

## Persistence of SARS-CoV-2–Specific IgG in Children 6 Months After Infection, Australia

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The duration of the humoral immune response in children infected with severe acute respiratory syndrome coronavirus 2 is unknown. We detected specific IgG 6 months after infection in children who were asymptomatic or had mild symptoms of coronavirus disease. These findings will inform vaccination strategies and other prevention measures.

Children <18 years of age account for ≈3% of coronavirus disease (COVID-19) cases worldwide (1). Most (70%) children with COVID-19 are asymptomatic or have mild illness; very few require hospitalization (2,3). The nature and persistence of the immune response generated by children after infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, is unknown. We investigated the humoral immune response to SARS-CoV-2 in children and adults as part of a longitudinal cohort study in Melbourne, Victoria, Australia.

Nasopharyngeal swab samples of persons with suspected SARS-CoV-2 infection and their close contacts were tested by reverse transcription PCR at The Royal Children's Hospital in Melbourne during May–October 2020. We invited SARS-CoV-2–positive patients and their household members to participate in this cohort study. We collected blood samples at the

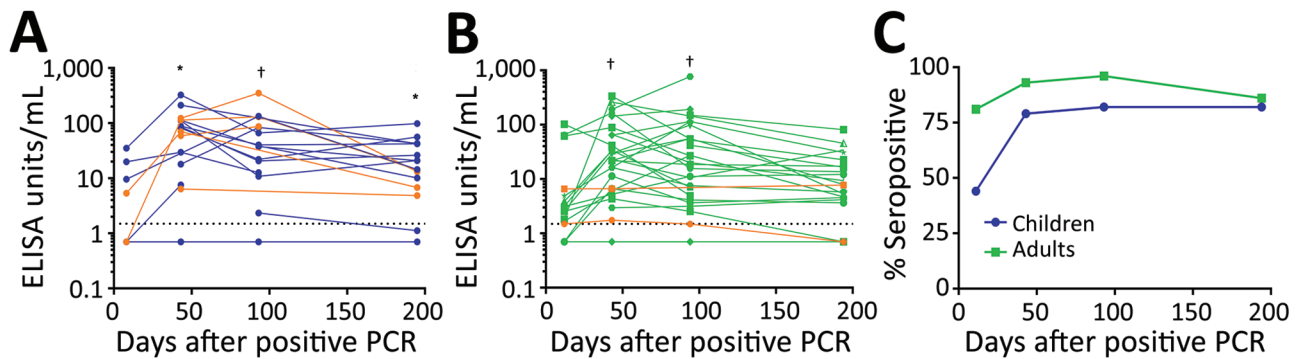
time of enrollment, as well as ≈28 days, 3 months, and 6 months later. We obtained written informed consent from parents/guardians and assent from children. The study was conducted with the approval of the Human Research Ethics Committee at The Royal Children's Hospital (approval no. HREC/63666/RCHM-2019).

To measure IgG, we used a modified 2-step ELISA based on the method described by Amanat et al. (4) and the LIAISON SARS-CoV-2 S1/S2 IgG assay (DiaSorin, <https://www.diasorin.com>). We also conducted a SARS-CoV-2 microneutralization assay on an available subset of samples. For the ELISA, we screened samples using the SARS-CoV-2 receptor-binding domain as the antigen; for potential positive samples, we confirmed results that tested positive by additional ELISA using S1 antigen. We calculated the results of S1-positive samples according to the World Health Organization SARS-CoV-2 pooled serum standard (standard provided by the National Institute for Biological Standards and Control, South Mimms, UK) and reported data as ELISA units per milliliter. We set a seropositivity cutoff at 1.5 ELISA units/mL on the basis of results of archived serum samples taken before the pandemic. We then conducted the LIAISON assay according to the manufacturer's instructions and the microneutralization assay as described by Tosif et al. (5) (Appendix, <https://wwwnc.cdc.gov/EID/article/27/8/21-0965-App1.pdf>).

During May 10, 2020–October 28, 2020, we recruited a cohort of 134 children (0–18 years of age) and 160 adults (19–73 years of age). We included only participants with a positive PCR result for SARS-CoV-2 or who were seropositive at the first timepoint (median 11 days after diagnosis, range 5–13 days) and had blood samples for ≥2 timepoints. At the first timepoint, 4 adults had negative PCR results but positive serologic results; of these adults, 3 had borderline seropositive antibody levels.

