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A serosurvey of SARS-COV-2 at a large public university

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Abstract

Objective: This study investigated the seroprevalence of SARS-CoV-2 antibodies among adults over 18 years

Design: Prospective cohort study.

Settings: a population-based study among the big university community

Participants: This study took volunteers over five days and recruited adult 1064 participants.

Primary outcome measures: We conducted a seroprevalence in our community with SARS-CoV-2-specific antibodies due to previous exposure to SARS-CoV-2 and/or vaccination.

Results: The seroprevalence of the anti-receptor binding domain (RBD) antibody was 90% by a lateral flow assay and 88% by a semi-quantitative chemiluminescent immunoassay. The seroprevalence for anti-nucleocapsid (NC) was 20%. In addition, individuals with previous natural COVID infection plus vaccination had higher anti-RBD antibody levels compared to those who had vaccination only or infection only. Individuals who had a breakthrough infection had the highest anti-RBD antibody levels.

Conclusion: Accurate estimates of the cumulative incidence of SARS-CoV-2 infection can inform the development of university risk mitigation protocols such as encouraging booster shots, extending mask mandates, or reverting to online classes. It could help us to have clear guidance to act at the first sign of the next surge as well, especially since there is a surge of COVID subvariant infections.

Strengths and limitations of this study:

- Conducting longitudinal studies in university settings will provide valuable information about vaccine efficacies, infection spread among vaccinated individuals and provide mitigation regarding policies that work when implemented appropriately, during current and future pandemics.
- The study has a large number of prospective participants during a short window. Study limitations need to be noted in this study, including samples with an unknown degree of selection bias due to convenience sample, self-reported COVID test results, and vaccine status.
- Participants were only tested once for antibodies, thus lacking longitudinal data to compare antibody waning rates in individuals.
- The number of breakthrough infections and infections only was relatively small.

Introduction

The COVID-19 pandemic has been a major challenge worldwide. COVID-19 is caused by a novel Betacoronavirus (SARS-CoV-2) and was first reported from Wuhan, China, on 8 December 2019. The World Health Organization (WHO) declared it a pandemic on

11 March 2020 [1 2]. The United States had recorded more than 101 million cases and 1,091,000 deaths by January 11, 2023. Since the end of 2019, communities around the world have had to fight against outbreaks, including physical distancing, staying at home, avoiding groups indoors, wearing masks, frequent testing, and contact tracing, etc [3]. Although the intensity of these measures has recently abated partially, activities have not fully returned to the pre-pandemic routine and there are still an estimated 2500 COVID-19 deaths weekly[4]. Scientists have developed rapid diagnostics tests [5-7] and many effective vaccines [8-10] that have reduced morbidity and mortality considerably. Throughout the pandemic, university life has represented a unique challenge because universities tried to maximize safe in-person learning opportunities and maintain safe school operations by implementing effective practices.

ASU shifted to online classes on March 16, 2020 while a team of researchers at the Biodesign Institute set up a clinical testing laboratory. At that time, Arizona was a worldwide COVID-19 hotspot. ASU students and employees were encouraged to do COVID test frequently at no charge. After a few months of monitoring COVID, ASU switched to hybrid classes in August 2020. However, COVID cases began surging in late November 2020, resulting in the implementation of a fully remote learning model in December 2020. On January 11, 2021, ASU switched back to hybrid learning model until Fall semester of 2021. During these months, COVID testing, and vaccines were available to all students and employees at ASU. The ASU community followed CDC guidelines by offering frequent qPCR saliva testing, rigorous contact tracing, and strong support during isolation. This allowed the safe return to a fully in-person class in the fall of 2021 (Figure 1).

A research project at Davidson College in North Carolina reported that almost 6000 four-year colleges and universities provided combinations of online and in-person classes, another 446 had “primarily in-person” courses, and 45 operated “fully in-person” during 2020[11]. Also, a survey was conducted by New America and Global Strategy Group with 1,002 college students nationwide from April 29 through May 13, 2021. In the survey, 62% of students claimed that their schools would provide combinations of online and in-person classes, 14% claimed that their schools will offer online classes only, and only 12% would provide fully in-person classes in the fall of 2021[12]. Arizona State University was among the 12% operating “fully in-person” and one of the country’s largest universities, with over 79,000 students who had returned to campus for in-person classes in the fall of 2021. ASU wanted to evaluate the success of the COVID-19 management strategy by monitoring SARS-CoV-2 seroprevalence to estimate immunity from prior infection, vaccination, or both. Thus, ASU conducted a serosurvey to collect self-reported experiences and to determine the number of people in our community with various SARS-CoV-2-specific antibodies. The university had anonymized information about the prevalence of positive qPCR tests in its community, but at the time of this study, it lacked information about the level of immunity and possible viral exposure rate. This study would help inform on deciding on safety protocols, vaccination recommendations, masking recommendations mandates, and

online vs in-person classes. At the time of this survey, September 2021, the SARS-CoV-2 subvariant Delta, a highly contagious variant, accounted for 65% of all cases in Arizona [4].

By assessing humoral immunity, seroprevalence studies estimate the percentage of a specific population who have been previously infected with a pathogen. Many seroprevalence studies of SARS-CoV-2 have been reported. Arnaud et al. showed that neutralizing and anti-RBD antibodies persisted for at least 6 months after a mild COVID infection from hospital workers[13]. A similar result was also demonstrated by Baker et al, who found that the antibodies against SARS-CoV-2 produced by health care workers or patients who have mild COVID infection were stable for up to six months and helped prevent recurrent infections [14]. Another group investigated anti-NC antibody levels in severe and mild patients at hospitals. The data indicated that anti-NC levels started to decline after 2 months after post PCR and antibody levels were lower in patients with mild compared to severe illness [15]. However, the seroprevalence studies at the educational institutions/ communities, where students and employees are in close contact on daily basis, are very limited.

SARS-CoV-2 induces antibodies with IgM, IgA, and IgG isotypes against spike protein, RBD of the spike protein, and NC protein. The antibodies produced by COVID vaccination (Pfizer, Moderna, J&J, AstraZeneca, and Covishield) are IgM, IgA, and IgG isotypes against spike protein, specifically the RBD of the spike protein. Whereas anti-spike antibodies do not distinguish between vaccination and infection, anti-NC positivity generally implies a previous infection; however, participants who received COVID vaccines from Sinopharm, Sinovac, and Covaxin, which contain inactivated SARS-CoV-2 viruses, and who attend an international university like ASU, may also have anti-NC antibodies from vaccination. By combining the self-reported vaccine and infection history with documented antibodies to SARS-CoV2 antigens, we estimated the number of: a) individuals with detectable anti-spike antibodies; b) individuals with likely previous SARS-CoV-2 exposure, even if they did not report COVID-19 symptoms; and c) individuals with no detectable antibodies after vaccination or previous infection.

Methods

Participants

We employed a two-stage sampling strategy. First, a random sample of current students were invited to participate in the serosurvey through email invitation. To increase the representativeness of the sample, targeted recruitment was made via social media advertising, as well as in-person recruitment from selected areas of the campus. Responses are time-stamped to allow for analysis according to the date of completion. This study took volunteers over five days (9/13-9/17 of 2021) at 4 campuses (Downtown Phoenix, Polytechnic, Tempe, and West) of ASU.

Survey Instruments

Demographics, COVID-19 vaccination, testing history, and COVID symptoms were self-reported by questionnaire.

Blood sample collection

The blood samples were collected by phlebotomists at ASU with serum tubes (Cat # 37988 from BD) and were placed into a cooler within 4 hours and transported within 6 hours of collection to the clinical testing laboratory at ASU. Samples were centrifuged at 1300 g for 20 minutes to separate the serum. 1064 serum samples and matching survey results were analyzed.

Serology testing

The main serological detection methods were all approved by Emergency Use Authorization from the US Food and Drug Administration for marketing are the chemiluminescence immunoassay (CLIA), enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), and lateral flow immunoassay (LFA) [16 17]. In this survey, the serological tests were done either at the ASU Biodesign Clinical Testing Laboratory (ABCTL) or the Center for Personalized Diagnostics (CPD). Samples were tested for antibodies against the RBD domain of the Spike protein using Access SARS-CoV-2 chemiluminescent IgG II and IgM assay (Beckman coulter) and Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid lateral flow assay (Sienna-Clarity) to estimate vaccine-induced SARS-CoV-2 seroprevalence. Samples also were tested for antibodies against table NC protein using Platelia SARS-CoV-2 Total Ab ELISA Assay and rapid COVID-19 IgM/IgG Combo lateral flow test kit (Megna Health Inc.) to estimate infection-induced SARS-Cov-2 seroprevalence. The manufacturer-reported sensitivity and specificity were reported in the Supplementary Table 1.

Access SARS-CoV-2 chemiluminescent IgG II and IgM assays from Beckman coulter were performed in this study to determine IgG and IgM antibody level of SARS-coV-2 RBD protein according to the manufacturer's instructions [18]. 5 different concentrations of calibrators and two different concentrations of controls were provided by the manufacture to ensure reagent integrity and proper assay performance before analyzing samples. The result is compared to the cut-off value defined during the calibration of the instrument.

Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid lateral flow assay is to detect IgG and/or IgM isotypes specific to the RBD portion of the S1 protein. 10 µL of serum and 2 drops of buffer were added, and test results were read after 10 min by the laboratory technician and the kits were photographed for a second independent reading by another laboratory technician.

Platelia SARS-CoV-2 total Ab ELISA assay from Bio-Rad is a qualitative diagnostic test. It is the detection of total antibodies (IgM/IgA/IgG) against SARS-CoV-2 NC. The result

was interpreted based on the manufacturer's recommendations: < 0.8, negative; between > 0.8 and < 1.0, equivocal; ≥1.0, positive.

COVID-19 IgM/IgG Combo lateral flow test kit from Megna Health Inc is to detect IgG and/or IgM isotypes specific to NC protein. 2 µL of serum and 2 drops of buffer were added and test results were read after 15 min by the laboratory technician and the kits were photographed for a second independent reading by another laboratory technician.

Meso scale discovery (MSD) coronavirus panel from Meso Scale Diagnostics is a multiplexed immunoassay to measure the IgG antibody response to SARS-CoV-2. A 96-well MSD plate has different antigens in each well. A calibration curve was created by using a reference standard with 4-fold serial dilution steps and a zero-calibrator blank for quantitation. Three levels of controls were also included in the assay to ensure the accuracy of the performance. First, the plate was blocked with Blocker A solution for 30 minutes at RT. The plate was washed 3 times with 150 µL/well of MSD wash buffer and then 50 µL of calibrator, controls, and diluted samples were dispensed into the plate and incubated with shaking for 2 hours at RT. After incubation and 3 x 150 µL/well washes with MSD wash buffer, detection antibody was added and then incubated with shaking for 1 hour. After detection antibody, the plate was washed with wash buffer following which reader buffer B was added and the plate reads using the MESO QuickPlex SQ 120 instrument.

Patient and public involvement

Patients or the public were not involved in the design, conduct, or its outcome measures or preparation of the manuscript in this study.

Results

Demographic

Overall, this survey included 1064 participants from the four different campuses of ASU. A total of 480 students were randomly selected to receive invitation emails, which led to 250 subjects (28% of the final student participants); the remaining participants responded to university wide advertising, learned of the survey by word of mouth or personally observed the collections and volunteered. The participants provided saliva samples for a qPCR diagnostic test and also donated blood. Of the 1064 participants, 893 (83.9%) subjects were students, 79 (7.4%) were employees, and 92 (8.6%) subjects did not provide information about their occupation status. 556 participants (52.3%) were female, and 467 participants (43.9%) were male. 762 participants (71.6%) were in the age group of 18-25 years, 190 (17.9%) were aged 26-40 years, 81 (7.6%) were aged 41-65 years, 31 (2.9%) were not reported. The demographic characteristics of the three different groups are presented in Table 1.

Table 1 Demographics of our serosurvey participants

		Students (n=893)	Employees (n=79)	Randomly Selected* (n=250)	Total Participants (n=1064)
Gender	Female	444 (49.7%)	51 (64.6%)	142 (56.8%)	556 (52.3%)
	Male	409 (45.8%)	28 (35.4%)	101 (40.4%)	467 (43.9%)
	Other	10 (1.1%)	NA	2 (0.8%)	11 (1.0%)
	Not Reported	30 (3.4%)	NA	5 (2%)	30 (2.8%)
Age	18-25	723 (81%)	4 (5.1%)	183 (73.2%)	762 (71.6%)
	26-40	120 (13.4%)	31 (39.2%)	50 (20%)	190 (17.9%)
	41-65	19 (2.1%)	44 (55.7%)	12 (4.8%)	81 (7.6%)
	Not Reported	31 (3.5%)	NA	5 (2%)	31 (2.9%)
Race	White	410 (45.9%)	62 (78.5%)	121 (48.4%)	528 (49.6%)
	Asian	270 (30.2%)	6 (7.6%)	66 (26.4%)	292 (27.4%)
	Mixed	39 (4.4%)	5 (6.3%)	13 (5.2%)	46 (4.3%)
	Black	24 (2.7%)	1 (1.3%)	7 (2.8%)	27 (2.5%)
	Native	13 (1.5%)	NA	6 (2.4%)	14 (1.3%)
	Other	99 (11.1%)	4 (5.1%)	31 (12.4%)	117 (11%)
	Prefer not to say	7 (0.8%)	1 (1.3%)	1 (0.4%)	9 (0.9%)
	Not Reported	31 (3.5%)	NA	5 (2%)	31 (2.9%)
Vaccination Status	Yes	822 (92.1%)	70 (88.6%)	239 (95.6%)	978 (91.9%)
	No	67 (7.5%)	9 (11.4%)	11 (4.4%)	82 (7.7%)
	Not Reported	4 (0.5%)	NA	NA	4 (0.4%)
Vaccine Source	Pfizer	424 (47.5%)	32 (40.5%)	137 (54.8%)	510 (47.9%)
	Moderna	248 (27.8%)	35 (44.3%)	70 (28%)	309 (29.0%)
	Janssen	86 (9.6%)	2 (2.5%)	18 (7.2%)	94 (8.8%)
	AstraZeneca	46 (5.2%)	NA	9 (3.6%)	46 (4.3%)
	Covaxin	9 (1.0%)	NA	NA	9 (0.9%)
	Sinopharm	2 (0.2%)	NA	2 (0.8%)	2 (0.2%)
	Sinovac	1 (0.1%)	NA	1 (0.4%)	1 (0.1%)
	Not Reported	77 (8.6%)	1 (1.3%)	13 (5.2%)	93 (8.7%)
previous self-reported Covid infection	Yes	174 (19.5%)	12 (15.2%)	32 (12.8%)	205 (19.3%)
	No	717 (80.3%)	67 (84.8%)	218 (87.2%)	857 (80.6%)
	Not Reported	2 (0.2%)	NA	NA	2 (0.2%)

*Randomly selected from enrolled students and invited by email

Self-reported COVID-19 infection and vaccine status

Asymptomatic carriers can be a potential source of infection outbreaks in the community. We therefore evaluated how many participants had active COVID without reporting symptoms on the day when they donated samples. We found the prevalence of PCR positivity in asymptomatic students and employees in the university community was 0.4% (n=4/1064) on the day of sample collection. Among the 1064 participants, nearly 20% (19.3%, n=205/1064) reported testing positive for COVID-19 test in the past, whereas 80.6% reported no history of a positive test (Table 2).

Table 2. Anti-RBD and Anti-NC antibody seroprevalence status of the population

Ab Sub-Type	Manufacturer	Antigen Detected	Name of the test	Assay type	Positives	Negative	Inconclusive
IgG	Beckman Coulter	RBD	Access SARS-CoV-2 IgG II (Semi-Quantitative)	Chemiluminescent Immunoassay (CLA)	938 (88.2%)	126 (11.8%)	0 (0%)
	Sienna-Clarity	RBD	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test	Lateral Flow (LFA)	954 (89.7%)	109 (10.2%)	1 (0%)
	MSD	RBD	V-PLEX COVID-19 Respiratory Panel 3 Kit	Electrochemiluminescent immunoassay	1032 (97%)	32 (3%)	0 (0%)
	Megna Health Inc.	Nucleocapsid	Rapid COVID-19 IgM/IgG Combo Test Kit	Lateral Flow (LFA)	975 (91.6%)	85 (8%)	4 (0.4%)
	MSD	Nucleocapsid	V-PLEX COVID-19 Respiratory Panel 3 Kit	Electrochemiluminescent immunoassay	171 (16.1%)	893 (83.9%)	0 (0%)
IgM	Beckman Coulter	RBD	ACCESS SARS-CoV-2 IgM (Qualitative)	Chemiluminescent Immunoassay (CLA)	87 (8.2%)	977 (91.8%)	0 (0%)
	Salofa Oy	RBD	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test	Lateral Flow (LFA)	8 (0.8%)	1054 (99%)	2 (0.2%)
	Megna Health Inc.	Nucleocapsid	Rapid COVID-19 IgM/IgG Combo Test Kit	Lateral Flow (LFA)	4 (0.4%)	1055 (99.2%)	5 (0.5%)
Total Ab (IgM, IgA, and IgG)	Bio-Rad	Nucleocapsid	Platelia SARS-CoV-2 Total Ab Assay	Enzyme-Linked Immunosorbent Assay (ELISA)	210 (19.7%)	841 (79%)	13 (1.2%)

*Excluded Megna Health LFA results in the further analysis due to a higher rate of false positives

More than 90% (91.9%, n=978/1064) of participants reported at least 1 dose of vaccine, whereas 7.7% of participants reported never receiving vaccine. Most participants received Pfizer (47.9%, n=510/1064) and Moderna (29.0%, n=309/1064) (Table 1). There was no significant difference in vaccine rate, nor reported history of COVID across age groups; however, we noticed that the lowest COVID rate among the 26-40 years group (14.2%, n=27/190) had the highest vaccine rate (93.2%, n=177/190) (Table 3).

Table 3. COVID and vaccine status by age group

Category	Age Group		
	18-25	26-40	41-65
COVID Exposed	155 (20.3%)	27 (14.2%)	16 (19.8%)
Vaccinated	702 (92.1%)	177 (93.2%)	72 (88.9%)
COVID Exposed & vaccinated	141 (18.5%)	24 (12.6%)	11 (13.6%)
Total	762	190	81

Seroprevalence

SARS-CoV-2 RBD of spike IgG and IgM antibodies

All serological assays were evaluated with the same set of 1064 serum samples (Table 4). Of 1064 individuals, the seroprevalence for anti-RBD IgG antibody was found to be 89.7% by Sienna-Clarity, 88.2% by Beckman, and 97% by MSD (Table 2). There were no significant differences in the seroprevalence of anti-RBD IgG antibodies cross the groups (all participants, students only, employee only, and randomly invited students).

Among 182 participants who self-reported COVID infection and were vaccinated, 179 (98.4%) tested positive by Beckman, 181 (99.5%) by Sienna-Clarity LFA, and 181 (99.5%) by MSD for anti-RBD antibody. Among 22 participants who self-reported COVID infection and were not vaccinated, 10 (45.5%) tested positive by Beckman immunoassay, 12 (54.5%) by Sienna-Clarity, and 21 (95.4%) by MSD for anti-RBD antibody. Among 789 participants who self-reported no-COVID infection and were vaccinated, 721 (91.4%) tested positive by Beckman, 735 (93.2%) by Sienna-Clarity, and 778 (98.6%) by MSD for anti-RBD antibody (Table 4).

