10 November 2021 1430-1600 CET

https://cepi-net.zoom.us/j/96520099315

Passcode: 473053

Recording is available at:
https://worldhealthorg.sharepoint.com/sites/COVID19AssaysdevelopmentforVaccines/Shared%20Documents/General/Webinar%20on%20Calibration%20of%20Secondary%20Standards%20Nov%2010%202021/Nov10CalibrationWorkshopFinal.mp4

Time (CET)	Presentation Title	Speaker
14:30-14:35	Welcome and meeting objectives	**Ivana Knezevic**, (WHO)
14:35-14:50	Calibration to the WHO IS for anti-SARS-CoV-2 immunoglobulin	**Peter Rigsby** (National Institute for Biological Standards and Control)
14:50-15:05	US SARS-CoV-2 Serology Standard: Background and Calibration to WHO IS 20/136	**Troy Kemp** (Frederick National Laboratory for Cancer Research)
15:05-15:20	Calibration of a Commercial Assay to the International Standard: VITROS Anti-SARS-CoV-2 IgG Quantitative Assay	**Paul Contestable** (Ortho Diagnostics)
15:20-15:35	UKHSA's experiences with the WHO International Standard for anti-SARS-CoV-2 immunoglobulin	**Kevin Bewley** (UK Health Security Agency)
15:35-16:00	Q&A	

Training webinar for the calibration of quantitative serology assays using the WHO International Standard for anti-SARS-CoV-2 immunoglobulin

Introduction

Dr Ivana Knezevic, WHO/MHP/HPS/TSS/NSB

10th November 2021

Zoom meeting

WHO standards for COVID-19 established by the ECBS in Dec 2020

World Health Organization

ECBS meeting on 9-10 Dec 2020 (focused on COVID-19): Technical Report Series 1030

on WHO web site: https://www.who.int/groups/expert-committee-on-biological-standardization:

- Executive Summary posted on WHO web site on 16 Dec 2020:

3 new WHO International reference preparations established

Standards for use in public health emergencies		
SARS-CoV-2 RNA for NAT-based assays	7.40 $\log_{10}$ IU/ampoule	First WHO International Standard
Anti-SARS-CoV-2 immunoglobulin	250 IU/ampoule (neutralizing antibody activity)	First WHO International Standard
Anti-SARS-CoV-2 immunoglobulin panel	[no assigned units]	First WHO International Reference Panel

- Proposal to develop a standard for SARS-CoV-2 antigens to support the development, assessment and comparability of antigen-based rapid diagnostic tests - endorsed.

- Update on written standards provided

WHO measurement standards for COVID-19: 2020-2022

World Health Organization

Aim: to facilitate: 1) the development, validation and assessment of molecular and antibody assays and 2) to allow the comparability of results from different assays/labs and help harmonizing the evaluation of diagnostics, vaccines and other products.

Milestones for development of 1st IS for anti-SARS-CoV-2 immunoglobulin	Timelines
Development of measurement standards start	Feb-March 2020
Sourcing of the candidate material	March-May 2020
Agreement to proceed with Measurement standards	April 2020
Formulation of the candidate Standard	June 2020
Collaborative study	July-Oct 2020
Progress report to ECBS meeting	Aug 2020
Data analysis and report published for PC	Oct-Nov 2020
Establishment by ECBS	December 2020

WHO International Standards for anti-SARS-CoV-2 immunoglobulin in 2021/2022:

1) **2nd WHO IS for anti-SARS-CoV-2 immunoglobulin: proposal endorsed by the ECBS in Oct 2021**
2) **WHO Reference Panel for antibodies for SARS-CoV-2 Variants of Concern: proposal endorsed by the ECBS in Oct 2021**

Role of WHO International Antibody Standards in clinical trials:

- **help interpreting results from vaccine CTs by providing the basis for the expression of the antibody titers in the International Units, particularly results from efficacy trials for various vaccine candidates.**
- **For instance, correlate of protection can be defined as IU/mL**

Distribution of the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin

Status at end July 2021:
2400 units shipped to 581 individual customers in 46 countries

Medicines & Healthcare products Regulatory Agency

WHO International Standard
First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human)
NIBSC code: 20/136
Instructions for use
(Version 2.0, Dated 17/12/2020)

1. INTENDED USE

The First WHO International Standard for anti-SARS-CoV-2 immunoglobulin is the freeze-dried equivalent of 0.25 mL of pooled plasma obtained from eleven individuals recovered from SARS-CoV-2 infection. The preparation has been evaluated in a WHO International Collaborative study (1). The intended use of the International Standard is for the calibration and harmonisation of serological assays detecting anti-SARS-CoV-2 neutralising antibodies. The preparation can also be used as an internal reference reagent for the harmonisation of binding antibody assays. The preparation has been solvent-detergent treated to minimise the risk of the presence of enveloped viruses (2).

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The assigned potency of the WHO International Standard for SARS-CoV-2 is 250 IU/ampoule for neutralising antibody activity. After reconstitution in 0.25 mL of distilled water, the final concentration of the preparation is 1000 IU/mL.

For binding antibody assays, an arbitrary unitage of 1000 binding antibody units (BAU)/mL can be used to assist the comparison of assays detecting the same class of immunoglobulins with the same specificity (e.g. anti-RBD IgG, anti-N IgM, etc.)

Way forward – technical assistance to the users of standards (1)

World Health Organization

1. **WHO technical assistance:**

1.1. Evaluation of vaccines by manufacturers and regulators – assistance with units IU and BAU

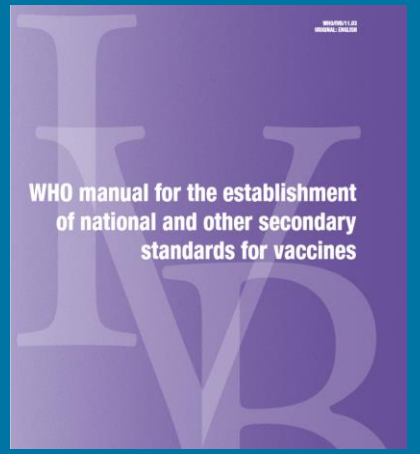
1.2. WHO EUL: "Considerations for the Assessment of COVID-19 Vaccines for Listing by WHO" (https://www.who.int/teams/regulation-prequalification/eul/covid-19):

> 4.1. The assays used for immunogenicity evaluation should be validated for their intended purpose and calibrated against WHO international standards, where available (section 3.2)
>
> 4.2. Assay results should be reported in international units wherever possible (section 3.3.8)

1.3. Manual for secondary standards for vaccines – *New* for antibody standards

(eg, anti-SARS-CoV-2 Ig, anti RSV etc): PC from 18 Oct to 30 Nov 2021:

https://www.who.int/health-topics/biologicals#tab=tab_1



1.4. Input to the new expert group of regulators TAG-CO-VAC

Way forward – technical assistance to the users of standards (2) through COVAX

World Health Organization

1. **Blueprint – workshops on immunobridging and variants of concern**

2. Collaboration through COVAX SWAT teams – Enabling Sciences, Clinical, Manufacturing

2.1 Enabling Sciences:

- https://epi.tghn.org/covax-overview/enabling-sciences/
- Research reagent prepared by NIBSC for anti-SARS-CoV-2 immunoglobulin: an interim solution!
- Standardization of immune response assays: webinars in January, March, May, August, October 2021
- Agility project – animal models and in vitro and in vivo evaluation of SARS-CoV-2 variants

2.2. CEPI Centralized Laboratory Network: opportunity for expressing results in the IU:

- Report from the webinar held on 31 Aug 2021: https://www.bebpa.org/conferences-2/2021-cepi/

2.3. Publications in scientific journals – broader impact:

- Call for use of standards and expression of results in the IU
- Intended use of the IS in the context of neutralization and binding assays used in vaccine CTs

1st call for expression of neutralization assay results from COVID-19 vaccine trials in the International Units

World Health Organization

WHO International Standard for anti-SARS-CoV-2 immunoglobulin

The development timeline of COVID-19 vaccines is unprecedented, with more than 300 vaccine developers active worldwide.[1] Vaccine candidates developed with various technology values were reported relative to the International Standard.

The International Standard and International Reference Panel for anti-SARS-CoV-2 immunoglobulins were adopted by the WHO Expert Committee on Biological Standardization on Dec 10, 2020.[2] The International Standard allows the accurate calibration of assays to an arbitrary unit, thereby reducing inter-laboratory variation trials expressed in IU would allow for the comparison of the immune responses after natural infection and induced by various vaccine candidates. This comparison is particularly important for the identification of correlates of protection against COVID-19; should neutralising antibodies be further supported as a component of the protective response, the expression of antibody responses in IU/mL is essential

Published Online March 23, 2021 https://doi.org/10.1016/S0140-6736(21)00527-4

Need for help from regulators and manufacturers

"Correspondence on the WHO International Standard for Ab for SARS-CoV-2" published in The Lancet: https://www.thelancet.com/action/showPdf?pii=S0140-6736%2821%2900527-4

2nd call for expression of neutralization assay results from COVID-19 vaccine trials in the International Units

World Health Organization

Personal View

WHO International Standard for evaluation of the antibody response to COVID-19 vaccines: call for urgent action by the scientific community

Ivana Knezevic, Giada Mattiuzzo, Mark Page, Philip Minor, Elwyn Griffiths, Micha Nuebling, Vasee Moorthy

The first WHO International Standard and International Reference Panel for anti-SARS-CoV-2 immunoglobulin were established by the WHO Expert Committee on Biological Standardization in December, 2020. The WHO International Antibody Standards are intended to serve as global reference reagents, against which national reference preparations or secondary standards can be calibrated. Calibration will facilitate comparison of results of assays (eg, of the neutralising antibody response to candidate COVID-19 vaccines) conducted in different countries. Use of these standards is expected to contribute to better understanding of the immune response, and particularly of the correlates of protection. This Personal View provides some technical details of the WHO Antibody Standards for SARS-CoV-2, focusing specifically on the use of these standards for the evaluation of the immune response to COVID-19 vaccines, rather than other applications (eg, diagnostic or therapeutic). The explanation with regard to why rapid adoption of the standards is crucial is also included, as well as how funders, journals, regulators, and ethics committees could drive adoption in the interest of public health.

Lancet Microbe 2021

Published Online
October 26, 2021
https://doi.org/10.1016/S2666-5247(21)00266-4

Department of Health Products Policy and Standards, Access to Medicines and Health Products (I Knezevic PhD) **and Research for Health Department, Science Division** (V Moorthy PhD), **World Health Organization, Geneva, Switzerland; National Institute for Biological Standards and Control, Potters Bar, UK** (G Mattiuzzo PhD, M Page PhD); **St Albans, UK** (P Minor PhD); **Kingston upon Thames, UK** (E Griffiths PhD); **Paul-Ehrlich-Institut, Langen, Germany** (Micha Nuebling, PhD)

Introduction

Developing, licensing, and rolling out vaccines against an emerging pathogen that is declared a global public health emergency presents many challenges, including accelerated time frames for evaluating safety and efficacy of candidate vaccines. Regulatory processes must be

A WHO International Standard for neutralising activity of anti-SARS-CoV-2 immunoglobulin has been available since December, 2020, but its usefulness in enabling comparability between vaccines, between laboratories, and over time can be realised only if the International Standard is used widely. To advance

Need for help from the entire scientific community

https://authors.elsevier.com/sd/article/S2666-5247(21)00266-4

Calibration to the WHO IS for anti-SARS-CoV-2 immunoglobulin

Peter Rigsby, Analytical & Biological Sciences, NIBSC

Medicines & Healthcare products Regulatory Agency

WHO International Standards (IS)

- Most IS define International Units (IU) of biological activity
  - Arbitrary units representing content of ampoule or vial; no uncertainty assigned
  - Often not dependent on assay method used

- Often lyophilized, giving highly stable preparations

- Not intended for routine use
  - Secondary standards calibrated directly against (and traceable to) the relevant IS are required

WHO IS 20/136

- Established by WHO ECBS in December 2020

Medicines & Healthcare products Regulatory Agency

WHO International Standard
First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (human)
NIBSC code: 20/136
Instructions for use
(Version 2.0, Dated 17/12/2020)

1. INTENDED USE

The First WHO International Standard for anti-SARS-CoV-2 immunoglobulin is the freeze-dried equivalent of 0.25 mL of pooled plasma obtained from eleven individuals recovered from SARS-CoV-2 infection. The preparation has been evaluated in a WHO International Collaborative study (1). The intended use of the International Standard is for the calibration and harmonisation of serological assays detecting anti-SARS-CoV-2 neutralising antibodies. The preparation can also be used as an internal reference reagent for the harmonisation of binding antibody assays. The preparation has been solvent-detergent treated to minimise the risk of the presence of enveloped viruses (2).

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The assigned potency of the WHO International Standard for SARS-CoV-2 is 250 IU/ampoule for neutralising antibody activity. After reconstitution in 0.25 mL of distilled water, the final concentration of the preparation is 1000 IU/mL.

For binding antibody assays, an arbitrary unitage of 1000 binding antibody units (BAU)/mL can be used to assist the comparison of assays detecting the same class of immunoglobulins with the same specificity (e.g. anti-RBD IgG, anti-N IgM, etc.)

