CEPI

Agility Program Biweekly Progress

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

Partners: UK Health Security Agency (UKHSA) Medicines and Healthcare products Regulatory Agency (MHRA)







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/#ref

Variants of Concern and Interest Monitored by the Agility Project

Delta - B.1.617.2Assessed2Omicron BA.1Assessed2Data 1 de construirAssessed2	Tested within Agility Alpha – B.1.1.7	Not tested within Agility
Omicron BA.1 Assessed ²	Alpha – B.1.1.7	
BA.1.1Assessed2BA.2Assessed2BA.2.12.1Assessed2BA.4Assessed2BA.5.2.1Assessed2BA.2.75.3Assessed2BA.4.6Assessed2BQ.1.22Assessed2BA.2.75.2Assessed2CH.1.1Assessed1	Beta – B.1.351 Gamma – P.1 Zeta (P.2) – sourced from Fiocruz Zeta (P.2) – sourced from BEI Kappa (B.1.617.1) Mu (B.1.621) Alpha + E484K Lambda (C.37)	Eta (B.1.525) Epsilon (B.1.427/B.1.429) Theta (P.3) lota (B.1.526+E484K) Omicron – BA.3

Superscripts denote assessed at 1 or 2 sites in vitro

Recombinants	Status*
Omicron x Delta recombinant (XD)	Sourced
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.1.1)	Assessed ¹
BJ.1 x BM.1.1 recombinant (XBB.1.5)	Assessed ¹

Gamma – P.1	Theta (P.3)
Zeta (P.2) – sourced from Fiocruz	lota (B.1.526+E484K)
Zeta (P.2) – sourced from BEI	Omicron – BA.3
Карра (В.1.617.1)	
Mu (B.1.621)	
Alpha + E484K	
Lambda (C.37)	
AY.1	
AY.4.2	

Questions? Reach us at agility@cepi.net

^ahttps://www.medrxiv.org/content/10.1101/2021.08.20.21262342v1 ^bhttps://www.medrxiv.org/content/10.1101/2021.09.14.21263564v1.full

Table updated as of March 23, 2023

Agility Project: Variant Growth/Testing for Neutralization Phenotype

	Va	ariant	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
		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
WHO VOCs	Omicron (B.1.1.529)	BA.5.2.1	Complete	Complete	Complete	In progress
	(,	BA.2.75.3	Complete	Complete	Complete	
		BA.4.6	Complete	Complete	Complete	
		BQ.1.22	Complete	Complete	In progress	
		BA.2.75.2	Complete	Complete	Complete	
		CH.1.1	Complete	In progress	In progress	
		Omicron x Delta recombinant (XF)	Complete	Complete	Complete	
		Alpha x Delta recombinant (XC)	Complete	In progress	In progress	
Other	Recombinants	BA.1 x BA.2 recombinant (XE)	Complete	Complete	Complete	
		BJ.1 x BM.1.1 recombinant (XBB.1.1)	Complete	Complete	In progress	
		BJ.1 x BM.1.1 recombinant (XBB.1.5)	Complete	In progress	In progress	

Table updated as of March 23, 2023

^ahttps://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 (<i>fast</i>)
Sequence analysis – Illumina NGS/SISPA	Examination of minor variants, absence of contaminants, fill in any 'missed' regions due to Arctic protocol primer mismatches (<i>detailed</i>)

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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 March 23, 2023

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 ND₅₀s across variants
 - Preliminary data for candidate replacement standard 21/338 shows high titres against BA.x variants
 - Omicron exhibit the largest drops in ND₅₀ 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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Live-virus *in vitro* antibody neutralization assay – Vaccinee panel



- Serum samples from 14 vaccinated individuals following 1 vaccination
- BA.2.75.2, BA.4.6, BQ.1.22, XBB.1.1 and XBB.1.5, which all contain the R346T mutation, display the most resistance to neutralisation from this panel
- Despite reduced potency, NIBSC 21/338 retains ability to neutralise BQ.1.22, XBB.1.1 and XBB.1.5
 - Pool of 265 individuals (vaccinated and convalescent pre-Omicron)

Updated as of March 23, 2023

Live-virus in vitro antibody neutralization assay - Vaccinee



- Serum samples from 10 vaccinated UKHSA staff volunteers following 3 vaccinations
 - Samples taken at a median of 72 days post booster.
- Variants BQ.1.22 and XBB.1.1 display the most resistance to neutralisation seen to date
 - N.b BQ.1.22 = BQ.1.1 + C26681T (synonymous)
 - Majority of samples <LLOD (cannot perform statistics)
- NIBSC WR 21/338 retains ability to neutralise BQ.1.22 and XBB.1.1

Variant assessment – Vaccinee panel (4 dose)



- Serum samples from 22 vaccinated UKHSA/MHRA staff volunteers following 4 vaccinations
- CH.1.1 displays the most resistance to neutralisation seen to date
 - CH.1.1 = BM.4.1.1 (BA.2.75 sub lineage) + S:K444T, S:L452R
 - 7 out of 22 samples <LLOD</p>
- NIBSC WR 21/338 retains ability to neutralise all of the most recent variants
 - Pool of 265 individuals (vaccinated and convalescent pre-Omicron)
- Preliminary data: BA.4, CH.1.1, BQ.1.22 are data from single labs



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 (<u>https://www.nc3rs.org.uk/the-3rs</u>)
- All research is done in compliance with CEPI's <u>Animals in Research Policy</u>

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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

CINITIANS Infection	VCRe-CIN 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 <u>https://www.biorxiv.org/content/10.1101/2021.12.24.474081v1</u>

- 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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Table updated as of March 23, 2023

Variant assessment – In vivo

Primary infection studies with omicron sub-variants demonstrated varying severity of disease in the hamster model. Hamsters were followed until 7and 28- days post infection \checkmark Viral shedding from the upper re

Initial Infection	Clinical signs infection?	Weight loss after infection?
BA.1	Yes	No
BA.2	Yes	Little
BA.2.12.1	Yes	Little
BA.4	Infrequent	No
BA.5.2.1	Yes	Yes

All studies were conducted in compliance with all UK government regulatory requirements.

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- Viral shedding from the upper respiratory tract was higher in BA.4 and BA.5.2.1 hamsters compared to earlier subvariants
- Convalescent sera conferred partial protection against emerging sub-variants
- Pathology data has identified more severe legions in BA.5.2.1 infected hamsters, similar to Victoria
- With the exception of BA.5.2.1, Omicron sub-variants have presented less severely in the hamster model

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

- Assessment of the Biological Impact of SARS-CoV-2 Genetic Variation Using an Authentic Virus Neutralisation Assay with Convalescent Plasma, Vaccinee Sera, and Standard Reagents Viruses 2023, 15(3), 633; https://doi.org/10.3390/v15030633
- 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 21Mar23: ECDC/WHO Euro SARS-CoV-2 virus characterisation working group meeting – N Coombes/ Y. Le Duff

- 24Jan23: ECDC/WHO Euro SARS-CoV-2 virus characterisation working group meeting S. Funnell
- 29Nov22: ECDC/WHO Euro SARS-CoV-2 virus characterisation working group meeting K. Bewley, L. Davies
- 18Oct22: UKHSA conference N. Coombes ٠
- 23Sep22: WHO Animal Models Group K. Bewley, L. Davies

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