Prevalence of SARS-CoV-2–Specific Antibodies, Japan, June 2020

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We used 2 commercially available antibody tests to estimate seroprevalence of severe acute respiratory syndrome coronavirus 2 infection in Japan during June 2020. Of 7,950 samples, 8 were positive by both assays. Using 2 reliable antibody tests in conjunction is an effective method for estimating seroprevalence in low prevalence settings.

During the first wave of the coronavirus disease (COVID-19) pandemic in Japan, a total of 16,884 persons tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by May 31, 2020, indicating a national cumulative incidence of 0.013% (*1*,*2*) (Appendix Figure, https://wwwnc.cdc.gov/EID/article/27/2/20-4088-App1.pdf). To establish a surveillance method in low prevalence settings, we assessed the seroprevalence of SARS-CoV-2 infection in Japan in early June 2020.

The Study

By October 2020, no standard antibody test or standardized method for estimating the seroprevalence of SARS-CoV-2 infection had been established. We used 2 serologic tests, a neutralizing antibody assay, and participant questionnaires to estimate the seroprevalence of SARS-CoV-2 infection in Japan.

We conducted a seroprevalence survey of SARS-CoV-2 infection in 3 prefectures of Japan during June 1–7, 2020. We selected 2 prefectures with a relatively high cumulative incidence of confirmed COVID-19 cases as of May 31, 2020: Tokyo, with an incidence of 0.039% (5,408 cases/13.9 million population) and Osaka, with an incidence of 0.020% (1,785 cases/8.8 million population). To better estimate the range of seroprevalence of SARS-CoV-2 infection in Japan, we also chose a prefecture with a relatively low cumulative incidence, Miyagi, with an incidence of 0.004% (88 cases/2.3 million population).

Each prefecture was responsible for using its civil registration data to randomly select participants. The Tokyo metropolitan government used random sampling stratified by age and sex in 3 cities with a cumulative incidence resembling the average of the Tokyo metropolitan area. The Miyagi prefectural government used its residence registry to conduct random sampling with stratification for age, sex, and geographic region. The Osaka prefecture used age-adjusted random sampling to select resident users of an existing smartphone application on general health (Figure).

Eligible participants were persons ≥20 years of age living in Japan. The Tokyo and Miyagi prefectures excluded otherwise eligible participants with temperatures ≥37.5°C. All participants provided written informed consent. The study was approved by the internal review boards of the Research Institute of Tuberculosis (approval no. RIT/IRB 2020–04, 2020–05) and the National Institute of Infectious Diseases (approval no. 1140).

First, we asked participants to complete a questionnaire (Appendix Table 1). Trained healthcare workers collected blood samples from the participants. After centrifuging the samples, the workers collected serum and tested the samples with 2 commercially available antibody tests to detect the SARS-CoV-2 nucleocapsid antigen: a chemiluminescent microparticle immunoassay with published specificity results of 99.6%–99.9% at a cutoff index of 1.4 (SARS-CoV-2 IgG assay; Abbott, https://www.abbott.com) (*3*,*4*) and an electrochemiluminescence immunoassay for the