Guidance documents

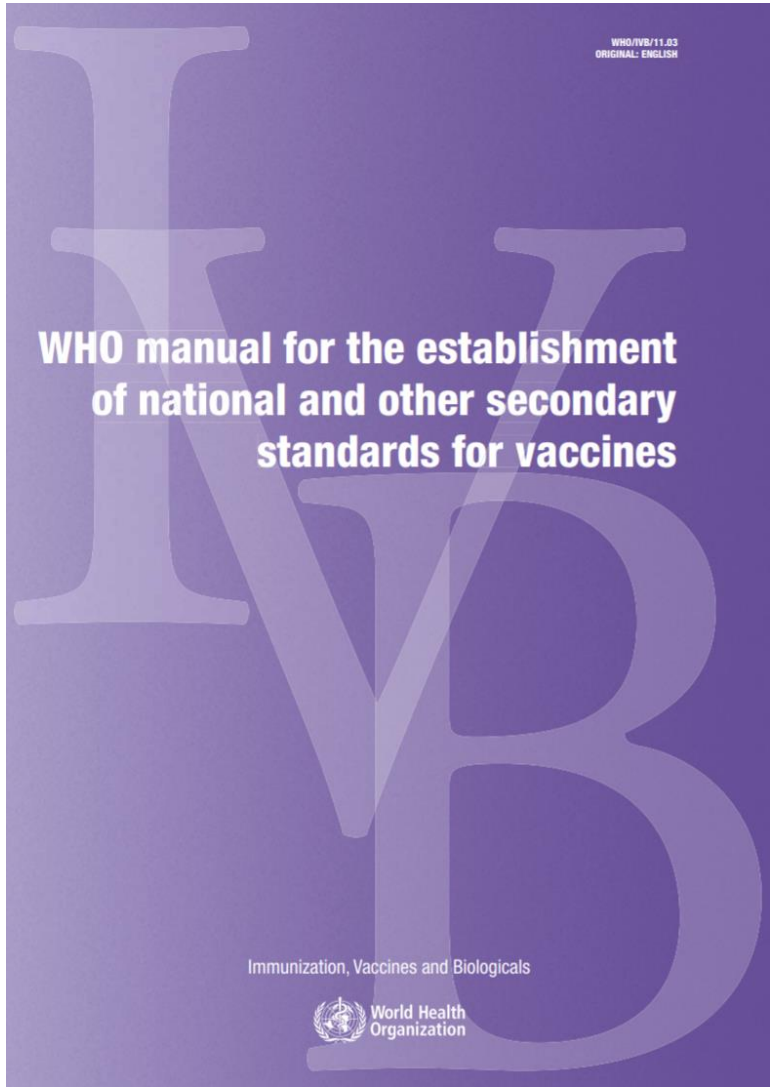
World Health Organization

Post ECBS version

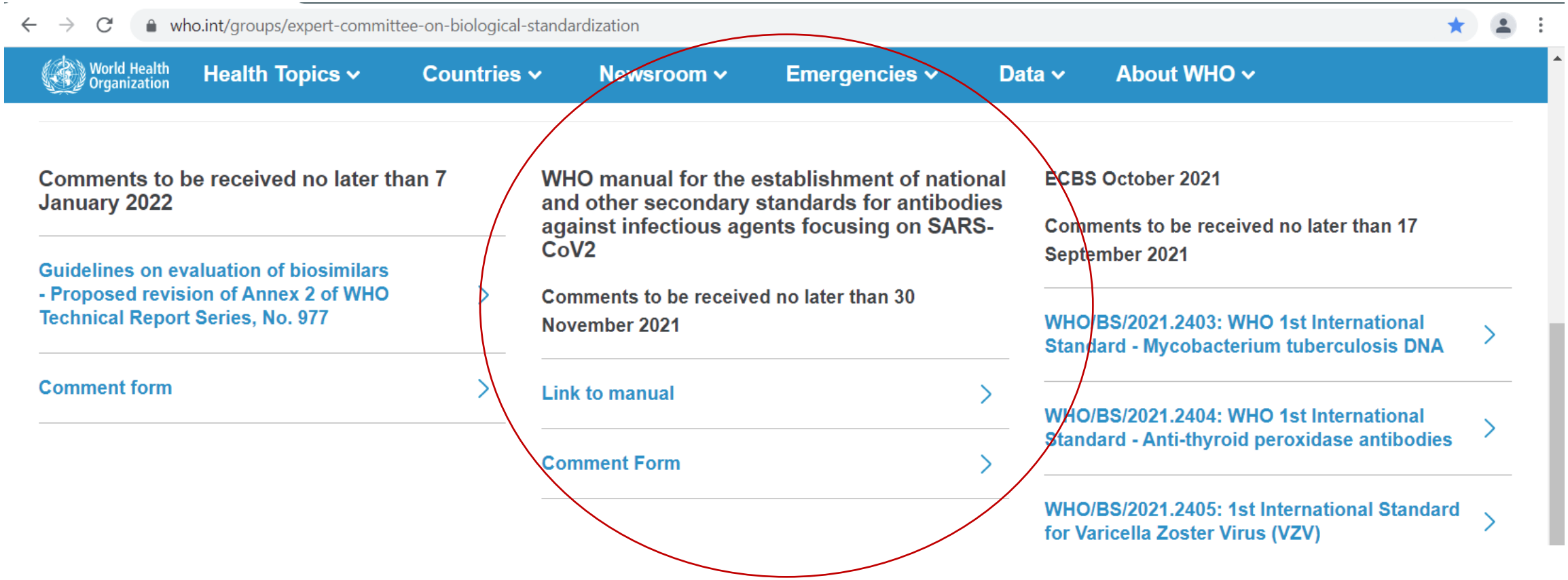
ENGLISH ONLY

MANUAL FOR THE PREPARATION OF SECONDARY REFERENCE MATERIALS FOR IN VITRO DIAGNOSTIC ASSAYS DESIGNED FOR INFECTIOUS DISEASE NUCLEIC ACID OR ANTIGEN DETECTION:

CALIBRATION TO WHO INTERNATIONAL STANDARDS



Guidance document - new



Key properties of different standards

	WHO International Standard	Secondary standard	Tertiary standard
Alternate names	Highest order, International calibrator	Regional or national working reference materials, working reagents, manufacturer's working calibrator	Working reagents, in-house calibrators, in-house controls, manufacturer's product calibrator
Calibration	Established by WHO international collaborative study	Calibrated against the WHO International Standard	Calibrated against the secondary standard
Unitage	IU/mL	IU/mL	IU/mL
Traceability	N/A	Yes	Yes
Uncertainty of measurement	No	Yes (assay specific)	Yes (assay specific)
Final format	Lyophilised (generally)	Lyophilised or liquid	Liquid (generally)
Usage	Calibration of secondary standards, Initial validation of new assay/platform	Calibration of tertiary standards, working reagent, limited use as run control or calibrator	Working reagent, run control, calibrator
Establishment of standard	International agreement through WHO international collaborative study including laboratories worldwide, different assays, different types of test laboratories (approximately 15-30 participants)	May be calibrated in several ways: • In parallel with a study to establish the International Standard • Regional or national collaborative study similar to the WHO collaborative study but with fewer participants from regional laboratories • Small study by a single or limited number of laboratories with a single or limited number of different assays/platforms	Assay-specific study, normally by a single laboratory for use with a specific test/platform Small study by a single or limited number of laboratories with a single or limited number of different assays/platforms

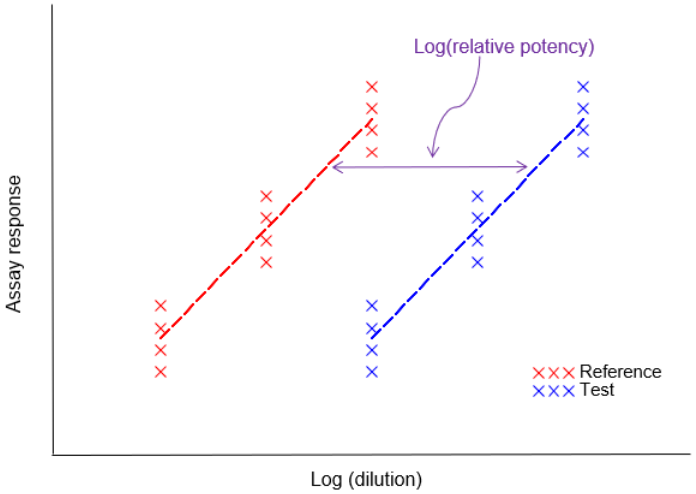
(WHO Technical Report Series, No. 1004, Annex 6, 2017)

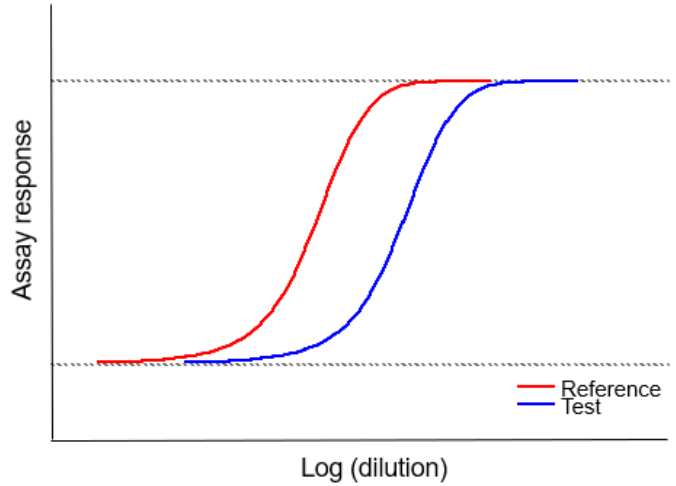
Calibration principles

- Test secondary standard multiple times on different occasions in parallel with the WHO IS under exact same test conditions
- Assess validity of individual assays e.g. linearity and parallelism
- Estimate potency (e.g. IU/mL) for secondary standard in all valid assays
- Combine estimates and assign combined estimate as potency

Data analysis

- Objectives of dose-response data analysis:
  - Assess assay validity e.g. linearity/parallelism for linear model
  - Estimate potency of IHS relative to WHO IS
- Various possible analysis methods, including:
  - Parallel line (parallel curve) analysis [recommended]
  - Interpolation from fitted dose-response curve for WHO IS
- Software options will depend on analysis method:
  - Specialised software for bioassay analysis
  - General statistical software packages, Excel





Notes on calibration

- Use optimal test system (e.g. commercial assay, validated laboratory test)
- Use only qualified operators, equipment etc.
- No general guidance regarding number of assay runs to perform
  - Decision will depend on various factors
  - E.g. sufficient testing may be performed to give Uncertainty of Measurement (UoM) that is negligible in comparison to the expected precision of the routine assay

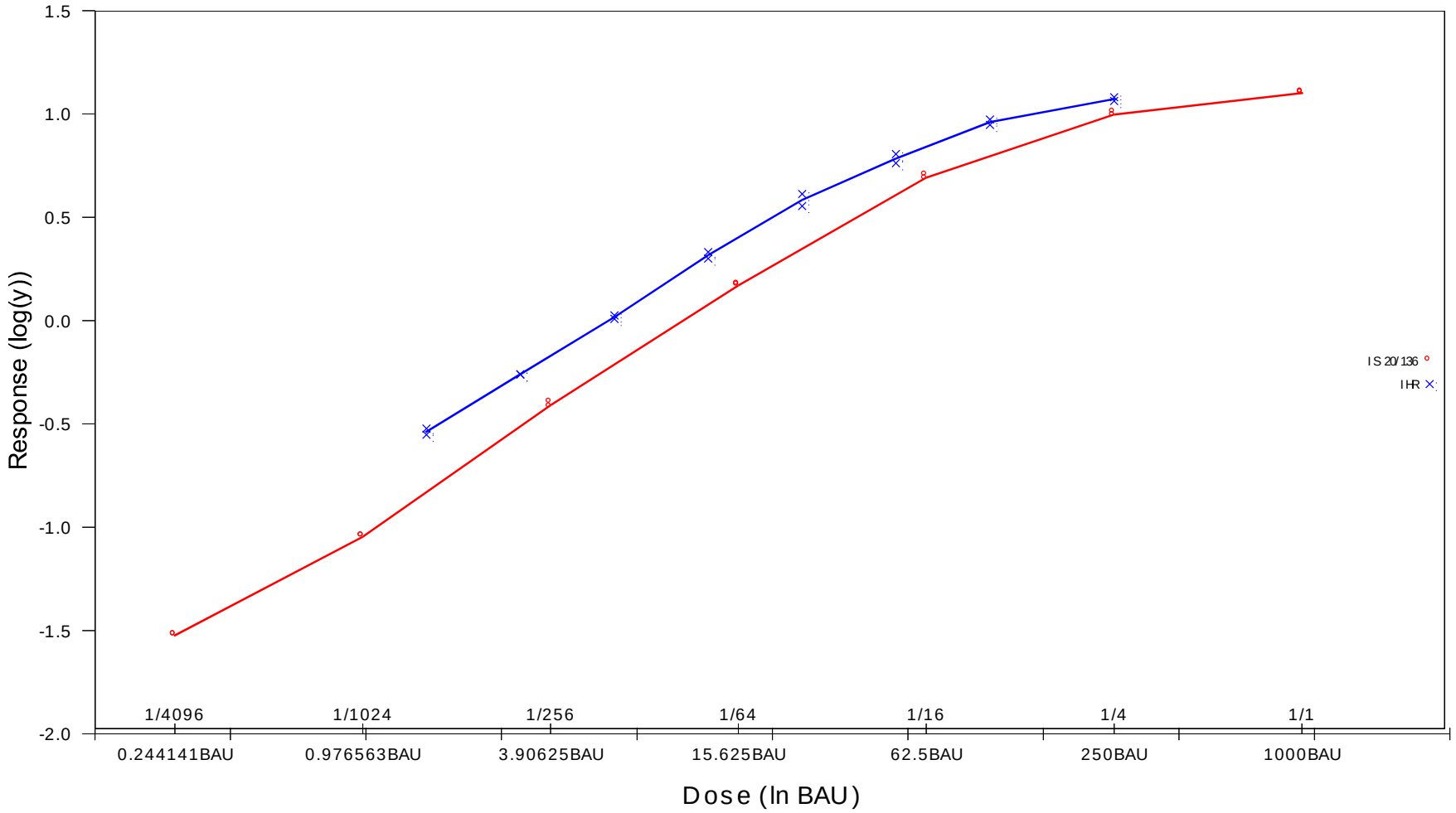
Calibration example

- Samples tested:
  - WHO IS 20/136 - *reconstituted to concentration of 1000 BAU/mL*
  - In-House Reference (IHR)
- Aim: Calibrate IHR in BAU/mL using IS
- COVID-19 ELISA; RDB SPIKE S1 & S2; IgG; semi-quantitative
- Samples initially tested in 3 independent assay runs

