Agility Program Biweekly Progress

CEPI

Agility Program: To enable the rapid assessment of the biological impacts of new variants of SARS-CoV-2

Partners:
UK Health Security Agency (UKHSA – formerly Public Health England)
National Institute for Biological Standards and Control (NIBSC)

Slideset provided on a regular basis to update latest in vitro neutralization activity and in vivo pathogenesis and cross protection data against SARS-CoV-2 virus variants

Find this slide set posted at:

https://epi.tghn.org/covax-overview/enabling-sciences/agility_epi/#ref1

Variants of Concern and Interest Monitored by the Agility Project

WHO Variants of Concern	Status*
Delta - B.1.617.2	Assessed[2]
Omicron	
BA.1	Assessed[2]
BA.1.1	Assessed[2]
BA.2	Assessed[2]
BA.2.12.1	Assessed[2]
BA.4	Assessed[2]
BA.5.2.1	Assessed[2]
BA.2.75.3	Assessed[2]
BA.4.6	Sourced
BQ.1.1	Sourced
BA.2.75.2	Sourced

Superscripts denote assessed at 1 or 2 sites in vitro

Recombinants	Status*
Omicron x Delta recombinant (XD)	Seeking
Omicron x Delta recombinant (XF)	Assessed[2]
Alpha x Delta recombinant (XC)	Sourced
BA.1 x BA.2 recombinant (XE)	Assessed[2]
BJ.1 x BM.1.1 recombinant (XBB)	Sourced

Deselected Variants	
Tested within Agility	**Not tested within Agility**
Alpha – B.1.1.7	Eta (B.1.525)
Beta – B.1.351	Epsilon (B.1.427/B.1.429)
Gamma – P.1	Theta (P.3)
Zeta (P.2) – sourced from Fiocruz	Iota (B.1.526+E484K)
Zeta (P.2) – sourced from BEI	Omicron – BA.3
Kappa (B.1.617.1)	
Mu (B.1.621)	
Alpha + E484K	
Lambda (C.37) (single lab evaluation)	
AY.1	
AY.4.2	

*Isolates provided by Alex Sigal, African Health Research Institute, pursued for reasons of interesting Spike mutations

[a]https://www.medrxiv.org/content/10.1101/2021.08.20.21262342v1

[b]https://www.medrxiv.org/content/10.1101/2021.09.14.21263564v1.full

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Table updated as of November 15, 2022

Agility Project: Variant Growth/Testing for Neutralization Phenotype

Variant			Sourcing or Propagation Seeking/In progress/Complete	Characterisation In progress/Complete/No longer required	In vitro (neutralisation) In progress/Complete/No longer required	In vivo Not selected/Planning/In progress/In-life complete
WHO VOCs	**Omicron (B.1.1.529)**	**BA.1**	Complete	Complete	Complete	In-life complete –manuscript link[a]
		BA.1.1	Complete	Complete	Complete	
		BA.2	Complete	Complete	Complete	In progress
		BA.2.12.1	Complete	In progress	Complete	In progress
		BA.4	Complete	Complete	Complete	In progress
		BA.5.2.1	Complete	Complete	Complete	In progress
		BA.2.75.3	Complete	Complete	Complete	
		BA.4.6	Complete	In progress	In progress	
		BQ.1.1	In progress			
		BA.2.75.2	In progress			
Other	**Recombinants**	**Omicron x Delta recombinant (XD)**	Seeking			
		Omicron x Delta recombinant (XF)	Complete	In progress	Complete	
		Alpha x Delta recombinant (XC)	Complete	In progress	In progress	
		BA.1 x BA.2 recombinant (XE)	Complete	In progress	Complete	
		BJ.1 x BM.1.1 recombinant (XBB)	In progress			

†No longer a WHO VOC

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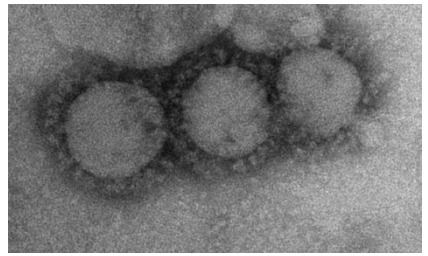
[a]https://www.biorxiv.org/content/10.1101/2021.12.24.474081v1

