Appendix 1. Live virus Microneutralization (vMN) assay

It was performed in 96-well plate. Serum samples were serially diluted in 2-folds with minimum essential medium (Gibco, Green Island, NY, USA) starting from 1:10. Diluted sera will be mixed with 100 TCID50 of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) HKU-001a (wild type; Genback accession number MT230904) and incubated at 37°C for 1.5 hours. The mixture was added to VeroE6 TMPRSS2 cells and incubated at 37°C and 5% CO_2. Cytopathic effect was determined by examination under inversion microscopy after 72 hours of incubation. The vMN antibody titer was the highest dilution with 50% inhibition of cytopathic effect, with standardization to the World Health Organization (WHO)'s International Standard for SARS-CoV-2 immunoglobulin (human). The pooled plasma (NIBSC code 20/136), the first WHO international standard for anti-SARS-CoV-2 immunoglobulin, was obtained from 11 coronavirus disease 2019 (COVID-19) recovered patients. It can be used in neutralization assay, and assigned potency is 250 IU/ampoule (https://www.nibsc.org/documents/ifu/20-136.pdf). vMN positivity (seroconversion) was defined as titer ≥10 (31.25 IU/mL).

SARS-CoV-2 and COVID-19 vaccines can elicit non-neutralizing and neutralizing antibody. Both antibodies can bind to virus, and neutralizing antibody can protect host from virus infection.

Receptor-binding domain (RBD) and N-terminal domain (NTD) of spike protein can induce anti-SARS-CoV-2 neutralizing antibody which can inhibit SARS-CoV-2 infection by blocking the viral entry.[1] There are many methods to evaluate the antibody level, for instance, enzyme-linked immunoassay (ELISA), immunofluorescence (IF), and vMN. ELISA and IF are used to determine antibody which can bind to viral antigen or virus, while vMN results represent the neutralizing activity against virus at protein expression level. Currently, there is an ELISA-based surrogate neutralizing antibody (sNAb) test which can be used to evaluate anti-RBD antibody specifically.[2] However, vMN results exhibit the total neutralizing activity, including anti-RBD and anti-NTD neutralizing antibody. Viral neutralization tests (VNTs) are often considered as the 'gold standard' for serological detection, as the results demonstrate inactivation of infectious virus, and as such,VNTs represent a strong correlative indicator of protection from disease.[3]

Supplementary References

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