COVID-19 Serology

IMPLEMENTATION, CLINICAL UTILITY, AND OUTSTANDING QUESTIONS

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Disclosures – Research Funding

Abbott Diagnostics
NowDiagnostics
Beckman Coulter

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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

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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
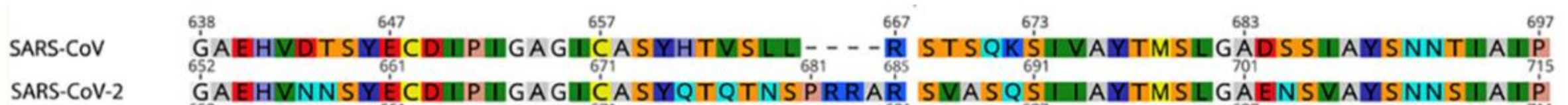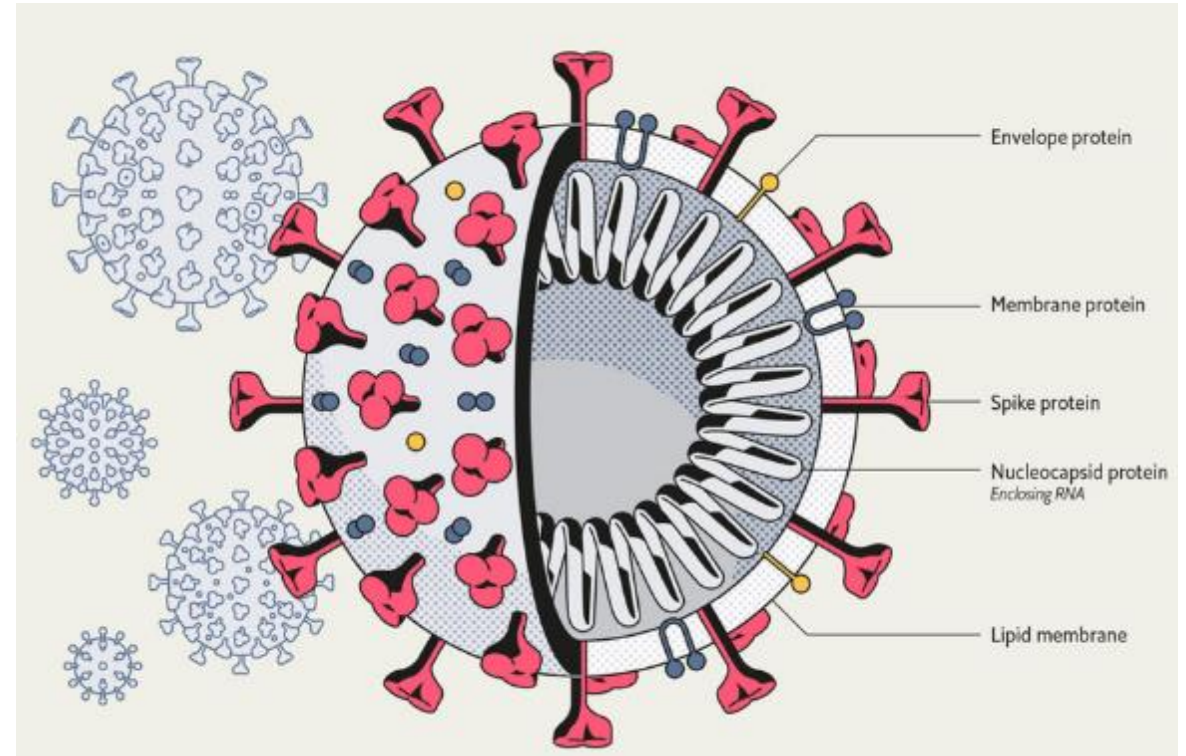
COVID-19 cases worldwide

