NIBSC

Assuring the suitability of International Standards for SARS-CoV-2 in Diagnostic Assays

Neil Almond

Medicines & Healthcare products Regulatory Agency

Acknowledgments

David Padley
Josh Duran
Sara Fabi
Rob Anderson
Sarah Kempster
Jacqueline Fryer
Clare Morris

Giada Mattiuzzo
Emma Bentley
Mark Page
Peter Rigsby

Nicola Rose/Chris Burns

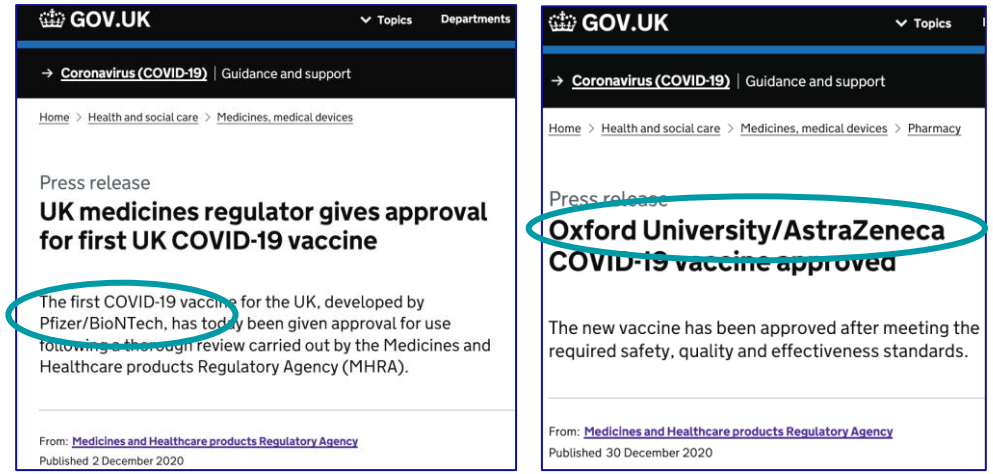
NHS Blood and Transplant
Heli Harvala-Simmonds
David Roberts
Kate Tettmar

PHE, Porton Down
Tim Brooks
Ashley Otter
Abbie Bown
Amanda Semper

COVID-19 Vaccine independent control testing

- Early contact was made with several vaccine developers (spring / summer 2020)
  - Based on progress of R&D, clinical trials, published Government contracts
  - Agreed process for ‘technical transfer’ of selected methods with manufacturers under ISO17025:2017
  - Established close contact; weekly technical and progress meetings
- Nominated technical experts to import and verify methods and validate reagents
  - Staff with many years’ experience with different types of biological medicines
  - Deployed staff based on their combination of technical, scientific and regulatory skills
  - Product-specific teams but with overlap for resilience
- Encouraged the manufacturer to do their testing in parallel with NIBSC
  - Benefit outweighed the risk of the batch not making it to market
  - Quick turnaround for final NCL certification of compliant batches

The first vaccines approved for use in UK


GOV.UK Topics Departments

Coronavirus (COVID-19) | Guidance and support

Home > Health and social care > Medicines, medical devices

Press release

UK medicines regulator gives approval for first UK COVID-19 vaccine

The first COVID-19 vaccine for the UK, developed by Pfizer/BioNTech, has today been given approval for use following a thorough review carried out by the Medicines and Healthcare products Regulatory Agency (MHRA).

From: Medicines and Healthcare products Regulatory Agency

Published 2 December 2020

GOV.UK Topics

Coronavirus (COVID-19) | Guidance and support

Home > Health and social care > Medicines, medical devices > Pharmacy

Press release

Oxford University/AstraZeneca COVID-19 vaccine approved

The new vaccine has been approved after meeting the required safety, quality and effectiveness standards.

From: Medicines and Healthcare products Regulatory Agency

Published 30 December 2020

- NIBSC was able to test and certificate batches on the same day as MHRA approval to initiate UK vaccine campaign
- First batch certificated 2 December 2020
  - >150 batches certificated to date
  - >135 million doses
- Kept ahead of the curve for dose volumes for vaccination campaign targets

- Actively testing batches of all 4 COVID-19 vaccines approved for use in UK:
  - AZ; Pfizer/BioNTech; Moderna; Janssen (J&J)
- Establishing tests for further vaccines that are in consideration for regulatory approval
- Advice, support, and product testing requested by, and provided to other countries


npj Vaccines www.nature.com/npjvaccines

PERSPECTIVE OPEN

Check for updates

National control laboratory independent lot testing of COVID-19 vaccines: the UK experience

Nicola J. Rose[1✉], Paul Stickings[2], Silke Schepelmann[1], Marc J. A. Bailey[3] and Chris Burns[4]

The past 18 months have seen an unprecedented approach to vaccine development in the global effort against the COVID-19 pandemic. The process from discovery research, through clinical trials and regulatory approval often takes more than 10 years. However, the critical need to expedite vaccine availability in the pandemic has meant that new approaches to development, manufacturing, and regulation have been required: this has necessitated many stages of product development, clinical trials, and manufacturing to be undertaken in parallel at a global level. Through the development of these innovative products, the world has the best chance of finding individual, or combinations of, vaccines that will provide adequate protection for the world's population. Despite the huge scientific and regulatory achievements and significant investment to accelerate vaccine availability, it is essential that safety measures are not compromised. Here we focus on the post regulatory approval testing by independent laboratories that provides an additional assurance of the safety and quality of a product, with an emphasis on the UK experience through the National Institute for Biological Standards and Control (NIBSC), an expert centre of the UK's Medicines and Healthcare products Regulatory Agency (MHRA).

npj Vaccines (2021)6:100 ; https://doi.org/10.1038/s41541-021-00368-7

Measuring Ag:Ab for Emerging Diseases

Measuring Antigen:Antibody Interactions in Infectious Disease is IMPORTANT

- Diagnostic assay detection
  - Specific antigen
  - Selected antibody response(s)
- Serological Immune Protection
  - Measurement of specific antibody response in calibrated assay

Harmonising measurement of polyclonal serological responses is A CHALLENGE

- Complex antigen
  - Multiple epitopes
  - Variable sequence (changing?)
- Serological response is determined by complex host genetics

Emerging Diseases – the Need for Speed

Consideration for Antibody Assay Design

1) Functional Antibodies – neutralising/complement fixing/ADCC
    + - Likely to focus on common target antigen
    + - Biological relevance for clinical management(?)
    - - Slow and Fiddly
2) Binding Antibodies – ELISA/High throughput platforms
    + - Fast
    - - Is it biologically relevant
    - - Different assays may focus of measuring different antibodies

