











# Cycle Threshold Values in the Context of Multiple RT-PCR Testing for SARS-CoV-2

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**Purpose:** Discharge or follow up of confirmed coronavirus disease 2019 (COVID-19) cases depend on accurate interpretation of RT-PCR. Currently, positive/negative interpretations are based on amplification instead of quantification of cycle threshold (Ct) values, which could be used as proxies of patient infectiousness. Here, we measured Ct values in hospitalized confirmed COVID-19 patients at different times and its implications in diagnosis and follow up.

**Patients and Methods:** Observational study between March 17th-May 12th, 2020 using multiple RT-PCR testing. A cohort of 118 Hispanic hospitalized patients with confirmed COVID-19 diagnosis in a reference hospital in Quito, Ecuador. Multiple RT-PCR tests were performed using deep nasal swab samples and the assessment of SARS-CoV-2 genes N, RdRP, and E.

**Results:** Patients' median age was of 49 years (range: 24–91) with a male majority (62.7%). We found increasing levels of Ct values in time, with a mean Ct value of 29.13 (n = 61, standard deviation (sd) = 5.55) for the first test and 34.38 (n = 60, sd = 4), 35.52 (n = 20, sd = 2.85), and 36.12 (n = 6, sd = 3.28), for the second, third, and fourth tests, respectively. Time to RT-PCR lack of amplification for all tests was of 34 days while time to RT-PCR Ct values >33 was of 30 days.

**Conclusion:** Cycle thresholds can potentially be used to improve diagnosis, management and control. We found that turnover time for negativity can be large for hospitalized patients and that 11% cases persisted with infectious Ct values for more time than the current isolation recommendations.

**Keywords:** SARS-CoV-2, coronavirus, pandemic, cycle thresholds, RT-PCR, diagnosis, COVID-19

## Introduction

Since the first cases of coronavirus disease-2019 (COVID-19) were detected in Latin America in late February 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread dramatically due to political inaction and established economical and societal vulnerabilities.<sup>1</sup> Not surprisingly, countries such as Brazil, Peru, or Ecuador have become large hotspots of COVID-19 in South America with 4,147,794; 689,977; and 110,092 confirmed cases, respectively, as of September 2020.<sup>2</sup>

In contexts of overwhelmed health systems, criteria for COVID-19 patient management should be revised to avoid depletion of hospital resources including laboratory supplies and health personnel. Recently, the World Health Organization (WHO) updated its guidelines to allow hospital discharge based on clinical findings

as an alternative of RT-PCR positive to negative conversions,<sup>3</sup> a decision that acknowledged in paper “long-lasting” local practices in low- and middle-income countries. Regardless, multiple institutions across the Americas still request a double RT-PCR negative test to assure a lack of infectiousness, which can be misleading if interpreted plainly as a positive/negative test.<sup>4,5</sup>

Recently, an alternative approach has been suggested to interpret RT-PCR COVID-19 diagnosis based on the cycle threshold (Ct) values, which are correlated with SARS-CoV-2 viral load and therefore viral dynamics such as replication and transmissibility.<sup>6,7</sup> However, there is a lack of consensus of whether a particular threshold for RT-PCR COVID-19 Ct values might be safely used to determine patient infection status with some considering infectious RT-PCR Ct values to those  $\leq 24$ ,<sup>8</sup>  $\leq 34$ ,<sup>7,9</sup> or  $\leq 38$ .<sup>10,11</sup>

Here, we (1) explored two Ct values (ie, 24 and 33) as avenues for patient diagnosis and follow up in a context of multiple RT-PCR testing in a South American cohort, (2) depict the dynamics of Ct values at different testing times, and (3) compare the differences of interpretation between Ct values and plain amplification for COVID-19 diagnosis. We also measured different demographic, clinical, and laboratory variables of the same population to explore their capacity to predict the profile of Ct values at different testing times.

## Patients and Methods

In this observational case-series, we studied 118 patients hospitalized in a public COVID-19 reference hospital (Hospital General del Sur de Quito) in Quito, Ecuador from March 17th to May 12th, 2020. Deep nasal swab samples were collected using inactivated viral preservative medium (IMPROVIRAL™) and stored at 2–8°C at Hospital Carlos Andrade Marín in Quito, Ecuador. All samples were processed within eight hours of collection. We extracted RNA using QIAamp® Viral RNA mini Kit (Qiagen, cat: 52,906) and the RT-PCR was performed using Allplex™ 2019-nCoV assay (Seegene Inc., Seoul, South Korea) targeting genes E, RdRP, and N, as recommended by the respective manufacturer. If more than one gene was positive, we obtained the average of those observations as a representation of overall Ct values; on the contrary, if only one gene was available, we used the available value to represent the observation instead of the average. Follow up sample collection was performed regardless of patient status (ie, hospitalized or discharged)

and was continued until RT-PCR no-amplification was observed.

