PAEDS Paediatric Active Enhanced Disease Surveillance An NCIRS led collaboration

# PAEDS COVID-19 serosurveillance 2023: Final report

## A national paediatric serosurvey of Omicron subtypes undertaken at hospitals within the PAEDS network

**Prepared by the National Centre for Immunisation Research and Surveillance (NCIRS)**

**20 August 2024**


### Summary

- This third paediatric national SARS-CoV-2 serosurvey conducted in Australian children and adolescents was done in the eight PAEDS network sites between 1 November 2023 and 5 December 2023 and sampled children and adolescents aged 0–15 years.
- Serum antibodies specific to SARS-CoV-2 spike variants (Ancestral, BA.2.86, BA.5, EG.5.1, FL.1.5.1, XBB.1.5, XBB.1.16, XBB.1.16.6, XBB.2.3) and ancestral N-antibody protein were measured with the Meso Scale Discovery (MSD) V-PLEX SARS-CoV-2 Panel 37 kit (Rockville, MD, USA).
- Cut-off values for spike (S) antibody positivity and nucleocapsid (N) antibody were determined using sera from participants prior to the onset of the pandemic (pre-pandemic cut-off). In addition, cut-off values were also determined for nucleocapsid antibody using samples tested for the paediatric serosurvey in 2022 on the Roche Elecsys assay.
- From 1,065 samples in children and adolescents aged 0–15 years, the estimated crude seroprevalence of SARS-CoV-2 ancestral S-antibody (using a pre-pandemic cut-off) was 94.4% (95% CI 92.8–95.6%; 1,005/1,065) and for ancestral N-antibody was 87.3% (95% CI 87.3–85.2%; 930/1,065) using the pre-pandemic cut-off and 63.4% (95% CI 60.5–66.3%; 676/1,065) using the Roche Elecsys cut-off.
- These ancestral spike seroprevalence estimates were similar across all jurisdictions across Australia and were similar to the second PAEDS seroprevalence estimates determined in 2022 (92.1 % (95% credible interval (CrI) 91.0–93.3%). Crude seroprevalence of Omicron subvariant S-antibodies were all high (>94%) and taken together were similar to the crude seroprevalence of ancestral S-antibody.
- Geometric mean concentrations (GMC) of ancestral and Omicron subvariant S-antibodies increased with age and vaccination doses, but nucleocapsid antibody GMC only increased with age, not with increasing vaccination dose receipt.
- A lower GMC was found in children aged 0–6 months compared to children aged 6–12 months, likely representing maternal antibody with postnatal decay over time.
- These data provide important insights into the population seroprevalence and immunity profile in relation to infection with a variety of SARS-CoV-2 variants and to vaccination in the paediatric population from 2020 to 2023.

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# PAEDS investigators, staff and serosurvey collaborators

| Name | Organisation |
|---|---|
| Dr Archana Koirala | National Centre of Immunisation Research and Surveillance and The Children's Hospital at Westmead |
| Prof Kristine Macartney | |
| A/Prof Phillip Britton | |
| Prof Nicholas Wood | |
| A/Prof Justin Skowno | |
| Ms Emma Carey | |
| Ms Shayal Prasad | |
| Dr Joanne Jackson | |
| Mr Ajay Jadhav | |
| Ms Fatima Gondalwala | |
| Ms Rosemary Joyce | |
| Ms Katheryn Meredith | |
| Prof Peter Richmond | Telethon Kids Institute – Vaccine Trials Group and Perth Children's Hospital |
| Dr Ushma Wadia | |
| Prof Christopher Blyth | |
| Prof Britta S von Ungern-Sternbeg | |
| Ms Carolyn Finucane | |
| Dr Christian Tjiam | |
| Dr Ruth Thornton | |
| Ms Zoe Ellis | |
| Ms Chantalia Tedja | |
| Ms Shania Tansil | |
| Ms Nina D'vaz | |
| Ms Cathy Pienaar | |
| Ms Erin van der Helder | |
| Ms Joanne Harvey | |
| Dr Brenden McMullan | The Sydney Children's Hospital, Randwick |
| Dr Adam Barlett | |
| Dr Shona Chung | |
| Dr Phoebe Williams | |

| Name | Organisation |
|---|---|
| Dr Jeremy Carr | Monash Children's Hospital |
| Dr Joe Speakman | |
| Ms Guillian Hunter | |
| Ms Annalisa Shine | |
| Ms Mai Khuu | |
| Prof Nigel Crawford | The Royal Children's Hospital |
| Prof Andrew Davidson | |
| Ms Alissa McMinn | |
| Ms Lauren Weston | |
| Ms Abigail Fernado | |
| Ms Jill Nguyen | |
| Ms Klara Glavacevic | |
| A/Prof Julia Clark | Queensland Children's Hospital |
| Dr Paul-Lee Archer | |
| Ms Sonia Dougherty | |
| Ms Sara Cook | |
| Ms Natsha Doran | |
| Ms Anjalee Panditha | |
| Ms Selina Ward | |
| Prof Helen Marshall | Women's and Children's Hospital |
| Dr Laura L Burgoyne | |
| Ms Natascha D'Angelo | |
| Ms Donna Reuterink | |
| Ms Mary Walker | |
| Dr Joshua Francis | Royal Darwin Hospital |
| Ms Laura Francis | |
| Dr Edith Waugh | |
| Prof William Rawlinson | NSW Health Pathology, The Prince of Wales Hospital |
| Dr Zin Naing | |
| Ms Malinna Yeang | |
| Dr Marnie Downes | Murdoch Children's Research Institute |

# Glossary

| | |
|---|---|
| ACT | Australian Capital Territory |
| ARIA+ | Accessibility/Remoteness Index of Australia Plus |
| ATAGI | Australian Technical Advisory Group on Immunisation |
| AU/mL | arbitrary units per millilitre |
| BAU/mL | binding antibody units per millilitre |
| COVID-19 | coronavirus disease 2019 |
| CI | confidence interval |
| CrI | credible interval |
| GMC | geometric mean concentration |
| IgG | immunoglobulin G |
| IRSD | Index of Relative Socio-economic Disadvantage |
| MSD | Meso Scale Discovery Platform |
| N-antibody | nucleocapsid antibody |
| NSW | New South Wales |
| NT | Northern Territory |
| OWS | Operation Warp Speed |
| PAEDS | Paediatric Active Enhanced Disease Surveillance |
| Qld | Queensland |
| REDCap | Research Electronic Data Capture Software |
| RPM | revolutions per minute |
| SA | South Australia |
| SARS-CoV-2 | severe acute respiratory syndrome coronavirus 2 |
| S-antibody | spike antibody |
| SEIFA | Socio-Economic Indexes for Areas |
| Vic | Victoria |
| VOC | variant of concern |
| VTG | Vaccine Trials Group |
| WHO | World Health Organization |

# Introduction

SARS-CoV-2 Omicron variant began to circulate in late 2021 and was designated a variant of concern (VOC) by the World Health Organization.[1] Since its emergence, more than 30 mutations in the spike protein of the Omicron variant have been detected.[2] Understanding SARS-CoV-2 Omicron subvariant population-level infection rates is important to inform infection-related risk and contextualise rates of severe outcomes, such as hospitalisation and death.

Children, in particular, are subject to under-reporting of infections due to higher rates of asymptomatic or mild infections and difficulties in testing. Serosurveillance, which measures serum antibodies in individuals within a population, can give insights into the cumulative prevalence of infection and/or vaccine uptake over time across a population.

In 2020, we conducted a national cross-sectional serosurvey to collect sera from children and adolescents undergoing an anaesthetic procedure in eight Australian paediatric tertiary hospitals, across the Paediatric Active Enhanced Disease Surveillance (PAEDS) network. We reported a low seroprevalence of SARS-CoV-2 spike antibody (S-antibody) (<0.60%) in children aged 0–19 years prior to the Delta variant outbreak.[3] A serosurvey was repeated in 2022, after the emergence of the Omicron variant, vaccine rollout and easing of public health restrictions in Australia, which reported an S-antibody seroprevalence of 84.2% and a nucleocapsid antibody (N-antibody) seroprevalence of 67.1% in unvaccinated children[4] (Table 1).

Recent SARS-CoV-2 infection rates in children post Omicron subvariant outbreaks are unknown as many children have asymptomatic or mild infection and are not tested. Wide capability of self-testing using rapid antigen tests has also meant that there is limited onward reporting occurring in those who are SARS-CoV-2 positive. No correlate of protection against infection or severe disease exists for SARS-CoV-2 but studies have suggested that higher concentration of neutralising antibodies[5] have been associated with lower risk of infection. Neutralisation studies are laborious and cost limiting to do in a serosurvey, and a validation study of the MSD assay in adults showed a strong correlation between the presence of neutralising activity and the amount of antibodies against the spike proteins in sera from both convalescent and vaccinated individuals.[6]

In this third national paediatric SARS-CoV-2 serosurvey, we aimed to estimate the levels of antibodies against the SARS-CoV-2 spike protein from both ancestral and variant strains, as well as the N-antibody, to explore the extent and level of antibody immunity among Australian children aged 0–16 years.

***Table 1:*** *Prior paediatric SARS-CoV-2 serosurveys conducted through the PAEDS network and the impact of these serosurveys*[3,4]

| Collection | Seroprevalence | Impact |
|---|---|---|
| **Nov 2020–Mar 2021**<br>Assay: WANTAI SARS-CoV-2 total Ab ELISA | **S-**antibody: 0.23% (95% credible interval [CrI], 0.06–0.57%). | Understanding that silent, unrecognised infection was not occurring in children<br><br>No population immunity |
| **June–Aug 2022**<br>Assay: Roche Elecsys Anti-SARS-CoV-2 | **S-**antibody: 92.1% (95% CrI 91–93.3%)<br>**N-**antibody: 67.0% (95% CrI 64.6–69.3%)<br><br>*Unvaccinated children:*<br>**Spike** antibody:<br>84.2% (95% CrI 81.9–86.5%)<br>**Nucleocapsid** antibody:<br>67.1% (95% CrI 64.0–69.8%) | Majority of children had been infected<br><br>Contributed to the national policy against universally vaccinating children <5 years |

# Methods

## Study population, locations and recruitment

Children and adolescents aged 0–16 years were invited to participate in this serosurvey prior to undergoing an elective surgical procedure requiring general anaesthesia at one of eight paediatric tertiary referral hospitals within the Paediatric Active Enhanced Disease Surveillance Network (PAEDS) across six of eight Australian jurisdictions.

Blood collection occurred during intravenous cannulation following anaesthetic induction.

**PAEDS recruitment sites**

1. The Children's Hospital at Westmead, Sydney, New South Wales
2. Sydney Children's Hospital Randwick, Sydney, New South Wales
3. Queensland Children's Hospital, Brisbane, Queensland
4. The Royal Children's Hospital, Melbourne, Victoria
5. Monash Medical Centre, Melbourne, Victoria
6. Women's and Children's Hospital, Adelaide, South Australia
7. Perth Children's Hospital, Perth, Western Australia
8. Royal Darwin Hospital, Darwin, Northern Territory

Children who were immunosuppressed or receiving intravenous immunoglobulin were excluded due to concerns about mounting an antibody response to infection, or detecting circulating donor antibody.

A questionnaire was administered to obtain demographic information (age, sex, Indigenous status, postcode of residence), history and timing of known SARS-CoV-2 infection, date of vaccination dose 1 and 2, and underlying medical conditions. An additional questionnaire was administered if infants were <1 year of age to ask about history of maternal SARS-CoV-2 infection and vaccination prior to delivery.

## Consent

Informed consent was provided by parents/guardians of children aged ≤16 years via a Research Electronic Data Capture (REDCap) survey for the purpose of a blood sample to be collected for subsequent serological testing of SARS-CoV-2 antibodies. In addition, assent was provided by adolescents aged ≥12 years.

## Sample size

The planned sample size, based on binomial sampling, was calculated to 385 samples per age group (0–4 years, 5–11 years and 12–15 years) to achieve 95% confidence intervals (CIs) of no more than +/– 5% (Table 2).

To obtain nationally representative estimates of seroprevalence by age group (0–4 years, 5–11 years and 12–15 years), the number of specimens to be tested is required to be proportionate to the population distribution across the six states and territories of Australia where testing will occur (NSW, Vic, Qld, SA, WA, NT) (Table 2). Of note, there are no PAEDS sites in Tasmania or ACT; however, we feel estimates are nonetheless reasonably nationally representative.

This sample size was *adjusted from* the sample size calculated and stated in the original methods described in the Australian Government Department of Health and Aged Care contract due to the following:

1. We found through the 2022 PAEDS serosurvey that the majority of children across Australia have been infected with SARS-CoV-2. We also found that children were uniformly infected across all jurisdictions and so a jurisdictional estimate would no longer prove insightful or necessary.
2. Collecting sera from children is labour intensive and difficult to do. As high S-antibody seroprevalence has been demonstrated, repeating a serosurvey to redemonstrate this would not provide further insight into our understanding of paediatric population immunity. Instead, at this stage of the pandemic we felt that better understanding of Omicron variant specific immunity across multiple Omicron variants in vaccinated and unvaccinated children would provide guidance on:
    a) breadth of immunity across Australian children by age and vaccination status
    b) insight into maternal antibody decay in infants.

***Table 2:*** *Sample sizes calculated to be tested in each jurisdiction to obtain estimates of overall nationally representative seroprevalence for each age group**

| Age group (years) | New South Wales | Victoria | Queensland | South Australia | Western Australia | Northern Territory | Total (six jurisdictions) |
|---|---|---|---|---|---|---|---|
| 0–4 | 127 | 101 | 81 | 26 | 45 | 5 | 385 |
| 5–11 | 125 | 101 | 84 | 26 | 45 | 4 | 385 |
| 12–15 | 125 | 99 | 86 | 27 | 44 | 4 | 385 |
| Total sample size | 377 | 301 | 251 | 79 | 134 | 13 | 1,155 |

**Using these sample sizes will not allow individual state and territory estimates of seroprevalence to be calculated. If any of the age groups are stratified further using the same overall sample size, degree of precision will be reduced. The calculated sample sizes have not considered the comparison of seroprevalence estimates from the previous PAEDS SARS-CoV-2 serosurvey.*

## Serological testing

Blood samples obtained from venepuncture were centrifuged at each site and sera were separated and extracted. The serum samples were coded and de-identified before sending to Telethon Kids Institute Vaccine Trials Group (VTG), Perth, Western Australia for testing.

