celerion
Translating Science to Medicine

Singlicate analysis for immunogenicity

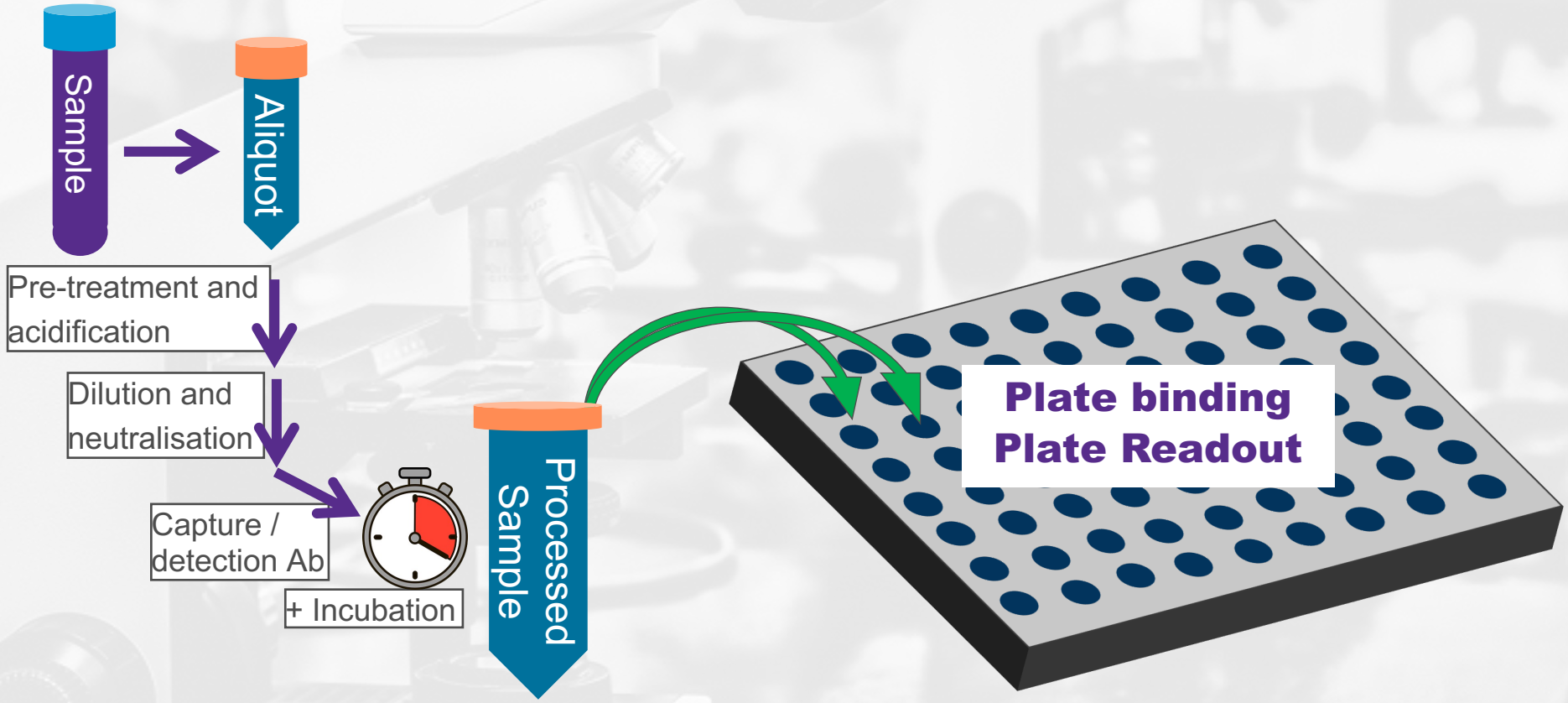
Johannes Stanta, PhD
Global Director Molecular and Cell Biology
Celerion