## Statistical Analysis

We explored the ability of demographic, laboratory, and epidemiological variables (eg, age, leucocytes, comorbidities, etc) to explain patterns of Ct values at different testing times. We assessed the normality of distributions and applied a *t*-test or Mann–Whitney-U accordingly to compare different groups. We used Pearson correlation tests to identify any relationship between quantitative variables; also, we dichotomized each result using a threshold of >33 to differentiate between non-infectious/infectious cases and explored different parameters using a Chi-square test or Fisher exact test depending on expected values being smaller than five.<sup>12</sup> We established the value of statistical significance considering an alpha <0.05. We repeated all the statistical explorations using a Ct value of 24 for the first test; we did not explore this threshold in further testing times due to the lack of samples for comparison.

## Ethics

The study was approved by the ethical committee of the Universidad San Francisco de Quito (P2020-023M) and the Ministry of Public Health of Ecuador. We confirm that all patients provided informed consent and that this study was conducted in accordance with the Declaration of Helsinki.

## Results

The median age of the population was 49 years (range: 24 to 91) with 74 males (62.7%) and 44 females (37.3%). Seven patients died during hospitalization (5.9%). The median since symptoms onset to hospital admission was six days (range: 0 to 28; [Table 1](#)). The median time from symptom onset to first test was of nine days (range: 0 to 53; [Figure 1](#)).

Averaged Ct values of the three genes from amplifying samples showed increasing values in time with a mean Ct value of 29.13 (n = 61, sd = 5.55) for the first test and 34.38 (n = 60, sd = 4), 35.52 (n = 20, sd = 2.85), and 36.12 (n = 6, sd = 3.28), for the second, third, and fourth tests, respectively ([Figure 1](#)). The overall median of no amplification was of 30 days (range: 4 to 69) with 50% of cases labeled as negative between days 22 and 37. Non-infectious Ct values (ie, >33) were found with a median

**Table 1** Clinical, Epidemiological, and Laboratory Characteristics of the 118 Patients Included in the Present Study

Patients	Counts	Summary Statistics
Age	49 y	24–91 (range)
Men	74	62.7%
Women	44	37.1%
Death	7	5.9%
Alive	111	94.1%
Beginning of symptoms to hospitalization	6 d	0–28 (range)
Beginning of symptoms to discharge	25 d	0–76 (range)
First attention to discharge	20.5 d	0–72 (range)
<b>Comorbidities</b>	33	30.28%
• Hypertension	22	19.8%
• Diabetes mellitus type 2	8	7.2%
• Hypothyroidism	7	6.3%
• Chronic kidney disease	3	2.7%
• Cancer	2	1.8%
• Chronic ischemic cardiomyopathy	1	0.9%
• Rheumatoid arthritis	1	0.9%
• Lupus	1	0.9%
• Hepatic cirrhosis	1	0.9%
• HIV	1	0.9%
<b>Symptomatology</b>		
• Fever	75	67.6%
• Dry cough	57	51.4%
• Asthenia	46	41.4%
• Dyspnea	35	31.5%
• Odynophagia	29	26.1%
• Arthralgia	23	20.7%
• Productive cough	22	19.8%
• Myalgia	21	18.9%
• Headache	18	16.2%
• Diarrhea	13	11.7%
• Runny nose	13	11.7%
• Abdominal pain	7	6.3%
• Anosmia	6	5.4%
• Dysphagia	6	5.4%
• Chest pain	3	2.7%
• Nausea	4	3.6%
• Vomit	4	3.6%
• Dysgeusia	4	3.6%
• Conjunctivitis	1	0.9%
• Dysuria	1	0.9%
• Increased frequency of urination	1	0.9%
<b>Laboratory parameters</b>		
White blood cells	6.1	1.6–6.41 (range)
Hemoglobin	15	8.7–49 (range)
Hematocrit	43.45	16–55.9 (range)

