Antibody reactivity against SARS-CoV-2 in adults from the Vancouver metropolitan area, Canada

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Abstract

Background: Quantifying antibody reactivity against multiple SARS-CoV-2 antigens at the population level may help understand individual differences in COVID-19 severity. Pre-existing low antibody cross-reactivity may be particularly prevalent among childcare providers, including pediatric health care workers (HCW) who may be more exposed to circulating coronaviruses.

Methods: Cross-sectional study that included adults in the Vancouver area in British Columbia (BC), Canada, between May 17 and June 19, 2020. SARS-CoV-2 seroprevalence was ascertained by measuring total SARS-CoV-2 IgG/M/A antibodies against a recombinant spike (S1) protein and adjusted for bias due to false-positive and false-negative test results. A novel, high sensitivity multiplex assay was also used to profile IgG against four SARS-CoV-2 antigens, SARS-CoV and four circulating coronaviruses.

Findings: Among 276 participants (71% HCW), three showed evidence of direct viral exposure, yielding an adjusted seroprevalence of 0.60% [95%CI 0% – 2.71%], with no difference between HCW and non-HCW, or between paediatric and adult HCW. Among the 273 unexposed individuals, 7.3% [95%CI 4.5% – 11.1%], 48.7 [95%CI 42.7% – 54.8%] and 82.4% [95%CI 77.4% – 86.7%] showed antibody reactivity against SARS-CoV-2 RBD, N or Spike proteins, respectively. SARS-CoV-2 reactivity did not significantly correlate with age, sex, did not significantly differ between HCW and non-HCW (prevalence 1.0% vs 1.0%; P=1.00) and between pediatric and adult HCW (0.7% vs 1.6%; P=0.54), and modestly correlated with reactivity to circulating coronaviruses (Spearman rho range: 0.130 to 0.224 for 7 significant (FDR 5%), out of 16 correlations, from 36 correlations tested).

Interpretation: A substantial proportion of individuals showed low, but detectable antibody reactivity against SARS-CoV-2 antigens in this population despite low evidence of direct SARS-CoV-2 exposure.

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Introduction

Coronavirus disease 2019 (COVID-19) was declared a global pandemic on March 11th, 2020 and has resulted in more than 36.3 million cases and 1.06 million deaths worldwide as of October 8, 2020. Although this virus has caused unacceptably high mortality, a majority of cases are asymptomatic (1). Seroconversion occurs within 2-3 weeks in >99% of individuals with COVID-19, with the spike and nucleocapsid proteins eliciting strongest antibody responses (2, 3). However, up to 40% of SARS-CoV-2-unexposed individuals showed T cell reactivity against SARS-CoV-2 antigens, suggesting a high degree of cross-reactivity from a previous exposure to unidentified heterologous antigens (4, 5).

Four seasonal coronaviruses pre-dated COVID-19 and are known to cause up to 30% of upper respiratory tract infections (6). Of these endemic coronaviruses, the beta-coronaviruses HKU1 and OC43 are more structurally similar to SARS-CoV-2, with 40% sequence similarity between their main surface antigen (the spike protein) and that of SARS-CoV-2, whereas the alpha-coronaviruses, NL63 and 229E, are less structurally related (7). Since the SARS-CoV and the current SARS-CoV-2 pandemics, interest in the natural history and antibody levels of circulating coronaviruses across seasons has increased (8, 9). The potential for pre-existing antibody reactivity against SARS-Cov-2 due to circulating coronaviruses has been debated (10-13). A main caveat of previous studies is that the assays used may not have been sensitive enough to detect the low SARS-CoV-2 antigen cross-reactivity in unexposed individuals (14).

Across the world, estimates for SARS-CoV-2 seroprevalence range from 0.26% to 24.4%, with some reports suggesting higher rates in health care workers (HCW) (15, 16). Vancouver, British Columbia (BC), is the third largest metropolitan area in Canada, with a population of 2.5 million people. The province of BC (population 5.017 million) reported its first COVID-19 case on January 25. In BC, the epidemiological peak of the pandemic occurred between the third week of March and late April, with the vast majority of cases occurring in the greater Vancouver area (17). This westernmost Canadian province has done comparatively well in controlling the spreading of SARS-CoV-2 within its territory (17). Indeed, a study from the BC Centre for Disease Control (BC CDC) estimated that only 0.55% of individuals in Vancouver had been directly exposed to SARS-CoV-2 after the first wave. Thus, with its low number of reported COVID-19 cases, and considering that immunity to circulating coronaviruses is only short-lived and seasonal (8, 9), BC, represents an interesting setting to compare antibody reactivity against SARS-CoV-2 arising from a direct exposure to COVID-19 versus the pre-existing SARS-CoV-2 antibody reactivity in unexposed individuals, within the same population and across the same time period.

The main objective of this study was to determine the population-level seroprevalence for SARS-CoV-2, and antibody reactivity against SARS and circulating coronaviruses in unexposed individuals within the greater Vancouver area. Understanding that pre-existing reactivity to circulating coronaviruses could be more prevalent among paediatric HCW (18), a secondary objective of this study was to compare the SARS-CoV-2 antibody reactivity profiles between adult, paediatric HCW and non-HCW.

Methods

Study design: Prospective cross-sectional seroprevalence study after the first pandemic wave in BC.

Participants: Adults over 18 years of age from the greater Vancouver metropolitan area were included if they did not have *active* COVID-19, did not require self-isolation as per BC provincial public measures or had recovered from COVID-19 at least 14 days prior to the study visit and blood sample collection.