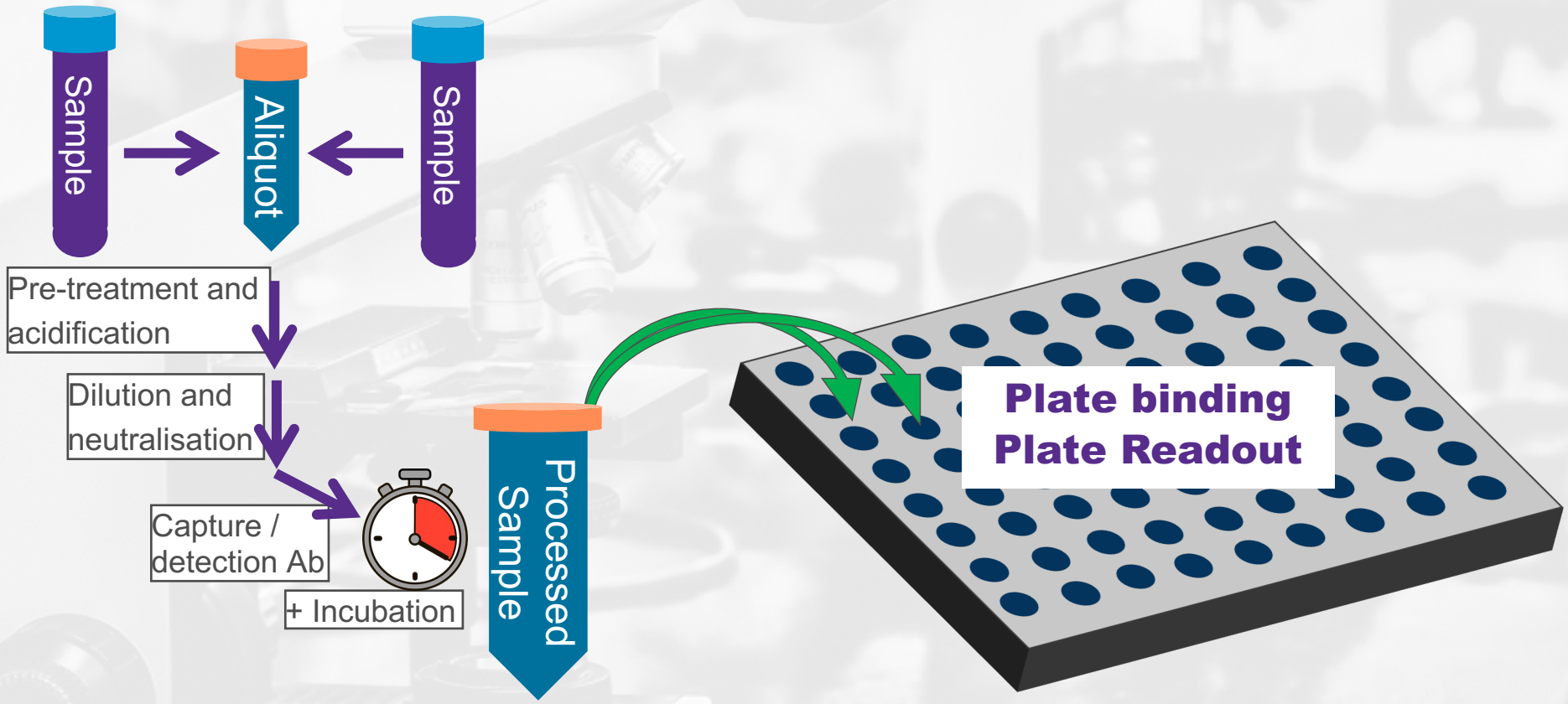
EIP Lisbon - April 2023

Fears and barriers

- Will the regulatory agencies accept it?
  - Yes. There is no mention of replicate use in any immunogenicity guideline
- Do I need to demonstrate in validation that singlicate is as good as duplicate?
  - No. You only need to demonstrate that you have a valid method
- What if the analyst makes an error
  - Duplicate analysis will not detect significant analyst errors

