UK Health Security Agency

“Biological assessment of SARS-CoV-2 variants in the CEPI funded Agility project.”

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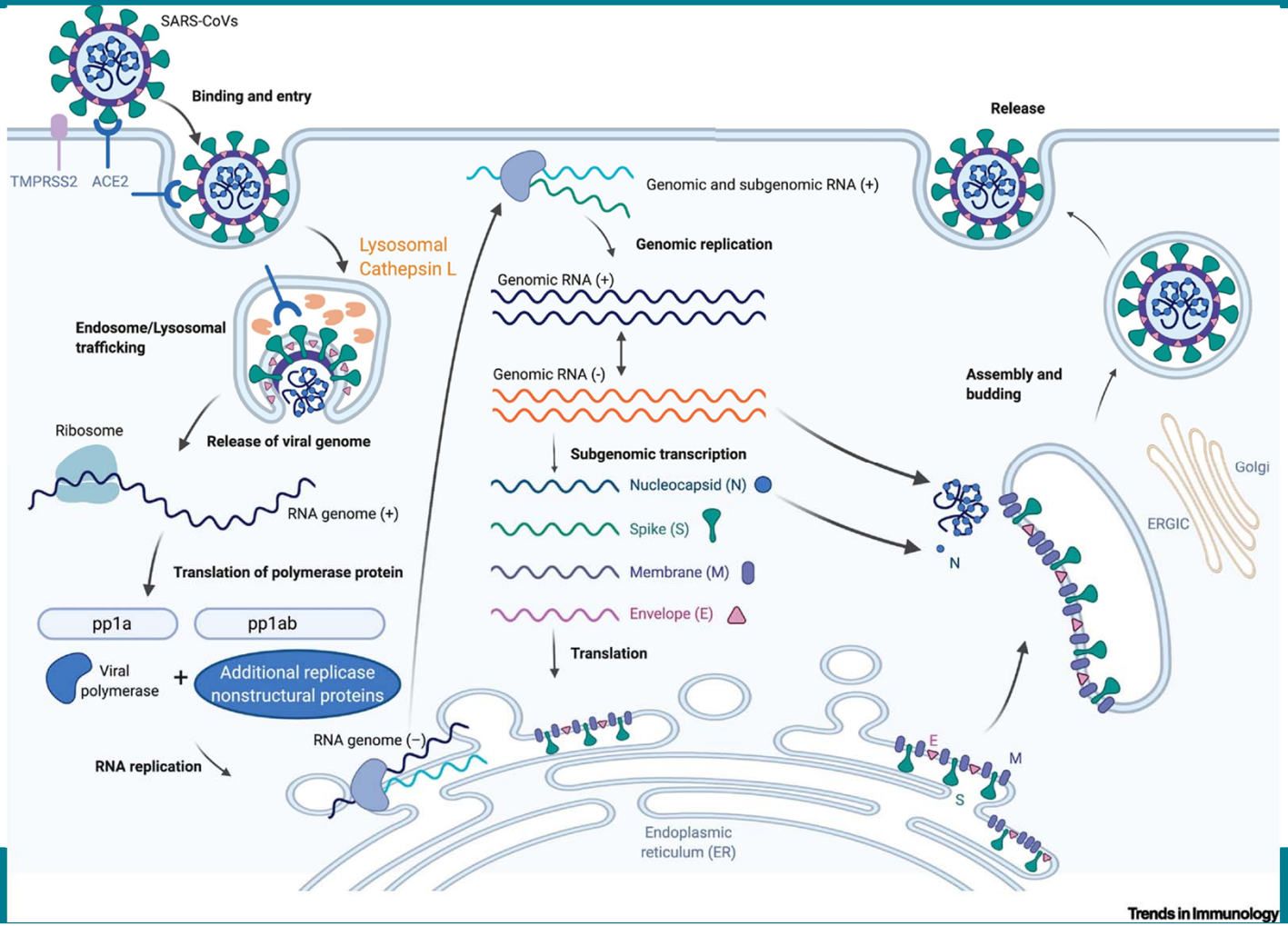
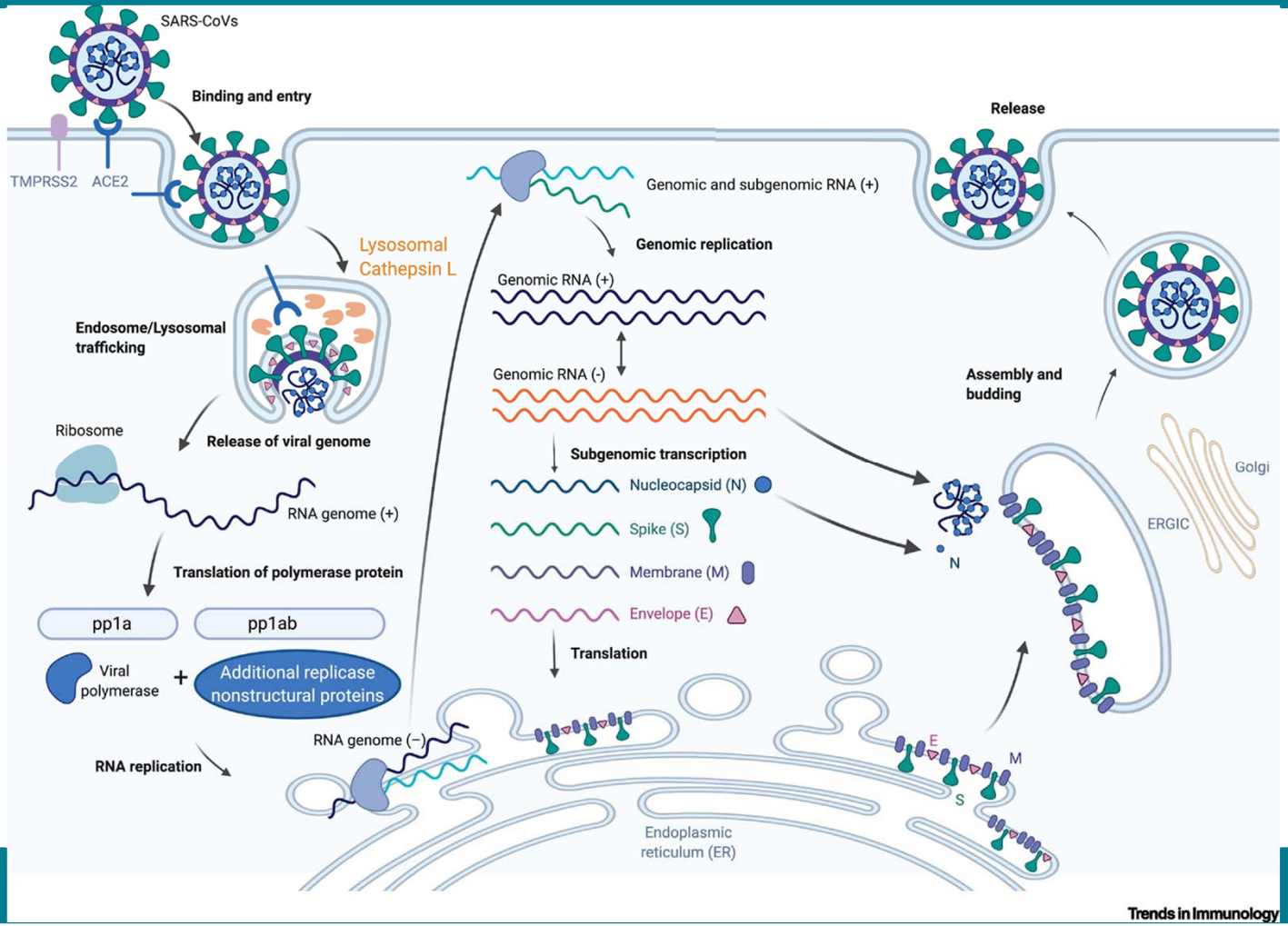
UK Health Security Agency

