Supplementary Methods

1）GeneSoC® SARS-CoV-2 N2 Detection Kit (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan; Kyorin assay)

The Kyorin assay employs primer-probe sets targeting the nucleoprotein (N)-gene of SARS-CoV-2 based on the principle of real-time reverse transcription-polymerase chain reaction (real-time RT-PCR). The procedure was performed in according to the manufacturer's instructions. After adding 5 μL of the extracted RNA sample to 15 μL of a mixture of PCR Buffer, Enzyme Mix, and Primer/Probe Mix, 19 μL was injected into a dedicated GeneSoC® measurement chip (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan) and measured using GeneSoC® (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan). The test results were deemed positive when an increase in the amplification curve was observed during the reaction time.

2）N2 assay using the Japanese National Institute of Infectious Diseases (NIID) assay

The NIID assay was performed using real-time RT-PCR. The Manual for the Detection of Pathogen 2019-nCoV Ver.2.6 [1] employs primer-probe sets for two regions: the N set and N2 set. Only the N2 set was evaluated in this study because previous analyses have suggested that the detection rate of the N set was low [2]. Real-time RT-PCR was performed using the QuantiTect Probe RT–PCR Kit (QIAGEN, Hilden, Germany) and LightCycler 480 System (Roche, Basel, Switzerland). The procedure was performed in according to the manufacturer's instructions. Cycling conditions were as follows: 30 min at 50 °C, 15 min at 95 °C, and 45 cycles of 15 s at 95 °C and 60 s at 60 °C. The

test results were deemed positive when an increase in the amplification curve was observed with a Ct value of <40 during reaction time. In addition, to measure the viral load of SARS-CoV-2, dilutions of "novel coronavirus positive control RNA" (Nihon Gene Research Laboratories, Inc., Miyagi, Japan) were measured with the samples with the NIID assay, and a calibration curve was generated.

3）Ampdirect™ 2019-nCoV Detection Kit (Shimadzu Corporation, Kyoto, Japan; SHIMADZU assay)

The SHIMADZU assay employs N1 and N2 primer-probe sets targeting the N-gene of SARS-CoV-2 based on the principle of real-time RT-PCR. The procedure was performed in according to the manufacturer's instructions. Briefly, a mixture of the sample (5 μL) and treatment solution (5 μL) was pretreated at 95 °C for 5 min and then added to the PCR solution. Amplification and real-time detection were performed on a LightCycler 480 System (Roche, Basel, Switzerland). Cycling conditions were as follows: 10 min at 42 °C, 1 min at 95 °C, and 45 cycles of 5 s at 95 °C and 15 s at 60 °C. The test results were deemed positive when an increase in the amplification curve was observed during the reaction time.

4）Lumipulse Presto SARS-CoV-2 Ag (Fujirebio Inc., Tokyo, Japan; FUJIREBIO assay)

The FUJIREBIO assay measures antigen levels quantitatively by targeting the N-protein of SARS-CoV-2 based on the principle of chemiluminescent enzyme immunoassay. The procedure was performed in according to the manufacturer's instructions. The samples were separated into other tubes and centrifuged at 2000×g for 5 min and tested using a fully automated Lumipulse® L2400 (Fujirebio Inc., Tokyo, Japan). Lumipulse® L2400 measures the

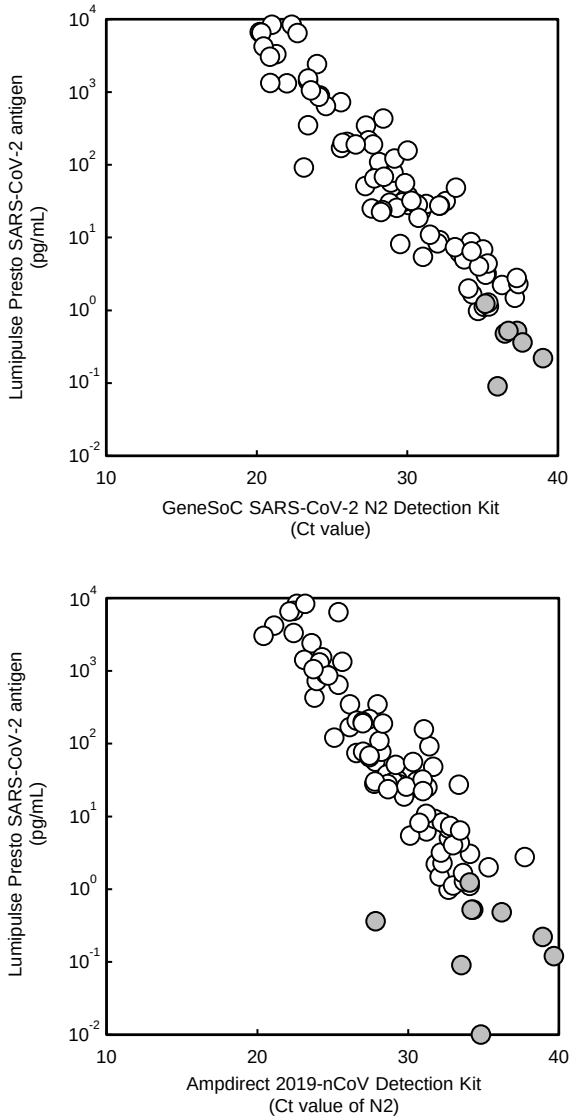
luminescence intensity emitted from a sample using a two-step sandwich method. The antigen concentration in the sample is calculated by automatically applying the luminescence intensity of the sample to a calibration curve created based on the luminescence intensity obtained from a standard SARS-CoV-2 Ag solution (Fujirebio Inc., Tokyo, Japan). The test results were deemed positive or judgment pending or negative by the antigen concentration in the nasopharyngeal (negative: less than 1.00 pg/mL, judgment pending: from 1.00 to less than 10.00 pg/mL, and positive: over 10.00 pg/mL) and saliva specimens (negative: less than 0.67 pg/mL, judgment pending: from 0.67 to less than 4.00 pg/mL, and positive: over 4.00 pg/mL), respectively. When the result of first test was within the judgment pending level, the sample was tested after re-centrifuged at 2000×g for 5 min. The results of retests were deemed positive when the antigen concentration exceeded 1.34 pg/mL in the nasopharyngeal specimens and 0.67 pg/mL in the saliva specimens.

Reference

1. National Institute of Infectious Diseases. Manual for the Detection of Pathogen 2019-nCoV Ver.2.6. 2020;1–16. Available at: https://www.niid.go.jp/niid/images/epi/corona/2019-nCoVmanual20200217-en.pdf Accessed: 28 November 2023.

2. Shirato K, Nao N, Katano H, Takayama I, Saito S, Kato F, et al. Development of Genetic Diagnostic Methods for Detection for Novel Coronavirus 2019 (nCoV-2019) in Japan. Jpn J Infect Dis 2020;73:304–7.

Supplemental Figure



Supplementary Figure: Correlation of antigen concentration with Ct values. Gray circles are negative for SARS-CoV-2 antigen.