Calibration example – assay 1 data

Assay Cut-off			0.278		
Acceptability Criteria			index <0.9 negative 0.9-1.1 equivocal ≥1.1 positive		
Study Sample	Reciprocal Dilution	+/-	Raw Data		
			replicate 1 S/Co	replicate 2 S/Co	replicate 3 S/Co
No sample (Diluent Only)			0.01	0.01	
Negative Control		NEG	0.11	0.10	
Positive Control		POS	9.15	9.16	
In-House Reference (IHR)	1:2	pos	12.07	11.57	
	1:5	pos	9.42	8.87	
	1:10	pos	6.40	5.79	
	1:20	pos	4.10	3.59	
	1:40	pos	2.15	2.00	
	1:80	equiv	1.06	1.02	
	1:160	neg	0.55	0.55	
	1:320	neg	0.30	0.28	
Titre / value			dil 1:40	dil 1:40	
WHO IS 20/136	N	pos	12.67	12.55	
	1:4	pos	10.13	9.76	
	1:16	pos	5.03	4.82	
	1:64	pos	1.47	1.49	
	1:256	neg	0.38	0.40	
	1:1024	neg	0.09	0.09	
	1:4096	neg	0.03	0.03	
	1:16384	neg	0.01	0.01	
Titre / value			dil 1:64	dil 1:64	

Calibration example – assay 1 plot



Calibration example – assay 1 analysis

Model: log(y)=d+a*(lgt(x)) where x=c.+b*ln(dose)
Design: Completely randomised
Weight function: w=1.0
Variance: Observed residuals

Common slope(factor): b = 0.541029 (0.535405 to 0.546653)
Correlation | r |: 0.998682 (Weighted), 0.999379 (Unweighted)
Asymptotes: -2.13212 and 1.31506

Source of variation	Degrees of freedom	Sum of squares	Mean square	Chi-square	Probability
Preparations	1	0.691970	0.691970	2172.37	0.000 (***)
Regression	1	7.97454	7.97454	25035.3	0.000 (***)
Non-parallelism	1	4.03172E-06	4.03172E-06	0.0126572	0.910
Non-linearity	11	0.0181154	0.00164686	56.8716	0.000 (***)
Standard	5	0.00756646	0.00151329	23.7542	0.000 (***)
Sample 1	6	0.0105490	0.00175816	33.1175	0.000 (***)
Treatments	14	8.68463	0.620331	27264.5	0.000 (***)
Residual error	15	0.00477798	0.000318532		
Total	29	8.68941	0.299635		

	Slope per Sample	Difference with Standard	Ratio with Standard
Standard	0.540728 (0.533590 to 0.547867)	0	1
Sample 1	0.541521 (0.532387 to 0.550656)	0.000792893 (-0.0107995 to 0.0123853)	1.00147 (0.980188 to 1.02309)

Sample 1			
Id.	IHR		
(BAU/ml)	Lower limit	Estimate	Upper limit
Potency	873.302	908.709	945.816
Rel. to Ass.	174.7%	181.7%	189.2%
Rel. to Est.	96.1%	100.0%	104.1%

Calibration example – assay 1 analysis

Model: log(y)=d+a*(lgt(x)) where x=c.+b*ln(dose)
Design: Completely randomised
Weight function: w=1.0
Variance: Observed residuals

Common slope(factor): b = 0.541029 (0.535405 to 0.546653)
Correlation | r |: 0.998682 (Weighted), 0.999379 (Unweighted)
Asymptotes: -2.13212 and 1.31506

Source of variation	Degrees of freedom	Sum of squares	Mean square	Chi-square	Probability
Preparations	1	0.691970	0.691970	2172.37	0.000 (***)
Regression	1	7.97454	7.97454	25035.3	0.000 (***)
Non-parallelism	1	4.03172E-06	4.03172E-06	0.0126572	0.910
Non-linearity	11	0.0181154	0.00164686	56.8716	0.000 (***)
Standard	5	0.00756646	0.00151329	23.7542	0.000 (***)
Sample 1	6	0.0105490	0.00175816	33.1175	0.000 (***)
Treatments	14	8.68463	0.620331	27264.5	0.000 (***)
Residual error	15	0.00477798	0.000318532		
Total	29	8.68941	0.299635		

	Slope per Sample	Difference with Standard	Ratio with Standard
Standard	0.540728 (0.533590 to 0.547867)	0	1
Sample 1	0.541521 (0.532387 to 0.550656)	0.000792893 (-0.0107995 to 0.0123853)	1.00147 (0.980188 to 1.02309)

Sample 1			
Id.	IHR		
(BAU/ml)	Lower limit	Estimate	Upper limit
Potency	873.302	908.709	945.816
Rel. to Ass.	174.7%	181.7%	189.2%
Rel. to Est.	96.1%	100.0%	104.1%

Calibration example – assay 1 analysis

Model: log(y)=d+a*(lgt(x)) where x=c.+b*ln(dose)
Design: Completely randomised
Weight function: w=1.0
Variance: Observed residuals

Common slope(factor): b = 0.541029 (0.535405 to 0.546653)
Correlation | r |: 0.998682 (Weighted), 0.999379 (Unweighted)
Asymptotes: -2.13212 and 1.31506

Source of variation	Degrees of freedom	Sum of squares	Mean square	Chi-square	Probability
Preparations	1	0.691970	0.691970	2172.37	0.000 (***)
Regression	1	7.97454	7.97454	25035.3	0.000 (***)
Non-parallelism	1	4.03172E-06	4.03172E-06	0.0126572	0.910
Non-linearity	11	0.0181154	0.00164686	56.8716	0.000 (***)
Standard	5	0.00756646	0.00151329	23.7542	0.000 (***)
Sample 1	6	0.0105490	0.00175816	33.1175	0.000 (***)
Treatments	14	8.68463	0.620331	27264.5	0.000 (***)
Residual error	15	0.00477798	0.000318532		
Total	29	8.68941	0.299635		

	Slope per Sample	Difference with Standard	Ratio with Standard
Standard	0.540728 (0.533590 to 0.547867)	0	1
Sample 1	0.541521 (0.532387 to 0.550656)	0.000792893 (-0.0107995 to 0.0123853)	1.00147 (0.980188 to 1.02309)

Sample 1			
Id.	IHR		
(BAU/ml)	Lower limit	Estimate	Upper limit
Potency	873.302	908.709	945.816
Rel. to Ass.	174.7%	181.7%	189.2%
Rel. to Est.	96.1%	100.0%	104.1%

Calibration example – assay 1 analysis

Model: log(y)=d+a*(lgt(x)) where x=c.+b*ln(dose)
Design: Completely randomised
Weight function: w=1.0
Variance: Observed residuals

Common slope(factor): b = 0.541029 (0.535405 to 0.546653)
Correlation | r |: 0.998682 (Weighted), 0.999379 (Unweighted)
Asymptotes: -2.13212 and 1.31506

Source of variation	Degrees of freedom	Sum of squares	Mean square	Chi-square	Probability
Preparations	1	0.691970	0.691970	2172.37	0.000 (***)
Regression	1	7.97454	7.97454	25035.3	0.000 (***)
Non-parallelism	1	4.03172E-06	4.03172E-06	0.0126572	0.910
Non-linearity	11	0.0181154	0.00164686	56.8716	0.000 (***)
Standard	5	0.00756646	0.00151329	23.7542	0.000 (***)
Sample 1	6	0.0105490	0.00175816	33.1175	0.000 (***)
Treatments	14	8.68463	0.620331	27264.5	0.000 (***)
Residual error	15	0.00477798	0.000318532		
Total	29	8.68941	0.299635		

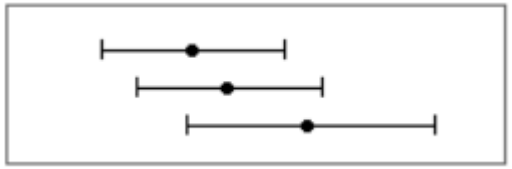
	Slope per Sample	Difference with Standard	Ratio with Standard
Standard	0.540728 (0.533590 to 0.547867)	0	1
Sample 1	0.541521 (0.532387 to 0.550656)	0.000792893 (-0.0107995 to 0.0123853)	1.00147 (0.980188 to 1.02309)

Sample 1			
Id.	IHR		
(BAU/ml)	Lower limit	Estimate	Upper limit
Potency	873.302	908.709	945.816
Rel. to Ass.	174.7%	181.7%	189.2%
Rel. to Est.	96.1%	100.0%	104.1%

Calibration example – combined estimate

- Combined estimate (BAU/mL) from assays 1-3:

Assay 1:
Assay 2:
Assay 3:



Sample	Estimate	95% LCL	95% UCL
IHR	925.4	899.1 *(97.2%)*	952.4 *(102.9%)*

- Assigned value to IHR is 925 IU/mL

Summary

- Expression of assay results in International Units (IU) requires use of the WHO International Standard (IS) directly, or the use of a secondary standard calibrated using the IS
- A calibration exercise can be performed to assign an IU value to the secondary standard
- Existing assay results already reported relative to a secondary standard (in µg, EU, relative titre etc.), can then be reported as IU
  - In most cases, existing assay analysis methods are unaffected

© Crown copyright

Frederick National Laboratory
for Cancer Research
sponsored by the National Cancer Institute

US SARS-CoV-2 Serology Standard: Background and Calibration to WHO IS 20/136

Troy Kemp, PhD, Scientific Manager

Ligia Pinto, PhD, Director, Vaccine, Immunity and Cancer Program

A US SARS-CoV-2 Serology Standard

- **What is it and why do we use it?**
- **How we made it and what does it contain?**
- **How do we assign unitage and evaluate suitability?**
- **Calibration to the WHO International Standard**

A US SARS-CoV-2 Serology Standard: What is it and why do we use it?

- The serology standard is to be used as **an assay calibrator** by laboratories conducting SARS-CoV-2 serology testing
  - to measure antibodies after infection or after vaccination

- The main goal of a serology standard is **to harmonize assays** that measure anti-SARS-CoV-2 antibodies **to increase comparability** of results from different studies, including different candidate vaccines

How was the standard generated?
Screening Study to Select Candidates

Four Laboratories screened 9 convalescent ACD plasma samples (high volume) donated by BARDA to select suitable samples to use as a SARS-CoV-2 standard

Testing laboratories

- CDC
- NIAID-Integrated Research Facility (IRF)
- NIH Clinical Center
- FNLCR

Assays

- Spike IgG and IgM ELISA
- Neutralization Assay
- Roche Nucleocapsid Total Antibody
- Nucleocapsid IgG and IgM

Sample ID	Sample Type	Sample Volume (mL)	IRF Neutralization	HSL Spike IgM (AU/mL)	HSL Spike IgG (AU/mL)	HSL Nucleocapsid IgG (AU/mL)	OD- FNL Krammer SOP RBD IgG	Titer- FNL CDC SOP IgM	Titer- FNL CDC SOP IgG	Titer- CDC Spike PanIg	Titer- CDC Spike IgG	Titer- CDC Spike IgM
Sample 1	Plasma	124	1:34	116.1	814.2	4146.9	2.1	100	400	400	400	NEG
Sample 2	Plasma	500	1:33	NEG	295.8	647.1	1.6	NEG	400	100	400	NEG
Sample 3	Plasma	500	1:80	610.8	12281.5	10813.7	2.8	1600	6400	6400	6400	100
Sample 4	Plasma	470	NEG	NEG	NEG	NEG	0.1	NEG	NEG	NEG	NEG	NEG
Sample 5	Plasma	400	1:99	201.6	4971.0	15838.9	2.8	100	6400	1600	1600	NEG
Sample 6	Plasma	110	1:843	124.7	31893.3	20297.7	2.8	400	6400	6400	25600	100
Sample 7	Plasma	400	1:40	230.2	923.3	6240.1	2.4	100	1600	400	400	NEG
Sample 8	Plasma	110	1:618	1195.0	13597.1	16618.8	3.3	1600	6400	6400	6400	400
Sample 9	Plasma	300	1:282	473.1	5319.1	12356.2	2.7	100	6400	1600	6400	NEG

How did we assign unitage and evaluated suitability across assay types?

COLLABORATIVE STUDY

Panel

16 samples (7 samples + US Standard) in duplicate, deidentified

Panel Testing

Three sets of the samples were sent to participating labs
Each set of 16 samples were tested on three separate days

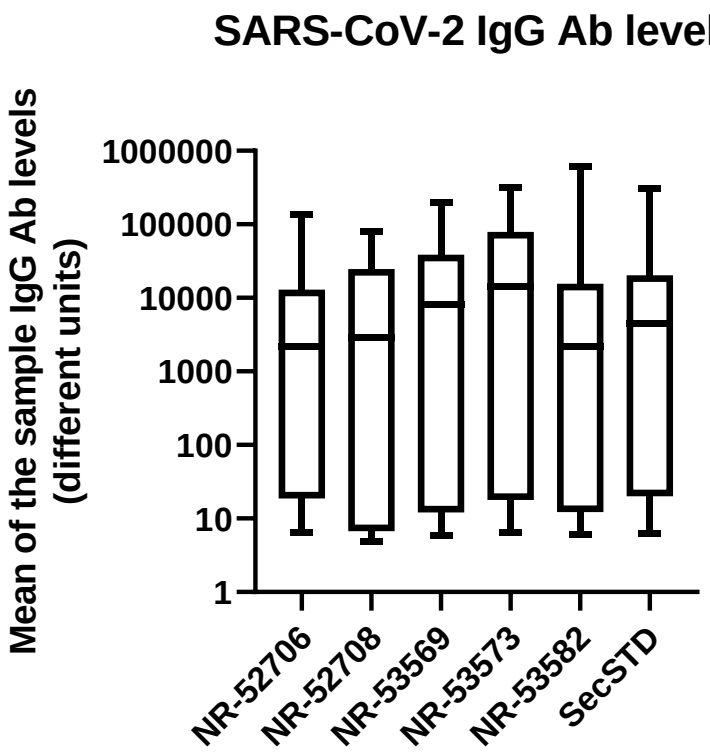
Testing laboratories

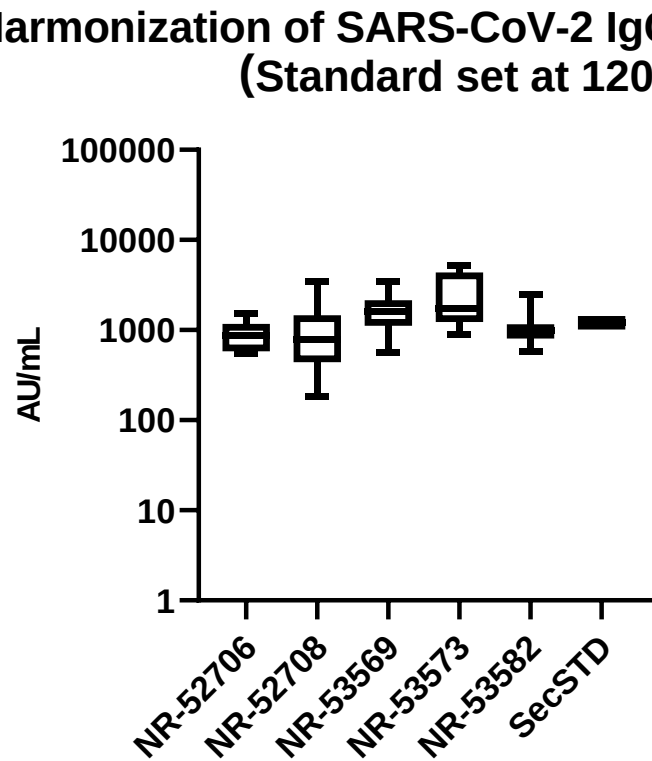
- CDC
- NIAID-IRF
- Mount Sinai
- NIH Clinical Center
- Quest Diagnostics
- FNLCR
- NIST
- NIAID-VRC