US Prevalence
~1.64%

as of

8/17/20

https://coronavirus.jhu.edu/map.html

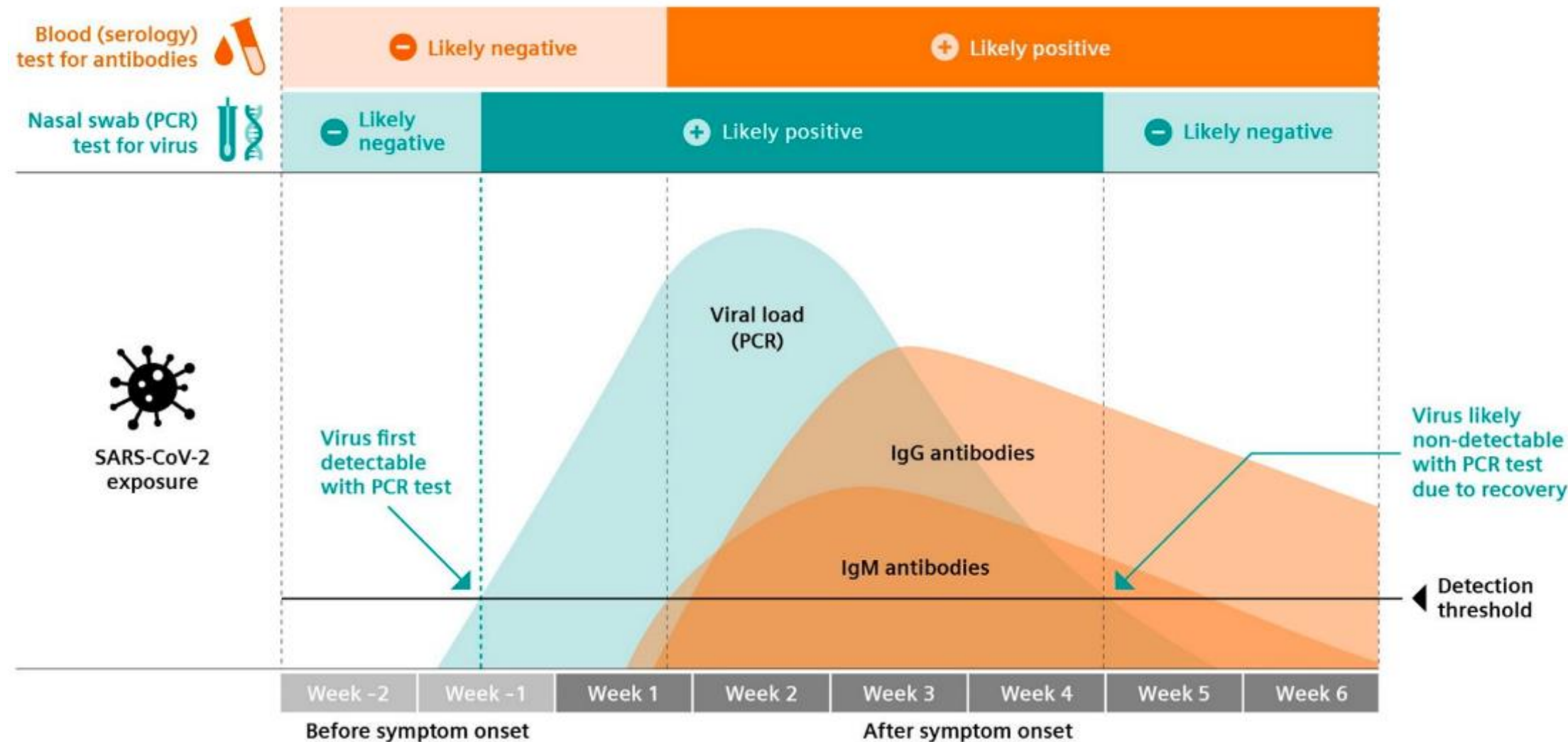CardinalHealth

Current testing for SARS-CoV-2

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

- Antigen Testing
  - Currently 2 available
  - Detect nucleocapsid protein from nasal or NP swab
  - Sensitivity ~ 80% relative to PCR

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Current testing for SARS-CoV-2

- Serology
  - Tests for the presence of antibodies to SARS-CoV-2
  - Originally > 200 tests available in the US
  - Require Emergency Use Authorization
  - Currently 35 tests with EUA
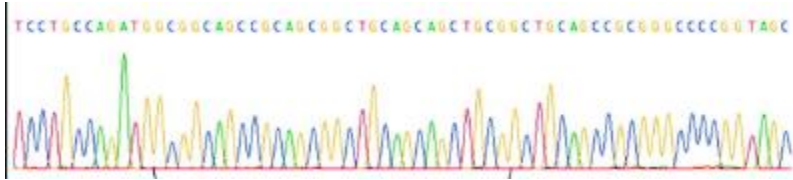 - Performance- highly variable