Recruitment: At the time this study was initiated, the SARS-CoV-2 seroprevalence in Canada was not known and there were little data around the world. To gain more insight, an invitation email was sent about this study to the following departments at BC Children's Hospital and BC Women's Hospital (together, C&W, the largest pediatric referral hospital centre in BC): pediatric intensive care; pediatric emergency; family medicine; obstetrics and gynecology; pediatric anesthesiology; and pediatric otolaryngology. The affiliated research institute, the BC Children's Hospital Research Institute (BCCHRI), was also contacted email. The email was also sent to hospitalists and the anesthesiology and critical care departments at Surrey Memorial Hospital (SMH), the most active hospital site for pandemic COVID-19 cases in BC. To minimize recruitment bias, all adults who responded to the email and returned their signed consent form were enrolled sequentially and were invited to give a blood sample, without triaging. Blood samples were collected at BCCHRI and SMH between May 17 and June 19, 2020 (2,794 COVID-19 cases had been reported in BC by the end of this enrollment period). Written informed consent was obtained from all participants. The study was approved by the University of British Columbia Children's & Women's Research Ethic Board (H20-01205).

Study size: Since there was very little SARS-CoV-2 seroprevalence data available at the time and none in BC or even Canada, no *a priori* sample size calculation was performed. The recruitment period was therefore defined by convenience over a three-week period of enrolment, in order to obtain baseline data.

Blood sampling: Blood was drawn in gold-top serum separator tube with polymer gel (BD, cat# 367989); after at least 30 minutes of clotting at room temperature, the blood sample was then centrifuged at 1,400 G to obtain serum aliquots that were frozen at -80 °C within four hours of collection.

Multiplex antibody assay: A highly sensitive 10-plex assay (Meso Scale Diagnostics, Gaithersburg, USA) where each antigen is 'spotted' into a single well of a 96-well plate (19) was used to measure antibodies against four SARS-CoV-2 antigens – S-2P native spike protein (20), receptor-binding domain (RBD), N-terminal domain (NTD) and nucleocapsid (N) protein; the SARS-CoV spike protein; and spike proteins from circulating beta- (HKU1, OC43) and alpha- (229E and NL63) coronaviruses. Briefly, after blocking wells with 5% BSA, sera were added at 4 dilutions (1:100, 1:800, 1:25 and 1:10,000) and incubated with shaking for two hours. Sulfo-tag-labelled anti-IgG detection antibody was added to the wells and the electrochemiluminescence signal was read using the MSD Sector 600 instrument. Results are dilution-corrected interpolated values from a standard curve with assigned Arbitrary Units (AU)/mL. Assignment of AU/mL of serum was performed by Meso Scale Diagnostics and is designed such that values are comparable to an International Standard Serum (ISS), and such that in the future, bridging to a WHO International Standard will be possible. In absence of a definable threshold for positive antibody reactivity for SARS-CoV-2 antigens in unexposed individuals, we used the positive/ negative thresholds derived from the ROC using likelihood ratios>10 from a comparison of symptomatic COVID-19 cases versus anonymized sera from healthy adults pre-dating 2019 (19), to estimate the proportion of individuals showing background antibody reactivity against SARS-CoV-2 antigens.

Commercial chemiluminescent (CLIA) antibody assay: Total antibody (IgA, IgG and IgM) against recombinant spike (S1) protein was determined using the VITROS 5600 analyser (Ortho-Clinical Diagnostics, Rochester, New York) according to manufacturer instructions. This is a Health Canada and FDA-licensed qualitative assay with reported performance and in-house validation indicating sensitivities >7 days post onset range between 96% and 100% and specificities from 99% to 100% (21, 22).

Variables: The following information about participants was collected by questionnaire: age, sex, the first three digits of their postal code, whether they were health care workers and the type of profession,

their history of travel outside BC since January 1, 2020, and history of COVID-19 symptoms and testing. SARS-CoV-2-exposed cases were defined by a positive result on the commercial CLIA assay, confirmed by antibody profiling on the multiplex assay. SARS-CoV-2-unexposed cases were defined by a negative result on the commercial CLIA assay, validated using the multiplex assay.

Statistical analyses: The seroprevalence for SARS-CoV-2 was calculated by dividing the number of SARS-CoV-2-exposed (positive) cases by the total number of participants recruited, and adjusted for bias due to false positive and false negative tests using the Greenland method (14). Differences in proportions were calculated using a Fisher exact test, with significance threshold at $P<0.05$. Hierarchical clustering of antibody levels (based on the multiple assay), was performed on log-transformed, z-score normalized serology data, using the complete linkage agglomeration method and Euclidean distance measures. Correlation between antibody levels and metavariables was computed using Spearman correlation, adjusted for multiple testing using the Benjamini-Hochberg false-discovery rate method (FDR 5%). Analyses were conducted in R version 4.0.2, R Studio version 3.6.2 and GraphPad Prism version 8.4.

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Results

Study population: In total, 276 participants were recruited. Their demographic characteristics are shown in **Table 1**, with their area of residence geographically mapped in **Supplemental Figure 1**. A majority (n = 196; 71%) of participants were HCW. Nearly half of the participants had travelled outside of BC in 2020, including the USA, Europe, Iran, the Caribbean, Australia, Mexico and Japan. Two study participants reported a history of PCR-confirmed COVID-19. About 25% of participants reported COVID-19-associated symptoms more than 14 days prior to the study visit (**Table 1**).

Serology outcomes: To gain insights into participants' antibody reactivity against SARS-CoV-2, we measured antibody levels against four SARS-CoV-2 antigens, SARS-CoV, and the circulating beta- (HKU1, OC43) and alpha- (229E and NL63) coronaviruses, in the 276 study participants plus three selected COVID-19 convalescent sera (A, B, C), included as positive controls (**Supplemental Figure 2**). All individuals showed high antibody reactivity against circulating coronaviruses, over background.

Three study participants tested positive (CW087, CW0150, FH0037) on the commercial CLIA assay, including the two individuals with a history of COVID-19 (**Supplemental Figure 3**). A clustering analysis based on the multiplex of antibody profiles against the four SARS-CoV-2 antigens of the 276 study participants plus the 3 positive control convalescent sera showed that the three sera that were reactive on the commercial CLIA assay clustered with the three convalescent sera, but distinctly from the 273 other participants (**Supplemental Figure 4**). Thus, we established a SARS-CoV-2 seroprevalence of 1.1% [95%CI 0.2% to 3.1%], with 3 seropositive and 273 unexposed participants. After adjustment for bias using point estimates of specificity and sensitivity of the CLIA assay, the adjusted seroprevalence estimate in this population was 0.60% [95%CI 0% to 2.71%].

