The dynamics of the humoral immune response following SARS-CoV-2 infection and the potential for reinfection.

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Purpose and recommendations

This document summarises knowledge about the antibody response to human coronavirus infections, and catalogues recent insights into SARS-CoV-2 serology, including non-peer reviewed studies, in humans and non-human primates. Its purpose is to provide a framework for the interpretation of serological testing during the current COVID19 pandemic, and to consider the potential for reinfection of individuals by SARS-CoV-2.

Top line messages:

1. **Most patients infected with SARS CoV2 mount an antibody response at 10-14 days after clinical infection, but a small proportion (30% in one recent study, often after mild disease) show a later response (first detected at 28 days) or no antibody response at all.**
2. **Low or absent immune response might be partly explained by poor sensitivity (70%) of the tests being employed, but nonetheless there is considerable variation in the amount of antibody produced by different individuals after infection.**
3. **There is no data in the public domain about how long antibody responses last after SARS CoV-2 infection, beyond about 2 weeks after recovery.**
4. **A single animal study (NHP) shows that antibody protected from reinfection with SARS CoV-2 at 28 days after infection and this supports antibody as a correlate of immunity.**
5. **Based on literature for other coronaviruses, mild infections can result in low antibody responses that wane over the months after infection.**
6. **People who have experienced mild infection with SARS-CoV2 may mount weak antibody responses, making it difficult to detect them using serological assays and such low responses may wane over months allowing them to be reinfected in a second wave.**

Recommendations:
1. **Longitudinal serology studies are urgently required to understand whether antibody to SARS CoV-2 will wane and over what time scale, especially from mild cases.**
2. **If immunity passports were issued to allow key workers to return to work, frequent (monthly) retesting would be important to ascertain antibody levels were maintained over time.**
3. **Seroepidemiology should take into account low sensitivity and slow time course of the antibody response when serological tests are used to detect mild cases.**

Supporting assessment of current knowledge about humoral responses to coronavirus infection.

Serological decline after MERS CoV and SARS CoV infection
A few studies have assessed antibody titres to MERS CoV and SARS CoV in the months and years following primary infection. Robust immune responses with long lived (> 1 year) functional antibodies were seen following severe MERS CoV infections or those with prolonged virus shedding (Choe et al. 2017; Alshukairi et al. 2016). This was also observed in a small study of MERS CoV, where neutralizing antibodies, were detectable in 6 (86%) of 7 persons who had previously had severe MERS (including 5 with pneumonia) for at least 34 months after infection. However, in this small group there was evidence of antibody waning; 4/7 showed 4 to 16-fold reduction in nucleocapsid binding titres and 4/7 show a 2 fold reduction in neutralising titres over 34 months, with 4/7 assessed as having a low neutralising tires throughout (Payne et al. 2016). After mild or asymptomatic MERS CoV infections, antibody responses were either limited or rapidly declined (Choe et al. 2017; Okba et al. 2019). Although numbers are small, no neutralizing antibody response was seen in 4/6 and 2/3 mild MERS CoV longitudinal samples either within three months and in some cases, not even immediately after infection (Choe et al. 2017; Alshukairi et al. 2016). In a separate study of 280 contacts of 26 confirmed MERS CoV index cases, 12 contacts likely to have been infected were identified. 7/12 contacts sampled within 4-14 days of index contact were virus genome positive by RT-PCR but serologically negative (actively infected), whereas 5/7 were virus genome negative, but had detectable binding and neutralising antibody titres (infected and recovered) (Drosten et al. 2014).

Similarly, although SARS CoV was largely associated with symptomatic disease, antibodies decline over time. In a 3-year follow-up of hospitalised SARS CoV patients, SARS CoV IgG binding titres were undetectable in 19.4% of people by 30 months post infection and neutralizing tires were undetectable in 11.1% of people at this time (Cao et al. 2007). Consistent with this observation, a study of 98 SARS patients over 2 years showed all had detectable antibody binding titres over 2 years but that, in a subset, titres declined over this period. 18 individuals with neutralizing antibodies had titres that peaked on day 30, then decayed gradually so that by 2 years 1/18 had no detectable neutralizing antibodies, and the remaining patients had a low antibody titres close to background levels (Mo et al. 2006). Similarly, in a study following 176 previously SARS CoV infected people, the ELISA optical densities (ODs) that indicate antibody titre reduced by 33% within one year, 46% by 2 years and ~75% by 3 years (Wu et al. 2007).