The VTG conducted serology testing on collected study specimens. Testing was conducted using the Meso Scale Discovery Platform (MSD): an electrochemiluminescent multiplex assay allowing users to assay a range of analytes in complex sample matrices with low sample volumes. The Reference Standard 1 in V-PLEX Serology kits is calibrated against the WHO International Standard (NIBSC code: 20/136), and results from the V-PLEX Serology Panels can be reported in international units (BAU/mL). The V-PLEX SARS-CoV-2 Panel 2 was chosen by Operation Warp Speed (OWS) as the basis of its standard binding assays for immunogenicity assessments in all funded Phase III clinical trials of vaccines.[7]

This assay was chosen in preference to the Roche Elecsys Anti-SARS-CoV-2 assay (used in 2022 serosurvey, measures total immunoglobulin) as it would provide a cost-effective method of determining seroprevalence of antibodies to recently circulating Omicron sublineages. A comparison of ancestral spike and nucleocapsid Roche Elecsys, MSD and Abbott assays were performed on 329 adult participants with known SARS-CoV-2 infection. The MSD spike assay had a 97% survival probability at 200 days (95% CI

95–99%) compared to the Roche Elecsys spike assay (95% survival probability at 200 days, 95% CI 93–97%). The Roche nucleocapsid assay had a survival probability at 150 days of 95% (95% CI 92–97%) compared to the MSD nucleocapsid assay survival probability of 91% (95% CI 87%–94%). Both were superior to the Abbott-N assay with a survival probability at 150 days of 66% (60–71%).[8]

The V-PLEX SARS-CoV-2 Panel 37 (IgG) Kit was used to measure IgG antibodies specific to N-antibody and S-antibody protein components of the SARS-CoV-2 virus, in both wildtype and Omicron variants.

Additional analysis was conducted to determine comparable serostatus reference values to that of the Roche Elecsys Anti-SARS-CoV-2 N-antibody and S-antibody assays. Pre-pandemic samples, as well as seropositive and negative samples as predetermined by the Elecsys Anti-SARS-CoV-2 N assay, were used to establish cut-off values for the MSD readouts. Analysis comparing antibody titres between each cohort determined N-antibody cut-off values of 1,000 AU/mL to readily distinguish seropositivity in both paediatric and adult cohorts.

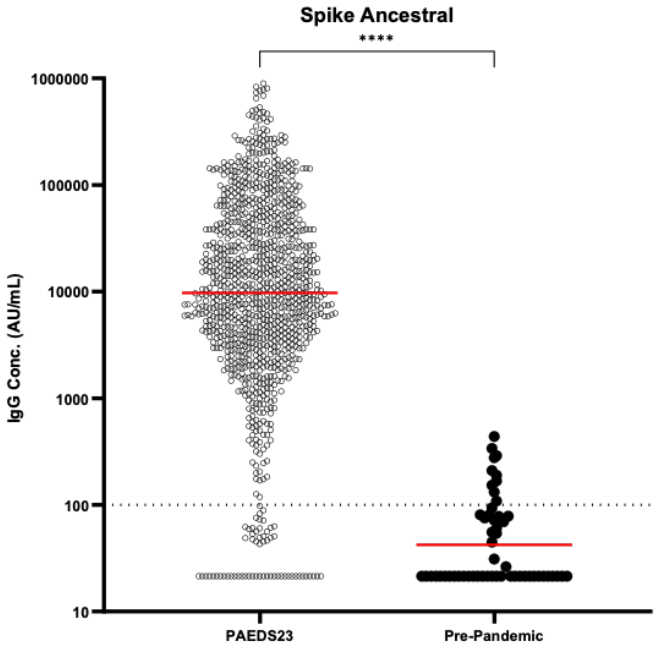
**Quantification of SARS-CoV-2 IgG with multiplexed electrochemiluminescence immunoassay**

Serum antibodies specific to SARS-CoV-2 spike variants (Ancestral, BA.2.86, BA.5, EG.5.1, FL.1.5.1, XBB.1.5, XBB.1.16, XBB.1.16.6, XBB.2.3) and ancestral-N-antibody protein were measured with the Meso Scale Discovery (MSD) V-PLEX SARS-CoV-2 Panel 37 kit (Rockville, MD, USA) according to the manufacturer's instructions.

Briefly, MSD plates were allowed to equilibrate to room temperature for 15 minutes and then removed from their packaging. MSD Blocker A solution was added to all wells of the MSD plates and incubated for 30 minutes at room temperature on an orbital shaker (700 rpm). Plates were washed three times with 1X MSD Wash Buffer. Standards were prepared by serially diluting MSD Reference Standard 1 (A0080286) four-fold in MSD Diluent-100, starting at neat and ending at 1/16,384, and added to each plate in duplicate. Serology controls 1.1 (A00C0825), 1.2 (A00C0826) and 1.3 (A00C0827) were added to plates undiluted to serve as internal controls. Serum samples were assayed at 1/5,000 dilution, and those that fell out of range of the standard curve were repeated at dilutions of 1/1,000 or 1/50,000. Standards, controls and samples were incubated for 2 hours at room temperature on an orbital shaker (700 rpm). Plates were washed three times with 1X MSD Wash Buffer and Sulfo-tagged anti-human IgG diluted 1/200 was added to all wells for 1 hour at room temperature on an orbital shaker (700 rpm). MSD plates were washed three times with 1X MSD Wash Buffer and then MSD GOLD Read Buffer B was added to all wells. An electrical current was applied to the plate to initiate electrochemiluminescence and the signal was recorded on a MESO SECTOR S 600MM plate reader with Methodical Mind software V1.0.38. Data were exported as text files and processed in MSD Workbench Software V4.0 to calculate IgG concentrations.

**Quantification of N-antibody and S-antibody seropositivity cut-offs using pre-pandemic paediatric sera**

Pre-pandemic sera collected before 2019 from children aged 1–16 years (n=50; collected between February 2004 and August 2009) were assayed with MSD Panel 37. In addition, cord bloods (n=5; October 2018–August 2019) were assayed on MSD Respiratory Panel 1. Geometric mean concentrations and corresponding 99% confidence intervals (CI) for N-antibody and ancestral S-antibody IgG were calculated in these samples and kits. A concentration ≥200 AU/mL for MSD N-antibody IgG and ≥100 AU/mL for MSD ancestral and Omicron sublineage S-antibody IgG were deemed as seropositive according to upper 99% CI values of pre-pandemic cohorts calculated for each assay (Figure 1, Table 3).




*GMC is represented by the red line; the black dotted line is the determined cut-off value.*

***Figure 1:*** *Serum positivity cut-offs for ancestral spike antibody determined using pre-pandemic samples*

***Table 3:*** *Determination of the Omicron subvariant seropositivity cut-off using pre-pandemic samples*

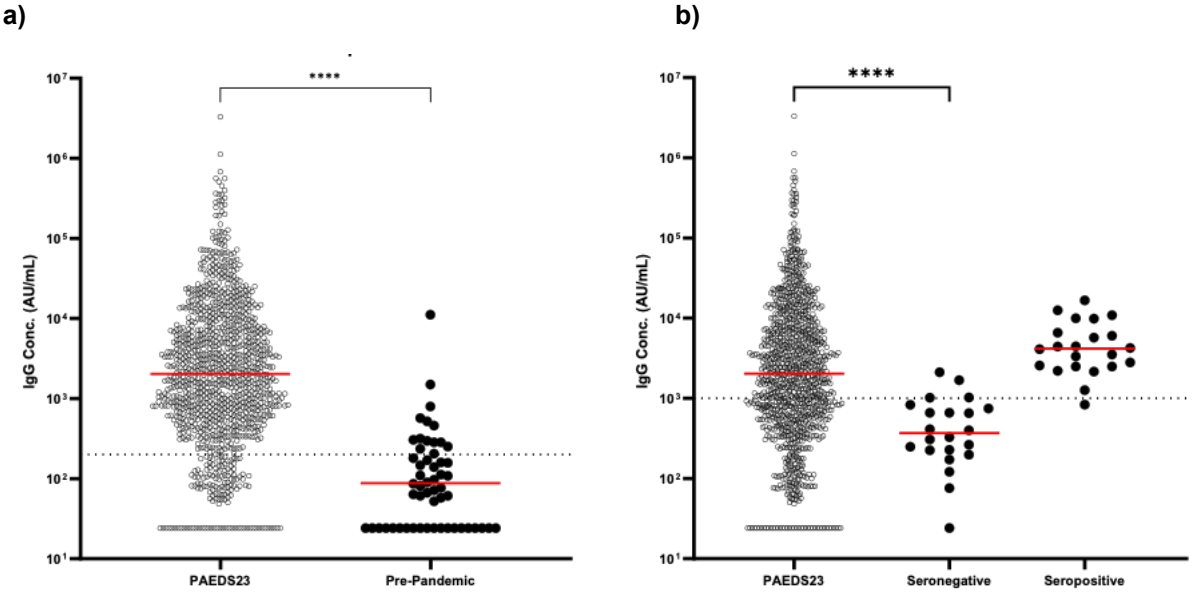
| Variant | Seropositivity cut-off |
|---|---|
| | Value (AU/mL) |
| **Spike (Ancestral) *** | 100 |
| **Spike BA.5** | 50 |
| **Spike XBB.1.5** | 40 |
| **Spike FL.1.5.1** | 40 |
| **Spike XBB.2.3** | 50 |
| **Spike XBB.1.16** | 50 |
| **Spike XBB.1.16.6** | 40 |
| **Spike EG.5.1** | 40 |
| **Spike BA.2.86** | 30 |

**A total of 55 pre-pandemic (n=50 serum and n=5 cord blood) samples were tested for ancestral spike. For all remaining variants a total of 50 sera were tested. Variants ordered from top to bottom according to their global emergence (oldest to newest).*

**Quantification of N-antibody seropositivity cut-offs with independent cohorts previously analysed with Elecsys SARS-CoV-2 N-antibody assays (Roche)**

Sera collected from children (aged 0–18 years) who participated in the 2022 PAEDS serosurvey (n=24) and adults (aged 50–70 years) from the Platform Trial in COVID-19 Boosting (PICOBOO; n=20) were tested on MSD SARS-CoV-2 Panel 37 kit. A total of 12/24 samples in the PAEDS22 cohort and 20/20 samples in PICOBOO were previously determined as seronegative for Elecsys SARS-CoV-2 N-antibody IgG assay (Roche). For the Elecsys SARS-CoV-2 S-antibody IgG assay, 3/24 samples in the PAEDS 2022 cohort and 0/20 samples in PICOBOO were determined as seronegative.

Geometric mean concentrations and corresponding 99% CIs for MSD N-antibody were calculated on samples previously deemed seronegative for both Elecsys SARS-CoV-2 N assays. A concentration of ≥1,000 AU/mL for MSD N-antibody IgG was deemed as seropositive, in accordance with upper 99% CI values (Figure 2). Insufficient numbers of samples were available for calculating an S-antibody seropositive value for these cohorts.




*GMC is represented by the red line; the black dotted line is the determined cut-off value.*

***Figure 2:*** *Serum positivity cut-offs for nucleocapsid antibody determined using a) pre-pandemic samples and b) results from 2022 SARS-CoV-2 paediatric serosurvey deemed positive and negative using the Roche Elecsys assay*

## Data management

Information on participants was entered into a REDCap online database hosted by The University of Sydney and managed by the PAEDS Coordinating Centre research team at The Children's Hospital at Westmead. Accessibility was provided to respective PAEDS sites in each state. Data linkage was performed to link serosurvey participants to PAEDS SARS-CoV-2 hospitalisation data.

De-identified information (e.g. gender, jurisdiction of residence/postcode, Aboriginal and/or Torres Strait Islander status, vaccination status, etc.) from the study database was extracted for analysis. Statistical analyses were undertaken using Microsoft Excel and R Statistical Software (V4.3.1).[9] 95% CI were calculated on the overall seroprevalence using the binomial Clopper-Pearson method.[10]

## Ethics

Ethics approval for this national study was provided by the Sydney Children's Hospitals Network Human Research Ethics Committee (HREC 18/SCHN/72).

## Funding

Funding was provided by the Australian Government Department of Health and Aged Care.

# Results

We consented 1,209 participants for inclusion across eight PAEDS hospitals. Samples were obtained from 1,070 participants and tested; see Figure 3 for more details. Five participants who provided blood samples were excluded from the analysis: one participant did not complete the questionnaire, two recruited were aged 16 years and two resided outside Australia.

## Study participant characteristics

The demographic characteristics of those tested are described in Table 4. Most of the participants resided in Queensland (n=253), Western Australia (n=200), New South Wales (n=207) and South Australia (n=180). There were sixteen participants from the Northern Territory and five participants each from the Australian Capital Territory and Tasmania. 104 participants (9.8%) identified as being of Aboriginal and Torres Strait Islander background. Most of the participants did not have an underlying medical condition (659/1,065, 61.9%) and most were unvaccinated (698/1,065, 65.5%).