SARS-CoV-2 replication schematic

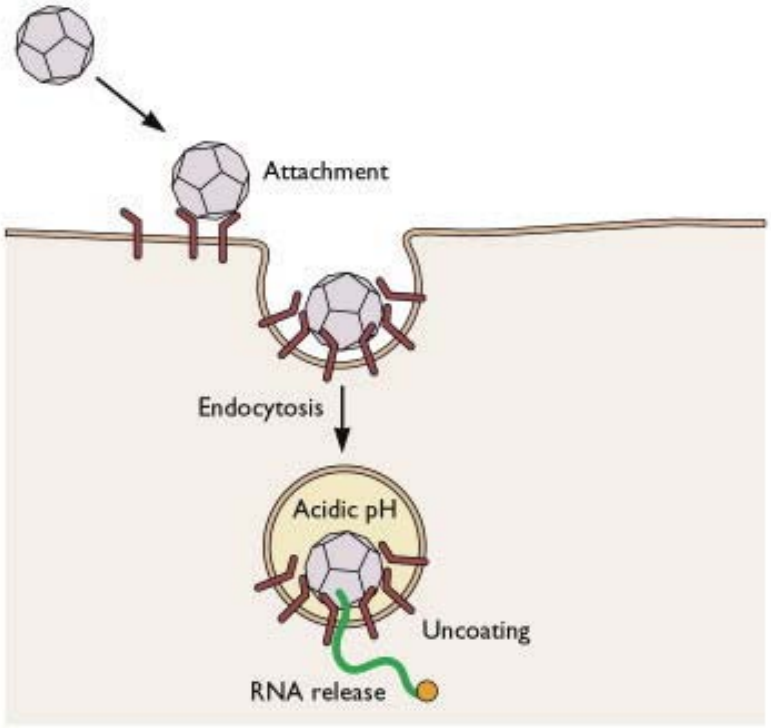


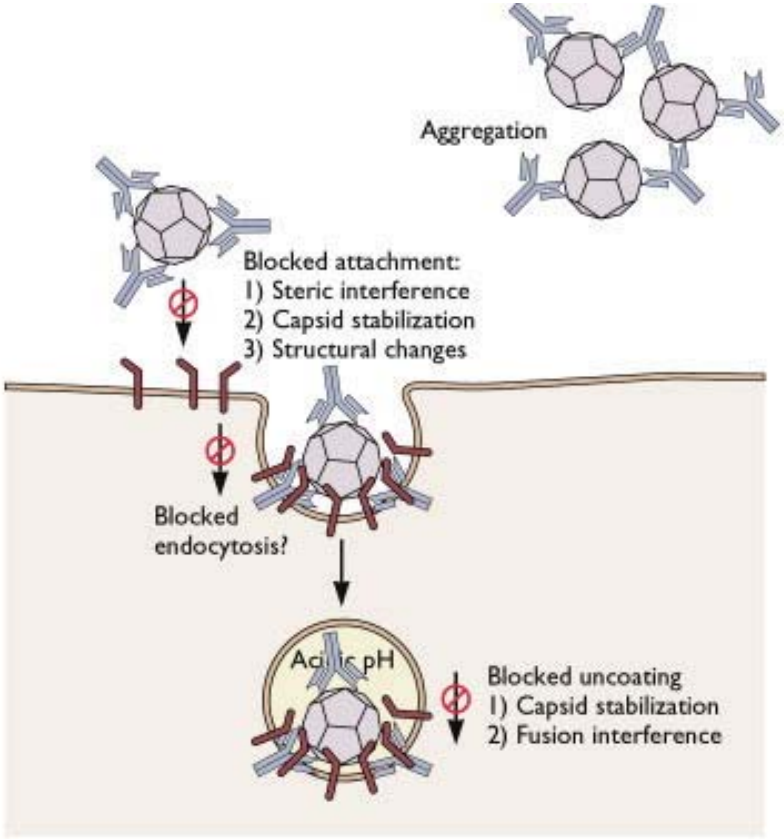
SARS-CoV-2 replication schematic



1. Viral release before cell lysis
2. Viral release during cell lysis
3. Viral infection of adjoining cell
4. Infected cell merger (syncytia)

