

Seropositivity in blood donors and pregnant women during 9-months of SARS-CoV-2 transmission in Stockholm, Sweden

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General abstract

Public health strategies to contain the pandemic continue to vary markedly across the world. In Sweden, compared to most advanced economies, social restrictions have primarily relied upon voluntary adherence to a set of recommendations and strict lockdowns have not been enforced. To better understand the development of humoral immunity to SARS-CoV-2 in the Stockholm population before the start of mass vaccinations, healthy blood donors and pregnant women ($n=4,100$) were sampled at random between 14th March-11th December 2020. All individuals ($n=200$ /sampling week) were screened for anti-SARS-CoV-2 spike (S) trimer- and RBD-specific IgG responses with highly sensitive and specific ELISA assays, and the results were compared with those from historical controls ($n=595$). Data were modelled using a probabilistic Bayesian framework that considered individual responses to both antigens. We found that after a steep rise at the start of the pandemic, the seroprevalence trajectory increased steadily in approach to the winter second-wave of infections, approaching 15% of all individuals surveyed by 11th December. In agreement with the high transmission rate observed in the Stockholm area, seroprevalence in this cohort of active adults increased during the 9 months from the start of the outbreak, but was far from that required for herd immunity at the end of 2020.

Structured abstract

Objectives: As Sweden did not enforce social lockdown in response to the pandemic, it is critical to establish seropositivity to SARS-CoV-2 in healthy, active adults – here represented by blood donors and pregnant women. Random sampling was carried out in Stockholm, the country’s most populous region, and the study ran from virus emergence (March 2020) until

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the end of 2020, shortly prior to the first round of vaccinations, allowing for an estimate of population seropositivity in response to natural infection.

Design: In this cross-sectional prospective study, otherwise-healthy blood donors ($n=2,100$) and pregnant women ($n=2,000$) were sampled at random for consecutive weeks (at three intervals) between 14th March and 11th December 2020. Sera from all participants and a large cohort of historical controls ($n=595$) were screened for IgG responses against the SARS-CoV-2 spike (S) trimer and the receptor-binding domain (RBD). As a complement to standard approaches to analyze the data, a probabilistic Bayesian approach that assigns likelihood of past infection was used to analyze the population data. The study was carried out in accordance with Swedish Ethical Review Authority registration no. 2020-01807.

Setting: Healthy participant samples were selected from their respective pools at random through the Karolinska University Hospital.

Participants: None of the participants were symptomatic at the time of sampling and none had previously been hospitalized for COVID-19. No additional metadata was available from the samples.

Results: Blood donors and pregnant women showed a similar seroprevalence. After a steep rise at the start of the pandemic, the seroprevalence trajectory increased steadily in approach to the winter second-wave of infections, approaching 15% of all individuals surveyed by 11th December 2020. Importantly, 96% of antibody-positive healthy donors screened ($n=56$) developed neutralizing antibody responses at titers comparable to or higher than those observed in clinical trials of SARS-CoV-2 spike mRNA vaccination, supporting that mild infection engenders a competent B cell response.

Conclusions: In agreement with currently rising COVID-19 cases and ICU occupancy during a second winter wave of infections, these data demonstrate that the metropolitan Stockholm area was far from herd immunity nine months after the outbreak, with approximately one-in-six persons in the examined cohort seropositive for SARS-CoV-2.

Introduction

Densely populated areas – such as Stockholm county – facilitate the spread of SARS-CoV-2 and evidence suggests that transmission can be curtailed by temporary restriction of leisure and business activities, and track and trace¹. In contrast to the vast majority of European, American and Asian countries, Sweden has favored a strategy in which individuals are encouraged to adhere to a set of basic public health instructions, while society has remained largely open – drawing numerous criticisms. The country has reported a significantly higher burden on the healthcare system than its Scandinavian neighbors (Fig. 1A).

Enumerating past infection status over time is critical for estimating viral spread and understanding characteristics of adaptive immune responses to a new pathogen. Antibody testing is also important for optimal planning of vaccination campaigns, especially when vaccines doses are limited. Therefore, to estimate seropositivity in the capital city, we developed robust SARS-CoV-2 antibody tests and statistical methods and applied them to healthy participants in the region throughout 2020.

Blood donors and pregnant women, studied throughout, represent two good proxies for adult population health, being generally working age and mobile members of society without being enriched for individuals at especially high-risk of SARS-CoV-2 infection, such as healthcare or public transportation employees, where seroprevalence may be higher. Similarly, seroprevalence may be lower in the unexposed and elderly, and higher in children.

