S1_ELISA of sera to detect for antibodies against 2019-nCoV protein (IgG)

jeabchanida Ruchisrisarod[1]

[1]Chanida Ruchisrisarod

jeabchanida Ruchisrisarod

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We use this protocol and it's working

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ABSTRACT

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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

S1_ELISA of sera to detect for antibodies against 2019-nCoV protein (Ig

1 S1_ELISA of sera to detect for antibodies against 2019-nCoV protein (IgG).docx Coat the 96 well plate with 2ug/ml of 2019-nCoV protein diluted in bicarbonate 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 1:100 in blocking buffer in duplicates, at 50ul per well. Incubate the plate at room temperature for 2 hours.

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

8 Add anti-human IgG-HRP diluted 1:2000 in blocking buffer, at 50ul per well. Incubate the plate at room temperature for 1 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 1 minute 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. TMB – 450um, background of plate – 570um.