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Neutralizing antibodies (NAbs) tests

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Protocol for detection

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We use this protocol and it's working

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Abstract

Antibody assays of IgM, IgG and surrogate isotype independent virus neutralizing antibody (sVNT) targeting receptor binding domain of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) were employed in 97 real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) confirmed Coronavirus Disease 2019 (COVID-19) patients with varying severity admitted to King Chulalongkorn Memorial Hospital. Concordance rate was 100% regardless of severity, onset of symptoms and magnitude of viral load. Per available samples, antibodies appeared on the same day of symptom onset in one patient; one day after in 18 patients and two days after in 19 patients. In two patients, antibodies appeared as early as 4 days after infection (exposure). IgM and IgG were evident in all patients' first assay (within two days of admission). sVNT was also evident within two days of admission in all but 3 patients. IgM usually remained positive during the entire course of hospital stay, where the longest in this study was 32 days. Antibody assays were also applied to samples collected at a State Quarantine premise from 77 asymptomatic Thais returning from Sudan in October. Virus was detected by real-time RT-PCR in 15 cases (day 0=6, day 3=4, day 5=4 and day 9=1). Twenty-nine (including 11 RT-PCR positive cases) were antibody positive on day 0, while 4 PCR positive with antibody negative on day 0 became antibody positive on day 14. Evaluation on antibody response at days 7 or 10 is needed to help build a case to shorten length of quarantine among negative cases.

Guidelines

For human serum, blood was collected into a serum separator tube and allowed to clot for 30 minutes at room temperature. Samples were then centrifuged at 3,500 × rpm for 5 minutes to separate the serum. Assays were performed immediately after preparation; otherwise, serum was aliquoted and store at ≤ −20 °C until analysis. Repeated freeze–thaw cycles were avoided.

Materials

Materials

- 96 well capture plate (8 wells x 12 strips) pre-coated with recombinant ACE2 protein
- Adhesive plates sheet (for covering plate)
- Positive control 1 vial (0.05 ml)
- Negative control 1 vial (0.05 ml)
- HRP conjugated RBD 1 vial (0.02 mL)
- HRP dilution buffer 1 bottle (10 ml)
- Sample dilution buffer 1 bottle (30 ml)
- 20× wash solution 1 bottle (40 ml)
- TMB ELISA substrate solution 1 bottle (12 ml)
- Stop solution 1 bottle (6 ml)
- Plate washer-automated or manual (manifold dispenser)
- Microtiter plater reader with software capable of measuring at 450 nm
- Deionized water

Safety warnings

- If samples will not be tested immediately, freeze them after collection.
- Avoid multiple freeze–thaw cycles of frozen samples.
- Thaw samples completely and mix gently (do not vortex) prior to analysis.

1 All reagents were equilibrated to room temperature (20–25 °C) prior to use for 30 min. After handling reagents were promptly returned to refrigerated storage.

2 All samples and controls were mixed thoroughly by vortex prior to use.

3 HRP-conjugated RBD was diluted 1:1000 in HRP dilution buffer. For instance, to prepare sufficient working solution for a 96-well plate, 10 µl of HRP-conjugated RBD was mixed with 10 ml of HRP dilution buffer.

4 The 20× wash Solution was diluted 1:19 with deionized water to prepare a 1X working solution. Prepared solution was stored at 2–8 °C when not in use.

5 Dilute the test serum positive and negative controls 1:9 in dilution buffer, then mix each with HRP-RBD at a 1:1 ratio in a tube. Incubate at 37 °C for 30 minutes at room temperature. It is recommended that all positive and negative controls be prepared in duplicate.

6 Add 100 µl of the mixture to each well of the 96-well capture plate and incubate at 37 °C for 15 minutes.

7 Wash the plate three times with 1X wash buffer, removing 260 µl of excess solution each time using the ELISA washer.

8 Add TMB substrate at 100µl per well was added and incubated in a dark room at room temperature for 15 minutes.

9 Add 50 µL of stop solution to each well to stop the reaction.

10 Read the absorbance in the microtiter plate reader at 450 nm immediately.

Protocol references

Genescript SARS-CoV-2 Surrogate Virus Neutralization Test Kit