The novel coronavirus: Defining terms

SARS-CoV-2 ("Severe Acute Respiratory Syndrome Coronavirus 2")

SARS-CoV-2 is the virus that causes COVID-19

COVID-19 ("CoronaVirus Disease 2019")

COVID-19 is the disease resulting from infection with the novel coronavirus

Clinical classification and transmission of COVID-19

Safe opening of society requires identification of patients with both active and prior exposure

Testing Options for COVID-19

Disclosure

Full Time Employment with Siemens Healthineers
Direct Testing for SARS-CoV-2

Viral RNA
- Detects presence of the virus by molecular method such as real-time RT-PCR (diagnostic). Common genetic targets are N gene, E gene, ORF1ab, S gene.
- Typical sample types: Nasopharyngeal or oropharyngeal swabs.

Antigen
- Detects presence of nucleocapsid protein from the virus (confirms infection but less sensitive than RNA testing).
- Typical sample types: Swabs.

The Roles of rt-PCR COVID-19 Patient Testing

- Exposed patient, may be symptomatic or asymptomatic.
- rt-PCR testing to detect SARS-CoV-2 RNA for active infection (virus presence).
- Positive results:
  - Highly sensitive for presence of viral RNA.
  - Current "gold standard" for diagnosis.
  - May be positive for up to 8 weeks post infection indicating presence of RNA remnants.
- Not specific for replication competent virus.
- Negative results:
  - Considered to be diagnostic negative.
  - May not have been exposed or may be in very early or late stages of infection.

CDC Highlights Non-Viable Viral RNA Detection:

Key Takeaways

1. Replication-competent virus has been recovered after 10 days for mild/moderate infections.
2. Recovery of replication competent virus has occurred between 10-20 days after symptom onset in patients with severe disease that, in some cases, was complicated by immunocompromise. Even in this group, 88% of patients no longer yielded viable virus specimens after 10 days, and 95% after 15 days were non-viable.
3. Recovery of non-viable SARS-CoV-2 RNA can occur for up to 12 weeks.

CDC Guidance for Antigen Testing

"Rapid antigen tests perform best when the person is tested in the early stages of infection with SARS-CoV-2 when viral load is generally highest."

Accessed Sept. 5, 2020

порядок визуализации: 
1. Direct Testing for SARS-CoV-2
2. The Roles of rt-PCR COVID-19 Patient Testing
3. CDC Highlights Non-Viable Viral RNA Detection:
4. CDC Guidance for Antigen Testing
**N Protein is Abundantly Expressed in SARS-CoV-2**

Antigen: Test for presence of N protein
PCR: Test for presence of viral RNA

---

**Testing Options for COVID-19**

Direct Detection: Molecular/Antigen
Indirect Detection: Serology

IgM Antibody
IgG Antibody

---

**Serologic Utility**

- Convalescent plasma screening
- Serial measurements for comparing relative concentrations
- In-patients: assessing acute disease course
- Out-patients: assessing durability of immunity
- Defining Conferring immunity
- Companion diagnostics for vaccine programs and monoclonal antibody therapy

---

**CDC Guidance on Serology Testing**

Late Presenters: "For persons who present 9–14 days after illness onset, serologic testing can be offered in addition to recommended viral direct detection methods such as polymerase chain reaction or antigen detection tests."  
MIS in Children: "Serologic testing should be offered as a method to help support a diagnosis when patients present with late complications of COVID-19 illness, such as multisystem inflammatory syndrome in children."

"Assure a high positive predictive value (e.g., 95%) by choosing tests with sufficiently high specificity (e.g., > 99.5%)."

<table>
<thead>
<tr>
<th>Alignment w/CDC and IDSA guidance re antibody testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDSA</td>
</tr>
<tr>
<td>Serology to support a late diagnosis (&gt; 14 days)</td>
</tr>
<tr>
<td>Serology in children with/without MIS</td>
</tr>
<tr>
<td>Serology for seroprevalence</td>
</tr>
</tbody>
</table>

---

**Heterogeneous Antibody Response Between Patients**

- "Antibodies" vary between patients

---

**Simplify detection of immune response with a total Ab assay**

Seroconversion: Timeline for Theoretic IgM and IgG Production

Timeline of IgM and IgG Antibody Levels to SARS-CoV-2 from Onset of Symptoms

---
**Bind vs. Neutralizing Antibodies:**

- Binding Antibody: Recognizes and binds to different parts (antigens) of the virus
- Neutralizing Antibody: Recognizes, binds, and interferes with viral infection