By February 2021, we had identified 54 SARS-CoV-2–positive participants: 22 children (median age of 4 years, range 0–18 years) and 32 adults (median age of 37 years, range 22–73 years). In total, 5 (23%) children and 2 (6%) adults were asymptomatic; the rest had mild symptoms, and none were hospitalized. The median duration of follow-up after diagnosis was 195 days (range 188–213 days) for children and 194 days (range 183–212 days) for adults.

By day 43 (range 27–79), 15/19 (79%) children and 26/28 (93%) adults had seroconverted. These participants remained seropositive for ≥90 days (Figure, panels A, B). By day 195 (≈6 months), 14/17 (82%) of children and 18/21 (86%) of adults were seropositive; however, from day 43 to 195, geometric mean antibody



**Figure.** Persistence of IgG responses against severe acute respiratory syndrome coronavirus 2 in children and adults, Australia, 2020–2021. Patients tested positive by PCR, ELISA, or both. A) Antibody responses of 22 children 0–18 years of age. B) Antibody responses of 32 adults 22–73 years of age. Orange points and lines indicate asymptomatic cases; blue points and lines indicate symptomatic cases in children; green points and lines indicate symptomatic cases in adults. Dotted lines indicate seropositivity cutoff. C) Seropositivity rates in 22 children and 32 adults. Blue points and lines indicate all children, regardless of symptoms; green points and lines indicate all adults, regardless of symptoms. \* $p < 0.05$ ; † $p < 0.01$  (compared with the first timepoint [day 11]).

concentration decreased  $\approx 2$ -fold in both groups (Figure, panel C). We observed no significant differences in geometric mean antibody concentration from day 43 (range 27–79) to day 194 (range 183–212), nor from 93 (range 27–79) to day 194 (range 183–212), for either children or adults (Figure, panels A, B). The seropositivity and antibody levels were also not significantly different between children and adults at all timepoints (Figure 1, panel C; Appendix Figure 1). Seropositive samples defined by our in-house ELISA correlated with results from the LI-AISON assay and neutralizing antibody assay (Appendix Figures 2, 3). In total, 4/19 (21%) children and 2/28 (7%) adults did not seroconvert; however, we could not rule out other SARS-CoV-2-related immune responses, such as cellular or mucosal mechanisms (5,6).

We found that, similar to the adults in this cohort and those in previous studies (7,8), SARS-CoV-2-positive children with no or mild symptoms mounted strong and durable humoral responses that persisted for  $\geq 6$  months. Our study was limited by the relatively small sample size; in addition, only a subset of samples was available for the microneutralization assay. In conclusion, our data indicate that SARS-CoV-2-positive children have a persistent antibody response for  $\geq 6$  months. The roles and durations of other components of the immune system (such as the cellular and mucosal responses) during SARS-CoV-2 infection remain undetermined. These results will inform vaccination strategies and other public health measures.

### Acknowledgments

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Dr. Toh is a research officer based at the Murdoch Children's Research Institute, Melbourne, Victoria, Australia. His primary research interests include infectious diseases, immunology, and vaccinology.

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## COVID-19 and the Consequences of Anchoring Bias

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Suspicion of coronavirus disease in febrile patients might lead to anchoring bias, causing misdiagnosis of other infections for which epidemiologic risks are present. This bias has potentially severe consequences, illustrated by cases of human granulocytic anaplasmosis and Lyme disease in a pregnant woman and human granulocytic anaplasmosis in another person.

Coronavirus disease (COVID-19) took the United States by force during the first quarter of 2020, affecting the economy, societal norms, and the delivery of medical care (1,2). As fear of COVID-19 has spread, diagnosing COVID-19 in febrile persons has been prioritized, and patients may be presumed to have COVID-19 pending results of testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This mindset has had unintended consequences, including delaying evaluations for other infectious diseases, potentially leading to adverse outcomes. We describe 2 cases that illustrate this point.

