Ct values:
What do they mean?
Can they be used?

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PCR is a method of amplifying a target DNA molecule. For SARS-CoV-2, the target is the virus’ genome; it is made of RNA; but it is an easy process to convert RNA into DNA.

-PCR takes place in cycles; each cycle, temperature is changed from cold to hot to warm and then back to cold.

With each cycle, the amount of target (theoretically) doubles. This is amplification, and gives PCR its extreme sensitivity.
PCR

--With each cycle, if target is present, the amount of target is (essentially) doubled

--amplified targets are measured by fluorescent light that they give off

--”positive” and “negative” specimens are differentiated by whether the amount of fluorescent light given off passes a threshold

--The cycle where that amount of fluorescence is reached is a “Ct” or “Cycle threshold”.
PCR

--lab tests therefore use Ct value as a measure of whether to call a specimen Positive or Negative

--low Ct values are achieved when there is a large amount of target present; high Ct values are achieved when there is a low amount of target present

--think of Ct as a measure of “effort” that the test has to make to detect a positive specimen:

if there is very little target (virus) in the sample, then you have to do a lot of cycles of amplification to find it; and vice versa
PCR testing, the components:

1. RNA (viral genome) extraction
2. Convert all RNA to DNA
3. Amplify by PCR

Positive or Negative Result based on Ct value
Ct values correlate with a specimen’s ability to infected cells in laboratory culture


- CDC, unpublished data

• I've personally done this/seen this myself, here at the NV State Public Health Lab
What is “culture”?  

→ Another kind of lab test

---**infectious** virus can be detected using what are called “cell culture” techniques

---Cell culture involves using cells derived from humans or animal tissue that is / was cancerous

---cancer cells live forever in culture

---viruses can be **detected** / **propagated** by adding them to cell cultures

---”Vero” cells are commonly used for SARS-CoV-2 infection/propagation

**Vero cells:** African Green Monkey Kidney cancer: have been growing since 1962;
“high” Ct specimen don’t grow in culture

--low amount of virus?

--“broken”, junk particles?

-CDC showed no ability to infect cells in Vero culture after Ct value 33.00 (on their PCR assay)

So...

--do such specimens present a public health threat?

--do PCR assays go too far?

No commercial or CDC assay used in Nevada with EUA uses cutoffs higher than 40.
Ct values are not ready to be used diagnostically, or routinely

Seven considerations in this regard, follow:
1. Different assays, different Ct

--NSPHL Ct data between two assays

--efficiencies of PCR vary

--where would you draw the line?

--e.g. what would you say about Ct values of, say, 32 or 34, if your cutoff was 33?

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40% of specimens show >4-fold difference in load (i.e. greater than 2 Ct differences)
2. Extraction methods affect Ct values

-swabs pulled out of noses and throats have viral loads on them

-to measure it, the viruses must be destroyed and their RNA molecules removed

-the RNA is removed, and ‘washed’ for PCR to follow

-this is called “extraction”

-there are many kits on the market for this

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Arrows indicate where different extraction methods led to different Ct values from the SAME specimens in the final PCR by at least a factor of 2 (est: a 4-fold change in measured viral load)
3. Is lab cell culture a proper surrogate for the real infectious process?

--cancer cells in a dish vs. primary human systems: are they equal?

--evolution of SARS-CoV-2 occurs(ed) in real tissue, not in cell cultures

--very hard to do actual infectivity experiments without volunteer human subjects

We don’t know yet. For other viruses (e.g. HIV), there are vast differences in infectivity
4. Collection and storage variability can cause Ct variability

Keep in mind:

What is tested by PCR may not reflect what was in the nose at the time of collection:

After collection, specimens are put into media, stored for 1-3 days, at room temperature or cold packs, sometimes they are transported long distances

So: what was an infectious virus at time of collection, may not be after PCR testing has occurred

Lots of handling steps.
5. Most positive specimens detected are in an “infectious” Ct range

--pandemic was not caused by high Ct values

--sampling 1,264 specimens from our CDC assay data*:
  mean Ct: 27.55
  SD: 6.11

So: ~84% of specimens tested have had a Ct value less than 33.66.

According to CDC data, this means that
The strong majority of specimens we have ascertained at NSPHL likely were infectious in cell culture

*using N-gene detection
6. Note: viral load doesn’t tell you whether infection is new or old

--high Ct, low load specimens can be “coming” or “going”

-misclassification of a new infection as an old infection could be catastrophic
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---high Ct, low load specimens can be “coming” or “going”

-misclassification of a new infection as an old infection could be catastrophic
7. Published Work showing Ct values >36 can harbor infectious virus


• They show that perhaps timing of specimen collection after symptoms can affect infectivity of specimen
What is going to happen?

- Truth: there is a correlation with infectivity!

Potential ways forward:

- Standardization of viral loads
- Antigen tests as “clearance” tests?
- Per-assay cutoffs?
- Whatever it is, the FDA will have a major say in how and when!