

Introduction

There are many factors that impact the use and interpretation of Ct values that are generated during real-time PCR testing. Diagnostic laboratories should not include Ct values on laboratory reports because it could be out of compliance with laboratory regulations and they should not be used to inform patient management. In some instances, Ct values may provide information that assists in prioritizing or informing public health surveillance, contact tracing and investigations, but APHL does not yet recommend this as a routine practice. This is an area that requires further investigation and gathering of data before that step is taken. Sharing and interpreting Ct values in the context of public health surveillance or a public health investigation should always be done in consultation with jurisdictional public health laboratory staff.

What are nucleic acid amplification tests (NAATs)?

There are several different kinds of diagnostic tests that detect the nucleic acid (DNA or RNA) of a pathogen in a patient specimen. The majority of these assays work by amplifying the target nucleic acid present, but they do not tell us whether the pathogen is infectious or alive.

NAATs can be based on different types of chemical reactions, including real-time polymerase chain reaction (real-time PCR), transcription-mediated amplification (TMA), loop-mediated isothermal amplification (LAMP) or other chemistries. Different types of NAATs may be reported differently.

NAATs may be developed as multiplex assays, meaning they can detect multiple pathogen targets in one test.

What are COVID-19 Diagnostic NAATs?

There are many different NAAT-based tests to detect SARS-CoV-2 RNA for the diagnosis of COVID-19, some based on real-time PCR (e.g., the CDC Diagnostic Panel, Cepheid Xpert Xpress SARS-CoV-2, Roche Cobas SARS-CoV-2) and others based on methods like TMA (e.g., Hologic Aptima SARS-CoV-2) or LAMP. A full list can be obtained on the [FDA's EUA website](#).

NAATs for COVID-19 diagnostic testing are generally very sensitive, meaning they can detect very low levels of viral RNA, and very specific, meaning they detect only SARS-CoV-2 RNA.

All of the commercially available diagnostic NAAT tests for COVID-19 in the US are “qualitative” tests— the test produces a qualitative result of positive or negative. The tests are NOT designed to provide a semi-quantitative or quantitative measurement of the level of viral RNA in the specimen.

What is a Ct value?

Many NAAT tests generate a number as part of the test result. For real-time PCR, this is called the Ct or “cycle threshold” value. A Ct value is defined as the number of amplification cycles required to reach a fixed background level of fluorescence at which the diagnostic result of the real-time PCR changes from negative (not detectable) to positive (detectable).

The total number of cycles required to exceed the established threshold to call a result positive is specific to that test platform, and generally ranges from about 15 to 45 cycles. Different tests calculate the Ct values differently, and different tests also count the number of cycles differently. Some tests generate the Ct value through software installed on the instrument itself, some require the operator to interpret and define the Ct value based on parameters set by the test manufacturer, while others do not generate a Ct value that is available or visible to the operator and simply provide a positive or negative test result. In addition, some tests have an established Ct “cutoff” beyond which the test result is considered negative; for others, the “cutoff” is the last cycle of the test. These parameters are determined by the test manufacturer and cannot be altered by the laboratory performing the test.

Is there variability in Ct values?

Short answer: Yes

The number of cycles required for detectable amplification of viral RNA is dependent on a long list of variables beyond simply how much viral RNA is present in a patient specimen. The relative impacts of these variables on the Ct value differs between test platforms and can vary widely. Variables that can impact Ct values include but are not limited to:

Pre-analytic Variables

- Efficiency of the collection of specimen
- Time of collection of specimen after onset of infection
- Specimen type—matrix effect
- Specimen type – level of viral RNA in different specimen types (e.g., upper vs. lower respiratory tract) can differ between specimens from the same patient at the same time
- Storage and transport conditions of specimen prior to testing
- Age of specimen

Analytic Variables

- Nucleic acid extraction efficiency
- Amount of viral RNA in the specimen
- Nature of the target RNA and design of the primer/probe sequences
- Efficiency of the real-time PCR chemistry in the assay (singleplex, multiplex)
- Method for defining/determining Ct value

I can get a quantitative test for HIV, why can't I get one for COVID-19?

Short answer: They are not currently commercially available in the US

Quantitative viral load assays are specifically designed for this purpose. They are run on specimen types that mitigate the impact of variables on the Ct value and include controls and calculations to assess viral load. For example, an HIV quantitative viral load assay is performed on a blood specimen. This specimen is homogenous and can be collected in a very standardized manner. The real-time PCR assay used to calculate viral load includes a set of controls to “standardize” the specimen (e.g., a control for specimen adequacy) and a set of standards (i.e., known dilutions of virus for calibration). Ct values of the patient specimen are compared to those of the standard curve to calculate the viral load in a standardized specimen.

This type of assay is not yet available for SARS-CoV-2. Respiratory specimens are not homogeneous and are challenging to standardize. The collection process of a respiratory specimen does not lend itself to quantifying the amount of virus present. Each swab collection is different and does not assure that the same amount of sample is collected. Quality of specimen collection is impacted by other variables including the skill of the collector, which nostril is swabbed first, or whether the patient recently ate or drank. Many COVID-19 diagnostic real-time PCR assays do not include specimen adequacy controls, and those that do still lack the standardization necessary to calculate viral load.

