Research Article

Viral kinetics in SARS-CoV-2 infected asymptomatic patients

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Abstract

Introduction: As the daily number of patients diagnosed with SARS-CoV-2 infection by PCR increases, the necessity to identify truly infectious cases becomes more significant. We aimed to identify a cut-off Ct value of the COVID-19 RT-PCR assay for likely infectivity by assessing the COVID-19 IgG status and investigating the utility of the Rapid Antigen Test (RAT) in identifying infectious cases among asymptomatic individuals.

Methods: Nasopharyngeal/throat swabs were simultaneously taken for COVID-19 RT-PCR and RAT from 552 asymptomatic individuals at De Soysa Maternity Hospital, Colombo, from 23rd of November to 19th of December 2020. In addition, SARS-CoV-2 IgG (against nucleoprotein) status in PCR positive individuals was evaluated when simultaneously taken sera was available.

Results: COVID-19 RT-PCR positive rate among asymptomatic individuals was 14.3% (n=79). The overall sensitivity of RAT was 30.4% but increased to 73.9% when Ct values below 25 were considered. The COVID-19 IgG response was evaluated in 37 PCR positive subjects and the overall seropositivity was 40.5%. The optimal Ct thresholds for discrimination of COVID-19 IgG status were 30.5 and 30.29 for the E and S gene respectively. There was a significant positive correlation between Ct values of the E gene and IgG ratio values ($r=0.345$, $p<0.05$). The Ct thresholds for RAT positivity were 26.5 and 26.06 for E and S genes respectively, with a significant negative correlation ($p<0.001$).

Conclusion: We conclude that it is possible to define a cut off Ct value in SARS-CoV-2 PCR (with some error margin for practical purposes) for likely non-infectivity. It was also deduced that positive COVID-19 rapid antigen result seems to be more predictive of infectivity in comparison to positive PCR result.

Keywords: *COVID-19, Ct value, PCR assay, Rapid antigen test, SARS-CoV-2*

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Introduction

Coronavirus disease 2019, caused by SARS-CoV-2, was initially identified in Wuhan, China, in December 2019.[1] The virus caused a pandemic that swept across the globe, posing enormous challenges to healthcare systems worldwide.

SARS-CoV-2 virus is an enveloped positive-sense single-stranded RNA virus. It is a member of the subgenus Sarbecovirus.[2] The SARS-CoV-2 has four structural proteins of which S (spike), E (envelope), and M (membrane) are viral envelope proteins and the other that is bound to the RNA genome is an N (nucleocapsid) protein.[3] This virus is transmitted by respiratory droplets.[4] The median incubation time to develop SARS-CoV-2 infection is 5 days and a long incubation period of more than 12 days is rare.[5] The outcome of SARS-CoV-2 infection varies from asymptomatic to mild infection with fever, cough, muscle pain and diarrhoea to severe infection presenting with severe respiratory distress and multi-organ dysfunction with high mortality.[6-8]

Though 80% of infected cases are completely asymptomatic, they substantially contribute to the spread of SARS-CoV-2 infection.[9-12] The median infectious period for asymptomatic cases was found to be 6.5 to 9.5 days.[13] The median persistence of viral nucleic acid among asymptomatic patients is 9 days, and a further 25% of patients demonstrate viral nucleic acid persistence beyond 20 days.[14]

A study found that the overall sensitivity of the rapid antigen test in the asymptomatic population is 64%. However, sensitivity rises to over 90% with a negative predictive value of 99.4% after applying an infective viral load cut off of >=5.2 log 10 gene copies/ml.[15]

The adaptive humoral immune response produces IgM, IgG, and IgA antibodies following SARS -CoV-2 infection. This can be detected by serological tests and the process is named seroconversion.[16] IgG production exceeds the IgM level after around 10 days of infection when the infectivity decreases. A multi-centre study found that IgG is detectable in all patients by 20 days of onset of disease, by which time the patient is no longer infectious.[13]

Asymptomatic patients who present with positive PCR results may actually be non-infectious, as the non-viable virus is detectable and well below the infectious viral load cut off. Thus, the utilization of PCR, rapid antigen and antibody testing in combination to develop a model to measure infectivity and define cut off Ct values, may mitigate these concerns. Accordingly, this study aimed to assess the antibody and viral kinetics in asymptomatic SARS-CoV-2 infected individuals, to understand the burden of active transmissible infection in a local setting.