Antibody mediated viral neutralisation



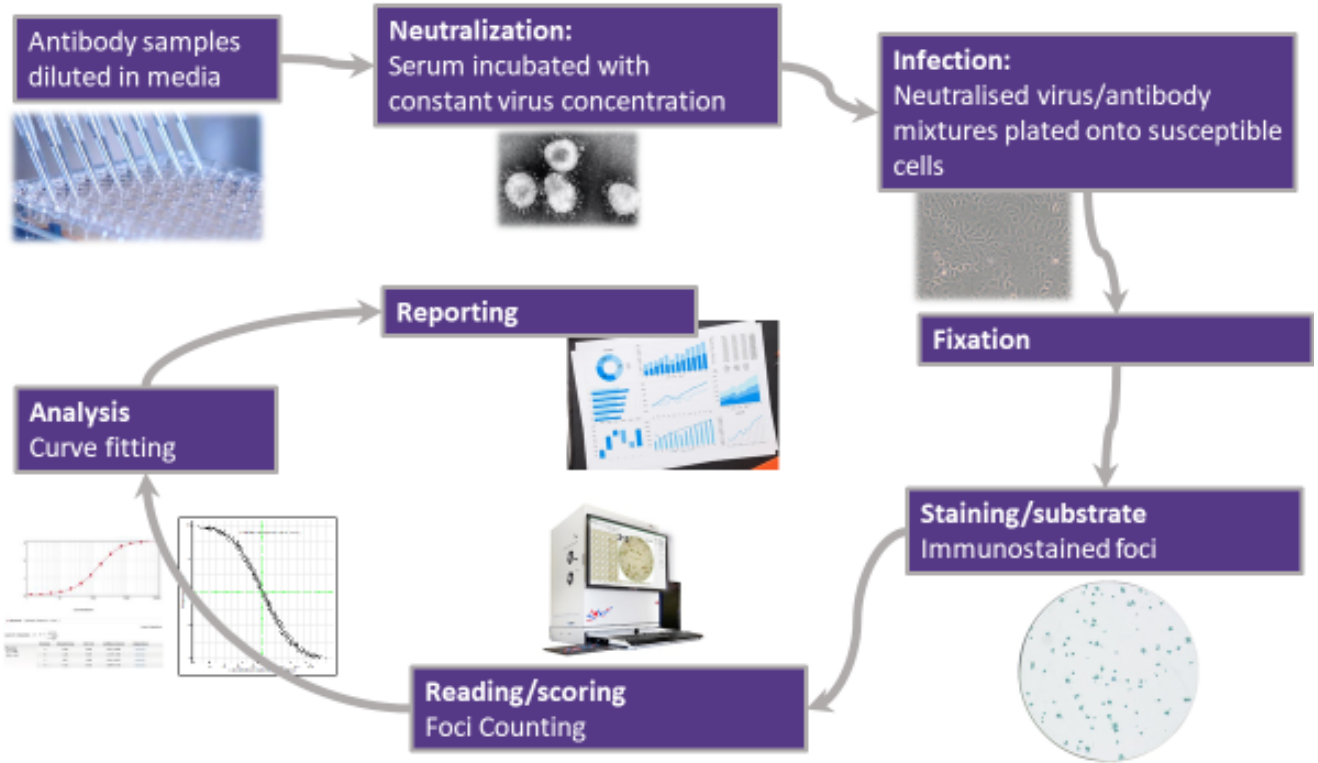


Humoral Ab only

Doesn't measure T cell immunity

SARS-CoV-2 antibody 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

Wild-type virus neutralisation

- Measures **functional neutralising antibodies**

  Which are a subset of the total population of antibodies against the virus

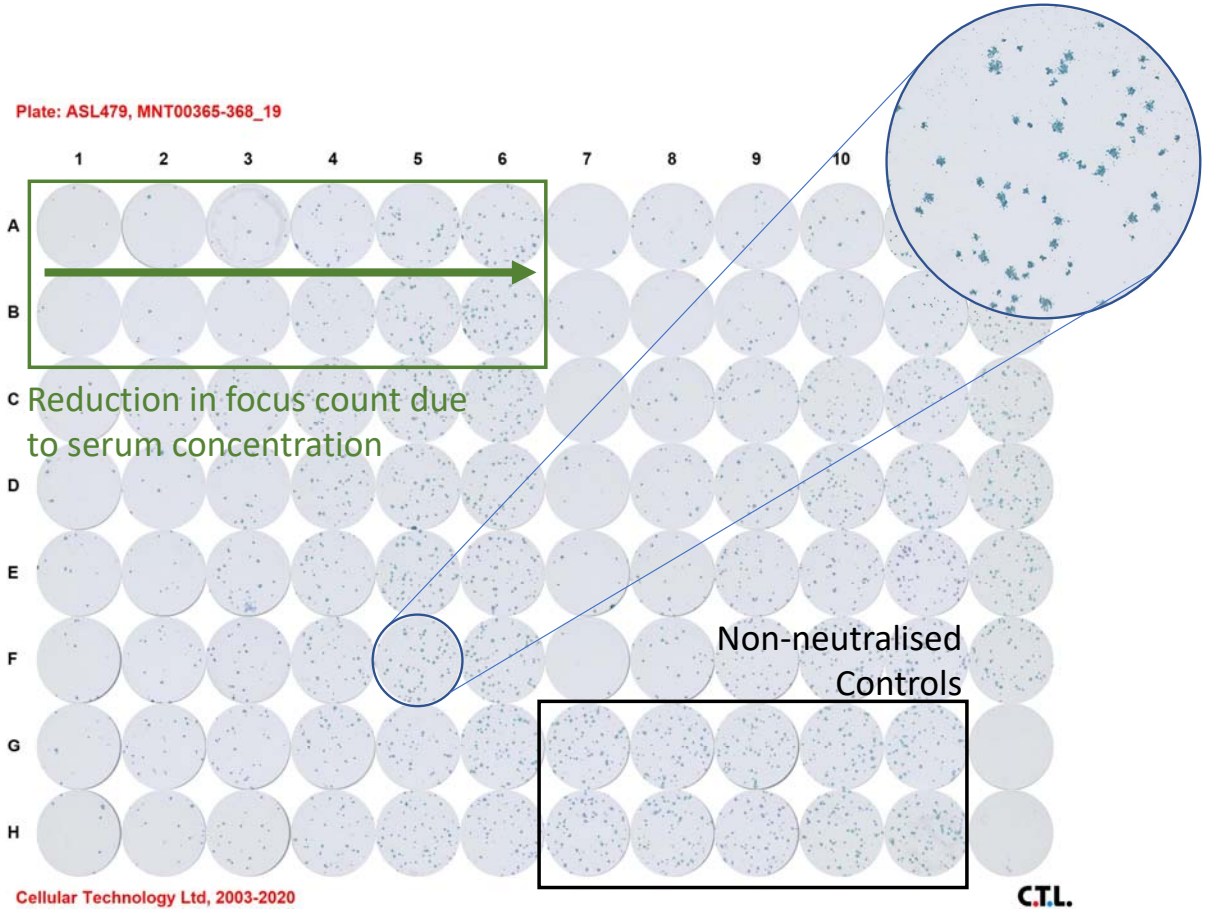
- Wild-type virus target includes **all** viral antigens
  - Not just the spike protein
  - Also Matrix, Nucleocapsid and Envelope

- Includes all components of the viral replication machinery (non structural proteins)

- Readily adaptable to analyse non-serological samples e.g. **anti-viral** compounds