The pattern of antibody reactivity for SARS-CoV-2 antigens was quite distinct between seropositive individuals, including the convalescent sera, and unexposed individuals. Exposed seroreactive individuals

showed high antibodies against Spike, RBD and the N protein (**Supplemental Figure 5A**). However, unexposed ones showed variably high antibodies against Spike or RBD (**Supplemental Figure 5B, C**).

The clinical characteristics of the three SARS-CoV-2-exposed participants are shown in **Table 2**. Two out of the three exposed seroreactive participants were HCW: one a pediatric HCW and one a non-pediatric HCW. There was no difference therefore in seroprevalence between HCW and non-HCW, or between pediatric and non-pediatric HCW (1.0% vs 1.0%; *P*=1.00 and 0.7% vs 1.6%; *P*=0.54, respectively).

Positive antibody reactivity against RBD, N and S-2P native spike proteins was estimated to occur in 7.3% [95%CI 4.5% to 11.1%], 48.7 [95%CI 42.7% – 54.8%] and 82.4% [95%CI 77.4% to 86.7%] of the 273 unexposed participants, respectively. A clustering analysis based on these data showed that antibody reactivity among these unexposed participants was evenly distributed according to age, sex, HCW status, travel history and on whether participants thought they had COVID-19 symptoms (**Figure 1**). Moreover, we detected no significant correlations between antibody reactivity for any SARS-CoV-2 antigens, age, sex, recruitment site or travel history (P values from 0.36 to 0.90 adjusted at 5% FDR). On the other hand, we did detect modest correlation between antibody reactivity to SARS-CoV-2, and HKU1, NL63 and X229E, with Spearman rho values ranging from 0.130 - 0.224 for 7 significant (FDR 5%), out of 16 relevant correlations with circulating coronaviruses (**Figure 2**; **Supplemental Table 1**).

Discussion

We estimated the seroprevalence from a direct SARS-CoV-2 exposure to be 0.60% [95%CI 0% to 2.71%] in this population mainly composed of HCW in the greater Vancouver area. Data were confirmed using both a multiplex and commercial CLIA assays with 100% (6/6) agreement. Reported SARS-CoV-2 seroprevalence data in populations after the first wave vary widely across the world because of various factors, including the timing of sampling in relation to the pandemic; how countries managed to control spread of the virus; the target population of the study; and the assay used. Highest rates were reported in Sweden in May (7.3%) (23), Geneva in April/early May (10.8%) (24), and Madrid in late April/early May (>10%) (25); lowest rates were reported in BC (17). Indeed, the SARS-CoV-2 seroprevalence estimate from the current study is consistent with data published by the BC Centre for Disease Control (BCCDC) and obtained between May 15 and 27, 2020, which reported a 0.55% seroprevalence among 885 residual sera obtained from an outpatient laboratory network in the Lower Mainland of BC; their study population represents a wider geographical catchment and did not specifically target HCW (17). The current study confirms that transmission of COVID-19 in BC after the first wave was low, even among HCW, contrasting with a high seroprevalence reported among HCW in other studies (16, 26, 27).

The antibody detection combining a commercial and highly sensitive multiplex assays to detect multiple SARS-CoV-2 antigens allowed us to distinguish antibody profiles between exposed and unexposed participants, and to identify that a significant proportion of unexposed individuals display antibody reactivity to SARS-CoV-2 antigens. This result is consistent with the highly prevalent frequency T cell reactivity detected in about 40% of non-SARS-CoV-2-exposed individuals in recent studies (4, 5), and with another smaller study where 12 out of 95 pre-pandemic sera exhibited cross-reactive IgG antibody reactivity with conserved epitopes in SARS-CoV-2 proteins (S2 and N), using a flow cytometry method validated by ELISA (28). Previous seroprevalence studies have either used single-antigen assays, focused on SARS-CoV-2, or, in the latter case, examined cross-reactivity in selected sera (28). However, the current study is remarkable by the detection of SARS-CoV-2 antibody reactivity at the population level.

The antigen source of SARS-CoV-2 antibody reactivity in a relatively high proportion of individuals in the study is unclear. About 25% of participants in this study thought that they had experienced COVID-19 symptoms in the past. However, because the number of reported COVID-19 cases in BC was low, it seems unlikely that this antibody reactivity results from a direct exposure to the virus; most likely, this antibody reactivity in unexposed individuals reflects heterologous antigen cross-reactivity. On the other hand, antibody cross-reactivity between SARS-CoV-2 and circulating coronaviruses was modest, although this is predicted to occur, particularly within beta-coronaviruses, owing to relatively high sequence similarity (35-40% for nucleocapsid and spike proteins) (7). Antibody cross-reactivity between SARS-CoV and SARS-CoV-2 is also expected, due to >75% sequence identity between the spike proteins from these two viruses (29). Notably, evidence of cross- neutralization between circulating coronavirus and SARS-CoV-2 antibodies (28), raises a possibility that this pre-existing immunity may alter the clinical course of COVID-19. However, answering this question requires large follow-up studies.

This study has limitations. First, its cross-sectional design and lack of follow-up makes it impossible to establish whether the antibody reactivity to SARS-CoV-2 antigens in unexposed individuals may confer any immune protection. Second, the non-random method of recruitment method is likely to introduce a selection bias towards people who wanted to get tested for COVID-19 antibodies. Third, there is a possibility that a previous SARS-CoV-2 exposure may be underestimated by serology because of lack of sensitivity, especially early on (<7 days) and possibly also in asymptomatic individuals (30). However, the low number of reported cases means that this is unlikely to have represented many individuals in BC. Fourth, the small sample size limited the power for correlation analyses and the precision of estimates.

In conclusion, this study reports frequent and variable antibody reactivity to SARS-CoV-2 antigens in adults from the greater Vancouver area despite a low overall seroprevalence and low evidence of direct exposure to the virus after the first wave.

