

Use of Rapid COVID-19 Antibody Testing to Evaluate Relative Risk of Infection in Campus Versus Non-campus Residents at a Government Institution in Saudi Arabia: a Cohort Study

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Research

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Abstract

Background: The World Health Organization confirmed in January 2020 that SARS-CoV-2 has become a pandemic infection. The first case in Saudi Arabia was reported on March 2, 2020. The Saudi Ministry of Health has authorized the use of anti-SARS-CoV-2 immunoglobulin M/immunoglobulin G (IgM/IgG) antibody testing, but serological test evaluations are still ongoing.

Methods: The primary study aim was to determine whether living on a government institution campus, thus limiting contact with the general public, protects against SARS-CoV-2 infection. A study population of 763 employees of the King Abdulaziz City for Science and Technology (KACST) in Saudi Arabia and their family members were asked about their age, nationality, residency on or off the KACST campus, chronic conditions, previous COVID-19 symptoms, exposure to infected individuals, and COVID-19 PCR test results. After informed consent was obtained, the VivaDiag™ COVID-19 IgM/IgG Rapid Test was administered. Statistical analysis was conducted of Pearson correlation coefficients for, and generalized linear regression model fitting for predictive ability of, several independent variables versus IgG status.

Results: While the study population was skewed towards male, Saudi nationality, and younger individuals, the age distribution was similar between on-campus residents and off-campus residents. Of the 763 study individuals 91.1% were non-campus residents and 8.9% were campus residents.

Discussion: As expected, being IgG+ strongly positively correlated with having the COVID-19 symptom of loss of smell ($r = 0.417052483$). On-campus residency weakly or somewhat correlated with being IgG positive (IgG+; $r = 0.187990064$) or IgM positive (IgM+; $r = 0.242302626$), indicating that residing on campus actually increased the risk of SARS-CoV-2 infection. Consistent with this, residency status was highly statistically significantly predictive ($p = 0.00002$) of IgG status, second only to contact with a COVID19 infected individual ($p = 0.00000$), and living on campus increased the likelihood of being IgG+. Blood type ($p = 0.01069$), loss of sense of smell ($p = 0.01079$); hypertension ($p = 0.01871$), nationality ($p = 0.02324$), and PCR test status ($p = 0.04243$) also statistically significantly predicted IgG status.

Conclusions: Contrary to the hypothesis, living on campus actually increased the risk of testing positive for IgG antibodies against SARS-CoV-2 infection.

Introduction

In January 2020, the World Health Organization confirmed that SARS-CoV-2 has become a pandemic infection^[1]. It is highly infectious and spreads rapidly^[2] by transmission primarily through respiratory droplets emitted from an infected person during a cough or sneeze. The resulting disease, COVID-19, has mild-to-moderate symptoms in most individuals, but can cause severe respiratory distress and death in some, particularly those with advanced age or other medical co-morbidities.

The first case was confirmed in Saudi Arabia on March 2, 2020^[3]. Since then, 367,267 confirmed cases have been identified in the country, with 6,366 deaths^[4]. This makes detection and tracking of infected

individuals vital to disease control and prevention efforts. Countries that were hit early by the virus, such as China, have used antibody assays and other tests for diagnosis and are building a more refined picture of the scale of community infection and of levels of acquired immunity in exposed populations. The Saudi Ministry of Health has authorized use of serological tests for anti-SARS-CoV-2 immunoglobulin M/immunoglobulin G (IgM/IgG) antibodies, but testing is not yet underway, and studies evaluating and comparing the various available serological tests are still ongoing.

The Kingdom of Saudi Arabia has begun to validate, in Ministry of Health hospitals, VivaDiag™ COVID-19 IgM/IgG Rapid Test, which the manufacturer reports to have a specificity of 100% and sensitivity of 97.1–100%. This test is also under trial by the Saudi Food and Drug Administration for more widespread population testing, in the hope of furthering economic and social recovery by speeding up the reopening of schools and businesses.

Serological tests detect the body's humoral immune response to viral infection, but do not detect the virus itself, so reverse-transcription polymerase chain reaction (RT-PCR) tests that specifically detect viral RNA are required^[5]. However, RT-PCR testing materials can be costly and difficult to obtain in needed quantities, especially given the global demand. Therefore, serological tests can be helpful, particularly for large population screening to identify individuals who need to undergo the RT-PCR test, or those who have recovered from infection but still maintain serum antibodies to the virus^[6] and thus may be able to donate convalescent plasma for possible treatment of seriously ill patients.

Nonetheless, serological SARS-CoV-2 tests may produce false negatives^[2]. While anti-SARS-CoV-2 IgG antibodies are generally detectable in blood several days after initial infection, they may not be detectable in early infection, before full immune response occurs^[7]. Additionally, the persistence of antibodies post-infection is not well characterized; some evidence suggests that the antibody response may fade quickly during recovery^[8]. Furthermore, it is not fully understood whether the presence of anti-SARS-CoV-2 IgM/IgG antibodies can prevent re-infection or whether their detectable absence is a marker for susceptibility. Moreover, there may be important differences between the IgG and IgM responses^[2]. Further study is required to understand when the antibody response starts, how long antibodies persist following recovery, and if the presence of antibodies confers immunity. It is also important to determine whether serological assays are sensitive enough to be used to differentiate high risk from low risk populations.

Therefore, an IgM/IgG Rapid Test was used to compare the levels of anti-SARS-CoV-2 IgG and IgM antibodies in two specific populations, one with a putative higher likelihood of exposure to the virus due to repeated interaction with the general population and the other more secluded and less exposed, to determine whether supposed seclusion is actually protective, and to ascertain the epidemiological characteristics of SARS-CoV-2 exposure in both groups. Thus, this study examined whether living in a mostly secluded environment, such as on a government institution campus, with limited contact with the outside world, protects against infection by SARS-CoV-2. The hypothesis is that the test will demonstrate,

based on antiviral IgM/IgG status, a difference in viral prevalence between the two populations, and will indicate that limiting contact with the general population reduces SARS-CoV-2 infection risk.

