Interpreting SARS-CoV-2 immune assay data involving variants and the use of the WHO International Standard for anti-SARS-CoV-2 immunoglobulin
• Please **turn on your video** during your assigned session. As a presenter / panelist, your video will be shown to the audience unless you turn it off.

• As a presenter, **you can mute / unmute yourself to speak**. Note that general attendees cannot do this – they can only speak if Dane/Judy identifies an individual to take themselves off mute. If you would like to call on an attendee to speak, please state their first **and** last name.

• Please **say “next slide”** to advance the slides. Judy will be sharing her screen with everyone’s presentations already loaded.

• If you do not see the correct slide on your screen, it may be due to internet connectivity issues. Please **say the name of the slide header** that you’d like to see on the screen. As a backup, please **open your slides separately in PowerPoint** to reference the materials in the event internet issues arise.

• We will be keeping time and **will issue reminders for 5 minutes to go and 2 minutes** to go in each session.

• During the discussion sessions, **Janet and Bill** will serve as moderator and **Karen** will be sifting through the Q&A and feeding questions to the main moderator.
Meeting Norms and Recording Disclaimer

• Throughout the workshop, please ask any questions in the “Q&A” function. If you see that your question is already asked, you can “like” the question in the “Q&A” function.

• This workshop will be recorded. Please be mindful of the diverse audience attending the meeting when participating in open discussions.
## Interpreting SARS-CoV-2 immune assay data involving variants and the use of the WHO International Standard for anti-SARS-CoV-2 immunoglobulin

<table>
<thead>
<tr>
<th>Time (CET)</th>
<th>Presentation Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:00-15:05</td>
<td>Welcome and meeting objectives</td>
<td>Ivana Knezevic, co-lead of ES SWAT team, (WHO)</td>
</tr>
</tbody>
</table>

### Session 1: Antibody Standards Chair: William Dowling (CEPI)

<table>
<thead>
<tr>
<th>Time (CET)</th>
<th>Presentation Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:05-15:20</td>
<td>Global review of Neutralization assays against SARS-CoV-2 variants</td>
<td>Henning Jacobsen (Helmholtz Centre for Infection Research) and Ioannis Sitaras (Johns Hopkins University)</td>
</tr>
<tr>
<td>15:20-15:30</td>
<td>WHO manual for the establishment of secondary standards for antibodies against infectious agents focusing on SARS-CoV2</td>
<td>Dianliang Lei (World Health Organization)</td>
</tr>
<tr>
<td>15:30-15:50</td>
<td>WHO International standard for VOCs; replenishment plans and new collaborative study</td>
<td>Giada Mattiuzzo (National Institute for Biological Standards and Control)</td>
</tr>
<tr>
<td>15:50-16:10</td>
<td>Harmonized approach to creating secondary standards and creation of a virtual biorepository</td>
<td>May Chu and Jon Windsor (Colorado School of Public Health)</td>
</tr>
<tr>
<td>16:10-16:35</td>
<td>Panel 1/Q&amp;A Session</td>
<td>Moderated by William Dowling (CEPI)</td>
</tr>
<tr>
<td>16:35-16:40</td>
<td>BREAK</td>
<td></td>
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</tbody>
</table>

### Session II: Immune assays and SARS-CoV-2 Variants Chair: Janet Lathey (NIAID)

<table>
<thead>
<tr>
<th>Time (CET)</th>
<th>Presentation Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:40-16:45</td>
<td>Introduction</td>
<td>Karen Makar (Bill and Melinda Gates Foundation)</td>
</tr>
<tr>
<td>16:45-17:05</td>
<td>Surrogate virus neutralization assays</td>
<td>Lin-Fa Wang (Duke-NUS Medical School)</td>
</tr>
<tr>
<td>17:05-17:25</td>
<td>Risk assessment of SARS-CoV-2 Variants of Concern and Impact on Vaccine Escape</td>
<td>Bassam Hallis (UK Health Security Agency)</td>
</tr>
<tr>
<td>17:25-17:45</td>
<td>Chimeric reporter Virus Neutralization assays</td>
<td>Pei-yong Shi (University of Texas Medical Branch)</td>
</tr>
<tr>
<td>17:45-18:05</td>
<td>SARS-CoV-2 Neutralization Assay Standardization and Variant Characterization</td>
<td>Shaunna Shen (Duke University)</td>
</tr>
<tr>
<td>18:05-18:25</td>
<td>Binding and functional assays using multiplex solid phase platform</td>
<td>David Goldblatt (University College London)</td>
</tr>
<tr>
<td>18:25-18:55</td>
<td>Panel 2/ Q&amp;A session</td>
<td>Moderated by Janet Lathey (National Institute of Allergy and Infectious Diseases)</td>
</tr>
<tr>
<td>18:55-19:00</td>
<td>Wrap up &amp; Next Steps</td>
<td>Ivana Knezevic, co-lead of ES SWAT team, (WHO)</td>
</tr>
</tbody>
</table>
Welcome & Meeting Objectives

Ivana Knezevic, co-lead of ES SWAT team, (WHO)
Background: 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.

Key questions:

- What are 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?
Global review of Neutralization assays against SARS-CoV-2 variants

Henning Jacobsen (Helmholtz Centre for Infection Research) and Ioannis Sitaras (Johns Hopkins University)
Global Review of Neutralisation Assays against SARS-CoV-2 Variants

Henning Jacobsen & Ioannis Sitaras

COVAX Workshop

28th of October, 2021
SARS-CoV2 Serology

- Distinguish infected from uninfected
- Track progress of infection in a population
- Identify presence of acquired immunity
- Find correlation between antibody titres and disease progress/severity
- Establish correlates of protection from further infection either by similar strains or their variants
- Quantify immunogenicity
- Describe functional aspects of antibodies important for protection

Find correlation between antibody titres and disease progress/severity
Serological assays
SARS-CoV2

Antibody Quantification
- ELISA
- Multiplex assays

Antibody Function
- Neutralisation Assays
- Antibody-dependent cellular immune functions
  - Antibody-dependent cellular cytotoxicity
  - Antibody-dependent cellular phagocytosis
  - Antibody-dependent complement deposition
Neutralisation Assays: Pros & Cons

PROS:
- Evaluation of vaccine protection against infection and disease.
- Possible correlate of protection (Earle, et al., Cromer, et al., Khourey et al., Feng et al., Gilbert et al.).
- Assessment of (humoral) immunogenicity against circulating strains.
- Assessment of immune waning.

CONS:
- Do not reflect cellular immunity.
- High intra- and inter-assay variability.
- Comparison is difficult without standardisation.
- Subjective interpretation of results.
- Relatively labour-intensive, resource-demanding.
WHO Working Group on SARS-CoV-2 Variants and Neutralisation

- Tasked to screen, understand, summarise and report to WHO literature on NT assays using post-vaccination sera against VoCs and Vols.

- More than 1000 papers screened.

- Collection and analysis of all available data for four VoCs.
  - Live vs pseudo-viruses
  - Vaccine platform
  - Individual vaccines
Neutralisation Data: Global Summary

https://view-hub.org/resources
Neutralisation Assays: Live vs Pseudo-viruses
Neutralisation Assays: Vaccine Platforms vs VoCs
Neutralisation Assays: Individual Vaccines vs VoCs

Fold Reduction in NAbS by SARS-CoV-2 Variant of Concern and Vaccine

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Alpha</th>
<th>Beta</th>
<th>Gamma</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhui ZL - Recombinant</td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
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<tr>
<td>AstraZeneca - Vaxzevria</td>
<td><img src="image" alt="Graph" /></td>
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<tr>
<td>Beijing CNBG - BBIBP-CoV</td>
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<tr>
<td>Bharat - Covaxin</td>
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<td>Gamaleya - Sputnik V</td>
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<tr>
<td>Jaarsen - Ad26.COVD.S</td>
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<tr>
<td>Moderna - mRNA-1273</td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
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</tr>
<tr>
<td>Moderna - mRNA-1273 + Pfizer BioNTech - Comirnaty</td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
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<tr>
<td>Novavax - Covax</td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
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</tr>
<tr>
<td>Pfizer BioNTech - Comirnaty</td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
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<tr>
<td>SII - Covshield</td>
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</tr>
<tr>
<td>Sinovac - CoronaVac</td>
<td><img src="image" alt="Graph" /></td>
<td><img src="image" alt="Graph" /></td>
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</tr>
</tbody>
</table>
Difficulties in Evaluating Neutralisation Data

- Large data variance
- Strong outliers
- Insufficient data
Identified 11 aspects (33 parameters) that are likely to affect the reliability / comparability of neutralization studies.