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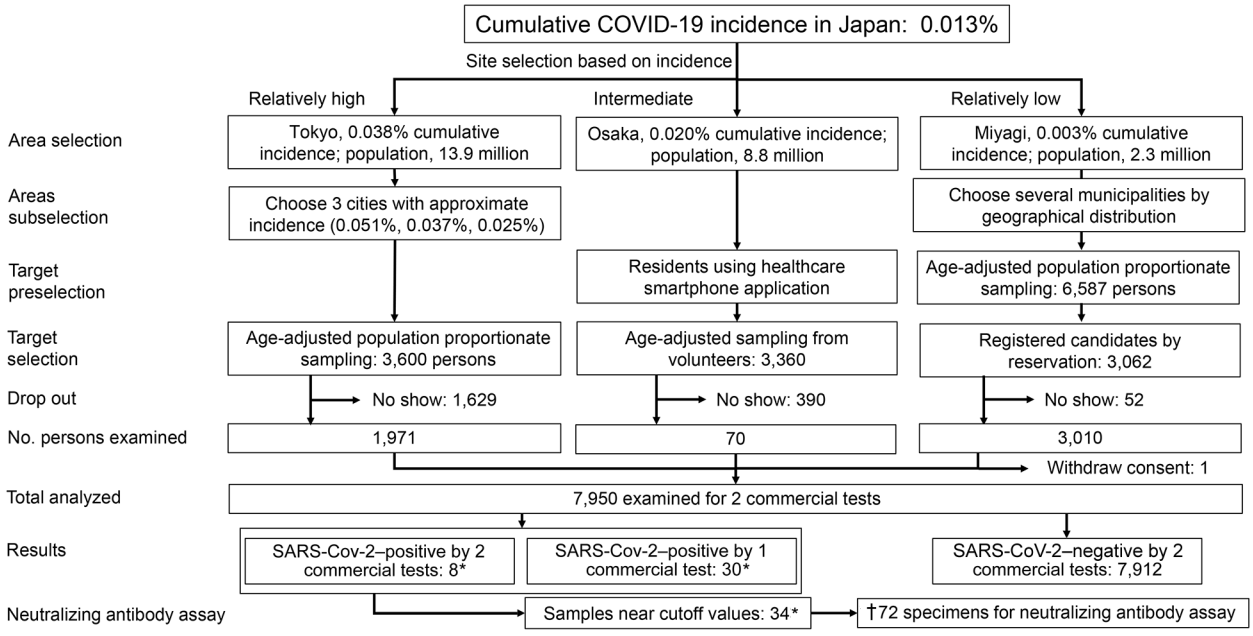

Figure. Flowchart of participants and results of SARS-CoV-2–specific antibody survey, Japan, 2020. Dagger (†) indicates sum of values marked with asterisks (*). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

qualitative detection of antibodies with 99.8% specificity and 100% (manufacturer determined) sensitivity (Elecsys Anti-SARS-CoV-2 immunoassay; F. Hoffmann-La Roche Ltd, https://www.roche.com) (*5*). Samples that were positive or borderline negative by ≥1 assay (reference range 1.20–1.39 for the Abbott test and 0.70–0.99 titer for the Roche test) were sent to Japan's National Institute of Infectious Diseases (Tokyo) for a neutralizing antibody assay with VeroE6/TMPRSS2 cells (JCRB Cell Bank accession no. JCRB1819) (*6*). For the neutralizing antibody assay, we used an in vitro cytopathic effect assay, which is more accurate than serologic tests and therefore well-suited for confirmation of results; however, only a few laboratories in Japan have the resources to conduct the assay.

We compared the 2 groups using the χ^2 test, considering values with $p<0.05$ to be significant. We compared ordinal scales by using the Mann-Whitney U test. We used Excel (Microsoft, https://www.microsoft.com) to conduct statistical analyses.

In total, 13,547 persons were invited to participate in the study; 7,950 (58.7%) accepted and gave informed consent. Of the participants, 3,660 (46.0%) were men and 4,290 (54.0%) were women. Persons 20–29 years of age (877 of 1,875 invitees) or 80–99 years of age (337 of 1,102 invitees) had the lowest response rate (Appendix Table 2). Participants from Osaka were more likely to have a history of fever within the past 4 months (2.7%) than participants from Tokyo (2.2%) and Miyagi (1.2%) (Appendix Table 1).

Of the 7,950 serum samples, 8 tested positive by both tests and 30 samples tested positive by only 1 test (15 by Abbott and 15 by Roche) (Table). All 8 specimens that were positive for both commercial tests also tested positive in the neutralizing antibody assay. No other specimens, including those that tested positive or borderline negative in 1 assay, tested positive by the neutralizing antibody assay.

The proportion of participants with 2 positive test results was significantly higher among those with fever (2.5%) than those without fever (0.05%; $p<0.001$). The proportion of participants with 1 positive test result was not significantly different among those with fever (1.2%) and those without fever (0.36%; $p = 0.25$) (Appendix Table 1). These findings, validated by the neutralizing antibody assay, indicated that 2 positive test results accurately identified seropositive participants. The proportion of participants that tested positive by both tests was 0.1% in Tokyo, 0.17% in Osaka, and 0.03% in Miyagi. The ratios of seroprevalence to cumulative incidence were 2.6 in Tokyo, 8.3 in Osaka, and 8.7 in Miyagi. Seropositivity rates were highest among participants 20–39 years of age.