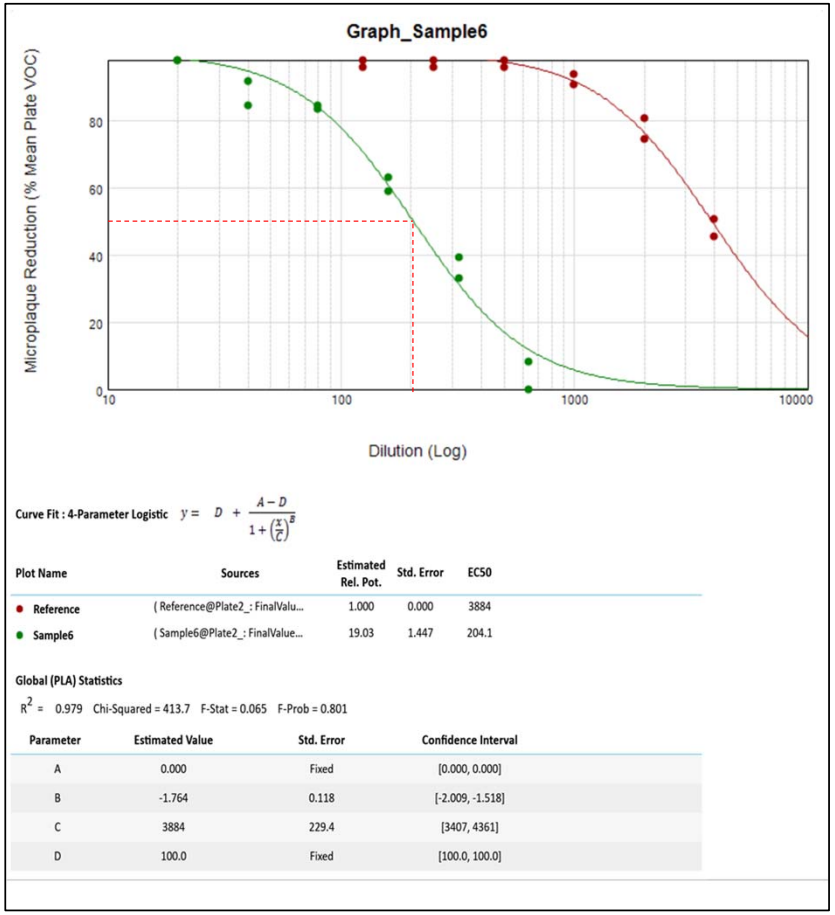
The UKHSA Microneutralisation Assay (MNA)

