

NERVTAG paper: Asymptomatic SARS-CoV-2 infection

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COVID- Dynamics of infectiousness and antibody responses

Outline

This document attempts to ascertain the duration of the infectious period for individuals infected with SARS-CoV-2, by reviewing data on duration of viral shedding measured by PCR positivity, Ct values and viable virus culture. Virus detection by RT-PCR is the key measurement for determination of infectivity of an individual, typically applied to upper or lower respiratory samples or secretions, but RT-PCR detection does not distinguish between infectious and non-infectious virus. The ability to recover infectious virus from clinical samples may be a better proxy for infectiousness, but this is not widely available. Hence it is important to review data which link these two measurements and consider to what extent RT-PCR Ct value can be used as a proxy for infectiousness. The document also considers the dynamics of the serological response, with the hypothesis that an antibody response may account for, or at least temporally correlate with, a fall in the potential ability of individuals to transmit the virus.

Key conclusions of the paper

8. Viable virus has been recovered from pre-symptomatic patients, supporting the hypothesis that patients are infectious in the pre-symptomatic phase.
9. Viral RNA dynamics (measured by RT-PCR) confirm a peak in viral load just prior to or around the time of symptom onset followed by a gradual decline in viral load.

10. RT-PCR detection can extend until day 43 post symptom onset in some individuals, but beyond 14 days post symptom onset most, **but not all**, infected people shed virus at amounts lower than can be cultured suggesting they are no longer infectious.
11. In hospitalized patients there is evidence of recovery of viable virus until day 20, but data suggests $\leq 5\%$ probability of culturable virus if ≥ 15 days since symptom onset.
12. Data in mildly ill subjects are limited, but viral culture data from early cohorts of SARS-CoV-2 infection, which include mildly ill subjects, indicate that virus cannot be cultured 7-9 days after symptoms onset.
13. Antibody responses are seen as early as day 10-14 in most individuals and might either coincide or even account for reduced infectivity. Measurements may improve as the antibody diagnostics become more robust.
14. There remains a lack of epidemiological transmission data in patients beyond day 7 or 14 to confirm true risk of infectivity to other individuals.

Conclusions (and level of confidence in these)

5. In mildly ill subjects who are recovering, there is a low probability of infectiousness 7-9 days after illness onset (moderate confidence).
6. In hospitalised patients, there is a low probability of infectiousness 14 days after illness onset (moderate confidence).
7. An RT-PCR cycle threshold of >35 is likely to correlate with a low probability of infectiousness (moderate confidence).
8. A neutralising antibody titre of $\geq 1:80$ is likely to correlate with a low probability of infectiousness (low confidence).

Recommendations

- Returning to work after mild COVID: Individuals can remain RT-PCR positive for more than 40 days after infection but this does not mean they are infectious to others. Provided symptoms are resolving, the probability of infectiousness is likely to be low, but not zero, 7 days after illness onset.
- Returning to work after asymptomatic PCR +ve result: For people who were found to be RT-PCR +ve through screening, the probability of infectiousness is likely to be low 7 days after screening, provided symptoms have not developed, in which case exclusion should be for 7 days after illness onset.
- Returning to work with vulnerable people: For this occupational group, consideration should be given to adopting a risk-based approach. Reassurance that it is safe to return could be obtained by measuring Ct values (viral load) in a swab taken at time of return, considering time since symptoms and severity of symptoms and perhaps also measuring antibody levels. Low viral load (high Ct value), longer times since symptoms, mild symptoms and the presence of antibody mitigates the risk of transmission.
- Discharge after COVID that required hospital care: A small number of hospitalised COVID patients (fewer than 5%) may continue to shed infectious virus beyond day 14. These do represent a small risk for onwards transmission to carers and cohabitants. A risk-based approach is recommended, especially for people who will be discharged to an environment where they will interact with vulnerable people (e.g. nursing homes). Assessment for these

people can be informed by considering the viral load indicated by the Ct value from RT-PCR testing (if it is available) and measurement of serum antibody. Low viral load and presence of antibody mitigates the risk of transmission.