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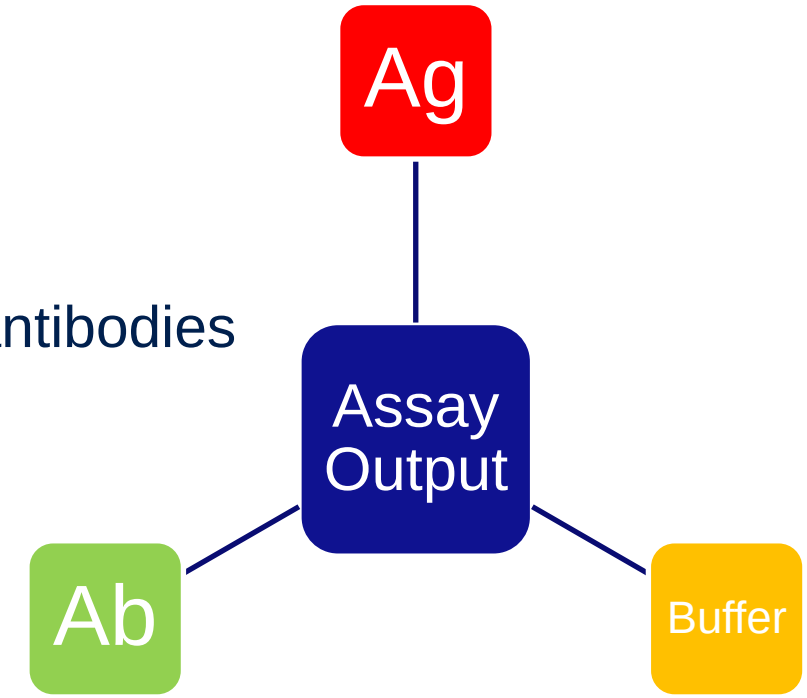

Tale of two viruses

Hepatitis B

- Established availability of relevant reference standard
- Robust measurement of Ab
- Protection at 10 mIU/ml
- Clinical management cut-off at 100 mIU/ml (booster)

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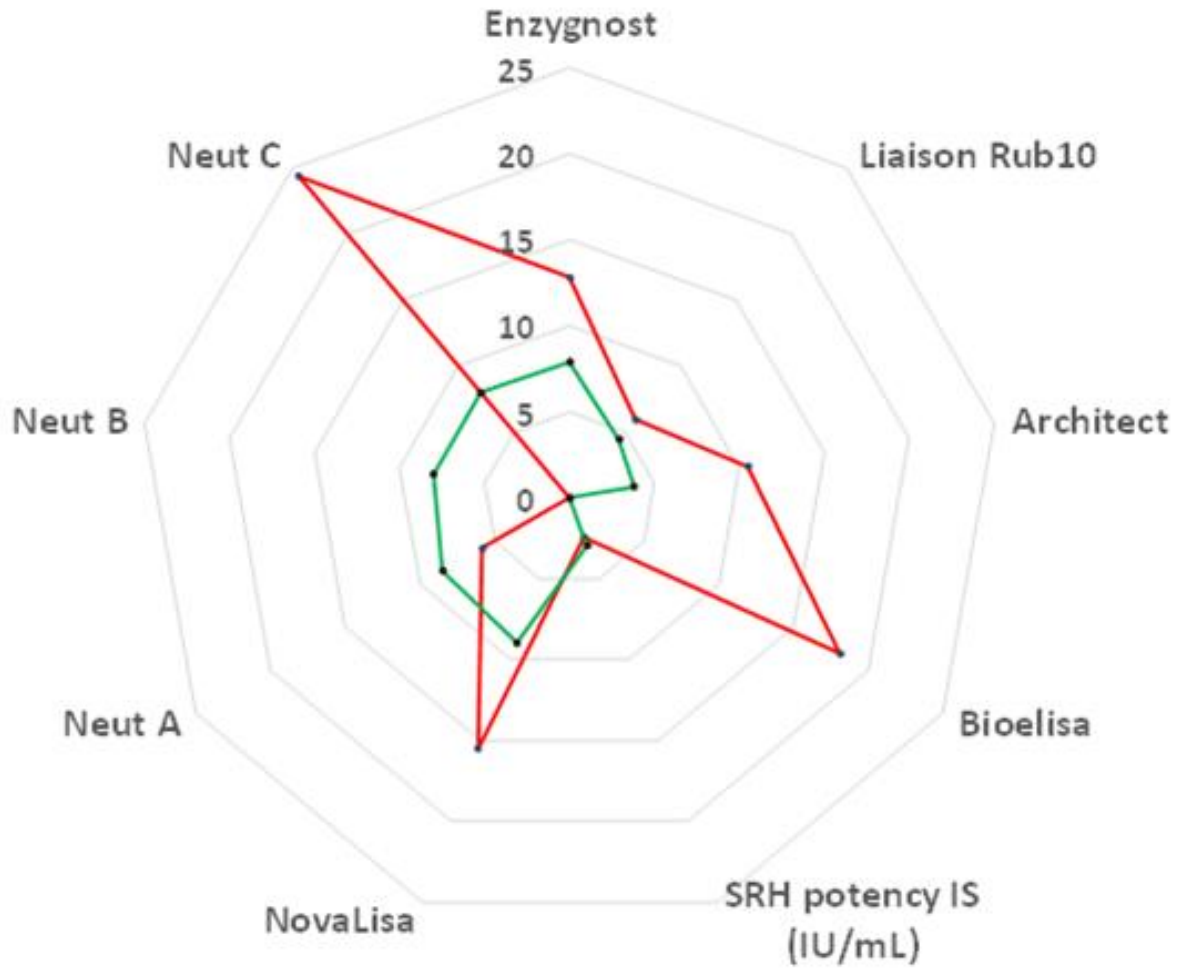
Rubella

- Established availability of relevant reference standard
- HOWEVER standard does not harmonise measurement in binding assays
- Relevance of 10 IU/ml cut-off of protection ?