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Declaration of interests: The authors declare no conflicts of interest.

Author's contributions: AM, CM and SD coordinated the study sample accrual and blood processing in Vancouver. JG and DM coordinated recruitment at SMH. CC collated the data and helped with data analysis. SEOC performed the multiplex ECLIA assays, with help from SN and MB. MG and DD provided important input into the study design. VEB and DMG supervised the commercial CLIA testing of samples. PML and ABM supervised the study in Vancouver and at the NAID/NIH, respectively. AM, CM, SD and PML wrote the manuscript first draft. All authors contributed to the study concept, design, data analysis and reviewing the manuscript and accept the article submission in its final form.

Table 1. Demographical characteristics of study participants.

Participants' characteristics	Total n = 276
Age, years, mean (standard deviation)	42.4 (11.9)
Sex, % female / male (n)	67.4 (186) / 32.6 (90)
Health worker status[£], % (n)	71.0% (196)
Travelled outside BC[¶], % (n)	50.3% (139)
Prior positive testing for COVID-19 (PCR), % (n)	0.72% (2)
COVID-19-associated symptoms, % (n)	25.0% (69)

BC: British Columbia, history of travel since January 1, 2020; [£] includes physicians; nurses; respiratory therapists; dieticians, genetic counsellors; psychologists; social workers; administrators; physiotherapists; occupational therapists; pharmacists; and pathologists.

Table 2. Clinical characteristics of three seroreactive study participants, plus three COVID-19 convalescent sera.

Participant ID#	**Age**	**Sex**	**Travel outside BC**	**Convalescence period (days)***	**Reported COVID-19-associated symptoms** (by open-ended question)
CW0087[£]	39	Female	No	57	Nasal congestion, nasal dripping, laryngitis, intermittent dry cough, fatigue, chills, anosmia/ageusia with gradual return over 3 months
CW0150[£]	24	Female	No	unknown[¶]	Asymptomatic
FH0037[£]	68	Male	Yes	72	Shortness of breath on exertion, generalized aching, fever, mild cough
A[€]	30	Female	No	75	Cough, rhinorrhea, generalized body ache, extreme fatigue, head congestion, fever, anosmia/ageusia
B[€]	35	Male	Yes	100	Fatigue, severe headache/body aches, shortness of breath, drenching night sweats, mild diarrhoea, very mild dry cough, pain in both feet persisting
C[€]	51	Male	Yes	96	Protracted cough, fatigue, mild coryza and conjunctivitis, head congestion, and mild sore throat

[£]Seroreactive study participants; [€]COVID-19 convalescent sera; *time between COVID-19 diagnosis by PCR and serology testing.
[¶]Retrospectively (after participant was made aware of positive serology testing) we were able to identify that she had a contact with a COVID-19 case 89 days prior to serology testing.

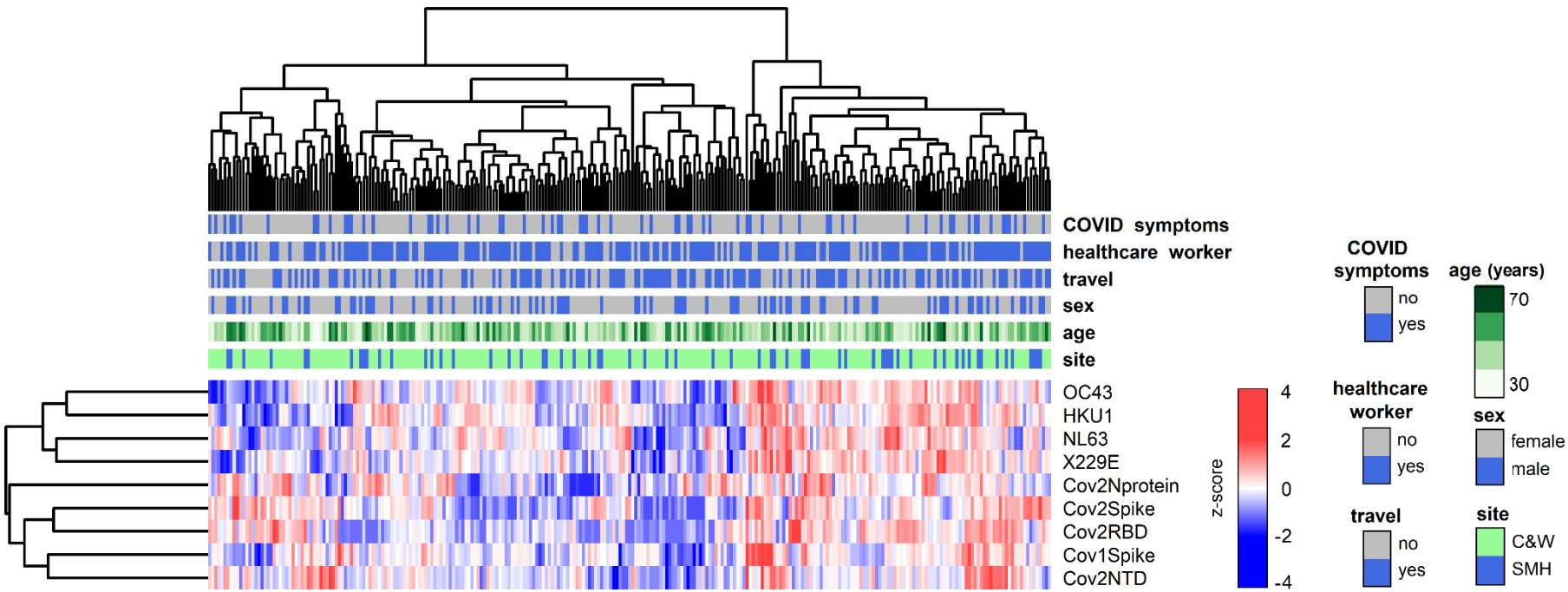


Figure 1. Hierarchical clustering based on antibody levels against 4 SARS-CoV-2, SARS-1 Spike antigens and human circulating coronaviruses from the 273 unexposed study participants. Colour scale represents antibody levels as a z-score.