<table>
<thead>
<tr>
<th>Sample Size</th>
<th>Required to assess the statistical strength, potential for spurious results and overall generalizability of results. Reduces probability of spurious results.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2 INFECTION</td>
<td></td>
</tr>
<tr>
<td>Reported</td>
<td>There is accumulating evidence that convalescent subjects develop a stronger immune response to vaccination compared to SARS-CoV-2 naive subjects. This is especially important for single-dose vaccinations and post-plate time points.</td>
</tr>
<tr>
<td>Confirmed</td>
<td>Because the potential impact of non-naive subjects, the cohort should be screened by for previous COVID-19 by highly sensitive methods (e.g., NGS/ELISA or by repeated PCR screening over the whole study period and pre-study period if applicable).</td>
</tr>
<tr>
<td>Breakthrough cases reported</td>
<td>Especially in long-term studies, breakthrough cases of COVID-19 might occur. These infections can affect the subject’s immune response and neutralization titers.</td>
</tr>
<tr>
<td>Breakthrough cases stratified</td>
<td>If breakthrough cases of COVID-19 are reported for the study cohort, neutralization results should be stratified for naive and infected subjects to acknowledge booster effects of the infection.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vaccination Regimen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosing interval reported</td>
<td>There is increasing evidence that the dosing interval for vaccines with a prime-boost regimen can affect the immune response including neutralization titers.</td>
</tr>
<tr>
<td>Immunization</td>
<td>Certain studies investigate neutralization titers from partially and fully vaccinated individuals. It is imperative that these cohorts are completely separated, as it is known that post-prime titers are significantly inferior to post-boost titers.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Collection Period</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2-7 days post last dose</td>
<td>Because of the kinetics of neutralizing antibody generation, no samples taken ≤ 7 days post immunization should be considered.</td>
</tr>
<tr>
<td>Stratified OR ≥ 14 days and ≤ 4 months post last dose</td>
<td>Peak neutralization titers are usually observed 14 days post immunization followed by a gradual decline of neutralization activity (waning). When assessing neutralization results and especially when comparing studies, it is important to acknowledge these kinetics by stratification of the results or by only including subjects sampled within a range of peak titers.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age distribution reported</td>
<td>As for many other pathogens, age is very likely to also affect neutralization titers against SARS-CoV-2, especially when imperfect responses are reported.</td>
</tr>
<tr>
<td>Stratified by age group</td>
<td>To acknowledge possible effects of age on neutralization titers, we recommend stratifying results based on age-groups, especially for older adults (&gt; 60 years) and children (&lt; 18 years).</td>
</tr>
<tr>
<td>Sex distribution reported</td>
<td>Although there is conflicting data, several studies suggest that the biological sex might also affect neutralization titers against SARS-CoV-2.</td>
</tr>
<tr>
<td>Stratified by sex OR equal sex distribution</td>
<td>To acknowledge possible effects of the biological sex on neutralization titers, we recommend stratifying results based on the subjects’ sex.</td>
</tr>
<tr>
<td>Cohort selection unbiased</td>
<td>If neutralization titers are generally assessed, it is essential that no biased pre-selection was performed on the study cohort.</td>
</tr>
<tr>
<td>Study period and geographic location reported</td>
<td>To correctly interpret SARS-CoV-2 infections occurring before or during the study, it is important to understand which SARS-CoV-2 variants caused infection, because variants can have differential effects on the neutralization response. If the variant distribution is not available, the study period and geogaphic location allow predicting a likely distribution of variants.</td>
</tr>
<tr>
<td>Variant prevalence reported</td>
<td>As described above, the prevalence of variants can help to understand and to correctly interpret data in the context of SARS-CoV-2 infections that occurred during or before the study period.</td>
</tr>
<tr>
<td>Stratified by variant prevalence</td>
<td>We recommend stratifying the results by the respective variants causing infection to acknowledge emerging data on potential effects of SARS-CoV-2 infection on cross-neutralization response in vaccines.</td>
</tr>
</tbody>
</table>
Assessment of Data Reliability

<table>
<thead>
<tr>
<th>Journal</th>
<th>Vaccine</th>
<th>SARS-CoV-2 variant</th>
<th>Fold-reduction in neutralization compared to parental strain</th>
<th>Sample size</th>
<th>SARS-CoV-2 infection</th>
<th>Sample collection period</th>
<th>Demographic characterization</th>
<th>Clinical characterization</th>
<th>Protocol</th>
<th>Dosage</th>
<th>Pseudovirus (if applicable)</th>
<th>Assay standardization</th>
<th>Final Risk</th>
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</thead>
<tbody>
<tr>
<td>Cell</td>
<td>BNT162b2</td>
<td>Beta</td>
<td>34.5</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>51</td>
<td>High</td>
<td>Exclusion</td>
<td>82</td>
</tr>
<tr>
<td>Nature</td>
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<td>14.0</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>91</td>
<td>High</td>
<td>Exclusion</td>
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<tr>
<td>Cell</td>
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<td>Beta</td>
<td>8.9</td>
<td>5</td>
<td>6</td>
<td>4</td>
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<td>2</td>
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<td>High</td>
<td>Exclusion</td>
<td>82</td>
</tr>
<tr>
<td>Cell</td>
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<td>Beta</td>
<td>7.6</td>
<td>5</td>
<td>6</td>
<td>4</td>
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<td>High</td>
<td>Exclusion</td>
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<tr>
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<tr>
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<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
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<td>73</td>
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<tr>
<td>Nat Med</td>
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<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>73</td>
<td>High</td>
<td>Exclusion</td>
<td>73</td>
</tr>
</tbody>
</table>

*Low risk categories*
Improving Neutralisation Data and Reporting

- Use of different testing protocols
  - **International antibody standards** greatly improve comparability and reliability of neutralization studies.

- Range in study design and technical performance
  - Important aspects within the **study design and technical performance** should be standardized.

- Insufficient details reported
  - **High reporting standards** are crucial for allowing proper evaluation.
Acknowledgments

Maria Deloria Knoll
Naor Bar Zeev
Melissa Higdon
Marley Jurgensmeyer
Kate O’Bien
Mick Mulders
Daniel Feikin
Anna-Lea Kahn
David Goldblatt
WHO manual for the establishment of secondary standards for antibodies against infectious agents focusing on SARS-CoV2

Dianliang Lei (World Health Organization)
WHO manual for the establishment of secondary standards for antibodies against infectious agents focusing on SARS-CoV2

Dianliang Lei
TSS/MHP
World Health Organization
to “develop, establish and promote international standards with respect to food, biological, pharmaceutical and similar products” is a core function of WHO

WHO international standards are established by ECBS with an assigned IU.
  - ISs serve as the primary standard for calibration of national and other secondary standards and are considered to be standards of highest order.

Secondary standards are normally established by the National Control Laboratories or manufacturers and are used as working standards.
WHO Provide Guidance on standards

- Recommendations for the preparation, characterization and establishment of international and other biological reference standards

- WHO manual for the establishment of national and other secondary standards for **vaccines**
  - (WHO/IVB/11.03)

- WHO manual for secondary reference materials for **in vitro diagnostic** assays
  - (WHO TRS 1004 Annex 6)
Request from member states

- WHO IS are widely used

- **Feedback** indicated that no manual addressing standards for antibodies: anti-Covid 19, anti-RSV, anti-HPV etc.

- To meet this need, WHO is developing a manual for the establishment and calibration of secondary standards for antibodies.

- It will provide guidance for users of IS in the preparation, calibration and use of their secondary standards.
Scope of the Manual

- Antibody reference standards are used to ensure uniformity in the designation of potency or activity to immune sera and antibody preparations and minimize systematic deviation of assays.

- 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 qualification or validation of serological test procedures is typically achieved using panels of low, medium and high titre sera calibrated against the IS and is beyond the scope of this document.
Content of the manual

- Introduction
- Use of Biological standards
- Scope of document
- Principles
- Planning
- Selection of candidate materials
- Processing of final container
- Characterization
- Calibration against IS
- Statistical analysis
- Stability
- Monitoring stability in storage
- Responsibilities of custodian lab
- IFU and Labelling
- Dispatch of standards
- Bach replacement
Principles for establishment of secondary standard

The calibration of a secondary reference material is a complex process and considerations that should be taken into account include:

- Traceability
- Uncertainty
- Value-assignment methodology
- Stability
- Commutability – the extent to which the reference standard is suitable as a standard for the various samples being evaluated.
Candidate materials

- Typically, is derived from a pool of human plasma or sera
- The pool may consist of plasma or serum from convalescent or vaccinated individuals depending on the intended application of the standard
  - Specificity of the antibodies (convalescent vs vaccines)
  - Safety
  - Sufficient volume
  - Homogeneous pooling
  - Stored frozen
Calibration against the IS

- Calibration is the process by which a unitage is assigned to a reference by the direct comparison of measurements with a higher order reference, and is one of the crucial stages of the establishment of a secondary standard.

- Calibration can be
  - by one laboratory (single assay calibration) and
  - by multiple laboratories in multiple methods (i.e. a collaborative study calibration).

- Several independent runs (same assay using the same test conditions)
Examples

- Collaborative study protocols
- Guidance for calibration of national standards
- Guidance for qualification and calibration of SARS-CoV 2, HPV, RSV.
- SOPs for ELISA micro neutralization and pseudovirus for anti-SARS CoV 2
- SOP for plaque reduction neutralization test (PRNT) of RSV
• Posted on WHO Biological website for public consultation by 30th Nov 2021

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

- **Drafting group**
  - (Dr Ian Feavers, Dr Micha Nuebling, Dr Peter Rigsby, Dr Giada Mattiuzzo, Dr Mark Page, Dr Ligia Pinto, Dr Troy Kemp, Dr Gagandeep Kang, Dr Youchun Wang, Dr Ivana Knezevic and Dr Dianliang Lei)

- **Institutions provided SOPs and study reports**
  - the National Institute for Biological Standards and Control, UK; National Institutes for Food and Drug Control, China;
  - Frederick National Laboratory for Cancer Research, USA;
  - CEPI, Norway;
  - PATH, USA;
  - Public Health England and
  - Nexelis, Canada
WHO International standard for VOCs; replenishment plans and new collaborative study

Giada Mattiuzzo (National Institute for Biological Standards and Control)
WHO International standard for VOCs; replenishment plans and new collaborative study

Giada Mattiuzzo
Timelines of COVID-19 and reference preparations

SARS-CoV-2 isolation
WHO declared COVID-19 pandemic

Jan’20

COVID-19

Mar

Research reagent SARS-CoV-2 RNA

Apr

Research reagent and reference panel for anti-SARS-CoV-2 Antibody

Jul

Launch of 2x collaborative studies for COVID19 standards

WHO ECBS meeting
Establishment of
IS for SARS-CoV-2 RNA
IS for anti-SARS-CoV-2 immunoglobulin
Reference Panel for anti-SARS-CoV-2 antibody

Aug’21

1st vaccine authorised
20/136 WHO IS depleted
SECONDARY STANDARDS AVAILABLE

NIBSC code
19/304
20/130
20/118
20/146
20/136
20/268

7818 cases
0.5 million cases 24000 deaths
17 million cases 670 000 deaths
64 million cases 1.5 million deaths
175 million cases 4 million deaths

UK Lockdown
UK Lockdown
UK Lockdown

3818 cases
64 million cases
1.5 million deaths
0.5 million cases
24000 deaths
17 million cases
670 000 deaths
175 million cases
4 million deaths

WHO declared COVID-19 PHE
Establishment of First WHO IS for anti-SARS-CoV-2 immunoglobulin

- Pool of convalescent plasma from 11 COVID-19 recovered individuals from UK
- Characterised by 44 laboratories from 15 countries worldwide, using 125 methods
- WHO IS established by WHO ECBS on 10th December 2020;
- Available in NIBSC catalogue on 18th December 2020;
Role of the WHO IS for anti-SARS-CoV-2 immunoglobulin
## Binding antibody assay - Spike ELISA

