NERVTAG paper: Asymptomatic SARS-CoV-2 infection

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COVID- Dynamics of infectiousness and antibody responses

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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 findings

- Viable virus has been recovered from pre-symptomatic patients, supporting the hypothesis that patients are infectious in the pre-symptomatic phase.
- Viral RNA dynamics (measured by RT-PCR) confirm a peak in viral load around the pre-symptomatic/symptomatic transition time point, followed by a gradual decline in viral load, with RT-PCR detection extending until day 43 in some individuals.

- Beyond 14 days most, but not all, infected people shed virus at amounts lower than can be cultured suggesting they are no longer infectious. Beyond day 21 post symptom onset viral loads decline to levels unlikely to yield infectious virus.
- Viral culture data indicating likely infectiousness is limited but suggests most people are not infectious 12 days after symptoms onset. However, a very small minority of hospitalised individuals might remain infectious until day 20.
- 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.
- There remains a lack of epidemiological transmission data, and a lack of data about shedding of infectious virus, in patients beyond day 7 post symptoms and in asymptomatic individualsto confirm true risk of infectivity to other individuals.

Recommendations:

- Returning to work after COVID: Individuals can remain RT-PCR positive for more than 40 days after infection but this does not mean they are infectious to others. Infectiousness is likely to be low enough 7 days after illness onset and with resolution of symptoms for safe return to work. However for people who care for vulnerable people, reassurance that it is safe to return could be obtained by measuring Ct values (viral load) in a swab taken just prior to return, considering time since symptoms and severity of symptoms and measuring antibody levels. Low viral load, longer times since symptoms, mild and resolving symptoms and presence of antibody mitigates the risk of transmission.
- Risk from patients after 14 days: Since 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 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.
- Risk of reseeding infections to the community from hospital: Similarly, 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 all patients by RT-PCR before discharge. A low Ct value and absence of antibody would indicate they may still be infectious.

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]

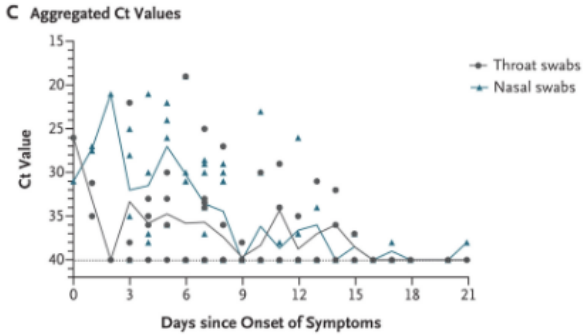
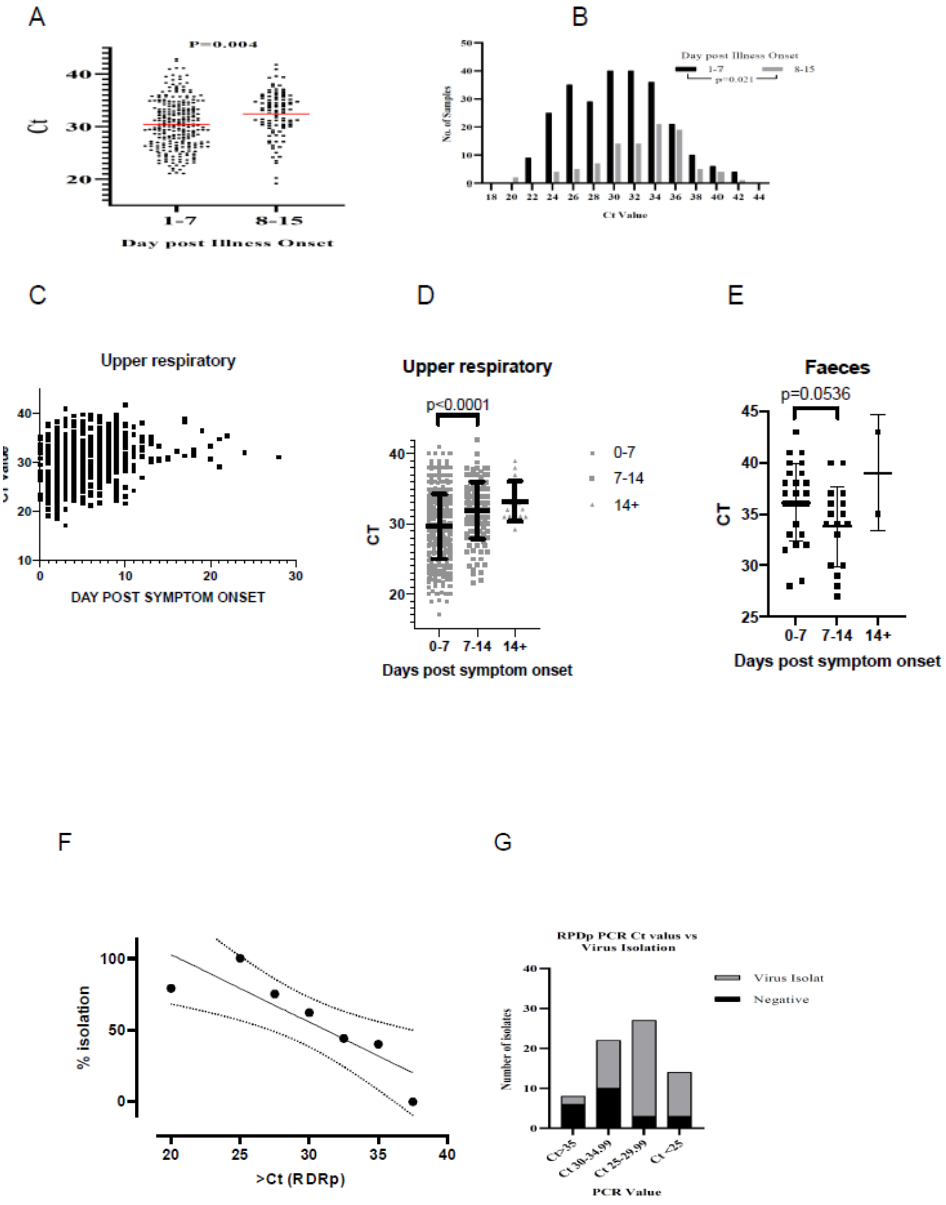


