Navigating through the quagmire of COVID-19 diagnostic testing

An overview of assay methods and result interpretation of COVID-19 diagnostic testing

Disclosures

- Roche Diagnostics, transplant viral testing advisory panel
- NIH RADx-UP, program member
- IDSA COVID-19 Diagnostic Guideline, panel member
Objectives

- Assess antigen, antibody, and molecular test options, and select the appropriate test
- Discuss the risks and benefits associated with PCR and isothermal molecular testing
- Explain the limitations of using Ct values and estimating viral load for clinical guidance

Types of common methods used for the detection of SARS-CoV-2

- Molecular: detects the presence of viral RNA, indicating active infection
- Antibody: detects presence of antibodies raised against the virus, indicating active or past infection
- Antigen: detects presence of viral proteins, indicating active infection

Note: none of the tests are FDA approved or cleared. Marketed tests are granted Emergency Use Authorization (EUA) status - essentially no clinical data on submission
Detection and Course of Infection


Antibody Detection

- Compared to PCR (positive and negative)
- IgM sensitivity at week 5, 75-80%
- IgA insufficient data, and specify issues (false positive)
- IgG maintains close to 95% sensitivity after week 3

Hanson et al., IDSA COVID-19 Diagnostic Guidelines Serology, 2020
Antigen Detection

- Commercially available antigen test performance:
  - Sensitivity is not good, 84-97% sensitive compared to PCR (some reporting < 50%), thus false negative is a concern
  - Specificity is very good, ≥99%, thus false positive results unlikely

- Detection window is very narrow to about 1 week after onset of symptoms (unclear in asymptomatic individuals)
  - > 1 week (5-7 days), can be below limit of detection

- Currently, negative antigen test results should be confirmed with a PCR test

- FDA has been actively pulling badly performing antigen (and antibody) tests off the market

Molecular Detection

- NAAT (nucleic acid amplification test), most common and the current "gold standard"
  - Technical note: RNA needs to be made into DNA (complementary DNA, cDNA), Reverse Transcription (RT)
  - NAAT comes in two flavors:
    - RT-qPCR (i.e. Roche, Cepheid), commonly referred to as just "PCR"
    - RT-isothermal (i.e. ID Now), commonly referred to as "LAMP" or "isothermal"

- ddPCR: droplet digital PCR, oil emulsion of 20,000 unique reactions

- CRISPR: 20 bp oligo binds to target and Cas13 cuts away a palindromic sequence that releases fluorophores for detection

- Next-Gen Sequencing (NGS), NextSeq or NovaSeq
PCR vs. Isothermal

- RT-qPCR: most laboratory-based test
  - Usually Turnaround Time (TAT) is several hours to days
    - Transit time
    - Aliquoting and batching
  - Cepheid Xpert, bioMérieux BioFire: most common PCR tests used as point-of-care
    - 45 minutes instrument runtime

- RT-isothermal: mainly deployed for point-of-care testing
  - Faster than traditional PCR
    - 5 to 15 minutes instrument runtime
  - ID Now, most common example in the US

Amplification
- PCR: Cycle of temp up and down, so Ct value = number of amplification
- Isothermal: Constant temp, no cycle values just continuous amplification (no Ct value)

Threshold
- Set by Tech (open)
- Set by manufacturer (Most tests)

Lower Ct value = Higher Viral Load
Higher Ct value = Lower Viral Load
# PCR vs. Isothermal

<table>
<thead>
<tr>
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<td><strong>Output detection</strong></td>
<td>Fluorescence</td>
<td>Varies, colorimetric, fluorescence</td>
</tr>
<tr>
<td><strong>Primer/Probes</strong></td>
<td>Specific, targeted</td>
<td>Multiple primers/probes</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>Set number of cycles (usually 40)</td>
<td>Until detected since there are no cycles; that is why it takes 5 to 15 minutes</td>
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<tr>
<td><strong>Throughput</strong></td>
<td>Can be very high by batching (i.e. run 96 samples at a time)</td>
<td>Single test use, so low throughput (need to run 1 at a time)</td>
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## Isothermal compared to PCR

