Agility Program Biweekly Progress

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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 biweekly 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

WHO Variants of Concern and Interest Monitored by the Agility Project

WHO Variants of Interest	Status*		WHO Variants of Concern	Status*
†Epsilon - B.1.427/B.1.429	Deselected		†Alpha - B.1.1.7	Assessed[2]
†Zeta – P.2	Assessed[1]		†Beta - B.1.351	Assessed[2]
†Eta – B.1.525	Seeking		†Gamma - P.1	Assessed[2]
†Theta – P.3	Deselected		Delta - B.1.617.2	Assessed[2]
†Iota – B.1.526+E484K or S477N	Seeking		Omicron – BA.1, BA.1.1 and BA.2[1]	Assessed[2]
†Kappa – B.1.617.1	Assessed[2]		Omicron x Delta recombinant	Seeking
†Lambda – C.37	Assessed[1]			
†Mu - B.1.621	Assessed[2]			
additionally sourcing AY sublineages based on geographical prevalence…				
AY.4, AY.23, AY.25, AY.30, AY.32 AY.4.2, AY.43	Seeking Sourced		AY.4.2 AY.1	Assessed[2] Assessed[2]

Link to the WHO weekly Epi report website:
https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

*From; Not selected/Seeking/Sourced/Assessed
†No longer a WHO VOC/VUI, may prompt deselection
Superscripts denote assessed at 1 or 2 sites in vitro

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Table updated as of March 25, 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	†Alpha (B.1.1.7)	Complete	Complete	Complete	In-life complete
	†Beta (B.1.351)	Complete	Complete	Complete	
	†Gamma (P.1)	Complete	Complete	Complete	
	Delta (B.1.617.2)	Complete	Complete	Complete	In-life complete –reporting underway
	Omicron (B.1.1.529) BA.1, BA.1.1, BA.2	Complete	In progress	Complete	In-life complete –manuscript link[c]
	Recombinant Delta x BA.x	Seeking			
	BA.3	Sourced	In progress		
WHO VOIs	†Eta (B.1.525)	Seeking			
	†Epsilon (B.1.427/B.1.429)	Sourced	No longer required		
	†Zeta (P.2) – sourced from Fiocruz †Zeta (P.2) – sourced from BEI	Complete Complete	Complete In progress	Complete Complete (single lab evaluation)	In-life complete NA
	†Theta (P.3)	Deselected	No longer required		
	†Iota (B.1.526+E484K)	Seeking			
	†Kappa (B.1.617.1)	Complete	Complete	Complete	
	†Lambda (C.37)	Complete	In progress	Complete (single lab evaluation)	
	†Mu (B.1.621)	Complete	Complete	Complete	In-life complete
UK	Alpha + E484K	Complete	In progress	Complete	
n/a	*C.1.2[a]	Complete	In progress	In progress	
n/a	*Isolate D190[b]	Complete	In progress	In progress	

†No longer a WHO VUI, *Isolates provided by Alex Sigal, African Health Research Institute, pursued for reasons of interesting Spike mutations.

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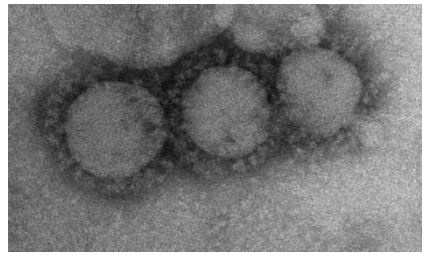
[a]https://www.medrxiv.org/content/10.1101/2021.08.20.21262342v1
[b]https://www.medrxiv.org/content/10.1101/2021.09.14.21263564v1.full
[c]https://www.biorxiv.org/content/10.1101/2021.12.24.474081v1

