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| Seroprevalence of SARS-CoV-2  Specific antibodies among Victorian blood donors |
| *Summary report for the Victorian Government Department of Health*  *03 May 2022* |
| OFFICIAL |

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# Overview

* Routine surveillance does not capture all people infected with SARS-CoV-2 because some are asymptomatic, not diagnosed, or not reported; therefore, estimating the proportion of the population with SARS-CoV-2 antibodies (i.e., seroprevalence) can improve understanding of the population-level incidence of infection.
* This report uses de-identified blood donor specimens to examine trends in infection- and vaccine-induced SARS-CoV-2 seroprevalence before and throughout the first Omicron epidemic wave in Victoria.
* Specimens were collected from Victorian blood product donations received during each of 4 time intervals: November 26–30, 2021 (n=1,000), December 24–28, 2021 (n=1,000), January 27–31, 2022 (n=1,000), and 24 February–March 2, 2022 (n=1,099) and tested using the Roche Elecsys anti-SARS-CoV-2 anti-spike and anti-nucleocapsid protein immunoassays. Crude seroprevalence estimates and 95% confidence intervals (CIs) were calculated.
* The presence of anti-spike antibodies indicates prior vaccination against SARS-CoV-2 infection and/or natural infection. The presence of anti-nucleocapsid protein antibodies indicates previous natural infection, most likely within the recent past.
* Prevalence of anti-spike antibody was very high (>98.0%) across all 4 time points, with little variation by age group and sex.
* Prevalence of anti-nucleocapsid seroprevalence was very low in November (0.2%), and December (0.7%), increasing to 9.5% in January and 22.5% in February. For both the January and February time points, anti-nucleocapsid seroprevalence was highest among donors aged 18–29 years (22.2% and 34.2%, respectively), decreasing steadily with increasing age-group to 0.0% in January and 11.1% in February, among donors aged 70­­–89 years.
* Seropositivity for anti-spike was modestly higher than in the general population based on vaccine coverage rates. This may reflect the presence of anti-spike antibodies induced by vaccination and those induced following infection, as it is not possible to distinguish between them. It may also be due to the behaviour of donors who may be more likely to be vaccinated.
* Seroprevalence for anti-nucleocapsid across time points was consistent with the epidemiology of notified cases, with the Omicron wave in Victoria beginning in late December. Of note, however, the November and December anti-nucleocapsid prevalence estimates were lower than cumulative case notifications for that period in people 18¬89 years (1.6% and 1.9%, respectively), while the January and February time points were higher (7.7% and 9.8%, respectively).
* Limited evidence suggests that as a marker of recent infection, anti-nucleocapsid antibodies may have lower sensitivity in vaccinated compared with unvaccinated persons who become infected. Additional work is underway to refine sensitivity estimates for the Roche anti-nucleocapsid immunoassay in this context and inform an analysis approach that accounts for a reduction in test sensitivity.

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# Background

Since the start of the COVID-19 pandemic, understanding the extent to which SARS-CoV-2 infection has been transmitted through the community has been critical to informing and evaluating infection control and prevention policies. Case reporting depends on several factors, including testing capacity and access, eligibility criteria, test reliability, and health-seeking behaviour of the population. Also, a substantial proportion of people who are infected with SARS-CoV-2 have few or no symptoms and will often not be detected by routine surveillance systems1.

However, infection leaves an ‘immunological signal’ in the form of anti-SARS-CoV-2 antibodies which can be detected in blood specimens. Large scale serosurveys of SARS-CoV-2 antibody prevalence conducted in the first year of the pandemic found very low levels in Australia, even after the so-called “second wave”in Victoria in 20202-5. Serosurveys are utilised routinely by many countries, including the USA, UK and South Africa6-8 to generate data on population exposure to infection and vaccination, and to inform and track the impact of the emergence of new, more transmissible variants such as Delta and Omicron. The highly transmissible Omicron variant of SARS-CoV-2 emerged in November 2021 and became the dominant variant in Australia by late December 2021. In Victoria, daily notified COVID-19 cases peaked in early-mid January 2022 (Figure 1).

However, the true incidence of COVID-19 during this wave is likely to have been much higher for several reasons. First, case ascertainment was reduced due to limited PCR testing capacity, with testing infrastructure unable to meet demand for the first 4–6 weeks of the Omicron surge. Second, rapid antigen tests (RATs) were recommended for most of Victoria’s population as the preferred case detection tool in early January 2022, but population-wide availability and use were limited for several weeks. Third, in contrast to PCR testing which is centralised through laboratories, individuals were required to self-report positive RAT results, with an unknown degree of compliance with this expectation. Finally, the proportion of Omicron infections that are asymptomatic is substantial, estimated at 27% in a recent South African study9 and likely higher in a highly vaccinated population. The Victorian Department of Health commissioned a retrospective survey of SARS-CoV-2 antibody prevalence in stored plasmapheresis specimens. This survey aimed to estimate seroprevalence of SARS-CoV-2 antibodies among Victorian blood donors prior to and during the Omicron wave to better understand infection spread in the population.

Figure 1. Count of COVID-19 (daily and cumulative) case notifications between 1 September 2021 and 9 March 2022 in Victoria among adults aged 18-89 years, and timing of specimen collections. See Appendix for full description.**Figure 1.** Count of COVID-19 (daily and cumulative) case notifications between 1 September 2021 and 9 March 2022 in Victoria among adults aged 18-89 years, and timing of specimen collections. See Appendix for full description.

