COVID-19 Serology
IMPLEMENTATION, CLINICAL UTILITY, AND OUTSTANDING QUESTIONS

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DEPARTMENT OF PATHOLOGY & IMMUNOLOGY
DIVISION OF LABORATORY MEDICINE
Disclosures – Research Funding

Abbott Diagnostics
NowDiagnostics
Beckman Coulter
Learning Objectives

1) Understand how to validate COVID-19 serological assays

2) Describe the shortcomings associated with COVID-19 serological testing

3) List the proposed utilities of serological testing for COVID-19
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

- Single Stranded RNA virus closely related to other coronaviruses
- Alphacoronavirus
  - 229E and NL63
- Betacoronavirus
  - OC43 and HKU1
  - SARS-CoV (2002)
  - MERS-CoV (2012)
  - SARS-CoV-2
COVID-19 cases worldwide

US Prevalence
~1.64%

as of
8/17/20

https://coronavirus.jhu.edu/map.html
Current testing for SARS-CoV-2

• Molecular Testing
  o Preferred method for diagnosis of SARS-CoV-2
  o Tests for the presence of viral RNA
  o Some issues with sensitivity
  o Supply chain and reagent shortages

• Antigen Testing
  o Currently 2 available
  o Detect nucleocapsid protein from nasal or NP swab
  o Sensitivity ~ 80% relative to PCR
Current testing for SARS-CoV-2

• Serology
  o Tests for the presence of antibodies to SARS-CoV-2
  o Originally > 200 tests available in the US
  o Require Emergency Use Authorization
  o Currently 35 tests with EUA
  o Performance- highly variable
COVID-19 Serology and PCR proposed kinetics

COVID-19 Serology Timeline

- December 31st - Wuhan, China reports first cases
- January 12th - first sequence released
- January 21 - February 23rd - First known cases spread to the US
- ~March - First known lateral flow test is marketed in the US
- May 4th - EUA Required in the US
- Late April - High throughput assays released
Emergency Use Authorization and Serology

Allows for the use of unapproved medical devices to be used in an emergency

Emergency Use Authorization of Medical Products and Related Authorities

Guidance for Industry and Other Stakeholders

U.S. Department of Health and Human Services
Food and Drug Administration
Office of the Commissioner
Office of the Chief Scientist
Office of Counterterrorism and Emerging Threats

Originally, serologic assays did not require an EUA

A: As stated in Section IV.D of the FDA's *Policy for Diagnostic Tests for Coronavirus Disease-2019*, the FDA does not intend to object to the development and distribution by commercial manufacturers, or development and use by laboratories, of serology tests to identify antibodies to SARS-CoV-2, where the test has been validated, notification is provided to FDA, and information along the lines of the following is included in the test

**Why?**

They weren’t meant to be diagnostic
They were meant to be used in high complexity labs
Mainly for seroprevalence / study purposes
But early serological assays did not deliver!

**Antibody Test, Seen as Key to Reopening Country, Does Not Yet Deliver**

The tests, many made in China without F.D.A. approval, are often inaccurate. Some doctors are misusing them. The rollout is nowhere close to the demand.

**U.K. Paid $20 Million for New Coronavirus Tests. They Didn’t Work.**

Facing a global scramble for materials, British officials bought millions of unproven kits from China in a gamble that became an embarrassment.
Early studies demonstrated numerous false positives

<table>
<thead>
<tr>
<th>Assay</th>
<th>Total N</th>
<th>False positive</th>
<th>%</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay 1</td>
<td>107</td>
<td>4</td>
<td>96.3</td>
<td>90.7 - 99.0</td>
</tr>
<tr>
<td>Assay 2</td>
<td>104</td>
<td>2</td>
<td>98.1</td>
<td>93.2 - 99.8</td>
</tr>
<tr>
<td>Assay 3</td>
<td>107</td>
<td>9</td>
<td>91.6</td>
<td>84.6 - 96.1</td>
</tr>
<tr>
<td>Assay 4</td>
<td>108</td>
<td>1</td>
<td>99.1</td>
<td>94.9 - 100.0</td>
</tr>
<tr>
<td>Assay 5</td>
<td>108</td>
<td>0</td>
<td>100.0</td>
<td>96.6 - 100.0</td>
</tr>
<tr>
<td>Assay 6</td>
<td>108</td>
<td>1</td>
<td>99.1</td>
<td>94.9 - 100.0</td>
</tr>
<tr>
<td>Assay 7</td>
<td>108</td>
<td>0</td>
<td>100.0</td>
<td>96.6 - 100.0</td>
</tr>
<tr>
<td>Assay 8</td>
<td>107</td>
<td>2</td>
<td>98.1</td>
<td>93.4 - 99.8</td>
</tr>
<tr>
<td>Assay 9</td>
<td>99</td>
<td>4</td>
<td>96.0</td>
<td>90.0 - 98.9</td>
</tr>
<tr>
<td>Assay 10</td>
<td>108</td>
<td>10</td>
<td>90.7</td>
<td>83.6 - 95.5</td>
</tr>
</tbody>
</table>

