Primer on cycle threshold (Ct) values for the QEII laboratory, Central Zone Nova Scotia Health

The QEII Microbiology Laboratory has received multiple requests for how our polymerase chain reaction (PCR) tests are interpreted based on cycle threshold (Ct) values. The following is a brief FAQ on Ct values used in our laboratory for the detection of the virus that causes COVID-19, SARS-CoV-2. For a full discussion on interpretation of Ct values, please refer to the Public Health Ontario's excellent document Ct(Public Health Ontario)

What is a Ct value? Most nucleic acid amplification tests (NAAT) (like Reverse transcriptase PCR (RT-PCR) detect viral RNA through a process of amplifying targeted, specific strands of viral RNA The presence of the virus in a clinical specimen is determined by copying it in an exponential fashion through a temperature cycling reaction of up to 45 times. The presence of the viral copies are detected by a florescent signal produced during the reaction, which increases with the product. The number of amplification cycles required to create enough copies of the viral RNA to be detected is called the cycle threshold or Ct value.

How are Ct values used? The fewer amplification steps it takes to pass this threshold (a low Ct value) the more viral RNA is likely to have been present in the initial specimen; conversely more cycles required to amplify the viral RNA above the threshold (a high Ct value) suggests a lower amount of virus present in the initial sample. There can be up to 45 total number of cycles for many NAATs, and non-specific reactions can occur near the end of the cycling process that can mistakenly be flagged as positive by the instrument. The Ct value cutoff for positivity is defined by the vendor or the laboratory during the validation process to ensure that PCR is correctly detecting the presence of the virus.

Below is a table outlining how the different testing methods used at the QEII use Ct values to define positive results:

	Lab Developed Test[¥]	GeneXpert SARS-CoV-2	Roche 6800 SARS-CoV-2	Panther
Total cycles	45	45	Not described	Not described
Genetic Targets	RdRp gene	E gene N2 gene	E gene ORF1 gene	ORF 1a/b gene
Definition of positive	RdRp Ct <35	Dual gene positive: E POS and Ct ≤ 37* N2 POS and Ct ≤ 37*	Dual gene positive: E POS and Ct ≤ 38* ORF1 POS and Ct ≤ 38*	As per instrument (does not produce a Ct value)
Definition of negative	RdRp Ct ≥38	E Neg N2 Neg	ORF1 Neg E Neg	As per instrument (does not

				produce a Ct value)
Definition of indeterminate**	RdRp Ct 35 - 38	Single gene positive: E POS and Ct ≤ 37* N2 Neg E Neg N2 POS and Ct ≤ 37*	Single gene positive: E POS and Ct ≤ 38* ORF1 Neg E Neg ORF1 POS and Ct ≤ 38*	As per instrument (does not produce a Ct value)

* Positive results with Ct above this value needs to be discussed with director who examines the amplification curve to help determine if this is a true or non-specific amplification

****Indeterminate report phrase:** SARS-CoV-2 (COVID 19) result indeterminate. This may represent early disease, late disease, or a false positive result. Please recollect once after 24 hrs if clinically warranted. If indeterminate result persist, please discuss with public health

¥ While RdRp is used for interpretation of the SARS-CoV-2 result in the LDT, the RT-PCR reaction also include a second target, the E gene (Corman et al) to increase specificity, and helps with the interpretation of specimens with high RdRp Ct values. A microbiologist would be notified of result where a single target detection with E gene only is observed, and the result would be interpreted using the same Ct value cutoffs as RdRp.

Important factors to consider in interpreting Ct values:

1) *Ct values will depend on the stage of infection* - During pre-symptomatic and early infection, the baseline viral load can be initially low which is associated with high Ct values i.e. >30 and above. This period may last hours to days. Ct value interpretation is further complicated by asymptomatic infections where the time of infection onset may be unknown. **Therefore, if clinically indicated, patients should undergo repeat testing within 24 to 48 hrs to determine if the Ct value is stable, rising or declining**.

2) *Individuals can shed detectable SARS-CoV-2 RNA for a prolonged period* – RT-PCR can be positive for over 100 days or more after infection, but in most cases are unlikely to transmit to others beyond 10 days post symptom onset.

3) *Ct values are affected by the type AND quality of the specimen* - Nasopharyngeal swabs (NPs) are the most sensitive specimen type in the outpatient setting; throat/nares swabs, and gargles may be less sensitive. Also in patient with lower tract infection (e.g. pneumonias), lower tract specimens are preferred as upper tract specimens may be negative. The quality of the sample collection directly impacts the amount of respiratory material collected and this directly affects the generated Ct value i.e., poorly collected samples can yield an artificially high Ct value (low RNA levels).

4) *Ct values are not comparable between different testing platforms* - The Ct ranges and distributions differ by the PCR technology used. There is no international standard to allow for comparison. Results of proficiency panels used in in other provinces where identical specimens were tested by different laboratories have seen variation of upto 8 Cts.

5) The impact of new variants on Ct values is not clear – While our current tests can detect the current SARS-CoV-2 variants identified in the UK and South Africa, ongoing surveillance is underway to identify novel variants and their potential impacts on diagnostic testing.

Does a certain Ct value predict who is infectious? This is a complex issue. There is good evidence that when more than 24 to 30 cycles are required to detect virus the virus concentration is so low that it becomes difficult to grow the virus in the laboratory (Bullard et al., 2020; Baslie et al., 2020; Singanayagam et al., 2020). However the cells used in the laboratory to grow the virus are different that cells in the back of the throat and nose (nasopharynx) or the lungs in people. So just because one can't grow the virus in a laboratory that does not mean that it won't transmit. Many

believe that with low copy numbers (high CT) values the virus is not likely to be transmitted. But we do not know how much virus is actually required to cause an infection in someone and there are other important factors that may influence infectiousness including the health of the person exposed and the type of exposure that has happened.

How does Public Health use Ct values? Considering the Ct values can be helpful when reviewing people with positive test results that are asymptomatic or in situations where there are concerns about potential false positive results.

References:

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2. Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, Boodman C, Bello A, Hedley A, Schiffman Z, Doan K, Bastien N, Li Y, Van Caeseele PG, Poliquin G. Predicting infectious **SARS**-**CoV**-2 from diagnostic samples. Clin Infect Dis. 2020 May 22:ciaa638. doi: 10.1093/cid/ciaa638. Online ahead of print.
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