| | Confirmed COVID-19 | | | | SARS-CoV-2 PCR negative | | | | |
|---------------------|--------------------|--------------|--------------|--------------|-------------------------|--------------|--------------|--------------|----------|
| Profile | symptomatic | | asymptomatic | | symptomatic | | asymptomatic | | Unscreen |
| | acute | convalescent | acute | convalescent | Acute | convalescent | acute | convalescent | ed |
| Ν | 42 | 24 | 6 | 2 | 3 | 53 | 2 | 121 | 229 |
| CRNT (%) | | | | | | | | | |
| Minimum | 0.0 | 35.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 25% Percentile | 0.0 | 64.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Median | 0.0 | 83.5 | 34.5 | 34.7 | 0.0 | 0.0 | 22.5 | 0.0 | 17.0 |
| 75% Percentile | 30.6 | 90.0 | 75.2 | 69.5 | 0.0 | 17.5 | 45.0 | 21.2 | 31.2 |
| Maximum | 95.5 | 98.5 | 86.5 | 69.5 | 0.0 | 91.0 | 45.0 | 92.0 | 19.0 |
| htCRNT (%) | | | | | | | | | |
| Minimum | 0.0 | 28.0 | 0.0 | 10.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 25% Percentile | 0.0 | 67.2 | 0.0 | 10.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Median | 0.0 | 83.5 | 45.5 | 25.5 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 |
| 75% Percentile | 15.0 | 91.0 | 86.0 | 31.0 | 0.0 | 0.0 | 4.0 | 0.0 | 11.0 |
| Maximum | 98.0 | 98.0 | 89.0 | 31.0 | 0.0 | 83.0 | 4.0 | 91.0 | 50.0 |
| Anti-RBD antibody (| (U/mL) | | | | | | | | |
| Minimum | 0.40 | 0.84 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| 25% Percentile | 0.40 | 7.63 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Median | 0.40 | 35.0 | 1.59 | 16.4 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| 75% Percentile | 0.46 | 137.0 | 7.55 | 32.4 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Maximum | 25.1 | 1640 | 12.3 | 32.4 | 0.40 | 66.4 | 0.40 | 294.0 | 0.40 |

Supplemental Table 1. Basic statistics of SARS-COV-2 serological tests

2 CRNT, chemiluminescent reduction neutralizing test; htCRNT, high-throughput CRNT, RBD, receptor-binding domain

4 Supplemental Table 2. Samples with discordant serological test results

5

| | CRNT | | htCRN | IT | Anti-RBD antibody | |
|----------------------------|--------------------------|----------|--------------------------|----------|-------------------|----------|
| Profile | Values (% Inhibition) | Results | Values (% Inhibition) | Results | Values (U/mL) | Results |
| Confirmed COVID-19 | | | | | | |
| symptomatic, convalescent | 35.5 | Negative | 28.0 | Negative | 5.06 | Positive |
| asymptomatic, convalescent | 69.5 | Positive | 41.0 | Negative | 32.4 | Positive |
| SARS-CoV-2 PCR negative | | | | - | | |
| symptomatic, convalescent | 53.5 | Positive | 0.0 | Negative | 0.40 | Negative |
| asymptomatic, convalescent | 85.0 | Positive | 37.0 | Negative | 2.05 | Positive |
| asymptomatic, convalescent | 58.5 | Positive | 22.0 | Negative | 0.40 | Negative |
| Unscreened | | | | | | |
| | 69.0 | Positive | 22.0 | Negative | 0.40 | Negative |
| | 66.0 | Positive | 37.0 | Negative | 0.40 | Negative |

6 CRNT, chemiluminescent reduction neutralizing test; htCRNT, high-throughput CRNT; RBD, receptor-binding domain

8 Supplemental Table 3. Testing in acute and convalescent phases

9

| Test | Desult | F | | | |
|------------------------|----------|-----------|------------------|-----------------------|--|
| Test | Result | Acute (n) | Convalescent (n) | - Fisher's exact test | |
| CONT | Positive | 13 | 21 | p < 0.01 | |
| CRNT | Negative | 38 | 2 | | |
| | Positive | 13 | 20 | p < 0.01 | |
| htCRNT | Negative | 38 | 3 | | |
| Anti DDD antihady toot | Positive | 13 | 22 | p < 0.01 | |
| Anti-RBD antibody test | Negative | 38 | 1 | | |

10 CRNT; chemiluminescent reduction neutralizing test, htCRNT; high-throughput CRNT, RBD; receptor binding domain

12 Supplemental Table 4. Test results and clinical symptoms of confirmed COVID-19 patients with positive serological tests

- 13 in the acute phase
- 14

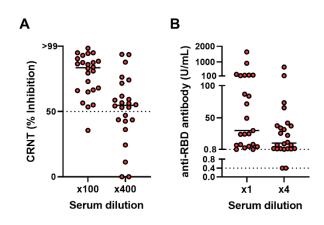
| Days after onset or the episode of close contact | Age range (years) | CRNT | | htCRNT | | Anti-RBD antibody test | | - Symptoms |
|--|----------------------|-----------------|----------|-----------------|----------|---------------------------|----------|---|
| | | % Inhibition | Results | % Inhibition | Results | U/mL | Results | Symptoms |
| 2 | 40-49 | 60.0 | Positive | 81.0 | Positive | 3.26 | Positive | Fever |
| 4 | ≥70 | 72.5 | Positive | 73.0 | Positive | 13.1 | Positive | Fever, dyspnea |
| 6 | ≥70 | 76.0 | Positive | 63.0 | Positive | 1.07 | Positive | Fever, cough |
| 7 | 60-69 | 80.5 | Positive | 77.0 | Positive | 3.40 | Positive | Fever |
| 8 | 60-69 | 95.5 | Positive | 91.0 | Positive | 1.72 | Positive | Fever, cough, sputum, loss of taste, loss of smell |
| 9 | 50-59 | 89.5 | Positive | 98.0 | Positive | 25.1 | Positive | Fever, cough |
| 9 | ≥70 | 88.5 | Positive | 78.0 | Positive | 1.09 | Positive | Fever |
| 2 | ≤19 | 65.5 | Positive | 79.0 | Positive | 2.77 | Positive | No symptom* |
| 3 | ≥70 | 86.5 | Positive | 89.0 | Positive | 5.97 | Positive | No symptom* |
| 5 | ≤19 | 71.5 | Positive | 85.0 | Positive | 12.3 | Positive | No symptom* |

