



NUVISAN

Diving deeper into data:
investigation of a CV% issue during
a biomarker study using Gyrolab

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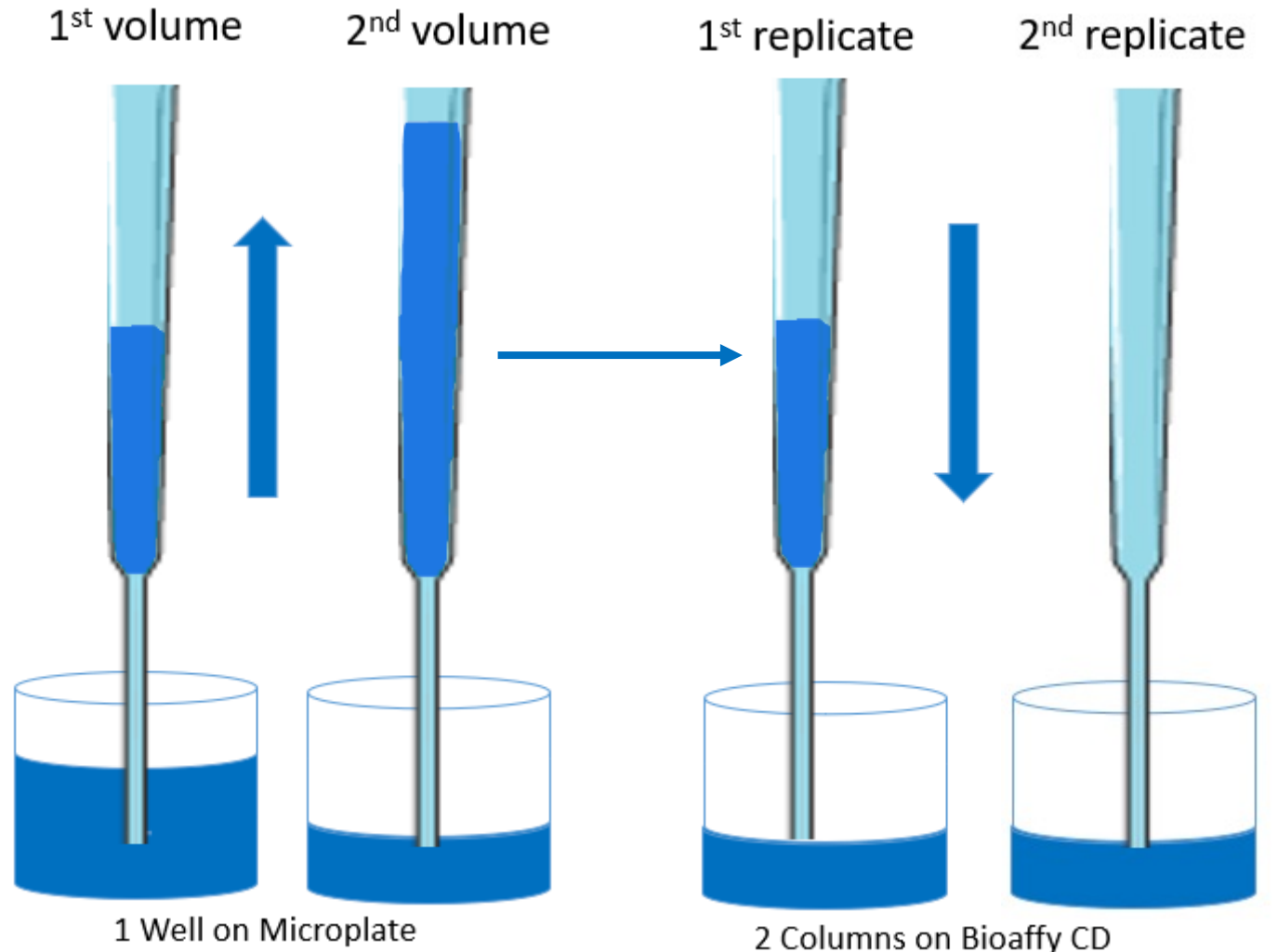
Basics: Gyrolab platform

Gyrolab Loading List

MP-1

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9			
B	10	11	12	13	14	15						
C	16	17	18	19	20	21	22	23	24	25	26	27
D	28	29	30	31	32	33	34	35	36			
E	37	38										
F	39	40	41	42	43							
G												
H												

Pipetting scheme for duplicates





Background information: Project Biomarker X

Biomarker X

- soluble, secreted protein
- isoelectric point around 5.4
- can form oligomers
- endogenous baseline levels ~0.7-7ngEq/mL

Context of use:

Biomarker X is target of a new biological entity,
free levels of Biomarker X thus will decrease
vs total levels are expected to increase during treatment



Background information: free Biomarker X assay requirements and setup

Development of free Biomarker X assay

Assay platform:	Gyrolab
Assay format:	Capture (Drug) – Biomarker X – Detection (anti-Biomarker X antibody)
Analyte:	recombinant human Biomarker X
Surrogate matrix:	PBS-BSA (2%)
Sensitivity:	as good as possible (context of use!)

