Navigating through the quagmire of COVID-19 diagnostic testing

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

Duke University School of Medicine

dcasip.medicine.duke.edu

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Disclosures

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

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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

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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

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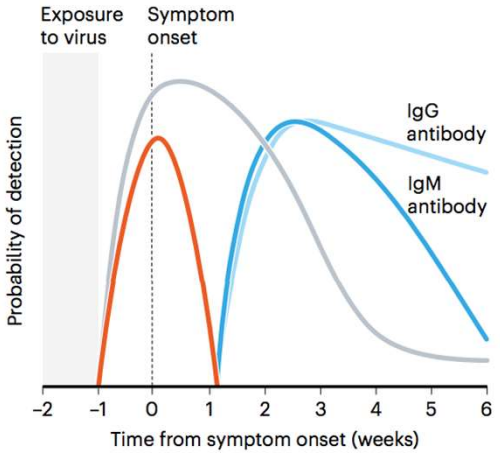
Detection and Course of Infection



PCR-based tests detect small amounts of viral genetic material, so a test can be positive long after a person stops being infectious.

Rapid antigen tests detect the presence of viral proteins and can return positive results when a person is most infectious.

Antibody tests detect the body's immune response to the virus and are not effective at the earliest phase of infection.

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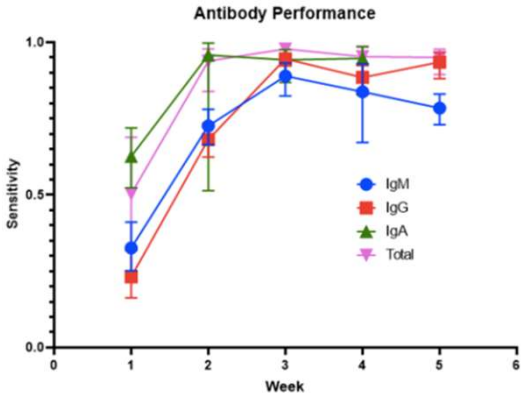
Guglielmi, Nature 2020, https://www.nature.com/articles/d41586-020-02661-2

Antibody Detection



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

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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
    - Aliquotting 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

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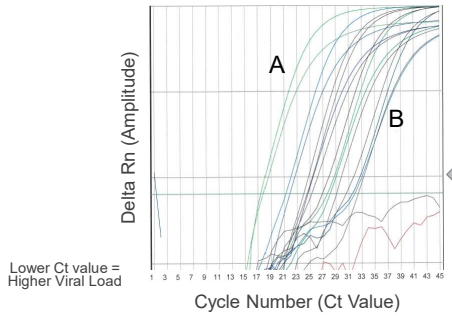
PCR vs. Isothermal

	PCR	Isothermal
Amplification	Cycle of temp up and down, so **Ct value** = number of amplification	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

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PCR vs. Isothermal

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

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Isothermal compared to PCR

	Basu et al.	Harrington et al.	Zhen et al.	Smithgall et al.	Rhoads et al.
Sample Type	Nasal Swab (dry swab)	Nasal Swab (foam swab)	NP Swab in VTM	NP Swab in VTM	NPS in VTM, saline, SC
Testing Location	Micro Lab	Point of Care	Micro Lab	Micro Lab	Micro Lab
Population	101 ED pts	524 ED and inpatients	108 symptomatic pts	113 ED and inpatients	96 unspecified pts
Sensitivity (PCR test)	54.8%	74.7%	87.7%	73.9%	94%

Basu et al., 2020 https://doi.org/10.1128/JCM.01136-20.
Harrington et al., 2020 https://doi.org/10.1128/JCM.00798-20.
Zhen et al., 2020 https://doi.org/10.1128/JCM.00783-20
Smithgall et al.,2020 https://doi.org/10.1016/j.jcv.2020.104428
Rhoads et al, 2020 https://doi.org/10.1128/JCM.00760-20

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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%

https://abbott.mediaroom.com/2020-10-07-Abbott-Releases-ID-NOW-TM-COVID-19-Interim-Clinical-Study-Results-from-1-003-People-to-Provide-the-Facts-on-Clinical-Performance-and-to-Support-Public-Health

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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?

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Characteristics of select assays

	Abbott m2000	Roche Cobas 6800	DiaSorin MDX	Cepheid GeneXpert	Abbott ID Now
SARS-2 Target(s)	RdRp, N	Orf1ab, E	Orf1ab, S	N2, E	RdRp
Probe	Combined (1 Ct value)	Separate (2 Ct values)	Separate (2 Ct values)	Separate (2 Ct values)	Mulitple (No Ct values)
Highest Ct value	32.5	40	40	45	N/A

Misleading to compare Ct values across platforms

Packet Insert Information, accessed Nov 1, 2020
Personal communications with each manufacturer

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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

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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

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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

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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

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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

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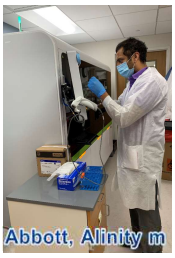
Platforms used at DUHS

Microbiology Lab



Point of Care

www.abbott.com

Mol Path/Vaccine Lab

www.suntimes.com

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DUHS Clinical Microbiology Lab

Thank You!

Questions?

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