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COVID-19 Serology and PCR proposed kinetics

https://www.siemens-healthineers.com/en-us/laboratory-diagnostics/assays-by-diseases-conditions/infectious-disease-assays/cov2g-assay
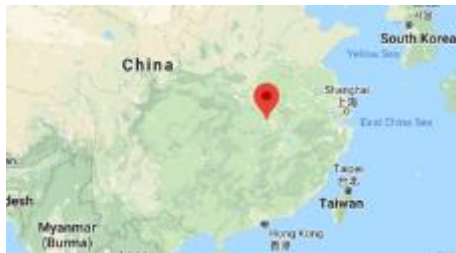
COVID-19 Serology Timeline

January 12th- first
sequence released

~March- First known lateral
flow test is marketed in the US

May 4th
EUA Required in the US

December 31st- Wuhan
China reports first cases

January 21- February 23rd-
First known cases spread
to the US

Late April- High
throughput assays
released

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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

https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization

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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

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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.

THE HILL

Just say 'no' to antibody testing

BY DR. REBEKAH DIAMOND, OPINION CONTRIBUTOR — 06/25/20 12:30 PM EDT
THE VIEWS EXPRESSED BY CONTRIBUTORS ARE THEIR OWN AND NOT THE VIEW OF THE HILL

153 COMMENTS

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Early studies demonstrated numerous false positives

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

Jeffrey D. Whitman et al. Test performance evaluation of SARS-CoV-2 serological assaysBiorxiv. 2020.

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Manufacturer validations pre-EUA

Group	Anti-SARS-CoV-2 ELISA IgG			Sensitivity
	positive	borderline	negative	
< 10 days after onset of symptoms	1	1	2	33.3%
> 10 days after onset of symptoms	4	0	1	80.0%

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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.

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So what does a positive result mean?
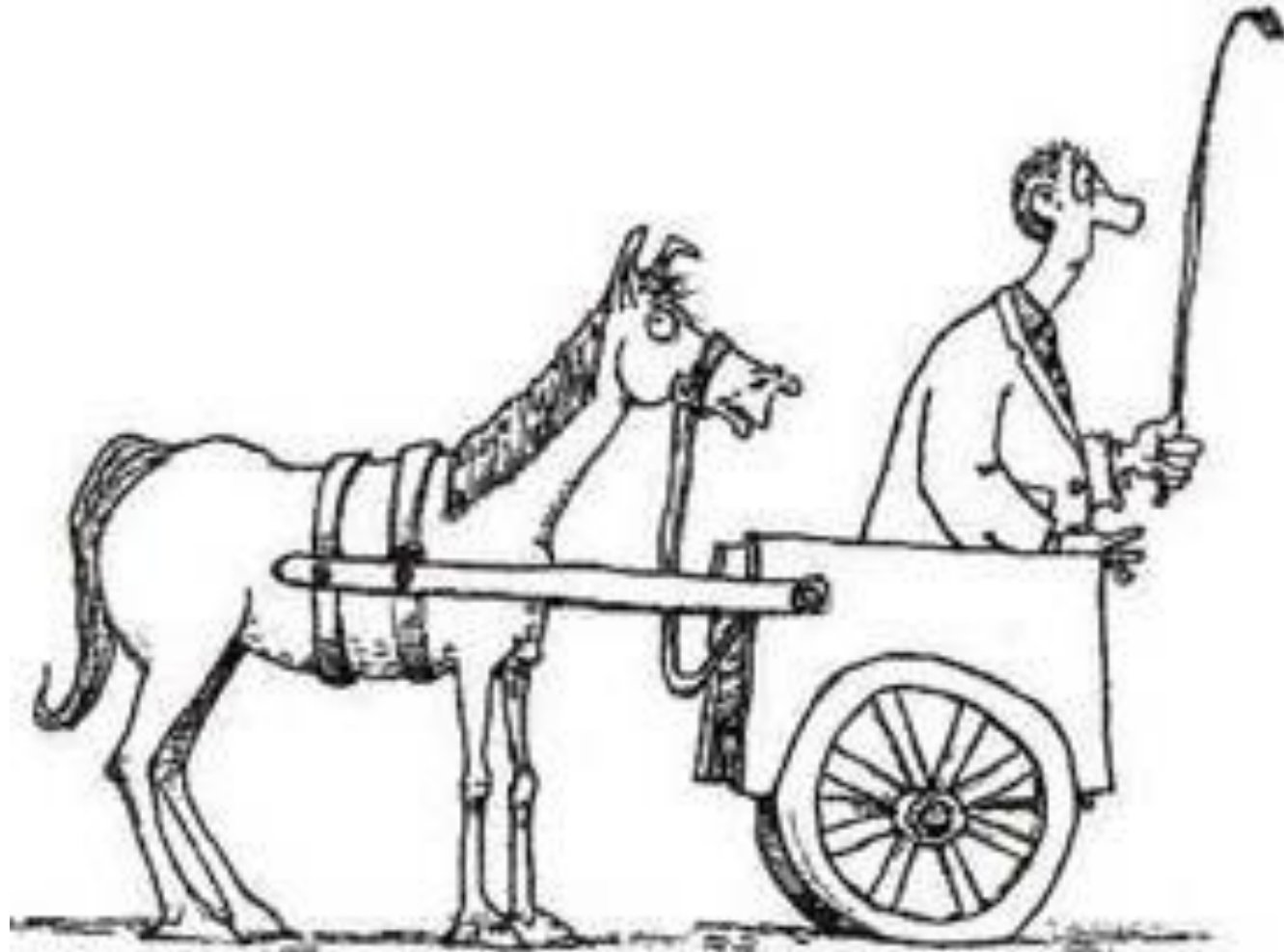
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Check for updates