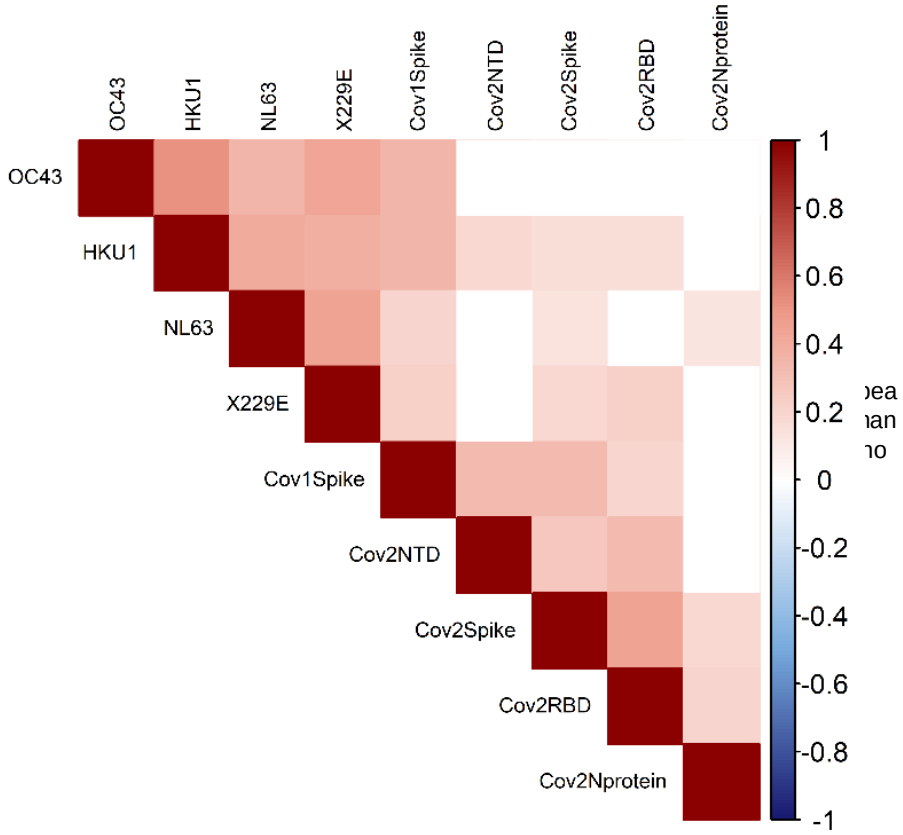
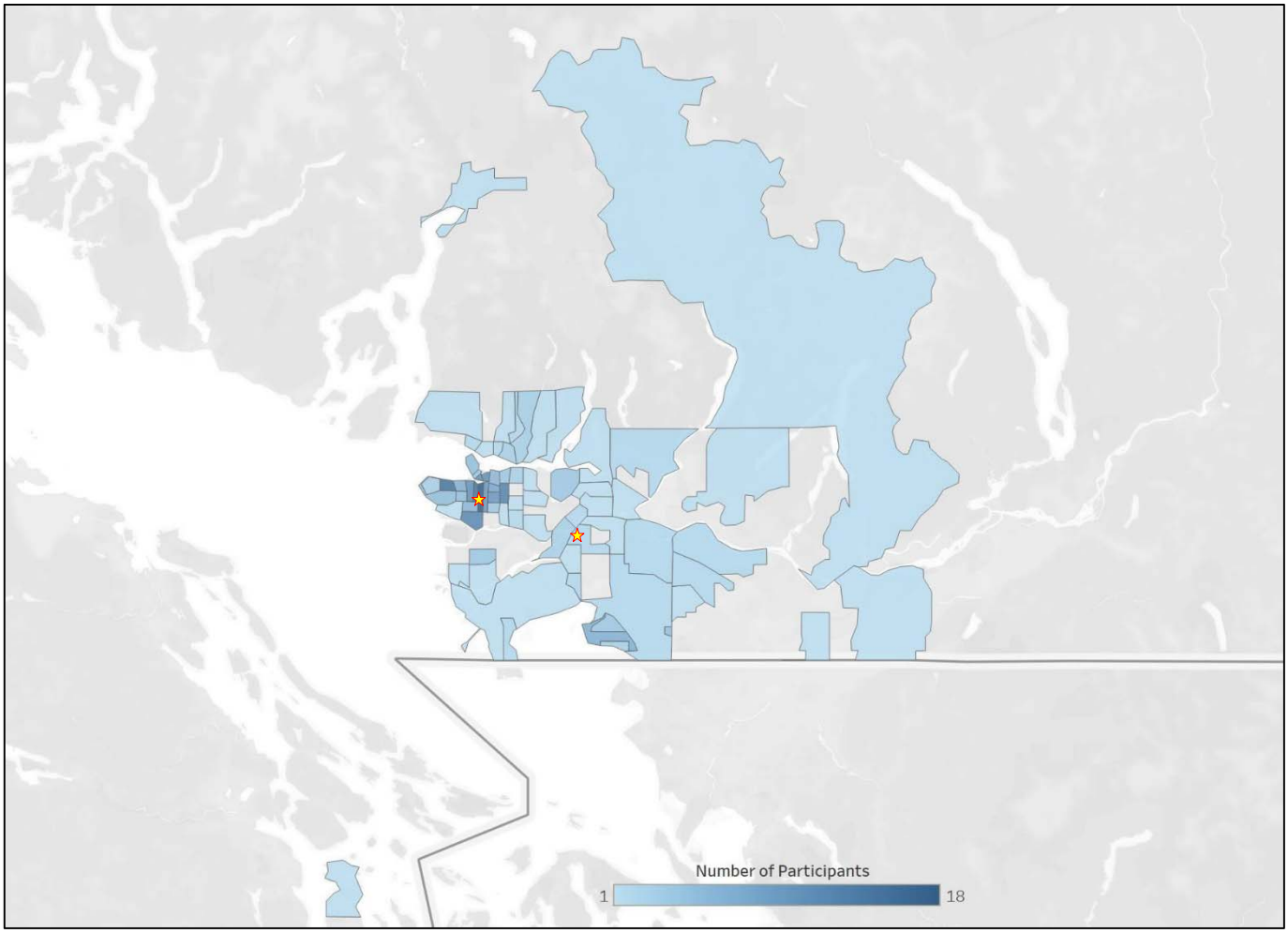
