NIBSC

Harmonising Calibration of Serological Assays for SARS-CoV-2

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Acknowledgments

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Kate Tettmar

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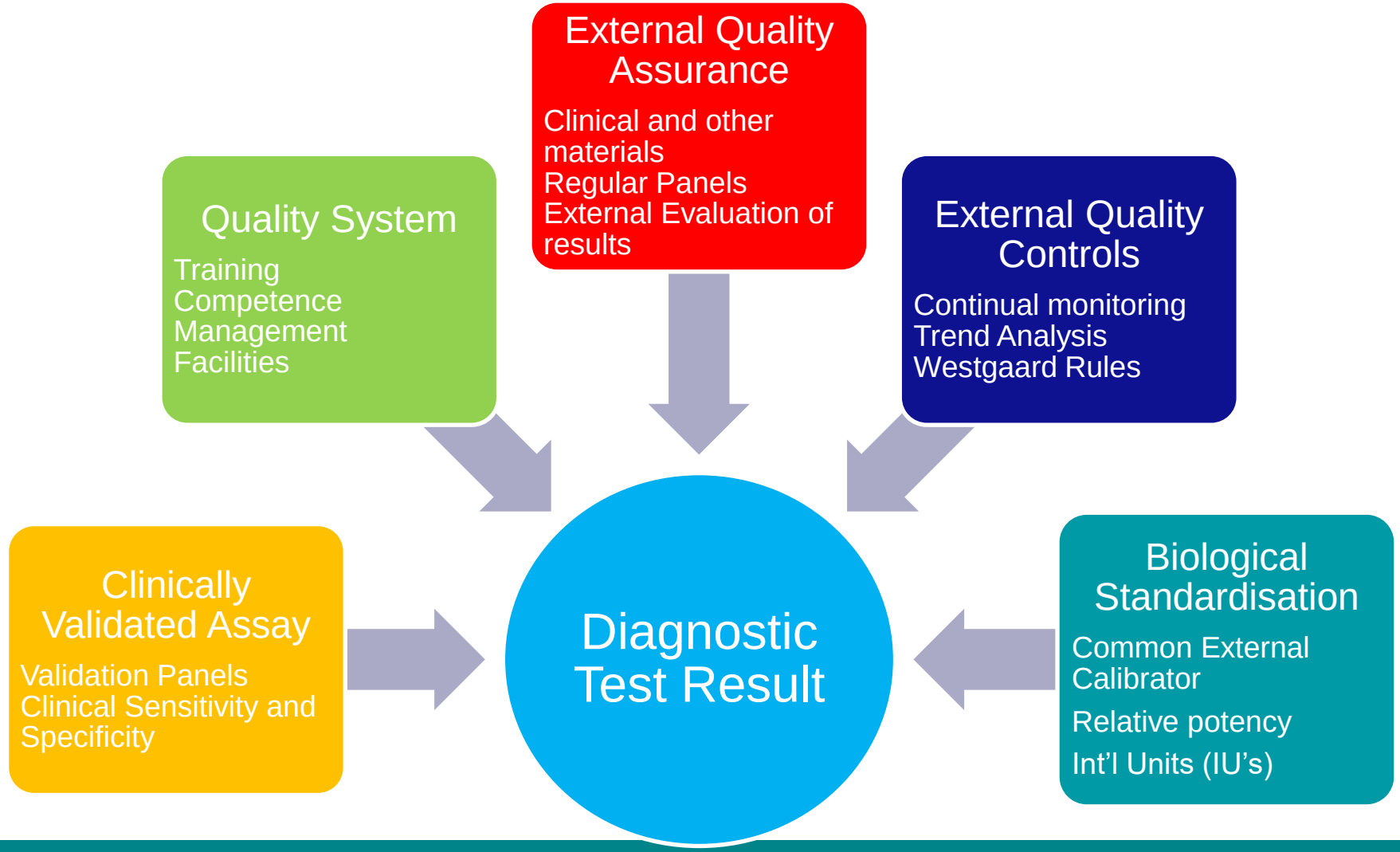
Tim Brooks

Ashley Otter

Abbie Bown

Assuring the Quality of Diagnosis



WHO Established Int'l Standards – SARS-CoV-2

1) 1st WHO IS for SARS-CoV-2 RNA (NIBSC code 20/146)

2) 1st WHO IS for anti-SARS-CoV-2 immunoglobulin, human (NIBSC code 20/136)

3) 1st WHO IRP for anti-SARS-CoV-2 immunoglobulin, human (NIBSC code 20/268)

Available from NIBSC

4) In progress – Reference Standard for SARS-CoV-2 Antigen
   Driven by WHO PQ team for Lateral Flow Devices

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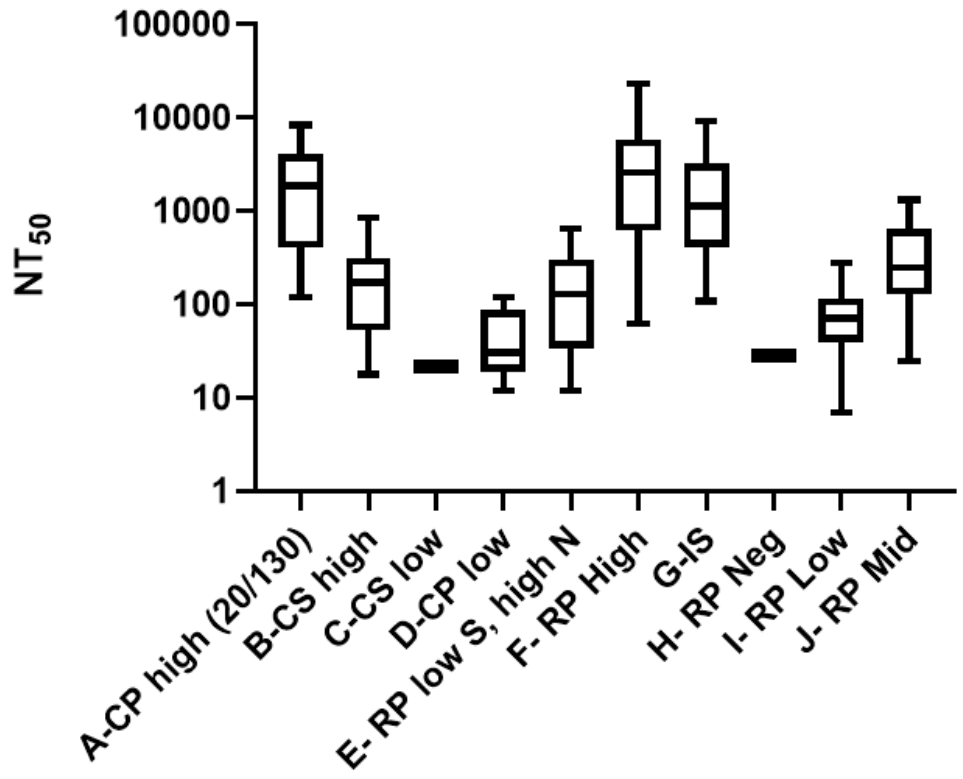
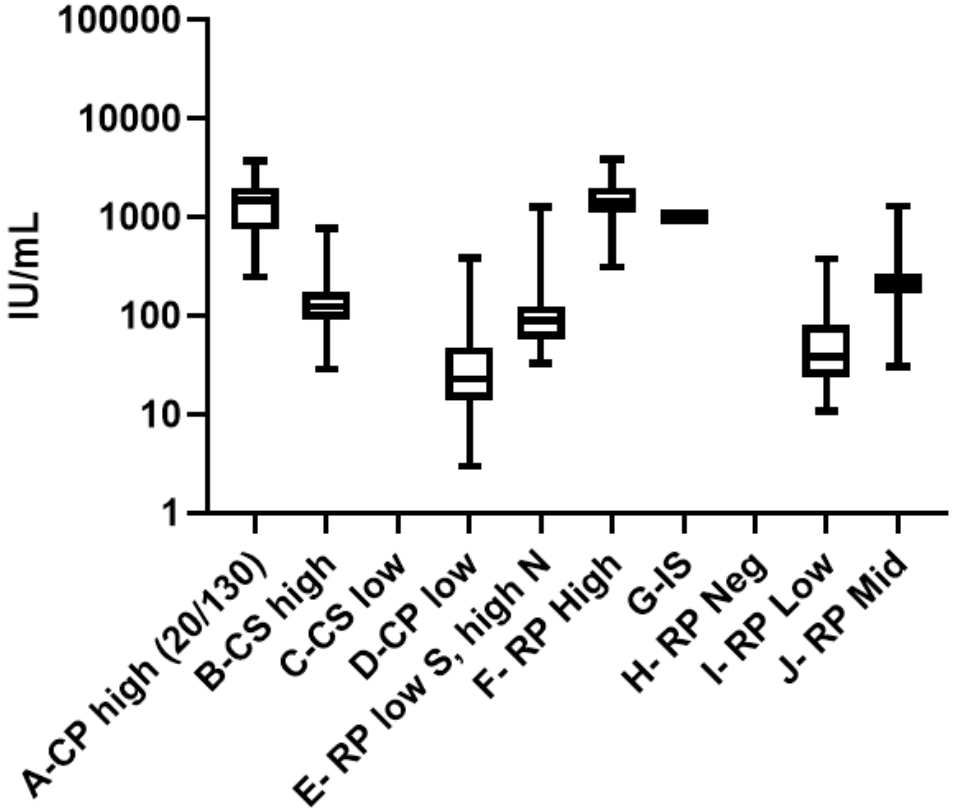
Harmonisation of Neutralisation Assays by 20/136