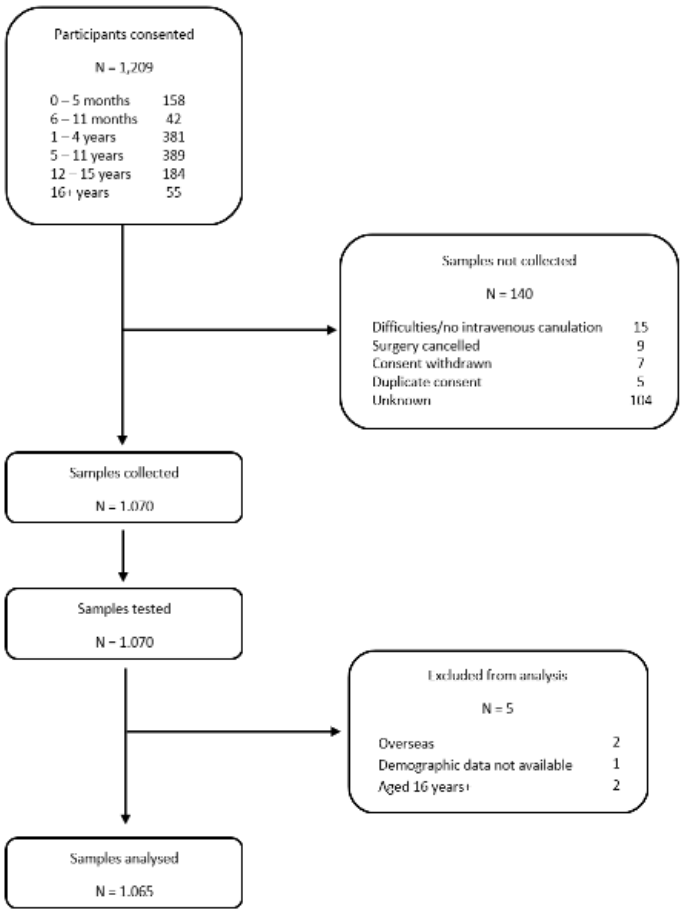
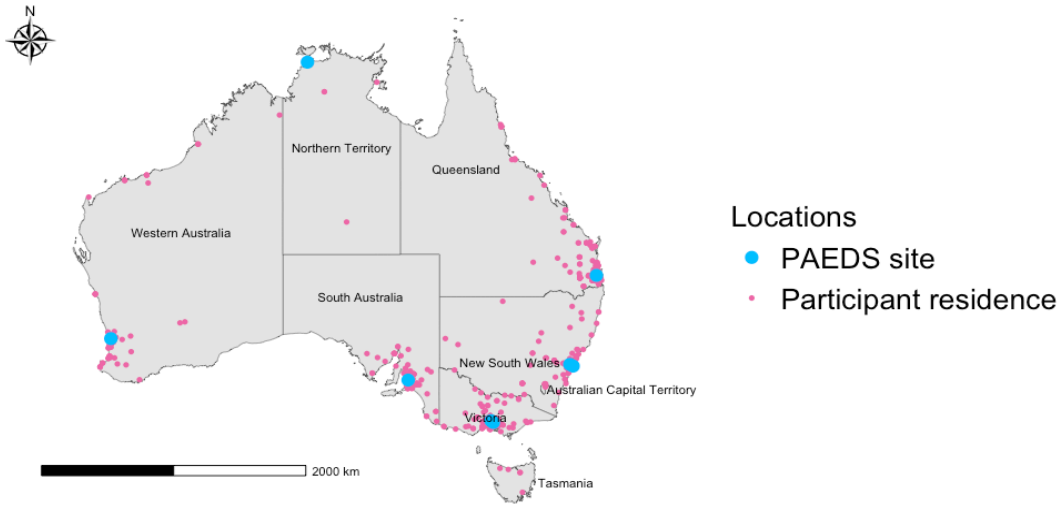


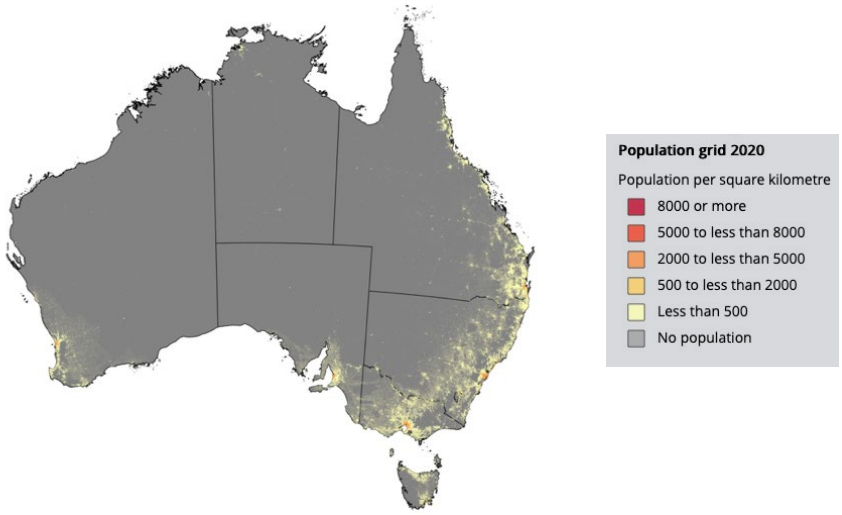

***Figure 3:*** *Participant recruitment and sample collection of children and adolescents aged 0–15 years for SARS-CoV-2 spike and nucleocapsid antibodies while undergoing an anaesthetic procedure in a PAEDS hospital, between 1 November 2023 and 7 December 2023 in Australia*

Figure 4 shows 788 (74%) participants resided in a major city, 251 (23.6%) in regional and 26 (2.4%) in remote areas. This is in line with the Australian population distribution: Australia's population is concentrated in the major cities, which are home to 73% of the total population. Around 1 in 4 (26%) live in inner regional and outer regional Australia, with the remainder (2%) living in remote and very remote areas.[11]

**a)**




**b)**




*Map A: The dots represent the centromere of the postcodes and do not indicate the exact location of the participant's residence.*

*Map B: Adapted from the Australian Bureau of Statistics website under Creative Commons Australian Bureau of Statistics (2021)*

*'Regional Population 2019–20: Population Grid' [https://absstats.maps.arcgis.com/apps/MapSeries/index.html?appid=b2fa123c0032456a8d47fbd0203a3dec]' Regional Population Growth, accessed 16 July 2024*

***Figure 4:** Map of a) postcode of residence of participants aged 0–15 years tested for SARS-CoV-2 spike and nucleocapsid antibodies while undergoing an anaesthetic procedure in a PAEDS hospital, between 1 November and 7 December 2023, in Australia compared to b) population geographic distribution of Australia in 2022, Australian Bureau of Statistics*

## Seroprevalence estimation

Using the pre-pandemic sample cut-off, the ancestral S-antibody crude seroprevalence in the overall sample was 1,005/1,065 (94.4%, 95% CI 92.8–95.6%). Using the pre-pandemic sample cut-off, the ancestral N-antibody crude seroprevalence was 930/1,065 (87.3%, 95% CI 87.3–85.2%). Using the Elecsys SARS-CoV-2 N-antibody assays (Roche), the crude seroprevalence was 676/1,065 (63.4%, 95% CI 60.5–66.3%).

The following estimates are exploratory and need to be adjusted to the Australian population distribution. Crude ancestral S- and ancestral N-antibody seroprevalence was similar across all jurisdictions, geographical remoteness and socio-economic status of area of residence (Tables 5 and 6).

Children and adolescents identifying as having an Aboriginal and/or Torres Strait Islander background had high crude S-seroprevalence (97/104, 93.3%), as did non-Indigenous children and adolescents (907/959, 94.6%). The ancestral N-antibody crude seroprevalence for Aboriginal and/or Torres Strait Islander children was 56/104 (53.8%) using the Roche sample cut-off and 91/104 (87.5%) using the pre-pandemic cut-off. In comparison, in non-Indigenous children, the ancestral N-antibody cut-off was 620/959 (64.7%) using the Roche sample cut-off and 839/959 (87.5%) using the pre-pandemic sample cut-off.

Crude ancestral S-antibody and crude ancestral N-antibody seroprevalence by age, sex, vaccination status, Indigenous status, remoteness index and socio-economic quintiles are presented in Table 5 and Table 6, respectively.

## Age

Crude ancestral S-antibody seroprevalence at 0–5 months of age was 100% (27/27). It decreased at 6–11 months (38/41, 92.7%) and 1–4 years (333/377, 88.3%), increased at 5–11 years (371/384, 96.6%), and returned to 100% (236/236) at 12–15 years. Ancestral crude N-antibody seroprevalence was lowest at 6–11 months of age using both the Roche cut-off method 11/41 (26.8%) and the pre-pandemic cut-off method 21/41 (51.2%).

## Vaccination

Crude ancestral S-antibody seroprevalence was high in unvaccinated children (639/698, 91.5%) but lower than in 1-, 2- or 3-dose vaccinated children (1-dose: 53/53, 100%; 2-dose: 295/296, 99.7%; 3+ doses 12/12, 100%).

Similarly, unvaccinated children had lower crude ancestral N-antibody seroprevalence (392/698, 56.2% using the Roche cut-off method, 579/698, 83.0% using the pre-pandemic cut-off method) compared to vaccinated children (Roche cut-off method: 1-dose: 38/53, 71%, 2-dose: 232/296, 78.4%, 3+ doses: 10/12, 83.3%; pre-pandemic cut-off method: 1-dose: 50/53, 94.3%, 2-dose: 285/296, 96.3%, 3+ doses: 11/12, 91.7%). Unvaccinated children without history of infection had crude ancestral S-antibody seroprevalence of 81.7% (237/290) (Table 5).

## Subvariants

Crude S-antibody seroprevalence to Omicron subvariants BA2.86, BA.5, EG.5.1, FL.1.5.1, XBB.1.16, XBB1.16.6, XBB.1.5, XBB.2.3 are listed in Table 5. Crude seroprevalence of Omicron subvariant S-antibodies were all high and taken together were similar to the crude seroprevalence of ancestral S-antibody.

### Geometric mean concentration of antibodies and trends

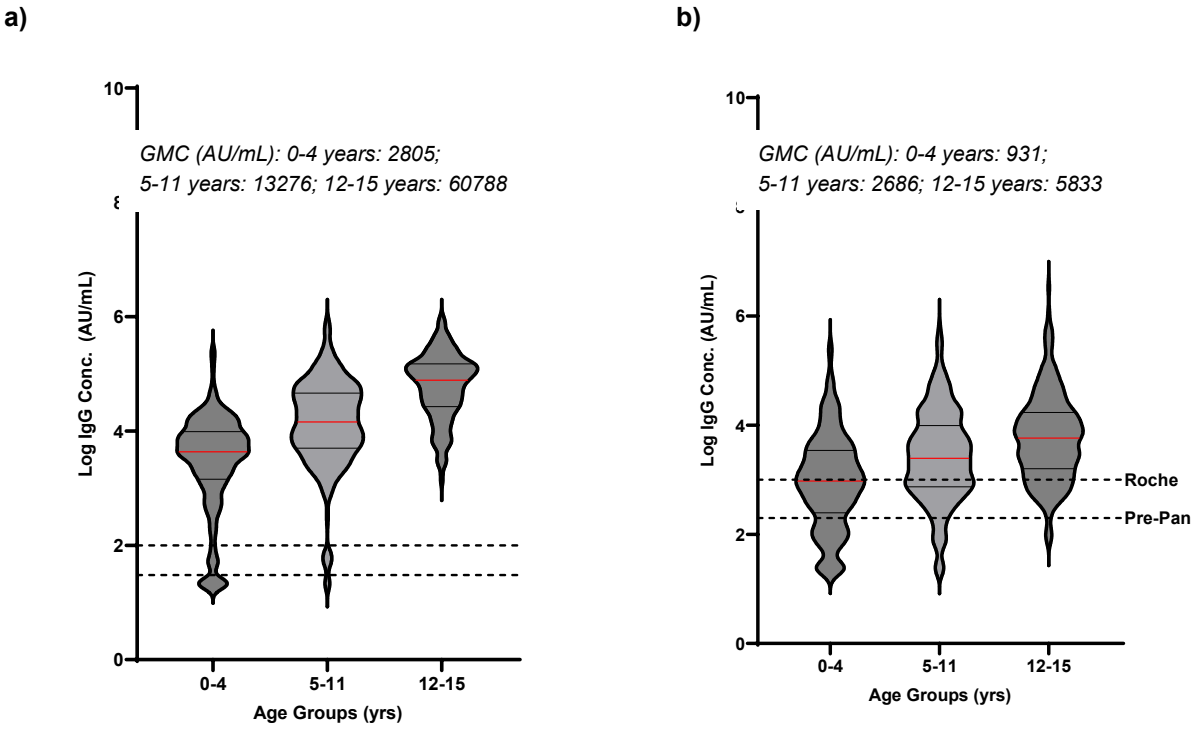
Geometric mean concentration of antibodies (GMC, AU/mL) against the ancestral variant (Figures 5 and 6) and Omicron subvariants were calculated for age and vaccine doses (Figures 7 and 8, Supplementary figures 1 and 2). The GMC increased with age and vaccination status. Ancestral S-antibody GMC was higher than Omicron subvariants in vaccinated children. In contrast, in unvaccinated children, ancestral S-antibody GMC was higher than Omicron subvariants in children <1 year but in unvaccinated children aged >1 year BA.5 S-antibody GMC were higher than ancestral S-antibody (Figure 9).

***Table 4:*** *Demographics of children and adolescents aged 0–15 years tested for SARS-CoV-2 spike and nucleocapsid antibodies while undergoing an anesthetic procedure in a PAEDS hospital, between 1 November and 7 December 2023, in Australia*

| | Number of samples (n) | Percentage of total (%) |
|---|---|---|
| **Total population** | 1,065 | |
| | | |
| **Jurisdiction**[a] | | |
| Australian Capital Territory | 5 | 0.5 |
| New South Wales | 207 | 19.4 |
| Northern Territory | 16 | 1.5 |
| Queensland | 253 | 23.8 |
| South Australia | 180 | 16.9 |
| Tasmania | 5 | 0.5 |
| Victoria | 199 | 18.7 |
| Western Australia | 200 | 18.8 |
| **Age** | | |
| 0–5 months | 27 | 2.5 |
| 6–11 months | 41 | 3.8 |
| 1–4 years | 377 | 35.4 |
| 5–11 years | 384 | 36.1 |
| 12–15 years | 236 | 22.2 |
| **Sex** | | |
| Male | 613 | 57.2 |
| Female | 449 | 42.2 |
| Other | 2 | 0.2 |
| Unknown | 1 | 0.1 |
| **Aboriginal and Torres Strait Islander status** | | |
| Non-Indigenous | 959 | 90.0 |
| Aboriginal and Torres Strait Islander | 104 | 9.8 |
| Unknown | 2 | 0.2 |
| **Vaccination status** | | |
| Unvaccinated | 698 | 65.5 |
| 1 dose | 53 | 5.0 |
| 2 doses | 296 | 27.8 |