Cts and infectiousness—can we infer one from the other?

Short answer: No

There are a number of reasons that Ct values should not be used to determine how infectious someone is. The first relate to the nature of the available testing methods and the inherent variability of Ct values:

- The available assays are qualitative, not quantitative. Qualitative tests are not designed to provide an indication of possible infectivity.
- There are many variables that impact Ct value that are unrelated to the amount of viral RNA in a specimen (see above).
- The only method available for determining the presence of live virus in a specimen is inoculating the virus into cell culture to determine if the virus can grow there. This is a very insensitive and qualitative method, may not detect low levels of infectious virus and does not necessarily correlate with infectiousness.

There are also simply not enough data at this time to infer a correlation between detectable SARS-CoV-2 viral RNA and infectiousness. We do not know how much virus (as measured by detecting viral RNA) is needed in a respiratory specimen for a person to be able to transmit it to someone else. We also do not know what the “cutoff” is for a person to no longer be infectious (i.e., at what point the amount of virus in a person’s respiratory specimen is too little for them to be able to infect others).

Do Ct values correlate with viral load?

Short Answer: Often, but not always

There is a relationship between Ct values and amount of virus in a patient specimen, but they are not equivalent. There are many variables that impact Ct values (see above). Although Ct may be used as a proxy for viral load, caution must be taken when interpreting in this manner. A high Ct value often correlates with a low viral load, but not always.

A specimen could have a very high viral load, but also a high Ct value (i.e., it took more cycles to detect the viral RNA) because the extraction was inefficient, the patient just drank something that inhibited the real-time PCR reaction, or the specimen was packaged inappropriately and reached a high temperature during transportation to the lab and the viral RNA in the specimen degraded in the heat.

Any specimen that generates a result that is defined as “positive” by the test manufacturer is considered positive. As with any diagnostic test, the result should be interpreted in the clinical context.

The process of viral replication and infection must be taken into consideration as well. If a specimen is collected very close to the time of the initial infection the viral load may be very low as the virus has not had a lot of time to replicate; a specimen collected in the coming days may have a much higher viral load. A specimen collected many days to weeks after the initial infection may have a low viral load, and viral RNA can be detectable for many weeks after infection in some patients. Limited epidemiological and culture data indicate that patients are not infectious more than 10-15 days post-onset of symptoms.

Can I compare a Ct value from one test method to another?

Short answer: No

Ct values and cutoffs are assay- and method-specific. A specimen with a Ct of 35 by one assay will not necessarily have the same Ct value by other assays. These values can vary up to two to three logs from test to test due to how the tests are designed.¹

There can be a difference in the relative sensitivities of FDA authorized tests which may also impact Ct values. According to comparison data recently published by FDA using a standard panel, there can be as much as a 1000-fold difference between the various assays.²

Why don't labs report Ct values on their reports for NAATS?

Short answer: This would be a regulatory violation

All currently-available nucleic acid tests for SARS-CoV-2 are FDA-authorized as qualitative tests, and Ct values from qualitative tests should never be used to direct or inform patient management decisions. Therefore, it would be a regulatory violation for laboratories to include Ct values on patient reports.

Can Ct values be used to inform infection control decisions?

Short Answer: We need additional data

The amount of detectable viral RNA in an infected individual is quite low in the first few days after infection, then rises exponentially for several days before dropping back off. It is reasonable to conclude that this period of peak viral load is when the infected individual is most capable of transmitting the virus to others, and when their specimens will have

¹ D. Rhoads, DR Peaper, RC She, FS Nolte, CM Wojewoda, NW Anderson, BS Pritt. College of American Pathologists microbiology committee perspective: Caution must be used in interpreting the cycle threshold (Ct) value. *Clin. Infect Dis.* (2020), [10.1093/cid/ciaa1199](#)

² FDA [SARS-CoV-2 Reference Panel Comparative Data](#) webpage.

their lowest Ct values. Therefore, a positive real-time PCR test result with a low Ct value can be interpreted as being from a person with a high viral load and high chance of transmissibility. However, most individuals would be considered non-infectious by 10 days post-symptom onset, although a NAAT may still be positive with a relatively high Ct value since the assay is detecting left-over fragments of the viral RNA. Additionally, correlates between viral load and infectiousness are not completely understood, including the interpretation of viral loads in asymptomatic individuals.

Additional data on when an individual is infectious and capable of transmitting virus are needed to further inform how Ct values may be used to inform public health decision making.

Additional Notes about Diagnostic Laboratories

All laboratories that perform diagnostic testing on human specimens must adhere to state and federal regulations and always perform rigorous evaluation of a new test—in addition to ongoing monitoring—to assure that tests are performing as expected. This involves testing known positive and negative samples to ensure the test is working properly, evaluating staff to make sure they are performing the test correctly and continual assessment of results.