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SPECIAL INTEREST GROUP OF THE SOUTH AFRICAN MEDICAL ASSOCIATION

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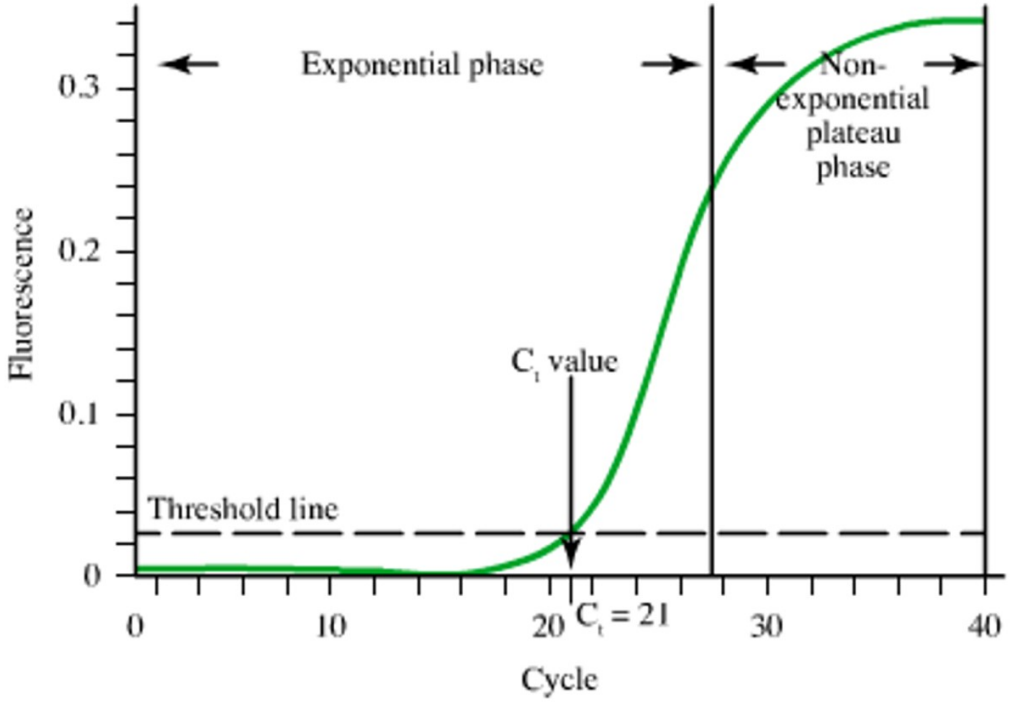
The interpretation of CT values in COVID19 RT-PCR

This communication has been written in response to numerous requests received at the laboratories to report on CT values as a proxy for viral load obtained in the performance of rtPCR assays used for the diagnosis of SARS-CoV-2. While there is some support in the literature for this approach (cid 2020:71 (15 October) : 2252-2254), there are equally articles warning against the use of CT values for this purpose (JID https://doi.org/10.1016/j.jinf.2020.10.017)

The gold standard in the detection of SARS-CoV-2 is the RT-PCR (reverse transcription polymerase chain reaction). This is a technique which enables a laboratory to amplify a portion, or several portions, of the genome of SARS-CoV-2, and then to detect specific targets of interest if they are present in the sample. This technique enables us to detect very small amounts of genetic material in a sample relatively quickly.

As the biochemical reaction in the assay progresses, if there is the genetic material of interest present in the sample, the target begins to amplify. The Cycle Threshold (CT value) is measured as the amplification reaction enters the exponential phase and crosses the threshold of detection. In other words, as the fluorescent signal exceeds the background, it crosses the threshold for positivity and this is the CT value. The lower the CT value, the earlier the exponential phase is reached. This is an indicator of a greater amount of genetic material present in the sample.



https://www.dnasoftware.com

Most of the assays in use detect multiple gene targets from SARS-CoV-2 . The gene segments most commonly detected in the assays available in the laboratory are as follows:

Limits of RT-PCR:

- While this technique detects viral nucleic acid, it cannot be used to determine if live virus is present in the sample, and therefore cannot be used to determine infectiousness of the sample or the patient.

