

Use of Cycle Threshold (Ct) Values in SARS-CoV-2 Molecular Testing

Situation: Current Covid-19 nucleic acid amplification tests approved by the FDA are QUALITATIVE tests only. This means any numbers generated (e.g. cycle threshold (Ct) values) should not be used to guide decision making or management, except in rare circumstances. Knowing the limitations of Ct values is important to avoid patient harm and inappropriate resource utilization.

Background: The most common tests used at YNHHS for the diagnosis of SARS-CoV-2 infection are molecular tests, which include real-time reverse transcriptase (RT) PCR. For real-time RT-PCR, viral detection is indicated by the cycle of amplification that a fluorescent signal crosses the threshold to a positive result. This is referred to as the cycle threshold (Ct) value. The Ct value is inversely correlated to viral amount; therefore the **lower the Ct value** (fewer amplification cycles to cross the threshold to positive), **the higher the viral amount** in the sample. While lower Ct values indicate higher levels of viral RNA detected, viable and nonviable viral particles are not distinguished. Ct values can generally be categorized into 3 groups:

Cycle Threshold	Strength of Positive Signal
<22.0	Strong positive
22.0-30.0	Moderate positive
>30.0	Low positive

It is important to note that different assays have different cutoffs for positive results (variably <37, <40, or <45 cycles). Not all molecular Covid-19 assays/instruments report Ct values and thus Ct values are frequently not available.

Assessment: The quantitative nature of cycle thresholds suggests a precision that could result in over-interpretation. **Cycle thresholds are not equivalent to viral load.** In particular, **Ct values are not directly comparable between assays** due to different sample input, gene targets, reagents, assay parameters, reference curves, and instruments. Sample type, quality, collection method, timing, and handling are variable and also affect the viral amount detected. Though there are limited scenarios where Ct values can be helpful, there is the potential for providers to misinterpret or misapply Ct values, especially when comparing serial testing using different assays.

Recommendation: Ct values should not be used in routine clinical care. Interpretation of Ct values is highly specific to certain clinical situations and results should be used with caution. Ct values from SARS CoV-2 real-time RT-PCR tests can provide information regarding high, moderate, or low viral RNA concentrations in a given sample and this may be helpful when evaluating for reinfection or for prolonged infection in a subgroup of severely immunocompromised hosts. However, **cycle threshold values should not be used for clinical decision making without expert input from Infectious Disease consultation or YNHHS Infection Prevention.**

- Covid-19 Testing Stewardship Committee

1. <https://www.cdc.gov/coronavirus/2019-ncov/lab/faqs.html#Interpreting-Results-of-Diagnostic-Tests>
2. Rhoads D, Peaper DR, She RC, et al. College of American Pathologists (CAP) Microbiology Committee Perspective: Caution must be used in interpreting the Cycle Threshold (Ct) value. Clin Infect Dis. 2020 Aug 12:ciaa1199. doi: 10.1093/cid/ciaa1199. Epub ahead of print. PMID: 32785682.
3. Humphries RM, Azar MM, Caliendo AM, et al. for IDSA. Test, perchance to Diagnose: Practical strategies for SARS-CoV-2 Testing across settings. Open Forum Infectious Diseases (in press).

This statement is based on current information, recommendations, and evidence and will be subject to revision or retraction based on continued monitoring by the Committee.