Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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VIROLOGY

SARS-CoV-2 viral load dynamics and real-time RT-PCR cycle threshold interpretation in symptomatic non-hospitalised individuals in New Zealand: a multicentre cross sectional observational study

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Summary

We conducted a multicentre cross sectional observational study of laboratory, public health and hospitalisation data for PCR-confirmed COVID-19 cases within the New Zealand Northern Region, between 12 February and 8 June 2020. The aim of this study was to describe population level SARS-CoV-2 upper respiratory tract (URT) viral load dynamics by stratifying positivity rates and polymerase chain reaction (PCR) cycle threshold (Ct) values of URT samples from COVID-19 cases by days since symptom onset, and to explore utility of Ct values in determining length of time post-infection and thus potential infectivity.

Of 123,124 samples tested for SARS-CoV-2 by PCR, 579 samples (407 positive and 172 negative) from 368 symptomatic non-hospitalised individuals with PCR-confirmed infection were included. Sample positivity rate was 61.5% (8/13) for pre-symptomatic samples, rising to 93.2% (317/340) for samples collected during the purported symptomatic infectious period (days 0–10 post-symptom onset), and dropping to 36.3% (82/226) for post-infectious period samples (day 11 onwards). URT viral load peaked shortly after symptom onset, with median Ct values ranging 20.00–29.99 until 15 days post-symptom onset, and >30.00 after this time. Of samples with a Ct value of <20.00, 96.1% were collected during the symptomatic infectious period. However, of samples with a Ct value $\geq$30.00 and $\geq$35.00, 46.9% and 18.5%, respectively, were also collected during the symptomatic infectious period.

The findings of this study indicate that at or soon after symptom onset represents the optimum time to test for SARS-CoV-2 in the URT, with median Ct values suggesting the useful testing window extends until around 15 days post-symptom onset. In asymptomatic individuals or those with unknown dates of symptom onset, Ct values <20.00 imply recent onset/potential infectivity, but Ct values $\geq$30.00 or $\geq$35.00 do not exclude recent onset/potential infectivity. Individual sample Ct values should not be used as an absolute marker of length of time post-infection or to exclude infectivity where date of symptom onset is unavailable.

Key words: COVID-19; SARS-CoV-2; PCR; cycle threshold; New Zealand.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), reached New Zealand on 28 February 2020 when the country identified its first case.[1] Following a rapid increase in cases the New Zealand Government declared a state of national emergency and mandated a nationwide social lockdown on 25 March ('Alert Level 4').[1] This lockdown formed part of an outbreak elimination strategy designed to prevent importation of new cases and halt viral transmission within the populace. An essential element of such a strategy is rapid detection of new cases through widespread testing.[1] The mainstay of COVID-19 testing consists of analysing clinical samples for the presence of SARS-CoV-2 nucleic acid using real time reverse transcription polymerase chain reaction technology (hereafter referred to as PCR).[2] When a PCR test for SARS-CoV-2 detects the specified nucleic acid target sequences, a positive result is

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associated with a cycle threshold (Ct) value: the number of PCR cycles needed to produce a detectable signal.[3] The lower the Ct value, the fewer PCR cycles needed to produce a positive result, the greater the quantity of viral nucleic acid in the sample tested, and the greater the quantity of viral nucleic acid (the viral load) in the anatomical site sampled.[3] Ct values do not equate to direct viral load measurement (which requires standardisation using a reference curve) but provide a useful surrogate measure of viral load.[4]

In the initial stages of SARS-CoV-2 infection there is a high viral load in the upper respiratory tract (URT), with URT samples favoured for detecting early infection.[2] Viral load in the URT decreases with time, however studies examining URT temporal viral load dynamics have primarily involved hospitalised patients,[5] and viral dynamics in non-hospitalised individuals require further elaboration.