Assays

- Spike IgG and IgM ELISA
- Neutralization Assay
- Orthogonal (RBD IgG and Spike IgG)
- Abbott Nucleocapsid IgG
- Roche Nucleocapsid Total Antibody
- Euroimmun Spike IgG
- Nucleocapsid IgG and IgM
- Inhibition Assay (RBD-ACE2)
- MSD (RBD, Spike, Nucleocapsid) IgG

Collaboration Study Analyses of IgG Assays according to NIBSC guidance





ID	Interlab GM	Interlab SD	Interlab %GCV	Max GM	Min GM
NR-52706	844.8	1.5	3416.3%	137642.1	6.4
NR-52708	822.3	1.7	4393.8%	80929.4	4.9
NR-53569	1498.9	1.7	5223.8%	197620.6	5.9
NR-53573	2096.9	1.8	6160.8%	321956.8	6.5
NR-53582	1001.3	1.7	4703.3%	614402.9	6.1
SecSTD	1241.1	1.6	4334.8%	303158.3	6.4
Mean	1250.9				

ID	Interlab GM	Interlab SD	Interlab %GCV	Max GM	Min GM
NR-52706	841.8	0.2	41.6%	1547.5	547.8
NR-52708	807.5	0.4	134.6%	3428.8	182.1
NR-53569	1490.5	0.2	60.6%	3508.8	571.7
NR-53573	2097.3	0.3	90.9%	5192.1	901.1
NR-53582	998.5	0.2	43.3%	2449.3	576.9
SecSTD	1200.0	0.0	0.0%	1200.0	1200.0

Calibration to WHO Serology
SARS-CoV-2 International Standard (IS)

Study Design

FNLCR tested the WHO IS along with the US SARS-CoV-2 serology standard in Spike and Nucleocapsid assays.

Testing Plan

Day 1: Reconstitute a single vial of WHO IS and test the material in triplicate in the four FNLCR assays (Spike IgG and IgM; Nucleocapsid IgG and IgM). The US SARS-CoV-2 serology standard was also included in the same plate and tested in triplicate.

-Repeat Day 1 testing for a total of three separate testing days.

Day 1	1	2	3	4	5	6	7	8	9	10	11	12
Plate 1	C_STD	C_STD	NEG	PC1	STD-C1	STD-C2	STD-C3	STD-T1	STD-T2	STD-T3	C_STD	C_STD
A	50	50	50	50	200	200	200	200	200	200	50	50
B	100	100	150	150	400	400	400	400	400	400	100	100
C	200	200	450	450	800	800	800	800	800	800	200	200
D	400	400	1350	1350	1600	1600	1600	1600	1600	1600	400	400
			No Sample	PC2								
E	800	800	50	150	3200	3200	3200	3200	3200	3200	800	800
F	1600	1600	150	450	6400	6400	6400	6400	6400	6400	1600	1600
G	3200	3200	450	1350	12800	12800	12800	12800	12800	12800	3200	3200
H	6400	6400	1350	4050	25600	25600	25600	25600	25600	25600	6400	6400

STD-C: WHO IS 1000 (IU/mL)

STD-T: US Serology Standard

C_STD: Assay Daily Use Standard (Internal Reference Standard)

Calibration to WHO Serology SARS-CoV-2 International Standard

Data Analyses

Dashed lines- Response range of WHO IS (20/136)

Notes:

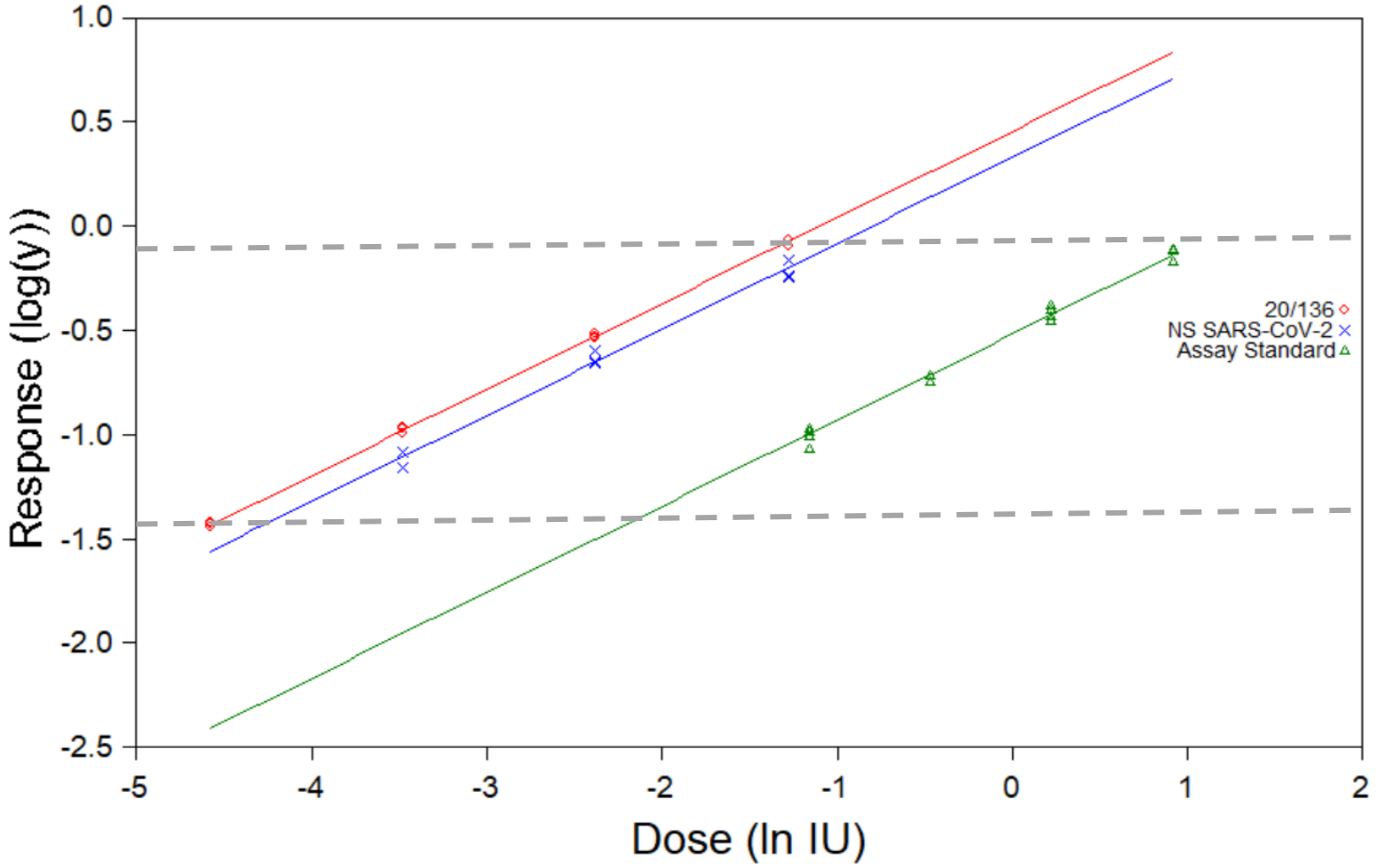
1. **Response range of Samples needs to fall within the calibrator response range**
2. **Dose-response lines should be parallel and linear**

Calibrator-WHO Serology SARS-CoV-2 International Standard (Red Line)

Test- US SARS-CoV-2 Serology Standard (Blue Line)

Assay Standard- Internal Reference Standard (Green Line)

Parallel Line Method: Combistats



Calibration to WHO Serology
SARS-CoV-2 International Standard

IgG Data Analyses- with Parallel Line Analysis (Combistats)

SPIKE

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean
STD-C	400	1000	1000	1000	1000
STD-T	400	740	783	770	764
C_STD	50	95	98	90	94

NUCLEOCAPSID

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean
STD-C	400	1000	1000	1000	1000
STD-T	400	714	655	676	681
C_STD	100	74	76	73	74

STD-C: WHO IS 1000 (BAU/mL)

STD-T: US Serology Standard

C_STD: Assay Daily Use Standard (Internal Reference Standard)

Calculation:
Set STD-C value as 1000 BAU/mL, and software calculates STD-T value

Calibration to WHO Serology
SARS-CoV-2 International Standard

IgM Data Analyses- with Parallel Line Analysis (Combistats)

SPIKE

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean
STD-C	100	1000	1000	1000	1000
STD-T	100	208	264	271	246
C_STD	100	843	975	919	911

NUCLEOCAPSID

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean
STD-C	50	1000	1000	1000	1000
STD-T	50	1132	950	1038	1037
C_STD	400	11454	10449	10256	10707

STD-C: WHO IS 1000 (BAU/mL)

STD-T: US Serology Standard

C_STD: Assay Daily Use Standard (Internal Reference Standard)

Calculation:
Set STD-C value as 1000 BAU/mL, and software calculates STD-T value

Calibration to WHO Serology
SARS-CoV-2 International Standard

Neutralization Data Analyses- with Sigmoid Model Analysis (Combistats)

Neutralization Assay (Wild-type virus, FRNA)

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean*
STD-C	40	1000	1000	1000	1000
STD-T	40	738	875	865	813

*- semi-weighted combination

STD-C: WHO IS 1000 (IU/mL)

STD-T: US Serology Standard

Calculation:
Set STD-C value as 1000 IU/mL, and software calculates STD-T value

Calibrated US SARS-CoV-2 Serology Standard

	Spike IgG (BAU/mL)	Nucleocapsid IgG (BAU/mL)	Spike IgM (BAU/mL)	Nucleocapsid IgM (BAU/mL)	Neutralization (IU/mL)
WHO International Standard (20/136)	1000	1000	1000	1000	1000
US Serology Standard	764	681	246	1037	813

US SARS-CoV-2 Serology Standard Promotion and Implementation

Promotion

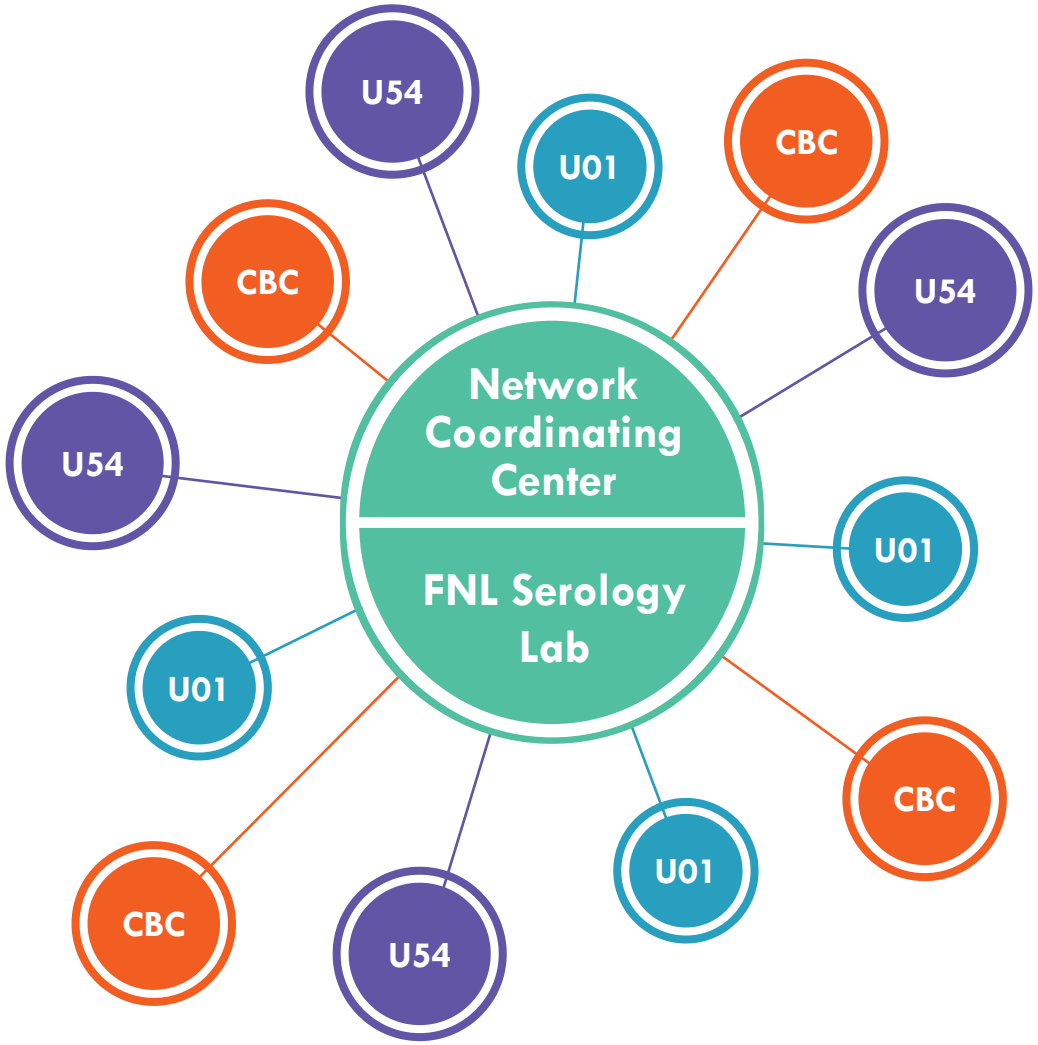
NCI and FNL have promoted the availability of the Serology Standard through various channels

