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 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	Sourced		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 (Including AY.1)	Assessed[2]
Iota – B.1.526+E484K or S477N	Seeking			
Kappa – B.1.617.1	Assessed[2]			
Lambda – C.37	Sourced			
Mu - B.1.621	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 VUI, may prompt deselection
Superscripts denote assessed at 1 or 2 sites in vitro

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Table updated as of Oct 8, 2021

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
WHO VOIs	†Eta (B.1.525)	Deselected			
	†Epsilon (B.1.427/B.1.429)	In progress	No longer required		
	†Zeta (P.2)	Complete	Complete	Complete (1 of 2 labs)	
	†Theta (P.3)	Deselected	No longer required		
	†Iota (B.1.526+E484K)	Deselected			
	†Kappa (B.1.617.1)	Complete	In progress	Complete	
	Lambda (C.37)	Complete	In progress	In progress	
	Mu (B.1.621)	Complete	In progress	Complete	
UK	**Alpha + E484K**	Complete	In progress	Complete	
n/a	**Cluster V (Denmark) and N439K**	Complete	No longer required	No longer required	*n/a*

†No longer a WHO VUI

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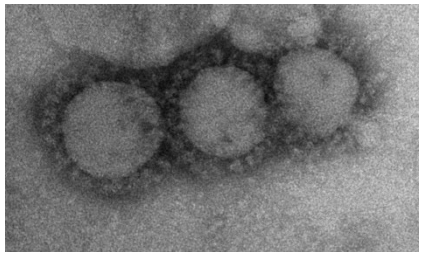
Wildtype virus Quality Control



- 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
- 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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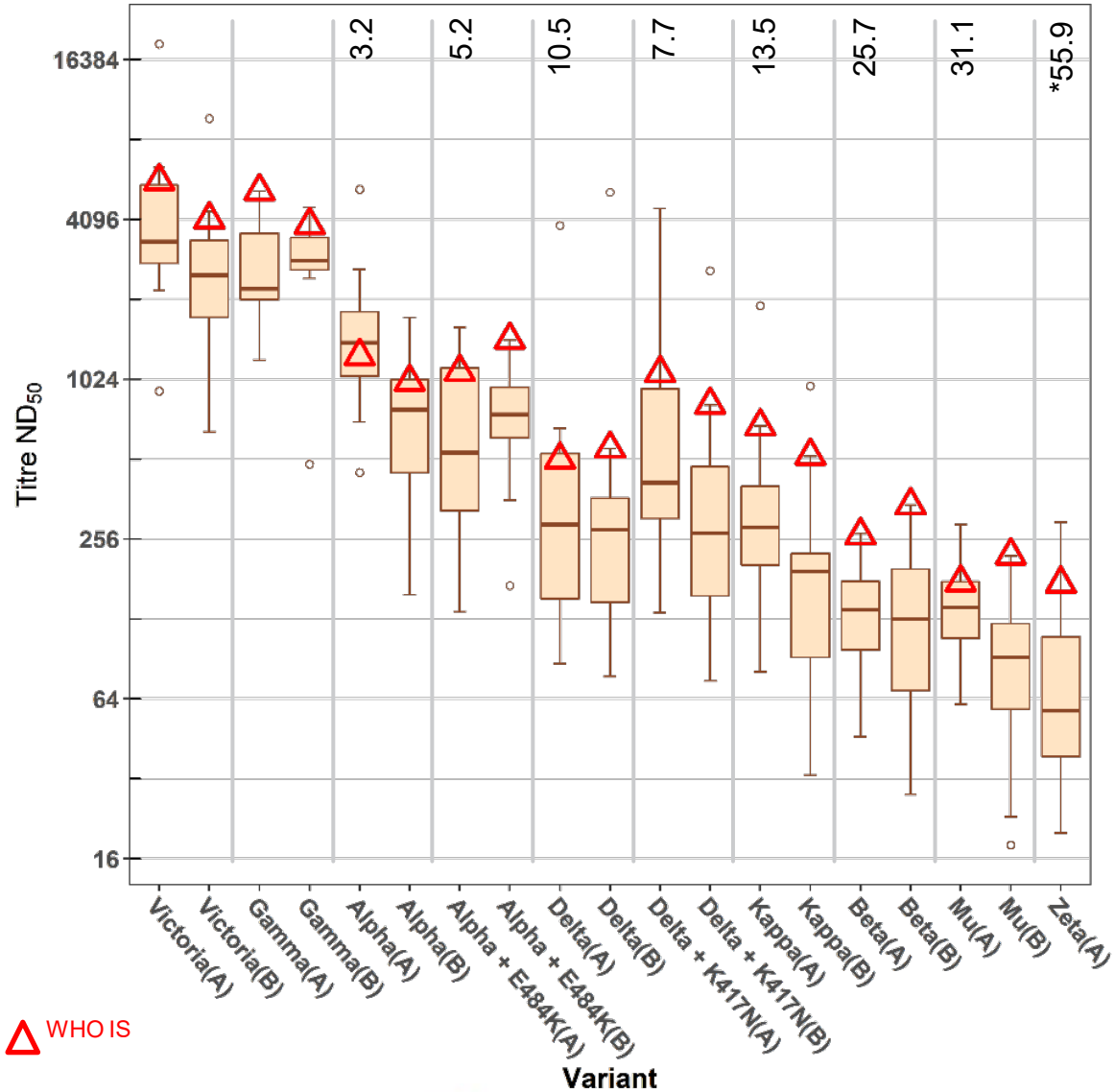
Updated as of Oct 8, 2021