The infectious period is generally regarded as ending at 10 days post-symptom onset, with replication competent virus rarely isolated after this time.[6–8] Increased screening of asymptomatic individuals (such as case contacts or returning travellers) poses a diagnostic and public health challenge, as with no clear date of symptom onset the infectious period cannot be easily delineated. This leads to the question of whether Ct values from positive results can predict infectivity and inform duration of isolation/quarantine.[9] Specifically, does a higher Ct value (e.g., $\geq$35.00) imply that the infectious period has passed?

In this multicentre cross sectional observational study, we reviewed laboratory, public health and hospital admission data for all PCR-confirmed SARS-CoV-2 cases of all ages within the 1.75 million population of the Auckland and Northland areas of New Zealand [the Northern District Health Board (DHB) Region].[10] This study had two main aims. Firstly, to describe SARS-CoV-2 URT viral load dynamics at a population level by comparing positivity rates and Ct values of samples from PCR-confirmed cases with time since symptom onset. A greater understanding of URT temporal viral load dynamics in symptomatic non-hospitalised individuals will enhance understanding of the testing window and interpretation of results in this group. Secondly, to describe the distribution of sample collection (split by purported symptomatic infectious period, days 0–10) in samples with given Ct value ranges. This will allow better understanding of the utility of Ct values in determining length of time post-infection and thus potential infectivity in the absence of a date of symptom onset.

MATERIALS AND METHODS

Laboratory data

Four laboratories are routinely responsible for SARS-CoV-2 PCR testing within the Northern DHB Region of New Zealand: LabPLUS Auckland City Hospital, Auckland DHB (Site A); Labtests, Community Pathology Services Provider (Site B); Middlemore Hospital, Counties Manukau DHB (Site C); and North Shore Hospital, Waitematā DHB (Site D). A retrospective review of laboratory data was undertaken for all SARS-CoV-2 PCR tests performed by these sites between 12 February 2020 (the date SARS-CoV-2 PCR testing commenced in the Northern DHB Region, at Site A) and 8 June 2020. Extracted laboratory data from each site for the above study period included: total number of samples tested; sample types; collection dates; SARS-CoV-2 PCR results; and Ct values for positive results. A variety of in-house and commercial SARS-CoV-2 PCR testing platforms were used by the four study sites during the study period. A comparison of Ct values between assays and targets was not an aim of the present study, as this has already been undertaken elsewhere [see local and regional external quality assurance (EQA) program reports], and thus data on the individual assays and targets used was not collected.

Public health, hospital admission, and demographic data

COVID-19 was made a notifiable disease in New Zealand on 30 January 2020. Routinely collected public health data for notified cases include presence or absence of clinically compatible symptoms and date of symptom onset. For individuals with PCR-confirmed SARS-CoV-2 infection, these data were collected from public health case notification forms, epidemiological surveillance databases, and case/contact management progress notes. For all individuals with PCR confirmed SARS-CoV-2 infection, hospital admission and crude demographic data were extracted from electronic clinical records. This consisted of sex, age, and the dates and reason for any inpatient admission episodes during the study period.

Analysis

The laboratory, public health and regional hospital admission data were integrated into an anonymised secure dataset for analysis. Positive and negative URT samples from individuals of all ages with PCR-confirmed symptomatic SARS-CoV-2 infection, who had not been admitted to hospital for >24 hours due to COVID-19, and were resident in the Northern DHB Region, were included in the analysis. Where multiple samples had been collected from a single individual on the same date, or where a single sample was tested multiple times or against multiple PCR targets, only the positive specimen/result or the specimen/result with lowest Ct value was included. This sample selection strategy was used to ensure that the included results/values most accurately reflected the true underlying viral load in the URT at any given time point post-symptom onset. For example, where a positive and a negative specimen was collected on the same date, the negative was assumed to be a false negative result, and where two positive samples were collected on the same date, the 'stronger' positive (lower Ct) was felt to be a truer indicator of the actual viral load. Samples were excluded from the analysis if they were from individuals without PCR-confirmed SARS-CoV-2 infection, from individuals residing outside of the Northern DHB Region, if they were lower respiratory tract samples, if they were from asymptomatic individuals (individuals never recorded as having developed symptoms), if they were from individuals hospitalised for >24 hours due to COVID-19, if they tested positive for SARS-CoV-2 but the Ct value was not recorded, or if either the sample collection date or the date of symptom onset was unavailable.

