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Immunogenicity Determination of SARS-CoV-2 Vaccine

1. Scope

This method is applicable to determine the titer of neutralization antibodies in serum of the COVID-19 vaccine immunized animals to against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

2. Method

Collect the serum from animals immunized with the COVID-19 vaccine and allow serum to interact with specified amounts of SARS-CoV-2, then inoculate with Vero E6 cells. After 3 to 5 days incubation, observe cytopathic effect (CPE) of cells by phase contrast microscope, record and calculate the neutralization titer at NT_{100} and NT_{50} of anti-SARS-CoV-2 antibodies.

NT_{100}: the dilution folds of the serum sample that completely blocks infectivity of a virus is defined as 100% neutralization titer.

NT_{50}: the dilution folds that blocks half-maximal infectivity of a virus is defined as 50% neutralization titer.

2.1. Work environment (note 1)

Experiments for determining anti-SARS-CoV-2 antibodies titer should be conducted in a biosafety level 3 (BSL-3) laboratory. Procedures that handle viruses, such as cell infection or serial dilution, should be performed in a certified class II, type B2 biological safety cabinet (BSC). All the process should follow the regulations approved by the Taiwan Centers for Disease Control (CDC) (note 2).

Note 1: SARS-CoV-2 is classified as a risk group 3 (RG3) human pathogen. Personnel who handle and process specimens associated with SARS-CoV-2 should follow related laboratory biosafety guidelines.

Note 2: Regulations Governing Management of Infectious Biological Materials; Operation Directions Governing Management of Infectious Biological Materials; Guidelines for the Implementation of Laboratory Biorisk Management; Safety Guidelines for the Use of Point-of-Care Testing (POCT) for SARS-CoV-2; Biosafety Guidelines for Laboratory Handling SARS-CoV-2.

2.2. Equipment

2.2.1. Biological safety cabinet (BSC): class II, type B2.
2.2.2. Autoclave: reach and maintain a temperature of 121°C or above.
2.2.3. Refrigerator: maintain a temperature of 5 ± 3°C.
2.2.4. Freezer: maintain a temperature of -30 ± 3°C.
2.2.5. Ultra-low temperature freezer: maintain a temperature of -80 ± 5°C.
2.2.6. CO_2 incubator: with temperature control at 35°C or 37°C, and the level of CO_2 at 5%.
2.2.7. Cell counter: Beckman Coulter cell counter Z2 or an equivalent product.
2.2.8. Phase contrast microscope: up to 400X magnification.
2.2.9. Thermo-controllable water-bath.
2.2.10. Refrigerated centrifuge: appropriate for 15 mL and 50 mL centrifuge tubes; centrifugal force ≥ 2000 ×g and with temperature control at 4 °C.

2.3. Reagents
2.3.1. Normal Saline (NS).
2.3.2. Phosphate buffered saline (PBS).
2.3.3. Trypsin.
2.3.4. Dulbecco's Modified Eagle's Medium (DMEM): Sigma-Aldrich D5796, or an equivalent product.
2.3.5. Fetal bovine serum (FBS).
2.3.6. Antibiotics: with 10,000 units/mL penicillin and 10,000 units/mL streptomycin.
2.3.7. Vero E6 cell line (ATCC® CRL-1586™): lower passage number (below 15) should be used. Cells are tested and found free of bacteria, fungi and mycoplasma.
2.3.8. SARS-CoV-2, clade L.

2.4. Materials and Labware
2.4.1. Syrian hamster.
2.4.2. Syringe with needle: 1 mL and 3 mL.
2.4.3. Micropipettes with volume ranges of 2 µL, 10 µL, 20 µL, 200 µL and 1000 µL.
2.4.4. Filter tips for micropipettes: 2 µL, 10 µL, 20 µL, 200 µL and 1000 µL.
2.4.5. Serological pipette: 5 mL, 10 mL and 25 mL.
2.4.6. Glass bottles: 100 mL, 250 mL, 500 mL and 1000 mL.

