Antibody Response in COVID-19 – Serology, Neutralizing Antibodies and Vaccine Breakthrough

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Disclosures

• I, Dr. Erin Kaleta, have no relevant financial relationships with ineligible companies to disclose.
Where are we now?

Total Cases
732,470
Up 1,524 since yesterday

Total Deaths
12,134
-13 since yesterday

Case Rates
208 per 100,000
High Transmission

Percent Positivity
10%
High Transmission

Maricopa County – Current as of 10/28/21 from week 10/17-10/23.

https://www.maricopa.gov/5460/Coronavirus-Disease-2019
Where are we now?

- Maricopa County Epidemic Curve

https://www.maricopa.gov/5460/Coronavirus-Disease-2019

Maricopa County Hospitalization
Where are we now?

• Vaccine Statistics:
  – Total doses administered: 4,775,993
  – Full series completed: 2,183,743

Percent of Maricopa Residents in Each Age Group*

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>62.0%</td>
</tr>
<tr>
<td>10–14</td>
<td>23.3%</td>
</tr>
<tr>
<td>15–24</td>
<td>50.8%</td>
</tr>
<tr>
<td>25–34</td>
<td>51.5%</td>
</tr>
<tr>
<td>35–44</td>
<td>59.9%</td>
</tr>
<tr>
<td>45–54</td>
<td>65.4%</td>
</tr>
<tr>
<td>55–64</td>
<td>73.7%</td>
</tr>
<tr>
<td>65–74</td>
<td>87.0%</td>
</tr>
<tr>
<td>&gt;=75</td>
<td>91.2%</td>
</tr>
</tbody>
</table>

https://www.maricopa.gov/5460/Coronavirus-Disease-2019
Types of Testing

• Molecular Tests
  – Rapid or 1-3 days
  – Best in acute period of infection

• Antigen Tests
  – Rapid, often lateral flow assays, 15-30 min
  – Sacrifices accuracy

• Serology Tests
  – Takes 1-3 weeks for antibody response
  – Best for identifying previous infection
Regulatory requirements for EUA testing

Regulatory issues exacerbated for Serology

EUA Initiated:
Allows unapproved medical products or unapproved uses of approved medical products to be used in an emergency to diagnose, treat, or prevent serious or life-threatening disease

Wild West:
Allows manufacturers to sell and distribute assays without receiving EUA, “as set forth in Section IV.D”

FDA Tightens Up:
To stay on the market, must submit EUA to demonstrate effectiveness

Hurdles to Sars-CoV-2 Testing
• Supplementing PCR for late-presenting cases
  – Particularly 3-4wks post-symptom onset
  – Identifying prior exposure

• Identifying convalescent plasma donors and potential for assessing vaccine response

• Tracking SARS CoV 2 exposure in the community
Sars-CoV-2 Antigens of Interest

Spike Protein: S1 + S2: Trimeric glycoprotein Essential for recognizing host ACE2 receptors (S1/RDB) and facilitate entry into host cells (S2)

N Protein: Nucleocapsid: Binds to viral RNA; non-glycosylated and more highly conserved than Spike Protein

Sars-CoV-2 Antibody Kinetics

Antibodies, but do they Neutralize?

ACE2/RBD Surrogate Neutralizing Ab Tests

• Types of Neutralizing Antibody tests
  – Virus Neutralizing Tests/PRNT
    • Live cells, BSL-3
  – Pseudotype-based Virus Neutralizing Tests
    • Live cells, BSL-2
  – Surrogate Virus Neutralizing Tests
    • Mimics the virus/host interaction
    • Uses purified ACE2 and S1-RBD
    • No live virus
    • ELISA format for high-throughput applications
Blocking of Binding Assays

RBD

ACE-2

Signal

[RBD]
Blocking of Binding Assays

- RBD
- ACE-2

Signal

Inhibition

[RBD]
Surrogate Neutralizing Antibody Assay

Surrogate Neutralizing Antibody Assay

Surrogate Neutralizing Antibody Assay

What Titer is Needed?