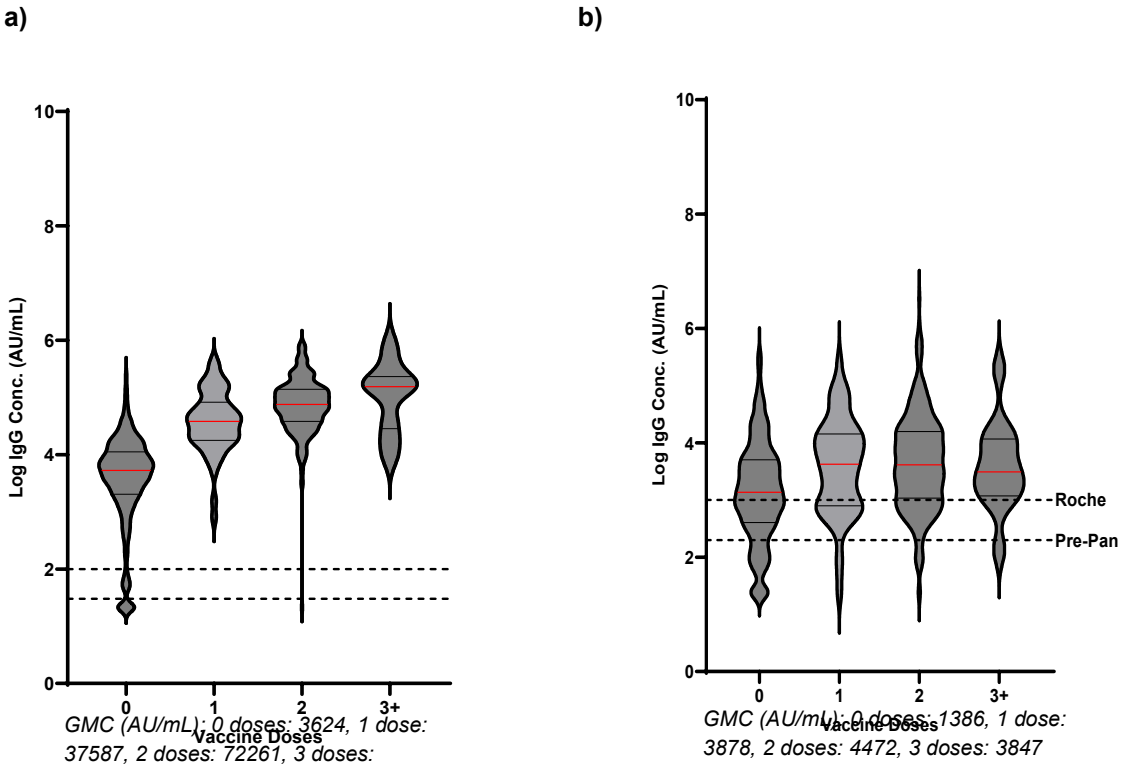
| | Number of samples (n) | Percentage of total (%) |
|---|---|---|
| 3 or more doses[b] | 12 | 1.1 |
| Unknown | 6 | 0.6 |
| **Resident by Area of Remoteness Index (ARIA)** | | |
| Major city | 788 | 74.0 |
| Regional | 251 | 23.6 |
| Remote | 26 | 2.4 |
| **Socio-economic status of area of residence (SEIFA, using IRSD[c])** | | |
| First (least advantaged) | 209 | 19.6 |
| Second | 173 | 16.2 |
| Third | 239 | 22.4 |
| Fourth | 236 | 22.2 |
| Fifth (most advantaged) | 208 | 19.5 |
| **Underlying medical condition(s)** | | |
| Present | 405 | 38.0 |
| None | 659 | 61.9 |
| Unknown | 1 | <0.001 |
| **Reported history of past infection[d]** | | |
| Yes | | |
| Vaccinated | 254 | 23.8 |
| Unvaccinated | 405 | 38.0 |
| None | | |
| Vaccinated | 105 | 9.9 |
| Unvaccinated | 290 | 27.2 |
| Unknown | 11 | 1.0 |

*[a]Jurisdiction determined by resident postcode; [b]10 participants received 3 doses, 1 participant received 4 doses, and 1 participant received 5 doses; [c]IRSD: Index of Relative Socio-economic Disadvantage; [d]parent/participant reported infection*



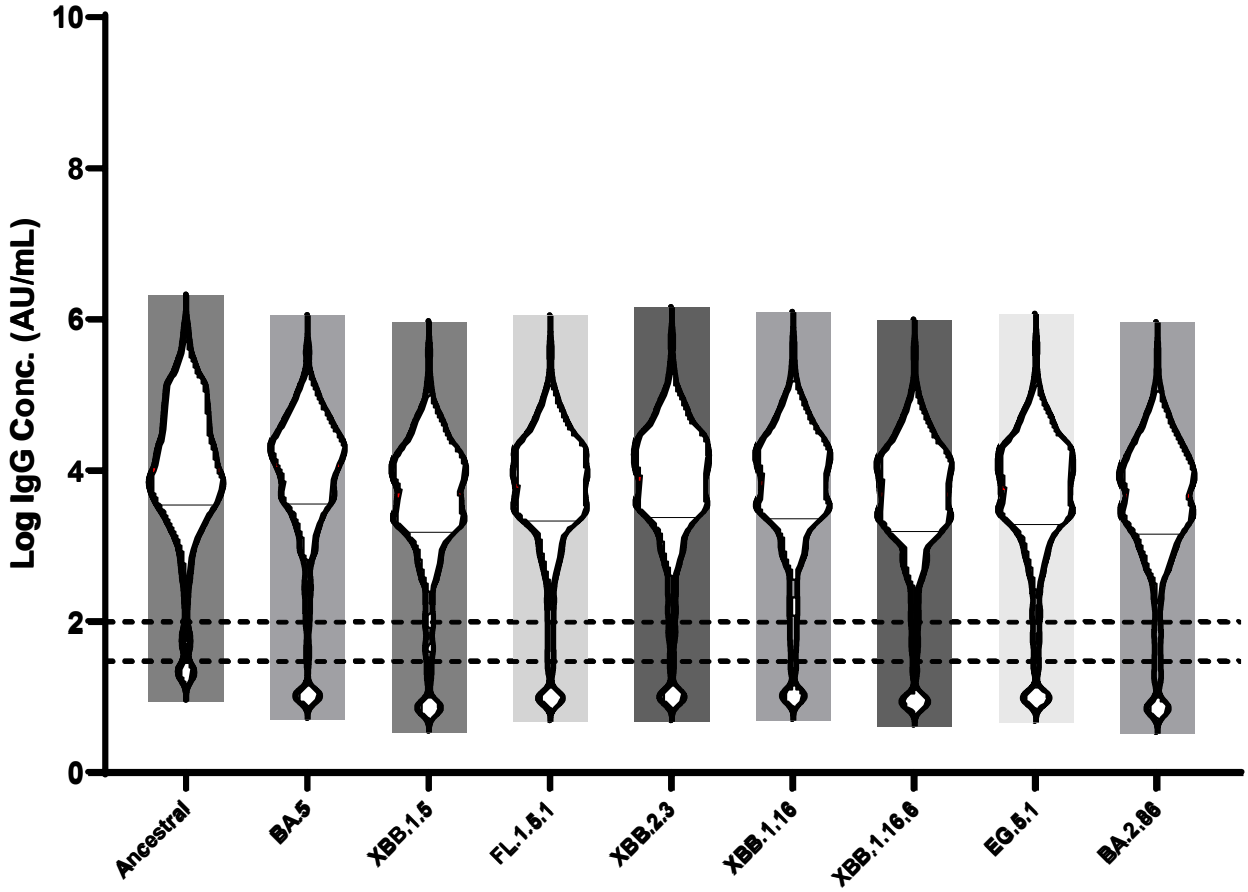

*Antibody titres were transformed to log scale and represented as violin plots, where red bars represent the median and black lines represent the interquartile range. Dotted lines indicate seropositivity cut-off range for the spike variants.*

***Figure 5:*** *The GMC of SARS-CoV-2 a) ancestral S-antibody and b) ancestral N-antibody by age groups: 0–4 years (n=447), 5–11 years (n=384) and 12–15 years (n=236)*




*Antibody titres were transformed to log scale and represented as violin plots, where red bars represent the median and black lines represent the interquartile range. Dotted lines indicate seropositivity cut-off range for the spike variants.*

***Figure 6:*** *The GMC of SARS-CoV-2 a) ancestral S-antibody and b) ancestral N-antibody by vaccine doses: 0 doses (n=706), 1 dose (n=53), 2 doses (n=299) and 3 or more doses (n=12)*




*Antibody titres were transformed to log scale and represented as violin plots, where red bars represent the median and black lines represent the interquartile range. Dotted lines indicate seropositivity cut-off range for the spike variants. Variants are ordered according to global emergence (left-right).*

***Figure 7:*** *The seroprevalence and GMC of ancestral and Omicron subvariant S-antibody*

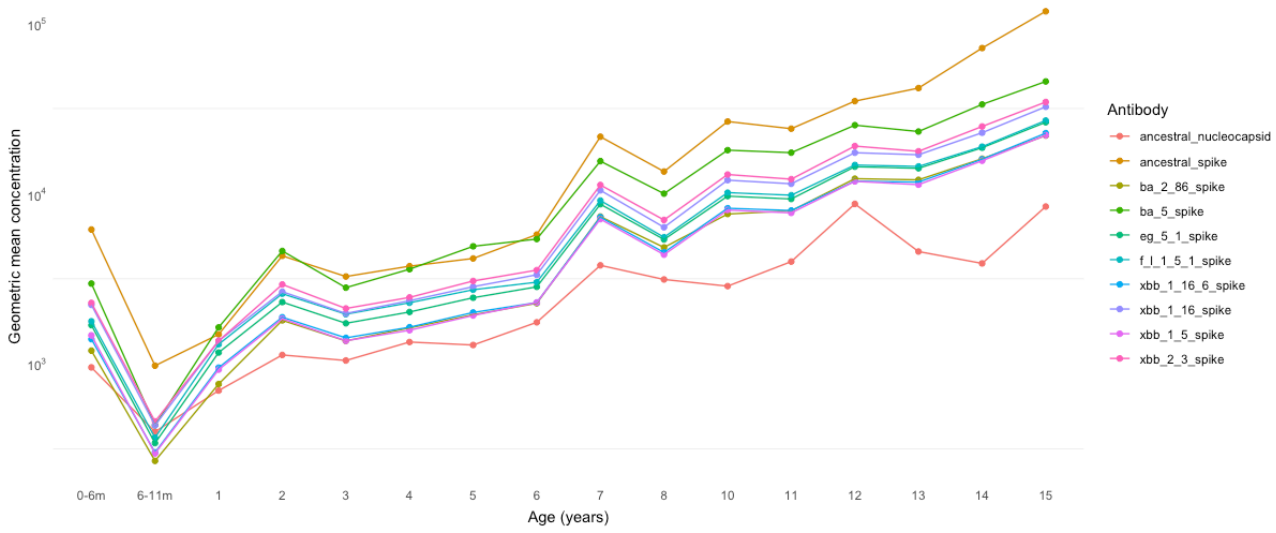
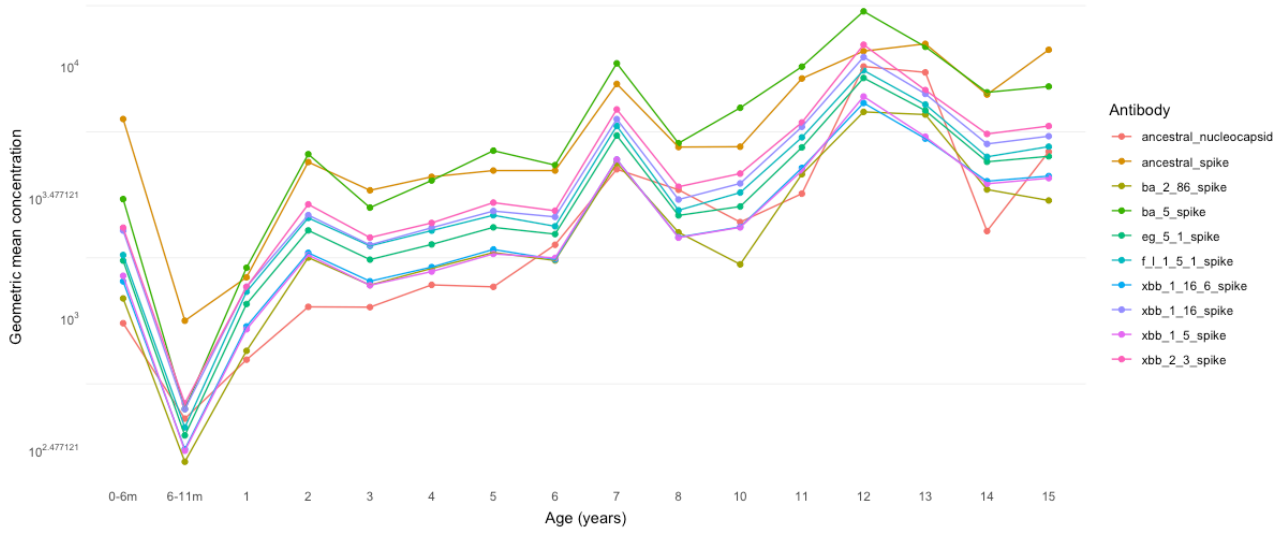
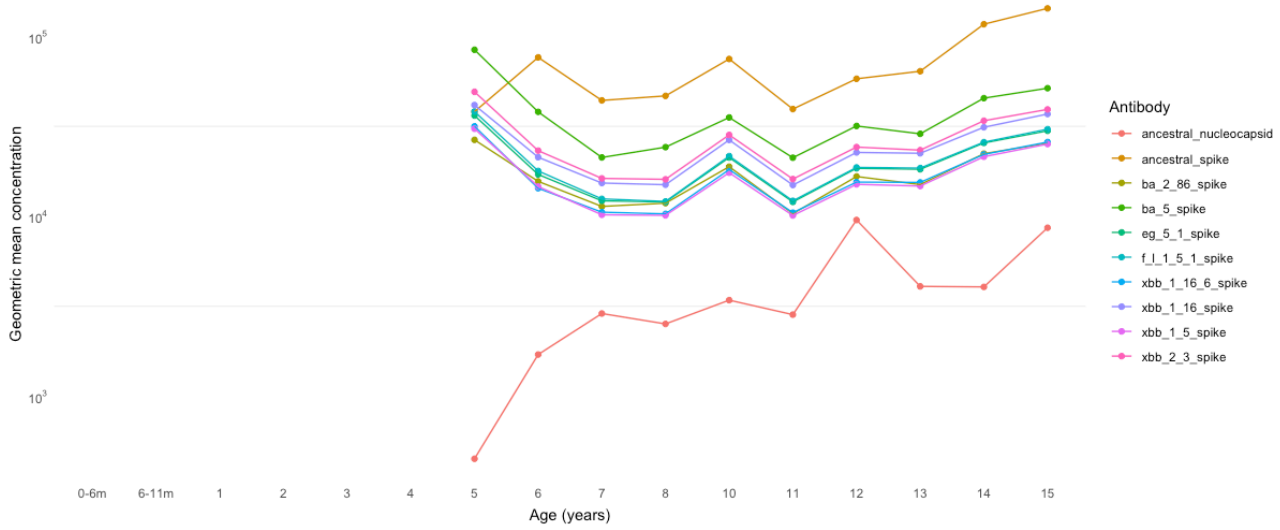



***Figure 8:*** *The GMC of ancestral and Omicron subvariant S-antibody and ancestral N-antibody by age*

a)




b)




**COVID-19 primary vaccination with/without a booster dose was recommended for all children aged 5 years and above (commencing 2021–2022). For children aged 6 months to 5 years, only those with select high-risk medical conditions were recommended for COVID-19 vaccination.*

***Figure 9**: The GMC of ancestral and Omicron subvariant S-antibody and ancestral N-antibody by age in a) unvaccinated participants and b) 2-dose vaccinated participants**

