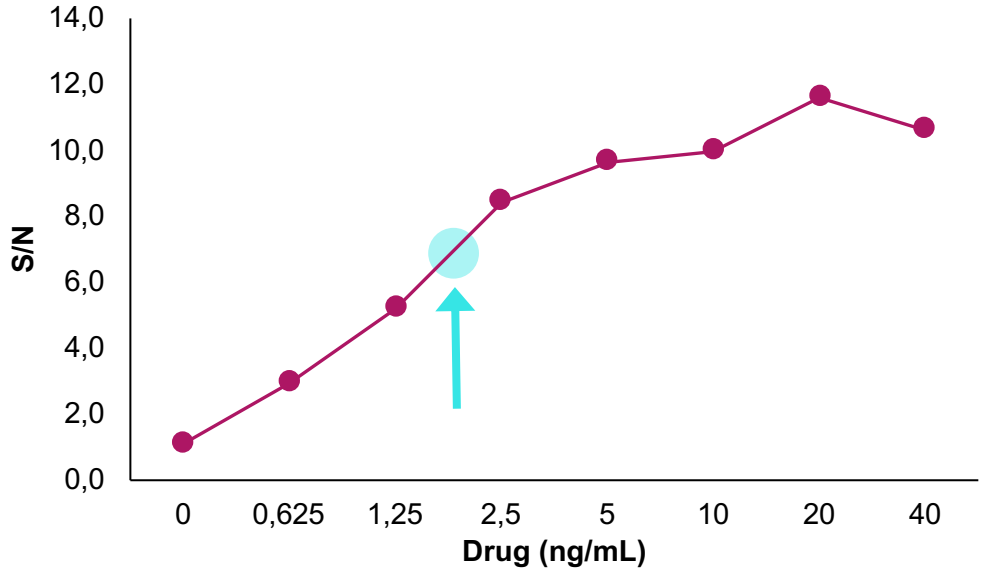
Sample	Average (S/NC)	%CV
NC	1.0	5.1
pLPC	0.6	2.3
MPC	0.5	0.8
HPC	0.2	7.6

rCP
0.86

- 2/30 false positives in a CP run
- 3/10 false positives in a selectivity run

Assay challenge: ◯ **Precision** ◯ **False positives**

1. CBA: Endogenous Glycoprotein



	NC	LPC	MPC	HPC
n	4	4	4	4
Mean	1.0	0.6	0.4	0.2
CV%	0.0	11	4.5	8.8
Intra-assay precision	8.4	11	9.8	10