Modelling data from vaccine trials can predict a titer necessary for protective immunity.

Normalizes vaccine trials against convalescent plasma to account for non-standardized assays.

Estimates that a titer of 20% convalescent plasma is required for protection (1:160 PRNT).

How Long Does Immunity Last?

• Depends on many factors
  – Neutralizing antibodies = “Sterilizing” immunity
  – Cell Mediated immunity
    • T-cells
    • B-cells
  – Dose of exposure
    • Initial dose
    • Duration of illness
    • Severity of illness
Immunity with mRNA Vaccines

1. Intramuscular immunization
2. mRNA/antigen uptake by APCs
3. Trafficking to lymph nodes
4. Priming of T lymphocytes
5a. CD4 activation
5b. CD8 activation
6. GC reaction

How Long Does Immunity Last?

Memory B-cells
- Increased over 120 days and remained high at ≥6m post infection
- Produced and maintained in all subjects with no apparent halflife

CD8$^+$ T Cells
- 50% recovered patients positive at ≥6m post infection
- Decline estimated $t_{1/2} = 125 – 190$ days or greater

CD4$^+$ T Cells
- 92% positive at ≥6m post infection
- Declined $t_{1/2} = 94$ days

• Immunity memory has interpatient variability
• 95% of individuals were positive for at least 3/5 markers 5-8m Post Symptom Onset

How Long Does Immunity Last?

- Many factors contribute:
  - As circulating sterilizing immunity wanes, memory-based immunity predominates
  - Memory can take 3-5 days to ramp up, during which virus can replicate
  - Transmission events can become more likely
How Long vs. Titers Needed

Various vaccines have a different starting neutralization level.

Studies show decay is predicted to be linear up to 8 months.

Durable plateau titer will vary dependent on initial titer.

Natural Infection vs. Vaccine Immunity

• mRNA Vaccines
  – Pfizer BNT162b2
  – Moderna mRNA-1273
### Natural Infection vs. Vaccine Immunity

<table>
<thead>
<tr>
<th>Antibody Results</th>
<th>Nucleocapsid Ab</th>
<th>Spike Ab</th>
<th>Neutralizing Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Infection</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vaccine-Induced</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Natural Infection vs. Vaccine Immunity

• Are serology assays useful for measuring vaccine response??
  – Most individuals seroconvert: 91-100%
  – Limited correlation to protective immunity

• What about immunocompromised patients?
  – Unknown value of positive result in U/mL
  – Negative results could be informative
Who is immunocompromised?

- HIV+ participants show equivalent antibody production
- Kidney Tx patients: 25%
- Solid organ Tx patients: 54%
- Autoimmune Disease: 80%
- Hematologic cancers: 55% or 66%

Doesn’t tell the whole story – Cell mediated immunity is higher.
Approved for Use in Immunocompromised Patients

Percent of subjects with antibody response after two mRNA vaccine doses by immunocompromising condition and study (n=63)

Healthy Controls: 95%-100%

Darker blue color is hematologic cancers

- Studies that compared response after 1st and 2nd dose demonstrated poor response to dose 1
- Antibody measurement and threshold levels vary by study protocol

Adapted from ACIP Data and Clinical Considerations for Additional Doses in Immunocompromised People
Sara Oliver ACIP July 22, 2021

Antibody Levels in Solid Organ Transplant Patients

Figure. Antibody Levels of Study Participants After 2-Dose Series of SARS-CoV-2 mRNA Vaccine

Vaccine Breakthrough

• Defined as:
  – Detection of SARS-CoV-2 RNA or antigen
  – ≥ 14 days after vaccine series completed
Through 5/1/2021:
- 10,262 vaccine breakthrough cases
- 27% asymptomatic infections
- 10% hospitalized
  - 29% of these were hospitalized for unrelated reasons
- 2% death
  - 18% of these died from unrelated causes