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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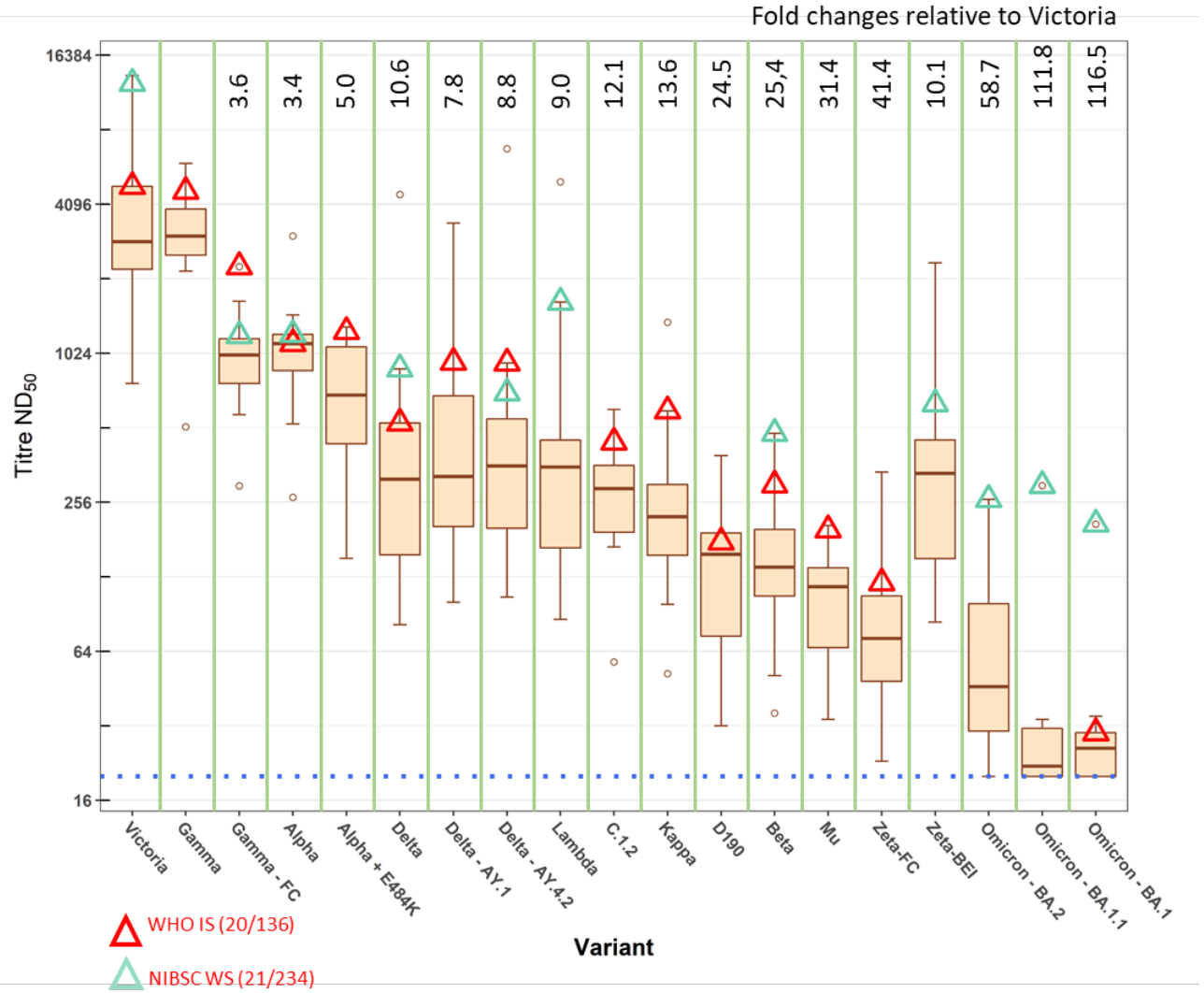
Live-virus *in vitro* antibody neutralization assay progress



- UKHSA and NIBSC neutralisation assays behave comparably across variants
  - Data are presented as the geometric mean of titres from both labs
- Variants shows 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*
- 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*