Standard ELISA and MSD methods



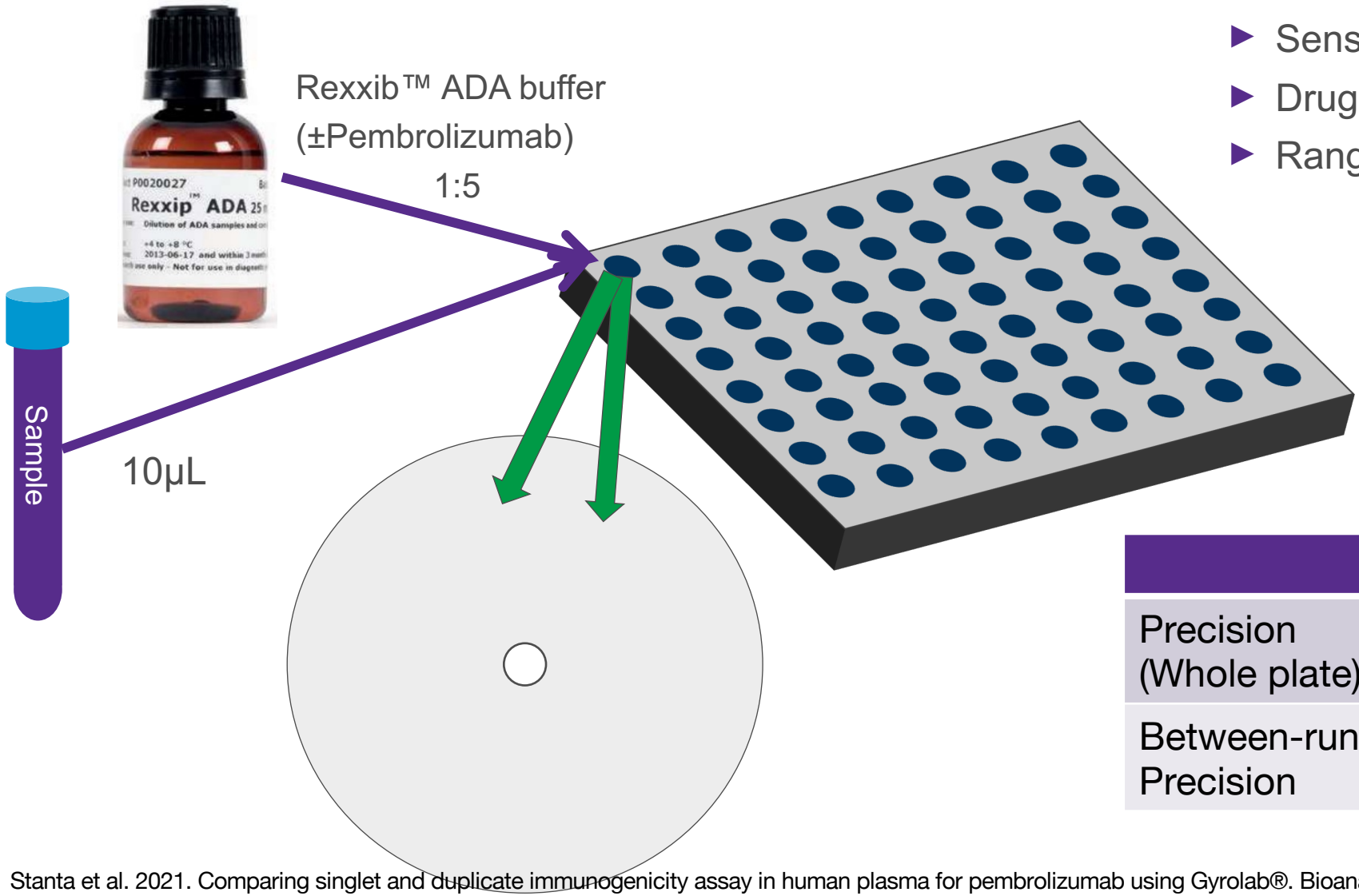

Sample
Aliquot
Sample
Pre-treatment and acidification
Dilution and neutralisation
Capture / detection Ab
+ Incubation
Processed Sample
Plate binding
Plate Readout

Fears and barriers

- We still see %CV failures and need to exclude samples
  - These failures would be picked up anyway (%Bias) or are irrelevant
- We split the sample at the end to avoid too many errors
  - The point and value of duplicate analysis is lost too
- Doing duplicates from the start is too much work
  - A pseudo-duplicate or technical replicate is extra work but no added value

Case study 1

Pembrolizumab ADA
Gyrolab® Mixing CD 96



Method performance characteristics

- Sensitivity: 50 ng/mL
- Drug Tolerance: 1000+ µg/mL
- Range: 50 – 20,000 ng/mL

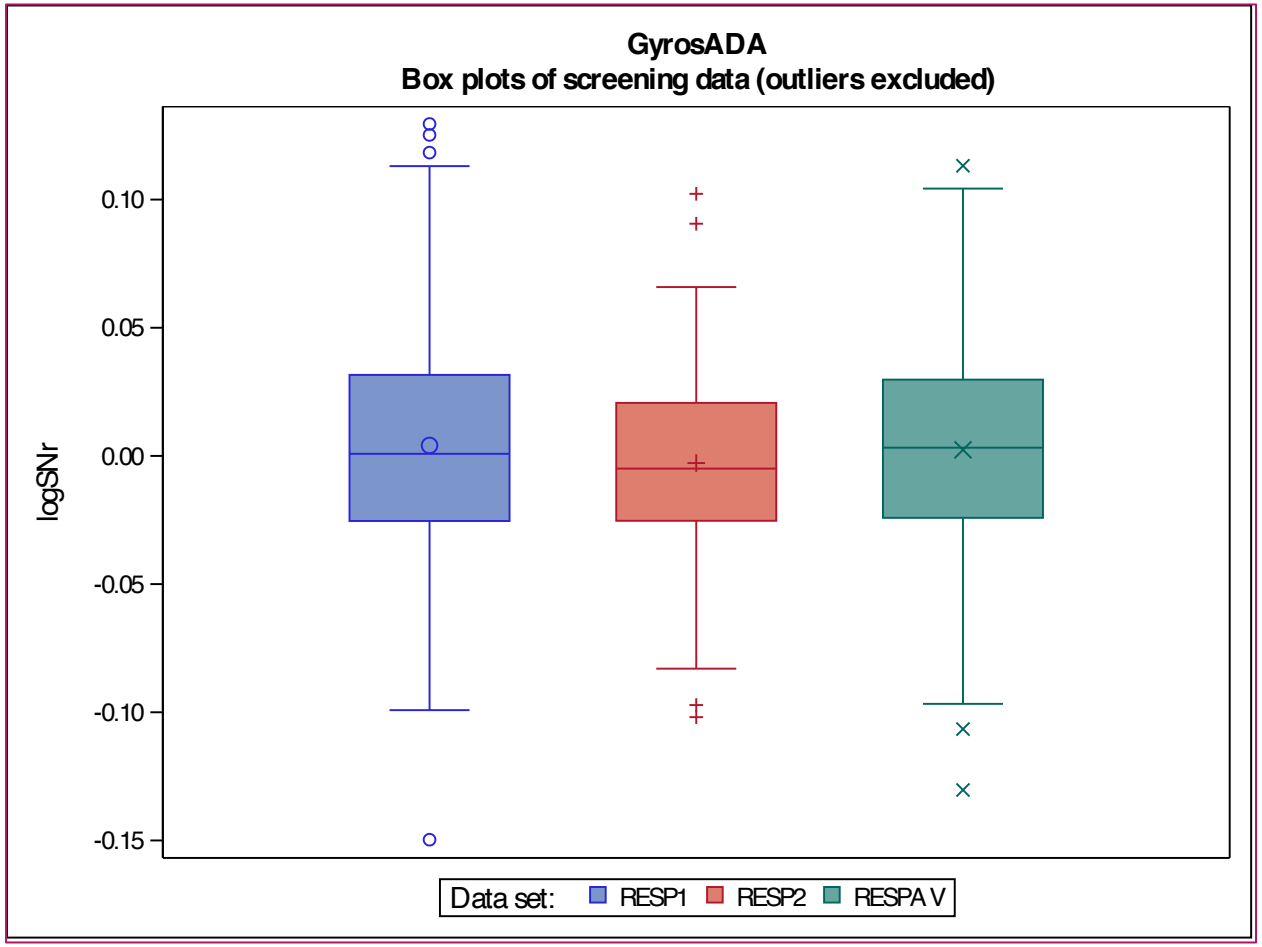
		Avg	Rep 1	Rep 2
Precision (Whole plate)	LPC	4.0%	7.1%	3.4%
	HPC	6.3%	6.0%	7.7%
Between-run Precision	LPC	7.9%	8.0%	8.4%
	HPC	5.6%	5.5%	6.1%