Assay setup

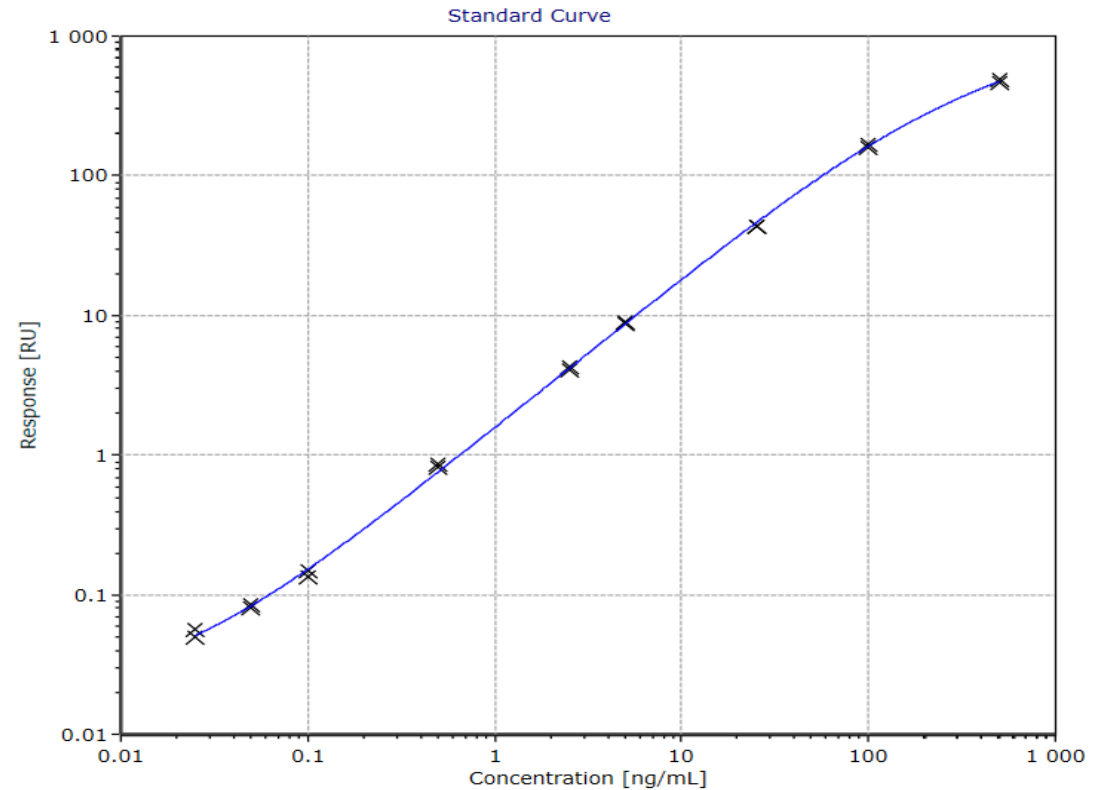
Bioaffy CD:	1000 or 4000
Method:	1000/4000-3W-001-A
Wash buffers:	2 (PBS-T and pH11)
MRD:	as low as possible
Rexxip buffer:	H or H max
Detection:	monoclonal antibody



Background information: Assay development

Sample Name	Expected Conc [ng/mL]	Response [RU]	Average Conc [ng/mL]	CV Conc [%]	CV Response [%]
▶ CAL_Blank	0.00	0.0180			24.5
▶ CAL_Blank	0.00	0.0127			24.5
▶ CAL_1	0.0250	0.0568	0.0268	15.7	9.74
▶ CAL_1	0.0250	0.0495	0.0268	15.7	9.74
▶ CAL_2	0.0500	0.0858	0.0495	5.11	4.84
▶ CAL_2	0.0500	0.0802	0.0495	5.11	4.84
▶ CAL_3	0.100	0.148	0.0927	6.82	6.50
▶ CAL_3	0.100	0.135	0.0927	6.82	6.50
▶ CAL_4	0.500	0.853	0.549	3.00	3.08
▶ CAL_4	0.500	0.817	0.549	3.00	3.08
▶ CAL_5	2.50	4.35	2.52	4.69	4.97
▶ CAL_5	2.50	4.05	2.52	4.69	4.97
▶ CAL_6	5.00	8.94	5.06	2.11	2.23
▶ CAL_6	5.00	8.66	5.06	2.11	2.23
▶ CAL_7	25.0	44.1	23.9	0.461	0.460
▶ CAL_7	25.0	43.8	23.9	0.461	0.460
▶ CAL_8	100	170	102	5.31	4.30
▶ CAL_8	100	160	102	5.31	4.30
▶ CAL_9	500	485	502	6.26	3.22
▶ CAL_9	500	463	502	6.26	3.22

Chart Settings



Final assay setup:

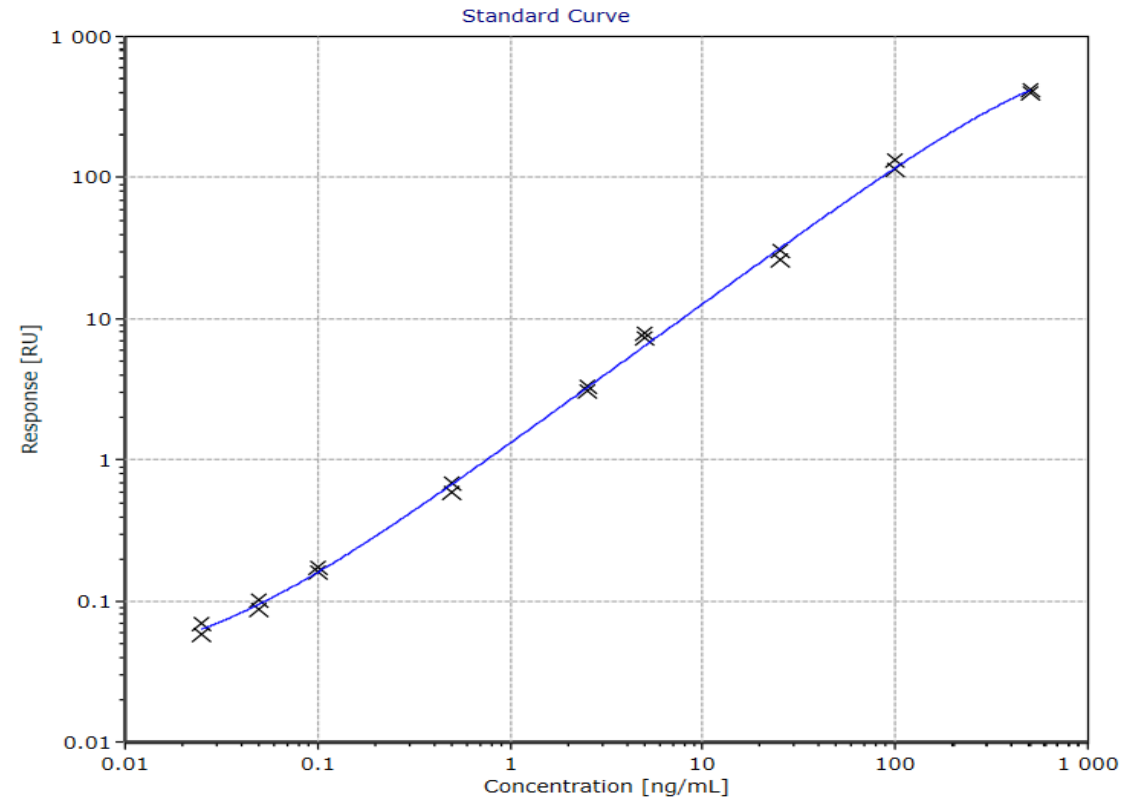
- Gyrolab Bioaffy 4000 CD
- MRD 1:2 (dilution buffer REXXIP H max)
- Surrogate Matrix PBS-BSA (2%)
- Detection range 0.0500 – 100 ng/mL at MRD, LLOQ 0.100 ng/mL



