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AACC Recommendation for Reporting SARS-CoV-2 Cycle Threshold (C_T) Values

This AACC recommendation advises against utilizing cycle threshold (C_T) values in the management of patients with COVID-19 and summarizes the limitations of reporting C_T values.

Molecular tests are useful to both diagnose disease and manage patients. When properly validated and/or approved by the FDA, molecular tests can provide qualitative, semi-quantitative or quantitative detection of an analyte. One commonly used method for the detection and quantification of RNA viruses is reverse-transcriptase polymerase chain reaction (RT-PCR). During the COVID-19 pandemic, many RT-PCR assays have been authorized by the FDA for emergency use or validated as laboratory developed tests (LDT) for the qualitative detection of SARS-CoV-2 nucleic acids. However, some healthcare providers and public health agencies have requested that laboratories report the numerical C_T value along with the qualitative result when a specimen has detectable SARS-CoV-2 nucleic acids. According to the Clinical and Laboratory Standards Institute (CLSI), the C_T value is the "number of cycles needed for an amplicon to become detectable above background"[1]. In other words, the C_T value is the lowest PCR cycle number at which the fluorescent probe signal for the amplified target sequence is greater than the minimal detection level determined during validation by the user[1,2]. Lower C_T values are associated with a higher amount of target viral sequence (copy number) in the sample tested.

General Considerations

The Infectious Diseases Society of America (IDSA) and the Association of Public Health Laboratories (APHL) have previously issued a paper discussing SARS CoV-2 C_T values[3]. More recently, the IDSA and Association for Molecular Pathology (AMP) posted a position paper regarding the potential utility and limitations of C_T values for patient management[4].

Regulatory Considerations

Only one qualitative COVID molecular test has been cleared by the FDA through the De Novo, 510K process at this time (March 17, 2021). However, a large number of tests have received emergency use authorization (EUA) from the FDA as a qualitative test or have been validated as an LDT. The current EUA molecular tests use a variety of methods (e.g., RT-PCR, transcription mediated amplification (TMA), loop-mediated isothermal amplification (LAMP), sequencing, etc.). Due to supply chain and inventory issues during the COVID-19 pandemic, many laboratories have verified/validated multiple methods for the qualitative detection of SARS-CoV-2, many of which generate a C_T value.

Validation of quantitative tests is more complex than validation of qualitative tests and requires more rigorous characterization of test performance (e.g., determination of lower limit of quantification (LoQ) as opposed to lower limit of detection (LoD)). Multiple calibrators, that follow the ISO 17025 guideline for reference materials, are required to determine the LoQ. Commutability of results can be obtained by having such controls or the designation of an international unit that serves to harmonize values across instruments; however, certified reference materials have only recently become available and are not yet incorporated into these assays[5]. For these reasons as well as the fact that manufacturers instruct laboratories, through their instructions for use, to report results qualitatively, most laboratories report qualitative results for SARS-CoV-2.

Factors Influencing C_T Values

According to the harmonized terminology database, CLSI and ISO 15189 designate three testing phases: preexamination (formerly, pre-analytic), examination (formerly, analytic), and post examination (formerly, post-analytic). Factors that can affect the C_T values in each of the three phases are listed in Tables 1, 2, and 3; respectively.

Preexamination

Various preexamination aspects that cause variability in C_T values have been discussed[3,4,6]. These include patient preparation, biological variance, specimen type, and transport and storage (Table 1).

Table 1. Preexamination Factors Affecting C_T Values

Factors	Explanation
Patient preparation	Not removing excess mucous prior to anterior nares, midturbinate or nasopharyngeal collection (i.e., blowing nose) or not refraining from food/drink prior to oral collection can lead to inaccurate results
Time between exposure and specimen collection.	False-negative results can be obtained, if specimen is collected too early (e.g., within first 3 days after exposure) or too late (e.g., >7 days after symptom onset)
Efficiency of specimen collection	Non-vigorous specimen collection can lead to inaccurate results
Biological variance	Differences between right and left nostrils, time of day, patient age and biogeographical ancestry (BGA) can affect results
Media utilized	Results may vary depending on the media (e.g., dry swab, phosphate buffered saline, viral transport media, universal transport media)
Specimen type	Results may vary depending on the source of the specimen (e.g., nasopharyngeal, midturbinate, anterior nares, saliva, sputum)
Transport and storage	Shipping conditions and temperature may affect results
Age of specimen	Specimen stability should be defined. If the time between collection and testing exceeds stability window, results may be inaccurate

Examination

Factors that affect the C_T values during actual test performance include extraction efficiencies, fluorescent probe selection and lot-to-lot variability (Table 2).