Conclusions

The US Centers for Disease Control and Prevention suggests using an orthogonal testing algorithm, which considers the results of 2 independent antibody tests, in settings with low SARS-CoV-2 prevalence (*7*). Some surveys in high SARS-CoV-2 prevalence areas such as

Spain (*8*), China (*9*), and Geneva, Switzerland (*10*) have not adopted this approach. We believe an orthogonal testing algorithm, such as the one used in this study, would be particularly valuable in our low prevalence setting. The 8 specimens that tested positive by both commercial antibody assays were confirmed to have neutralizing activity against SARS-CoV-2 with a neutralizing antibody assay. These results support our use of the neutralizing assay to confirm the validity of the commercial tests. Any 2 commercial tests with high sensitivity and specificity would be appropriate to use in this orthogonal testing strategy.

Our prefecture-level seroprevalence:cumulative case detection ratios (2.6–8.7) resemble those of the United States, which are ≈10 (*11*), and are lower than those of Switzerland (≈20–50) (*10*). These results indicate that Japan has monitored the pandemic as accurately as have other countries.

This study has several limitations. First, participant selection in Osaka was based on a volunteer population (i.e., users of a particular smartphone application) rather than the general community. In addition, the prefectures of Tokyo and Miyagi excluded otherwise eligible participants with temperatures ≥37.5°C. As a result, Osaka had the highest proportion of participants with fevers at the time of the survey and the highest seroprevalence. These factors might have introduced participation bias, skewing the results. Another limita-

Table. Patient characteristics and serologic results of 2 antibody tests for severe acute respiratory syndrome coronavirus 2, Japan, June 2020*

Characteristic	Both +	Roche –, Abbott +	Roche +, Abbott –	Both –	Subtotal	% Patients positive by both tests (95% CI)
Total	8	15	15	7,912	7,950	0.10 (0.04–0.20)
Area						
Tokyo	2	2	4	1,963	1,971	0.10 (0.01–0.37)
Osaka	5	11	5	2,949	2,970	0.17 (0.05–0.39)
Miyagi	1	2	6	3,000	3,009	0.03 (0.00–0.19)
Sex						
M	3	7	5	3,643	3,658	0.08 (0.02–0.24)
F	5	8	10	4,269	4,292	0.12 (0.04–0.27)
Age, y						
20–29	3	0	0	875	878	0.34 (0.07–1.00)
30–39	3	2	1	1,210	1,216	0.25 (0.05–0.72)
40–49	0	3	7	1,589	1,599	0 (0.00–0.23)
50–59	0	2	4	1,457	1,463	0 (0.00–0.25)
60–69	1	4	0	1,315	1,320	0.08 (0.00–0.42)
70–79	1	4	1	1,128	1,134	0.09 (0.00–0.49)
≥80	0	0	2	338	340	0 (0.00–1.08)
Job setting						
Working as before	4	4	3	3,091	3,102	0.13 (0.04–0.33)
Working at home	0	3	2	432	437	0 (0.00–0.84)
Working as before and at home	1	1	5	1,974	1,981	0.05 (0.00–0.28)
Not working	3	7	5	2,410	2,425	0.12 (0.03–0.36)
No information	0	0	0	5	5	0 (0.00–52.20)
Time spent outside the home, h						
0	1	4	1	1,153	1,159	0.09 (0.00–0.48)
<2	1	5	6	2,871	2,883	0.03 (0.00–0.19)
2–4	3	5	5	1,182	1,195	0.25 (0.05–0.73)
>4	3	1	3	2,704	2,711	0.11 (0.02–0.32)
No information	0	0	0	2	2	0 (0.00–84.20)
Fever at time of study						
Yes	0	0	0	16	16	0 (0.00–20.60)
No	8	15	15	7,886	7,924	0.10 (0.04–0.20)
No information	0	0	0	10	10	0 (0.00–30.90)
History of fever lasting >4 days in past 4 months						
Yes	4	1	1	155	161	2.48 (0.68–6.24)
No	4	14	14	7,756	7,788	0.05 (0.01–0.13)
No information	0	0	0	1	1	0 (0.00–97.50)
Previous PCR result						
Positive	1	0	0	0	1	100.00 (2.50–100.00)
Negative	0	0	0	33	33	0 (0.00–10.60)
Not applicable	7	15	15	7,879	7,916	0.09 (0.04–0.18)