Data taken from ECBS Report – Giada Mattiuzzo, Emma Bentley,,,,Mark Page

a) 50% Neutralisation Data Returned



b) Calculated Relative Potencies vs G-IS



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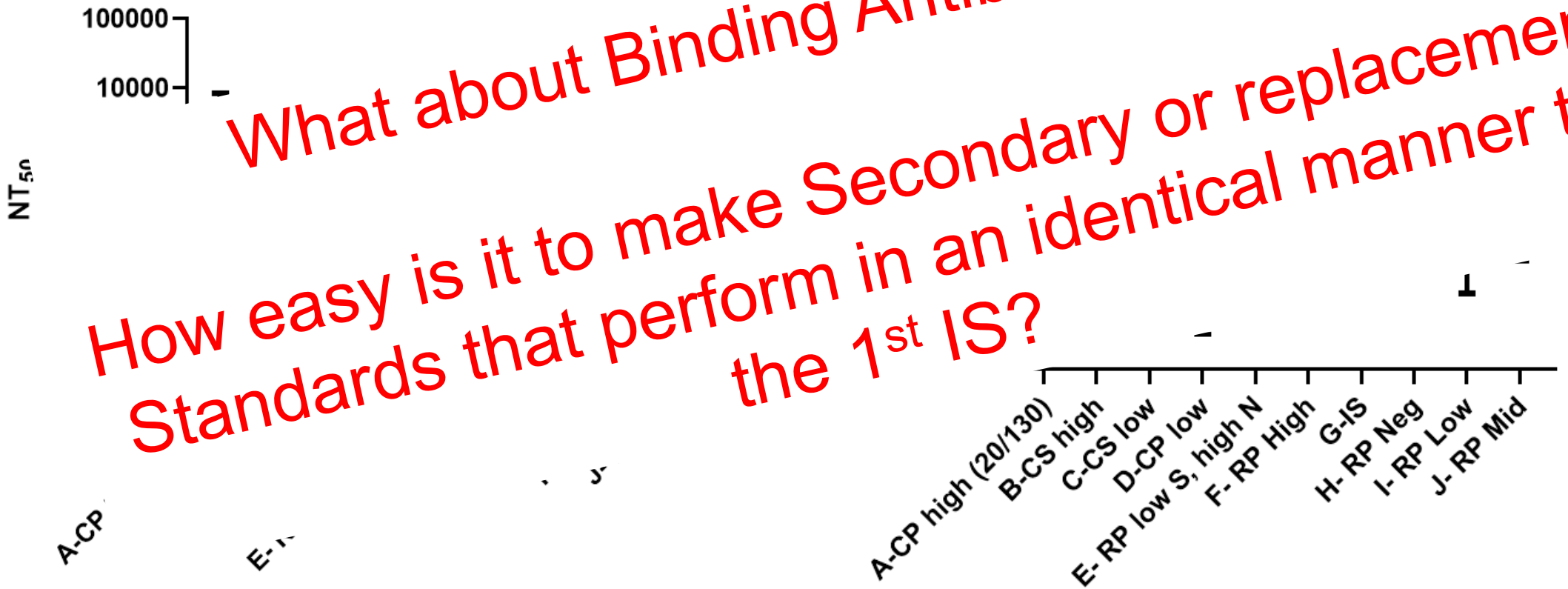

What about Binding Antibody Assays?

How easy is it to make Secondary or replacement Standards that perform in an identical manner to the 1st IS?

Other Reference Materials for SARS-CoV-2 Diagnosis

1) CE Marked Molecular Quality Control (NIBSC code 20/110)
- Low positive run control used for monitoring assay performance

2) CE Marked Serology Control (NIBSC code 20/B764)
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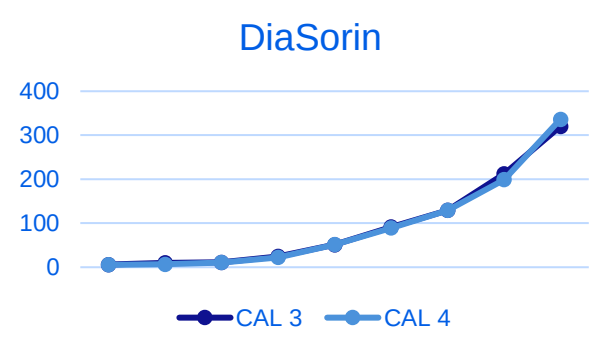
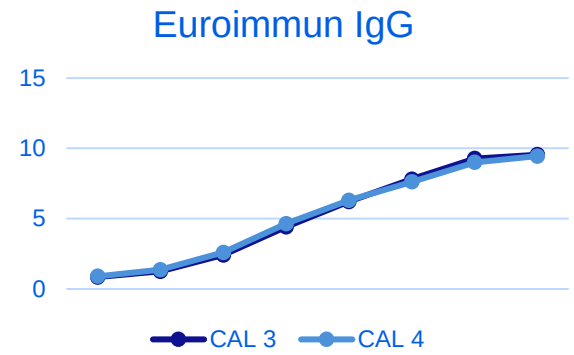
- **Pool of high Ab titre convalescent plasma with low neutralising activity**

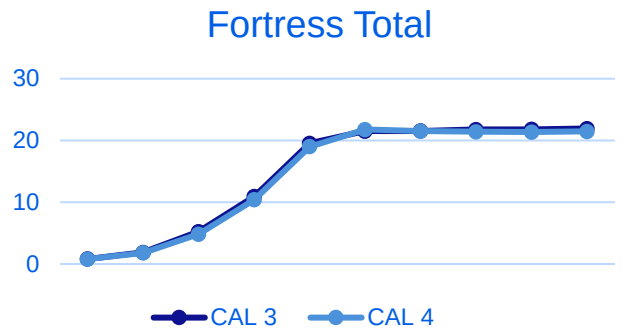
4) Verification Panel for Diagnostic Assays (NIBSC code 20/B770)
- 37 members: 23 +ve & 14 -ve