- Discharge back to settings with vulnerable people: Since there continues to be ongoing acquisition of SARS CoV2 infections in hospitals, patients admitted for other reasons may be presymptomatic or asymptomatic for COVID on discharge and might reseed infections into the community. Consideration should be given to screening patients before discharge back to vulnerable settings. A low Ct value and absence of antibody would indicate they may still be infectious.
- Re-testing or screening patients or staff who have recovered from COVID-19 and present a second time: In the setting of staff or patients who re-present with an illness compatible with COVID-19, it is likely that detection of RT-PCR positivity will persist from the prior diagnosis;
 - until 21 days for those who have had mild symptoms, but at values below which virus is likely to be cultured
 - until 28 days for those with severe symptoms but at values below which virus is likely to be cultured

Therefore, antibody detection in conjunction with low viral load (Ct >35), in patients/staff with a prior COVID-19 diagnosis within the last 28 days, could be used to exclude that these are active COVID-19 reinfections and confirm these individuals are unlikely to be infectious to others.

Viral dynamics/duration of viral shedding measured by RT-PCR

SARS-CoV-2 virus can initially be detected in upper respiratory samples 1–2 days prior to symptom onset, most studies on sequential RT-PCR testing demonstrate high viral load soon after symptom onset, then followed by a gradual decline, as anticipated in the viral dynamics of other coronaviruses. Characterisation of the length of viral shedding is key to understanding the potential ability of infected individuals to transmit the virus.

One early study on hospitalised patients in Guangdong, China, demonstrated duration of shedding of 137 patients ranged from 8-37 days, with a median 20 days. A minority of patients displayed high viral load (Ct <35) between day 7 and 12. Beyond day 15 nose and throat swabs had a cycle threshold (ct) >35 indicating low viral load (Zou et al).¹

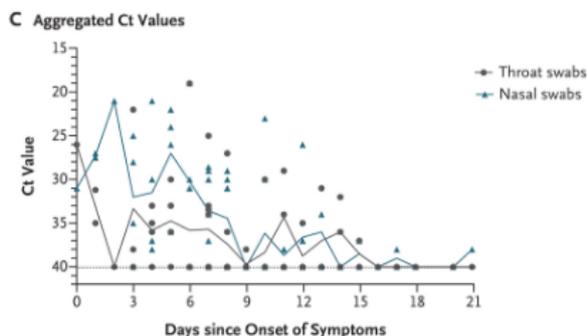
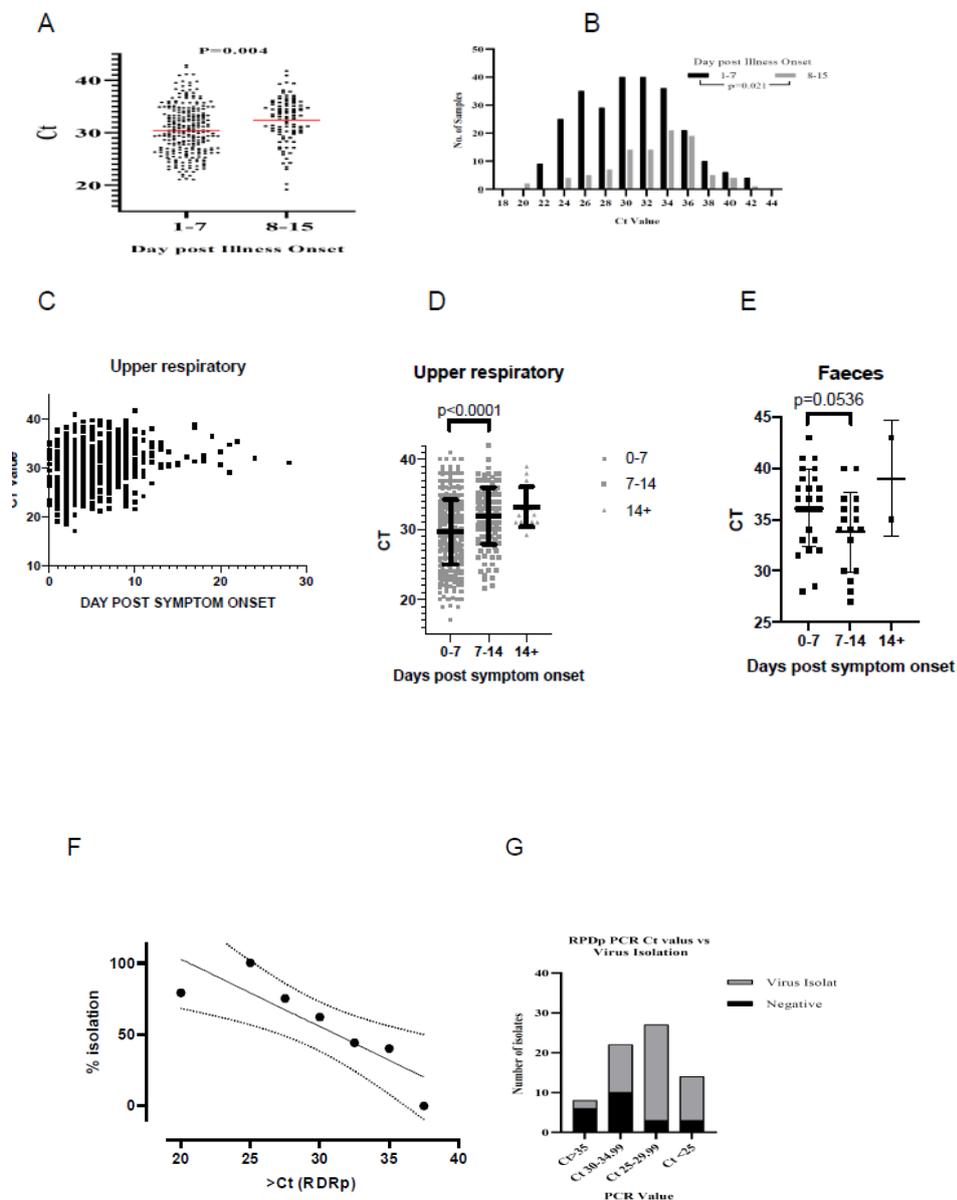