Wildtype virus Quality Control



- Most viruses isolated from clinical material through UKHSA's network
- Some have been isolated elsewhere and donated by other institutes
  - G2P consortium
  - Barclay 'flu lab (Imperial College, London)
  - Oxford University, UK
  - Fiocruz, Brazil
  - Sheba Medical Centre, Israel
  - AHRI, South Africa
- All are grown into working banks and quality control assessments are performed
  - CoAs issued
  - Virus stocks available from NIBSC and EVAg

Criteria	Result
Passage history, cell line(s) used, MOI and harvest details	Recorded
Morphology	Transmission electron microscopy
Cytopathic effect	Record appearance
Viable titre	Plaque forming units on Vero E6 (and additionally/alternatively VAT or foci)
Usage dilution in micro-neutralisation assay (MNA)	For ~130 focus forming units/well in non-neutralisation control
Sterility	7 days in TSB & Thioglycollate at 22° and 37°C
Absence of mycoplasma	ECACC validation PCR test
Sequence analysis – Nanopore/Arctic v3	Confirm presence of furin cleavage site, identity, lineage (*fast*)
Sequence analysis – Illumina NGS/SISPA	Examination of minor variants, absence of contaminants, fill in any 'missed' regions due to Arctic protocol primer mismatches (*detailed*)

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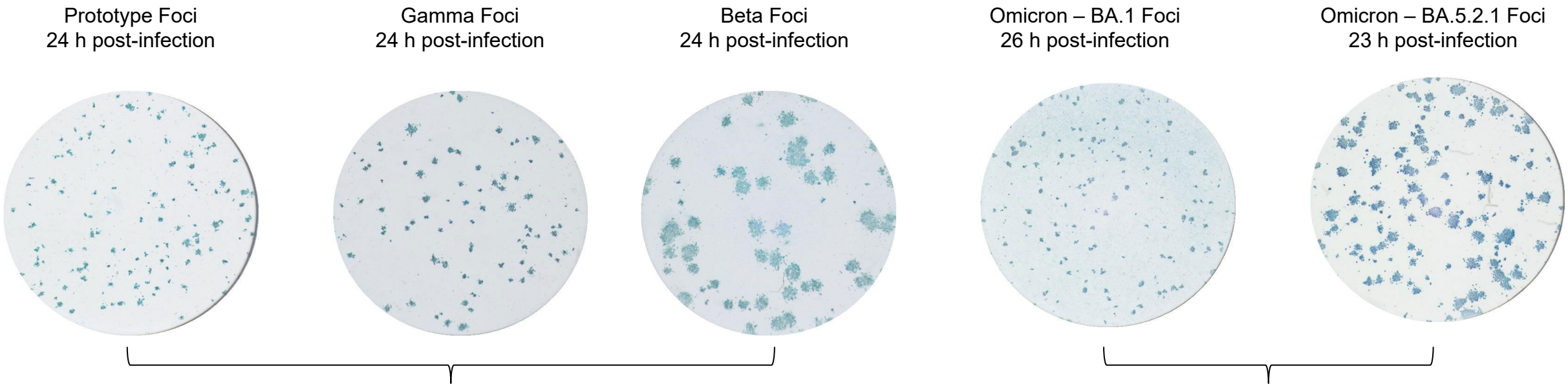
Differences in foci phenotype between variants

- Immunostaining of foci formation from different variants over time has revealed changes in phenotype
- Incubation times have been modified to permit accurate counting of foci
- The emergence of Omicron necessitated changes in the immunostaining protocol (RBD antibody no longer recognised infected cells)
  - Permeabilisation and nucleocapsid staining required for Omicron subvariants