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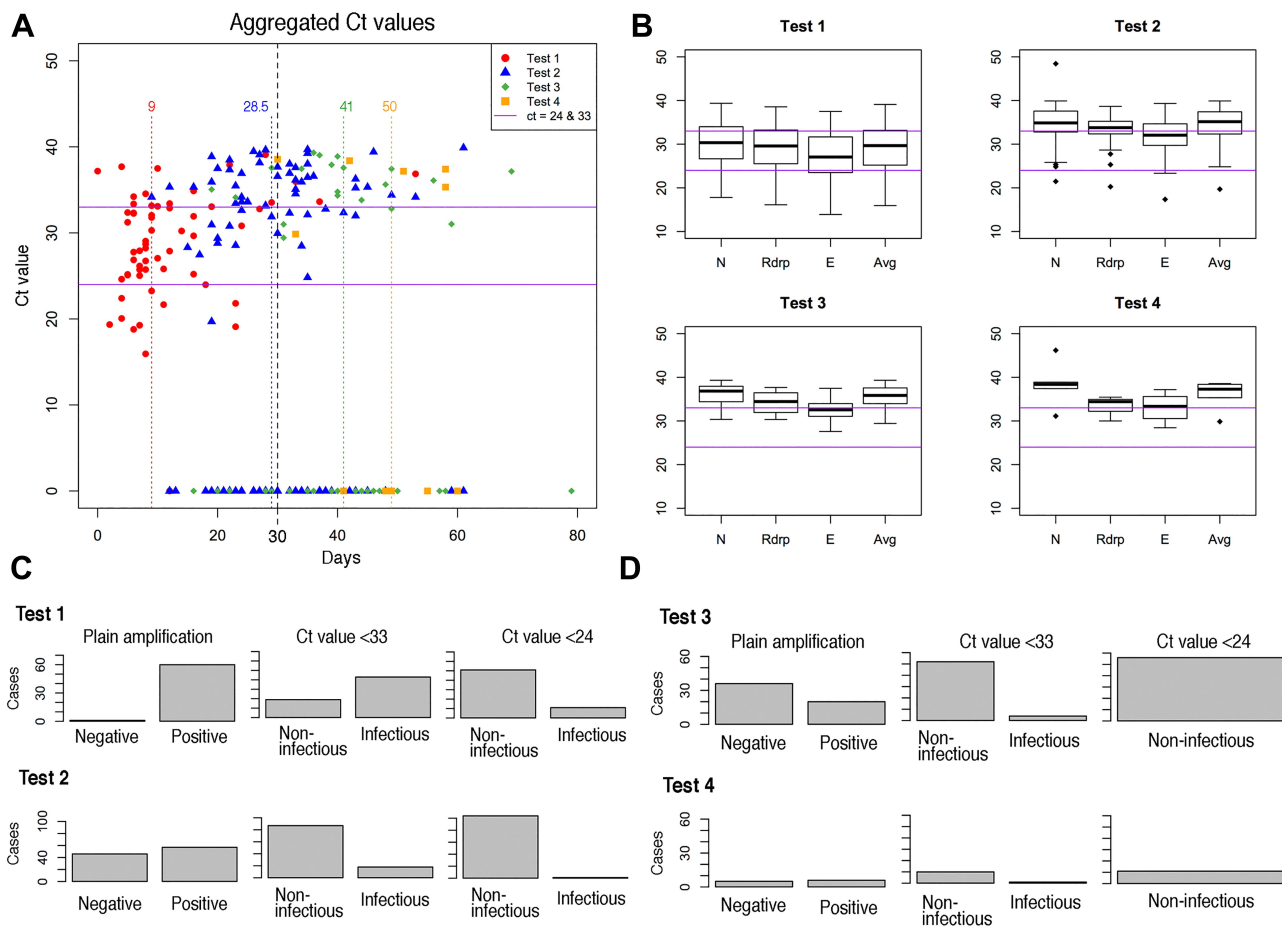
**Table 1** (Continued).

Patients	Counts	Summary Statistics
Platelets	225	2.26–588 (range)
Red blood cells	4.99	3.13–6.32 (range)
Monocytes %	7.6	0.4–17.3 (range)
Eosinophils %	0.2	0–20 (range)
Lymphocytes %	20.9	0.5–61 (range)
Neutrophils %	68	2.8–94.7 (range)
Basophils %	0.4	0–2.3 (range)
Glucose	97	56–468 (range)
BUN	13	5–55 (range)
Urea	25.68	0.77–117.7 (range)
Creatinine	0.81	0.45–66 (range)

of 12 days (range: 4–53) since the start of symptoms to the first test (Table 2).