Background information: Assay validation - I

Sample Name	Expected Conc [ng/mL]	Response [RU]	Average Conc [ng/mL]	CV Conc [%]	CV Response [%]
▶ CAL_Blank	0.00	0.0373			33.5
▶ CAL_Blank	0.00	0.0230			33.5
▶ CAL_1	0.0250	0.0700	0.0259	24.4	13.3
▶ CAL_1	0.0250	0.0579	0.0259	24.4	13.3
▶ CAL_2	0.0500	0.101	0.0498	14.4	9.84
▶ CAL_2	0.0500	0.0882	0.0498	14.4	9.84
▶ CAL_3	0.100	0.172	0.106	5.59	4.41
▶ CAL_3	0.100	0.161	0.106	5.59	4.41
▶ CAL_4	0.500	0.693	0.475	12.2	11.6
▶ CAL_4	0.500	0.588	0.475	12.2	11.6
▶ CAL_5	2.50	3.35	2.49	5.69	5.62
▶ CAL_5	2.50	3.09	2.49	5.69	5.62
▶ CAL_6	5.00	7.87	5.93	5.32	5.27
▶ CAL_6	5.00	7.31	5.93	5.32	5.27
▶ CAL_7	25.0	30.5	22.6	10.5	10.3
▶ CAL_7	25.0	26.4	22.6	10.5	10.3
▶ CAL_8	100	134	108	11.5	10.3
▶ CAL_8	100	116	108	11.5	10.3
▶ CAL_9	500	424	497	5.86	3.55
▶ CAL_9	500	403	497	5.86	3.55

Chart Settings



Higher CV% than usual, but nothing to worry about (?)