rCP
0.77

- No false positives

Assay challenge: Precision, False positives

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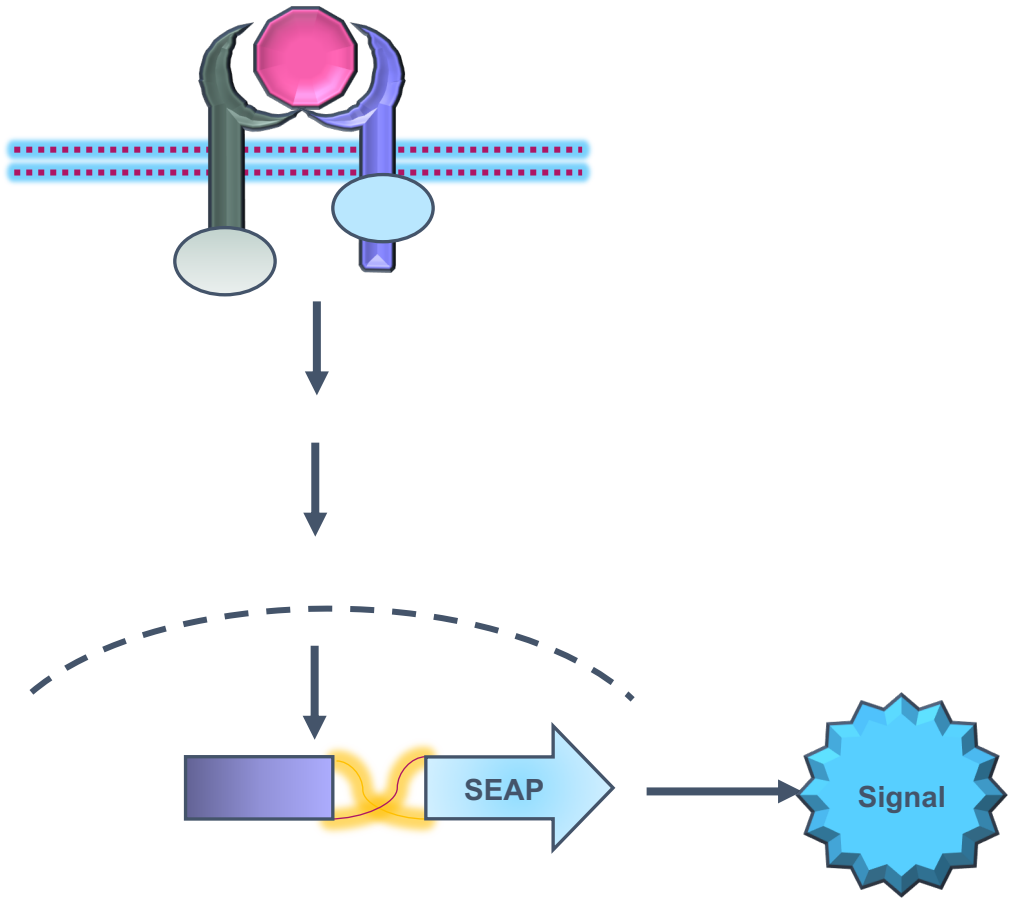
02

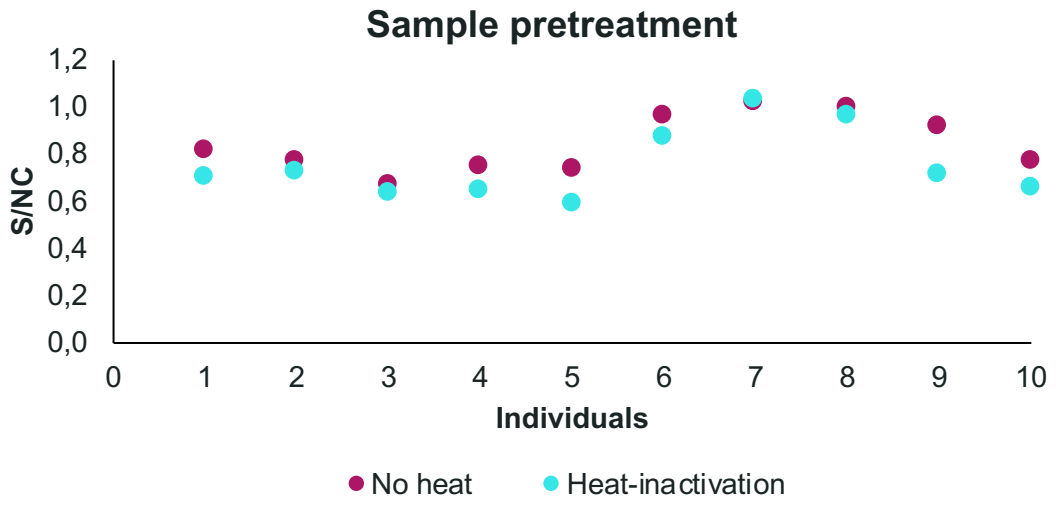
CBA: Fusion antibody conjugated to a cytokine