The number of days since symptom onset was calculated by subtracting the date of symptom onset (day 0) from the date of sample collection. For the purposes of plotting data and calculating medians and interquartile ranges (IQR), included negative and indeterminate specimens were assigned a Ct value of 40.00. Positive, negative and indeterminate results were defined on an individual assay basis, either by each testing laboratory for in-house assays, or by the manufacturer for commercial assays. Ct values for included samples were plotted against days since symptom onset to visualise the temporal viral load dynamics. Descriptive statistics and graphical data representation were undertaken using the R statistical software package.[11] Variations in Ct value over time were visualised by fitting LOESS (locally weighted smoothing) curves with a span of 0.8.[12] This study was approved by the Auckland Health Research Ethics Committee (AHREC, AH1391).

RESULTS

Between 12 February and 8 June 2020 inclusive, a total of 123,124 samples underwent SARS-CoV-2 PCR testing by the four study sites. Of the total number of samples tested, 122,628 (99.6%) tested negative (or indeterminate) and 496 (0.4%) tested positive. Taking positive and negative samples from individuals with PCR-confirmed SARS-CoV-2 infection, 708 samples were assessed for study inclusion. Subsequently, 129 samples were excluded, leaving 579 samples [407 (70.3%) positive and 172 (29.7%) negative] from 368 symptomatic non-hospitalised individuals included in the final analysis (Fig. 1). Included positive and negative samples by days since symptom onset are shown in Supplementary Fig. 1 (Appendix A).

Included samples were from 368 individuals aged from 4–97 years, with a median age of 37 years (IQR 27–55) and 160/368 (43.5%) male. Sampling times for included samples ranged from 20 days prior to symptom onset to 104 days after. The earliest positive sample was collected 8 days before symptom onset, and the latest positive sample was collected 80 days after symptom onset.

The positivity rate of samples collected during the pre-symptomatic period was 61.5% (8/13), rising to 93.2% (317/340) for samples collected during the purported symptomatic infectious period (days 0–10), and dropping to 36.3% (82/226) for samples collected in the post-infectious period (day 11 onwards).

The lowest Ct value of included samples was 8.36 (three samples from three separate individuals), with a median Ct value of 27.82 (IQR 21.51–40.00). Raw and median Ct values by days since symptom onset are shown in Fig. 2 and Supplementary Fig. 2 (Appendix A), respectively. A comparison of median Ct values by study site is shown in Supplementary Fig. 3 (Appendix A).

Taking only positive samples collected from the day of symptom onset (day 0) onwards (n=399), for given Ct ranges the number and proportion of samples collected during the purported symptomatic infectious period (days 0–10) and the post-infectious period (day 11 onwards) was calculated (Table 1).

To illustrate the URT viral load dynamics at an individual level, Ct values by days since symptom onset were plotted separately for individuals with ≥5 samples included in the study (n=10) (Fig. 3). These plots illustrate that positive samples may have fluctuating Ct values over the course of infection (Fig. 3E,G); individuals may alternate between positive and negative results (Fig. 3B,E,H); and samples with higher Ct values (e.g., ≥30.00) may be collected at the start of the infectious period (Fig. 3A,F).

DISCUSSION

In this study of symptomatic non-hospitalised individuals with PCR-confirmed COVID-19 in New Zealand, we examined population level results, including Ct value trends relative to time since symptom onset, to provide a surrogate measure of the URT viral load dynamics in mild SARS-CoV-2 infection.