Figure 1 Aggregated ct values for Viral Load Detected in Nasal and Throat Swabs Obtained from Patients Infected with SARS-CoV-2 Zhou et al [1]

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]

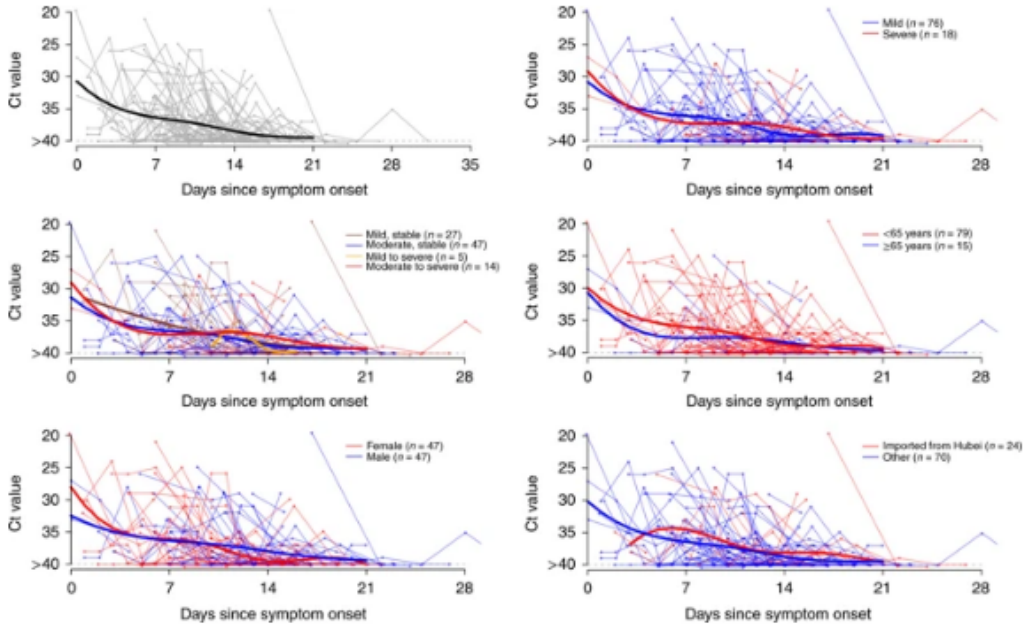


Figure 3 Viral load detected by RT–PCR in throat swabs from patients infected with SARS-CoV-2 (N = 94), overall and stratified by disease severity, sex, age group and link to Hubei province. The thick lines show the trend in viral load, using smoothing splines.

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]

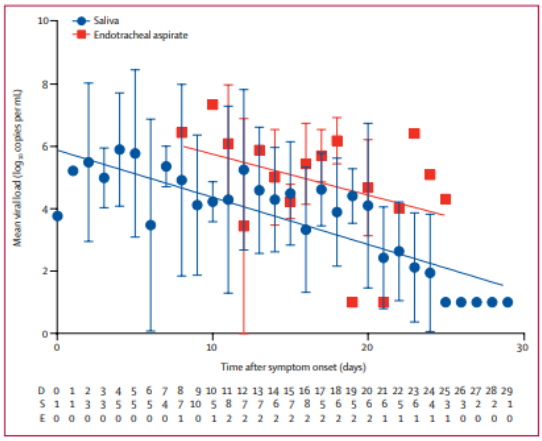


Figure 4 Temporal profile of serial viral load from all patients (n=23) Data points denote the mean; error bars indicate SD; slope represents best fit line. D=days after symptom onset. S=saliva. E=endotracheal aspirate.