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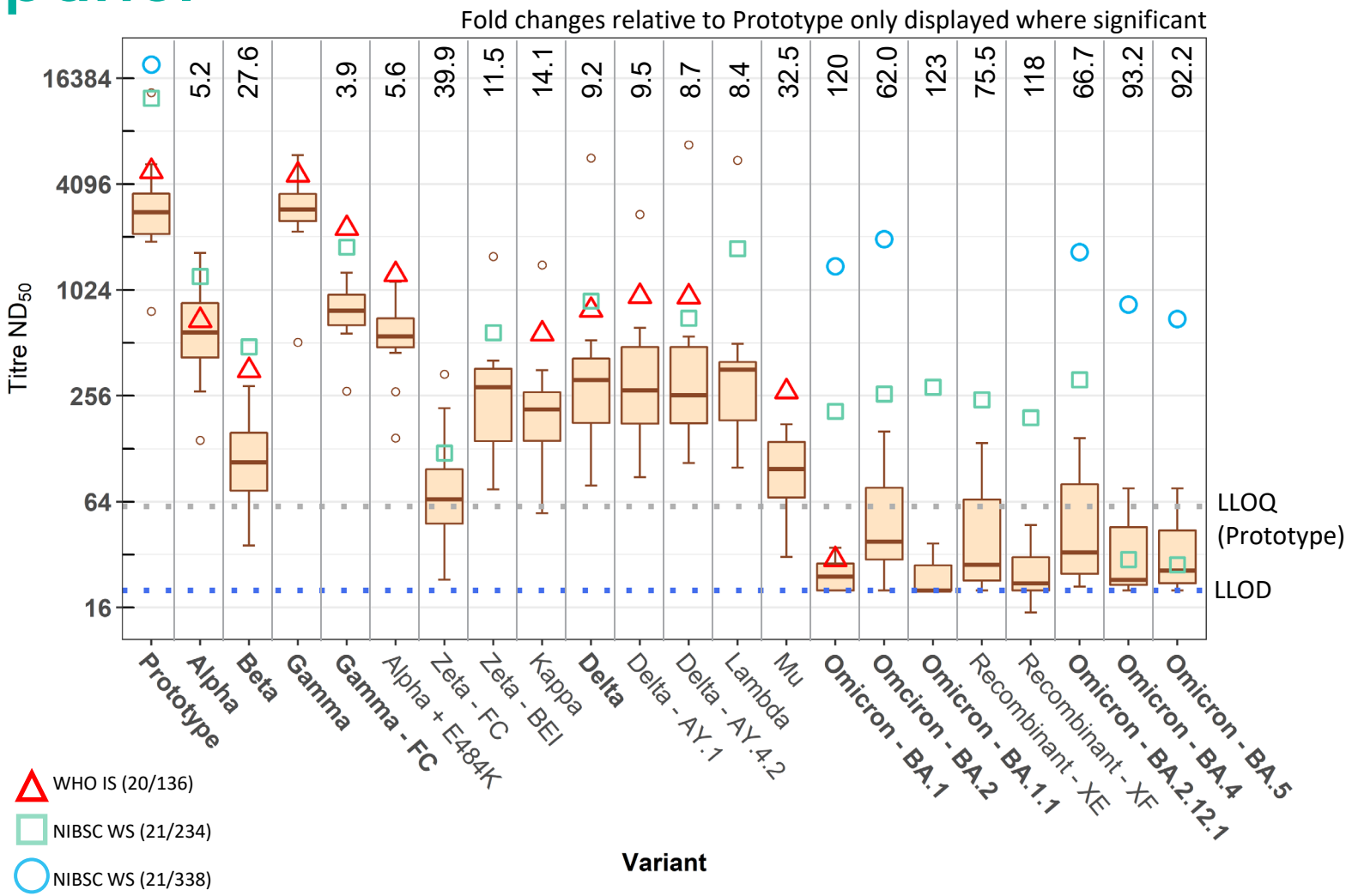
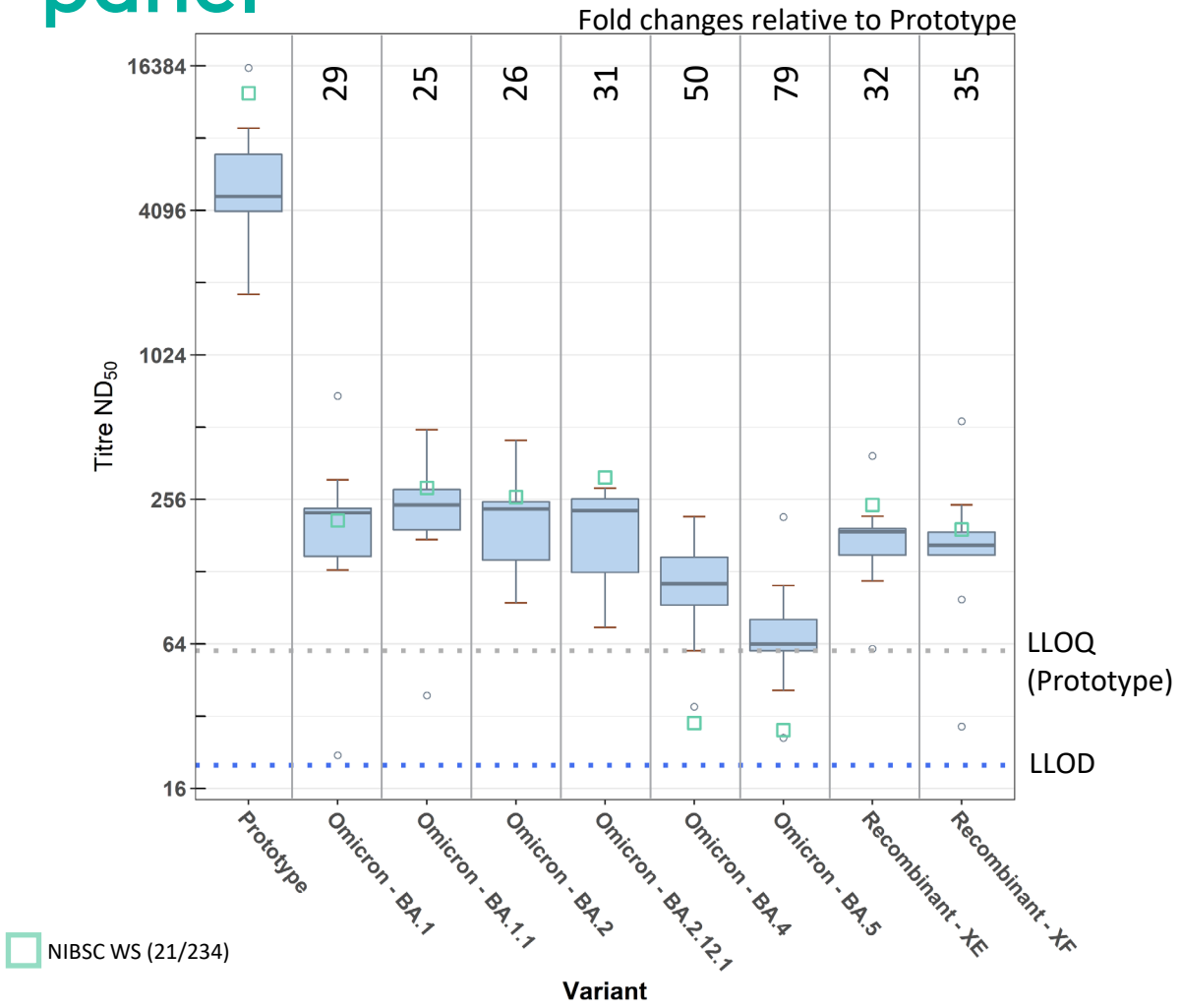
Updated as of November 15, 2022