# Methods

Blood specimens were obtained for testing through 2 separate mechanisms:

1. Retrospective plasmapheresis specimens: A total of 1000 specimens were retrieved from storage, from plasmapheresis donors who had donated in Victoria during 3 time intervals: 26–30 November 2021, 24–28 December 2021, and 27–31 January 2022.
2. The Australian COVID-19 Serosurveillance Network conducted the first round of a series of regular, national prospective serological surveys among blood donors of SARS-CoV-2 antibody prevalence. In this round, 5,187 specimens were collected nationally, including 1,099 samples from Victoria, from donations made between 24 February and 2 March (Figure 1).

All samples are from Lifeblood donors aged 18 years or over who meet routine donor eligibility criteria, as per the Australia Red Cross Lifeblood (<https://www.lifeblood.com.au/blood/eligibility>). Data were collected for each specimen on age, sex, and residential postcode. The plasmapheresis samples are routinely stored as plasma for four months at –30°C for regulatory purposes, and were retrieved for the study from the Lifeblood archive facility in Tullamarine. The specimens from the national survey were fresh blood samples from any blood donor (plasmapheresis or whole blood), and were collected from the Melbourne Lifeblood processing centre prior to discard.

Samples were tested for the presence of antibodies to spike and nucleocapsid proteins using the Roche Elecsys Anti-SARS-CoV-2 anti-Spike and anti-nucleocapsid immunoassays. This diagnostic system was selected for high sensitivity, specificity and throughput capacity 10,11.

Population data were obtained from the Australian Bureau of Statistics 2021 mid-year estimated residential population (ERP), and blood donor panel statistics were obtained from Lifeblood.

Reported seroprevalence estimates and 95% confidence intervals (CIs) were based on crude seropositivity for anti-spike and anti-nucleocapsid, with 95% confidence intervals estimated by the binomial exact method. Estimates were presented stratified by age-groups, sex, Statistical Area Level 4 (SA4) and donation type (plasmapheresis versus other). In sensitivity analyses, crude seropositivity age-standardised to the Victorian ERP were also calculated.

Ethics approvals were granted by the Sydney Children’s Hospital Network Human Research Ethics Committee ([SCHN HREC] HREC 2022/ETH00187), Lifeblood Ethics Committee (2022#07) and UNSW HREC (2022/ETH00187).

# Results

## Characteristics of the survey population

Valid results were available for 4,085/4,099 (99.7%) samples collected, including 996 for November, 994 December, 997 January and 1,098 February. The mean age of donors was 44.9 years (range 18–82) and was similar across the time points. Males and females were sampled equally (51.1% male). All SA4s (the largest sub-region smaller than state level, with a minimum of 100,000 population) were sampled and distributions were broadly similar across time points, with metropolitan SA4’s slightly oversampled in the February time point (Figure 2).

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| **Figure 2.** Distribution of demographic characteristics, age group (A), sex (B), and geographic area1 (C), for the 4 survey time points, the broader Victorian blood donor population2 and Estimated Residential Population (ERP)3 . See Appendix for full description. |
| Figure 2. Distribution of demographic characteristics, age group (A), sex (B), and geographic area1 (C), for the 4 survey time points, the broader Victorian blood donor population2 and Estimated Residential Population (ERP)3 . See Appendix for full description. |
| **1:** ABS Statistical Area Level 4 (SA4). SA4 regions are the largest sub-state regions have a minimum population of 100,000 persons. **2:** All persons donating blood to Australian Lifeblood between 1 January 2021 – 31 December 2021. **3:** ABS data on the estimated residential population of persons aged 18-89 years in Australia (excluding Other Territories) as of 30 June 2021. |

## Anti-spike protein seroprevalence

Prevalence of anti-spike antibody was very high across all 4 time points (99.2% [98.4–99.7] in November, 98.9% [98.0–99.4] in December, 98.8% [97.9–99.4] in January and 98.8% [98.0–99.4] in February) with little variation by age-groups (Figure 3A and Appendix Table 1).

Seroprevalence was similar for males and females at each time point.

Among samples positive for anti-spike antibody, the majority had titres >250 U/ml, with this proportion increasing at the January and February time points, which may be due to boosting effects from natural infection and/or vaccination.

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| **Figure 3.** Crude SARS-CoV-2 anti-spike protein seroprevalence among Victorian blood donors over time, by age group (A), sex (B), and semi-quantitative antibody concentration levels over time (C). See Appendix for full description. |
| Crude SARS-CoV-2 anti-spike protein seroprevalence among Victorian blood donors over time, by age group (A), sex (B), and semi-quantitative antibody concentration levels over time (C). See Appendix for full description. |

## Anti-nucleocapsid protein seroprevalence

Prevalence of anti-nucleocapsid seroprevalence was very low in November (0.2% [0-0.7]), and December (0.7% [0.3–1.4]), increasing to 9.5% [7.8­–11.5] in January and 22.5% [20.1–25.1] in February (Figure 4A).

A trend of decreasing anti-nucleocapsid seroprevalence with increasing age is present in the January and February time points. No differences in seroprevalence across time points were observed following age-adjustment compared with unadjusted seroprevalence. Males and females had similar anti-nucleocapsid seroprevalence at each time point.

The proportion of anti-spike positive samples that were anti-nucleocapsid negative decreased over the time points, from >99% in November and December to 90.4% in January and 77.1% in February.

Anti-nucleocapsid seroprevalence was compared with cumulative case notifications (aged 18–89 years, as a proportion of the Victorian population of the same age) reported up to 14 days prior to the median date of collection at each time point. The November and December seroprevalence estimates are lower than the population proportion of cumulative case notifications (1.6% in November and 1.9% in December), while the January and February time points are higher (7.7% in January and 9.8% in February).

Anti-nucleocapsid seroprevalence by SA4 is presented in Table S4.

Within the February time point, plasmapheresis donors aged 18–29 and 30–39 had lower anti-nucleocapsid seroprevalence (31.9% [23.4–41.3] and 20.8% [13.5–29.7]) compared to other blood donor types (36.5% [27.7–46.0] and 29.8% [20.8­–40.1]). This difference was not observed in older age groups (Table S5 and Figure S1).