In summary, studies of MERS and SARS CoV indicate total binding antibodies and neutralising antibodies decrease to a level where by 2-3 years everyone previously infected will have minimal detectable antibody response, but with those suffering more severe disease having the highest titre responses for longer. Although the time dependent decay of neutralizing antibody titers implies a lack of protection from reinfection, this cannot be concluded unequivocally, due to the possibility of other protective mechanisms, perhaps from disease rather than infection, through other arms of the immune response (memory and cytotoxic T cells). It is, however, suggestive of a population level reduced protection from reinfection by epidemic CoVs over a short period of time.

Seroconversion rates to seasonal human coronaviruses
One indication of the strength of immune protection from coronavirus infection is to consider what is known for the endemic seasonal CoVs, namely the genetically related alphacoronaviruses NL63 and 229E, and the genetically related betacoronaviruses HKU1 and OC43. There is some evidence for antigenic cross protection between the Human CoVs in the same genetic group (see below). A cross sectional seroprevalence study for seasonal human alphacoronaviruses NL63 or 229E, showed 75% and 65% of children in the age group 2.5-3.5 years are seropositive for NL63 and 229E

respectively, and most children are seropositive by 6 years (Dijkman et al. 2008). In adults, respiratory infection by human seasonal CoVs accounted for 22% (43/195) (Gorse et al. 2020) and 25% (Ambrosioni et al. 2014) acute respiratory illness. The ability of human seasonal coronaviruses to infect adults who have likely been infected as children can be accounted for either by:

a) virus escape from neutralization (drift),
b) reinfection with a heterologous CoV of a different genotype (alpha followed by betacoronavirus infections, or vice versa) due to lack of cross protective antibodies,
c) reinfection with homologous coronavirus due to sub-protective/waning antibody responses.

The lack of extensive time resolved virus genetic data and a lack of extensive serology studies against extant and historic strains of the 4 seasonal coronaviruses makes the contribution of virus genetic drift to escape from pre-existing protective immune response difficult to judge. One paper describes genetic drift mapping to sugar binding domains in S protein of CoV OC43 suggesting drift may account for persistence of this genotype in the human population (Ren et al. 2015). Similar studies on other genotypes are lacking.

In the absence of drift, bearing in mind we only identify 4 genotypes of CoV endemic in humans and estimate they account for 20% clinical colds and likely many more asymptomatic infections each year, we can infer that each person gets infected at least once every 5 years by a coronavirus, and so homologous reinfection must take place, otherwise we would not get reinfected after the age of 20 or so.

Reinfection by seasonal human coronaviruses in the community

A small number of studies have attempted to detect reinfections in the community. In a cohort study of community acquired and childhood pneumonia admissions to hospital in Kenya, reinfections by human coronavirus NL63 were detected over a 6 month period (Dec-May 2010) in 46 of 163 patients (28%) (Kiyuka et al. 2018). Most reinfections resulted in low virus titres and decreased disease. However, for a small number (11%), reinfection resulted in higher virus shedding compared to the previous infection, with the caveat that peak of virus in first infection could have been missed. When reinfections occurred up to 80 days after first infection, the virus load was usually low. However, reinfection after 80 days sometimes resulted in high viral genome load, compatible with such viruses being capable of onwards transmission. Sequence analysis of paired viral samples from the same individual reinfected after 80 days suggested reinfection was by a homologous CoV (Kiyuka et al. 2018). No antibody levels were measured in this study.
In a recent population study from the FLUWATCH project, over 5 seasons 2006-7 to 2010-11, the seasonal CoVs NL63, 229E and OC43, were detected at a rate of 390 infections (95% CI 338-448) per 100,000 person-weeks. The rates of infection stratified by age showed a bimodal distribution with peaks at ages 0–4 and ages 16–44 consistent with previous serology studies. Importantly, 8 subjects had more than one consecutive coronavirus infection. Of these, no participants had the same coronaviruses strain twice; modelling suggests this provides some evidence for lasting immunity. Nonetheless, analysis of the CoV infection pairs per person shows these small numbers partition into 4/8 having a reinfection within 7-15 weeks whereas 4/8 have a reinfection between 23-56 weeks. The former group all comprise infection-reinfection with heterologous alpha (NL63 or 229E) and beta (OC43) CoVs consistent with lack of serological cross protection, whereas 3/8 of the latter group had homologous reinfection of alphacoronaviruses (Aldridge et al. 2020). Although too small in numbers to be definitive, this suggests that serological protection from reinfection does exist but that it declines over a year, when infection with a virus of the same genotype becomes possible.