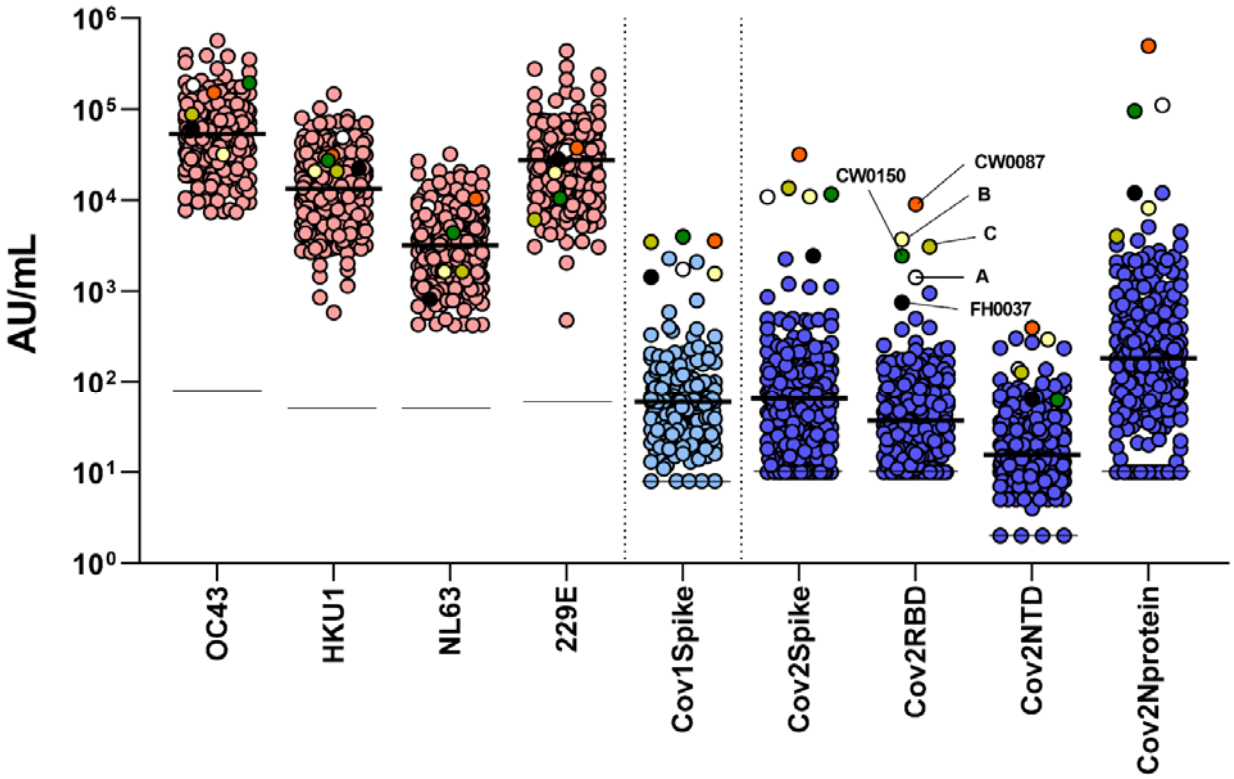