Materials And Methods

Subject Recruitment and Sampling:

This cohort study compared the prevalence of IgG and IgM antibodies against the SARS-CoV-2 virus in two populations: 1) individuals who live on the campus of King Abdul-Aziz City for Science and Technology (KACST) in Riyadh, Saudi Arabia, including doing daily activities (shopping, dining, etc.) on campus and 2) KACST employees who work on campus but live off campus, and who therefore must interact with the general population on a regular basis. There were no exclusions based on age, gender, or nationality; however, individuals who were symptomatic at the time of interview were excluded from this study, as were family members of the latter cohort.

Over a four-month period, finger-stick blood samples were taken from a total of 763 individuals, consisting of 68 campus residents and 695 non-campus residents, and analyzed using the VivaDiag™ COVID-19 IgM/IgG Rapid Test. Prior to sample collection, each subject was interviewed to collect demographic information and medical history regarding symptoms of COVID-19 within the past 3 months, as well as pre-existing diabetes, hypertension, asthma, or obesity. A clinical examination of blood pressure, body temperature, and heart rate was also conducted by a licensed nurse.

Institutional Review Board approval was obtained from King Fahad Medical City (KFMC). All subjects signed informed consent forms and were privately notified of their test results. The collected study data were de-identified to protect the subjects' medical privacy.

Testing for Anti-SARS-CoV-2 Antibodies:

The antibody test used in this study, the VivaDiag™ COVID-19 IgM/IgG Rapid Test, is a single-use rapid diagnostic test for the qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human blood. The test devices include a conjugate pad, containing recombinant SARS-CoV-2 antigen labeled with colloidal gold and a quality control antibody gold marker, and a nitrocellulose membrane containing IgM and IgG detection lines as well as a quality control line (C line). Any IgM or IgG antibodies against SARS-CoV-2 present in a blood sample added to the test device sample well will bind to the viral antigen labeled with colloidal gold. The sample will then flow to the IgM and then IgG lines, which are coated, respectively, with mouse anti-human IgM and IgG monoclonal antibodies that will bind the patient antibody. The resulting formation of a "sandwich complex" will generate a purplish-red color change at the IgM or IgG line, indicating a positive response. A lack of color on either the IgM or IgG line corresponds to the absence of that type of anti-SARS-CoV-2 antibody. The positive control C line should always display the purplish red color; the absence of color in the C line indicates an invalid test and required retest^[9].

Statistical analysis:

Pearson correlation coefficients were calculated (using Microsoft Excel) to determine the degree of correlation between independent variables and IgG or IgM status, between IgG or IgM status and PCR status, and between IgG status and IgM status. JMP software (version 15, SAS) was used for model fitting via generalized linear regression with a binomial distribution forced onto the numerical and nominal data. The software ranked, by statistical significance, the effectiveness of the independent variables as predictors of IgG status and provided a parameter estimate for each independent variable that quantified its contribution to the probability that the subject was IgG negative. Due to the high Pearson correlation coefficient (0.865) between IgG and IgM status, IgM data was removed from the JMP model fitting to avoid co-linearity. Additionally, Firth Bias-Adjusted Estimates were used, since the software indicated a lack of coalescence of the analysis when generalized linear regression was calculated without the adjustment.

Results

Of the 763 individuals recruited into the study, 72.7% were male and 27.3% were female (Fig. 1); in terms of nationality, 86.8% were Saudi and 13.2% were non-Saudi (Fig. 2). Most importantly, 91.1% were non-campus residents and 8.9% were campus residents (Fig. 3). The study subjects ranged from 18 to 82 years of age, with a median age of 35 (Table 1). When categorized into 5 age groups, 54.7% were 31–43 years of age, 27.1% were 18–30 years of age, 15.2% were 44–56 years of age, and 2.6% were 57–69 years of age, with only 0.4% in the 70–82 years of age group (Fig. 4A). The age distributions in the campus resident and non-campus resident cohorts were roughly similar (Fig. 4B-D).

Table 1
Age distribution of study subjects by residence status and total.

Ages	18–30	31–43	44–56	57–69	70–82	Total	Median
Campus	16	27	20	4	1	68	37
Non-Campus	191	390	96	16	2	695	35
Total	207	417	116	20	3	763	35

Of the 763 individuals, 57 (7.5%) were positive for both anti-SARS-CoV-2 IgG and IgM antibodies (Fig. 5); of these, 16 (28.1%) were campus residents, while 41 (71.9%) were non-campus residents (Fig. 6A). Although these individuals were currently asymptomatic, the antibody status indicated that they had recovered recently^[10]. Of the 763 total subjects, 16 (2.1%) were IgM-positive but IgG-negative (Fig. 5), out of which 6 (37.5%) were campus residents and 10 (62.5%) were non-campus residents (Fig. 6A). These patients were also asymptomatic, but likely had been infected more recently than the previous group^[10].

Among all 763 individuals, there was only one (0.1%; Fig. 5), a non-campus resident (Fig. 6A), who was IgG-positive but IgM-negative, indicative of a cleared past infection^[10].

Only 42 of the 763 subjects had previously been confirmed by a PCR-based test to have been infected with SARS-CoV-2. Based on the reported high accuracy of the PCR tests^[11], these subjects were considered to have been Covid-19 positive. Out of these 42 cases, only one (2.4%) was a campus resident, while 41 (97.6%) were non-campus residents. Of note, only 21 (50%) of these 42 PCR-positive patients were also positive by the antibody test, although all of these 21 were positive for both IgG and IgM antibodies against SARS-CoV-2.