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Basu et al., 2020 [https://doi.org/10.1128/JCM.01136-20](https://doi.org/10.1128/JCM.01136-20)
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- Variable collection (NS vs. NPS vs. VTM vs. swab types)
- Time of onset of symptoms or type of Sx unknown
- Bias in using PCR as both reference and comparison

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**Isothermal compared to PCR**

Abbott post-EUA authorization studies (ID Now compared to 2 or more PCR platforms):

- **Urgent Care Clinic Study** (5 urgent care sites: NJ, TN, LA, TX, SC), 430 patients, on-going
  - Symptoms (2 or more), 96.2% sensitivity
- **The Everett Clinic Study**, 974 patients
  - 763 symptomatic, 192 asymptomatic, Tu et al. (AMP presentation)
  - 91.3% sensitivity
- **Hospitalized patients**, 518 patients, on-going
  - Symptomatic patients > 7 days from onset of symptoms showed 71.1% sensitivity, and < 7 days from onset showed 86.7%

How to put all this into context?

- Analytical Performance vs. Clinical Performance
  - Detection ≠ Prediction of Disease
  - Assay can be:
    - Too sensitive (false positive)
    - Not specific enough (false negative)
  - IVD (in vitro diagnostic) test development usually takes years to roll out (+ robust clinical trials)
  - Need to look at prevalence to understand Positive and Negative Predictive Agreement (PPA, NPA), i.e. 5% vs. 30%

- For Infection Prevention and Control professionals, there is another question to ask:
  - Does detection = transmissibility?


Characteristics of select assays

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Misleading to compare Ct values across platforms

Packet Insert Information, accessed Nov 1, 2020
Personal communications with each manufacturer
### Characteristics of select assays

- Ct values of different targets from the same assay can be vastly different
  - Target A 26.7 vs. Target B 31.2, and this is NOT consistent across patients, some Target A < Target B, others Target A > Target B

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Target A Ct value ≠ Target B Ct value

### Characteristics of select assays

- Ct values of different targets using the same Probe read out can be misleading
  - Can’t assume Target A and Target B contributes equally
  - Some will be 50:50, some 40:60, some 60:40 etc…

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Target A abundance ≠ Target B abundance
Other considerations

- Regulatory compliance: per FDA EUA status, need to use these assays as qualitative detection
  - Reporting Ct values, makes them quasi quantitative

- Translating Ct values to a quantification (Log10 copies/mL or IU) is even more challenging
  - Calibration material is NOT universal, viral load between assays will be inconsistent
    - Synthetic materials don’t cover the full genome
  - Assays were developed as qualitative tests, so Ct values are not linear at higher Ct values (lower viral load)

- Sample type challenges

Can we quantitate like CMV, HIV, HCV?

- Sample type: homogenous vs. heterogeneous matrix

- Blood is a homogenous (evenly distributed)

- NP swab is heterogeneous (not evenly distributed): collection bias, impact of swab, stabilizing media (VTM, saline, others)
  - Sufficient for qualitative detection but inconsistent for viral load

- Virology and viral shedding: uncertain whether viral shedding in the nasopharynx can be used to monitor viral shedding elsewhere like lower respiratory/lungs
Take Home Message

- Molecular method should be used to diagnose active infection

- Unlike in most viral infections, anti-SARS-CoV-2 IgG and IgM come up around the same time

- IgM only lasts 2-3 weeks

- Due to major limitations and pitfalls, Ct values should not be used as basis for clinical decision making

Platforms used at DUHS

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<tr>
<td>Dr. Sara MDX</td>
<td>Abbott ID Now</td>
</tr>
<tr>
<td>Thomas Fisher</td>
<td><a href="http://www.abbott.com">www.abbott.com</a></td>
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DUHS Clinical Microbiology Lab

Thank You!
Questions?