***Table 5:*** *Crude seroprevalence of 1,065 children and adolescents aged 0–15 years tested for SARS-CoV-2 spike antibodies (ancestral and Omicron subvariants) in a PAEDS hospital, between 1 November and 7 December 2023, in Australia.*

| | **Ancestral spike n/N (%)** | **BA.2.86 spike n/N (%)** | **BA.5 spike n/N (%)** | **Eg.5.1 spike n/N (%)** | **Fl.1.5.1 spike n/N (%)** | **Xbb.1.16 spike n/N (%)** | **Xbb.1.16.6 spike n/N (%)** | **Xbb.1.5 spike n/N (%)** | **Xbb.2.3 spike n/N (%)** |
|---|---|---|---|---|---|---|---|---|---|
| | **Cut-off pre-pandemic** | **Cut-off pre-pandemic** | **Cut-off pre-pandemic** | **Cut-off pre-pandemic** | **Cut-off pre-pandemic** | **Cut-off pre-pandemic** | **Cut-off pre-pandemic** | **Cut-off pre-pandemic** | **Cut-off pre-pandemic** |
| | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) |
| **Total population** | 1,005/1,065 (94.4) | 1,004/1,065 (94.3) | 1,002/1,065 (94.1) | 1,002/1,065 (94.1) | 1,003/1,065 (94.2) | 1,004/1,065 (94.3) | 1,001/1,065 (94) | 1,001/1,065 (94) | 1,002/1,065 (94.1) |
| | | | | | | | | | |
| **Jurisdiction** | | | | | | | | | |
| Australian Capital Territory | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) |
| New South Wales | 195/207 (94.2) | 195/207 (94.2) | 193/207 (93.2) | 194/207 (93.7) | 195/207 (94.2) | 195/207 (94.2) | 193/207 (93.2) | 194/207 (93.7) | 192/207 (92.8) |
| Northern Territory | 16/16 (100) | 16/16 (100) | 16/16 (100) | 16/16 (100) | 16/16 (100) | 16/16 (100) | 16/16 (100) | 16/16 (100) | 16/16 (100) |
| Queensland | 236/253 (93.3) | 236/253 (93.3) | 237/253 (93.7) | 235/253 (92.9) | 236/253 (93.3) | 236/253 (93.3) | 236/253 (93.3) | 235/253 (92.9) | 236/253 (93.3) |
| South Australia | 170/180 (94.4) | 166/180 (92.2) | 167/180 (92.8) | 169/180 (93.9) | 169/180 (93.9) | 169/180 (93.9) | 168/180 (93.3) | 169/180 (93.9) | 169/180 (93.9) |
| Tasmania | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) | 5/5 (100) |
| Victoria | 185/199 (93) | 186/199 (93.5) | 187/199 (94) | 186/199 (93.5) | 185/199 (93) | 186/199 (93.5) | 187/199 (94) | 186/199 (93.5) | 187/199 (94) |
| Western Australia | 193/200 (96.5) | 195/200 (97.5) | 192/200 (96) | 192/200 (96) | 192/200 (96) | 192/200 (96) | 191/200 (95.5) | 191/200 (95.5) | 192/200 (96) |
| **Age** | | | | | | | | | |
| 0–5 months | 27/27 (100) | 25/27 (92.6) | 27/27 (100) | 26/27 (96.3) | 26/27 (96.3) | 27/27 (100) | 26/27 (96.3) | 27/27 (100) | 27/27 (100) |
| 6–11 months | 38/41 (92.7) | 34/41 (82.9) | 34/41 (82.9) | 35/41 (85.4) | 36/41 (87.8) | 35/41 (85.4) | 34/41 (82.9) | 34/41 (82.9) | 35/41 (85.4) |
| 0–4 years | 333/377 (88.3) | 335/377 (88.9) | 332/377 (88.1) | 331/377 (87.8) | 332/377 (88.1) | 332/377 (88.1) | 332/377 (88.1) | 331/377 (87.8) | 331/377 (87.8) |
| 5–11 years | 371/384 (96.6) | 374/384 (97.4) | 373/384 (97.1) | 374/384 (97.4) | 373/384 (97.1) | 374/384 (97.4) | 373/384 (97.1) | 373/384 (97.1) | 373/384 (97.1) |
| 12–15 years | 236/236 (100) | 236/236 (100) | 236/236 (100) | 236/236 (100) | 236/236 (100) | 236/236 (100) | 236/236 (100) | 236/236 (100) | 236/236 (100) |
| **Sex** | | | | | | | | | |
| Male | 577/613 (94.1) | 576/613 (94) | 576/613 (94) | 574/613 (93.6) | 574/613 (93.6) | 576/613 (94) | 573/613 (93.5) | 573/613 (93.5) | 576/613 (94) |
| Female | 425/449 (94.7) | 425/449 (94.7) | 423/449 (94.2) | 425/449 (94.7) | 426/449 (94.9) | 425/449 (94.7) | 425/449 (94.7) | 425/449 (94.7) | 423/449 (94.2) |
| Other | 2/2 (100) | 2/2 (100) | 2/2 (100) | 2/2 (100) | 2/2 (100) | 2/2 (100) | 2/2 (100) | 2/2 (100) | 2/2 (100) |
| **Aboriginal and Torres Strait Islander status** | | | | | | | | | |
| Non-Indigenous | 907/959 (94.6) | 905/959 (94.4) | 904/959 (94.3) | 904/959 (94.3) | 905/959 (94.4) | 906/959 (94.5) | 903/959 (94.2) | 903/959 (94.2) | 904/959 (94.3) |

| | Ancestral spike n/N (%) | BA.2.86 spike n/N (%) | BA.5 spike n/N (%) | Eg.5.1 spike n/N (%) | Fl.1.5.1 spike n/N (%) | Xbb.1.16 spike n/N (%) | Xbb.1.16.6 spike n/N (%) | Xbb.1.5 spike n/N (%) | Xbb.2.3 spike n/N (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic |
| | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) |
| Aboriginal and Torres Strait Islander | 97/104 (93.3) | 98/104 (94.2) | 97/104 (93.3) | 97/104 (93.3) | 97/104 (93.3) | 97/104 (93.3) | 97/104 (93.3) | 97/104 (93.3) | 97/104 (93.3) |
| Unknown | 1/2 (50) | 1/2 (50) | 1/2 (50) | 1/2 (50) | 1/2 (50) | 1/2 (50) | 1/2 (50) | 1/2 (50) | 1/2 (50) |
| **Total population** | 1,005/1,065 (94.3) | 1,004/1,065 (94.3) | 1,002/1,065 (94.1) | 1,004/1,065 (94.3) | 1,003/1,065 (94.2) | 1,004/1,065(94.3) | 1,004/1,065 (94.3) | 1,004/1,065 (94.3) | 1,004/1,065 (94.3) |
| **Vaccination status** | | | | | | | | | |
| Unvaccinated | 639/698 (91.5) | 637/698 (91.3) | 635/698 (91) | 635/698 (91) | 636/698 (91.1) | 637/698 (91.3) | 634/698 (90.8) | 634/698 (90.8) | 635/698 (91) |
| 1 dose | 53/53 (100) | 53/53 (100) | 53/53 (100) | 53/53 (100) | 53/53 (100) | 53/53 (100) | 53/53 (100) | 53/53 (100) | 53/53 (100) |
| 2 doses | 295/296 (99.7) | 296/296 (100) | 296/296 (100) | 296/296 (100) | 296/296 (100) | 296/296 (100) | 296/296 (100) | 296/296 (100) | 296/296 (100) |
| 3 or more doses | 12/12 (100) | 12/12 (100) | 12/12 (100) | 12/12 (100) | 12/12 (100) | 12/12 (100) | 12/12 (100) | 12/12 (100) | 12/12 (100) |
| Unknown | 6/6 (100) | 6/6 (100) | 6/6 (100) | 6/6 (100) | 6/6 (100) | 6/6 (100) | 6/6 (100) | 6/6 (100) | 6/6 (100) |
| **Resident by Area of Remoteness Index (ARIA)** | | | | | | | | | |
| Major cities of Australia | 743/788 (94.3) | 742/788 (94.2) | 740/788 (93.9) | 742/788 (94.2) | 744/788 (94.4) | 743/788 (94.3) | 740/788 (93.9) | 740/788 (93.9) | 740/788 (93.9) |
| Regional Australia | 236/251 (94) | 236/251 (94) | 236/251 (94) | 234/251 (93.2) | 233/251 (92.8) | 235/251 (93.6) | 235/251 (93.6) | 235/251 (93.6) | 236/251 (94) |
| Remote Australia | 26/26 (100) | 26/26 (100) | 26/26 (100) | 26/26 (100) | 26/26 (100) | 26/26 (100) | 26/26 (100) | 26/26 (100) | 26/26 (100) |
| **Socio-economic status of area of residence (SEIFA, using IRSD)** | | | | | | | | | |
| First (least advantaged) | 198/209 (94.7) | 194/209 (92.8) | 196/209 (93.8) | 196/209 (93.8) | 198/209 (94.7) | 196/209 (93.8) | 196/209 (93.8) | 196/209 (93.8) | 196/209 (93.8) |
| Second | 162/173 (93.6) | 164/173 (94.8) | 162/173 (93.6) | 162/173 (93.6) | 162/173 (93.6) | 162/173 (93.6) | 162/173 (93.6) | 162/173 (93.6) | 162/173 (93.6) |
| Third | 225/239 (94.1) | 225/239 (94.1) | 227/239 (95) | 224/239 (93.7) | 223/239 (93.3) | 226/239 (94.6) | 226/239 (94.6) | 225/239 (94.1) | 227/239 (95) |
| Fourth | 223/236 (94.5) | 222/236 (94.1) | 221/236 (93.6) | 222/236 (94.1) | 222/236 (94.1) | 222/236 (94.1) | 221/236 (93.6) | 222/236 (94.1) | 222/236 (94.1) |
| Fifth (most advantaged) | 197/208 (94.7) | 199/208 (95.7) | 196/208 (94.2) | 198/208 (95.2) | 198/208 (95.2) | 198/208 (95.2) | 196/208 (94.2) | 196/208 (94.2) | 195/208 (93.8) |
| **Underlying medical conditions** | | | | | | | | | |
| Present | 379/405 (93.6) | 383/405 (94.6) | 379/405 (93.6) | 380/405 (93.8) | 380/405 (93.8) | 380/405 (93.8) | 379/405 (93.6) | 378/405 (93.3) | 379/405 (93.6) |
| None | 625/659 (95.0) | 620/659 (94.2) | 622/659 (94.5) | 621/659 (94.4) | 622/659 (94.5) | 623/659 (94.7) | 621/659 (94.4) | 622/659 (94.5) | 622/659 (94.5) |
| Unknown | 1/1 (100) | 1/1 (100) | 1/1 (100) | 1/1 (100) | 1/1 (100) | 1/1 (100) | 1/1 (100) | 1/1 (100) | 1/1 (100) |
| **Reported history of past infection** | | | | | | | | | |
| Yes | | | | | | | | | |
| Vaccinated | 254/254 (100) | 254/254 (100) | 254/254 (100) | 254/254 (100) | 254/254 (100) | 254/254 (100) | 254/254 (100) | 254/254 (100) | 254/254 (100) |

| | Ancestral spike n/N (%) | BA.2.86 spike n/N (%) | BA.5 spike n/N (%) | Eg.5.1 spike n/N (%) | Fl.1.5.1 spike n/N (%) | Xbb.1.16 spike n/N (%) | Xbb.1.16.6 spike n/N (%) | Xbb.1.5 spike n/N (%) | Xbb.2.3 spike n/N (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic | Cut-off pre-pandemic |
| | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) | Crude n/N (%) |
| Unvaccinated | 399/405 (98.5) | 399/405 (98.5) | 399/405 (98.5) | 400/405 (98.8) | 400/405 (98.8) | 400/405 (98.8) | 399/405 (98.5) | 399/405 (98.5) | 399/405 (98.5) |
| None | | | | | | | | | |
| Vaccinated | 104/105 (99.0) | 105/105 (100) | 105/105 (100) | 105/105 (100) | 105/105 (100) | 105/105 (100) | 105/105 (100) | 105/105 (100) | 105/105 (100) |
| Unvaccinated | 237/290 (81.7) | 235/290 (81) | 233/290 (80.3) | 232/290 (80) | 233/290 (80.3) | 234/290 (80.7) | 232/290 (80) | 232/290 (80) | 233/290 (80.3) |
| Unknown | 11/11 (100) | 11/11 (100) | 11/11 (100) | 11/11 (100) | 11/11 (100) | 11/11 (100) | 11/11 (100) | 11/11 (100) | 11/11 (100) |

***Table 6:*** *Crude seroprevalence of 1,065 children and adolescents aged 0–15 years tested for SARS-CoV-2 ancestral nucleocapsid antibodies in a PAEDS hospital, between 1 November and 7 December 2023, in Australia*

| | **N-antibody n/N(%)** | **N-antibody n/N(%)** |
|---|---|---|
| | **Cut-off pre-pandemic[a]** | **Roche cut-off[b]** |
| | Crude n/N (%) | Crude n/N (%) |
| **Total population** | 930/1,065 (87.3) | 676/1,065 (63.5) |
| | | |
| **State/territory** | | |
| Australian Capital Territory | 5/5 (100) | 5/5 (100) |
| New South Wales | 175/207 (84.5) | 131/207 (63.3) |
| Northern Territory | 14/16 (87.5) | 10/16 (62.5) |
| Queensland | 217/253 (85.8) | 145/253 (57.3) |
| South Australia | 164/180 (91.1) | 124/180 (68.9) |
| Tasmania | 4/5 (80) | 2/5 (40) |
| Victoria | 171/199 (85.9) | 122/199 (61.3) |
| Western Australia | 180/200 (90) | 137/200 (68.5) |
| **Age** | | |
| 0–5 months | 23/27 (85.2) | 14/27 (51.9) |
| 6–11 months | 21/41 (51.2) | 11/41 (26.8) |
| 0–4 years | 299/377 (79.3) | 190/377 (50.4) |
| 5–11 years | 355/384 (92.4) | 263/384 (68.5) |
| 12–15 years | 232/236 (98.3) | 198/236 (83.9) |
| **Sex** | | |
| Male | 521/613 (85) | 366/613 (59.7) |
| Female | 407/449 (90.6) | 309/449 (68.8) |
| Other | 2/2 (100) | 1/2 (50) |
| Unknown | 0/1 (0.0) | 0/1 (0.0) |
| **Aboriginal and Torres Strait Islander status** | | |
| Non-Indigenous | 839/959 (87.5) | 620/959 (64.7) |
| Aboriginal and Torres Strait Islander | 91/104 (87.5) | 56/104 (53.8) |
| Vaccination status | | |
| Unknown | 0/2 (0.0) | 0/2 (0.0) |
| Unvaccinated | 579/698 (83) | 392/698 (56.2) |
| 1 dose | 50/53 (94.3) | 38/53 (71.7) |
| 2 doses | 285/296 (96.3) | 232/296 (78.4) |
| 3 or more doses | 11/12 (91.7) | 10/12 (83.3) |
| Unknown | 5/6 (83.3) | 4/6 (66.7) |
| | **N-antibody n/N(%)** | **N-antibody n/N(%)** |

| | N-antibody n/N(%) | N-antibody n/N(%) |
|---|---|---|
| | **Cut off pre-pandemic[a]** | **Roche cut-off[b]** |
| | Crude n/N (%) | Crude n/N (%) |
| **Total population** | 930/1,065 (87.3) | 676/1,065 (63.5) |
| **Resident by Area of Remoteness Index (ARIA)** | | |
| Major cities of Australia | 685/788 (86.9) | 518/788 (65.7) |
| Regional Australia | 221/251 (88) | 142/251 (56.6) |
| Remote Australia | 24/26 (92.3) | 16/26 (61.5) |
| **Socio-economic status of area of residence (SEIFA, using IRSD)** | | |
| First (least advantaged) | 180/209 (86.1) | 128/209 (61.2) |
| Second | 150/173 (86.7) | 105/173 (60.7) |
| Third | 211/239 (88.3) | 154/239 (64.4) |
| Fourth | 203/236 (86) | 152/236 (64.4) |
| Fifth (most advantaged) | 186/208 (89.4) | 137/208 (65.9) |
| **Underlying medical condition** | | |
| Present | 347/405 (85.7) | 259/405 (64) |
| None | 583/659 (88.5) | 417/659 (63.3) |
| Unknown | 0/1 (0) | 0/1 (0) |
| **Reported history of past infection** | | |
| Yes | | |
| Vaccinated | 248/254 (97.6) | 207/254 (81.5) |
| Unvaccinated | 375/405 (92.6) | 270/405 (66.7) |
| None | | |
| Vaccinated | 96/105 (0) | 71/105 (0) |
| Unvaccinated | 202/290 (69.7) | 121/290 (41.7) |
| Unknown | 9/11 (81.8) | 7/11 (63.6) |