<table>
<thead>
<tr>
<th>output</th>
<th>lab</th>
<th>A-20/130</th>
<th>B-CS High</th>
<th>C- CS Low</th>
<th>D- CP low</th>
<th>E-low S,high N</th>
<th>F-High</th>
<th>G-IS</th>
<th>H- neg</th>
<th>I - low</th>
<th>J- mid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dil fact</td>
<td>22b</td>
<td>10159</td>
<td>5081</td>
<td>-</td>
<td>100</td>
<td>1270</td>
<td>12800.0</td>
<td>18102.0</td>
<td>-</td>
<td>635</td>
<td>2851</td>
</tr>
<tr>
<td>AU/mL</td>
<td>37a</td>
<td>3464</td>
<td>1764</td>
<td>-</td>
<td>42</td>
<td>510</td>
<td>5284</td>
<td>7215</td>
<td>-</td>
<td>340</td>
<td>1812</td>
</tr>
<tr>
<td>ug/mL</td>
<td>9a</td>
<td>25.2</td>
<td>13.3</td>
<td>0.4</td>
<td>0.8</td>
<td>4.5</td>
<td>47.4</td>
<td>55.4</td>
<td>-</td>
<td>2.6</td>
<td>13.3</td>
</tr>
<tr>
<td>ratio S/CO</td>
<td>38</td>
<td>18.7</td>
<td>13.9</td>
<td>-</td>
<td>-</td>
<td>4.1</td>
<td>21.4</td>
<td>23.7</td>
<td>-</td>
<td>3.1</td>
<td>11.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>output</th>
<th>lab</th>
<th>A-20/130</th>
<th>B-CS High</th>
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<th>E-low S,high N</th>
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<th>G-IS</th>
<th>H- neg</th>
<th>I - low</th>
<th>J- mid</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAU/mL</td>
<td>22b</td>
<td>457</td>
<td>284</td>
<td>-</td>
<td>8</td>
<td>114</td>
<td>949</td>
<td>1000</td>
<td>-</td>
<td>47</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>37a</td>
<td>480</td>
<td>244</td>
<td>-</td>
<td>6</td>
<td>71</td>
<td>732</td>
<td>1000</td>
<td>-</td>
<td>47</td>
<td>251</td>
</tr>
<tr>
<td></td>
<td>9a</td>
<td>463</td>
<td>246</td>
<td>8</td>
<td>13</td>
<td>85</td>
<td>836</td>
<td>1000</td>
<td>-</td>
<td>47</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>787</td>
<td>585</td>
<td>-</td>
<td>-</td>
<td>175</td>
<td>900</td>
<td>1000</td>
<td>-</td>
<td>131</td>
<td>503</td>
</tr>
</tbody>
</table>
Comparison of WHO IS with clinical samples

- Serial dilutions of the WHO IS fit within a spread of clinical samples
- Run in parallel to the NIBSC SARS-CoV-2 verification panel (n=266) in 7 commercial platforms

Data provided by David Padley, IDD Division, NIBSC
Uptake of the WHO IS 20/136

Over 2400 units 20/136 were shipped to 581 individual customers

Kit manufacturers have adopted the WHO IS units

Correlate of protection studies are reporting values using the WHO IS units
Khoury et al, Nat Med, 2021
Feng et al, Nat Med, 2021
Gilbert at al, medRxiv, 2021
Goldbaltt et al, research square, 2021

Other immunogenicity studies have been reported in WHO IS units.
Replacement WHO IS - Challenges

- Activity against the VOC
- Titre – kit manufacturers required higher value
- Source of the material
- Unitage
- Timelines
## Variants of Concern

<table>
<thead>
<tr>
<th>WHO label</th>
<th>Pangolinage*</th>
<th>GISAIDclade</th>
<th>Nextstrainclade</th>
<th>Additional amino acid changes monitored*</th>
<th>Earliest documented samples</th>
<th>Date of designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>B.1.1.7 #</td>
<td>GRY</td>
<td>20I (V1)</td>
<td>+S:484K +S:452R</td>
<td>United Kingdom, Sep-2020</td>
<td>18-Dec-2020</td>
</tr>
<tr>
<td>Beta</td>
<td>B.1.3 51</td>
<td>GH/501Y.V2</td>
<td>20H (V2)</td>
<td>+S:L18F</td>
<td>South Africa, May-2020</td>
<td>18-Dec-2020</td>
</tr>
<tr>
<td>Gamma</td>
<td>P.1</td>
<td>GR/501Y.V3</td>
<td>20J (V3)</td>
<td>+S:681H</td>
<td>Brazil, Nov-2020</td>
<td>11-Jan-2021</td>
</tr>
</tbody>
</table>

https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/

## Variants of Interest

<table>
<thead>
<tr>
<th>WHO label</th>
<th>Pangolinage*</th>
<th>GISAIDclade</th>
<th>Nextstrainclade</th>
<th>Earliest documented samples</th>
<th>Date of designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eta</td>
<td>B.1.525</td>
<td>G/484K.V3</td>
<td>21D</td>
<td>Multiple countries, Dec-2020</td>
<td>17-Mar-2021</td>
</tr>
<tr>
<td>Iota</td>
<td>B.1.526</td>
<td>GH/253G.V1</td>
<td>21F</td>
<td>United States of America, Nov-2020</td>
<td>24-Mar-2021</td>
</tr>
<tr>
<td>Kappa</td>
<td>B.1.617.1</td>
<td>G/452R.V3</td>
<td>21B</td>
<td>India, Oct-2020</td>
<td>4-Apr-2021</td>
</tr>
<tr>
<td>Lambda</td>
<td>C.37</td>
<td>GR/452Q.V1</td>
<td>21G</td>
<td>Peru, Dec-2020</td>
<td>14-Jun-2021</td>
</tr>
<tr>
<td>Mu</td>
<td>B.1.621</td>
<td>GH</td>
<td>21H</td>
<td>Colombia, Jan-2021</td>
<td>30-Aug-2021</td>
</tr>
</tbody>
</table>

### What’s the impact on vaccines and therapeutics efficacy?

Serological assays needed
Use of First WHO IS for VOC

- The International unit is an arbitrary value, it does not equal to a physical measurement, therefore cannot be “calculated” per variant
- In this cases, the potency of the IS should be reported specific per isolate used
Candidate material – initial plan

Candidate material will be selected between the convalescent plasma/serum sourced for the Reference Panel for VOC

Ideal candidate will have an high antibody titer against all VOC

Pool of convalescent plasma/serum from different VOC could also be investigated in the collaborative study

Quantity will be enough to produce ideally 5000 ampoules
Convalescent plasma/serum from infected and vaccinated individuals has

- Higher titres
- Broader responses
### WHO Reference Panel for VOC

<table>
<thead>
<tr>
<th>VOC</th>
<th>source</th>
<th>sequenced</th>
<th>status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wuhan-like</td>
<td>USA-2020</td>
<td>no</td>
<td>filled</td>
</tr>
<tr>
<td>Alpha</td>
<td>UK- Jan 2021</td>
<td>no</td>
<td>filled</td>
</tr>
<tr>
<td>Beta</td>
<td>UK</td>
<td>yes</td>
<td>too little</td>
</tr>
<tr>
<td>Gamma</td>
<td>Brazil- Jan 2021</td>
<td>no</td>
<td>collected</td>
</tr>
<tr>
<td>Delta</td>
<td>Kenya-2021</td>
<td>yes</td>
<td>filling</td>
</tr>
</tbody>
</table>

Purpose of the panel is to assist assay development – act as positive control
Epidemiology + serology data enough?

- **Wuhan-like panel member**
  - USA sample 2020
  - UK sample Jan-2021

- **Alpha panel member**
  - Beta-PCR confirmed
  - Beta-sequenced samples
Unitage: BAU or IU?

The Committee indicated its satisfaction with the proposed assignment of units for neutralizing antibodies to the candidate material 20/136 but expressed concern that the assignment of the same unitage for antibody binding assays based on different antigens would allow for the inappropriate use of the standard to compare relative antibody titres against different antigens. However, recognizing its potential utility in the harmonization of such assays, the Committee requested that further statistical analysis be conducted to confirm the suitability of the material for this purpose, with a view to recommending the assignment of a unitage for antibody binding activity at its next meeting. Given the urgent need for such a standard during the ongoing COVID-19 pandemic, the Committee recommended that the material be made available immediately via the custodian laboratory as an NIBSC working reagent for the harmonization of antibody binding assays. Data to support its use in antibody binding assays should be provided in the IFU and webinar-based and other technical assistance provided to users.
Should a potency being assigned for each VOC?

*Neutralisation*

- Data from the CS will be used to assigned an unitage to the 2\textsuperscript{nd} IS based on a comparison with the 1\textsuperscript{st} WHO IS.
- Should the value being calculated for each of the VOC in the study?
- For new VOC an arbitrary value can be assigned

*Binding*

- VOC less problematic than different antigens (?)
Collaborative study timelines

- collaborative study end Nov 2021  →  50 labs recruited
- Results returned in mid-Jan 2022
- Report submitted mid-Feb 2022
- ECBS Spring 2022

Issues:
Possible delay in the study
Sourcing material from infected/vaccinated
Next steps

• Uptake of the WHO IS has been higher than anticipated
• Misuse of the WHO IS - more education on the use is needed
• Challenges in the replacement strategy – data from CS?

• Need of secondary standards and validation panels
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Harmonized approach to creating secondary standards and creation of a virtual biorepository

May Chu and Jon Windsor
(Colorado School of Public Health)
Harmonized approach to creating secondary standards and creation of a virtual biorepository

May C. Chu, William J. Windsor, Judith Giri, Thomas Jaenisch
Colorado School of Public Health
28 October 2021

Funded by Bill and Melinda Gates Foundation
Background and Needs:

• Early in the pandemic, we quickly realized the need for QC materials as diagnostics and vaccines were being developed

• Unprecedented start up on all fronts but how could public health ensure reliability and accuracy?

• Sharing samples was not a problem but how to efficiently share them was a challenge

• We identified 2 work areas that could address these gaps:
  • COVID-19 Serology Control Panel (CSCP)
  • Virtual Federated Biorepository (VFBR)

• Align with WHO and other entities to be synergistic not duplicative
  • Qualify secondary reference materials to give greater access to multiple efforts being undertaken on test results assurance

• Build durable infrastructure with a “big tent” up from the grass-roots approach

• Organize a systematic and geographically-representative pipeline of samples for future needs
Harmonized approach to creating secondary standards
# Reference Material Providers

Inclusion requirements: All panels and reference materials must be publicly available beyond this study.

