The Medical Journal of Australia – **Pre-print – 11 November 2020**

Seroprevalence of SARS-CoV-2-specific antibodies in Sydney, Australia following the first epidemic wave in 2020

Heather F Gidding
Senior Research Fellow
1, National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases
Westmead, New South Wales, Australia

NHMRC Career Development Fellow and Principal Research Fellow
2, The University of Sydney
Faculty of Medicine and Health
The University of Sydney Northern Clinical School, Women and Babies Research
Sydney, New South Wales, Australia

Dorothy A Machalek
University of New South Wales
3, The Kirby Institute
Sydney, New South Wales, Australia

Alexandra J Hendry
Senior Research Officer - Coverage & Surveillance
4, Children's Hospital at Westmead
National Centre for Immunisation Research & Surveillance
Westmead, New South Wales, Australia

Helen E Quinn
Senior Research Fellow
4, The Children's Hospital at Westmead
National Centre for Immunisation Research and Surveillance of Vaccine Preventable
Westmead, New South Wales, Australia

2, The University of Sydney
Sydney, New South Wales, Australia

Kaitlyn Vette
Senior Research Fellow
1, National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases
Westmead, New South Wales, Australia

Frank H Beard
Staff Specialist - Public Health Physician
4, The Children's Hospital at Westmead
National Centre for Immunisation Research and Surveillance
Westmead, New South Wales, Australia

Senior Lecturer
School of Public Health

University of Sydney
Sydney, New South Wales, Australia

Hannah Shilling
5, The Royal Women's Hospital Melbourne
Centre for Women's Infectious Diseases
Melbourne, Victoria, Australia

Rena Hirani
Research Fellow
6, Australian Red Cross Blood Service
Research and Development
Alexandria, New South Wales, Australia

Iain B Gosbell
Research Fellow
6, Australian Red Cross Blood Service
Clinical Services & Research
Alexandria, New South Wales, Australia

7, Western Sydney University
School of Medicine
Penrith, New South Wales, Australia

David O Irving
Director of Research
6, Australian Red Cross Blood Service
Research and Development
Alexandria, New South Wales, Australia

8, University of Technology Sydney
Faculty of Health
Sydney, New South Wales, Australia

Linda Hueston
Principal Scientist
Institute of Clinical Pathology and Medical Research
9, Centre for Infectious Diseases and Microbiology Laboratory Services (CIDMLS)
Westmead, New South Wales, Australia

Marnie Downes
Post Doctoral Fellow
10, Murdoch Children's Research Institute
Clinical Epidemiology & Biostatistics Unit
Melbourne, Victoria, Australia

John B Carlin
Director
10, Murdoch Children's Research Institute
Clinical Epidemiology and Biostatistics Unit
Royal Children's Hospital
Parkville, Victoria, Australia

11, University of Melbourne
Department of Paediatrics & School of Population and Global Health
Melbourne, Victoria, Australia

Matthew NV O'Sullivan
Staff Specialist in Infectious Diseases and Microbiology
Westmead Hospital and University of Sydney
12, Centre for Infectious Disease and Microbiology & Marie Bashir Institute for Infectious Diseases and Biosecurity
Westmead, New South Wales, Australia

The University of Sydney
Sydney, New South Wales, Australia

Dominic E Dwyer
Institute of Clinical Pathology and Medical Research
13 Westmead Hospital
Centre for Infectious Diseases and Microbiology Laboratory Services
New South Wales Health Pathology
Westmead, New South Wales, Australia

The University of Sydney
Sydney, New South Wales, Australia

John M Kaldor
Professor and Program Head of Public Health Interventions Research Group
14, University of New South Wales
Kirby Institute
Kensington, New South Wales, Australia

Kristine Macartney
Director
Children's Hospital at Westmead
National Centre for Immunisation Research and Surveillance (NCIRS)
Westmead, New South Wales, Australia

Professor
University of Sydney
Department of Paediatrics and Child Health
Sydney, New South Wales, Australia

Competing interests: No relevant disclosures

Acknowlegements:

We would like to thank everyone who contributed to this project, especially the laboratory staff who collected the specimens and the scientific staff who performed the IFA and neutralisation assays. This includes Katherine Tudo, Melanie Lograsso and Bassam Al Zahroon (NSW Health Pathology-ICPMR); Andrew Cullen, Ian Chambers, Annabelle Farnsworth, Karen Wagner and Daniel Clifford (Douglass Hanly Moir Pathology); Agustin Franco, Pratibha James and Michael Mark (4Cyte Pathology); Juliette Holland and Kartik Naidu (Laverty Pathology); Rebecca Burrell, Philip Britton and Alex Micati (The Children's Hospital at Westmead); Elizabeth Knight (Australian Red Cross Lifeblood); Darren Croese (NSW Health Pathology Incident Management Team). We are grateful to Lucy Armstrong for her help co-ordinating the study (NCIRS); Heather Whitaker and Nick Andrews (Public Health England) for their statistical advice and sharing their analysis code; James McCaw and Jodie McVernon (The Peter Doherty Institute) for their input into the initial study design; and Michelle Cretikos, Roy Byun, Sheena Adamson and Laura Collie for their helpful advice (NSW Ministry of Health)

Abstract

Objectives, Setting, Participants: To estimate SARS-CoV-2-specific antibody seroprevalence among three subpopulations in Sydney (20-39-year-old women undergoing antenatal screening, 20-69-year-old plasmapheresis blood donors, and people of all ages having blood tests at selected diagnostic pathology services—general pathology) following the first epidemic wave of COVID-19 in Australia.

Design: Cross-sectional, involving de-identified residual blood specimens from public and private laboratories and Australian Red Cross Lifeblood collected April to June 2020, sampled by geographic location across 10-year age groups.