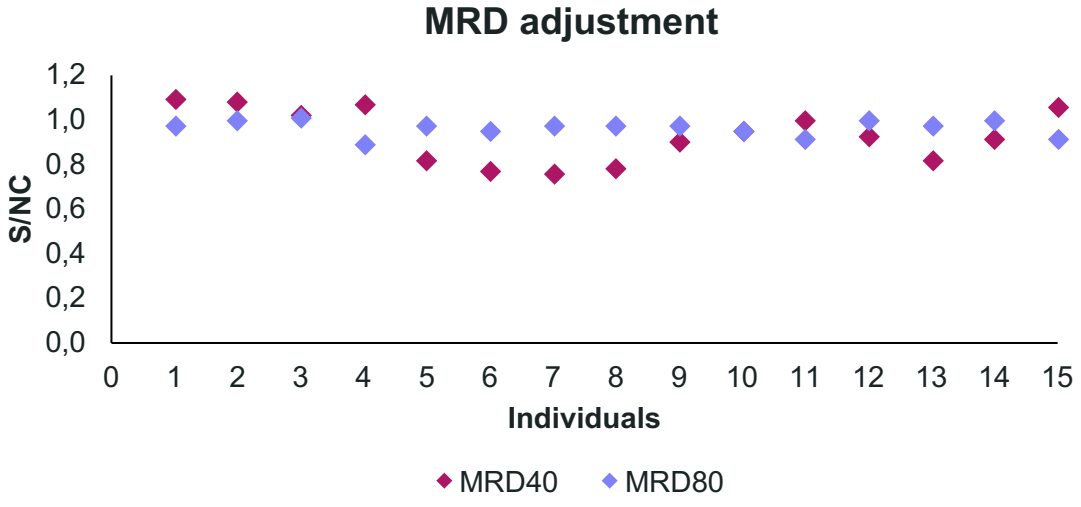
2. CBA: Fusion Antibody Conjugated to a Cytokine



Assay challenges:
Individual variability
Practicality

2. CBA: Fusion Antibody Conjugated to a Cytokine





Treatment	% CV
No heat	15 %
Heat	20 %

MRD	% CV
40	13 %
80	4 %

Assay challenges: Individual variability

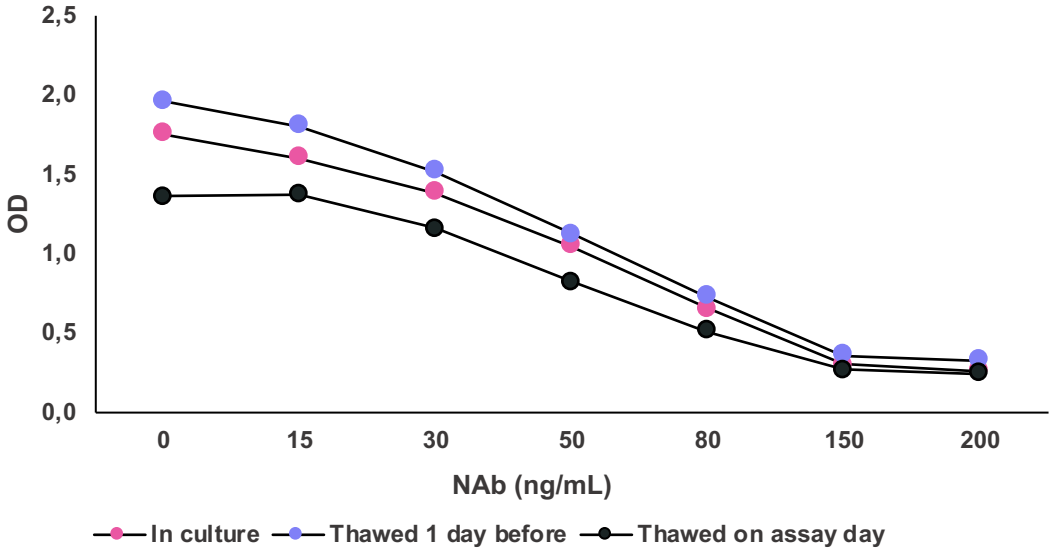
2. CBA: Fusion Antibody Conjugated to a Cytokine

