Workshop Report

COVAX Enabling Sciences SWAT Team Workshop on *"Interpreting SARS-CoV-2 immune assay data involving variants and the use of the WHO International Standard for anti-SARS-CoV-2 immunoglobulin"*

October 28, 2021

Meeting report prepared by

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Executive summary

On 28 October 2021, the COVAX Enabling Sciences SWAT Team, co-led by the Coalition for Epidemic Preparedness Innovations (CEPI) and the World Health Organization (WHO), and including members from the Bill & Melinda Gates Foundation (BMGF), the National Institute of Allergy and Infectious Diseases (NIAID) and industry, hosted a workshop entitled "*Interpreting SARS-CoV-2 immune assay data involving variants and the use of the WHO International Standard for anti-SARS-CoV-2 immunoglobulin*."

The background that prompted the convening of this workshop is the recognition that different immune assays, particularly neutralization assays, have produced a range of results when applied to SARS-CoV-2 viral variants. The fold-reduction results in neutralization against a particular variant when compared to a prototypic Wuhan-like strain have varied considerably by assay and by laboratory in published reports.

The overall objectives of this workshop were to address the following key questions:

- What are some of the challenges in the interpretation of data for SARS-CoV-2 variants?
- How can immune assays be utilized to provide actionable information to vaccine developers and regulators on the effect of SARS-CoV-2 variants?
- How can results be interpreted over different assay types and laboratories?
- How is the WHO International Standard appropriately used to assess the effects of SARS-CoV-2 variants on assay performance?

The first half of the workshop featured presentations focused on the development, proper use, and equitable distribution of antibody standards. Key points included:

A. A tool for the assessment of Data Reliability has been developed and is being prepared for publication and distribution for public use, free of charge, to enable standardization of studies.

B. The most effective tool for data comparison may be the WHO international antibody standard to and with which study design and technical performance should be standardized.

C. The *WHO manual for the establishment of secondary standards for antibodies against infectious agents focusing on SARS-CoV2* is posted for public comment until 30November2021 and can be found at the following link: https://www.who.int/health-topics/biologicals#tab=tab_1

D. The WHO IS and Reference Panel were established December 2020; stock of WHO IS was depleted within eight months. However, the IS unit can still be used because secondary standards are available. In addition, source(s) of the replacement candidate material has been discussed at the last WHO ECBS meeting.

E. Efforts are underway to prepare a COVID-19 Serology Control Panel (CSCP). Aligning with WHO guidance on qualifying secondary materials is currently available for public comment at: https://www.who.int/health-topics/biologicals#tab=tab_1

F. The creation of a Virtual Federated Biorepository (VFBR) is also underway as part of the solution to acquire samples for the pipeline quickly and to "get ahead" of any future public health challenge such as the SARS-CoV-2 pandemic.

The second half of the workshop focused on immune assays and SARS-CoV-2 variants and introduced a variety of neutralization assays that when taken as whole, highlight the need for reliable immune correlates of protection in animal models that support the identification and use of serological surrogate markers of efficacy in humans. Assays presented included:

A. A surrogate virus neutralization test (sVNT), the GenScript cPass™ kit, has been calibrated with the WHO IS 20/136 (IU/mL) and assay reliability has been demonstrated. While the PRNT is the reference standard, it requires BSL-3 containment and 3-5 days to complete. The cPass kit requires one hour, is US FDA approved, and correlated to the PRNT with a coefficient of determination (R2) of 0.95. Details can be found in Tan, *et al*. Nat Biotech 2020.

B. (Live) Virus Neutralization Assays (VNA) employ standardised viral materials treated with solutions of NAbs at varying concentrations to characterize the ability of these antibodies to prevent infection. Details can be found in Bewley, *et al.*, 2021 Nature Protocols.

C. A stable mNeonGreen reporter SARS-CoV-2 (icSARS-CoV-2-mNG) has been developed and can be used to screen antiviral inhibitors such as interferon and accelerate vaccine development. This work was described in Xie, *et al.*, 2020, Cell Host Microbe. In addition, a trans-complementation system for SARS-CoV-2 at BSL-2 has been developed. Recognizing that BSL-3 requirements can cause bottlenecks, this model was developed to produce single-round infectious SARS-CoV-2 that recapitulates authentic viral replication. This is described in a pre-print that can be found at https://pubmed.ncbi.nlm.nih.gov/33501436/.

D. V-PLEX COVID-19 Serology Kits, manufactured by Meso Scale Discovery®, are a series of panels used to detect antibodies to antigens from various coronavirus or other respiratory pathogens. Modifications have allowed development of a sero-epidemiology tool to measure human IgG to four SARS-CoV-2 antigens simultaneously (full-length trimeric S, RBD and NTD of spike and N protein).

The final set of presentation materials from the workshop have been posted on the COVAX Epi Hub and can be found here: https://epi.tghn.org/covax-overview/enabling-sciences/

The Agenda for this workshop, entitled *Interpreting SARS-CoV-2 immune assay data involving variants and the use of the WHO International Standard for anti-SARS-CoV-2 immunoglobulin*, is shown below:

Time (CET)	October 28, 2021	Speaker(s)
15:00–15:02	**Opening Remarks**	**Karen Makar** (BMGF)
15:00–15:05	**Welcome and meeting objectives**	**Ivana Knezevic,** co-lead of ES SWAT team (WHO)
Session 1: Antibody Standards *Chair: William Dowling (CEPI)*		
15:05-15:20	Global review of Neutralization assays against SARS-CoV-2 variants	**Henning Jacobsen (**Helmholtz Centre for Infection Research) and **Ioannis Sitaras** (Johns Hopkins University)
15:20-15:30	WHO manual for the establishment of secondary standards for antibodies against infectious agents focusing on SARS-CoV2	**Dianliang Lei** (WHO)
15:30-15:50	WHO International standard for VoCs; replenishment plans and new collaborative study	**Giada Mattiuzzo** (National Institute for Biological Standards and Control)
15:50-16:10	Harmonized approach to creating secondary standards and creation of a virtual biorepository	**May Chu and Jon Windsor** (Colorado School of Public Health)
16:10-16:35	**Panel Discussion 1 / Q&A Session**	Moderated by: **William Dowling** (CEPI)
16:25-16:30	**BREAK**	ALL

Session 2: Immune assays and SARS-CoV-2 Variants *Chair: Janet Lathey (NIAID)*		
16:40-16:45	Introduction	**Karen Makar** (BMGF)
16:45-17:05	Surrogate virus neutralization assays	**Lin-Fa Wang** (Duke-NUS Medical School)
17:05-17:25	Risk assessment of SARS-CoV-2 Variants of Concern and Impact on Vaccine Escape	**Bassam Hallis** (UK Health Security Agency)BEI
17:25-17:45	Chimeric reporter Virus Neutralization assays	**Pei-yong Shi** (University of Texas Medical Branch)
17:45-18:05	SARS-CoV-2 Neutralization Assay Standardization and Variant Characterization	**Shaunna Shen** (Duke University)
18:05-18:25	Binding and functional assays using multiplex solid phase platform	**David Goldblatt** (University College London)
18:25-18:55	**Panel Discussion 2 / Q&A Session**	Moderated by: **Janet Lathey** (NIAID)
17:00-17:10	**Wrap Up & Next Steps**	**Ivana Knezevic,** co-lead of ES SWAT team (WHO)

Opening Remarks, Welcome and meeting objectives

Karen Makar, Senior Program Officer at the Bill and Melinda Gates Foundation (BMGF), opened the meeting, welcomed participants, and presented the agenda with an overview of general housekeeping items including how best to interact with presenters via the chat and the Q&A.

Ivana Knezevic, WHO Team Leader of the Norms and Standards for Biologicals Group, and co-leader of the Enabling Sciences (ES) SWAT team stepped in for co-leader Paul Kristiansen of CEPI who was unable to attend and thanked the workshop organizers.

She then reminded the group that COVAX (which is the abbreviated form of *COVID-19 Vaccines Global Access*), is the vaccines pillar of the *Access to COVID-19 Tools* (ACT) *Accelerator*, co-led by Gavi, The Vaccine Alliance (Gavi), CEPI, and the WHO with the aims to accelerate the development and manufacture of COVID-19 vaccines, and to guarantee fair and equitable global access to appropriate, safe and efficacious vaccines, with special attention to Low and Middle Income Countries (LIMCs).

This workshop is one in a series sponsored by several COVAX SWAT teams and is an extension of the WHO working group on assays and standards lead by William (Bill) Dowling, PhD, Non-Clinical Vaccine Development Leader at CEPI.

The background which has prompted the convening of this workshop is the recognition that different immune assays, particularly neutralization assays, have produced a range of results when applied to SARS-CoV-2 viral variants. The fold-reduction results in neutralization against a particular variant when compared to a prototypic Wuhan-like strain have varied considerably by assay and by laboratory in published reports.