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]

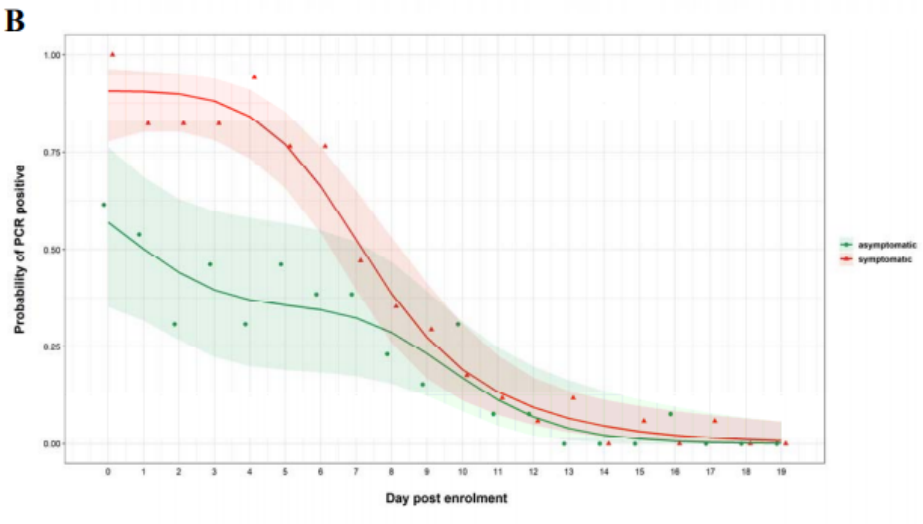


Figure 5 Dynamics of viral detection probability from enrolment onward

Several more recent preprints and publications have addressed the length of shedding and amount of virus shed in asymptomatic infections. These papers show very small differences in viral load and length of RT-PCR positivity between asymptomatic and presymptomatic groups.

Xioa et al reported a study from Shenzen where they enrolled 56 infected people, 23 of whom remained asymptomatic and 33 developed symptoms. The viral load at start was similar in both groups. This could be important since at the most infectious day it might suggest both groups were equally likely to transmit onwards. However virus was resolved faster in the asymptomatic group by 4 days (9.6 days to negative vs 13/6 for the presymptomatic group).

Yan et al. reported that asymptomatic people did not shed virus for more than 9 days.

Zhan et al. found no difference in time to clearing virus between asymptomatic and presymptomatics, both 7 or 8 days but the viral load was lower in asymptomatic group and this was significant in a small subset measured at 7-13 days.

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
- Asymptomatic infections have been sometimes reported to have higher Ct values and shed virus for fewer days, but the differences are small.

- 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 a small proportion of these 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

Viable/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 RNA 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. The PHE data have now been collated and are presented as a supplementary paper with this one. They show:

1. No difference between viral load (Ct) between asymptomatic, presymptomatic and symptomatic groups.

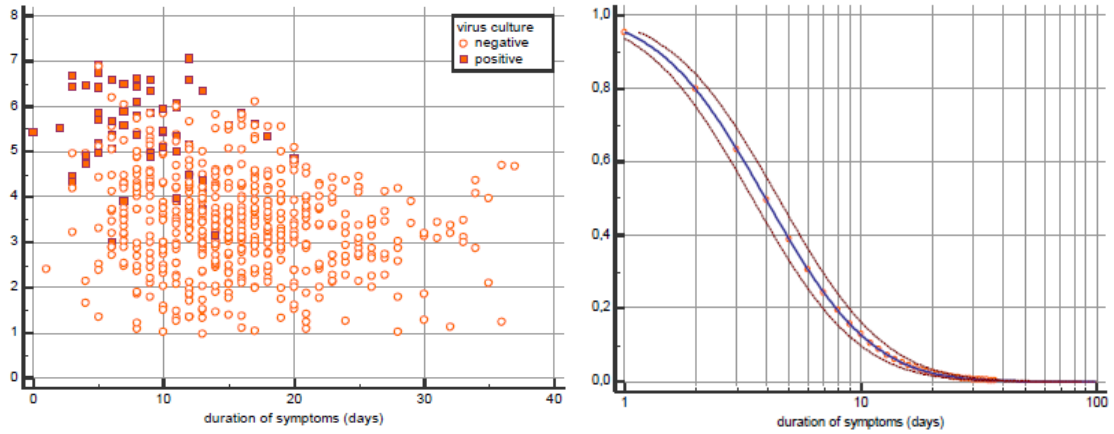
2. Culture of infectious virus in <10% of swabs with ct>35.

3. Culture of infectious virus from swabs taken early after infection or symptom onset, but 20% swabs taken between 8 and 12 days were als culture positive.

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 (now available on MedRix[2]) 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 log 10 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.

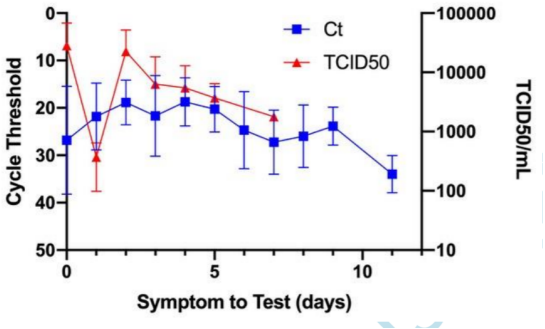


Figure 5: From Bullard et al. CID 2020. No infectious virus was cultured in samples taken later than 7 days after symptom onset or in samples with Ct higher than 24.