Live-virus *in vitro* antibody neutralization assay – Convalescent panel



- UKHSA and MHRA neutralisation assays behave comparably across variants
  - Data are presented as geometric mean of titres from both labs (XE are the results from a single lab)
- Variants show various degree of resistance to the panel - only statistically significant ($p<0.001$) fold changes relative to Victoria are shown)
- IS & *WS generally* show the least neutralisation reduction compared to individual samples – can't be used to correct ND_{50}s *across variants*
  - *Preliminary data for candidate replacement standard 21/338 shows high titres against BA.x variants*
- Omicron exhibit the largest drops in ND_{50} seen to date
  - *Many of the titres below assay limit of detection for BA.x variants which is likely to make this fold-change an underestimate*

Live-virus *in vitro* antibody neutralization assay – Vaccinee panel



- Serum samples from 10 vaccinated UKHSA staff volunteers following 3 vaccinations
  - 4 participants received Cominarty for their initial 2 doses
  - 6 participants received Vaxzevria for their initial 2 doses
  - All received an mRNA booster vaccine
  - 1 participant had COVID-19 between the 2nd dose and booster
- Samples taken at a median of 72 days post booster.
- **Serum from all vaccinees who received three doses of a vaccine (regardless of initial course) had detectable neutralisation titres against all variants**
- The difference between BA.4 and BA.5 is not significant
- BA.5 and BA.4 fold-reduction relative to the rest of the Omicron subvariants is significant ($p<0.0001$)

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Updated as of November 15, 2022

The broader scientific community is currently collecting biological infection data to understand disease severity and immune reponse to variants of concern in the following ways, plus many others:

- Human clinical studies assessing vaccine effectiveness against variant infections
- Animal studies in various laboratory model species to evaluate effectiveness of original vaccines against variants, and new vaccines, need for boosters, etc.

