

Letter to the Editor

Insufficient Number of Controls for Low SARS-CoV-2 Seroprevalence: Are the Positive Rates Statistically Different between Pre-pandemic and Post-pandemic Samples?

Dear Sir,

We read the article by Nguyen et al.¹ in *The American Journal of Tropical Medicine and Hygiene* with great interest. The authors surveyed the seroprevalence of neutralizing antibodies against SARS-CoV-2 in Vietnam in 2020 and considered the positives in plaque reduction neutralization tests (PRNT) as SARS-CoV-2 infection. SARS-CoV-2 neutralizing antibodies were identified in two blood donors in samples collected in 2020 ($N = 885$; 0.23%; 95% confidence interval: 0.06–0.82).

We have comments on this finding. The PRNT is considered specific unless there is a cross-reactivity from neutralizing antibodies to other coronaviruses. Antibodies to seasonal common cold coronaviruses (CCC; 229E, NL63, HKU1, or OC43) might cross-react with SARS-CoV-2. A study reported that two of 40 CCC convalescent sera had inconclusive results in a SARS-CoV-2 micro-focus reduction neutralization test.² Another study showed SARS-CoV-2 PRNT titers of 1:20 and 1:10 for OC43 and 229E convalescent sera, respectively.³

The authors conducted the PRNT for 46 pre-pandemic stored sera and found no positive result. The false-positive rate was 0%, but the 95% confidence interval was 0 to 7.7%. As already mentioned, PRNT for SARS-CoV-2 could show cross-reactivity to anti-CCC antibodies, and therefore, the false-positive rate should be determined accurately. In this case, the number of positive results should be statistically different between pre-pandemic (2017) and post-pandemic (2020) samples. To verify this point, we analyzed the rates by Fisher's exact test (Table 1), which showed no significant difference in SARS-CoV-2 positivity between the two sample sets ($P = 0.90$). The number of controls is apparently too small to provide adequate power to evaluate the difference. In our previous study, 400 pre-pandemic samples were assayed by lateral flow assay and ELISA and showed 1.5% and 1.8% SARS-CoV-2 positives, respectively.⁴

We think that the results in the paper by Nguyen et al. should be interpreted with caution. In particular, it should be noted that the seroprevalence in a low-prevalence situation of COVID-19 would be biased by the specificity of the test.

TABLE 1
The number of positive and negative plaque reduction neutralization tests in blood donors (2017 and 2020)

	2017	2020
PRNT positive	0	2
PRNT negative	46	883

PRNT = plaque reduction neutralization tests.
 $P = 0.90$ by Fisher's exact test.

TOMOKO FUJITANI and KOUJI H. HARADA
*Department of Health Environmental Sciences
Kyoto University Graduate School of Medicine
Kyoto, Japan
E-mail: kharada-hes@umin.ac.jp.*

Published online October 31, 2022.

This is an open-access article distributed under the terms of the Creative Commons Attribution (CC-BY) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

REFERENCES

1. Nguyen HHH et al., 2022. Seroprevalence of SARS-CoV-2 neutralizing antibodies among blood donors in Ho Chi Minh City, Vietnam, August–November 2020. *Am J Trop Med Hyg* 106: 891–895.
2. Dhochak N, Agrawal T, Shaman H, Khan NA, Kumar P, Kabra SK, Medigeshi GR, Lodha R, 2022. Humoral cross-reactivity towards SARS-CoV-2 in young children with acute respiratory infection with low-pathogenicity coronaviruses. *J Clin Virol Plus* 2: 100061.
3. Kohmer N, Westhaus S, Rühl C, Ciesek S, Rabenau HF, 2020. Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG antibody assays. *J Clin Virol* 129: 104480.
4. Lyu Z, Harada Sassa M, Fujitani T, Harada KH, 2020. Serological tests for SARS-CoV-2 coronavirus by commercially available point-of-care and laboratory diagnostics in pre-COVID-19 samples in Japan. *Diseases* 8: 36.