Development of a Flow Cytometry-based Micro-Neutralisation Assay to Evaluate Humoral Immunity against SARS-CoV-2 Variants of Concern in Vaccine Trials

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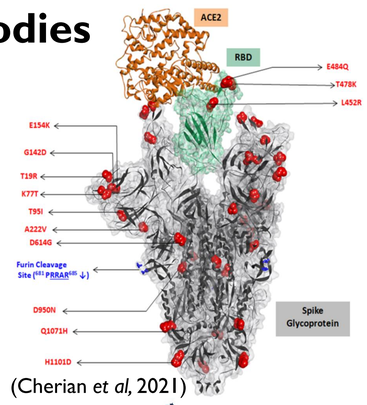
1. Objectives

The relationship between SARS-CoV-2 antibody titres and a protective immune response is poorly understood.
Here we aim to:

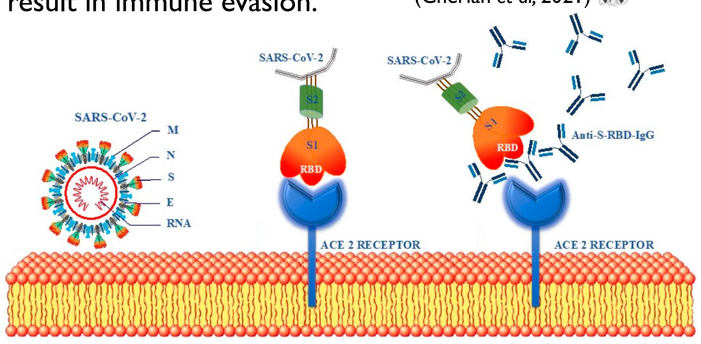
1) Develop an assay to measure neutralising capacity of high numbers of SARS-CoV-2 clinical samples
2) Use our assay to measure potential immune evasion by SARS-CoV-2 Variants of Concern (VOCs)

2. Neutralising Antibodies

Neutralising Antibodies bind a virus in such a way that prevents infection, e.g., blocking the Receptor Binding Domain (RBD) interacting with the cellular ACE2 receptor. Mutations in the Spike protein particularly in the RBD can result in immune evasion.



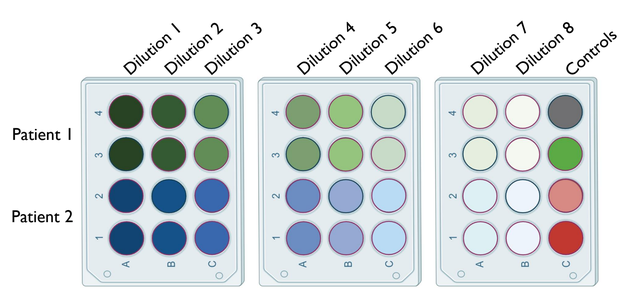
(Cherian *et al*, 2021)



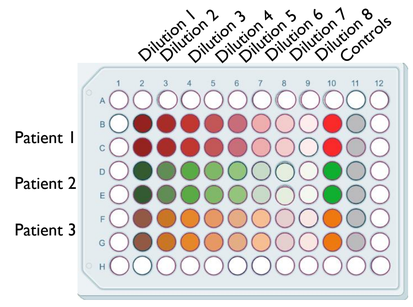
(Lo Sasso *et al*, 2021)

3. Plaque-Reduction Neutralisation Test (PRNT) Vs. Micro-Neutralisation Test (Micro-NT)

1. Serially dilute vaccinated/convalescent plasma samples
2. Co-incubate diluted plasma with SARS-CoV-2 for 1 hour at 37˚C



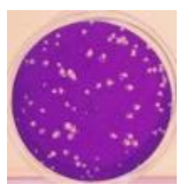
Throughput: 6 samples (9 plates)