*N-antibody: nucleocapsid antibody; [a]cut-off determined using pre-pandemic sera collected before 2019 from children aged 1–16 years (n= 50; Feb 2004–Jan 2009) and cord blood (n=5; Oct 2018–Aug 2019) available to study team; [b]cut-off determined using samples previously tested on the Elecsys SARS-CoV-2 N-antibody IgG assay (Roche) 2022 PAEDS serosurvey (n=24) and adults (aged 50–70 years) from the Platform Trial in COVID-19 Boosting[12] (PICOBOO; n=20)*

# Discussion

In Australia, by December 2023, the estimated seroprevalence in children and adolescents of SARS-CoV-2 ancestral S-antibody was 1,005/1,065 (94.4%, 95% CI 92.8–95.6%). We found 395 children reported no known infection, yet S-antibody was high at 81.7% even in the unvaccinated cohort, which is consistent with many children having mild or asymptomatic infection. The ancestral S-antibody seroprevalence was similar to the estimates from our serosurvey conducted between June and August 2022,[4] and although unpowered to statistically compare jurisdictions, crude values were high and similar across jurisdictions, socio-economic quintiles and geographic remoteness.

A systematic review of serosurveys in children between 1 December 2019 and 10 July 2022 showed that lowest seroprevalence was estimated for the Western Pacific region (0.01–1.01%);[13] but our studies have shown, since July 2022 onwards, approximately 6 months after public health measures began to be withdrawn and there was evidence of widespread SARS-CoV-2 transmission nationally, seroprevalence to SARS-CoV-2 antibodies in Australian children has been high. By 2023, multiple lines of evidence support understanding children have been reinfected several times with SARS-CoV-2 with the majority of infections not diagnosed.

Two methods were used to estimate the ancestral N-antibody seroprevalence, using a cut-off determined by samples from participants prior to the pandemic (pre-pandemic cut-off) and by using a cut-off determined by the Roche Elecsys assay (Roche Elecsys cut-off). Using the pre-pandemic cut-off value, the seroprevalence was determined to be higher, at 87.3%, (95% CI 87.3–85.2%) compared to 63.4% (95% CI 60.5–66.3%) using the Roche Elecsys cut-off. Validation of these assays in children is typically limited, but nucleocapsid antibody is known to wane 6–12 months after exposure, and it is possible that, in our setting, the MSD assay may be more sensitive than the Roche Elecsys. Regardless of the method of estimation, nucleocapsid antibody remains high (between 60–80%) among children and adolescents in Australia.

Of the 13 Omicron subvariants that have circulated in Australia, our assay was able to test for six (BA.2.86, BA.5, EG.5.1, XBB.1.5, XBB.1.16, XBB.2.3) in addition to two other subvariants (FL.1.5.1, XBB.1.16.6). This is important as testing antibodies against ancestral spike antibody alone may be inadequate to determine immunity against recently circulating Omicron variants. A study conducted between 29 April and June 2022, after the emergence of early Omicron variants in Switzerland, showed that although children had high seroprevalence of ancestral S-antibodies (76.7% [69.7–83.0] for ages 0–5 years, 90.5% [86.5–94.1] for ages 6–11 years), neutralising antibody for children aged <12 years was substantially lower against Omicron subvariants.[14] This is particularly important from 2023 onwards as multiple Omicron subvariants have circulated simultaneously and for short periods of time in Australia.[15] We found uniformly high seroprevalence of S-antibody against Omicron subvariants, which suggests that Australian children have developed a broad heterotypic antibody signature against multiple variants of SARS-CoV-2 early in life.

High antibody levels in the population do not necessarily indicate that everyone has been infected with multiple variants. Instead, shared epitopes lead to antibody cross-reactivity with various antigenically diverse viral variants, even without prior infection.[14] Whether this cross-reactivity provides a protective immune response against infection and symptomatic illness may be linked to the level of circulating antibody and is a separate consideration.

The geometric mean concentration (GMC) of ancestral and Omicron subvariant S-antibody increased with age, although the rise was less dramatic in unvaccinated individuals compared to vaccinated individuals. Ancestral N-antibody levels were also higher in older children. As N-antibody is only produced post infection

and wanes within 6–12 months, high levels are likely reflective of repeated recent infection in older children, perhaps due to increased social mixing in adolescence. We found, similar to other studies, higher antibody levels in children who have been vaccinated and infected (hybrid immunity) compared to children who have only been infected.[16-18] The implication of this is uncertain, as no correlate of protection exists. Nevertheless, studies have shown that people with hybrid immunity have both higher neutralising antibody and lower reinfection rates, but the effect is not necessarily long lasting.[19]

We also demonstrated that vaccinated children exhibited higher antibody concentrations to ancestral S compared to all other S variants tested. However, in unvaccinated children, levels of BA.5 S-antibody superseded ancestral S-antibody levels in children >1 year. Consistent with these antibody signatures, vaccinated individuals in our study received priming vaccines consisting mostly of ancestral spike, whereas unvaccinated individuals in this cohort were likely first infected with SARS-CoV-2 Omicron strains that were predominantly circulating when Australian borders were initially opened in 2022. The disparity in signatures of S variant reactivity between these groups is therefore likely representative of antigenic imprinting.[20] Longitudinal studies have shown that antibody production or a boost to the antibody level occurs to a wide range of Omicron subvariants following infection or vaccination,[19,21] and it is likely that regular boosting is occurring among children. It is unclear, however, whether boosting will overcome antigenic imprinting/bias in S variant-reactivity signatures.

Lower GMC was found in children aged 0–6 months compared to children aged 6–12 months, likely representing maternal antibody decay. There is then a sharp rise in antibody levels between ages 1–2 years, with GMC remaining stable and slowly rising thereafter in unvaccinated children.

## Impact on policy

Our study is unique in that no prior large-scale serosurveys on Omicron subvariants have been conducted in children. Vaccination policy in Australia has recently been modified to not recommend booster COVID-19 vaccination in children aged <18 years without severe immunocomprimise,[22] and only to consider primary vaccination in this age group if they have a chronic medical condition,[23] on the basis that most Australian children were no longer SARS-CoV-2 immune naïve, infections are mainly mild or asymptomatic, most children are infected early in life, and paediatric hospitalisation and deaths remained very low.[24] Importantly, iterative data analyses of this and the two prior PAEDS SARS-CoV-2 serosurveys contributed data that helped form these recommendations from the Australian Technical Advisory Group on Immunisation (ATAGI).

Of the paediatric hospitalisations, children aged 0–6 months are overrepresented[25,26] and vaccination in pregnancy to boost levels in infants may be protective[27-31] and a consideration to future policy.

## Limitations

Our study was necessarily pragmatic and cross-sectional in design. Nevertheless, we were able to obtain sera from over 1,000 children and adolescents, the majority of whom were healthy and did not have an underlying medical condition, across Australia's vast geographic range. In addition, survey data on vaccination and self/parent-reported infection history was provided. Together we believe that the results are both valuable and broadly representative of SARS-CoV-2 antibody seroprevalence in Australian children. The assay did not test for JN.1, which is the dominant variant circulating in 2024. We also did not test the samples for neutralising antibodies against circulating variants.

Unexpected challenges encountered

This study was our third SARS-CoV-2 seroprevalence study in Australian children and adolescents. We were unsure about the willingness of Australian families to participate in this study at this stage of the pandemic. In respect of this, we were keen to ensure recruitment only lasted 4–5 weeks, given resource limitations, and to also ensure we did not burden families during the December/January holiday period. We were pleased at how many families agreed to participate and are in the process of feeding both individual and overall summary study results back to participants. We will also be asking for feedback on the process and on participants' understanding of the importance of cross-sectional serosurvey research in children.

We chose not to include late adolescents (aged 16–19 years) in the current serosurvey. In previous serosurveys, we attempted to recruit this age group, but recruitment through paediatric centres resulted in small numbers. We recognise a gap, as adult serosurveys typically include individuals aged 18 years and above.

## Further analysis

Using the data obtained through this serosurvey, we aim to further explore the effects of age and vaccination on the antibody and protective response to SARS-CoV-2 infection. Although clinical disease necessitating hospitalisation is rare in children, it most commonly occurs in the first year of life, and understanding antibody kinetics could inform prevention strategies. Additionally, it may be helpful to closely examine whether antibody titres are uniformly high or low across subvariants in individuals, or if distinct peaks within individuals can be identified to determine recent infection.

A total of 81.6% of the participants have consented for their samples to be used for future ethically approved studies. The sera and participant records are being stored within the PAEDS network for future serologic studies.

## Conclusions

Australian children have high ancestral S and N-antibody levels, and broad heterotypic immunity against Omicron subvariants circulating in 2023. Seroprevalence was high even in unvaccinated children without reported infection. These findings support ATAGI's current recommendation against primary vaccination and boosters in healthy children <18 years. Vaccinated children had higher antibody levels across all Omicron subvariants. Levels of antibodies decreased during the first year of life but increased with age thereafter and there may be a therapeutic benefit in vaccinating women during pregnancy to protect infants against disease.