*Roche, Elecsys Anti SARS-CoV-2 (F. Hoffmann-La Roche Ltd, https://www.roche.com); Abbott, ARCHITECT SARS-CoV-2 IgG assay (Abbott, https://www.abbott.com); +, positive; –, negative.

tion is that Tokyo had the lowest participation of participants 20–29 years of age. Because seroprevalences were higher in younger age groups, this sampling distribution might have reduced the seropositivity rate and prevalence:cumulative incidence ratio found in Tokyo. Furthermore, this study did not include participants <20 years of age. Although patients <20 years of age make up <10% of COVID-19 cases (*1*), excluding these patients might lead to an overestimation of SARS-CoV-2 infection prevalence. Finally, antibodies against SARS-CoV-2 might disappear after 60 days (*12*); however, the elapsed time might not affect levels of nucleocapsid protein antibody (*13*). Further studies on antibody levels after disease onset and recovery are essential for monitoring the course of infections.

We estimate that SARS-CoV-2 seroprevalence ranged from 0.03%–0.17% in Japan in early June 2020. Public health officials in low prevalence areas should consider using 2 antibody tests in conjunction for accurate surveillance.

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Appendix

Sample Size Calculations

Based on an estimated 0.5% seropositive prevalence and a margin of error of 0.3%, we projected that a sample size of 2,124 participants would provide 80% power with an α error of 0.05. Each prefecture was asked to recruit 3,000 persons.

Methods for Neutralizing Antibody Assay

The cells were cultured as monolayers in Dulbecco modified Eagle medium supplemented with 5% fetal calf serum, 50 IU/mL penicillin G, and 50 μg/mL streptomycin. The SARS-CoV-2 strain 2019-nCoVJPN/TY/WK-521/2020 (GISAID ID: EPI_ISL_408667), originally isolated with VeroE6/TMPRSS2 cells from a COVID-19 infected patient, was used as the challenge virus. Serial 2-fold dilution of the test serum (from 1:5 to 1:320) and equal amounts of the prepared challenge virus (100 units of 50% tissue culture infectious dose) solution were mixed at 37°C for 1 hour, followed by the addition of 100 μL of VeroE6/TMPRSS2 cells (10^4 cells).

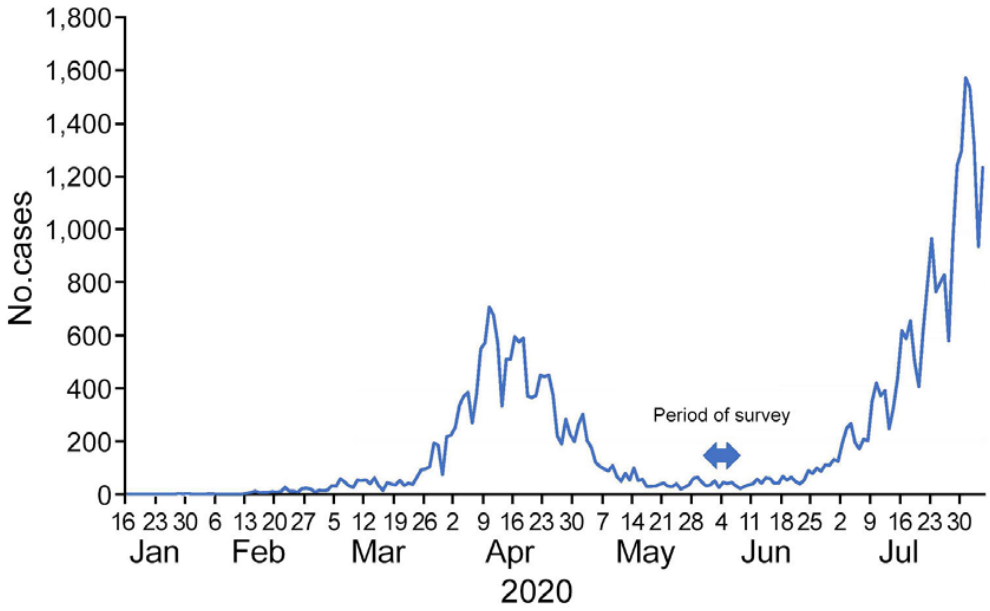
After 5 days of incubation at 37°C, the presence or absence of a cytopathic effect in each well was observed by using an inverted microscope. After formalin fixation, the cells were stained with a crystal violet solution for the final evaluation.