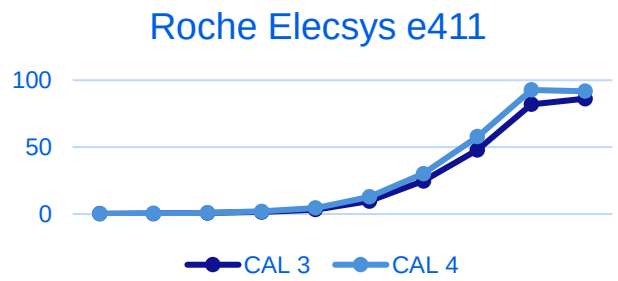
5) Validation Panel for Evaluation of NEW Assays
- 466 members: 266 +ve & 200 –ve
- A common validation panel used in UK DHSC serology assay evaluation process

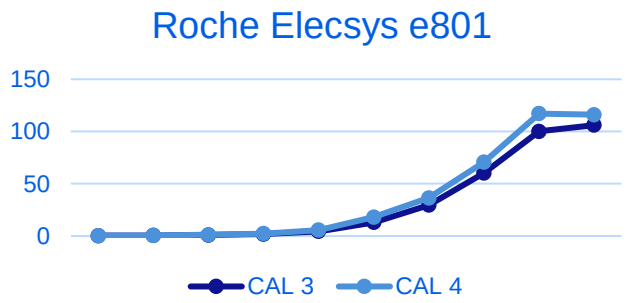
Analysis of NIBSC 20/162 July 2020

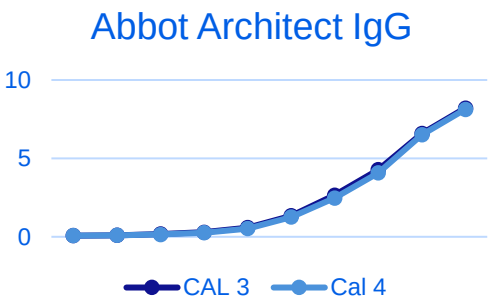


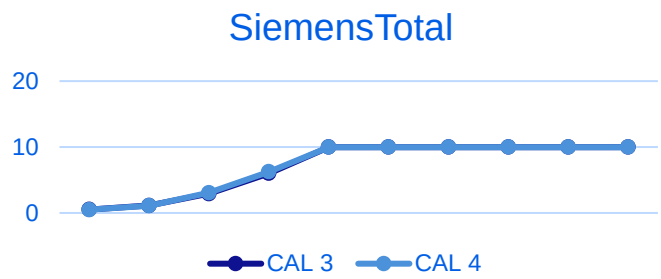


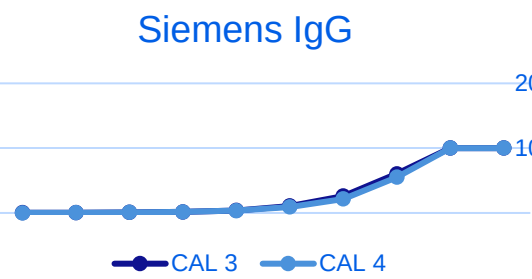


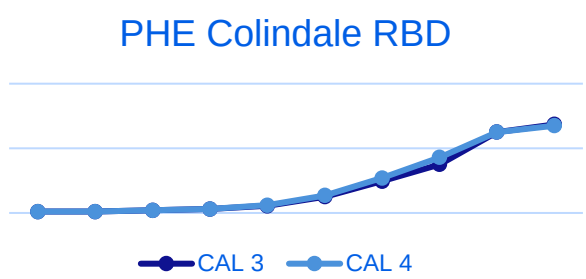












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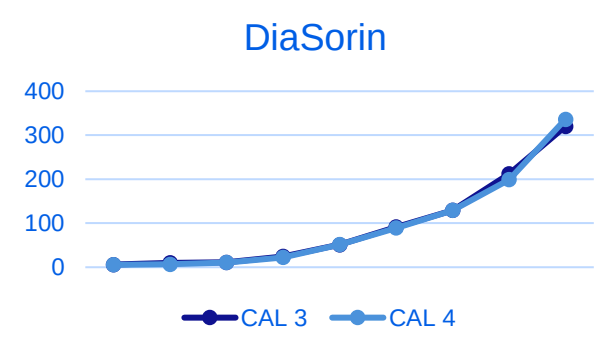
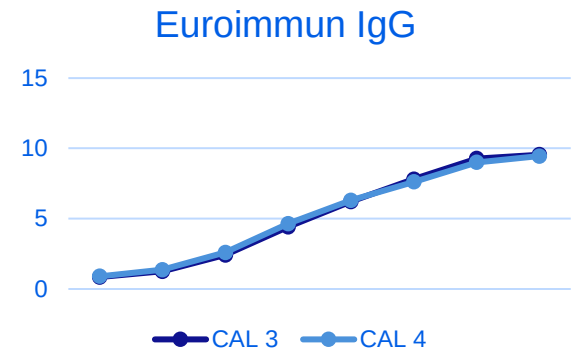
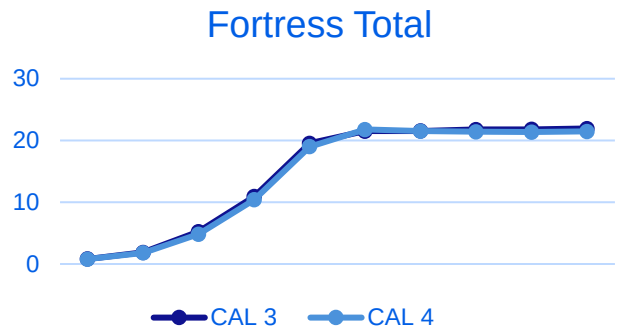
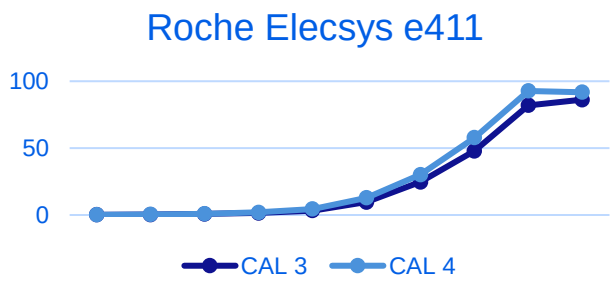
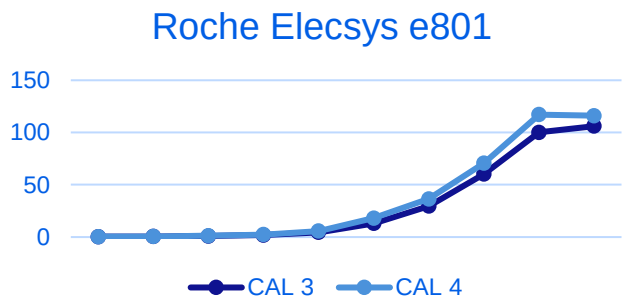
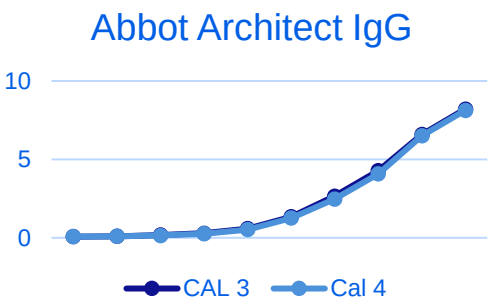


