



Kinetics of the SARS-CoV-2 antibody response and serological estimation of time since infection

Stéphane Pelleau, Tom Woudenberg, Jason Rosado, Françoise Donnadieu, Laura Garcia, Thomas Obadia, Soazic Gardais, Yasmine Elgharbawy, Aurelie Velay, Maria Gonzalez, et al.

► To cite this version:

Stéphane Pelleau, Tom Woudenberg, Jason Rosado, Françoise Donnadieu, Laura Garcia, et al.. Kinetics of the SARS-CoV-2 antibody response and serological estimation of time since infection. Journal of Infectious Diseases, 2021, pp.jiab375. 10.1093/infdis/jiab375 . pasteur-03319587v1

HAL Id: pasteur-03319587

<https://pasteur.hal.science/pasteur-03319587v1>

Submitted on 12 Aug 2021 (v1), last revised 2 May 2022 (v2)

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Kinetics of the SARS-CoV-2 antibody response and serological estimation of time since infection

Stéphane Pelleau^{1,2}, Tom Woudenberg^{1,2}, Jason Rosado^{1,2,3}, Françoise Donnadieu^{1,2}, Laura Garcia^{1,2}, Thomas Obadia^{1,2,4}, Soazic Gardais², Yasmine Elgharbawy², Aurelie Velay^{5,6}, Maria Gonzalez⁷, Jacques Yves Nizou⁸, Nizar Khelil⁸, Konstantinos Zannis⁸, Charlotte Cockram⁹, Sarah Hélène Merklings¹⁰, Annalisa Meola¹¹, Solen Kerneis^{12,13,14}, Benjamin Terrier^{15,16}, Jerome de Seze¹⁷, Delphine Planas¹⁸, Olivier Schwartz¹⁸, François Dejardin¹⁹, Stéphane Petres¹⁹, Cassandre von Platen²⁰, Sandrine Fernandes Pellerin²⁰, Laurence Arowas²¹, Louise Perrin de Facci²¹, Darragh Duffy²², Cliona Ní Cheallaigh^{23,24}, Jean Dunne^{25,26}, Niall Conlon^{25,26}, Liam Townsend^{23,24}, Veasna Duong²⁷, Heidi Auerswald²⁷, Laurie Pinaud²⁸, Laura Tondeur²⁸, Marija Backovic¹¹, Bruno Hoen²⁹, Arnaud Fontanet^{28,30}, Ivo Mueller^{2,31,32}, Samira Fafi-Kremer^{5,6}, Timothée Bruel^{18,33}, Michael White^{1,2*}

1. Infectious Disease Epidemiology and Analytics Unit, Department of Global Health, Institut Pasteur, Paris, France
2. Malaria: Parasites and Hosts Unit, Department of Parasites and Insect Vectors, Institut Pasteur, Paris, France
3. Sorbonne Université, ED 393, F-75005 Paris, France
4. Hub de Bioinformatique et Biostatistique, Département Biologie Computationnelle, Institut Pasteur, Paris, France
5. CHU de Strasbourg, Laboratoire de Virologie, F-67091 Strasbourg, France
6. Université de Strasbourg, INSERM, IRM UMR_S 1109, Strasbourg, France
7. CHU de Strasbourg, Service de Pathologies Professionnelles, F-67091 Strasbourg, France
8. Institut Mutualiste Montsouris, Paris, France
9. Spatial Regulation of Genomes Unit, Department of Genomes and Genetics, Institut Pasteur, Paris, France
10. Insect-Virus Interactions Unit, Department of Virology and CNRS UMR 2000, Institut Pasteur, Paris, France
11. Structural Virology Unit, Department of Virology and CNRS UMR 3569, Institut Pasteur, Paris, France
12. Equipe de Prévention du Risque Infectieux, AP-HP, Hôpital Bichat, Paris, France
13. Université de Paris, INSERM, IAME, Paris, France
14. Epidemiology and Modelling of Antibiotic Evasion (EMAE), Institut Pasteur, Paris, France

© The Author(s) 2021. Published by Oxford University Press for the Infectious Diseases Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

15. Department of Internal Medicine, National Referral Center for Rare Systemic Autoimmune Diseases, Assistance Publique Hôpitaux de Paris-Centre (APHP-CUP), Université de Paris, Paris, France
16. PARCC, INSERM U970, Paris, France
17. Centre d'Investigation Clinique - INSERM CIC-1434, Strasbourg, France
18. Virus and Immunity Unit, Department of Virology, Institut Pasteur, Paris, France
19. Production and Purification of Recombinant Proteins Technological Platform, Center for Technological Resources and Research, Institut Pasteur, Paris, France
20. Center for Translational Science, Institut Pasteur, Paris, France
21. Investigation Clinique et Accès aux Ressources Biologiques (ICAREB), Center for Translational Research, Institut Pasteur, Paris, France.
22. Translational Immunology Lab, Institut Pasteur, Paris, France
23. Department of Infectious Diseases, St James's Hospital, Dublin, Ireland
24. Department of Clinical Medicine, School of Medicine, Trinity Translational Medicine Institute, Trinity College Dublin, Ireland
25. Department of Immunology, St James's Hospital, Dublin, Ireland
26. Department of Immunology, School of Medicine, Trinity College Dublin, Ireland
27. Virology Unit, Institut Pasteur du Cambodge, Institut Pasteur International Network, Phnom Penh, Cambodia
28. Epidemiology of Emerging Diseases Unit, Department of Global Health, Institut Pasteur, Paris, France
29. Direction de la Recherche Médicale, Centre de Recherche Translationnelle, Institut Pasteur, Paris, France
30. Conservatoire National des Arts et Métiers, Paris, France.
31. Division of Population Health and Immunity, The Walter and Eliza Hall Institute, Melbourne, Australia
32. Department of Medical Biology, University of Melbourne, Melbourne, Australia
33. Vaccine Research Institute, Creteil, France

***Correspondence**

Dr Michael White (michael.white@pasteur.fr);

Infectious Disease Epidemiology and Analytics Unit,

Department of Global Health,

Institut Pasteur,

25-28 Rue du Docteur Roux,

Paris 75015,

France

Summary: Longitudinal follow-up of individuals infected with SARS-CoV-2 reveals differences in the duration of antibodies to multiple antigens. Mathematical models of antibody waning and statistical algorithms can estimate an individual's time since SARS-CoV-2 infection, and previous transmission waves in a population.

Abstract

Background

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) induces a complex antibody response that varies by orders of magnitude between individuals and over time.

Methods

We developed a multiplex serological test for measuring antibodies to five SARS-CoV-2 antigens and the Spike proteins of seasonal coronaviruses. We measured antibody responses in cohorts of hospitalized patients and healthcare workers followed for up to eleven months after symptoms. A mathematical model of antibody kinetics was used to quantify the duration of antibody responses. Antibody response data were used to train algorithms for estimating time since infection.