Methods

Study design

A descriptive cross-sectional study was designed to assess the antibody and viral kinetics in asymptomatic SARS-CoV-2 infections.

Study setting
De Soysa Maternity Hospital, Colombo, and Medical Research Institute, Colombo 8, Sri Lanka

Study period
23rd of November to 19th of December 2020

Data collection
We received respiratory samples from 552 asymptomatic individuals at De Soysa Maternity Hospital, Colombo, to determine SARS-CoV-2 infection status for the purpose of screening. Two nasopharyngeal/throat swabs were taken simultaneously for COVID-19 RT-PCR and rapid antigen test (RAT) from each individual from 23rd of November to 19th of December 2020. RAT was performed onsite using commercial SARS-CoV-2 rapid antigen kit (SD Biosensor, Korea). The kit showed 86.8% and >99% of sensitivity and specificity respectively. The test results were interpreted according to the manufacturer's instructions.

The nasopharyngeal/throat swab specimens were transported to the laboratory in VTM maintaining the cold chain. Samples were tested for SARS-CoV-2 RNA by real-time polymerase chain reaction (RT-PCR) assay in the laboratory. The specimens were extracted manually using an IVD approved commercial kit (Bioflux, China) and amplified with a commercial kit (Altona, Germany) that detects the spike (S) and envelope (E) genes by real-time PCR technology.

In addition, we evaluated the SARS-CoV-2 IgG status in PCR positive individuals if simultaneously taken sera were available. Sera from 37 participants were subjected to the SARS-CoV-2 IgG test. Two milliliters of venous blood were transported to the laboratory while maintaining the cold chain after keeping the specimen undisturbed at room temperature for 30 minutes to allow clotting. In the laboratory, the sample was centrifuged at 3000 rpm and chemiluminescent immunoassay technology was used with a commercially available kit (Elecsys, Germany) to detect SARS-CoV-2 IgG antibodies against the nucleocapsid protein of the virus. This kit has demonstrated 60.3% - 85.3% of sensitivity within 14days of initial PCR positivity and 99.5% of specificity. The test was performed according to the manufacturer's instructions.

Data analysis
The collected data was entered into an Excel® sheet and statistical analysis was performed by SPSS 17® software. The COVID-19 PCR positive rate was calculated by getting the percentage of RT-PCR positive persons. Rapid antigen test sensitivity was defined as the fraction of PCR positive specimens which tested positive on the COVID-19 rapid antigen test (RAT). IgG seroprevalence was calculated by the fraction of the tested population with IgG antibodies. Pearson correlation coefficient was used for correlation analysis and statistical significance was considered for p values of <0.05.

Results

Real-time PCR analysis of studied population

The study population included 552 patients of whom 79 were found to be infected with the SARS-CoV-2 virus by real-time PCR analysis. The COVID-19 PCR positive rate in the studied asymptomatic population was 14.3%.

Demographics of PCR positive cases

Characteristics of 79 asymptomatic persons with acute infection are presented in Table 1. The age range of the studied population was 18 to 60 years. The mean age of participants was 28.29 years (95% CI: 11.34-45.24 years). All individuals were females and 58 of the PCR positive cases were pregnant. 75% of them were in their third trimester. The mean POA of pregnant participants was32.13 weeks.

