

# Your Coronavirus Test is Positive. Maybe It Shouldn't Be.

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Discussion from a hospital laboratory perspective  
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## Points raised in NY Times article

- Standard tests diagnose large numbers of people carrying insignificant amounts of virus.
- Most are not likely to be contagious. If Ct >33, virus not grown in culture.
- A cycle threshold >35 is too sensitive.
- A more reasonable cutoff is Ct 30-35 or even Ct  $\leq$ 30.
- In NY state lab, **50% of recent positives had Ct >35.**
- In MA, **85-90% of positives in July had Ct >30.**
- Cycle threshold is never included in the results sent to clinicians.
- For **outbreak tracing**, cheap and abundant rapid tests are needed, even if less sensitive

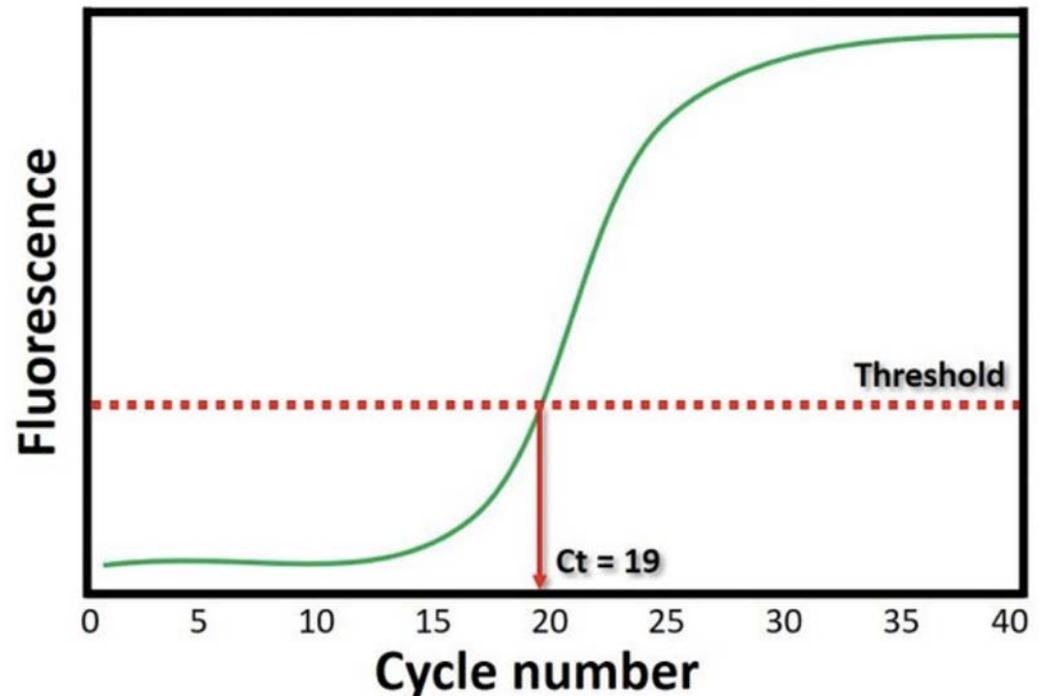
# The basics of Ct values

Correlation with amount of virus in sample

**What is a cycle threshold (Ct) value?** The cycle of amplification that the fluorescence crosses the threshold to positive. The Ct value correlates with viral load. A lower Ct value indicates a higher viral load in the sample, and vice versa.