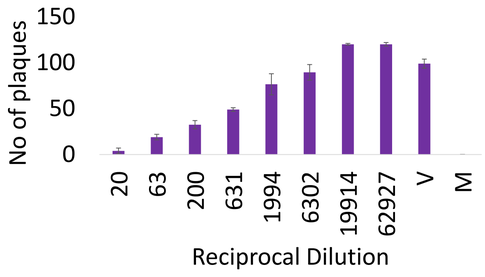
Throughput: 36 samples (12 plates)

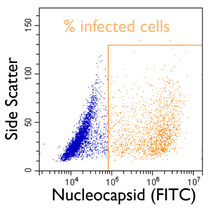
3. Incubate at 37˚C, shaking every 10 mins (90 mins)
4. Add CMC overlay (1%)
5. Incubate until Plaques become visible (48-96 hours)
6. Fix cells in 4% PFA (18 hours)
7. Stain with Crystal Violet
8. Image wells and count using ImageJ script

3. Incubate overnight (18-hours)
4. Trypsinise and fix cells in 4% PFA (8+ hours)
5. Stain with anti-SARS-CoV-2 Nucleocapsid antibody
6. Analyse cells using flow cytometry

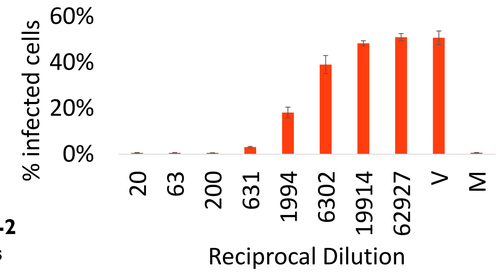


Output: Plaques





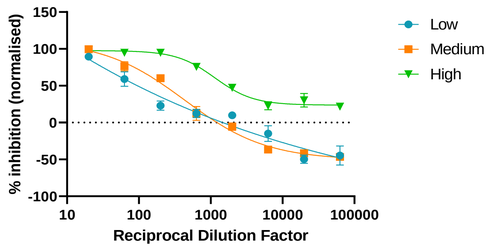
Output: SARS-CoV-2 Nucleocapsid + cells



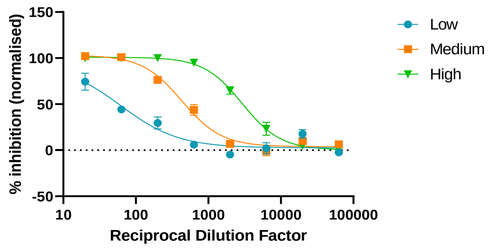
50% Neutralisation Titre (NT50): The plasma dilution factor that results in a 50% reduction in infected cells

4. Micro-NT Assay Matches PRNT Neutralisation Titres

A. PRNT



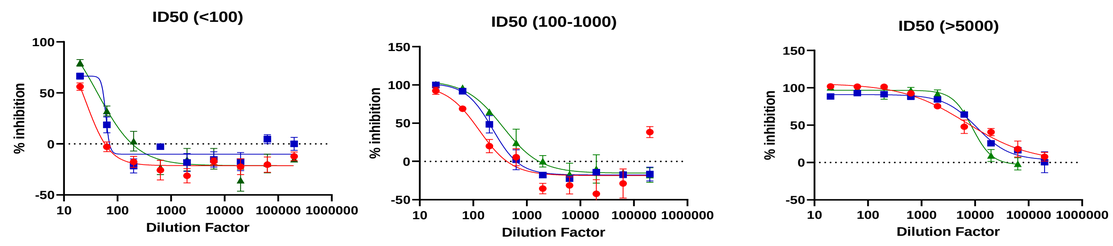
B. Micro-NT

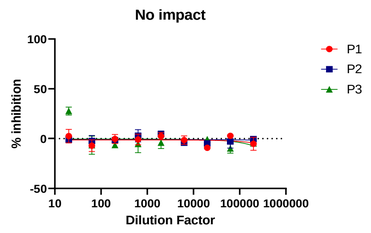


Graphs show Neutralisation of WT (B lineage) SARS-CoV-2, with WHO SARS-CoV-2 IgG standards, measured using PRNT (A) or Micro-NT (B) on Vero E6 cells.
PRNT: MOI 0.003, 96-hr infection
Micro-NT: MOI 0.05, 18-hr infection
The half-maximal Neutralisation Titres (NT50) were determined using four-parameter logistic regression.