Table 1: Demographic details of PCR positive cases

Characteristics	Study population n=79	
	n	%
Age distribution		
18 to 34 years	67	84.8
35 to 50 years	9	11.4
>50 years	3	3.8
Mean age in years	28.29	
range	18-65 years	
Pregnancy status		
Pregnant	58	73.4
Not pregnant	21	26.6
Trimester of pregnant women* (n=32)		
First trimester	4	12.5
Second trimester	4	12.5
Third trimester	24	75
Mean POA	32.13 weeks	
Range	7-40 weeks	

***Out of 58 PCR positive pregnant women, pregnancy trimester data were available only in 32

Analysis of PCR results in positive cases

The summary of the range of Ct values of 79 positive cases is illustrated in Table 2. Available Ct values include amplification of the E gene and S gene. The ranges of Ct values were 8.90 to 37 for the E gene and 8.79-35.70 for the S gene. The mean Ct value of asymptomatic patients was 25.80 for the E gene (C.I:12- 39.60) and 25.94 (C.I: 12.19- 39.69) for the S gene. The median Ct value of our asymptomatic population was around 28 and the interquartile range (IQR) was 23-31.

Twenty-four (24) PCR positive individuals with Ct value equal or less than 25 were found in this study.

Table 2: The summary of PCR results of positive cases

Gene	Ct value range	Number	%
E gene	<=20	17	21.5
	20.1- 30	40	50.6
	>30	22	27.8
S gene	<=20	16	20.3
	20.1- 30	38	48.1
	>30	25	31.6

Analysis of rapid antigen test results in the studied population

Rapid antigen test was positive in 24 PCR positive asymptomatic individuals with a sensitivity of 30.4%. The rapid antigen test was positive in 20 individuals out of a total of 27 asymptomatic individuals with Ct values of at or below 25. Therefore, the sensitivity of RAT was 74% compared to the PCR positive results at or below the Ct value of 25. All PCR negatives were found to be negative on RAT with a specificity of 100%.

Analysis of serology results in the studied cohort

Thirty-seven PCR positive subjects were tested for IgG response against SARS-CoV-2 nucleoprotein. Fifteen asymptomatic individuals were found to have SARS-CoV-2 antibodies with an overall seropositivity rate of 40.5%.

Figure 1 illustrates the relationship of Ct values of E and S genes with the SARS-CoV-2 IgG ratio in asymptomatic individuals. The upward trend of IgG ratio was noted with the rise of Ct values of both genes.