Main outcome measure: Proportion of participants in each subpopulation testing positive for anti-SARS-CoV-2-specific IgG antibody after adjustment for test sensitivity and specificity.

Results: Of 5,339 specimens, 38 were positive; there were no apparent patterns by age group, sex, or geographic area. Adjusted seroprevalence estimates were 0.15% (95% credible interval [CI] 0.04-0.41%) for people of all ages having a general pathology blood test, 0.79% (95% CI: 0.04-1.88%) for women aged 20-39 years undergoing antenatal screening and 0.29% (95% CI: 0.04-0.75%) for blood donors aged 20-69 years. When restricted to 20-39 year olds, the age group common to all three collections, estimates were 0.24% (95% CI: 0.04-0.80%) for general pathology, 0.79% (95% CI: 0.04-1.88%) for antenatal screening and 0.69% (95% CI: 0.04-1.59) for blood donors.

Conclusions: Seroprevalence well under 1% in all three subpopulations indicates limited community transmission during the first COVID-19 epidemic wave in Sydney. These findings indicate early and successful control of COVID-19, but also highlight the need to maintain efforts to mitigate further transmission.

The known: The first epidemic wave of COVID-19 in Australia was centred in Sydney, predominantly among returned travellers.

The new: Prevalence of SARS-CoV-2-specific IgG antibodies in Sydney following the first wave was very low in the three populations examined; 0.15% for people of all ages having a pathology test; 0.79% for women undergoing antenatal screening aged 20-39 years; and 0.29% for blood donors aged 20-69 years.

The implications: There was very limited community transmission during the first wave in Sydney.

Background

The SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) was first detected as causing human disease (COVID-19: coronavirus disease of 2019) in December 2019 in Wuhan, China, and a pandemic was declared by the World Health Organization on 11 March 2020.[1] In Australia, the first recorded case was diagnosed in Sydney from a sample collected on 22 January 2020 in a traveller from Wuhan.[2] In the following months, the number of daily COVID-19 diagnoses, as detected by nucleic acid testing (NAT) for SARS-CoV-2, increased to a peak of 469 nationally on 28 March followed by a rapid decline as various restrictions were imposed. During this 'first wave' in March-April, NSW reported nearly half of Australia's 6,932 confirmed COVID-19 cases to the end of April. Of the NSW cases, 60% were detected in Sydney among returned travellers, many of whom were in quarantine. Community-based transmission occurred but appeared to be sporadic rather than wide-spread.[3,4]

Reported COVID-19 cases represent an underestimate of the true number of infections in the population. The main reason is that a proportion of people with SARS-CoV-2 infection do not develop symptoms, or have mild symptoms, so do not attend clinical services for virus testing and diagnosis by NAT; others do not access testing when unwell. As a consequence, these infections are missing in case-based reporting.[5] Serological surveys (serosurveys) that measure the prevalence of SARS-CoV-2-specific antibodies in blood specimens from people in the community can help estimate how many people across the population have been previously infected with SARS-CoV-2 regardless of symptomatology or presentation for testing.[5] They have been undertaken in many countries, using a variety of sample collection and testing methodologies.[6]

The Australian Government has endorsed serosurveys as a core component of the national COVID-19 Surveillance Plan.[7] In line with this strategy and New South Wales Health's enhanced surveillance plan for COVID-19,[8] the first large population-based serosurvey was conducted in Sydney, just after the initial wave of reported COVID-19 cases. The survey aimed to estimate seroprevalence of SARS-CoV-2-specific antibodies in metropolitan Sydney using residual blood specimens, originally collected for other purposes, from three populations.

Methods

Data sources and selection of study participants

De-identified residual blood specimens (sera and heparinised plasma) were collected for testing from three metropolitan Sydney target subpopulations:

1) people of all ages having a blood test at selected diagnostic pathology services (general pathology);

2) pregnant women aged 20-39 years undergoing routine antenatal screening (antenatal); and

3) Australian Red Cross Lifeblood plasmapheresis donors aged 20-69 years.

General pathology specimens were sourced from multiple NSW Health Pathology laboratories and three large private pathology services (Douglass Hanly Moir Pathology, 4Cyte Pathology, and Laverty Pathology). Antenatal screening specimens were sourced from the same private laboratories as the general pathology specimens. Blood donor specimens were sourced from the Australian Red Cross Lifeblood processing centre in Sydney.

Eligible specimens were those from individuals living in postcodes assigned to the Statistical Area level 4 (SA4) regions of the Sydney Greater Capital City Statistical Area.[9] For the general pathology collection, specimens were excluded if they were collected from hospital inpatients (to exclude those potentially admitted due to COVID-19) or if SARS-CoV-2 antibody testing was specifically requested as part of the specimen collection. Blood donors actively recruited to donate convalescent plasma after a prior COVID-19 diagnosis were excluded. For each specimen, data on date of birth, sex, postcode of residence, and date of specimen collection were collected and used to ensure only one specimen per individual was included.

Target sample size and distribution

A target sample size of 350 specimens per 10-year age group in each collection was estimated to exclude a seroprevalence of greater than 2.0% at the 95% confidence level if observed seropositivity was at most 2/350 (0.6%). To obtain geographic representation, we aimed to collect specimens across the 14 SA4 regions of the Sydney Greater Capital City Statistical Area in numbers proportional to the size of the population in each SA4.[9] Allocation of specimens to a SA4 was based on postcode of residence.

