Comparison of biological and biochemical tests to detect neutralizing antibodies against SARS-CoV-2

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MOLECULAR BIOTECHNOLOGY AND DIAGNOCTICS

Introduction

The analysis of the neutralizing capacity of anti-SARS-CoV-2 antibodies is important since they correlate with protective immunity at individual and population level. Due to the SARS-CoV-2 pandemic, increasing number of serological tests have been developed. However, many of the new tests still need clinical validation and formal approval. Comparison of the results is also hampered by the fact that traditional tests are quite heterogeneous, which leads to considerable uncertainty in the interpretation of the results.

Our goal was to determine whether an in-house developed high-throughput surrogate virus neutralization test (sVNT) can replace the time-consuming virus microneutralization test (MNT). Although MNT remains the gold standard for detecting neutralizing antibodies, in the case of SARS-CoV-2 it is not practical due to its low throughput, high cost and stringent biosafety requirement. The novel sVNT is operated without cells, viruses or highly skilled users, and can be used to diagnose SARS-CoV-2 infections and measure protective immunity. The test can potentially be extended to diagnose other highly pathogenic virus infections.

Materials and methods

Human sera: Serum samples with known MNT values were selected from Pfizer-BioNTech COVID-19 (COMIRNATY®) vaccinated individuals (Southwest Finland health district ethical permission ETMK 19/1801/2020) and COVID-19 patients at convalescent phase (samples collected between 14 days to 6 weeks after the positive RT-qPCR result, ethical permission HUS/1238/2020).

Microneutralization test (MNT): Neutralizing antibody levels were measured in duplicate from patient sera using 1:20 - 1:1280 dilutions. Virus and serum specimens were incubated for 1 hour at +37 °C followed by addition of VeroE6-TMPRSS-H10 cells. After incubation (+37 °C for 4 days) cells were fixed with 10 % formaline and stained with crystal violet. MNT results were analyzed using the $TCID_{50}$ endpoint which was defined as the reciprocal of the viral dilution resulting in 50 % of the inhibition of SARS-CoV-2 infection.

Surrogate virus neutralization test (sVNT): To validate the sVNT, three purified ACE2 receptor constructs were coated on to plates: wild type ACE2 (wtACE2), dimeric ACE2 molecule fused with mouse IgG2a Fc-part (ACE2-wt-mFc) and a 8 x His-tagged ACE2 with five mutations (ACE2-5mut-8xHis). Test plates for validation of sVNT is described in Figure 1.

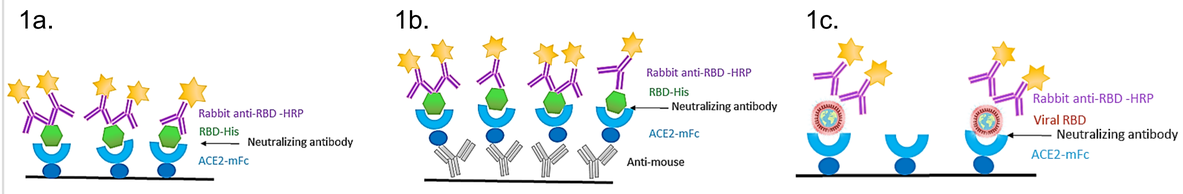

Figure 1. Test plates for sVNT. Plate 1a was coated with ACE2-wt-mFc and used for optimizing the test format. Plate 1b was an anti-mouse coated commercial plate to investigate whether binding can be enhanced for mFc-tagged ACE2. Plate 1c was for testing the ACE2-wt-mFc coated plate with live SARS-CoV-2.

Results and conclusions

In this study, we developed a biochemical test, which mimics the antibody-mediated blockage of the interaction between the host ACE2 (SARS-CoV-2 receptor), and the receptor binding domain (RBD) of the SARS-CoV-2. The buffers and the concentrations of the ACE2 coated on the plate and the RBD in the neutralization mixture were optimized. The optimal buffer was PBS supplemented with 0.1 % Tween-20 and 5 % swine serum. Optimized sVNT protocol is presented in Figure 2.