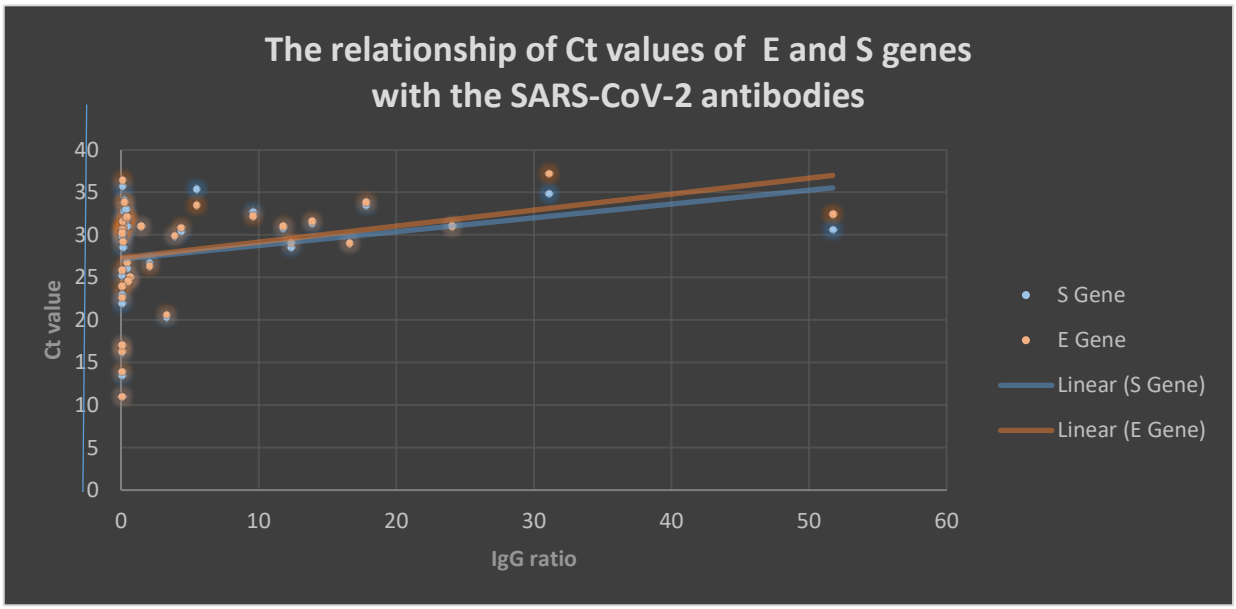

Figure 1: The relation of Ct values of E and S genes with the SARS-CoV-2 antibodies

Seropositivity and Ct values

There was a significant positive correlation between the Ct values of the E gene and SARS-CoV-2 IgG ratio (r=o.345, n=37, p<0.05). Though there was a positive correlation between the Ct

value of the S gene and the IgG ratio, we were not able to elicit a significance at a P-value of 0.05 (r = 0.291, n = 37, p = 0.081).

The diagnostic performance of SARS-CoV-2 PCR Ct values with regard to the detection of serostatus of positivity and negativity was analyzed using ROC analysis. Ct value of 30.5 with a sensitivity and specificity of 60% and 68.2% was the optimal Ct threshold for the E gene with respect to the discrimination between IgG positive and negative results among PCR positive asymptomatic individuals (Figure 2a). The optimal threshold for the S gene concerning the above discrimination was 30.29, with a sensitivity and specificity of 66.7% and 68.2% respectively (Figure 2b).

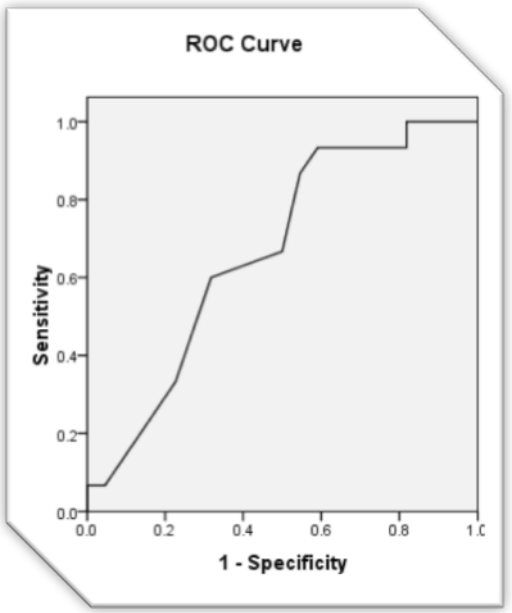

Figure 2a: ROC curve (Ct value of E gene and serostatus for COVID-19)

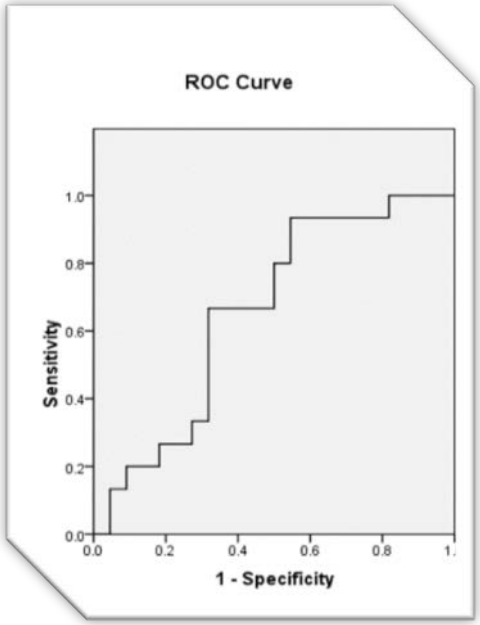

Figure 2b: ROC curve (Ct value of S gene and serostatus for COVID-19)

Rapid antigen test results and Ct values

There was a significant negative correlation between the Ct values of the E and S genes and rapid antigen test results. The r values were –0.630 (n=79, $p < 0.001$) and -0.641 (n = 79, $p < 0.001$) for E and S genes respectively.