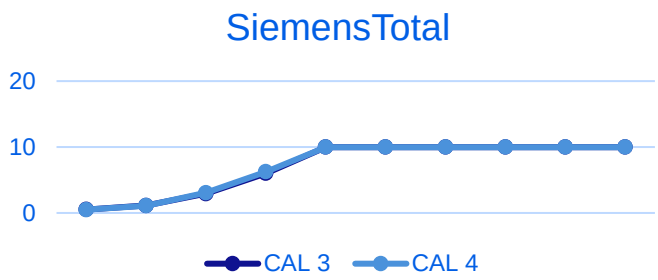


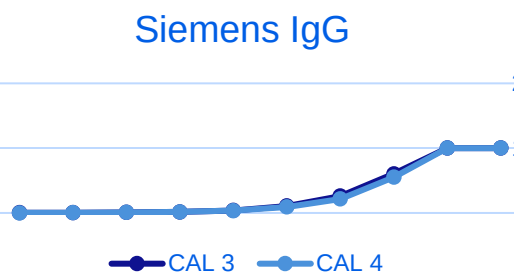


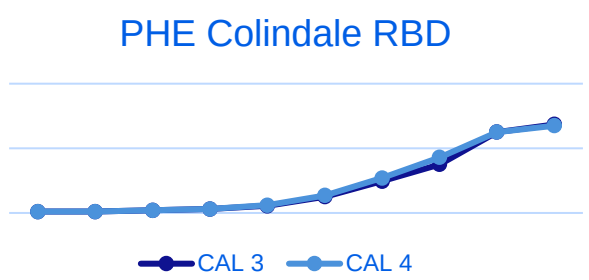












50x difference between LOD of most and least sensitive assays

Commutability of Reference Standards

The capacity of a reference material to perform in an assay (bio-assay, physico-chem measurement or diagnostic assay) in the same manner as clinical samples being measured

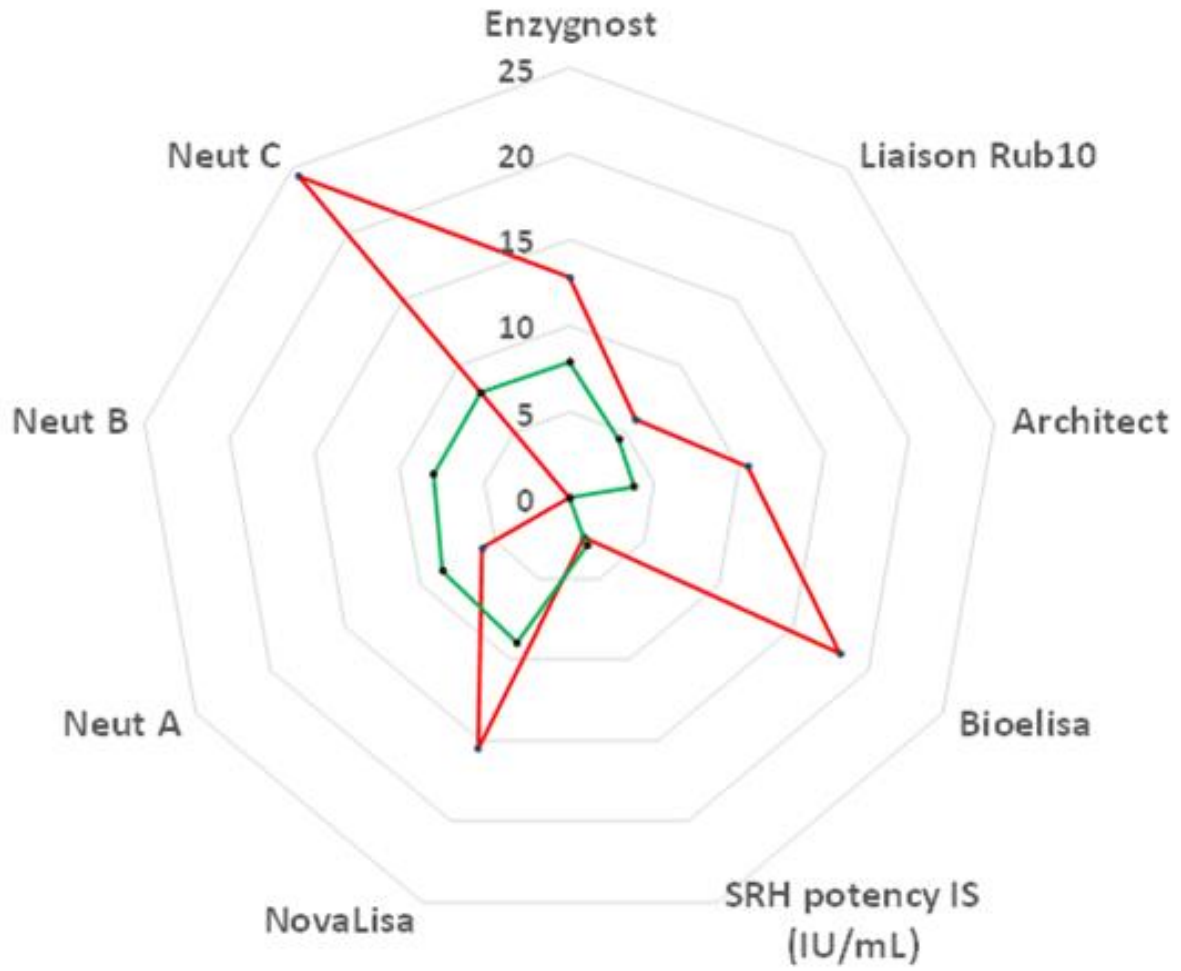
Goal for calibrant materials:

- to quantify the value measurement of clinical samples in a consistent manner.
- the net effect is harmonising measurement of assays performed over time and across space

CHALLENGE for calibrant materials:

- when it is used to calibrate two or more (bio)assays
- Is there a linkage between the measurand of Assay A and Assay B?
- Linkage may be direct (probably immuno-chemical) or indirect (immuno-biological)

Impact of failure to define Specific Assay Target



Spiderplot of anti-rubella sero-reactivity in two human sera measured by 9 different assays

Data supplied by Sarah Kempster

Accurate measurement of 10 IU/ml important when applied as a serological correlate of protection

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What we are doing to address commutability of 20/136

WHO IS for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136

- Prepare a dilution series

Anti-SARS-CoV-2 Antibody Diagnostic Calibrant (NIBSC code:20/162)