FNL Serology site for request

https://frederick.cancer.gov/seronet/serology-standard

Serological Sciences Network (SeroNet)

Widespread emails



Acknowledgments

NCI

- Douglas Lowy
- Sean Hanlon
- Jim Cherry

Frederick National Laboratory for Cancer Research

Serology Laboratory

- Troy Kemp
- David Pan
- Angelina Richards
- Jack Quesinberry
- Jordan Metz
- Sarah Loftus
- Marissa Blackburn
- Brooke Georgetti
- Aaron Bouk

NIAID

- Cristina Cassetti

FDA

- Brendan O'Leary
- Ribhi Shawar
- Pamela Gallagher
- Steve Gitterman

BARDA

- Rosemary Humes
- John Lee
- Charles Daitch

CDC

- S. Michele Owen
- Nathalie Thornburg

Icahn School of Medicine at Mount Sinai

- Florian Krammer
- Carlos Cordon-Cardo

NIBSC

- Mark Page

Frederick National Laboratory
for Cancer Research

sponsored by the National Cancer Institute

DEPARTMENT OF HEALTH AND HUMAN SERVICES • National Institutes of Health • National Cancer Institute

Frederick National Laboratory is a Federally Funded Research and Development Center operated by Leidos Biomedical Research, Inc., for the National Cancer Institute

Ortho Clinical Diagnostics

Because Every Test Is A Life™

Calibration of a Commercial Assay to the International Standard: VITROS Anti-SARS-CoV-2 IgG Quantitative Assay

November 10, 2021

VITROS SARS-CoV-2 IgG Quantitative Assay

Assay Background

- The VITROS SARS-CoV-2 IgG Quantitative is a Spike S1 assay modified from the VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG (qualitative) by the addition of a set of calibrators value assigned off the 1st WHO SARS-CoV-2 antibody standard.
- The VITROS SARS-CoV-2 Quantitative assay is calibrated to the 1st WHO International Standard Anti-SARS-CoV-2 Immunoglobulin (Human), NIBSC 20/136 with results reported in the WHO Anti-SARS-CoV-2 standard units of Binding Antibody Units/mL (BAU/mL).
- A set of nine internal Reference Calibrators (Standards) have been value assigned off the 1st WHO International Standard and are used to value assign sets of three Customer Calibrators on a reagent lot-to-lot basis for customer lab use.
- A qualitative cutoff for reactive or nonreactive has also been established for the assay for samples from individuals with unknown infection or vaccination status.

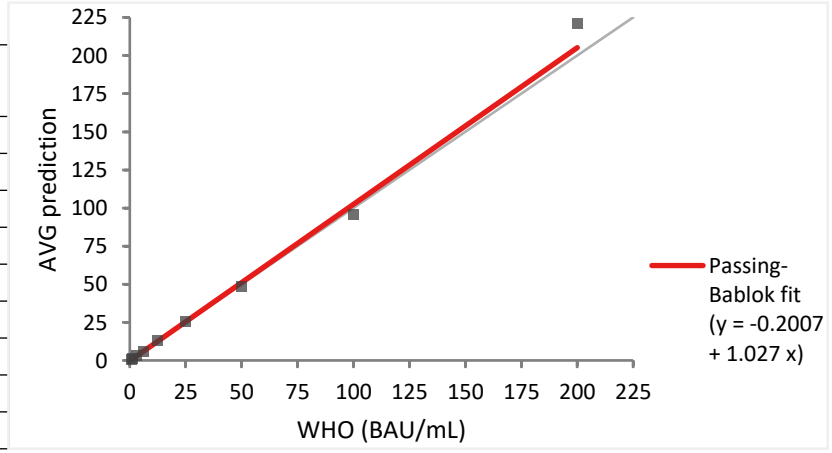
VITROS IgG Quant Reference Calibrator Value Assignment

Calibration Traceability

- Reference Calibrators were assigned values through a traceability study conducted with the WHO International Standard (NIBSC 20/136). A 10-member titration panel of the standard was run in duplicate on both a VITROS 3600 and VITROS ECi instrument (n=4) and used to assign values to an average calibration curve generated through 10 measurements of Reference Calibrators on 2 VITROS ECi's or 2 VITROS x600 instruments (n=20).
- This WHO-traceable reference calibrator panel was then used to back-predict the dose of the WHO-standard titration panel.
- This analysis demonstrated close alignment between the WHO-traceable Reference Calibrators and a titration of the WHO standard

RCAL	BAU/mL
R1b	0
R2	2.100
R4	8.446
R5	14.56
R6	34.78
R7	50.62
R8	75.92
R9	111.9
R10	141.5
R11	203.0

WHO (BAU/mL)	AVG pred. (BAU/mL)	AVG % Bias
200	221.0	10.5
100	96.0	-4.0
50	48.6	-2.9
25	25.8	3.1
12.5	12.9	3.1
6.25	6.13	-1.9
3.13	3.00	-4.0
1.56	1.51	-2.9
0.78	0.60	-23.0



VITROS IgG Quantitative Performance with US Standard

US Standard – Frederick National Laboratory

- US Standard was prepared and diluted on three separate occasions. Each preparation was tested (using a customer calibration) in triplicate over two days each for a total of 18 results with a %CV of <5% for each dilution across all preparations, testing days and replicates.
- Stock standard was at 764 BAU/mL and dilutions were made at 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640 for expected values shown in the table below.
- Excellent dilution recovery was observed demonstrating linearity and accuracy of the calibration

US SARS-CoV-2 Antibody Standard Dilution	Actual BAU/mL	VITROS IgG Quantitative Result - BAU/mL	Bias from Actual Concentration
Stock	764	741*	-3.1%
1:5	152	148	-3.1%
1:10	76.4	69.0	-9.7%
1:20	38.2	36.5	-4.4%
1:40	19.1	18.9	-1.3%
1:80	9.55	9.33	-2.3%
1:160	4.78	4.47	-6.5%
1:320	2.39	2.23	-6.8%
1:640	1.19	<2.00	NA

*calculated from 1:5 dilution

Note: Dilution performance not authorized by US FDA

VITROS SARS-CoV-2 IgG Quantitative

Qualitative Cutoff Placement and Clinical Performance

- For screening purposes, a qualitative cutoff was established based on clinical testing.
- Samples with a result of 17.8 BAU/mL or greater are considered reactive for Anti-SARS-CoV-2 IgG.
- Results will be reported both with a numerical result and a qualitative reactive/non-reactive result

Assay measuring range **is 2.0-200 BAU/mL**

- Clinical Performance
  - Observed specificity of 100%.

Number of Subjects Tested	IgG Non-reactive	IgG Reactive	IgG Specificity	Specificity (95% CI)
541	541	0	100.0%	99.3% - 100.0%

  - Observed percent positive agreement with PCR of 92.7%.

Number of Subjects Tested	IgG Reactive	IgG Non-reactive	IgG PPA	95% CI
264	244	20	92.7%	88.5% - 95.3%

Vaccine Induced Antibody Response

Vaccinated Individuals

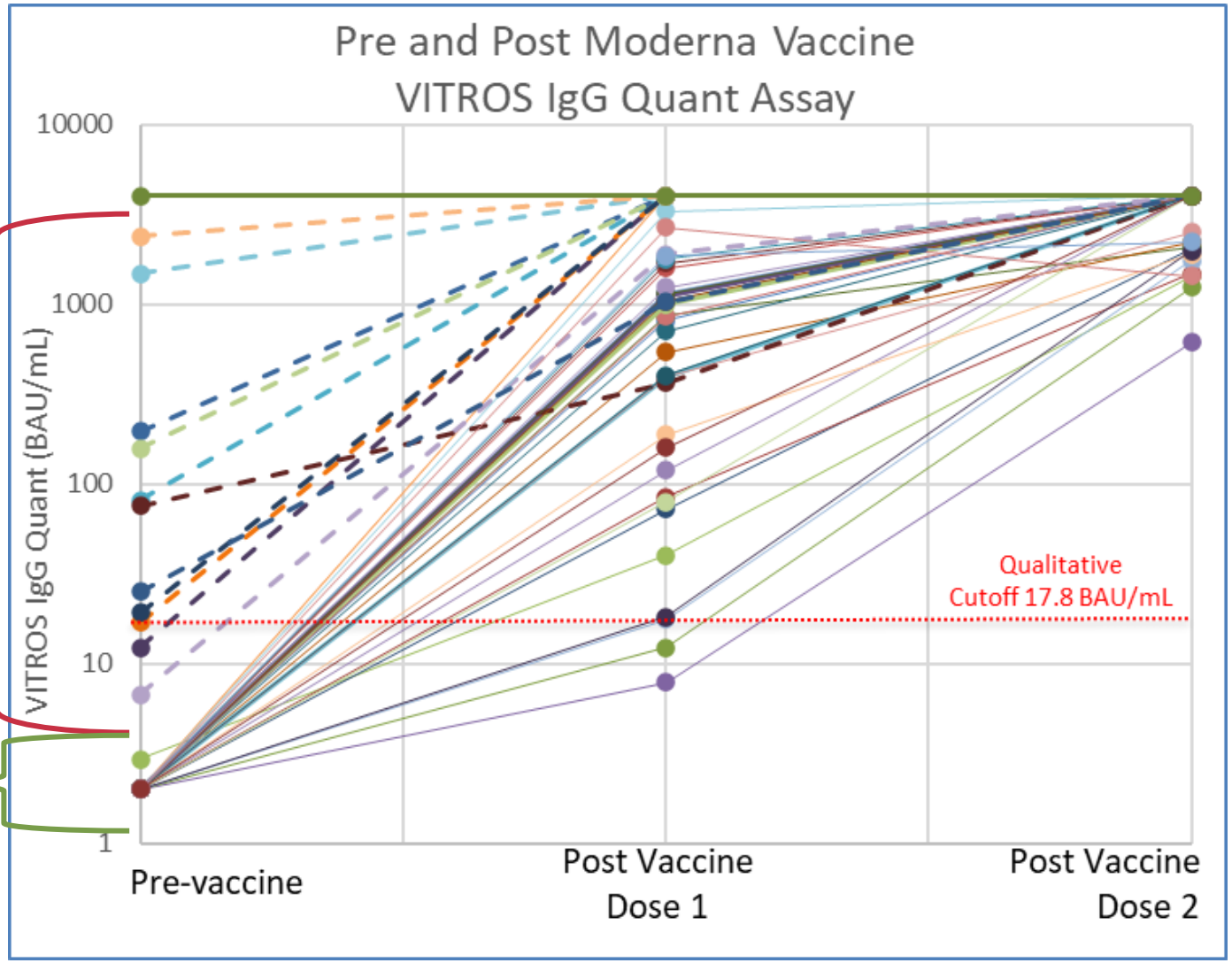
- Samples were collected from 45 donors. Each donor provided a sample collected prior to vaccination, after the 1st Moderna vaccine dose (22-59 days post 1st vaccine), and after the 2nd Moderna vaccine dose (12-17 days post 2nd vaccine dose). Each sample was analyzed on the VITROS SARS-CoV-2 IgG Quant assay.
- Of the 45 donor's panels, antibodies were detected in every panel. The post 2nd-dose sample yielded results over the top of the assay's range for every donor.
- Note: Twelve samples had elevated (>5 BAU/mL) CVGQN results prior to vaccination. All but one sample were reactive with the VITROS Nucleocapsid Total antibody assay indicating previous infection with SARS-CoV-2 in 11 of the individuals. Several of the individuals without previous SARS-CoV-2 exposure demonstrated slower response to the vaccine.

Vaccine Induced Antibody Response

Vaccinated Individuals (with and without previous infection)

VITROS Total N Antibody
Pre-vaccine Reactive
(Previously Infected Individuals)

VITROS Total N Antibody
Pre-vaccine Non-Reactive



Note: Vaccine performance not authorized by US FDA.

Ortho Clinical Diagnostics

Because Every Test Is A Life™

UK Health Security Agency

UKHSA's experiences with the WHO International Standard for anti-SARS-CoV-2 immunoglobulin

Dr Kevin Bewley (Senior Virologist)
Medical Interventions Group (MIG), UK Health Security Agency

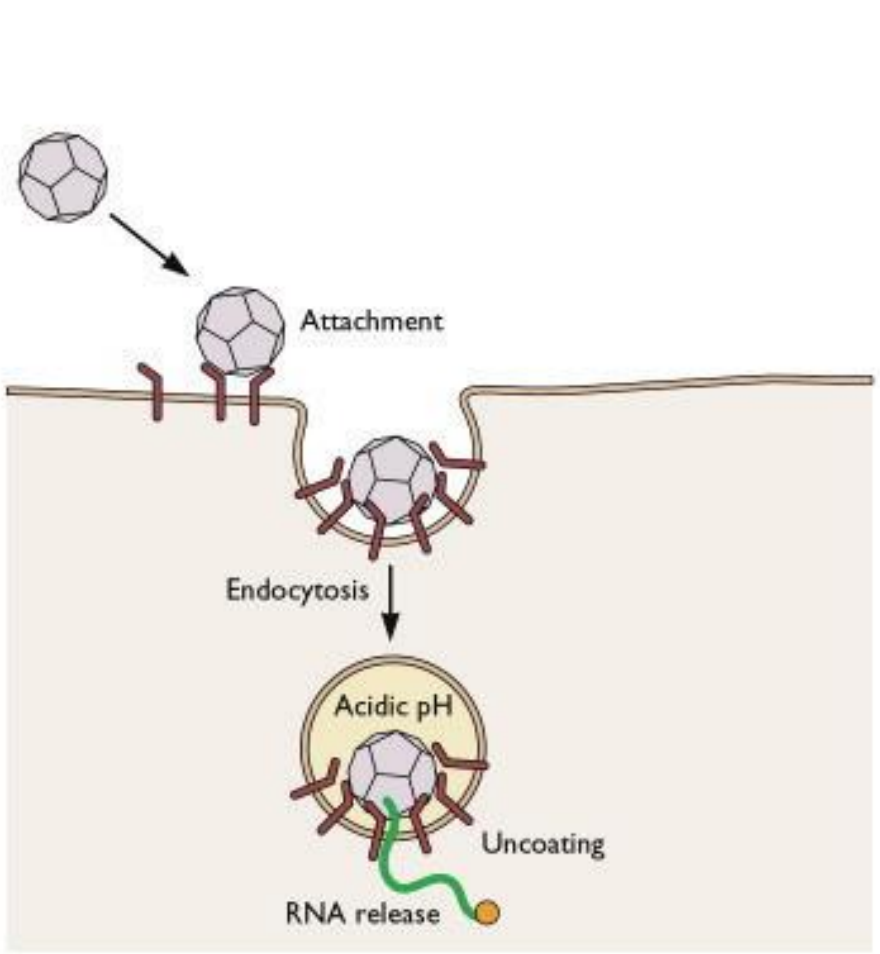
How do we use microneutralisation assays?

