

### Wild type virus neutralization

Heat-inactivated (56°C for 30 min) serum samples are serially diluted and incubated with approximately 60 PFU of wild type SARS-CoV-2 (2019-nCoV/Victoria/1/2020), for 1 h at 37°C in 5% CO<sub>2</sub>. Samples are incubated with Vero E6 monolayers in 96-well plates under MEM containing 1.5% carboxymethylcellulose (Sigma), 5% (v/v) foetal calf serum and 25mM HEPES buffer.

After incubation, at 37°C for 24 hours, plates will be fixed overnight with 20% (v/v) formalin/PBS, washed with tap water and patches of infected cells (microplaques) detected via an antibody pair specific for the SARS-CoV-2 RBD Spike protein.

Microplaques are visualized using KPL TrueBlue™ peroxidase substrate and counted on an Immunospot analyzer. Neutralisation dilution at 50% (ND50) and neutralising factor at 50% (NF50), normalised against the reference serum, is reported.