Discussion

This is the first large-scale community-based prevalence study for SARS-CoV-2 infection in Saudi Arabia using antibody testing kits. No statistically significant Pearson correlation was found between IgG status and any co-morbidity tested, i.e., hypertension, obesity, diabetes, or asthma, which have been associated with a greater severity of disease and need for hospitalization^{[12][13][14][15]}. Pearson correlation coefficients were also calculated between other independent variables and anti-SARS-CoV-2 IgG or IgM antibody status. There was a weak positive correlation between being a campus resident and being IgG positive (IgG+; $r = 0.187990064$) and a slightly stronger correlation ($r = 0.242302626$) between being a campus resident and being IgM positive (IgM+), which indicates that residing on campus may actually increase the risk of infection with SARS-CoV-2. However, for comparison, there is a stronger positive correlation between being IgG + and having loss of smell ($r = 0.417052483$); thus, loss of smell as a symptom is a reliable indicator of COVID-19 infection, and therefore of having IgG antibodies against the virus. Hence, according to Pearson correlation analysis, living on campus may increase one's risk for COVID-19, but only to a limited extent.

Next, generalized linear regression model fitting was conducted to identify independent variables that could predict the results of testing for anti-SARS-CoV-2 IgG antibody. IgM status was removed from consideration as such an independent variable because a high Pearson correlation coefficient between IgM status and IgG status (0.865) indicated possible high co-linearity, which might have skewed the results. Campus or non-campus residency status was highly statistically significantly predictive ($p = 0.00002$) of IgG status (Fig. 7A) and was second in statistical significance of predictive effectiveness only to contact with another individual who had COVID19 ($p = 0.00000$). This supports a difference in prevalence of IgG + individuals between campus and non-campus residents, as detected by the antibody test, in agreement with the first half of the hypothesis. However, based on the corresponding parameter estimates (Fig. 7B), being a non-campus resident contributed to the probability of being negative for the IgG against SARS-CoV-2, signifying that being a campus resident, rather than being protective, increased the likelihood of being IgG+, contradicting the second part of the hypothesis. In comparison, not being in contact with an individual who had COVID-19 increased the likelihood of being IgG negative (Fig. 7B). Thus, as expected, exposure to individuals with this contagious disease increases the likelihood of being infected and therefore developing antibodies against the virus. Hence, living in the close confines of the

campus may increase the likelihood of exposure to someone who was infected during an off-campus outing.

Other statistically significant predictors of IgG status (Fig. 7A) were blood type ($p = 0.01069$), loss of sense of smell ($p = 0.01079$); hypertension ($p = 0.01871$), nationality (i.e. Saudi or non-Saudi; $p = 0.02324$), and, finally, PCR test result status ($p = 0.04243$). Based on the corresponding parameter estimates (Fig. 7B), having an A+ blood type increased the likelihood of being IgG+; similarly, experiencing the COVID-19 symptom of loss of smell slightly increased the probability of being IgG+. Unexpectedly, the parameter estimates for hypertension indicated that having this chronic condition contributed to the probability of being IgG negative (Fig. 7B). It is possible that some individuals have hypertension because of reduced expression of the ACE2 receptor, which converts angiotensin II, a vasoconstrictive peptide that drives up blood pressure, into angiotensin, a vasodilator that reduces blood pressure^{[16][17][18]}. Since SARS-CoV-2 infects cells via this receptor, people with reduced ACE2 levels are less likely to be infected by, and therefore are more likely to be negative for IgG antibodies against, the virus. It is surprising that nationality was predictive of IgG status. The parameter estimates for non-Saudi nationality (Fig. 7B) indicated that non-Saudi individuals were more likely than Saudi individuals to be IgG+. It is possible that non-Saudi individuals were more likely to leave the campus to meet family or friends, dine out, etc., increasing their likelihood of exposure to SARS-CoV-2 and consequent generation of antibodies against the virus. It is also possible that Saudi individuals have genetic differences in the IgG heavy chain such that, while they may express anti-SARS-CoV-2 IgG antibody, the test does not detect it because the IgG constant domain lacks the exact epitope needed for binding of the secondary antibody used for detection. It was highly unexpected that nationality was more statistically significant than PCR status as a predictor of IgG status (Fig. 7A), since PCR status is usually considered the same as SARS-CoV-2 infection status, but this may be due to the very small number of individuals (42) who tested positive by PCR. Based on the parameter estimate (Fig. 7B) for PCR status, being negative by PCR increased the likelihood of being negative for IgG antibodies against SARS-CoV-2, as expected.

Only 42 out of the 763 study subjects (5.5%) were positive for SARS-CoV-2 by PCR. For each of these 42 study subjects, the interval, in days, from PCR confirmation of SARS-CoV-2 infection (which could be considered the date of COVID-19 onset) to antibody test administration was calculated. This interval may be relevant to the finding that, of these 42 individuals, only 21 were IgG+, which is only a 50% sensitivity of the IgG test. This is much lower than the maximum sensitivity (97.1–100%) of the test as reported by the testing kit manufacturer, but nearly in line with the range of 57.5–72.5% sensitivity, and the mean sensitivity of 65.4%, reported elsewhere^[19]. Nevertheless, in the current study, the lower sensitivity may be partly due to delay in IgG testing, relative to the date of detection of infection by PCR. The Pearson correlation coefficient between the PCR testing to IgG testing interval and the IgG status was -0.244 , indicating that, as the length of time between tests increased, the likelihood that the IgG test would be positive decreased slightly, causing false negative results.

This study had some limitations. The small sample size limits the applicability of the findings to a larger or more global population, as does the fact that most of the study participants were Saudi. Furthermore,

most of the patients who had COVID-19 symptoms did not get tested for SARS-CoV-2 by PCR, hindering the ability to truly compare the IgG and PCR test results and determine if the sensitivity of the IgG test may be higher than 50%.

Conclusion

The analysis of 763 subjects indicated that, while a difference in the prevalence of anti-SARS-CoV-2 IgG positive status was detectable between campus and non-campus residents, contrary to the hypothesis, living on campus was not protective, and, instead, increased the likelihood of being infected by the virus and thus being IgG positive.

List Of Abbreviations

ACE2 = acetylcholinesterase type 2; COVID-19 = Coronavirus disease of 2019; KACST = King Abdulaziz City for Science and Technology; KFMC = King Fahad Medical City; IgM/IgG = Class M/G Immunoglobulin; RT-PCR = reverse transcription polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

Declarations

Ethics approval and consent to participate:

All study subjects provided informed consent and data was depersonalized for analysis and publication. The study design was reviewed and approved by an Internal Review Board (IRB) from the King Fahad Medical City Hospital (IRB log number 20-V66).