Manufacturer validations pre-EUA

<table>
<thead>
<tr>
<th>Group</th>
<th>positive</th>
<th>borderline</th>
<th>negative</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 days after onset of symptoms</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>33.3%</td>
</tr>
<tr>
<td>&gt; 10 days after onset of symptoms</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>80.0%</td>
</tr>
</tbody>
</table>
The FDA reversed course on EUA for serology

- May 4th, 2020 new guidance:
  - Manufacturers must submit validation data for EUA w/in 10 days from
  - FDA provided specific performance threshold requirements

30 confirmed SARS-CoV-2 Ab positive samples/Ab type

80 Ab negative and/or pre-COVID-19 samples (10 HIV positive)
EUA also required the following language

• Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

• Results from antibody testing should not be used to diagnose or exclude acute SARS-CoV-2 infection.

• Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
So what does a positive result mean?
The Role of Antibody Testing for SARS-CoV-2: Is There One?

Elitza S. Theel, a Patricia Slev, b,c Sarah Wheeler, d Marc Roger Couturier, b,c Susan J. Wong, e Kamran Kadkhoda f

Clinical Chemistry 66:7
875-877 (2020)

SARS-CoV-2 Serology: Much Hype, Little Data

Christopher W. Farnsworth* and Neil W. Anderson
Classes of antibodies detected by anti-SARS-COV-2 assays

IgG
IgA
IgM
Total Ab

Variations in design of serological SARS-CoV-2 assays

- Spike Protein
  - S1
  - S2
  - Receptor Binding Domain

- Nucleocapsid

[Link: https://www.economist.com/briefing/2020/03/12/understanding-sars-cov-2-and-the-drugs-that-might-lessen-its-power]
How do SARS-CoV-2 serological assays generally work

2\textsuperscript{nd} Antibody with conjugate

Patient plasma with antibodies

SARS-CoV-2 antigen
How do SARS-CoV-2 serological assays generally work

Patient plasma with antibodies 

SARS-CoV-2 antigen 

2nd Antibody with conjugate 

Biotin 

Streptavidin + conjugate 

Streptavidin + conjugate
Types of assays:

Lateral Flow

ELISA

CLIA
Validating new SARS-CoV-2 serological assays

1. Analytic measuring range
2. Precision
3. Interferences
4. Accuracy
1) Analytic measuring range

- Only necessary for quantitative assays
- Must show accuracy and precision across reportable range
- Likely don’t correlate between assays

![Graph showing dilution vs signal for Assay 1 and Assay 2](image)
2) Precision

- Test intra-assay and inter-assay
- Sources of imprecision
  - Timing, temperature, reagent etc
- Ideally test at or near the cutoff

3) Interferences

- Effects of other compounds that impact measurement of analyte
- I.e. hemoglobin, triglycerides, bilirubin
- Perform near the cutoff for positive
- Labs may use data from manufacturers

<table>
<thead>
<tr>
<th>Hemolysis Index</th>
<th>Result</th>
<th>% of Original</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1.69</td>
<td>100.0%</td>
</tr>
<tr>
<td>47</td>
<td>1.70</td>
<td>100.6%</td>
</tr>
<tr>
<td>81</td>
<td>1.70</td>
<td>100.9%</td>
</tr>
<tr>
<td>150</td>
<td>1.69</td>
<td>100.3%</td>
</tr>
<tr>
<td>284</td>
<td>1.70</td>
<td>100.6%</td>
</tr>
<tr>
<td>563</td>
<td>1.68</td>
<td>99.4%</td>
</tr>
<tr>
<td>1089</td>
<td>1.70</td>
<td>100.6%</td>
</tr>
</tbody>
</table>
4) Accuracy