After 5/1/2021:
- CDC only monitoring hospitalized breakthrough

https://www.cdc.gov/mmwr/volumes/70/wr/pdfs/mm7021e3-H.pdf
Vaccine Breakthrough Cases

• Since 5/1/2021

<table>
<thead>
<tr>
<th>Hospitalized or Fatal Vaccine Breakthrough cases reported to CDC</th>
<th>8,054</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>3,856</td>
</tr>
<tr>
<td>(48%)</td>
<td></td>
</tr>
<tr>
<td>≥ 65 years</td>
<td>5,928</td>
</tr>
<tr>
<td>(74%)</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>1,400</td>
</tr>
<tr>
<td>(17%)</td>
<td></td>
</tr>
<tr>
<td>Hospitalizations*</td>
<td>7,608</td>
</tr>
<tr>
<td>(94%)</td>
<td></td>
</tr>
<tr>
<td>Deaths**</td>
<td>1,587</td>
</tr>
<tr>
<td>(20%)</td>
<td></td>
</tr>
</tbody>
</table>

* 1,883 (25%) of hospitalized cases were not related to COVID-19
** 341 (21%) of fatal cases were not related to COVID-19

https://www.cdc.gov/vaccines/covid-19/health-departments/breakthrough-cases.html
Vaccine Breakthrough Cases

• If breakthrough is happening, why bother vaccinating?
Vaccine Breakthrough Cases

- HEROES RECOVER study
  - 3975 Frontline Workers
    - Phoenix/Tucson AZ, Miami FL, Duluth MN, Portland OR, Temple TX, Salt Lake City UT
    - 67.5% fully vaccinated, 80% at least 1 dose
  - Monitored by weekly molecular testing
    - 5% COVID+ (204 participants)
    - 5 fully vaccinated, 11 partially vaccinated
    - 156 unvaccinated

## Vaccine Breakthrough Cases

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unvaccinated</th>
<th>Partially or Fully Vaccinated</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral RNA load</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. assessed</td>
<td>155</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>Mean — log₁₀ copies/ml†</td>
<td>3.8±1.7</td>
<td>2.3±1.7</td>
<td>40.2 (16.3–57.3)‡</td>
</tr>
<tr>
<td>Duration of viral RNA detection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. assessed</td>
<td>155</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>Mean — days</td>
<td>8.9±10.2</td>
<td>2.7±3.0</td>
<td>6.2 (4.0–8.4)</td>
</tr>
<tr>
<td>Detection of viral RNA for &gt;1 week — no./total no. (%)</td>
<td>113/156 (72.4)</td>
<td>4/16 (25.0)</td>
<td>0.34 (0.15–0.81)§</td>
</tr>
<tr>
<td>Febrile symptoms — no./total no. (%)¶</td>
<td>94/149 (63.1)</td>
<td>4/16 (25.0)</td>
<td>0.42 (0.18–0.98)‖</td>
</tr>
<tr>
<td>Total days of symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. assessed</td>
<td>148</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>Mean — days</td>
<td>16.7±15.7</td>
<td>10.3±10.3</td>
<td>6.4 (0.4–12.3)</td>
</tr>
<tr>
<td>Days spent sick in bed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. assessed</td>
<td>147</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td>Mean — days</td>
<td>3.8±5.9</td>
<td>1.5±2.1</td>
<td>2.3 (0.8–3.7)</td>
</tr>
</tbody>
</table>
POCT Rapid Antigen Tests

- Immunoassay for viral protein
- Less sensitive
- May detect active virus vs. PCR
- Best in symptomatic individuals
- Confirmatory testing with molecular method recommended
Point of Care Testing Options

• Many options ranging from Direct-to-Consumer mail in kits to in home rapid PCR
• Sars-CoV-2 diagnostics have come a long way in a short period of time
• Serology is best utilized for identifying previous exposures
  – Not ready for mainstream immunity testing
• Breakthrough infections are happening and were expected
• Our greatest tool is VACCINATION!
Thank you!