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| **Figure 4.** Crude SARS-CoV-2 anti-nucleocapsid protein seroprevalence among Victorian blood donors over time, by age group (A) and sex (B). See Appendix for full description. |
| Figure 4. Crude SARS-CoV-2 anti-nucleocapsid protein seroprevalence among Victorian blood donors over time, by age group (A) and sex (B). See Appendix for full description. |

# Interpretation and data considerations

* Findings from this report showed that seroprevalence of anti-spike antibody was very high (>98.0%) across all 4 time points, with little variation by age group and sex. These estimates are modestly higher than what would be expected in the general population based on vaccine coverage rates. At the end of February 2022, an estimated 94.8% of the eligible Victorian population had received at least 1 dose of a COVID-19 vaccine, with the lowest rates reported among young adults, in whom coverage was 83.4%, 86.3% and 90.8% for those aged 20–24, 25–29 and 30–34 years, respectively12.
* The very high prevalence of anti-spike antibodies likely reflects a combination of vaccine recipients and natural infection among a minority of donors who had not been vaccinated. The vaccines used in Australia, and natural infection with SARS-CoV-2 produce antibody responses against the SARS-CoV-2 spike protein that are indistinguishable, on the basis of anti-spike protein positivity alone. Other markers of antibody responses specific to a range of virus-variant epitopes can be examined to better understand immune profiles.
* Blood donors may be more highly vaccinated than the general population. It is well recognised that blood donors are a distinct population. They have been shown to have a higher average income and education and be healthier than the general population13,14. These factors have been shown to impact health-seeking behaviour, including COVID-19 vaccination15,16.
* Crude anti-nucleocapsid seroprevalence estimates were somewhat lower than expected, particularly for the November and December time points, where estimates were lower than the cumulative case notifications following the peak of the Delta wave. There is evidence to suggest that anti-nucleocapsid antibodies are produced at lower levels and wane faster in people who acquire infection following vaccination than those who have not been vaccinated17,18. Available data on the sensitivity of serology assays to detect anti-nucleocapsid antibodies in vaccinated persons who become infected with the Alpha or Delta variants differ considerably, with estimates ranging from as low as 26% and as high as 86%. Preliminary local data on breakthrough infections with the Omicron variant among vaccinated Australian healthcare workers suggests sensitivity of 74% (Table S6).
* While estimates of nucleocapsid protein prevalence that have not been adjusted for test performance will underestimate the true cumulative SARS-CoV-2 attack rate in the population, the general pattern across time points is consistent with the epidemiology of notified cases, with the Omicron wave in Victoria beginning in late December.
* In the UK and USA, crude estimates (i.e. without adjustment) have been used to track changes in infection rates over time using the Roche assay. Steady increases in anti-nucleocapsid protein seroprevalance have been reported, consistent with the reported SARS-CoV-2 epidemiology in these countries7,8,19.
* The lower seroprevalence observed among plasmapheresis donors in the February time point compared with other donor types suggests the presence of some systematic differences between plasmapheresis donors, which may also have contributed to lower, biased estimates in the November to January time points.
* Statistical approaches have been developed to produce adjusted seroprevalence estimates that incorporate assay-specific test performance characteristics4,5. Additional work is underway to estimate the sensitivity of the Roche anti-nucleocapsid immunoassay in this context and inform an analysis approach that accounts for a reduction in test performance (Table S6).

# Recommendations & additional planned work

* We recommend that additional statistical modelling be undertaken to produce adjusted estimates of anti-nucleocapsid seroprevalence if reliable test sensitivity and specificity data becomes available. As mentioned in the previous section, available estimates on the sensitivity of anti-nucleocapsid assays in vaccinated populations vary substantially and are based on breakthrough infections with either the Alpha or Delta variants in overseas populations. Local data on infections following the Omicron wave are needed to inform the analysis approach for this study. These are in the process of being generated.
* We do not recommend testing additional stored plasmapheresis specimens from the February time point for two reasons. First, the lower seroprevalence observed among plasmapheresis donors in the February time point compared with other donor types was also observed in other jurisdictions, suggesting a real effect. Testing additional samples would increase the precision (i.e., reduce the width of the confidence intervals) of the February estimates for plasmapheresis donors but is unlikely to change the overall study findings. Second, Lifeblood indicated that the retrospective collections turned out to be more demanding than they had anticipated, and they were strongly hesitant about retrieving more samples.
* Ongoing serosurveillance among blood donors will continue to provide crucial information on the impact of the pandemic. The Australian COVID-19 Serosurveillance Network will commence the next National blood donor serosurvey on 13 June, reflecting donation dates from 9 June. This survey time point will provide an estimate of SARS-CoV-2 antibody prevalence in Victoria following the spread of the Omicron BA.2 variant and capture any further surges leading up to winter. Results will be made available to the Department of Health as soon as they are available.

# Supplementary data

#### Table S1. Crude SARS-CoV-2 anti-spike protein seroprevalence among Victorian blood donors at each time point, by age group and sex. See Appendix for full description.