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]

Opinion
(F) (podcast icon)

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

Christopher W. Farnsworth* and Neil W. Anderson

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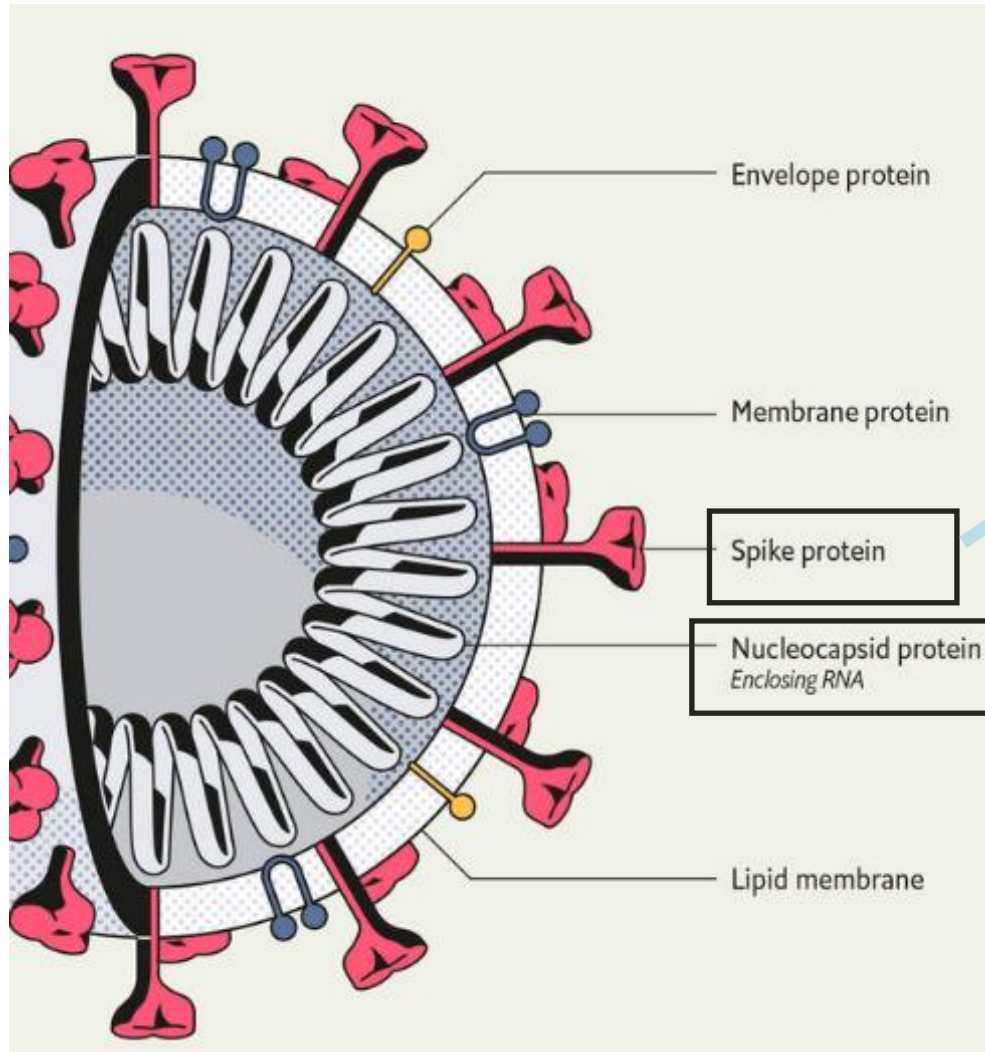
Classes of antibodies detected by anti-SARS-COV-2 assays