<table>
<thead>
<tr>
<th>Institution Source</th>
<th>Panel name</th>
<th># Samples</th>
<th>Material Type</th>
<th>Recommended Storage</th>
<th>Antibodies (if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermo Fisher</td>
<td>MAS™ SARS-CoV-2 IgG Positive Control Kit (Cat# 10028305)</td>
<td>1</td>
<td>plasma</td>
<td>2-8C</td>
<td>IgG</td>
</tr>
<tr>
<td>Colorado School of Public Health</td>
<td>COVID-19 Serology Control Panel</td>
<td>3</td>
<td>pooled plasma</td>
<td>-20-22C</td>
<td>IgM, IgG, IgA</td>
</tr>
<tr>
<td>INSTAND</td>
<td>INSTAND Serology panel</td>
<td>3</td>
<td>single human plasma</td>
<td>-80C</td>
<td>IgM, IgG, IgA</td>
</tr>
<tr>
<td>OneWorld Accuracy</td>
<td>SARS-CoV-2 Serology</td>
<td>4</td>
<td>single human plasma</td>
<td>2-8C</td>
<td>IgM, IgG</td>
</tr>
<tr>
<td>NIBSC</td>
<td>WHO IS (NIBSC 20/136)</td>
<td>1</td>
<td>pooled plasma</td>
<td>-20</td>
<td>IgM, IgG</td>
</tr>
<tr>
<td>NIH/NIC</td>
<td>Human SARS-COV-2 Serology Standard</td>
<td>1</td>
<td>pooled plasma</td>
<td>-20</td>
<td>IgM, IgG</td>
</tr>
</tbody>
</table>
## Testing Laboratories

Requirements: representative quantitative test platforms already in use for clinical, research and diagnostic test development

<table>
<thead>
<tr>
<th>Institution</th>
<th>Type of lab</th>
<th>Platform</th>
<th>Method</th>
<th>Antigen targets</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biodesix Laboratory</td>
<td>Commercial</td>
<td>Manual and semi-automated Tecan EVO</td>
<td>Neutralization</td>
<td>Spike, RBD</td>
<td>total Ig</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bio-Rad Platelia</td>
<td>ELISA</td>
<td>N</td>
<td>total Ig</td>
</tr>
<tr>
<td>Brigham and Women's Hospital</td>
<td>Academic/Clinical</td>
<td>Quanterix Simoa serological assay</td>
<td>multiplexed single molecule array</td>
<td>S1, spike, RBD, N</td>
<td>IgG, IgM, IgA</td>
</tr>
<tr>
<td>Wadsworth Center, David Axelrod Institute</td>
<td>Reference/Public Health</td>
<td>Luminex</td>
<td>Multiplexed microsphere</td>
<td>Spike, RBD, N</td>
<td>IgG, IgM, IgA</td>
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<tr>
<td>University of Colorado</td>
<td>Academic/Research</td>
<td>LDT</td>
<td>SARS-CoV Focus Reduction Neutralization Titer</td>
<td>whole virus</td>
<td>total Ig</td>
</tr>
<tr>
<td></td>
<td>Academic/Research</td>
<td>LDT</td>
<td>Multiplex bead immunoarray</td>
<td>N, RBD</td>
<td>IgG</td>
</tr>
</tbody>
</table>
Analyses

• Parallel line Assay Method

• Calculate the relative potency of samples to WHO IS
  • Determine Binding Antibody Units/mL in samples

• Present the BAU of each sample compared to the WHO IS
Current Status

- Received all data
- Harmonizing data to single report format
- Reproduce the analysis method in R
  - Will make the script open access
  - Compare the results to other traditional methods used
- Align with WHO guidance on qualifying secondary materials currently available for public comment

https://www.who.int/health-topics/biologicals#tab=tab_1
PLA Results for CSCP samples

Windsor et. al. https://www.medrxiv.org/content/10.1101/2021.07.07.21260101v1.full
### Table 3: Potency of CSCP Standards in International Binding Antibody Units

<table>
<thead>
<tr>
<th></th>
<th>High Reactive</th>
<th>Low Reactive #1</th>
<th>Low Reactive #2</th>
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</thead>
<tbody>
<tr>
<td><strong>Ig</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>212.1 (185.3-241.9)†</td>
<td>49.7 (38.7-62.5)</td>
<td>58.4 (47.7-70.5)</td>
</tr>
<tr>
<td>Spike</td>
<td>215.8 (190.0-244.3)</td>
<td>50.5 (43.2-58.6)</td>
<td>58.0 (49.9-67.1)</td>
</tr>
<tr>
<td>RBD</td>
<td>295.1 (260.3-333.6)</td>
<td>72.1 (62.0-83.3)</td>
<td>81.3 (70.2-93.6)</td>
</tr>
<tr>
<td><strong>IgM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Spike</td>
<td>231.8 (206.9-259.2)</td>
<td>54.7 (47.6-62.5)</td>
<td>64.4 (56.2-73.4)</td>
</tr>
<tr>
<td>RBD</td>
<td>145.8 (129.5-163.6)</td>
<td>35.3 (29.7-41.6)</td>
<td>39.5 (33.5-46.2)</td>
</tr>
<tr>
<td><strong>IgG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>257.7 (222.6-297.0)</td>
<td>55.8 (43.4-70.0)</td>
<td>55.4 (44.4-68.1)</td>
</tr>
<tr>
<td>Spike</td>
<td>246.1 (215.9-279.7)</td>
<td>57.0 (48.8-66.2)</td>
<td>57.6 (49.2-67.1)</td>
</tr>
<tr>
<td>RBD</td>
<td>408.3 (364.0-457.3)</td>
<td>101.0 (88.4-114.8)</td>
<td>97.3 (85.1-110.8)</td>
</tr>
</tbody>
</table>

† Binding antibody units based on WHO SARS-CoV-2 serology international standard as determined by parallel line analysis (+/- confidence interval).
Virtual Biorepository
Biorepository Models
For specimen collection and sharing

- Private Collector
- Museum
- Bank
- Bookstore
- Thrift Shop

- Assumes people are collecting samples of interest to them or their collaborators
- Mix and match possible
- What is the workable model(s) for you?

Source: Dr. Tony Moody, Duke University
Our Concept

Federated Virtual Biorepository (VFBR) Network

Coordination of access to qualified specimens

Sets of sharable characterized specimens within the coalition of institutes, industry, research labs in a public-private partnership

Directory of Specimen Resources
(in collaboration with other entities)
Operational Principles Established

A public good biorepository is critical to break open access to quality specimens from validated partners in a trusted operational environment.

Changes have to be made towards how professional achievements are credited, may be outside our purview but has a key impact.

Regulatory controls should not be punitive, should be used to facilitate sharing and maintain source origins.

New paradigm shifts to be prepared for pandemics, build trust and equity.
Virtual Biorepository Knowledge Hub

Objectives:

• Understand the virtual biorepository approach for access to well characterized specimens
• Identify the benefits of participating in the Virtual Biorepository network
• Consider current barriers to access and examples of working solutions

Website in collaboration with TGHN

Webinar: December 10, 2020

2nd Biorepository Workshop, December 7, 2021 to continue this discussion and implement the plans. Sponsored by CSPH, PATH, ReCoDID

https://globalbiorepository.tghn.org/
What roles VFBR envisions to provide:

- Act as a trusted exchange broker
  - Secretariat, webpage, links
  - Aligned with WHO BioHub
- Governance (Board)
- Code of conduct
- Durable business model (not-for-profit model)
- Standard, harmonized templates: MTA, PIC, LoA, MoU (aligned with WHO formats)
- Directory (in partnership with those who are also listing this)
- QC products that support/complement WHO and other authoritative entities used for training, inter-lab comparison, study controls (cost recovery model)
  - QC panels
  - Biological materials
  - Reference standards
- Coalition benefits package (examples)
  - Training, Infrastructure, Equipment
  - Travel
  - GCLP compliance
Pocket slides
1. Log-transform the dilution series.

2. Test for linearity between sample and WHO IS using R^2 test for non-linearity
   - If not linear, we log transform the results and re-evaluate
   - If linear, move to next step.

3. If they still are not parallel, we remove outliers until they are.

4. Once linear, determine the slope equation of each line.


6. Divide relative potency / 1000 BAU/mL = the secondary standard's r BAU/mL
   - Calculate 95% CI.
Preliminary results from interviews: Key barriers to access to specimens and associated metadata

Small diagnostics companies:
- identifying reliable sources of specimens, especially early in outbreak
- quality (most frequently identified) and quantity (in some cases) of specimens and especially sufficient data for to meet regulatory requirements

Mid to large companies, in addition to above:
- Sourcing from LMICs
- Biosafety/biosecurity
Preliminary results from interviews: Key barriers to access to specimens and associated metadata

**Non-profit and government associated/academic:**

- Availability of needed specimen types
- Access to clinical data
- Negotiations/legal hurdles and coordination of logistics
- Support by regional organizations; coordination of efforts

**Shared:** standardization (templates for MTAs and data sharing; SOPs)

Need for sustainable infrastructure, to be achieved by providing benefits to participants/partners
Panel Discussion

Moderated By:
William Dowling (CEPI)
Discussion Panel Members and Example Questions

<table>
<thead>
<tr>
<th>Panel Members</th>
<th>Potential Discussion Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Henning Jacobsen</strong>, Helmholtz Centre for Infection Research, Germany</td>
<td>1. How is the WHO International Standard appropriately used to assess the effects of SARS-CoV-2 variants on assay performance?</td>
</tr>
<tr>
<td><strong>Ioannis Sitaras</strong>, Johns Hopkins University, USA</td>
<td>2. 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?</td>
</tr>
<tr>
<td><strong>Dianliang Lei</strong>, World Health Organization, Switzerland</td>
<td>3. From reviews of the literature and/or collaborative studies, which assay format demonstrates the least variability?</td>
</tr>
<tr>
<td><strong>Giada Mattiuzzo</strong>, National Institute for Biological Standards and Control, UK</td>
<td>4. Are variant specific antibody panels available? Are they needed?</td>
</tr>
<tr>
<td><strong>May Chu</strong>, Colorado School of Public Health, USA</td>
<td></td>
</tr>
<tr>
<td><strong>Jon Windsor</strong>, Colorado School of Public Health, USA</td>
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<tr>
<td><strong>Mark Page</strong>, National Institute for Biological Standards and Control, UK</td>
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<tr>
<td><strong>Youchun Wang</strong>, National Institutes for Food and Drug Control, China</td>
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</tbody>
</table>
BREAK
Introduction
Session II: Immune assays and SARS-CoV-2 Variants