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

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)

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

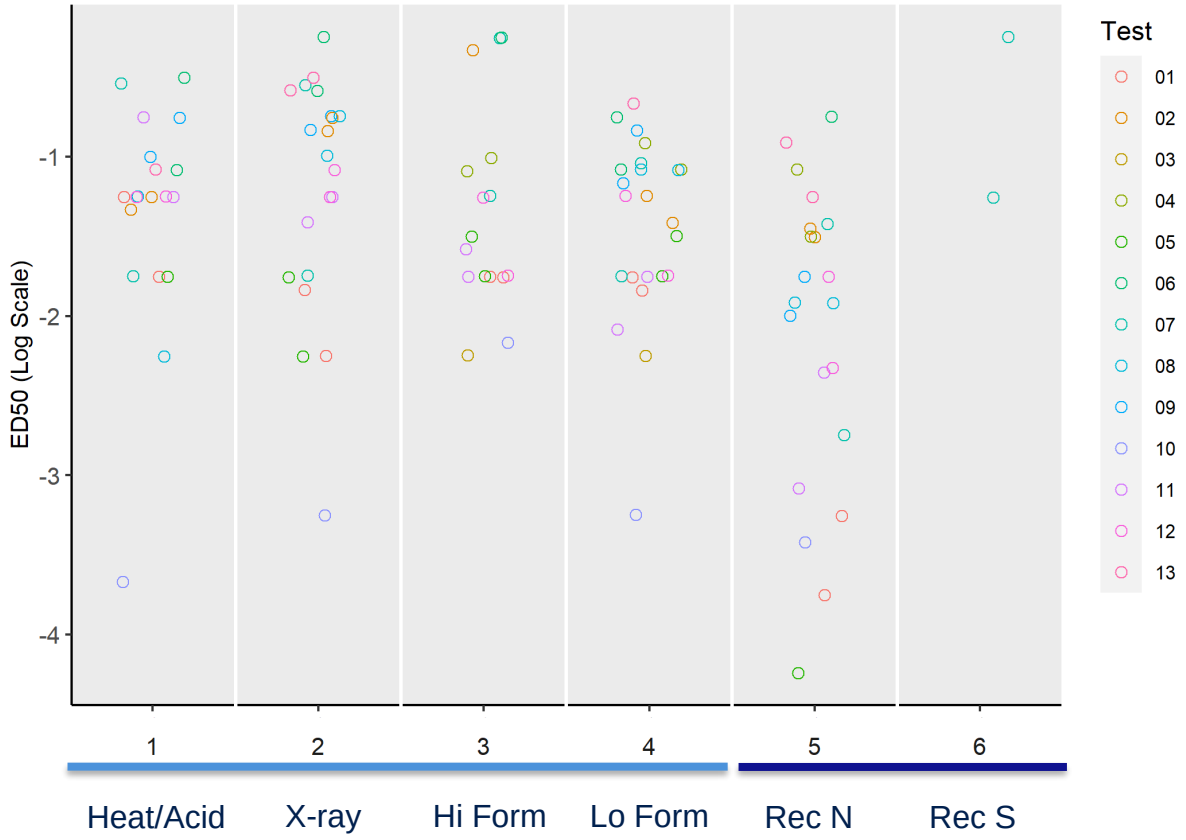
4) In progress – Reference Standard for SARS-CoV-2 Antigen

   Driven by WHO PQ team for Lateral Flow Devices

Pilot Study to develop WHO IS SARS-CoV-2 Antigen

Sample	Antigen preparation	Strain/sequence	Concentration (ng/mL)
1	Virus, heat/acetic acid inactivated	Australia/VIC/01/2020	15.8
2	Virus, x-ray irradiated	England/2/2020	15.8
3	Virus, 4% formaldehyde inactivated	Australia/VIC/01/2020	15.8
4	Virus, 0.01% formaldehyde inactivated	Australia/VIC/01/2020	15.8
5	Recombinant N protein, E. coli expressed, native	YP_009724397	316
6	Recombinant S protein, mammalian expressed, trimeric	NC_045512.2	316

Evaluation of Multiple LFD's with Different Antigens



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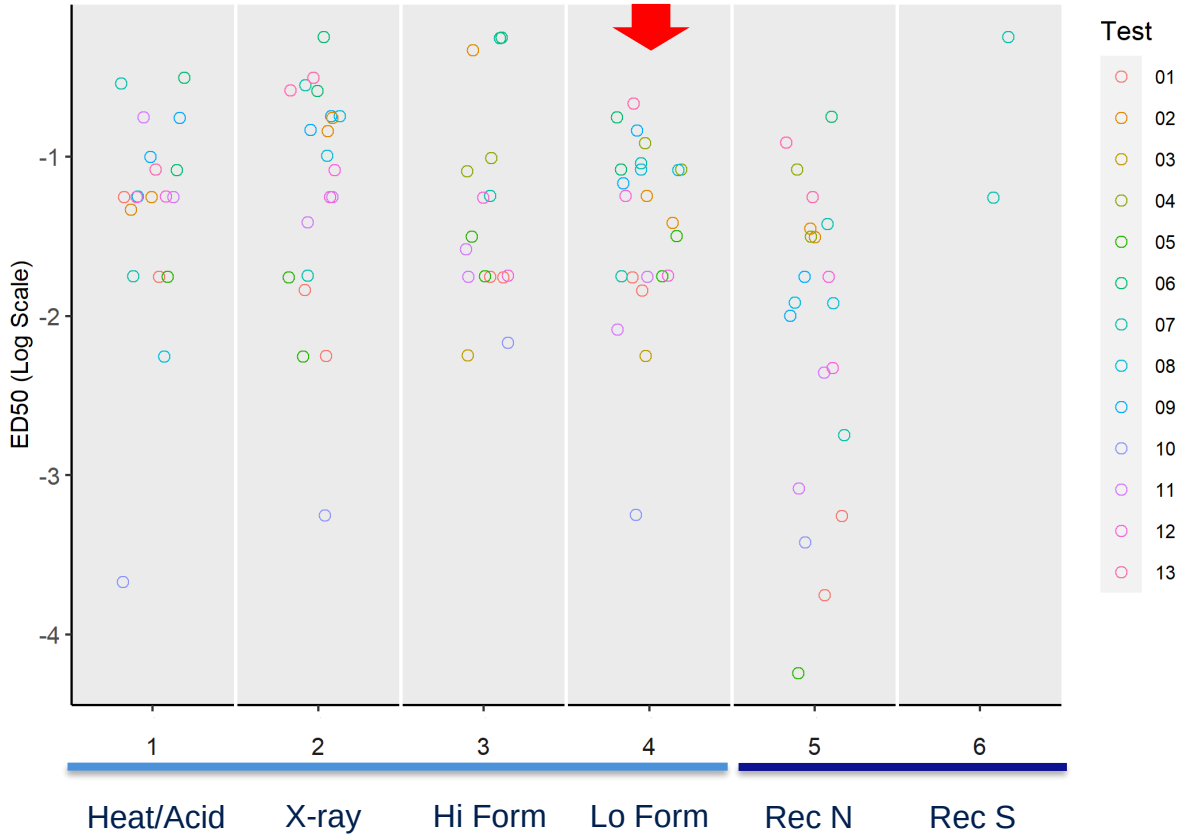
Material from Sample 4 will soon be available as an Interim Reference Material
BUT the proposed Int'l Standard will be inactivated (Lo Form.) DELTA Virus