IgG
IgA
IgM

Total
Ab
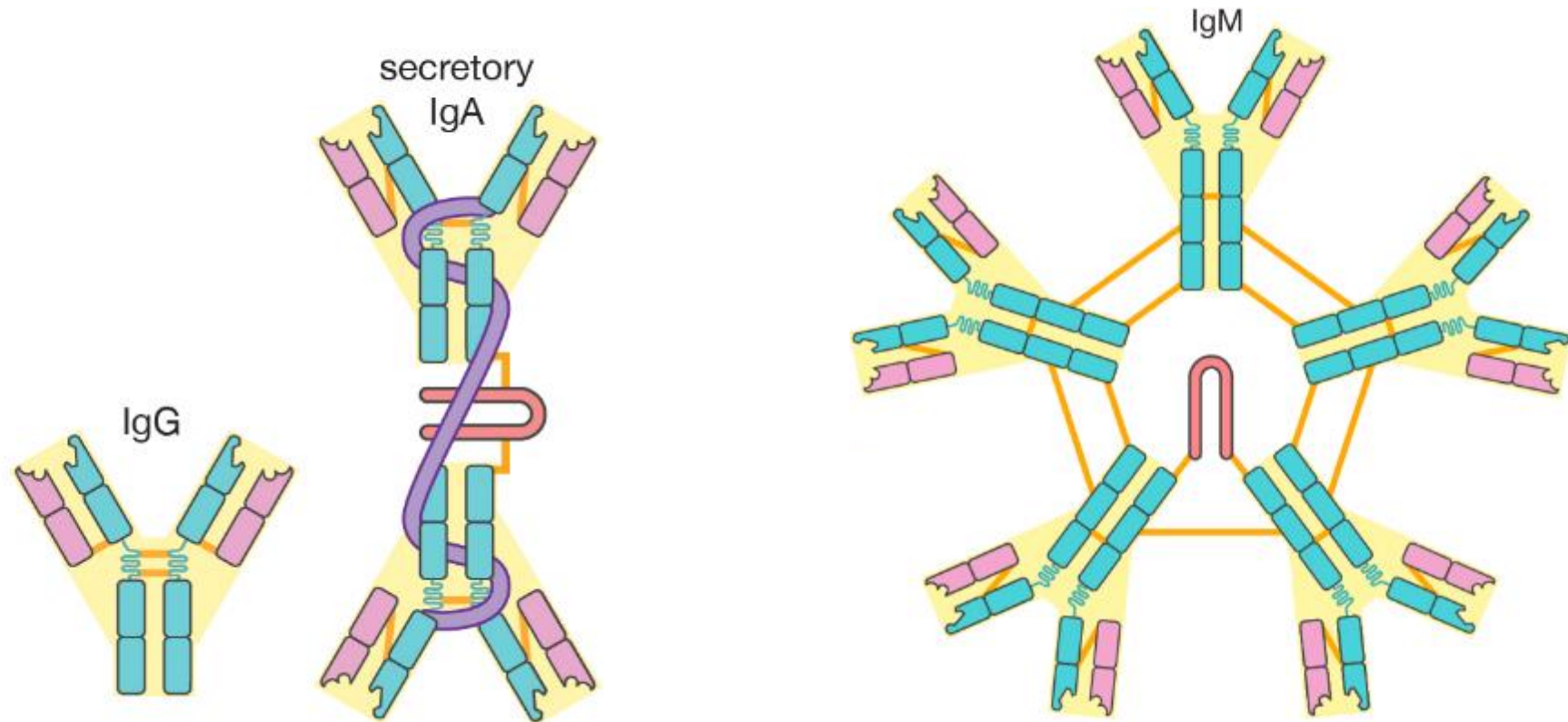
Jorden MA et al. MMWR 2020;69:680-84.

