Medicines & Healthcare products Regulatory Agency

NIBSC

Harmonisation and increased comparability of SARS-CoV-2 serological assays by WHO IS

Project leader: Giada Mattiuzzo



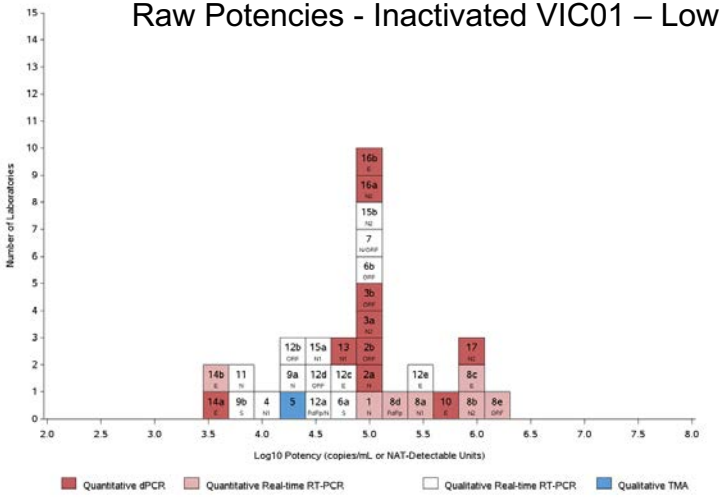
First WHO International Standard for SARS-CoV-2 RNA

Intended use: calibration and harmonisation of NAT assay for the detection of SARS-CoV-2 RNA

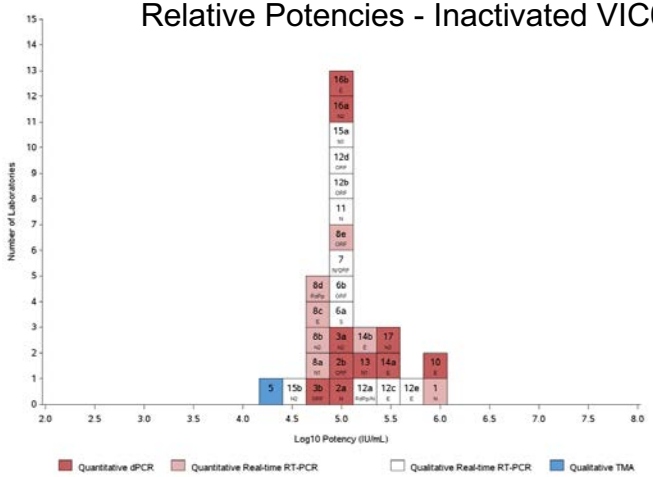
- Acid/heat inactivated England isolate (20/146) with an assigned potency of 7.4 Log_{10} IU/ampoule

  Approximately 2500 ampoules available for distribution

Raw Potencies - Inactivated VIC01 – Low



Relative Potencies - Inactivated VIC01 – Low



Why we make it (use of the antibody Standard)

- Serological assays are needed to understand the real impact of COVID-19, as asymptomatic cases or those with mild symptoms are often undetected
- Evaluation and comparison of vaccine responses
- Evaluation and comparison of other therapeutics (mAbs, CP)
- Immunological surveillance

How we make it….

- Collaborative study
- Establishment by WHO Expert Committee on Biological Standarization

Source Material

Convalescent plasma was provided by:

- ISARIC4C through University of Liverpool, UK
- NHS Blood and Transplant, UK
- Oslo University Hospital, Norway

Convalescent sera was provided by:

- Papworth Hospital, Cambridge, UK

All donors gave written informed consent.

All material has been collected >28 days post diagnosis and solvent-detergent treated at NIBSC; blood virology report negative for usual blood borne viruses

Candidate International Standard and Reference Panel

IS- Pool of plasma from 11 donors from UK
0.25 mL plasma per ampoule, freeze-dried