Table 4. Cohort characteristics and serological positive results by different assays

Cohort			IgG (RBD from Spike Protein)			IgG (Nucleocapsid Protein)	
Infection	Vaccine	n	CLIA (Beckman)	LFA (Sienna-Clarity)	MSD (MSD)	ELISA (Bio-Rad)	MSD (MSD)
YES	YES[A]	180	177 (98.3%)	179 (99.4%)	179 (99.4%)	101 (56.1%)	69 (38.3%)
	YES[B]	2	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)
	NO	22	10 (45.5%)	12 (54.5%)	21 (95.4%)	13 (59.1%)	9 (40.9%)
	NA	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
NO	YES[A]	779	719 (92.3%)	732 (94.0%)	770 (98.8%)	70 (8.9%)	73 (9.4%)
	YES[B]	10	2 (20%)	3 (30%)	8 (80%)	3 (30%)	3 (30%)
	NO	60	21 (35%)	19 (31.7%)	41 (68.3%)	20 (33.3%)	14 (23.3%)
	NA	1	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
NA	NA	2	2 (100%)	2 (100%)	2 (100%)	0 (0 %)	0 (0%)
Total	1064		938 (88.2%)	954 (89.7%)	1032 (97%)	210 (19.7%)	171 (16.1 %)

[A]Pfizer/BioNTech, Moderna, Janssen, AstraZeneca, Covishield; [B]Sinopharm, Sinovac, Covaxin

SARS-CoV-2 NC antibodies

Overall, the seroprevalence for total anti-NC was 19.7% by Bio-Rad and 16.1% for anti-NC IgG by MSD, but 91.6 % by Megna. We excluded results from the latter due to high false-positive results (91.6%) (Table 2) since the seroprevalence was estimated at 34.2% in September 2021 from the nationwide commercial lab in Arizona based on the CDC website [4].

Among 205 participants who self-reported COVID infection regardless of vaccination status, 117 (57.1%) by Bio-Rad and 81 (39.5%) by MSD tested positive for anti-NC antibody levels (Table 4). Interestingly, almost 80% (n=840) of the participants reported no known history of infection regardless of vaccine status (excluding 10 participants who received attenuated parasite vaccines*[B]). However, 10.7% (n=20+70=90) and 10.4% (n=73+14=87) tested positive for anti-nucleocapsid antibodies by the ELISA and the MSD assay without recalling at least one SAS-CoV-2 infection (Table 4), presumably representing occult infections.

Comparison of assays performances

The Venn diagrams show the overlapping distribution of positive results for each assay. For seropositive responses to the RBD of the spike protein, 926 specimens were positive by all three of Sienna-Clarity, Beckman, and MSD, whereas 75 specimens were positive only by MSD (Figure 2A). Based on the same sample population, the percentage of positive results for all three assays for anti-RBD IgG were comparable (90%, 88%, and 97% respectively; Figure 2). However, only Beckman and MSD provided the antibody levels provided a quantitative number which allowed us to track antibody levels post vaccination and monitor how long immunity persisted. In addition, Figure 3A showed the correlation of the values of anti-RBD IgG between two assays. The anti-RBD antibody results by Beckman correlated strongly with the results by MSD (r=0.79).

For seropositive NC, 130 specimens were positive by both Bio-Rad and MSD, whereas 80 specimens were positive only by Bio-Rad and 39 specimens by MSD (Figure 2B). The correlation of the values of anti-NC antibody level between Bio-Rad and MSD was weak (r=0.34) (Figure 3B).

Anti-RBD IgG antibody levels after vaccination

Anti-SARS-CoV-2 antibody persistence in the first six months after COVID vaccination decreased over time [19-21]. Here, we examined the relationship between the anti-RBD antibody titers of participants who received COVID vaccines and the number of days after vaccination using linear regression and summarized in Figure 4. As indicated in Figure 4, antibody titers varied widely, but there was clear trend towards lower titers over time. All vaccines have the same trend; we only report Modern and Pfizer in Figure 4; the other vaccines are reported in supplementary Figure 1. Participants who

received 2 doses of Moderna vs. Pfizer trended towards higher antibody titers, which lasted longer, although these results were not statistically separable.

RBD Antibody responses following vaccination/infection

Participants were first classified into different groups based on their vaccine and infection status (vaccine only, previous COVID infection only, and both) and then further categorized them based on the time between their most recent vaccination/infection date and the collection date (0-3, 4-6, ≥7 months). In each group, the median level of anti-RBD antibody levels was higher in the subgroups of vaccinated participants with COVID infection than those with vaccination or infection only. In every group, the lowest median anti-RBD antibody level was detected in the participants who were never vaccinated. There were no samples in the group of participants with infection after 4-6 months. Although anti-RBD antibody levels declined over time for all groups, median antibody levels in both vaccinated and infected or vaccination-only groups remained above the cut-off 7 months after either infection and/or vaccination, whereas median antibody levels in the infection only group dropped below the cut-off by 7 months post infection (Figure 5).

Increased anti-RBD IgG levels after breakthrough infection

Next, we investigated whether breakthrough COVID was associated with improved immune response. Participants were classified into three groups (breakthrough infection, hybrid immunity which is the participant who received vaccination after SARS-CoV-2 infection, and vaccine only). We had 645 fully vaccinated individuals, 19 individuals with 2 doses of vaccine after COVID infection (hybrid immunity), and 12 fully vaccinated individuals with breakthrough infection. Anti-RBD IgG values were significantly increased in both breakthrough and hybrid immune groups compared to vaccine only. In addition, the breakthrough infection group had significantly higher antibody levels compared to the hybrid immunity group, showing an association between breakthrough and enhanced immune response (Figure 6).

Increased anti-NC IgG antibody levels after infection

We also analyzed the antibody response to the SARS-CoV-2 NC protein in previously infected participants. However, the EUA-approved ELISA for NC antibodies that we used (Bio-Rad) is only a qualitative assay. To understand the trend of NC antibody levels post-infection, the MSD (Meso Scale Discovery) immunoassay from Meso Scale Diagnostics was applied, which uses ELISA-based quantitative detection. This assay was already verified with clinical samples, even though it is not an EUA-approved assay. Participants were categorized into different groups (0-1, 2-3, 4-5, 6-7, 8-9, 10-11, 12-13, 14-15, >15 months) depending on the interval between their infection date and collection date. Like RBD antibody levels, the NC antibody levels decreased over time, dropping somewhat faster than the ant-RBD antibodies in these data (Figure 7 & Supplementary Figure 2).

Discussion

In the fall of 2021, over 79,000 students returned to campus for in-person classes coincident with a large increase in COVID incidence during the Delta wave in Maricopa County, AZ. We observed only 0.4% (4 positives out of 1064) active COVID positivity based on saliva qPCR on the day of sample collection from the serosurvey study in the ASU community. Notably, those with symptoms were asked not to participate.

Vaccination compliance among the participants was very high

In the ASU community, 92% of participants have self-reported to had at least one dose of a COVID-vaccine. By comparison, only 85% of college students in the U.S. enrolled in spring or fall 2022 were vaccinated based on a nationally representative survey by the American College Health Association [22]. We believe, AUS's proactive communications to parents and students helped with increased rate of vaccination. Most of the vaccinated participants at ASU received the Moderna and Pfizer mRNA vaccines that have shown great effectiveness after the second dose. However, as previously noted, the antibodies produced by Pfizer's COVID-19 vaccine decline faster than those produced by the Moderna vaccine after 6 months of vaccination [23]. We observed a similar trend in our study. Based on anti-RBD antibodies levels from Beckman it showed that a higher anti-RBD IgG antibody level lasted longer in the participants who received 2 doses of Moderna compared to those who received 2 doses of Pfizer. This is probably due to the higher amount of RNA in Moderna (Figure 4).

Interestingly, 7 out of 978 participants who self-reported having received a COVID vaccine, tested negative for anti-RBD antibodies by all three assays in our study. Three out of 7 participants were vaccinated for more than 5 months (165, 168, and 216 days) with Pfizer vaccines leading to potential antibody decay based on figure 4. One out of 7 participants only received Pfizer for 7 days and antibody was likely not generated. It is known that there is substantial variation between individuals in the immune response to vaccination [24]. Other two out of 7 participants received Covaxin for 56 and 80 days and the level of anti-RBD antibodies in Covaxin was significantly lower than other vaccines. Another one out of 7 participants received AstraZeneca for more than 3 months (101 days), showing that Anti-RBD antibody levels from AstraZenecca started to wane after 2 months (Supplementary Figure 1) which was similar to previously reported result from other group [25].

The participants were tested negative for NC antibody after 6 months of post infection with COVID.

In the ASU community, 19.3% of participants (n=205) self-reported they had previous COVID infection; however, we only found 57% (n=117/205) and 39.5% (n=81/205) of participants from this group tested positive for NC antibody by Bio-Rad and MSD, respectively (Table 4). This could be due to antibody decay since their SARS-CoV-2 exposure. The median NC antibody levels fell below the positivity cutoff 6 months after infection, based on our MSD data (Figure 7). Also, by 8 months post infection, 50% of

participants from this group had undetectable NC antibodies. This finding was common with other serological studies, where the NC antibodies started to decline after a few months post infection and half the of participants have undetectable NC antibodies by 8 months post infection [26 27].

Among 847 self-reported no previous COVID infection participants (excluding 10 participants who received attenuated parasite vaccines*[B]), 10.6% (n=90) and 10.3% (n=87) tested positive for anti-NC antibody by Bio-Rad and MSD which means these 10% of participants had a COVID infection in the past without realizing it (Table 4). It could be these participants had mild or asymptotic previous COVID-19 infections.

SARS-CoV-2 antibodies and breakthrough infections

A main finding of this serological survey was that the participants who had breakthrough infection had higher anti-RBD IgG compared to those who were fully-vaccinated and also had prior infection (Figure 6), which agrees with previous studies [28 29]. Considering that the antibody developed by B cells multiply after each exposure through infection or vaccination, these results were expected. First, the highest anti-RBD antibody levels were in the combined vaccination and infection group and most likely represent an accumulation of antibodies produced after each exposure. Second, the anti-RBD antibody level in the infection only group decayed faster than the participants who received vaccines only. The participants here predominantly received the Pfizer and Moderna vaccines, which may be particularly efficient at evoking a durable anti-RBD response. Similar observations were made by Dashdor et al, that participants who received the Sinopharm vaccine (whole virus) had lower antibody levels compared to Pfizer/Moderna vaccine (spike protein) [30].

Author Contributions MJ, JL, and VM initiated the study and design. VH, BN, PS, TL, and MM developed the design. CH and VM contributed project administration, supervision, and analysis. CH, KT, VB, BB, JK, KN, AM, and VM designed and conducted the experiments. SW and YC performed statistical analysis. CH, VM, JL, MJ, and YC wrote and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Competing interests None declared.

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Data sharing statement Data may be available upon reasonable request. Contact information: Vel Murugan, Ph.D., Virginia G. Piper Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ, USA e-mail Vel.Murugan@asu.edu

Ethical approval The study was approved by ASU's institutional Review Board (IRB)(STUDY00014505).

Participant Consent All participants are 18+ years old and consented to participating in the study and were willing to provide their samples for the research.

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Figure 1. The timeline between the outbreak of the COVID-19 pandemic and serosurvey at ASU. In response to COVID, ASU rotated from in-person to remote to hybrid learning several times during the pandemic depending on the prevalence of infection in the community.

Figure 2. Comparison of assay performances. Venn diagrams showing overlap of positive results of (A) RBD of Spike and (B) Nucleocapsid from different assays.

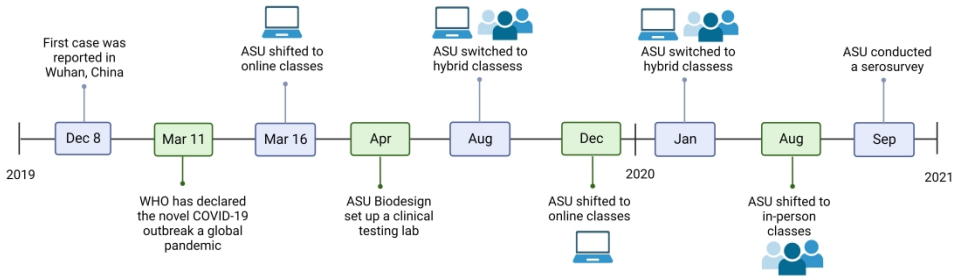
Figure 3. Correlations between antibody results by different assays. (A) Correlation between the value of anti-RBD IgG by Beckman and the MSD assay. (B) Correlation between the value of total anti-NC by Bio-Rad assay and the value of anti-NC IgG by the MSD assay. A red dotted line indicated the cut-off line. All test values equal to or greater than this line is considered positive.

Figure 4. **Anti-RBD antibody decay post-vaccination.** The antibody level is determined by the Beckman immunoassay. The linear regression of different vaccines to estimate vaccine decay.

Figure 5. Anti-RBD antibodies in participants who had previous COVID infection or COVID vaccines or both. Participants were categorized by the vaccine or COVID infection they got and the number of months between their vaccination/infection and their blood sample collection. Anti-RBD IgG level is measured by Beckman immunoassay. Cut-off defined per manufacturer. *P value is calculated by the Mann-Whitney test

Figure 6. Anti-RBD antibodies after breakthrough infection, hybrid immunity, and vaccine only. (A) Participants were categorized based on the order and approximate time scale of COVID infection and vaccination for each group. The blue bottle indicates a dose of vaccine, the virus indicates natural infection with SARS-CoV-2 based on the participant's self-reported, and the red vial indicates blood collection. (B) Anti-RBD IgG level is measured by Beckman immunoassay *P value is calculated by the Mann-Whitney test

Figure 7. Anti-nucleocapsid antibodies after COVID infection. Participants were categorized by the COVID infection they got and the number of months between their infection and their blood sample collection. Anti- nucleocapsid IgG levels were measured by ELISA. *P value is calculated by the Mann-Whitney test



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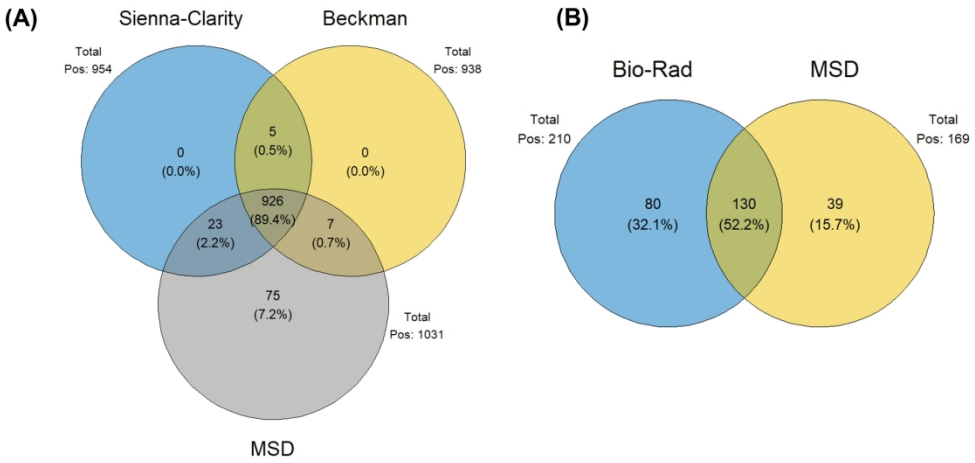

Assay	Antigen	Positive	Negative	Inconclusive	Missing
Sienna-Clarity	RBD	954 (89.7%)	109 (10.2%)	1 (0%)	0 (0%)
Beckman		938 (88.2%)	126 (11.8%)	0 (0%)	0 (0%)
MSD		1032 (97%)	32 (3%)	0 (0%)	0 (0%)
Bio-Rad	Nucleocapsid	210 (19.7%)	841 (79%)	13 (1.2%)	0 (0%)
MSD		171 (16.1%)	893 (83.9%)	0 (0%)	0 (0%)

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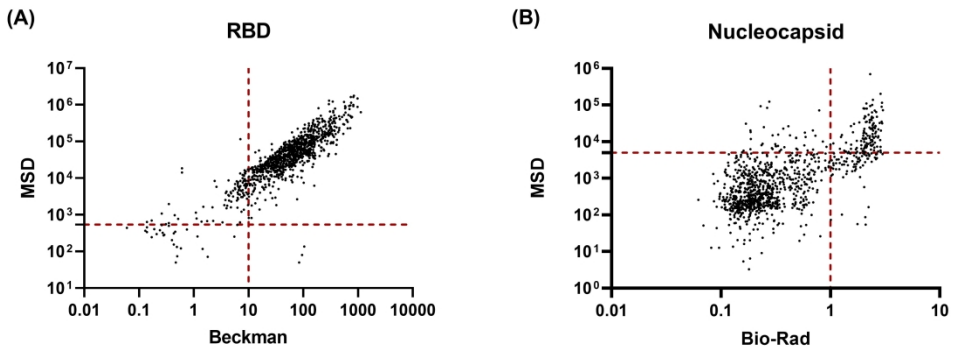

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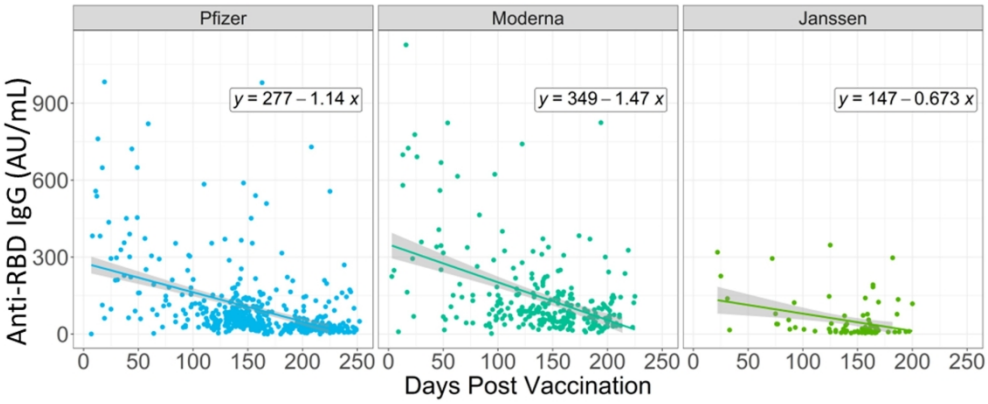

Linear Regression Analysis						
Vaccine	**n**	**Y-Intercept**	**X- Intercept**	**Slope**	**95% CI**	**P-value**
Moderna	274	349.37	237.14	-1.47	(-1.81, -1.13)	<0.001
Pfizer	458	277.17	242.84	-1.14	(-1.35, -0.93)	<0.001

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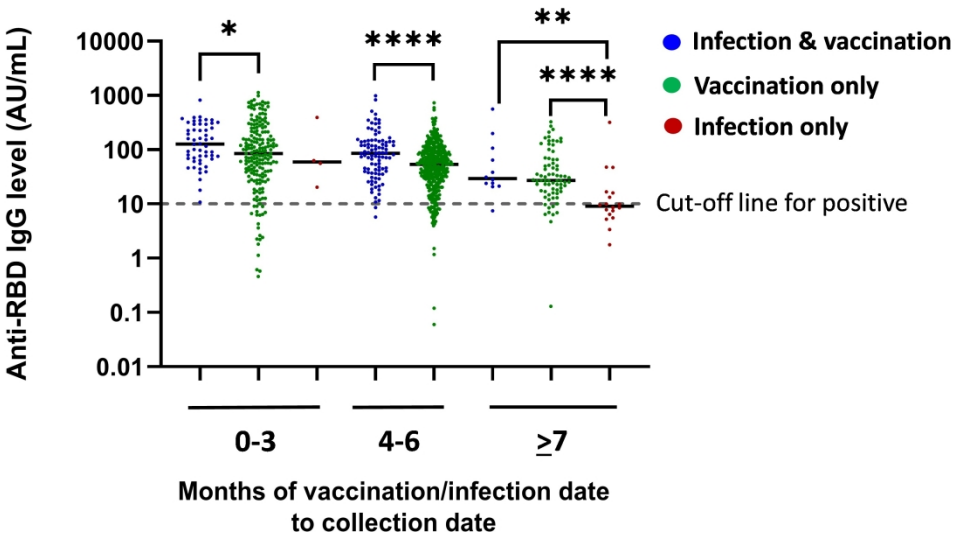