¹⁵ * No symptoms but consecutive positives in follow-up SARS-CoV-2 PCR tests. Discharged after confirming a PCR-negative result.

16 CRNT; chemiluminescent reduction neutralizing test, htCRNT; high-throughput CRNT, RBD; receptor binding domain

(A) CRNT using 100-fold and 400-fold dilutions of sera from patients convalescing following
symptomatic COVID-19. (B) Anti-RBD antibody test using 100-fold and 400-fold dilutions of
sera from patients convalescing following symptomatic COVID-19. Bars indicate medians.

22

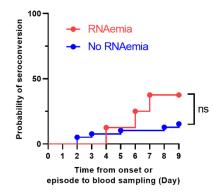


Supplemental Fig. 1

- 25 Relationship between seroconversion and RNAemia in confirmed COVID-19 patients in the
- acute phase. The Kaplan-Meier analysis was performed only for patients who had RNAemia.
- 27 ns, not significant.

28

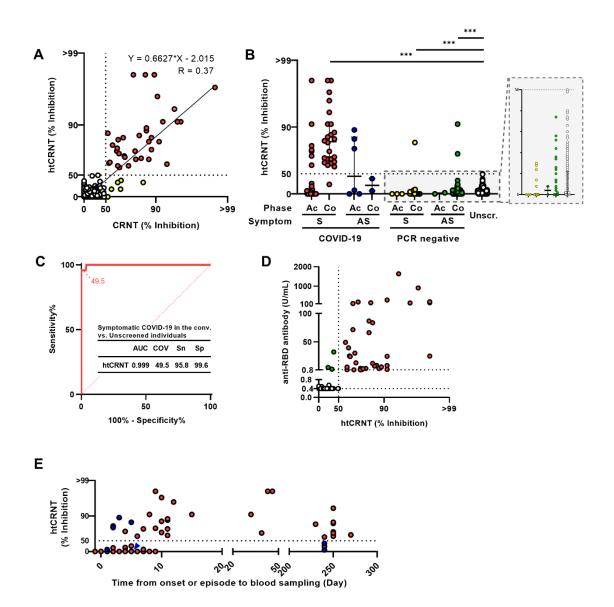
Supplemental Fig. 2



(A) Correlation between htCRNT and CRNT. Neutralization was assessed using serum 31 samples diluted 1:100. Correlation was expressed using Pearson's correlation coefficients. 32 (B) Neutralization of pseudotyped viruses measured using htCRNT. (C) ROC curve of 33 htCRNT for classification of symptomatic confirmed COVID-19 patients in the convalescent 34 35 phase and unscreened individuals. (D) Comparison of htCRNT neutralization levels and anti-RBD antibody results. Concordant samples are red (positive for both tests) or white (negative 36 for both tests). Discordant samples are green (positive for anti-RBD antibodies). The dotted 37 line for htCRNT indicates 50% inhibition (IC₅₀). (E) Relationship between htCRNT results and 38 39 time from symptom onset or a close-contact episode to blood sampling in patients with 40 COVID-19. Symptomatic and asymptomatic individuals are presented in red and blue (blue 41 arrowhead for overlapped), respectively. Error bars indicate medians (middle lines) and 42 interguartile range. ***p< 0.001 by unpaired Kruskal-Wallis test and Dunn's multiple comparison (unscreened group as a control). Ac, acute phase; Co, convalescent phase; S, 43 symptomatic; AS, asymptomatic; Unscr., unscreened; PCR negative, SARS-CoV-2 PCR-44 negative; AUC, area under the curve; COV, cut-off value; Sn, sensitivity; Sp, specificity. 45

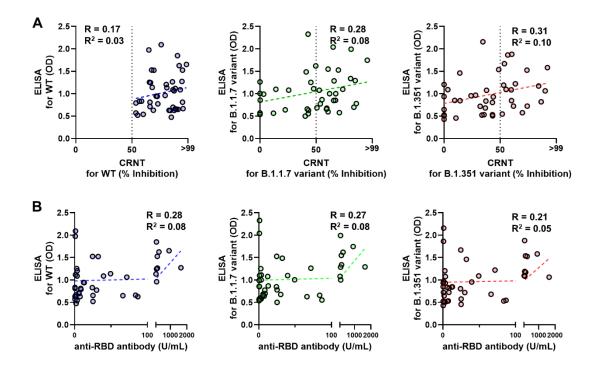
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Supplemental Fig. 3



Relationships between (A) ELISA and (B) CRNT values from sera that neutralize WT pseudotyped virus. The results from neutralization of WT (left), B.1.1.7 variant (middle), and B1.351 variant (right) were plotted for each. Dotted lines represent linear regression. Correlations were measured using Pearson's correlation coefficients.

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Supplemental Fig. 4