[2] https://www.medrxiv.org/content/10.1101/2020.06.08.20125310v1

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 ≤5% probability of culturable virus if ≥15 days since symptom onset, and PHE data shows only 20% samples obtained at 8-12 days were cultured, and probability of recovery of virus from samples with Ct >35 is less than 10%.
- 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 may be unable to culture from samples that are still containing infectious virus. The ID50 for human infection is not known so the 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]

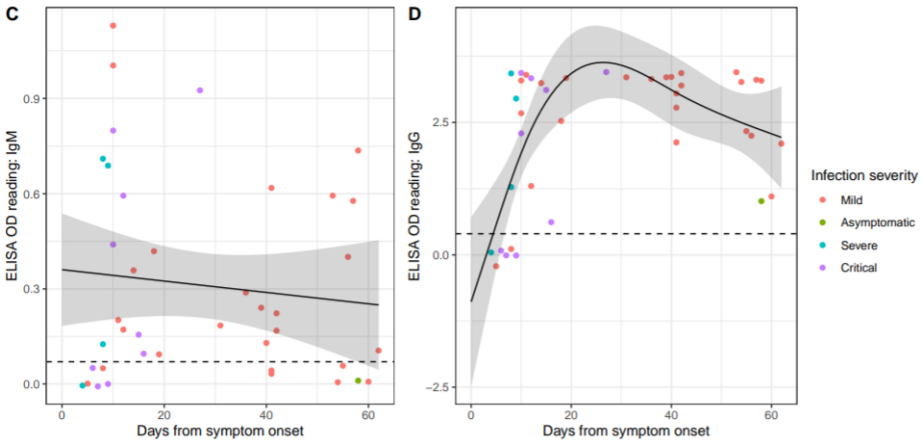


Figure 6

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 ≤5% probability of culturable virus was observed if the observed neutralising antibody titre was ≥ 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.

References

1. SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients.
   Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, Yu J, Kang M, Song Y, Xia J, Guo Q, Song T, He J, Yen HL, Peiris M, Wu J N Engl J Med. 2020;382(12):1177. Epub 2020 Feb 19

2. Are asymptomatic people with COVID-19 infectious? Anika Singanayagam, Maria Zambon (Virology Cell, PHE Colindale), with input from John Edmunds and Gavin Dabrera
   NERVTAG Paper Date: 02 April 2020

3. Temporal dynamics in viral shedding and transmissibility of COVID-19. Xi He, View ORCID Profile Eric HY Lau, Peng Wu, Xilong Deng, Jian Wang, Xinxin Hao, Yiu Chung Lau, Jessica Y Wong, Yujuan Guan, Xinghua Tan, Xiaoneng Mo, Yanqing Chen, Baolin Liao, Weilie Chen, Fengyu Hu, Qing Zhang, Mingqiu Zhong, Yanrong Wu, Lingzhai Zhao, Fuchun Zhang, Benjamin J Cowling, Fang Li, Gabriel M Leung medRxiv doi: https://doi.org/10.1101/2020.03.15.20036707

4. The natural history and transmission potential of asymptomatic 2 SARS-CoV-2 infection Nguyen Van Vinh Chau et al MedRxiv preprint doi:https://doi.org/10.1101/2020.04.27.20082347 April 29, 2020.

5. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study Kelvin Kai-Wang To*, Owen Tak-Yin Tsang*, Wai-Shing Leung, Anthony Raymond Tam, Tak-Chiu Wu, David Christopher Lung, Cyril Chik-Yan Yip, Jian-Piao Cai, Jacky Man-Chun Chan, Thomas Shiu-Hong Chik, Daphne Pui-Ling Lau, Chris Yau-Chung Choi, Lin-Lei Chen, Wan-Mui Chan, Kwok-Hung Chan, Jonathan Daniel Ip, Anthony Chin-Ki Ng, Rosana Wing-Shan Poon, Cui-Ting Luo, Vincent Chi-Chung Cheng, Jasper Fuk-Woo Chan, Ivan Fan-Ngai Hung, Zhiwei Chen, Honglin

Chen, Kwok-Yung Yuen Lancet Infect Dis 2020; 20: 565–74 https://doi.org/10.1016/ S1473-3099(20)30196-1

6. Humoral immune response and prolonged PCR positivity in a cohort of 1343 SARS-CoV 2 patients in the New York City region
Ania Wajnberg, Mayce Mansour, Emily Leven, Nicole
M Bouvier, Gopi Patel, Adolfo Firpo, Rao Mendu, Jeffrey Jhang, Suzanne Arinsburg, Melissa G itman, Jane Houldsworth, Ian Baine, Viviana Simon, Judith Aberg, Florian Krammer, David Rei ch, Carlos Cordon-Cardo medRvix 5 May 2020 https://doi.org/10.1101/2020.04.30.20085613

7. Virological assessment of hospitalized patients with COVID-2019.
Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirglmaier K, Drosten C, Wendtner C Nature. 2020

8. Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States. The COVID-19 Investigation Team.
Nature Medicine (2020) 23 April 2020

9. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility
Melissa M. Arons, R.N., Kelly M. Hatfield, M.S.P.H., Sujan C. Reddy, M.D., Anne Kimball, M.D., Allison James, Ph.D., Jesica R. Jacobs, Ph.D., Joanne Taylor, Ph.D., Kevin Spicer, M.D., Ana C. Bardossy, M.D., Lisa P. Oakley, Ph.D., Sukarma Tanwar, M.Med., Jonathan W. Dyal, M.D., et al., for the Public Health–Seattle and King County and CDC COVID-19 Investigation Team* NEJM April 24, 2020 DOI: 10.1056/NEJMoa2008457

10. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019
Juanjuan Zhao Jr., Quan Yuan, Haiyan Wang, Wei Liu, Xuejiao Liao, Yingying Su, Xin Wang, Jing Yuan, Tingdong Li, Jinxiu Li, Shen Qian, Congming Hong, Fuxiang Wang, Yingxia Liu, Zhaoqin Wang, Qing He, Zhiyong Li, Bin He, Tianying Zhang, Shengxiang Ge, Lei Liu, Jun Zhang, Ningshao Xia, Zheng Zhang Clinical Infectious Diseases doi: 10.1093/cid/ciaa344

11. Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel. Adams et al on behalf of the serology group from ISARIC CCP Study. MedRxiv May 7 2020

12. The Dynamics of Humoral Immune Responses Following SARS-CoV-2 Infection and the Potential for Reinfection. Kellam, P. Barclay, W .Preprints 2020, 2020040377 (doi: 10.20944/preprints202004.0377.v1).

13. Predicting infectious SARS-CoV-2 from diagnostic samples. Bullard et al. CID 2020

14. Viral dynamics in asymptomatic patients with COVID IJID. Zhou et al. 2020 96: 288-290

15. Duration of SARSCoV2 RNA in asymptomatic carriers. Yan et al. Crit Care. 2020 24:245.

16. Early viral clearance and antibody kinetics of COVID-19 among asymptomatic carriers. Xiao et al. MedRix 2020.

Supplementary Data 9th June 2020

Source - PHE

Analysis of virus isolation data

Isolation of SARS-CoV-2 virus may be considered a proxy for infectiousness. This paper is an updated summary of SARS CoV -2 live virus isolation work carried out at PHE Colindale under CL3 laboratory containment. Virus isolation has been attempted on **n=328** samples (up to 07/06/2020) from 257 cases of SARS CoV-2, predominantly samples taken from the upper respiratory tract. These are materials which have come directly to the laboratory, using defined sampling and virus transport media and transport arrangements, so as to ensure a reasonable degree of consistency in technical approaches to virus isolation

Virus isolation is performed by inoculation of Vero-E6 cells and culture for 14 days. A positive result signifying the presence of live virus indicates that cytopathic effect (cpe) with microscopic appearances indicative of coronavirus infection were observed, with subsequent confirmatory testing using RT PCR or ELISA.

Of 329 samples on which virus isolation was attempted, 135 (**41%)** were culture positive, 194 were culture negative and 1 sample was cytotoxic (excluded from analysis).

Different clinical scenarios

The samples included in this analysis (Table 1) were taken as part of clinical diagnostic work up and represent a real-world observational dataset and originate from a wide range of clinical case scenarios.

Hospitalised cases:

- Diagnostic samples received during the early epidemic/containment phase. These included symptomatic persons with mild-to-moderate disease who were isolated in HCID centres and those followed as part of the "First Few XX Cases". Sequential samples were received from some of these cases.
- Cases that required intensive care support or resulted in fatal outcome are grouped separately. These included those identified during the containment phase for whom diagnostic testing was performed at PHE Colindale and any additional cases from later in the pandemic where samples were sent directly to Colindale for analysis.

Community cases:

- (a)Outbreaks – As part of outbreak response, PHE performed widescale sampling of symptomatic and asymptomatic persons from a military barracks in London and from 6 care homes known as the "Easter six or London care homes".
- (b)Community testing/surveillance – samples were received from symptomatic cases via the RCGP sentinel surveillance scheme, from symptomatic cases followed up in the community as part of the "First Few XX Cases", and includes early samples from a PHE household contact (HOCO) study.

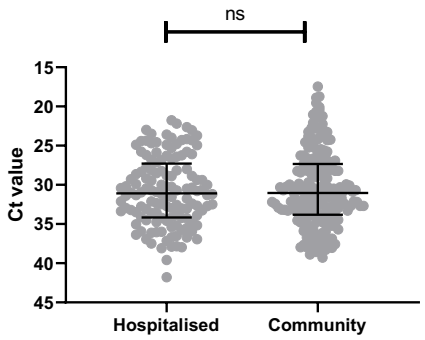
- (c) Healthcare workers (HCWs) – includes snapshot sampling of 1152 staff from six UK hospital trusts between 29th April and 7th May, of whom 2% were PCR positive. Positive samples from five of the six trusts were analysed for virus isolation