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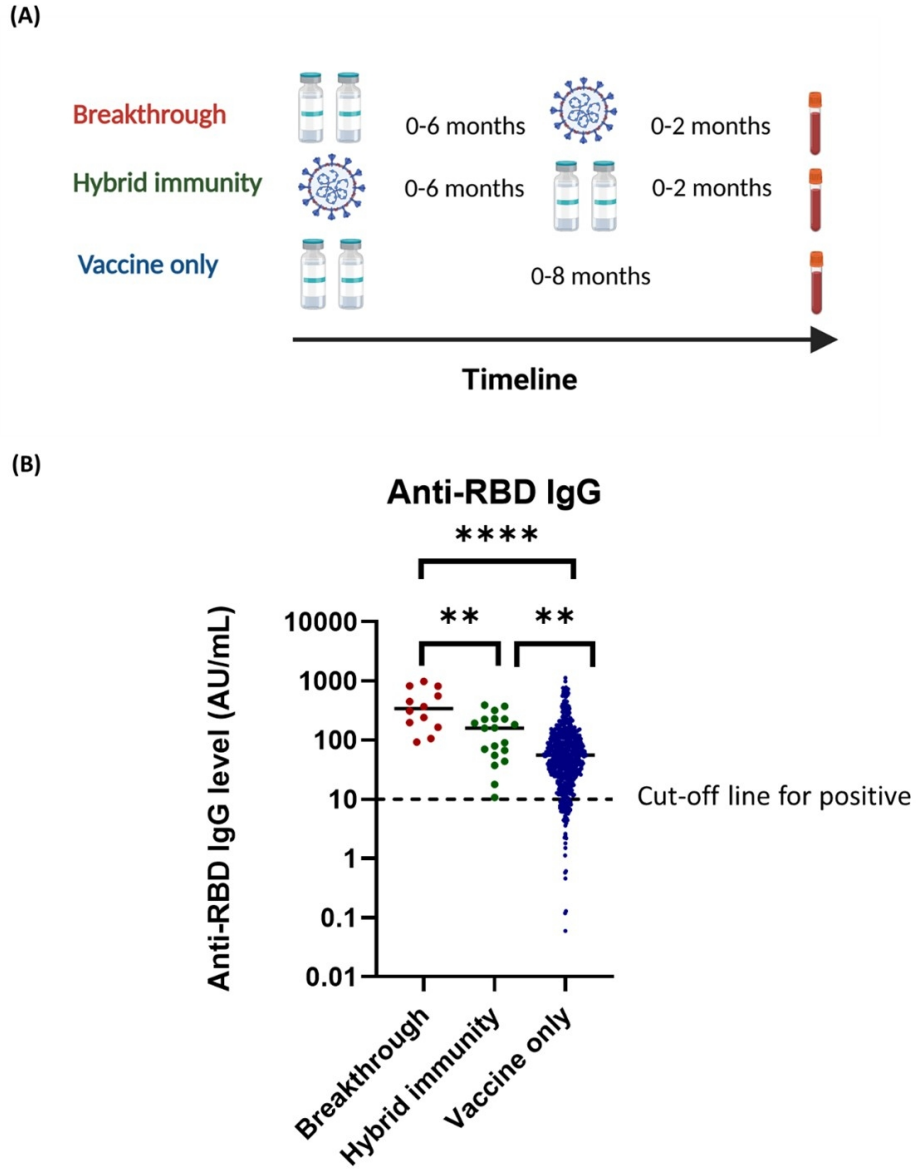

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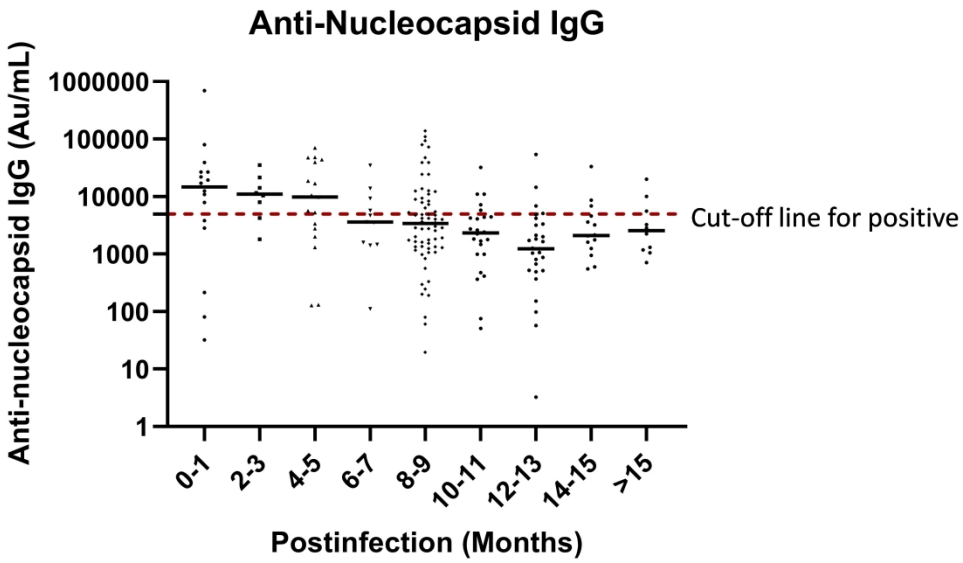

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Supplemental data for

A serosurvey of SARS-COV-2 at a large public university

Ching-Wen Hou[1], Stacy Williams[1], Kylee Taylor[1], Veronica Boyle[1], Bradley Bobbett[1], Joseph Kouvetakis[1], Keana Nguyen[1], Aaron McDonald[1], Valerie Harris[2], Benjamin Nussle[1], Phillip Scharf[3], Megan Jehn[4], Timothy Lant[2], Mitch Magee[1], Yunro Chung[1,5], Joshua Labaer[1], Vel Murugan [1]*

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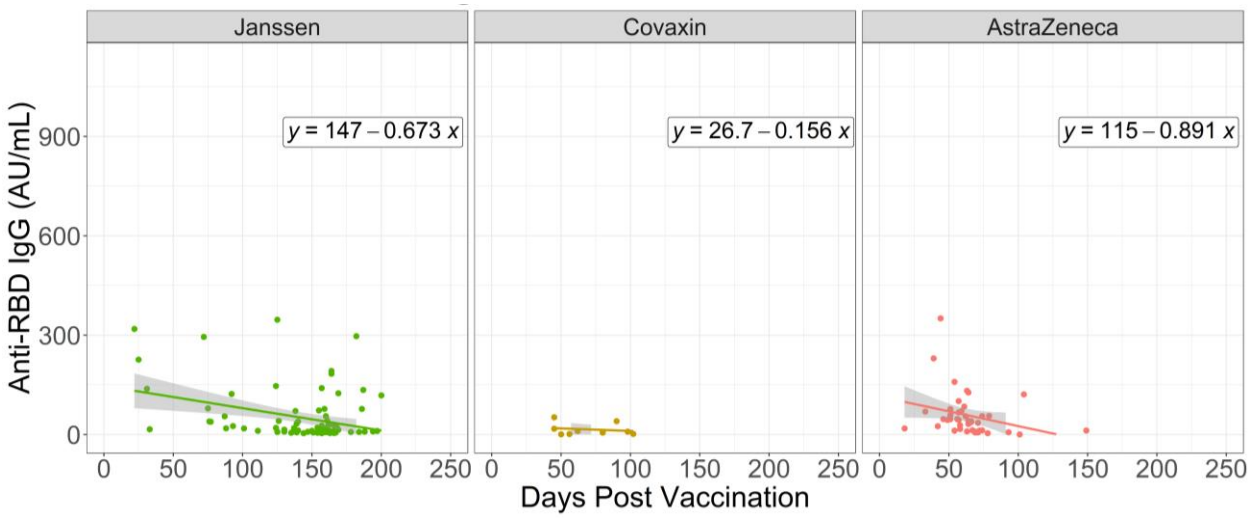
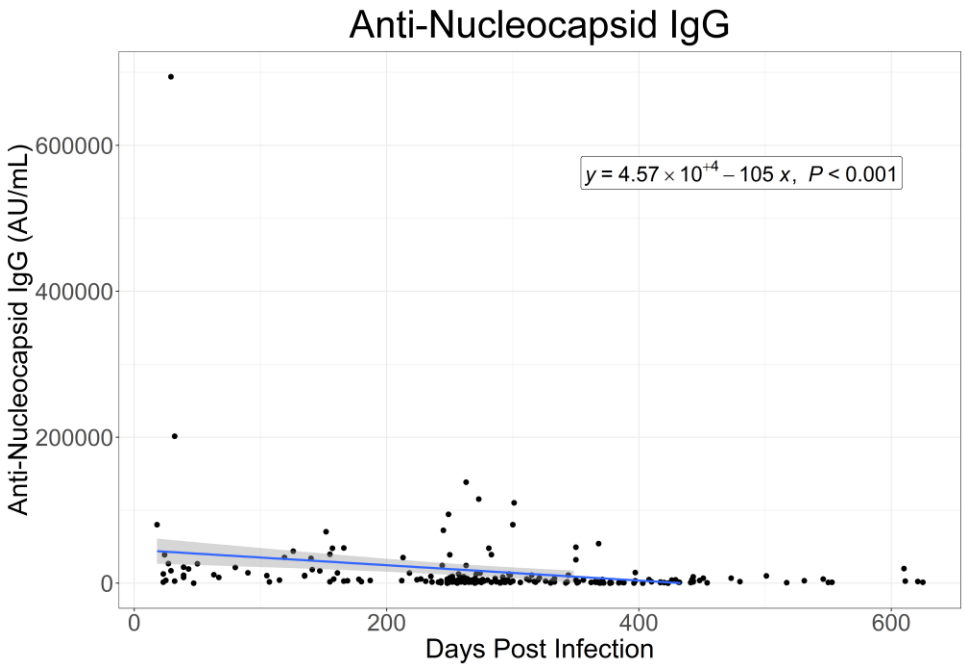
Supplementary Table 1. The manufacturer reported the sensitivity and specificity of EUA-authorized tests used in this study

Assay	Sensitivity (positive percent agreement at >/= 15 days post symptom onset)	Specificity
Chemiluminescent assay: Beckman (Anti-RBD IgG)	98.9%	100%
Later flow assay: Clarity (Anti-RBD IgG)	96.15%	100%
Later flow assay: Megna (Anti-NC IgG)	95%	99.3%
ELISA: Bio-Rad (Anti-NC IgM/IgG/IgA)	100%	98.86%
Electrochemiluminescence assay: MSD* (Anti-NC IgG)	93.8%	100%
Electrochemiluminescence assay: MSD* (Anti-RBD IgG)	98.3%	98.5%

*MSD is not a EUA-authorized test. It is a validated assay that meets the clinical laboratory standards institute guidelines.



Supplementary Figure 1. **Anti-RBD antibody decay post-vaccination.** The antibody level is determined by the Beckman immunoassay. The linear regression of different vaccines to estimate vaccine decay.



Supplementary Figure 2. **Anti-Nucleocapsid antibody decay post-infection.** The antibody level is determined by the MSD. The linear regression was to estimate antibody decay.

BMJ Open

Serological survey to estimate SARS-CoV-2 infection and antibody seroprevalence at a large public university; a cross sectional study

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Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Infectious diseases, Public health, Global health
Keywords:	COVID-19, SARS-CoV-2, Epidemiology < INFECTIOUS DISEASES

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Serological survey to estimate SARS-CoV-2 infection and antibody seroprevalence at a large public university; a cross sectional study

Ching-Wen Hou[1], Stacy Williams[1], Kylee Taylor[1], Veronica Boyle[1], Bradley Bobbett[1], Joseph Kouvetakis[1], Keana Nguyen[1], Aaron McDonald[1], Valerie Harris[2], Benjamin Nussle[1], Phillip Scharf[3], Megan Jehn[4], Timothy Lant[2], Mitch Magee[1], Yunro Chung[1,5], Joshua Labaer[1], Vel Murugan [1]*

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Abstract

Objective: This study investigated the seroprevalence of SARS-CoV-2 antibodies among adults over 18 years

Design: Prospective cohort study.

Settings: a population-based study among the big university community

Participants: This study took volunteers over five days and recruited adult 1064 participants.

Primary outcome measures: We conducted a seroprevalence in our community with SARS-CoV-2-specific antibodies due to previous exposure to SARS-CoV-2 and/or vaccination.

Results: The seroprevalence of the anti-receptor binding domain (RBD) antibody was 90% by a lateral flow assay and 88% by a semi-quantitative chemiluminescent immunoassay. The seroprevalence for anti-nucleocapsid (NC) was 20%. In addition, individuals with previous natural COVID infection plus vaccination had higher anti-RBD antibody levels compared to those who had vaccination only or infection only. Individuals who had a breakthrough infection had the highest anti-RBD antibody levels.

Conclusion: Accurate estimates of the cumulative incidence of SARS-CoV-2 infection can inform the development of university risk mitigation protocols such as encouraging booster shots, extending mask mandates, or reverting to online classes. It could help us to have clear guidance to act at the first sign of the next surge as well, especially since there is a surge of COVID subvariant infections.

Strengths and limitations of this study:

- We investigated both active infection and seroprevalence for the university population at the same time.
- Our study was strengthened by the data available on participants from their self-report and the independent validation by an EUA authorized diagnostic test.
- Our study was performed within the university setting therefore it only reflects the COVID-19 situation within that community.
- Our study lacks longitudinal data to compare antibody waning rates in individuals.
- The number of breakthrough infections was small requiring confirmation.

Introduction

The COVID-19 pandemic has been a major challenge worldwide. COVID-19 is caused by a novel Betacoronavirus (SARS-CoV-2) and was first reported from Wuhan, China, on 8 December 2019. The World Health Organization (WHO) declared it a pandemic on 11 March 2020 [1 2]. The United States had recorded more than 101 million cases and

1,091,000 deaths by January 11, 2023. Since the end of 2019, communities around the world have had to fight against outbreaks, including physical distancing, staying at home, avoiding groups indoors, wearing masks, frequent testing, and contact tracing, etc [3]. Although the intensity of these measures has recently abated partially, activities have not fully returned to the pre-pandemic routine and there are still an estimated 2500 COVID-19 deaths weekly[4]. Scientists have developed rapid diagnostics tests [5-7] and many effective vaccines [8-10] that have reduced morbidity and mortality considerably. Throughout the pandemic, university life has represented a unique challenge because universities tried to maximize safe in-person learning opportunities and maintain safe school operations by implementing effective practices.

ASU shifted to online classes on March 16, 2020 while a team of researchers at the Biodesign Institute set up a clinical testing laboratory. At that time, Arizona was a worldwide COVID-19 hotspot. ASU students and employees were encouraged to do COVID test frequently at no charge. After a few months of monitoring COVID, ASU switched to hybrid classes in August 2020. However, COVID cases began surging in late November 2020, resulting in the implementation of a fully remote learning model in December 2020. On January 11, 2021, ASU switched back to hybrid learning model until Fall semester of 2021. During these months, COVID testing, and vaccines were available to all students and employees at ASU. The ASU community followed CDC guidelines by offering frequent qPCR saliva testing, rigorous contact tracing, and strong support during isolation. This allowed the safe return to a fully in-person class in the fall of 2021 (Supplementary Figure 1).

A research project at Davidson College in North Carolina reported that almost 6000 four-year colleges and universities provided combinations of online and in-person classes, another 446 had “primarily in-person” courses, and 45 operated “fully in-person” during 2020[11]. Also, a survey was conducted by New America and Global Strategy Group with 1,002 college students nationwide from April 29 through May 13, 2021. In the survey, 62% of students claimed that their schools would provide combinations of online and in-person classes, 14% claimed that their schools will offer online classes only, and only 12% would provide fully in-person classes in the fall of 2021[12]. Arizona State University was among the 12% operating “fully in-person” and one of the country’s largest universities, with over 79,000 students who had returned to campus for in-person classes in the fall of 2021. ASU wanted to evaluate the success of the COVID-19 management strategy by monitoring SARS-CoV-2 seroprevalence to estimate immunity from prior infection, vaccination, or both. Thus, ASU conducted a serosurvey to collect self-reported experiences and to determine the number of people in our community with various SARS-CoV-2-specific antibodies. The university had anonymized information about the prevalence of positive qPCR tests in its community, but at the time of this study, it lacked information about the level of immunity and possible viral exposure rate. This study would help inform on deciding on safety protocols, vaccination recommendations, masking recommendations mandates, and online vs in-person classes. At the time of this survey, September 2021, the SARS-

CoV-2 subvariant Delta, a highly contagious variant, accounted for 65% of all cases in Arizona [4].

By assessing humoral immunity, seroprevalence studies estimate the percentage of a specific population who have been previously infected with a pathogen. Many seroprevalence studies of SARS-CoV-2 have been reported. Arnaud et al. showed that neutralizing and anti-RBD antibodies persisted for at least 6 months after a mild COVID infection from hospital workers[13]. A similar result was also demonstrated by Baker et al, who found that the antibodies against SARS-CoV-2 produced by health care workers or patients who have mild COVID infection were stable for up to six months and helped prevent recurrent infections [14]. Another group investigated anti-NC antibody levels in severe and mild patients at hospitals. The data indicated that anti-NC levels started to decline after 2 months after post PCR and antibody levels were lower in patients with mild compared to severe illness [15]. However, the seroprevalence studies at the educational institutions/ communities, where students and employees are in close contact on daily basis, are very limited.

SARS-CoV-2 induces antibodies with IgM, IgA, and IgG isotypes against spike protein, RBD of the spike protein, and NC protein. The antibodies produced by COVID vaccination (Pfizer, Moderna, J&J, AstraZeneca, and Covishield) are IgM, IgA, and IgG isotypes against spike protein, specifically the RBD of the spike protein. Whereas anti-spike antibodies do not distinguish between vaccination and infection, anti-NC positivity generally implies a previous infection; however, participants who received COVID vaccines from Sinopharm, Sinovac, and Covaxin, which contain inactivated SARS-CoV-2 viruses, and who attend an international university like ASU, may also have anti-NC antibodies from vaccination. The main objective of this study is to estimate the seroprevalence of IgG and IgM antibodies to the 'SARS-CoV-2' coronavirus in the university population, both through vaccination and through exposures to SARS-CoV-2 virus. Serosurvey would also answer questions like; a) Does universities have similar infection rates like the general community? b) Is immunization by the university community better or worse than the population at large? In addition to providing data on the university population's exposure to COVID-19, this study would shed light on risk factors for developing SARS-CoV-2 infection. Overall, we expect that the estimates would provide us ample evidence to gauge the population-level scenario of COVID-19 at the university as well as provide insights into other epidemiological aspects of the disease, including the risk factors for developing SARS-CoV-2 infection. We also intend to compare multiple assays and their performance characteristics in real-world scenario. By combining the self-reported vaccine and infection history with documented antibodies to SARS-CoV2 antigens, we intend to estimate the number of: a) individuals with detectable anti-spike antibodies; b) individuals with likely previous SARS-CoV-2 exposure, even if they did not report COVID-19 symptoms; and c) individuals with no detectable antibodies after vaccination or previous infection.

Methods

Participants

We employed a two-stage sampling strategy. First, a random sample of current students were invited to participate in the serosurvey through email invitation. To increase the representativeness of the sample, targeted recruitment was made via social media advertising, as well as in-person recruitment from selected areas of the campus. Responses are time-stamped to allow for analysis according to the date of completion. This study took volunteers over five days (9/13-9/17 of 2021) at 4 campuses (Downtown Phoenix, Polytechnic, Tempe, and West) of ASU.

Survey Instruments

Demographics, COVID-19 vaccination, testing history, and COVID symptoms were self-reported by questionnaire.

Blood sample collection

The blood samples were collected by phlebotomists at ASU with serum tubes (Cat # 37988 from BD) and were placed into a cooler within 4 hours and transported within 6 hours of collection to the clinical testing laboratory at ASU. Samples were centrifuged at 1300 g for 20 minutes to separate the serum. 1064 serum samples and matching survey results were analyzed.