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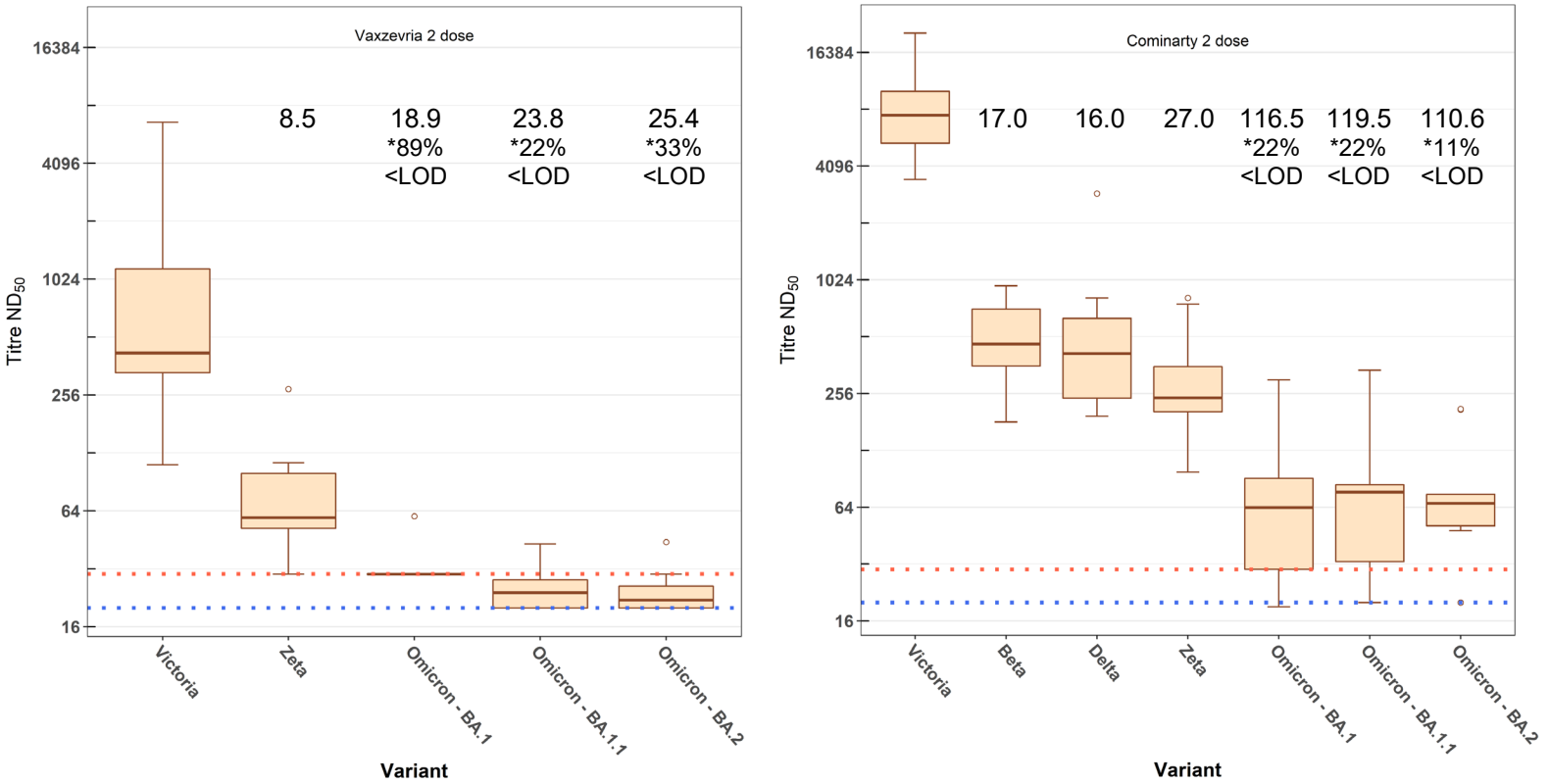
Cross-neutralisation results from the UKHSA ESCAPE staff vaccinee study

Fold changes relative to Victoria



Red line indicates assay limit of detection (LOD) for Omicron (BA.1) and Zeta
Blue line indicates LOD for all other viruses

* Many titres below LOD for BA.x variants which is likely to make fold-change an underestimate. Percentage below LOD indicated

- Sera samples from vaccinated UKHSA staff volunteers following 2 doses of the specified vaccine. Small panel, n=9 per vaccine
  - Vaxzevria time post 2nd vaccination (median = 84 days; IQR = 50)
  - Cominarty time post 2nd vaccination (median = 18 days; IQR = 7.5)
- Lower titres against Vic for Vaxevria mean fold-change for BA.x variants appear lower
- Convalescent panel data is in agreement with neutralisation data for vaccinee serum following 2 doses of approved vaccine