Results

One year after symptoms, we estimate that 36% (95% range: 11%, 94%) of anti-Spike IgG remains, 31% (9%, 89%) anti-RBD IgG remains, and 7% (1%, 31%) anti-Nucleocapsid IgG remains. The multiplex assay classified previous infections into time intervals of 0–3 months, 3–6 months, and 6–12 months. This method was validated using data from a sero-prevalence survey in France, demonstrating that historical SARS-CoV-2 transmission can be reconstructed using samples from a single survey.

Conclusions

In addition to diagnosing previous SARS-CoV-2 infection, multiplex serological assays can estimate the time since infection which can be used to reconstruct past epidemics.

Key words

SARS-CoV-2; sero-prevalence; antibody kinetics; surveillance; time since infection

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), has led to widespread morbidity and mortality since its emergence. The response to the SARS-CoV-2 pandemic is critically dependent on surveillance data, most notably numbers of COVID-19 associated hospital admissions and deaths recorded through health systems surveillance, and cases confirmed SARS-CoV-2 positive by PCR-based testing¹. Serology, based on the detection of antibodies induced by infection with SARS-CoV-2, represents another category of surveillance information^{2,3}. Appropriately designed sero-prevalence studies can provide estimates of the previously infected proportion of a population. Although no substitute for health systems surveillance, sero-prevalence studies have the advantage of accounting for asymptomatic cases, and symptomatic individuals who do not present to health systems. Sero-prevalence studies also provide information on the status of SARS-CoV-2 epidemics in situations where record keeping by health systems is not possible, a common challenge in many low and middle-income countries⁴.

Infection with SARS-CoV-2 induces diverse humoral and cellular immune responses^{5,6}. Humoral immunity includes antibodies of several immunoglobulin isotypes targeting SARS-CoV-2 proteins, most notably Spike (S) and Nucleocapsid (N). The concentration of antibodies in blood varies substantially between individuals, and with time since infection^{6,7,8,9,10,11}. Studies of the duration of immunity to SARS-CoV-1 demonstrated that antibodies remain detectable six years after infection¹². Longitudinal follow-up of individuals infected with SARS-CoV-2 indicates a pattern of waning antibody responses consistent with other coronaviruses^{13,14}. Within the first three months, antibody levels boost sharply and wane rapidly. Over a longer interval of eight months, antibody levels wane more slowly⁶. These observations can be explained by the bi-phasic nature of antibody kinetics¹⁵.

Serological diagnostics typically classify a sample as positive if a measured antibody level is greater than a defined cutoff. Analysis of quantitative rather than binary antibody levels provides additional information, for example antibody levels are associated with time since infection, symptom severity, and sex¹⁶. However, large variation in antibody levels between individuals prevents this from having predictive value: detected antibodies could be from a recent infection, or due to immunological memory of older infections. This limitation has recently been overcome for a range of pathogens through the combination of multiplex assays and classification algorithms. Using machine learning algorithms to analyse quantitative antibody responses to multiple antigens, the time since previous infection can be estimated for *Plasmodium falciparum* malaria^{17,18}, *P. vivax* malaria¹⁹, and cholera²⁰.

In this study, we use multiplex assays to measure antibodies to SARS-CoV-2 in healthcare workers and hospitalized patients followed for up to eleven months after infection, and apply mathematical models to characterize antibody kinetics in the first year following infection. Classification algorithms were developed that minimize the reduction in the sensitivity of serological tests over time, in addition to estimating time since previous infection from a single blood sample. Finally, we present a method for serological reconstruction of past SARS-CoV-2 transmission and validate its use with samples collected from a cross-sectional survey.

Methods

Samples

A panel of 407 negative control serum or plasma samples was assembled from pre-pandemic cohorts (before December, 2019) with ethical approval for broad antibody testing (Table 1). A panel of 407 positive control serum samples was assembled from individuals with recent SARS-CoV-2 infection. This included 72 samples from Paris hospitals that cared for patients with COVID-19^{21,22}; 161 samples from healthcare workers in Strasbourg University Hospital who developed symptomatic PCR-confirmed COVID-19²³; and 174 samples from community members of Crépy-en-Valois, France, collected between February and March 2020, and confirmed seropositive by flow cytometry based testing^{24,25}.

The duration of antibody responses following SARS-CoV-2 infection was studied in longitudinal cohorts of hospitalized patients and healthcare workers. 213 serum samples from 194 patients in Dublin hospitals were collected, with date post symptom onset extending up to four months. 724 serum samples from 347 healthcare workers in Strasbourg hospitals were collected, with date post symptom onset extending up to nine months²⁶.

In April 2020, our team implemented a study of the sero-prevalence of SARS-CoV-2 in healthcare workers from Institut Mutualiste Montsouris, a hospital in Paris. Serum samples were collected from 769 healthcare workers, and tested with our multiplex assay (Supplementary Figure 1). Healthcare workers who tested sero-positive in April 2020 were invited to present a second sample in January 2021. In total we obtained follow-up samples from 29 healthcare workers.

To demonstrate the serological reconstruction of past COVID-19 epidemics, 725 samples were collected from residents of the town of Crépy-en-Valois in France from 13th November to December 17th 2020. Past transmission levels estimated from serological data were validated using an independent source of data on ICU admissions for COVID-19 in Oise (the department containing the town of Crépy-en-Valois) extracted from the national public data on daily hospital admissions related

to Covid-19 published by Sante Publique France, available on data.gouv.fr (file downloaded 18th April 2021 at https://www.data.gouv.fr/fr/datasets/donnees-hospitalieres-relatives-a-lepidemie-de-covid-19/#_). Daily records cover the period from 18th March 2020 to 18th April 2021.

Serological assays

We established a 9-plex bead-based assay allowing simultaneous detection of antibodies to five SARS-CoV-2 antigens and four seasonal coronaviruses (Spike proteins of NL63, 229E, HKU1, OC43) in 1µL serum or plasma samples that were heat-inactivated at 56°C for 30 minutes. SARS-CoV-2 antigens were from Spike (whole trimeric Spike (S), its Receptor Binding Domain (RBD) and Spike subunit 2 (S2)), nucleocapsid protein (N) and a Membrane-Envelope fusion protein (ME). ME and S2 antigens were purchased from Native Antigen, Oxford, UK and all other antigens were produced as recombinant proteins at Institut Pasteur. The mass of proteins coupled on beads was optimized to generate a log-linear standard curve with a pool of 27 positive sera prepared from RT-qPCR-confirmed SARS-CoV-2 patients. We measured the levels of IgG, IgA and IgM of each sample in three separate assays. Briefly, serum was diluted 1/200 for IgG or 1/400 for IgA and IgM, and incubated with mixed antigen-coupled beads for 30 minutes. Secondary antibodies conjugated to R-phycoerythrin (Jackson ImmunoResearch) were used at 1/120, 1/200 or 1/400 for detection of specific IgG, IgA and IgM respectively. All dilutions and cycles of washing steps were done in phosphate buffer saline supplemented with 1% bovine serum albumin and 0.05% (v/v) Tween-20. On each assay plate, two blanks (only beads, no serum) were included to control for background signal as well as a standard curve prepared from two-fold serial dilutions (1/50 to 1/102,400) of a pool of positive controls. Plates were read using a Luminex® MAGPIX® system and the median fluorescence intensity (MFI) was used for analysis. A 5-parameter logistic curve was used to convert MFI to relative antibody unit (RAU), relative to the standard curve performed on the same plate to account for inter-assay variations. The data from our multiplex assay was compared against data from two different neutralization assays with live virus using a subset of serum samples²⁶ (Supplementary Methods).