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| --- | --- | --- | --- | --- |
|  | **Nov 2021** | **Dec 2021** | **Jan 2022** | **Feb 2022** |
|  | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** |
| **Overall** | 988/996  (99.2; 98.4-99.7) | 983/994  (98.9; 98.0-99.4) | 985/997  (98.8; 97.9-99.4) | 1085/1098  (98.8; 98.0–99.3) |
| **Age group (years)** |  |  |  |  |
| 18–29 | 215/216  (99.5; 97.4-100) | 211/212  (99.5; 97.4-100) | 196/198  (99.0; 96.4-99.9) | 228/228  (100; 98.3–100) |
| 30–39 | 216/219  (98.6; 96.0-99.7) | 207/210 (98.6; 95.9-99.7) | 184/185 (99.5; 97.0-100) | 196/200  (98.0; 95.0–99.2) |
| 40–49 | 175/175 (100; 97.9-100) | 168/170 (98.8; 95.8-99.9) | 184/188 (97.9; 94.6-99.4) | 201/202  (99.5; 97.2–100) |
| 50–59 | 179/181 (98.9; 96.1-99.9) | 184/188 (97.9; 94.6-99.4) | 204/207 (98.6; 95.8-99.7) | 217/222  (97.7; 94.8–99.0) |
| 60–69 | 157/159 (98.7; 95.5-99.8) | 162/163  (99.4; 96.6-100) | 172/173 (99.4; 96.8-100) | 180/183  (98.4; 95.3–99.4) |
| 70–89 | 46/46 (100; 92.3-100) | 51/51  (100; 93.0-100) | 45/46 (97.8; 88.5-99.9) | 63/63  (100; 94.3–100) |
| **Sex** |  |  |  |  |
| Male | 495/500  (99.0; 97.7-99.7) | 456/463  (98.5; 96.9-99.4) | 484/490  (98.8; 97.4-99.5) | 624/633  (98.6; 97.3–99.3) |
| Female | 493/496  (99.4; 98.2-99.9) | 527/531 (99.2; 98.1-99.8) | 501/507 (98.8; 97.4-99.6) | 461/465  (99.1; 97.8–99.7) |

#### Table S2. Crude SARS-CoV-2 anti-spike protein seroprevalence among Victorian blood donors at each time point, by geographic area (Statistical Area Level 4). See Appendix for full description.

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| --- | --- | --- | --- | --- |
|  | **Nov 2021** | **Dec 2021** | **Jan 2022** | **Feb 2022** |
|  | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** |
| **Overall** | 988/996  (99.2; 98.4-99.7) | 983/994  (98.9; 98.0-99.4) | 985/997  (98.8; 97.9-99.4) | 1085/1098  (98.8; 98.0–99.3) |
| Inner | 119/120 (99.2; 95.4-100) | 124/125  (99.2; 95.6-100) | 108/108 (100; 96.6-100) | 186/187  (99.5; 97.1-100) |
| Inner East | 45/45 (100; 92.1-100) | 38/38 (100; 90.7-100) | 45/45  (100; 92.1-100) | 89/89  (100; 95.9-100) |
| Inner South | 87/87 (100; 95.8-100) | 77/77  (100; 95.3-100) | 107/108 (99.1; 94.9-100) | 93/94  (98.9; 94.2-100) |
| North-East | 76/76 (100; 95.3-100) | 59/59  (100; 93.9-100) | 86/86 (100; 95.8-100) | 163/165 (99.4; 96.6-100) |
| North-West^ | 46/48 (95.8; 85.7-99.5) | 45/46 (97.8; 88.5-99.9) | 41/41  (100; 91.4-100) | 74/76  (97.4; 90.8-99.7) |
| Outer East | 81/82 (98.8; 93.4-100) | 67/67  (100; 94.6-100) | 61/64 (95.3; 86.9-100) | 199/202 (98.5; 95.7-99.7) |
| South-East | 101/102 (100; 96.4-100) | 68/68  (100; 94.7-100) | 85/87 (97.7; 91.9-99.7) | 96/98  (98.0; 92.8-99.8) |
| West | 118/120 (98.3; 94.1-99.8) | 115/118  (97.5; 92.7-99.5) | 80/80  (100; 95.5-100) | 86/86  (98.8; 90.8-100) |
| Mornington Peninsula | 80/80 (100; 95.5-100) | 77/77  (100; 95.3-100) | 70/71 (98.6; 92.4-100) | 57/58  (98.3; 90.8-100) |
| Ballarat | 42/42  (100; 91.6-100) | 36/36  (100; 90.3-100) | 33/34  (97.1; 84.7-99.9) | 3/3  (100; 29.2-100) |
| Bendigo | 33/34  (97.1; 84.7-99.9) | 54/56  (96.4; 87.7-99.6) | 64/64 (100; 94.4-100) | 2/2  (100; 15.8-100) |
| Geelong | 65/65 (100; 94.5-100) | 100/101  (99.0; 94.6-100) | 70/70  (100; 94.9-100) | 11/11  (100; 71.5-100) |
| Hume | 14/14  (100; 76.8-100) | 29/29  (100; 88.1-100) | 40/40 (100; 91.2-100) | 13/14  (92.9; 66.1-99.8) |
| Latrobe - Gippsland | 44/44  (100; 92.0-100) | 38/40  (95.0; 83.1-99.4) | 54/57  (94.7; 85.4-100) | 3/3  (100; 29.2-100) |
| North-West# | Nil | 4/4 (100; 39.8-100) | 2/2  (100; 15.8-100) | Nil |
| Shepparton | 18/19  (94.7; 74.0-99.9) | 15/15 (100; 78.2-100) | 23/23  (100; 95.2-100) | 2/2  (100; 15.8-100) |
| Warrnambool and Southwest | 14/14 (100; 74.8-100) | 30/30  (100; 88.4-100) | 13/14  (92.9; 66.1-99.8) | 2/2  (100; 15.8-100) |
| Missing | 5/5 | 7/8 | 3/3 | 7/7 |

^ Melbourne city north-west. # Regional north-west.**Table S3. Crude SARS-CoV-2 anti-nucleocapsid protein seroprevalence among Victorian blood donors at each time point, by age group and sex. See Appendix for full description.**

