Pseudo typed virus neutralization

This pseudo typed virus neutralization assay is used to quantify the titer of neutralizing antibody for SARS-CoV-2. Pseudo typed virus particles are made using a genetically modified Vesicular Stomatitis Virus from which the glycoprotein G was removed (VSVΔG). The VSVΔG virus is transduced in HEK293T cells previously transfected with the spike glycoprotein of the SARS-CoV-2 coronavirus (Wuhan strain) for which the last 19 amino acids of the cytoplasmic tail were removed (ΔCT). The pseudo particles generated (VSVΔG – Spike ΔCT) contain a luciferase reporter which can be quantified in relative luminescence units (RLU).

Heat-inactivated samples are serially diluted (7-serial 2-fold dilution) in a 96-well plate and a pre-determined amount of pseudo typed virus (corresponding to approximately 150,000 RLU/well) is applied to the plate and incubated with serum/plasma to allow binding of the neutralization antibodies to the pseudo typed virus.

After the incubation of the serum/plasma-pseudo typed virus complex, media is aspirated from Vero E6 cells in a 96-well plate and the serum/plasma-pseudo typed virus complex is transferred to the plate containing Vero E6 cells. Test plates are incubated at 37°C with 5% CO2 overnight. Once the incubation is complete, luciferase substrate is added to the plates which are then read using a plate reader detecting luminescence. The intensity of the light being emitted is inversely proportional to the amount of anti-SARS-CoV-2 Pre-Spike antibodies bound to the VSVΔG – Spike ΔCT particles.