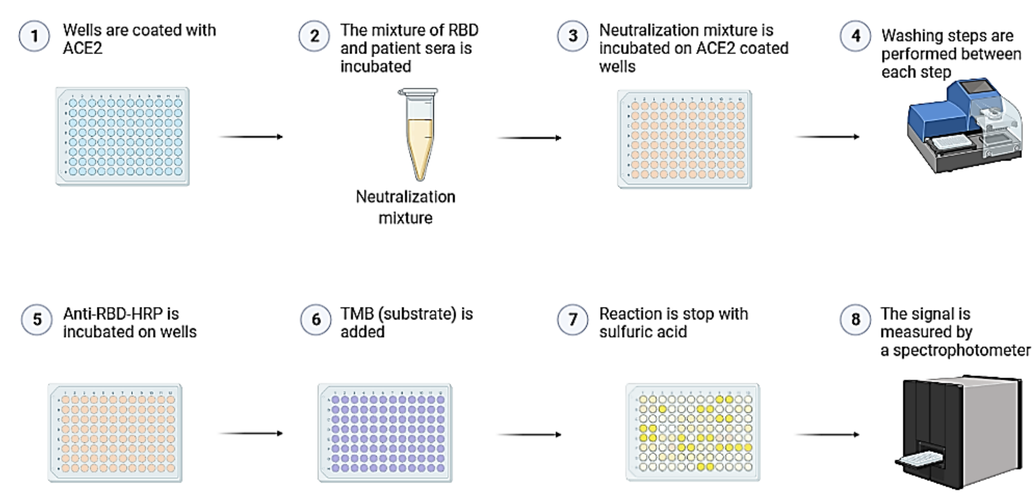

Figure 2. sVNT procedure. 1. 96-well plates are coated with ACE2 protein; 2. RBD and sample dilution is incubated to allow the binding of neutralizing antibodies to RBD and; 3. Mixture of RBD and sample is incubated on ACE2 coated plate to allow RBD to bind to ACE2 receptor; 4. Plates are washed after each incubation step; 5. Anti-RBD-HRP is applied to detect ACE2-bound RBD; 6. Substrate for HRP is added; 7. Reaction is stopped by sulphuric acid; 8. Absorbance is measured at 450 nm. Figures are prepared using BioRender.

Our results from 64 samples from COVID-19 patients at convalescent phase and Pfizer-BioNTech COVID-19 (COMIRNATY®) vaccinees, indicate that the new sVNT can be used instead of the tedious and expensive MNT. We observed a strong correlation (r=0.7804) between sVNT and MNT with Wuhan-like SARS-CoV-2 strain and a moderate correlation (r=0.4604) with Delta variant. Initial results show a good clinical sensitivity (98.4 %) that will be confirmed in the future studies. An international standard from WHO will be used to enable the harmonization of the two tests and the quantitation of the sVNT.

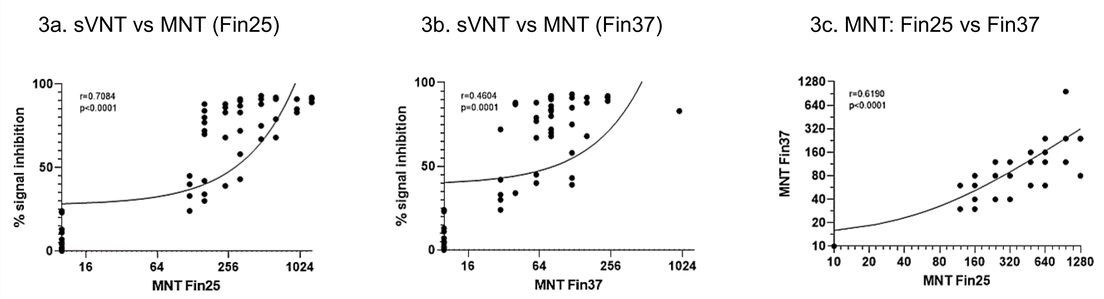

Figure 3. Comparison of MNT and sVNT using 3a Fin25 (Wuhan-like strain) and 3b Fin37 (Delta variant). A comparison of MNT with Fin25 and MNT with Fin37 is shown in Figure 3c. % signal inhibition of sVNT was calculated: (1 - sample OD450nm/neg pool OD450nm) *100. 30 % signal inhibition was determined as a cutoff. Correlation analysis from 64 sera by sVNT and MNT was done by GraphPad Prism using Pearson´s correlation coefficient. Statistical significance was calculated using the two-tailed test. MNT results are presented as log of the MNT titers (10-1280) and inhibition as % (0-100).

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