The overall objectives of this workshop were to address the following key questions:

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Session 1: Antibody Standards

Chair: William Dowling (CEPI)

Unit 1: Global review of neutralization assays against SARS-CoV-2 variants

Henning Jacobsen, PhD, Postdoctoral Fellow at the Helmholtz Centre for Infection Research and Ioannis Sitaras, PhD, Research Associate of the Johns Hopkins University Bloomberg School of Public Health, serve as external technical experts for the WHO in matters of SARS-CoV-2 neutralization assay data.

Dr. Sitaras introduced serology as a field that can help us understand SARS-CoV-2 epidemiology and better assess vaccination-induced immunity. By differentiating infected from uninfected people, this technology allows us to track the progress of infection in a given population. This is especially important in scenarios where infected (and infectious) persons are often asymptomatic, such as the case with SARS-CoV-2 infections. A comprehensive overview of available neutralization data and the global status of COVID-19 vaccines and programs can be found at https://view-hub.org/resources.

Serological assays, though subject to pros and cons, can be used quite broadly to:

1. Identify and quantify acquired immunity derived from infection, vaccination, or both
2. Establish corelates of protection from further infection by variants or similar strains
3. Develop correlates between antibody titers and disease progress/severity
4. Describe and quantify functional aspects of antibodies that may be important for protection from disease

Since March of this year, Jacobsen and Sitaras have been screening the literature concerning neutralization assays using post-vaccination sera against the 4 most prevalent SARS-CoV-2 Variants of Concern (VoC), alpha, beta, gamma, and delta. This body numbers over 1000 studies including preprints on the servers (see https://asapbio.org/preprints-and-covid-19 for current index).

Inclusion criteria were that (a) comparison was made using the vaccine seed strain or one that is antigenically very similar; and (b) any pseudovirus used had to contain the full complement of spike mutations that characterized a given VoC. The analytic algorithm employed was (1) live virus versus pseudovirus, (2) vaccine platform, and (3) specific vaccine.

Summary:

1) Neutralization assays using well-characterized pseudoviruses are a valuable alternative to those using live viruses. The benefits of pseudovirus assays include a broader network of labs to perform such assays since the global availability of BSL3 facilities is low. Fold-reduction for variants between the two methods correlated well (see slide 14 of workshop deck).

2) Vaccine platforms were stratified as inactivated, mRNA, protein subunit and vector. mRNA vaccine data was the most plentiful and may bias the analysis in its favour, but the overall trend of fold reductions was similar within each VoC analysis. Highest fold reduction (4-6 fold) was seen against the beta variant, followed by gamma and delta (~3 fold) and lastly, alpha (</~ 2 fold) (see slide 15 of the workshop deck).
3) Stratifying across 12 vaccines authorized for use in most parts of the world, resulted in same patterns as seen in vaccine platforms, noting that the alpha variant follows the vaccine seed strain quite closely (see slide 16 of the workshop deck).

Dr. Jacobsen then discussed the difficulties in evaluating available neutralization data for SARS-CoV-2 variants. These include large variances in data ranging from no effect of the variant to over-exaggerated effect (i.e., 30-fold reduction against the beta variant), strong outliers, and paucity / insufficiency of data from which to draw conclusions. This is compounded by the inability to directly compare neutralization data across studies due to the lack of standardization in quantitative studies or methodologies. Meta analysis is not currently an option since inclusion / exclusion criteria have not yet been established.

Summary:

1) Assessment of reliability and comparability of neutralization assays is difficult. Engagement with a group of experts led to identification of 11 aspects encompassing 33 parameters that might affect such assessment.
2) A tool for the assessment of Data Reliability has been developed and is being prepared for publication and distribution for public use, free of charge, to enable standardization of studies. Criteria include, for example: sample size, presence or absence of SARS-CoV-2 infection, vaccination regimen, sample collection period, demographic characterization, protocol, live virus / pseudovirus (if applicable), assay standardization, and data.
3) Validation of this tool – which scores a study based on the overall risk of low reliability of the data – was initially performed by evaluating 10 randomly selected studies assessing neutralizing antibody titers elicited by the Pfizer-BioNTech vaccine against the beta variant (see slide 19 of the workshop deck). This preliminary evaluation showed that the assessment tool developed by Dr. Sitaras and Dr. Jacobsen correctly identifies as high risk and excludes studies with missing or incorrect methodology and studies that result in outlier data.
4) The most effective tool for data comparison may be the WHO international antibody standard with which study design and technical performance should be standardized. Standards for reporting are crucial for proper evaluation of any study.

Unit 2: WHO manual for the establishment of secondary standards for antibodies against infectious agents focusing on SARS-CoV2

This session was presented by Dianliang Lei, PhD, Scientist at the WHO. Lei presented the work being conducted by the WHO to guide laboratories in establishing and calibrating secondary standards for SARS-CoV-2 using a primary international standard of titrated immunized serum.

Summary:

1) The WHO has developed and provided guidance and international standards for biologicals for more than 70 years. The Recommendations for the preparation, characterization and establishment of international and other biological reference standards was developed in 1978, with updates through 2004. Following that the WHO manual for the establishment of national and other secondary standards for vaccines was published in 2011 and the WHO manual for secondary reference materials for in vitro diagnostic assays, annex 6 in 2017.
2) While WHO international standards are widely used, feedback from users indicated the need for a manual to guide the proper use of the standards. Therefore, a manual for the establishment and calibration of secondary standards for antibodies is being developed.
3) The scope of this document is limited to the calibration secondary standards for use in evaluating antibody responses elicited by natural infection or vaccination. The contents of the manual are found on slide 28 of the workshop deck.
4) Secondary standard calibration is complex and points to consider include: traceability, uncertainty, value-assignment methodology, stability, and commutability.
5) Candidate materials are pools of human plasma or sera derived from convalescent or vaccinated individuals (depending on intended use). Points to consider here include: specificity, safety, sufficient quantity, homogeneous pooling and frozen storage.
6) The WHO manual for the establishment of secondary standards for antibodies against infectious agents focusing on SARS-CoV2 is posted for public comment until 30November2021 and can be found at the following link: https://www.who.int/health-topics/biologicals#tab=tab_1

Unit 3: WHO International standard for VoCs; replenishment plans and new collaborative study

This topic was presented by Giada Mattiuzzo, PhD, Senior Scientist at the National Institute for Biological Standards and Controls (NIBSC) and began with a timeline presentation of the SARS-CoV-2 pandemic and preparation of International Standard (IS) and other reference materials (see slide 37 of the workshop deck). The normal 2-3 year timeline was compressed to nine months. A research reagent was made available in April 2020

The WHO IS and Reference Panel were established December 2020; stock of WHO IS was depleted within eight months. However, the IS unit can still be used because secondary standards are available, including the NIBSC secondary standard which was calibrated to the WHO IS and the serology standard from the Frederick National Lab in the USA that can be requested using this Serology Material Request Form.

Summary:

1) The first WHO IS was a pool of convalescent plasma from 11 SARS-CoV-2 recovered individuals in the UK, characterized by 44 laboratories from 15 countries using 125 methods (see: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7987302/pdf/main.pdf).

2) Ten samples were provided with the goal of ensuring that the WHO IS was fit-for-purpose, performed well and reduced inter-laboratory variability when assessing SARS-CoV-2 antibodies. Neutralizing titres were measured and showed wide variability but when reported as relative to the IS, the variability was reduced (see slide 39 of the workshop deck).
3) For binding antibody assays, 4 different ELISAs showed over 500-fold variability. When the WHO IS was applied, that variability was abrogated (see slide 40 of the workshop deck).
4) Serial dilutions of the WHO IS (starting dilution of 1:10) were applied to clinical samples and run in parallel to the NIBSC SARS-CoV-2 verification panel of 266 samples from individuals infected with SARS-CoV-2 in 7 commercial platforms. Data fit very well for both IgG and IgM results (see slide 41 of the workshop deck).
5) Global distribution of 2400 units to 581 customers with limit of 5 ampoules was completed. Some kit manufacturers have adopted or provide conversion formulae to the WHO IS.
6) The literature is starting to show potential correlates of protection using the WHO IS (see slide 42 of the workshop deck).
7) Challenges to replacing the WHO IS include questions of activity against VoCs, kit manufacturers requiring higher titers in the context of people being vaccinated, what is right source of material in these cases and how should the unitage be assigned – all in the context of tight timelines.
8) Serological assays are needed to assess the impact on efficacy of vaccines and therapeutics against VoCs (see also https://www.who.int/en/activities/tracking-SARS-CoV-2-variants).
9) The WHO IS has an assigned arbitrary value of International Unit (IU), and is not an absolute value attached to a physical measurement. It cannot be “calculated” per VoC. The potency of the IS should be reported per isolate used and its dose-response performance allows it to be used as a calibrant in each assay.
10) Sources of the replacement candidate material have been discussed at the last WHO ECBS meeting – possible candidates are: i) COVID-19 convalescent plasma similar to the first WHO IS, as 1st wave in UK, and ii) convalescent plasma from infected and vaccinated individuals; the latter displays high titres and broad responses across the VIC-01, alpha, beta and delta variants (gamma was unavailable for these studies).
11) A “VoC Reference Panel” derived from convalescent individuals that have not been vaccinated is being procured to act as positive controls (see slide 48 of the workshop deck).The combination of epidemiology and serology data may suffice in selecting the candidate material for the new IS and reference panel.
12) There is some concern over unitage and consideration is ongoing:
    a. For Neutralization Assays: each iteration of the IS can be compared to its predecessor in order to maintain consistency and allow comparison of assays using the IU/mL unitage. . For circulating VoC the new IS will have to be traceable to the first WHO IS. For new identified VoC an arbitrary unitage can be assigned.

b. For Binding Assays: the current unitage, Binding Antibody Unit (BAU/mL) has to be specific for each viral antigen; from data in the literature it seems that the impact of the VoC is less significant than for neutralization activity; this begs the question of whether VoCs are less a problem than different antigens for assigning an unitage to the binding activity.