We found that the positivity rate of samples differed by time since onset of symptoms, with approximately 60% of

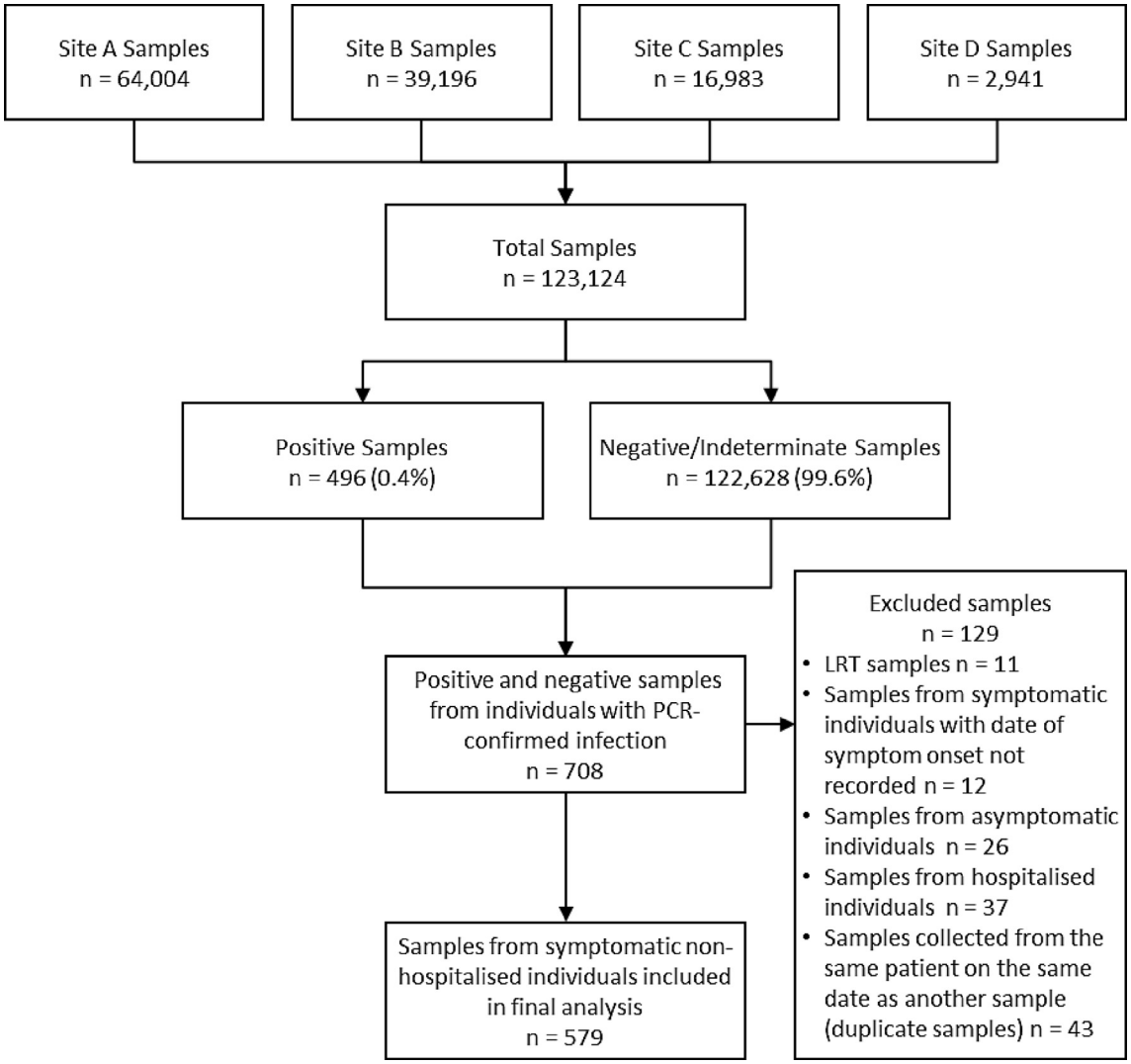

Fig. 1 Summary of samples included in the study. LRT, lower respiratory tract.