- The CT values are reflective of the amount of intact nucleic acid in the SAMPLE, and this is not necessarily an accurate reflection of the amount of viral particles (viral load) present in the patient.

Factors that affect the amount of viral nucleic acid present in the sample:

- The quality of the sample collection. This might be affected by some of the following factors:
    - Different sample types yield different amounts of virus depending on the stage of infection. E.g sputum might yield a better result than a nasopharyngeal swab in a patient in ICU who has been symptomatic for 8 days, while a nasopharyngeal swab would potentially have a better yield at day 2 of symptomatic disease.
    - The type of swab used with some materials collecting a larger amount of biological material than others. E.g. flock swabs may absorb more material than a nylon swab.
    - The collection technique
    - Transport time to the laboratory, especially from remote locations, as some nucleic acid degradation may be observed in samples with prolonged time from collection to processing.
- The amount of virus present at the anatomical site of collection
    - Fewer viral particles are shed very early in the course of infection, and towards the end of the disease progression, and also intermittent shedding is encountered later in the course of the disease. If the sample is collected at the wrong time and the CT value is used to determine viral load, the level of infectiousness of the patient could be underestimated. If, for example, the sample is collected early in the infection and high CT values are used to decide that the patient is not infectious, an outbreak amongst his or her contacts will occur as the disease course progresses.
- Whether or not inhibitors are present at the anatomical site. The presence of PCR inhibitors might lessen the sensitivity of the assay used, thus affecting the reported CT value, or yield an invalid result.

Factors within the laboratory that can affect the quantity of virus detect/CT value:

- The volume of sample collected
  - Very small amounts of biological material will have fewer viral particles present.
  - Combining multiple swabs during testing may yield more nucleic acid compared to a single swab.
- The extraction method utilized
  - Heating and lysis methods will yield lower quantities of pure nucleic acid than more formal (and less available) nucleic acid extraction techniques.
- The reagent and sample input volumes used
  - Some sample preparation steps require larger input volumes, and these will be likely to have greater diagnostic sensitivity than those requiring lower input volumes. The same sample will yield different CT values depending on factors like this!
- The RT-PCR kit used
  - Different laboratories use different kits, and some laboratories use many different PCR kits in order to process the large numbers of samples received each day during a pandemic. Each kit will have a different sensitivity (limit of detection) and so, on the same sample, Kit A might have a CT value for the N gene of 35, while kit B might have a CT value for the N gene of 31. Therefore, the conclusions reached if CT value as a proxy for viral load would be different depending on the diagnostic kit used.
- The gene targets selected
  - Different targets have different sensitivities (limits of detection) in each assay. Assays detecting the N gene, for example, are generally more sensitive than those detecting the RdRP gene.

CT values can be semi-quantitative in measuring the amount of virus in a cell, but this requires the following criteria to be met:

- There has to be a fixed input sample volume (this is impossible to achieve with respiratory swabs).
- The cellular content of the swab needs to be quantified, which may be done if the internal control run in the assay is cellular nucleic acid. Many of the assays in use, however, utilize phage or other sources of internal controls and therefore are not necessarily appropriate for this type of quantification.

The conclusion reached is that CT values should only be reported if linearity, limit of detection, and standard quantification curves are available. The normal PCR tests run for the diagnosis of SARS-CoV-2 do not satisfy these criteria, and the CT values should not be reported upon or used as a proxy for the amount of virus present in the patient. If serial testing is to be used in order to monitor response to management of a patient, it is important to ensure standardized sample collection and to liaise with the laboratory to ensure that exactly the same methodology is used for each sample. Serial testing should also not be used for infection control decision processes.