13) Uptake of the IS has been higher than anticipated but misuse has been common and more education is needed for its proper use. In addition, secondary standards and validation panels are needed. There is a training webinar planned for Nov 10 on the use of the International Standard. Bill sent out the calendar invitation to all meeting participants.

14) Coronavirus (Covid-19)-related research reagents are available here: NIBSC standards.

Unit 4: Harmonized approach to creating secondary standards and creation of a virtual biorepository

This topic was presented by May Chu, PhD, Clinical Professor and William J. (Jon) Windsor, MPH, MLS Epidemiologist/Laboratorian at the Colorado School of Public Health. Dr. Chu began setting the stage by acknowledging that the need for Quality Control (QC) materials for diagnostics and vaccines was recognized very early in the pandemic. Unprecedented world events and efforts for vaccine development made ensuring reliability and accuracy a challenge. While sharing samples was not an issue, how to do so efficiently was a challenge.

Two areas were recognized to address the gaps:

a) The preparation of a COVID-19 Serology Control Panel (CSCP)

b) The creation and maintenance of a Virtual Federated Biorepository (VFBR)

Another factor considered was that global efforts, such as the WHO IS should not be duplicated but rather synergistic efforts should be made. Attention was given to durable infrastructure with "big tent" up from a grass-roots approach organized systematically with a pipeline that included samples with geographic representation.

Dr. Windsor first explained that the goal of the current work to prepare a COVID-19 Serology Control Panel (CSCP) is to bring together the multiple of SARS-CoV-2 reference materials in order qualify them as secondary standards against the WHO IS. The most important inclusion criterion was that all panels and reference materials had to be publicly available beyond the scope of the study. The reference material providers are listed below (note that NIH is also known as the US Serology Standard from the Frederick National Laboratory):

Institution	Panel Name	# of samples	Material type	Recommended Storage	Antibodies (if applicable)
ThermoFisher	MAS™ SARS-CoV-2 IgG Positive Control Kit (Cat# 10028305)	1	Plasma	2-8C	IgG
Colorado School of Public Health	COVID-19 Serology Control Panel	3	Pooled plasma	-20-22C	IgM, IgG, IgA
INSTAND	INSTAND Serology panel	3	Single human plasma	-80C	IgM, IgG, IgA
OneWorld Accuracy	SARS-CoV-2 Serology	4	Single human plasma	2-8C	IgM, IgG
NIBSC	WHO IS (NIBSC 20/136)	1	Pooled plasma	-20C	IgM, IgG
NIH/NIC	Human SARS-COV-2 Serology Standard	1	Pooled plasma	-20C	IgM, IgG

Each sample was then sent to the five testing laboratories shown below with the requirements that representative quantitative test platforms be established in use for clinical, research and diagnostic test development. A diversity of platforms was sought.

Institution	Type of Lab	Platform	Method	Antigen targets	Antibodies
Biodesix Laboratory	Commercial	Manual & semiautomated Tecan EVO	Neutralization	Spike, RBD	Total Ig
		Bio-Rad Platelia	ELISA	N	
Brigham & Women's Hospital	Academic / Clinical	Quanterix Simoa serological assay	Multiplexed single molecule array	S1, Spike, RBD, N	IgM, IgG, IgA
Wadsworth Center, David Axelrod Institute	Reference / Public Health	Luminex	Multiplexed microsphere	Spike, RBD, N	IgM, IgG, IgA
University of Colorado	Academic / Research	LDT	SARS-CoV Focus Reduction Neutralization Titer	Whole virus	Total Ig
			Multiplex bead immunoassay	N, RBD	IgG

Summary:

1) The Parallel Line Assay method, developed to compare secondary standards to a respective WHO IS, was applied and facilitated determining the Binding Antibody Units (BAU/mL) as well as the calculation of relative potency of samples versus WHO IS
2) The study is ongoing, all data have been received and are being harmonized to single report format. The analysis method will be in R to allow open access and enable comparison to other traditional methods used. Aligning with WHO guidance on qualifying secondary materials currently available for public comment which can be found at the following link: (https://www.who.int/health-topics/biologicals#tab=tab_1).
3) Preliminary results showing potency of the CSCP Standards in International BAUs are available on the medrxiv server (Windsor, *et al*.) and findings have been submitted to American Journal of Tropical Medicine and Hygiene.
4) The COVID-19 Serology Control Panel Request Form should be used for any requests. Additional reference material is also available.

Dr. Chu went on to introduce the second proposal to harmonize SARS-CoV-2 work by the creation and maintenance of a Virtual Federated Biorepository (VFBR).

Summary:

1) The VFBR is being proposed as part of the solution to acquire samples for the pipeline quickly and to "get ahead" of any future public health challenge such as the SARS-CoV-2 pandemic. Models of the VFBR are likened to Private Collectors, Museums, Banks, Bookstores and Thrift Shops (see slide 66 or the workshop deck).

2) The goal of VFBR is that each member would agree to set aside a defined set of samples within their institutes that are fully characterized in the parameters described in Units 2 and 3 of this workshop. These parameters include sequence information, safety, sufficient quantity, etc., and all would reside in a Directory of Specimen Resources similar to those already developed by other groups.

3) Operational Principals have been defined as:

a. A public good biorepository is critical to break open access to quality specimens from validated partners in a trusted operational environment
b. Regulatory controls should not be punitive, should be used to facilitate sharing and maintain source origins
c. Changes have to be made towards how professional achievements are credited, may be outside our purview but has key impact
d. New paradigm shifts to be prepared for pandemics, build trust and equity.

4) A 2nd Biorepository Workshop, co-sponsored by CSPH, PATH and ReCoDID, is scheduled for 07December2021 to continue the discussion and implement the plans.

5) There has been collaboration with Oxford University and the WHO bio-hub (conducting a concurrent session) to scope out the kind of samples and collections to gather.

Panel discussion for Session 1: Antibody Standards

A panel discussion, moderated by Bill Dowling, included each of the presenters as well as Mark Page, PhD, Head of Emerging Viruses Group at the NIBSC and Youchun Wang, MD, PhD, Chief Scientist at the National Institutes for Food and Drug Control (NIFDC), China. The panel addressed the following questions and topics with respective key points given below:

A. How is the WHO International Standard appropriately used to assess the effects of SARS-CoV-2 variants on assay performance?

In other words, when assessing a new or different variant, how should the WHO IS be applied?
[**Comments from Giada**]

- To compare a sample potency against the variants, use the IS unit to compare your data with another dataset looking at exactly the same VoC.
- If the data are against a VoC comparing to the original strain or to another VoC then the IS units should not be used as one will not be able to compare the data – there is no algorithm to convert potency to a VoC to the other for the WHO IS.

A follow-up that Bill introduced (posed in the Q&A) was requesting further comment on the **misuse of the WHO IS** and the common misconceptions seen in the literature Giada mentioned during her presentation.

- Giada has seen several types of issues:
  i. **Using the WHO IS as a validation tool**: the most common mistake is the use of the WHO IS as a validation tool. I have seen leaflets from kit manufacturers which state they have reproducible results in testing the WHO IS and therefore their assay is validated or standardised. The WHO IS is a calibrant, not a validation tool.
  ii. **Using the WHO IS as a comparator for different targets:** the intended use of the WHO IS is not to harmonize among binding assays when looking at different viral antigens

iii. **Using the WHO IS as a conversion factor:** technically this is possible but it must be done properly; the assay standard and WHO IS curves must be parallel and the clinical sample must fall within the range of the standard; if it goes over, the conversion factor will not be linear; if the dose response curve is sigmoidal it may be more useful to develop an algorithm to convert each point to the WHO IS unit (rather than one single conversion factor).