SARS-CoV-2-specific antibody testing

Testing was performed at the Institute of Clinical Pathology and Medical Research (ICPMR) using a validated in-house immunofluorescent antibody (IFA) assay.[10] Specimens were considered seropositive if the SARS-CoV-2-specific IgG titre was ≥10 by IFA. The sensitivity

of this test when performed on sera collected from individuals with suspected COVID-19 ≥14 days post illness onset (compared to the 'reference standard' of SARS-CoV-2 NAT performed on upper respiratory tract swabs at the time of presentation) was 90.7% (95% confidence interval: 83.9-95.3%) and specificity was 99.3% (95% confidence interval: 98.9-99.6%). Seropositive specimens were further tested for IgA and IgM antibodies using IFA and for SARS-CoV-2-specific neutralising antibodies, via a microneutralisation assay also developed by ICPMR, with a titre of ≥10 considered positive.

Statistical analysis

First, the age, sex and SA4 distribution of each collection was compared with the following reference populations as appropriate: 2019 Australian Bureau of Statistics (ABS) estimated residential population (ERP);[9] estimated numbers of women undergoing antenatal care (based on the ERP for females in 2019 multiplied by fertility rates in 2016-2018);[11] and numbers of Lifeblood plasmapheresis donors in 2019. Then, for each collection, the crude proportion of participants with an IFA IgG titre ≥10 was calculated. In addition, the distribution of seropositive participants by age group, sex and SA4 was described. The proportions with high (≥160) and low (10) titres and those also positive for IgA, IgM and neutralising antibodies were compared between collections.

A Bayesian analysis was undertaken to estimate the true proportion seropositive in each subpopulation after adjustment for test sensitivity and specificity, incorporating the uncertainty in their estimates (see Supporting Information, Supplementary methods for details). Further adjustment for sampling to obtain population weighted estimates was planned but not possible due to the small number of seropositive participants. Seroprevalence was summarised using the median and 95% credible interval (CI; highest posterior density interval) of its posterior distribution. The base case prior distribution for seroprevalence assumed uniform probability density between a seroprevalence of 0.04% (lower bound established from the ratio of notified cases to the ABS ERP) and 100%; this assumption is conservative and reflects the common preference to 'noninformative' prior assumptions. A sensitivity analysis was undertaken with an alternative prior distribution that assigned a relatively higher probability to lower seroprevalence estimates.

An estimate of the cumulative number of SARS-CoV-2 infections in the population was obtained by multiplying the seroprevalence point estimate and 95% credible interval (CI) for the general pathology collection by the 2019 ABS ERP for Sydney.[9] The general pathology collection was chosen as it included all ages and had the largest sample size and number of seropositive participants. The estimated ratio of infections to notified NAT-positive cases ("infection to case ratio") was then calculated using the cumulative number of notified cases 14 days prior to the mid-point of the collection period (30th April 2020); data supplied by NSW Health.

Ethics approval

Ethics approvals were obtained from The Sydney Children's Hospital Network Human Ethics Committee (HREC/17/SCHN/245) and the Australian Red Cross Lifeblood Ethics Committee (2020#07).

Results

Specimens were collected from participants between 20 April and 2 June, 2020. The timing of the collections in relation to SARS-CoV-2 case notifications is presented in Figure 1. In total, 5,339 eligible specimens were collected, including 3,231 general pathology, 560 antenatal screening and 1,548 blood donor specimens (Table 1). The target sample size (n=350) was met in most age groups, with the largest shortfall in children 0-9 years old and 60-69-year-old blood donors. Compared with the 2019 ABS and target populations, the actual distributions of the collections were broadly representative by sex and geographic location, although the antenatal collection had proportionally more from the Outer West and Blue Mountains and fewer from Blacktown and the Inner South West regions than the corresponding target population (Table 1).

SARS-CoV-2-specific IgG antibodies were detected by IFA in 38 individuals: 19 general pathology, 7 antenatal screening and 12 Lifeblood participants. Seropositive participants came from across the Sydney metropolitan region, with the highest number across all collections combined coming from Sydney's South-West and neighbouring Parramatta SA4 (Figure 2). Most of the SARS-CoV-2-specific IgG positive participants had low titres with 11 of the general pathology participants having the minimum titre of 10; 6 were also positive for IgM or IgA (Table 2). Overall 63% (24/38) of the IgG positive participants also had neutralising antibodies, with a lower proportion of general pathology participants having neutralising antibody than antenatal participants and blood donors (Table 2).

Crude seroprevalence ranged from 0.6% to 1.3% across the three populations (Table 3). The numbers seropositive were insufficient to indicate any patterns by age group or sex within each collection (Table 3). However, 20-29-year-old blood donors had a higher number seropositive than other age groups and there were no seropositive children <10 years of age (in the general pathology collection). After adjustment for test sensitivity and specificity, using the base case prior distribution, the median estimated seroprevalence ranged from 0.15% to 0.79% (Table 4). Estimates were lower when using the alternative prior distribution (Table 4).

Based on the seroprevalence point estimate of 0.15% from the general pathology collection (covering all age groups), the estimated cumulative number of SARS-CoV-2 infections in the Sydney population of 5 million people was 7,450 (or 1 in every 670 people infected), giving an estimated infection to case ratio of 3.5 when comparing to the number of notified cases up to 30 April 2020 (n=2,118), with a plausible range from 2,118 to 20,370 based on the credible interval around the 0.15% estimate.

Discussion

We report the first comprehensive assessment of SARS-CoV-2 seroprevalence in Australia, based on a survey in three distinct populations in the city most affected by the first epidemic wave. Seroprevalence was below 1% and we did not observe any differences by age group, sex or geographic location. The low seroprevalence estimates are consistent with other data, particularly case-based notifications, indicating that there was limited community transmission of SARS-CoV-2 during this period.