Karen Makar (Bill and Melinda Gates Foundation)
Surrogate virus neutralization assays

Lin-Fa Wang (Duke-NUS Medical School)
Surrogate virus neutralization test (sVNT)

Linfa WANG
Programme in Emerging Infectious Diseases
Outline

• Surrogate: biochemical vs live system
• Singleplex sVNT: from cPass to dPass
• Multiplex sVNT: VOC-adaptive (and pan-sarbeCoV ready)
• WHO International Standard for SARS-CoV-2 NAbs – calibration using sVNT and a pan-sarbecovirus NAb calibrator ?!
• IS + harmonized test kit: the ultimate goal?!
VNT  The serum virus neutralization test is a serological assay used to detect the presence and magnitude of functional systemic antibodies that prevent infectivity of a (NATIVE) virus (in susceptible cells)

SURROGATE  A substitute

SURROGATE VNT
- Based on a live (virus–cell) system: pseudovirus VNT and reporter virus VNT
- Based on a biochemical system: (receptor binding protein–receptor) blocking assay
A biochemical surrogate (simulation)

**a** Virus Neutralization Test (VNT)

- Neutralizing antibody
- Spike protein
- ACE2 receptor
- RBD of spike protein binds to ACE2 receptor

**b** surrogate Virus Neutralization Test (sVNT)

- Neutralizing antibody
- HRP-conjugated RBD
- Signal
- TMB + H₂O₂
- ELISA plate

*Tan et al. Nat Biotech (2020)*
RBD-targeted vs non-RBD-targeted NAbs

Tan et al. Nat Biotech (2020)
Singleplex sVNT

(US patent granted and FDA approved)
Correlation of PRNT and cPASS

$R^2 = 0.9446$

$Y = 0.9353X + 0.08183$

$P < 0.0001$

$n = 50$
RBD-specific binding vs neutralizing antibodies

**Graphs:**

**a** Binding

- **Absorbance 450 nm**
  - 4A1D10
  - 6D11F2
  - 5B7D7
  - 9B1E8

- **Log concentration (pM)**
  - -4 to 6

**b** Inhibition

- **Inhibition (%)**
  - 4A1D10
  - 6D11F2
  - 5B7D7
  - 9B1E8

- **Log concentration (pM)**
  - 0 to 6
Converting cPass to “dPass” (cPass for VOC Delta)

Delta patient sera

- cPass
- dPass

Neutralizing antibody

HRP-conjugated RBD

TMB + H₂O₂

ACE2 receptor

ELISA plate
• Reversing the liquid-solid phase configuration: RBD on beads and PE-ACE2 in liquid
• Use of biotinylated RBD to achieve uniform coating in a multiplex system
• Presence of equimolar RBDs creates “in-tube competition”
Strategic selection of sarbecovirus RBDs

Tan et al. NEJM (2021)

PhyML tree of ACE2 binding Sarbecoviruses RBD:

ACE2 binding

- SARS-CoV-2
- SARS-CoV-2 B.1.1.7
- SARS-CoV-2 B.1.351
- SARS-CoV-2 B.1.617.2
- Bat CoV RaTG13
- Pangolin CoV GX-P5L
- Pangolin CoV GD-1
- Bat CoV LyrRa11
- Bat CoV Rs4231
- Bat CoV RsSHC014
- Bat CoV Rs2018B
- Bat CoV WIV-1
- SARS-CoV-1
Serum panels:

Parental

Beta

Delta

WT > Alpha > Delta > Beta = Gamma
Beta = Gamma > Alpha > WT > Delta
Delta > WT > Alpha > Gamma > Beta
WHO IS (IU/ml) and sVNT calibration
EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 9 - 10 December 2020

Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody

Giada Mattiuzzo1#, Emma M. Bentley1, Mark Hassall1, Stephanie Routley1, Samuel Richardson1, Valentina Bernasconi2, Paul Kristiansen2, Heli Harvala2, David Roberts3, Malcom G Semple3, Lance CW Turtle4, Peter JM Openshaw5 and Kenneth Baillie6 on behalf of the ISARIC4C Investigators, Lise Sofie Haug Nissen-Meyer7, Arne Broch Brantsæter8, Helen Baxendale9, Eleanor Atkinson10, Peter Rigsby10, David Padley11, Neil Almond11, Nicola J. Rose1, Mark Page1 and the collaborative study participants*
Figure 2. Harmonisation of SARS-CoV-2 antibody titres in all of the neutralisation assays when reported as relative to the candidate International Standard. A) 50% neutralisation titres reported by participants and B) antibody potencies expressed as relative to the candidate International Standard, sample G with an arbitrary assigned unitage of 1000 International Units per mL. The range of the values for each samples from each laboratory is represented as a box; the black line within the box marks the median; the boundary of the box indicate minimum (lower bar) and maximum (upper bar) value.
Table 11. Geometric mean of SARS-CoV-2 Total/IgG commercial ELISA methods, as reported by the participants

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<tr>
<th>Antigen</th>
<th>Lab</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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<th>F</th>
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<td>-</td>
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<td>&gt;5</td>
<td>2.8</td>
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<td>76.6%</td>
<td>&lt;20%</td>
<td>&lt;20%</td>
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<tr>
<td>Surrog</td>
<td>20c</td>
<td>85.3%</td>
<td>76.9%</td>
<td>&lt;20%</td>
<td>&lt;20%</td>
<td>38.8%</td>
<td>85.9%</td>
<td>91.7%</td>
<td>&lt;20%</td>
<td>28.5%</td>
<td>79.3%</td>
</tr>
</tbody>
</table>
First WHO International Standard for anti-SARS-CoV-2 immunoglobulin, human (NIBSC code: 20/136)

**Material:** Antibody, human, convalescent plasma, WHO IS  

**Intended use:** Primary calibrant for serological assays  

**Description:** Pool of convalescent plasma from recovered COVID-19 patients, containing high titre antibodies against SARS-CoV-2. Plasma has been solvent detergent treated to minimise the risk of presence of enveloped viruses.  

**Enquiries:** standards@nibsc.org
IS and cPass calibration

(A) Inhibition % vs IU/ml for different locations: China, Monaco, Singapore.

(B) Inhibition formula: $\text{Inhibition} = \frac{100}{1 + e^{0.105 - 2.242\log(IU)}}$ with $R^2 = 0.978$.
An IS calibrator for all VOC/sarbecoviruses?!
Option one: Singleplex sVNT kits + calibrator

Option two: A multiplex VOC sVNT kit + calibrator

Option three: A multiplex pan-sarbeCoV sVNT kit + calibrator
Acknowledgments

Duke-NUS Team

NCID Team

DxD Hub

NIBSC

GenScript
• Co-inventor of the sVNT platform which is commercialized under the trade name cPass
• Co-inventor of a novel cross-clade vaccination method and 3GCoVax candidates
• Co-inventor of a novel method for production of highly potent pan-sarbecovirus neutralizing mAbs
Risk assessment of SARS-CoV-2 Variants of Concern and Impact on Vaccine Escape

Bassam Hallis (UK Health Security Agency)
Risk assessment of SARS-CoV-2 Variants of Concern and Impact on Vaccine Escape

Dr Bassam Hallis (Head of Pre-clinical Development)
Medical Interventions Group (MIG), UK Health Security Agency
What are neutralisation studies?

- Neutralising antibodies prevent / mitigate severity of infection
- They are a product of adaptive immunity and generated in response to infection and/or immunisation
- They function through a number of mechanisms that include interference with cellular binding to prevent host cell invasion and subsequent replication
- Neutralisation studies investigate the effects of treatment of standardised viral materials with solutions of neutralising antibodies at varying concentrations to characterise the ability of these antibodies to prevent infection
UKHSA Live Virus Neutralisation Assay Application

- Assay run manually (increase throughput from 150 to 700 samples/week by staff deployment)
- Two-stage investment from VTF/BEIS increasing capacity to 1500 (stage 1) and subsequently 3000 samples/week (stage 2)
- Supporting vaccine licensure
- Risk assessment of virus variants.
How do we use MNA studies to plan SARS-CoV2 epidemic response?

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 variant strains of SARS-CoV2 as judged by potential for vaccine escape

• To examine the potential for cross-immunity between infections with variant strains using convalescent sera

MNAs may also be helpful in determining the therapeutic potential for novel biological medicines that interact directly with the SARS-CoV2 virus
Neutralisation

UKHSA Microneutralisation Assays
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
## Validation of MNA

<table>
<thead>
<tr>
<th>Example Parameter</th>
<th>Acceptance Criteria</th>
<th>Results</th>
<th>Validation Acceptance</th>
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</thead>
<tbody>
<tr>
<td>Precision</td>
<td>≤ 50% GCV repeatability&lt;br&gt;≤ 50% GCV intermediate precision</td>
<td>Repeatability: 29%&lt;br&gt;Inter-assay: 8%&lt;br&gt;Intermediate Precision: 30%</td>
<td>All %GCV ≤ 50% Pass</td>
</tr>
<tr>
<td>Specificity</td>
<td>SARS-CoV-2 positive sera should show neutralisation;&lt;br&gt;negative sera should be ≤LLOD&lt;br&gt;% relative recovery must be 50 – 200% for the positive mixed 1:1 with a negative sample</td>
<td>GMT of positive samples: ND50 = 1922&lt;br&gt;Negative samples: ≤LLOD&lt;br&gt;Geomean of %relative recovery = 112%</td>
<td>Pass</td>
</tr>
<tr>
<td>Linearity</td>
<td>Data fitted through a regression line must have coefficient of multiple determinations (R²) ≥ 0.75 and a slope between 0.75 to 1.25</td>
<td>R² = 0.91&lt;br&gt;Slope: 0.79 (90% CI 0.73 – 0.85)</td>
<td>Pass</td>
</tr>
<tr>
<td>Relative Accuracy</td>
<td>80% of points must lie between the range of 50% to 200% relative recovery</td>
<td>GMT % recovery between 70 – 111%</td>
<td>Pass</td>
</tr>
</tbody>
</table>