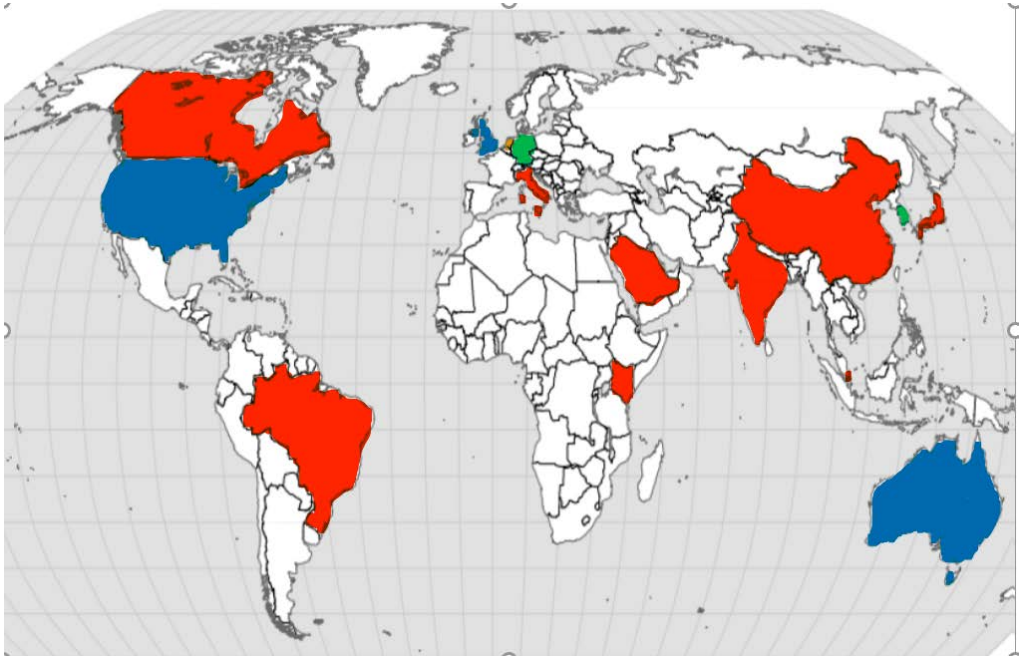
Reference Panel- 0.25 mL plasma pools per ampoule, freeze-dried
High titer
Mid titer
Low anti-S, high anti-N
Low
Negative

Collaborative study samples

Sample	Description	formulation/vol (mL)
A-CP high (20/130)	20/130, Convalescent plasma from one patient, positive	liquid 0.1
B-CS high	Convalescent sera pool, positive	liquid 0.2
C-CS low	Convalescent sera pool, very weak positive	liquid 0.2
D-CP low	Convalescent plasma from one donor, weak positive	liquid 0.2
E-RP low S, high N	20/144, Reference Panel member, weak S, high N	f/d 0.25
F-RP high	20/150, Reference Panel member, high	f/d 0.25
G-IS	20/136, Candidate WHO IS	f/d 0.25
H-RP neg	20/142, Reference Panel member, negative	f/d 0.25
I-RP low	20/140, Reference Panel member, low	f/d 0.25
J-RP Mid	20/148, Reference Panel member, mid	f/d 0.25

Collaborative study participants

- 44 participants from 15 countries
- Vaccine developers, NCL/NRL, diagnostic labs, kit manufacturers, non-profit vaccine research organisation and academic laboratories



Methods: 125 data sets

NEUTRALISATION ASSAY (27)

LIVE SARS-CoV-2* (15)
PRNT/FRNT
CPE
MN

PSEUDOTYPED VIRUS(12)

VSV(Luc)
HIV(Luc)

* 9 different isolates

ELISA (78)

IgG:

In house (44)
Commercial kit * (18)

IgM (7), IgA (9)

RBD, S1, Spike, N, M, E and S2

* 13 different kits

OTHERS (20)