Titre	WHO (IU/ml)	NT50 (PRNT)	NT50 (Flow)
Low	44	86	62
Medium	210	435	431
High	1473	1144	2962

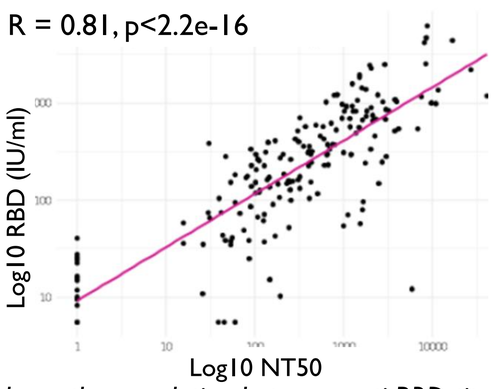
5. Micro-NT Assay Demonstrates Broad Dynamic Range Across a Range of NT50s





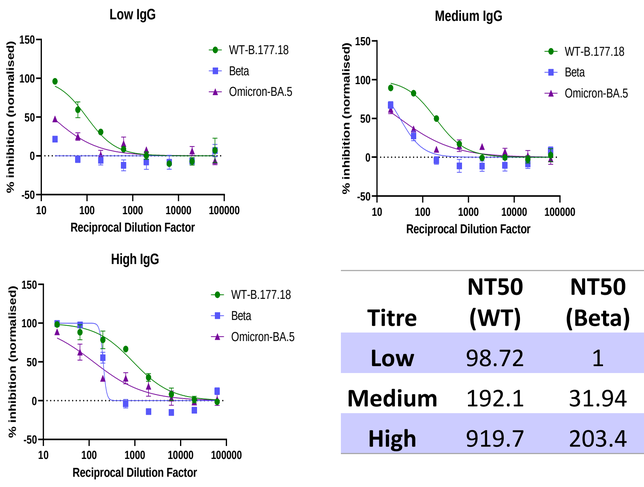
Graphs show representative data of individuals with a range of neutralising capacities. 190 COVID-19 convalescent plasma samples were analysed using Micro-NT. No impact samples are COVID-19 naïve individuals.
MOI 0.05, 18-hr infection
The half-maximal Neutralisation Titres (NT50) were determined using four-parameter logistic regression.

6. NT50 correlates strongly with Anti-RBD Titre



Graph shows the correlation between anti-RBD titre in IU/ml, and NT50 for190 plasma samples from SARS-CoV-2 positive individuals.
Binding IgG concentrations against RBD were measured using a multiplex electro-chemiluminescence assay.
NT50 values were measured using Micro-NT (Vero E6 cells infected with WT (B lineage) SARS-CoV-2

7. Reduced Neutralisation Capacity against SARS-CoV-2 VOCs



Titre	NT50 (WT)	NT50 (Beta)	NT50 (BA.5)
Low	98.72	1	1
Medium	192.1	31.94	31.38
High	919.7	203.4	125.4

Graphs show the neutralisation of SARS-CoV-2 WT D614G (B.177.18 lineage), Beta and Omicron (BA.5) by WHO SARS-CoV-2 IgG standards with Low, Medium of High IgG Titres.

Conclusions

1. Micro-NT is higher-throughput, requires less labour and resources than the gold standard Neutralising Assay, PRNT
2. Micro-NT shows high correlation with PRNT and with anti-RBD titres
3. Micro-NT can demonstrate reduced neutralisation capacity against VOCs
4. Selected for EU VACCELERATE clinical COVID-19 vaccine trials

Acknowledgements

With thanks to the participants and investigators of the All-Ireland Infectious Disease (AIID) Cohort.

References

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