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| --- | --- | --- | --- | --- |
|  | **Nov 2021** | **Dec 2021** | **Jan 2022** | **Feb 2022** |
|  | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** |
| **Overall** | 2/996 (0.2; 0.0-0.7) | 7/994 (0.7; 0.3-1.4) | 95/997 (9.5; 7.8-11.5) | 248/1098  (22.6; 20.1–25.2) |
| **Age group (years)** |  |  |  |  |
| 18–29 | 1/216  (0.5; 0.0-2.6) | 4/212 (1.9; 0.5-4.8) | 44/198 (22.2; 16.6-28.7) | 78/228  (34.2; 28.4–40.6) |
| 30–39 | 0/219 (0.0; 0.0-1.7) | 0/210 (0.0; 0.0-1.7) | 28/185 (15.1; 10.3-21.1) | 50/200  (25.0; 19.5–31.4) |
| 40–49 | 0/175 (0.0; 0.0-2.1) | 1/170 (0.6; 0.0-3.2) | 10/188 (5.3; 2.6-9.6) | 42/202  (20.8; 15.8–26.9) |
| 50–59 | 0/181 (0.0; 0.0-2.0) | 1/188 (0.5; 0.0-2.9) | 5/207 (2.4; 0.8-5.5) | 41/222  (18.5; 13.9–24.1) |
| 60–69 | 1/159 (0.6; 0.0-3.5) | 1/163 (0.6; 0.0-3.4) | 8/173 (4.6; 2.0-8.9) | 30/183  (16.4; 11.7–22.4) |
| 70–89 | 0/46 (0.0; 0.0-7.7 | 0/51  (0.0; 0.0-7.0) | 0/46 (0.0; 0.0-7.7) | 7/63  (11.1; 5.5–21.2) |
| **Sex** |  |  |  |  |
| Male | 0/500 (0.0; 0.0-7.4) | 3/463 (0.6; 0.1-1.9) | 55/490 (11.2; 8.6-14.4) | 143/633  (22.6; 19.5–26.0) |
| Female | 2/496  (4.0; 0.0-1.4) | 4/521 (0.8; 0.2-1.9) | 40/507 (7.9; 5.7-10.6) | 105/465  (22.6; 19.0–26.6) |

#### Table S4. Crude SARS-CoV-2 anti-nucleocapsid protein seroprevalence among Victorian blood donors at each time point, by geographic area. See appendix for full description.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Nov 2021** | **Dec 2021** | **Jan 2022** | **Feb 2022** |
|  | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** |
| **Overall** | 988/996  (99.2; 98.4-99.7) | 983/994  (98.9; 98.0-99.4) | 985/997  (98.8; 97.9-99.4) | 1085/1098  (98.8; 98.0–99.3) |
| Inner | 0/120  (0; 0-3.0) | 1/125  (0.8; 0-4.4) | 24/108  (22.2; 14.8-31.2) | 43/187  (23.0; 17.2-29.7) |
| Inner East | 0/45  (0; 0-7.9) | 1/38  (2.6; 0.1-13.8) | 5/45  (11.1; 3.7-24.1) | 18/89  (20.2; 12.4-30.1) |
| Inner South | 0/87 (0; 0-4.2) | 0/77  (0; 0-4.7) | 11/108  (10.2; 5.2-17.5) | 25/94  (26.6; 18.0-36.7) |
| North-East | 1/76  (1.3; 0-7.1) | 0/59  (0; 0-6.1) | 6/86  (7.0; 2.6-14.6) | 40/165  (24.4; 18.0-31.7) |
| North-West^ | 0/48  (0; 0- 7.4) | 0/46  (0; 0-7.7) | 6/41  (14.6; 5.6-29.2) | 19/76  (25.0; 15.8-36.7) |
| Outer East | 0/82  (0; 0-4.4) | 0/67  (0; 0-5.4) | 2/64  (3.1; 0.4-10.8) | 28/202  (13.9; 9.4-19.4) |
| South-East | 0/102  (0; 0-3.6) | 1/68  (1.5; 0-7.9) | 12/87  (13.8; 7.3-22.9) | 23/98  (23.5; 15.5-33.1) |
| West | 0/120  (0; 0-3.0) | 2/118  (1.7; 0.2-6.0) | 9/80  (11.3; 5.3-20.3) | 30/86  (34.9; 24.9-45.9) |
| Mornington Peninsula | 0/80  (0; 0-4.5) | 1/77  (1.3; 0-7.0) | 3/71  (11.3; 5.3-20.3) | 12/58  (20.7; 11.2-33.4) |
| Ballarat | 0/42  (0; 0-8.4) | 0/36  (0; 0-9.7) | 2/34  (5.9; 0.7-19.7) | 1/3  (33.3; 0.8-90.6) |
| Bendigo | 0/34  (0; 0-10.3) | 0/56  (0; 0-6.4) | 2/64  (3.1; 0.4-10.8) | 0/2  (0; 0-84.2) |
| Geelong | 0/65  (0; 0-5.5) | 1/101  (1.0; 0-5.4) | 5/70  (7.1; 2.4-15.9) | 1/11  (9.1; 0.2-41.3) |
| Hume | 0/14  (0; 0-23.2) | 0/29  (0; 0-11.9) | 2/40  (5.0; 0.6-16.9) | 2/14  (14.2; 1.8-42.8) |
| Latrobe – Gippsland | 1/44  (2.3; 0.1-12.0) | 0/40  (0; 0-8.8) | 4/57  (7.0; 1.9-17.0) | 1/3  (33.3; 0.8-90.6) |
| North-West# | Nil | 0/4  (0; 0-60.2) | 0/2  (0; 0-84.2) | Nil |
| Shepparton | 0/19  (0; 0-17.6) | 0/15  (0; 0-21.8) | 1/23  (4.3; 0.1-21.9) | 0/2  (0; 0-84.2) |
| Warrnambool and Southwest | 0/14  (0; 0-23.3) | 0/30  (0; 0-11.6) | 0/14  (0; 0-23.2) | 0/2  (0; 0-84.2) |
| Missing | 0/5 | 0/8 | 1/3 | 5/7 |