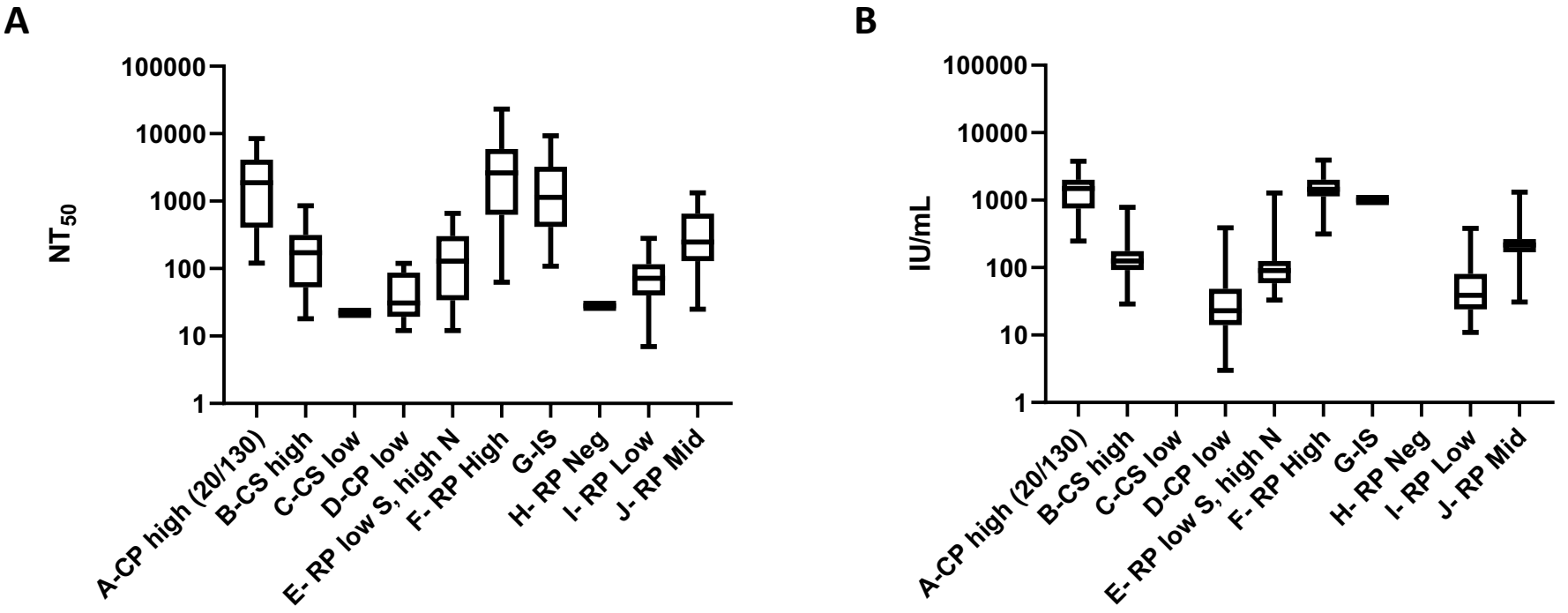
Flow cytometry-based binding Ab assay

Lateral flow immunoassay

Fusion inhibitory assay

ACE2 binding inhibitory assay

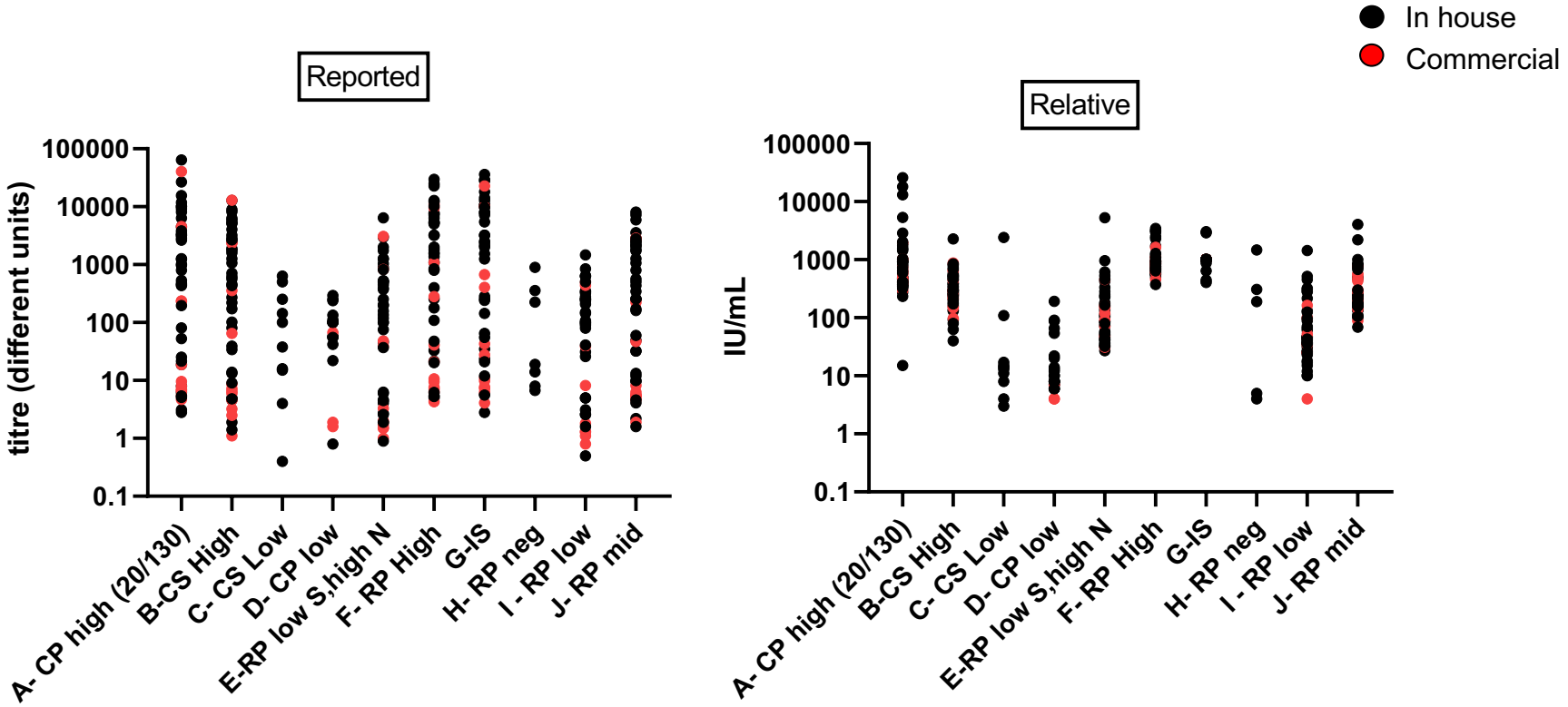
Neutralisation assays



Neutralisation assays harmonisation

		A-CP high (20/130)	B- CS high	C-CS low	D-CP, low	E- RP low S, high N	F- RP high	G- IS	H- RP neg	I-RP low	J-RP mid
%GCV	reported	249	179	n/a	116	231	281	241	n/a	152	161
	Relative to sample G	94	95	n/a	250	119	67	-	n/a	150	93
	Relative to sample F	118	115	n/a	218	149	-	65	n/a	182	115
GM:Med<2	reported	37%	44%	n/a	50%	46%	26%	22%	n/a	70%	46%
	Relative to sample G	67%	74%	n/a	56%	65%	85%	-	n/a	55%	81%
	Relative to sample F	63%	70%	n/a	44%	58%	-	85%	n/a	50%	69%
UQ/LQ	reported	4.039	3.759	n/a	4.118	4.094	8.177	6.765	n/a	2.671	3.851
	Relative to sample G	2.607	1.781	n/a	3.148	1.888	1.666	-	n/a	2.638	1.503
	Relative to sample F	2.887	2.350	n/a	3.348	3.050	-	1.604	n/a	4.537	2.690