Statistical methods

SARS-CoV-2 antibody kinetics are described using a previously published mathematical model of the immunological processes underlying the generation and waning of antibody responses following infection (Supplementary Methods)²⁷.

For the estimation of time since previous infection, measurements of antibodies of three isotypes (IgG, IgM, IgA) to multiple SARS-CoV-2 antigens were used to create a training dataset. Samples where the time post symptom onset was ≤ 14 days or unknown were excluded. In total we had 407 samples from pre-pandemic negative controls and 1402 positive samples. A Random Forests multiway classification algorithm was developed for categorizing samples into four classes: (i) negative; (ii) infected < 3 months ago; (iii) infected 3 – 6 months ago; and (iv) infected 6 – 12 months ago. The algorithm was calibrated to have 99% specificity for correctly classifying negative samples. Positive samples were classified according to the maximum number of votes. Uncertainty in classification performance was assessed via cross-validation with a training set comprising two thirds of the data and a disjoint testing set comprising a third of the data, repeated 1000 times. Classification algorithms were implemented in R (version 3.4.3).

Ethics

Serum samples were biobanked at the Clinical Investigation and Access to BioResources platform at Institut Pasteur (Paris, France). Samples were obtained from consenting individuals through the CORSER study (ClinicalTrials.gov Identifier: NCT04325646), directed by Institut Pasteur and approved by the Comité de Protection des Personnes Ile de France III (on February 19th 2020), and the French COVID cohort (ClinicalTrials.gov Identifier: NCT04262921), sponsored by Inserm and approved by the Comité de Protection des Personnes Ile de France VI. Samples from French blood donors were approved for use by Etablissement Français du Sang (Lille, France) and approved through the CORSER study by the Comité de Protection des Personnes Ile de France VI. Sample collection in Hôpital Cochin was approved by the Research Ethics Commission of Necker-Cochin Hospital. Samples from healthcare workers in Strasbourg University Hospitals followed longitudinally were collected as part of an ongoing clinical trial (ClinicalTrials.gov Identifier: NCT04441684) which received ethical approval from the Comité de Protection des Personnes Ile de France III. Samples collected from patients in Dublin received ethical approval for study from the Tallaght University Hospital (TUH)/St James's Hospital (SJH) Joint Research Ethics Committee (reference REC 2020-03). Use of the Peruvian negative controls was approved by the Institutional Ethics Committee from the Universidad Peruana Cayetano Heredia (SIDISI 100873). The Human Research Ethics Committee at the Walter and Eliza Hall Institute of Medical Research and the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand, approved the use of the Thai negative control samples. Informed written consent was obtained from all participants or their next of kin in accordance with the Declaration of Helsinki.

Results

SARS-CoV-2 antibodies over time

Antibodies of three isotypes (IgG, IgM, IgA) to nine coronavirus antigens were measured in 407 pre-pandemic serum samples, and 1402 serum samples from individuals with previous SARS-CoV-2 infection. 961/1402 of the positive samples were from individuals with SARS-CoV-2 infection confirmed by PCR-based testing with available data on time post symptoms. Figure 1 presents the IgG, IgM and IgA antibody responses to SARS-CoV-2 S, RBD, and N measured over time. Supplementary Figures 2 and 3 presents the antibody responses to S2, ME, and the Spike proteins of the four human seasonal coronaviruses. Notably there is substantial inter-individual variation in antibody responses, with antibody levels varying by orders of magnitude between individuals. As a measure of functional immunity, we applied live virus neutralizing assays to a subset of samples. There was substantial variation in neutralizing activity between individuals and over time (Supplementary Figure 4).

Modelled SARS-CoV-2 antibody kinetics

Our data on measured SARS-CoV-2 antibody responses were supplemented with data from six other longitudinal studies of the SARS-CoV-2 antibody response⁶⁻¹¹, and one longitudinal study of the SARS-CoV-1 antibody response¹². Supplementary Figure 5 provides a comparison of the measured antibody responses between the eight studies. The data were rescaled so that the mean antibody response for each study equals one at day 14 after symptom onset. In addition to the large inter-individual variation, there is notable variation in antibody levels between studies. A mathematical model of antibody kinetics was simultaneously fitted to data from all eight studies. Supplementary Figure 6 provides an overview of the model fit to the data. Figure 2a provides example fits to data from one representative individual from each of the eight studies. Figure 2b provides model predictions of the percentage antibody level remaining over the first two years post infection, where 100% is assumed to be the antibody response at day 14 following symptom onset. There are considerable differences in the pattern of waning between isotypes and between antigens. Table 2 summarises the duration of the antibody response. One year following symptom onset, we estimate that 36% (95% range: 11%, 94%) anti-S IgG remains, 31% (9%, 89%) anti-RBD IgG remains, and 7% (1%, 31%) anti-N IgG remains. The uncertainty represents the considerable degree of inter-individual variation in the duration of the antibody responses. Antibodies of the IgM isotype waned more rapidly, with 6% (0%, 27%) anti-S IgM remaining after one year, 9% (2%, 32%) anti-RBD IgM

remaining after one year, and 15% (4%, 50%) anti-N IgM remaining after one year. Antibodies of the IgA isotype also waned rapidly, with 18% (4%, 67%) anti-S IgA remaining after one year, 10% (3%, 38%) anti-RBD IgA remaining after one year, and 3% (0%, 13%) anti-N IgA remaining after one year.

We also observed comparable reductions in titres for viral neutralization over time (Supplementary Figure 4). The small number of samples prevented application of the antibody kinetic model to viral neutralization data, but measuring the reduction in paired samples we estimate a rate of decrease of -0.94 (inter-quartile range (IQR): $-2.41, 0.26$) year^{-1} . Assuming simple exponential decay in neutralization activity over time, this corresponds to 39.3% (IQR: 8.9%, 125.2%) neutralizing activity remaining after one year, broadly consistent with the long-term kinetics of anti-RBD IgG.