#### Table S5. Crude SARS-CoV-2 anti-spike and anti-nucleocapsid protein seroprevalence among Victorian blood donors at the February timepoint, by donation type. See Appendix for full description.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Anti-spike** | | **Anti-nucleocapsid** | |
|  | **Plasmapheresis** | **Other\*** | **Plasmapheresis** | **Other\*** |
|  | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** | **n/N (%; 95% CI)** |
| **Overall** | 575/ 584 (98.5; 97.1-99.3) | 510/514 (99.2; 98.0-99.8) | 120/ 584 (20.5; 17.3-24.1) | 128/514 (24.9; 21.2-28.9) |
| 18–29 | 113/113  (100; 96.8-100) | 115/115  (100; 96.8-100) | 36/113  (31.9; 23.4-41.3) | 42/115  (36.5; 27.7-46.0) |
| 30–39 | 102/106  (96.2; 90.6-99.0) | 94/94  (100; 96.2-100) | 22/106  (20.8; 13.5-29.7) | 28/94  (29.8; 20.8-40.1) |
| 40–49 | 95/96  (99.0; 94.3-100) | 106/106  (100; 96.6-100) | 20/96  (20.8; 13.2-30.3) | 22/106  (20.8; 13.5-29.7) |
| 50–59 | 120/123  (97.6; 93.0-99.5) | 97/99  (98.0; 92.9-99.8) | 20/123  (16.3; 10.2-24.0) | 21/99  (21.2; 13.6-30.6) |
| 60–69 | 108/109  (99.1; 95.0-100) | 72/74  (97.3; 90.6-99.7) | 18/109  (16.5; 10.1-24.8) | 12/74  (16.2; 8.7-26.6) |
| 70-89 | 37/37  (100; 90.5-100) | 26/26  (100; 86.8-100) | 4/37  (10.8; 3.0-25.4) | 3/26  (11.5; 2.4-30.2) |

\* Includes plateletpheresis samples (17/514) and samples where the volume collected was too low to determine collection type (7/514).

|  |
| --- |
| Figure S1. Crude SARS-CoV-2 anti-spike (A) and anti-nucleocapsid (B) protein seroprevalence among Victorian blood donors at the February timepoint, by donation type. See Appendix for full description. |
| Figure S1. Crude SARS-CoV-2 anti-spike (A) and anti-nucleocapsid (B) protein seroprevalence among Victorian blood donors at the February timepoint, by donation type. See Appendix for full description. |

#### Table S6. Summary of available data on the sensitivity of anti-nucleocapsid antibodies in vaccinated persons who become infected. See Appendix for full description.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Source** | **Time period** | **Number N-Ab positive1** | **Number tested2** | **Sensitivity**  **(Calculated 95% CI)** | **Assay** |
| *Roche insert* | *March–June 2020* |  | *496* | *99.5%* | *Roche* |
| **Publications** | | | | | |
| Allen et al. 202120 | April 2021 | 6 | 23 | 26.1%  (10.2–48.4%) | Roche |
| Pollett et al. 202221 | March 2020– May 2021 | 4 | 6 | 66.7%  (22.3–95.7%) | MMIA LakePharma |
| Whitaker et al. 202222 | February – August 2021 | Single dose  Alpha: 125  Delta: 20  Two doses  Alpha: 21  Delta: 42 | Single dose  Alpha: 142  Delta: 20  Two doses  Alpha: 27  Delta: 44 | Single dose   * Alpha: 88.0% * (81.5–92.9%) * Delta: 100% * (83.2–100%)   Two doses   * Alpha: 77.7%   (57.7–91.4%)   * Delta: 95.5%   (84.5–99.4%) | Roche |
| Follmann et al. 202223 | June 2020–March 2021 | 21 | 52 | 40.4% (27.0–54.9%) | Roche |
| **Internal validations** | | | | | |
| ICPMR Healthcare worker study | January–February 2022 | 23 | 31 | 74.2% (55.4–88.1) | Roche |

1Breakthrough infections confirmed positive by NAT. 2Total number of SARS-COV-2 vaccinated people tested.