Binding antibodies-ELISA

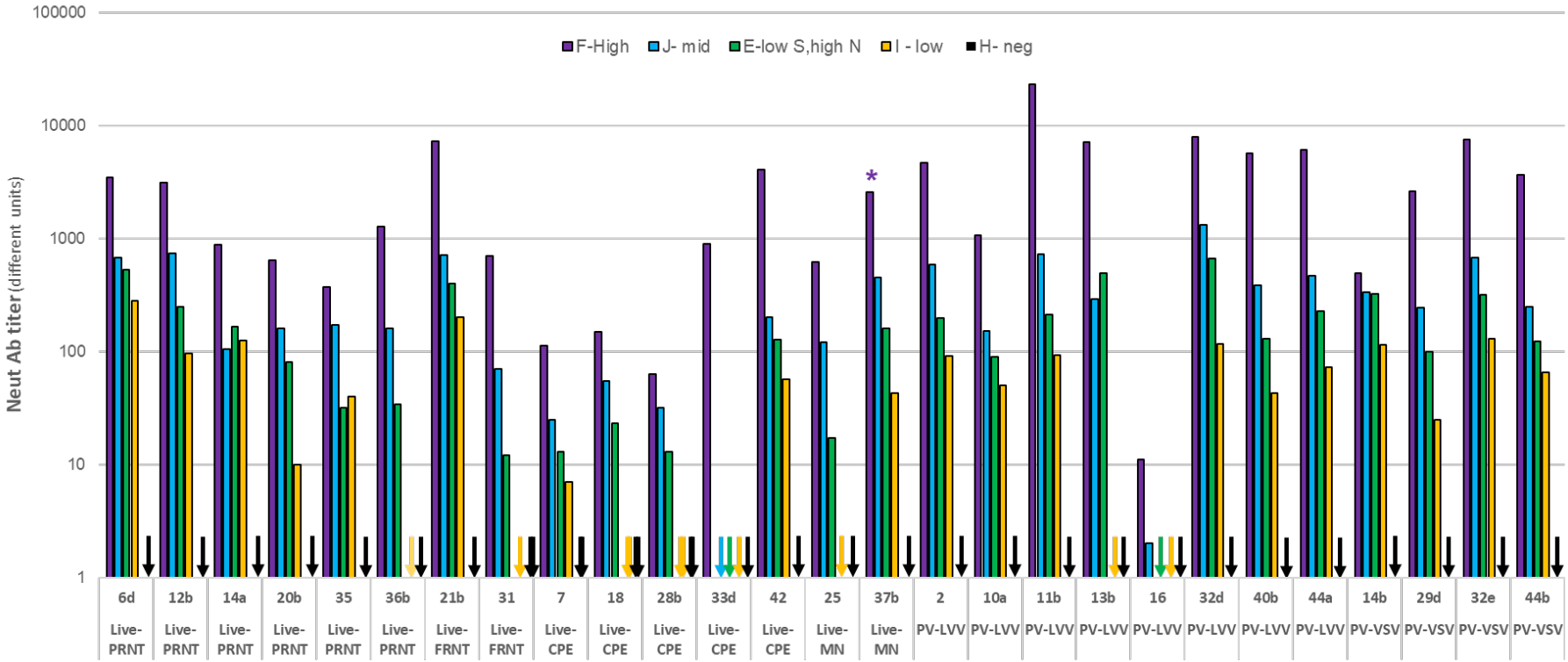


Harmonisation ELISA titres

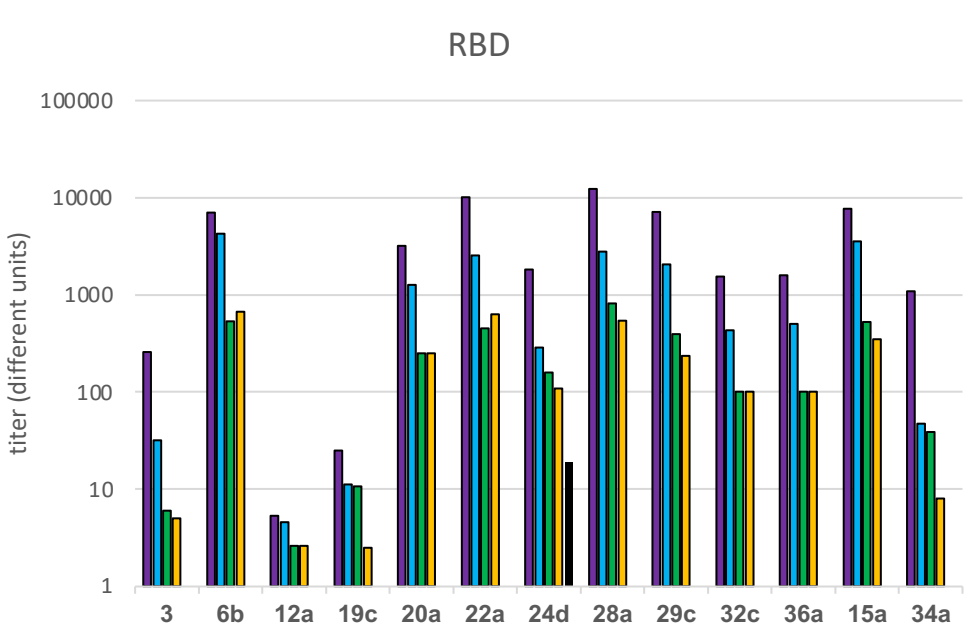
		A-20/130, CP high	B- CS high	C-CS low	D-CP low	E-RP low S , high N	F-RP high	G- IS	H-RP neg	I-RP low	J-RF mid
GM	reported	826	485	153	63	131	1217	1411	79	83	381
	relative to G*	550	316	9	10	83	790	-	15	39	241
%GCV	reported	1842	1437	307	604	1171	1557	1698	545	913	1174
	relative to G	121	72	96	151	107	30	-	811	134	66
Lab GM: Med <2	Reported	20%	24%	50%	52%	26%	26%	31%	25%	28%	20%
	relative to G	77%	79%	75%	81%	63%	98%	-	67%	69%	77%
UQ/LQ	Reported	150.37	101.99	2.95	3.66	67	30.53	45.02	16.56	35.47	78.52
	relative to G	2.01	2.03	2.27	1.41	3.04	1.39	-	7.34	1.94	1.38