Estimating time since previous SARS-CoV-2 infection

Using a dataset on measured IgG, IgM and IgA antibody levels from pre-pandemic negative controls and samples from individuals with SARS-CoV-2 infection confirmed by PCR-based testing and followed for up to eleven months after symptom onset, Random Forests binary classification algorithms were trained to identify individuals with previous SARS-CoV-2 infection (Supplementary Figures 7 and 8). Next, a Random Forests multi-way classification was trained to simultaneously identify previous infection and estimate the time since infection (Figure 3). The diagnostic algorithm identified samples from individuals with previous SARS-CoV-2 infection with 99% specificity and 98% (95% confidence interval: 94%, 99%) sensitivity (Figure 3a). The diagnostic test classified samples from individuals infected within the previous 3 months (Figure 3b). It was easier to distinguish recent infections (<3 months) from older infections (6 – 12 months), compared to infections that occurred 3 – 6 months ago. There was limited statistical signal to distinguish between infections that occurred 3 – 6 months ago, and older infections occurring more than 6 months ago (Figure 3c). A breakdown of classification performance by time since infection is provided in Figure 3D. The diagnostic test had predictive power for samples of all categories, with the exception of samples from individuals infected 3 – 6 months ago. Many of these samples were incorrectly classified in the neighbouring infection categories of 0 – 3 months or 6 – 12 months.

Serological reconstruction of past COVID-19 transmission in Oise Department, France

One of the first reported clusters of SARS-CoV-2 transmission in France occurred in Crépy-en-Valois in Oise Department from late February to early March 2020²⁴. A team of scientists led by Institut

Pasteur are conducting repeat cross-sectional surveys in this community. 725 serum samples collected between November 13th and December 17th 2020 were analyzed with our serological assay. 34.6% (251/725) of samples were sero-positive indicating previous infection with SARS-CoV-2.

Statistical algorithms for estimation of time since infection were applied to data from the 251 sero-positive individuals. We estimated that 80.2% (95% CI: 47.3%, 94.5%) were infected in the six months from December 2019 to May 2020, 0.0% (95% CI: 0.0%, 52.1%) were infected in the three months from June to August, and 12.6% (95% CI: 0.0%, 29.2%) were infected in the three months from September to November (Figure 4). The serological estimates are consistent with a large first wave before June 2020, and a smaller second wave (or the beginning of a larger one) between September and December 2020. To assess the validity of these estimates, we compared with data on reported intensive care unit (ICU) admissions in Oise Department between March 18th 2020 and December 1st 2020. Of the ICU admissions, 56.0% were reported in the three months from March to May, 8.7% were reported in the three months from June to August, and 35.3% were reported in the three months from September to November.

Discussion

Based on data from a range of studies with up to eleven months follow up after symptom onset, we estimate that 31% (95% CrI: 9%, 89%) of anti-RBD IgG antibody levels remain one year after infection. Antibodies of other isotypes waned more rapidly, with 9% (95% CrI: 2%, 32%) of anti-RBD IgM antibody levels remaining after one year, and 10% (95% CrI: 3%, 38%) of anti-RBD IgA antibody levels remaining after one year. There was considerable variation in kinetic profiles between different SARS-CoV-2 antigens, with 7% (95% CrI: 1%, 31%) of anti-N IgG antibody levels remaining after one year. Although the determinants of the duration of antigen-specific antibody responses remain poorly understood¹⁶, the diversity in patterns of antibody kinetics can be quantified using epidemiological studies, yielding valuable information for serological diagnostics.

The majority of commercially available serological tests classify individuals as having previous SARS-CoV-2 infection if a measured antibody response is greater than a defined cutoff^{28,29}. Instead of reducing a complex antibody response to a binary data point, a more detailed serological signature based on quantitative measurements of multiple antibody responses provides two notable advantages⁵. Firstly, the reduction in diagnostic sensitivity associated with waning antibodies is minimized – no reduction in sensitivity over the eleven months of follow up was observed. Secondly, the time since previous infection can be estimated providing additional epidemiological information.

For the current assay, time since previous infection was categorised into intervals of 0 – 3 months, 3 – 6 months, and 6 – 12 months. More precise classification is possible in theory, but this must be balanced against the statistical signal. For example, there was little statistical signal to discriminate between infections that occurred 6 – 9 months ago, versus infections that occurred 9 – 12 months ago. It is anticipated that further assay improvements such as the incorporation of new antigens, more training samples with a range of time since infection, and better algorithms will lead to improvements in accuracy.

Existing sero-prevalence studies estimate the proportion of a population previously infected with SARS-CoV-2. The addition of a diagnostic tool capable of estimating time since infection allows for the serological reconstruction of past incidence trends. Thus, using samples from a sero-prevalence study collected at a single time point, we can discriminate between a scenario of constant SARS-CoV-2 transmission and a scenario where transmission occurs in distinct epidemic waves. This diagnostic technology has a range of possible applications. For countries that experienced double wave SARS-CoV-2 epidemics, it has been challenging to quantify the relative magnitude of these waves due to the challenges of implementing PCR-based diagnostic testing³⁰. Furthermore, there are many epidemiological settings where it is unknown if SARS-CoV-2 transmission was constant over time, or occurred as distinct epidemic waves⁴.

Estimates of time since previous SARS-CoV-2 infection in individual samples can be combined to provide a serological reconstruction of previous SARS-CoV-2 transmission in a population. Following validation of our statistical method on simulated data, we demonstrated that our test could accurately identify the two epidemic peaks in the town of Crépy-en-Valois using samples collected in a single cross-section. This finding was consistent with daily reported ICU admissions data collected continuously over one year in the Oise Department. The minor differences between the serologically reconstructed epidemic profile and that from public health surveillance data may be because the town of Crépy-en-Valois is not necessarily representative of the wider Oise Department, as the town was specifically targeted by the study team because of its early levels of SARS-CoV-2 transmission. Furthermore, we did not analyse data on COVID-19 specific ICU admissions before March 2020, and ICU admissions may not necessarily be representative of community level infection events³¹.

There are several limitations to this study. Estimates of the duration of antibody responses are based on data from multiple studies, each using a unique immunoassay^{6,7,8,9,10,11,12}. Every immunoassay may differ in terms of background reactivity, cross-reactivity with other pathogens, protein formulation, dynamic range and reproducibility. We believe that the benefit of drawing on multiple data sources outweighs the benefit of having a smaller more homogeneous database, especially

since the mathematical model of antibody kinetics is sufficiently flexible to incorporate data from multiple assays. Our selection of a mechanistic mathematical model of antibody kinetics is a potential limitation. The model is based on a mechanistic understanding of the immunological processes underlying the generation and persistence of antibodies, and imposes a flexible functional form on how antibody levels change over time. Although this approach has been validated in a range of applications^{16,17,18,19}, there will be instances where the model fails to capture the true pattern of antibody kinetics, for example in immune-deficient individuals. An advantage of a mechanistic model versus a non-parametric statistical model is the ability to make projections forward in time. We have provided predictions up to two years following infection, for example, by estimating that 16% (5%, 48%) of anti-RBD IgG antibodies remain after two years. There is a risk to providing predictions beyond the timescale of the data, but these predictions can be easily falsified via continued longitudinal studies.

Sero-prevalence studies are playing a critical role in monitoring the progress of the SARS-CoV-2 pandemic. In the early stages of the pandemic, immunoassays had the advantage of measuring high antibody levels in the initial months after infection. As the pandemic progresses, sero-prevalence will become more challenging to accurately measure due to waning antibody responses and increased vaccine-induced immunity. Multiplex assays and algorithms accounting for how antibody levels change over time may be an important tool for ensuring the ongoing utility of sero-surveillance.