Live-virus *in vitro* antibody neutralization assay progress



- Variants assessed in neutralisation assay to date against a "pre-Alpha" serum panel
- PHE and NIBSC neutralisation assays behave comparably across variants
- Most serum in panel neutralise all tested variants
- Figures shown are fold-reduction in neutralisation titre (ND_{50}) relative to Victoria – only significant ($P<0.05$) data shown
- Greatest resistance to neutralisation seen for Beta, Mu and Zeta

* Zeta results provisional; subject to repeat at second site

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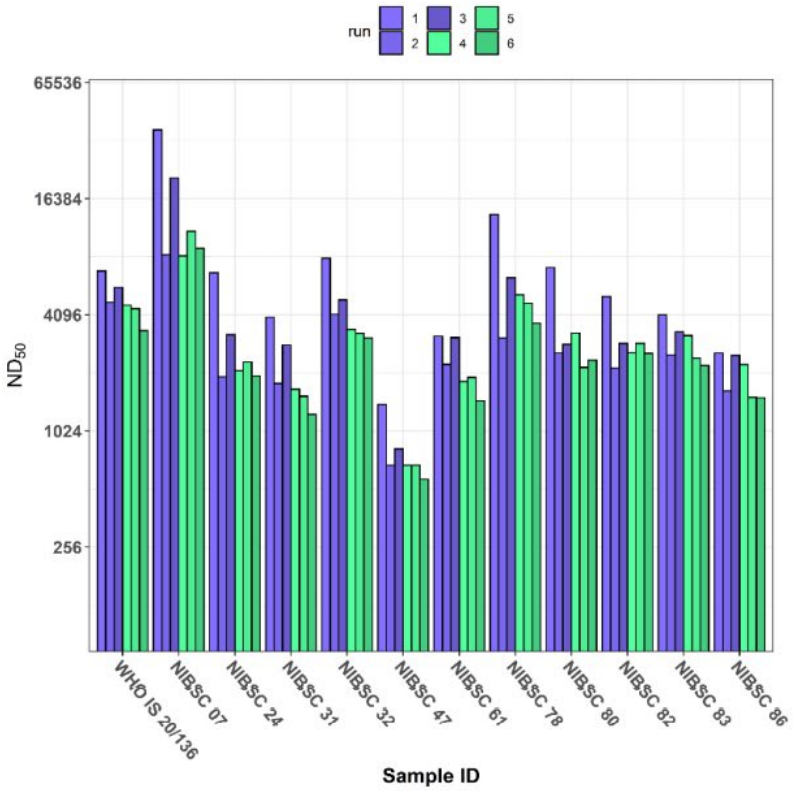
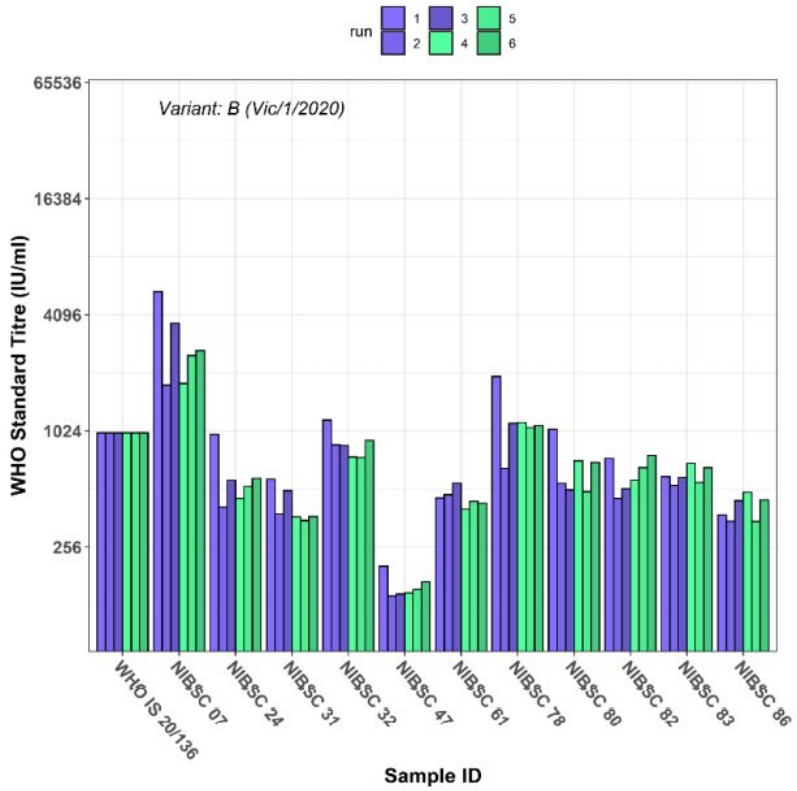
PHE and NIBSC Inter-lab assay agreement testing Wuhan-type SARS-CoV-2 (Victoria-OI)





- Run 1-3 lab A
  Run 4-6 lab B
- ND_{50} = 40.4%GCV
  IU/mL = 22.5 %GCV
- Improvement 17.9%, p<0.001
- Conversion to IU/mL further reduce variability of already comparable data

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

- ✓ **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.**

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.

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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
- WHO International Antibody 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

- 08 September 2021: WHO SARS-CoV-2 Assay Working Group
- 19 August 2021: WHO SARS-CoV-2 Animal Modeling Working Group
- 19 May 2021: WHO SARS-CoV-2 Assays Working Group