Evaluation of Multiple LFD's with Different Antigens

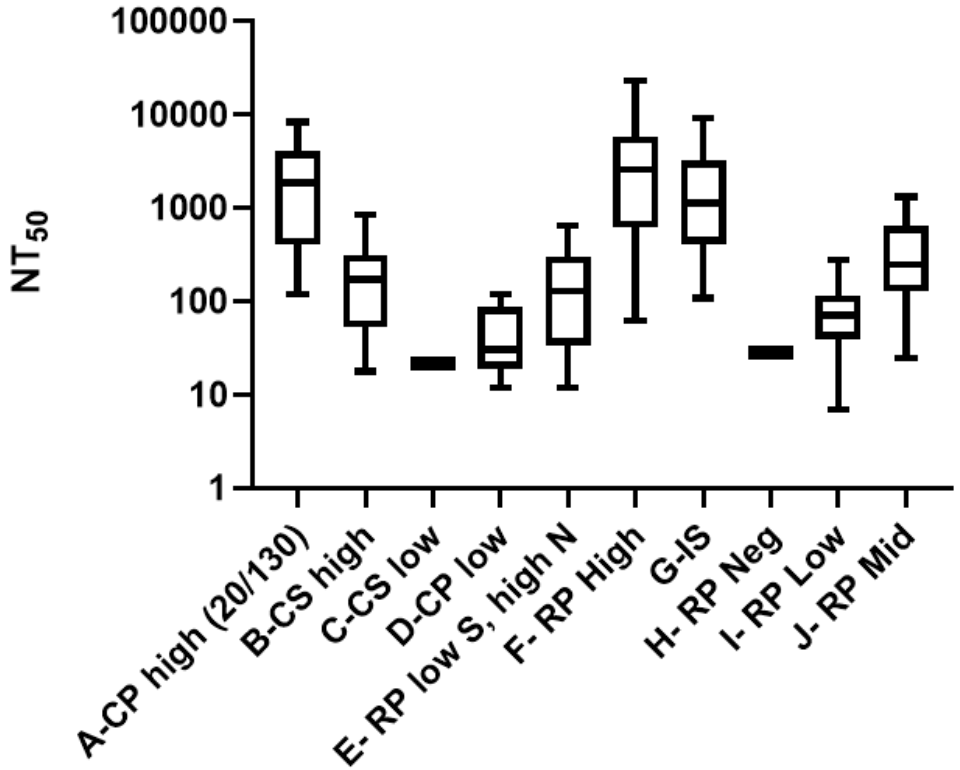


Harmonisation of Neutralisation Assays by 20/136

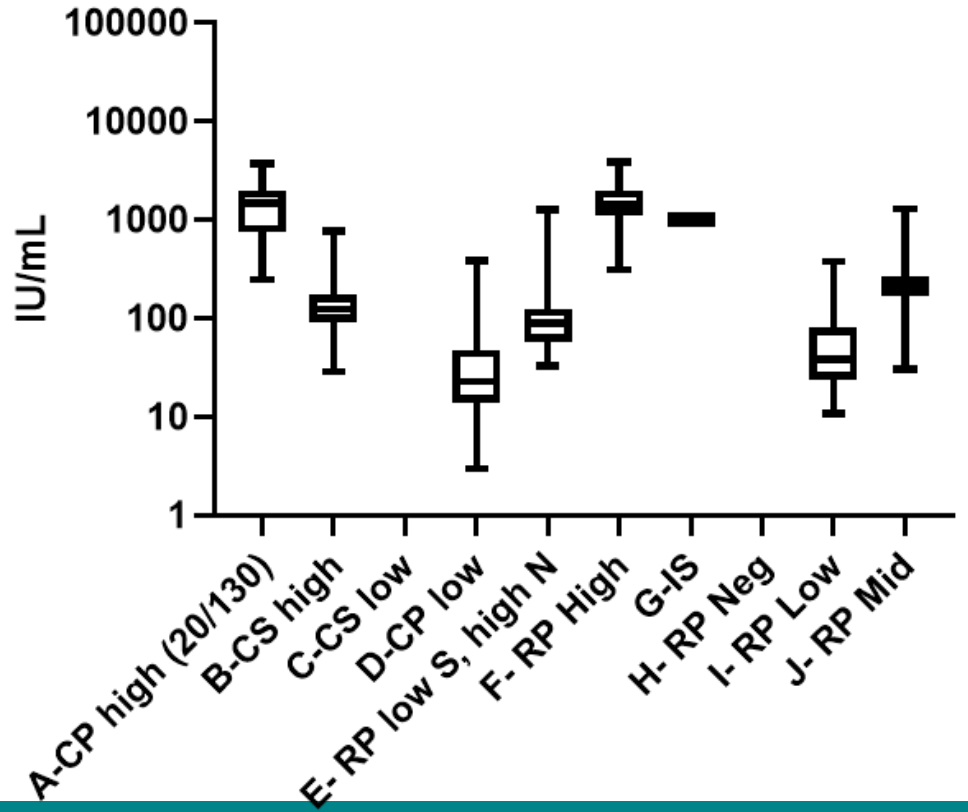
Data taken from ECBS Report – Giada Mattiuzzo, Emma Bentley,,,,Mark Page

The International Standard (sample G) was prepared from pooling hi-titre neut convalescent plasma