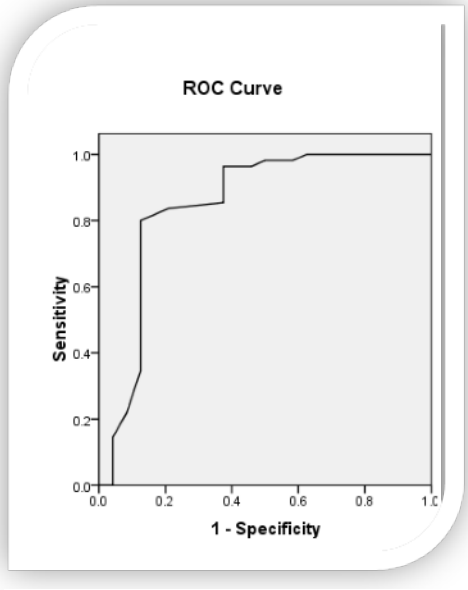

Figure 3a: ROC curve (Ct value of E gene and rapid antigen test result)

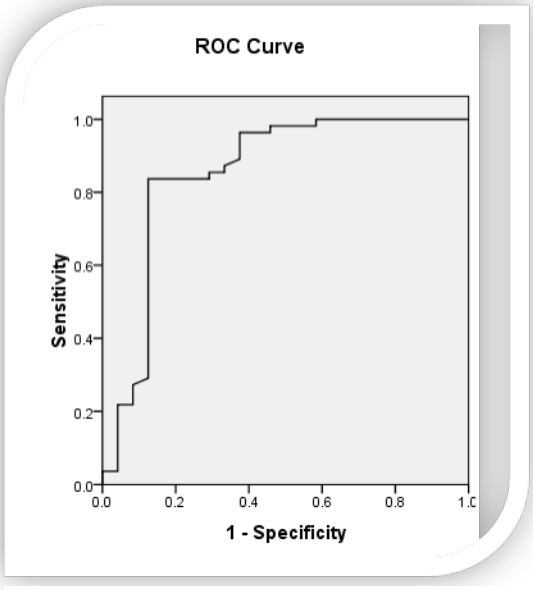

Figure 3b: ROC curve (Ct value of S gene and rapid antigen test result)

The diagnostic performance of Ct values with regard to the detection of infection by rapid antigen testing was analyzed using ROC analysis. CT value of 26.5 with a sensitivity and specificity of 80% and 87.5% respectively was the Ct threshold for the E gene concerning the rapid antigen test negativity (Figure 3a). The S gene's optimal threshold with respect to the above discrimination was 26.06, with a sensitivity and specificity of 83.6% and 87.5% respectively (Figure 3b). The AUC of the ROC curve for E gene was 0.847 (95 % CI = 0.733 – 0.962, $p < 0.05$) and the AUC of the ROC curve of S gene was 0.854 (95 % CI = 0.743 – 0.965, $p < 0.05$).

Discussion

Our study indicates that the median Ct value of the SARS-CoV-2 PCR assay for the asymptomatic population is 28 and IQR is 23-31. The mean Ct values of the asymptomatic studied population were 25.80 and 25.94 for the E and S gene respectively. The Ct values of the asymptomatic population studied here are slightly lower when compared with previous studies.[10,17] A study involving 37 asymptomatic subjects has shown that median Ct values were around 32 with IQR 30 to 35.[10] However, it must be noted that the aforementioned study used the N gene and the ORF 1b as their targets.[10]

The rapid antigen test sensitivity is 30.4% in asymptomatic individuals. However, the sensitivity of RAT was 73.9% compared to PCR positive results below the Ct value 25. Only 30.4% of our

studied population had Ct values equal or less than 25. The lower limit of detection in the rapid antigen test is higher than PCR tests. Generally RAT has a sensitivity of 97% in comparison to positive RT-PCR results with Ct values less than 25, but sensitivity drops to 29.4% when RT-PCR results with Ct values more than 25 are considered.[15,18] When people are most infectious, Ct values tend to be less than 25.[17] The Ct thresholds obtained in our study for rapid antigen positivity are 26.5 and 26.06 for E and S gene respectively with a significant negative correlation ($p<0.001$). Therefore, rapid antigen testing would be helpful in identifying the most infectious individuals.