**PCR commonly uses 40 cycles of amplification, and each cycle doubles the target DNA.  
3.3 cycles = a 10-fold change**

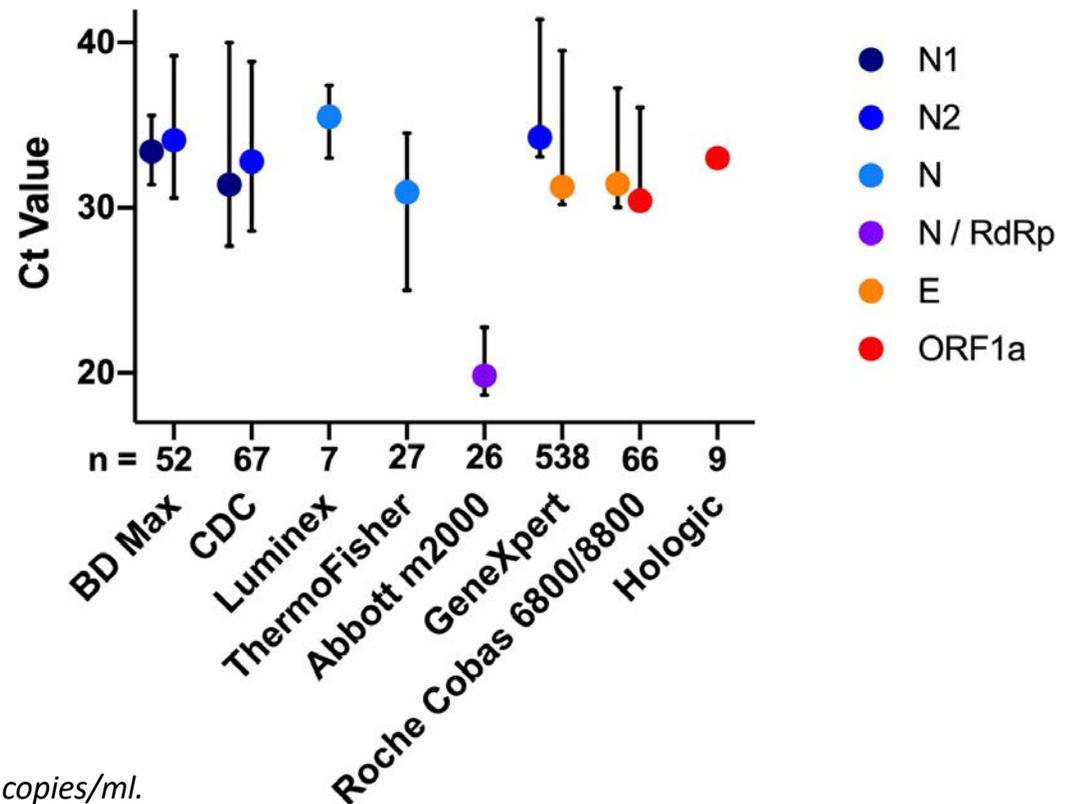
*In Real-time PCR, a fluorescence signal emitted during amplification can be seen “in real time”, and can provide Ct values.*



# How consistent are Ct values?

Ct values vary 3-12 cycles within and between viral gene targets, PCR tests and Labs.

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 [published online ahead of print, 2020 Aug 12]. *Clin Infect Dis.* 2020;ciaa1199. doi:10.1093/cid/ciaa1199



Note: Similar variations can be seen with quantitative viral loads in copies/ml.