- 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**
Application of MNA

- Convalescent sera
- Pre-clinical samples
- Clinical trial samples
- Risk assessment of Variants of Concern
- Antiviral/Therapeutics inc monoclonal antibodies
Isolation of SARS-CoV-2 variants

1. Nasal swab from infected individual
2. Spin down particulates
3. Infect Vero/hSLAM & VAT* (+ 2x ABM)
4. Harvest and aliquot virus

- EM analysis
- NGS Sequence
- Morphology
- Sterility & mycoplasma

* VAT cells – Vero E6 overexpressing hACE2 and hTMPRSS2
SARS-CoV-2 Variants

- Rapid optimisation for novel variants
- Isolation from clinical sample to first neutralisation results in ~3 weeks
Variant assessment

- Monitor variants using a pre-Alpha convalescent serum panel – combining results from two laboratories (A) and (B)
- Statistically significant fold-changes relative to Victoria responses shown
Effect of IS normalisation *across variants*

- Majority of fold-changes between variants are ‘lost’ when normalising in this manner
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:** 
  \[ \text{ND}_{50} = 40.4 \% \text{GCV} \]
- **Normalised** (to IS): 
  \[ \text{IU/mL} = 22.5 \% \text{GCV} \]
- An improvement in inter-lab variability of 17.9%; \( p < 0.001 \)
- **Conversion** to IU/mL further reduces variability of already comparable data
Variant assessment - Vaccines

- Donated serum assessed after 1- or 2-doses of a vaccine
- After second dose; response against Victoria virus increases 24-fold
- After 2-doses
  - Titres (Vic, Beta & Delta) greater than ND$_{50}$ = 1/128
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: Nine 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
How do we use VNA studies to plan SARS-CoV2 epidemic response?

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 variant strains of SARS-CoV2 as judged by potential for vaccine escape

• To examine the potential for cross-immunity between infections with variant strains using convalescent sera

VNAs may also be helpful in determining the therapeutic potential for novel biological medicines that interact directly with the SARS-CoV2 virus.
Benefits of VNA studies

• VNA studies can be performed relatively quickly, provided the primary biological materials (virus and source of neutralising antibodies) are available; and before observational studies of epidemiological benefit can be obtained.

• VNA studies are semi-quantitative and can be correlated to a threshold for minimum likely effect for a therapeutic benefit to be achieved.

• VNA studies allow comparison between different therapeutic options, such as cross-comparison of immunogenicity of different vaccines.

• VNA studies can give early demonstration of potential differences in vaccine scheduling options.
Disadvantages of VNA studies

• The laboratory testing environment has to be compliant with the hazard classification of the virus being studied (which is why many SARS-CoV2 studies are conducted on pseudo-viruses)

• They only reflect one element of the adaptive immune system

• When conducted with vaccine volunteer plasma or convalescent sera they have a threshold floor below which it is not easy to distinguish between NAb activity and other viricidal plasma elements
Challenges of VNA studies

- Obtaining suitable biological materials, especially newly emerging variants quickly
- Growing virus stock without inducing mutation for study
- The growing diversity of virus exposure and immunisations (doses / intervals / vaccine types) that means that ‘clean’ donors who have never been infected / only received a single vaccine type / been exposed to a single variant to provide convalescent plasma is being lost
- Correlating VNA results with clinical effect
Conclusions

VNA studies in the UK have allowed:

• The selection and deployment of credible vaccines before data from observational studies of vaccine effectiveness became available

• Have given re-assurance that vaccines in use in the UK programme remain fit for purpose as new variants of SARS-CoV2 infections have emerged

VNA studies are also part of informing the design of the booster programme

VNA studies are giving insights into the potential effectiveness of some novel biological medicines

The SARS-CoV2 VNA research infrastructure built for this pandemic will remain an important part of future resilient response infrastructure
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 20/126 use
  • Distorts the *between variant* fold-changes (incorrect usage)
  • Within variant reduces inter-lab variation significantly
Acknowledgment

• UK HSA Porton Down Team
• UK DCMO
• UK Vaccine Task Force
• NIBSC
• CEPI
• Vaccine Sponsors
• NISEC
• Collaborators
Chimeric reporter
Virus
Neutralization
assays

Pei-yong Shi (University of Texas Medical Branch)
Chimeric Reporter Virus Neutralization Assays

Pei-Yong Shi, Ph.D.
University of Texas Medical Branch at Galveston
A reporter SARS-CoV-2 neutralization assay

Day 0  Plating cells
1.2×10^4 cells/well
Vero cells

Day 1  Infection
Mixing serum & reporter viruses
Serum

Day 2  Data acquisition & analysis
High-content imaging
1) Counting positive cells
2) Calculate infection rate & NT_{50}

Xie et al., 2020, Cell Host Microbe

Muruato et al., 2020, Nat. Commun.
Enable Pfizer/BioNTech’s vaccine development

Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults
COVID-19 vaccine BNT162b1 elicits human antibody and T_\text{H}_1 T cell responses
Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates

Enable Moderna’s vaccine study

Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination
Antibody Persistence through 6 Months after the Second Dose of mRNA-1273 Vaccine for Covid-19
Pfizer vaccine-elicited neutralization of variants

Liu et al., 2021, Nature; Xie et al., 2021; Nat. Med.; Liu et al., 2021a, NEJM; Liu et al., 2021b, NEJM; Chen et al., 2021, Nat. Med.
Neutralization results support booster strategy

Liu et al., 2021, *NEJM*
A *trans*-complementation system for SARS-CoV-2 at BSL-2

Zhang et al., 2021, *Cell*
Acknowledgement

Xuping Xie  Camila Fontes  Tony Muruato  Hongjie Xia

Xianwen Zhang  Jing Zou  Yang Liu  Jianying Liu

Xianwen Zhang  Camila Fontes  Tony Muruato  Hongjie Xia

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John Bilello
Tomas Cihlar

UT Health
Zhiqiang An
Ningyan Zhang
Zhiqiang Ku

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Pfizer
Phil Dormitzer
David Cooper
Kathrin Jasen

BioNTech
Ugur Sahin
Alex Muik

IGM Biosciences
Steve Carrol

UTMB
Scott Weaver
Vineet Menachery
Tina Wang
Jia Zhou
Shinji Makino
Chien-Te K. Tseng
Mariano Garcia-Blanco
SARS-CoV-2 Neutralization Assay Standardization and Variant Characterization

Xiaoying (Shaunna) Shen (Duke University)
SARS-CoV-2 Neutralization Assay Standardization and Variant Characterization

Xiaoying (Shaunna) Shen, D.V.M., Ph.D.
David C. Montefiori, Ph.D.

Duke University Medical Center
Durham, NC

WHO COVAX Workshop
October 28, 2021
Introduction to assay technology
SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells (Duke Assay)

- Adapted from Barney Graham, Kizzmekia Corbett, Nicole Doria-Rose, John Mascola- VRC, NIH
- 293T/ACE2 cells from Mike Farzan and Huihui Mou
Formally optimized, qualified and validate lentivirus-based PsV assay (Master File #26862 with FDA)

1. Assay Set-up

2. Plates Read

3. Dose-response curves

4. Results & IQC

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<th>Control</th>
<th>IQC</th>
<th>Acceptable Ranges</th>
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<td>ID50</td>
<td>ID80</td>
</tr>
<tr>
<td>DH1043NHS</td>
<td>Plate Control</td>
<td>1,510-13,589</td>
<td>300-2,697</td>
</tr>
<tr>
<td>MDP3-High</td>
<td>Run Control</td>
<td>3,206-28,851</td>
<td>933-8,393</td>
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<tr>
<td>MDP3-Med</td>
<td>Run Control</td>
<td>923-8,307</td>
<td>193-1,740</td>
</tr>
<tr>
<td>MDP3-Low</td>
<td>Run Control</td>
<td>120-1,080</td>
<td>44-397</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>&lt;10</td>
<td>&lt;10</td>
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</table>
Calibration of PsV neutralization titers between Duke and Monogram laboratories
Duke and Monogram are both assessing neutralizing antibodies in clinical trials.

Both assays are validated and FDA approved.

Though similar technologies, the Monogram assay generates ~3-fold higher titers.

Calibration is needed to aid decision-making based on data from these two assays.
Duke and Monogram PsV Assays

**Similarities:**
- Lentivirus (HIV) backbone
- SARS-CoV-2 full-length Spike (D614G variant)
- Firefly luciferase reporter gene readout (Luminescence)
- HEK-293T cells used for pseudovirus production (transfection)

**Differences:**
- Duke utilizes TMPRSS2 during pseudovirus production
- Monogram utilizes TMPRSS2 on the target cells for assay
Calibration of Two Validated SARS-CoV-2 Pseudovirus Neutralization Assays for COVID-19 Vaccine Evaluation

- Three sample sets:
  - 248 convalescent sera (early pandemic, D614G dominant)
  - Sera from 30 recipients of Moderna mRNA-1273
    - post 1st (D29) and post 2nd (D57) (100 µg dose)
  - WHO International Standard (20/136)

- Three calibration approaches:
  - Approach 1: Calibrate to the WHO-IS using an arithmetic mean-based calibration factor
  - Approach 2: Calibrate to convalescent sera using a bivariate normal distribution model
  - Approach 3: Calibrate to convalescent sera using a linear regression model

- Apply the calibration to test equivalency of nAb titers in vaccine sera between the two labs.
Distributions of ID50 titers measured by the Duke and Monogram labs of convalescent sera, vaccine sera, and the WHO IS
Three calibration approaches applied to vaccine sera collected 4 wks post first (turquoise) and second (orange) mRNA-1273 dose.