Interestingly, the overall seropositivity was 40.5% in asymptomatic individuals in our study which is comparable to findings in the existing literature. One study showed that 30.4% of asymptomatic PCR-positive persons had IgG antibodies.[19]

In our study, the optimal Ct thresholds for discrimination of COVID- 19 IgG status were 30.5 and 30.29 for the E and S gene respectively and there was a significant positive correlation between Ct values of the E gene and IgG ratio values. Ct value is one potential marker of contagiousness and is inversely correlated with the quantity of viral RNA. A linear relationship between Ct and RNA rate in upper respiratory tract samples when Ct value <34 was observed in a study by Yu et al.[20] Another study found that the virus cannot be cultivated when the Ct value is over 30. Our study found that the optimal threshold of antibody development is at the range of 30-30.5 Ct values. We were not able to directly demonstrate that the virus is not cultivable at Ct values beyond 30-30.5 in our patients to demonstrate that they are non-infectious. A multi-centre study by Qin et al found that IgG is detectable in 100% of patients by day 20 after onset of disease, by which time the patient is no longer infectious.[13] Therefore, antibody development may indirectly indicate that the patient is no longer excreting viable viruses, and therefore, the individuals with Ct values beyond 30-30.5 can be considered mostly non-infectious in our study population.

One of the strengths of this study is that it is the first published study on cutoff Ct for true infectivity by analyzing antibody ratio in a Sri Lankan sub population. Our study population consists of an asymptomatic population which is more important with regard to an epidemiological point of view. Nevertheless, there are some limitations present in our study. Though our study population is well defined, specimens were not prospectively acquired. In addition, we cannot exclude differences related to the time point of sampling specimens and individual differences in the sampling of nasopharyngeal swabs. Since our cohort in the antibody study is small, further studies involving a large number of participants are needed to determine a threshold for serological response with good sensitivity and specificity. In addition, considering the emerging variants of the SARS-CoV-2 virus, our findings may not be attributed to all SARS-CoV-2 variants being circulated in a population at all times, and the parameters might also change according to the performance of PCR and antigen kits used. Nevertheless, our study can be used as a model to define one's own criteria on infectivity using combined COVID-19 antibody assay with PCR and rapid antigen assay in a local setting.

Conclusion

Our study showed that the presence of SARS-CoV-2 IgG antibodies and rapid antigen negativity are associated with Ct values at a viral load which is considered as non-cultivable and therefore, non-infectious. It is therefore possible to define a cutoff Ct value (with some error margin for practical purposes) for likely non-infectivity. It was also deduced that positive COVID rapid antigen assay seems to be more predictive of infectivity in comparison to PCR.

Declarations

- Acknowledgement: Not applicable
- Funding: Not applicable
- Conflict of Interest: There are no conflicts of interest
- Ethics statement: The ethical clearance was obtained from Ethics Review Committee of Faculty of Medicine, University of Colombo: Protocol No EC 21-089
- Author contributions: All authors contributed to the work presented in this paper.