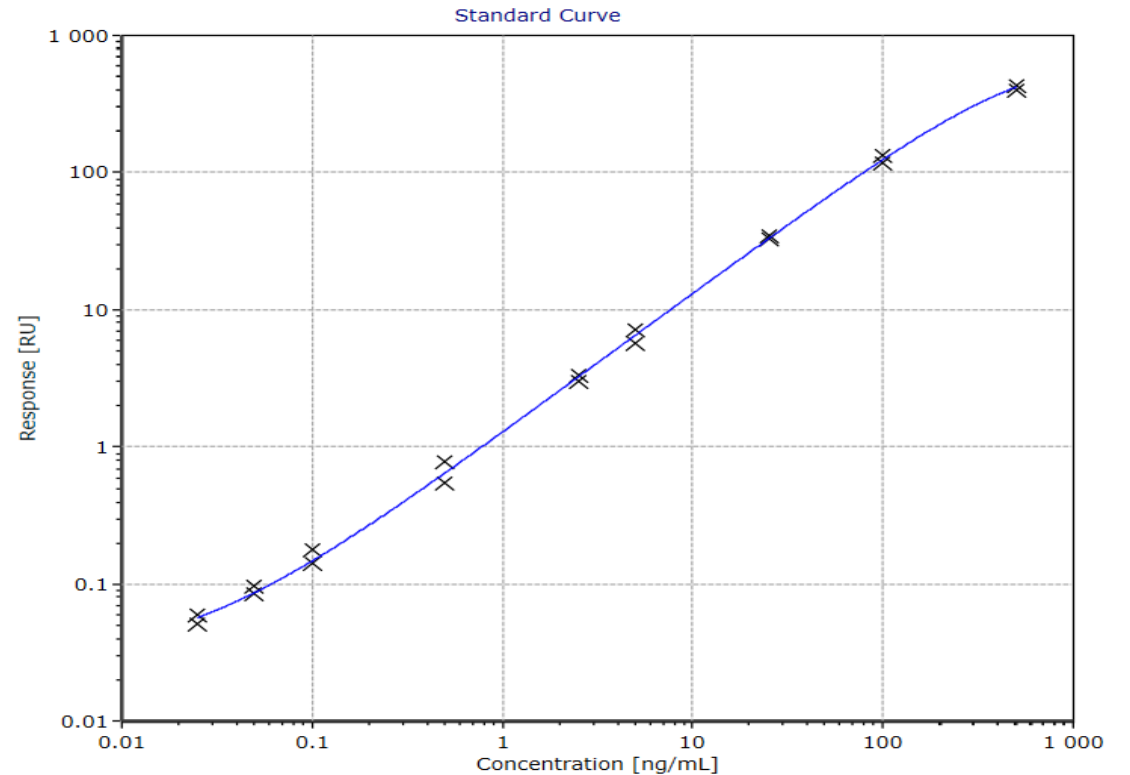
CAL_1 = anchor point



Background information: Assay validation - II

Sample Name	Expected Conc [ng/mL]	Response [RU]	Average Conc [ng/mL]	CV Conc [%]	CV Response [%]
▶ CAL_Blank	0.00	0.0220			10.0
▶ CAL_Blank	0.00	0.0253			10.0
▶ CAL_1	0.0250	0.0589	0.0235	19.7	9.57
▶ CAL_1	0.0250	0.0515	0.0235	19.7	9.57
▶ CAL_2	0.0500	0.0957	0.0533	11.9	8.46
▶ CAL_2	0.0500	0.0849	0.0533	11.9	8.46
▶ CAL_3	0.100	0.176	0.110	17.6	14.9
▶ CAL_3	0.100	0.142	0.110	17.6	14.9
▶ CAL_4	0.500	0.770	0.511	24.2	23.7
▶ CAL_4	0.500	0.549	0.511	24.2	23.7
▶ CAL_5	2.50	3.34	2.47	6.91	7.00
▶ CAL_5	2.50	3.02	2.47	6.91	7.00
▶ CAL_6	5.00	7.22	4.96	16.8	17.0
▶ CAL_6	5.00	5.67	4.96	16.8	17.0
▶ CAL_7	25.0	35.1	26.0	3.86	3.83
▶ CAL_7	25.0	33.2	26.0	3.86	3.83
▶ CAL_8	100	135	102	9.94	9.00
▶ CAL_8	100	119	102	9.94	9.00
▶ CAL_9	500	436	501	10.5	5.55
▶ CAL_9	500	403	501	10.5	5.55

Chart Settings



Single not acceptable calibration curve samples

Action cleaning of instrument

Result: nice CV% again

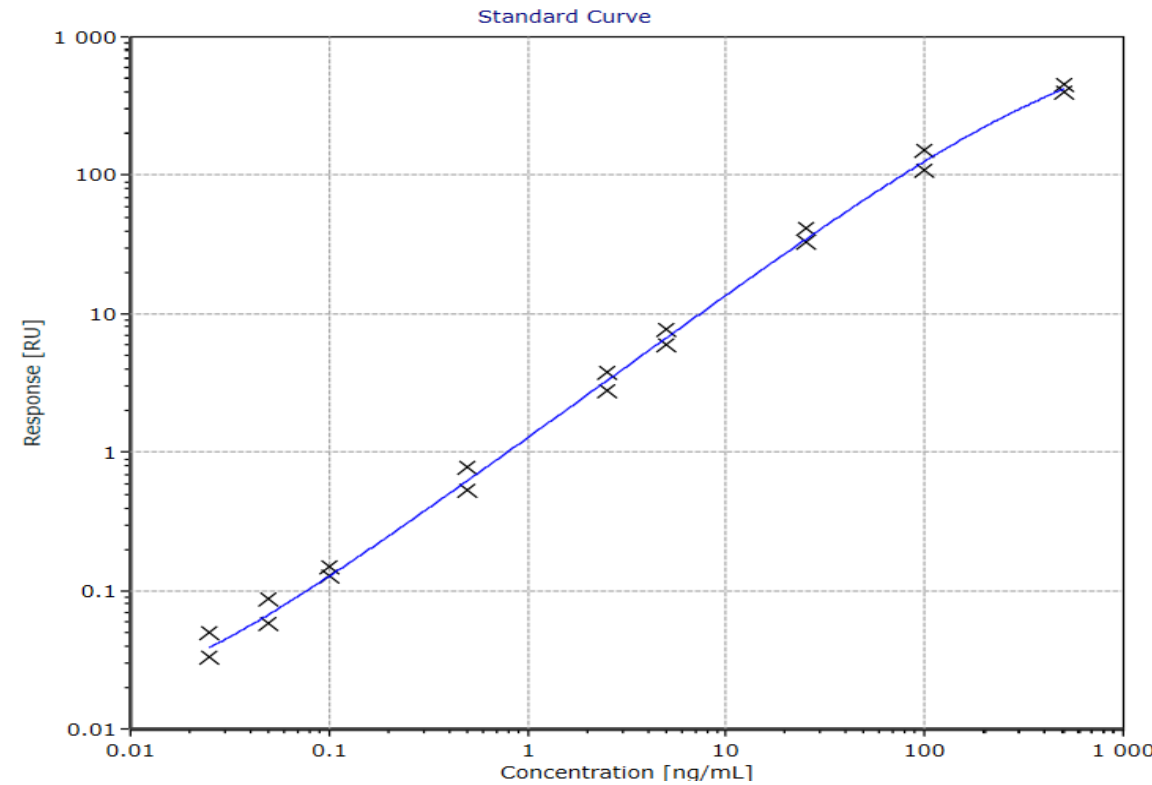
→ Assay validation was completed



The issue

Sample Name	Expected Conc [ng/mL]	Response [RU]	Average Conc [ng/mL]	CV Conc [%]	CV Response [%]
CAL_Blank	0.00	0.0145			4.41
CAL_Blank	0.00	0.0136			4.41
CAL_1	0.0250	0.0499	0.0274	36.9	28.4
CAL_1	0.0250	0.0332	0.0274	36.9	28.4
CAL_2	0.0500	0.0865	0.0539	32.0	28.3
CAL_2	0.0500	0.0577	0.0539	32.0	28.3
CAL_3	0.100	0.148	0.109	11.6	10.7
CAL_3	0.100	0.127	0.109	11.6	10.7
CAL_4	0.500	0.772	0.519	26.2	26.8
CAL_4	0.500	0.526	0.519	26.2	26.8
CAL_5	2.50	3.74	2.50	19.6	20.2
CAL_5	2.50	2.80	2.50	19.6	20.2
CAL_6	5.00	7.80	5.18	17.5	18.0
CAL_6	5.00	6.04	5.18	17.5	18.0
CAL_7	25.0	41.3	27.1	16.4	16.2
CAL_7	25.0	32.8	27.1	16.4	16.2
CAL_8	100	152	105	26.8	23.4
CAL_8	100	109	105	26.8	23.4
CAL_9	500	449	509	13.9	8.43
CAL_9	500	399	509	13.9	8.43

Chart Settings



During sample analysis CV% issue occurred again

Action cleaning of instrument

Result: still bad CV% 😞

→ Sample analysis was paused to have a closer look at the data and find a solution



Diving deeper into data: what do we know?