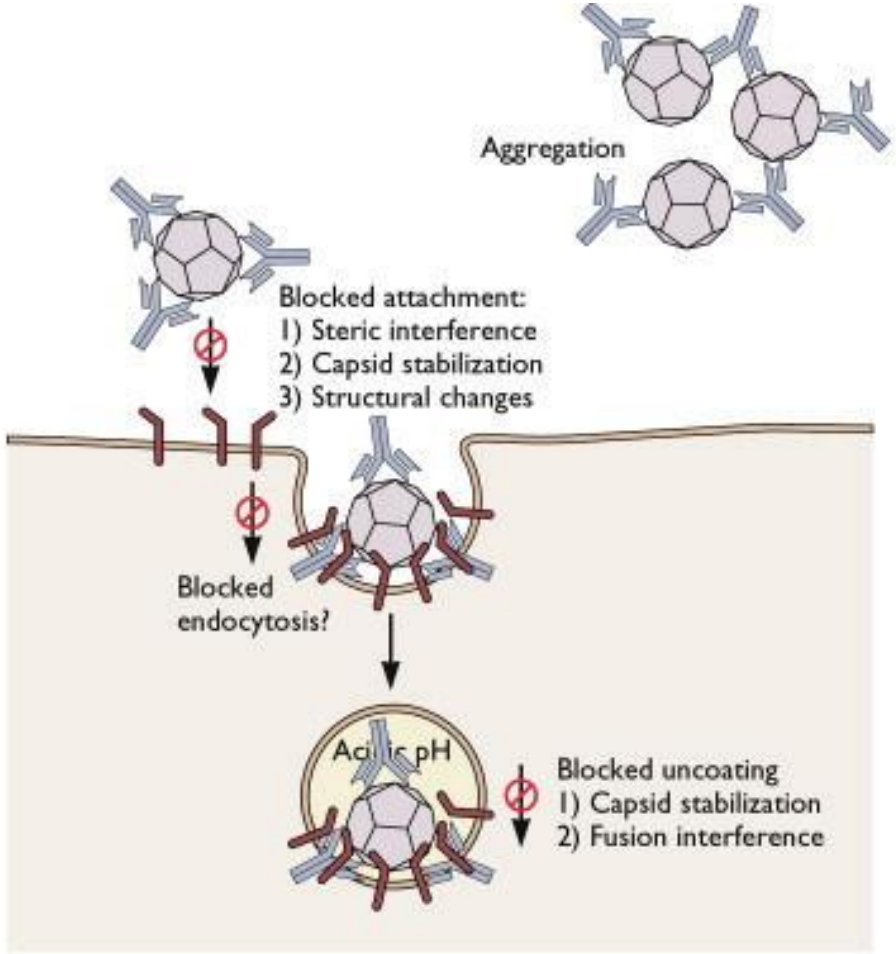
The principal use of neutralising studies in the UK have been:

- To give biologically plausible evidence of the probable **therapeutic effect of vaccines**
- To **risk-assess** emergent SARS-CoV-2 **variants** as judged by potential for convalescent/vaccine escape

MNA “format” may also be helpful in determining the **therapeutic potential** for novel biological medicines that interact directly with the SARS-CoV2 virus

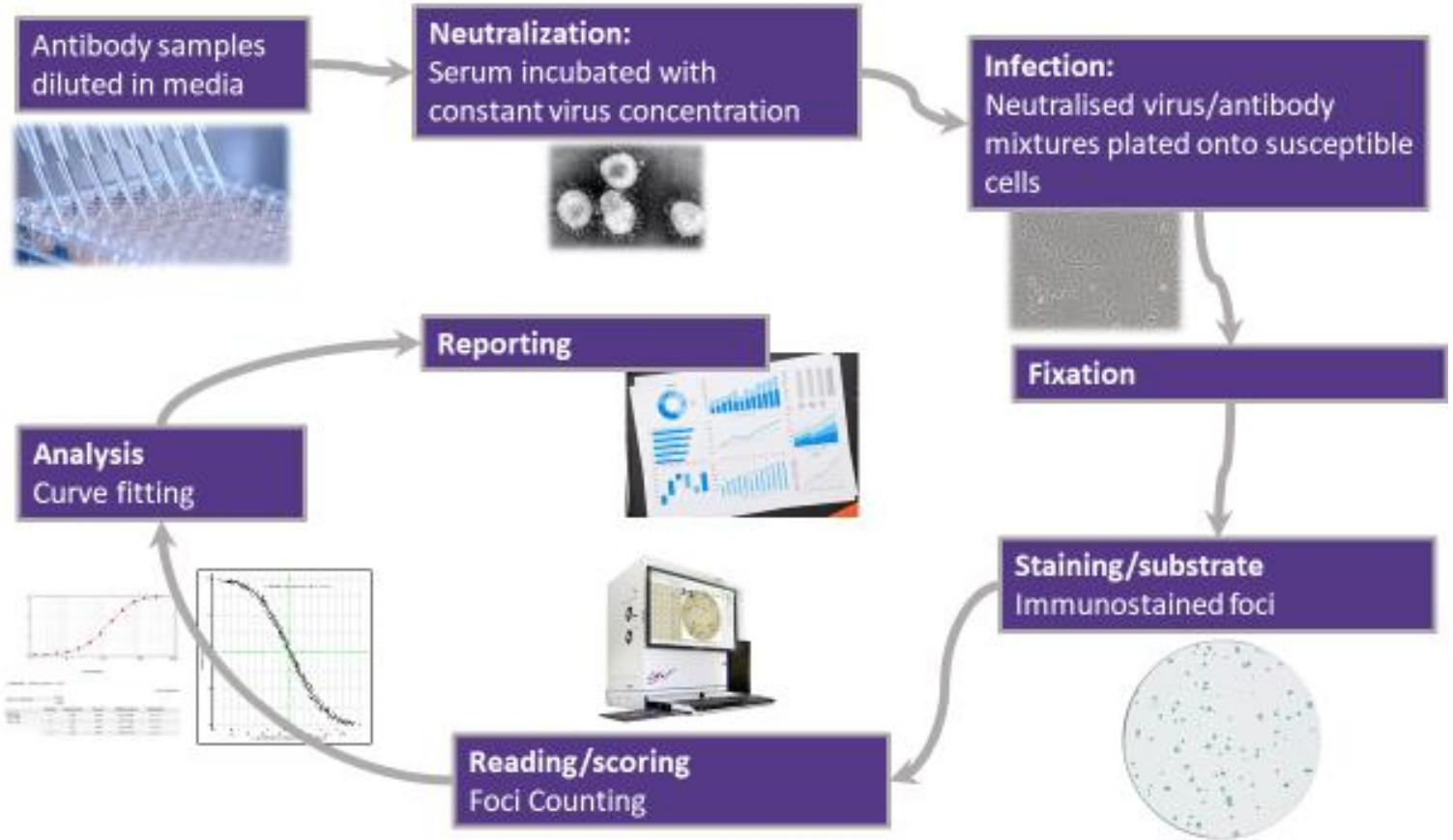
Neutralisation





Neutralisation assays

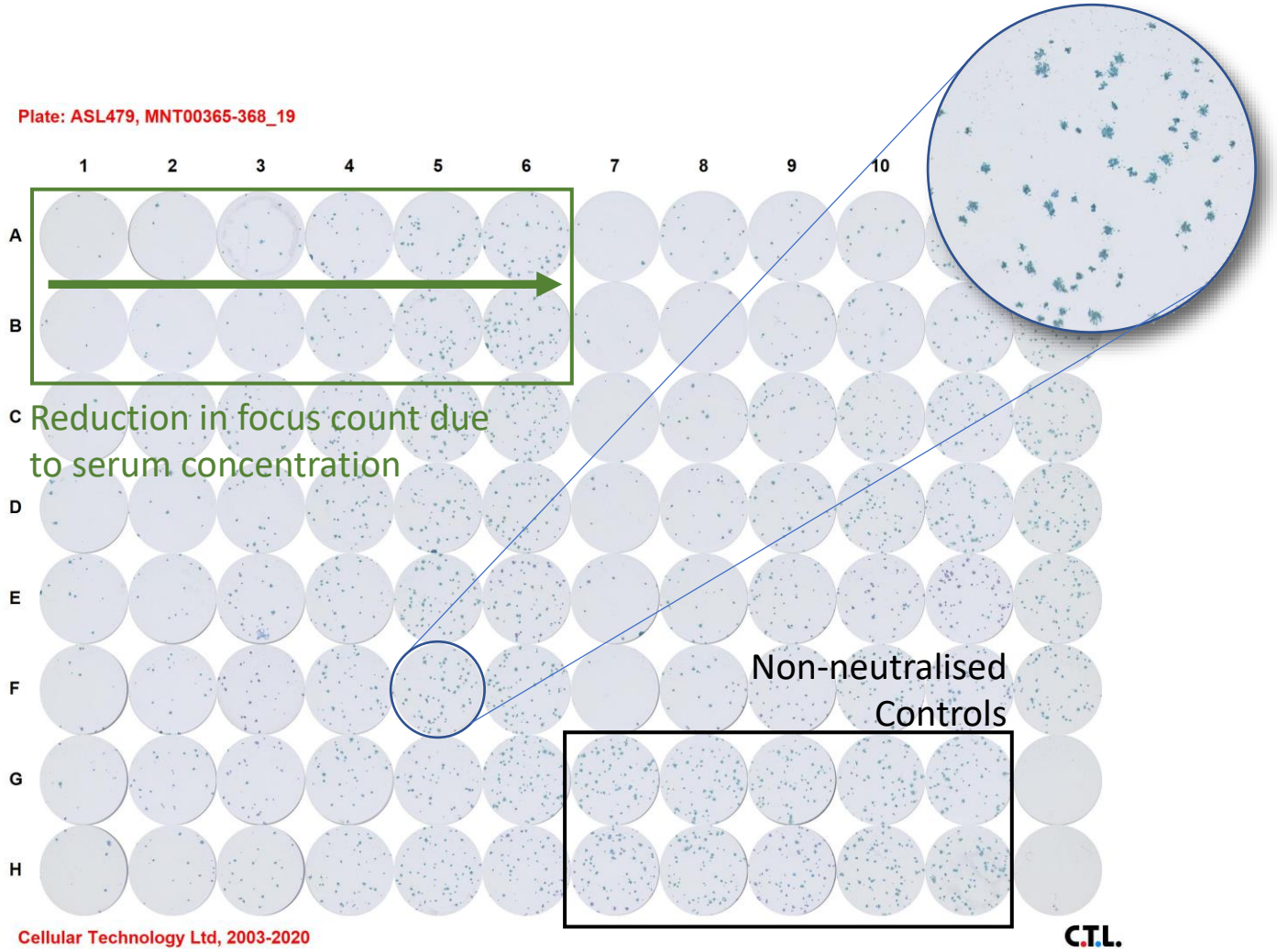
Live virus neutralisation assay – focus-reduction method (adapted)



Bewley *et al.* (2021) Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. *Nature Protocols.* **16**; 3114–3140

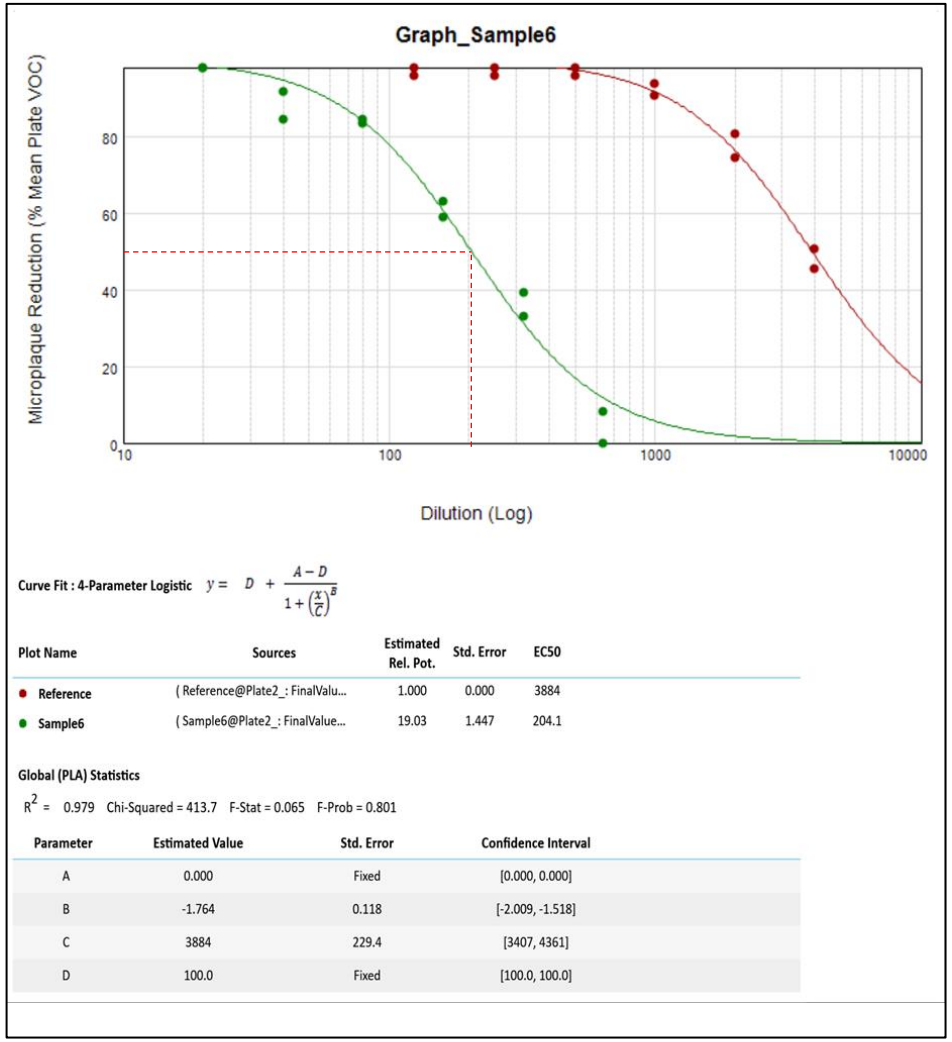
The UKHSA Microneutralisation Assay (MNA)