2.4.7. Microcentrifuge tube: 1.5 mL.

2.4.8. Centrifuge tube: 15 mL and 50 mL, PP.

2.4.9. Cryogenic vial: 2.0 mL.

2.4.10. Cell culture flask/plate: T75 flask, T150 flask and 96-well plate.

2.4.11. 96 deep well plate for dilution.

2.5. Preparation of reagents

2.5.1. DMEM containing 10% FBS: Add 100 mL of FBS and 10 mL of antibiotics to 890 mL of DMEM, and mix with gently shaking.

2.5.2. DMEM containing 2% FBS: Add 20 mL of FBS and 10 mL of antibiotics to 970 mL of DMEM, and mix with gently shaking.

2.6. Animal immunization

2.6.1. Before immunization, collect 200 μL blood serum from hamsters of 5-8 weeks old and at least 6 hamsters per experiment. After centrifugation, collect serum samples as the pre-immunization control (Day 0) and store at -20℃. Then, each hamster is given an intramuscular injection of vaccine equivalent to the human dose, and followed with the second dose injection after 14 days.

2.6.2. Seven days after the second boost, collect blood from the immunized hamsters (Day 21) and centrifuge the blood at 860 ×g at 4°C for 10 minutes. Collect the serum samples and store in microcentrifuge tubes at -20°C for later use.

2.7. Refer to the analytical method "SARS-CoV-2 Neutralization Assay" published by Taiwan FDA for cell culture, virus preparation, virus titration, neutralization procedure, back titration, and calculation of neutralization titer.

2.8. Validity evaluation of the vaccine immunogenicity assay

2.8.1. The viral titer of the diluted virus used in the neutralization antibody test should be confirmed. The values of $CCID_{50}$ of the inoculated virus must be within 50-150 to consider as a valid viral titer to evaluate the neutralizing antibody test. (note 3)

2.8.2. When the following condition meet is a validated experiment. To use the national standard of neutralizing antibody or a calibrated in-house standard of neutralizing antibody with known titer as a positive and valid control for each experiment. The deviation of each neutralization

antibody test and positive control must be within ±2 dilution folds. (note 4)

2.8.3. To determine the immunogenicity of different batches of vaccines, the titer of the neutralizing antibodies from individual hamster serum should be test after completely vaccination. The titer of neutralizing antibody from hamster serum tested within the same batch is calculated to obtain the geometric mean titer (GMT). The NT_{50} of serum before immunization (Day 0) should be zero, and NT_{50} of Day 21 is set tentatively at 640, which is referred to the titer of neutralizing antibody from naturally infected and standard vaccinated hamsters. (note 5). (A vaccine batch is considered to possess effective immunogenicity while the assay result of the batch is greater than or equal to the titer of 640.)

Note 3: For the suggestive criteria in Section 2.8.1. and Section 2.8.2, please refer to the analytical method " SARS-CoV-2 Neutralization Assay" published by the Taiwan FDA.

Note 4: The viruses used by Taiwan FDA for neutralizing antibody test are the third passage of SARS-CoV-2 (CDC NO.4, hCoV-19/Wuhan/WIV04/2019). After NGS sequencing (illumina), the virus genome was analyzed with the reference sequence (GenBank Accession, NC_045512.2), and the results showed that the 50-60% of SARS-CoV-2 viruses harbor mutant spike protein lacking amino acid fragment from 68 to 76. 60-70% of SARS-CoV-2 viruses posses mutant spike protein lacking amino acid fragment from 675 to 679. Therefore, the SARS-CoV-2 virus used in testing laboratory should be sequenced to confirm the mutation profile of the spike gene. If the viral genome sequence mutates, the virulence of the mutant virus should be confirmed.

Note 5: The titer of neutralizing antibody will be under rolling adjustment based on the accumulative data from different vaccine batches.

References

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Experiment flow