B. *What Secondary Antibody Standards are calibrated in IU and/or BAU and widely available? Are there particular standards recommended until the WHO IS is replenished? What about other national standards?*

[Comments from Giada]

- There are two that I know have been calibrated:
  - The Frederick National Laboratory has the COVID-19 Serology Control Panel and the Request Form should be used.
  - The NIBSC repurposed one of the high titer samples used in the WHO international reference panel, and it is coded 21/234 as a working reagent for anti-SARS-CoV-2 immunoglobulin.

[Comments from Jon]

- Release of the Colorado School of Public Health COVID-19 Serology Control Panel (CSCP) is pending completion of the publication process; this will then be officially qualified as a secondary standard.
- Other reference materials included in the overall serology harmonization work are still in process of conversion to IU/mL with the goal of qualification as secondary standards.

[Comments from May]

- The CSCP is available as dry tube specimens shipped at room temperature free of charge upon request.

Bill then asked Youchan Wang to comment based on mentions of other national standards that have been or will be calibrated to the WHO IS during the first session.

[Comments from Youchan]

- National Standards in China had been set up in September of 2020 and more than ten laboratories participated in calibration efforts to 1000 U/ml and this was widely used domestically as QC standards
- When WHO IS became available, the National Standard was calibrated to 630 IU/mL
- The supply of the National Standard is almost exhausted and efforts are underway to create a new National Standard to be calibrated to the International Standard.

Bill then asked the group and invited contributions via the chat on use or availability of other secondary standards; mentioning specifically that a national Standard in India had been mentioned also in the first session. This standard was developed by DBT India Consortium for Covid-19 Research and is described in Chaudhuri, et al., 2020.

Bill presented a question posed by Adrian McDermott in the Q&A:

Does the international standard accommodate for high sensitivity antibody detection methodologies?

Bill asked Mark Page to comment

[Comments from Mark]

- Yes, the IS allows / accommodates for high sensitivity (i.e., the low end of the range) which is actually a characteristic of the assay, not the IS.
- Mark learned recently of a human serum available from the European Commission that is not yet calibrated to WHO IS. Perhaps this meeting will raise awareness of the need to have these secondary standards and to take the effort to calibrate them.

C. *From reviews of the literature and/or collaborative studies, which assay format demonstrates the least variability?*

Bill presented this question to Henning and Ioannis

[Comments from Henning]

- This is a difficult question. Due to the many different methodologies used, comparing different studies is no simple matter. There are many confounding factors such as whether the samples studied are from vaccinees or convalescents, or the timing sampling took place.
- There have been a few studies directly comparing different methodologies using the same sample sets and small but persistent differences were observed.
- When considering all available data on neutralization assays using pseudo viruses versus live viruses, there are no striking differences seen. There have been some recent studies that directly compare those assays and they see that, for example, there might be some differences in their sensitivity but not in the variability. However, these studies are few in number.

[Comments from Ioannis]

- The correlation between antigens and spike-based ELISAs and neutralization assays is reasonable.
- Currently I am involved in an effort to try to correlate ELISA results with the neutralization assays. Results are very preliminary and we have seen some variation but the work is only approximately 10% complete and results already in the literature are also preliminary.

Bill then asked for any additional comment from Mark or Giada or Jon with respect to variability.

[Comments from Giada]

- There is a caveat: If I just look at the numbers, I can tell you that neutralization assays are much closer in results than binding assays. But that is because binding assays have a much more diverse output in terms of results and data is reported out as relative to a dilution factor or they may have their own arbitrary units. This

has created the biggest discrepancy. I cannot say if one is actually better than the other because of the way the data are reported.

[Comments from Jon]

- We have the same conclusion that Giada has. It is very dependent on the reporting formats and the just the nuances from actual commercial provider to commercial provider, even within a single methodology. So it's kind of hard to say, but when we compile and analyze all of the data from our harmonization study, we might actually be able to tease out some of that information on the back end, so stay tuned.

Bill picked up a question from the chat for a live answer from Giada again ***reviewing how to use the WHO IS properly*** – she again described its use as a calibrant especially for clinical samples due to the need for individual conversion factors (as noted above).

Bill then presented another related question from the chat:

If I establish relation of IS to a secondary standard used in my lab to each VoC individually, can I keep using secondary standard for all assays moving forward and converting it to IS for VoC pseudovirus neut assay?

[Comments from Giada & Mark]

- If I understand correctly, they have their own secondary standard and it was calibrated to the WHO IS, then, yes, they can keep using the secondary standard and not the WHO IS. Mark concurred – if you have calibrated and there is a unit – then the unit is the unit going forward.

Bill responded to **Ivana** who had hand raised in the chat to ask another related question from the chat:

Do National Regulatory Authorities use WHO standards?

[Comments from Ivana]

- Well it is actually quite a complex question because national regulatory authorities, those that actually have national controlled laboratories, as part of their organization they use WHO standards for potency, for instance, and when it comes to the vaccine lot release, then these standards, are used. These labs know how to develop secondary standards and calibrate (see again the guidance manual). The example that Youchan mentioned is exactly this.
- But in this case, we are calling on the scientific community, including regulators, to help. And the role of regulators is to require expression of the results from neutralization assays in the clinical trials in the international units.

Bill responded to **May** who also had hand raised in the chat

[Comments from May]

- May began by saying she was going to change the subject a little bit. Appealing to the assembled audience of experts, May asked for all to consider and ensure that the earliest convalescent samples and pooled plasma collected and used to begin the process of developing assays be retained because those are “pure” – unaffected by vaccine, unaffected by secondary infections and VoCs - and will be important for benchmarking new assays and emerging VoCs.

- "Just a plea and comment to others to make sure that we have that and that perhaps if NIBSC or WHO is collating or collecting, who has what samples, this might be a place that we share that information."

Bill opened floor to the panel while he looked in the chat for any additional questions.

[Comments from Mark]

- I would just like to reiterate what Giada said about how you report data and this really relates to binding antibody assay formats.
- Results must be reported in Binding Antibody Units and then specify the antigen target. This is to avoid inappropriate use of data especially as we have so many different diagnostic assays (nucleoprotein based and those that are reporting on vaccine response and therefore largely spike based) The distinction between anti-spike and anti-N is important. The data will be different, and if they get confused, we may end up in a situation where an anti-N assay has been used to report for vaccine response – clearly not appropriate. This has happened in the past with respect to Rubella. That is why we are asking for BAU reporting and specifying the antigen targets.

Bill read the question in the chat from Richard Tedder and opened to the panel. The question was "*I have taken a different approach to antibody detection by constructing a hybrid double antigen binding assay which measure anti-RBD and predicts/quantifies neutralising antibody. It produces a good calibration against the WHO IS. The assay is class and species neutral which could allow an immunized animal as a secondary standard, not available to most conventional immunoassays, has anyone done anything similar?"*

[Comments from Mark]

- This is an ideal situation – advisedly using the word "surrogate" in this case (as the terms "surrogate marker" and "correlate of protection" are being debated/clarified). Such an assay would be easier, faster, and safer than neutralizing assays. It has been adopted for the flu and must demonstrate an increasing titer, which is a surrogate marker and not a correlate of protection.

Bill moved to the final question for the panel:

D. *Are variant specific antibody panels available? Are they needed?*

Giada mentioned that there is a panel being put together and is the only one of which Bill has learned but Giada qualified that this is a work in progress.

Bill adjourned Session 1 due to time constraints and closed for a short break.

Session 2: Immune assays and SARS-CoV-2 Variants

Chair: Janet Lathey (NIAID)

The second session began with an Introduction by Karen Makar who thanked the first session presenters, noted the good progress on the international comparative neutralization assays and the expected availability of secondary standards, and reminded all that this SWAT team will continue to provide updates on how to access the materials including the WHO manual to support appropriate use of the various standards.

Dr. Makar went on to set the framework for the second session focused on immune assays and SARS-CoV-2 variants. There are a number of challenges in this area including how to interpret fold reductions for a particular variant and what that means in terms of immune escape. This difficulty, along with the inter-lab and inter-assay variations discussed in the first session, directly impact the ability to develop vaccines and therapeutics as safely and rapidly as possible.

The session was then turned over to the Chair, Janet Lathey, PhD, Program Officer in the Office of Biodefense, Research Resources, and Translational Research of NIAID.

Unit 5: Surrogate virus neutralization test (sVNT)

This topic was presented by Lin-Fa Wang, PhD, Director of the Programme in Emerging Infectious Diseases at Duke-NUS Medical School, Singapore. He began by defining surrogate as a biochemical substitute for the live virus plaque-reduction neutralization test (PRNT) and a description of the assay which reduces the time to result from 3-5 days down to one hour (see slide 83 of the workshop deck and Tan, *et al*. Nat Biotech 2020).