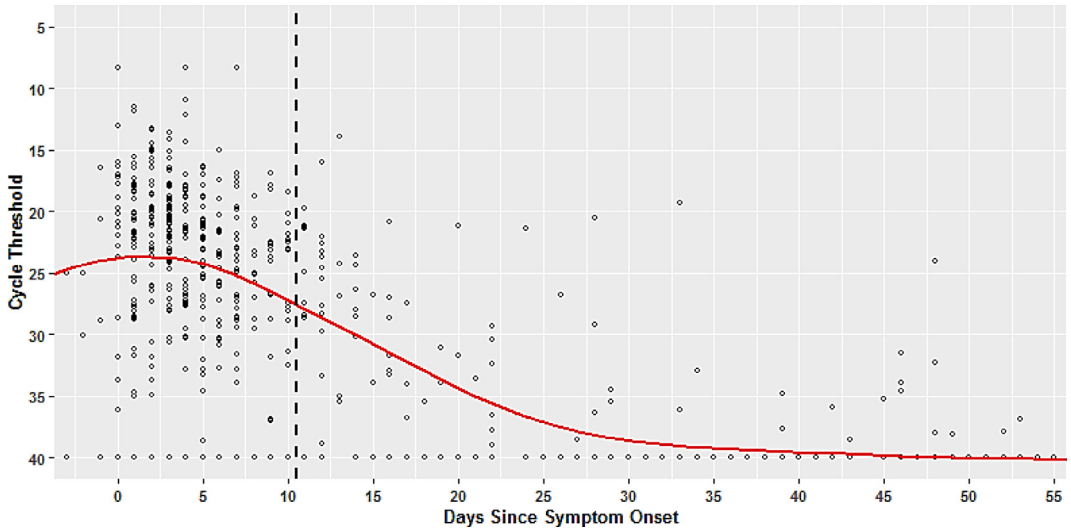

Fig. 2 Raw cycle threshold (Ct) values by days since symptom onset for included positive and negative samples from symptomatic non-hospitalised individuals with PCR-confirmed SARS-CoV-2 infection (n=579), with LOESS (locally weighted smoothing) curves fitted to visualise viral load dynamics over time. Day 0 = day of symptom onset. Dashed vertical line represents end of symptomatic infectious period (after day 10 post-symptom onset). A Ct value of 40.00 indicates a negative sample.

samples positive when collected in the pre-symptomatic period, rising to >90% of samples positive when collected during the first 10 days post-symptom onset, and declining to <40% positive when collected from day 11 onwards. Although there is no diagnostic gold standard comparator, these data are consistent with findings from repeat PCR studies elsewhere, that suggest a single PCR test collected in the first week after symptom onset demonstrates approximately 90% sensitivity for detection of SARS-CoV-2,[13–15] and thus support a single sample testing strategy in low risk symptomatic individuals in the community who present early in illness.

In this population, the median Ct values of URT tract samples were approximately 30.00 in the pre-symptomatic period, with median Ct values ranging between 20.00 and 29.99 from time of symptom onset to around day 15, and then rising to >30.00 after day 15. The median Ct value was lowest, indicating peak URT viral load, at 2 days post-symptom onset. A peak URT viral load close to the start of the symptomatic period is consistent with the majority of previous studies in hospitalised patients.[5] These findings, coupled with the high sample positivity rate above, suggest that the optimum time to test for early SARS-CoV-2 infection is as soon as possible after symptom onset.

Whilst the present study includes a limited number of samples collected in the pre-symptomatic period (n=13), the lower median Ct and positivity rate of these samples suggest that the viral load is lower in the pre-symptomatic period compared with soon after symptom onset and demonstrates that a negative COVID-19 PCR result in an individual without symptoms should not preclude repeat testing if they subsequently develop a clinically compatible illness.[16]

Visualisation of raw Ct values by days since symptom onset shows that a broad range of Ct values, including both positive and negative results, are observed throughout the course of the infection. Notable outliers can be seen, with Ct values ≥35.00 obtained as early as the day of symptom onset (day 0), and Ct values <20.00 from samples collected greater than 30 days post-symptom onset. Of samples with Ct values <20.00, 96.1% were collected in the purported symptomatic infectious period (days 0–10), suggesting that a Ct value of <20.00 could act as a proxy for infectivity in asymptomatic individuals. However, the converse was not the case, with a substantial proportion of samples with Ct values ≥30.00 and ≥35.00 having been collected during the purported symptomatic infectious period (46.9% and 18.5%, respectively).