a) 50% Neutralisation Data Returned



b) Calculated Relative Potencies vs G-IS

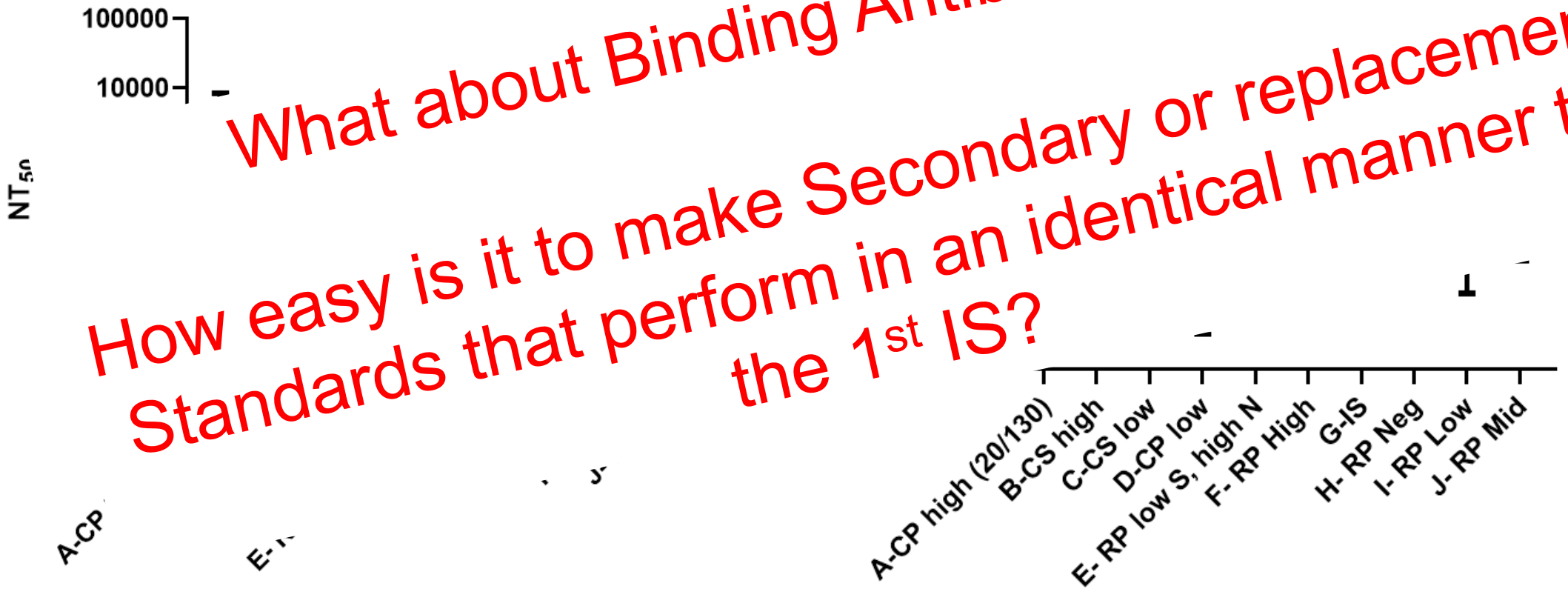


Harmonisation of Neutralisation Assays by 20/136

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

a) 50% Neutralisation Data Returned

b) Calcu



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)
- Low positive run control used for monitoring assay performance

3) Secondary standard for calibration of diagnostic assays (NIBSC code 20/162)
- **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**

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

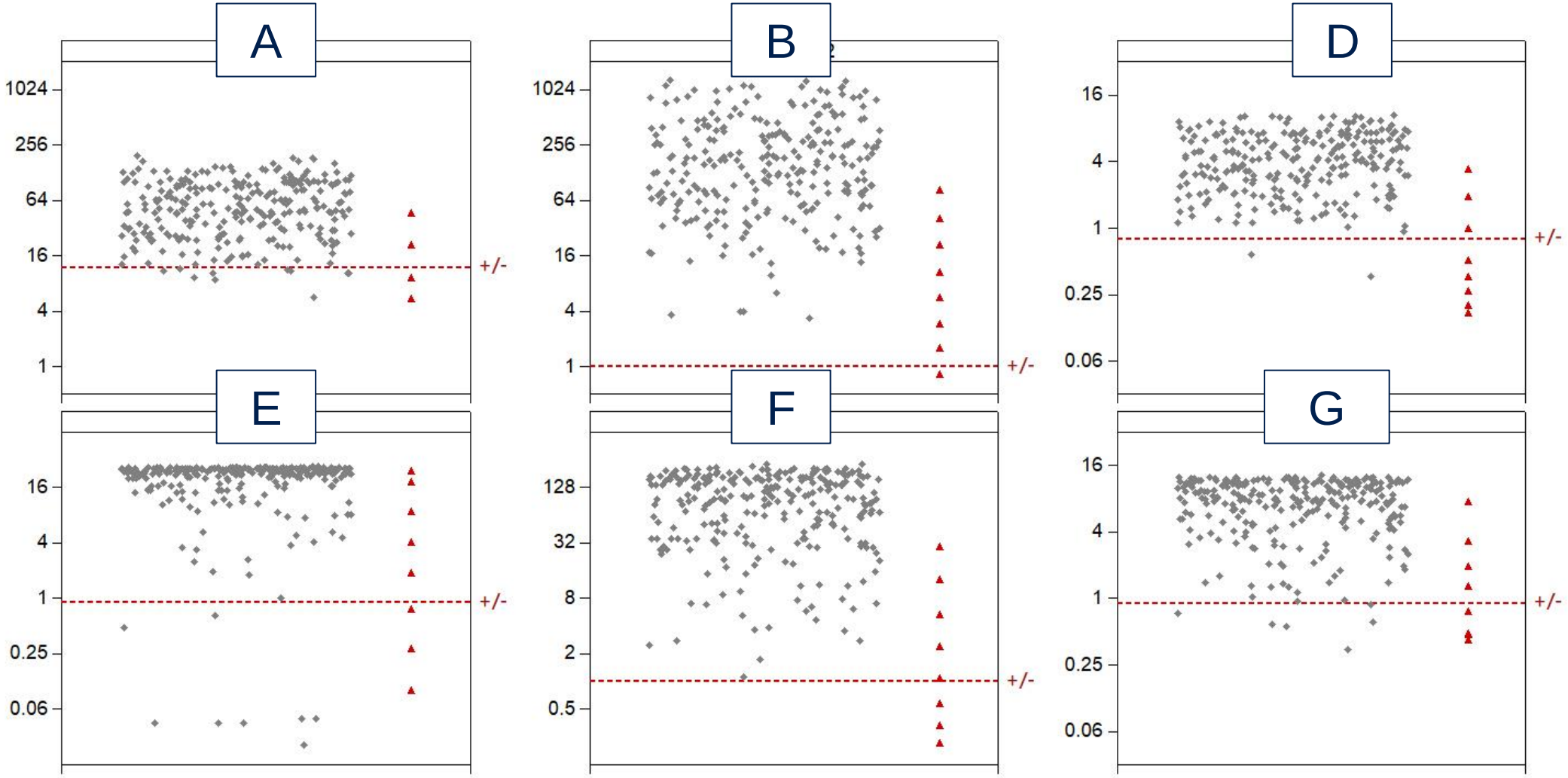
➢ Run all of these materials in as many diagnostic assay platforms as possible