Facts:

1. The issue of having high CV% occurred occasionally, but could be solved by cleaning the instrument
2. 38 sample analysis runs were performed without having issues with high CV%
3. Cleaning does not help anymore
4. Issue only occurs with calibration samples; study samples and QCs seem not to be affected
5. All used materials were tested (different lots, new preparation, etc.) but issue remained

Conclusions:

1. Needles seem to be affected
2. The issue seems to build up over time
3. ??? Theory: intense cleaning led to super smooth surface
4. Only recombinant protein in combination with surrogate matrix seems to be affected
5. Specific issue within assay setup



Diving deeper into data – what do we miss?

Sample Name	Expected Conc [ng/mL]	Response [RU]	Average Conc [ng/mL]	CV Conc [%]	CV Response [%]
CAL_Blank	0.00	0.0145			4.41
CAL_Blank	0.00	0.0136			4.41
CAL_1	0.0250	0.0499	0.0274	36.9	28.4
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CAL_2	0.0500	0.0577	0.0539	32.0	28.3
CAL_3	0.100	0.148	0.109	11.6	10.7
CAL_3	0.100	0.127	0.109	11.6	10.7
CAL_4	0.500	0.772	0.519	26.2	26.8
CAL_4	0.500	0.526	0.519	26.2	26.8
CAL_5	2.50	3.74	2.50	19.6	20.2
CAL_5	2.50	2.80	2.50	19.6	20.2
CAL_6	5.00	7.80	5.18	17.5	18.0
CAL_6	5.00	6.04	5.18	17.5	18.0
CAL_7	25.0	41.3	27.1	16.4	16.2
CAL_7	25.0	32.8	27.1	16.4	16.2
CAL_8	100	152	105	26.8	23.4
CAL_8	100	109	105	26.8	23.4
CAL_9	500	449	509	13.9	8.43
CAL_9	500	399	509	13.9	8.43



→ Second value is always lower than the first value!

Reason for high CV%:
Analyte seems to get stuck in needles

Data acquired during sample analysis



Diving deeper into data – what we „missed“?

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▶ CAL_Blank	0.00	0.0220			10.0
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Data acquired during assay validation



Diving deeper into data – what we „missed“?

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Data acquired during assay validation



Diving deeper into data – what we „missed“?

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▶ CAL_1	0.0250	0.0495	0.0268	15.7	9.74
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▶ CAL_8	100	160	102	5.31	4.30
▶ CAL_9	500	485	502	6.26	3.22
▶ CAL_9	500	463	502	6.26	3.22



→ Effect was always more or less visible even if the CV% values were good!

Data acquired during assay development



Diving deeper into data: what is going on?

→ Performance of carry-over tests



Confirmation of analyte loss

→ Checking for different surrogate matrices



Confirmation of first value as being correct

Recombinant Biomarker X in combination with surrogate matrix PBS-BSA leads to high CV% values!

→ Change of surrogate matrix



Need of re-validation

→ Change of method settings
(inclusion of needle washes, etc.)