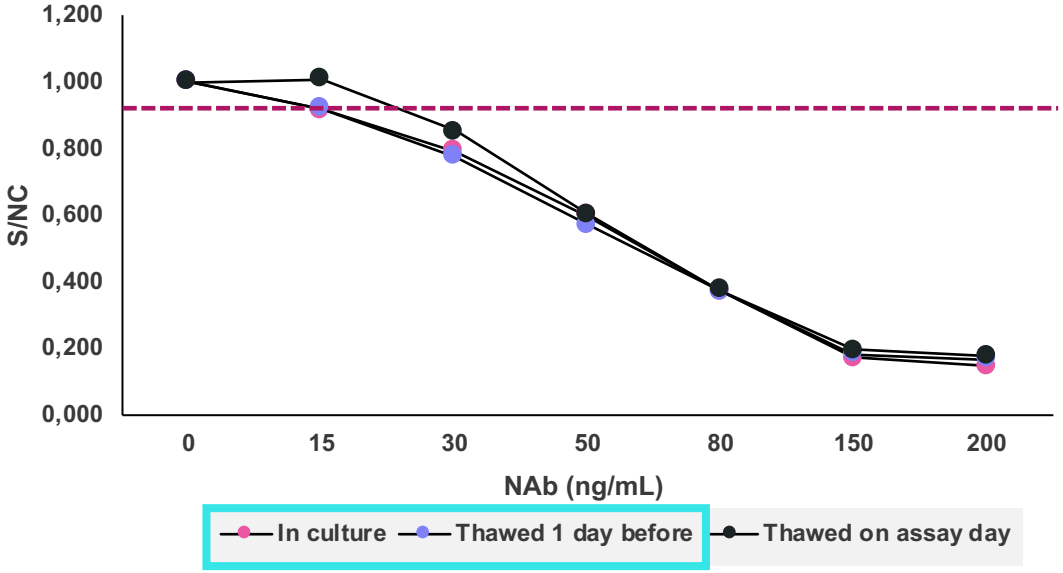
Kept in culture

Thawed 1 day before

Thawed on assay day

Assay challenges:
Practicality





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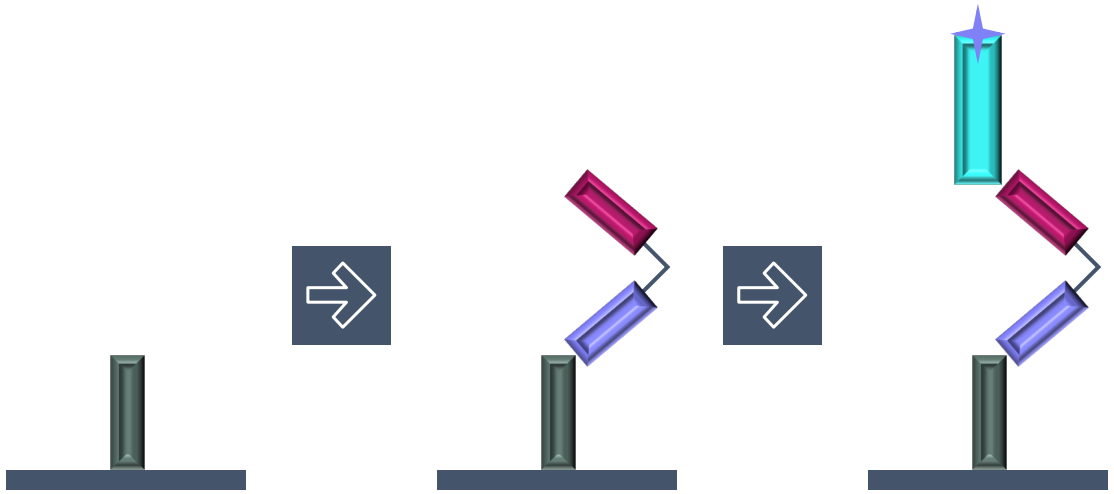
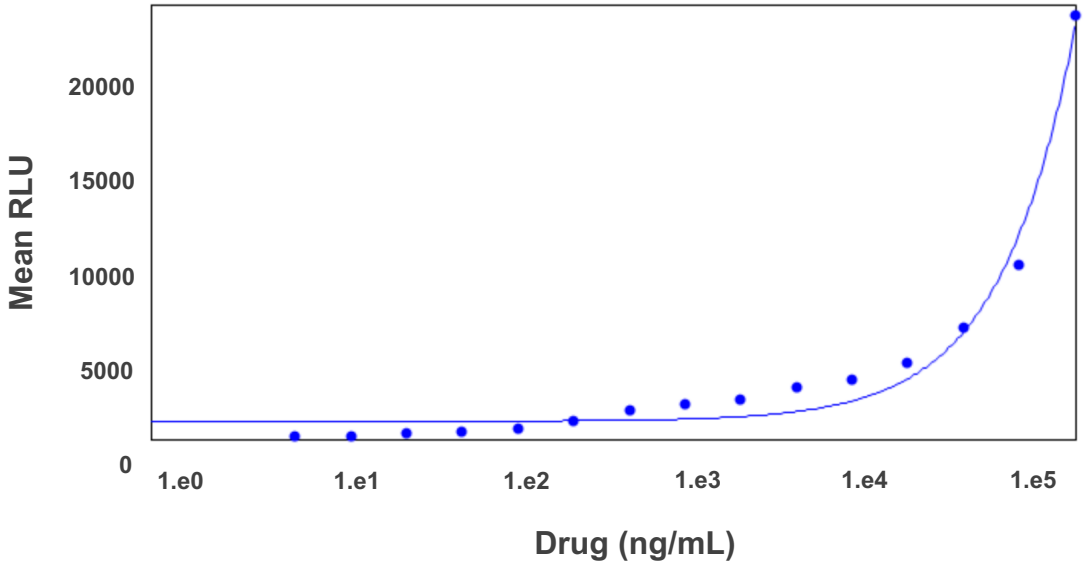
03