Summary:

1) Previous discussion around RBD-targeted versus non-RBD targeted (i.e., whole spike) neutralizing antibodies (NAbs) notwithstanding, the vast majority of NAbs (90-95%) are RBD-targeted and these outperform S1-targeted assays in the same format (see publication cited above).
2) While the PRNT is the reference standard, it requires BSL-3 containment and 3-5 days to complete. The GenScript cPass™ kit is US FDA approved and commercially available and correlated to the PRNT with a coefficient of determination (R^2) of 0.95.
3) Converting the cPASS to dPASS (i.e., adapting the test to the delta variant) presented a challenge with respect to dilution factors that was successfully addressed by developing a multiplex assay based on the Luminex platform. (NOTE: the Luminex assay has also been adapted for use with other prevalent VoCs).
4) The modifications required for the multiplex assay included:
    a. Reversing the liquid-solid phase configuration by placing RBD on beads and PE-ACE2 in liquid
    b. Use of biotinylated RBD to achieve uniform coating
    c. Presence of equimolar variant RBDs to create “in-tube competition” and allows differentiation of NAbs against variants
5) The multiplex sVNT correlated again with live virus showing parental serum neutralization of [WT > Alpha > Delta > Beta = Gamma]; Beta serum neutralization of [Beta = Gamma > Alpha > WT > Delta]; and Delta serum neutralization of [Delta > WT > Alpha > Gamma > Beta]

6) Calibration of the sVNT with the WHO IS 20/136 (IU/mL) was performed and assay reliability has been demonstrated (see slide 95 of the workshop deck). Twenty-one biological replicates were run in 3 different labs in 3 different nations. Reproducibility was high and an algorithm has been developed to transform cPass units to IU/mL. Various titrations and dilutions were tested with consistent results. The value is great because this is a biochemical test not requiring live virus and commercial production generally conveys greater quality assurance over LDTs.
7) It has been demonstrated that potent cross-clade pan-sarbecovirus NAbs are induced in survivors of SARS-CoV-1 infection who have been immunized with the BNT162b2 (Pfizer-BioNTech) mRNA vaccine. The NAbs are high-level and broad-spectrum, neutralize all currently known VoCs, as well as sarbecoviruses found in bats and pangolins and that have the potential to cause human infection. These findings, demonstrating the feasibility of a pan-sarbecovirus vaccine strategy, are detailed in Tan, *et al*., 2021 NEJM.
8) Monoclonal antibodies from these individuals were cloned and 2 were selected and mixed to a cocktail. Potency correlated well (see slide 100 of the workshop deck).
9) With the goal of "a test + IS calibrator" kit, three options were presented:
    a. Singleplex sVNT kits + calibrator
    b. Multiplex (5) VoC sVNT kit + calibrator
    c. Multiplex (16) pan-sarbeCoV sVNT kit + calibrator

Two questions were taken so that Dr. Wang could take his leave. First question was can the multiplex assay determine which VoC caused infection. The answer was yes if it is a primary infection. Vaccinations and antibody therapy can interfere with this ability to differentiate. The second question was whether the broader Ab response to vaccination following recovery was dependent on the severity of COVID. Dr. Wang explained that the patients were infected with SARS-CoV-1 17 years ago and had severe disease. Following vaccination with the Pfizer-BioNTech mRNA vaccine, and NAbs against all VoCs were high.

Unit 6: Risk Assessment of SARS-CoV-2 Variants of Concern and Impact on Vaccine Escape

This topic was presented by Bassam Hallis, PhD, Head of Pre-Clinical Development at UK Health Security Agency (UKHSA). In the interest of time, Dr. Hallis skipped material that introduced the concept of neutralization studies which can be found on slide 106 of the workshop deck. Briefly, NAbs are products of adaptive immunity generated in response to infection and/or immunization, that prevent / mitigate severity of infection. They function through a number of mechanisms that include interference with cellular binding to prevent host cell invasion. (Live) Virus Neutralization Assays (VNA) employ standardised viral materials treated with solutions of NAbs at varying concentrations to characterize the ability of these antibodies to prevent infection. Investments from the Vaccine Task Force have enabled capacity to expand to 3000 samples per week. The assay has been used in several clinical trials and is supporting vaccine licensure and risk assessment of SARS-CoV-2 variants.

Summary:

1) At the UKHSA up to 700 samples per week are run manually through a live virus microneutralization assay (MNA). This is a Focus Reduction Neutralization Test (FRNT) run on 96-well plates (6 samples per plate with reference sera and VoC wells on each plate) and take 4 days to complete. Details can be found in Bewley, *et al.*, 2021 Nature Protocols.
2) In addition to Precision, Specificity, Linearity and Relative Accuracy, Qualification and Validation, the assay passed the parameters of Dilutability, Analytical Range, LLOQ and ULOQ verification, LLOD, Sample stability (serial freeze thaws and refrigeration of samples), and Robustness.
3) Applications have included convalescent sera, pre-clinical samples of hamsters, ferrets, and non-human primates and clinical trial VoCs and antivirals, including MAbs.
4) VoCs have been gathered through generous global sharing of nasal swab samples from which VoCs are isolated, used to infect Vero/hSLAM or VAT cells and then characterized with Next-Generation Sequencing (NGS) and morphologic EM analysis followed by sterility & mycoplasma checks. The process typically takes 3 weeks and a Certificate of Analysis are generated and examples of the variant can be seen on slide 116 of the workshop deck.
5) A collaboration with NIBSC and the Agility project of CEPI have allowed variant assessment using a pre-Alpha convalescent serum panel (i.e., Wuhan-like strain) – combining results from two laboratories as fold-change relative to Victoria NIBSC and UKHSA data correlated closely with each and calibrated similarly against the WHO IS, which was generated against Victoria (see slide 117 of the workshop deck).
6) The majority of fold-changes are flattened or "lost" when normalizing against the WHO IS and this may not be the best way to analyze the data (see slide 118 of the workshop deck). However, when used to normalize data between laboratories when looking at the same variant, the WHO IS performs very well (see slide 119 of the workshop deck).
7) Variant assessment for vaccines appears robust (see slide 120 of the workshop deck).
8) The Pros, Cons and Challenges of VNAs are summarized on slides 123-125 of the workshop deck.

Unit 7: Chimeric reporter Virus Neutralization assays

This topic was presented by Pei-yong Shi, PhD, Professor and John Sealy Distinguished Chair in Innovations in Molecular Biology at the University of Texas Medical Branch. Dr. Shi's team developed a recombinant SARS-CoV-2 that replicates as efficiently as the original clinical isolate. A stable mNeonGreen reporter SARS-CoV-2 (icSARS-CoV-2-mNG) has also been developed and can be used to screen antiviral inhibitors such as interferon and accelerate vaccine development. This work was described in Xie, *et al.*, 2020, Cell Host Microbe. In addition, a unique collaboration with Pfizer-BioNTech helped to deal with the effect of VoCs on the vaccine-elicited neutralizing activity. Last, Dr. Shi described some new platforms that allow authentic neutralization assays at BSL-2.

Summary:

1) The reporter virus assay involves an overnight incubation to adhere Vero cells to a 96-well plate, serum sample are mixed with appropriate dilutions of reporter virus and added to plate, 16-hr post-infection and using Hoechst staining, plates are counted using high-content imaging. This work is described in Muruato, *et al.*, 2020, Nat. Commun.
2) This assay, demonstrating robustness and reliability, has been adopted by Pfizer-BioNTech for clinical development of their vaccine. Moderna subsequently utilized the assay to characterize neutralization kinetics post-vaccination (see slide 132 of the workshop deck).
3) Multiple references noted on slide 133 of the workshop deck demonstrate the utility of the chimeric reporter. The same 20 patient sera panel is used for every variant to allow horizontal comparison of variants. The Washington variant is also used in every assay to allow comparison across the variants. Moreover, the Washington variant backbone is used for every construct swapping only the complete spike gene. As long as the spike sequence is published, the chimeric variant can be constructed. Conventional plaque-reduction neutralization test (PRNT) was used in these variant studies.
4) This approach does not assess any mutations outside the spike which may affect other aspects of infection. However, for vaccine development, the targets are the spike and this allows efficient assessment of vaccine-elicited neutralization against different variants.
5) For all studies thus far, traditional BSL-3 PRNT assays are conducted in parallel to minimize the variations between experiments. As seen on slide 133 of the workshop deck, and consistent with other presentations, the beta variant is most concerning because it reduces neutralizing titers more than all other variants tested.
6) The data thus far indicate no immediate need to change the sequence of the mRNA vaccines as the wild-type spike elicits neutralizing antibody levels against all the variants.
7) Longitudinal studies of 11 individuals aged 18-55 years showed dramatic decline of neutralizing activity over 8 months using the PRNT assay. A booster dose after 8 months significantly increased NAbs levels. This and additional data support booster strategies to enhance the overall neutralizing activities. This work is described in Falsey, *et al.*, 2021, NEJM.
8) A trans-complementation system for SARS-CoV-2 at BSL-2 is presented in slide 135 of the workshop deck. Recognizing that BSL-3 requirements can cause bottlenecks, this model was developed to produce single-round infectious SARS-CoV-2 that recapitulates authentic viral replication. This is described in a pre-print that can be found at https://pubmed.ncbi.nlm.nih.gov/33501436/.
9) All reagents are available for industry and academic use.