Table 2. Examination Factors Affecting C_T Values

Factors	Explanation
Nucleic acid recovery efficiency (non-extraction methods)	The absence of concentrating capability, potential for inhibition and matrix effects can affect results
Nucleic acid extraction efficiency (extraction methods)	Variable levels of extraction efficiency and matrix effects will affect results
Inhibition detection	Unavailability of controls to detect assay inhibition may produce false negative results or falsely high C_T values
Gene target (multiple vs. single)	C_T values may vary depending on gene target(s) and their different amplification kinetics leading to result variability in the same or different specimens
Design of fluorescent detection	Probes for the same gene target labeled with differing fluorophores will increase result variability
Impact of variants	Binding of primers/probes may be affected by variants leading to altered C_T values
Defining cycle thresholds	There is potential for false positive and negative results based on how the C_T threshold is defined (e.g., use of a calibrator fixed to a C_T value), or how the threshold is calculated (i.e., manual vs. automated)
Access to certified reference materials	Absence of certified reference materials such as calibrators, makes it challenging to confirm result accuracy and validate an assay as a quantitative LDT
Commutability of C_T values	Platforms have different sensitivities and cutoff values. There is no international unit established. Consequently, results cannot be standardized across platforms
C_T Range Reliability	C_T values outside of linear range should not be reported due to lack of accuracy
Lot-to-Lot variability	C_T values may vary between reagent lots. Unless new reagent lots are verified using calibrated or previously characterized material, the degree of change is unknown
Within instrument variability	Results may vary depending on the operator and time of day particularly for manual methods

Postexamination

In the postexamination process C_T values are relayed to the healthcare provider in addition to factors such as the purpose of testing, reportable units, and assay targets (Table 3).

Table 3. Postexamination Factors Affecting C_T Values

Factors	Explanation
Purpose of testing	Interpretation and utility of C_T value depends on the purpose of testing, e.g., screening, surveillance, diagnosis, monitoring, return to work, pre-procedural, patient discharge management, infectiousness
Test ordering	Monitoring a given patient longitudinally with a different PCR-based test is discouraged due to assay-to-assay variability
Infectivity versus viral detection	Viral detection does not equate to infectivity. The range of C_T values that indicate infectious virus needs to be determined by cell culture studies
Patient specific factors	Immune and/or vaccination status may confound the utility of C_T values
Reportable units	Lack of correlation between C_T (cycle number), ng/uL, TCID50/mL, genomic copies/mL, RNA NAAT detectable units (NDU)/mL, can complicate result interpretation
Manual reporting	Manual methods are more prone to errors (e.g., data entry errors) particularly if quality steps are not followed
Trending	Lack of LIS or quality flags for unexpected changes (i.e., significant changes in C_T relative to other measurements) may affect results
Re-infection/relapse	Secondary infections with same or different variants may occur and interpretation of C_T values is unclear
Assay Targets	There may be inconsistency between initial and repeat test with PCR-target drop-out/target sequence variation

Considerations for the Laboratory Compelled to Report C_T Values

AACC acknowledges that laboratories have been placed in a difficult position with regards to C_T values. As summarized in this document, there are many limitations associated with reporting C_T values. While laboratories are aware of these limitations, there may also be pressure from their clinical colleagues to report or, at a minimum, have access to C_T values. For laboratories that decide to report C_T values, AACC suggests including the interpretative comment below in italics to mitigate potential risks associated with reporting C_T values. AACC recommends that this comment be appended to the result and prominent in the laboratory report. Laboratories may also want to consider assay specific C_T ranges, assuming acceptable precision, providing semi-quantitative results (e.g., low, medium, high viral RNA) and/or calculating C_T values based on delta from a calibrator. ■

The utilization of C_T values to guide patient management is discouraged. Correlation with viral load, viral burden, or infectivity has not been established for qualitative SARS-CoV-2 tests. Numerous factors such as biological variance, adequacy of sample, time of exposure, instrumentation, methodology, lack of certified reference material, and regulatory factors influence the C_T values detected in qualitative SARS-CoV-2 assays. Therefore, AACC discourages reporting or disclosing C_T values to guide patient management. For more discussion on this topic, read the IDSA statement: https://www.idsociety.org/globalassets/idsa/public-health/covid-19/idsa-amp-statement.pdf.

References

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