Stanta et al. 2021. Comparing singlet and duplicate immunogenicity assay in human plasma for pembrolizumab using Gyrolab®. Bioanalysis

Cut-point

Screening cut-point assessment with 98 individuals in 2 labs

Dataset	N	Mean	STD	Parametric SCPF
Rep 1	207	0.00408	0.046	1.1997
Rep 2	208	0.00278	0.035	1.1343
Avg	211	0.00239	0.042	1.1787



Singlicate Plate layout for Cut-point assessment

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	Drug + NC	S4	Drug + S4	S12	Drug + S12	NC	Drug + NC	S26	Drug + S26	S34	Drug + S34
B	NC	Drug + NC	S5	Drug + S5	S13	Drug + S13	S19	Drug + S19	S27	Drug + S27	S35	Drug + S35
C	LPC	Drug + LPC	S6	Drug + S6	S14	Drug + S14	S20	Drug + S20	S28	Drug + S28	S36	Drug + S36
D	LPC	Drug + LPC	S7	Drug + S7	S15	Drug + S15	S21	Drug + S21	S29	Drug + S29	NC	Drug + NC
E	HPC	HPC	S8	Drug + S8	S16	Drug + S16	S22	Drug + S22	S30	Drug + S30	NC	Drug + NC
F	S1	Drug + S1	S9	Drug + S9	S17	Drug + S17	S23	Drug + S23	S31	Drug + S31	LPC	Drug + LPC
G	S2	Drug + S2	S10	Drug + S10	S18	Drug + S18	S24	Drug + S24	S32	Drug + S32	LPC	Drug + LPC
H	S3	Drug + S3	S11	Drug + S11	NC	Drug + NC	S25	Drug + S25	S33	Drug + S33	HPC	HPC

Singlicate Balanced design and plate layout

Analyst	Run	Plate	Spl 1 - 18	Spl 19 - 36	Spl 37 - 54
Analyst 1	Run 1	1	X	X	
		2	X		X
		3		X	X
	Run 2	1		X	X
		2	X	X	
		3	X		X
Analyst 2	Run 1	1	X	X	
		2	X		X
		3		X	X
	Run 2	1		X	X
		2	X	X	
		3	X		X

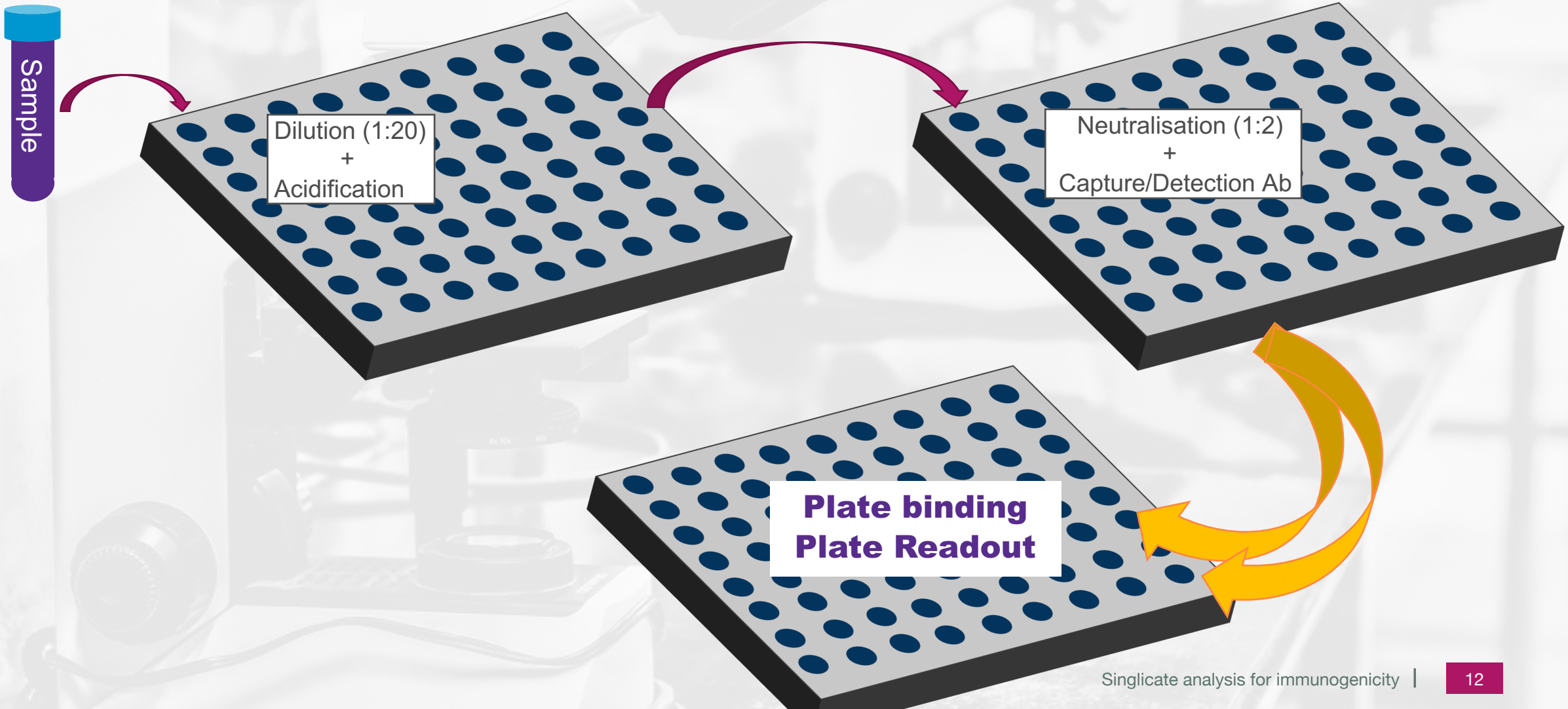
Every sample tested twice by every analyst in every run