# What are the Ct value cutoffs for the 6 SARS CoV-2 tests offered at YNHH?

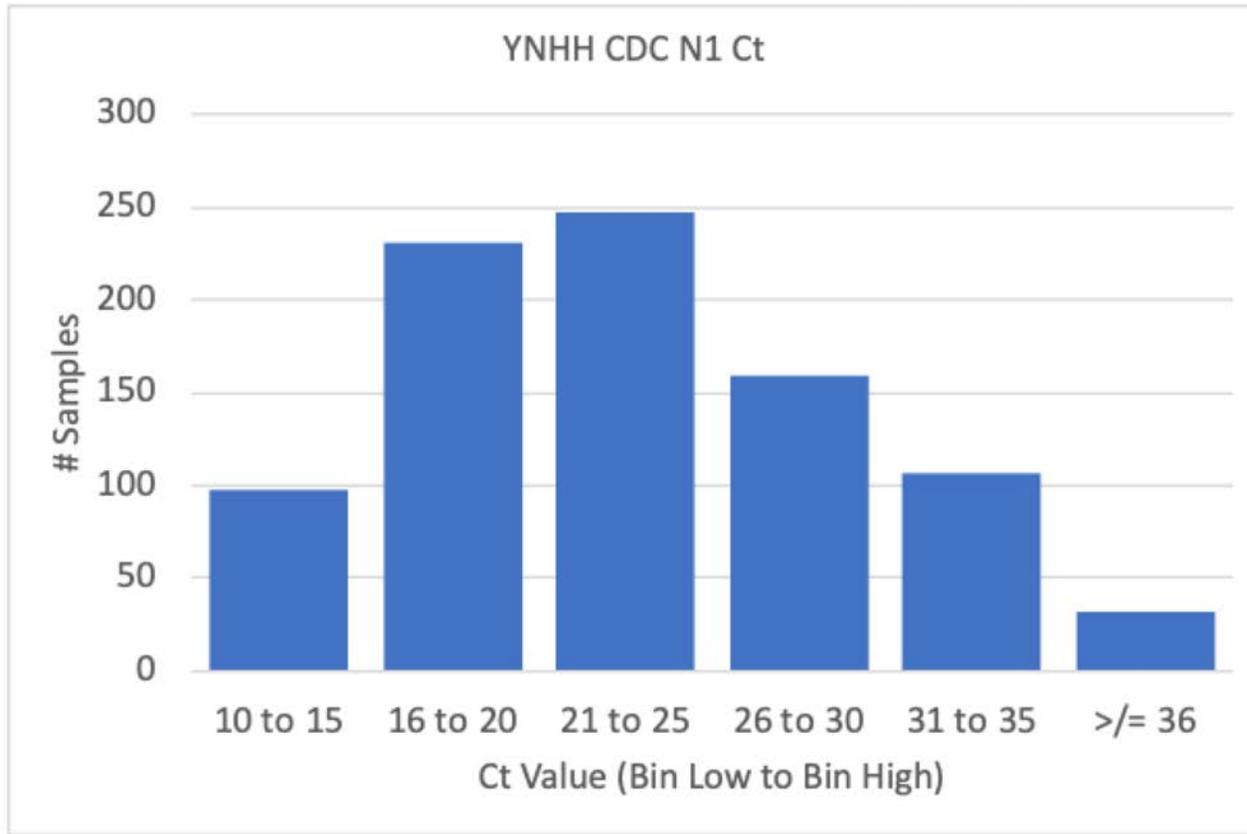
Test	Method	Cycle threshold cutoffs	Lab can access in EPIC Beaker	SARS CoV-2 Gene targets
CDC- lab developed	Real-time RT-PCR	<40	Yes	N1, N2
Simplexa (Diasorin)	Real-time RT-PCR	<40	No	ORF1ab, S
BD Max (Becton Dickinson)	Real-time RT-PCR	<40	No	N1, N2
<b>GeneXpert (Cepheid)</b>	<b>Real-time RT-PCR</b>	<b>&lt;45</b>	<b>Yes</b>	<b>N2, E</b>
<b>TaqPath (Thermofisher)</b>	<b>Real-time RT-PCR</b>	<b>&lt;37</b>	<b>Soon</b>	<b>ORF1ab, N, S</b>
Panther TMA (Hologic)	Transcription mediated amplification	N/A	No	Two regions ORF1ab

**GeneXpert and TaqPath are the most commonly used platforms with Ct values available for review.**  
 Lab developed CDC assay is the gold standard, but uses 3 singleplex PCRs and has limited use at present.

What is the distribution of Ct values at YNHH?

Should we report not report results of Ct >30 or Ct >35?