- Extent to which a method compares to a reference method
- Ideally RT-PCR confirmed SARS-CoV-2 infection

Basic lab statistics: sensitivity and specificity

<table>
<thead>
<tr>
<th></th>
<th>No Infection</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody (-)</td>
<td>True neg.</td>
<td>False neg.</td>
</tr>
<tr>
<td>Antibody (+)</td>
<td>False pos.</td>
<td>True pos.</td>
</tr>
</tbody>
</table>

sensitivity = \[
\frac{\# \text{ true positives}}{\# \text{ true positives} + \# \text{ false negatives}}
\]
Basic lab statistics: sensitivity and specificity

<table>
<thead>
<tr>
<th></th>
<th>No Infection</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody (-)</td>
<td>True neg. 98</td>
<td>False neg. 10</td>
</tr>
<tr>
<td>Antibody (+)</td>
<td>False pos. 2</td>
<td>True pos. 90</td>
</tr>
</tbody>
</table>

sensitivity = \( \frac{90}{90+10} = 90\% \)
Basic lab statistics: sensitivity and specificity

<table>
<thead>
<tr>
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<th>No Infection</th>
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</thead>
<tbody>
<tr>
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<td>False neg.</td>
</tr>
<tr>
<td>Antibody (+)</td>
<td>False pos.</td>
<td>True pos.</td>
</tr>
</tbody>
</table>

Specificity = \[
\frac{\text{# true negatives}}{\text{# true negatives} + \text{# false positives}}\]
### Basic lab statistics: sensitivity and specificity

<table>
<thead>
<tr>
<th></th>
<th>No Infection</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody (-)</td>
<td>True neg. 98</td>
<td>False neg. 10</td>
</tr>
<tr>
<td>Antibody (+)</td>
<td>False pos. 2</td>
<td>True pos. 90</td>
</tr>
</tbody>
</table>

**Specificity =** \[
\frac{98}{98 + 2} = 98\%
\]
Sample of sensitivity and specificity analysis

Specificity: 98.69% (95.63-99.84)

<table>
<thead>
<tr>
<th></th>
<th>Healthy donors</th>
<th>Cross-reactivity panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay 1</td>
<td>100% (149/149)</td>
<td>99% (104/105)</td>
</tr>
<tr>
<td>Assay 2</td>
<td>100% (149/149)</td>
<td>99% (104/105)</td>
</tr>
<tr>
<td>Assay 3</td>
<td>99.3% (148/149)</td>
<td>96.2% (101/105)</td>
</tr>
</tbody>
</table>

Sample of sensitivity and specificity analysis

<table>
<thead>
<tr>
<th>Pos</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>2</th>
<th>0</th>
<th>8</th>
<th>15</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>50</td>
<td>9</td>
<td>14</td>
<td>80</td>
<td>12</td>
<td>20</td>
<td>23</td>
<td>47</td>
</tr>
</tbody>
</table>

**Specificity:** 98.69% (95.63-99.84)

**Sensitivity:**
- **<3d:** 0.0% (0.00-26.47)
- **3-7d:** 40.0% (19.12-63.95)
- **8-13d:** 65.22% (42.73-83.62)
- **14d+:** 89.36% (76.90-96.45)

Sensitivity will vary based on how it is calculated.

Expected low sensitivity early in disease.

Overestimated sensitivity early in disease.

<table>
<thead>
<tr>
<th>Time From Symptom Onset</th>
<th>Time From PCR (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos/Total</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>9</td>
</tr>
<tr>
<td>Ratio</td>
<td>125</td>
</tr>
</tbody>
</table>

Specificity: 98.69% (95.63-99.84)

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>0.0% (0.00-25.47)</th>
<th>&lt;3d: 50.0% (34.19-65.81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-7d: 40.0% (19.12-63.95)</td>
<td>3-7d: 72.73% (49.78-89.27)</td>
<td></td>
</tr>
<tr>
<td>8-13d: 65.22% (42.73-83.62)</td>
<td>8-13d: 72.73% (49.78-89.27)</td>
<td></td>
</tr>
<tr>
<td>14d+: 89.36% (76.90-96.45)</td>
<td>14d+: 75.0% (47.62-92.73)</td>
<td></td>
</tr>
</tbody>
</table>

So what matters the most, sensitivity or specificity?