Evidence to support seroprotection against homologous virus genotypes exists in children, using serology assays specific for carboxyl-terminal region of the nucleocapsid protein of each of the four viruses. Seroconversion to NL63 (alphacoronavirus) and OC43 (betacoronavirus) occurs more frequently in children in both households and in hospitals. When examining small numbers of reinfections, seroconversion to NL63 was correlated with protection from infection by 229E, both alphacoronaviruses. Seroconversion to OC43 can protect from reinfection by HKU1, both betacoronaviruses. However, the reciprocal protection (229E protects against NL63 and HKU1 against OC43) did not occur (Dijkman et al. 2012), suggesting that even homologous protection by genetically related CoV is not immunological simple.

Reinfection by seasonal human coronaviruses in controlled human infection models (CHIM).
One way to distinguish between infection due to virus escape from neutralization, including heterologous challenge or infection in the presence of sub-protective antibody responses, is to attempt to experimentally infect adult volunteers with seasonal human coronavirus either in the presence of their preexisting immunity or by re-challenge with a homologous virus. Inoculation of healthy adult volunteers with human coronavirus 229E led to infection in 10/15 people and clinical symptoms in 8 of those 10 infected people, even though most must have already experienced 229E infection previously. All those infected had increased antibody titres within 3 weeks of infection, which rapidly declined by 12 weeks and returned to baseline by 52 weeks. When re-challenged at 1 year, 66% (6/9) became re-infected but none developed clinical symptoms (Callow et al. 1990). There are no data about the levels of virus shedding after the first or second challenge. These data were different to earlier studies where reinfection by a homologous coronavirus after 1 year did not occur, but reinfection with heterologous virus produced symptoms of infection. However, in the absence of sequence information about these heterologous '229E-like' CoVs and the possibility that Reed's volunteers were more robustly infected initially, so their antibody titre took longer to decay these data are not easy to interpret (Reed 1984).

Serological responses to SARS-CoV-2.

Antibody responses to SARS-CoV-2 infection in humans and animal models have been reported in very recently published papers and non-peer reviewed preprints. These early studies suggest the immune response to SARS-CoV-2 is similar to that for SARS-CoV and MERS-CoV. Most infected individuals (RT-PCR positive) seroconvert 10-14 days after symptoms, but antibody levels in some mild cases take longer to appear and are low or undetectable. There is no data at all on how long the antibodies remain and what level of antibody is associated with immune protection. In comparing studies, caution should be exercised because many of the studies use different assays to measure the serological response and these are not yet calibrated against each other.

Different tests to measure SARS CoV2 antibodies:
The gold standard test for antiviral antibody is the **virus neutralization** test. This demonstrates that antibodies in a serum sample can prevent susceptible cells from being infected when mixed with a standard challenge dose of virus. However, using this test for SARS CoV-2 requires work inside a high containment (Containment Level 3) laboratories with infectious virus. A surrogate neutralization test uses **pseudotyped virus** particles (PV) that bear the Spike protein of the SARS CoV-2 virus. This test can be performed at containment level 2 and is read out with a suitable reporter such as luciferase. However, it is still not suitable for high throughput or point of care testing.
Immunofluorescent test (IF) also use virus-infected cells, detecting antibody present in the patient blood sample through its reaction with a viral antigens expressed in the fixed cells.
Enzyme-linked immunosorbent assays (ELISA) tests and point **of care lateral flow assays** are suitable for high throughput butdo not measure the function of the antibody, only that antibody can bind to a given antigen. The antigen is usually a recombinant protein such as whole Spike protein or a fragment thereof. Some tests are using just the spike subdomain (S1), and others even only use the receptor binding domain (RBD) a small piece of S. It is possible that the smaller the spike fragment used, the less likely it is that antibodies in the sera raised against other human coronaviruses will cross react, however, this may come at the price of sensitivity. Some tests use the virus nucleoprotein N as the antigen. This is rather more conserved amongst human coronaviruses and SARS CoV2 and so these tests may lack specificity.