Serology testing

The main serological detection methods were all approved by Emergency Use Authorization from the US Food and Drug Administration for marketing are the chemiluminescence immunoassay (CLIA), enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), and lateral flow immunoassay (LFA) [16 17]. In this survey, the serological tests were done either at the ASU Biodesign Clinical Testing Laboratory (ABCTL) or the Center for Personalized Diagnostics (CPD). Samples were tested for antibodies against the RBD domain of the Spike protein using Access SARS-CoV-2 chemiluminescent IgG II and IgM assay (Beckman coulter) and Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid lateral flow assay (Sienna-Clarity) to estimate vaccine-induced SARS-CoV-2 seroprevalence. Samples also were tested for antibodies against NC protein using Platelia SARS-CoV-2 Total Ab ELISA Assay and rapid COVID-19 IgM/IgG Combo lateral flow test kit (Megna Health Inc.) to estimate infection-induced SARS-Cov-2 seroprevalence. Beckman Access SARS-CoV-2 IgG II is EUA authorized as semi-quantitative assay. All LFA are qualitative. Beckman Access SARS-CoV-2 IgM, and Platelia SARS-CoV-2 Total Ab ELISA Assay gives a quantitative readout, but they are authorized as qualitative tests. The manufacturer-reported sensitivity and specificity were reported in the Supplementary Table 1.

Access SARS-CoV-2 chemiluminescent IgG II assay from Beckman coulter were performed in this study to determine IgG antibody level of SARS-coV-2 RBD protein

according to the manufacturer's instructions [18]. 5 different concentrations of calibrators and two different concentrations of controls were provided by the manufacturer to ensure reagent integrity and proper assay performance before analyzing samples. The result is compared to the cut-off value in arbitrary units (AU/mL) defined during the calibration of the instrument.

Access SARS-CoV-2 chemiluminescent IgM assay from Beckman coulter was utilized to measure the IgM antibody level of SARS-coV-2 RBD protein, following the manufacturer's instructions [18]. To ensure reagent integrity and proper assay performance before analyzing samples, two different concentrations of calibrators and controls were provided by the manufacture, which were analyzed prior to testing the samples. The obtained results were compared to the instrument-defined cut-off value, expressed as signal to cut-off (S/Co).

Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid lateral flow assay is to detect IgG and/or IgM isotypes specific to the RBD portion of the S1 protein. 10 µL of serum and 2 drops of buffer were added, and test results were read after 10 min by the laboratory technician and the kits were photographed for a second independent reading by another laboratory technician.

Platelia SARS-CoV-2 total Ab ELISA assay from Bio-Rad is a qualitative diagnostic test. It is the detection of total antibodies (IgM/IgA/IgG) against SARS-CoV-2 NC. The result was interpreted based on the manufacturer's recommendations: < 0.8, negative; between > 0.8 and < 1.0, equivocal; ≥1.0, positive.

COVID-19 IgM/IgG Combo lateral flow test kit from Megna Health Inc is to detect IgG and/or IgM isotypes specific to NC protein. 2 µL of serum and 2 drops of buffer were added and test results were read after 15 min by the laboratory technician and the kits were photographed for a second independent reading by another laboratory technician.

Meso scale discovery (MSD) coronavirus panel from Meso Scale Diagnostics is a multiplexed immunoassay to measure the IgG antibody response to SARS-CoV-2. A 96-well MSD plate has different antigens in each well. A calibration curve was created by using a reference standard with 4-fold serial dilution steps and a zero-calibrator blank for quantitation. Three levels of controls were also included in the assay to ensure the accuracy of the performance. First, the plate was blocked with Blocker A solution for 30 minutes at RT. The plate was washed 3 times with 150 µL/well of MSD wash buffer and then 50 µL of calibrator, controls, and diluted samples were dispensed into the plate and incubated with shaking for 2 hours at RT. After incubation and 3 x 150 µL/well washes with MSD wash buffer, detection antibody was added and then incubated with shaking for 1 hour. After detection antibody, the plate was washed with a wash buffer following which reader buffer B was added and the plate reads using the MESO QuickPlex SQ 120 instrument. MSD's multiplexed immunoassay provides quantitative antibody responses to antigens of interest. The result is in AU/mL defined during the calibration of the instrument.

Patient and public involvement

Patients or the public were not involved in the design, conduct, or its outcome measures or preparation of the manuscript in this study.

Statistical Analysis:

We performed descriptive statistics for demographic variables, self-reported vaccination-related variables, and antibody test results. We performed correlation analysis to assess relationships between different assays. We used linear regressions to investigate trends in anti-RBD antibody titers over time across different vaccine types. These antibody tilters were further compared between infections and vaccinations groups using Mann-Whitey tests. We used R version 4.2.0 and GraphPad Prism 9.5.1 for statistical analysis.

Results

Demographic

Overall, this survey included 1064 participants from the four different campuses of ASU. A total of 480 students were randomly selected to receive invitation emails, which led to 250 subjects (28% of the final student participants); the remaining participants responded to university wide advertising, learned of the survey by word of mouth or personally observed the collections and volunteered. The participants provided saliva samples for a qPCR diagnostic test and also donated blood. Of the 1064 participants, 893 (83.9%) subjects were students, 79 (7.4%) were employees, and 92 (8.6%) subjects did not provide information about their occupation status. 556 participants (52.3%) were female, and 467 participants (43.9%) were male. 762 participants (71.6%) were in the age group of 18-25 years, 190 (17.9%) were aged 26-40 years, 81 (7.6%) were aged 41-65 years, 31 (2.9%) were not reported. The demographic characteristics of the three different groups are presented in Table 1.

Table 1 Demographics of our serosurvey participants

		Students (n=893)	Employees (n=79)	Randomly Selected* (n=250)	Total Participants (n=1064)
Gender	Female	444 (49.7%)	51 (64.6%)	142 (56.8%)	556 (52.3%)
	Male	409 (45.8%)	28 (35.4%)	101 (40.4%)	467 (43.9%)
	Other	10 (1.1%)	NA	2 (0.8%)	11 (1.0%)
	Not Reported	30 (3.4%)	NA	5 (2%)	30 (2.8%)
Age	18-25	723 (81%)	4 (5.1%)	183 (73.2%)	762 (71.6%)
	26-40	120 (13.4%)	31 (39.2%)	50 (20%)	190 (17.9%)
	41-65	19 (2.1%)	44 (55.7%)	12 (4.8%)	81 (7.6%)
	Not Reported	31 (3.5%)	NA	5 (2%)	31 (2.9%)
Race	White	410 (45.9%)	62 (78.5%)	121 (48.4%)	528 (49.6%)
	Asian	270 (30.2%)	6 (7.6%)	66 (26.4%)	292 (27.4%)
	Mixed	39 (4.4%)	5 (6.3%)	13 (5.2%)	46 (4.3%)
	Black	24 (2.7%)	1 (1.3%)	7 (2.8%)	27 (2.5%)
	Native	13 (1.5%)	NA	6 (2.4%)	14 (1.3%)
	Other	99 (11.1%)	4 (5.1%)	31 (12.4%)	117 (11%)
	Prefer not to say	7 (0.8%)	1 (1.3%)	1 (0.4%)	9 (0.9%)
	Not Reported	31 (3.5%)	NA	5 (2%)	31 (2.9%)
Vaccination Status	Yes	822 (92.1%)	70 (88.6%)	239 (95.6%)	978 (91.9%)
	No	67 (7.5%)	9 (11.4%)	11 (4.4%)	82 (7.7%)
	Not Reported	4 (0.5%)	NA	NA	4 (0.4%)
Vaccine Source	Pfizer	424 (47.5%)	32 (40.5%)	137 (54.8%)	510 (47.9%)
	Moderna	248 (27.8%)	35 (44.3%)	70 (28%)	309 (29.0%)
	Janssen	86 (9.6%)	2 (2.5%)	18 (7.2%)	94 (8.8%)
	AstraZeneca	46 (5.2%)	NA	9 (3.6%)	46 (4.3%)
	Covaxin	9 (1.0%)	NA	NA	9 (0.9%)
	Sinopharm	2 (0.2%)	NA	2 (0.8%)	2 (0.2%)
	Sinovac	1 (0.1%)	NA	1 (0.4%)	1 (0.1%)
	Not Reported	77 (8.6%)	1 (1.3%)	13 (5.2%)	93 (8.7%)
Previous self-reported Covid infection	Yes	174 (19.5%)	12 (15.2%)	32 (12.8%)	205 (19.3%)
	No	717 (80.3%)	67 (84.8%)	218 (87.2%)	857 (80.6%)
	Not Reported	2 (0.2%)	NA	NA	2 (0.2%)

*Randomly selected from enrolled students and invited by email

Self-reported COVID-19 infection and vaccine status

Asymptomatic carriers can be a potential source of infection outbreaks in the community. We therefore evaluated how many participants had active COVID without reporting symptoms on the day when they donated samples. We found the prevalence of PCR positivity in asymptomatic students and employees in the university community was 0.4% (n=4/1064) on the day of sample collection. Among the 1064 participants, nearly 20% (19.3%, n=205/1064) reported testing positive for COVID-19 test in the past, whereas 80.6% reported no history of a positive test (Supplementary Table 2).

Table 2. Anti-RBD and Anti-NC antibody seroprevalence status of the population

Ab Sub-Type	Manufacturer	Antigen Detected	Name of the test	Assay type	Positives	Negative	Inconclusive
IgG	Beckman Coulter	RBD	Access SARS-CoV-2 IgG II (Semi-Quantitative)	Chemiluminescent Immunoassay (CLA)	938 (88.2%)	126 (11.8%)	0 (0%)
	Sienna-Clarity	RBD	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test	Lateral Flow (LFA)	954 (89.7%)	109 (10.2%)	1 (0%)
	MSD	RBD	V-PLEX COVID-19 Respiratory Panel 3 Kit	Electrochemiluminescent immunoassay (ECL)	1032 (97%)	32 (3%)	0 (0%)
	Megna Health Inc.	Nucleocapsid	Rapid COVID-19 IgM/IgG Combo Test Kit	Lateral Flow (LFA)	975 (91.6%)	85 (8%)	4 (0.4%)
	MSD	Nucleocapsid	V-PLEX COVID-19 Respiratory Panel 3 Kit	Electrochemiluminescent immunoassay (ECL)	171 (16.1%)	893 (83.9%)	0 (0%)
IgM	Beckman Coulter	RBD	ACCESS SARS-CoV-2 IgM (Qualitative)	Chemiluminescent Immunoassay (CLA)	87 (8.2%)	977 (91.8%)	0 (0%)
	Salofa Oy	RBD	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test	Lateral Flow (LFA)	8 (0.8%)	1054 (99%)	2 (0.2%)
	Megna Health Inc.	Nucleocapsid	Rapid COVID-19 IgM/IgG Combo Test Kit	Lateral Flow (LFA)	4 (0.4%)	1055 (99.2%)	5 (0.5%)
Total Ab (IgM, IgA, and IgG)	Bio-Rad	Nucleocapsid	Platelia SARS-CoV-2 Total Ab Assay	Enzyme-Linked Immunosorbent Assay (ELISA)	210 (19.7%)	841 (79%)	13 (1.2%)

*Excluded Megna Health LFA results in the further analysis due to a higher rate of false positives

More than 90% (91.9%, n=978/1064) of participants reported at least 1 dose of vaccine, whereas 7.7% of participants reported never receiving vaccine. Most participants received Pfizer (47.9%, n=510/1064) and Moderna (29.0%, n=309/1064) (Table 1). There was no significant difference in vaccine rate, nor reported history of COVID across age groups; however, we noticed that the lowest COVID rate among the 26-40 years group (14.2%, n=27/190) had the highest vaccine rate (93.2%, n=177/190) (Supplementary Table 2).

Seroprevalence

SARS-CoV-2 RBD of spike IgG and IgM antibodies

All serological assays were evaluated with the same set of 1064 serum samples. Of 1064 individuals, the seroprevalence for anti-RBD IgG antibody was found to be 89.7% by Sienna-Clarity, 88.2% by Beckman, and 97% by MSD (Table 2). There were no significant differences in the seroprevalence of anti-RBD IgG antibodies cross the groups (all participants, students only, employee only, and randomly invited students).

Among 182 participants who self-reported COVID infection and were vaccinated, 179 (98.4%) tested positive by Beckman, 181 (99.5%) by Sienna-Clarity LFA, and 181 (99.5%) by MSD for anti-RBD antibody. Among 22 participants who self-reported COVID infection and were not vaccinated, 10 (45.5%) tested positive by Beckman immunoassay, 12 (54.5%) by Sienna-Clarity, and 21 (95.4%) by MSD for anti-RBD antibody. Among 789 participants who self-reported no-COVID infection and were vaccinated, 721 (91.4%) tested positive by Beckman, 735 (93.2%) by Sienna-Clarity, and 778 (98.6%) by MSD for anti-RBD antibody (Supplementary Table 3).

SARS-CoV-2 NC antibodies

Overall, the seroprevalence for total anti-NC was 19.7% by Bio-Rad and 16.1% for anti-NC IgG by MSD, but 91.6 % by Megna. We excluded results from the latter due to high false-positive results (91.6%) (Table 2) since the seroprevalence was estimated at 34.2% in September 2021 from the nationwide commercial lab in Arizona based on the CDC website [4].

Among 205 participants who self-reported COVID infection regardless of vaccination status, 117 (57.1%) by Bio-Rad and 81 (39.5%) by MSD tested positive for anti-NC antibody levels (Supplementary Table 3). Interestingly, almost 80% (n=840) of the participants reported no known history of infection regardless of vaccine status (excluding 10 participants who received attenuated parasite vaccines*[B]). However, 10.7% (n=20+70=90) and 10.4% (n=73+14=87) tested positive for anti-nucleocapsid antibodies by the ELISA and the MSD assay without recalling at least one SAS-CoV-2 infection (Supplementary Table 3), presumably representing occult infections.

Demographic variables and seroconversion:

Seroconversion is the transition from the point of viral infection and/or vaccination to when antibodies of the virus become present in the blood. We performed sub-group analysis and looked for any association between seroconversion rates in ASU community and factors such as race, gender, age, employment status and vaccine types using linear and logistic regressions. In case of race, all other races except whites and Asians are grouped into one due to small sample numbers. The result indicates that there are no significant differences between different races, age groups, gender, and employment status for their ability to produce anti-RBD antibodies upon self-reported vaccination or anti-NC antibodies upon self-reported exposures to SARS-CoV-2 virus. As expected, people who received mRNA vaccines had significantly more seroconversion compared to other vaccines (supplemental table 4). There are no significant differences in the rate of anti-RBD antibody decay among these groups (supplemental table 5). Participants who received mRNA vaccines (Pfizer or Moderna) had significantly higher seroconversion rate and slower decay compared to other vaccine types (supplemental table 4 and 5).

Comparison of assays performances

The Venn diagrams show the overlapping distribution of positive results for each assay. For seropositive responses to the RBD of the spike protein, 926 specimens were positive by all three of Sienna-Clarity, Beckman, and MSD, whereas 75 specimens were positive only by MSD (Figure1A). Based on the same sample population, the percentage of positive results for all three assays for anti-RBD IgG were comparable (90%, 88%, and 97% respectively; Figure 1). However, only Beckman and MSD provided the antibody levels provided a quantitative number which allowed us to track antibody levels post vaccination and monitor how long immunity persisted. The anti-RBD antibody results by Beckman correlated strongly with the results by MSD (Figure 1C; r=0.79).

For seropositive NC, 130 specimens were positive by both Bio-Rad and MSD, whereas 80 specimens were positive only by Bio-Rad and 39 specimens by MSD (Figure 1B). The correlation of the values of anti-NC antibody level between Bio-Rad and MSD was weak (r=0.34) (Figure 1D).

Anti-RBD IgG antibody levels after vaccination

Anti-SARS-CoV-2 antibody persistence in the first six months after COVID vaccination decreased over time [19-21]. Here, we examined the relationship between the anti-RBD antibody titers of participants who received COVID vaccines and the number of days after vaccination using linear regression and summarized in Figure 2. As indicated in Figure 2, antibody titers varied widely, but there was clear trend towards lower titers over time. All vaccines have the same trend; we only report Modern and Pfizer in Figure 2; the other vaccines are reported in supplementary Figure 2. Participants who received 2 doses of Moderna vs. Pfizer trended towards higher antibody titers, which lasted longer, although these results were not statistically separable.

RBD Antibody responses following vaccination/infection

Participants were first classified into different groups based on their vaccine and infection status (vaccine only, previous COVID infection only, and both) and then further categorized them based on the time between their most recent vaccination/infection date and the collection date (0-3, 4-6, ≥7 months). In each group, the median level of anti-RBD antibody levels was higher in the subgroups of vaccinated participants with COVID infection than those with vaccination or infection only. In every group, the lowest median anti-RBD antibody level was detected in the participants who were never vaccinated. There were no samples in the group of participants with infection after 4-6 months. Although anti-RBD antibody levels declined over time for all groups, median antibody levels in both vaccinated and infected or vaccination-only groups remained above the cut-off 7 months after either infection and/or vaccination, whereas median antibody levels in the infection only group dropped below the cut-off by 7 months post infection (Figure 3A).

Increased anti-NC IgG antibody levels after infection

We also analyzed the antibody response to the SARS-CoV-2 NC protein in previously infected participants. However, the EUA-approved ELISA for NC antibodies that we used (Bio-Rad) is only a qualitative assay. To understand the trend of NC antibody levels post-infection, the MSD (Meso Scale Discovery) immunoassay from Meso Scale Diagnostics was applied, which uses ELISA-based quantitative detection. This assay was already verified with clinical samples, even though it is not an EUA-approved assay. Participants were categorized into different groups (0-1, 2-3, 4-5, 6-7, 8-9, 10-11, 12-13, 14-15, >15 months) depending on the interval between their infection date and collection date. Like RBD antibody levels, the NC antibody levels decreased over time, dropping somewhat faster than the ant-RBD antibodies in these data (Figure 3B & Supplementary Figure 3).

Increased anti-RBD IgG levels after breakthrough infection

Next, we investigated whether breakthrough COVID was associated with improved immune response. Participants were classified into three groups (breakthrough infection, hybrid immunity which is the participant who received vaccination after SARS-CoV-2 infection, and vaccine only). We had 645 fully vaccinated individuals, 19 individuals with 2 doses of vaccine after COVID infection (hybrid immunity), and 12 fully vaccinated individuals with breakthrough infection. Anti-RBD IgG values were significantly increased in both breakthrough and hybrid immune groups compared to vaccine only. In addition, the breakthrough infection group had significantly higher antibody levels compared to the hybrid immunity group, showing an association between breakthrough and enhanced immune response (Figure 3C).

Discussion

Estimating the cumulative proportion of the population infected with SARS-CoV-2 is crucial for effective planning and targeting public health responses during future pandemic. Understanding the current status of the pandemic and assessing the susceptibility of different populations and their behavior is also vital for implementing any policy changes towards mitigating the spread of the virus. Since the beginning of SARS-CoV-2 pandemic, CDC relied on commercial laboratories to gather nationwide seroprevalence data [22 23]. These survey, along with other representative studies, provided real-time estimate of proportion of individuals exposed to SARS-CoV-2, at least once before the sampling (see: https://covid19serohub.nih.gov/). However, there is a lack of reported seroprevalence studies from communities like universities, where the student population experiencing different social dynamics compared to the general public.

In the fall of 2021, over 79,000 students returned to campus for in-person classes coincident with a large increase in COVID incidence during the Delta wave in Maricopa County, AZ. We observed only 0.4% (4 positives out of 1064) active COVID positivity based on saliva qPCR on the day of sample collection from the serosurvey study in the ASU community. Notably, those with symptoms were asked not to participate. Although Nasopharyngeal swabs for SARS-CoV-2 gene detection via reverse transcriptase polymerase chain reaction (RT-PCR) testing is considered as gold standard, saliva has been identified as potential alternative [24 25]. We used TaqPath COVID-19 Combo Kit to test for SARS-CoV-2 infection in saliva samples. In a limited cross validation study, we did not see any significant differences between NP swabs and saliva for their ability to detect the presence of SARS-CoV-2 virus using TaqPath COVID-19 Combo Kit among asymptomatic populations. Czumbel et al reported 91% (CI 80-99%) sensitivity for saliva tests and 98% (CI 89-100%) sensitivity for NPS tests among previously confirmed COVID-19 patients [26], and concluded Saliva tests as an alternative to NPS for COVID-19 diagnosis. It is possible that 0.4% positivity that we report here could be an underestimation.