Figure 1 Aggregated ct values for Viral Load Detected in Nasal and Throat Swabs Obtained from Patients Infected with SARS-CoV-2 Zhou et al¹

UK data, presented to NERVTAG on 74 UK HCID cases, demonstrated viral RNA remains detectable until day 28 in upper respiratory tract secretions (though may persist for longer in some individuals, including in faeces Figure 2). Comparison of Day 1-7 versus day 8-15 Ct values (Figure 2 graph A and B) suggests a significant fall in viral load, this trend continues after day 14 but no statistical analysis is given beyond day 14. (Figure 2 graph D).²

Bearing in mind that virus was unlikely to be cultured from samples with Ct values higher than 35 (Figure 2F), this suggests that infectiousness falls with time. However, it should be noted that some patient samples in figure 2A collected between day 8 and 15 have low Ct values. Data is not given about whether any virus was cultured from samples taken late.

All UK data shown is at a preliminary stage of analysis and may be subject to change.



Preliminary virological shedding data from UK cases. (A) Virus detection over time from $n=352$ respiratory samples from $n=74$ UK HCID cases (B) Observed virus detection over time from $n=352$ respiratory samples from $n=74$ UK HCID cases. (C) Upper respiratory tract samples from $n=569$ samples from $n=262$ UK cases (D) Upper respiratory tract samples from $n=569$ samples from $n=262$ UK cases grouped over time (E) Faecal samples from $n=46$ samples from $n=21$ UK cases; (F and G) Virus isolation from $n=73$ respiratory samples.

Figure 2 Preliminary UK data on 74 HCID COVID positive patients presented to NERVTAG

A further study by He et al looked at a total of 414 throat swabs from 94 patients, from symptom onset up to 32 days after onset, illustrating a rising Ct towards the detection limit as defined as a Ct= 40, at about day 21. In this data set there appeared to be a rise in Ct (>38) beyond day 14 (hence a reduction in potential viral load). There was no obvious difference in Ct values across sex, age groups and disease severity. ³