Unit 8: SARS-CoV-2 Neutralization Assay Standardization and Variant Characterization

This topic was presented by Xiaoying (Shaunna) Shen, DVM, PhD, Associate Professor in the Department of Surgery, Division of Surgical Sciences of the Duke University Medical Center on behalf of herself and David Montefiori, PhD, Professor and Director of the Laboratory for AIDS Vaccine Research and Development, also in the Department of Surgery, Division of Surgical Sciences of the Duke University Medical Center. Dr. Shen's laboratory specializes in the performance of **validated cGCP assays** to monitor vaccine-elicited neutralizing antibodies in preclinical and clinical vaccine studies. Dr. Shen introduced the SARS-CoV-2 Spike-

Pseudotyped Virus (PsV) Neutralization Assay in 293T/ACE2 Cells (Duke Assay) through co-transfection of 293 T cells with a lentivirus backbone, SARS-CoV-2 spike protein and plasmids expressing luciferase, which is the read-out.

Summary:

1) The assay is high-throughput and performed under **cGCP and cGLP** with a typical 96-well plate layout. Validated and approved by the US FDA, ID50 and ID80 titers are reported based on titration curves.
2) Calibration of the PsV neutralization titers was conducted between the Duke Assay and a similar assay, also validated and FDA approved developed at LabCorp-Monogram Biosciences (Monogram Assay) with participation of Fred Hutchinson Cancer Research Center. Though similar, the latter generates ~3-fold higher titers and calibration is needed to allow inter-assay comparison.
3) The assays are similar in that both utilize (a) lentivirus (HIV) backbone, (b) SARS-CoV-2 full-length Spike (D614G variant), (c) firefly luciferase reporter gene readout, and (d) HEK-293T cells for PsV production via transfection. They differ in the utilization of TMPRSS2: Duke during PsV production and Monogram on the target cells for assay.
4) Sample sets included Convalescent sera, Moderna vaccinee sera and the WHO IS. While (uncalibrated) concordance was good, Monogram was consistently higher. All three sets were subjected to 3 calibration approaches. The WHO IS was subjected to analysis using arithmetic mean, geometric mean and median titers with the first performing best. Overall, the pooled convalescent sera subjected to bivariate normal distribution was the superior calibrant but it was acknowledged that the WHO IS has greater feasibility going forward. A preliminary report is available here: Calibration of Two Validated SARS-CoV-2 Pseudovirus Neutralization Assays for COVID-19 Vaccine Evaluation.
5) The team next investigated the use of D614G neutralization for immunobridging across VoCs. Serum samples were collected from 2,213 recipients of 2 or 3 doses of the Moderna mRNA vaccine and 46 individuals actively infected or recovered from infection with SARS-CoV-2 (15 with D614G, 19 with Beta, and 12 with Delta). Correlations were strong (see slides 151-155 of the workshop deck). Conclusion is that neutralization titers against D614G can be used to predict titers against variants.
6) Antigenic mapping or cartography for SARS-CoV-2 variants was developed in collaboration with a team led by Derek Smith of the Centre for Pathogen Evolution and is based on studies of the influenza virus where the method was compared to GPS tracking.
    a. For SARS-CoV-2 variant antigenic cartography the relative locations of the samples and variants are fixed by the lines representing different distances from each sample to each variant; the closer a sample is to a variant, the better it neutralizes it. (see slides 159-162 of the workshop deck).
    b. Variants that share key mutations are antigenically clustered and these include E484K/Q, F490S, K417N/T, N501Y, L452R.
7) Antibody landscape, also developed though collaboration with Dr. Derek Smith's team, suggests that sera from Beta and Delta infection provide complementary coverage for neutralization of SARS-CoV-2 variants.

Unit 9: Binding and functional assays using multiplex solid phase platform

This topic was presented by David Goldblatt, MD, PhD, Professor of Vaccinology and Immunology at the Great Ormond Street Institute of Child Health, University College London. Dr. Goldblatt also serves as Consultant Paediatric Immunologist at the Great Ormond Street Hospital for Children NHS Trust and is the Director of Clinical Research and Development for the joint Institution.

Dr. Goldblatt first pointed out that despite the strong predilection toward the use of neutralizing assays as protective correlates for COVID-19 vaccine development and licensing, that, in fact, binding assays display greater correlation for protection with rank correlation p values of 0.79 and 0.93, respectively. Evidence supporting the use of post-immunization antibody titers to establish a correlate of protection for COVID-19 vaccines was published earlier this year.

Summary:

1) V-PLEX COVID-19 Serology Kits, manufactured by Meso Scale Discovery®, are commercially available as a series of panels used on a Research Use only platform to detect antibodies to antigens from various coronavirus or other respiratory pathogens. Originally designed as a serology assay with the conjugated antibody carrying a chemiluminescent tag, Dr. Goldblatt's team utilized the assay as a sero-epidemiology tool *to measure human IgG to four SARS-CoV-2 antigens simultaneously* (full-length trimeric S, RBD and NTD of spike and N protein). The assay utilizes a 96-well based solid-phase antigen printed plate and an electro-chemiluminescent detection system.
    a. Qualification of this assay: This study was published early on in the pandemic before any vaccines were in use and showed the specificity and sensitivity of the binding IgG assay was highest for S protein. An adaptation of this assay utilizing tagged human ACE2 protein can measure the ability of serum to inhibit the interaction between spike protein components and soluble ACE2. IgG concentration to S and RBD correlated strongly with percentage inhibition measured by the pseudo-neutralization assay (see slide 174 of the workshop deck).
2) This served well for a number of sero-epidemiology studies and when the WHO IS was made available, the internal working standard was calibrated to the WHO 20/136 IS. All three antigens (S, N, RBD) calibrated to 1000 BAU/mL
3) Meta-analyses of relationship between vaccine efficacy and in vitro neutralizing and binding antibody titers of 7 vaccines showed variations due to the lack of assay standardization. Prior to the WHO IS, Dr. Goldblatt's team used a ratio of the vaccine response to the titers measured in the same studies to human convalescent sera. The data were published in May of this year (see slide 177 of the workshop deck) and show remarkable correlation due to the use of a single platform and noting that the sera were derived from individuals who were not part of clinical trials.
4) Next, the team applied the assay to the VoCs and found that concentrations had to be adjusted based on the raw signal derived from chemiluminescence. The MSD platform plate is in generation 19 and has the following composition of spike from all the following variants:

Spot Location	Lineages	Antigens	Common Designation
Spot 1	A (WT)	SARS-CoV-2 Spike	Wuhan
Spot 2	B.1.621	SARS-CoV-2 Spike (B.1.621)	Mu
Spot 3	AY.2	SARS-CoV-2 Spike (AY.2) **Alt Seq 1**	**Delta** sub-lineage
Spot 4	B.1.617.2; AY.4	SARS-CoV-2 Spike (B.1.617.2; AY.4) **Alt Seq 2**	**Delta** sub-lineages
Spot 5	C.37	SARS-CoV-2 Spike (C.37)	Lambda
Spot 6	AY.12	SARS-CoV-2 Spike (AY.12)	**Delta** sub-lineage
Spot 7	P.1	SARS-CoV-2 Spike (P.1)	Gamma
Spot 8	AY.1	SARS-CoV-2 Spike (AY.1) **Alt Seq 1**	**Delta** sub-lineage
Spot 9	B.1.351	SARS-CoV-2 Spike (B.1.351)	Beta
Spot 10	B.1.617.2; AY.3; AY.5; AY.6; AY.7; AY.14	SARS-CoV-2 Spike (B.1.617.2; AY.3; AY.5; AY.6; AY.7; AY.14) **Alt Seq 1**	**Delta** sub-lineages

