

COVID-19 Associated Inference of Cycle Threshold (Ct) Value by RT-PCR: A Rapid Review

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Abstract: Currently, all SARS-CoV-2 detection measures make use of RT-PCR test, and its results are generally reported as either positive or negative, which tells us if a person is infected. However, to contain the pandemic, what we need to know is whether that person is infectious or in other words, 'can he spread the disease?' It's critical to know that the RT-PCR test does give an extra degree of the viral load within the test. This perusing is called the cycle threshold (Ct) value. [1] Prove recommends that detailing this Ct value (calculated viral load) can help in a higher elucidation of the condition, additionally in taking clinical choices. The conclusion, screening, and reconnaissance depend on an extremely intense respiratory disorder coronavirus 2 (SARS-CoV-2) reverse transcriptase polymerase chain response (RT-qPCR) tests, and comes about being for the most part detailed to the requesting physician as positive or negative. [2] In any case, the test does give a degree of the viral load within the test, in what is called the cycle threshold (Ct) value. Through this article we suggest guidelines which announcing this Ct value, or a calculated viral load, can help in the elucidation and clinical choices in conjunction with its importance and interference.

Keywords: Cycle threshold (Ct); Real time Polymerase Chain reaction (RT-PCR); COVID-19, Viral Load.

INTRODUCTION

Real-time PCR has revolutionized the way clinical microbiology laboratories diagnose many human microbial infections [1]. Reverse transcription polymerase chain reaction (RT-PCR) is an established laboratory technique that can be used to identify the presence of specific genetic material through a biochemical process of amplification using enzymes and is based on specific target recognition [2]. This testing method combines PCR chemistry with fluorescent probe detection of amplified product in the same reaction vessel. In general, both PCR and amplified product detection are completed in an hour or less, which is considerably faster than conventional PCR detection methods [3]. The combination of excellent sensitivity and specificity, low contamination risk, and speed has made real-time PCR technology an appealing alternative to culture- or immunoassay-based testing methods for diagnosing many infectious diseases [4]. Modern applications of RT-PCR allow the reaction to be monitored during each stage, known as real time RT-PCR. RT-PCR testing can tell us whether there is a detectable virus present in an individual [5]. Still, it

does not accurately tell us whether that individual is infectious or is capable of spreading the disease. Infectivity in cell culture is the standard for determining whether a patient is contagious [5]. In the absence of viral culture data, one can use viral load or cycle threshold (Ct) values derived from RT-PCR as a proxy for the likelihood of transmission. The Ct is the number of replication cycles required for a signal of RT-PCR product to cross a determined threshold.

Cycle threshold (Ct) is a semi-quantitative value that can broadly categorize the concentration of viral genetic material in a patient sample following testing by RT PCR as low, medium or high that is, it tells us approximately how much viral genetic material is in the sample [6]. A typical RT-PCR assay will have a maximum of 40 thermal cycles. The lower the Ct value the higher the quantity of viral genetic material in the sample (as an approximate proxy for viral load). Ct values obtained in this way are semi-quantitative and are able to distinguish between high and low viral loads [7]. A 3-point increase in Ct value is roughly equivalent to a 10-fold decrease in the quantity of viral genetic material. In some circumstances, Ct values can be used

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as a more quantitative technique to accurately measure the number of viral copies per cell in the original sample – however, this requires that the sample is tested alongside verified standard dilutions and there is fixed sample input alongside quantification of cellular content of a swab sample [7]. A low Ct indicates a high concentration of viral genetic material, which is typically associated with high risk of infectivity. A high Ct indicates a low concentration of viral genetic material which is typically associated with a lower risk of infectivity [6]. In the context of an upper respiratory tract sample a high Ct may also represent scenarios where a higher risk of infection remains for example, early infection, inadequately collected or degraded sample [8]. A single Ct value in the absence of clinical context cannot be relied upon for decision making about a person's infectivity. Ct values cannot be directly compared between assays of different types not all laboratories use the same assay, and some may use more than one [9]. The Ct value for a given sample will be different in different laboratories depending on the test platform. In general terms for this report a Ct value of 30 or greater is considered a high Ct value and a value of 35 or greater is considered a very high Ct value. [8] It is appropriate for laboratories to adjust these thresholds based on their experience with the platform they are using [10].