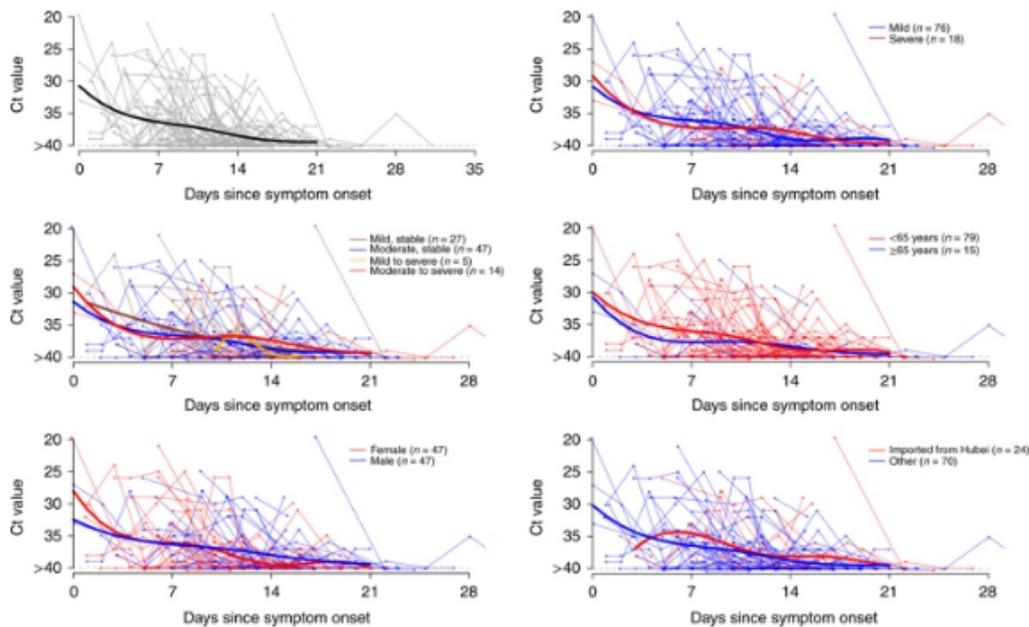


Figure 3 Viral load detected by RT–PCR in throat swabs from patients infected with SARS-CoV-2 (N = 94), overall and stratified by disease severity, sex, age group and link to Hubei province. The thick lines show the trend in viral load, using smoothing splines.

To et al performed a cohort study in Hong Kong including 23 hospitalised patients and looked at the viral load in posterior oropharyngeal saliva of the 21 patients who survived. Of these, seven (33%) had viral RNA detected for 20 days or longer after symptom onset. As demonstrated in figure 4 mean viral load >2 log genome copies per ml was detected until day 24 post symptom onset. ⁴

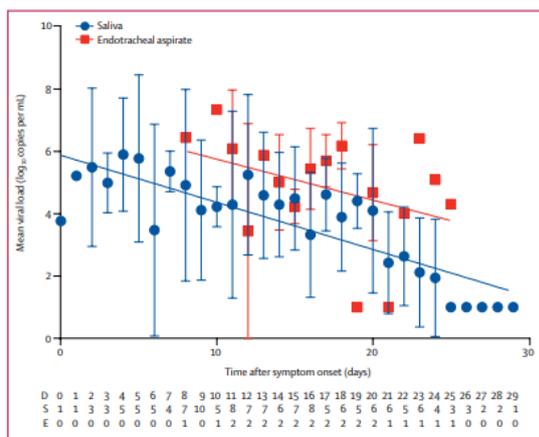


Figure 4 Temporal profile of serial viral load from all patients (n=23) Data points denote the mean; error bars indicate SD; slope represents best fit line. D=days after symptom onset. S=saliva. E=endotracheal aspirate.

A study from Chau et al in Vietnam prospectively followed 30 quarantined participants and the analysis of the probability of RT-PCR positivity showed asymptomatic participants had faster viral clearance than symptomatic participants ($P < 0.001$ for difference over first 19 days). This difference was most pronounced during the first week of follow-up. (Figure 5)⁴