- **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

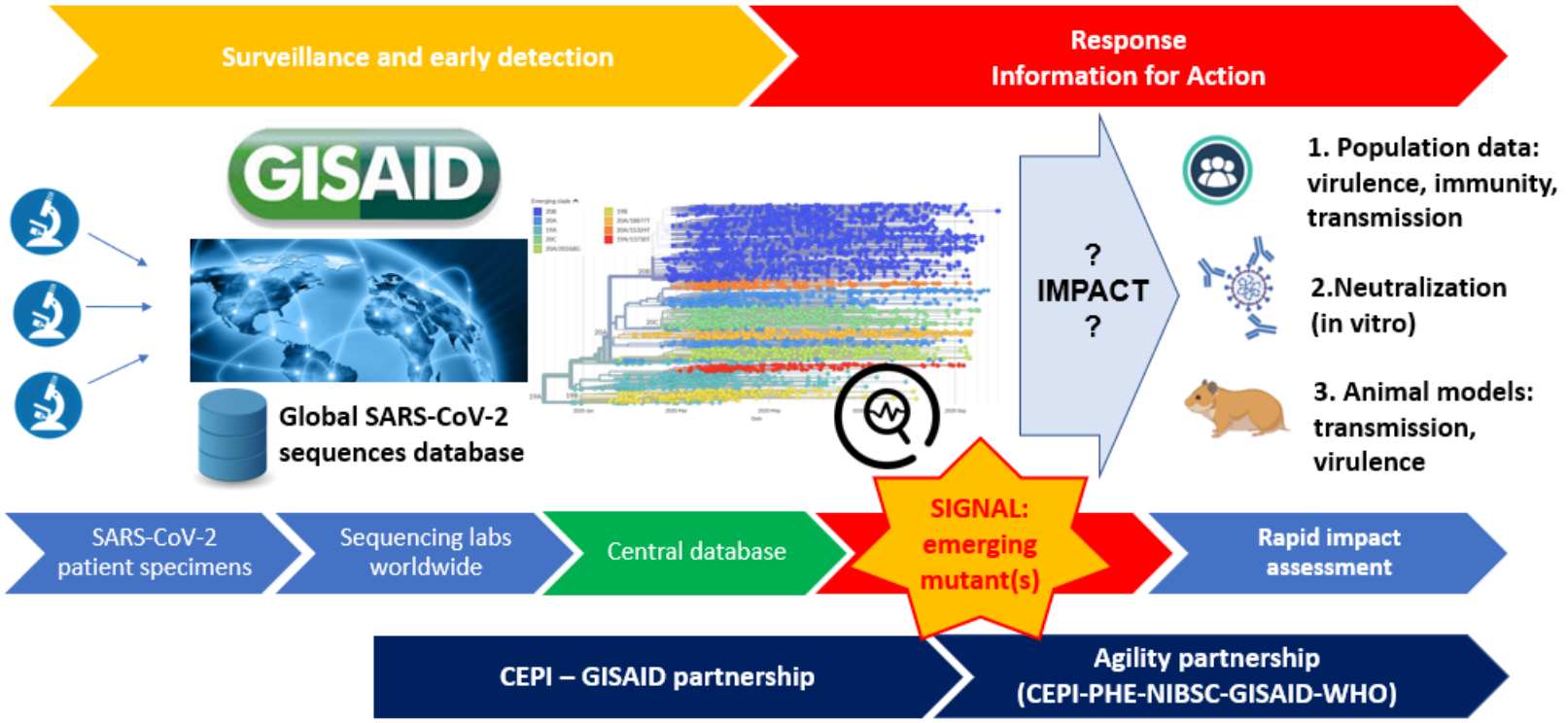


Calculation of median neutralising dose ND_{50}

- Automated spots counting on **CTL scanner** with fixed parameters
- Excel data input directly into **SoftMax Pro (SMP)**
- Curve fitted to a four parameter logistic (**4PL**) nonlinear regression model
- SoftMax Pro – GxP approved software
  - Acceptability by regulators
- Data used in several clinical trials



Agility project: Genomic surveillance and response for COVID-19 R&D

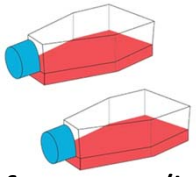


COVAX Enabling Science SWAT team

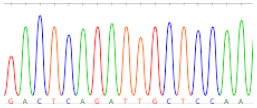
Isolation of SARS-CoV-2 variants

Nasal swab from infected individual

Spin down particulates

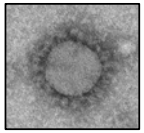


Infect Vero/hSLAM & VAT* (+ 2x ABM)



