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 <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.


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?

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

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 or not report results of Ct >30 or Ct >35?
Onset of Pandemic: Ct value distribution 3/13-4/4 for admitted symptomatic patients

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

~14% had Ct > 30

All were acute infections requiring hospitalization

Obtaining the most sensitive result was deemed essential

CDC assay used

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

<table>
<thead>
<tr>
<th>PCR day</th>
<th>Ct value N1 gene</th>
<th>Ct value N2 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Negative</td>
<td>38.4</td>
</tr>
<tr>
<td>Day 2</td>
<td>34.6</td>
<td>35.4</td>
</tr>
<tr>
<td>Day 5</td>
<td>26</td>
<td>25.3</td>
</tr>
<tr>
<td>Day 7</td>
<td>15.1</td>
<td>14.6</td>
</tr>
<tr>
<td>Day 10</td>
<td>18.8</td>
<td>18.4</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Test (gene target)</th>
<th>Category</th>
<th>N</th>
<th>Acute Infection&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Prior Diagnosis</th>
<th>Uncertain&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermofisher</td>
<td>Ct 30-34.9</td>
<td>14</td>
<td>8 (57%)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>(N gene)</td>
<td>Ct &gt;35-36.9</td>
<td>3</td>
<td>1 (33%)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Xpert</td>
<td>Ct 30-34.9</td>
<td>2</td>
<td>2 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N2 gene)</td>
<td>Ct &gt;35-39.9</td>
<td>5</td>
<td>2&lt;sup&gt;b&lt;/sup&gt; (40%)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ct &gt;40-44.9</td>
<td>17</td>
<td>0 (0%)</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

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

<sup>b</sup> One patient in hypoxic respiratory failure and admitted from ED to ICU had Xpert Ct value = 37.2

<sup>c</sup> 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.