**Approach 1:**
- WHO-IS, calibration factor based on arithmetic mean
- CCC: 0.75 (95% CI: 0.60, 0.85)

**Approach 2:**
- Convalescent sera, bivariate normal distribution model
- CCC: 0.87 (95% CI: 0.85, 0.87)

**Approach 3:**
- Convalescent sera, linear regression model
- CCC: 0.77 (95% CI: 0.71, 0.82)

**CCC:** concordance correlation coefficient
Correlation between neutralization titers for SARS-CoV-2 prototype versus variants
Rationale for D614G Neutralization as a Comparator for Immunobridging

- D614G was the dominant variant for initial estimates of efficacy in phase 3 vaccine trials
- D614G neutralization correlates with vaccine efficacy and is a major comparator for immunobridging
- The pandemic is now driven by other variants, mostly Delta
- How to proceed with immunobridging?
  Do D614G nAb titers correlate with titers against Delta and other variants?
Serum Samples Used for Variant Correlation Analysis

- Recipients of 2 or 3 doses of Moderna mRNA vaccine
  - n= 2,213

- People actively infected or recovered from infected with SARS-CoV-2
  - D614G (n= 15)
  - Beta (n= 19)
  - Delta (n= 12)
Pearson Correlation of ID50 Titers, D614G vs Beta or Delta

**Beta**

- $R = 0.92, p < 0.001$

**Delta**

- $R = 0.9, p < 0.001$

**Beta**

- $R = 0.9, p < 0.001$

**Delta**

- $R = 0.83, p < 0.001$

1283 or 1273 x2
1273 x2 or x3
1273 prime x2, 1273 boost
1273 prime x2, 1273.211 boost

1273: prototype
1283: next generation (RBD+NTD)
1273.211: bivalent prototype + Beta
Pearson Correlation of ID80 Titers, D614G vs Beta or Delta

<table>
<thead>
<tr>
<th>Beta</th>
<th>Beta</th>
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<tbody>
<tr>
<td>$R = 0.88$, $p &lt; 0.001$</td>
<td>$R = 0.89$, $p &lt; 0.001$</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Delta</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R = 0.91$, $p &lt; 0.001$</td>
<td>$R = 0.84$, $p &lt; 0.001$</td>
</tr>
</tbody>
</table>

- 1283: next generation (RBD+NTD)
- 1273: prototype
- 1273.211: bivalent prototype + Beta
- 1283 or 1273 x2
- 1273 x2 or x3
- 1273 prime x2, 1273 boost
- 1273 prime x2, 1273.211 boost

Legend:
- P101_1283
- P201_Part B
- P205.1273 100ug
- P205.211 50ug
<table>
<thead>
<tr>
<th>Variant Name</th>
<th>Pango Lineage</th>
<th>Mutations in Spike</th>
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<tbody>
<tr>
<td>D614G</td>
<td>B.1</td>
<td>D614G</td>
</tr>
<tr>
<td>Epsilon</td>
<td>B.1.429</td>
<td>S13I,W152C,L452R,D614G</td>
</tr>
<tr>
<td>Iota</td>
<td>B.1.526</td>
<td>L5F,T95I,D253G,E484K,D614G,A701V</td>
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</tbody>
</table>
Good Correlation between D614G and Variant titers for Prototype Vaccine and Convalescent Sera

R and p values are for Pearson correlation.
Good Correlation Between D614G and Variant Titers for Beta and Delta Sera

**Beta Convalescent Sera**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Correlation Coefficient ($R$)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.89, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Alpha/E484K</td>
<td>0.88, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td>0.83, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Delta_AY.1</td>
<td>0.88, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Delta_AY.2</td>
<td>0.82, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Delta_AY.3</td>
<td>0.84, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>AY_3_E484Q</td>
<td>0.86, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>AY_3_K417N</td>
<td>0.9, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Epsilon</td>
<td>0.91, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Gamma</td>
<td>0.73, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Iota</td>
<td>0.88, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Kappa</td>
<td>0.86, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Lambda</td>
<td>0.92, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Mu</td>
<td>0.77, $p &lt; 0.001$</td>
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</tbody>
</table>

**Delta Infected Sera**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Correlation Coefficient ($R$)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Alpha</td>
<td>0.94, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Alpha/E484K</td>
<td>0.91, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td>0.86, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Delta_AY.1</td>
<td>0.33, $p = 0.418$</td>
<td></td>
</tr>
<tr>
<td>Delta_AY.2</td>
<td>0.86, $p = 0.026$</td>
<td></td>
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<tr>
<td>Delta_AY.3</td>
<td>0.73, $p = 0.064$</td>
<td></td>
</tr>
<tr>
<td>AY_3_E484Q</td>
<td>0.94, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>AY_3_K417N</td>
<td>0.41, $p = 0.317$</td>
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</tr>
<tr>
<td>Epsilon</td>
<td>0.89, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Gamma</td>
<td>0.93, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Iota</td>
<td>0.93, $p &lt; 0.001$</td>
<td></td>
</tr>
<tr>
<td>Kappa</td>
<td>0.89, $p &lt; 0.001$</td>
<td></td>
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</tbody>
</table>

$R$ and $p$ values are for Pearson correlation.
Antigenic cartography for SARS-CoV-2 variants
## Sample Sets Used in Analysis

<table>
<thead>
<tr>
<th>Infection Lineage</th>
<th>Source</th>
<th>Infection Stage</th>
<th>Sequence Confirmation</th>
<th>Vaccination Status</th>
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<tbody>
<tr>
<td>Alpha</td>
<td>BC CDC/Jassem</td>
<td>Convalescent</td>
<td>Yes</td>
<td>Unvaccinated</td>
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<tr>
<td>N=14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td>CoVPN/Hural</td>
<td>Convalescent</td>
<td>Yes</td>
<td>Unvaccinated</td>
</tr>
<tr>
<td>N=19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>Duke Clinics/Datto</td>
<td>Active infection</td>
<td>Yes</td>
<td>Unvaccinated?</td>
</tr>
<tr>
<td>N=12</td>
<td>Duke ICU/Denny</td>
<td>Active infection</td>
<td>Yes</td>
<td>Unvaccinated?</td>
</tr>
<tr>
<td></td>
<td>BC CDC/Jassem</td>
<td>Convalescent</td>
<td>Yes</td>
<td>Unvaccinated</td>
</tr>
<tr>
<td></td>
<td>UW-Madison/Kawaoka</td>
<td>Active infection</td>
<td>Yes</td>
<td>?</td>
</tr>
<tr>
<td>Gamma</td>
<td>BC CDC/Jassem</td>
<td>Convalescent</td>
<td>Yes</td>
<td>Unvaccinated</td>
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<tr>
<td>N=17</td>
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<tr>
<td></td>
<td>UW-Madison/Kawaoka</td>
<td>Active infection</td>
<td>Yes</td>
<td>?</td>
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<tr>
<td>Iota</td>
<td>CDC/C-HEaRT/Veguilla</td>
<td>Convalescent/Longevity</td>
<td>Yes</td>
<td>Unvaccinated</td>
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<tr>
<td>N=11</td>
<td>Mt. Sinai/Krammer/Simon</td>
<td>Convalescent</td>
<td>Yes</td>
<td>Unvaccinated</td>
</tr>
<tr>
<td>Lambda</td>
<td>St. Jude/Webby</td>
<td>Active infection/Convalescent</td>
<td>Yes</td>
<td>Unvaccinated</td>
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<tr>
<td>N=9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ID50 Fold-Reduction Relative to D614G for Prototype and Variant Sera
Mapping the Antigenic and Genetic Evolution of Influenza Virus

Derek J. Smith,1,2* Alan S. Lapedes,3* Jan C. de Jong,2 Theo M. Bestebroer,2 Guus F. Rimmelzwaan,2 Albert D. M. E. Osterhaus,2 Ron A. M. Fouchier2*

[Diagram showing connections and nodes labeled as 'Variant' and 'Serum Sample']
Hypothesis Testing: Effect of K417N on the Antigenic Map
Antibody Landscape- Different Coverage of Variants Depending on the Infecting Variant

Modern (mRNA 1273)  Alpha (B.1.1.7) sera  Beta (B.1.351) sera  Gamma (P.1) sera

Prototype sera  Delta (B.1.617.2) sera  Lambda (C.37) sera

D Smith; B Mühlemann; E LeGresley; S Wilks; S Tureli; A Netzl
Black: prototype convalescent sera
Yellow: Beta (B.1.351) sera
Brown: Delta (B.1.617.2) sera
Major Findings

- Successful calibration of neutralization titers from two independent laboratories using the WHO-IS

- Neutralization titers against D614G predict titers against variants
  - Slope of regression can vary with variant and vaccine regimen

- Variants sharing certain key mutations cluster together antigenically.
## Acknowledgements

### BC CDC
- Agatha Jassem
- Citlali Marquez
- Samantha Kaweski
- Monika Saran

### UW-Madison
- Yoshihiro Kawaoka
- Peter Halfman

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- Olivia Widman
- Kendall Bradley
- Jiayu Chen
- Xiaoju Daniell
- Leihua Liu
- Elizabeth Domin
- Rodolfo Martinez

### CDC
- Vic Veguilla
- Fatimah Dawood
- Melissa Rolfes
- Ashton Dixon

### C-HEaRT Cohort
- Melissa Stockwell
- Priyam Thind
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- Franklin Sosa
- Liqun Wang

### BC CDC
- Jin Tong
- Sarah Hiles
- Haili Tang
- Wenhong Feng
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- Hongmei Gao
- Francesca Suman
- Kelli Greene

### Mt. Sinai
- Yoshihiro Kawaoka
- Peter Halfman

### U Cambridge
- Derek Smith
- Barbara Mühlemann
- Eric LeGresley
- Sam Wilks
- S. Tureli
- Antonia Netzli

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- Michael Datto
- Marcella Sarzotti-Kelsoe
- Thomas Denny
- Thomas Burke
- Anna Mazur
- Christina Barkauskas

### FHCRC
- John Hural
- Yunda Huang
- Peter Gilbert
- Sara Thiebaud

### U Cambridge
- Derek Smith
- Barbara Mühlemann
- Eric LeGresley
- Sam Wilks
- S. Tureli
- Antonia Netzli
“Bottom-right” vs. “Upper-right” RBD positions

Inner RBD surface

Outer RBD surface

RBD in blue

ACE2-interacting residues of the RBD in yellow

“Bottom-right” positions

“Upper-right” positions
ID50 Titers for Neutralization of Variants

- mRNA-1273
- Prototype
- Alpha
- Beta
- Delta
- Gamma
- Iota
- Lambda
Impact of E484K and E484Q

Variants containing E484K:
- Beta
- Gamma
- Lambda

Caveat: titers for Beta and Gamma sera are lower for Beta and Gamma against for Alpha and Delta variants.
Binding and functional assays using multiplex solid phase platform

David Goldblatt (University College London)
Interpreting SARS-CoV-2 immune assay data involving variants and the use of the WHO International Standard for anti-SARS-CoV-2 immunoglobulin