The data presented here follow seropositivity in across the first and second waves of infections in Stockholm, providing critical information about population immunity over time and prior to the introduction of SARS-CoV-2 vaccines.

Materials and methods

Human samples and ethical declaration

Anonymized samples from blood donors ($n=100$ /sampling week) and pregnant women ($n=100$ /sampling week) were randomly selected from their respective pools by the department of Clinical Microbiology, Karolinska University Hospital. No metadata, such as age or sex information were available for the samples in the study. Pregnant women were sampled as part of routine screening for infectious diseases during the first trimester of pregnancy. Blood donors ($n=595$) collected through the same channels a year previously (Spring 2019) were randomly selected for use as assay negative controls. The use of study samples was approved by the Swedish Ethical Review Authority (registration no. 2020-01807) and no study subject was hospitalized for COVID-19 or symptomatic at sample collection. Blood donors are required to be a healthy for a minimum of two weeks before donation.

Stockholm County death and Swedish mortality data was sourced from the ECDC, EU (<https://www.ecdc.europa.eu/en/covid-19/data>) and the Swedish Public Health Agency (<https://www.folkhalsomyndigheten.se/folkhalsorapportering-statistik/>), respectively, and were current with the date of publication.

Serum sample processing

Blood samples were collected by the attending clinical team and serum isolated by the department of Clinical Microbiology. Samples were anonymized, barcoded and stored at -20°C until use. Serum samples were not heat-inactivated for ELISA protocols.

SARS-CoV-2 antigen generation

The plasmid for expression of the SARS-CoV-2 prefusion-stabilized spike ectodomain with a C-terminal T4 fibritin trimerization motif was obtained from². The plasmid was used to transiently transfect FreeStyle 293F cells using FreeStyle MAX reagent (Thermo Fisher Scientific). The ectodomain was purified from filtered supernatant on Streptactin XT resin (IBA Lifesciences), followed by size-exclusion chromatography on a Superdex 200 in 5 mM Tris pH 8, 200 mM NaCl. The RBD domain (RVQ – QFG) of SARS-CoV-2 was cloned upstream of a Sortase A recognition site (LPETG) and a 6xHIS tag, and expressed in 293F cells as described above. RBD-HIS was purified from filtered supernatant on His-Pur Ni-NTA

resin (Thermo Fisher Scientific), followed by size-exclusion chromatography on a Superdex 200. The nucleocapsid was purchased from Sino Biological.

Anti-SARS-CoV-2 ELISA

96-well ELISA plates (Nunc MaxiSorp) were coated with SARS-CoV-2 S trimers, RBD or nucleocapsid (100 µl of 1 ng/µl) in PBS overnight at 4°C. Plates were washed six times with PBS-Tween-20 (0.05%) and blocked using PBS-5% no-fat milk. Human serum samples were thawed at room temperature, diluted (1:100 unless otherwise indicated), and incubated in blocking buffer for 1h (with vortexing) before plating. Serum samples were incubated overnight at 4°C before washing, as before. Secondary HRP-conjugated anti-human antibodies were diluted in blocking buffer and incubated with samples for 1 hour at room temperature. Plates were washed a final time before development with TMB Stabilized Chromogen (Invitrogen). The reaction was stopped using 1M sulphuric acid and optical density (OD) values were measured at 450 nm using an Asys Expert 96 ELISA reader (Biochrom Ltd.). Secondary antibodies (from Southern Biotech) and dilutions used: goat anti-human IgG (2014-05) at 1:10,000. All assays were developed for their fixed time and negative control samples were run alongside test samples in all assays. Raw 450nm optical density data were log transformed for statistical analyses.

In vitro virus neutralisation assay

Pseudotyped viruses were generated by the co-transfection of HEK293T cells with plasmids encoding the SARS-CoV-2 spike protein harboring an 18 amino acid truncation of the cytoplasmic tail²; a plasmid encoding firefly luciferase; a lentiviral packaging plasmid (Addgene 8455) using Lipofectamine 3000 (Invitrogen). Media was changed 12-16 hours post-transfection and pseudotyped viruses harvested at 48- and 72-hours, filtered through a 0.45 µm filter and stored at -80°C until use. Pseudotyped neutralisation assays were adapted from protocols validated to characterize the neutralization of HIV, but with the use of HEK293T-ACE2 cells. Briefly, pseudotyped viruses sufficient to generate ~100,000 RLUs were incubated with serial dilutions of heat-inactivated serum for 60 min at 37°C. Approximately 15,000 HEK293T-ACE2 cells were then added to each well and the plates incubated at 37°C for 48 hours. Luminescence was measured using Bright-Glo (Promega) according to the manufacturer's instructions on a GM-2000 luminometer (Promega) with an integration time of 0.3s. The limit of detection was at a 1:45 serum dilution.