- **Focus Reduction Neutralisation Test (FRNT)**
- 96 well format – 6 samples per plate
- Reference sera and VOC wells on every plate
- Immunostaining of foci (spots):
  - Primary antibody: Anti-spike-RBD
  - Secondary antibody: HRP-conjugated
  - Substrate: TrueBlue
- 4 days from cell seeding to results
- Routinely testing several thousand samples per month

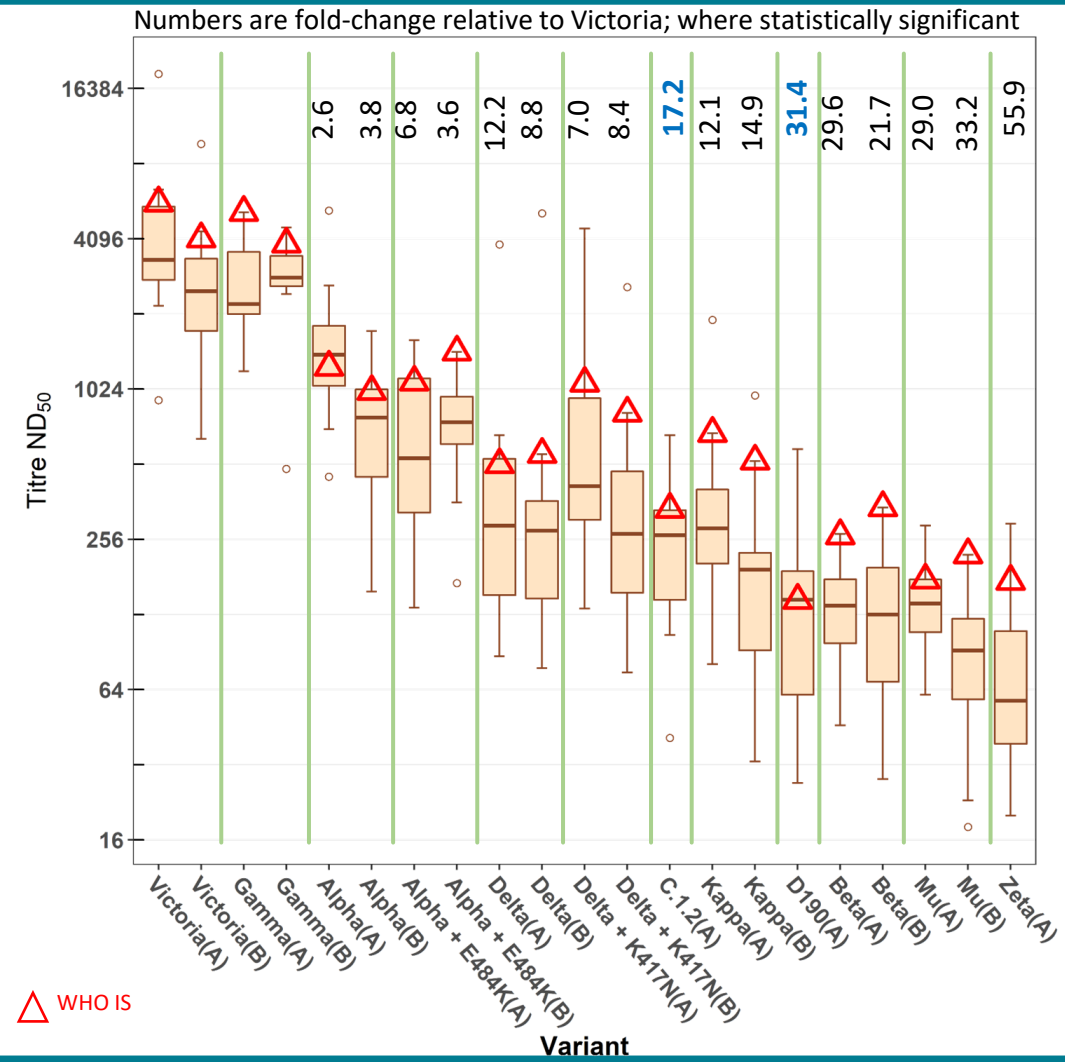


Calculation of median neutralising dose ND_{50}

- Automated spots counting on **CTL scanner** with fixed parameters
- Excel data input into **SoftMax Pro (SMP)**
- Curve fitted to a four parameter logistic (**4PL**) nonlinear regression model
- SoftMax Pro – GxP approved software
- Assay used in several clinical trials

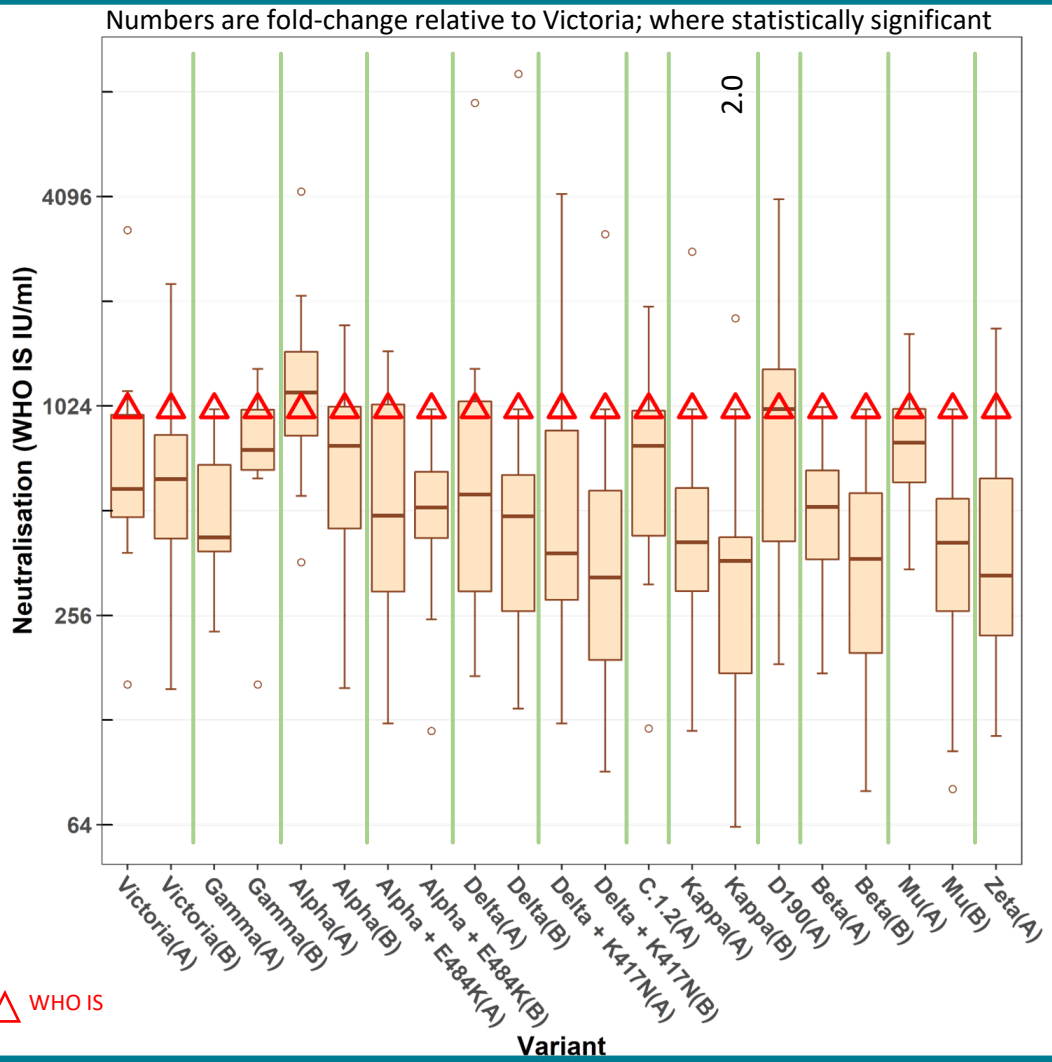


Variant assessment



- Monitor variants using a **pre-Alpha convalescent serum panel** – combining results from two laboratories (A) and (B)

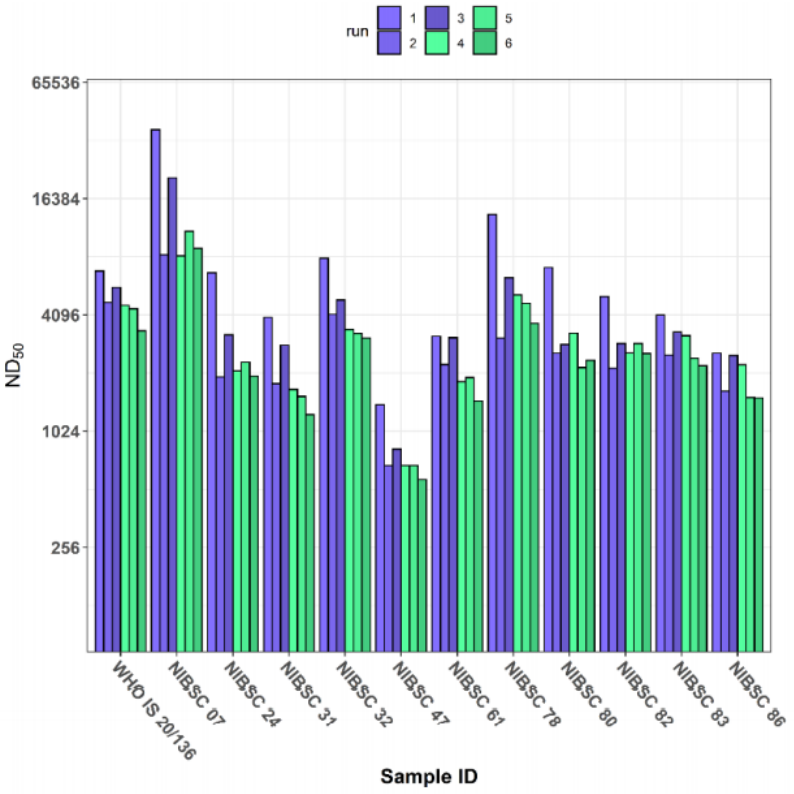
Effect of IS normalisation *across variants*

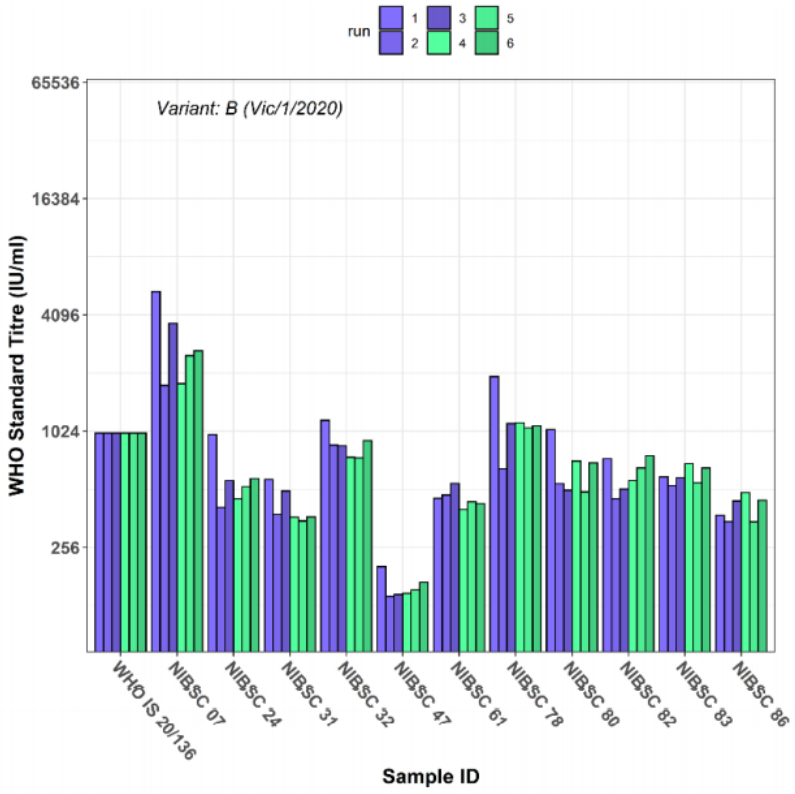


- Run IS in same assay
  - Work out what IS ND_{50} needs to be multiplied by to normalise it to 1,000 IU/ml*
  - Multiply all 'unknown' ND_{50} in that assay by that factor
- This plot of the data from the previous slide illustrates that normalising *inter-variant* titres **removes majority of observed fold-changes**
- "Incorrect use"

* stated activity of IS 20/136

Utility of IS normalisation *between labs*
Same variant (Vic-01)