Consent for publication:

Since all data was depersonalized prior to publication, even in Supplementary Data, no consent for publication is required.

Availability of data and materials:

All materials used in the study, besides patient samples, are commercially available, and the depersonalized patient data is available for analysis in the Supplementary Data section.

Competing interests:

None of the authors have any conflicts of interest or competing interests to declare.

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Authors' contributions:

FSH was the project manager for the study, wrote the manuscript (as first author) and conducted the statistical analysis of the results. MF and MA conceived of the study and obtained funding for it. AM and MSS helped accelerate the recruitment of study participants. SM, EAS, and SSO bravely volunteered to administer the antibody tests to potentially infected study participants and recorded the results of the tests. MF, AM, and MSS also provided suggestions for improvement of the manuscript.

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Figures

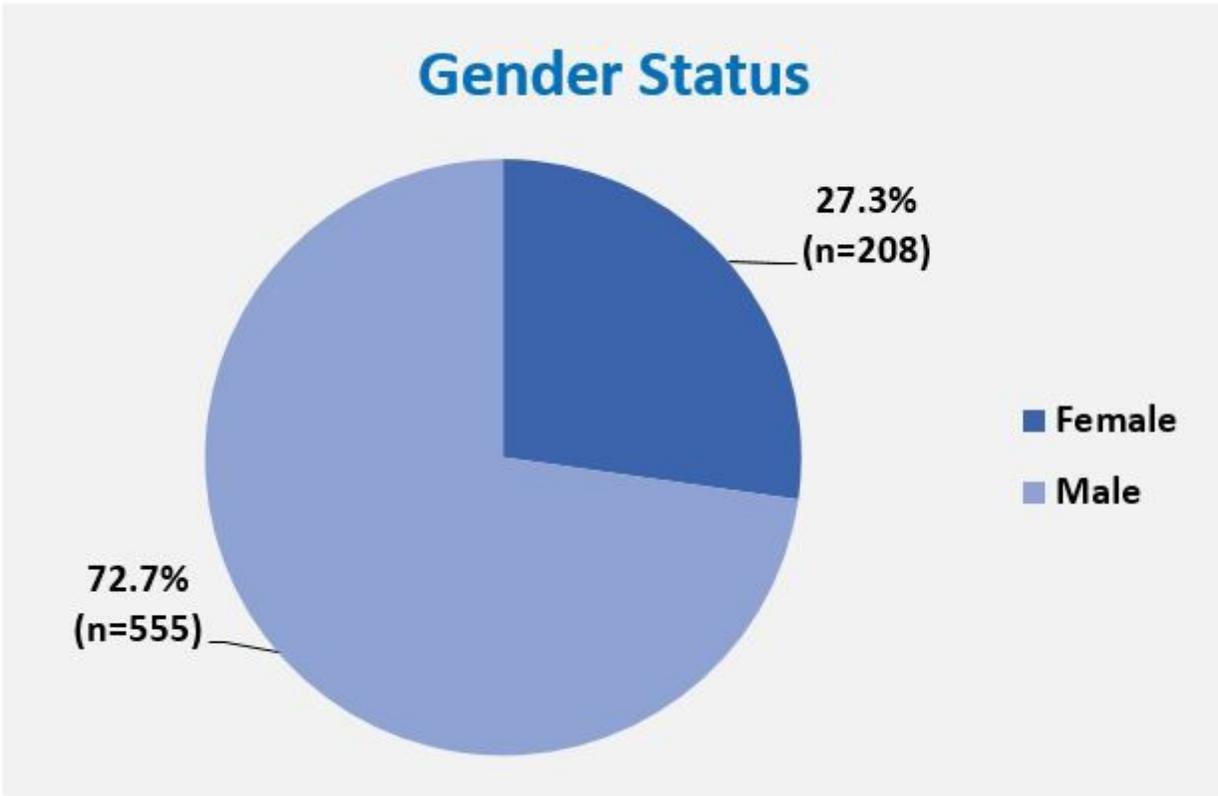


Figure 1

Gender status (assuming a binary gender distribution)