Guidance

In general, someone who has had a previous positive test should not be retested within 12 weeks unless they develop symptoms [9]. This statement encompasses people who are identified as close contacts of COVID-19 cases but who are noted to have tested positive in the previous 12 weeks [11]. The application of this guidance should take account of the epidemiological situation (time and place) in which the sample is taken. In general terms, a high Ct value PCR result in an asymptomatic person is more likely to represent residual RNA detection of no public health or infection prevention and control (IPC) significance in a situation in which the incidence of infection in the population is low and falling [12]. Such a result is more likely to represent an early pre-symptomatic RNA detection that is of public health and IPC significance in a situation in which the incidence of infection is high and increasing. Interpretation of results is dependent on the availability of Ct values. Laboratories may not report Ct values routinely but may be able to provide them on request. [10] If Ct values are not available the default is to assume a positive result represents a significant result and that the person is infectious [13]. When reporting confirmed positive results with high or very high Ct values in settings where case by case evaluation is not practical it is appropriate, where possible, to include an interpretive comment indicating in general terms that the result may not reflect current infectious COVID-19 [14].

If they report relevant symptoms with a date of onset of more than 10 days prior to the test OR if they report no symptoms at any time, the following approach is appropriate:

1. If the Ct value is high, but not very high they should be provisionally managed as an infectious case (with self-isolation, notification and contact tracing) pending further evaluation [15].
2. If the Ct value is very high they should be advised to self-isolate, but notification and initiation of contact tracing may await the outcome of further evaluation or change in the clinical condition [15].

Further evaluation must include a repeat test on the second day after the initial test. Ideally the second test should be performed on the same platform as the initial test [15].

1. If the Ct value remains in the high or very high range on second day after the initial test the person may generally be considered as a remotely acquired infection and non-infectious at the time of testing. If contact tracing was initiated (high Ct value) it can be stood down and the person need no longer self-isolate
2. If the Ct value has fallen below the high /very high range on repeat testing they should be regarded as a recent onset infectious case.
3. In the event of a major change in Ct value within the high to very high range (for example Ct value changes from 39 to 31 it may be appropriate to take a further test 2 days later.

The threshold level and C_q value on a real-time PCR amplification curve

Despite the fact that real-time PCR fluorescent dyes and probes should be sequence-specific, a considerable amount of background fluorescence occurs during most real-time PCR experiments [16]. It is critical to bypass or account for this background signal in order to glean meaningful information about your target. This issue is addressed by two values in real-time PCR: the threshold line and the C_t value.

1. **The threshold line** is the level of detection or the point at which a reaction reaches a fluorescent intensity above background levels. Before conducting PCR, you (or the software in your cycler) set a threshold level [16]. This is literally a line in your graph that represents a level above background fluorescence that also intersects your reaction curve somewhere at the beginning of its exponential phase (Figure-1).
2. **The C_q value** is the PCR cycle number at which your sample's reaction curve intersects the threshold line. This value tells how many cycles it took to detect a real signal from your samples [16]. Real-Time PCR runs will have a

reaction curve for each sample and therefore many C_q values. Your cyclers's software

calculates and charts the C_q value for each of your samples.