5) Data involving variants and use of the WHO IS for anti-SARS-CoV-2 immunoglobulin showed that mRNA vaccines elicited greater spike antibodies and functional viral inhibition than viral vector vaccines for wild-type (WT), alpha, and delta with trends consistent for all four vaccines were WT > alpha > delta. Extending analysis to efficacy against beta, in particular, is confounded by lack of data.
6) The team then investigated whether the assay can be used to show immunogenicity of new vaccine candidates when efficacy data are not yet available. The Clover vaccine was plotted among the previous 4 for comparison (see slide 183 of the workshop deck and preprint). Such studies could provide insight into antigen composition in developing new vaccines in response to emerging VoCs. The group has been in discussion with regulators about using this approach in the licensing package for vaccines when efficacy trials are not possible.
7) Key question to be addressed is what the relationship between the binding antibody and functional antibody. In collaboration with Bassam Hallis (see Unit 6 of this workshop) and Shaunna Shen / David Montefiori (see Unit 7 of this workshop) the data of various cohorts were interrogated and showed strong correlation between IgG binding antibody titers and live virus or pseudovirus neutralization titers and is the basis of confidence in using binding antibodies as a true correlate of protection in making regulatory decisions.
8) Next, Dr. Goldblatt turned to comparative ACE2 Receptor Blocking (Inhibition). Across the board, trends looked the same with mRNA vaccines exhibiting higher activity than viral vectored vaccines with same hierarchy of WT > alpha > delta and as well as relatively poor performance of the latter against delta.
9) Correlations with the Hallis and Shen/ Baltimore labs again demonstrate that this assay is a robust alternative especially when access to BSL-3 facilities is limited.
10) One potential drawback brought up by members of US FDA is that binding antibodies cannot distinguish between the variants. In fact this can be done in naïve individuals undergoing a primary infection (also noted in Unit 6 by Dr. Wang). Dr. Goldblatt presented serology studies wherein the strains could be distinguished, despite cross-reactivity – which is why vaccines with spike derived from wild-type virus cross protect against VoCs. Within the same variant, longitudinal analyses can be done that allow identification of the variant (see slide 187 of the workshop deck).
11) Conclusions included:

a. Binding antibodies are important markers of exposure to and protection from SARS-CoV-2
b. Binding IgG to Spike and RBD correlate well with neutralization activity following vaccination
c. Standardization of assays and availability of standard reagents is needed – with a focus on VoCs
d. Binding assays are robust, high throughput and can be standardized; they are also amenable to rapid adjustment and can incorporate measurement to emergent variants – and should be paid more attention in vaccine development.

Panel discussion for Session 2: *Immune assays and SARS-CoV-2 Variants*

A panel discussion, moderated by Janet Lathey, included each of the presenters as well as Jenny Hendriks, PhD, Director of Biomarkers Viral Vaccines at The Janssen Pharmaceutical Companies of Johnson & Johnson, Lou Fries, MD, of Novavax and Beth Kelly, PhD, Director of Clinical Virology at AstraZeneca. The panel addressed the following questions and topics with respective key points given below:

A. What are the largest challenges in the interpretation of immune assay data for SARS-COV-2 variants?

Lou Fries opened by expressing agreement with David Goldblatt that there has been intense focus, perhaps detrimentally, on neutralization assays – both at the regulatory level and by vaccine developers. While neutralization remains a reasonable endpoint, the interpretation of neutralization data is difficult and complicated by many variables including:

- different endpoints
- use of different virus stocks
- different behavior of VoCs (such as kinetics of infection)
- different readouts ranging from cytopathic effect up to the intensity of expression of various exogenous reporter genes

Antigen binding assays are simpler and have their own sources of variability, however they are much more in-line with observed differences in efficacy across VoCs, compared with the dramatic variability in *in vitro* neutralization assays that may or may not be relevant *in vivo*.

Pei Yong Shi joined the conversation by stating that different labs have different purposes for their studies that are not necessarily aligned with vaccine developers. The latter have stringent criteria and standards for every assay they perform. I am sure all the individual vaccine developers have controls for each sample but not necessarily the universally accepted standards. The research community has different objectives which lead to variations in approaches, reagents, and assays.

Vaccine developers, as Lou pointed out, must do what they can in terms of neutralizing assays because it is the obvious and (relatively) easy to do but there are so many things we do not know in terms of immune protective parameters. The vaccine developers must

look at Real World Data and that will ultimately guide decision making about assays of choice and whether we need to switch to new sequences / strains when new VoCs emerge.

It is multi-faceted and multi-pronged in that researchers and vaccine developers need to work together toward the common goal.

Lou clarified in response that he was not denigrating any assays that are not binding assays – they all have utility in understanding immunity to a virus. But given that I am talking about the development of interventions to induce immunity to the virus, we should keep in mind that something as simple as a binding essay might be able to overcome a lot of the differences that are embedded in various neutralization assays.

Bassam Hallis next opined that what we are really looking at is the large challenges in interpretation of the data. Relying on data from different assays, done in different labs under different conditions, with different virus stocks and analytical methods including assay endpoints, presents challenges that can perhaps be overcome with efforts such as the CEPI centralized lab or what the UK government's vaccine task force has done by standardizing the assay. Standardizing as much as possible, running head to head comparisons, running proficiency panels between labs are some of the steps that can be taken to reduce these challenges.

Beth Kelly joined and stated that these may not be the biggest challenges – as we move toward correlates of protection work. What everybody is trying to do is draw that statistical interrelationship between the humoral immunogenicity and clinical vaccine efficacy. What we are seeing now with COVID is a little different than the viruses in the past where thresholds of antibody titers were not associated with vaccine efficacy.

Some of the things that we're talking about where people are fixated on binding versus neutralizing assays may end up coming out in the wash when we get more work done on correlates of protection (see publication from Peter Gilbert and his group with the mRNA1273 vaccine). Some of the early associations that the Oxford-AstraZeneca team have seen as well, where we may not have those kind of defined thresholds, may shed light on the data. Regulators are going to see that data; they are going to look at those close relationships between those different assays and examine what the associations to clinical efficacy look like.

It becomes harder if you know we have to change the paradigm a little bit and think that there may not actually be a threshold of antibodies that works for all products. There are many factors associated with clinical efficacy, more than just a single titer of neutralizing antibodies that we can say are associated with efficacy.

David Goldblatt suggested that the reason we are not finding a threshold is because the two papers that Beth has mentioned are both looking at individuals who have had breakthrough infections. The problem with individuals who have had breakthrough infections is the large overlap in antibody levels and neutralization titers in this group. If one gets a large dose of SARS-CoV-2 virus, no matter how much antibody is produced, infection will ensue (as is seen in fully vaccinated individuals who are still getting

infected). Trying to find a threshold using only individuals with breakthrough infection is fundamentally flawed.

However, with a new virus, initially the focus is on neutralization assays because there has to be some confidence that the antibodies being measured are functional. It is unfortunate that the field has not really progressed because there is a lot of information and incredible work done so quickly on neutralization. This has helped understand the immunity to the virus. But I think what we also know, particularly from some of the graphs of Dr. Shi and (I think) Dr. Wang is that, after the first dose of vaccine there are relatively low neutralization antibody titers. Yet these (mRNA) vaccines are quite efficacious after a single dose – and this not reflected in the level of neutralizing antibodies.

While national regulatory authorities focus on functional anti bodies for new pathogens at the beginning of a pandemic, now almost 2 years in, there is a lot of data on additional antibodies. Perhaps it is not a fixation on binding versus neutralization but rather an iterative approach to understanding and using all the information available.

There are clearly mechanisms which are protective and mediated by antibodies that are not effective in neutralization and are not reflected if the neutralization target is just the Spike antibody on another framework. There are other antibodies that contribute.

We need to be more open minded rather than the focus on the way we are evaluating these vaccines and, indeed, natural immunity.

Pei Yong then expressed agreement and pointed out that neutralizing antibody titers are just one aspect of the immune response that shows up within 10 days (or sooner) after the first dose. Because we do not understand the mechanism, we must explore other options to develop a set of parameters, while closely watching the real world data to make better decisions.

Janet interjected to move on to the next topic but added an observation that if we are using vaccines to find a correlate of protection, we may find the correlate to be different for each vaccine and that is another challenge and another complication if we are just using one assay.

She then introduced the next question as having come up in a few of the presentations and was not one of the prepared questions for the panel.

Is there data concerning peak responses, particularly when looking at the VoCs, and how the intensity and breadth of responses change over time?

Shaunna Shen stated that her group does not, but that it would be wonderful to have a set of samples that can be longitudinally followed from the infection through to recovery for all variants and original strain. **Lou** commented that he does not have anything to share but believes the data are evolving. **David** added that there are two issues. As Shaunna said, we do need individuals being followed after peak immunization (and there are some natural experiments such as the Israeli experience with so many samples). Then we can explore responses properly with a variety of assays and get to a level which would be associated, at least for that vaccine, with individuals becoming more susceptible to a second infection.

However, much of the data on second infections ("breakthrough") is confusing or unclear. People are getting sick, or are just PCR positive. As a global community, we need to better define what a breakthrough infection really is and if it is meaningful to put so much effort into preventing people from becoming PCR positive. The focus should be on people not getting sick.
Lastly, the other misnomer around the correlate of protection approach is people think that because antibody measurements are done at the peak of immunization, then efficacy over time has no validity. In other words, *durability* of protection is not appreciated. When the early studies were set up, that was not the major focus. Vaccine efficacy, as you saw from the slides I showed and many other slides that people have put together, shares a *relationship* with antibody titers reached immediately after immunization – and we need to learn how to better understand and exploit that relationship.