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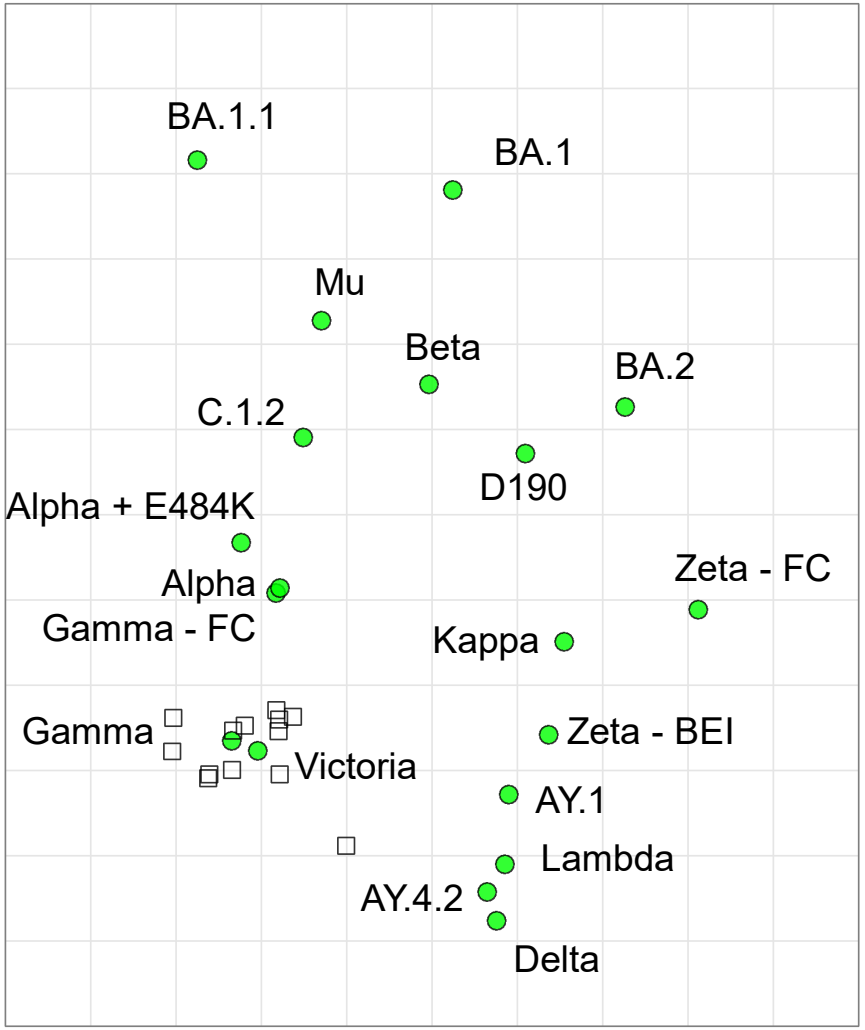
https://manchestercrf.nihr.ac.uk/about/our-impact-case-studies/escape/

Updated as of Mar 25, 2022

Antigenic cartography – Agility panel



- Generated from UKHSA & NIBSC ND_{50} data in R
  - Using the “Racmacs” package -
  - Wilks S (2022). *Racmacs: R Antigenic Cartography Macros*. https://github.com/acorg/Racmacs.
  - Developed by Dr. Sam Wilks, part of Prof. Derek Smith’s Team at Cambridge, UK
- Boxes represent individual plasma panel members
- Circles represent “viral antigens”

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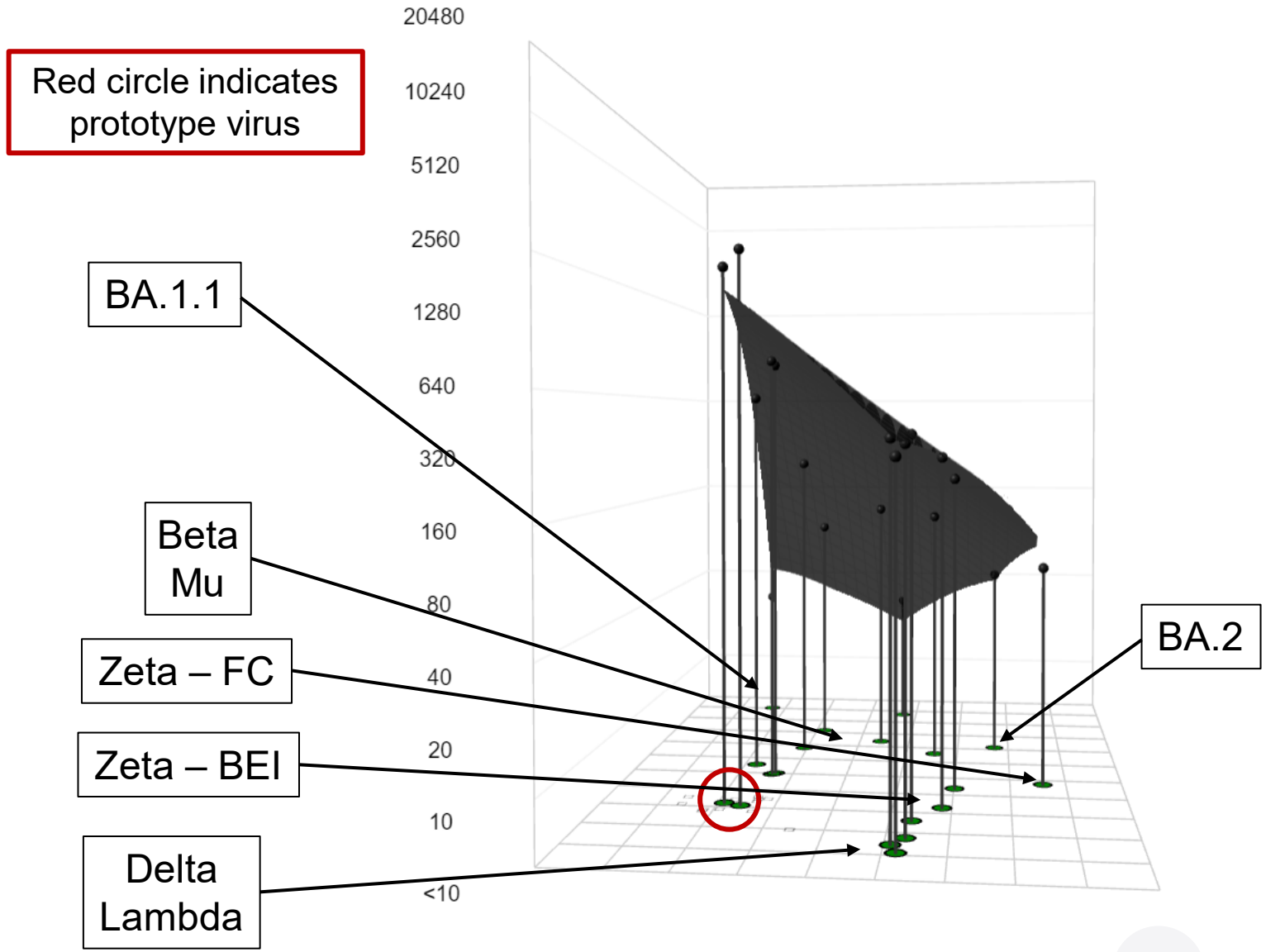
Antibody landscape – Agility panel