CLBA: Bispecific antibody

3. CLBA: Bispecific Antibody

1 Assay type





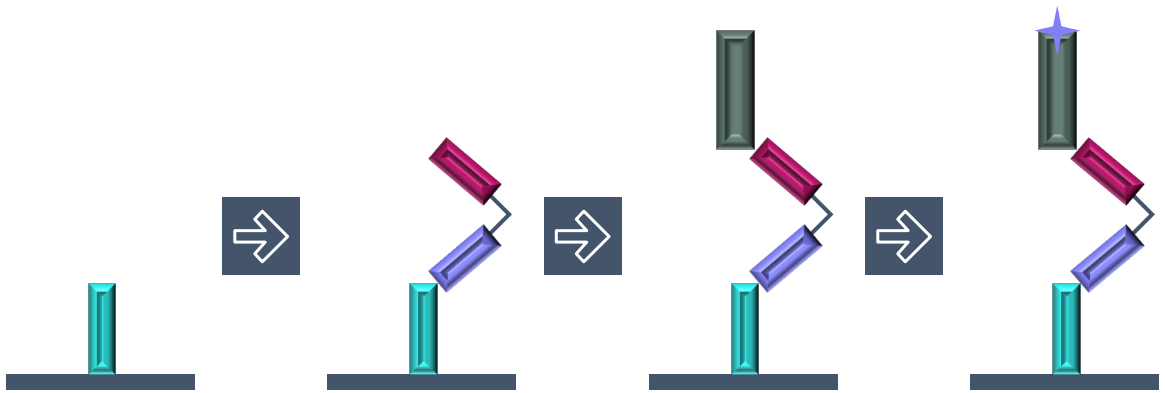
: Target 1 | : Drug | : SulfoTAG-Target 2