HOSPITALISED					
	Number of samples	Number of cases	Culture positive samples (%)	Symptomatic cases (%)	Asymptomatic cases (%)
Early epidemic/ containment phase	109	55 *(3 cases ≤16yo)*	60 (55%)	53 (96%)	2 (4%)
Severe/fatal	23	14	4 (17%)	14 (100%)	0
COMMUNITY					
Military (outbreak)	15	13	7 (47%)	2 (15%)	13 (87%)
Care home (outbreak)	96	95	33 (34%)	49 (52%) *(10 fatal)*	47 (48%)
Community testing/surveillance	61	56 *(5 cases ≤16yo)*	30 (49%)	56 (100%)	0
Healthcare workers (snapshot survey & sporadic)	24	24	1 (4%)	22 (92%) *(1 unknown)*	1 (4.5%)
TOTAL	**328**	**257**	**135 (41%)**	**196**	**63**

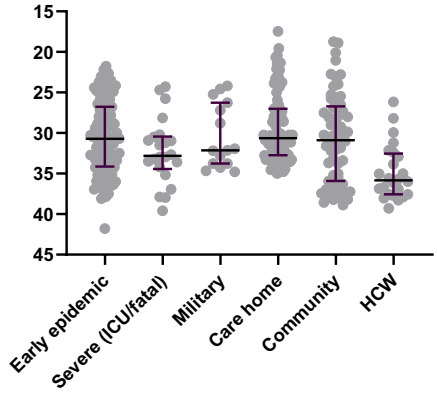
Table 1. Clinical categories of samples received at PHE Colindale where virus isolation was attempted

There was no difference in the Ct values of samples received from hospitalised versus community cases (Figure 1A). The analysis accounted for multiple samples from the same individuals. The random intercept for individuals was not statistically significant, providing no evidence for dependencies within person, thus each individual sample is treated as being independent in many of the following analyses. Ct values from samples received from HCWs were higher (lower viral load) than those categorised as early epidemic hospitalised cases, care home or community cases (Figure 1B). There was no difference seen in Ct values across samples received from different age groups, sex, or severity of illness (Figures 1C-E).

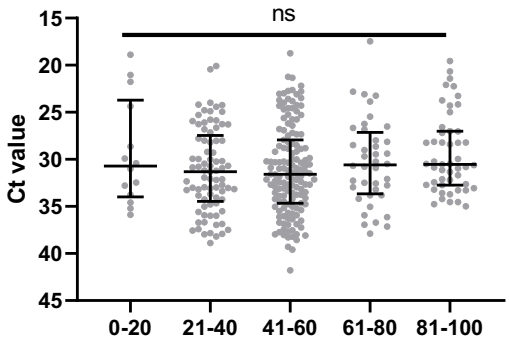
A


ns
Ct value
15
20
25
30
35
40
45
Hospitalised
Community

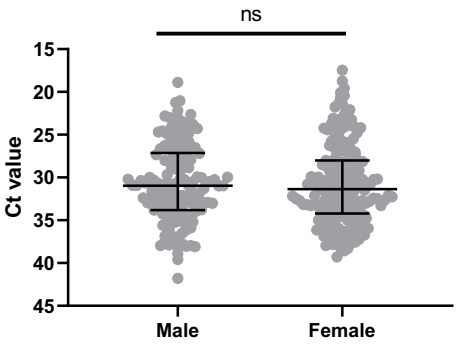
B


15
20
25
30
35
40
45
Early epidemic
Severe (ICU/fatal)
Military
Care home
Community
HCW

C


ns
Ct value
15
20
25
30
35
40
45
0-20
21-40
41-60
61-80
81-100

D


ns
Ct value
15
20
25
30
35
40
45
Male
Female

E

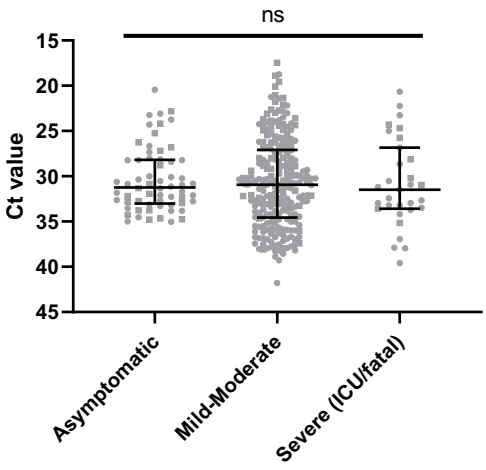
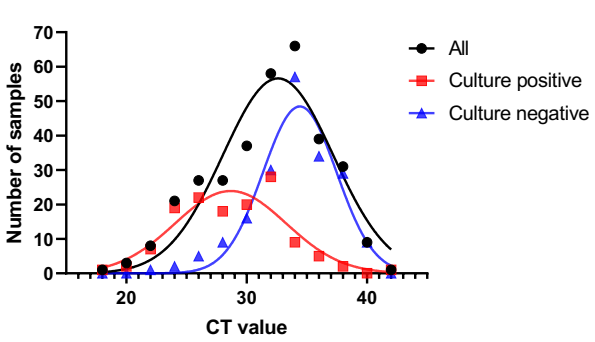


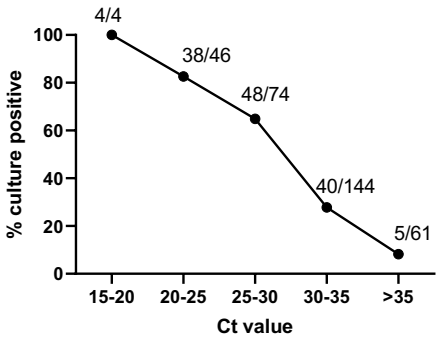
Figure 1. Comparison of Ct values across clinical categories. Median and IQR shown. Mann Whitney test used to compare 2 groups and one-way ANOVA used where >2groups.