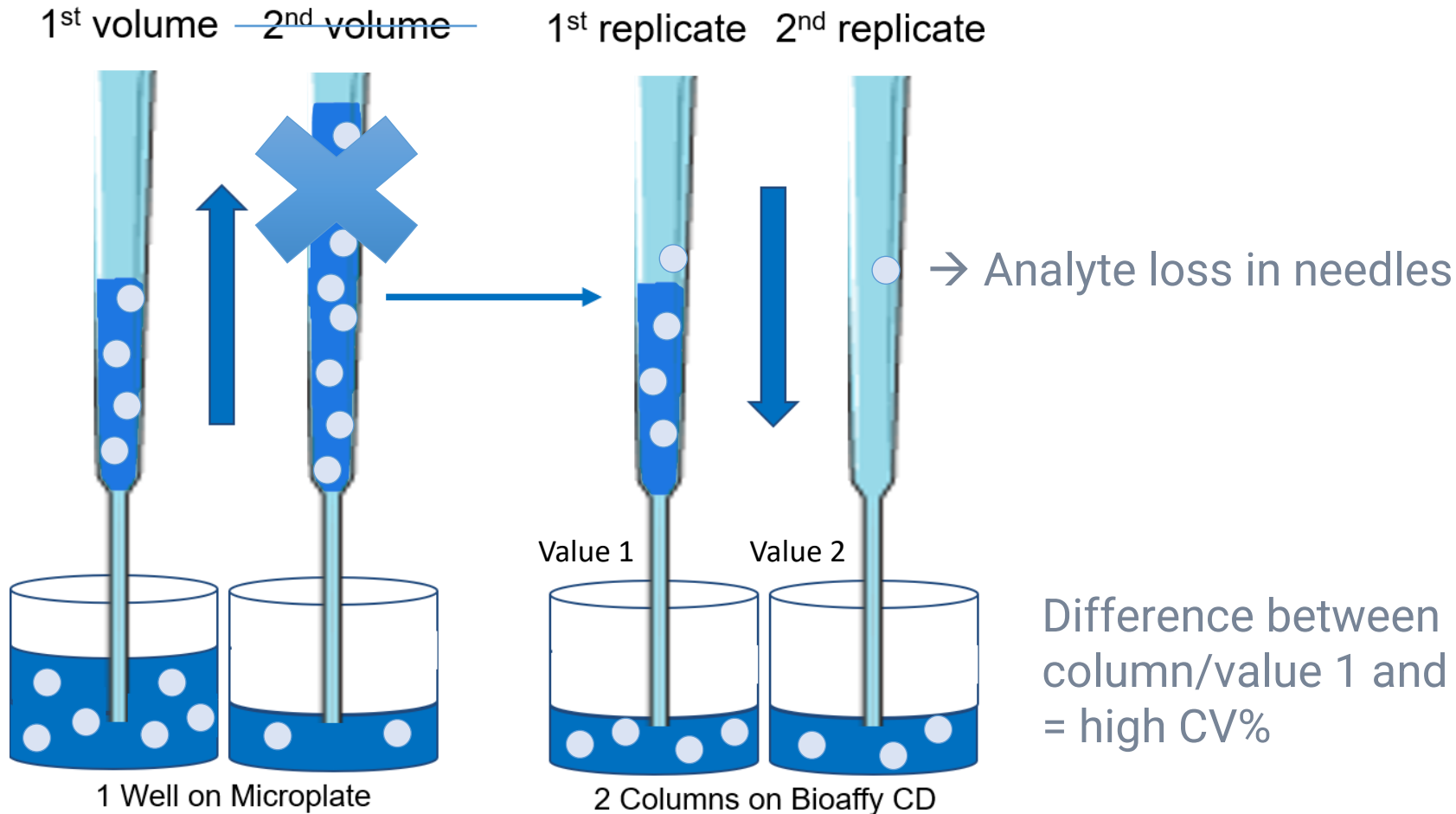


Need of re-validation



How to deal with it – the short-term solution

Pipetting scheme for duplicates



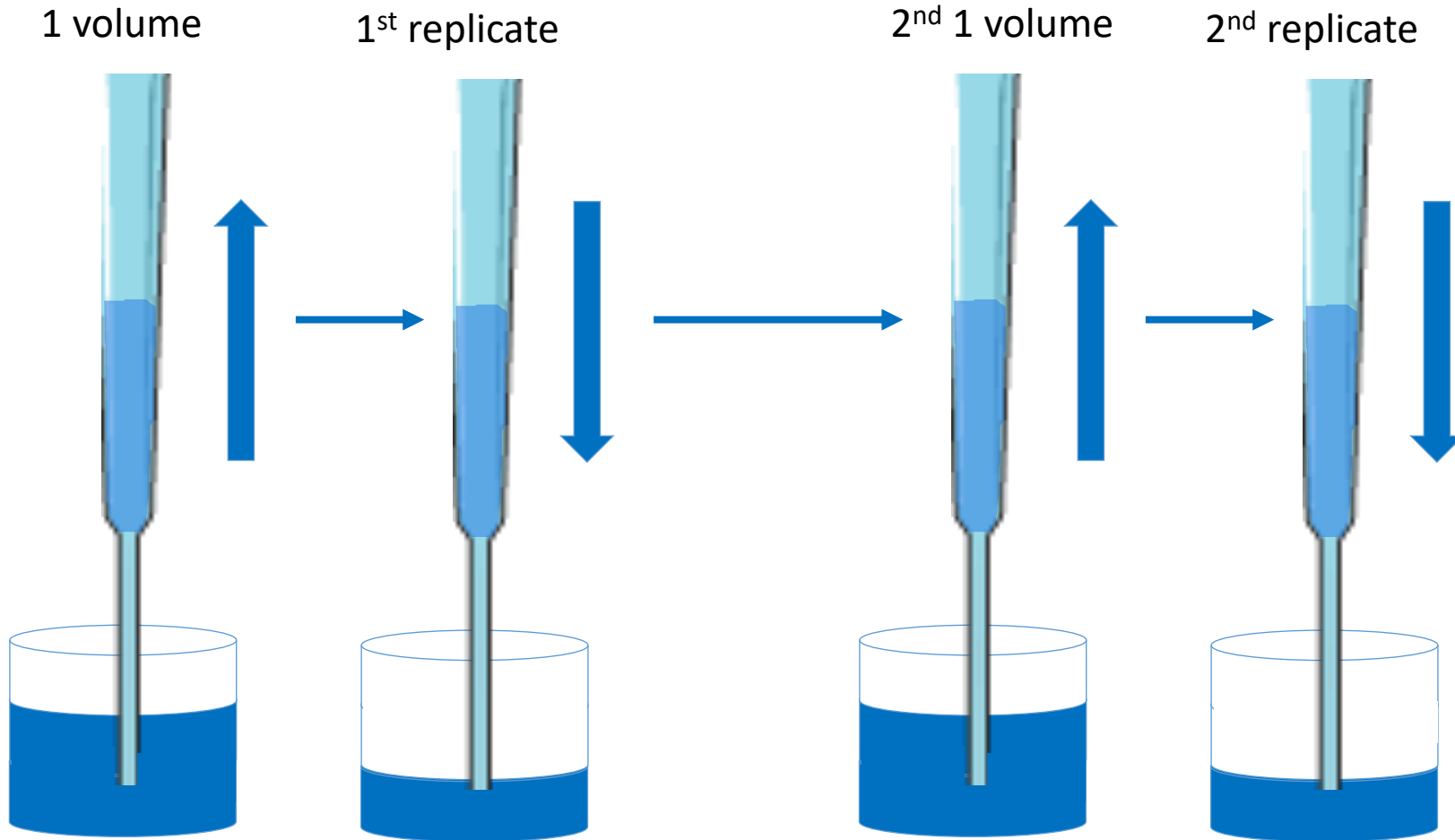
Difference between column/value 1 and 2 = high CV%



→ Interaction between analyte and needles needs to be reduced!



How to deal with it – the short-term solution



Gyrolab Loading List

MP-1

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9			
B	10	11	12	13	14	15						
C	16	17	18	19	20	21	22	23	24	25	26	27
D	28	29	30	31	32	33	34	35	36			
E	37	38										
F	39	40	41	42	43							
G												
H												

