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Information on Cycle threshold (Ct) values for SARS-CoV-2

Many commercial, academic, reference and public health laboratories conducting diagnostic testing for COVID-19 (SARS-CoV-2 virus) use a molecular test method known as real-time polymerase chain reaction (RT-PCR) to look for the genetic material (nucleic acids) of the SARS-CoV-2 virus in patient samples. RT-PCR is the "gold standard" for SARS-CoV-2 diagnostic testing in the U.S. and worldwide and has the highest sensitivity and specificity of any known test for this novel infection.

What is a Ct value?

- Cycle threshold (Ct) is a numerical value generated during a RT-PCR test. It refers to the number of cycles needed for a sample to amplify and cross a threshold (cut-off) to be considered detected/positive.
- Most RT-PCR tests use Ct cutoffs of 35-40 cycles, so any sample with a Ct value below the cutoff, would be considered a true positive.

Who determines the Ct cutoff?

- The Ct cutoff is determined by the manufacturer of the test, not the state or laboratory performing the test. The cutoffs are reviewed during the submission process for the FDA's Emergency Use Authorization (EUA). Once a test receives the EUA, the Ct cutoffs are set and cannot be changed by laboratories.
- Not all test manufacturers use the same Ct cutoffs, each test differs based on how it is designed and what part of the SARS-CoV-2 genetic material it targets for detection. Test manufacturers establish the cutoffs based on evaluation of their test with known positive and negative samples.
- Laboratories that perform clinical testing, including the Tennessee Public Health Laboratory (TN PHL) are federally regulated and always perform rigorous in-house evaluation and verification of each new assay before using it to test patient samples. This involves testing known positive and negative SARS-CoV-2 samples to ensure the test is working properly and not producing false results.

What variables can alter a Ct value?

- Viral load can vary based on the type of specimen collected (ex. nasal swab vs bronchoalveolar lavage) and how much specimen is collected.
- External variables can influence Ct values, including specimen transport, specimen storage conditions, how many times a specimen is frozen/thawed, and the instrument on which testing is performed.
- It is still unknown how much virus is needed to transmit the virus from person-to-person and cause new infections. Since Ct values can vary based on many factors, it is not a good indicator of how infectious a person is or how much virus is present in a person (known as viral load).
- The Ct value can also change based on the stage of infection, as the amount of virus present in a person can vary during the course of their illness. A specimen may have a higher Ct value (low viral load) if the patient is early in their infection and the virus is still increasing in their body, or later in infection when the viral load is decreasing. In both these examples, a high Ct value still represents detection of the virus and would still be considered positives for SARS-CoV-2.

Why isn't the Ct value reported on SARS-CoV-2 tests?

- The FDA EUA limits molecular diagnostic tests to report qualitative (positive/negative) of SARS-CoV-2 results and not quantitative (Ct value) results.
- Ct values and cutoffs differ by test and thus cannot be compared from one test to another. A specimen with a Ct=36 may be considered positive by one test but produce a different Ct value and be considered negative or indeterminate on another.
- Some RT-PCR tests do not use Ct values, but produce different values like relative light units (RLU) or cycle numbers (CN). No matter the method, all SARS-CoV-2 tests are reported qualitatively (positive/negative, etc).

The TN PHL uses 3 main molecular test platforms for COVID-19, all of which have received Emergency Use Authorization (EUA) from the FDA. While some of the platforms generate Ct values, results for all 3 test platforms are reported in qualitative form (positive/negative), as specified in the intended use of the EUAs. All test platforms are rigorously evaluated and verified for accuracy prior to use on patient samples.

References:

Association of Public Health Laboratories & Wisconsin State Laboratory of Hygiene