A recent study compared 3 CE-marked commercial ELISA assays and 6 POC tests that were available in Denmark (Lassaunière et al., 2020). Thirty serum samples from severe COVID patients were assessed, along with 10 negative sera and another 71 sera from people with other viral infections to test for specificity. The Wantai SARS CoV2 total antibody ELISA that has Spike RBD as the antigen was the most sensitive test, 100% day 10 samples were positive. The Euroimmun IgG test was less sensitive and only detected 78% of the same samples. In addition, the Euroimmun IgG ELISA showed poor specificity because it detected antibodies in 3 sera from patients not infected by SARS CoV2.

Antibody responses reported in SARS CoV2 patients.
A study of 173 people admitted into hospital in China with acute respiratory infection syndromes and/or abnormalities in chest CT images (Zhao et al 2020) used three different assays to measure seroconversion. Similar to the Wantai commercial test above, one measured total antibody to the Spike receptor binding domain (RBD), the second measured IgM to the same Spike RBD antigen and the third assay measured IgG against nucleoprotein (N). The first assay detected positive sera in 93.1% (161/173) with a median response time of 11 days, the second measured a seroconversion rate of 82.7% (143/173), median response time 12 days, and the response rate for IgG to the nucleoprotein was lower at 64.7% (112/173) and took longer to appear, with median response time of 14 days. In later samples collected between 15-39 days from disease onset, the assay that measured Spike RBD antibodies detected seronconversion in 100% patients, whereas the other assays were less sensitive: RBD IgM in 94.3% and N IgG in 79.8%. This study showed that SARS-CoV-2

seroconversion occurs on a time course that is consistent with other epidemic CoVs and antibodies to Spike RBD were the most reliable for case counting. At 2 weeks post symptom onset, antibody titres were statistically higher in critical compared to non-critical patients, possibly due to different rates to a maximal antibody response or reflecting similar disease severity observations from MERS-CoV and SARS-CoV patients as described above (Zhao et al. 2020).

In a separate European collaborative study, in-house and commercial ELISAs together with a virus neutralization assay were used to measure antibodies in a total of 19 severe and mild cases. A temporal study of seroconversion in three patients showed the patient with severe disease became antibody positive earlier than the other two who had mild disease, indeed, one mild patient only gave a positive serum sample using the nucleocapsid ELISA or the neutralization test at 28 days after symptoms (Okba et al., 2020). In 9 mild cases from early in the German outbreak, antibody responses were measured by neutralization assay and by immunofluorescence detecting IgG and IgM binding antibodies. There was incomplete correlation between the titres in the different tests. Seroconversion occurred by day 7 in 50% patients and in all patients by 14 days after symptom onset. The onset of the antibody response did not result in a rapid decline in virus shedding (Wölfel et al. 2020). In contrast, the timing and functionality of the immune response to SARS CoV-2 infection was considered in a detailed study of a single female patient with moderate disease in Australia. The appearance of antibody secreting cells, T follicular helper cells and CD8 positive T cells in the blood of this patient at day 7-9 was coincident with a fall in virus titre and recovery (Thevarajan et al., 2020). The antibody response was also investigated in 23 patients in Hong Kong (To et al. 2020). In this study, the correlation between virus neutralisation activity and IgG titres to nucleoprotein and the S1 RBD were excellent. Antibody trajectories over 20 days from this small number of severe and mild cases again demonstrate variability in individual early antibody responses, that did not correlate with disease severity. A further study from a recovered cohort of 175 patients in Shanghai, measured neutralizing antibody (Nab) titres by the ability of sera to block pseudotyped virus entry (Wu et al., 2020). The average time for seroconversion was 10-15 days. The typical pattern was observed: patients with more severe illness showed higher NAb. Importantly, in this study around 30% patients showed very low Nab titre, and 10 patients (6%) who were confirmed to have been infected from having an RT-PCR positive respiratory sample did not show any antibody response at all even at a later time point 2 weeks after hospital discharge. In the positive samples taken 2 weeks after hospital discharge there was no evidence of antibody waning. The authors comment that the individuals with no antibody measured clearly recovered from COVID without any antibody help, but whether they are at risk of reinfection is not known. Wu et al also emphasize the importance of screening convalescent plasma if it is to be used from prevention or treatment. Indeed, the same PV neutralization assay was used to measure potent antibodies raised in rats immunized with a potential SARS CoV-2 vaccine based on the spike protein RBD fragment. The antibodies were as potent at inhibiting PV entry as ACE2- Ig, a potent SARS CoV-2 entry inhibitor (Quinlan et al., 2020).