MP-1

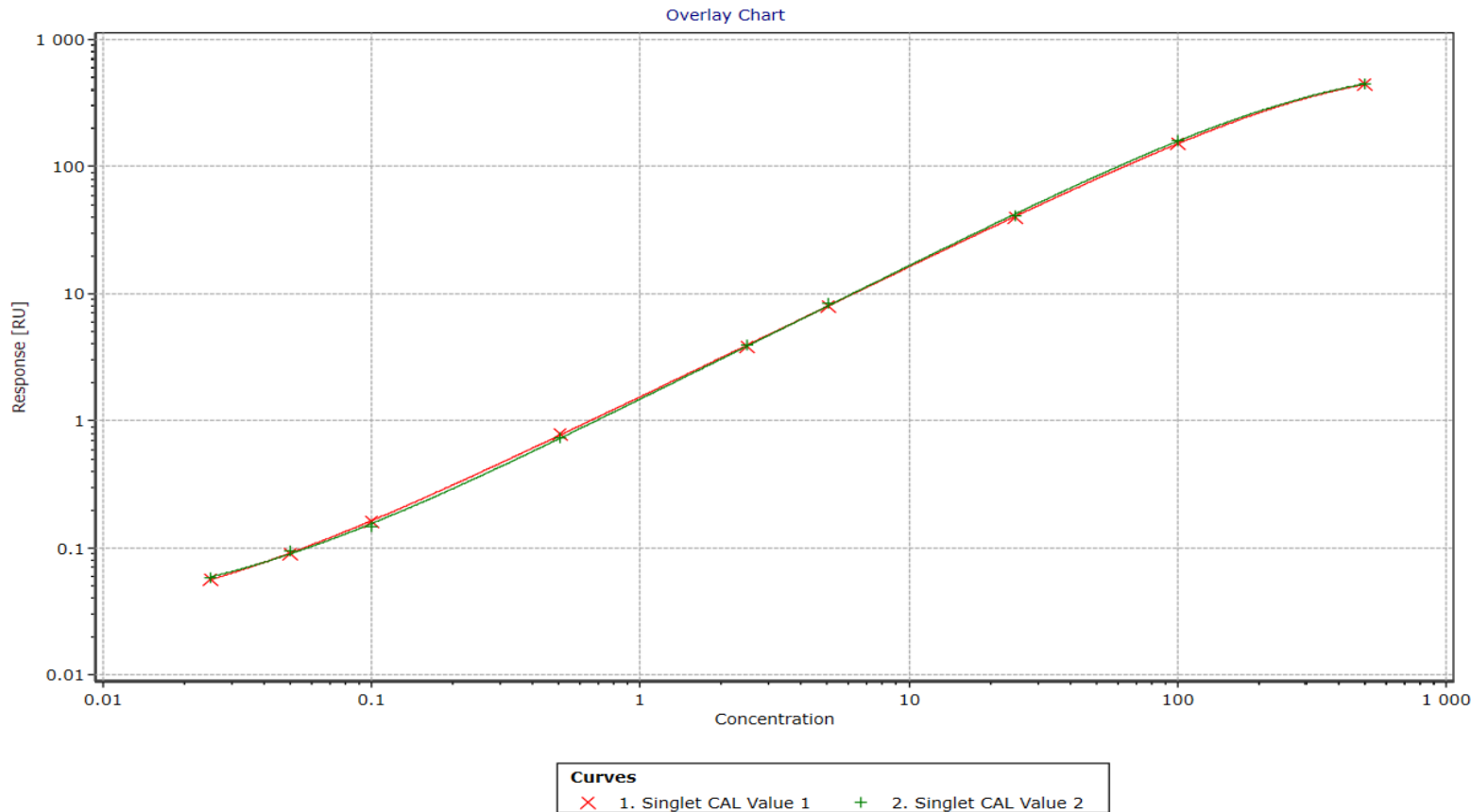
	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10		
B	11	12	13	14	15	16	17	18	19	20		
C	21	22	23	24	25	26						
D	27	28	29	30	31	32	33	34	35	36	37	38
E	39	40	41	42	43	44	45	46	47	48	49	50
F	51	52	53	54	55	56	57					
G	58	59										
H	60	61	62	63								

- Blank
- Standard
- QC
- NC/PC



How to deal with it – the short-term solution

Two singlet calibration curves were combined to have a regular one with duplicates:



- Overlay with previous calibration curves showed good results!
- Quality controls were acceptable!



How to deal with it – the long-term solution

Short-term solution was fine for the moment to finish sample analysis, but...