In the first case, a 35-year-old man left New York, New York, USA, to go hiking in Maryland during June 5–June 7, 2020. He experienced fever, body aches, and fatigue during June 10–13 that resolved but left him fatigued and weak. He was seen on June 19; laboratory results were unremarkable, but lymphopenia was detected. He tested negative for SARS-CoV-2 on June 19 and June 25 by PCR. On June 25, ELISA for Lyme disease was positive, and reflex to Western blot revealed IgM 41-kD, 39-kD, and 23-kD bands but no IgG bands. Fever up to 38°C recurred on June 22 and lasted until June 29; he also experienced persistent fatigue and myalgia. Further testing on July 6 revealed serologic results for Lyme similar to results from June 25 and *Anaplasma phagocytophilum* titers of IgM 1:320 and IgG 1:1260. *Anaplasma* PCR was negative on that date. He was treated with doxycycline for 10 days and recovered.

In the second case, a 31-year-old woman who was 6 months pregnant left New York at the end of May 2020 to rent a house in Ulster County, New York. On June 3, she removed a tick from her neck. On June 9, she experienced severe headaches and the next day had low-grade fever, chills, and body aches. She had no cough, shortness of breath, or sore throat. On June 10, she tested negative for SARS-CoV-2 by PCR. She continued to have extreme fatigue, myalgia, and low-grade fever. She was prescribed oseltamivir by her obstetrician on June 11. On June 14, she felt better. Repeat PCR testing for SARS-CoV-2 on June 15 was negative. She continued to improve until June 23, when she experienced recurrent fever up to 38.9°C, chills, and lethargy. She contacted her obstetrician and was told she had

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## Appendix

### Appendix Methods

#### ELISA

We used a modified 2-step ELISA based on the method described by Amanat et al. (4) but used S1 instead of the trimeric spike protein to determine the antibody response against SARS-CoV-2. This assay has 100% specificity and 93% sensitivity for SARS-CoV-2 antibodies and was granted Emergency Use Authorization from the US Food and Drug Administration (Reference 1 in Appendix). Briefly, 96-well high binding plates (Thermo Fisher Scientific, <https://www.thermofisher.com>) were coated with SARS-CoV-2 receptor-binding domain (RBD) or S1 (Sino Biological Inc., <https://www.sinobiological.com>) diluted in phosphate-buffered saline (PBS) at 2 µg/mL and then incubated at 4°C overnight. The next day, plates were washed with PBS containing 0.1% (v/v) Tween20 (PBS-T)(MP Biomedicals, <https://www.mpbio.com>) and blocked with PBS containing 0.1% Tween and 10% (w/v) skim milk (PBS-TSM) for 1 h at room temperature (RT). Serum samples prepared in PBS-TSM were first screened at 1:50 dilution in duplicates, and potential seropositive samples were then confirmed with either RBD or S1 titration assay starting at 1:80 with 4-fold serial dilutions. The blocking solution was removed and 50 µl of each serial dilution was added to the plates for 2 h at RT. The plates were then washed 3 times with 200 µl per well of PBS-T. Goat anti-human IgG horseradish peroxidase conjugated secondary antibody (Southern Biotech, <https://www.southernbiotech.com>) was prepared in PBS-TSM (1:10,000), and 50 µl of this secondary antibody was added to each well for 1 h. Plates were washed with PBS-T followed by distilled water and 50 µL of 3,3', 5,5'-tetramethylbenzidine (SeraCare, <https://www.seracare.com>) substrate solution was added for 9 min. The reaction was stopped by the addition of 50 µL of 1M phosphoric acid (Sigma Aldrich, <https://www.sigmaaldrich.com>) and optical densities measured using a microplate reader (BioTek Instruments, Inc., <https://www.biotek.com>) at 450 nm (630 nm reference filter).

Seropositive samples were titrated and calculated based on a World Health Organization SARS-CoV-2 pooled serum standard obtained from the National Institute of Biologic Standards and Controls, United Kingdom. Samples with optical density readings (at 450 nm) that exceeded a cutoff of 0.5 units based on the RBD screening assay (based on 40 pre-pandemic sera) were considered to be potentially positive and were subjected to sample titration using S1 protein. Seropositive cutoff for the confirmatory assay was set at 1.5 ELISA Units/mL. Seronegative samples from the screening assay were assigned half of the seropositive cutoff value.

#### **Liaison SARS-CoV-2 S1/S2 IgG Assay**

The quantitative commercial assay for the detection of IgG antibodies against S1/S2 antigens of SARS-CoV-2 was performed according to the manufacturer instructions (LIAISON SARS-CoV-2 S1/S2 IgG assay; DiaSorin, <https://www.diasorin.com>). Data was reported as Assay Units/mL. Seronegative samples by Diasorin were assigned half the cutoff value (<12 AU/mL).