NGS Sequence

EM analysis

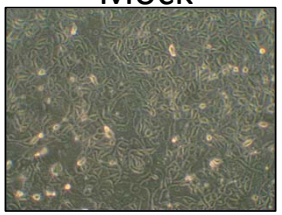


Morphology

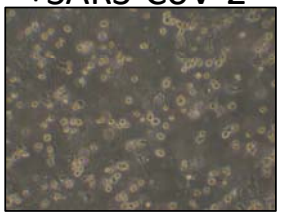
Sterility check

Sterility & mycoplasma

Mock



+SARS-CoV-2



Harvest and aliquot virus

* VAT cells – Vero E6 overexpressing hACE2 and hTMPRSS2

SARS-CoV-2 **Variants**

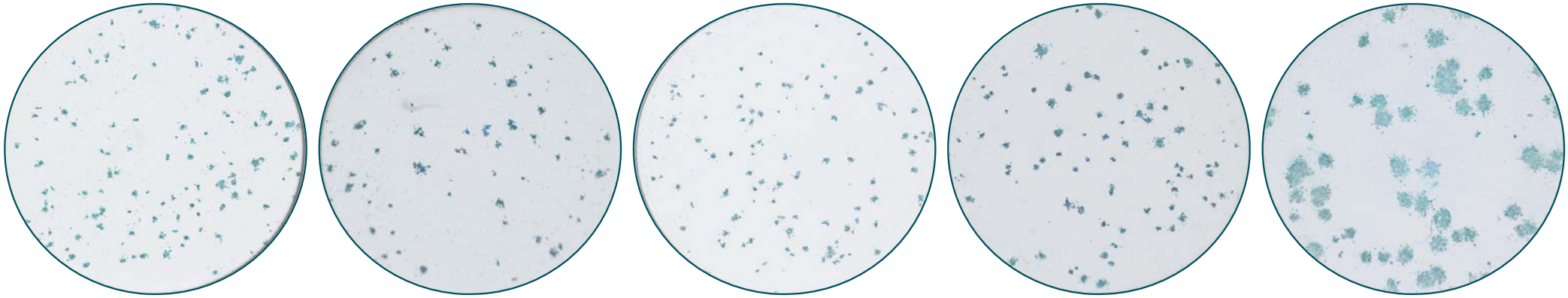
Victoria B.1 Foci
24 h post-infection

Danish “cluster 5” Foci
24 h post-infection