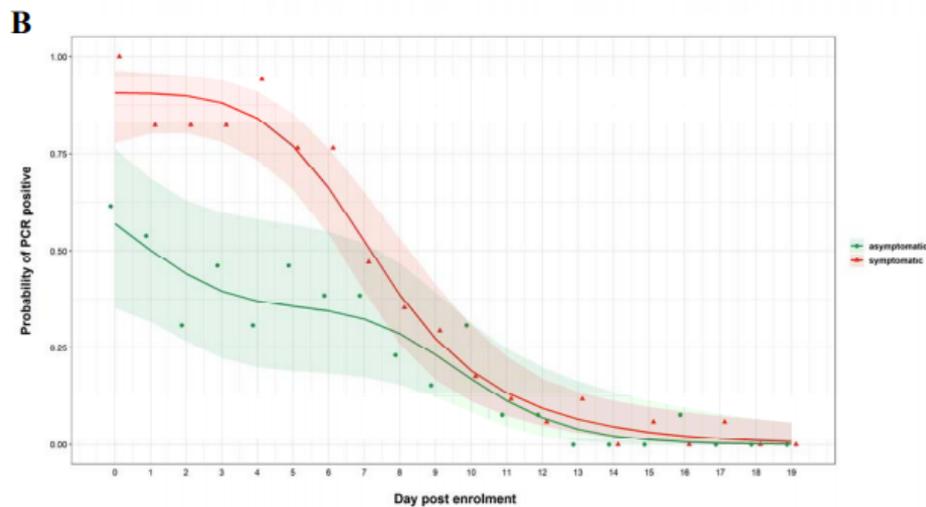


Figure 5 Dynamics of viral detection probability from enrolment onward

Further data from an outreach program in New York by Wajnberg et al identified people recovered from COVID. They included participants in the community with mild disease. In total, 1,343 participants were recruited, with an average age of 40 (17-76), all self-reported complete resolution of symptoms 3-14 days prior to testing. Of these 249 (19%) were RT-PCR positive, the maximum time was 43 days from symptom onset and 28 days from symptom resolution.⁶

Summary

- Viral dynamics confirm a gradual decline following a peak around symptom onset. RT-PCR detection has been confirmed until day 43. Importantly the majority of RT-PCR positivity appears to be above the limit of detection (ct >40) beyond day 21 post symptom onset.
- The PHE UK data confirms a significant increase in Ct value after day 7, when comparing day 1-7 and day 8-14. However, some viral loads at day 8-14 are within the range for which viable virus has been cultured.
- Beyond day 14 Ct values tend to rise to >35 -38
- Wajnberg et al observed only 19% were RT-PCR positive beyond day 14 from symptom onset, which is an important community based data set in mildly symptomatic individuals.
- To et al observed 33% were RT-PCR positive beyond day 20 for severe hospitalised patients but not beyond day 24.
- We recommend caution when interpreting these trends in ct data from clinical data as opposed to systematic clinical trial data. Currently the quality of clinical sampling is very variable (including swab location and type), human genomic targets are not routinely used, there is observed wide variation in diagnostic targets and sensitivity, direct RT-PCR without extraction has been adopted to overcome supply challenges, plus recognised stochastic variation of Ct values in sequential samples.
- Re-testing or screening patients or staff who have recovered from COVID-19 is likely to detect RT-PCR positivity
 - after 14 days and some of these are at values from which virus might be cultured

- until 21 days for those who have had mild symptoms, but at values below which virus is likely to be cultured
- until 28 days for those with severe symptoms but at values below which virus is likely to be cultured

Viabile/cultured virus data

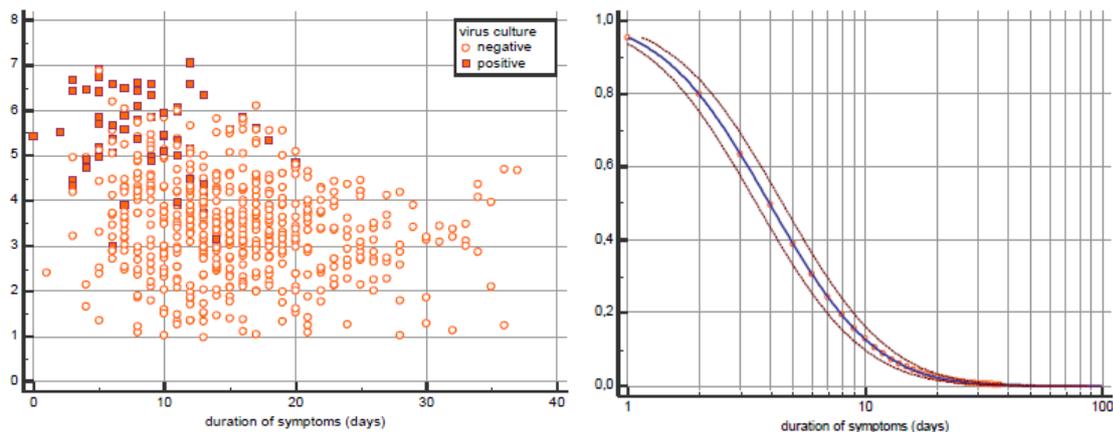
Wölfel et al presented some early viral culture data on a small cohort of hospitalised patients and virus was readily isolated during the first week of symptoms from a considerable fraction (16.66% of swabs and 83.33% of 14 sputum samples), no isolates were obtained from samples taken after day 8 in spite of ongoing high viral loads. ⁷

Early PHE data presented to NERVTAG found the latest time point in the data set presented was a sample where virus was recovered at Day 8 post illness onset and suggested a Ct >35 demonstrated a reduction in number of isolates (Figure 2 graph G). ² However, this data was limited and there were few samples from later in illness, all of which had very high Cts.