Case study 2 – anti-mAb ADA

Electrochemiluminescence Assay

Validation and Sample analysis

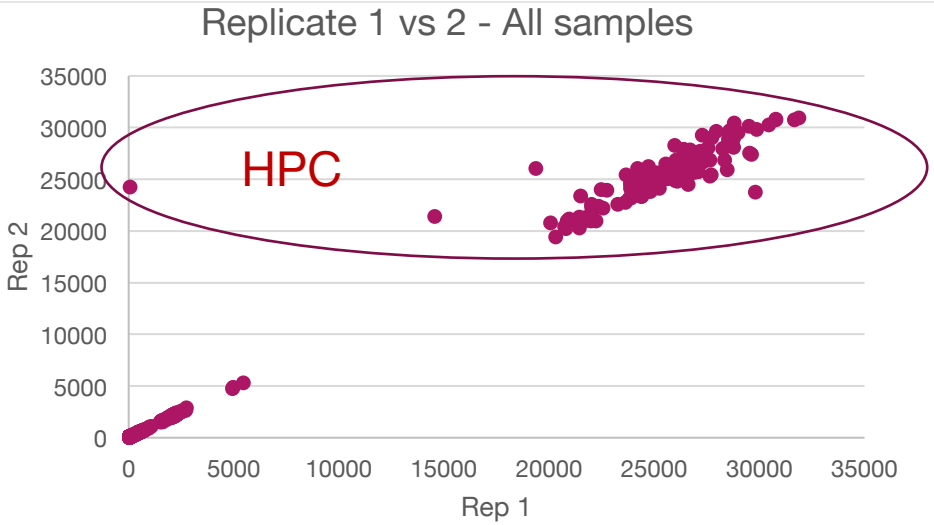
Standard MSD method

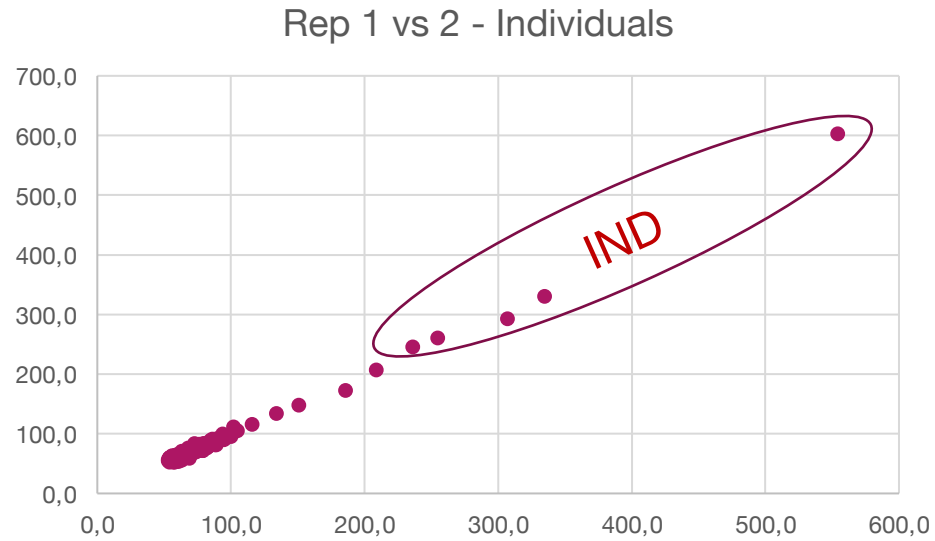


Validation – Mean vs singlicate

Dataset	N	Mean	sCF	tCF	iCP
Rep 1	306	0.120	1.2467	1.4944	8.8
Rep 2	306	0.136	1.2363	1.4762	10.6
Avg	306	0.130	1.2292	1.4583	8.8