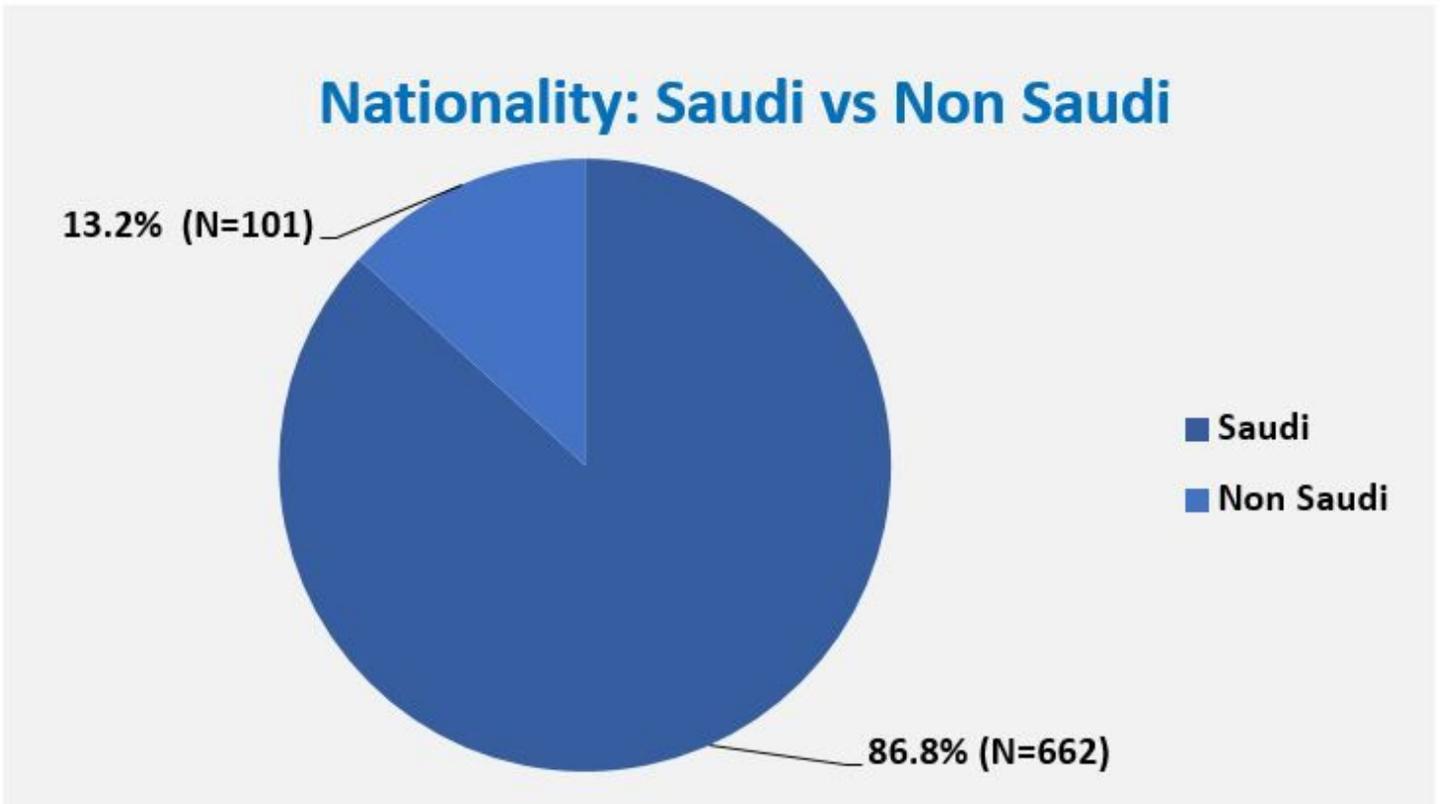


Figure 2

Nationality status

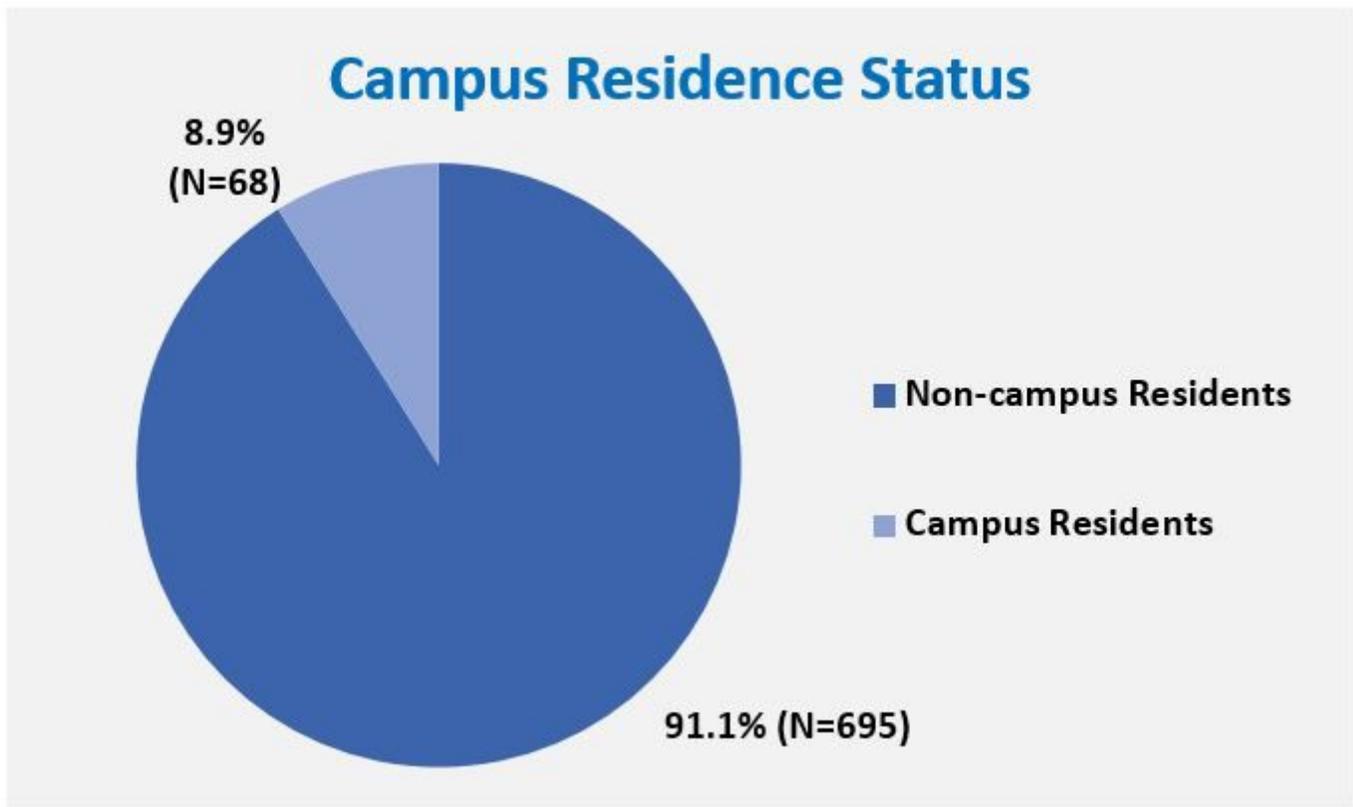


Figure 3

Campus residence status Campus residents were defined as King Abdul-Aziz City for Science and Technology (KACST) employees and/or their family members who lived exclusively on campus, including doing most daily activities (shopping, dining, etc.) on campus. Non-campus residents were defined as KACST employees who lived, shopped, and dined outside of the KACST campus. The family members of non-campus residents were excluded from the study.