References

1. Zhou P, Yang XL, Wang XG et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; 579:270–273. *doi: https://doi.org/10.1038/s41586-020-2012-7*
2. Kaul D. An overview of coronaviruses including the SARS-2 coronavirus - Molecular biology, epidemiology and clinical implications. *Current Medicine Research and Practice* 2020; 10(2):54–64. *doi: https://doi.org/10.1016/j.cmrp.2020.04.001*
3. Wu C, Liu Y, Yang Y et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica* 2020; 10(5):766-88. *doi: https://doi.org/10.1016/j.apsb.2020.02.008*
4. Stadnytskyi V, Bax CE, Bax, A et al. The airborne lifetime of small speech droplets and their potential importance in SARS-CoV-2 transmission. *Proceedings of the National Academy of Sciences of the United States of America* 2020; 117:11875– 11877. *doi: https://doi.org/10.1073/pnas.2006874117*
5. Lauer SA, Grantz KH, Bi Q et al. The incubation period of coronavirus disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Annals of Internal Medicine* 2020; 172:577–582. *doi: https://dx.doi.org/ 10.7326/M20-0504*
6. Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395:497–506. *doi: https://doi.org/10.1016/s0140-6736(20)30183-5*
7. Mizrahi B, Shilo S, Rossman H et al. Longitudinal symptom dynamics of COVID-19 infection. *Nature Communications* 2020; 11:6208. *doi: https://doi.org/10.1038/s41467-020-20053-y*
8. Rothe C, Schunk M, Sothmann P et al. Transmission of 2019-nCoV Infection from an asymptomatic contact in Germany. *New England Journal of Medicine* 2020; 382:970–971 *doi: https://doi.org/10.1056/nejmc2001468*
9. Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: A narrative review. *Annals of Internal Medicine* 2020; 173:362–367. *doi: https://doi.org/10.7326/m20-3012*
10. Long QX, Tang XJ, Shi QL et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nature Medicine* 2020; 26:1200–1204. *doi: https://doi.org/10.1038/s41591-020-0965-6*
11. Gandhi M, Yokoe DS, Havlir DV. Asymptomatic transmission, the Achilles' heel of current strategies to control Covid-19. *New England Journal of Medicine* 2020; 382: 2158–2160. *doi: https://doi.org/10.1056/nejme2009758*

12. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). *Science* 2020; 368:489–493. *doi: https://doi.org/10.1126/science.abb3221*
13. Byrne AW, McEvoy D, Collins AB et al. Inferred duration of infectious period of SARS-CoV-2: rapid scoping review and analysis of available evidence for asymptomatic and symptomatic COVID-19 cases. *British Medical Journal* Open 2020; 10:e039856. *doi: https://doi.org/10.1136/bmjopen-2020-039856*
14. Saurabh S, Kumar R, Gupta MK et al. Prolonged persistence of SARS-CoV-2 in the upper respiratory tract of asymptomatic infected individuals. *International Journal of Medicine* 2020; 113:556–60. *doi: https://doi.org/10.1093/qjmed/hcaa212*
15. Schuit E, Veldhuijzen IK, Venekamp RP et al. Diagnostic accuracy of rapid antigen tests in pre-/asymptomatic close contacts of individuals with a confirmed SARS-CoV-2 infection 2021;374:1676 *doi: https://doi.org/10.1101/2021.03.18.21253874*
16. Vabret N, Britton GJ, Gruber C et al. Immunology of COVID-19: current state of the science. *Immunity* 2020; 52:910–941. *doi: https://doi.org/10.1016/j.immuni.2020.05.002*
17. Zhou R, Li F, Chen F et al. Viral dynamics in asymptomatic patients with COVID-19. *International Journal of Infectious Diseases* 2020; 96:288-290. *doi: https://doi.org/10.1016/j.ijid.2020.05.030*
18. Fernandez-Montero A, Argmeri J, Rodriguez JA et al. Validation of a rapid antigen test as a screening tool for SARS-CoV-2 infection in asymptomatic populations. Sensitivity, specificity and predictive values *eClinical Medicine* 2021; 37:100954. *doi: https://doi.org/10.1016/j.eclinm.2021.100954*
19. Batchi-Bouyou AL, Ingoba LL, Ndounga M et al. High SARS-CoV-2 IgG/IGM seroprevalence in asymptomatic Congolese in Brazzaville, the Republic of Congo *International Journal of Infectious Diseases* 2021; 106: 3–7. *doi: https://doi.org/10.1016/j.ijid.2020.12.065*
20. Yu P, Zhu J, Zhang Z et al. A familial cluster of infection associated with the 2019 novel coronavirus indicating possible person-to-person transmission during the incubation period. *The Journal of Infectious Diseases* 2020; 221:1757–61. *doi: https://doi.org/10.1093/infdis/jiaa077*