		Avg	Rep 1	Rep 2
Between-run Precision	HPC	6.5	6.1	7.0
	MPC	5.1	4.8	5.5
	LPC	5.4	5.2	5.7
	NC	12.8	12.6	13.0





Sample Analysis - Screen

- Samples Screened: 337

	Mean	Rep 1	Rep 2
Negative	309	310	308
Positive	28	27	29
%Reactive	9.1%	8.7%	9.4%
%CV	2.5% (0 – 14.4%)		

- Screen: Discrepancy

Sample ID	Mean Response	%CV	AVG	Rep 1	Rep 2
Patient 1 8 month	125	6.8	negative	negative	reactive
Patient 2 4 month	118	3.6	reactive	reactive	negative
Patient 3 1 month	108.5	0.7	negative	negative	reactive
Patient 4 12 month	120	1.8	reactive	negative	negative
Patient 5 12 month	107.5	0.7	negative	negative	reactive

Confirmed Samples

Sample ID	Mean Response	%CV	Screen			Confirmation		
			AVG	Rep 1	Rep 2	AVG	Rep 1	Rep 2
Patient 1 8 month	125	6.8	negative	negative	reactive	negative	negative	negative
Patient 2 4 month	118	3.6	reactive	reactive	negative	negative	negative	negative
Patient 3 1 month	108.5	0.7	negative	negative	reactive	negative	negative	negative
Patient 4 12 month	120	1.8	reactive	negative	negative	negative	negative	negative
Patient 5 12 month	107.5	0.7	negative	negative	reactive	negative	negative	negative

Titer assessment

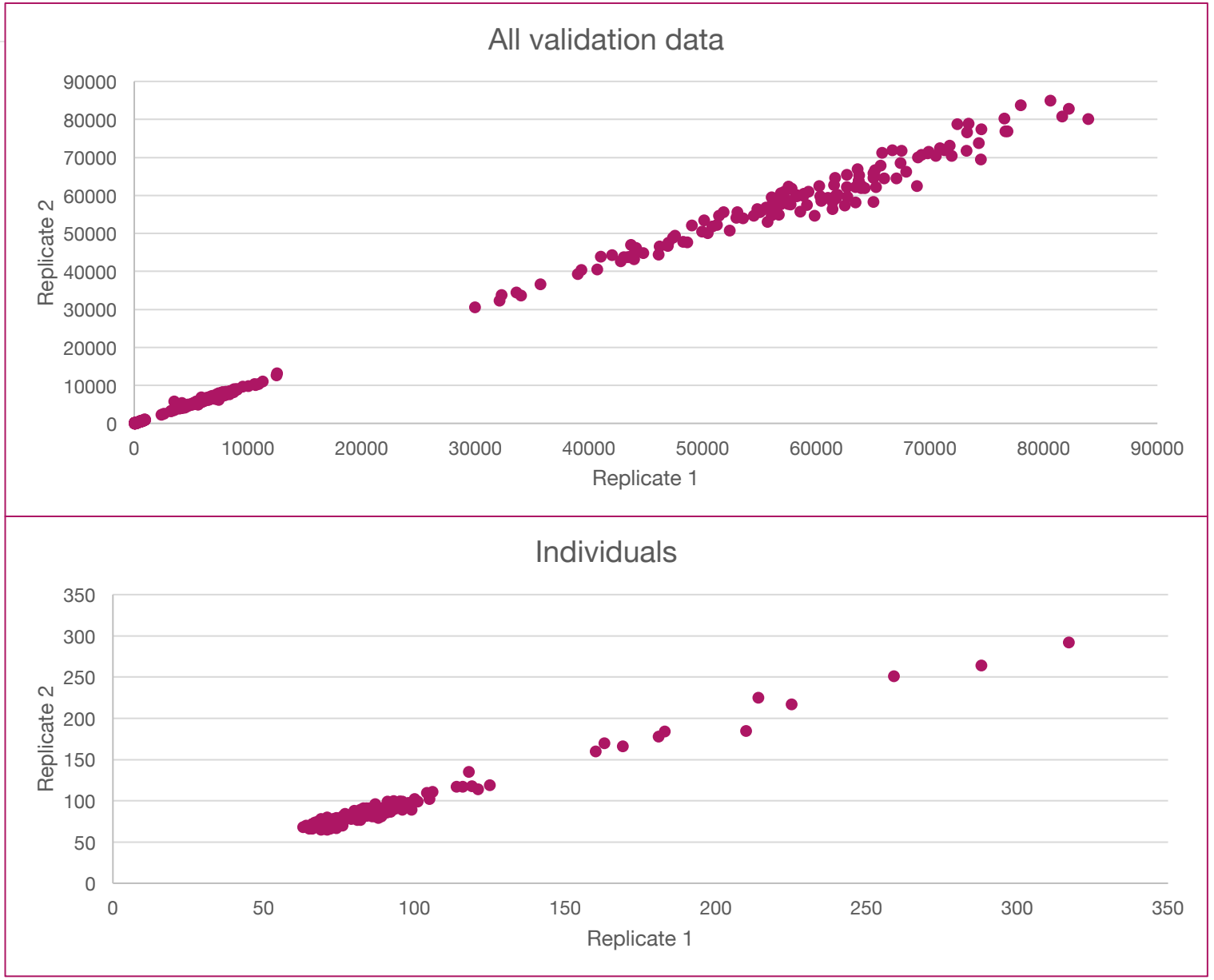
4 samples confirmed positive and were tittered