Studies on SARS-CoV2 antibodies in experimental animal infections.

Animal studies have found several species to be susceptible to SARS CoV-2 infection including non-human primates, ferrets and cats (Chen 2020; Munster et al. 2020; Kim 2020). Infected ferrets had serum neutralizing antibodies at 12 days post infection, but so far, no re-challenge experiments were reported (Chen 2020). Rhesus macaques are susceptible to SARS-CoV-2, where infection causes a respiratory disease lasting 8-16 days, with detectable high viral loads in the nose, throat and bronchoalveolar lavages. All animals seroconverted to the Spike protein and showed neutralising antibodies by 10 days post infection (Munster et al. 2020). Rhesus macaques were productively infected by SARS-CoV-2, by clinical, virology and serological assessment. At 28 days from the primary infection, when anti-spike antibodies were detectable, two animals were rechallenged with virus

and neither became infected (Bao et al. 2020). This is unsurprising as the animals were most likely at or near the peak of their seroconversion but suggests that immediate reinfection in the face of robust neutralising antibodies to SARS-CoV-2 is not possible.
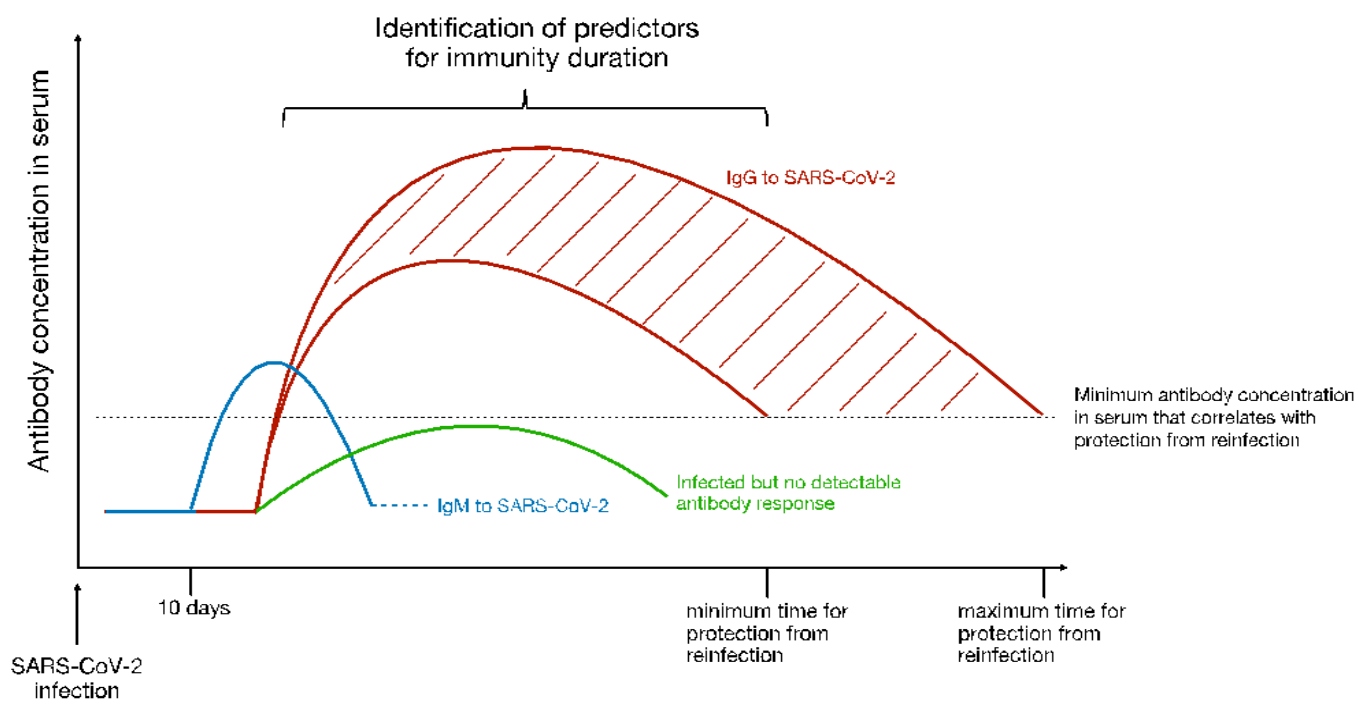
Concluding remarks:

It is clear most people infected with SARS-CoV-2 display an antibody response between 10 and 14 days after infection. In some mild cases, detection of antibodies requires a longer time after symptoms, and in a small number of cases, antibodies are not detected at all, at least during the time scale of the reported studies. There is a paucity of information about the longevity of the antibody response to SARS-CoV-2, but it is known that antibodies to other human coronaviruses wane over time, and there are some reports of reinfection with homologous coronaviruses after as little as 80 days. Thus, the possibility of reinfection of previously mild SARS-CoV-2 cases is a realistic possibility, and should be considered. Such reinfection may be less likely to result in clinical disease, unless antibody dependent disease enhancement by sub-neutralising antibody titres occurs. It is unclear if such reinfections will result in onward transmission, but that cannot be excluded. The potential effect of this should be explored in epidemiological models. Obtaining longitudinal serological data where both binding titres and functional neutralisation titres stratified by age groups and previous disease severity status should be undertaken as a matter of urgency.

We recommend that:

1) The possibility for an individual to be reinfected by SARS-CoV2 is introduced into the epidemiological models, acknowledging that a proportion of those reinfected may go on to develop disease.
2) The reinfection parameter could be applied taking into account the following: the time when reinfection becomes possible is likely correlated with viral load or symptoms in the initial infection. This is because people with symptomatic disease are likely to mount a high antibody titre that decays over time until it crosses a threshold which is no longer protective against infection, whereas people known or predicted to have had mild (non-hospitalised) infections mount lower or even non-existent antibody response that decays at the same rate but will cross the protection threshold sooner.
3) The delay from primary infection recovery to being susceptible for reinfection should range from 30-720 days, with parameters to explore different starting antibody titres related to disease severity proportions but with a constant rate of serological decline.
4) Scenarios of the proportion of people susceptible to reinfection should range from $0 - 66\%$ (the upper boundary here based on small numbers from a MERS study; the seasonal NL63 study would suggest 28% as a reasonable number) and in some scenarios anchored on disease severity proportions.
5) It should be borne in mind for future modelling, based on wide scale serology, that a proportion of people with mild or asymptomatic first infections may not seroconvert at all and therefore serology may not reveal the total number of infections that have occurred.

What studies should be established to assess the risk or reinfection?
- The effect of waning antibody titers and the possibility of reinfection and recurrent disease should be modelled.
- People with low antibody titres after mild disease should be followed up for evidence of reinfection and recurrent disease by regular clinical monitoring and by Q-RT-PCR. If a case of reinfection is detected, serial viral load by Q-RT-PCR should be performed and measures of antibody status at the time of reinfection established.
- Studies should be initiated to determine the relationship between serological antigen binding titres and functional virus neutralisation titres to interpret the likely level and length of seroprotection in the UK population, and to inform correlates for vaccine seroprotection to be used in Phase 1 clinical studies for vaccines in the UK.

Identification of predictors
for immunity duration

Antibody concentration in serum

IgG to SARS-CoV-2

Minimum antibody concentration
in serum that correlates with
protection from reinfection

Infected but no detectable
antibody response

IgM to SARS-CoV-2

10 days

SARS-CoV-2
infection

minimum time for
protection from
reinfection

maximum time for
protection from
reinfection

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