# References

1. Buitrago-Garcia D, Egli-Gany D, Counotte MJ, et al. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: A living systematic review and meta-analysis. PLOS Medicine 2020; 17(9): e1003346.
2. al KAe. Seroprevalence of SARS-CoV-2-specific antibodies in Australian children between November 2020 and March 2021. MJA (In Press). 2022.
3. Gidding HF, Machalek DA, Hendry AJ, et al. Seroprevalence of SARS-CoV-2-specific antibodies in Sydney after the first epidemic wave of 2020. Med J Aust 2021; 214(4): 179-85.
4. Machalek DA, Vette KM, Downes M, et al. Serological testing of blood donors to characterist the impact of COVID-19 in Melbourne, Australia, 2020. medRxiv 2022: 2022.03.11.22272185.
5. Vette KM, Machalek DA, Gidding HF, et al. Seroprevalence of Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibodies in Australia After the First Epidemic Wave in 2020: A National Survey. Open Forum Infect Dis 2022; 9(3): ofac002.
6. Chen X, Chen Z, Azman AS, et al. Serological evidence of human infection with SARS-CoV-2: a systematic review and meta-analysis. Lancet Glob Health 2021; 9(5): e598-e609.
7. Jones JM, Stone M, Sulaeman H, et al. Estimated US Infection- and Vaccine-Induced SARS-CoV-2 Seroprevalence Based on Blood Donations, July 2020-May 2021. Jama 2021; 326(14): 1400-9.
8. Whitaker HJ, Elgohari S, Rowe C, et al. Impact of COVID-19 vaccination program on seroprevalence in blood donors in England, 2021. J Infect 2021; 83(2): 237-79.
9. Garrett N, Tapley A, Andriesen J, et al. High Rate of Asymptomatic Carriage Associated with Variant Strain Omicron. medRxiv 2022.
10. Bond KA, Williams E, Nicholson S, et al. Longitudinal evaluation of laboratory-based serological assays for SARS-CoV-2 antibody detection. Pathology 2021; 53(6): 773-9.
11. Peluso MJ, Takahashi S, Hakim J, et al. SARS-CoV-2 antibody magnitude and detectability are driven by disease severity, timing, and assay. Sci Adv 2021; 7(31).
12. Australian Government Department of Health. COVID-19 Vaccine Roll-out Jurisdictional Breakdown. 28 February 2022. Available at: www.health.gov.au/sites/default/files/documents/2022/03/covid-19-vaccine-rollout-update-jurisdictional-breakdown-28-february-2022.pdf.
13. Karki S, Gemelli CN, Davison TE, et al. Willingness of blood donors in Australia to provide additional data and blood sample for health research. Transfusion 2021; 61(10): 2855-61.
14. Burgdorf KS, Simonsen J, Sundby A, et al. Socio-demographic characteristics of Danish blood donors. PLoS One 2017; 12(2): e0169112.
15. Reedman CN, Drews SJ, Yi QL, Pambrun C, O'Brien SF. Changing Patterns of SARS-CoV-2 Seroprevalence among Canadian Blood Donors during the Vaccine Era. Microbiol Spectr 2022; 10(2): e0033922.
16. Edwards B, Biddle N, Gray M, Sollis K. COVID-19 vaccine hesitancy and resistance: Correlates in a nationally representative longitudinal survey of the Australian population. PLoS One 2021; 16(3): e0248892.
17. Allen N, Brady M, Riain UN, et al. Prevalence of Antibodies to SARS-CoV-2 following natural infection and vaccination in Irish Hospital Healthcare Workers; changing epidemiology as the pandemic progresses. medRxiv 2021: 2021.11.04.21265921.
18. Demmer RT, Baumgartner B, Wiggen TD, et al. Identification of natural SARS-CoV-2 infection in seroprevalence studies among vaccinated populations. medRxiv 2021: 2021.04.12.21255330.
19. Clarke KEN, Jones JM, Deng Y, et al. Seroprevalence of Infection-Induced SARS-CoV-2 Antibodies - United States, September 2021-February 2022. MMWR Morb Mortal Wkly Rep 2022; 71(17): 606-8.
20. Allen N, Brady M, Carrion Martin AI, et al. Serological markers of SARS-CoV-2 infection; anti-nucleocapsid antibody positivity may not be the ideal marker of natural infection in vaccinated individuals. J Infect 2021; 83(4): e9-e10.
21. Pollett SD, Richard SA, Fries AC, et al. The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) mRNA Vaccine-Breakthrough Infection Phenotype Includes Significant Symptoms, Live Virus Shedding, and Viral Genetic Diversity. Clin Infect Dis 2022; 74(5): 897-900.
22. Whitaker HJ, Gower C, Otter AD, et al. Nucleocapsid antibody positivity as a marker of past SARS-CoV-2 infection in population serosurveillance studies: impact of variant, vaccination, and choice of assay cut-off. medRxiv 2021: 2021.10.25.21264964.
23. Follmann D, Janes HE, Buhule OD, et al. Anti-nucleocapsid antibodies following SARS-CoV-2 infection in the blinded phase of the mRNA-1273 Covid-19 vaccine efficacy clinical trial. medRxiv 2022: 2022.04.18.22271936.

# Appendixes

## Figure 1

Figure 1 is a combination graph showing the timing of the four serosurvey collections along with numbers of new daily notified cases in bars and cumulative case numbers as a line. The X-axis shows date of notification by single day from 1 September 2021 to 9 March 2022. The primary Y-axis shows daily notified cases on scale from 0 to 34,000. The secondary Y-axis shows cumulative notified cases on a scale from 0 to 550,000. The figure shows that Survey 1, from 26¬–30 November 2021 occurred at a time when daily case numbers were low and relatively stable following the Delta peak in 2021. The figure shows that Survey 2, from 24–28 December 2021, occurred at the start of the Omicron wave as case numbers were beginning to rise sharply. The figure shows that Survey 3, from 27¬–31 January 2022, occurred following the peak of the Omicron wave as new case numbers were slowing and the cumulative case curve was levelling out. The figure shows that Survey 4, from 24 February–2 March 2022 as case numbers were lower and had stabilised following the peak of the Omicron wave in January.

## Figure 2

Figure 2 is a panel of three figures.

Figure 2A shows the distribution of the serosurvey sample at each of the four survey time points, as well as the overall Lifeblood population and estimated resident population (ERP) for Victoria as stacked bar graphs by age group (18–29, 30–39, 40–49, 50–59, 60–69, and 70–89 years). The X-axis shows the month and year of the four surveys as well as the Lifeblood and the Victorian ERP, and the Y-axis shows the proportion of the sample from 0–100%. Figure 2A shows that age distribution of the sample was stable across survey time points. The survey age distribution was similar to the Lifeblood population, with younger age groups slightly under-sampled and older age groups oversampled. The survey age distribution was similar to the Victorian ERP, with the 50–59 and 60–69 year age groups slightly oversampled and the 70–89 year age group slightly under-sampled.

Figure 2B shows the distribution of the serosurvey sample at each of the four survey time points, as well as the overall Lifeblood population and estimated resident population (ERP) for Victoria as stacked bar graphs by sex. The X-axis shows the month and year of the four surveys as well as the Lifeblood and the Victorian ERP, and the Y-axis shows the proportion of the sample from 0–100%. Figure 2B shows that the distribution of the samples at the first three time points were similar to each other and to the Lifeblood population and Victorian ERP, at approximately 50%, while the fourth time point in February 2022 slightly oversampled males.