#### **SARS-CoV-2 Microneutralisation Assay**

SARS-CoV-2 isolate CoV/Australia/VIC01/202027 passaged in Vero cells was stored at  $-80^{\circ}\text{C}$ . Serial 2-fold dilutions of heat-inactivated plasma were incubated with 100 50% tissue culture infectious dose of SARS-CoV-2 for 1 h and residual virus infectivity was assessed in quadruplicate wells of Vero cells; viral cytopathic effect was read on day 5. The neutralizing antibody titer is calculated using the Reed/Muench method (Reference 2 in Appendix).

#### **Statistical Analysis**

The S1-specific IgG antibody levels between children and adults, as well as between each timepoint within children or adult were compared using Mann-Whitney U test. For correlation analysis, antibody titers and concentrations were log-transformed and analyzed using Pearson's correlation analysis. Fisher exact test was used to compare the seropositivity rate. All analyses were performed with GraphPad Prism version 7.0 (GraphPad Software, <https://www.graphpad.com>). A  $p < 0.05$  was considered significant.

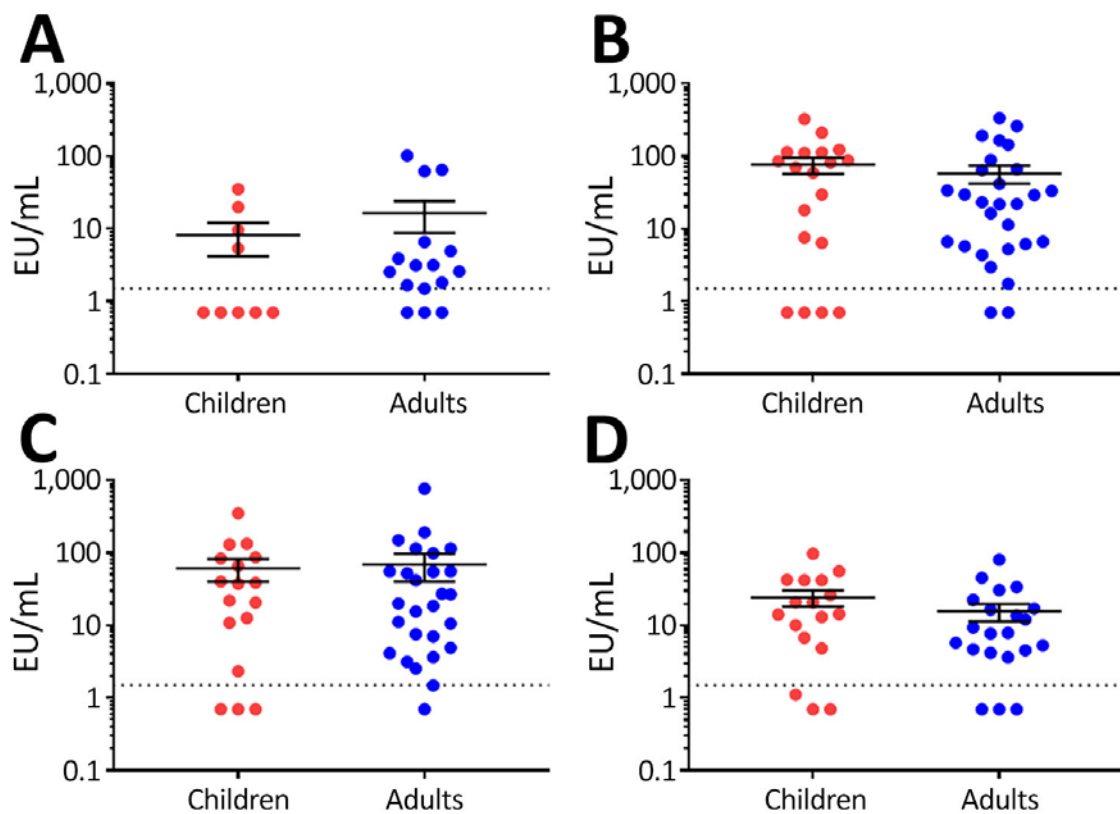
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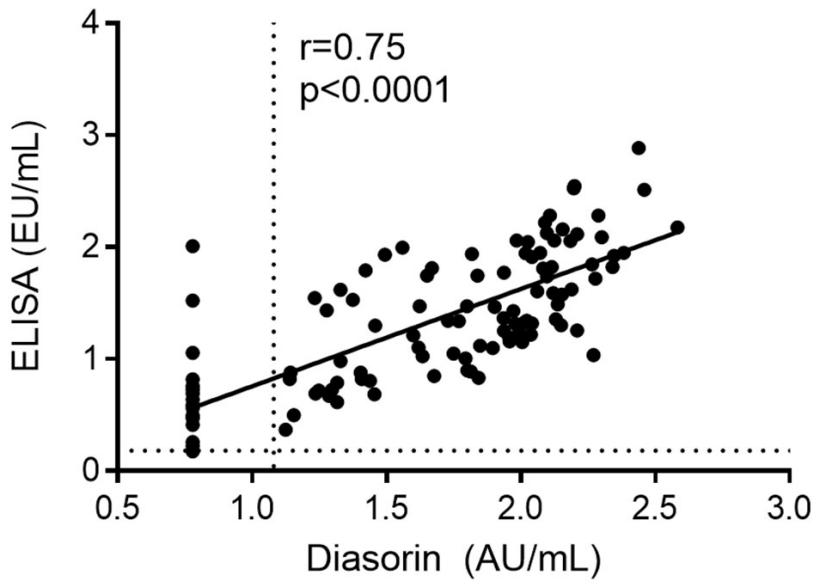