A report of the first 12 cases in the US, viral culture was attempted on initial respiratory specimens from nine patients and was successful for all nine, including two patients who were not hospitalized, viable SARS-CoV-2 was cultured at day 9 of illness in 1 patient, but again was not attempted on later specimens. ⁸

Evidence of the high infectivity in the pre-symptomatic phase was demonstrated in a nursing facility in the US where 76 residents who participated in point-prevalence surveys, 48 (63%) tested positive. Of these 48, 27 (56%) were asymptomatic at the time of testing; 24 subsequently developed symptoms (median time to onset, 4 days). Samples from these 24 pre-symptomatic residents had a median RT-PCR Ct value of 23.1, and viable virus was recovered from 17 residents. ⁹

NERVTAG have been provided with unpublished data (slides shown below) from van Kampen et al on infectiousness of 129 hospitalised patients who underwent parallel and serial sampling using RT-PCR, virus culture and neutralised antibody. Importantly isolation of infectious virus was seen until day 20 (slide 1) with a significant decline from day 15 of symptom onset (slide 2).



They made the following conclusions:

- ≤5% probability of culturable virus if ≥15 days since symptom onset (95% CI 13.4 – 17.2)
- ≤5% probability of culturable virus if ≤3.95 log₁₀ viral RNA copies/ml by RT-PCR (95% CI 3.66 – 4.18)
- ≤5% probability of culturable virus if neutralising antibody titre ≥ 1:80

A recent publication described attempts at virus isolation from 90 PCR positive samples collected during routine care and surveillance in Manitoba Canada (Bullard et al COD). Infectious virus was only recovered from swabs collected 8 days or less from onset of symptoms and with low Ct values (Ct of 24 or lower). This level suggest a less sensitive virus isolation capability than at PHE but confirms the picture that infectiousness declines with viral load indicated by Ct and with time after symptom onset.

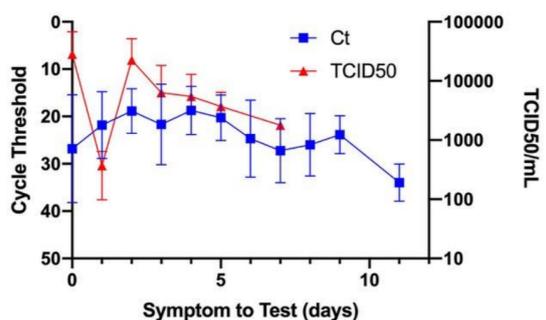


Figure 5: From Bullard et al. CID 2020. No infectious virus was cultured in samples taken later than 7 days after symptom onset or in samples with Ct higher than 24.

Summary

- Viable virus was recovered from 70% of pre-symptomatic patients, supporting the hypothesis that patients are likely infectious in the pre-symptomatic phase.
- There is evidence of recovery of viable virus until day 20, but the Dutch data suggests $\leq 5\%$ probability of culturable virus if ≥ 15 days since symptom onset.
- Although viral culture is an important method to evaluate viral infectivity and activity, it is unavailable in clinical practice and has challenges of its low sensitivity and long turn-around time for virus detection. This may account for the lack of data in this area. Coronaviruses can be hard to culture, many cell types are refractive to infection, and some labs be unable to culture from samples that are still containing infectious virus. The ID50 for human infection is not known so this potential for individuals with such low titre samples to infect others is not clear.

Serological responses to SARS-CoV-2.