Probabilistic seroprevalence estimations

Prior to analysis, each sample OD was standardized by dividing by the mean OD of “no sample controls” on that plate or other plates run on the same day. This resulted in more similar distributions for 2019 blood donor samples with 2020 blood donors and pregnant volunteers. Our Bayesian approach is presented in detail in Christian *et al*³. Briefly, we used a logistic regression over anti-RBD and -S training data (from $n=595$ historical blood donor controls and $n=138$ SARS-CoV-2 PCR+ individuals across the clinical spectrum) to model the relationship between the ELISA measurements and the probability that a sample is antibody-positive. We adjusted for the training data class proportions and used these adjusted probabilities to inform the seroprevalence estimates for each time point. Given that the population seroprevalence cannot increase dramatically from one week to the next, we constructed a prior over seroprevalence trajectories using a transformed Gaussian Process, and combined this with the individual class-balance adjusted infection probabilities for each donor to infer the posterior distribution over seroprevalence trajectories. We validated our Bayesian approach by comparing the output with that of more established probabilistic algorithms (support vector machines and linear discriminant analysis) that we have previously developed for ELISA measurements⁴, finding strong congruency between the approaches.

Results

Blood donor and pregnant women serum samples ($n=100$ of each per sampling week) were selected at random from their respective pools and the IgG response against SARS-CoV-2 S glycoprotein trimers and the smaller RBD subunit was measured in all sera using established ELISA assays⁴ (Fig. 1B-E). Test samples were run alongside historical (negative) control sera ($n=595$ blood donors from spring 2019) throughout the study.

Using conventional 3 or 6 standard deviations (SD) from the mean of negative control sera as assay cut-offs for positivity, and simple linear regression, between 17-21% and 14-19% of individuals were IgG-positive against spike or the RBD, respectively, at the last sampling point (11th December) (Fig. 1F).

However, the many measurements between the 3 and 6 SD cut-offs for both or a single antigen highlight the problem of assigning case to low values. Therefore, to provide an accurate seropositivity estimates for this metric and to model population-level changes over time, we

developed and validated a cut-off-independent, probabilistic Bayesian framework that models the log odds a sample is antibody-positive based on responses in training data; in this case SARS-CoV-2 PCR+ COVID-19 patients across the clinical spectrum that we have extensively characterized^{3,4}. The correct classification of low titers is important, since a number of cases that have experienced asymptomatic or mild infection generate antibody titers closer to the assay boundary, and antibody levels decline with time in all seropositive individuals, meaning that incorrect classification can skew seroprevalence estimates⁴⁻⁷.

Using this more quantitative approach that considers the wide range of responses present in the population and shares information between sampling weeks, we found seropositivity to increase sharply at the start of the pandemic (Fig. 1G). By the time COVID-19 deaths in the country were at very low levels during August, the seroprevalence trajectory increased at a slower rate approaching to the winter second wave (Fig. 1A and G), in agreement with continued viral spread in the Stockholm population, and consistent with persistent antibody responses over a 9-month period.

By week 50 (11th December), our probabilistic approach identified 14.8% (95% Bayesian CI [12.2-18.0]) of the cohort to have been previously infected (Supp. Table 1). We validated the estimates from our Bayesian approach with an equal-weighted (cut-off-independent) learner from the output of support vector machines and linear discriminant analysis that we previously optimized for ELISA measurements (Fig. 1G)⁴, which showed highly consistent results.

Importantly, 96% of antibody-positive blood donors and pregnant women randomly subsampled from the entire cohort ($n=56$) had virus neutralizing responses in their sera ($ID_{50}=600$; 95% CI [357 – 1,010] and $ID_{50}=350$; 95% CI [228 - 538], respectively, Fig. 1H-I), with titers comparable to those engendered by recently-approved COVID-19 mRNA vaccines that were shown to be protective in clinical phase 3 trials^{8,9}. These observations support that asymptomatic/mild infection generates an antibody response that provides a first line of defense against potential re-exposures¹⁰, although inter-individual heterogeneity will have a role in outcomes¹¹⁻¹³.