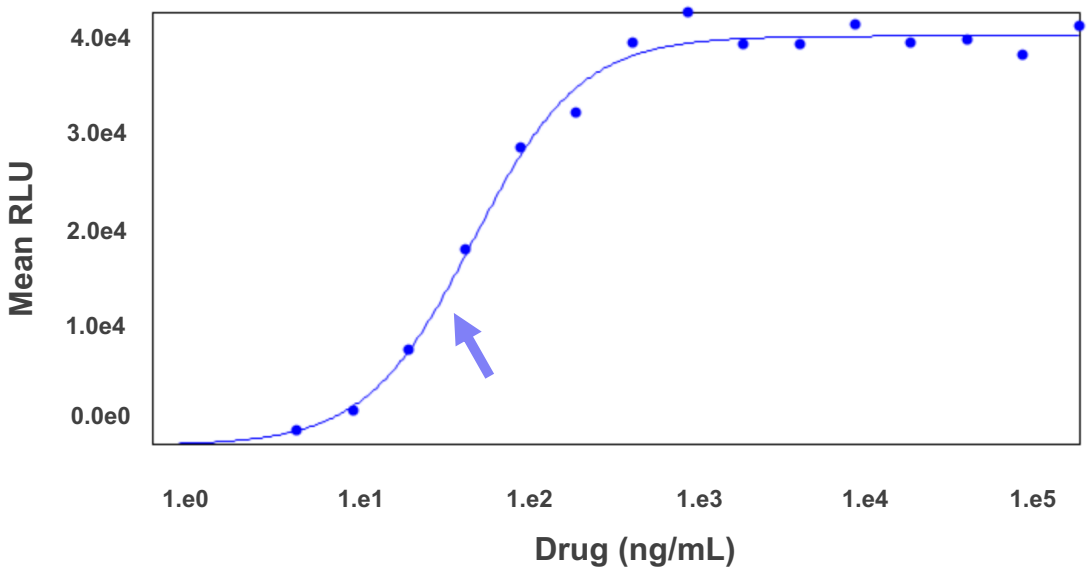
Target 1: (ca. 33.3 kDa)
Target 2: (ca. 109.9 kDa)

Assay challenge:
Appropriate dose-response of the drug

3. CLBA: Bispecific Antibody

2 Assay type





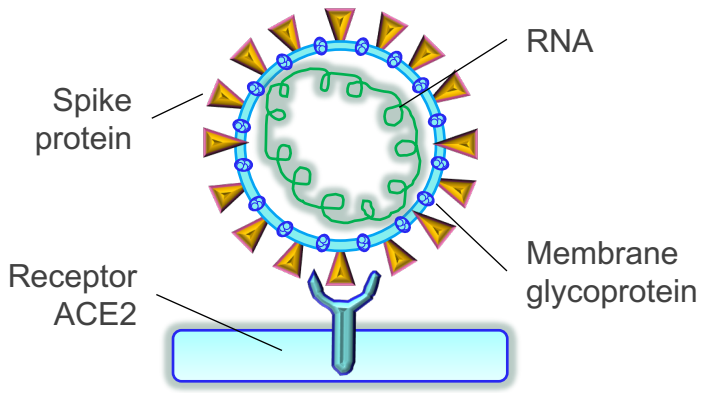
: Target 2 | : Drug | Biotin-Target 1 | : SA-STAG

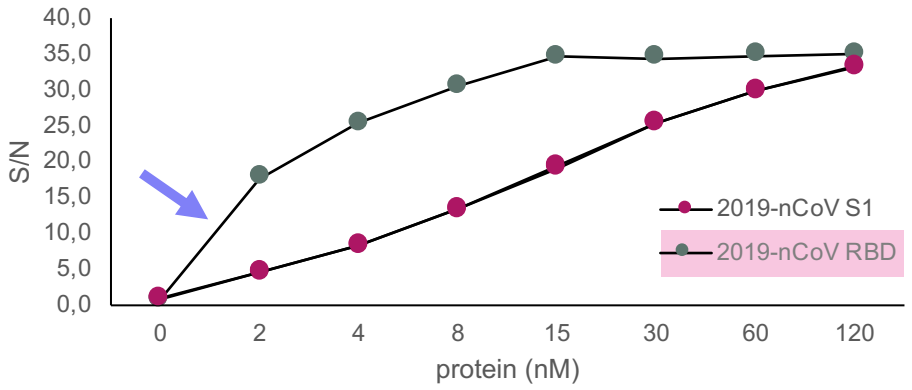
Target 1: (ca. 33.3 kDa)
Target 2: (ca. 109.9 kDa)