Declaration of interests

MTW, IM, JR, SPel, MB, and SPet are inventors on provisional patent PCT/US 63/057.471 on a serological antibody-based diagnostics of SARS-CoV-2 infection. TB and OS are coinventors on provisional patent PCT/US 63/020,063 entitled “S-Flow: a FACS-based assay for serological analysis of SARS-CoV-2 infection” submitted by Institut Pasteur. SK reports personal fees from Accelerate Diagnostics, Astellas, Merck Sharp & Dohme, Pfizer, and Menarini, and grants and personal fees from bioMérieux, outside of the submitted work.

Acknowledgements

The French COVID cohort is supported by the REACTing consortium and by the French Directorate General for Health. Jessica Vanhomwegen (Institut Pasteur) is thanked for provision of a MAGPIX

machine. Jérôme Hadjadj and Laura Barnabei (Institut Imagine, Paris) are thanked for their work on the Hôpital Cochin study. Dionicia Gamboa (Cayetano Heredia University, Lima) is thanked for sharing negative control samples from Peru. Jetsumon Sattabongkot (Mahidol University, Bangkok) is thanked for sharing negative control samples from Thailand. Marie-Noelle Ungeheuer and Blanca Liliana Perlaza are thanked for processing samples at the Clinical Investigation and Access to BioResources platform in Institut Pasteur. We thank all patients and healthcare workers who kindly agreed for samples to be used for medical research purposes.

Data availability

All data and code used for reproducing the results is freely available on GitHub:

https://github.com/MWhite-InstitutPasteur/SARSCoV2_AB_kinetics.

Funding

This work was supported by the European Research Council (MultiSeroSurv ERC Starting Grant 852373; MW), l'Agence Nationale de la Recherche and Fondation pour la Recherche Médicale (CorPopImm; MW), and the Institut Pasteur International Network (CoronaSeroSurv; MW). JR was supported by the Pasteur Paris University (PPU) International PhD Program. CC was supported by the European Research Council 771813. MB, AL and HA were supported by the « URGENCE COVID-19 » fundraising campaign of Institut Pasteur. HA is supported by Centre for International Migration and Development. NC and CNC are part-funded by a Science Foundation Ireland (SFI) grant, Grant Code 20/SPP/3685. LT has been awarded the Irish Clinical Academic Training (ICAT) Programme, supported by the Wellcome Trust and the Health Research Board (Grant Number 203930/B/16/Z), the Health Service Executive, National Doctors Training and Planning and the Health and Social Care, Research and Development Division, Northern Ireland (<https://icatprogramme.org/>). MB and AM were supported by Institut Pasteur TaskForce funding (TooLab project). IM is supported by a NHMRC Principal Research Fellowship. SFK lab is funded by Strasbourg University Hospitals (SeroCoV-HUS; PRI 7782), the Agence Nationale de la Recherche (ANR-18-CE17-0028), Laboratoire d'Excellence TRANSPLANTEX (ANR-11-LABX-0070_TRANSPLANTEX), and Institut National de la Santé et de la Recherche Médicale (UMR_S 1109).

Table legends

Table 1: Panels of samples. Age and days post symptom onset are presented as median and ranges.

NA = not available.

Table 2: Estimated duration of antibody responses following SARS-CoV-2 infection. The percentage antibody level remaining over time is compared to the measured antibody level 14 days after symptom onset. Estimates are presented as the population median, with the 95% range due to inter-individual variation.

Figure legends

Figure 1: Antibody kinetics in the first year following infection with SARS-CoV-2. A bead-based multiplex Luminex assay used to measure antibodies of multiple isotypes (IgG, IgM, IgA) to multiple antigens in serum samples from individuals with PCR-confirmed SARS-CoV-2 infection and pre-pandemic negative controls.

Figure 2: Modelled SARS-CoV-2 antibody kinetics. A mathematical model of SARS-CoV-2 antibody kinetics was simultaneously fitted to data from seven studies of SARS-CoV-2 and one study of SARS-CoV-1 (Tang *et al.*). **(A – top row)** Examples of the model fit to the data for one individual from each study. Data are represented as points, posterior median model prediction as lines, and 95% credible intervals as shaded areas. **(B – middle and bottom rows)** Model predicted duration of antibodies within the first 2 years following infection. Antibody levels are shown relative to the expected antibody level at day 14 following symptom onset. Each point represents the prediction from an individual at 6, 12, 18 and 24 months post symptoms. The median predictions for each of the eight studies are presented as lines.

Figure 3: Classification of time since previous SARS-CoV-2 infection. A cross-validated multi-way classification algorithm was trained to estimate time since infection. **(A)** The algorithm can differentiate between positive and negative samples. **(B)** The algorithm can classify individuals infected within the previous 3 months. **(C)** There is limited diagnostic power to distinguish between infections that occurred 3-6 months ago versus 6-12 months ago. **(D)** Breakdown of classification performance according to time since previous infection. Colours represent model predicted classification. >99% of negative samples are correctly classified as negative (blue). For the positive samples, the distribution shows the time since previous infection. Samples with time since infection <3 months are mostly classified in the 0 – 3 month category (red). Samples with time since infection >6 months ago are mostly classified in the 6 – 12 month category (purple). There is a substantial degree of misclassification of samples with time since infection 3 – 6 months ago. This is due to the temporal imbalance in the training data.

Figure 4: Serological reconstruction of past COVID-19 transmission in Oise Department. **(A)** Of 725 samples collected from residents of the Oise Department in France between November 13th and December 17th 2020, 65% (474/725) were SARS-CoV-2 sero-negative. For the 251 sero-positive individuals, we estimated that 80.2% (95% CI: 47.3%, 94.5%) were infected in the 6 months from December 2019 to May 2020 0.0% (95% CI: 0.0%, 52.1%) were infected in the 3 months from June to August, and 12.6% (95% CI: 0.0%, 29.2%) were infected in the 3 months from September to November. Proportions are presented as median estimates and do not necessarily sum to 100%. **(B)** Reported intensive care unit (ICU) admissions in Oise Department between March 18th 2020 and Dec 1st 2020. Of the ICU admissions 56.0% were reported in the 3 months from March to May, 8.7% were reported in the 3 months from June to August, and 35.3% were reported in the 3 months from September to November.