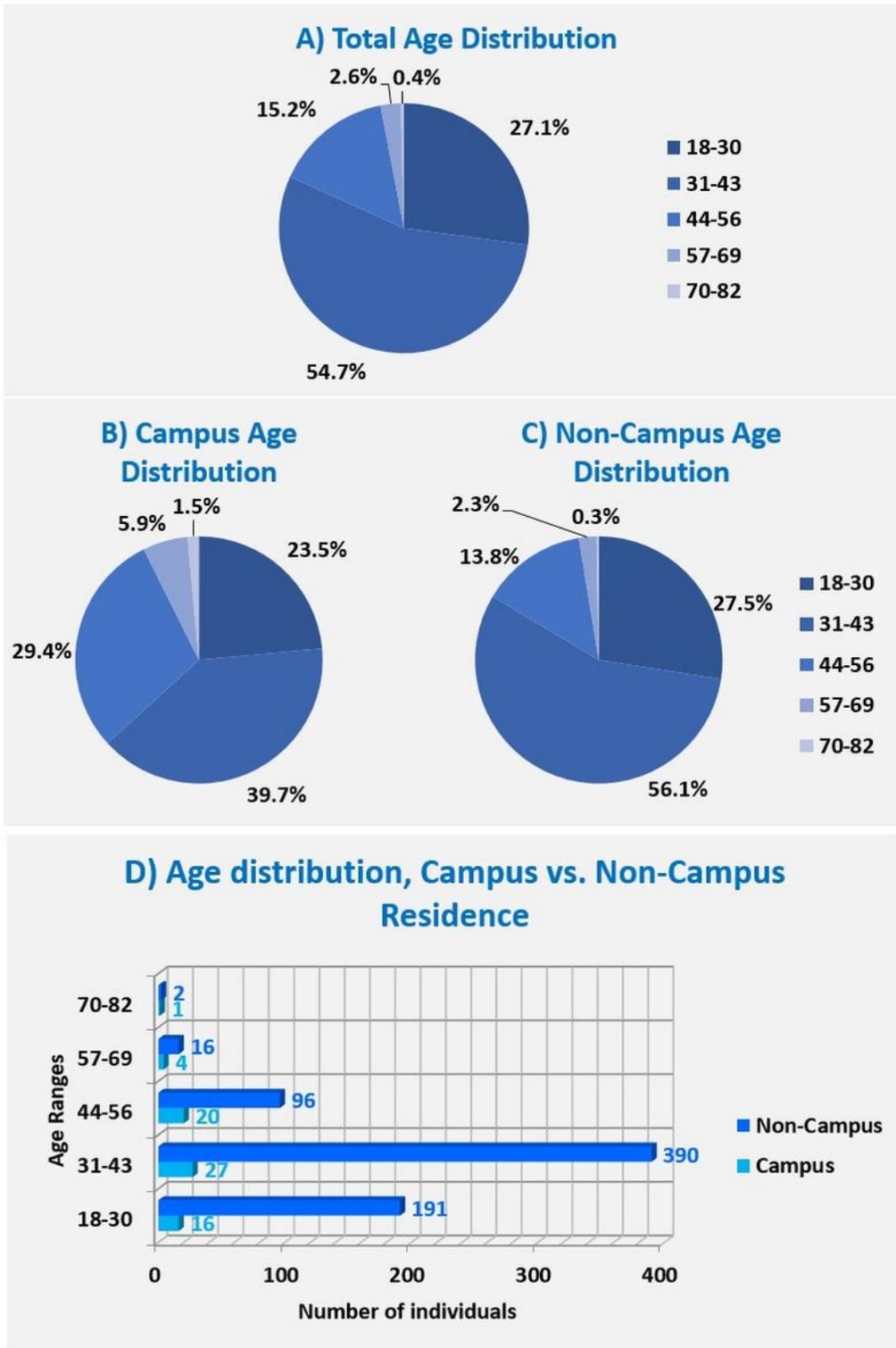


Figure 4

Age Distribution. A) Total age distribution of all study subjects, categorized into 5 groups consisting of 13 year ranges each to account for the overall 65 year age range. B) Age distribution of campus residents only. C) Age distribution of non-campus residents only. D) Comparison of age distributions between campus and non-campus residents.

COVID-19 antibody status	IgG ⁻ , IgM ⁻	IgG ⁻ , IgM ⁺	IgG ⁺ , IgM ⁻	IgG ⁺ , IgM ⁺	Total
Number	689	16	1	57	763