1. Relationship between Ct value and virus isolation

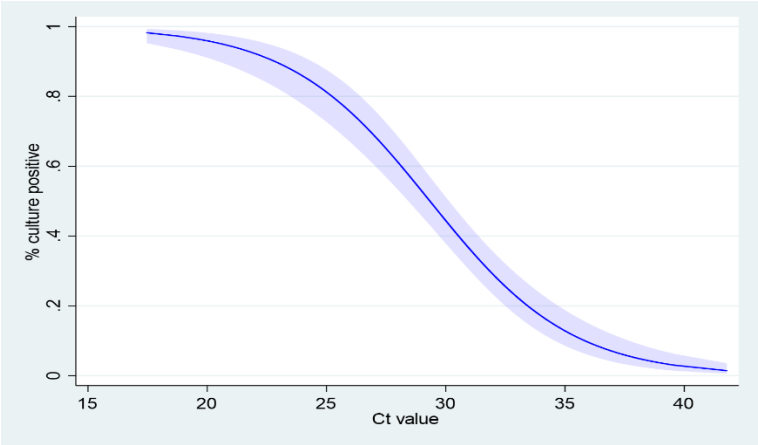
Culture positivity was observed more frequently from samples with lower Ct value (Figure 2A,B,C). There is a strong relationship between Ct value and ability to recover infectious virus. The odds ratio of recovering infectious virus decrease by 0.67 for each unit increase in Ct value (CIs 0.58-0.77). At Ct value>35, virus isolation was successful from 5/61 (8%) of samples. Mixed effects regression analysis for these data are shown in Figure 2C. the estimated probability of recovery of virus from samples with Ct >35 is less than 10%



A



B



C

Figure 2. Analysis of relationship between Ct value and virus isolation

2. Relationship between "symptom to test" interval and virus isolation

There were **n=250 samples** from **n=179 cases** where the date of onset of symptoms was known. The majority of samples received were from the 1st and 2nd week (day 0-14) of illness (Figure 2A). Culture positivity rate was significantly higher during week 1 than week 2 (Figure 2B). Culture positivity peaked shortly after symptom onset. 7/13 pre-symptomatic cases were culture positive (Figures 2C-E).

Of n=132 samples (from 92 cases) received >7 days after symptom onset, 28 samples (21%) (from 20 cases) were culture positive. The latest time point that virus was successfully isolated was 12 days after symptom onset. Of these 28 culture-positive late samples, 25/28 (89%) were from "early epidemic" hospitalised cases, cultured between days 8-10, and none were known to have had severe illness (Figure 2E). Data on whether symptoms had resolved or were still present at the time of sample collection is not currently available for these cases. Two care home residents that were PCR and culture positive on days 11 and 12 respectively had ongoing symptoms (cough) at the time of sampling.