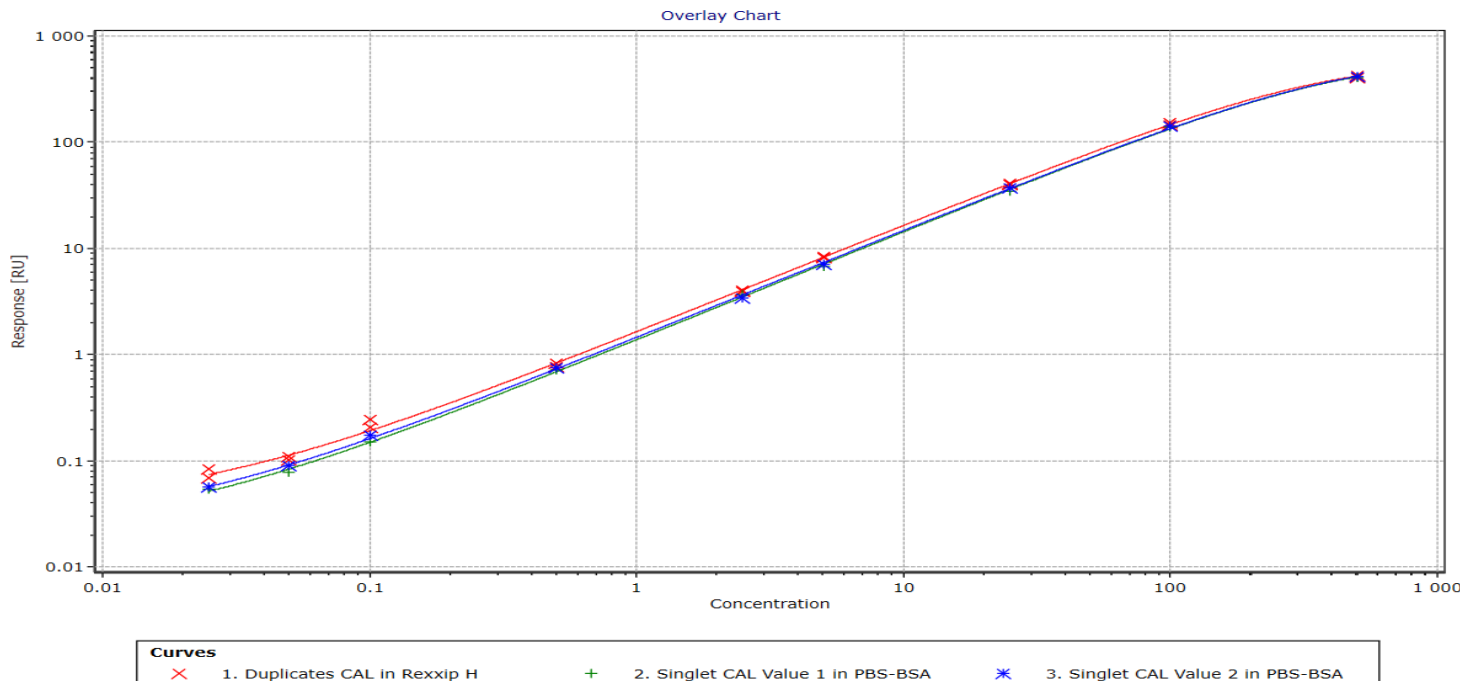


Gyrolab device needs more time to pipette samples



More material is needed which isn't sustainable (and more expensive)

→ Search for a new surrogate matrix! Requirement: results must be comparable!



→ REXXIP H was identified.
 → Following sample analysis was performed with REXXIP H as surrogate matrix without having any CV% issues!



Lessons learned/what should be emphasized?

- Check your data for trends – even if they are small!
- Check your column profiles.
- Test for carry-over.
- Perform your maintenance.
#lagom (Swedish for “just the right amount”)



THANK YOU FOR YOUR ATTENTION!

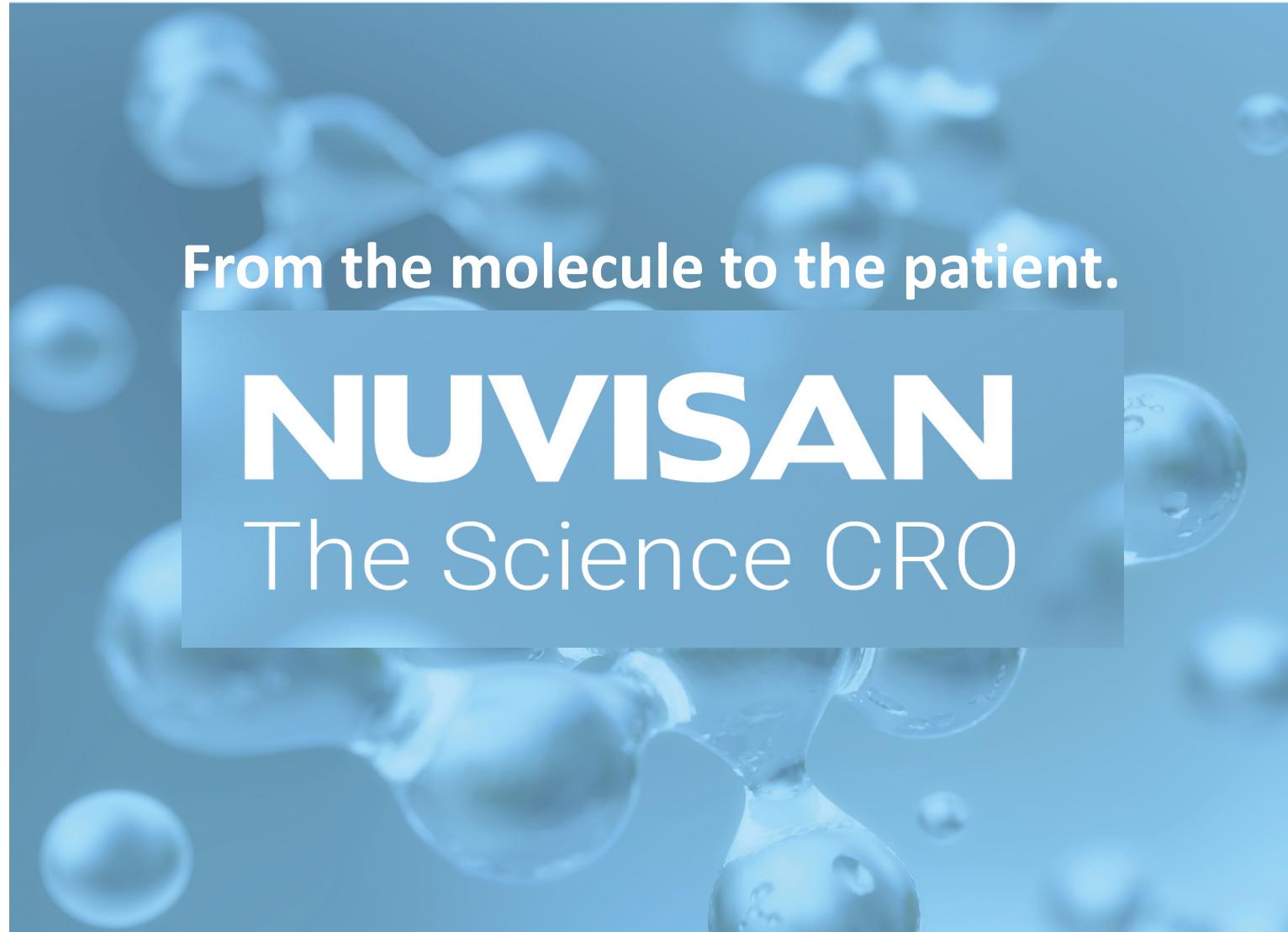
Any questions?

Feel free to ask!

Acknowledgement

Dr. Michaela Golob, Dr. Uwe Kärcher,
Jennifer Müller, and Ilona Stelzer
Nuvisan, Department Immunoassays

Gyros Support
(especially Dr. Linda Klauss,
Jan Dussel, and John Chappell)
Gyros Protein Technologies



From the molecule to the patient.

NUVISAN
The Science CRO