Sample ID	Average		Replicate 1		Replicate 2	
	Dilution	Titer	Dilution	Titer	Dilution	Titer
Patient A 2 week	2	80	2	80	2	80
Patient A 1 week	5	200	4	160	5	200
Patient B 1 week	5	200	4	160	6	240
Patient C 1 week	7	280	6	240	7	280

Case Study 3 – mAb with ECL

Validation

- %CV average 2.46%
- %CV range 0 – 34.7%
- All data: n = 1987
- Individuals: n = 306



Case Study 3 – mAb with ECL

Sample analysis

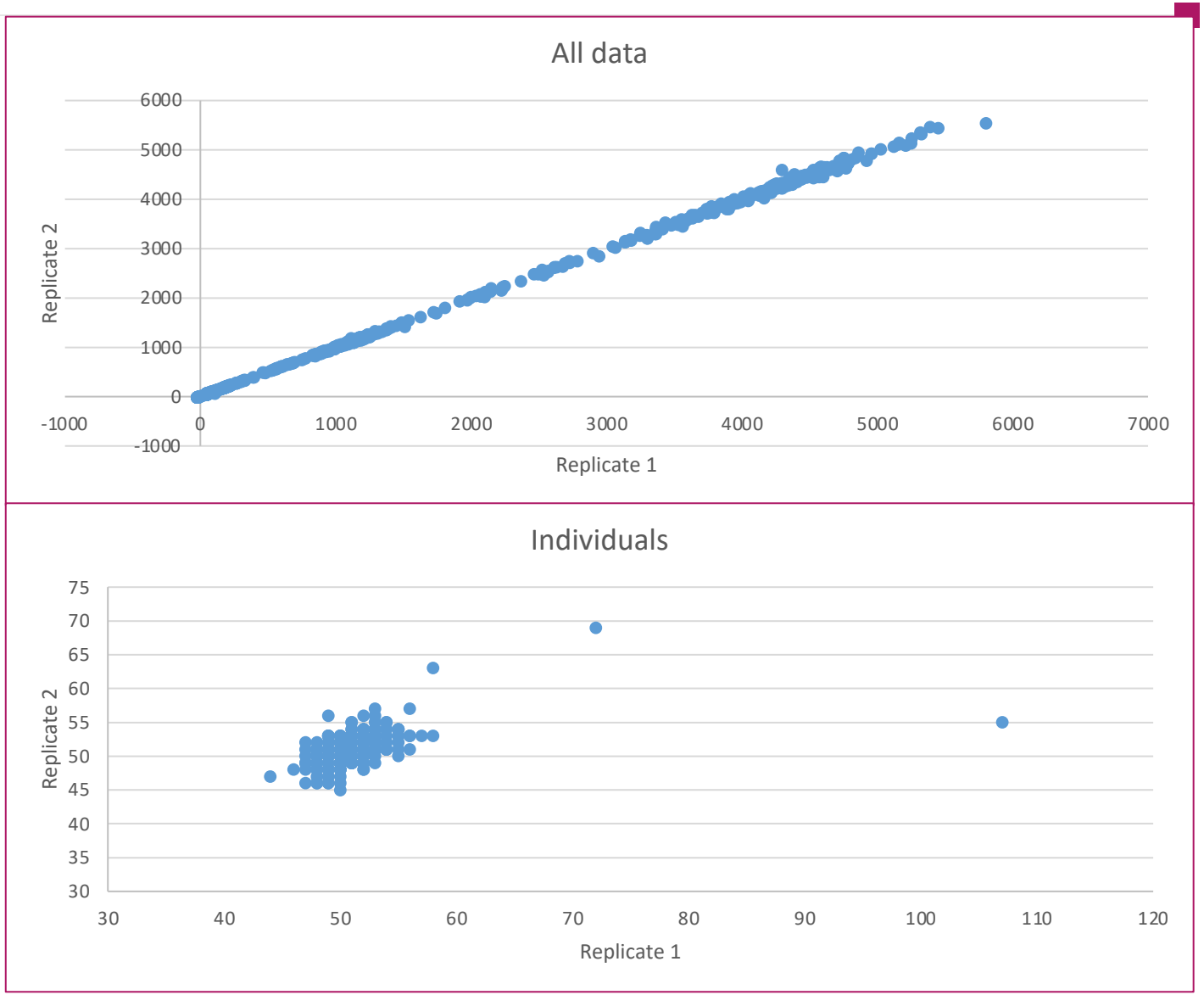
- %CV average: 3.31%
- %CV range: 0 – 140%
- All data: n = 4205