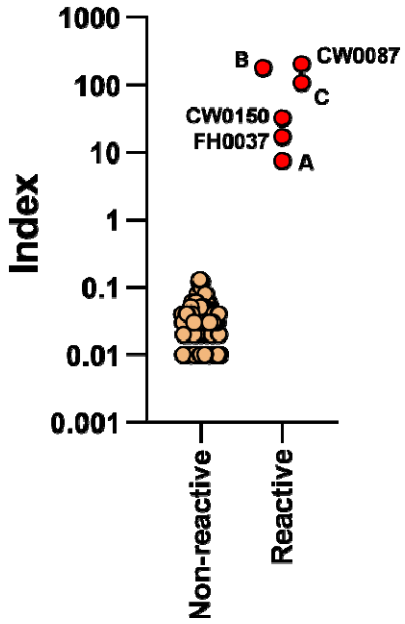
Figure 2. Correlations among SARS-CoV-2 and circulating coronaviruses antibody levels. Correlations among SARS-CoV-2 and circulating coronavirus antibody levels; Spearman rho values for significant correlations are shown on colour scale (FDR 5%; P values provided in Supplemental Table 1).



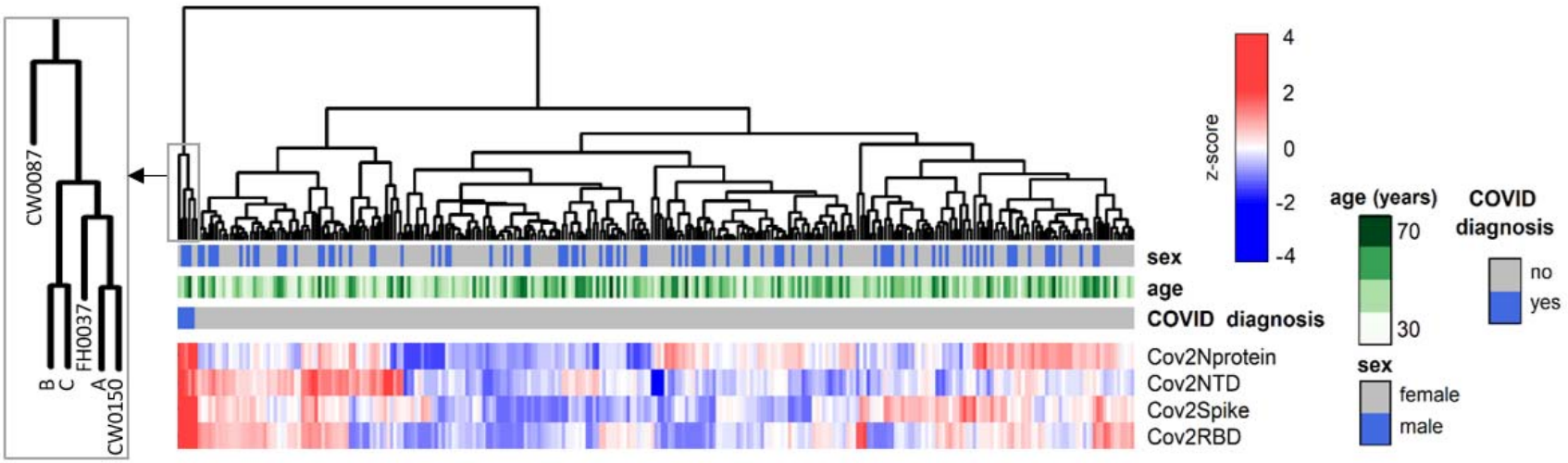
Supplemental Figure 1. Geographical distribution. Data are from 276 study participants, based on postal code information. Stars indicate the location of the two recruitment sites (BC Children's Hospital Research Institute on the left and Surrey Memorial Hospital on the right).



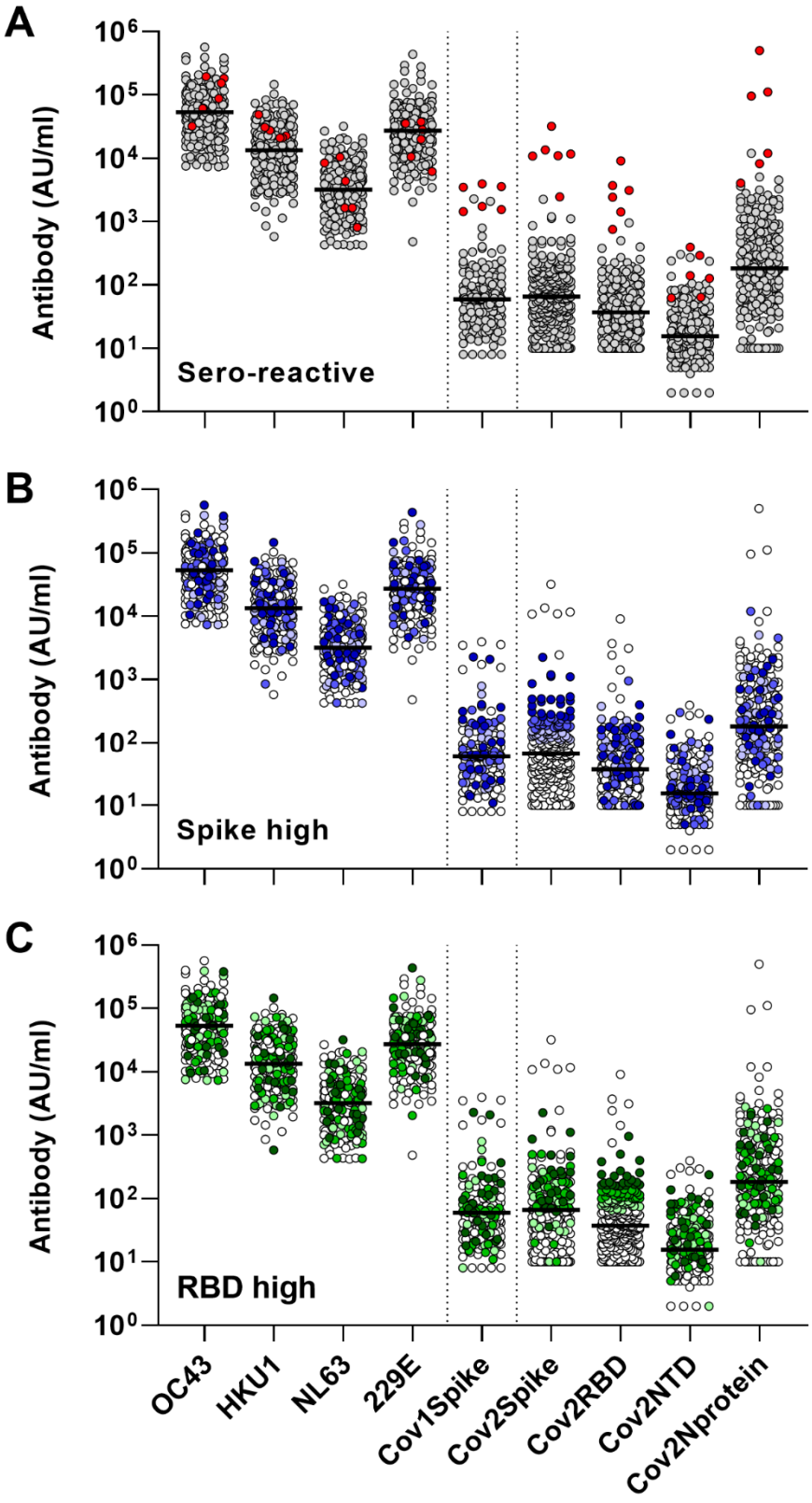
Supplemental Figure 2. Multiplex assay data. Data from 276 study participants including three exposed seroreactive participants, plus three additional COVID-19 convalescent sera (A, B and C). The six seroreactive sera are multicolour-coded, whereas antibody levels are also grouped according to whether they represent circulating coronaviruses (pink), SARS-CoV (light blue) and SARS-CoV-2 antigens (dark blue). Thick black line = median. Thin black line = lower detection limit of assay for particular antigen.



Supplemental Figure 3. Seroreactivity on commercial CLIA assay among 222 of 273 study participants who tested above the mean for any SARS-CoV-2 antigen in the multiplex ECLIA assay, identifies three reactive sera (red) and 219 non-reactive sera (orange).