- Prepare a dilution series

Validation Panel for Evaluation of NEW Assays

- 466 members: 266 +ve available as individual patient plasma samples

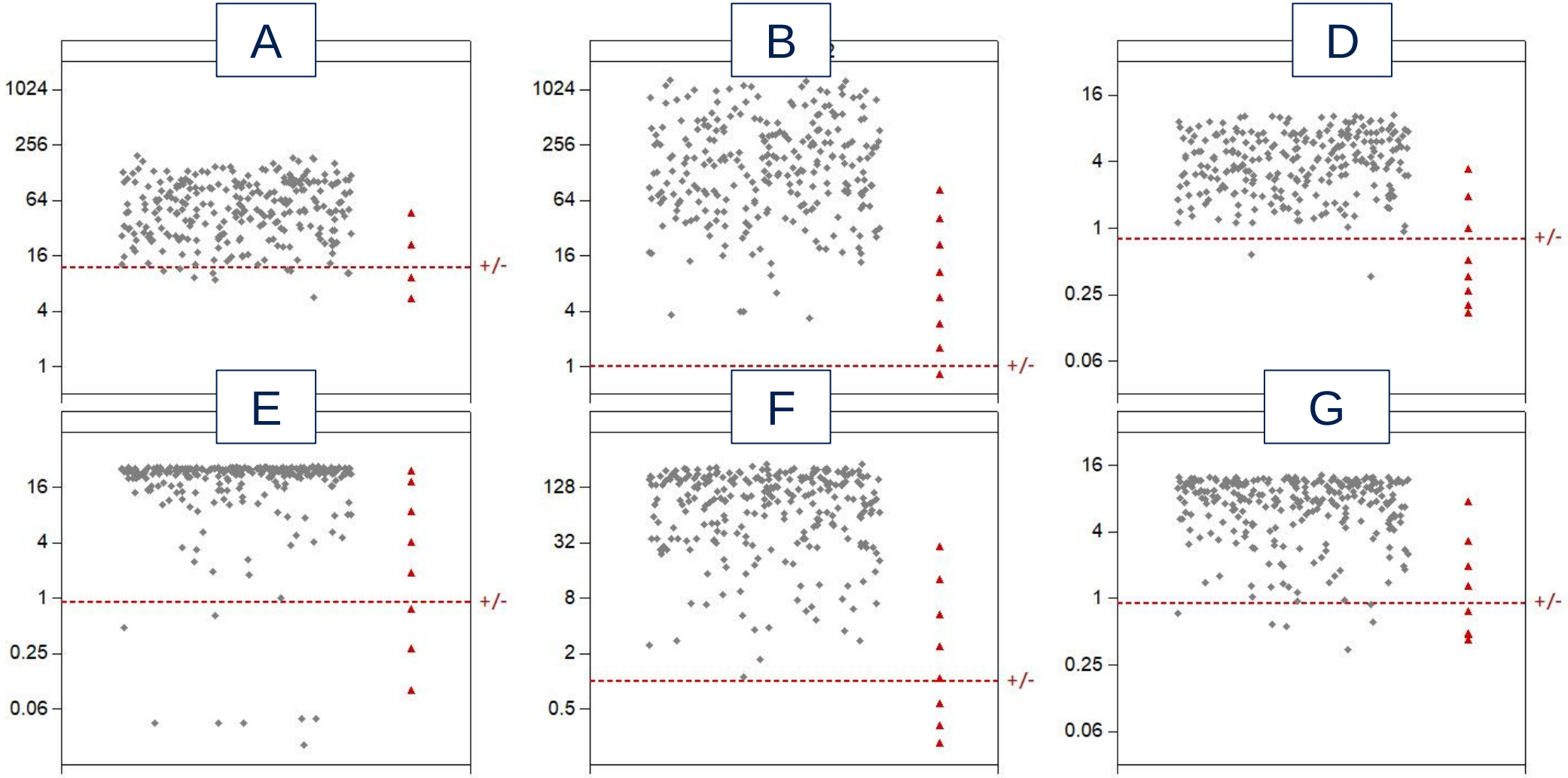
➢ Run all of these materials in as many diagnostic assay platforms as possible

Assays performed & samples tested

Assay	Assay Code	Cut-off	Unitage
DiaSorin Liaison IgG	A - S1/S2	12	AU/ml
Roche Elecsys Spike/RBD	B - S1/S2	1.0	U/ml
DiaSorin Liaison TrimericS IgG	C - S1/S2	12	AU/ml
Euroimmun IgG	D - S1	0.8	OD/CO
Fortress Total	E - N	0.9	OD/CO
Roche Elecsys	F - N	1.0	COI
DiaPro IgG	G - S1/S2/N	0.9	S/CO

- SARS-CoV-2 serum panel samples (n = 266)
- WHO IS for anti-SARS-CoV-2 immunoglobulin (20/136) at 8 dilutions
- NIBSC anti-SARS-CoV-2 diagnostic calibrant (20/162) at 8-10 dilutions

Results overview – 266 sero+ve and IS dilution series



Results for IS 20/136

Dilution	Assay						
	A - S1/S2	B - S1/S2	C - S1/S2	D - S1	E - N	F - N	G - S1/S2/N
	AU/ml	U/ml	AU/ml	OD/CO	OD/CO	COI	S/CO
10	45.1	78.7	48.6	3.32	23.6	28.8	7.35
20	20.2	39.1	23.7	1.86	17.8	12.4	3.26
40	8.84	20.3	11.9	0.951	8.60	5.13	1.89
80	5.21	9.95	5.99	0.497	3.93	2.29	1.26
160	<3.8	5.31	3.27	0.347	1.84	1.04	0.740
320	<3.8	2.76	1.93	0.256	0.732	0.550	0.468
640	<3.8	1.50	<1.85	0.191	0.273	0.316	0.406
1280	<3.8	0.77	<1.85	0.164	0.100	0.206	0.455

Positive	Equivocal	Negative

Results for Diagnostic Calibrant 20/162

Dilution	Assay						
	A - S1/S2	B - S1/S2	C - S1/S2	D - S1	E - N	F - N	G - S1/S2/N
	AU/ml	U/ml	AU/ml	OD/CO	OD/CO	COI	S/CO
Neat	336	2464	>800	9.45	21.5	116	-
2	199	1230	720	9.00	21.5	117	-
5	129	512.7	336	7.61	21.8	70.5	-
10	89.2	245.3	-	6.30	21.4	36.5	-
20	51.3	119.7	75.8	4.64	21.4	16.0	-
50	22.2	46.87	29.6	2.59	19.0	5.58	-
100	10.7	22.40	14.1	1.37	10.4	2.31	-
200	5.44	11.53	7.00	0.898	4.81	1.11	-
500	-	4.403	3.48	-	1.78	0.46	-
1000	-	2.183	1.93	-	0.78	0.27	-