Janet then moved on to the second question:

B. How can a vaccine candidate demonstrate* in vitro *immunological non-inferiority across variants?

There was some initial confusion regarding the intent of the question and **Beth** suggested a clarification that the *in vitro* part may be causing the confusion. Is the idea here to comment on clinical immunobridging studies for vaccines against VoCs or is it slightly different? **Bassam** noted that he, too, did not fully understand the question and thanked Beth for the attempt to clarify.

Beth went on to propose the converse which would be "can *in vitro* neutralizing antibody potency against a single prototype vaccine be used to then decide when you need to trigger a new one?"

Janet replied that the intention was to be more open-ended, she went on to define the question as "how can we use the *in vitro* data to either augment the vaccine against different variants or totally change it, because it's just not working anymore?" Can the *in vitro* data help us do that and how?

David joined the conversation by suggesting looking at the data for Lambda and Mu, it is obviously difficult to kill them in the lab but they are not spreading wildly and seem to be out-competed by Delta. In the lab, Lambda and Mu appear virulent but in the real world, they are not dominant. David then invited Shaunna to the conversation and asked her opinion.

Shaunna commented that other than neutralization susceptibility, virus infectivity is another factor. Lambda and Mu are not neutralized by the antibodies *in vitro* but they are not spreading. This could be because they are not pathogenically infectious enough. Both infectivity / transmissibility and immune susceptibility affect the spread of a virus in the population.

Jenny Hendriks joined the discussion and said that this highlights one of the problems that we have raised. Namely, interpreting the *in vitro* immunological data for extrapolation of expectations towards efficacy. If that does not align and certainly does not consider any

pharmacological properties of the variants then that is a difficult extrapolation to do directly. Shaunna concurred.

David added that perhaps the most powerful way of using non-inferiority is, do head to head immunogenicity studies with a vaccine with proven efficacy (e.g., against Delta), to a new vaccine. Then you can use a variety of assays to look at non-inferiority and confer confidence that the new vaccine is efficacious against the variant. Non-inferiority and immunological assays in the absence of any biological demonstration of efficacy is much more difficult.

Lou commented that that this would work as long as you have an assay to run the two vaccines head-to-head and know something about the efficacy within one variant. He concluded by stating that we are likely not at a place where we can extrapolate efficacy to any given level in a particular assay yet.

Janet agreed and used this as a segue to the next question:

C. ***What are the key factors in each assay format that lead to variable results between different operators/ laboratories?***

Bassam started out by being careful to understand the differences between the two big elements of accuracy and precision but the biggest factors for success are the operators. Clear standard operating procedures, clear detailed work instructions, and rigorous training will reduce variability. After that, the critical reagents and proper bridging between lots or batches are the next most important factor.

Lou added that while speaking highly of binding assays, what goes into those assays is of great importance. Is there a full spike, or an RBD? In ACE2 binding inhibition, the source of human ACE2 can impact the assay. How the assays are constructed, how the plates are adsorbed, so many variables can impact the quality of assay.

Shaunna agreed and pointed out that what Lou mentioned with respect to kinetics is quite important; in addition, target cells, how much ACE2, and so many other variables can affect the quality of the assay.

Lou went to express astonishment at the generally high level of correlation between various binding assays, various human ACE2 binding inhibition assays, and various neutralization formats. Shaunna concurred.

Janet interjected to note that this conversation related to a question in the chat that was directed to Bassam about the difference in plaque sizes he found and cell lines he used.

Bassam explained that his team standardizes the assay to start with a fixed amount of virus and that amount varies depending on the variant. The specific conditions will therefore vary. Vero/hSLAM cells are used for all variants because they exhibit low mutation rates. More recently, his team uses VAT cells which over-express hACE2, and have low rates of mutation during the isolation process.

Janet used the pause and introduced a comment that appeared in the chat from **Peter Gilbert.**

> ***A comment on correlates. It is not necessary to have a truly clear separation of antibody levels in vaccine breakthrough cases vs. non-cases to be able to have an applicable correlate. The needed output is the relationship between vaccine efficacy and the post-vaccination antibody level. From this curve, one can select the antibody level associated with whatever degree of vaccine efficacy is considered high enough for public health applications. From the Moderna COVE trial correlates analysis, an ID50 titer of 10 IU50/ml was associated with >= 90% vaccine efficacy, and an MSD bAb Spike level of 33 BAU/ml was associated with >= 85% vaccine efficacy. These set benchmark threshold that could potentially be used (which can be put to the test in future studies).***

Janet then put forth a related question that appeared in the chat from **Wellington Sun** on Question 2:

> ***Does the Panel agree with the current FDA serologic criteria for demonstrating non-inferiority of neutralizing antibody response, i.e. using GMT ratio of 1.5 (and seroconversion rate difference of 10%?)***

And a second question that appeared in the chat:

> ***If we do not have a true threshold, a true correlate of protection if it turns out to be specific for specific vaccines, then, how do we do non inferiority if we do not have that correlate or it is not agreed upon by the regulatory agencies?***

David answered:

First: using the GMT ratio of 1.5
If we went head-to-head with Moderna versus AstraZeneca or Janssen, likely that neither of the vector vaccine would be approved based on non-inferiority…yet they are efficacious vaccines and absolutely critical to the global effort to prevent SARS-CoV-2. FDA, using its own criteria, would be rejecting those vaccines in the face of the competitor mRNA vaccine.

Second: a seroconversion rate of 10%
If we start with a sero-negative community, the antibody level is zero. So if there is an antibody level of .4, the community has seroconverted. So the seroconversion rate of 10% is also meaningless because essentially everybody seroconverts if you start out as sero-negative.

Perhaps these parameters are based on earlier vaccines or previous vaccines. A logical path forward without excluding efficacious vaccines is difficult, without having a slightly different approach.

Peter Gilbert made the point that you can look at the curve of antibody titers and the peak of immunization and predict, point by point, how efficacious a vaccine is likely to be. So

Peter gave the example of Moderna. At level 33, 85% of individuals are likely to be protected. So we do need to use the sophisticated statistical methods that Peter and others are developing to help FDA (and other NRAs that follow FDA) with a more logical way of evaluating some of the new vaccines, particularly from the smaller manufacturers who do not have the capacity to do large efficacy trials, helping them to get the likely efficacious vaccines licensed.

Beth commented that she thinks we will see an evolution in our regulatory agencies. We have just seen the FDA EUA for the mRNA booster shot in the absence of meeting FDA guidance on sero-response. EMA and MHRA are also evolving in that direction. She agreed with David that these thresholds are probably not required for something that is clinically efficacious and meaningful. The hope is that the regulatory agencies are seeing this as well, and signs point in that direction.

Janet closed the session due to time limitations and introduced Ivana Knezevic for closing comments.

Wrap-up and next steps

Dr. Ivana Knezevic thanked the presenters and attendees for their participation in the workshop and provided the following comments:

A. Wonderful presentations and rich discussions took place with active engagement through the chat and Q&A with valuable links shared so that resources could be accessed.

B. Many ideas shared for additional work and potential collaborations to be done.

C. The Enabling Science SWAT team is looking for additional material for the panel of VoCs, so if anybody has anything to offer, please let us know.

D. At peak attendance more than three hundred individuals joined the call.

E. Highlights of the workshop:

- There is momentum now and we must reflect on where we have come since the beginning of the pandemic and think about where we want to be in 6-12 months from now.
- In the first session, the challenges with the standardization of the assays, and development of secondary standards were addressed. Secondary standards are being developed, and some of them are calibrated against the WHO IS. It is important to continue this work.
  - The WHO manual for secondary standards is open for public comments and suggestions until 30 November 2021 at WHO web site for biological standardization (https://www.who.int/health-topics/biologicals#tab=tab_1) so please feel free to contribute your thoughts.
  - It was interesting to see that people are also asking about the misuse of WHO IS. That is not going to be covered in the manual, but perhaps we can devise other ways of addressing this so we continue to promote best practices.

        - Normalizations with the WHO IS is not a good idea when working with variants. However, the WHO IS is particularly useful for normalizing results across different labs when looking at the same variant.
    - In the second session, we heard an overview of the broad range of different assays in use, what the difficulties associated with the interpretation of results and the issues and solutions under consideration.
    - Repositories were introduced as a potential means to share virtual resources.
    - Antigenic cartography as another way to look at data was discussed.
    - The relationship between binding antibodies and functional assays is being intensely explored.
    - The vaccine regulatory environment is evolving in light of new understandings derived from the development of new vaccines.

F. All registrants of this workshop will be invited to join the online training seminar for the calibration of quantitative serology assays using the WHO International Standard for anti-SARS-CoV-2 immunoglobulin scheduled for 10Nov2021.

G. Resources related to this meeting are shared here: https://epi.tghn.org/covax-overview/enabling-sciences/.

H. The COVAX Enabling Sciences SWAT plan to continue sharing learnings across developers as we pursue our common goal – a global supply of safe and effective vaccines.