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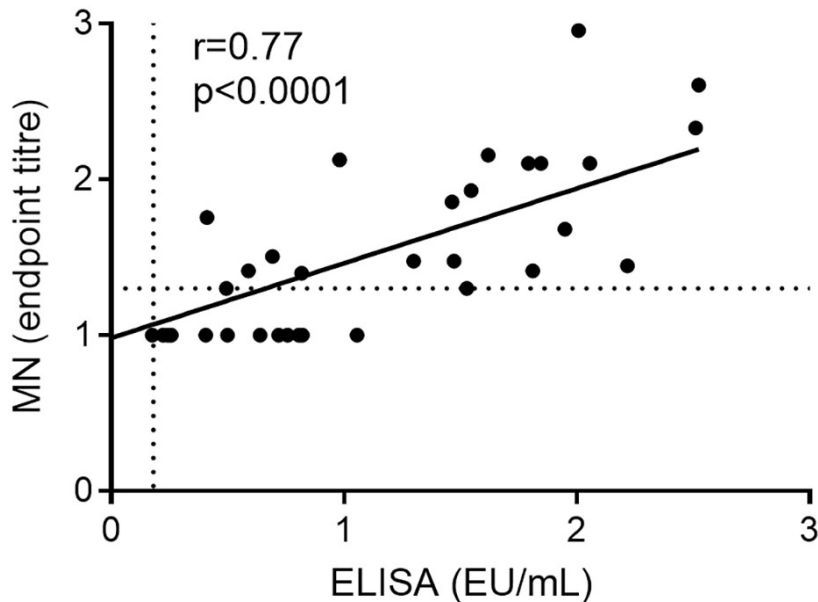
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**Appendix Figure 1.** Comparison of IgG responses against severe acute respiratory syndrome coronavirus 2 between children and adults at each timepoint, Australia, 2020–2021. Dotted lines indicate seropositivity cutoff. EU, ELISA units.



**Appendix Figure 2.** Correlation between log-transformed results of 2-step ELISA and LIAISON SARS-CoV-2 S1/S2 IgG assay (DiaSorin, <https://www.diasorin.com>) using Pearson's correlation analysis, Australia, 2020–2021. Data shown for 113 samples from 53 persons. Horizontal dotted lines indicate seropositivity cutoffs for Diasorin; vertical dotted lines indicate seropositivity cutoffs for ELISA. AU, assay units; EU, ELISA units.



**Appendix Figure 3.** Correlation between log-transformed results of in-house ELISA and microneutralization assay for severe acute respiratory syndrome coronavirus 2 using Pearson's correlation analysis, Australia, 2020–2021. Paired data shown for 47 samples. Horizontal dotted lines indicate seropositivity cutoffs for neutralization assay; vertical dotted lines indicate seropositivity cutoffs for ELISA. EU, ELISA units; MN, microneutralization.