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S2_Capture ELISA to detect for IgM antibodies against 2019-nCoV protein

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jeabchanida Ruchisrisarod[1]

[1]Chanida Ruchisrisarod

S2_Capture ELISA to det... S1_ELISA of sera to dete...

jeabchanida Ruchisrisarod
Thai Redcross

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We use this protocol and it's working

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Abstract

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 in March-April 2020. 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.

KEYWORDS (3-6):COVID-19, SARS-CoV-2, Antibodies, neutralising antibodies, Thailand

1 Coat the 96 well plate with 10ug/ml of goat anti-human IgM antibody diluted in bicarbonate buffer (for 1 full plate, add 50ul of antibody to 5ml of coating buffer) at 50ul/well in 4^{O}C overnight.

2 The next day, remove the coating solution as biohazard waste. Wash the plate 5x by filling each well with 150ul of PBST and remove the PBST wash as biohazard waste.

3 Remove remaining solutions by tapping it hard against a paper towel.

4 Block the remaining protein binding sites with 150ul blocking buffer per well. Incubate the plate at room temperature for 2 hours.

5 Wash the plate 1x with PBST wash buffer and remove excess solutions as steps 2-3.

6 Add heat-inactivated serum diluted 1:50 in blocking buffer in duplicates, at 50ul per well. Include negative controls. Incubate the plate at room temperature for 2 hours or at 37 deg for 1 hour.

7 Wash plate 5x with PBST wash buffer and remove excess solutions as steps 2-3.

8 Add RBD-HRP diluted to 4ug/ml in blocking buffer (stock concentration = 0.38mg/ml, for 1 full plate, add 52ul to 5ml of blocking buffer), at 50ul per well. Incubate the plate at room temperature for 1 hour or 37 deg for half an hour.

9 Wash plate 5x with PBST wash buffer and remove excess solutions as step 2-3.

10 Add 50ul of TMB substrate per well. Observe the chromogenic reaction and stop after 2 minutes before it reaches the maximum readable range of the Cytation5 plate reader (i.e. OD450 of 2).

11 Stop reaction by addition of 50ul per well of Stop solution.

12 Read the absorbance on the Cytation5 plate reader immediately after stopping plate. TMB – 450um, background of plate – 570um. *Note: if there are multiple plates, you should work on one plate at a time. Leave the last wash buffer in the other plates and only proceed with adding TMB solution one plate at a time.