SARS-CoV-2 Diagnostic Testing

Kimberly Hanson, MD, MHS
Professor of Medicine
Section Head, Clinical Microbiology Laboratory
Medical Director, Transplant Infectious Diseases

University of Utah and ARUP Laboratories
Conflicts of Interest

• None relevant to this topic
Overview

• Brief review of SARS-CoV-2 virology

• Review diagnostic methods and test performance
  - Molecular diagnostics
  - Antigen testing
  - Serology

• Highlight testing recommendations
SARS-CoV-2
Protein and genome structure

- Enveloped β-coronavirus
  - ss +RNA

- Diagnostic targets
  - Nucleic acid amplification
    - ORFab, RdRp, S, E and/or N genes
  - Antigen
    - S or N protein
  - Antibody
    - S and/or N proteins

Alanagreh et al. Pathogens 2020, 9(5), 331
Nucleic acid amplification testing (NAAT)

Analytical test performance

• RNA detection is the diagnostic standard to confirm infection

• > 180 FDA emergency use authorization (EUA) molecular tests
  - Various methodologies – isothermal NAAT, PCR, TMA
  - Analytical test characteristics are variable
    ✓ FDA limit of detection comparison NAAT detectable units (NDU)/mL
      - Swabs in transport media 180 – 1800 NDU/mL
      - Dry swabs 60,000 – 540,000 NDU/mL
      - Saliva 600 – 180,000 NDU/mL
    ✓ Specificity is high > 95%
Nucleic acid amplification testing (NAAT)

Clinical test performance

• Clinical performance difficult to establish in the absence of a gold-standard
  ✓ 70% sensitivity range vs. clinical composite
  ✓ 94% sensitivity vs. NAAT test comparison

Woloshin et al. NEJM 2020; 383(6): e38
Laboratory-based PCR Platforms

*Crossing thresholds (Ct values)*
Viral load and kinetics

Importance of time post-symptom onset, host and specimen type

Temporal dynamics of virus shedding throat swabs

Duration of viral shedding in upper tract 17 days, maximum 83 days
- typically longer in critically ill, immunocompromised and lower respiratory tract samples

Virus loads in asymptomatic individuals roughly equivalent to symptomatic patients

Temporal dynamics upper versus lower respiratory tract shedding


He et al. Nat Med 2020 26, 672–675 (2020)


Lee et al. JAMA Internal Med 2020; Ra et al. Thorax 2020
Infectious Diseases Society of America Guidelines on the Diagnosis of Coronavirus Disease 2019

Symptomatic Individuals***

- Suspicion for COVID-19 is high
  - Non-Hospitalized
    - Lower respiratory tract symptoms
      - • Known exposure
      - • High prevalence area
  - Hospitalized

- Suspicion for COVID-19 is low

Asymptomatic Individuals

- • Exposed and testing is available
- • Immunosuppressive procedure
- • Major time-sensitive surgery
- • Time-sensitive aerosol-generating procedures when PPE is limited and testing is available
- • Admission to the hospital when prevalence high

Direct SARS-CoV-2 nucleic acid amplification testing

Nasopharyngeal, Nasal, or Mid-turbinate over Oropharyngeal or Saliva specimen
Provider-collected or self-collected specimens acceptable for different specimen types except nasopharyngeal

If negative, repeat testing
If negative, repeat testing (from lower tract if possible)
If negative, and high suspicion, repeat testing
If negative, do not repeat testing

Update underway - performance of different specimen types and rapid NAAT and immunocompromised patients

***Note:
- Testing should be prioritized for symptomatic patients first.
- When resources are adequate, testing for selected asymptomatic individuals can also be considered.
Antigen Testing

• 7 EUA immunoassays
  - Nasal or NP swab
  - Difficult to compare LODs
• Low complexity and low cost with rapid turn around (~ 15 minutes)
• Less sensitive than NAAT (~ 85% vs. PCR)
  - Best when viral load is highest
    - 1st 7 days of symptoms
    - Ct cut-offs ≤ 25-30 range
    - Less sensitive in children?
    - High clinical suspicion or medium-high prevalence back up with NAAT!
• FDA issued an alert about false positive results

Viral Culture

• BSL3 pathogen
• Likely grows in routine clinical virology cell lines (R-mix, RhMK) without CPE
• Vero E6 cells display highest titers and CPE

Uninfected VERO cells

SARS CoV cytopathic effect (CPE) 24 hrs
The RNA or Antigen detection vs. culture

• Animal models – culture positivity correlates with transmissibility
  - No direct human studies