Discussion

To characterize immunological responses to SARS-CoV-2 and safeguard public health, it is critical to monitor the level of population immunity after natural infection¹⁴, especially in settings with different public health measures – allowing for concurrent and retroactive evaluation of different strategies. As Sweden has taken a unique public health approach to mitigate the effects of the virus, data from the country provide an important contrast to comparable settings and may help inform future pandemic management.

Serology is amenable to studies of large cohorts and is the *gold standard* for determining previous exposure to pathogens. Importantly, increasing evidence suggests that most persons infected with SARS-CoV-2 develop virus-specific antibodies, including following mild or asymptomatic infections^{4,15,16}. Studies have highlighted the protective role B cells play in controlling SARS-CoV-2 infection in humans¹⁰ and animal models^{17,18}, while potent neutralizing antibodies were rapidly isolated from infected donor samples^{19–21}. Critical future research is required to determine the duration of B cell memory in those infected, as well as following vaccination. Early results show that infected individuals can maintain a detectable virus-specific B cell response for at least eight months^{20,22}, which together with the steadily increasing seropositivity observed in the study (and in NYC²³), help allay early fears that immunity waned in the short-term; although individuals with mild infection can have titers close to the assay boundary that may decline from peak responses at different rates between individuals. Neutralizing titers in our study samples were comparable to those engendered by the first mRNA vaccines, supporting that natural infection generates immunological memory. Given these observations, antibody testing should be used for optimal coordination of vaccine interventions, especially in the near future when vaccine doses are expected to be limited.

Epidemiologically, the data presented here indicate that nine months after viral emergence in Stockholm, Sweden – and in the midst of a winter second wave – approximately one-in-six active adults (represented by blood donors and pregnant women) have been infected with the virus. Although this is a considerable number, which is associated with a significant public health burden²⁴, this proportion remains far from that required for herd immunity. This situation in Stockholm is similar to that observed in New York City, USA, where despite high rates of infection and mortality²⁵, herd immunity was not attained within six months of the outbreak, reaching *ca.* 20% of the general population²³. Compared to other European regions, our study

supports that Stockholm has fared worse in terms of infections (following the first wave) than the majority of study locations^{26–28}, with the exception of Lombardy, Italy (23% seropositivity, an early European center of infection)²⁹, and comparable to London, UK (13%)³⁰ and Geneva, Switzerland (11%)³¹. However, direct comparisons between sites are complicated by differences in the demographics of the study subjects as well as the sensitivity of the assay used for antibody detection³². Longitudinal studies over longer timescales (first, second and future waves), using robust antibody tests and different cohorts are urgently needed for improved comparisons and understanding COVID-19.

Together, the results presented here highlight the continued need for measures to curtail virus dissemination and protect the most vulnerable in society. Understanding seroprevalence changes in the population (and individuals) over time - including following the introduction of vaccines - is of fundamental importance for informing public health approaches.

Author contributions

GKH and XCD designed the study, analyzed the data and wrote the manuscript with input from co-authors. JA, TA, SM and GB provided the study samples. XCD generated the ELISA data. LH, DJS, GM and BM generated SARS-CoV-2 antigens and pseudotyped viruses. DJS performed the neutralization assay. MC, CW, N.F.G, and BM carried out statistical analyses. MC and BM developed the Bayesian framework.

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Conflict of interest

The study authors declare no competing interests related to the work.

Data and code availability statement

Data generated as part of the study, along with custom code for statistical analyses, is openly available via our GitHub repositories: <https://github.com/MurrellGroup/DiscriminativeSeroprevalence/> and <https://github.com/chr1swallace/seroprevalence-paper>.