# Onset of Pandemic: Ct value distribution 3/13-4/4 for admitted symptomatic patients



## CDC assay used

1,016 positive (2 genes) or inconclusive (1 gene)

~14% had Ct  $\geq$  30

All were acute infections requiring hospitalization

Obtaining the most sensitive result was deemed essential

*Graph courtesy D. Peaper*

## Case example: Diagnosis of acute infection

- 43 year old, with fever, cough, SOB for 8 days. Presented to ED at outside hospital.
  - **CXR showed ground glass opacities**
  - SARS CoV-2 RT-PCR negative [GeneXpert]
  - Sent home
- Patient returned to 2 days later with worsening SOB and O2 sats
- **SARS CoV-2 RT-PCR positive** [CDC assay]
  - **Ct values: 35.2 N1 /37.7 N2**
- Patients with **pneumonia may have little virus in upper airway and using a sensitive assay is essential.**
  - PCR of sputum or BAL preferred, but often not available.

## Case example 2: Low and rising viral load

- 66 year old with hypertension, diabetes, on dialysis, anemic, admitted with **weakness**, no fever, no cough or SOB.
- Patient improved. SARS CoV-2 RT-PCR was ordered prior to discharge to a SNF, and was “inconclusive” with only 1 or 2 genes positive.
- Serial PCR results over 10 days shown:
- Patient remained without symptoms
- If less sensitive test was used, diagnosis would have been missed and patient discharged to SNF as COVID negative

PCR day	Ct value N1 gene	Ct value N2 gene
Day 0	Negative	38.4
Day 2	34.6	35.4
Day 5	26	25.3
Day 7	15.1	14.6
Day 10	18.8	18.4

