Frequently Asked Questions (FAQs) for PCR Testing Cycle Threshold (Ct) Value Interpretation

Updated April 5, 2021

1. **What is the Ct value and what does it mean?**
   The Ct value is the “cycle threshold” or number of temperature cycles at which the fluorescence produced by a polymerase chain reaction (PCR) assay crosses from “negative” to “positive.” Generally, the more template (e.g., SARS-CoV-2) that is in the original sample, the fewer the cycles that are needed before the target is detected and crosses this threshold.

2. **What factors affect the Ct value generated by an assay and is the Ct value standardized across all test methods?**
   Assay independent factors that can affect Ct value include:
   - the type of specimen collected (e.g., anterior nasal swab versus nasopharyngeal swab)
   - 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.

   Not all molecular test methods provide a Ct value. In addition, 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. Extraction may remove 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 cutoffs can vary. The FDA also has published notice that currently there is no consensus as to whether or not particular Ct values correlate with a person being or not being infectious or risk level for disease severity.

3. **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 also
have high Ct values. PCR result is not an indication of presence or absence of live infectious virus.

4. **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.

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 (i.e., 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. **Should I ask my ministry’s Clinical Laboratory to routinely include Ct value for results if available?**

   No. Because Ct values are not standardized across various brands of testing methods, the System Laboratory Clinical Leadership Group and Clinical Lab Leader Network do not recommend this value be included as part of result reporting. Instead providers should follow existing guide that recommends providers consider this on a case by case basis [refer to Flu/COVID Testing System]. For example, a provider of a patient who recovered from initial infection and then two months later has new onset of symptoms can be retested. If virus is detected again the System guide recommends consulting an infectious disease specialist who can request the Ct from the clinical lab and assist with interpretation of and management of this case.

   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. 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. **If I’ve ordered a molecular test for my patient and it detects SARS-CoV-2, how can I find out the Ct value?**

   Because the interpretation of the value is not always straightforward, it is recommended the ordering provider consult their ministry’s infectious disease specialist to assist with this. As indicated in other questions, the Ct is not needed for all tests that detect this virus; however, there are some unique patient specific scenarios, e.g., recovered from initial infection and weeks or months later now needs elective surgery and was tested preoperatively and has detected virus again. The infectious disease specialist can determine the need and consult
with the clinical director of the performing laboratory to discuss a specific test’s Ct value. If an infectious disease specialist is not available and the result may alter a time sensitive need, the ordering provider should contact the Clinical Director of the performing lab and request CORONAVIRUS DISEASE 2019 (COVID-19) © 2020 Trinity Health, All Rights Reserved 3 the Ct value. The Clinical Director of the performing Lab may be able to connect the provider with a colleague at the ministry who can assist with Ct value interpretation and application.

8. Can Ct values be used to determine infectivity?
Lower Ct values have been associated with higher chance of viral recovery by culture. However, clinical impact and infection control measures associated with this finding are not clear. CDC has guidance around the role of PCR testing to discontinue strict infection prevention measures and the FDA also has stated there is no consensus on relative infectivity of a person based on Ct value.

Current CDC guidance for assessment of recovery and removal from isolation does not recommend a testing based approach, and sets minimum and maximum times (10 days after symptom onset, or 20 days after symptom onset in the context of severe disease or underlying immunocompromised condition) for removal from isolation. The guidance does not recommend retesting within 90 days of symptom onset to determine infectivity; however, the guidance acknowledges that this approach may be less ideal for immunocompromised individuals or individuals with suspicion of re-infection. Re-infection is not widely prevalent, but this prevalence may change with the spread of novel variants.

9. Should Ct values be routinely reported for SARS-CoV-2 PCR testing?
No. 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, although the FDA allows Ct value reporting with guidance on the context described in the previous FAQs. 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. Ministries should follow any local and/or state requirements that Ct value be included in reporting results to public health agencies.

References:
College of American Pathologists. Cycle threshold (Ct) Values Questions & Answers.


System Office. testing-algorithm-flu-reinfection.pdf (trinity-health.org)