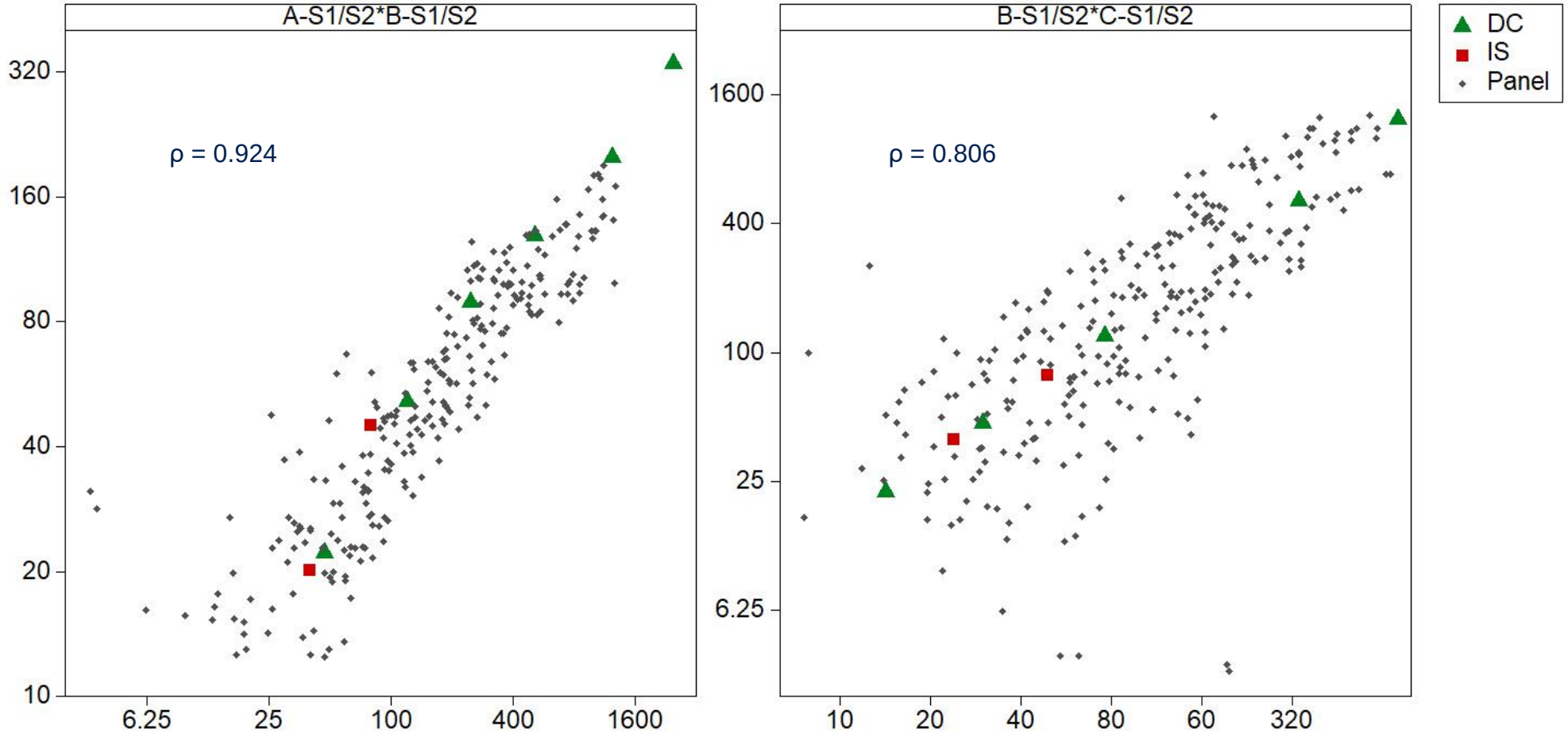
Positive	Negative

Assay correlations

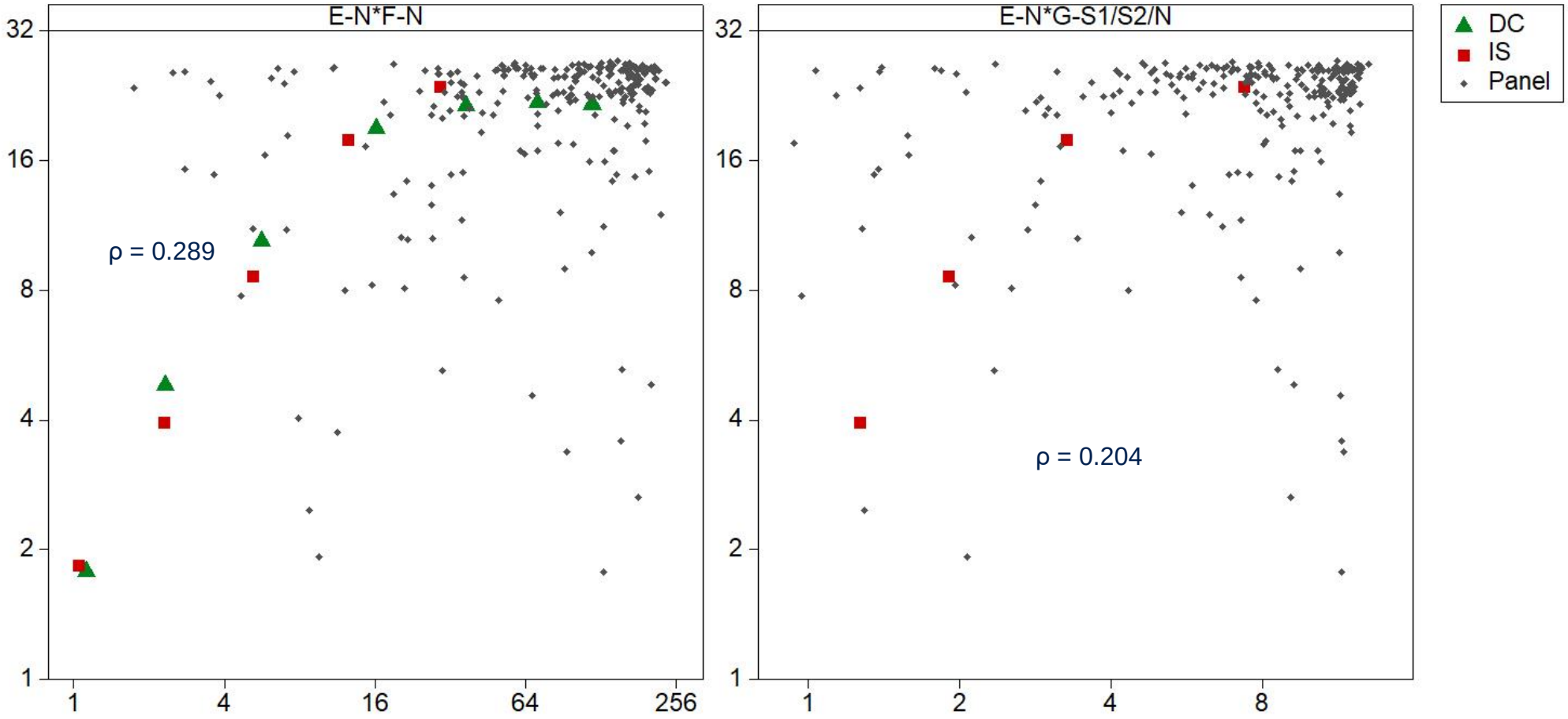
Spearman ρ:

	A - S1/S2	B - S1/S2	C - S1/S2	D - S1	E - N	F - N	G - S1/S2/N
A - S1/S2	1						
B - S1/S2	0.924	1					
C - S1/S2	0.878	0.806	1				
D - S1	0.875	0.781	0.845	1			
E - N	0.402	0.595	0.396	0.392	1		
F - N	0.390	0.444	0.385	0.362	0.289	1	
G - S1/S2/N	0.448	0.404	0.477	0.535	0.204	0.685	1

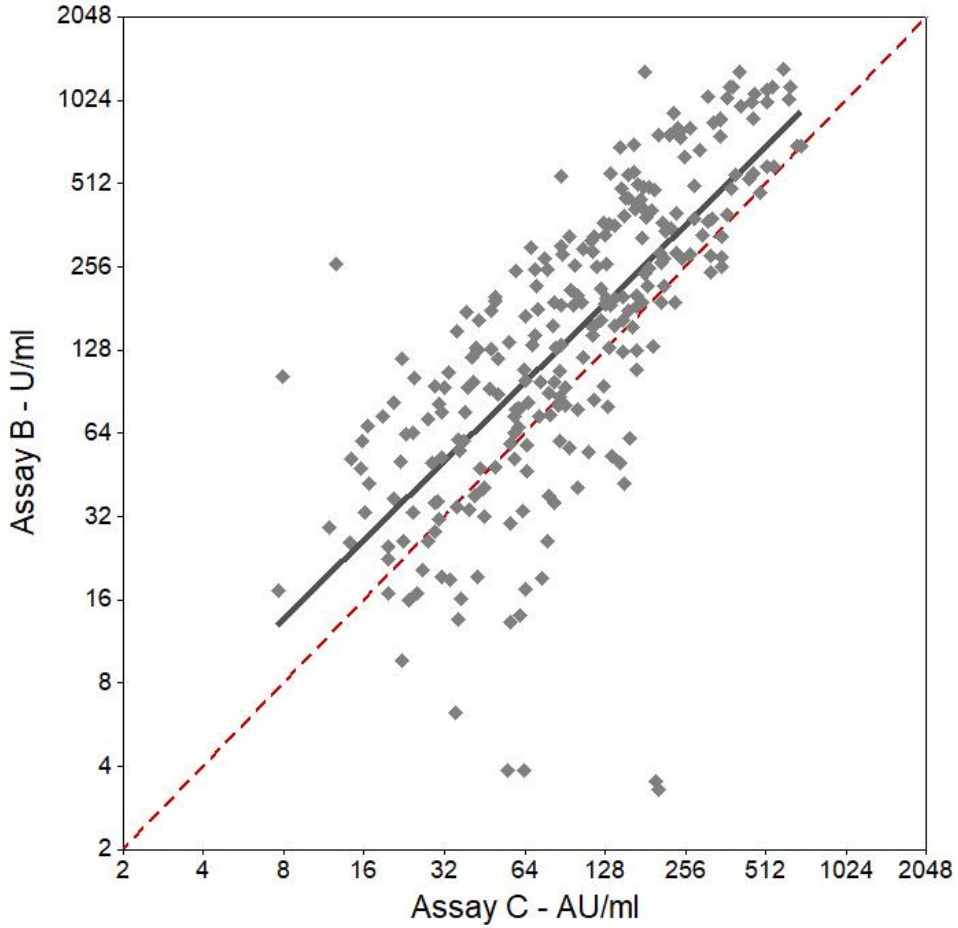
Assay correlations (A,B,C – S1/S2)