Table 1 Proportion of samples of given cycle threshold (Ct) value ranges collected during the symptomatic infectious period (days 0–10 since symptom onset) and the post-infectious period (day 11 onwards)

Ct value range	Number of samples (%)		Total
	Symptomatic infectious period (0-10 days since symptom onset)	Post-infectious period (≥11 days since symptom onset)	
<15.00	15 (93.8)	1 (6.3)	16
<20.00	98 (96.1)	4 (3.9)	102
20.00–29.99	181 (83.8)	35 (16.2)	216
≥30.00	38 (46.9)	43 (53.1)	81
≥35.00	5 (18.5)	22 (81.5)	27

For samples of given cycle threshold (Ct) ranges, the proportion of positive samples collected during the symptomatic infectious period (days 0–10) and post-infectious period (day 11 onwards) is shown. Includes only positive samples collected post-symptom onset. Note that the Ct value range <20.00 is inclusive of all samples within the Ct value range <15.00, and the Ct value range ≥30.00 is inclusive of all samples within the Ct value range ≥35.00, thus totals have not been provided.

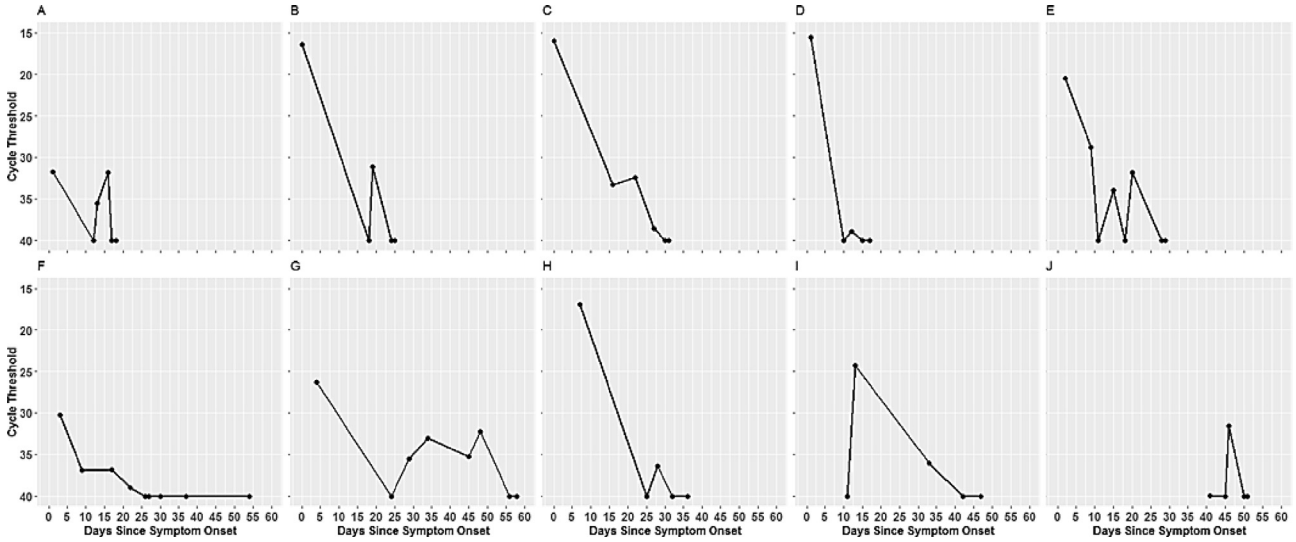

Fig. 3 (A–J) Cycle threshold (Ct) values by days since symptom for individuals with five samples or more included in the study (n=10). Day 0 = day of symptom onset. A Ct value of 40.00 indicates a negative sample.