Individual variation in the immune response to vaccination

In the ASU community, 92% of participants have self-reported to had at least one dose of a COVID-vaccine. By comparison, only 85% of college students in the U.S. enrolled in spring or fall 2022 were vaccinated based on a nationally representative survey by the American College Health Association [27]. As of September 07, 2021, two weeks before this study date, only 58% of Arizonan's received at least one dose of COVID-19 vaccine [28]. We believe, AUS's proactive communications to parents and students helped with increased rate of vaccination. Most of the vaccinated participants at ASU received the Moderna and Pfizer mRNA vaccines that have shown great effectiveness after the second dose. However, as previously noted, the antibodies produced by Pfizer's COVID-19 vaccine decline faster than those produced by the Moderna vaccine after 6 months of vaccination [29]. We observed a similar trend in our study. Based on anti-RBD antibodies levels from Beckman it showed that a higher anti-RBD IgG

antibody level lasted longer in the participants who received 2 doses of Moderna compared to those who received 2 doses of Pfizer. This is probably due to the higher amount of RNA in Moderna (Figure 2).

Interestingly, 7 out of 978 participants who self-reported having received a COVID vaccine, tested negative for anti-RBD antibodies by all three assays in our study. Three out of 7 participants were vaccinated for more than 5 months (165, 168, and 216 days) with Pfizer vaccines leading to potential antibody decay based on figure 2. One out of 7 participants only received Pfizer for 7 days and antibody was likely not generated. It is known that there is substantial variation between individuals in the immune response to vaccination [30]. Other two out of 7 participants received Covaxin for 56 and 80 days and the level of anti-RBD antibodies in Covaxin was significantly lower than other vaccines. Another one out of 7 participants received AstraZeneca for more than 3 months (101 days), showing that Anti-RBD antibody levels from AstraZenecca started to wane after 2 months (Supplementary Figure 2) which was similar to what was previously reported from other group [31].

Seroconversion was found to be associated with days after the symptoms, increasing severity of the disease and the presence of co-morbidity [32]. The severe/moderate cases of COVID-19 tended to have earlier seroconversion than the asymptomatic/mild cases [32]. Children were less likely to have seroconversion than adults despite having similar viral loads [32]. Unlike other previously reported studies [33], in this study race and gender did not show any significant differences in their ability to produce anti-RBD antibodies after receiving primary vaccination regimen or upon exposures to SARS-CoV-2 virus. This could be due to a relatively young cohort of participants in our serosurvey. There was no difference in seroconversion between students and staff suggesting that the similar working environment did not contribute to variations in seroconversion rate. This is different compared to the seroconversion differences observed in the occupational risk of exposure to SARS-CoV-2 between hospital departments and healthcare workers [34].

The participants were tested negative for NC antibody after 6 months of post infection with COVID.

Serological tests enable detection of past SARS-CoV-2 infection and may detect cases of SARS-CoV-2 infection that were missed by earlier diagnostic tests. It is important to note that the diagnostic accuracy of different serological test can vary significantly depending on the cohorts of interest (asymptomatic, symptomatic, hospitalized) and the times of sampling post exposure/vaccination [35]. Several studies reported that the initial immune response in asymptomatic individuals is not as strong as in patients with more severe disease [36].

In the ASU community, 19.3% of participants (n=205) self-reported they had previous COVID infection; however, we only found 57% (n=117/205) and 39.5% (n=81/205) of participants from this group tested positive for NC antibody by Bio-Rad and MSD,

respectively (Supplementary Table 3). This could be due to antibody decay since their SARS-CoV-2 exposure. The median NC antibody levels fell below the positivity cutoff 6 months after infection, based on our MSD data (Figure 3B). Also, by 8 months post infection, 50% of participants from this group had undetectable NC antibodies. This finding was common with other serological studies, where the NC antibodies started to decline after a few months post infection and half the of participants have undetectable NC antibodies by 8 months post infection [37 38]. In September 2021, 14.6% (95% CI; 14% – 15.2%) of Arizona population tested positive for both NC and Spike antibodies, suggesting <15% exposed to SARS-CoV-2, ~4% less than our study population [39]. We recognized that the time of sampling (time since exposure/vaccination), method of testing (ELISA, CL, ECL, LFA), SARS-CoV-2 antigen(s) used (NC, RBD, Spike) and reference standard used to set cut-off for non-SARS-CoV-2 cases could potentially contribute to slight variations in our estimations of positive and negative cases.

Asymptomatic infections have been widely reported for COVID-19. Increasing evidence of greater asymptomatic in children and younger adults compared with the elderly [40]. Similar results were observed in cases with comorbidities compared to cases with no underlying medical conditions [40]. Among 847 self-reported no previous COVID infection participants (excluding 10 participants who received attenuated parasite vaccines*[B]), 10.6% (n=90) and 10.3% (n=87) tested positive for anti-NC antibody by Bio-Rad and MSD which means these 10% of participants had a COVID infection in the past without realizing it (Supplementary Table 3). It could be the participants in our study, most of them are young and with no underlying health conditions, had mild or asymptotic previous COVID-19 infections.

SARS-CoV-2 antibodies and breakthrough infections

SARS-CoV-2 infection induces a robust humoral and cellular immune response [41 42]. Similar to infection, vaccines result in early production of serum IgA, IgM, and IgG antibodies [43 44]. There is substantial immunologic and epidemiologic evidence suggests that the vaccination following infection further increases protection against subsequent illness among those who have been previously infected [45 46]. Neutralizing antibody and memory B cell response elicited by mRNA vaccination following previous exposure with SARS-CoV-2 results in an increased antibody titer compared to individuals who were not previously infected [47-50]. An important finding of this serological survey was that the participants who had breakthrough infection had higher anti-RBD IgG compared to those who were fully-vaccinated and also had prior infection (Figure 3C), which agrees with previous studies [51 52]. Although the number of breakthrough infections reported in this study is small, it was observed that previous COVID-19 infection resulted in the generation of robust and sustained levels of SARS-CoV-2 antibodies in vaccinated individuals. Considering that the antibody developed by B cells multiply after each exposure through infection or vaccination, these results were expected. First, the highest anti-RBD antibody levels were in the combined vaccination and infection group and most likely represent an accumulation of antibodies produced after each exposure.

Second, the anti-RBD antibody level in the infection only group decayed faster than the participants who received vaccines only. The participants here predominantly received the Pfizer and Moderna vaccines, which may be particularly efficient at evoking a durable anti-RBD response. Similar observations were made by several studies, that participants who received the Sinopharm vaccine (whole virus) had lower antibody levels compared to Pfizer/Moderna vaccine (spike protein) [53 54]. mRNA vaccine candidate also induces higher cellular immune responses than the recombinant protein vaccine.

Author Contributions MJ, JL, and VM initiated the study and design. VH, BN, PS, TL, and MM developed the design. CH and VM contributed project administration, supervision, and analysis. CH, KT, VB, BB, JK, KN, AM, and VM designed and conducted the experiments. SW and YC performed statistical analysis. CH, VM, JL, MJ, and YC wrote and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Competing interests None declared.

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Data sharing statement Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi: 10.5061/dryad.hhmgqnkn5

Ethical approval The study was approved by ASU's institutional Review Board (IRB)(STUDY00014505).

Participant Consent All participants are 18+ years old and consented to participating in the study and were willing to provide their samples for the research.

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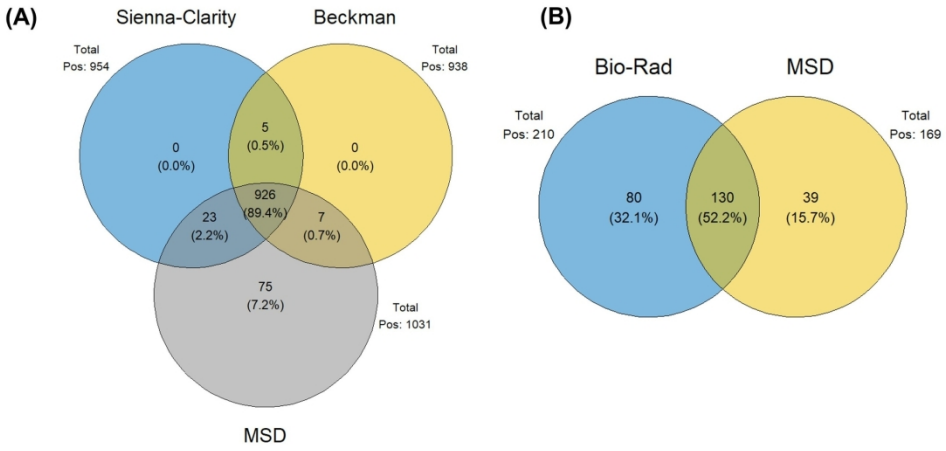
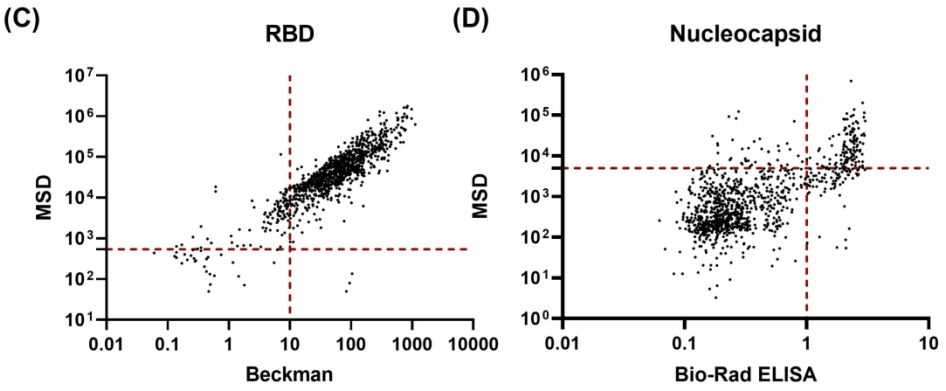
Figure 1. Comparison of assays. (A) Venn diagrams showing overlap of positive results of RBD and (B) Nucleocapsid from different assays. (C) Correlation between the anti-RBD IgG by Beckman and the MSD assays. (D) Correlation between anti-NC by Bio-Rad assay and MSD assays. A red dotted line indicates the cut-off line where test values equal or greater than this line are considered positive.

Figure 2. **Anti-RBD antibody decay post-vaccination.** The antibody level is determined by the Beckman semi-quantitative immunoassay. The linear regression of different vaccines to estimate vaccine decay.

Figure 3. Antibody response in participants with or without previous COVID infection, vaccination, or Both: (A) Anti-RBD antibodies measured using Beckman immunoassay in participants who had previous COVID infection or COVID vaccines or both. Participants were categorized by the vaccine or COVID infection and time interval from vaccination/infection to blood collection. **(B)** Anti-nucleocapsid antibodies measured using MSD in participants who had previous COVID infection. Participants were categorized by the COVID infection and time interval from infection to blood collection. **(C)** Anti-RBD antibodies after breakthrough infection, hybrid immunity, and vaccine only. Participants were categorized based on the order and the time of COVID infection and vaccination for each group. The blue bottle indicates vaccination, the virus indicates natural infection based on participant self-reporting, and the red vial indicates blood collection. Anti-RBD IgG level is measured by Beckman immunoassay. Cut-off is defined by the manufacturer. *P value is calculated by the Mann-Whitney test



Assay	Antigen	Positive	Negative	Inconclusive	Missing
Sienna-Clarity	RBD	954 (89.7%)	109 (10.2%)	1 (0%)	0 (0%)
Beckman		938 (88.2%)	126 (11.8%)	0 (0%)	0 (0%)
MSD		1032 (97%)	32 (3%)	0 (0%)	0 (0%)
Bio-Rad	Nucleocapsid	210 (19.7%)	841 (79%)	13 (1.2%)	0 (0%)
MSD		171 (16.1%)	893 (83.9%)	0 (0%)	0 (0%)



Comparison of assays. (A) Venn diagrams showing overlap of positive results of RBD and (B) Nucleocapsid from different assays. (C) Correlation between the anti-RBD IgG by Beckman and the MSD assays. (D) Correlation between anti-NC by Bio-Rad assay and MSD assays. A red dotted line indicates the cut-off line where test values equal or greater than this line are considered positive.

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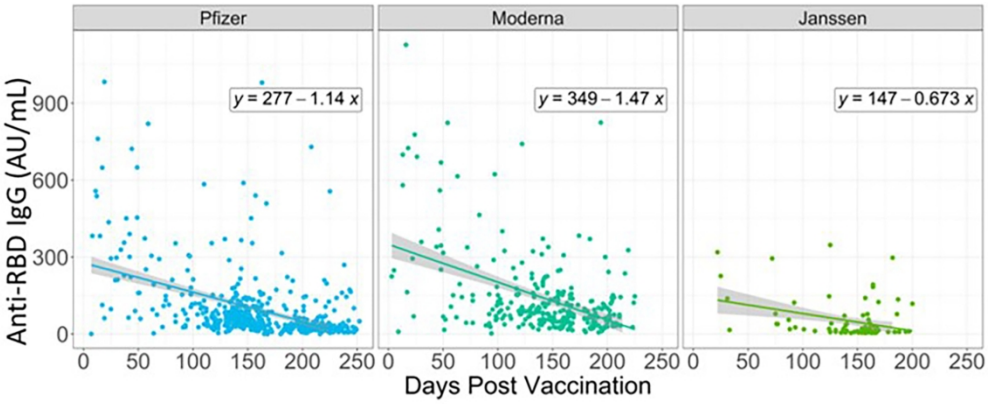

Linear Regression Analysis

Vaccine	n	Y-Intercept	X- Intercept	Slope	95% CI	P-value
Moderna	274	349.37	237.14	-1.47	(-1.81, -1.13)	<0.001
Pfizer	458	277.17	242.84	-1.14	(-1.35, -0.93)	<0.001

Anti-RBD antibody decay post-vaccination. The antibody level is determined by the Beckman semi-quantitative immunoassay. The linear regression of different vaccines to estimate vaccine decay.

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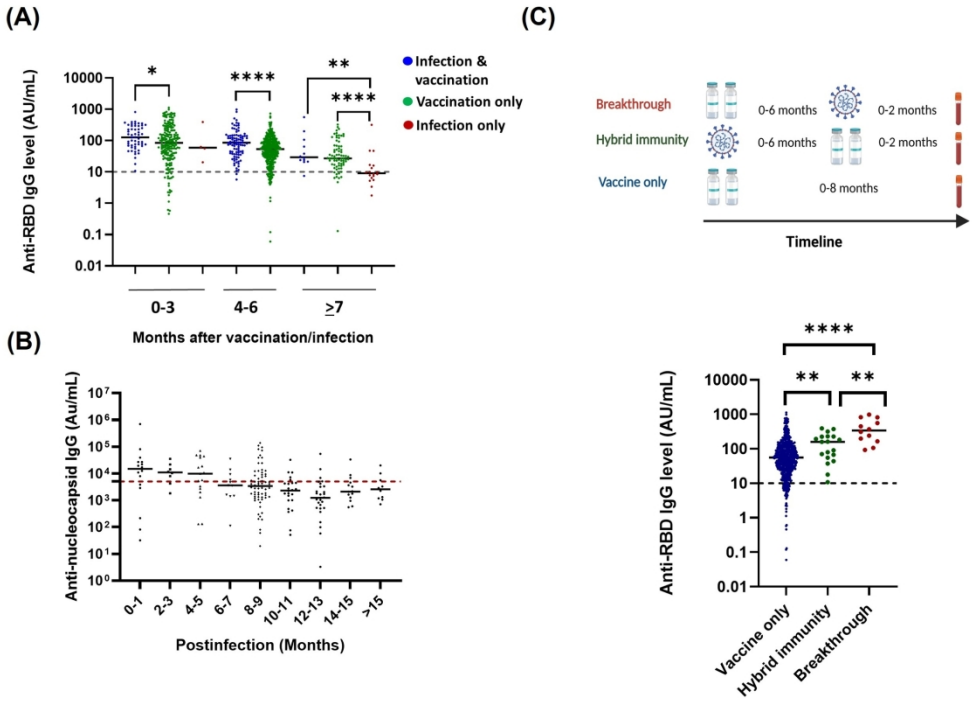

Antibody response in participants with or without previous COVID infection, vaccination, or Both: (A) Anti-RBD antibodies measured using Beckman immunoassay in participants who had previous COVID infection or COVID vaccines or both. Participants were categorized by the vaccine or COVID infection and time interval from vaccination/infection to blood collection. (B) Anti-nucleocapsid antibodies measured using MSD in participants who had previous COVID infection. Participants were categorized by the COVID infection and time interval from infection to blood collection. (C) Anti-RBD antibodies after breakthrough infection, hybrid immunity, and vaccine only. Participants were categorized based on the order and the time of COVID infection and vaccination for each group. The blue bottle indicates vaccination, the virus indicates natural infection based on participant self-reporting, and the red vial indicates blood collection. Anti-RBD IgG level is measured by Beckman immunoassay. Cut-off is defined by the manufacturer. *P value is calculated by the Mann-Whitney test

196x140mm (300 x 300 DPI)

Supplemental data for

Serological survey to estimate SARS-CoV-2 infection and antibody seroprevalence at a large public university; a cross sectional study

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Supplementary Table 1: Manufacturer reported sensitivity and specificity of EUA authorized tests used in this study

Assay	Sensitivity (positive percent agreement at >/= 15 days post symptom onset)	Specificity
Chemiluminescent assay: Beckman (Anti-RBD IgG)	98.9%	100%
Later flow assay: Clarity (Anti-RBD IgG)	96.15%	100%
Later flow assay: Megna (Anti-NC IgG)	95%	99.3%
ELISA: Bio-Rad (Anti-NC IgM/IgG/IgA)	100%	98.86%
Electrochemiluminescence assay: MSD* (Anti-NC IgG)	93.8%	100%
Electrochemiluminescence assay: MSD* (Anti-RBD IgG)	98.3%	98.5%

*MSD is not an EUA authorized test. It is a validated assay that meets the clinical laboratory standards institute guidelines.