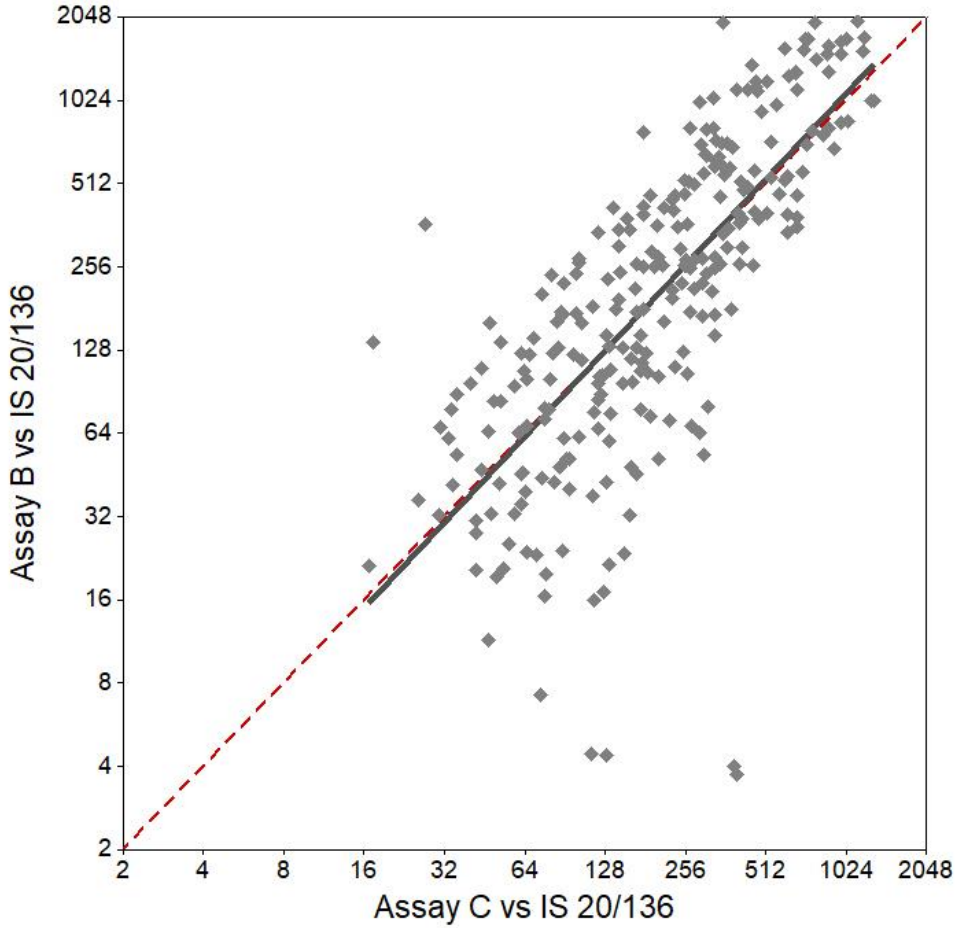


Assay correlations (E – N, F – N, G – S1/S2/N)