A B

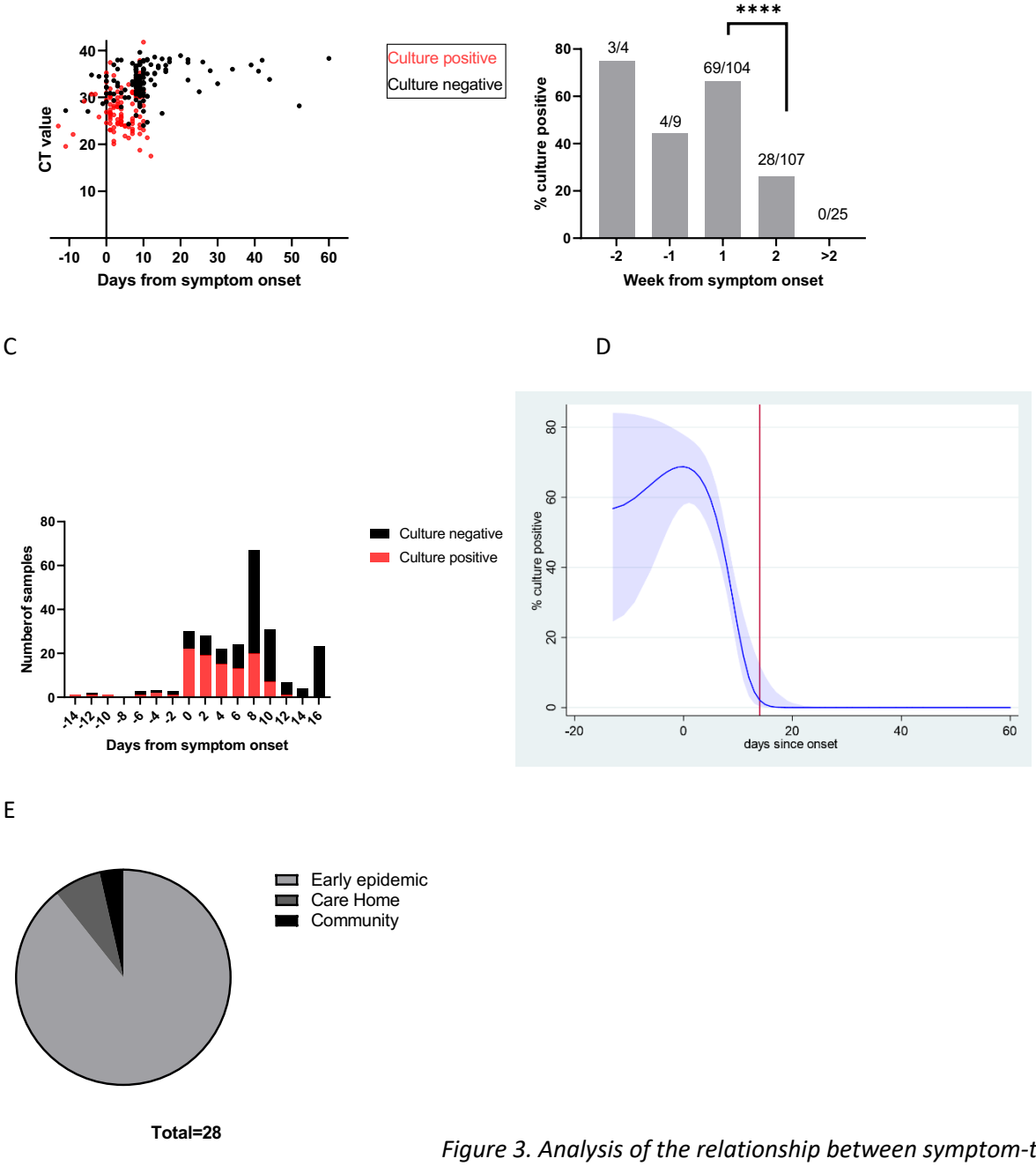


Figure 3. Analysis of the relationship between symptom-to-test interval and virus isolation. Fishers exact test used to compare proportions of culture positive and negative samples in (B). (D)Regression analysis of Ct values, culture positivity vs symptoms indicates that presymptomatic samples are at least as equivalent as samples taken during symptomatic phases. There is an approximately linear decrease in Ct values until about day 20. Red line on Figure 3D is at day 14 post symptom onset

3. Asymptomatic cases

The majority of asymptomatic cases in this dataset are derived from outbreak settings (care home and military barracks). 21/62 (34%) of samples from asymptomatic persons were culture positive, compared with 113/266 (42.5%) from symptomatic cases (p=0.068, non significant). The proportion

of asymptomatic cases was similar across age groups, except for ages 81-100 where a larger proportion of asymptomatic cases was seen (42.5%), likely biased by the care home data. The interpretation of this finding requires further consideration as it may reflect real differences in response to infection in this age group (lower response to fever, higher CFR with symptoms) or bias in ascertainment of symptoms

There was no difference in proportion of asymptomatic cases between males (23%) and females (25%), (p=0.76 non significant).

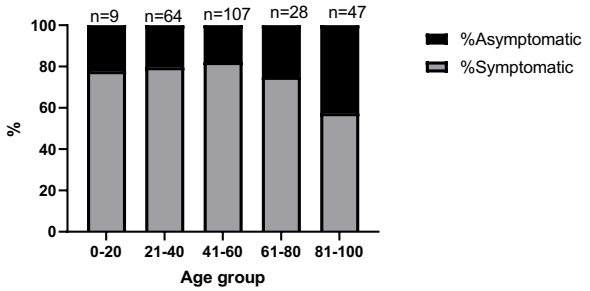


Figure 4. Proportion of asymptomatic versus symptomatic cases by age group

4. Limitations of the dataset

- Real world data
- Heterogenous population and sampling arrangements
- Limited numbers of asymptomatic and pre-symptomatic cases
- Sequential samples from same patient are treated as independent data for analysis of virus isolation, although analyses that did account for sequential samples on the same individuals found no strong evidence of dependencies between these samples

5. Conclusions

- No difference in median Ct values was observed across age groups, sex, severity of illness or clinical category.
- There was no difference in Ct value or culture positivity when comparing asymptomatic and symptomatic cases.
- Strong relationship between Ct values and ability to recover infectious virus, with narrow confidence intervals
- Cultivable virus was obtained more frequently from samples with low Ct value and during the first week after symptom onset.
- Virus was obtained from 7/13 (54%) pre-symptomatic cases.
- Culture positivity rate declined after the first week post symptom onset.
- Cultivable virus was obtained from 28 samples (21% of 132 tested) between days 8-12.
- No viable virus was obtained >12 days post symptom onset.
- Assessment of this data with knowledge of antibody titre will assist the interpretation of the infectious risk posed by late symptoms