The Agility Program is leveraging CEPI Preclinical Laboratory Network Partners to perform hamster modeling studies under high ethical standards

- CEPI Network of Partners was established in 2019 via a call for proposals to engage laboratories with high animal ethics standards, biocontainment laboratory capabilities and high-quality research methods that meet regulatory requirements
- All animal studies are performed in accordance with UK NC3Rs guidelines (https://www.nc3rs.org.uk/the-3rs)
- All research is done in compliance with CEPI's Animals in Research Policy

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Variant assessment – *In vivo*

Primary infection studies confirmed typical coronavirus disease; and Re-Infection Studies showed solid protection from disease in hamsters, even across variants

Initial Infection	*Re-infection*	*Clinical signs after re-infection?*	*Weight loss after re-infection?*	*Protection against re-infection?*
Alpha	Delta	No	No	Yes
Victoria	Delta	No	No	Yes
Beta	Gamma	No	No	Yes
Beta	Beta	No	No	Yes
Gamma	Beta	No	No	Yes
Gamma	Gamma	No	No	Yes
Victoria	Mu	No	No	Yes
Victoria	Zeta	No	No	Yes
Victoria	Omicron	No	No	Yes*

*All studies were conducted in compliance with all UK government regulatory requirements. In-life phase complete: full data analysis is underway, with ELISA, microneutralization and pathology data pending. *pre-print released Dec 24 https://www.biorxiv.org/content/10.1101/2021.12.24.474081v1*

- ✓ **For all VOCs tested, prior infection was able to protect against secondary infection 28 days later.**
- ✓ **None of the combinations of VOCs tested showed escape from immunity.**
- ✓ **Preliminary pathology data has not identified any difference between VOCs with the exception of Omicron for which similar lesions in the lung and upper respiratory tract were present, but with lower severity.**

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Variant assessment – *In vivo*

Primary infection studies with omicron sub-variants demonstrated varying severity of disease in the hamster model. Hamsters were followed until 7- and 28- days post infection

Initial Infection	***Clinical signs after infection?***	***Weight loss after infection?***
BA.1	Yes	No
BA.2	Yes	Yes
BA.2.12.1	Yes	Yes
BA.4	Infrequent	No
BA.5	Yes	Yes

All studies were conducted in compliance with all UK government regulatory requirements. In-life phase complete: full data analysis is underway, with ELISA, microneutralization and pathology data pending.

Important considerations for laboratory methods

- Serial propagation of SARS-CoV-2 variants in Vero E6 or other cell types may lead to furin cleavage site mutations that affect how the virus grows and behaves in vitro or in vivo. Propagation of unwanted mutations can be mitigated by growth in cells such as Vero/hSLAM and by frequent sequence confirmation (deep sequence methods preferred). link
- NIBSC Working Standard should be used for neutralization assays, but it performs differently for each variant. Any data presented comparing the WHO IS should always identify the variant under test.

Recent relevant publications

- Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays Nature Protocols **16**, 3114-3140 (2021)
- A cautionary perspective regarding the isolation and serial propagation of SARS-CoV-2 in Vero cells NPJ Vaccines **6**:83 (2021)

Recent online conference presentations

- 18Oct22: UKHSA conference 23Sep22: WHO Animal Models Group
- 30 August 2022: ECDC/WHO Euro SARS-CoV-2 virus characterisation working group meeting
- 20 July 2022: WHO Assays Working Group
- 11 April 2022: Keystone Symposia (Lessons from the Pandemic: Responding to Emerging Zoonotic Viral Diseases)
- 13 January 2022: WHO Animal Models Working Group meeting
- 22 Feb 2022: Joint ECDC and WHO lab assay working group meeting
- 17 March 2022: New Variant Assessment Platform (NVAP) module on SARS-CoV-2 Risk Assessment and Virology