Assay challenge:
Appropriate dose-response of the drug

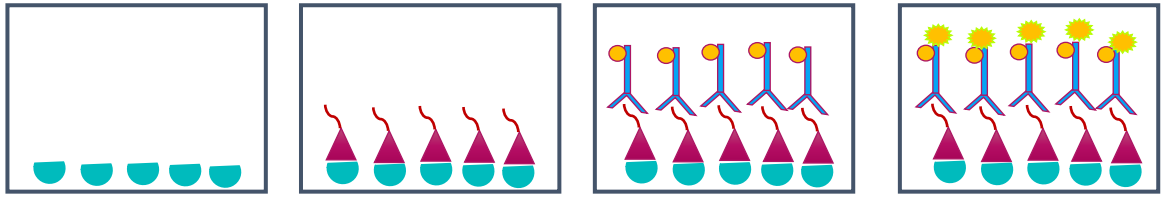
04 CLBA: COVID-19

4. CLBA: COVID-19



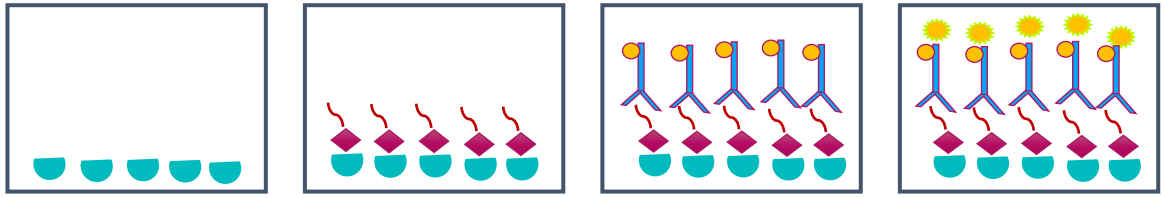


1 Assay type



ACE 2 | nCoV Spike S1 – His TAG | Anti – His TAG – HRP | HRP substrate

2 Assay type



ACE 2 | nCoV Spike RBD – His TAG | Anti – His TAG – HRP | HRP substrate

4. CLBA: COVID-19

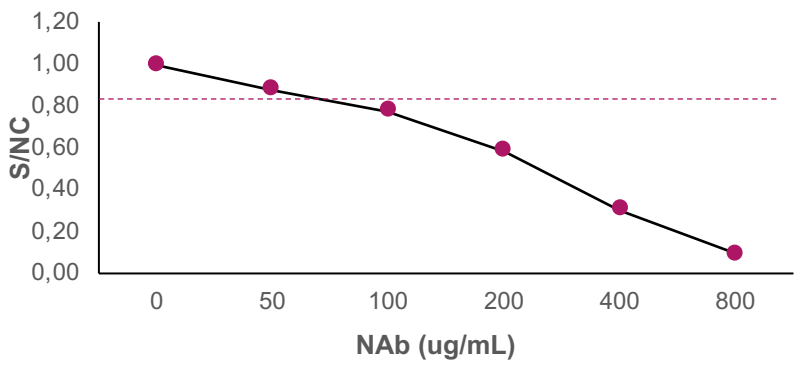
Precision

	0 ng/mL	200 ng/mL	400 ng/mL	800 ng/mL
	NC	LPC	MPC	HPC
n	4	3	3	3
mean	1	0.7	0.4	0.2
%cv	1.8	7.4	14	17
intra-assay precision	2.7	1.9	4.6	2.3

Selectivity

Sample	Accepted
Blank	10 / 10
200 ng/mL	10 / 10
800 ng/mL	10 / 10

Sensitivity



Estimated sensitivity
87 ng/mL

COVID-19 individuals

Individual	S/NC	<rCP?
1	0.10	Yes
2	0.04	Yes
3	0.16	Yes
4	0.94	No
5	0.22	Yes
6	0.10	Yes
7	0.02	Yes
8	0.03	Yes
9	0.04	Yes
10	0.03	Yes

rCP 0.84

Conclusions

Cell-based assays

- Careful adjustment of the stimulatory drug concentration
- Heat-inactivation
- MRD selection
- Single-use vials or cells kept in culture?

Competitive ligand -binding assays

- Target molecule selection for capture and detection

Acknowledgements

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THANK YOU