Prevalence Of IgG And IgM Antibodies Against SARS-CoV-2

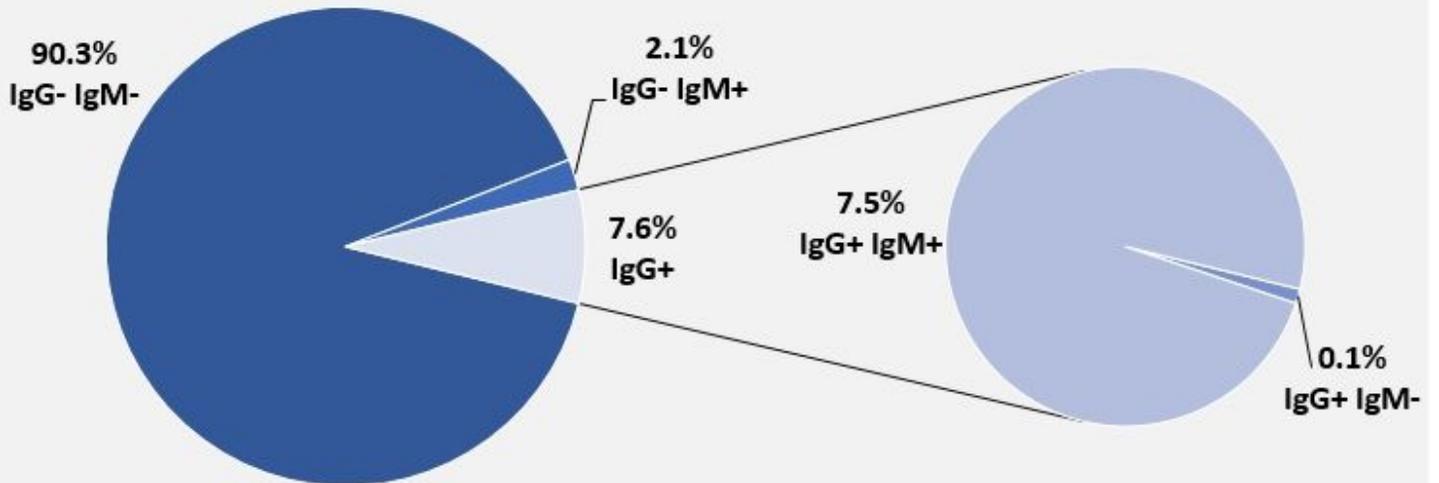
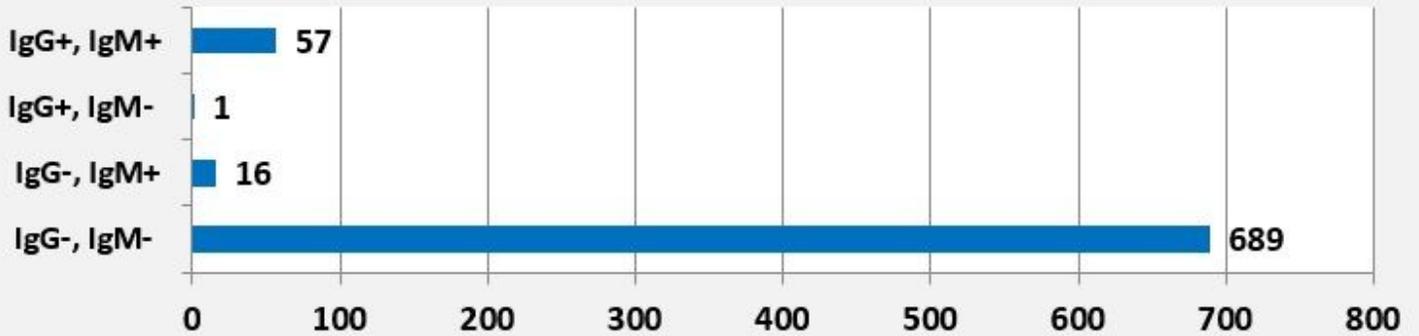
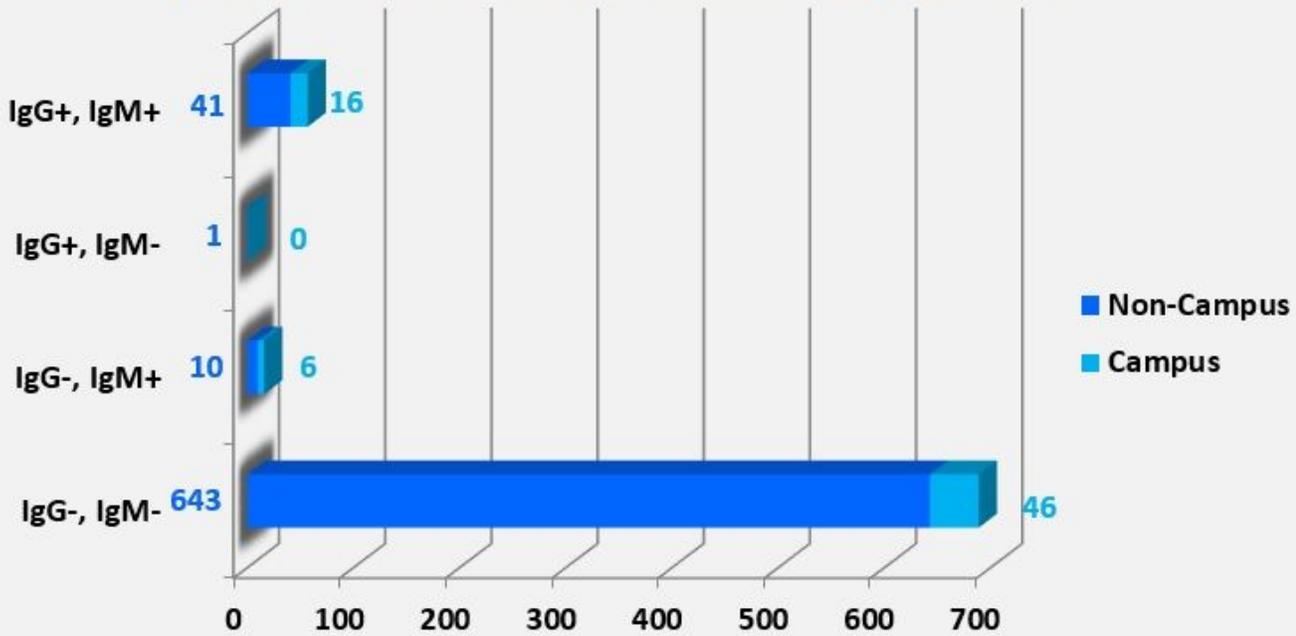


Figure 5

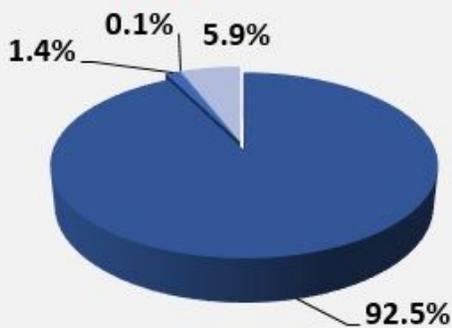
Prevalence of IgG and IgM Antibodies against SARS-CoV-2. Distribution of study subjects into groups based on the status (positive or negative) of test results for IgG and IgM antibodies against SARS-CoV-2, including percentages of IgG negative (bottom left) and IgG positive (bottom right) subgroups.