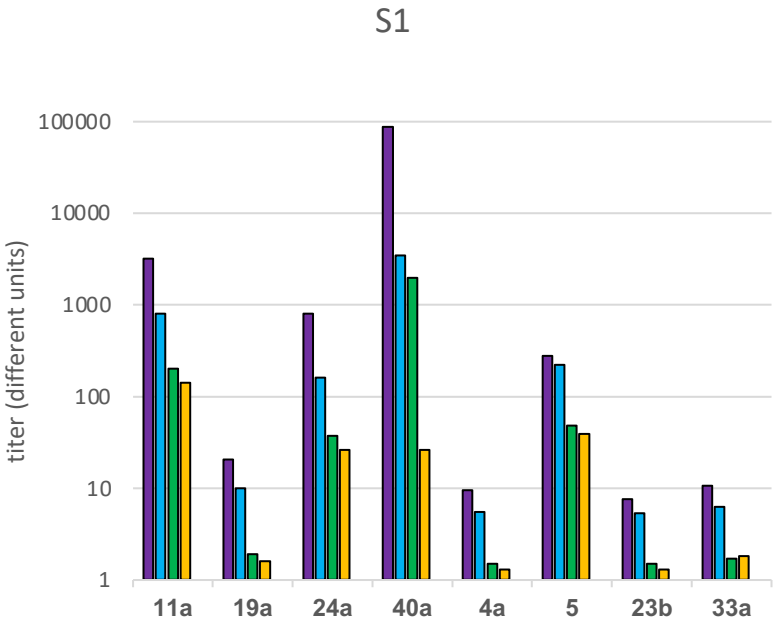
*with an arbitrary value of 1000 IU/mL

Reference Panel neutralising antibodies

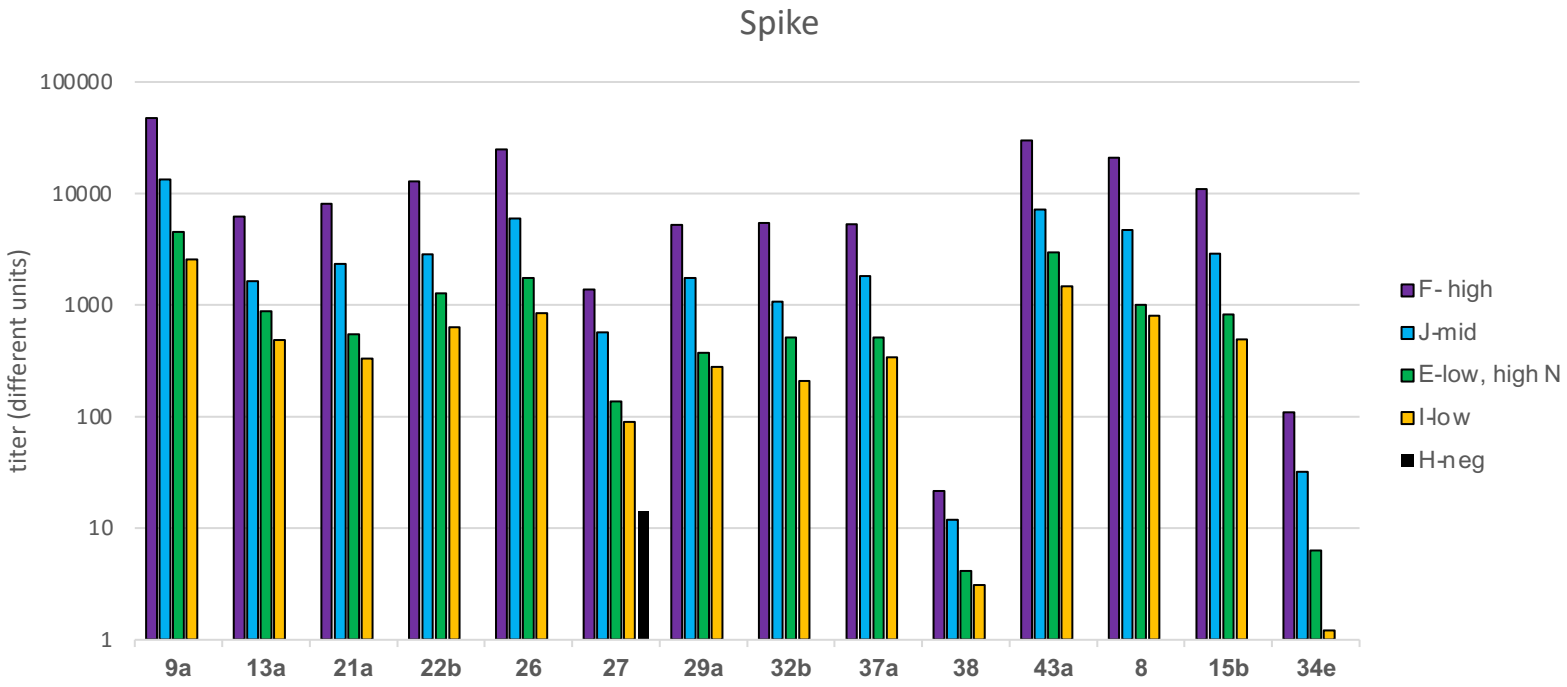


Reference Panel binding antibodies

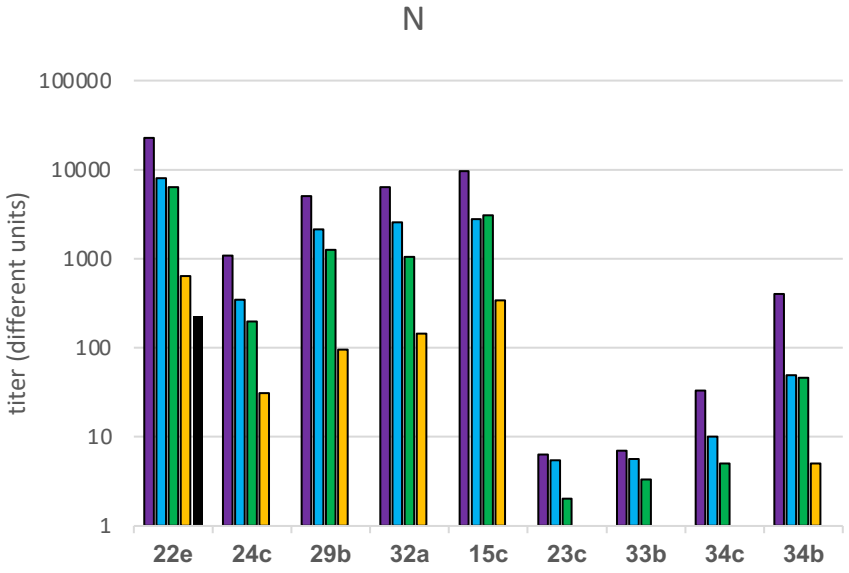


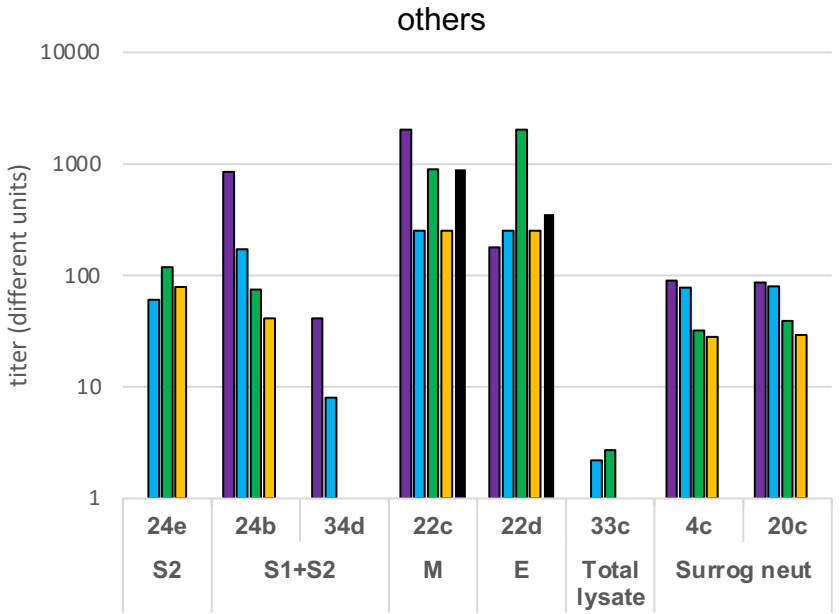


Reference Panel binding antibodies-II



Reference Panel binding antibodies-III





Other assays

Lateral flow immunoassay :

- IgG results provided same ranking of samples as ELISA;
- IgM detected in at least sample A, F, G and J

Binding antibody detection by Flow cytometry analysis:

- IgG results provided same ranking of samples as ELISA;
- IgM detected in sample A, F and G against RBD, S1, Spike and N, and in sample E, I and J for Spike (antibody against S2 and E detected in all samples including the negative)
- IgA (flow cytometry only) detected in sample A, F, G for S1, S2, Spike and N, and in sample J for S2 and Spike, sample E for S2 only

Inhibition assays:

- Results provided same ranking of samples as neutralisation assays, with F>G;
- Cell fusion inhibition assay detected all positive plasma samples, but not the serum samples