Whilst several reports based on epidemiological and viral culture data show that people are likely non-infectious >10 days after symptom onset and that viable virus is generally unculturable from samples with Ct values >30.00–35.00,[6–8] our data (Table 1, Fig. 3) clearly illustrate that a Ct value cannot be used as an absolute marker of time since onset of infection. Recently, in New Zealand and elsewhere, there is an increasing focus on PCR testing of asymptomatic individuals.[17–26] However, in the absence of epidemiological and viral culture studies looking specifically at infectivity in those with higher Ct values who are pre-symptomatic, asymptomatic, or in the first few days after the onset of symptoms, any assumptions that high Ct values imply old infection or non-infectivity are ill advised in an individual with an unknown date of symptom onset.

As has been observed in other studies and case reports, SARS-CoV-2 RNA can persist in the URT long after resolution of infection:[6–8] 80 days post-symptom onset in this study. This has important implications for individuals or populations undergoing repeated testing to screen for re-exposure to COVID-19, as PCR cannot discriminate between active replicating virus and residual viral nucleic acid. Additionally, it may lead to misleading positive SARS-CoV-2 testing results in individuals who have had undiagnosed COVID-19 in the recent past. For example, an individual that has mild self-limiting COVID-19 and 2 months later presents with a clinically similar viral illness may still have detectable SARS-CoV-2 RNA in the URT, and the cause of their current illness may be erroneously attributed to COVID-19.

In the event of a positive COVID-19 test in an asymptomatic individual, or an individual simultaneously testing positive for COVID-19 and one or more other respiratory viruses, serology may play a useful role in elucidating the chronicity of the COVID-19 infection. In such individuals, repeated SARS-CoV-2 PCR testing is unlikely to be of any value, since Ct value trends from a single individual do not accurately reflect viral load dynamics, as we have shown in Fig. 3, and thus should not be used for 'clearance' purposes.

This study has some notable limitations. The dates of symptom onset as notified to public health may have inaccuracies, as they rely on an individual's ability to recollect the start of what are often mild and non-specific symptoms. The samples included in this study were tested by different laboratories using a variety of testing platforms and gene targets, and thus there is likely to be Ct value variation both within laboratories, and between laboratories, as shown in Supplementary Fig. 3 (Appendix A). For example, more efficient nucleic acid extraction methods and higher extraction volumes may produce lower Ct values, certain gene targets generally yield lower Ct values than others, and some assays perform a pre-amplification/nested PCR step, producing lower Ct values compared with other single PCR step assays. Nonetheless, this reflects the reality of SARS-CoV-2 testing globally, where a combination of high testing volumes and resource constraints mean that samples from a given population are rarely tested by a single laboratory using a single testing platform with a single gene target. By using unadjusted Ct values 'as they would be reported', we have demonstrated both the usefulness of examining a large pool of samples to visualise population level viral load dynamics, and also the lack of utility in examining Ct value trajectories for single individuals (i.e., repeated PCR testing), or using individual Ct values to estimate length of time post-infection. In the present study, individual samples had rarely been tested in parallel using different methods, and thus a comparison of Ct values between different methods was not attempted and has already been undertaken by local and regional EQA programs.

CONCLUSION

In this study of SARS-CoV-2 temporal viral load dynamics in symptomatic non-hospitalised individuals with COVID-19 in New Zealand, we have shown that viral load peaks shortly after symptom onset, sample positivity rate is highest during the symptomatic infectious period (days 0–10), and median Ct values indicate that PCR is likely to be reliable for detecting SARS-CoV-2 infection in the first 15 days post-symptom onset, but diagnostic yield may drop after this time. Samples with Ct values <20.00 may indicate recent onset of infection, but positive samples with Ct values of ≥30.00 or ≥35.00 are frequently obtained during the

symptomatic infectious period; therefore, for asymptomatic individuals or symptomatic individuals where the date of symptom onset is unclear, Ct values from individual samples should not be used as an absolute marker of length of time post-infection or to exclude infectivity.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2021.01.007.

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