Alpha B.1.1.7 Foci
24 h post-infection

Gamma P.1 Foci
24 h post-infection

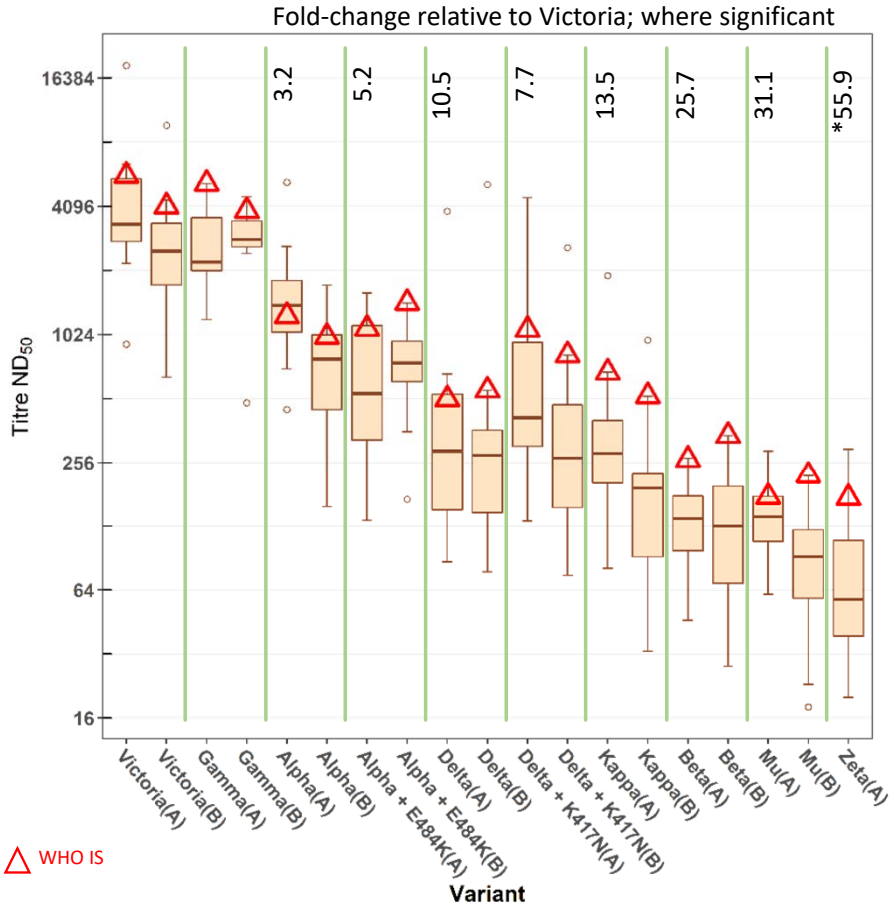
Beta B.1.351 Foci
24 h post-infection



Post-infection fixation time (Gamma, Beta) optimised further to standardise foci appearance for automated counting

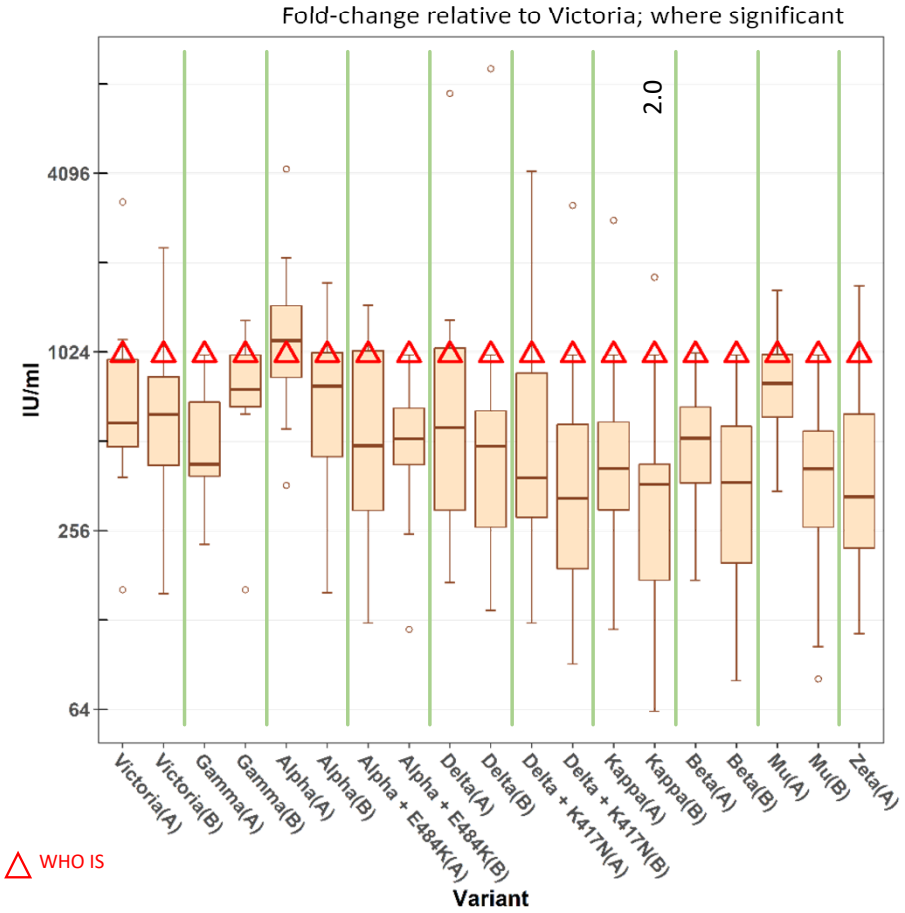
- Rapid optimisation for novel variants
- Isolation from clinical sample to first neutralisation results in ~3 weeks