Supplemental Figure 4. Hierarchical clustering analysis according to antibodies against SARS-CoV-2 antigens. This figure combines data from 276 study participants plus the three COVID-19 convalescent sera. Colour scale represents antibody detection as a z-score.



Supplemental Figure 5. Antibodies levels against circulating coronavirus and SARS antigens in the combined group of 273 study participants, plus three convalescent control sera, highlighting (**A**) SARS-CoV-2-exposed individuals (red), and (**B**, **C**) 273 unexposed participants with antibody reactivity against Spike or RBD at the top 90th (darker tone), 80th (mid-tone) or 70th (lighter tone) centiles. Line = median.

References

1. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020.

2. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med. 2020;26(6):845-8.

3. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020;20(5):565-74.

4. Mateus J, Grifoni A, Tarke A, Sidney J, Ramirez SI, Dan JM, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. Science. 2020.

5. Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature. 2020.

6. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends Microbiol. 2016;24(6):490-502.

7. Hicks J, Klumpp-Thomas C, Kalish H, Shunmugavel A, Mehalko J, Denson JP, et al. Serologic cross-reactivity of SARS-CoV-2 with endemic and seasonal Betacoronaviruses. medRxiv. 2020.

8. Monto AS, DeJonge PM, Callear AP, Bazzi LA, Capriola SB, Malosh RE, et al. Coronavirus Occurrence and Transmission Over 8 Years in the HIVE Cohort of Households in Michigan. J Infect Dis. 2020;222(1):9-16.

9. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. Epidemiology and infection. 1990;105(2):435-46.

10. Sun ZF, Meng XJ. Antigenic cross-reactivity between the nucleocapsid protein of severe acute respiratory syndrome (SARS) coronavirus and polyclonal antisera of antigenic group I animal coronaviruses: implication for SARS diagnosis. J Clin Microbiol. 2004;42(5):2351-2.

11. Che XY, Qiu LW, Liao ZY, Wang YD, Wen K, Pan YX, et al. Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43. J Infect Dis. 2005;191(12):2033-7.

12. Patrick DM, Petric M, Skowronski DM, Guasparini R, Booth TF, Krajden M, et al. An Outbreak of Human Coronavirus OC43 Infection and Serological Cross-reactivity with SARS Coronavirus. The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale. 2006;17(6):330-6.

13. Khan S, Nakajima R, Jain A, de Assis RR, Jasinskas A, Obiero JM, et al. Analysis of Serologic Cross-Reactivity Between Common Human Coronaviruses and SARS-CoV-2 Using Coronavirus Antigen Microarray. bioRxiv. 2020.

14. Speer CP. Inflammation and bronchopulmonary dysplasia. Semin Neonatol. 2003;8(1):29-38.

15. Eckerle I, Meyer B. SARS-CoV-2 seroprevalence in COVID-19 hotspots. Lancet. 2020.

16. Houlihan CF, Vora N, Byrne T, Lewer D, Kelly G, Heaney J, et al. Pandemic peak SARS-CoV-2 infection and seroconversion rates in London frontline health-care workers. Lancet. 2020.

17. Skowronski D, Sekirov I, Sabaiduc S, Zou M, Morshed M, Lawrence D, et al. Low SARS-CoV-2 sero-prevalence based on anonymized residual sero-survey before and after first wave measures in British Columbia, Canada, March-May 2020. medRxiv. 2020:1-26.

18. Cummings DAT, Radonovich LJ, Gorse GJ, Gaydos CA, Bessesen MT, Brown AC, et al. Risk Factors for Healthcare Personnel Infection with Endemic Coronaviruses (HKU1, OC43, NL63, 229E): Results from the Respiratory Protection Effectiveness Clinical Trial (ResPECT). Clin Infect Dis. 2020.

19. Johnson M, Wagstaffe HR, Gilmour KC, Mai AL, Lewis J, Hunt A, et al. Evaluation of a novel multiplexed assay for determining IgG levels and functional activity to SARS-CoV-2. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2020;130:104572.

20. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020;367(6483):1260-3.

21. [Available from: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/eua-authorized-serology-test-performance.

22. Garnett E, Jung J, Tam E, Rajapakshe D, Cheney S, Brown C, et al. Clinical validation and performance evaluation of the automated Vitros Total Anti-SARS-CoV-2 Antibodies assay for screening of serostatus in COVID-19. medRxiv [Internet]. 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.06.09.20126474v1.

23. Sweden. PHA. Första resultaten från pågående undersökning av antikroppar för covid-19-virus. nyhetsarkiv/2020/maj/forsta-resultaten-fran-pagaende-undersokning-avantikroppar-for-covid-19-virus. 2020.

24. Stringhini S, Wisniak A, Piumatti G, Azman AS, Lauer SA, Baysson H, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. Lancet. 2020.

25. Pollan M, Perez-Gomez B, Pastor-Barriuso R, Oteo J, Hernan MA, Perez-Olmeda M, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet. 2020.

26. Stubblefield WB, Talbot HK, Feldstein L, Tenforde MW, Rasheed MAU, Mills L, et al. Seroprevalence of SARS-CoV-2 Among Frontline Healthcare Personnel During the First Month of Caring for COVID-19 Patients - Nashville, Tennessee. Clin Infect Dis. 2020.

27. Garcia-Basteiro AL, Moncunill G, Tortajada M, Vidal M, Guinovart C, Jimenez A, et al. Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospital. Nature communications. 2020;11(1):3500.

28. Ng K, Faulkner N, Cornish G, Rosa A, Earl C, Wrobel A, et al. Pre-existing and de novo humoral immunity to SARS-CoV-2 in humans. bioRxiv. 2020:1-16.

29. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature communications. 2020;11(1):1620.

30. Lynch KL, Whitman JD, Lacanienta NP, Beckerdite EW, Kastner SA, Shy BR, et al. Magnitude and kinetics of anti-SARS-CoV-2 antibody responses and their relationship to disease severity. Clin Infect Dis. 2020.