Supplementary Table 2: COVID and vaccine status by age group

Category	Age Group		
	18-25	**26-40**	**41-65**
COVID Exposed	155 (20.3%)	27 (14.2%)	16 (19.8%)
Vaccinated	702 (92.1%)	177 (93.2%)	72 (88.9%)
COVID Exposed & vaccinated	141 (18.5%)	24 (12.6%)	11 (13.6%)
Total	762	190	81

Supplementary Table 3: Cohort characteristics and serological positive results by different assays

Cohort			IgG (RBD from Spike Protein)			IgG (Nucleocapsid Protein)	
Infection	Vaccine	n	CLIA (Beckman)	LFA (Sienna-Clarity)	MSD (MSD)	ELISA (Bio-Rad)	MSD (MSD)
YES	YES[A]	180	177 (98.3%)	179 (99.4%)	179 (99.4%)	101 (56.1%)	69 (38.3%)
	YES[B]	2	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)
	NO	22	10 (45.5%)	12 (54.5%)	21 (95.4%)	13 (59.1%)	9 (40.9%)
	NA	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
NO	YES[A]	779	719 (92.3%)	732 (94.0%)	770 (98.8%)	70 (8.9%)	73 (9.4%)
	YES[B]	10	2 (20%)	3 (30%)	8 (80%)	3 (30%)	3 (30%)
	NO	60	21 (35%)	19 (31.7%)	41 (68.3%)	20 (33.3%)	14 (23.3%)
	NA	1	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
NA	NA	2	2 (100%)	2 (100%)	2 (100%)	0 (0 %)	0 (0%)
Total	1064		938 (88.2%)	954 (89.7%)	1032 (97%)	210 (19.7%)	171 (16.1 %)

[A]Pfizer/BioNTech, Moderna, Janssen, AstraZeneca, Covishield; [B]Sinopharm, Sinovac, Covaxin

Supplementary Table 4: Seroconversion by race, age, gender, employment status, and the types of vaccines:

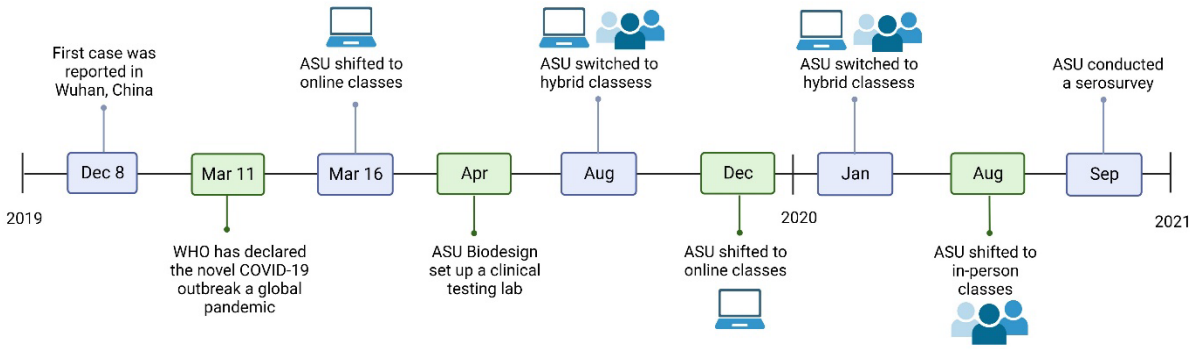
Variable	Classifiers	Anti RBD Antibody (Access SARS-CoV-2 IgG II				Anti NC antibody (Platelia NC total Ab)			
		n	beta	95% CI	P-value	n	OR	95% CI	P-value
Race	White vs Other	345 vs 142	-18.14	(-45.76, 9.47)	0.20	95 vs 42	0.75	(0.34, 1.65)	0.48
	Asian vs Other	217 vs 142	-21.39	(-52.27, 9.48)	0.17	38 vs 42	1.70	(0.64, 4.63)	0.29
	White vs Asian	345 vs 217	3.25	(-22.01, 28.50)	0.20	95 vs 38	0.44	(0.18, 1.04)	0.48
Age	20-30 vs <20	393 vs 214	-22.26	(-46.41, 1.90)	0.07	112 vs 43	0.73	(0.34, 1.54)	0.41
	30-40 vs <20	51 vs 214	-29.01	(-74.03, 16.01)	0.21	6 vs 43	1.07	(0.18, 8.70)	0.94
	40-50 vs <20	25 vs 214	-54.03	(-121.52, 13.46)	0.12	8 vs 43	1.03	(0.16, 7.52)	0.97
	50>= vs <20	21 vs 214	-43.28	(-115.57, 29.01)	0.24	6 vs 43	3.31	(0.39, 73.67)	0.33
	30-40 vs 20-30	51 vs 393	-6.75	(-49.62, 36.12)	0.21	6 vs 112	1.47	(0.26, 11.24)	0.94
	40-50 vs 20-30	25 vs 393	-31.77	(-97.44, 33.89)	0.12	8 vs 112	1.41	(0.24, 9.49)	0.97
	50>= vs 20-30	21 vs 393	-21.02	(-91.74, 49.69)	0.24	6 vs 112	4.52	(0.58, 97.19)	0.33
	40-50 vs 30-40	25 vs 51	-25.02	(-95.27, 45.23)	0.12	8 vs 6	0.96	(0.07, 11.61)	0.97
	50>= vs 30-40	21 vs 51	-14.27	(-88.96, 60.42)	0.24	6 vs 6	3.08	(0.18, 92.05)	0.33
	50>= vs 40-50	21 vs 25	10.75	(-70.60, 92.09)	0.24	6 vs 8	3.21	(0.28, 79.51)	0.33
Gender	Male vs Female	325 vs 379	11.40	(-10.12, 32.91)	0.30	90 vs 85	1.64	(0.86, 3.17)	0.13
Employment Status	Student vs Employee	647 vs 57	27.80	(-22.30, 77.90)	0.28	163 vs 12	0.72	(0.13, 3.66)	0.69
Vaccine Group	mRNA vaccine vs Other	614 vs 90	58.91	(26.93, 90.90)	**<0.001**	119 vs 36	2.18	(0.97, 5.05)	0.06

*Bold indicates statistically significant differences

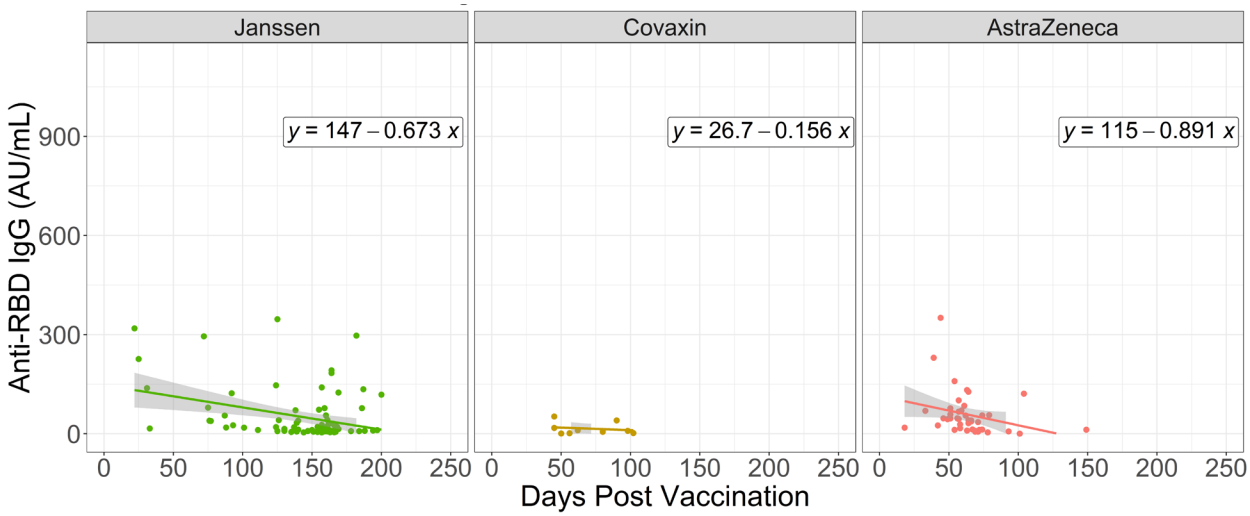
Supplementary Table 5: Anti-RBD antibody decay by race, age, gender, employment status, and the types of vaccines:

Variable	Classifiers	Anti-RBD antibody decay post vaccination			
		n	beta	95% CI	P-value
Race	White vs Other	368 vs 149	-10.40	(-34.67, 13.86)	0.40
	Asian vs Other	242 vs 149	-27.26	(-54.07, -0.46)	0.047
	White vs Asian	368 vs 242	16.86	(-5.09, 38.80)	0.40
Age	20-30 vs <20	420 vs 230	-15.88	(-36.80, 5.04)	0.14
	30-40 vs <20	58 vs 230	-15.99	(-54.31, 22.33)	0.41
	40-50 vs <20	28 vs 230	-27.29	(-84.17, 29.60)	0.35
	50>= vs <20	23 vs 230	-10.35	(-73.13, 52.43)	0.75
	30-40 vs 20-30	58 vs 420	-0.11	(-36.41, 36.19)	0.41
	40-50 vs 20-30	28 vs 420	-11.41	(-66.73, 43.92)	0.35
	50>= vs 20-30	23 vs 420	5.53	(-55.84, 66.91)	0.75
	40-50 vs 30-40	28 vs 58	-11.30	(-70.42, 47.83)	0.35
	50>= vs 30-40	23 vs 58	5.64	(-58.50, 69.79)	0.75
	50>= vs 40-50	23 vs 28	16.94	(-52.65, 86.53)	0.75
Sex	Male vs Female	356 vs 403	0.48	(-18.23, 19.18)	0.96
Employment Status	Student vs Employee	696 vs 63	-5.01	(-47.99, 37.97)	0.82
Vaccine Group	mRNA vaccine vs Other	635 vs 124	100.59	(74.95, 126.22)	**<0.001***

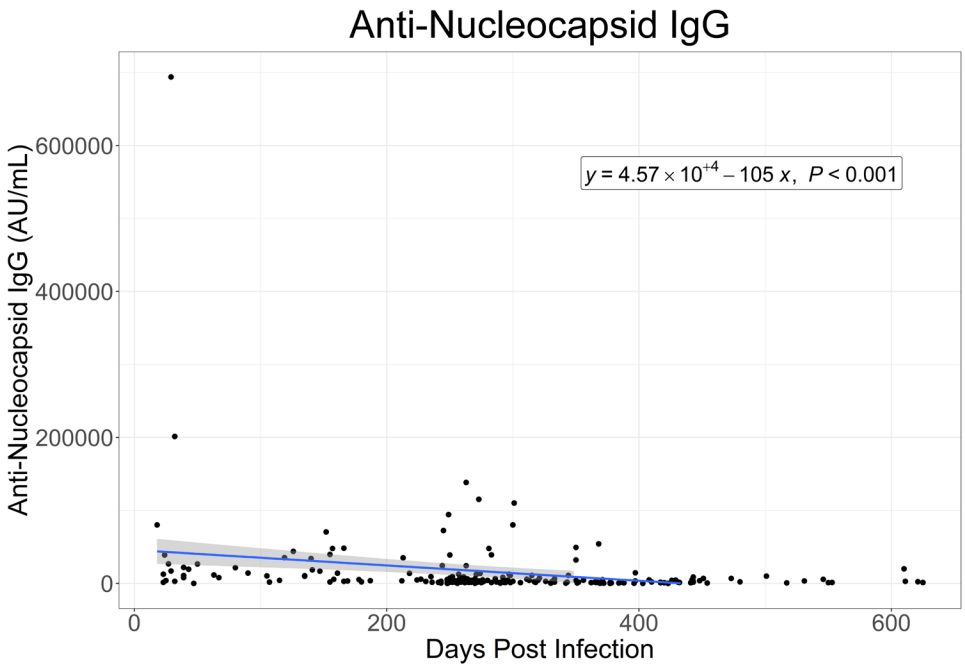
*Bold indicates statistically significant differences



Supplementary Figure 1: The timeline between the outbreak of the COVID-19 pandemic and serosurvey at ASU. In response to COVID, ASU rotated from in-person to remote to hybrid learning several times during the pandemic depending on the prevalence of infection in the community.



Supplementary Figure 2: **Anti-RBD antibody decay post-vaccination.** The antibody level is determined by the Beckman immunoassay. The linear regression of different vaccines to estimate vaccine decay.



Supplementary Figure 3: **Anti-Nucleocapsid antibody decay post-infection.** The antibody level is determined by the MSD. The linear regression was to estimate antibody decay.

Serological survey to estimate SARS-CoV-2 infection and antibody seroprevalence ~~A serosurvey of SARS-COV-2~~ at a large public university; a cross sectional study

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Abstract

Objective: This study investigated the seroprevalence of SARS-CoV-2 antibodies among adults over 18 years

Design: Prospective cohort study.

Settings: a population-based study among the big university community

Participants: This study took volunteers over five days and recruited adult 1064 participants.

Primary outcome measures: We conducted a seroprevalence in our community with SARS-CoV-2-specific antibodies due to previous exposure to SARS-CoV-2 and/or vaccination.

Results: The seroprevalence of the anti-receptor binding domain (RBD) antibody was 90% by a lateral flow assay and 88% by a semi-quantitative chemiluminescent immunoassay. The seroprevalence for anti-nucleocapsid (NC) was 20%. In addition, individuals with previous natural COVID infection plus vaccination had higher anti-RBD antibody levels compared to those who had vaccination only or infection only. Individuals who had a breakthrough infection had the highest anti-RBD antibody levels.

Conclusion: Accurate estimates of the cumulative incidence of SARS-CoV-2 infection can inform the development of university risk mitigation protocols such as encouraging booster shots, extending mask mandates, or reverting to online classes. It could help us to have clear guidance to act at the first sign of the next surge as well, especially since there is a surge of COVID subvariant infections.

Strengths and limitations of this study:

- We investigated both active infection and seroprevalence for the university population at the same time.
- Our study was strengthened by the data available on participants from their self-report and the independent validation by an EUA authorized diagnostic test.
- Our study was performed within the university setting therefore it only reflects the COVID-19 situation within ~~the~~that ~~-~~community.
- ~~Conducting longitudinal studies in university settings will provide valuable information about vaccine efficacies, infection spread among vaccinated individuals and provide mitigation regarding policies that work when implemented appropriately, during current and future pandemics.~~
- ~~The study has a large number of prospective participants during a short window. Study limitations need to be noted in this study, including samples with an unknown degree of selection bias due to convenience sample, self-reported COVID test results, and vaccine status.~~
- ~~Participants were only tested once for antibodies, thus lacking~~ Our study lacks longitudinal data to compare antibody waning rates in individuals.

- ~~One of the limitations of this study is the s~~~~Small~~The number of breakthrough infections was small requiring confirmation.
-
- ~~The number of breakthrough infections and infections only was relatively small.~~

Introduction

The COVID-19 pandemic has been a major challenge worldwide. COVID-19 is caused by a novel Betacoronavirus (SARS-CoV-2) and was first reported from Wuhan, China, on 8 December 2019. The World Health Organization (WHO) declared it a pandemic on 11 March 2020 [1 2]. The United States had recorded more than 101 million cases and 1,091,000 deaths by January 11, 2023. Since the end of 2019, communities around the world have had to fight against outbreaks, including physical distancing, staying at home, avoiding groups indoors, wearing masks, frequent testing, and contact tracing, etc [3]. Although the intensity of these measures has recently abated partially, activities have not fully returned to the pre-pandemic routine and there are still an estimated 2500 COVID-19 deaths weekly[4]. Scientists have developed rapid diagnostics tests [5-7] and many effective vaccines [8-10] that have reduced morbidity and mortality considerably. Throughout the pandemic, university life has represented a unique challenge because universities tried to maximize safe in-person learning opportunities and maintain safe school operations by implementing effective practices.

ASU shifted to online classes on March 16, 2020 while a team of researchers at the Biodesign Institute set up a clinical testing laboratory. At that time, Arizona was a worldwide COVID-19 hotspot. ASU students and employees were encouraged to do COVID test frequently at no charge. After a few months of monitoring COVID, ASU switched to hybrid classes in August 2020. However, COVID cases began surging in late November 2020, resulting in the implementation of a fully remote learning model in December 2020. On January 11, 2021, ASU switched back to hybrid learning model until Fall semester of 2021. During these months, COVID testing, and vaccines were available to all students and employees at ASU. The ASU community followed CDC guidelines by offering frequent qPCR saliva testing, rigorous contact tracing, and strong support during isolation. This allowed the safe return to a fully in-person class in the fall of 2021 (Supplementary Figure 1).

A research project at Davidson College in North Carolina reported that almost 6000 four-year colleges and universities provided combinations of online and in-person classes, another 446 had “primarily in-person” courses, and 45 operated “fully in-person” during 2020[11]. Also, a survey was conducted by New America and Global Strategy Group with 1,002 college students nationwide from April 29 through May 13, 2021. In the survey, 62% of students claimed that their schools would provide combinations of online and in-person classes, 14% claimed that their schools will offer online classes only, and only 12% would provide fully in-person classes in the fall of 2021[12]. Arizona State University was among the 12% operating “fully in-person” and one of the country’s largest universities, with over 79,000 students who had returned to

campus for in-person classes in the fall of 2021. ASU wanted to evaluate the success of the COVID-19 management strategy by monitoring SARS-CoV-2 seroprevalence to estimate immunity from prior infection, vaccination, or both. Thus, ASU conducted a serosurvey to collect self-reported experiences and to determine the number of people in our community with various SARS-CoV-2-specific antibodies. The university had anonymized information about the prevalence of positive qPCR tests in its community, but at the time of this study, it lacked information about the level of immunity and possible viral exposure rate. This study would help inform on deciding on safety protocols, vaccination recommendations, masking recommendations mandates, and online vs in-person classes. At the time of this survey, September 2021, the SARS-CoV-2 subvariant Delta, a highly contagious variant, accounted for 65% of all cases in Arizona [4].

By assessing humoral immunity, seroprevalence studies estimate the percentage of a specific population who have been previously infected with a pathogen. Many seroprevalence studies of SARS-CoV-2 have been reported. Arnaud et al. showed that neutralizing and anti-RBD antibodies persisted for at least 6 months after a mild COVID infection from hospital workers[13]. A similar result was also demonstrated by Baker et al, who found that the antibodies against SARS-CoV-2 produced by health care workers or patients who have mild COVID infection were stable for up to six months and helped prevent recurrent infections [14]. Another group investigated anti-NC antibody levels in severe and mild patients at hospitals. The data indicated that anti-NC levels started to decline after 2 months after post PCR and antibody levels were lower in patients with mild compared to severe illness [15]. However, the seroprevalence studies at the educational institutions/ communities, where students and employees are in close contact on daily basis, are very limited.

SARS-CoV-2 induces antibodies with IgM, IgA, and IgG isotypes against spike protein, RBD of the spike protein, and NC protein. The antibodies produced by COVID vaccination (Pfizer, Moderna, J&J, AstraZeneca, and Covishield) are IgM, IgA, and IgG isotypes against spike protein, specifically the RBD of the spike protein. Whereas anti-spike antibodies do not distinguish between vaccination and infection, anti-NC positivity generally implies a previous infection; however, participants who received COVID vaccines from Sinopharm, Sinovac, and Covaxin, which contain inactivated SARS-CoV-2 viruses, and who attend an international university like ASU, may also have anti-NC antibodies from vaccination. The main objective of this study is to estimate the seroprevalence of IgG and IgM antibodies to the 'SARS-CoV-2' coronavirus in the university population, ~~either~~both through vaccination ~~or~~and through exposures to SARS-CoV-2 virus. Serosurvey would also answer questions like; a) Does universities have similar infection rates like the general community? b) Is immunization by the university community better or worse than the population at large? In addition to providing data on ~~regarding the exposure of~~ the university population's exposure to COVID-19, this study would shed light on ~~some~~ risk factors for developing SARS-CoV-2 infection. Overall, we expect that the estimates would provide us ample evidence to gauge the population-

level scenario of COVID-19 at the university as well as provide insights into other epidemiological aspects of the disease, including the risk factors for developing SARS-CoV-2 infection. We also intend to compare multiple assays and their performance characteristics in real-world scenario. By combining the self-reported vaccine and infection history with documented antibodies to SARS-CoV2 antigens, we ~~estimated~~ intend to estimate the number of: a) individuals with detectable anti-spike antibodies; b) individuals with likely previous SARS-CoV-2 exposure, even if they did not report COVID-19 symptoms; and c) individuals with no detectable antibodies after vaccination or previous infection.

Methods

Participants

We employed a two-stage sampling strategy. First, a random sample of current students were invited to participate in the serosurvey through email invitation. To increase the representativeness of the sample, targeted recruitment was made via social media advertising, as well as in-person recruitment from selected areas of the campus. Responses are time-stamped to allow for analysis according to the date of completion. This study took volunteers over five days (9/13-9/17 of 2021) at 4 campuses (Downtown Phoenix, Polytechnic, Tempe, and West) of ASU.

Survey Instruments

Demographics, COVID-19 vaccination, testing history, and COVID symptoms were self-reported by questionnaire.