All antibodies are binding but only a subset are also neutralizing

---

**Choice of antigen target varies:**

<table>
<thead>
<tr>
<th>Homology of SARS-CoV-2 protein to:</th>
<th>SARS-CoV</th>
<th>MERS-CoV</th>
<th>HCoV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike (S)</td>
<td>77%</td>
<td>74% (S1 RBD)</td>
<td>90% (S2)</td>
</tr>
<tr>
<td>Nucleocapsid (N)</td>
<td>89%</td>
<td>48%</td>
<td>27–35%</td>
</tr>
<tr>
<td>Membrane / Matrix (M)</td>
<td>89%</td>
<td>39%</td>
<td>27–39%</td>
</tr>
<tr>
<td>Envelope (E)</td>
<td>94–96%</td>
<td>36%</td>
<td>17–30%</td>
</tr>
</tbody>
</table>

---

**IgG antibody alone does not confirm a resolved infection**

“Our results suggest that SARS-CoV-2 might be excreted at low levels despite clinical recovery. Thus, both serial viral load monitoring and antibody response should be considered when making decisions about infection control measures…”

“Serological assay can complement RT-qPCR.”

---

**All Antibodies are Binding; Only a Subset are also Neutralizing**

- Anti-N detect binding antibodies
- Anti-RBD detect neutralizing antibodies (which are also binding)
S1 RBD binds the ACE2 Receptor to start infection in SARS-CoV-2

The S1 RBD of spike binds ACE2

S1 RBD antibodies block the virus entry into cells

Guo et al. Military Medical Research (2020) 7:11

Image from: https://www.prosci-inc.com/ace2-antibodies/

Spike is divided into 2 regions: S1 and S2

- RBD comprises the majority of the S1 region
- S1 RBD binds ACE2 on the human host cell
- S2 mediates fusion and viral entry

Correlates of Protection: Humoral and Cellular Immunity

“Recent reports that antibodies to SARS-CoV-2 are not maintained in the serum following recovery from the virus have caused alarm. However, the absence of specific antibodies in the serum does not necessarily mean an absence of immune memory.”

Anti-N Antibodies Decay more Quickly

“Taken together, these results indicate that anti-N antibody responses may substantially (i.e. 30% to 45%) underestimate the proportion of SARS-CoV-2 exposed individuals compared to anti-S antibody responses in population-based seroprevalence studies.”
Antibodies to N Decline Rapidly in Many Patients

"In contrast to other reports, we conclude that immunity is durable for at least several months after SARS-CoV-2 infection."

Does Mild Disease Produce Longitudinal Immunity?

"At Visit 2, all CoV2+ individuals maintained anti-RBD IgG levels above the negative threshold."

RBD-Based Antibody Assays as a Correlate to Neutralization

"In conclusion, we have successfully cloned two human blocking mAbs using SARS-CoV-2 RBD-specific memory B cells isolated from recovered COVID-19 patients. These two mAbs can specifically bind to SARS-CoV-2 RBD, block the interaction between SARS-CoV-2 RBD and hACE2 receptor, and lead to efficient neutralization of SARS-CoV-2 S protein pseudotyped virus infection."

Quality and accuracy first

Test accuracy is paramount to minimize risks for communities and employees. There are numerous tests that claim to detect antibodies to the SARS-CoV-2 virus; only a few are highly accurate.

An antibody test with high positive predictive value is one that has specificity of 99.5% or above, which shows better performance even in populations with low disease prevalence.

All serology tests are not created equally.....
1. Testing options include direct and indirect methods
2. Direct methods (rt-PCR, antigen) are preferred for diagnosis
3. Indirect methods (serology) confirm previous infection or exposure
4. Antibodies detected may be binding and/or neutralizing
5. Serology testing supplies are more widely available than some direct methods, but introduce complexities:
   - Longevity/potency of antibody detected varies
   - Assay performance characteristics vary greatly by manufacturer

Specificity, Prevalence, and Predictive Value

Specificity impacts PPV across prevalence settings

Summary