References

1. Salje H, Tran Kiem C, Lefrancq N, Courtejoie N, Bosetti P, *et al.* Estimating the burden of SARS-CoV-2 in France. *Science*. 2020; 369(6500): 208-211
2. Le Vu S, Jones G, Anna F, Rose T, Richard JP, *et al.* Prevalence of SARS-CoV-2 antibodies in France: results from nationwide serological surveillance. *medRxiv*. 2020; <https://doi.org/10.1101/2020.10.20.20213116>
3. Ward H, Cooke G, Atchison C, Whitaker M, Elliott, *et al.* Declining prevalence of antibody positivity to SARS-CoV-2: a community study of 365,000 adults. *medRxiv*. 2020; <https://doi.org/10.1101/2020.10.26.20219725>
4. Kagucia EW, Gitonga JN, Kalu C, Ochomo E, Ochieng B, *et al.* Seroprevalence of anti-SARS-CoV-2 IgG antibodies among truck drivers and assistants in Kenya. *medRxiv*. 2020; <https://doi.org/10.1101/2021.02.12.21251294>
5. Rosado J, Pelleau S, Cockram C, Merkling SH, Demeret C, *et al.* Multiplex assays for the identification of serological signatures of SARS-CoV-2 infection: an antibody-based diagnostic and machine learning study. *Lancet Microbe*. 2020; 2(2): E60-E69
6. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, *et al.* Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science*. 2021; 371(587)
7. 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; S1473-3099(20)30196-1.
8. Iyer AS, Jones FK, Nodoushanian A, Kelly M, Beckera M, *et al.* Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Science Immunol*. 2020; 10.1126/sciimmunol.abe0367
9. Isho B, Abe KT, Zuo M, Jamal AJ, Rathod B, *et al.* Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Science Immunol*. 2020; 10.1126/sciimmunol.abe5511
10. Röltgen K, Powell AE, Wirz OF, Stevens BA, Hogan CA, *et al.* Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. *Science Immunol*. 2020; 10.1126/sciimmunol.abe0240
11. Seow J, Graham C, Merrick B, Acors S, Pickering S, *et al.* Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbio*. 2020; 5: 1598-1607
12. Tang F, Quan Y, Xin ZT, Wrammert J, Ma MJ, Lv H, *et al.* Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. *J Immunol*. 2011; 186 (12) 7264-7268
13. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. *Epidemiol Infect*. 1990; 105(2):435-46.
14. Choe PG, Perera RAPM, Park WB, Song KH, Bang JH, Kim ES, *et al.* MERS-CoV antibody responses 1 year after symptom onset, South Korea, 2015. *Emerg Infect Dis*. 2017; 23(7):1079-1084.
15. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. *Immunity*. 1998; 8: 363-72

16. Sceanovic P, Alanio C, Hammer C, Hodel F, Bergstedt J, *et al.* Human genetic variants and age are the strongest predictors of humoral immune responses to common pathogens and vaccines. *Genome Med.* 2018; 10(59)
17. Helb DA, Tetteh KK, Felgner PL, Skinner J, Hubbard A, Arinaitwe E, *et al.* Novel serologic biomarkers provide accurate estimates of recent *Plasmodium falciparum* exposure for individuals and communities. *Proc Natl Acad Sci USA.* 2015; 112(32):E4438-47
18. Yman V, Tuju J, White MT, *et al.* Distinct kinetics in antibody responses to 111 *Plasmodium falciparum* antigens identifies novel serological markers of recent malaria exposure. *medRxiv.* 2020; <https://doi.org/10.1101/2020.12.14.20242768>
19. Longley RJ, White MT, Takashima E, Brewster J, Morita M, Harbers M, *et al.* Development and validation of serological markers for detecting recent exposure to *Plasmodium vivax* infection. *Nature Med.* 2020; 26: 741-749
20. Azman AS, Lessler J, Luquero FJ, Bhuiyan TR, *et al.* Estimating cholera incidence with cross-sectional serology. *Sci Transl Med.* 2019; 11(480)
21. Lescure FX, Bouadma L, Nguyen D, Parisey M, Wicky PH, *et al.* Clinical and virological data of the first cases of COVID-19 in Europe: a case series. *Lancet Inf Dis.* 2020; 20(6): 697-706
22. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, *et al.* Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science.* 2020; 369(6504): 718-724
23. Fafi-Kremer S, Bruel T, Madec Y, Grant R, Tondeur L, *et al.* Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France. *EBioMed.* 2020; 59:102915
24. Grzelak L, Temmam S, Planchais C, Demeret C, Tondeur L, *et al.* A comparison of four serological assays for detecting anti-SARS-CoV-2 antibodies in human serum samples from different populations. *Sci Transl Med.* 2020; 12(559):eabc3103
25. Dufloo J, Grzelak L, Staropoli I, Madec Y, Tondeur L, *et al.* Asymptomatic and symptomatic SARS-CoV-2 infections elicit polyfunctional antibodies. *medRxiv.* 2020; <https://doi.org/10.1101/2020.11.12.20230508>
26. Planas D, Bruel T, Grzelak L, Guivel-Benhassine F, Staropoli I, *et al.* Sensitivity of infectious SARS-CoV-2 B.1.1.7 and B.1.351 variants to neutralizing antibodies. *medRxiv.* 2021; <https://doi.org/10.1101/2021.02.12.430472>
27. White MT, Griffin JT, Akpogheneta O, *et al.* Dynamics of the antibody response to *Plasmodium falciparum* infection in African children. *J Infect Dis* 2014; 210: 1115–22.
28. National SARS-CoV-2 Serology Assay Evaluation Group. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. *Lancet Inf Dis.* 2020; 20(12): 1390-1400
29. Peluso MJ, Takahashi S, Hakim J, Kelly JD, Torres L, *et al.* SARS-CoV-2 antibody magnitude and detectability are driven by disease severity, timing, and assay. *medRxiv.* 2021; doi: <https://doi.org/10.1101/2021.03.03.21251639>
30. Pullano G, Di Domenico L, Sabbatini CE, Valdano E, Turbelin C, *et al.* Underdetection of cases of COVID-19 in France threatens epidemic control. *Nature.* 2021; 590:134-139
31. Hozé N, Paireau J, Lapidus N, Tran Kiem C, Salje H, *et al.* Monitoring the proportion of the population infected by SARS-CoV-2 using age-stratified hospitalisation and serological data: a modelling study. *Lancet Public Health.* 2021; S2468-2667 (21) 00064-5.

Table 3: Panels of samples.

| cohort | status | participants | samples | PCR positive | age (years) | sex (% male) | days post symptoms |
|--|--|--------------|---------|--------------|-------------|--------------|--------------------|
| Établissement Français du Sang 1 | negative panel: pre-pandemic controls | 45 | 45 | | > 18 | | |
| Établissement Français du Sang 2 | negative panel: pre-pandemic controls | 213 | 213 | | 42 (18,81) | 40% | |
| Thai Red Cross | negative panel: pre-pandemic controls | 68 | 68 | | > 18 | | |
| Peruvian donors | negative panel: pre-pandemic controls | 81 | 81 | | > 18 | | |
| Hôpital Bichat (Paris, France) | positive panel: hospitalized patients | 2 | 8 | 2 | 31 (30, 32) | 100% | 14 (8, 24) |
| Hôpital Cochin (Paris, France) | positive panel: hospitalized patients | 64 | 64 | 64 | 55 (25, 79) | 76% | 17 (10, 28) |
| Strasbourg hospitals 1 | positive panel: infected healthcare workers | 161 | 161 | 161 | 32 (20, 62) | 31% | 24 (13, 39) |
| Crépy-en-Valois, February – March 2020 | positive panel: infected community members (flow cytometry positive) | 154 | 174 | 0 | 17 (15, 56) | 34% | |
| Dublin hospitals | hospitalized patients | 194 | 213 | 194 | 55 (21, 92) | 47% | 13 (1, 126) |
| Strasbourg hospitals 2 | follow-up of infected healthcare workers | 347 | 724 | 347 | 41 (21,74) | 23% | 132 (11, 284) |
| Institut Mutualiste Montsouris (Paris, France) | sero-prevalence survey in healthcare workers (unknown status) | 769 | 769 | 20 | 41 (18, 72) | 27% | |
| Institut Mutualiste Montsouris (Paris, France) | follow-up of sero-positive healthcare workers | 29 | 29 | 12 | 37 (24, 63) | 41% | 304 (285, 336) |
| Crépy-en-Valois, November – December 2020 | sero-prevalence survey in community members (unknown status) | 725 | 725 | NA | NA | NA | NA |

Age and days post symptom onset are presented as median and ranges. NA = not available.