Early studies suggest SARS-CoV-2 behaves similarly to SARS-CoV and MERS-CoV. In a study of 173 people, the seroconversion rate for total antibody to the spike receptor binding domain (RBD) was 93.1% (161/173), and for IgM to the Spike RBD was 82.7% (143/173) and IgG to the nucleoprotein 64.7% (112/173). The median time to seroconversion for total antibodies was 11 days, IgM 12 days and IgG 14 days, although some of these differences could be due to ELISA assay format. For samples collected between 15-39 days from disease onset, seroconversion for total antibodies was detected in 100%, IgM in 94.3% and IgG in 79.8% of patients to the RBD and nucleoprotein respectively.¹⁰

Following this earlier data reporting a trend of higher antibody levels with severe compared to mild disease this was not found by Wajnberg et al, in whom over 99% of the patients with self-reported or laboratory documented infection developed IgG (FDA approved 2 step ELISA) in this community cohort with mild disease. Their findings suggest IgG developed over 7-50 days from symptom onset with a medium of 24 days, suggesting the optimal testing for is 3 -4 weeks post symptom onset and at least 2 weeks after symptom resolution.

Further work UK work from the National COVID-19 Scientific Advisory Panel which included mild, severe and asymptomatic patients (Figure 6) detected IgM or IgG in 34/40 individuals with a confirmed history of COVID infection (sensitivity 85%, 95%CI 70-94%), vs. 0/50 pre-pandemic controls (specificity 100% [95%CI 93-100%]) and demonstrated high sensitivity for IgG from day 10 following symptom onset.¹¹

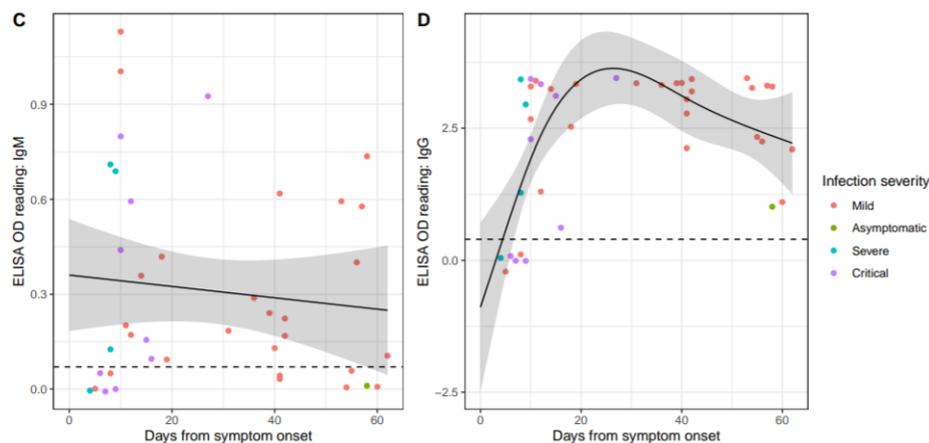


Figure 6

In a comprehensive summary on the humoral immune responses it is noted that antibody responses are detected in most individuals between 10-14 days after infection. There remains a lack of data on the longevity of antibody responses and protection against reinfection.¹²

Summary

- Antibody responses are observed in most people 10-14 days after infection, with peaks around 21-28 days post infection onset. Suggesting a potential fall in infectivity from day 14.
- The Dutch unpublished data suggests $\leq 5\%$ probability of culturable virus was observed if the observed neutralising antibody titre was $\geq 1:80$

- Antibody detection in conjunction with PCR positivity (Ct >35), in patients/staff with a prior COVID-19 diagnosis within the last 28 days, re-presenting with COVID-19 like symptoms could be used to exclude COVID-19 reinfection.

Recommendations for further studies

Current data between 7 and 14 days after onset of symptom is sparse and variable.

1. Duration of shedding infectious virus: We recommend systematically attempting virus isolation on sequential samples from individual's first positive sample until viral culture is negative, including capture of serological responses. We recommend including both upper respiratory samples and lower/sputum samples to compare recovery of viable virus between different sample types. Saliva and faeces could also be included since routes of transmission are not robustly established. Both hospitalised and community/mildly symptomatic individuals should be included to observe if there is a difference in disease severity and duration of infectiousness. For mildly symptomatic individuals (including healthcare workers) we recommend analysing data on Ct values and viral culture data specifically between day 7 and 14, to understand the proportion of those with milder symptoms and their potential infectiousness.

2. Evidence of onward transmission to contacts: To study the true risk of infectivity to other individuals we recommend thorough epidemiological transmission studies capturing patients beyond day 7 of symptom onset to ascertain onward transmission to close/household contacts.

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