# Thermofisher and Xpert RT-PCR at YNHH: Ct values obtained from 8/11-8/31/20

## Thermofisher (24 h TAT)

Used for outpatients

**47 positives**

**17 (36.2%) Ct >30**

**3 (6.4%) had Ct >35**

## GeneXpert (2 hr TAT)

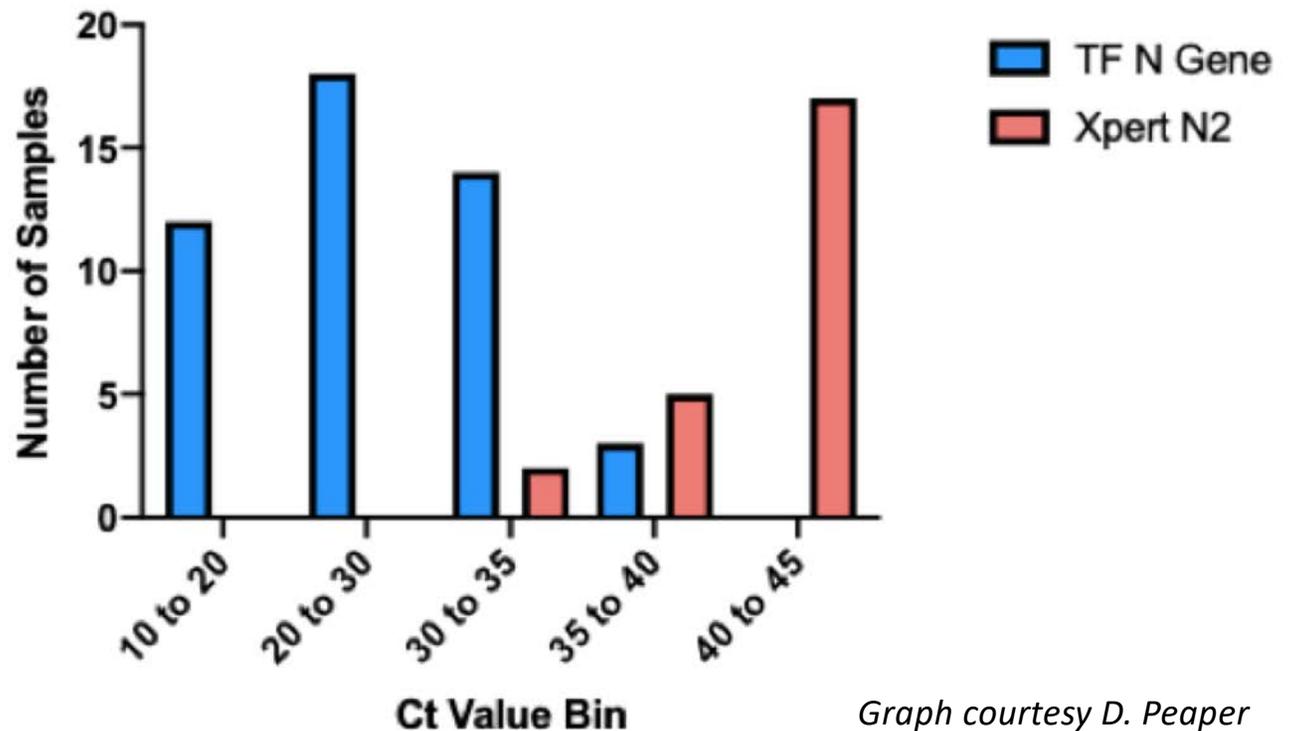
Used for admitted

patients if rapid result  
needed

**24 positives**

**24 (100%) Ct >30**

**17 (70.8%) had Ct >40**



*Graph courtesy D. Peaper*

# High Ct values can diagnose acute infections

Test (gene target)	Category	N	Acute Infection <sup>a</sup>	Prior Diagnosis	Uncertain <sup>c</sup>
Thermofisher	<b>Ct 30-34.9</b>	<b>14</b>	<b>8 (57%)</b>	6	
(N gene)	Ct $\geq$ 35-36.9	3	1 (33%)	2	
Xpert	<b>Ct 30-34.9</b>	<b>2</b>	<b>2 (100%)</b>		
(N2 gene)	Ct $\geq$ 35-39.9	5	2 <sup>b</sup> (40%)	3	
	Ct $\geq$ 40-44.9	17	0 (0%)	8	9

a, Some symptomatic or COVID-exposed patients on initial diagnosis had high Ct values, sometimes due to delays in being able to obtain testing.

b, One patient in hypoxic respiratory failure and admitted from ED to ICU had Xpert Ct value = 37.2

c, No symptoms, no prior diagnosis, no reported exposures. Either past infection (most likely) or false positive.

## Proposed: Addition of Test Result Comments for positives Ct >30 to assist interpretation

- **Low positive:** This sample was positive with a **Ct 30-34.9**. A low positive can be seen either very early or later in infection, with suboptimal sample collection, or with lower respiratory tract disease.
- **Very low positive:** This sample was positive with a **Ct >35-39.9**. A low positive can be seen either very early or later in infection, with suboptimal sample collection, or with lower respiratory tract disease.
- **Borderline positive:** This sample was positive with a **Ct >40-44.9**. A borderline positive is most likely due to recent past infection, but rarely could be a very early infection, or a false positive.

## Conclusion: Response to NY Times article from the perspective of a hospital COVID testing laboratory

- **Highly sensitive** tests are essential for **acutely ill hospitalized** patients as virus titers in the upper airway may be low (Ct >30 or Ct >35). However, recovering patients, now non-infectious, may also have a very low positive PCR result.
- For diagnostic testing in the **community, delays in obtaining testing**, as well as sample type and quality, **can lead to higher Ct values at diagnosis**. Not reporting positive results with Ct >30 would be a disservice to these patients.
- Reporting Ct values alone can be misleading, especially since **Ct values can vary significantly between various tests and labs**. However, a result comment for low positive results may be helpful. Ct values >40 may be of questionable value.
- It is essential to **confirm actual test sensitivity**, determine the **goals of testing** and understand the **tradeoffs** in various groups: e.g. asymptomatic screening, symptomatic patients, pre procedure, L&D, high risk nursing home residents.
- Tests with rapid but **somewhat less sensitive results may be acceptable in some outpatient settings**, especially when frequent repeat testing is performed.