The SARS-CoV-2 antibody profiles seen in seropositive participants were consistent with available evidence regarding antibody kinetics. We found few specimens had high titres of SARS-CoV-2 specific IgG antibodies, consistent with evidence that most infections would have been mild or asymptomatic, and therefore generate lower antibody levels than cases with more severe disease.[12] As expected, given the timing of our collection, few IgG positive specimens were also positive for IgM or IgA. Peak IgM and IgA levels are lower than for IgG and wane faster, declining to undetectable levels in most cases by 6 weeks.[10,12,13] Overall 63% of individuals seropositive by IFA also had neutralising antibody to SARS-CoV-2, although there were some differences between the three collections. Elsewhere, most studies have reported that over 90% of subjects with SARS-CoV-2 developed neutralising antibodies.[14] However, these results came primarily from studies of hospitalised patients[12] and might reflect that higher levels of antibodies are generated in more severe disease .[12,13,15] Alternatively, it is possible that some samples positive by IFA, but not demonstrating neutralising antibody, may have been false positives.

A key strength of our study was the inclusion of samples from three diverse populations with complementary characteristics. Women undergoing antenatal care are a relatively healthy population that is stable over time and are likely to have sought care in a manner that was not substantially affected by the pandemic. Blood donors are a healthy adult population, including both sexes, but have the limitation that they would have been ineligible to donate for 28 days after symptoms compatible with COVID-19 resolve. People having a blood test at a diagnostic pathology service have the strength of including all ages, but could over-represent people with underlying illnesses, who may have been more likely to have self-isolated to reduce their infection risk. Our samples were reasonably representative geographically and by sex of these three populations. Another strength was the high sensitivity and specificity of the assay used,[10] validated across a broad range of ages and symptomatology, including mostly non-hospitalised cases from the same catchment population.

On the other hand, the relatively small number of seropositive participants limited our ability to detect differences between subgroups, and did not enable us to reliably estimate an infection-fatality ratio or to perform population-weighted adjustments of the seroprevalence estimates. We only included plasmapheresis blood donors, as specimens appropriate for the requirements of the IFA were not available for other blood donors. In addition, the estimated

infection to case ratio derived from our study needs to be interpreted with caution. The plausible range is wide due to the small numbers of antibody positive specimens. Also, there have been changes in NAT testing over time. The initial focus was on returned travellers and close contacts of confirmed cases, whereas later testing criteria were broadened to include anyone with mild respiratory symptoms or unexplained fever, and this has been supported by increased testing capacity.[4] It is likely that the proportion of cases detected by NAT will have increased due to the expanded criteria, potentially lowering the ratio of infections to notified cases over time.

Comparisons with other SARS-CoV-2 serosurveys are not straightforward due to differences in target populations and age groups sampled, laboratory tests used, and timing in relation to the stage of the pandemic. However, our results are broadly consistent with the only other Australian serosurvey to date, which examined adult elective surgery patients from selected hospitals in four states (NSW, VIC, SA, WA) admitted in June-July 2020 (seroprevalence 0.28%; 95%CI: 0 to 0.72%); infection: case ratio 7-10).[16] The results of these two Australian serosurveys contrast with estimates from many countries in Europe, Asia (particularly India) and the America's where pandemic control has been less effective, and reported seroprevalence has exceeded 10%.[6]

In conclusion, our study provides robust evidence that there was limited community transmission during the first epidemic wave of COVID-19 in Sydney. This is undoubtedly due to the early and successful implementation of national and state-based public health measures, including rapid upscaling of capacity to test and contact trace, strict border controls and quarantining of overseas travellers, movement and mixing restrictions, and a high degree of compliance with these measures by the public.[17] While our findings highlight the successful control of COVID-19 in Sydney they also demonstrate the need to maintain strong efforts to mitigate the spread of SARS-CoV-2 to limit subsequent epidemics as well as the need for vaccination programs. Our serosurveillance approach provides a feasible framework for repeated examination of SARS-CoV-2 transmission over time. Similar methods are being used in a national serosurvey and may also be important in evaluating population-level immune responses following the introduction of COVID-19 vaccines.

References:

1. World Health Organization. WHO Timeline - COVID-19. Geneva: WHO; 2020. https://www.who.int/news-room/detail/08-04-2020-who-timeline---covid-19 (accessed Oct 2020).
2. Shaban R, Li C, O'Sullivan M, et al. COVID-19 in Australia: Our national response to the first cases of SARS-CoV-2 infection during the early biocontainment phase. Intern Med J. 2020; in press. DOI:10.1111/imj.15105.
3. Australian Government Department of Health. COVID-19, Australia: Epidemiology Report 13: Reporting week ending 26 April 2020 Canberra: Australian Government Department of Health; 2020. https://www1.health.gov.au/internet/main/publishing.nsf/Content/novel_coronavirus_2019_ncov_weekly_epidemiology_reports_australia_2020.htm (accessed Oct 2020).
4. NSW Ministry of Health. COVID-19 Weekly surveillance in NSW. New South Wales, Australia: NSW Ministry of Health. https://www.health.nsw.gov.au/Infectious/covid-19/Pages/weekly-reports.aspx (accessed Oct 2020).
5. Peeling RW, Wedderburn CJ, Garcia PJ, et al. Serology testing in the COVID-19 pandemic response. Lancet Infect Dis. 2020; 20: e245-e249. 10.1016/s1473-3099(20)30517-x.
6. Serotracker 2020. https://serotracker.com/Dashboard (accessed 10 October).
7. Australian Government Department of Health. Australian National Disease Surveillance Plan for COVID-19 Canberra: Australian Government Department of Health; 2020. https://www.health.gov.au/resources/publications/australian-national-disease-surveillance-plan-for-covid-19 (accessed Oct 2020).
8. NSW Ministry of Health. Enhanced surveillance plan for COVID-19 in NSW. New South Wales: NSW Ministry of Health; 2020. https://www.health.nsw.gov.au/Infectious/covid-19/Pages/surveillance-plan.aspx (accessed Oct 2020).
9. Australian Bureau of Statistics. Estimated Resident Population (ERP) and components by SA2 and above (ASGS 2016), 2017 onwards 2020. http://stat.data.abs.gov.au/ (accessed Oct 2020).
10. Hueston L, Kok J, Guibone A, et al. The antibody response to SARS-CoV-2 infection. Open Forum Infectious Diseases. 2020; 7: 1-8.
11. Australian Bureau of Statistics. 3301.0 - Births, Australia, 2018. Canberra: Australian Bureau of Statistics; 2019. https://www.abs.gov.au/AUSSTATS/abs@.nsf/Lookup/3301.0Main+Features12018?OpenDocument (accessed Oct 2020).
12. Post N, Eddy D, Huntley C, et al. Antibody response to SARS-CoV-2 infection in humans: a systematic review. medRxiv. 2020. https://doi.org/10.1101/2020.08.25.20178806.
13. Brochot E, Demey B, Touze A, et al. Anti-spike, anti-nucleocapsid and neutralizing antibodies in SARS-CoV-2 inpatients and asymptomatic carriers. medRxiv. 2020. https://doi.org/10.1101/2020.05.12.20098236.
14. Health Information and Quality Authority. Evidence summary of the immune response following infection with SARSCoV-2 or other human coronaviruses. Cork/Dublin: HIQA; 2020. https://www.hiqa.ie/reports-and-publications/health-technology-assessment/evidence-summary-immunity-response-following (accessed Oct 2020).
15. Robbiani DF, Gaebler C, Muecksch F, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. Nature. 2020; 584: 437-442. 10.1038/s41586-020-2456-9.
16. Hicks SM, Pohl K, Neeman T, et al. A dual antigen ELISA allows the assessment of SARS-CoV-2 antibody seroprevalence in a low transmission setting. J Infect Dis. 2020. 10.1093/infdis/jiaa623.
17. McAnulty JM, Ward K. Suppressing the epidemic in New South Wales. N Engl J Med. 2020; 382: e74. DOI: 10.1056/NEJMc2011592.

Table 1: Demographic characteristics of the three collections, their target populations and the Australian Bureau of Statistics estimated residential population for 2019[9]

Characteristic	Category	General pathology sample		2019 ABS population		Antenatal sample		Antenatal population		Blood donor sample		Blood donor population	
		N	%	N	%	N	%	N	%	N	%	N	%
Age group (years)	0-<10	201	6.2	628678	12.7								
	10-<20	469	14.5	576303	11.6								
	20-<30	419	13.0	810753	16.3	224	40.0	21557	34.4	364	23.5	8561	34.2
	30-<40	282	8.7	814907	16.4	336	60.0	41060	65.6	389	25.1	6762	27.0
	40-<50	357	11.0	657063	13.2					317	20.5	4271	17.1
	50-<60	367	11.4	566991	11.4					287	18.5	3380	13.5
	60-<70	349	10.8	444479	8.9					191	12.3	2070	8.3
	70-<80	350	10.8	291759	5.9								
	80+	437	13.5	177214	3.6								
Sex	Males	1518	47.0	2473581	49.8	0	0	0		741	47.9	13117	52.4
	Females	1713	53.0	2494566	50.2	560	100	62617		807	52.1	11927	47.6
Region (SA4)	Baulkham Hills and Hawkesbury	182	5.6	251477	5.1	14	2.5	2352	3.8	85	5.5	1281	5.1
	Blacktown	184	5.7	377280	7.6	29	5.2	5791	9.2	91	5.9	1276	5.1
	City and Inner South	121	3.7	368263	7.4	43	7.7	3863	6.2	158	10.2	2810	11.2
	Eastern Suburbs	197	6.1	295054	5.9	43	7.7	3352	5.4	109	7.0	1673	6.7
	Inner South West	358	11.1	626859	12.6	56	10.0	8572	13.7	149	9.6	2117	8.5
	Inner West	182	5.6	324421	6.5	36	6.4	3900	6.2	119	7.7	1898	7.6
	North Sydney and Hornsby	316	9.8	441358	8.9	41	7.3	4635	7.4	172	11.1	2784	11.1
	Northern Beaches	215	6.7	273499	5.5	30	5.4	2900	4.6	80	5.2	1126	4.5
	Outer South West	206	6.4	289282	5.8	24	4.3	4089	6.5	61	3.9	957	3.8
	Outer West and Blue Mountains	288	8.9	327457	6.6	76	13.6	4259	6.8	123	8.0	1949	7.8
	Parramatta	361	11.2	503305	10.1	65	11.6	7933	12.7	129	8.3	2199	8.8
	Ryde	178	5.5	204909	4.1	21	3.8	2193	3.5	76	4.9	1044	4.2
	South West	320	9.9	454801	9.2	64	11.4	6250	10.0	91	5.9	1699	6.8
	Sutherland	123	3.8	230182	4.6	18	3.2	2527	4.0	105	6.8	2231	8.9
Total		3231	100	4968147	100	560	100	62617	100	1548	100	25044	100

Table 2: Characteristics of SARS-CoV-2-specific IgG antibody positive participants by collection

Collection	N IgG pos	IgG titre=10		IgG titre ≥160		IgA or M positive*		Neutralising antibodies*	
		N	%	N	%	N	%	N	%
General pathology	19	11	57.9	3	15.8	6	31.6	8	42.1
Antenatal screening	7	3	42.9	0	0	0	0	5	71.4
Lifeblood donors	12	1	8.3	1	8.3	0	0	11	91.7
Total	38	15	39.5	4	10.5	6	15.8	24	63.2
P value**		0.019		0.805		0.020		0.021	

* Only participants who were SARS-CoV-2-specific IgG seropositive were tested for SARS-CoV-2-specific IgA, IgM and neutralising antibody