Residence	IgG ⁻ , IgM ⁻	IgG ⁻ , IgM ⁺	IgG ⁺ , IgM ⁻	IgG ⁺ , IgM ⁺
Non-Campus	643	10	1	41
Campus	46	6	0	16
Total	689	16	1	57

A) Campus vs Non-campus residents IgG/IgM status



B) Non-Campus IgG/IgM status



C) Campus IgG/IgM status

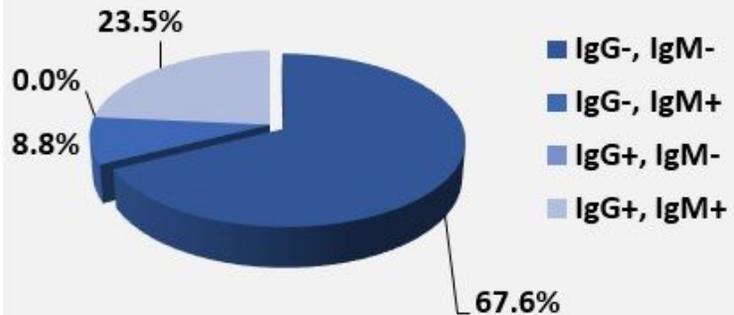
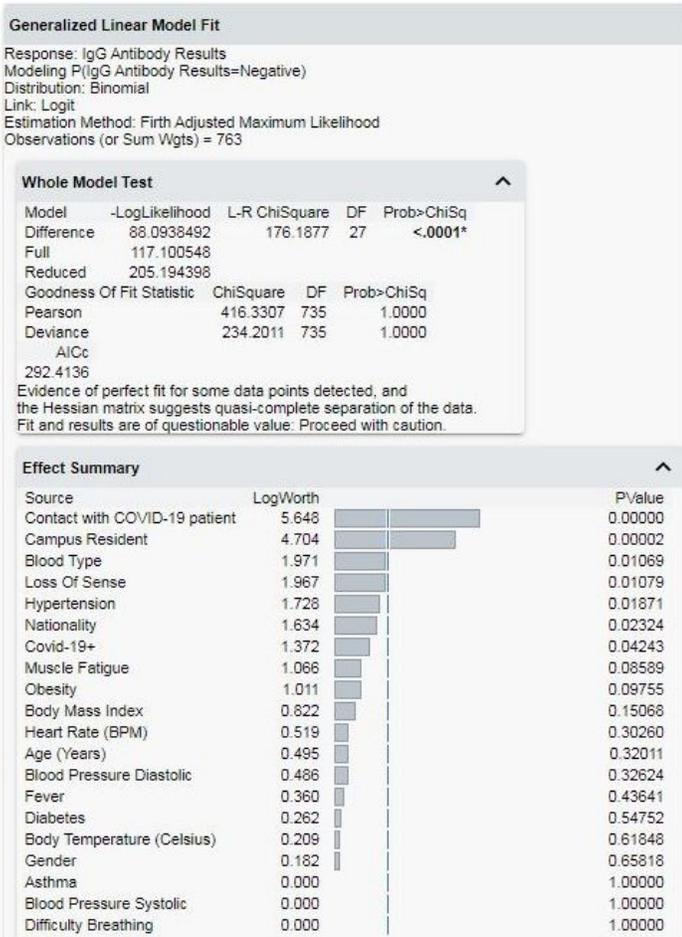


Figure 6

IgM/IgG prevalence in campus versus non-campus residents. Comparisons of distributions, between campus and non-campus residents, of positive or negative status of test results for IgG and IgM antibodies against SARS-CoV-2, among study subjects.

A)



B)

Parameter Estimates

Term	Estimate	Std Error	L-R ChiSquare	Prob>ChiSq
Intercept	11.421875	12.621567	0.9370089	0.3330
Asthma[FALSE]	0.0816006	0.3707789	0	1.0000
Campus Resident[No]	1.0532446	0.2544222	18.210364	<.0001*
Age (Years)	-0.019928	0.0193668	0.9892007	0.3199
Gender[Female]	0.1268664	0.2313591	0.1958622	0.6581
Nationality[Non-Saudi]	-0.523508	0.2216851	5.1508878	0.0232*
Blood Type[A-]	-0.209781	1.1248099	0	1.0000
Blood Type[A+]	-0.849526	0.5485186	4.6662318	0.0308*
Blood Type[AB-]	-2.11576	2.0987609	0	1.0000
Blood Type[AB+]	1.5071296	0.9184765	0	1.0000
Blood Type[B-]	2.8293835	2.129827	3.1338941	0.0767
Blood Type[B+]	-0.716551	0.5782542	2.9345343	0.0867
Blood Type[O-]	-0.705234	0.6224613	2.2472611	0.1339
Body Mass Index[High]	0.1639688	0.3563637	0	1.0000
Body Mass Index[Low]	-0.865575	0.5949848	1.6975794	0.1926
Body Temperature (Celsius)	-0.179208	0.3410459	0.2480699	0.6184
Heart Rate (BPM)	-0.018289	0.0144793	1.0631481	0.3025
Blood Pressure Systolic	-0.002317	0.00179	0	1.0000
Blood Pressure Diastolic	-0.018243	0.0171993	0.9638804	0.3262
Fever[FALSE]	0.2159792	0.2525492	0.6057099	0.4364
Difficulty Breathing[FALSE]	-0.059159	0.3366075	0	1.0000
Loss Of Sense[FALSE]	0.6234106	0.2657791	6.4995892	0.0108*
Muscle Fatigue[FALSE]	0.3992635	0.2350091	2.9496604	0.0859
Hypertension[FALSE]	-0.822935	0.4210387	5.5281554	0.0187*
Obesity[FALSE]	-0.540176	0.3815498	2.7448751	0.0976
Diabetes[FALSE]	0.4194866	0.437906	0.3619105	0.5474
Contact with COVID-19 patient[FALSE]	0.9178543	0.1970515	22.369964	<.0001*
Covid-19+[No]	0.5846347	0.2902042	4.117559	0.0424*

Figure 7

Generalized linear regression model fit, comparing multiple independent variables (but not IgM status) with IgG status, modeling prediction of anti- SARS-CoV-2 IgG negative: A) Effect summary and B) parameter estimate table

Supplementary Files

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- [Datainbrief.xlsx](#)