Variant assessment



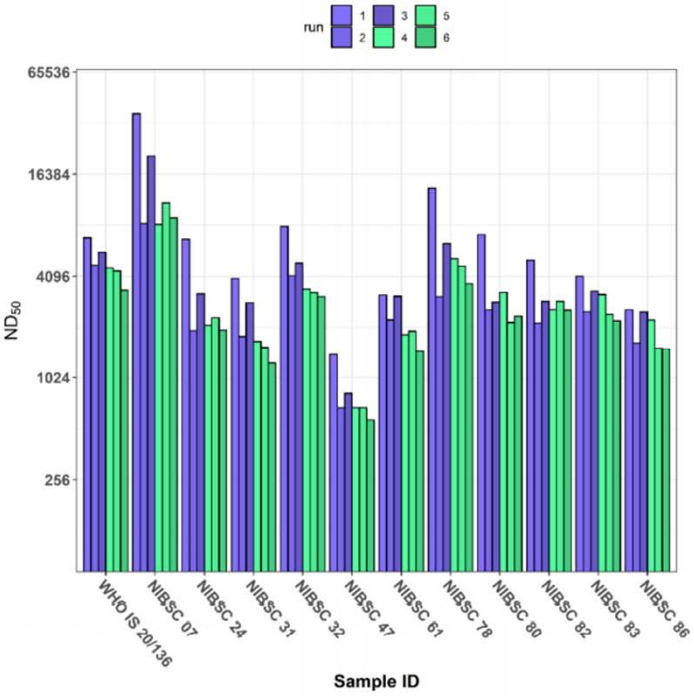
- Monitor variants using a **pre-Alpha convalescent serum panel** – combining results from two laboratories (A) and (B)
- Statistically significant fold-changes in ND_{50} relative to Victoria responses shown

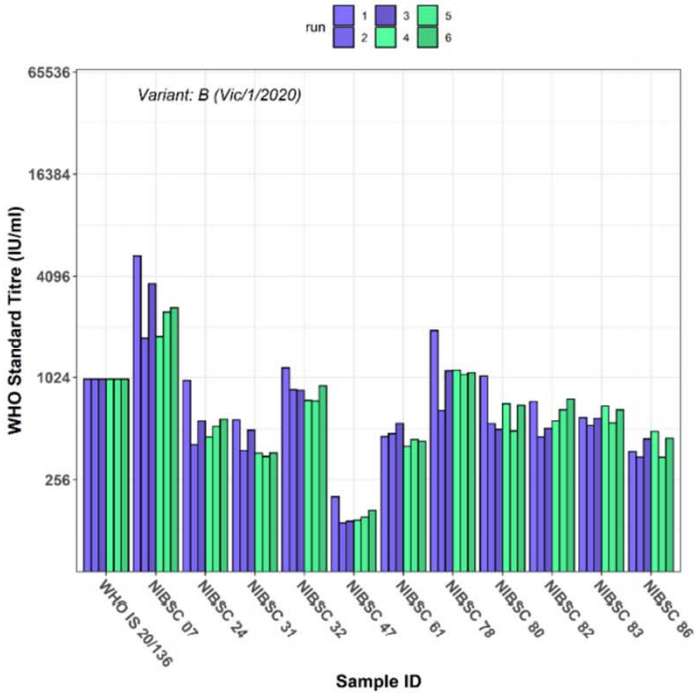
Effect of IS normalisation *across variants*



- Majority of fold-changes between variants are ‘lost’ when normalising in this manner
- IU/ml expression needs to include details of which variant was used
- Only useful for “within variant” comparison

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:
  ND_{50} = **40.4 %GCV**
- **Normalised** (to IS):
  IU/mL = **22.5 %GCV**
- An **improvement** in inter-lab variability of **17.9%**; p<0.001
- **Conversion** to IU/mL further **reduces variability** of already comparable data

Validation of the UKHSA MNA Used at GCLP for clinical trial materials

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

- 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**

Assay Utilisation

- MNA for prototype virus; RCT samples from vaccine developers
  - > 10 developers
  - Includes trials investigating/supporting human challenge studies, winter booster, ‘flu/covid co-vaccination
  - Many thousands of samples processed

- 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 breadth of protection against virus variants for vaccine developers

- Adapted MNA used to assess *in vitro* efficacy of:
  - Monoclonal antibody-based therapeutics
  - Antiviral compounds

Summary

- UKHSA has developed a microneutralisation test
  - Qualified and validated for use in regulated clinical trials
- The assay has been adapted for use with VOCs and VUIs
  - Assessments performed using a pre-alpha serum panel to assess ‘concern’
  - WHO IS 20/136 used in addition to this panel (also a pre-Alpha pool)
- WHO IS 20/126 use
  - User needs to specifiy which variant – usage reduces inter-lab variation significantly
  - Permits inter-laboratory data comparison per variant
  - Distorts the *between variant* fold-changes (incorrect usage)

- **Sender:William Dowling (william.dowling@cepi.net)**

  Subject: Training Webinar for the calibration of quantitative serology assays using the WHO International Standard for anti-SARS-CoV-2 immunoglobulin
  When: Wednesday, November 10, 2021 8:30 AM-9:30 AM (UTC-05:00) Eastern Time (US & Canada).
  Where: Zoom TBD; https://cepi-net.zoom.us/j/91271954785