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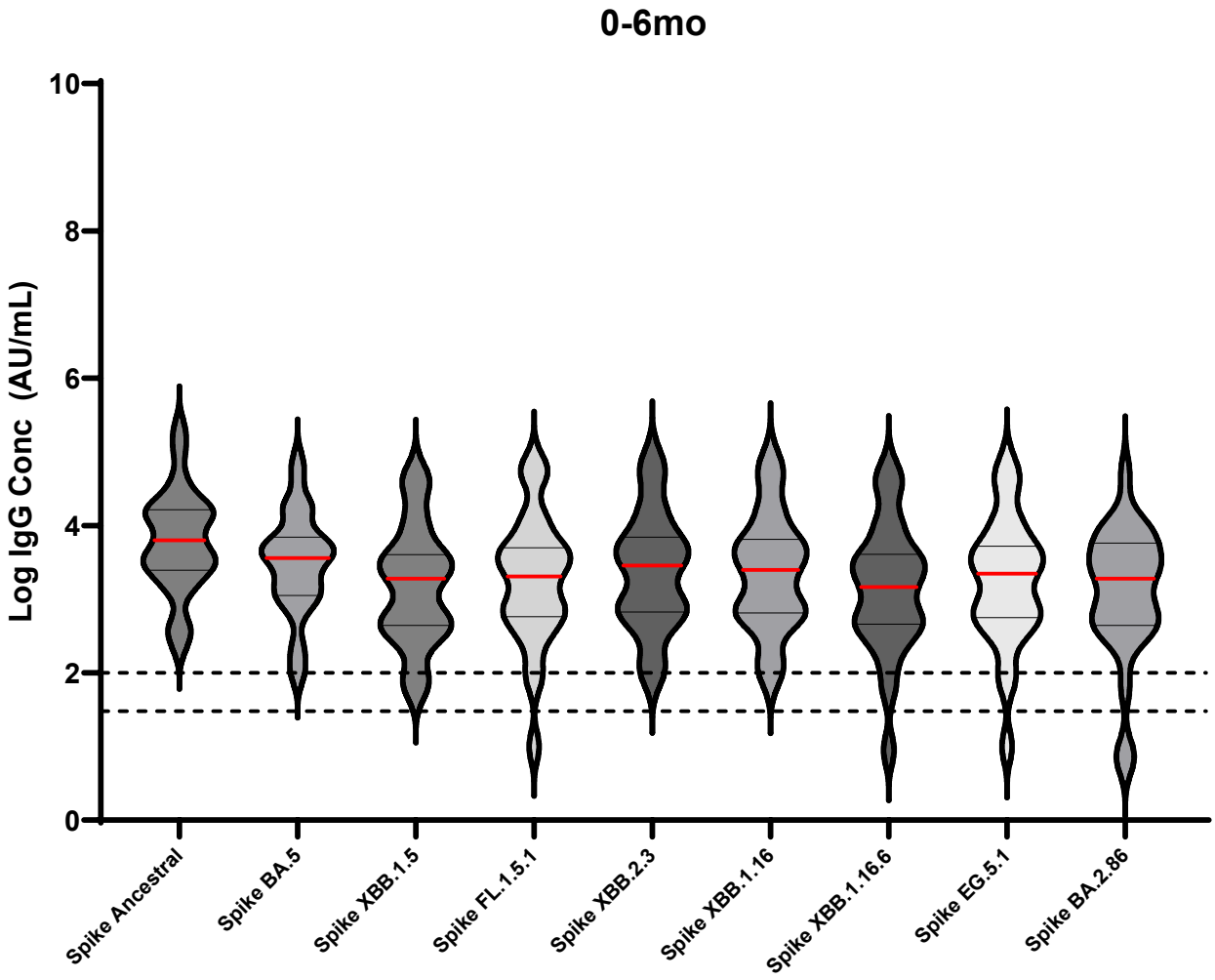
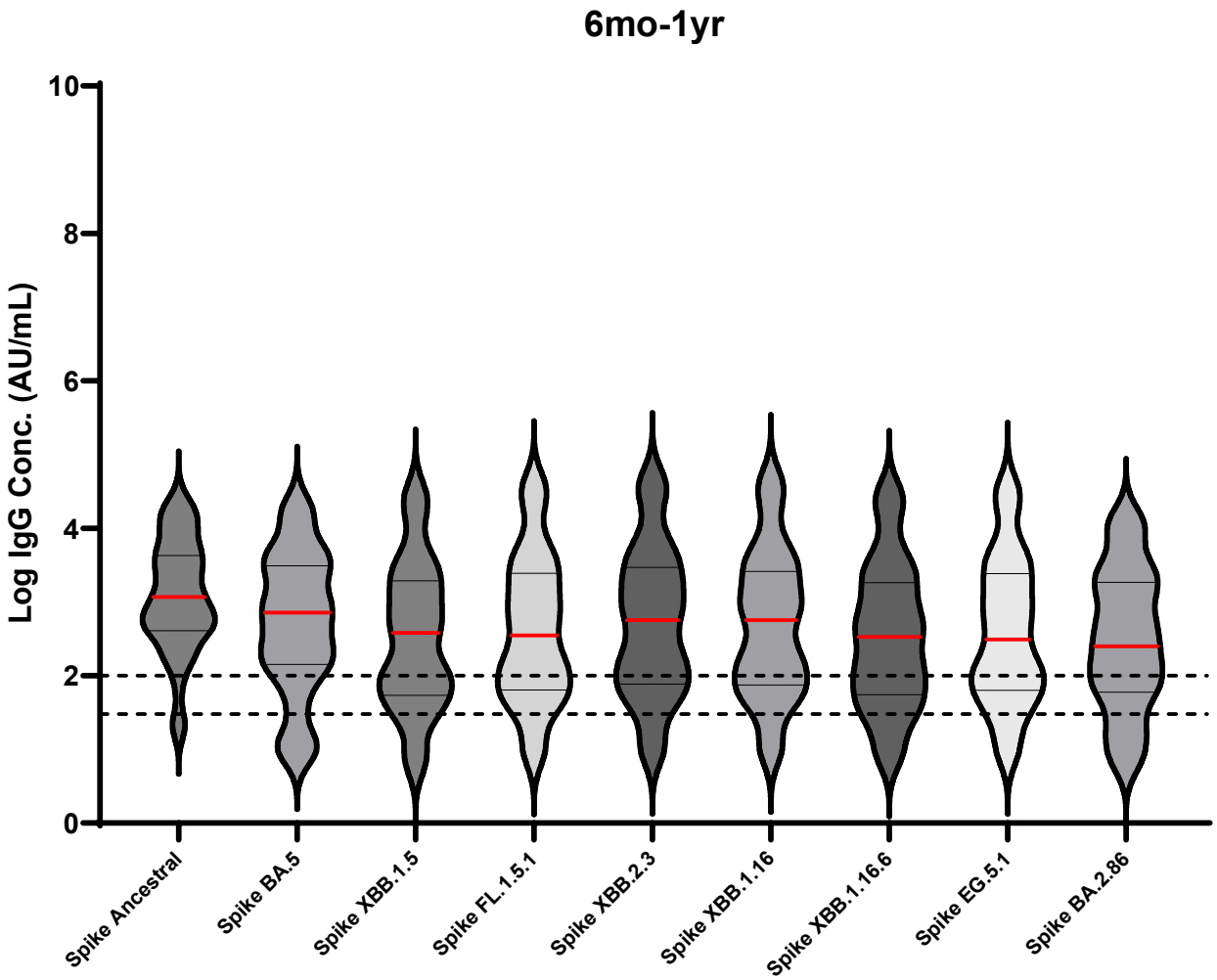
# Supplementary materials

***Supplementary Figure 1:*** *The GMC of ancestral and Omicron subvariant S-antibody in different age groups, a) 0–6 months (n=27), b) 6–12 months (n=41), c) 1–4 years (n=377), d) 5–11 years (n=385), e) 12–15 years (n=236). Antibody titres were transformed to log scale and represented as violin plots. Red bars represent the median, whereas dotted lines represent the interquartile range. Variants are ordered according to their global emergence*

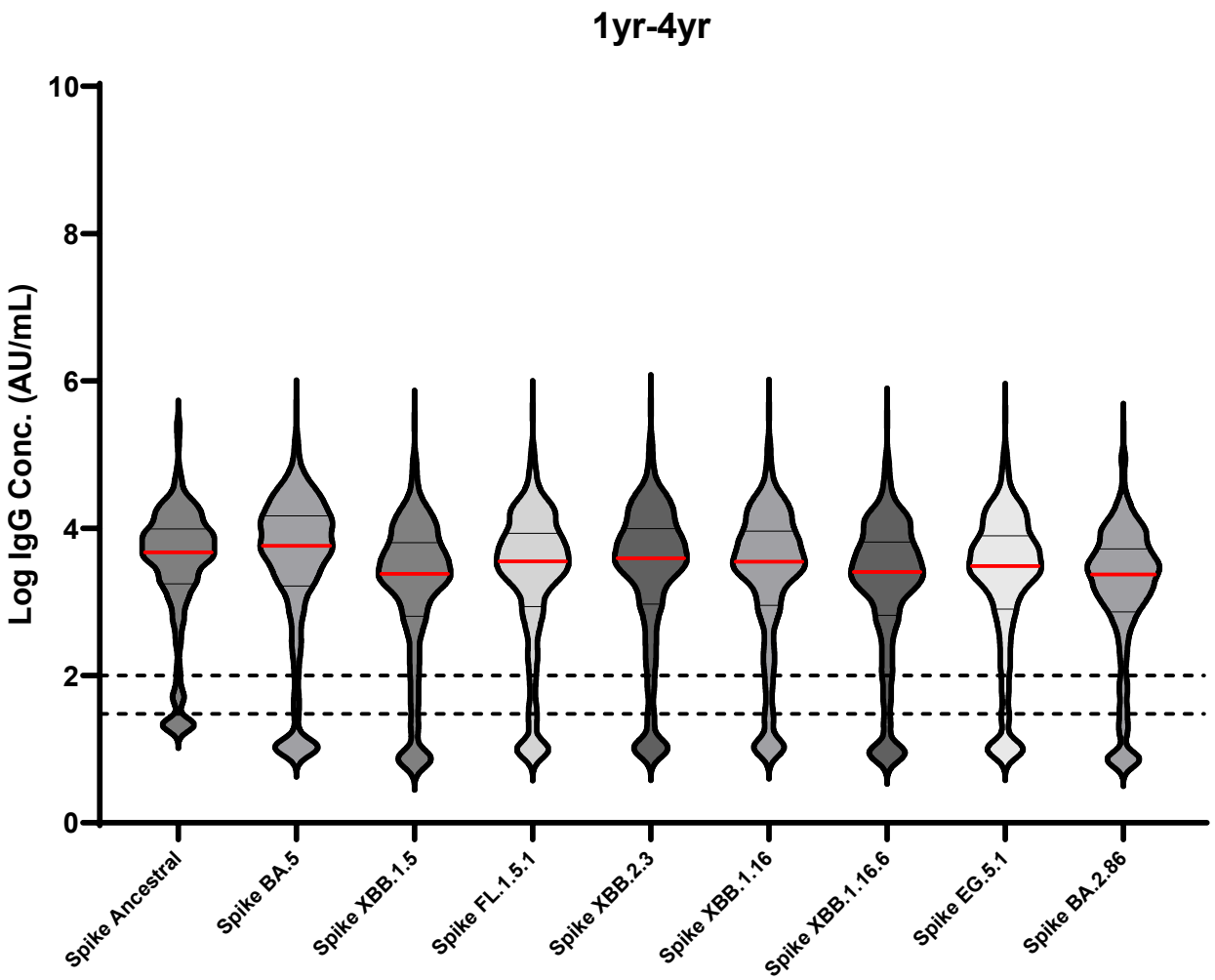
**a)**



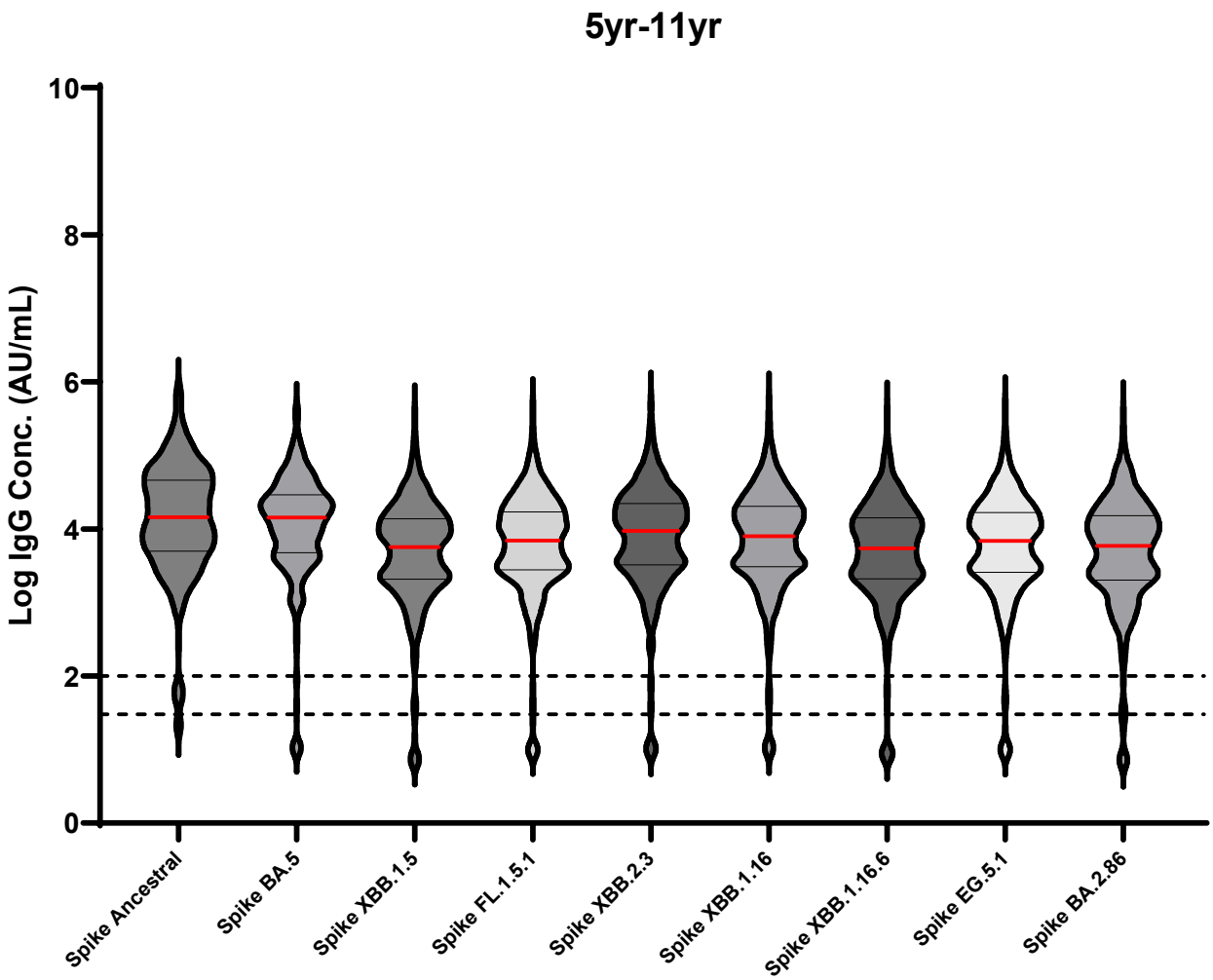
**b)**


6mo-1yr
Log IgG Conc. (AU/mL)
10
8
6
4
2
0
Spike Ancestral
Spike BA.5
Spike XBB.1.5
Spike FL.1.5.1
Spike XBB.2.3
Spike XBB.1.16
Spike XBB.1.16.6
Spike EG.5.1
Spike BA.2.86

**c)**


1yr-4yr
Log IgG Conc. (AU/mL)
10
8
6
4
2
0
Spike Ancestral
Spike BA.5
Spike XBB.1.5
Spike FL.1.5.1
Spike XBB.2.3
Spike XBB.1.16
Spike XBB.1.16.6
Spike EG.5.1
Spike BA.2.86

**d)**


5yr-11yr
Log IgG Conc. (AU/mL)
10
8
6
4
2
0
Spike Ancestral
Spike BA.5
Spike XBB.1.5
Spike FL.1.5.1
Spike XBB.2.3
Spike XBB.1.16
Spike XBB.1.16.6
Spike EG.5.1
Spike BA.2.86