Blood sample collection

The blood samples were collected by phlebotomists at ASU with serum tubes (Cat # 37988 from BD) and were placed into a cooler within 4 hours and transported within 6 hours of collection to the clinical testing laboratory at ASU. Samples were centrifuged at 1300 g for 20 minutes to separate the serum. 1064 serum samples and matching survey results were analyzed.

Serology testing

The main serological detection methods were all approved by Emergency Use Authorization from the US Food and Drug Administration for marketing are the chemiluminescence immunoassay (CLIA), enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay (IFA), and lateral flow immunoassay (LFA) [16 17]. In this survey, the serological tests were done either at the ASU Biodesign Clinical Testing Laboratory (ABCTL) or the Center for Personalized Diagnostics (CPD). Samples were tested for antibodies against the RBD domain of the Spike protein using Access SARS-CoV-2 chemiluminescent IgG II and IgM assay (Beckman coulter) and Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid lateral flow assay (Sienna-Clarity) to estimate

vaccine-induced SARS-CoV-2 seroprevalence. Samples also were tested for antibodies against ~~table~~ NC protein using Platelia SARS-CoV-2 Total Ab ELISA Assay and rapid COVID-19 IgM/IgG Combo lateral flow test kit (Megna Health Inc.) to estimate infection-induced SARS-Cov-2 seroprevalence. Beckman Access SARS-CoV-2 IgG II is EUA authorized as semi-quantitative assay. All LFA are qualitative. Beckman Access SARS-CoV-2 IgM, and Platelia SARS-CoV-2 Total Ab ELISA Assay gives a quantitative readout, but they are authorized as qualitative tests. The manufacturer-reported sensitivity and specificity were reported in the Supplementary Table 1.

Access SARS-CoV-2 chemiluminescent IgG II ~~and IgM~~ assay~~s~~ from Beckman coulter were performed in this study to determine IgG ~~and IgM~~ antibody level of SARS-coV-2 RBD protein according to the manufacturer's instructions [18]. 5 different concentrations of calibrators and two different concentrations of controls were provided by the ~~manufacture~~manufacturer to ensure reagent integrity and proper assay performance before analyzing samples. The result is compared to the cut-off value in arbitrary units (AU/mL) defined during the calibration of the instrument.

Access SARS-CoV-2 chemiluminescent IgM assay from Beckman coulter was utilized to measure the IgM antibody level of SARS-coV-2 RBD protein, following the manufacturer's instructions [18]. To ensure reagent integrity and proper assay performance before analyzing samples, two different concentrations of calibrators and controls were provided by the manufacture, which were analyzed prior to testing the samples. The obtained results were compared to the instrument-defined cut-off value, expressed as signal to cut-off (S/Co).

Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid lateral flow assay is to detect IgG and/or IgM isotypes specific to the RBD portion of the S1 protein. 10 µL of serum and 2 drops of buffer were added, and test results were read after 10 min by the laboratory technician and the kits were photographed for a second independent reading by another laboratory technician.

Platelia SARS-CoV-2 total Ab ELISA assay from Bio-Rad is a qualitative diagnostic test. It is the detection of total antibodies (IgM/IgA/IgG) against SARS-CoV-2 NC. The result was interpreted based on the manufacturer's recommendations: < 0.8, negative; between > 0.8 and < 1.0, equivocal; ≥1.0, positive.

COVID-19 IgM/IgG Combo lateral flow test kit from Megna Health Inc is to detect IgG and/or IgM isotypes specific to NC protein. 2 µL of serum and 2 drops of buffer were added and test results were read after 15 min by the laboratory technician and the kits were photographed for a second independent reading by another laboratory technician.

Meso scale discovery (MSD) coronavirus panel from Meso Scale Diagnostics is a multiplexed immunoassay to measure the IgG antibody response to SARS-CoV-2. A 96-well MSD plate has different antigens in each well. A calibration curve was created

by using a reference standard with 4-fold serial dilution steps and a zero-calibrator blank for quantitation. Three levels of controls were also included in the assay to ensure the accuracy of the performance. First, the plate was blocked with Blocker A solution for 30 minutes at RT. The plate was washed 3 times with 150 µL/well of MSD wash buffer and then 50 µL of calibrator, controls, and diluted samples were dispensed into the plate and incubated with shaking for 2 hours at RT. After incubation and 3 x 150 µL/well washes with MSD wash buffer, detection antibody was added and then incubated with shaking for 1 hour. After detection antibody, the plate was washed with ~~washa~~ wash buffer following which reader buffer B was added and the plate reads using the MESO QuickPlex SQ 120 instrument. MSD's multiplexed immunoassay provides quantitative antibody responses to antigens of interest. The result is in AU/mL defined during the calibration of the instrument.

Patient and public involvement

Patients or the public were not involved in the design, conduct, or its outcome measures or preparation of the manuscript in this study.

Statistical Analysis:

We performed descriptive statistics for demographic variables, self-reported vaccination-related variables, and antibody test results. We performed correlation analysis to assess relationships between different assays. We used linear regressions to investigate trends in anti-RBD antibody titers over time across different vaccine types. These antibody tilters were further compared between infections and vaccinations groups using Mann-Whitey tests. We used R version 4.2.0 and GraphPad ~~Prism XXX for~~Prism 9.5.1 for statistical analysis.

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Results

Demographic

Overall, this survey included 1064 participants from the four different campuses of ASU. A total of 480 students were randomly selected to receive invitation emails, which led to 250 subjects (28% of the final student participants); the remaining participants responded to university wide advertising, learned of the survey by word of mouth or personally observed the collections and volunteered. The participants provided saliva samples for a qPCR diagnostic test and also donated blood. Of the 1064 participants, 893 (83.9%) subjects were students, 79 (7.4%) were employees, and 92 (8.6%) subjects did not provide information about their occupation status. 556 participants (52.3%) were female, and 467 participants (43.9%) were male. 762 participants (71.6%) were in the age group of 18-25 years, 190 (17.9%) were aged 26-40 years, 81 (7.6%) were aged 41-65 years, 31 (2.9%) were not reported. The demographic characteristics of the three different groups are presented in Table 1.

Table 1 Demographics of our serosurvey participants

		Students (n=893)	Employees (n=79)	Randomly Selected* (n=250)	Total Participants (n=1064)
Gender	Female	444 (49.7%)	51 (64.6%)	142 (56.8%)	556 (52.3%)
	Male	409 (45.8%)	28 (35.4%)	101 (40.4%)	467 (43.9%)
	Other	10 (1.1%)	NA	2 (0.8%)	11 (1.0%)
	Not Reported	30 (3.4%)	NA	5 (2%)	30 (2.8%)
Age	18-25	723 (81%)	4 (5.1%)	183 (73.2%)	762 (71.6%)
	26-40	120 (13.4%)	31 (39.2%)	50 (20%)	190 (17.9%)
	41-65	19 (2.1%)	44 (55.7%)	12 (4.8%)	81 (7.6%)
	Not Reported	31 (3.5%)	NA	5 (2%)	31 (2.9%)
Race	White	410 (45.9%)	62 (78.5%)	121 (48.4%)	528 (49.6%)
	Asian	270 (30.2%)	6 (7.6%)	66 (26.4%)	292 (27.4%)
	Mixed	39 (4.4%)	5 (6.3%)	13 (5.2%)	46 (4.3%)
	Black	24 (2.7%)	1 (1.3%)	7 (2.8%)	27 (2.5%)
	Native	13 (1.5%)	NA	6 (2.4%)	14 (1.3%)
	Other	99 (11.1%)	4 (5.1%)	31 (12.4%)	117 (11%)
	Prefer not to say	7 (0.8%)	1 (1.3%)	1 (0.4%)	9 (0.9%)
	Not Reported	31 (3.5%)	NA	5 (2%)	31 (2.9%)
Vaccination Status	Yes	822 (92.1%)	70 (88.6%)	239 (95.6%)	978 (91.9%)
	No	67 (7.5%)	9 (11.4%)	11 (4.4%)	82 (7.7%)
	Not Reported	4 (0.5%)	NA	NA	4 (0.4%)
Vaccine Source	Pfizer	424 (47.5%)	32 (40.5%)	137 (54.8%)	510 (47.9%)
	Moderna	248 (27.8%)	35 (44.3%)	70 (28%)	309 (29.0%)
	Janssen	86 (9.6%)	2 (2.5%)	18 (7.2%)	94 (8.8%)
	AstraZeneca	46 (5.2%)	NA	9 (3.6%)	46 (4.3%)
	Covaxin	9 (1.0%)	NA	NA	9 (0.9%)
	Sinopharm	2 (0.2%)	NA	2 (0.8%)	2 (0.2%)
	Sinovac	1 (0.1%)	NA	1 (0.4%)	1 (0.1%)
	Not Reported	77 (8.6%)	1 (1.3%)	13 (5.2%)	93 (8.7%)
Previous self-reported Covid infection	Yes	174 (19.5%)	12 (15.2%)	32 (12.8%)	205 (19.3%)
	No	717 (80.3%)	67 (84.8%)	218 (87.2%)	857 (80.6%)
	Not Reported	2 (0.2%)	NA	NA	2 (0.2%)

*Randomly selected from enrolled students and invited by email

Self-reported COVID-19 infection and vaccine status

Asymptomatic carriers can be a potential source of infection outbreaks in the community. We therefore evaluated how many participants had active COVID without reporting symptoms on the day when they donated samples. We found the prevalence of PCR positivity in asymptomatic students and employees in the university community was 0.4% (n=4/1064) on the day of sample collection. Among the 1064 participants, nearly 20% (19.3%, n=205/1064) reported testing positive for COVID-19 test in the past, whereas 80.6% reported no history of a positive test (Supplementary Table 2).

Table 2. Anti-RBD and Anti-NC antibody seroprevalence status of the population

Ab Sub-Type	Manufacturer	Antigen Detected	Name of the test	Assay type	Positives	Negative	Inconclusive
IgG	Beckman Coulter	RBD	Access SARS-CoV-2 IgG II (Semi-Quantitative)	Chemiluminescent Immunoassay (CLA)	938 (88.2%)	126 (11.8%)	0 (0%)
	Sienna-Clarity	RBD	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test	Lateral Flow (LFA)	954 (89.7%)	109 (10.2%)	1 (0%)
	MSD	RBD	V-PLEX COVID-19 Respiratory Panel 3 Kit	Electrochemiluminescent immunoassay (ECL)	1032 (97%)	32 (3%)	0 (0%)
	Megna Health Inc.	Nucleocapsid	Rapid COVID-19 IgM/IgG Combo Test Kit	Lateral Flow (LFA)	975 (91.6%)	85 (8%)	4 (0.4%)
	MSD	Nucleocapsid	V-PLEX COVID-19 Respiratory Panel 3 Kit	Electrochemiluminescent immunoassay (ECL)	171 (16.1%)	893 (83.9%)	0 (0%)
IgM	Beckman Coulter	RBD	ACCESS SARS-CoV-2 IgM (Qualitative)	Chemiluminescent Immunoassay (CLA)	87 (8.2%)	977 (91.8%)	0 (0%)
	Salofa Oy	RBD	Sienna-Clarity COVIBLOCK COVID-19 IgG/IgM Rapid Test	Lateral Flow (LFA)	8 (0.8%)	1054 (99%)	2 (0.2%)
	Megna Health Inc.	Nucleocapsid	Rapid COVID-19 IgM/IgG Combo Test Kit	Lateral Flow (LFA)	4 (0.4%)	1055 (99.2%)	5 (0.5%)
Total Ab (IgM, IgA, and IgG)	Bio-Rad	Nucleocapsid	Platelia SARS-CoV-2 Total Ab Assay	Enzyme-Linked Immunosorbent Assay (ELISA)	210 (19.7%)	841 (79%)	13 (1.2%)

*Excluded Megna Health LFA results in the further analysis due to a higher rate of false positives

More than 90% (91.9%, n=978/1064) of participants reported at least 1 dose of vaccine, whereas 7.7% of participants reported never receiving vaccine. Most participants received Pfizer (47.9%, n=510/1064) and Moderna (29.0%, n=309/1064) (Table 1). There was no significant difference in vaccine rate, nor reported history of COVID across age groups; however, we noticed that the lowest COVID rate among the 26-40 years group (14.2%, n=27/190) had the highest vaccine rate (93.2%, n=177/190) (Supplementary Table 23).

Table 3. COVID and vaccine status by age group

Category	Age Group		
	18-25	26-40	41-65
COVID Exposed	155 (20.3%)	27 (14.2%)	16 (19.8%)
Vaccinated	702 (92.1%)	177 (93.2%)	72 (88.9%)
COVID Exposed & vaccinated	141 (18.5%)	24 (12.6%)	11 (13.6%)
Total	762	190	81

Seroprevalence

SARS-CoV-2 RBD of spike IgG and IgM antibodies

All serological assays were evaluated with the same set of 1064 serum samples (Table 4). Of 1064 individuals, the seroprevalence for anti-RBD IgG antibody was found to be 89.7% by Sienna-Clarity, 88.2% by Beckman, and 97% by MSD (Table 2). There were no significant differences in the seroprevalence of anti-RBD IgG antibodies cross the groups (all participants, students only, employee only, and randomly invited students).

Among 182 participants who self-reported COVID infection and were vaccinated, 179 (98.4%) tested positive by Beckman, 181 (99.5%) by Sienna-Clarity LFA, and 181 (99.5%) by MSD for anti-RBD antibody. Among 22 participants who self-reported COVID infection and were not vaccinated, 10 (45.5%) tested positive by Beckman immunoassay, 12 (54.5%) by Sienna-Clarity, and 21 (95.4%) by MSD for anti-RBD antibody. Among 789 participants who self-reported no-COVID infection and were vaccinated, 721 (91.4%) tested positive by Beckman, 735 (93.2%) by Sienna-Clarity, and 778 (98.6%) by MSD for anti-RBD antibody (Supplementary Table 34).

Table 4. Cohort characteristics and serological positive results by different assays

Cohort			IgG (RBD from Spike Protein)			IgG (Nucleocapsid Protein)	
Infection	Vaccine	n	CLIA (Beckman)	LFA (Sienna-Clarity)	MSD (MSD)	ELISA (Bio-Rad)	MSD (MSD)
YES	YES[A]	180	177 (98.3%)	179 (99.4%)	179 (99.4%)	101 (56.1%)	69 (38.3%)
	YES[B]	2	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)
	NO	22	10 (45.5%)	12 (54.5%)	21 (95.4%)	13 (59.1%)	9 (40.9%)
	NA	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
NO	YES[A]	779	719 (92.3%)	732 (94.0%)	770 (98.8%)	70 (8.9%)	73 (9.4%)
	YES[B]	10	2 (20%)	3 (30%)	8 (80%)	3 (30%)	3 (30%)
	NO	60	21 (35%)	19 (31.7%)	41 (68.3%)	20 (33.3%)	14 (23.3%)
	NA	1	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)
NA	NA	2	2 (100%)	2 (100%)	2 (100%)	0 (0 %)	0 (0%)
Total	1064		938 (88.2%)	954 (89.7%)	1032 (97%)	210 (19.7%)	171 (16.1 %)

[A]Pfizer/BioNTech, Moderna, Janssen, AstraZeneca, Covishield; [B]Sinopharm, Sinovac, Covaxin

SARS-CoV-2 NC antibodies

Overall, the seroprevalence for total anti-NC was 19.7% by Bio-Rad and 16.1% for anti-NC IgG by MSD, but 91.6 % by Megna. We excluded results from the latter due to high false-positive results (91.6%) (Table 2) since the seroprevalence was estimated at 34.2% in September 2021 from the nationwide commercial lab in Arizona based on the CDC website [4].

Among 205 participants who self-reported COVID infection regardless of vaccination status, 117 (57.1%) by Bio-Rad and 81 (39.5%) by MSD tested positive for anti-NC antibody levels (Supplementary Table 34). Interestingly, almost 80% (n=840) of the participants reported no known history of infection regardless of vaccine status (excluding 10 participants who received attenuated parasite vaccines*[B]). However, 10.7% (n=20+70=90) and 10.4% (n=73+14=87) tested positive for anti-nucleocapsid antibodies by the ELISA and the MSD assay without recalling at least one SAS-CoV-2 infection (Supplementary Table 34), presumably representing occult infections.

Demographic variables and seroconversion:

Seroconversion is the transition from the point of viral infection and/or vaccination to when antibodies of the virus become present in the blood. We performed sub-group analysis and looked for any association between seroconversion rates in ASU community and factors such as race, gender, age, employment status and vaccine types using linear and logistic regressions. In case of race, all other races except whites and Asians are grouped into one due to small sample numbers. Regression analysisThe result indicates that there are no significant differences between different races, age groups, gender, and employment status for their ability to produce anti-RBD antibodies upon self-reported vaccination or anti-NC antibodies upon self-reported exposures to SARS-CoV-2 virus (supplemental table 4). As expected, people who received mRNA vaccines had significantly more seroconversion compared to other vaccines (supplemental table 42). There are no significant differences in the rate of anti-RBD antibody decay among these groups (supplemental table 53). Participants who received mRNA vaccines (Pfizer or Moderna) had significantly higher seroconversion rate and slower decay compared to other vaccine types (supplemental table 4 and 5).

Comparison of assays performances

The Venn diagrams show the overlapping distribution of positive results for each assay. For seropositive responses to the RBD of the spike protein, 926 specimens were positive by all three of Sienna-Clarity, Beckman, and MSD, whereas 75 specimens were positive only by MSD (Figure 12A). Based on the same sample population, the percentage of positive results for all three assays for anti-RBD IgG were comparable (90%, 88%, and 97% respectively; Figure 12). However, only Beckman and MSD provided the antibody levels provided a quantitative number which allowed us to track

antibody levels post vaccination and monitor how long immunity persisted. ~~In addition, Figure 1C~~3A showed the correlation of the values of anti-RBD IgG between two assays. The anti-RBD antibody results by Beckman correlated strongly with the results by MSD (Figure 1C; r=0.79).

For seropositive NC, 130 specimens were positive by both Bio-Rad and MSD, whereas 80 specimens were positive only by Bio-Rad and 39 specimens by MSD (Figure 1~~2~~B). The correlation of the values of anti-NC antibody level between Bio-Rad and MSD was weak (r=0.34) (Figure 1D~~3B~~).

Anti-RBD IgG antibody levels after vaccination

Anti-SARS-CoV-2 antibody persistence in the first six months after COVID vaccination decreased over time [19-21]. Here, we examined the relationship between the anti-RBD antibody titers of participants who received COVID vaccines and the number of days after vaccination using linear regression and summarized in Figure 24. As indicated in Figure 24, antibody titers varied widely, but there was clear trend towards lower titers over time. All vaccines have the same trend; we only report Modern and Pfizer in Figure 24; the other vaccines are reported in supplementary Figure 2~~1~~. Participants who received 2 doses of Moderna vs. Pfizer trended towards higher antibody titers, which lasted longer, although these results were not statistically separable.

RBD Antibody responses following vaccination/infection

Participants were first classified into different groups based on their vaccine and infection status (vaccine only, previous COVID infection only, and both) and then further categorized them based on the time between their most recent vaccination/infection date and the collection date (0-3, 4-6, ≥7 months). In each group, the median level of anti-RBD antibody levels was higher in the subgroups of vaccinated participants with COVID infection than those with vaccination or infection only. In every group, the lowest median anti-RBD antibody level was detected in the participants who were never vaccinated. There were no samples in the group of participants with infection after 4-6 months. Although anti-RBD antibody levels declined over time for all groups, median antibody levels in both vaccinated and infected or vaccination-only groups remained above the cut-off 7 months after either infection and/or vaccination, whereas median antibody levels in the infection only group dropped below the cut-off by 7 months post infection (Figure 3A~~5~~).