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	-

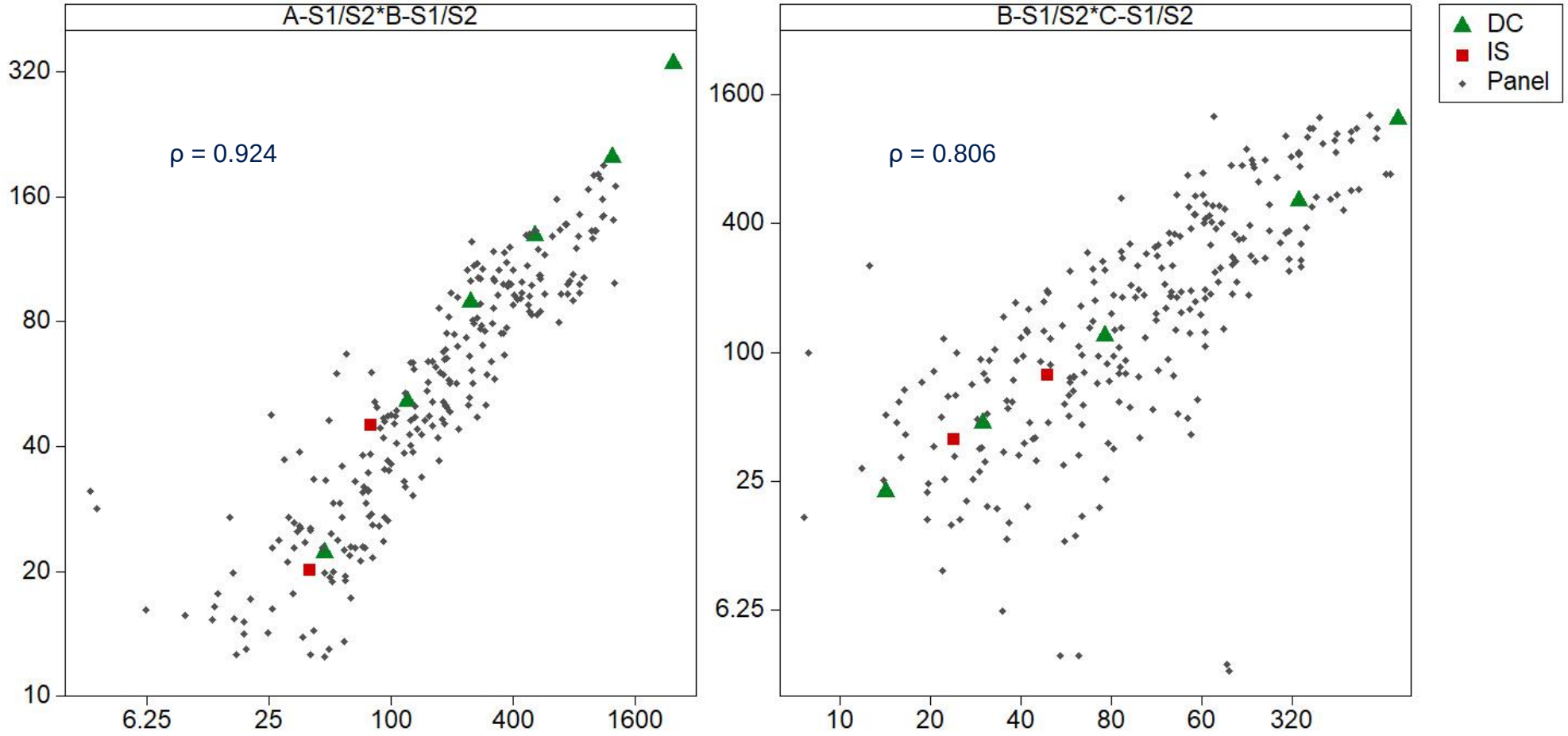
Positive	Negative

Assay correlations

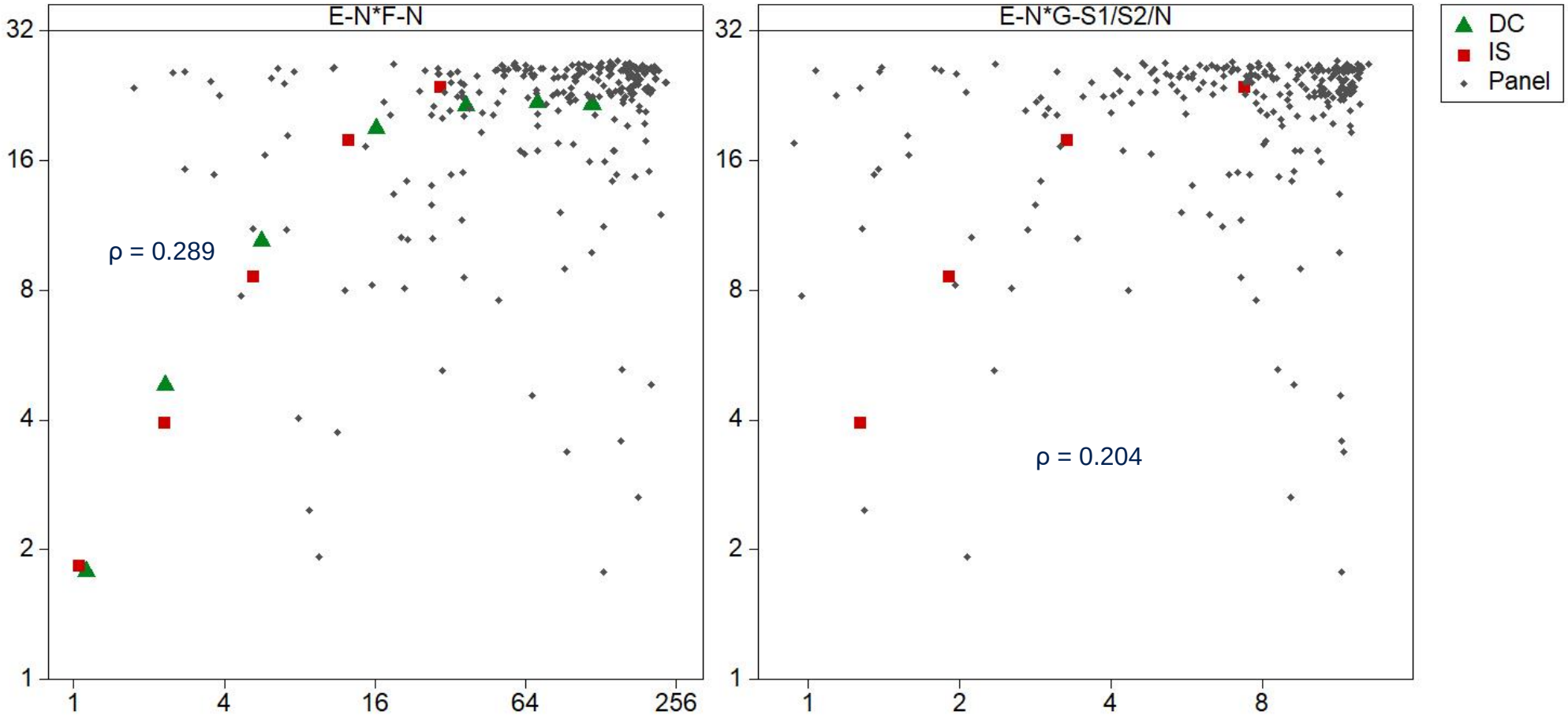
Spearman ρ:

	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

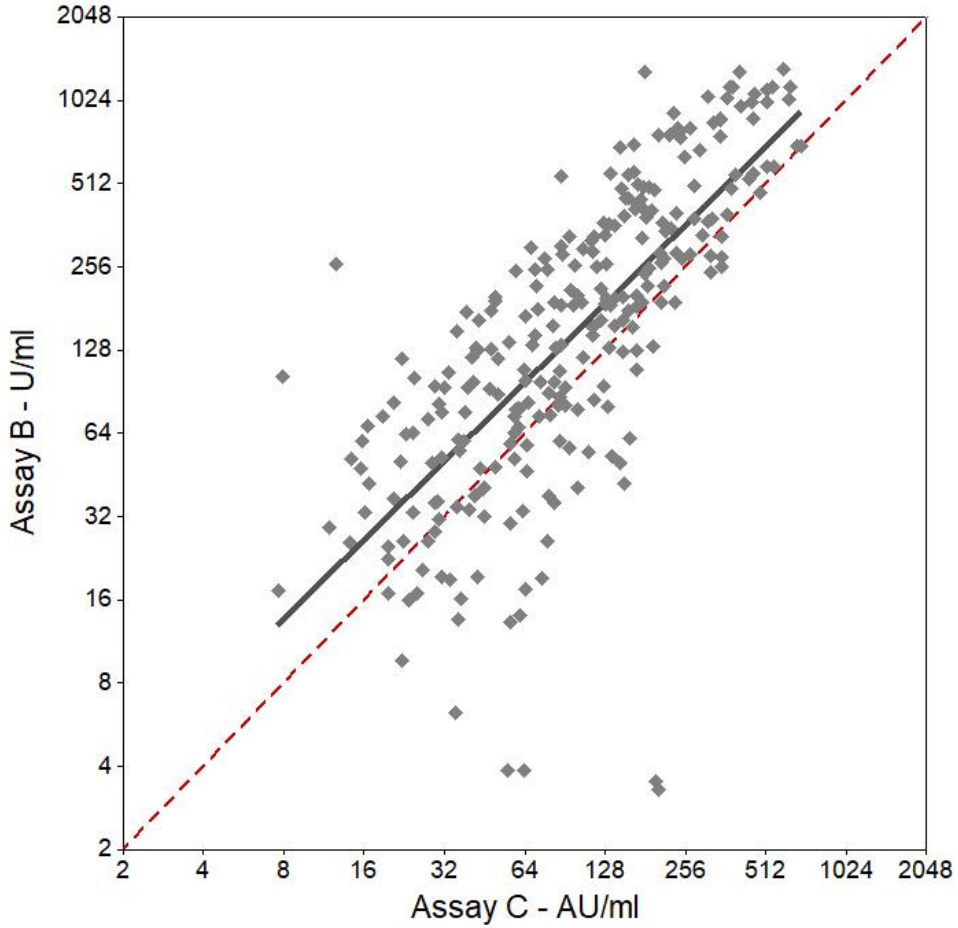
Assay correlations (A,B,C – S1/S2)

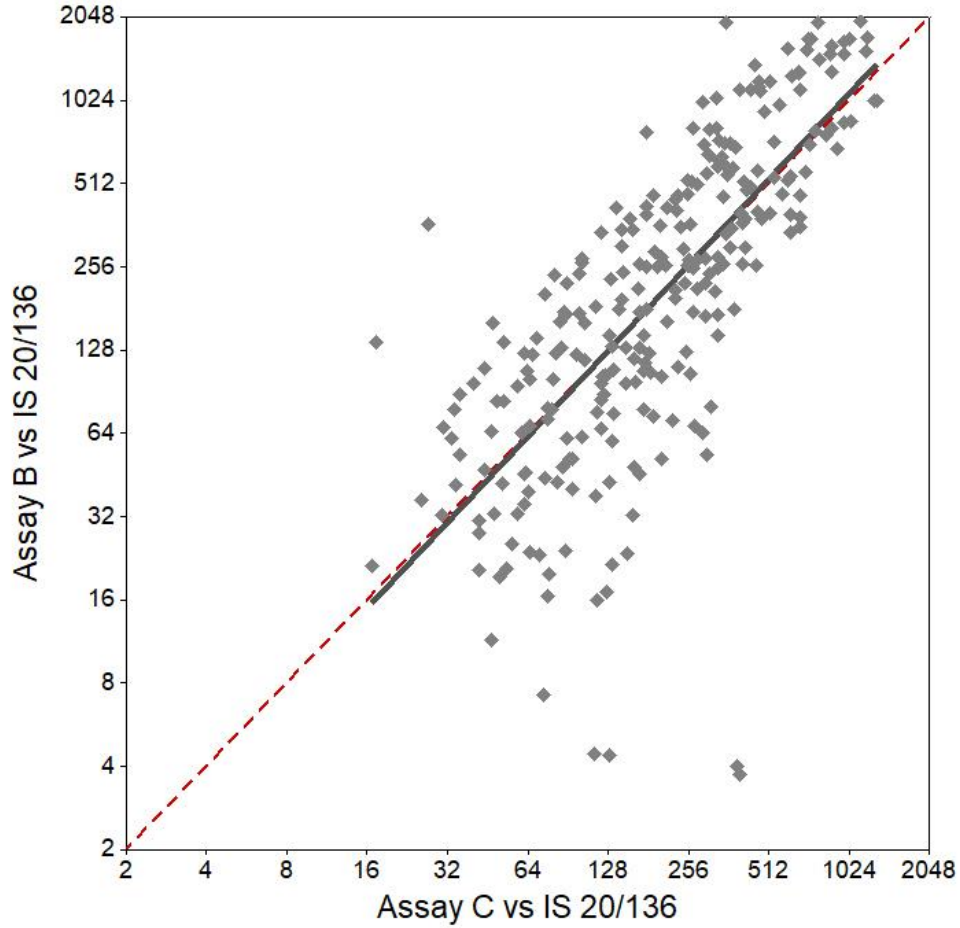


Assay correlations (E – N, F – N, G – S1/S2/N)