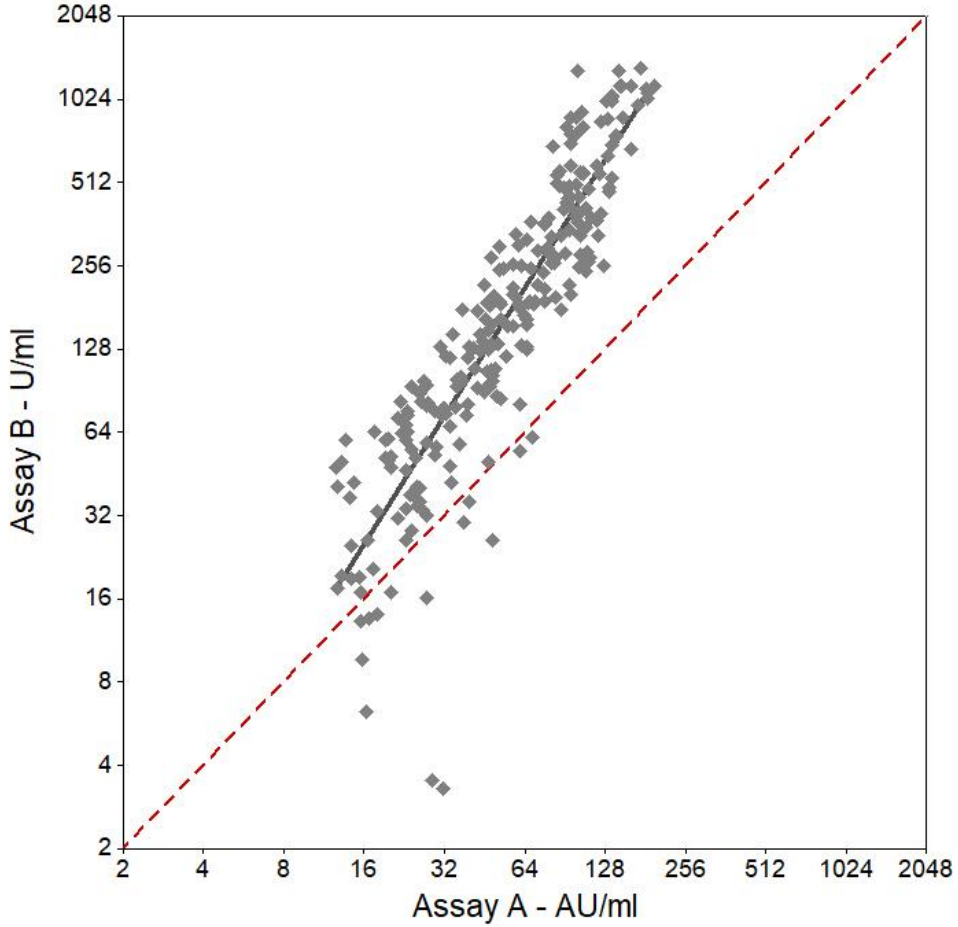


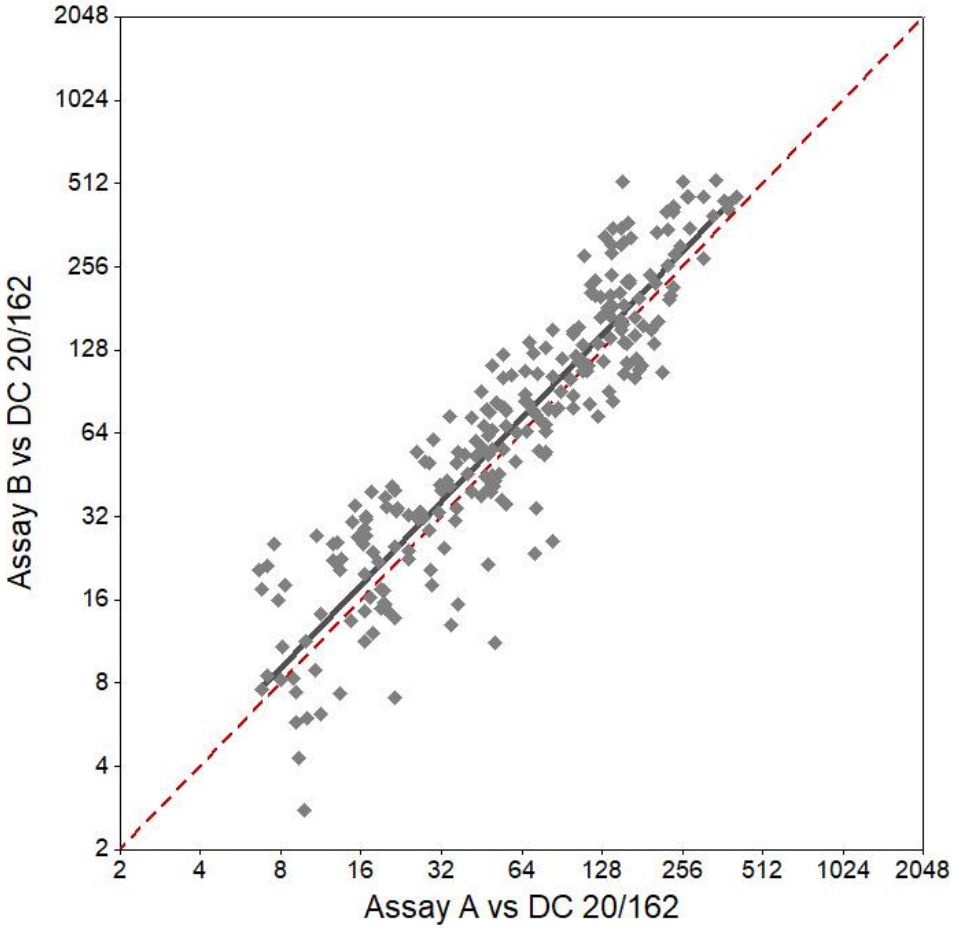
Assay harmonization using IS 20/136 (B,C – S1/S2)





Assay harmonization using DC 20/162 (A,B – S1/S2)





Current Conclusions

Harmonising the quantitative measurement of serological response to viruses by different assays is extremely challenging:

1) An individual's polyclonal antibody response is
   - driven by complex immuno-genetics
   - impacted by prior exposure to related antigens
   - dynamic (acute, convalescent, vaccine)

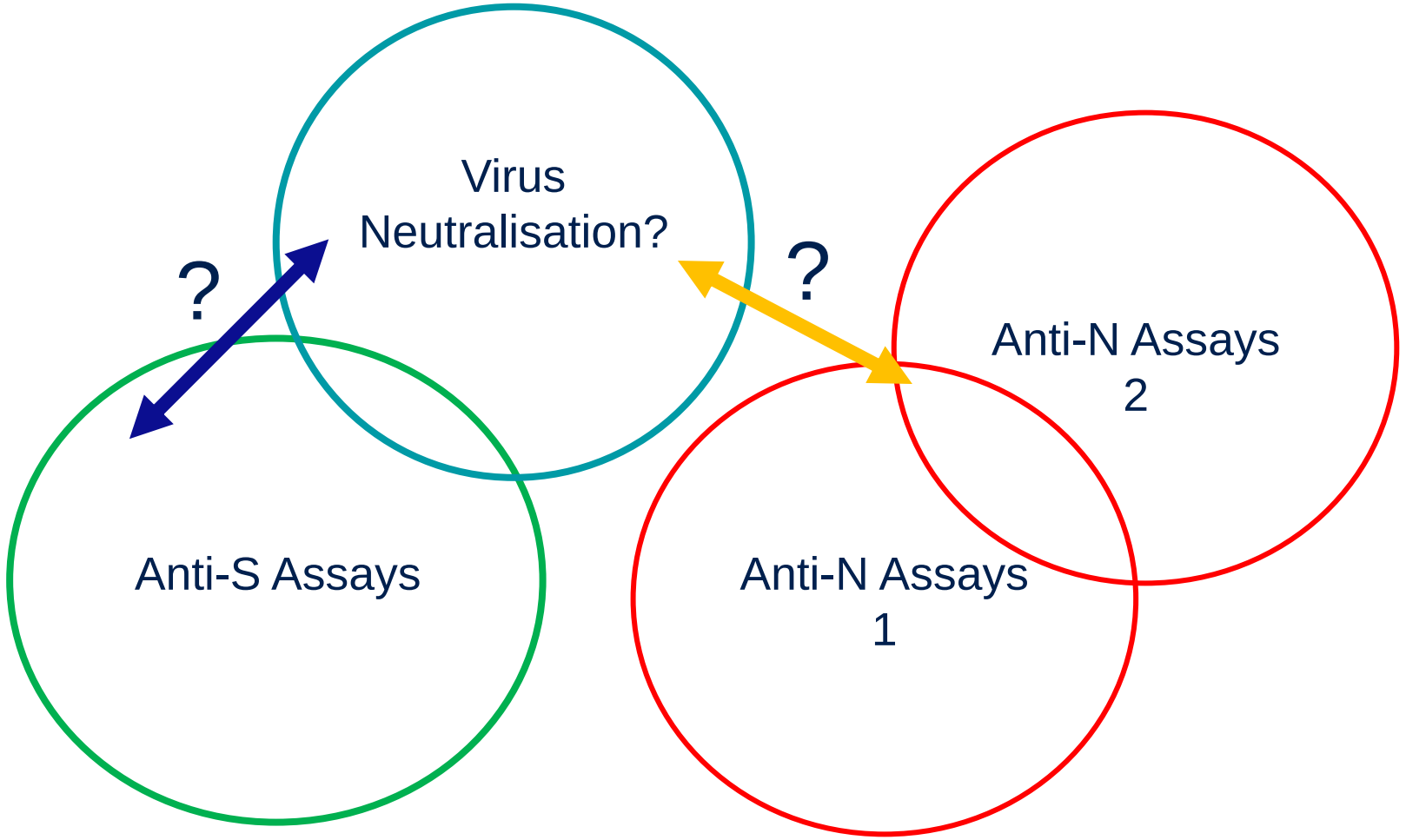
2) Different serological assays measure distinct sero-reactivities against SARS-CoV-2 antigens even when targeting the same viral protein

3) There is variable and sometimes limited association between the detection of anti-viral response in one assay and a second

4) Nevertheless, it is possible to develop biological standards (both primary and secondary) that harmonise the calibration between assays

5) HOWEVER, this capability CANNOT BE ASSUMED and NEEDS TO BE DEMONSTRATED

Current Concept



Future Work

1) Complete the Analysis of the Validation Panel and IS on further Assay Platforms
2) Complete Analysis of the NIBSC Diagnostic Calibrant (20/162) in the same series of assays
3) Establish the Neutralisation (and Pseudo-neutralisation) profiles of Validation panel and 20/162
4) Develop, if needed, Panels of Acute Infection and/or Vaccine Recipient Panels to complement Validation Panel
5) Passive Protection Studies with Convalescent and Vaccine Plasma Pools - (Is there a robust serological correlate of protection)

- ➢ Establish a framework for developing secondary standards that harmonise measurement of serological assays where it is possible
- ➢ Establish whether antibody responses correlate with protection against disease