- Two labs (A – Blue; B – Green)
- Panel of convalescent serum assessed in triplicate at each lab
- **Raw** data:
  ND_{50} = **40.4 %GCV**
- **Normalised** (to IS):
  IU/mL = **22.5 %GCV**
- An **improvement** in inter-lab variability of **17.9%**; p<0.001
- **Conversion** to IU/mL further **reduces variability** of already comparable data

Assay Utilisation

- MNA for prototype virus; RCT samples from vaccine developers
  - > 10 developers
  - Includes trials investigating/supporting human challenge studies, Com-Cov, Cov-Boost, ComFluCov etc.
  - Many thousands of samples processed
- Adapted MNA used to assess breadth of protection against virus variants for vaccine developers
- Adapted MNA used to assess virus variant immune escape
  - CEPI-Agility: **Twelve variants** assessed (including all VOCs)
  - https://epi.tghn.org/covax-overview/enabling-sciences/agility_epi/
- Adapted MNA used to assess in vitro efficacy of:
  - Monoclonal antibody-based therapeutics
  - Antiviral compounds

Summary

- UKHSA has developed a microneutralisation test
  - Qualified and validated for use in clinical trials

- The assay has been adapted for use with VOCs and VUIs
  - Assessments performed using a pre-alpha serum panel
  - WHO IS 20/136 used in addition to this panel (also a pre-Alpha pool)

- WHO IS 20/136 use
  - Distorts the *between variant* fold-changes (incorrect usage)
  - *Within* variant reduces inter-lab variation significantly (correct usage)

Acknowledgments

- UK HSA Porton Down Team
- UK DCMO
- UK Vaccine Task Force
- NIBSC
- CEPI
- Vaccine Sponsors
- NISEC
- Collaborators

Validation of MNA

Example Parameter	Acceptance Criteria	Results	Validation Acceptance
Precision	≤ 50% GCV repeatability ≤ 50% GCV intermediate precision	Repeatability: 29% Inter-assay: 8% Intermediate Precision: 30%	All %GCV ≤ 50% Pass
Specificity	SARS-CoV-2 positive sera should show neutralisation; negative sera should be ≤LLOD % relative recovery must be 50 – 200% for the positive mixed 1:1 with a negative sample	GMT of positive samples: ND_{50} = 1922 Negative samples: ≤LLOD Geomean of %relative recovery = 112%	Pass
Linearity	Data fitted through a regression line must have coefficient of multiple determinations (R^2) ≥ 0.75 and a slope between 0.75 to 1.25	R^2 = 0.91 Slope: 0.79 (90% CI 0.73 – 0.85)	Pass
Relative Accuracy	80% of points must lie between the range of 50% to 200% relative recovery	GMT % recovery between 70 – 111%	Pass

- Qualification and Validation also investigated **Dilutability**, **Analytical Range**, **LLOQ** and **ULOQ** verification, **LLOD**, **Sample stability** (serial freeze thaws and refrigeration of samples), and **Robustness**
  - All parameters **passed**

Training Webinar for the calibration of quantitative serology assays using the WHO International Standard for anti-SARS-CoV-2 immunoglobulin
10 Nov 21

Q&A

Q: Jon Windsor
When you run a parallel line assay between a WHO IS and an unknown sample, is it still preferrable to continue using Fieller's theorem to calculate confidence intervals for the final BAU? Or are more robust methods, such as bootstrapping or classic CI methods more fitting?
A: Mark Page
For the confidence interval from an individual assay the EP / USP guidance and many software packages (e.g. CombiStats) would still use Fieller's theorem. For the final combined estimates (which may use intra-assay variability, inter-assay variability or a combination of both) I'd also suggest following the Pharmacopeia methods. Of course any other suitably justified methods can be justified and used.

Q: Nora Pisanic
When you have a multiplex assay, would you have to do this exercise for every target in the assay, e.g. N, RBD, full spike? And if the in-house standard has a slightly different composition (e.g. more anti-N in comparison the to WHO std), how would you reconcile that for the stock in-house standard?
A: Giada Mattiuzzo
Thank you Nora, that is a very important question. Yes, the calibration is per viral antigen. This is because we expect that the proportion of specific antibodies for viral antigen is indeed different from the WHO IS, therefore each one of them as to be calculated separately. The WHO IS has an arbitrary unitage of 1000 BAU /mL for each antigen, this doesn't mean that the proportion of anti-N , anti-RBD etc is the same, but the same value can be used and it is not meant to calculate the proportion of the different antibodies species in the sample.

Q: Anonymous Attendee
What is the expected [or ideal] 95% confidence interval for the slope between samples and standard?
A; Mark Page
There is no general answer. Some assays may be precise and expected to have a slope-ratio confidence interval within 0.90-1.11. I'm aware of some ELISAs where only 0.80-1.25 is achievable. Some assay may need even wider limits, so needs to be considered on an assay-specific basis.

Q: Alison H
WHO IS 20/136 is now completely consumed right?
A: This question has been answered live
Mark Page
Yes, it is exhausted, we advise using a secondary standard which NIBSC provides (code 21/234) but also see following talk by Troy Kemp who will describe a US/NIH standard
Giada Mattiuzzo
The WHO IS stocks have been depleted, and NIBSC/WHO are working towards a replacement, but in the meantime, there are secondary standards calibrated to 20/136 which can be used for the calibration. One of these secondary standards is the US national serology standard and another is available at NIBSC under code 21/234
https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=21/234

Q: Raquel Binder
Is there an IgA standard?
A: Giada Mattiuzzo
From the collaborative study, the WHO IS contained IgA , therefore 20/136 could have been used to calibrate anti-IgA assays in the same manner than IgG assays, but specifying the class of immunoglobulin

Q: Anonymous Attendee
Re. expressing potency of antisera in IU against VOC, what is the best approach to compare potency of antisera against different VOC?. Since expressing the potency in IU for a VOC is relative to the potency of IS against that VOC, one cannot compare IU across different VOC.
A: Kevin Bewley (UKHSA)
This is something I'll try to illustrate in my talk later today

Q: Gaurav Batra
Can there be differences due to the matrix e.g. serum vs plasma
A: Giada Mattiuzzo
in the collaborative study for the evaluation of the WHO IS we had serum samples as well as plasma samples. Usually, we haven't observed any pattern except for one assay who couldn't detected serum samples, but we have not conducted a formal analysis (ie same quantity of Ab spike into either serum or plasma)

Q: Raquel Binder
How would the cut off on slide 11 have been determined?
A: Mark Page
I don't have that information I'm afraid as the data are from a collaborating lab.

Q: Charles Brandon Stauft
What level of uncertainty is unacceptable?
A: Mark Page
There is no general answer. For example, it will depend on the measurement uncertainty of the assay and the allowable uncertainty on the final results for routine test samples. This is another aspect that needs to be considered on an assay- (and product-) specific basis.

Q: Anonymous Attendee
What does it mean that "Dilution performance not authorized by US FDA"
A: Paul Contestable
FDA did not allow the claim in our IFU for dilution recovery. Ex-US we do have a dilution claim

Q: Alison H
Is there published data comparing 21/136 to 21/234?
A: Giada Mattiuzzo
21/234 was calibrated to 21/136 has part of a collaborative study. The report is available in the WHO website: https://www.who.int/publications/m/item/WHO-BS-2020.2403
Giada Mattiuzzo
In the collaborative study 21/234 was code 20/150, which is the high titre sample of the WHO Reference Panel. Also, the proposal in the report was discussed and the ECBS recommendations are available here: https://www.who.int/publications/i/item/9789240024373

Q: Nora Pisanic
I understand that the Vitros IgG quant considers 17.8 BAU/mL the cutoff but is there a consensus in the scientific community about how many anti-S, RBD and N WHO BAU/mL are considered "positive"?
A: Paul Contestable
There is a difference between screening and monitoring. For monitoring any result in the measuring range is likely to have some antibody binding. When screening unknown antibody status there is always a balance between sensitivity and specificity and the cutoff is going to be slightly different for every assay based on the separation between true positive and true negative for each individual assay. Assays with similar clinical sensitivity/specificity should have similar clinical cutoffs

Q: Camila Macedo Cincotta
Any comments on how the naive "previously non-infected" subjects developed N response after Moderna vaccine, detected by Orthos? Is this cross reactivity of the assay?
A: Paul Contestable
The previously non-infected subjects were all negative for N antibody after vaccination. The only N reactive subjects were N reactive before the first vaccine dose
Camila Macedo Cincotta
Thank you! I though the graph showed after second dose those 3 subjects have N response.
Paul Contestable
The graph only showed Spike S1 results, sorry for the confusion

Q: Marcelo N de Medeiros
In your experience plasma samples containing blood bag preservatives are a problem for preparation secondary standards?
A: Kevin Bewley (UKHSA)
If they're converted to Sera before use that generally overcomes any issues due to the preservatives

Q: Anonymous Attendee
For someone new to these statistical analysis methods, is there there any video available showing details on how to perform the analysis, and/or the combistat software, starting from raw data to the final output. Can any example datasets be made available, with expected results for that set. So one c perform the analysis themselves and cross check if they get the correct output. Just to make sure they are conducting the correct analysis steps appropriately, before applying to new data they would like t generate. Thank you.
A: Mark Page
Software vendors should provide several examples. I also believe some may provide training events et It may be possible for a full example to be presented at a future webinar if there is sufficient demand.

Q: Ugwu Alphonsus
Hi Kevin please how do you correlate a secondary serum to the WHO standard if you run out of the WHO standard?
A: Kevin Bewley (UKHSA)
https://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=21/234
There is a calibrated working reagent supplied by NIBSC - see link above

Q: Ruta Kulkarni
Should the WHO standard be used with the same unitage (1000 IU) against all the different variants?
A: Kevin Bewley (UKHSA)
For lab to lab comparisons, within the same variants, then normalisation to 1000 IU/ml helps make lab-to-lab titre comparisons

Q: Anonymous Attendee
How were the qualitative cut-off in the vitros assay determined?
A: Paul Contestable
Based on clinical samples and the separation between true positive and negative subjects. Then validated with 541 positive and several thousand negative samples
Raquel Binder
Can you define separation? Do you use 3 STDV above the mean of the negatives or ROC curve?

Q: Mike Busch
Will the new standards have higher antibody levels to increase the upper ranges of quantitation?
A: This question has been answered live

Q: Gaurav Batra
Any comment on using the international standard for RBD-ACE2 assay (Surrogate Neutralization assay)
A: This question has been answered live
Linfa Wang
Linfa Wang here: we have done a detailed calibration and a report on this work will be out in Lancet Mirobe soon. Thanks

Q: Arnaud Drouin
How can the WHO standard be used for titration of serum from subjects who received an infusion of the anti spike monoclonal infusion of Regeneron ?
A: This question has been answered live

Q: Anonymous Attendee
Can someone comment on value drift if you calibrate a secondary standard against another already calibrated standard ie recommend that this is not done.
A: This question has been answered live

Q: Anonymous Attendee
Will values for the WHO IS against VOCs be provided when the IS is provided?
A: This question has been answered live

Q: Anonymous Attendee
IS generation does seem to be something to be planned for the next several years. Have you planned the schedule for new standards beyond this?
A: This question has been answered live

Q: Arnaud Drouin
can you use the WHO standard pure? If you have a matrix effect if you use it pure and obtain a titration curve including the highest titer point?
A: This question has been answered live

Q: Anonymous Attendee
can parallelism be accessed equally in the different neutralization assays? wtVNA, psVNA
A: This question has been answered live

Q: carl hanson 08:54 AM
Some VOC have dozens of "sub variants". Does this complicate the interpretation?
A: This question has been answered live

Q: Prem Lakshmanane
Any recommendations for mAb standards for assay calibration, such as Spike/RBD-based binding and neutralization assays?
Mark Slifka group beautifully showed CR3022 (Cat# NR-52392, BEI resources) and (Cat#10–2005, Abeomics) are useful mAb standard. Any thoughts?
Thomas A, Messer WB, Hansel DE, Streblow DN, Kazmierczak SC, Lyski ZL, Lu Z, Slifka MK. Establishment of Monoclonal Antibody Standards for Quantitative Serological Diagnosis of SARS-CoV-2 in Low-Incidence Settings. Open Forum Infect Dis. 2021 Feb 2;8(3):ofab061. doi: 10.1093/ofid/ofab061. PMID: 33723513; PMCID: PMC7928679
A: This question has been answered live

Chat

From Ligia Pinto:
Requests of the US SARS-CoV-2 Serology Standard can be made at the following link: https://frederick.cancer.gov/initiatives/seronet/serology-standard

From Amy C. Shurtleff
This is the Agility data link that Kevin just presented... https://epi.tghn.org/covax-overview/enabling-sciences/agility_epi/#ref1

From Giada Mattiuzzo:
link 21/234: http://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=21/234

From Giada Mattiuzzo
link to the WHO report on the characterisation of 20/136 and 21/234 (20/150 in the collaborative study): https://www.who.int/publications/m/item/WHO-BS-2020.2403

From May Chu
This is an announcement to invite anyone interested in biorepository discussion to create/maintain pipeline for access to samples. December 7, 9-11 am EST.Link: https://zoom.us/webinar/register/WN_2Z9hTmkeTQOW5p5QnmAtiw

From Prem Lakshmanane to Hosts and panelists:

Any recommendations for mAb standards for assay calibration, such as Spike/RBD-based binding and neutralization assays?

From Arnaud Drouin to Hosts and panelists:

have you evaluated the WHO standard with plasma of subjects infused with the Regeneron anti spike monoclonals?

From Prem Lakshmanane to Hosts and panelists:

Mark Slifka group beautifully showed CR3022 (Cat# NR-52392, BEI resources) and (Cat#10–2005, Abeomics) are useful mAb standard. Any thoughts?

Thomas A, Messer WB, Hansel DE, Streblow DN, Kazmierczak SC, Lyski ZL, Lu Z, Slifka MK. Establishment of Monoclonal Antibody Standards for Quantitative Serological Diagnosis of SARS-CoV-2 in Low-Incidence Settings. Open Forum Infect Dis. 2021 Feb 2;8(3):ofab061. doi: 10.1093/ofid/ofab061. PMID: 33723513; PMCID: PMC7928679.

From Giada Mattiuzzo to Everyone:

link 21/234: http://www.nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=21/234