- hACE2 binding inhibition assay only detected the high and mid titre samples

Summary

- Candidate IS sample G was evaluated in 125 methods including ELISA and neut assay
- Candidate IS was scored as one of the top three highest titre samples in every assay
- Expression of the titre as relative to the candidate IS reduced inter-laboratory variation in both neutralisation assay and IgG-based ELISA
- The harmonization was more pronounced in the ELISA methods possibly because original results are reported in different units
- The candidate IS was tested for IgA and IgM and found positive against RBD, S1, Spike and N
- The candidate Reference Panel samples were ranked similarly in almost all the assays used with very few exceptions

		A	B	C	D	E	F	G	H	I	J
Titer* (IU/mL)	Neutralisating Ab	1299	126	-	26	95	1473	1000	-	44	210
	Binding antibody	550	316	9	10	83	790	1000	-	39	241

First WHO IS for SARS-CoV-2 immunoglobulin (20/136)

Assigned potency of 250 IU/ampoule for neutralising antibody activity

Approximately 3000 ampoules available for distribution

Recommended storage -20˚C, and suitably stable for shipping at ambient temperature

Also, recommended as reference reagent for calibration of assays detecting binding antibody

1000 ELISA unit (EU)/mL **for specific target**

NIBSC Research reagent 20/130

1300 IU/mL neutralising antibody titre;

502 EU/mL anti-RBD IgG

588 EU/mL anti-S1 IgG

476 EU/mL anti-Spike IgG

747 EU/mL anti-N IgG

WHO Reference Panel (20/268)

Reference Panel will comprise:

sample F, High (NIBSC code 20/150)

sample J, Mid (NIBSC code 20/148)

sample E, low S, high N (NIBSC code 20/144)

sample I, low (NIBSC code 20/140)

sample H, negative (NIBSC code 20/142).

Representative data

	High	Mid	low S, high N	low	
	20/150	20/148	20/144	20/140	
Neut Ab	1473	210	95	44	**IU/mL**
anti-RBD	817	205	66	45	**EU/mL**
anti-S1	766	246	50	46	**EU/mL**
anti-Spike	832	241	86	53	**EU/mL**
anti-N	713	295	146	12	**EU/mL**

- No unitage will be proposed for the Reference panel
- Approximately 2500 panels are available for distribution
  Recommended storage -20˚C, and suitable stable for shipping at ambient temperature

Acknowledgements

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