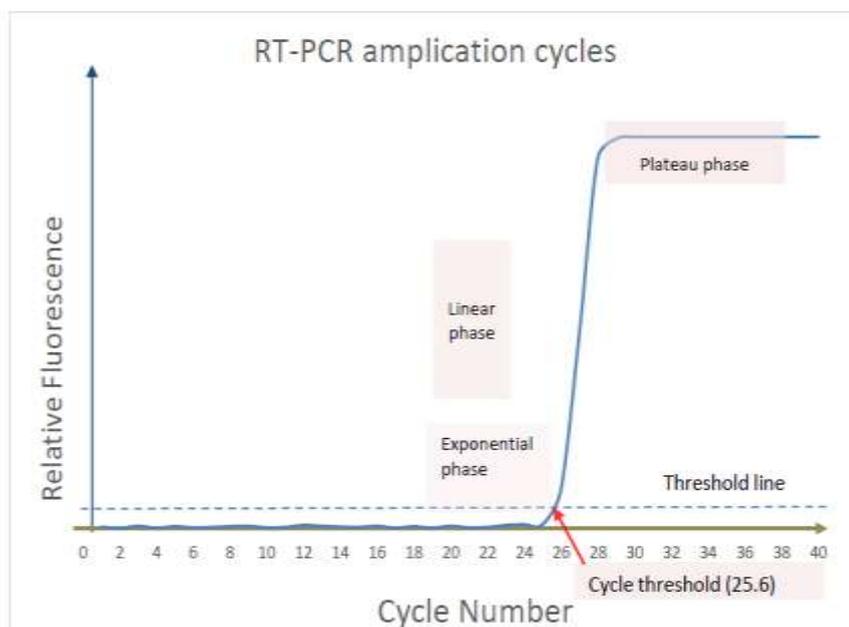


Fig-1: The threshold level and C_q value on a real-time PCR amplification curve

C_q values are inverse to the amount of target nucleic acid that is in your sample, and correlate to the number of target copies in your sample. Lower C_q values (typically below 29 cycles) indicate high amounts of the target sequence [17]. Higher C_q values (above 38 cycles) mean lower amounts of your target nucleic acid. High C_q values can also indicate problems with the target or the PCR set-up, as outlined later in the pitfalls section of this article [17].

PCR instrument will collect fluorescence data during each cycle. After about 15 cycles, a good idea of background fluorescence level will appear as a straight line starting from the zero cycle point [18]. The threshold level will be just above this, but at the point where the samples start moving into the exponential phase of PCR amplification [19]. Today, computer software calculates this exact point and all modern real-time cyclers have an automatic threshold line setting.

Real-Time PCR records the amount of fluorescence emitted during the reaction where all PCR components are abundant [16]. In this way, C_q values are usually consistent across replicates in real-time PCR. By the time the PCR reaction endpoint is reached, accumulated inhibitors, inactivated polymerases and limiting reagents create a lot of variation in endpoint values, and this is why conventional PCR cannot be used quantitatively [14].

The Importance and Determining Inference from CT Value

The medical practitioner seems to take the Ct result in settings and decide when the patient can cease

confinement [20]. This may abbreviate the term of isolation and, for healthcare workers and other fundamental specialists, would give a more evidence-based, testing-informed pathway for faster return to work [21]. Taking the Ct value under consideration may moreover offer assistance legitimize symptom-based techniques suggested by the CDC including time-since-illness-onset-and-resolution-based approaches (ie, lifting of segregation after 10 days taking after onset of indications and 3 days taking after determination of side effects) [22]. In conclusion, there may be suggestions for open wellbeing screening, empowering contact tracers to center on people most likely to be irresistible. This will gotten to be progressively imperative as asymptomatic screening expands [20].

The Ct value may be high as a result of early malady and the Ct value would have to be considered in the clinical setting [23]. An individual with a high Ct value tried early within the disease course may well be or gotten to be irresistible and this would present as a noteworthy diminish within the Ct value 24 hours taking after the primary test [24]. A quiet with settled indications and 2 Ct values both near to the cutoff is likely recouping and not irresistible [25]. Prove from both viral isolation and contact following studies supports a brief, early period of transmissible. By bookkeeping for the Ct value in setting, RT-qPCR results can be used in a way that's personalized, profoundly delicate, and more particular [25]. To actualize this, the genuine Ct values might be detailed together with reference ranges or changed over to viral stack and/or categorized as high, medium, or low [26].

CONCLUSION

As COVID-19 cases fill the hospitals, among the sickest and most likely to die are those whose body's react in a signature catastrophic way [27]. A positive RT-qPCR result may not fundamentally mean the individual is still irresistible or that he or she still has any significant illness [28]. The numbers of patients suffering from active COVID-19 infections are rising drastically across the world [29]. Till now seven strains have been found affecting the humans including the new COVID-19 [30]. To begin with, the RNA may well be from nonviable or killed virus. Live infection is frequently isolable as it were amid the primary week of side effects but not after day 8, indeed with positive RT-qPCR tests. [28] Moment, there may have to be a least sum of reasonable infection for forward transmission. For disease control purposes, the utility of the measure is most noteworthy when recognizing individuals who are positive and at hazard of advance transmission [31]. As long as asset restrictions on testing and PPE exist, we accept that time-since-symptom-resolution- and test-based techniques ought to proceed to coexist and complement one another [32]. Healthcare specialists, who may have less demanding get to testing and who may be most vital to induce back to work more rapidly, might advantage by test-based clearance, especially in case the Ct value is considered. Especially when testing within the non-attendance of side effects for COVID-19, we accept that announcing the Ct value or extend may offer assistance to superior clinical choices [33].

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