It depends on what you want to use it for!

Not All FDA-Authorized COVID-19 Antibody Tests Are Equally Reliable

Crucial findings published in AACC's Clinical Chemistry journal
Proposed Utilities for SARS-CoV-2 serology:

1) Diagnosis

2) Identifying Convalescent plasma donors

3) Population screening
Validation will depend on clinical use

1) Diagnosis
   • **Requires an assay that can detect Ig early after infection**

2) Identifying Convalescent plasma donors

3) Population screening
Diagnosis is attractive, because testing supplies are scarce
Low Sensitivity <7d from symptom onset

Assay 1

Assay 2

Assay 3

Sensitivity = <7d

18%

25%

9%

Tang MS, Clin Chem. 2020; hvaa120. doi: 10.1093/clinchem/hvaa120
Serology should not be used for acute diagnosis!

**FDA**: “Do not use serological (antibody) tests as the sole basis to diagnose COVID-19 but instead as information about whether a person may have been exposed.”

**IDSA**: “Antibody tests may be better suited for public health surveillance and vaccine development than for diagnosis.”

**WHO**: “Serologic tests cannot be used to diagnose acute infection with the COVID-19 virus.”

IDSA COVID-19 Antibody Testing Primer Updated: May 4, 2020
https://www.who.int/news-room/q-a-detail/q-a-serology-and-covid-19
Utility in symptomatic patients outside of PCR window

Serology helpful for dx?

At Wash U- 10 patients have been positive by serology but negative by PCR

IDSA COVID-19 Antibody Testing Primer Updated: May 4, 2020
© 2020 Cardinal Health. All Rights Reserved.
Validation will depend on clinical use

1) Diagnosis
   • Requires an assay that can detect Ig early after infection

2) Identifying Convalescent plasma donors
   • Positive result must be highly associative with protection or at least neutralizing titers

3) Population screening
Use of serology to identify convalescent plasma donors

Effect of Convalescent Plasma on Mortality among Hospitalized Patients with COVID-19: Initial Three-Month Experience

Use of serology to identify convalescent plasma donors

- Potentially Ideal donors
- Not clear?
- Not ideal plasma donors

Farnsworth Lab internal Data
Validation will depend on clinical use

1) Diagnosis
   • Requires an assay that can detect Ig early after infection

2) Identifying Convalescent plasma donors
   • Positive result must be associated with protection

3) Population screening
   • Must have high specificity with high confidence
Positive predictive value, it’s a matter of the stats!

<table>
<thead>
<tr>
<th>Antibody (−)</th>
<th>No Infection</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>True neg.</td>
<td>False neg.</td>
</tr>
<tr>
<td>Antibody (−)</td>
<td>760,000</td>
<td>2,000</td>
</tr>
<tr>
<td>Antibody (+)</td>
<td>False pos.</td>
<td>True pos.</td>
</tr>
<tr>
<td></td>
<td>40,000</td>
<td>198,000</td>
</tr>
</tbody>
</table>

**Specificity =** \[ \frac{TN}{TN + FP} = \frac{760,000}{800,000} = 95\% \]

**Sensitivity =** \[ \frac{TP}{TP + FN} = \frac{198,000}{200,000} = 99\% \]
Positive predictive value, it’s a matter of the stats!

<table>
<thead>
<tr>
<th></th>
<th>No Infection</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody (-)</td>
<td>True neg. 760,000</td>
<td></td>
</tr>
<tr>
<td>Antibody (+)</td>
<td>False pos. 40,000</td>
<td>True pos. 198,000</td>
</tr>
</tbody>
</table>

Positive predictive value = \[
\frac{\# \text{ true positive}}{\# \text{ true positive} + \# \text{ false positives}}
\]
Positive predictive value, it’s a matter of the stats!