Increased anti-NC IgG antibody levels after infection

We also analyzed the antibody response to the SARS-CoV-2 NC protein in previously infected participants. However, the EUA-approved ELISA for NC antibodies that we used (Bio-Rad) is only a qualitative assay. To understand the trend of NC antibody levels post-infection, the MSD (Meso Scale Discovery) immunoassay from Meso Scale Diagnostics was applied, which uses ELISA-based quantitative detection. This assay was already verified with clinical samples, even though it is not an EUA-approved

assay. Participants were categorized into different groups (0-1, 2-3, 4-5, 6-7, 8-9, 10-11, 12-13, 14-15, >15 months) depending on the interval between their infection date and collection date. Like RBD antibody levels, the NC antibody levels decreased over time, dropping somewhat faster than the ant-RBD antibodies in these data (Figure 3B & Supplementary Figure 3).

Increased anti-RBD IgG levels after breakthrough infection

Next, we investigated whether breakthrough COVID was associated with improved immune response. Participants were classified into three groups (breakthrough infection, hybrid immunity which is the participant who received vaccination after SARS-CoV-2 infection, and vaccine only). We had 645 fully vaccinated individuals, 19 individuals with 2 doses of vaccine after COVID infection (hybrid immunity), and 12 fully vaccinated individuals with breakthrough infection. Anti-RBD IgG values were significantly increased in both breakthrough and hybrid immune groups compared to vaccine only. In addition, the breakthrough infection group had significantly higher antibody levels compared to the hybrid immunity group, showing an association between breakthrough and enhanced immune response (Figure 3CC~~6~~).

~~**Increased anti-NC IgG antibody levels after infection**~~

~~We also analyzed the antibody response to the SARS-CoV-2 NC protein in previously infected participants. However, the EUA-approved ELISA for NC antibodies that we used (Bio-Rad) is only a qualitative assay. To understand the trend of NC antibody levels post-infection, the MSD (Meso Scale Discovery) immunoassay from Meso Scale Diagnostics was applied, which uses ELISA-based quantitative detection. This assay was already verified with clinical samples, even though it is not an EUA-approved assay. Participants were categorized into different groups (0-1, 2-3, 4-5, 6-7, 8-9, 10-11, 12-13, 14-15, >15 months) depending on the interval between their infection date and collection date. Like RBD antibody levels, the NC antibody levels decreased over time, dropping somewhat faster than the ant-RBD antibodies in these data (Figure 3B7 & Supplementary Figure 32).~~

Discussion

Estimating the cumulative proportion of the population infected with SARS-CoV-2 is crucial for effective planning and targeting public health responses during future pandemic. Understanding the current status of the pandemic and assessing the susceptibility of different ~~population~~populations and their behavior is also vital ~~during pandemic is also important~~ for implementing any policy changes towards mitigating the spread of the virus. Since the beginning of SARS-CoV-2 pandemic, CDC relied on commercial laboratories to gather ~~for~~ nationwide seroprevalence data [22 23]. These survey, along with ~~and~~ other representative studies, provided real-time estimate of proportion of individuals exposed to SARS-CoV-2, at least once before the sampling

(see: https://covid19serohub.nih.gov/). However, there is a lack of reported seroprevalence studies from communities like universities, where the student population experiencing different social dynamics compared to the general public.

In the fall of 2021, over 79,000 students returned to campus for in-person classes coincident with a large increase in COVID incidence during the Delta wave in Maricopa County, AZ. We observed only 0.4% (4 positives out of 1064) active COVID positivity based on saliva qPCR on the day of sample collection from the serosurvey study in the ASU community. Notably, those with symptoms were asked not to participate. Although Nasopharyngeal swabs for SARS-CoV-2 gene detection via reverse transcriptase polymerase chain reaction (RT-PCR) testing is considered as gold standard, saliva has been identified as potential alternative [24 25]. We used TaqPath COVID-19 Combo Kit to test for SARS-CoV-2 infection in saliva samples. In a limited cross validation study, we did not see any significant differences between NP swabs and saliva for their ability to detect the presence of SARS-CoV-2 virus using TaqPath COVID-19 Combo Kit among asymptomatic populations. Czumbel et al reported 91% (CI 80-99%) sensitivity for saliva tests and 98% (CI 89-100%) sensitivity for NPS tests among previously confirmed COVID-19 patients [26], and concluded Saliva tests as an alternative to NPS for COVID-19 diagnosis. It is possible that 0.4% positivity that we report here could be an underestimation.

Individual variation in the immune response to vaccination~~Vaccination compliance among the participants was very high~~

In the ASU community, 92% of participants have self-reported to had at least one dose of a COVID-vaccine. By comparison, only 85% of college students in the U.S. enrolled in spring or fall 2022 were vaccinated based on a nationally representative survey by the American College Health Association [27]. As of September 07, 2021, two weeks before this study date, only 58% of Arizonan's received at least one dose of COVID-19 vaccine [28]. We believe, AUS's proactive communications to parents and students helped with increased rate of vaccination. Most of the vaccinated participants at ASU received the Moderna and Pfizer mRNA vaccines that have shown great effectiveness after the second dose. However, as previously noted, the antibodies produced by Pfizer's COVID-19 vaccine decline faster than those produced by the Moderna vaccine after 6 months of vaccination [29]. We observed a similar trend in our study. Based on anti-RBD antibodies levels from Beckman it showed that a higher anti-RBD IgG antibody level lasted longer in the participants who received 2 doses of Moderna compared to those who received 2 doses of Pfizer. This is probably due to the higher amount of RNA in Moderna (Figure 24).

Interestingly, 7 out of 978 participants who self-reported having received a COVID vaccine, tested negative for anti-RBD antibodies by all three assays in our study. Three out of 7 participants were vaccinated for more than 5 months (165, 168, and 216 days) with Pfizer vaccines leading to potential antibody decay based on figure 24. One out of 7 participants only received Pfizer for 7 days and antibody was likely not generated. It is

known that there is substantial variation between individuals in the immune response to vaccination [30]. Other two out of 7 participants received Covaxin for 56 and 80 days and the level of anti-RBD antibodies in Covaxin was significantly lower than other vaccines. Another one out of 7 participants received AstraZeneca for more than 3 months (101 days), showing that Anti-RBD antibody levels from AstraZenecca started to wane after 2 months (Supplementary Figure 21) which was similar to what was previously reported ~~result~~ from other group [31].

Seroconversion was found to be associated with days after the symptoms, increasing severity of the disease and the presence of co-morbidity [32]. The severe/moderate cases of COVID-19 tended to have ~~an~~ earlyier seroconversion ~~as compared to~~than the asymptomatic/mild cases [32]. Children ~~are~~were less likely to have seroconversion than adults despite having similar viral loads [32]. Unlike other previously reported studies [33], in this study race and gender did not show any significant differences in their ability to produce anti-RBD antibodies after receiving primary vaccination regimen or upon exposures to SARS-CoV-2 virus. This could be due to a relatively young cohort of participants in our serosurvey. There was no difference in seroconversion between students and staff suggesting that the similar working environment did not contribute to variations in seroconversion rate.~~was unlikely to produce and differences in seroconversion~~ This is different compared to the seroconversion differences observed in the occupational risk of exposure to SARS-CoV-2 between hospital departments and healthcare workers [34].

The participants were tested negative for NC antibody after 6 months of post infection with COVID.

Serological tests enable detection of past SARS-CoV-2 infection and may detect cases of SARS-CoV-2 infection that were missed by earlier diagnostic tests. It is important to note that the d~~D~~iagnostic accuracy of different serological test can vary~~ries~~ significantly depending on the cohorts of interest (asymptomatic, symptomatic, hospitalized) and the times of sampling post exposure/vaccination [35]. Several studies reported that the initial immune response in asymptomatic individuals is not as strong as in patients with more severe disease [36].

In the ASU community, 19.3% of participants (n=205) self-reported they had previous COVID infection; however, we only found 57% (n=117/205) and 39.5% (n=81/205) of participants from this group tested positive for NC antibody by Bio-Rad and MSD, respectively (Supplementary Table 34). This could be due to antibody decay since their SARS-CoV-2 exposure. The median NC antibody levels fell below the positivity cutoff 6 months after infection, based on our MSD data (Figure 3~~7~~B). Also, by 8 months post infection, 50% of participants from this group had undetectable NC antibodies. This finding was common with other serological studies, where the NC antibodies started to decline after a few months post infection and half the of participants have undetectable NC antibodies by 8 months post infection [37 38]. In September 2021, 14.6% (95% CI; 14% – 15.2%) of Arizona population tested positive for both NC and Spike antibodies,

suggesting <15% exposed to SARS-CoV-2, ~4% less than our study population [39]. We recognized that the time of sampling (time since exposure/vaccination), method of testing (ELISA, CL, ECL, LFA), SARS-CoV-2 antigen(s) used (NC, RBD, Spike) and reference standard used to set cut-off for non-SARS-CoV-2 cases could potentially contribute to slight variations in our estimations of positive and negative cases.

Asymptomatic infections have been widely reported for COVID-19. Increasing evidence of greater asymptomaticity in children and younger adults compared with the elderly [40]~~35~~. Similar results were observed in cases with comorbidities compared to cases with no underlying medical conditions [40]. Among 847 self-reported no previous COVID infection participants (excluding 10 participants who received attenuated parasite vaccines*[B]), 10.6% (n=90) and 10.3% (n=87) tested positive for anti-NC antibody by Bio-Rad and MSD which means these 10% of participants had a COVID infection in the past without realizing it (Supplementary Table 3~~4~~). It could be the~~se~~ participants in our study, most of them are young and with no underlying health conditions, had mild or asymptotic previous COVID-19 infections.

SARS-CoV-2 antibodies and breakthrough infections

SARS-CoV-2 infection induces a robust humoral and cellular immune response [41 42]. Similar to infection, vaccines result in early production of serum IgA, IgM, and IgG antibodies [43 44]. There is substantial immunologic and epidemiologic evidence suggests that the vaccination following infection further increases protection against subsequent illness among those who have been previously infected [45 46]. Neutralizing antibody and memory B cell response elicited by mRNA vaccination following previous exposure with SARS-CoV-2 results in an increased antibody titer compared to individuals who were not previously infected [47-50]. An [43]important ~~main~~ finding of this serological survey was that the participants who had breakthrough infection had higher anti-RBD IgG compared to those who were fully-vaccinated and also had prior infection (Figure 3C~~6~~), which agrees with previous studies [51 52]. Although the number of breakthrough infections reported in this study is small, it was observed that previous COVID-19 infection resulted in the generation of ~~appeared to elicit~~ robust and sustained levels of SARS-CoV-2 antibodies in vaccinated individuals. Considering that the antibody developed by B cells multiply after each exposure through infection or vaccination, these results were expected. First, the highest anti-RBD antibody levels were in the combined vaccination and infection group and most likely represent an accumulation of antibodies produced after each exposure. Second, the anti-RBD antibody level in the infection only group decayed faster than the participants who received vaccines only. The participants here predominantly received the Pfizer and Moderna vaccines, which may be particularly efficient at evoking a durable anti-RBD response. Similar observations were made by several studies~~Dashdor et al~~, that participants who received the Sinopharm vaccine (whole virus) had lower antibody levels compared to Pfizer/Moderna vaccine (spike protein) [53 54]. mRNA vaccine candidate also induces higher cellular immune responses than the recombinant protein vaccine.

Author Contributions MJ, JL, and VM initiated the study and design. VH, BN, PS, TL, and MM developed the design. CH and VM contributed project administration, supervision, and analysis. CH, KT, VB, BB, JK, KN, AM, and VM designed and conducted the experiments. SW and YC performed statistical analysis. CH, VM, JL, MJ, and YC wrote and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Ethical approval The study was approved by ASU's institutional Review Board (IRB)(STUDY00014505).

Participant Consent All participants are 18+ years old and consented to participating in the study and were willing to provide their samples for the research.

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~~**Figure 1. The timeline between the outbreak of the COVID-19 pandemic and serosurvey at ASU.** In response to COVID, ASU rotated from in-person to remote to hybrid learning several times during the pandemic depending on the prevalence of infection in the community.~~

Figure 12. Comparison ~~and correlation~~ of assays ~~performances~~. (A) Venn diagrams showing overlap of positive results of ~~(A)~~ RBD ~~of Spike~~ and (B) Nucleocapsid from different assays. (C) Correlation between the ~~value of~~ anti-RBD IgG by Beckman and the MSD assays. (D) Correlation between ~~the value of total~~ anti-NC by Bio-Rad assay and ~~the value of anti-NC IgG by the~~ MSD assays. A red dotted line indicates~~d~~ the cut-off line where ~~. All test~~test values equal ~~to~~ or greater than this line are~~is~~ considered positive.

~~**Figure 3. Correlations between antibody results by different assays.** (A) Correlation between the value of anti-RBD IgG by Beckman and the MSD assay. (B) Correlation between the value of total anti-NC by Bio-Rad assay and the value of anti-NC IgG by the MSD assay. A red dotted line indicated the cut-off line. All test values equal to or greater than this line is considered positive.~~

Figure 24. Anti-RBD antibody decay post-vaccination. The antibody level is determined by the Beckman semi-quantitative immunoassay. The linear regression of different vaccines to estimate vaccine decay.

Figure 35. Antibody r~~R~~esponse in p~~P~~articipants with or without p~~P~~revious COVID i~~I~~nfection, v~~V~~accination, or Both: ~~Insights into Breakthrough Infection, Hybrid Immunity, and Vaccine Response.~~ (A) Anti-RBD antibodies measured using Beckman immunoassay in participants who had previous COVID infection or COVID vaccines or both. Participants were categorized by the vaccine or COVID infection and time interval from vaccination/infection to blood ~~sample~~ collection. ~~they got and the number of months between their vaccination/infection and their blood sample collection. Anti-RBD IgG level is measured by Beckman immunoassay.~~ **(B)** Anti-nucleocapsid antibodies measured using MSD in participants who had previous COVID infection. Participants were categorized by the COVID infection and time interval from infection to blood ~~sample~~ collection. **(C)** Anti-RBD antibodies after breakthrough infection, hybrid immunity, and vaccine only. Participants were categorized based on the order and ~~approximate~~ the time ~~scale~~ of COVID infection and vaccination for each group. The blue

bottle indicates ~~a dose of~~vaccination ~~vaccine~~, the virus indicates natural infection based on participant self-reporting, and the red vial indicates blood collection. Anti-RBD IgG level is measured by Beckman immunoassay. Cut-off ~~defined~~is defined by the~~per~~ manufacturer. *P value is calculated by the Mann-Whitney test

~~**Figure 6. Anti-RBD antibodies after breakthrough infection, hybrid immunity, and vaccine only.** (A) Participants were categorized based on the order and approximate time scale of COVID infection and vaccination for each group. The blue bottle indicates a dose of vaccine, the virus indicates natural infection with SARS-CoV-2 based on the participant's self-reported, and the red vial indicates blood collection. (B) Anti-RBD IgG level is measured by Beckman immunoassay *P value is calculated by the Mann-Whitney test~~

~~**Figure 7. Anti-nucleocapsid antibodies after COVID infection.** Participants were categorized by the COVID infection they got and the number of months between their infection and their blood sample collection. Anti- nucleocapsid IgG levels were measured by ELISA. *P value is calculated by the Mann-Whitney test~~

Commented [Y(C3]: I cannot find * in the figure. If none of them are significant, I suggest to remove this sentence.

Commented [CWH4R4]: Thank you! Removed it

Biodesign Institute
Arizona State University

June 1, 2023

To,

Shona Reeves
Research Editor
BMJ Open

Dear Dr. Reeves,

Reg: bmjopen-2023-072627 – Response to reviewers' comments

Thank you for giving me the opportunity to submit a revised draft of our manuscript titled "Serosurvey to estimate SARS-CoV-2 infection and antibody seroprevalence at a large public university" to BMJ Open. We appreciate the time and effort that you and the reviewers dedicated to providing your valuable feedback on our manuscript. We have been able to incorporate changes to reflect all suggestions provided by the editor and both reviewers. We extended our analysis to include regression analysis to identify any association between seroconversion and the factors ethnicity, vaccine dosage and type, age, and sex.
I also reduced the total number of figures and table to 5 in total as outlined in the Information for Authors.
I submitted two versions of the same manuscript.

1. A clean copy (without tracked or highlighted changes) of our revised article.
2. Edited version of our original article, including edits to address reviewers' comments. Changes have been highlighted using a track change function, in blue-colored text.

Here is a point-by-point response to the editorial and reviewers' comments and concerns:

Response to editorial comments:

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Comment 1: *Please revise the title to indicate the research question, setting, and study design. This is the preferred format for the journal*.
Response: Thanks for the suggestion. We changed the title to match the preferred format for the journal. (See page 1, title)

Comment 2: *Please revise the 'Strengths and limitations' section of your manuscript (after the abstract). This section should contain up to five short bullet points, no longer than one sentence each, that relate specifically to the methods. The novelty, aims, results or expected impact of the study should not be summarised here*.
Response: We agree with the comment and addressed it by revising the strength and limitation section. (See page 2)

Comment 2: *Please ensure your Introduction section ends in a clear research question.*
Response: We agree with the comment. Changed the last paragraph of the introduction with a clear research question that was answered in this manuscript (See page 4, last paragraph).

Response to Reviewers' comments:

Reviewer: 1

Comment 1: *It is important to note that the antibody detection tests used in this study were both semi-quantitative and quantitative, which may pose a challenge when interpreting the results.*
Response: Agree with the reviewer that the quantitative and semi-quantitative nature of the data may pose a challenge. We addressed this by adding additional details in serology testing within the methods section (see page 5, paragraph 4 and page 6, paragraph 2).

Comment 2: *In addition, there may be other factors that could impact the seroconversion rates in the ASU community, such as ethnicity, vaccine dosage and type, age, and sex, among others. A regression model could help to identify these factors and determine their association with seroconversion rates. It would be valuable to explore this possibility in future studies to better understand the complex dynamics of COVID-19 immunity in the ASU community and beyond.*
Response: We agree with the reviewers' suggestion. We did not see any significant differences in seroconversion by race, gender, and age. In the case of race, we grouped all other races except whites and Asians into one group due to the small sample numbers. We added detailed descriptions in the results (page 13, paragraph 3) and

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discussion (page 16, last paragraph) sections of the manuscript. We also added a statistical analysis section in methods (page 7, paragraph 2)

Reviewer: 2

Comment 1: *Results section: increased anti-RBD IgG levels after breakthrough infection: in the discussion this result is framed as a main finding however it is based on low numbers (12 fully vaccinated individuals with breakthrough infection. Indeed, it is an interesting result, but it should be mentioned that the low number is a limitation of this study and may compromise the strongly formulated statement*.
Response: Agree with the reviewer and revised our statements and included a low sample number in our discussion (page 17, last paragraph, and page 18, first paragraph) and have included it as a part of our study's limitations.

Comment 2: *Discussion section: A meta-analysis showed that saliva qPCR had a sensitivity of 91% compared to the gold standard of PCR detection of the virus in nasopharyngeal swabs. (Czumbel et al., 2020; Front. Med.) Could this at least explain part of the low active COVID positivity rate based on saliva qPCR.*
Response: Agree with the reviewer. Although we did not see a significant difference in TaqPath COVID-19 combo test performance between saliva and NPS samples among the asymptomatic population, other studies have reported seeing some differences. We acknowledge that in our revised discussion (page 15, last paragraph).

Comment 3: *Discussion section: the subtitle "vaccination compliance among participants was very high" is not covering the message in the text which is discussing mainly the antibody response to vaccination and different types of vaccination*
Response: We appreciate the reviewer's feedback and have revised the subtitle to "Individual variation in the immune response to vaccination" (Page 16, paragraph 2 title).

Comment 4: *The two issues at the end of the discussion section are rather a presentation of results. There is no comparison between these findings and results in other studies.*
The discussion section lacks a discussion about the impact of the study results on future measures to be taken. The limitations of the study are not mentioned nor discussed.
Response: This was an oversight on our part, and we increased the discussion about the impact of the study results in discussion sections (page 17 and page 18).

Comment 5: *Please define Au/mL (cfr fig 4-7)*

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Response: Thank you for the suggestion. We have now defined Au/mL in the methods section (page 6, paragraph #1 and 6).

Sincerely,

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Biodesign Center for Personalized Diagnostics

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