** P value comparing proportion positive by collection (Fisher's exact method)

Table 3: Number of specimens positive for SARS-CoV-2-specific IgG and total tested by age group, sex and collection

Age-group	General pathology		Antenatal screening		Blood donors	
(yrs)	N pos	N tested	N pos	N tested	N pos	N tested
0-9	0	201	-			-
10-19	2	469	-			-
20-29	3	419	3	224	8	364
30-39	0	282	4	336	1	389
40-49	3	357	-		3	317
50-59	2	367	-		0	287
60-69	5	349	-		0	191
70-79	2	350	-			-
80+	2	437	-			-
Males	10	1518	-	-	5	741
Females	9	1713	7	560	7	807
Total	19	3231	7	560	12	1548

Table 4: Estimated seroprevalence (per cent) and 95% credible intervals (CI) by age group and collection for the base case and alternative case prior seroprevalence distributions*

Age group (years)	Collection	Base case		Alternative case	
		%	95% CI	%	95% CI
20-39	General pathology	0.24	0.04-0.80	0.12	0.04-0.53
	Blood donors	0.69	0.04-1.59	0.37	0.04-1.23
	Antenatal screening	0.79	0.04-1.88	0.41	0.04-1.43
20-69	General pathology	0.25	0.04-0.68	0.14	0.04-0.50
	Blood donors	0.29	0.04-0.75	0.15	0.04-0.57
All ages	General pathology	0.15	0.04-0.41	0.09	0.04-0.32

*See Supporting Information, Supplementary methods for details

Figure 1: Frequency of SARS-CoV-2 case notifications in residents of Sydney Greater Capital City Statistical Area, and serosurvey specimen numbers by notification/collection date

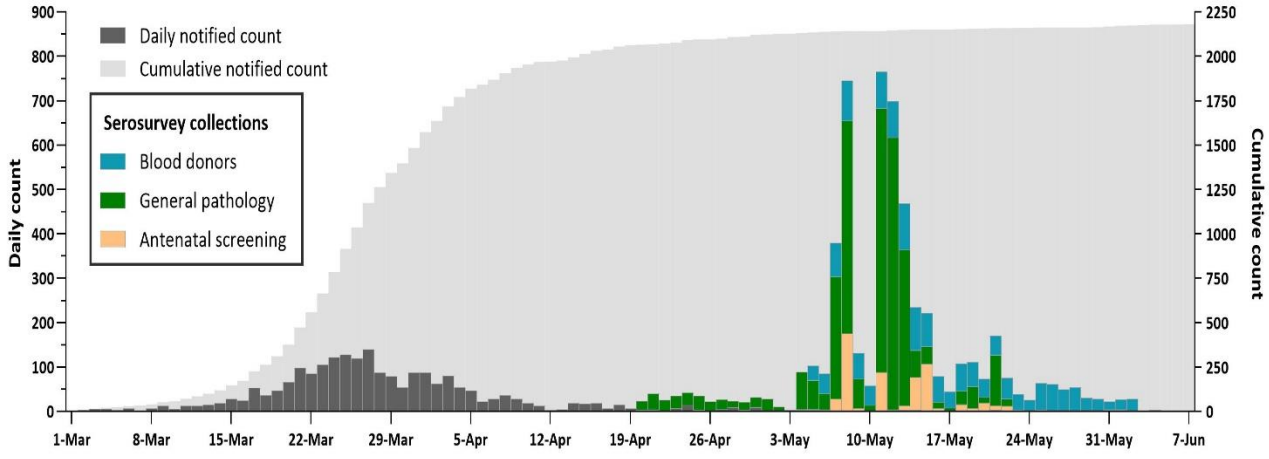
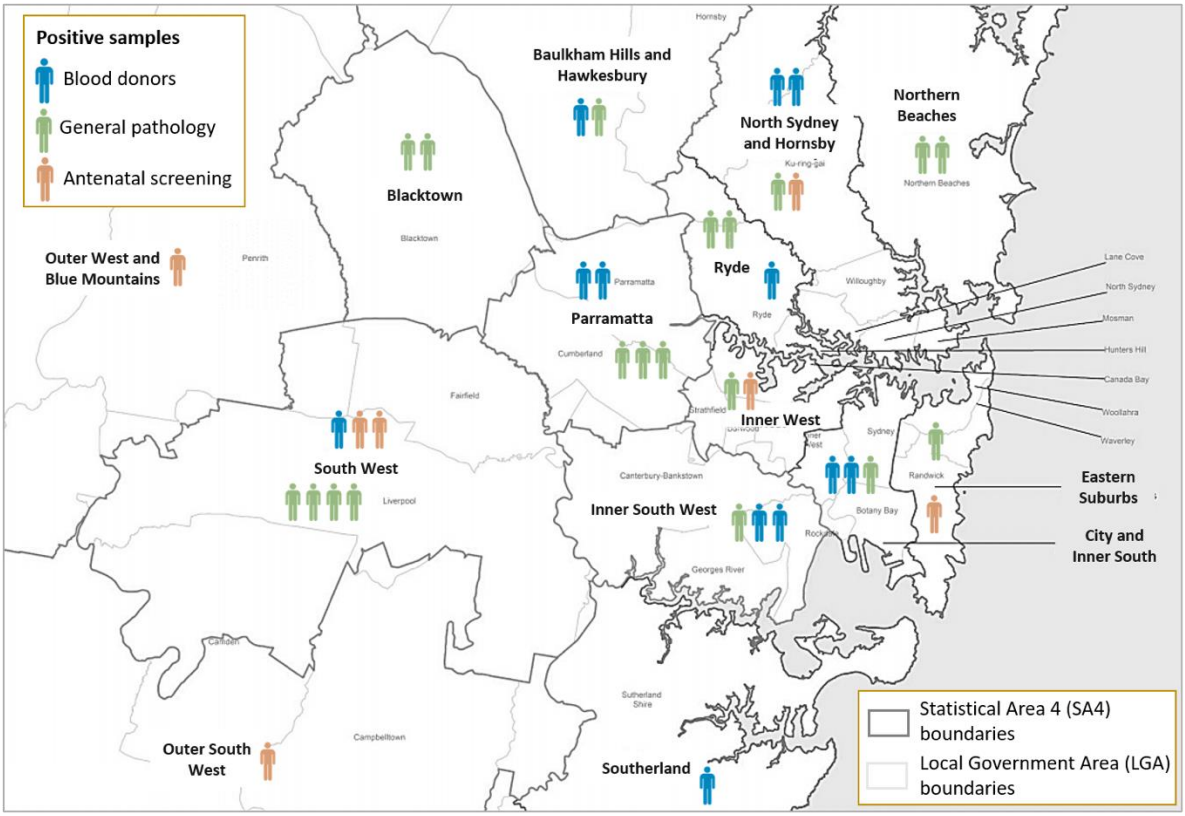