• Detectable RNA does not equal culture positivity or infectiousness
  - Envelope lysis and/or virus particle aggregation prevents subsequent infection, but does not eliminate nucleic acid, which degrades slowly over time

  - RNA fragments associated with intracellular vesicles protected from degradation

• Antigen detection may correlate better than PCR with culture

Pekosz et al. medRxiv 2020: 2020.10.02.20205708
Duration of culture positivity and infectivity

Evidence base for isolation recommendations

- Rare replication competent virus > 9 days after symptom onset (longest reported 20 days)
  - majority of transmission events within 5 days of symptom onset
- Culture negative when Ct > 35 (Ct values across platforms not directly comparable)
- Current recommendation - wait 10 to 20 days after symptom onset to discontinue transmission-based precautions
  - Note of caution - relapsing infection (and culture positivity) reported in severely immunocompromised after 20 days

Anti-SARS-CoV-2 antibody testing

• Types of antibodies
  - Binding antibodies (Abs)
    ▪ IgM, IgG, IgA and total
  - Neutralizing antibodies (nAbs)
    ▪ Block cell infection (S protein)

• 56 EUA tests detect anti-S and/or N Abs
  - Do not differentiate binding vs. neutralizing
  - Variable test performance across assays

• Neutralization assays research/reference lab
  - Plaque reduction assays with natural or pseudovirus
  - Variable nAb titer correlation with commercial EUA tests


Plaque Reduction Neutralization Testing

JCI Insight. 2020;5(22):e143213. https://doi.org/10.1172/jci.insight.143213
Pooled test characteristics

Sensitivity IgM week 2 = 73%
Sensitivity IgG week 4 = 88%
Specificity >97%

No difference by antigen targeted
Antibody Testing

Clinical observations and recommendations

• Duration of the Ab response and protection
  - Animal studies – infection provides at least short-term immunity and anamnestic response
  - Ab titers correlate with severity of illness
  - NYC study anti-S Ab titers relatively stable for at least 5 months (n=121 volunteers)

![IgG concentrations by symptom severity](attachment:LongQXetal_NatureMedicine2020.png)

![Overall titers](attachment:Wajnbergetal_Science2020.png)

![Correlation of neutralization versus ELISA at day 148 time point](attachment:Wajnbergetal_Science2020.png)
Antibody Testing

Clinical utility for diagnosis

- Individuals with a history of COVID-19-like illness
  ▪ when clinical suspicion is high and repeated NAAT negative to support diagnosis
  ▪ for defining multisystem inflammatory syndrome in children (MIS-C)

- Titer that correlates with protection not yet identified

- Identifying convalescent serum donors

- No role for donor/recipient screening or pre-chemo
Reinfection

- Repeat testing positive > 90 days after initial test
- Confirmation requires paired whole genome sequencing (WGS)
- Ct <33-35 in second sample or seroconversion is suggestive

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>First Infection (Ct)</th>
<th>Second Infection (Ct)</th>
<th>Intervening period (days)</th>
<th>Antibody after first infection</th>
<th>Antibody after reinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong Kong</td>
<td>Male</td>
<td>33</td>
<td>Mild (N/A)</td>
<td>Asymptomatic (27)</td>
<td>142</td>
<td>Negative</td>
</tr>
<tr>
<td>Nevada, USA</td>
<td>Male</td>
<td>25</td>
<td>Mild (35)</td>
<td>Hospitalised (35)</td>
<td>48</td>
<td>N/A</td>
</tr>
<tr>
<td>Belgium</td>
<td>Female</td>
<td>51</td>
<td>Mild (26-27)</td>
<td>Milder (33)</td>
<td>93</td>
<td>N/A</td>
</tr>
<tr>
<td>Ecuador</td>
<td>Male</td>
<td>46</td>
<td>Mild (37)</td>
<td>Worse (N/A)</td>
<td>63</td>
<td>IgM+ and IgG-</td>
</tr>
</tbody>
</table>

Data were obtained Sept 14, 2020, for reinfection cases confirmed by viral genome sequences. Ct=cycle threshold. N/A=not available. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

Iwasaki Lancet Infect Dis 2020; DOI:https://doi.org/10.1016/S1473-3099(20)30783-0
Conclusions

• NAAT remains the diagnostic reference test to confirm infection

• Know the tests your laboratory is performing

• Many unanswered diagnostic questions remain
  - Predictors of infectious/replication competent virus
  - Immune correlates of protection
  - Test performance in children and asymptomatic individuals