Figure 2C shows the distribution of the serosurvey sample at each of the four survey time points, as well as the overall Lifeblood population and estimated resident population (ERP) for Victoria by Statistical Area level 4 (SA4). The X-axis shows column graph with each of the four survey time points, the Lifeblood population, and the Victorian ERP grouped by each of the 17 Victorian SA4s. The Y-axis shows the proportion of the sample from 0–100%. Figure 2C shows that a broad range of SA4s were sampled, with sampling occurring from SA4s, with SA4s in metropolitan Melbourne oversampled in the fourth time point in February.

## Figure 3

Figure 3 is a panel of three figures.

Figure 3A shows crude anti-spike protein seroprevalence at each at each of the four survey time points by age group (18–29, 30–39, 40–49, 50–59, 60–69, and 70–89 years) as points with 95% confidence intervals as error bars. The X-axis shows the month and year of the collection, and the Y-axis shows the crude seroprevalence on a scale from 85.0% to 100%. Figure 3A shows that all age groups across all four time points had consistently high crude seropositivity with no significant differences seen in any age group.

Figure 3B shows crude anti-spike protein seroprevalence at each of the four survey time point by sex (Female and Male) as points with 95% confidence intervals as error bars. The X-axis shows the month and year of the collection, and the Y-axis shows the crude seroprevalence on a scale from 85.0% to 100%. Figure 3B shows that seropositivity was similar between males and females at all four time points with no significant differences seen between them at any point.

Figure 3C shows the proportion of the samples by semi-quantitative anti-spike concentration levels over the four survey time points, with the month and year of each collection on the X-axis and cumulative proportion from 0 to 100% on the Y-axis. Semi-quantitative levels are split into four groups: <0.8 units (negative), 0.8–<25.0 units, 25–<250 units, and ≥250 units. It shows the majority of samples at each time point have anti-spike concentration levels above 250 units, at approximately 80% of samples in November and December, increasing to >90% in January and February.

## Figure 4

Figure 4 is a panel of two graphs.

Figure 4A shows crude anti-nucleocapsid protein seroprevalence at each of the four survey time points by age group (18–29, 30–39, 40–49, 50–59, 60–69, and 70–89 years) as points with 95% confidence intervals as error bars. The X-axis shows the month and year of the collection and the Y-axis shows the crude seroprevalence on a scale from 0% to 45%. Figure 4A shows that anti-nucleocapsid protein seroprevalence was very low (<1%) overall and across all age groups in November and December, Seroprevalence increases in January, and increases further in February, with a trend in age emerging in these time points of decreasing seroprevalence with age.

Figure 4B shows crude anti-nucleocapsid protein seroprevalence at each of the four survey time points by sex as points with 95% confidence intervals as error bars. The X-axis shows the month and year of the collection and the Y-axis shows the crude seroprevalence on a scale from 0% to 30%. Figure 4B shows that seropositivity was similar between males and females at all four time points with no significant differences seen between them at any point.

## Figure S1

Figure S1 is a panel of two graphs.

Figure S1A shows crude anti-spike seroprevalence by age-group (18–29, 30–39, 40–49, 50–59, 60–69, and 70–89 years) and donation type (plasmapheresis and other) as points with 95% confidence intervals as error bars. Age group is on the X-axis and seroprevalence is on the Y-axis on a scale from 85.0–100%. Figure S1A shows slightly lower seroprevalence overall and within the 30–39 year and 40–49 year age groups, although confidence intervals overlap at all these points.

Figure S2A shows crude anti-nucleocapsid seroprevalence by age-group (18–29, 30–39, 40–49, 50–59, 60–69, and 70–89 years) and donation type (plasmapheresis and other) as points with 95% confidence intervals as error bars. Age group is on the X-axis and seroprevalence is on the Y-axis on a scale from 0–50%. Figure S2A shows lower seroprevalence overall and within the 18–29 year, 30–39 year and 50¬–59 year age groups, although confidence intervals overlap at all these points.

## Table S1

Table S1 shows the crude SARS-CoV-2 anti-spike protein seroprevalence at each time point by age group and sex. The table shows the number of seropositive samples out of the total tested, with the seroprevalence as a proportion and 95% confidence intervals in brackets.

## Table S2

Table S2 shows the crude SARS-CoV-2 anti-spike protein seroprevalence at each time point by geographic area at Statistical Area Level 4 (SA4). The table shows the number of seropositive samples out of the total tested, with the seroprevalence as a proportion and 95% confidence intervals in brackets.

## Table S3

Table S3 shows the crude SARS-CoV-2 anti-nucleocapsid protein seroprevalence at each time point by age group and sex. The table shows the number of seropositive samples out of the total tested, with the seroprevalence as a proportion and 95% confidence intervals in brackets.

## Table S4

Table S4 shows the crude SARS-CoV-2 anti-nucleocapsid protein seroprevalence at each time point by geographic area at Statistical Area Level 4 (SA4). The table shows the number of seropositive samples out of the total tested, with the seroprevalence as a proportion and 95% confidence intervals in brackets.

## Table S5

Table S5 shows crude anti-spike and anti-nucleocapsid protein seroprevalence at the February time point by donation type (plasmapheresis or other) and age group. The table shows the number of seropositive samples out of the total tested, with the seroprevalence as a proportion and 95% confidence intervals in brackets.

## Table S6

Table S6 shows a summary of available data on the sensitivity of anti-nucleocapsid assays in vaccinated persons who become infected, including the source of the data, the time period, the number of antibody-positive samples, the number tested, the sensitivity with 95% confidence intervals, and the assay used.