<table>
<thead>
<tr>
<th></th>
<th>No Infection</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody (-)</td>
<td>True neg. 760,000</td>
<td>False neg. 2,000</td>
</tr>
<tr>
<td>Antibody (+)</td>
<td>False pos. 40,000</td>
<td>True pos. 198,000</td>
</tr>
</tbody>
</table>

\[
\text{Positive predictive value} = \frac{198,000}{198,000 + 40,000} = 83.2\%
\]
Positive predictive value is impacted by prevalence

Sensitivity 99%
Population 1,000,000

<table>
<thead>
<tr>
<th>Specificity</th>
<th>TP</th>
<th>FP</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.5%</td>
<td>198,000</td>
<td>4,000</td>
<td>98.0%</td>
</tr>
<tr>
<td>95%</td>
<td>198,000</td>
<td>40,000</td>
<td>83.2%</td>
</tr>
</tbody>
</table>
Positive predictive value is impacted by prevalence

Sensitivity 99%
Population 1,000,000

<table>
<thead>
<tr>
<th>Specificity</th>
<th>TP</th>
<th>FP</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.5%</td>
<td>198,000</td>
<td>4,000</td>
<td>98.0%</td>
</tr>
<tr>
<td>95%</td>
<td>198,000</td>
<td>40,000</td>
<td>83.2%</td>
</tr>
</tbody>
</table>

200,000 Cases

Disease  □
No disease □

<table>
<thead>
<tr>
<th>Specificity</th>
<th>TP</th>
<th>FP</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.5%</td>
<td>19,800</td>
<td>4,000</td>
<td>83.2%</td>
</tr>
<tr>
<td>95%</td>
<td>19,800</td>
<td>40,000</td>
<td>33.3%</td>
</tr>
</tbody>
</table>

20,000 Cases
Prevalence will vary by location!

Missouri Prevalence

0.2% prevalence

<table>
<thead>
<tr>
<th>Specificity</th>
<th>TP</th>
<th>FP</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.5%</td>
<td>1,980</td>
<td>4,980</td>
<td>39.8%</td>
</tr>
</tbody>
</table>

Assumes sensitivity 99%, population of 1,000,0000

https://coronavirus.jhu.edu/map.html
Confidence in your specificity matters!

COVID-19 Antibody Seroprevalence in Santa Clara County, California

Eran Bendavid¹, Bianca Mulaney², Neeraj Sood³, Soleil Shah², Emilia Ling², Rebecca Bromley-Dulfano², Cara Lai⁶, Zoe Weissberg⁷, Rodrigo Saavedra-Walker⁴, Jim Tedrow⁵, Dona Tversky⁸, Andrew Bogan⁷, Thomas Kupiec⁹, Daniel Eichner⁹, Ribhav Gupta¹⁰, John P.A. Ioannidis¹¹, Jay Bhattacharya¹

Version 2, April 27, 2020
(revised in response to comments received. This remains a preliminary report of the work.)

~2.4 % prevalence of antibodies in 3,000 screened patients,

~10 fold higher than the prevalence at the time in Santa Clara

Test Specificity of 99.5% (95 CI 98.3-99.9%)

How they got this specificity: 30 samples of their own + 369 from manufacturer

If specificity closer to 98%, prevalence would be <1%
Use test with high specificity or orthogonal approach!

“In most of the country…. the prevalence of SARS-CoV-2 antibody is expected to be low, ranging from <5% to 25%, so that testing at this point might result in relatively more false-positive results and fewer false-negative results”

<table>
<thead>
<tr>
<th>Prevalence of COVID-19 in the population</th>
<th>PPV for one test (SE=90%, SP=99.8%)</th>
<th>PPV for two orthogonal tests (SE=90%, SP=95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td>90.2%</td>
<td>26.9%</td>
</tr>
<tr>
<td>5%</td>
<td>95.9%</td>
<td>48.6%</td>
</tr>
<tr>
<td>10%</td>
<td>98.0%</td>
<td>66.7%</td>
</tr>
<tr>
<td>30%</td>
<td>99.5%</td>
<td>88.5%</td>
</tr>
</tbody>
</table>

https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html#table1
How are physicians using serology?

How many days has it been since the beginning of the patient’s symptoms?

- <3 days
- 3-7 days
- 8-13 days
- >14 days
- Never symptomatic

Order Validation

You can proceed and sign these orders, but the following information is missing or might require your attention:

This test has a high likelihood of false negative results based on time of testing:
- <3 days from symptoms: <10% sensitivity
- 3-7 days from symptoms: <33% sensitivity
- 8-13 days from symptoms: <45% sensitivity

False positive results can also occur secondary to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E. A positive result may not ensure immunity from reinfection.
### How physicians are using serology at our hospital?