Figure 2: Geographical distribution of 38 SARS-CoV-2-specific IgG antibody positive participants by collection (total n=5,339)



Supporting Information: Bayesian inference for IFA-positive population seroprevalence accounting for uncertainty in test sensitivity and specificity

The target parameter of interest was the IFA-positive population seroprevalence π, which is related to the observable IFA-positive proportion, *p*, via the sensitivity δ and specificity γ of the IFA test, as follows:

$$\pi = (p + \gamma - 1)/(\delta + \gamma - 1)$$

(by solving the equation $p = (1 - \gamma)(1 - \pi) + \delta\pi$).

Bayesian inference was undertaken to account for multiple sources of uncertainty including sampling error in the outcome and uncertainty in the test sensitivity and specificity. The outcome model for y, the observed number of IFA positive tests from a sample size of n total tests, was

$$y \sim \text{Binomial}(n, p),$$

with prior distributions required for the parameters π, δ and γ, all constrained to lie between 0 and 1.

Priors for sensitivity (δ) and specificity (γ) were derived from validation study results superimposed on uniform prior distributions (true positives (TP) = 107, false negatives (FN) = 11, true negatives (TN) = 2605, false positives (FP) = 18, adapted from Hueston *et al.*,[1] see main text for details) as follows:

$$\delta \sim \text{Beta}(TP + 1, FN + 1)$$

$$\gamma \sim \text{Beta}(TN + 1, FP + 1)$$

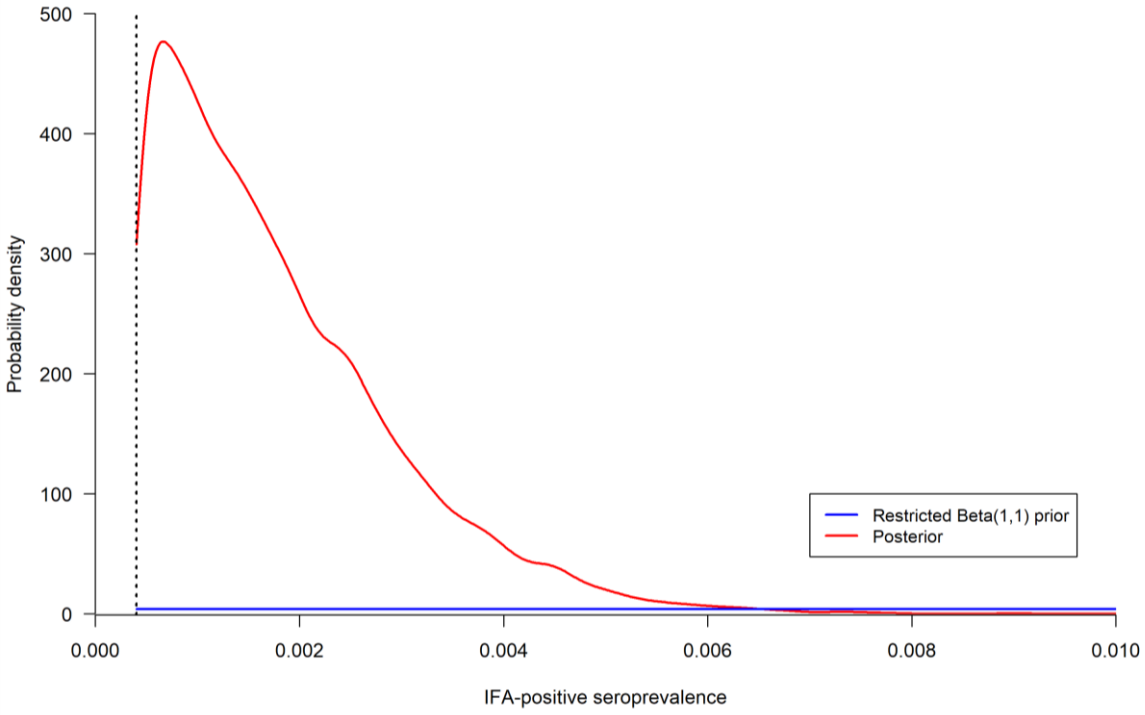
For the primary analysis, the prior distribution for π, the IFA-positive seroprevalence, was assumed to be uniform, i.e. Beta(1,1), restricted to values greater than 0.0004 (0.04%; Figure 1A). The lower bound was established from the ratio of notified cases to the ABS Sydney Estimated Resident Population, ABS 2019.[2] Although substantively unrealistic, the assumption of a uniform prior is a conservative approach that reflects the common preference for 'noninformative' prior assumptions. A sensitivity analysis was performed assuming an alternative prior distribution, Beta(0.2,10), also restricted to values greater than 0.0004 (0.04%; Figure 1B). This prior was more consistent with expert judgement that the true seroprevalence was unlikely to be more than 10-20 times greater than the cumulative incidence of notified cases; thus it assigned much greater weight to low values.