<table>
<thead>
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<th>DATE</th>
<th>TIME</th>
<th>LOCATION</th>
</tr>
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<tbody>
<tr>
<td>Thursday October 28th, 2021</td>
<td>06:00 – 10:00 PT / 15:00 – 19:00 CET</td>
<td>Zoom Webinar</td>
</tr>
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</table>

Binding and functional assays using multiplex solid phase platform

David Goldblatt
Professor of Vaccinology and Immunology
University College London
Evidence for antibody as a protective correlate for COVID-19 vaccines

Kristen A. Earle, Donna M. Ambrosino, Andrew Fiore-Gartland, David Goldblatt, Peter B. Gilbert, George R. Siber, Peter Dull, Stanley A. Plotkin

Neutralisation

$\text{Rank corr } p = 0.79$

Variance explained by linear model: 77.5%

Binding

$\text{Rank corr } p = 0.93$

Variance explained by linear model: 94.2%
Talk outline

• Meso Scale Discovery platform and simultaneous measurement of multiple antigens
• Use of the WHO standard serum 20/136 to calibrate a secondary standard
• Application to sero-epidemiology and vaccine evaluation
• Measuring immunity to variants
• Agreement between binding and functional assays
• Distinguishing between Variants causing infection using binding assays
• Agreement between ACE2 receptor blocking assays and virus neutralisation
V-PLEX COVID-19 Serology Assay

SULFO-TAG™ Conjugated Anti-Human Ig Antibody
Antigen-specific Antibody
Antigen
Working Electrode

MSD MULTI-SPOT® 96-Well 10-Spot Plate

MSD MULTI-SPOT® 384-Well 4-Spot Plate
Evaluation of a novel multiplexed assay for determining IgG levels and functional activity to SARS-CoV-2

Marina Johnson¹, Helen R. Wagstaffe², Kimberly C. Gilmour³, Annabelle Lea Mai², Joanna Lewis², Adam Hunt, Jake Sire⁴, Christopher Bengt⁵, Louis Grandjean⁶,⁷,⁸,⁹, David Goldblatt²,⁵,⁸,⁹

¹ Great Ormond Street Institute of Child Health, University College London, 30 Guilford Street, London, WC1N 1NB, UK
² Great Ormond Street Children’s Hospital MIB Foundation Trust, Great Ormond Street, London, WC1N 3JH, UK

Matrix and control samples are shown at each concentration.

CoV-2 S % Inhibition

CoV-2 RBD % Inhibition

CoV-2 N % Inhibition

CoV-2 NTD % Inhibition

p = <0.0001

r = 0.805

r = 0.834

97.4%

92.3%

92.8%
THE LANCET

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

Paul A Kristiansen  •  Mark Page  •  Valentina Bernasconi  •  Giada Mattiuzzo  •  Peter Dull  •  Karen Makar  •  et al.
Show all authors

Published: March 23, 2021  •  DOI: https://doi.org/10.1016/S0140-6736(21)00527-4
Long-Term Persistence of Spike Protein Antibody and Predictive Modeling of Antibody Dynamics After Infection With Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Healthcare Workers in Eight Countries: Cross-Sectional Prevalence of SARS-CoV-2 Antibodies in Eight Countries.
Evidence for antibody as a protective correlate for COVID-19 vaccines

Kristen A. Earle a, Donna M. Ambrosino b, Andrew Fiore-Gartland c, David Goldblatt d, Peter B. Gilbert e, George R. Siber f, Peter Dull a,v, Stanley A. Plotkin f

B

Vaccine efficacy (%) Voltage efficacy (%) Risk ratio (vaccine/placebo)

Ratio of vaccine response to titre in Human Convalescent Serum

Rank corr p = 0.93
Variance explained by linear model: 94.2%

SARS-CoV-2 spike IgG ELISA (geometric mean endpoint titer)

(geometric mean titer ratio of vaccinees to convalescent patients)
Goldblatt et al doi: 10.21203/rs.3.rs-832531/v1
Adjusting Concentrations For Variants of Concern

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

Wild Type
Alpha
Beta
Gamma
<table>
<thead>
<tr>
<th>Spike IgG BAU/ml</th>
<th>mRNA-1273</th>
<th>BNT162b2</th>
<th>ChadOx nCoV-19</th>
<th>Ad26.COV2.S</th>
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<tbody>
<tr>
<td>WT</td>
<td>5530</td>
<td>3890</td>
<td>1957</td>
<td>3890</td>
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<tr>
<td>Alpha</td>
<td>2667</td>
<td>1801</td>
<td>1061</td>
<td>1801</td>
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<tr>
<td>Delta</td>
<td>196</td>
<td>108</td>
<td>52</td>
<td>108</td>
</tr>
<tr>
<td>GMC (IgG BAU/ml)</td>
<td>61</td>
<td>37</td>
<td>32</td>
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</tr>
</tbody>
</table>

Goldblatt et al doi: 10.21203/rs.3.rs-832531/v1
Correlation between Spike IgG and published vaccine efficacy against Wt, alpha and delta

Goldblatt et al doi: 10.21203/rs.3.rs-832531/v1
Immunogenicity of SCB-2019 COVID-19 Vaccine Compared to Four Approved Vaccines

https://doi.org/10.21203/rs.3.rs-902086/v1
PHE Cohort: 48 Naïve post dose 1 (Pfizer) Vaccine

Spearman Correlation

Live Virus Neutralisation (Victoria strain) correlation with S and RBD titre

PseudoVirus Neutralisation 614G and correlation with S and RBD titre

Collaboration with Bassam Hallis

Collaboration with David Montefiori (Duke)
Comparative ACE2 Receptor Inhibition

<table>
<thead>
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<th>Vaccine</th>
<th>WT</th>
<th>Alpha</th>
<th>Delta</th>
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</thead>
<tbody>
<tr>
<td>mRNA-1273</td>
<td>201</td>
<td>106</td>
<td>65</td>
</tr>
<tr>
<td>ChadOx nCoV-19</td>
<td>100</td>
<td>60</td>
<td>3.5</td>
</tr>
<tr>
<td>Ad26.COV2.S</td>
<td>4.5</td>
<td>3.5</td>
<td>0.12</td>
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</table>

Comparison: ACE2 Receptor Inhibition GMT SPIKE
Live Virus Neutralisation (Victoria strain) correlation with ACE2R inhibition

PseudoVirus Neutralisation 614G and Delta strain correlation with Spike ACE2R Inhibition

Spike

Live Virus Neutralisation Titre (Victoria Strain) ND50

Spike 614G

Log WT ACE-2 Inhibition

Log 614G ID50

Spike Delta

Log Delta ACE-2 Inhibition

Log Delta ID50

Bassam Hallis Collaboration

David Montefiori Collaboration

r=0.80

r=0.78

r=0.84
UK HOSPITALISED PATIENTS DURING WAVE 1: Dominated by Original Spike

Number and cumulative number of laboratory-confirmed cases of COVID-19 by province and date of specimen collection, South Africa, 3 March 2020 – 22 May 2021 (n=1 635 465)

Post Wave 1 Seroconversion (n) | Spike IgG Beta:WT ratio | Post Wave 2 Seroconversion (n) | Spike IgG Beta:WT ratio
--- | --- | --- | ---
Mothers | 193/367 | 0.46 | 87/174 | 1.52
Children | 132/385 | 0.44 | 84/253 | 1.45

SOUTH AFRICAN SEROPREVALENCE: 3 participants during WAVE 2 (dominated by Variant Beta)

Serology to distinguish infecting Viral strain in naïve individuals?
Conclusions

• Binding antibodies are important markers of exposure and protection to SARS CoV-2
• Binding IgG to Spike and RBD correlate well with neutralization activity following vaccination
• Standardisation of assays as well as the availability of standard reagents is desirable with a focus on Variants of Concern
• Binding assays are robust, high throughput and standardizable as well as being amenable to rapid adjustment to incorporate the measurement of immunity to new variants as they arise and should be paid more attention in vaccine evaluation
Acknowledgements

Prof Heather Zar,
Red Cross Children’s Hospital
Cape Town, South Africa
(Ad26.COV2.S vaccinees)

Prof Dace Zavadska,
Children’s Clinical Hospital
Riga, Latvia
(BNT162b2 and mRNA1273 vaccinees)

All UK vaccinees (BNT162b2 and ChAdOX1) who volunteered to provide serum to help evaluate serological assays

Prof Bassam Hallis

Shaunna Shen, David Montefiori

Jim Wilbur
MesoScale Discovery

Marina Johnson, Adam Hunt,
Chris Bengt, Jake Sirr and
the WHO Pneumococcal Serology Reference Laboratory,
University College London
### Discussion Panel Members and Example Questions

<table>
<thead>
<tr>
<th>Panel Members</th>
<th>Potential Discussion Questions</th>
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<tbody>
<tr>
<td><strong>Lin-Fa Wang</strong>, Duke-NUS Medical School, Singapore</td>
<td>1. What are the largest challenges in the interpretation of immune assay data for SARS-COV-2 variants?</td>
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<tr>
<td><strong>Bassam Hallis</strong>, UK Health Security Agency, UK</td>
<td>2. How can a vaccine candidate demonstrate <em>in vitro</em> immunological non-inferiority across variants?</td>
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<tr>
<td><strong>Pei-yong Shi</strong>, University of Texas Medical Branch, USA</td>
<td>3. What are the key factors in each assay format that lead to variable results between different operators/laboratories?</td>
</tr>
<tr>
<td><strong>Xiaoying (Shaunna) Shen</strong>, Duke University, USA</td>
<td>4. In the experience of the panel members, have there been preferences expressed for particular assay formats by regulatory bodies? What have been their chief concerns?</td>
</tr>
<tr>
<td><strong>David Goldblatt</strong>, University College London, UK</td>
<td>5. Are variant specific antibody panels available? Are they needed?</td>
</tr>
<tr>
<td><strong>Jenny Hendriks</strong>, Janssen Pharmaceutical Company, The Netherlands</td>
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<tr>
<td><strong>Lou Fries</strong>, Novavax, USA</td>
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<tr>
<td><strong>Beth Kelly</strong>, AstraZeneca, USA</td>
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</table>
Wrap Up & Next Steps

Ivana Knezevic, co-lead of ES SWAT team, (WHO)
Closing remarks

• Thank you all for your participation and engagement today

• Workshop report distributed shortly to summarize today’s conversation

• We will continue to share resources at the website here: https://epi.tghn.org/covax-overview/enabling-sciences/

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