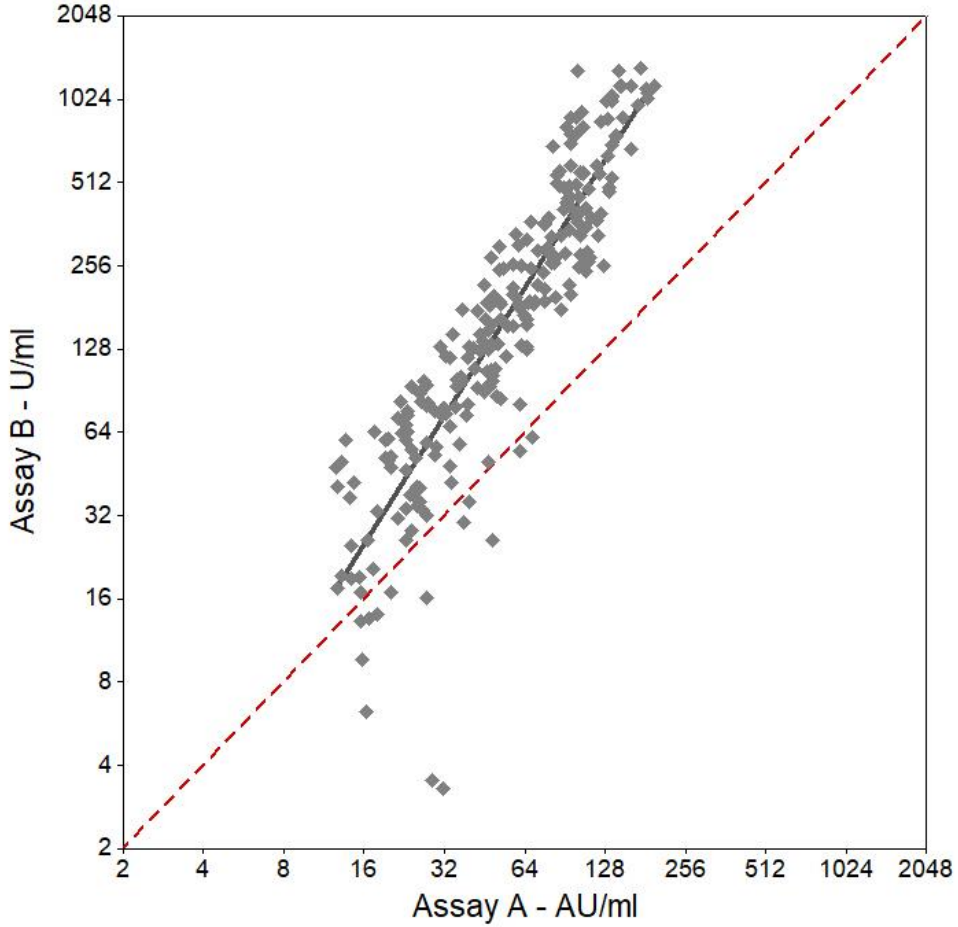


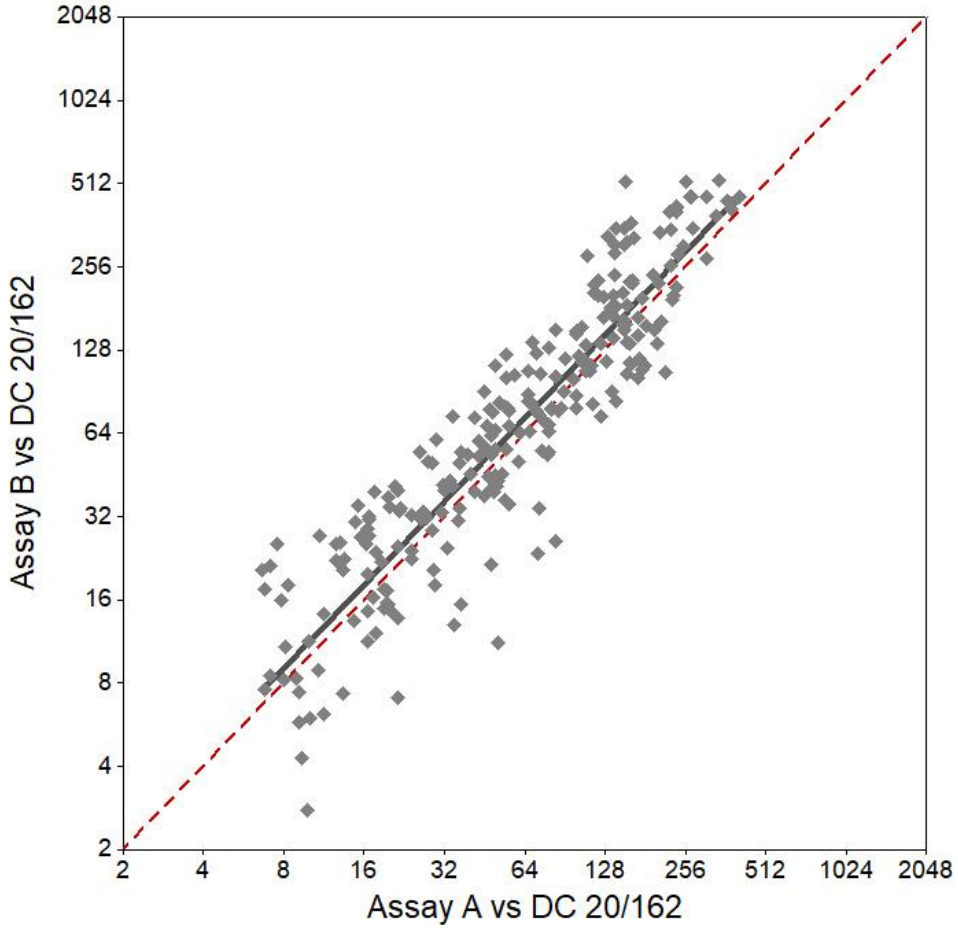
Assay harmonization using IS 20/136 (B,C – S1/S2)





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 of different binding assays

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

Lessons Learned and Future Challenges

- Clinical management of Emerging Diseases needs rapid development of Diagnostics
- Assays based on Ag:Ab interactions most at risk (especially early in pandemic response)

- Urgent need to move away from clinical sensitivity and specificity of assays
  - Identification of key interactions of clinical relevance (diagnosis, protection)
  - Early provision of antibody reagents (Mabs, Convalescent Serum)
  - Early development and Provision of Reference Standards, Common Validation Panels, Verification Panels to Mfrs and Testing Labs
  - ➢ BETTER MEASUREMENT OF KEY ASSAY PROPERTIES AND PARAMETERS

- Education of Clinicians, Laboratory Scientist, Regulators
- ➢ BETTER ASSAYS and BETTER MANAGEMENT OF PATIENTS AND POPULATIONS

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