Table 2: Estimated duration of antibody responses following SARS-CoV-2 infection.

| | 6 months | 12 months | 24 months |
|------------------|-----------------|------------------|------------------|
| Spike IgG | 55% (16%, 100%) | 36% (11%, 94%) | 16% (5%, 55%) |
| RBD IgG | 43% (13%, 100%) | 31% (9%, 89%) | 16% (5%, 48%) |
| Nucleocapsid IgG | 30% (8%, 92%) | 7% (1%, 31%) | 0.8% (0%, 7%) |
| Spike IgM | 12% (1%, 52%) | 6% (0%, 27%) | 2% (0%, 9%) |
| RBD IgM | 16% (4%, 51%) | 9% (2%, 32%) | 4% (1%, 16%) |
| Nucleocapsid IgM | 23% (6%, 75%) | 15% (4%, 50%) | 7% (2%, 24%) |
| Spike IgA | 21% (4%, 82%) | 18% (4%, 67%) | 12% (3%, 47%) |
| RBD IgA | 12% (4%, 49%) | 10% (3%, 38%) | 6% (2%, 24%) |
| Nucleocapsid IgA | 6% (1%, 30%) | 3% (0%, 13%) | 0.6% (0%, 4%) |

The percentage antibody level remaining over time is compared to the measured antibody level 14 days after symptom onset. Estimates are presented as the population median, with the 95% range due to inter-individual variation.