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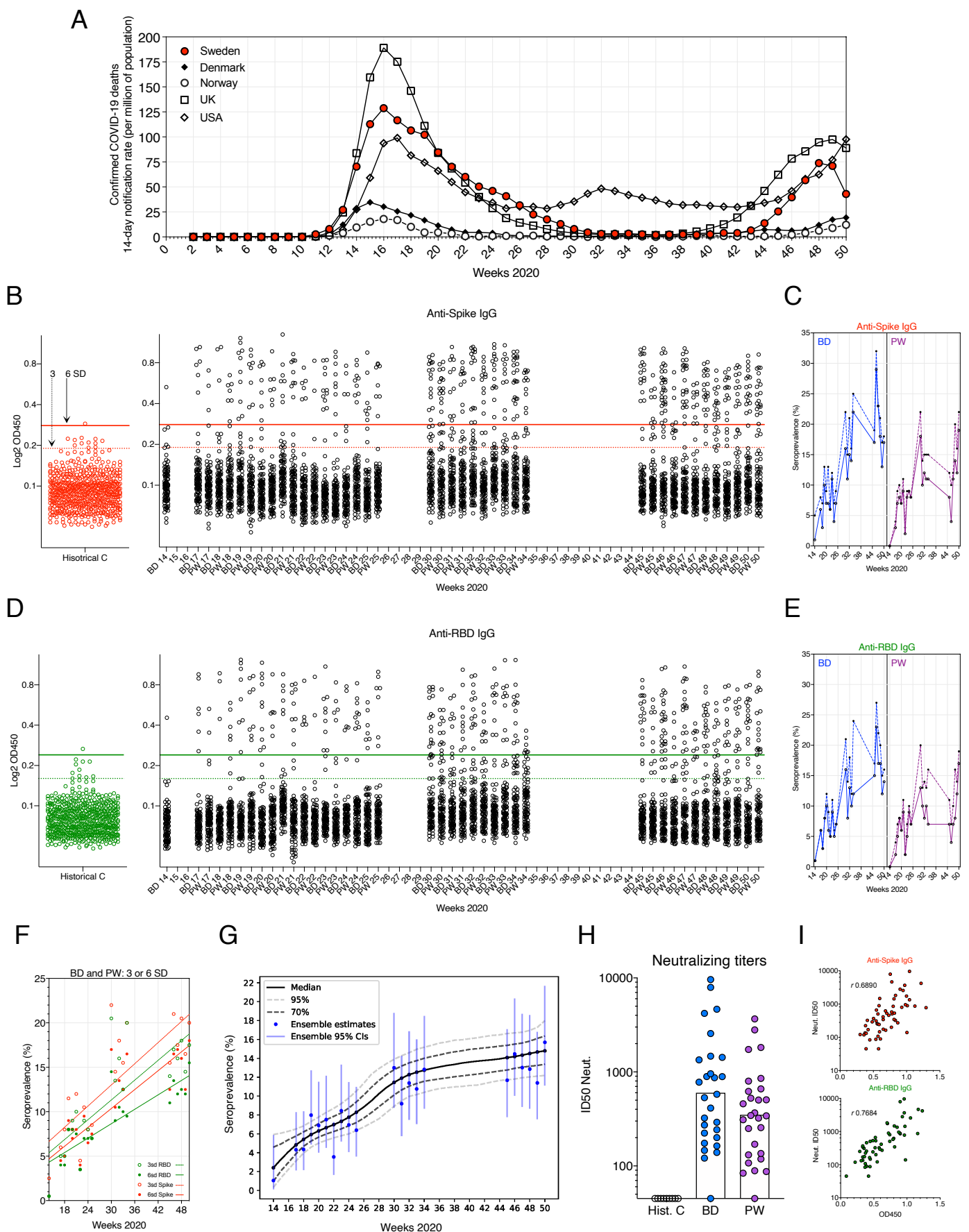


Figure 1: SARS-CoV-2 seropositivity estimates in Stockholm: March-December 2020. (A) Population-adjusted COVID-19 deaths for selected countries during the pandemic. (B) Anti-S IgG responses in blood donors (BD), pregnant women (PW) and $n=595$ historical control sera. 100 BD and 100 PW samples were analyzed per sampling week. Conventional 3 and 6 SD assay cut-offs are shown as dashed and solid lines, respectively. (C) Spike seropositivity in BD and PW according to 3 and 6 SD cut-offs. (D) Anti-RBD IgG responses in blood donors (BD), pregnant women (PW) and $n=595$ historical control sera. Conventional 3 and 6 SD assay cut-offs are shown as dashed and solid lines, respectively. (E) RBD seropositivity in BD and PW according to 3 and 6 SD cut-offs. (F) Seropositivity estimates in BD and PW combined according to 3 and 6 SD cut-offs. Simple linear regression was applied to each estimate. (G) Cut-off-independent Bayesian modelling of population seropositivity. A frequentist, equal-weighted ensemble learner from the output of support vector machines and linear discriminant analysis was used to validate the Bayesian framework. (H) *In vitro* pseudotyped virus neutralizing titers in a subset of antibody-positive BD and PW. Bars represent the geometric mean. (I) Binding and neutralization - for samples in (H) - are highly correlated.