Appendix Table 1. Characteristics of participants in serologic survey for severe acute respiratory coronavirus 2 infection, Japan, June 2020

Characteristics	Prefecture			Subtotal
	Tokyo	Osaka	Miyagi	
Total	1,971	3,009	2,970	7,950
Work setting				
Working outside of home	510	1,181	1,411	3,102
Working at home	192	149	96	437
Working both outside and at home	685	820	476	1,981
Not working	580	819	1,026	2,425
No information	4	1	0	5
Time spent outside the home each day, h				
0	352	385	422	1,159
<2	724	986	1,173	2,883
2–4	319	504	372	1,195
>4	574	1,095	1,042	2,711
No information	2	0	0	2
Fever at time of study				
Yes	1	4	11	16
No	1,960	2,966	2,998	7,924
No information	10	0	0	10
History of fever lasting >4 days in past 4 months				
Yes	43	82	36	161
No	1,927	2,888	2,973	7,788
No information	1	0	0	1
Previous PCR result				
Positive	1	0	0	0
Negative	11	17	5	33
Not applicable	1,959	2,953	3,004	7,916

Appendix Table 2. Participants in serologic survey for severe acute respiratory coronavirus 2 infection, Japan, June 2020

Location, age range	M			F			Total		
	Invited	Participated	%	Invited	Participated	%	Invited	Participated	%
Tokyo									
20–29	292	92	31. 5	299	132	44. 1	591	224	37. 9
30–39	298	127	42. 6	301	201	66. 8	599	328	54. 8
40–49	323	172	53. 3	311	217	69. 8	634	389	61. 4
50–59	288	177	61. 5	275	196	71. 3	563	373	66. 3
60–69	251	157	62. 5	250	170	68. 0	501	327	65. 3
70–79	194	103	53. 1	201	121	60. 2	395	224	56. 7
≥80	145	54	37. 2	172	52	30. 2	317	106	33. 4
Total	1,791	882	49. 2	1,809	1,089	60. 2	3,600	1,971	54. 8
Osaka									
20–29	237	194	81. 9	249	210	84. 3	486	404	83. 1
30–39	248	219	88. 3	261	235	90. 0	509	454	89. 2
40–49	325	289	88. 9	340	308	90. 6	665	597	89. 8
50–59	287	259	90. 2	301	282	93. 7	588	541	92. 0
60–69	235	208	88. 5	246	226	91. 9	481	434	90. 2
70–79	250	214	85. 6	263	228	86. 7	513	442	86. 2
≥80	50	42	84. 0	68	56	82. 4	118	98	83. 1
Total	1,632	1,425	87. 3	1,728	1,545	89. 4	3,360	2,970	88. 4
Miyagi									
20–29	408	103	25. 2	390	146	37. 4	798	249	31. 2
30–39	500	192	38. 4	494	243	49. 2	994	435	43. 8
40–49	599	284	47. 4	579	330	57. 0	1,178	614	52. 1
50–59	502	238	47. 4	502	308	61. 4	1,004	546	54. 4
60–69	508	248	48. 8	530	314	59. 2	1,038	562	54. 1
70–79	423	226	53. 4	485	244	50. 3	908	470	51. 8
≥80	244	62	25. 4	423	71	16. 8	667	133	19. 9
Total	3,184	1,353	42. 5	3,403	1,656	48. 7	6,587	3,009	45. 7



Appendix Figure 1. Case detection of coronavirus disease, Japan, June 2020.