- Antibody landscape depicting average titres for Agility panel against variants

- All plasma in the Agility panel are pre-alpha therefore as expected the landscape is highest in the region of the prototype virus



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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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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 to 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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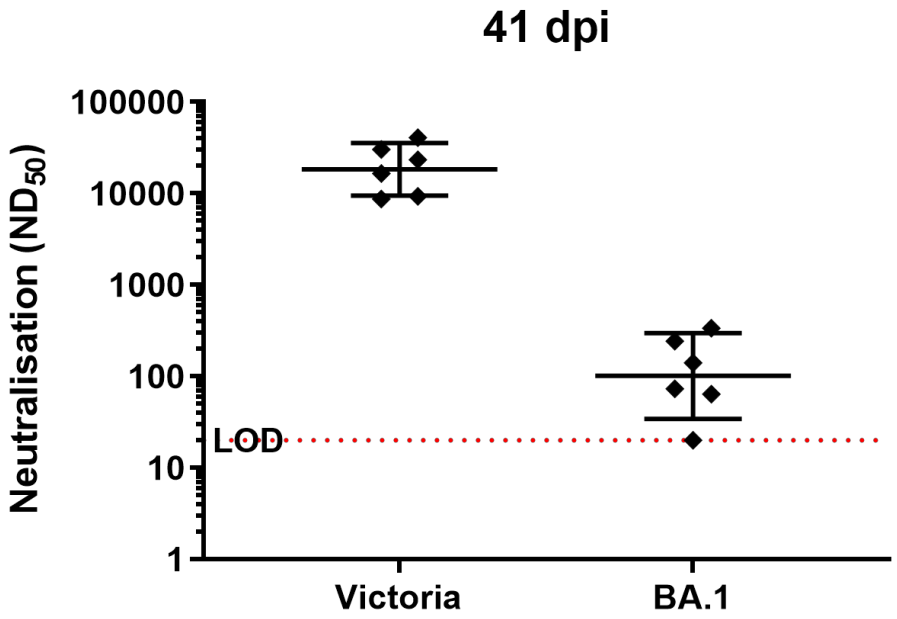
Variant assessment – *In vivo*

Preliminary data



Hamsters infected with Vic and sera taken 41 days pi

Similar fold-change to human convalescent sera with a 150-fold drop

Omicron neutralisation titres above the LOD

Ryan et al 2021, Convalescence from prototype SARS-CoV-2 protects Syrian hamsters from disease caused by the Omicron variant
https://www.biorxiv.org/content/10.1101/2021.12.24.474081v1

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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

- 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 https://www.gov.uk/guidance/new-variant-assessment-platform