#### Ordering Patterns over Thirty Days of Testing

<table>
<thead>
<tr>
<th>Time From Symptom Onset</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 days</td>
<td>18 (3%)</td>
</tr>
<tr>
<td>3-7 days</td>
<td>21 (4%)</td>
</tr>
<tr>
<td>8-13 days</td>
<td>8 (1%)</td>
</tr>
<tr>
<td>&gt;14 days</td>
<td>423 (76%)</td>
</tr>
<tr>
<td>Never symptomatic</td>
<td>87 (16%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>557</strong></td>
</tr>
</tbody>
</table>

#### Prevalence Studies

- Mostly outpatients seeing PCPs
  - ~70%
  - ~20%
  - ~10%

- Prevalence Studies
- In-patient
- Out-patient
I have antibodies to SARS-CoV-2, am I immune?

In the future, this may potentially be used to help determine, together with other clinical data, whether these individuals may be less susceptible to infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity.

Macaques protected from reinfection by SARS-CoV-2


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Durability of antibody response is undefined

But clearly decreases with time

https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30517-X/fulltext
Using SARS-CoV as a surrogate for SARS-CoV-2 antibodies?

Antibodies stick around for a while

**Fig. 1.** The percentage of patients who expressed specific IgG Abs/NAbs against SARS-CoV in recovered patients\(^2-4\).
Antibodies types and protection

• Antibodies can be binding or neutralizing
  o Binding (non-neutralizing) Abs
    – Produced at high levels,
    – unable to independently prevent infection
    – Bind and flag pathogen as ‘invader’
    – Good markers of prior infection
  o Neutralizing Abs (NAbs)
    – NAbs bind virus leading to loss of infectivity and blocking viral entry into host cells
    – Function independent of other immune system components
• Commercially available assays do not distinguish NAbs from non-NAbs
Assays for neutralizing antibodies are laborious

- Inoculation onto cells
- Plaque assay
- Focus assay
- TCID<sub>50</sub> assay

**B**

- antibody
- virus

Incubation (37°C, 1h)

SARS-CoV-2 Infected
Negative Control

% Relative Infection

\[
\log_{10}[\text{sera dilution}]
\]

[https://bio-protocol.org/e2855](https://bio-protocol.org/e2855)

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Neutralizing antibodies are present after SARS-CoV-2 infection

Tang MS et al. biorxiv. https://www.biorxiv.org/content/10.1101/2020.07.01.182220v1
Worse outcomes associated with higher neutralizing titer

Klein SL et al. JCI 2020, https://doi.org/10.1172/JCI142004.
People with less severe disease have reduced antibodies


Ab’s to SARS-CoV-2 does not equate to neutralizing Ab’s

Outbreak on USS Roosevelt
228 were serological positives
135 (59.7%) had neutralizing Abs

https://www.cdc.gov/mmwr/volumes/69/wr/mm6923e4.htm
Poor correlation between serological and neutralizing assays

Commercial Assay
(Sig/Cutoff)

Neutralizing Antibodies
(1 /Log_{10} Plasma Dil)

1. Positive cutoff

1.0 1.5 2.0 2.5 3.0 3.5
0 2 4 6 8

Dilution: 32 64 128 256

AUC = 0.90 (0.83-0.97)

100% - NPA%

PPA%

Tang MS et al. biorxiv. https://www.biorxiv.org/content/10.1101/2020.07.01.182220v1
Take home:

- Previous infection seems to provide some amount of protection

- Unclear how long protection lasts

- Unclear degree of protection from mild and asymptomatic infections
Conclusions

1) Validation of SARS-CoV-2 serological assays requires:
   • Precision, interferences, linearity, comparisons

2) Some (but not all) serological assays suffer from poor specificity

3) The clinical utility of serology is still relatively unknown, but numerous ongoing utilities for translational research and may be pivotal in the future

4) Proposed utilities include acute diagnosis, seroprevalence, and identifying convalescent plasma donors

5) Assay selection and validation will depend on the planned use
Questions?

Thank you to Dr. Neil Anderson and Dr. Mei San Tang for shared figures