Bayesian models were fitted using the probabilistic programming language Stan (see below for code).[3,4] Point seroprevalence estimates were median values from posterior distributions and 95% credible intervals were calculated as 95% highest posterior density intervals, since posterior distributions were not symmetrical.

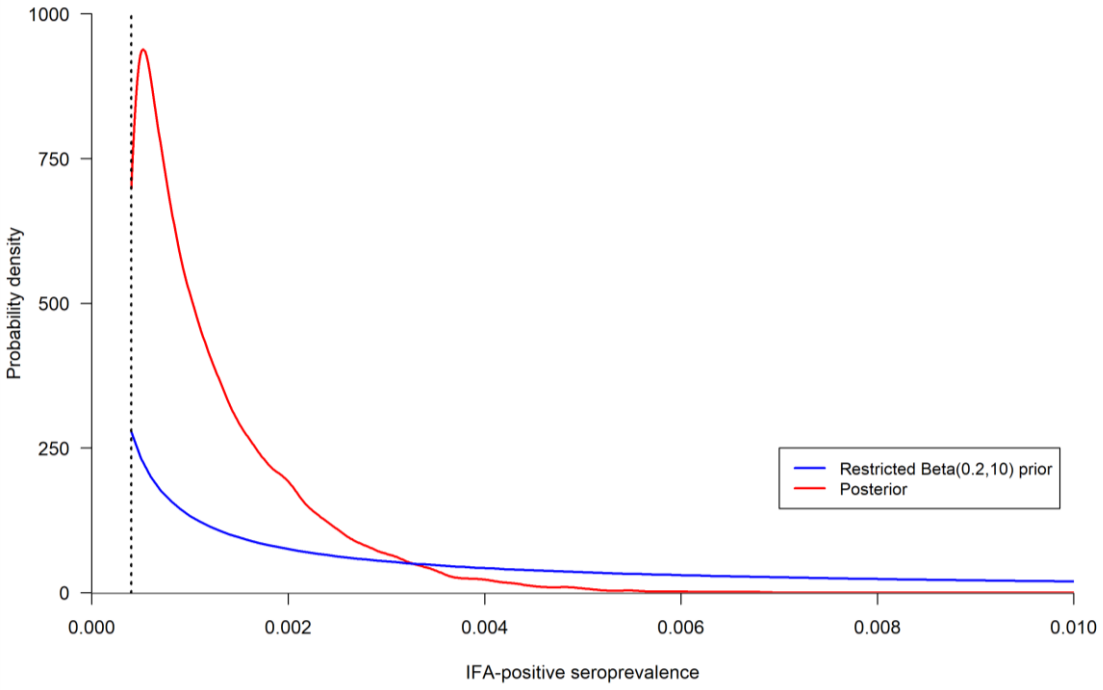
Prior and posterior probability density functions obtained from the primary analysis and sensitivity analysis of the general pathology subpopulation for estimation of IFA-positive seroprevalence are shown in Figures 1A and B.

Figure 1: Prior and posterior probability density functions for IFA-positive seroprevalence based on the general pathology subpopulation using: A) uniform Beta(1,1) prior restricted to π>0.04%; and B) informative Beta(0.2,10) prior restricted to π>0.04%.

A)



B)



1 Stan code

```
// Bayesian estimation of IFA-positive seroprevalence pooled across
age
      groups and sex
// Three collections considered separately (blood donors, general
pathology,
      antenatal screening)
// Accounting for uncertainty in test sensitivity and specificity

data{
  int<lower=0> N_pos;                      // observed number of
positive tests
  int<lower=0> N_tests;                    // number of total tests
  int<lower=0> TP;                         // number of true positives
from
                                               validation study (for
                                           test sens)
  int<lower=0> FN;                         // number of false
negatives from
                                               validation study (for
                                           test sens)
  int<lower=0> FP;                         // number of false
positives from
                                               validation study (for
                                     test spec)
  int<lower=0> TN;                         // number of true negatives
from
                                               validation study (for
                                     test spec)
}
parameters{
  real<lower=0,upper=1> sens;              // estimated test
sensitivity
  real<lower=0,upper=1> spec;              / estimated test
specificity
  real<lower=0.00037,upper=1> theta_true;  // estimated true
seroprevalence,
                        lower bound reflects ratio of case numbers to
                  ABS 2019
                         Sydney population size (1,840/4,968,147)
}
transformed parameters{
  real theta_obs = theta_true * sens + (1 - theta_true) * ( 1 - spec);
}
model{
  sens ~ beta(TP+1, FN+1);
  spec ~ beta(TN+1, FP+1);
  theta_true ~ beta(1,1);
// prior distribution for true seroprevalence, must change to
beta(0.2,10) for
      sensitivity analysis
  N_pos ~ binomial(N_tests, theta_obs);
}
```

References

1. Hueston L, Kok J, Guibone A, et al. The antibody response to SARS-CoV-2 infection. Open Forum Infectious Diseases. 2020; 7: 1-8.
2. Australian Bureau of Statistics. Estimated Resident Population (ERP) and components by SA2 and above (ASGS 2016), 2017 onwards 2020. http://stat.data.abs.gov.au/ (accessed Oct 2020).
3. Carpenter B, Gelman A, Hoffman MD, et al. Stan: A probabilistic programming language. J Statist Software. 2017; 76: 1–32.
4. Stan Development Team. Stan User's Guide, version 2.23. https://mc-stan.org/docs/2 23/stan-users-guide/index.html (accessed Oct 2020).