Run	Tier	CP	Replicate 1	Replicate 2	%CV	Comment
20	Conf	iCP 6.4	134	41578	141	Run failed on PCs
			93	2311	130	
			91	151	35	
77	Screen	sCP 77	3246	10057	72	Run failed on PCs
			66	962	123	
			67	360	97	
			104	204	46	
84	Screen	sCP 87	75	130	38	Negative
32	Screen	sCP 78	398	291	22	Positive
			405	272	28	
122	Conf	sCP 74	69	67	2.1	Negative
		iCP 6.4	63	87	22.6	
66	Conf	sCP 75	96	66	26.2	Negative
		iCP 6.4	60	67	7.8	

Case study 4 – Peptide (4 KDa)

Validation

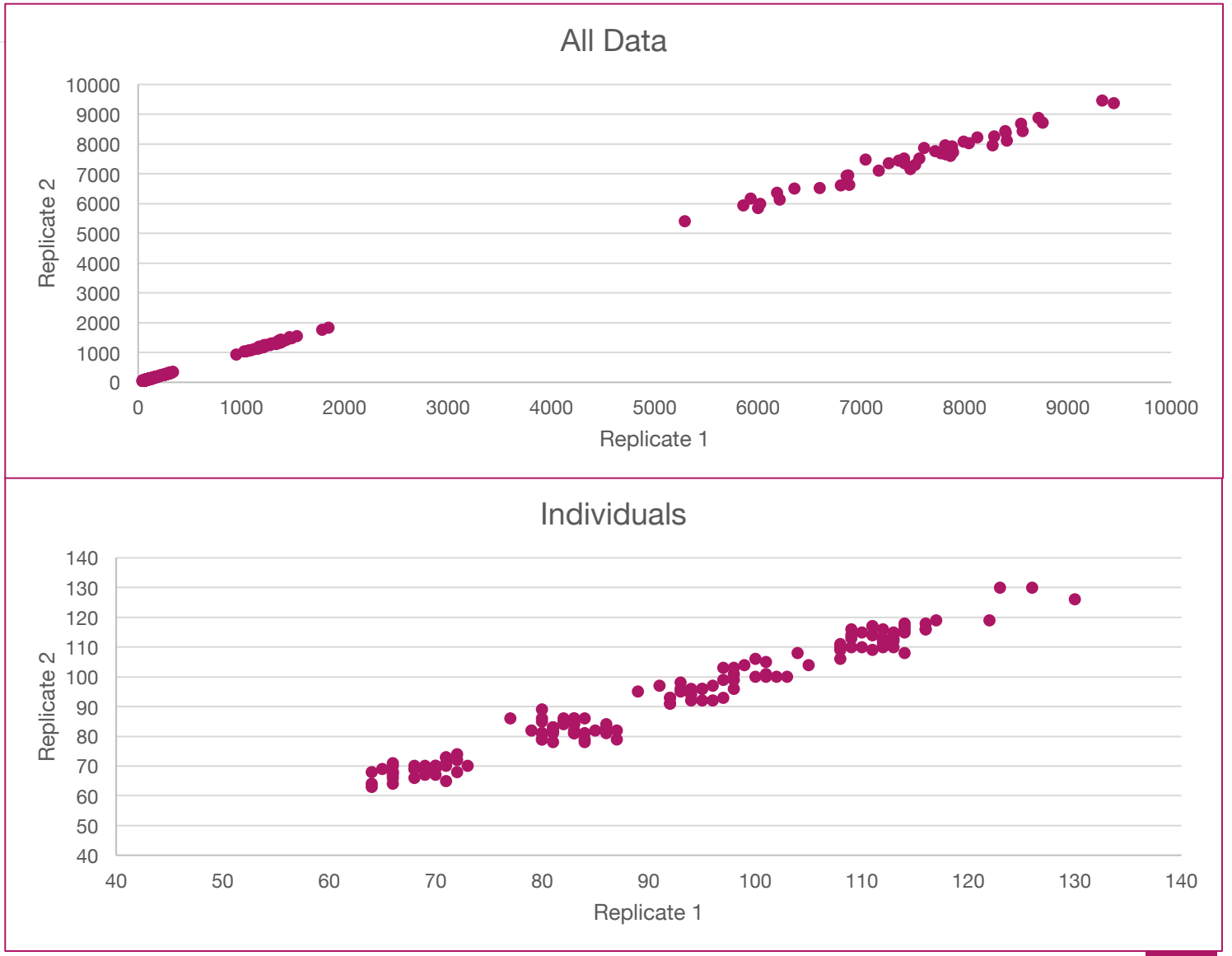
- %CV average 2.00%
- %CV range 0 – 45.4%
- All data: n = 4123
- Individuals: n = 231



Case study 5 – mAb on ECL

Validation

- %CV average 1.87%
- %CV range 0 – 21.6%
- All data: n = 960
- Individuals: n = 145



Recommendation for implementation

New Method

- Start ADA method development in singlicate
- Review data for precision and outliers. Is the assay performance acceptable?
  - Yes -> continue with singlicate
  - No -> will a second measurement fix it?
    - YES: implement duplicate assessment
    - NO: re-develop the assay (start with singlicate again)

Existing Method

- During reagent update (+ve control, new disease population)
- When new cut-point assessment or re-validation is done

Conclusion

- Singlicate analysis works well for ADA assays
- No regulatory requirement to generate 2 measurements from 1 sample
- Every result Confirmed and Titerd
- Efficiency gains are enormous >40%
- Should be a consideration for every method
  - Implementation with other technologies PCR and flow assays

THANK YOU