Five patients presented lower Ct values (ie, <33) during the third (n = 4) and four tests (n = 1). One case remained below the Ct (average = 31.33) on the sixth nasopharyngeal swab test after 53 days of symptoms onset (not shown in Figure 1). Further, while considering the second test, seven patients had a Ct value <33 after 30 days since symptoms onset (Supplementary material). The median Ct value of gen E remained below the threshold of 33 up to the third test (Figure 1). Using a less restrictive threshold (ie, Ct of 24),<sup>8</sup> all but one case might be considered non-infectious on the second test (Figure 1).

Univariate statistical explorations of demographic variables, predictors of severity, and different laboratory parameters were statistically non-significant for the third test and were not calculated for the four test due to lack of samples (Figure 1 and Supplementary material). For the first test, C reactive protein (CRP) worked as a predictor of infectious versus not infectious patients considering a Ct value of 33 ( $\chi^2 = 3.691$ ,  $p = 0.033$ ) with the majority of infectious patients with abnormal CRP measures. Also, for the first test, we found a correlation between platelet counts and Ct values (Pearson = 0.396,  $p = 0.003$ ). For the second test, we found a negative correlation between age and Ct values (Pearson = -0.276,  $p = 0.033$ ) as well as neutrophils and Ct values (Pearson = -0.259,  $p = 0.047$ );



**Figure 1** Cycle threshold (Ct) values obtained at different testing times and COVID-19 status based on RT-PCR plain amplification and Ct values. **(A)** Distribution of Ct values in relation of symptom to test time (x-axis), depicting the median symptom-to-test time for each testing scenario (vertical lines with numbers representing days) and two Ct thresholds (horizontal lines = 24 and 33). Day 30 was used previously as a guide for patient discharge and is depicted here for reference (black line). **(B)** Distribution of Ct values for each testing scenario for the three genes and their average (Avg) as analyzed in this study. **(C)** The difference of interpretation of COVID-19 status based on plain amplification, Ct values <33, and <24 for tests one and two. With a Ct of 24, almost all cases are non-infectious at the second test. **(D)** Same as **C** for test three and four.

moreover, we found a statistical significant difference between patients admitted versus those not admitted to the intensive care unit ( $\chi^2 = 6.094$ ,  $p = 0.013$ ). When using a Ct value of 24 to differentiate between infectious versus non-infectious patients, we found a statistically significant gender difference ( $\chi^2 = 6.579$ ,  $p = 0.04$ ), which was non-significant when evaluating a Ct value of 33 ([Supplementary material](#)).

## Discussion

In line with recent research, we encourage the use of Ct values to complement positive/negative SARS-CoV-2 diagnosis and as a potential guide for patient management.<sup>13</sup> Theoretically, viral replication peaks during the first days of symptoms onset, which argues in favor of presymptomatic transmission.<sup>14</sup> Here, we recorded Ct values on different testing scenarios in a cohort of inpatients with a median of 20.05 (range: 0 to 72)

hospitalization days, it might be the case that patients diagnosed with milder infections and treated outside the hospital would present higher Ct values on follow up tests and therefore show a lower transmission risk for the community.

In our study, the median from symptom onset to first testing equals nine days, which argues on a population that has received their first test after a relatively large period. This is a fair representation of the health capacity of Ecuador, which has seen a reduced ability to respond to the pandemic. Furthermore, we expect that the infectious to non-infectious Ct value turnover of the first test shown here (=12 days with a Ct of 33; [Table 2](#)) is a feature of a population that presented late on their disease course. Regardless, for the start of the second test, only 18/105 (17.14%) amplifications had a Ct value below 33 and therefore were labeled as infectious, whereas 87/105 (82.86%) values were above 33 and were identified as not infectious with the earliest non-infectious second test on day

**Table 2** Number of Cases per Test and Status Based on Proxies of Infectiousness at Two RT-PCR Cycle Threshold Values (ie, 24 and 33), and Plain Amplification. Turnover Represent the Median of Time Required to Obtain a Negative Sample

