



**Cycle threshold (Ct) Values Questions & Answers  
December 29, 2020**

**1. What is the Ct-value and what does it mean?**

The Ct-value is the “cycle threshold” at which the fluorescence produced by the assay crosses from “negative” to “positive.” The Ct-value can also be called the “crossing point.” The Ct-value is the number of temperature cycles needed to cross the fluorescence threshold from “negative” to “positive.” Generally, the more template (eg, virus) that is in the original sample, the fewer the cycles that are needed before the target is detected and crosses this threshold.

**2. How could reporting Ct-values aid the patient care team?**

Some patients that have recovered from COVID-19 may continue to have low amounts of viral RNA in their bodies for weeks or months after recovery. When this is the case, typically Ct-values are very high. Some providers find the Ct-value helpful in trying to discern the clinical scenario in which a positive SARS-CoV-2 test has occurred. In contrast, lower Ct-values are commonly seen in acute disease, but individuals with acute disease can have high Ct-values.

**3. What are the downsides of reporting Ct-values?**

Ct-values are not standardized across specimen sources, testing platforms, or laboratories. Although Ct-values have been correlated with prognosis and infectivity in some studies, there is an opportunity to over-interpret results or attribute false precision to a Ct-value. Other studies have identified infectious virions can be present in specimens that yield high Ct-values. Additionally, a significant portion of SARS-CoV-2 nucleic acid amplification testing is performed using methods other than PCR, which do not produce a Ct-value, and a reliance on Ct-values may complicate or delay management decisions or duplicate testing.

**4. What factors affect the Ct-value generated by an assay?**

Assay independent factors that can affect Ct value include the type of specimen collected (eg, anterior nasal swab versus nasopharyngeal swab), the training of collectors and resultant quality of specimen collected, the presence of mucus and other potentially inhibitory substances in the specimen, the timing of specimen collection compared to duration of infection and symptom onset, the type of swab used, the type of transport media used, and the conditions and time for specimen storage and transport prior to testing.

Among RT-PCR assays that generate Ct values there are several differences between assays that can give rise to different Ct values even for the same specimen. Some RT-PCR assays involve a nucleic acid extraction step while others do not. While extraction potentially adds time, complexity, and cost to an assay, it removes potentially inhibitory substances and potentially concentrates nucleic acids. Extraction methods can vary substantially among assays, and differences in extraction performance may be more pronounced at different viral burdens. The volumes of sample and reagent used in assays may differ, and the precise cycling conditions can vary substantially among assays or even for the same assay performed on different instruments. Between assays, the number of cycles monitored, and assay cut-offs can vary.

**5. Why don't all assays generate Ct values?**

Ct values are most commonly generated by real-time reverse transcriptase PCR (RT-PCR) reactions. In these assays, the presence of amplified genetic material is monitored by fluorescence after each temperature cycle. Other types of PCR assays use different underlying methods that may not continuously monitor for amplification and simply identify the presence or absence of amplification at the end of the assay (ie, end-point detection). Other methods may use two sequential PCR reactions, and non-PCR amplification methods may use different methods to both amplify and detect nucleic acids that don't involve fluorescence and/or regular cycles.



**6. If my laboratory is being asked to report Ct values, what factors should I consider providing the most appropriate information?**

Patient care teams expect that differences in Ct value reflect underlying disease status, but, as discussed above, many other factors can contribute to variations in Ct value. If Ct values are going to be reported and incorporated in clinical decision making, all efforts should be made to reduce other variables associated with COVID-19 testing including consistent sample collection practices, swab and transport media, and assay used. It is important to report the genetic target and testing method used when reporting a Ct-value because these variables impact the Ct-value.

**7. Can Ct-values be used to determine infectivity?**

Lower Ct-values have been associated with higher chance of viral recovery by culture (see [article](#) in *Clinical Infectious Diseases*). However, clinical impact and infection control measures associate with this finding are not clear. CDC has [guidance](#) around the role of PCR testing to discontinue strict infection prevention measures.

**8. Should Ct-values be reported for SARS-CoV-2 PCR testing?**

There is not currently formal guidance as to whether the Ct-value result of a PCR test used to detect SARS-CoV-2 should or should not be reported along with the interpretation of the result. All FDA EUA IVDs for the detection of SARS-CoV-2 are currently authorized for only qualitative interpretation. Generally, lower Ct-values are associated with higher viral burden in a sample, but inferring clinically meaningful information based on a Ct-value can be challenging (see [letter to the editor](#) in *Clinical Infectious Diseases*).