Figure 1

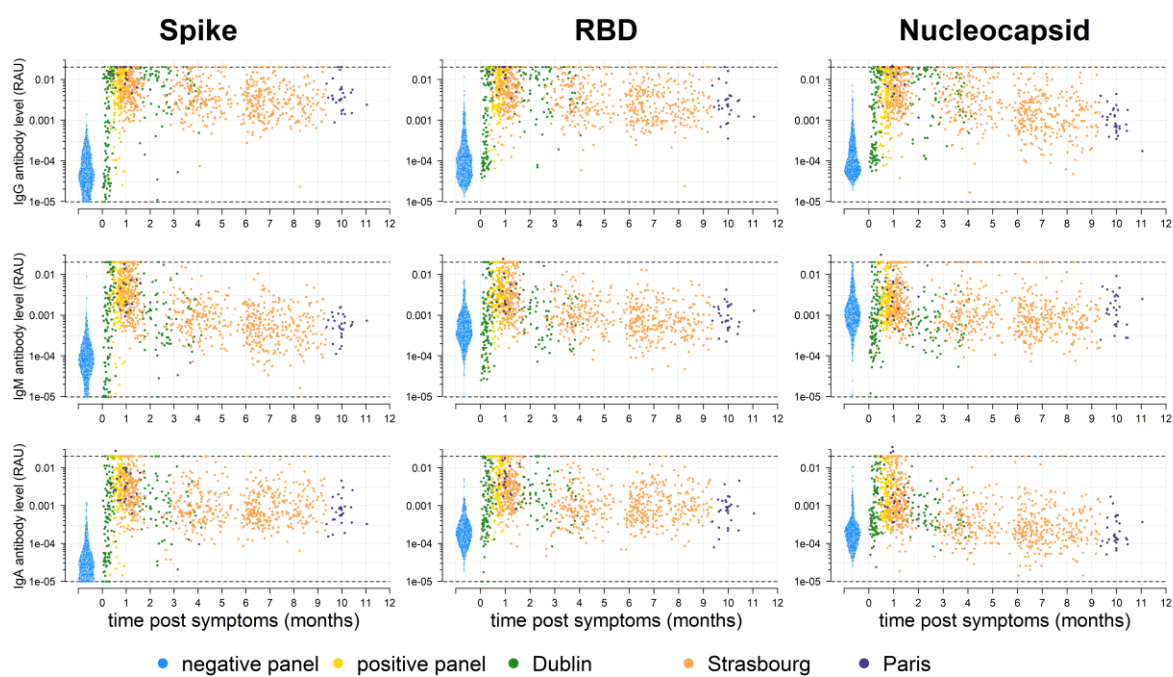


Figure 2

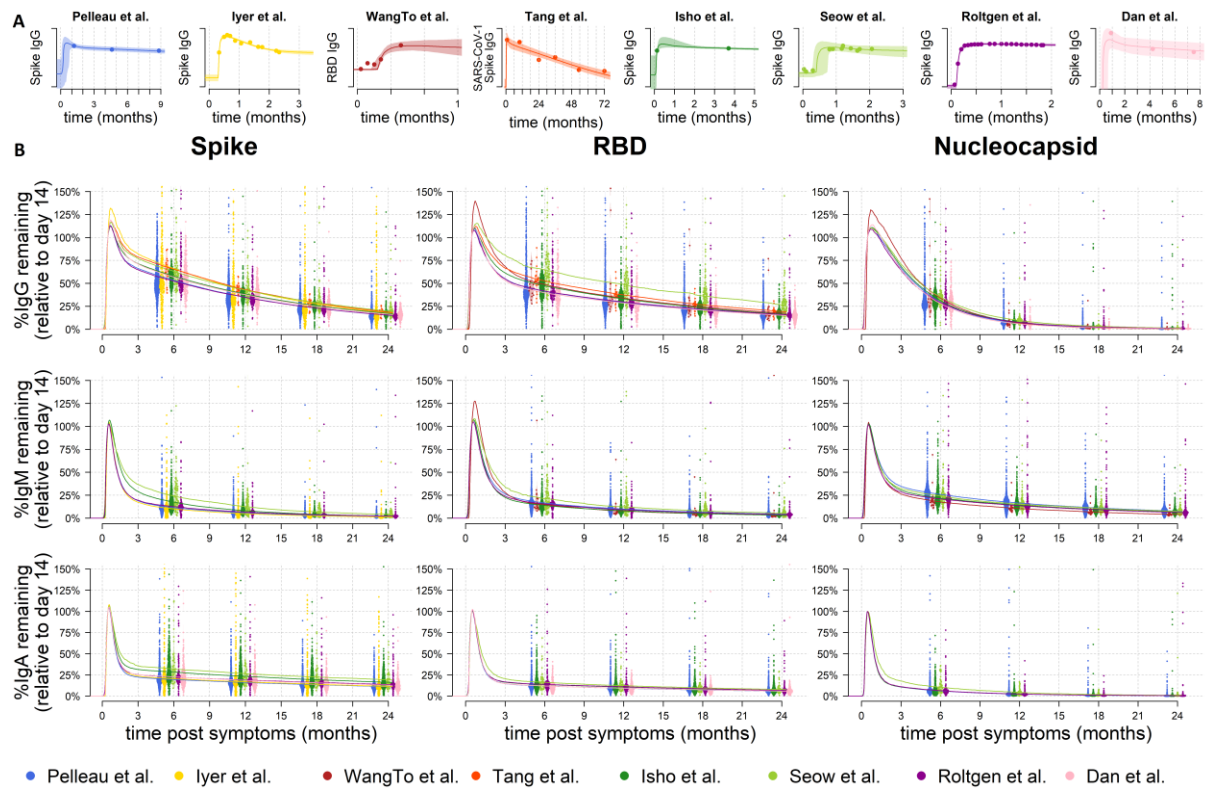


Figure 3

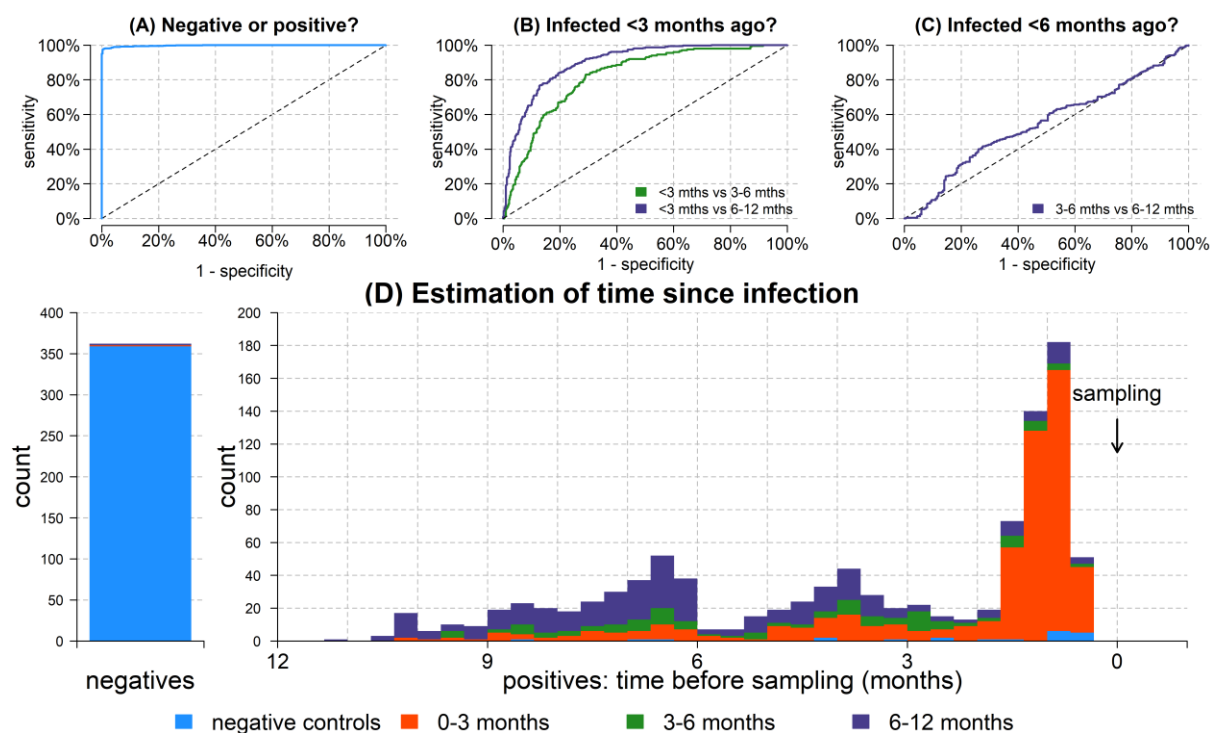
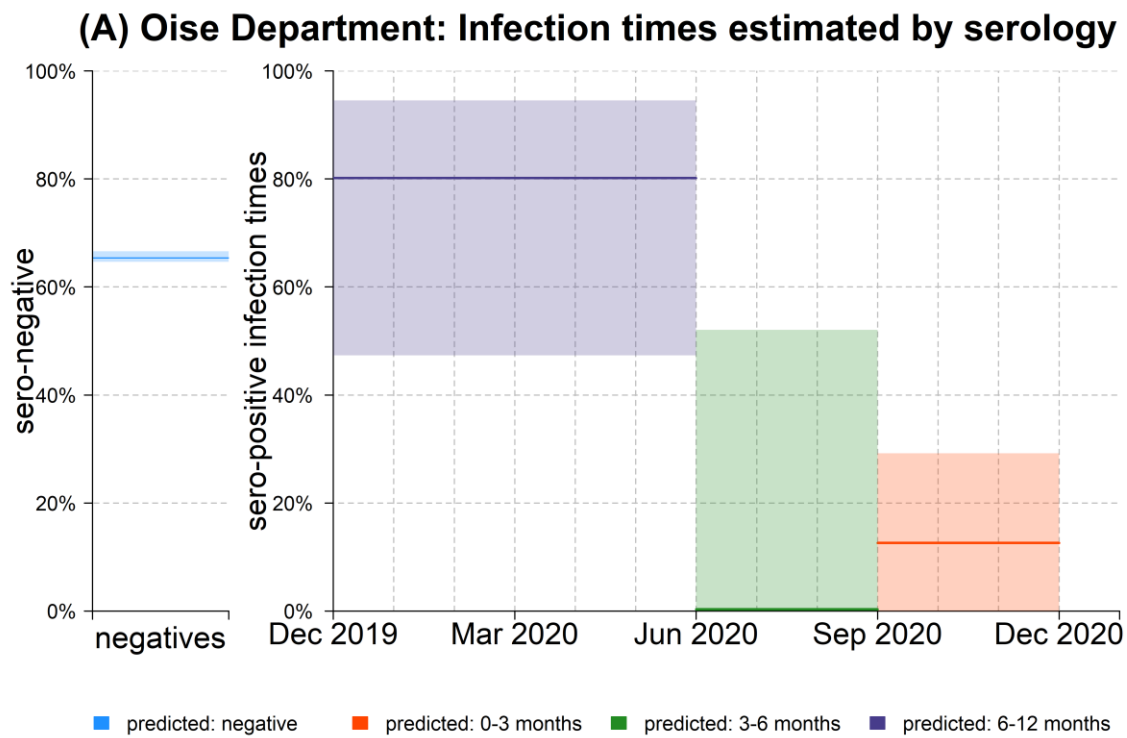


Figure 4



(B) Oise Department: Daily ICU admissions