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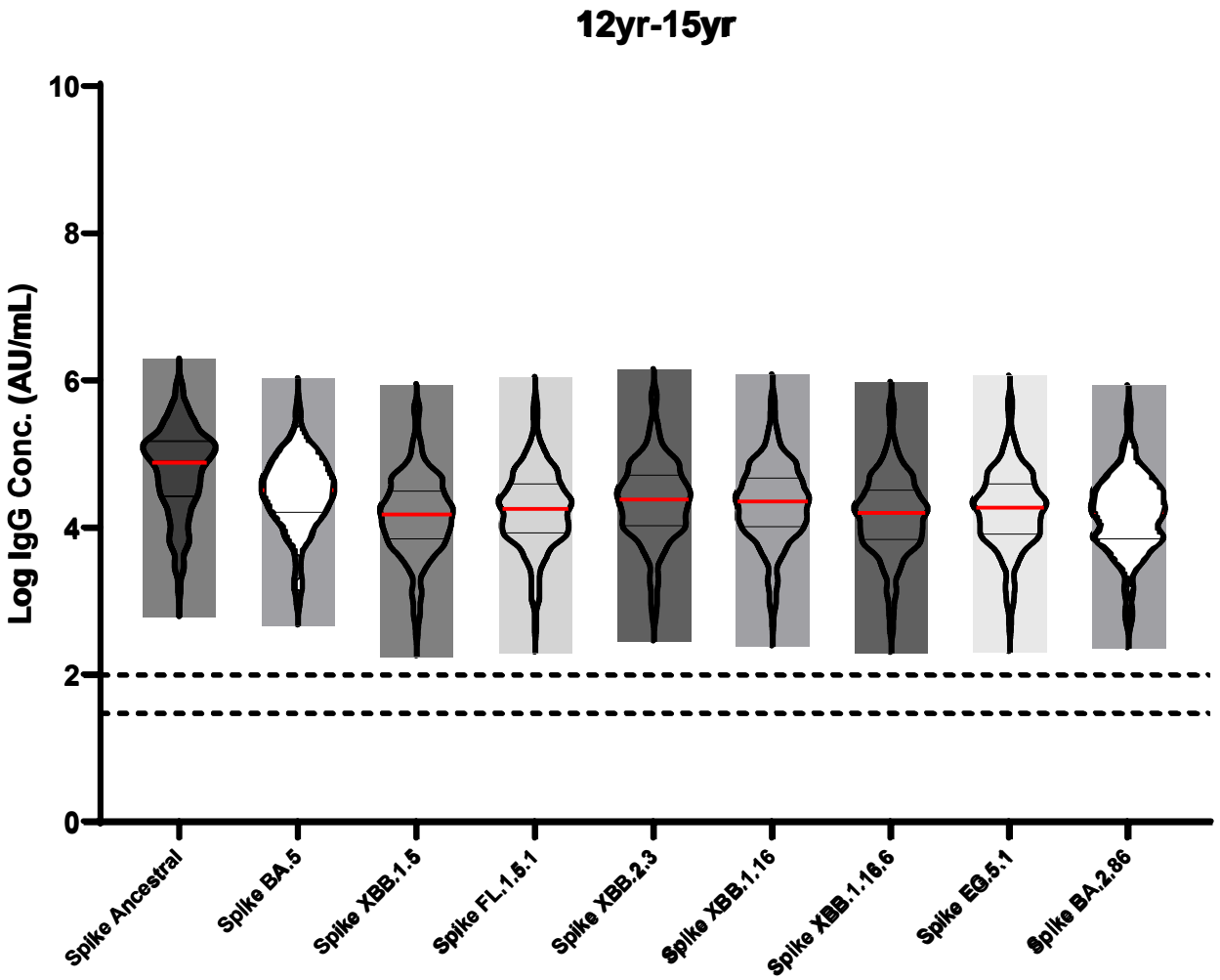
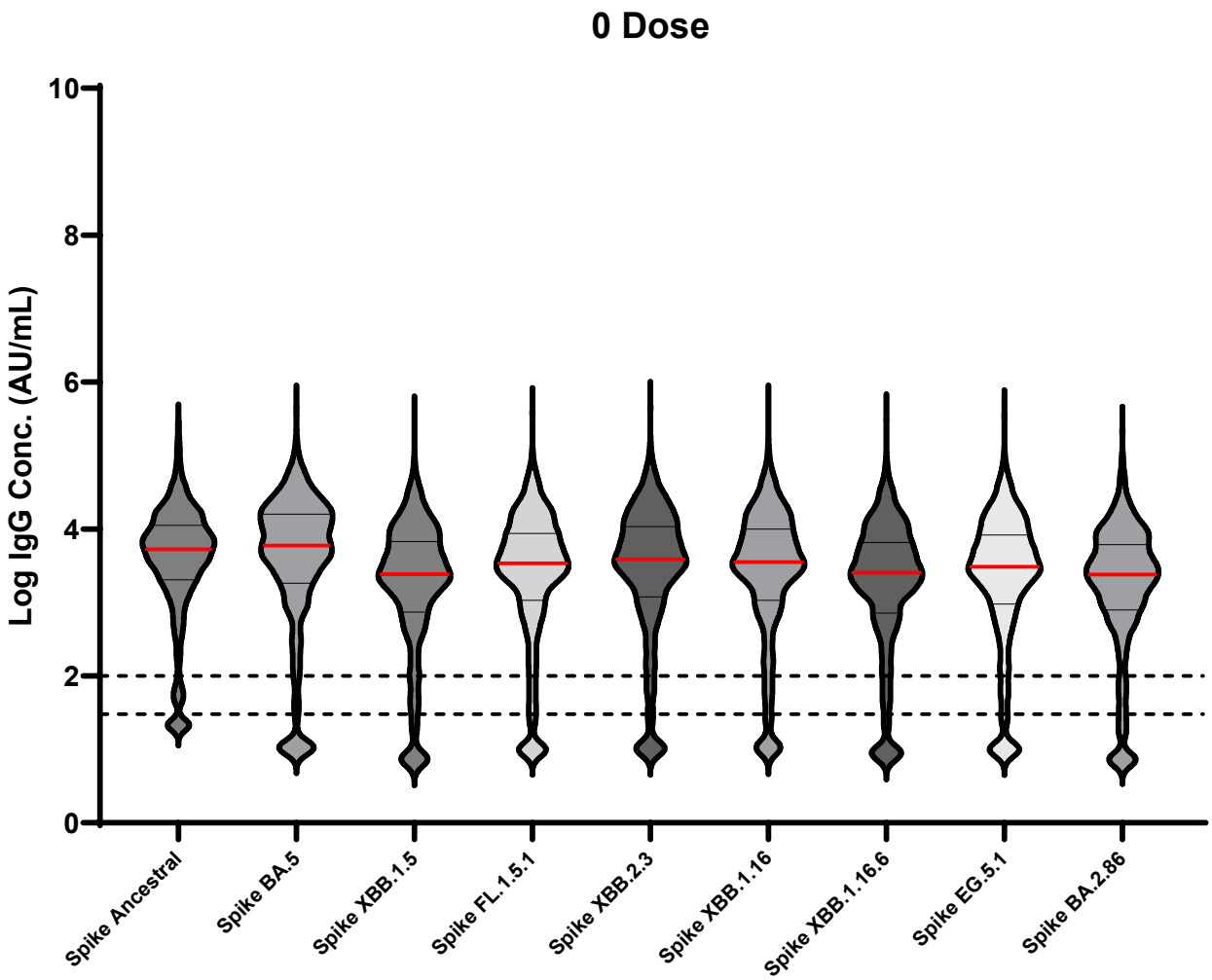
e)

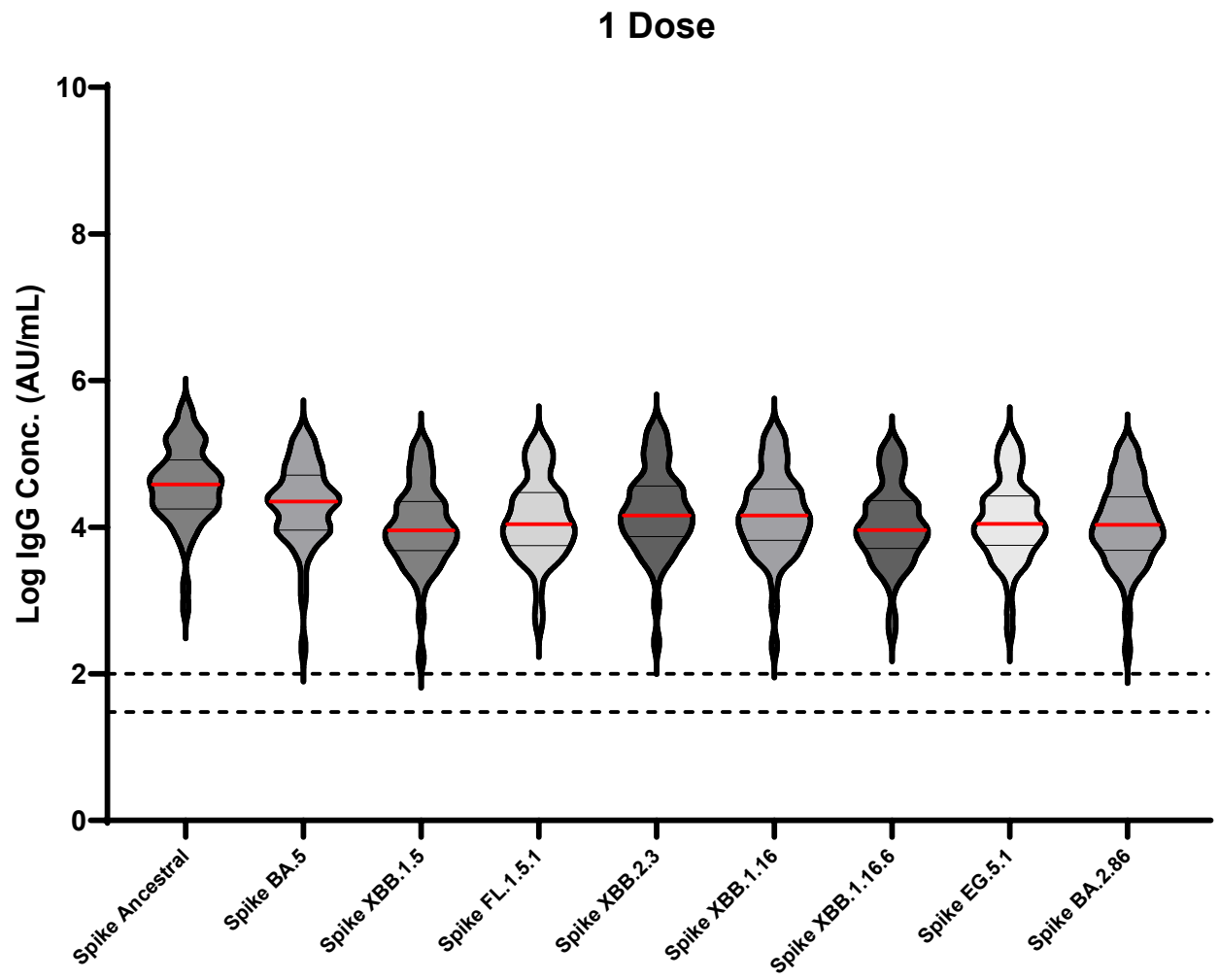

12yr-15yr
Log IgG Conc. (AU/mL)
10
8
6
4
2
0
Spike Ancestral
Spike BA.5
Spike XBB.1.5
Spike FL.1.5.1
Spike XBB.2.3
Spike XBB.1.16
Spike XBB.1.16.6
Spike EG.5.1
Spike BA.2.86

***Supplementary Figure 2:*** *The GMC of ancestral and Omicron subvariant S-antibody by vaccination status by a) 0 dose (n= 704), b) 1 dose (n=53) and c) 2 doses (n=296). Antibody titres were transformed to log scale and represented as violin plots. Red bars represent the median, whereas dotted lines represent the interquartile range. Variants are ordered according to their global emergence*

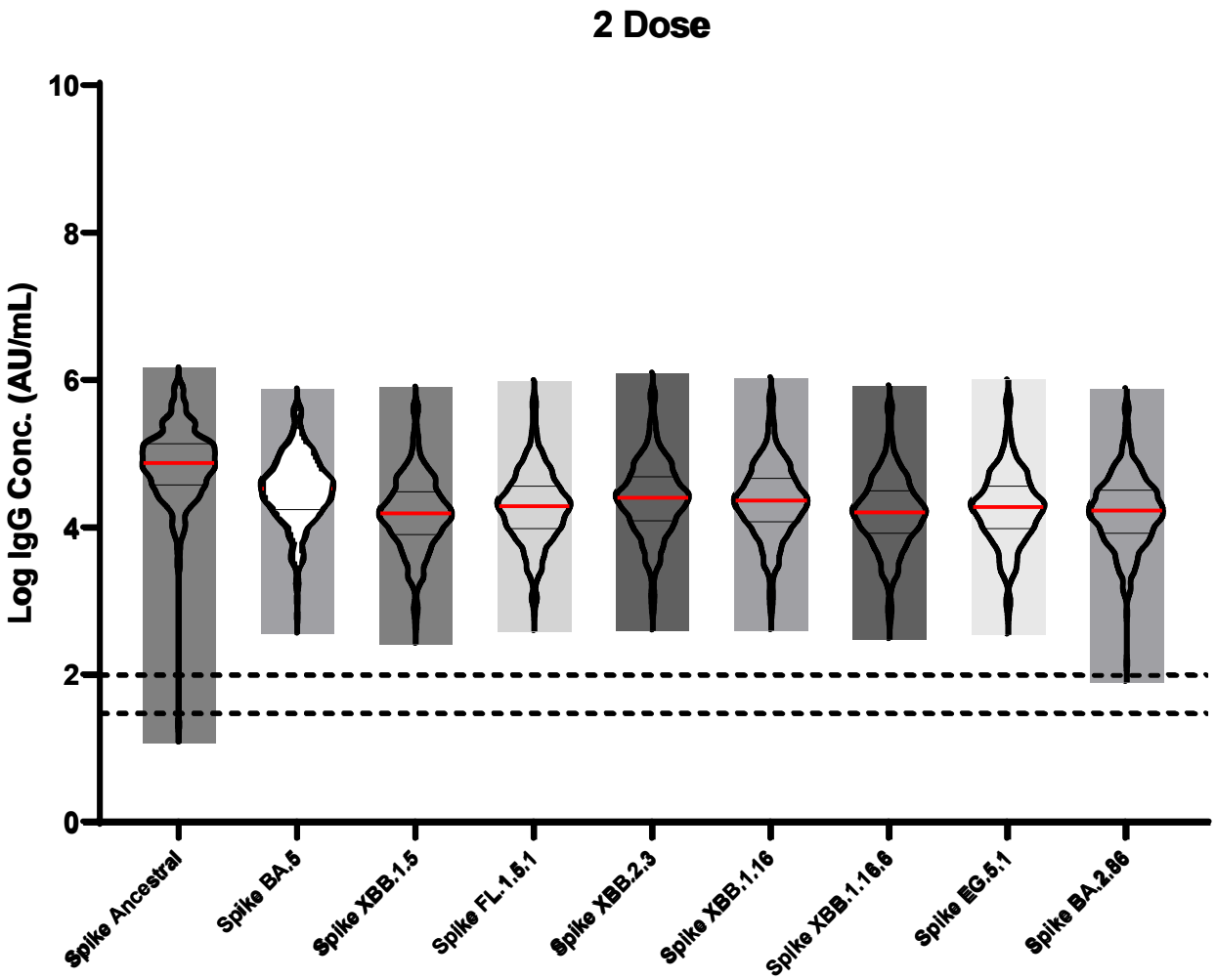
**a)**



**b)**


1 Dose
Log IgG Conc. (AU/mL)
10
8
6
4
2
0
Spike Ancestral
Spike BA.5
Spike XBB.1.5
Spike FL.1.5.1
Spike XBB.2.3
Spike XBB.1.16
Spike XBB.1.16.6
Spike EG.5.1
Spike BA.2.86

c)


2 Dose
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8
6
4
2
0
Spike Ancestral
Spike BA.5
Spike XBB.1.5
Spike FL.1.5.1
Spike XBB.2.3
Spike XBB.1.16
Spike XBB.1.16.6
Spike EG.5.1
Spike BA.2.86