Number of Test	Infectious Cases (Ct <33)	Non-Infectious Cases (Ct>33)	Time to Turnover Ct 33 (Median, Range)	Infectious Cases (Ct <24)	Non-Infectious Cases (Ct>24)	Time to Turnover Ct 24 (Median, Range)	Amplifying Cases	Non-Amplifying Cases	Time to Amplification Turnover (Median, Range)
Test 1 (n = 62)	43 (69.35%)	19 (30.65%)	12 (4–53)	11 (17.74%)	51 (82.26%)	9 (4–53)	61 (98.39%)	1 (1.61%)	12
Test 2 (n = 105)	18 (17.14%)	87 (82.86%)	28 (4–61)	1 (0.95%)	104 (99.05%)	22 (4–53)	60 (57.14%)	45 (42.86%)	29 (12–61)
Test 3 (n = 56)	4 (7.14%)	52 (92.86%)	33 (9–58)	0 (0%)	56 (100%)	28 (6–48)	20 (35.71%)	36 (64.29%)	39 (16–59)
Test 4 (n = 11)	1 (9.09%)	10 (90.91%)	38.5 (12–55)	0 (0%)	11 (100%)	25 (12–49)	6 (54.55%)	5 (45.45%)	49 (32–60)
Total time		30 (4–58)		23 (4–53)				34 (12–69)	We stop recording at day 69.

nine after symptoms onset (Figure 1 and Table 2). On the contrary, the Ct value below 24 identified only one patient as infectious in the second test (1/105; 0.95%) and no infectious cases for the third and four testing scenarios (Figure 1 and Table 2). Using a Ct >24 to categorize patients as non infectious might be too optimistic in real-world circumstances where the burden of false negatives might account for further lack of control.<sup>8</sup>

Despite guidelines of the Centers of Disease Control and Prevention (CDC), United States, and the WHO<sup>3,15</sup> we identified 13 cases (11%) with Ct values <33 after day 30 from symptoms onset and therefore labeled as infectious cases (Figure 1, Supplementary material). Although small, this sample of cases might be an important factor for SARS-CoV-2 spread and contribute to the persistence of the epidemic by seeding outbreaks with unrecognized sources.<sup>16,17</sup>

In our studied population, only a handful of variables explained the patterns of Ct values while using a Ct of 33; specifically, CRP and platelet for the first test, and age, neutrophils, and ICU admission for the second test. On the contrary, only gender was statistically significant in anticipating Ct values below 24 for the first test. The laboratory parameters including CRP, neutrophils, and platelet count denote a systemic state of infection; the first two have been statistically correlated with Ct values in other studies.<sup>13</sup> Similarly, age above 65 years old and ICU admission have been found as predictors of severity in other publications.<sup>4,18</sup> If these variables are altered on the first examination, Ct values are more likely to be under 33 and aid diagnosis during the first test, and might anticipate infectiousness during the second test. These and other predictors should be further studied using larger sample sizes to assess their ability to predict COVID-19 status<sup>13</sup> (Supplementary material). As a semi-quantitative proxy of viral loads, RT-PCR Ct values have been used for evaluating clinical outcomes and transmissibility for acute respiratory tract infections, influenza, and the Middle-East Respiratory Syndrome (MERS), the latter caused by another coronavirus<sup>19–21</sup> Despite the evidence supporting the role of RT-PCR Ct values on point of care evaluations,<sup>13,22</sup> variability among different RT-PCR diagnostic kits, and local viral load interpretability,<sup>23–25</sup> supports its application in tandem with other cues for COVID-19 diagnosis or management.

We acknowledge that our analysis is constrained to samples obtained from deep nasal swab examination and might be an underestimation considering higher concentrations reported in throat swabs or sputum samples;<sup>10,26</sup> if this is the case, turnover times as depicted in Table 2 might be

larger complicating indications for lack of infectivity.<sup>3,15</sup> Sources of uncertainties such as different devices, kits, and techniques might account for local variations and should be considered in order to generalize the present or any other study dealing with RT-PCR Ct values.<sup>26–28</sup>

## Conclusions

As proxies of viral load, Ct values offer an avenue as an improved criterion for discharge, interpretation of COVID-19 clinical phases, and as tools for individual clinical assessment regarding infectiousness or investment on multiple testing. In this study, 11% of patients persisted with RT-PCR Ct values <33 and were labeled as potential infectious cases for more than 30 days.

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## Author Contributions

All authors made substantial contributions to conception and design, acquisition or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit the manuscript to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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