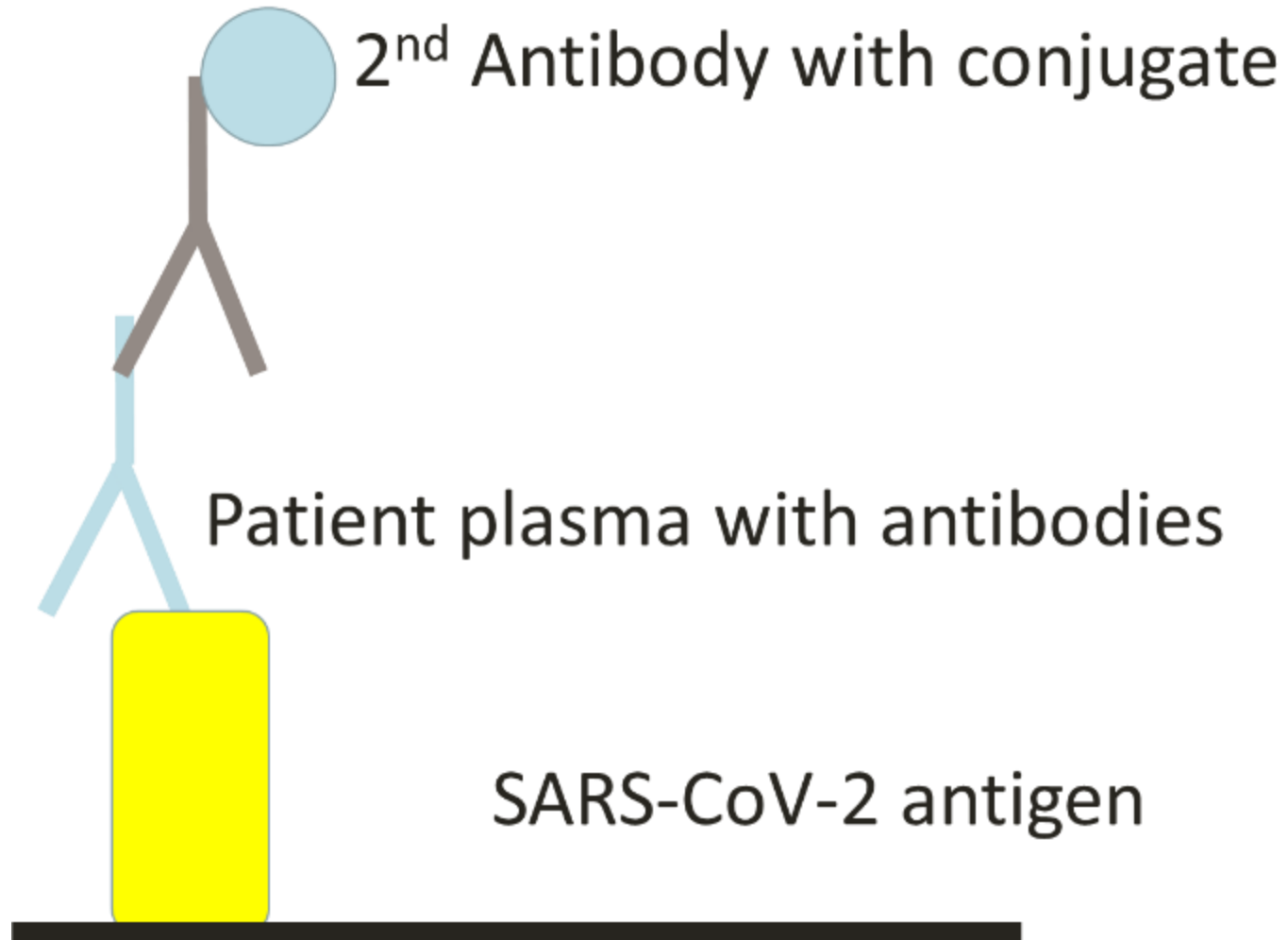**Cardinal**Health

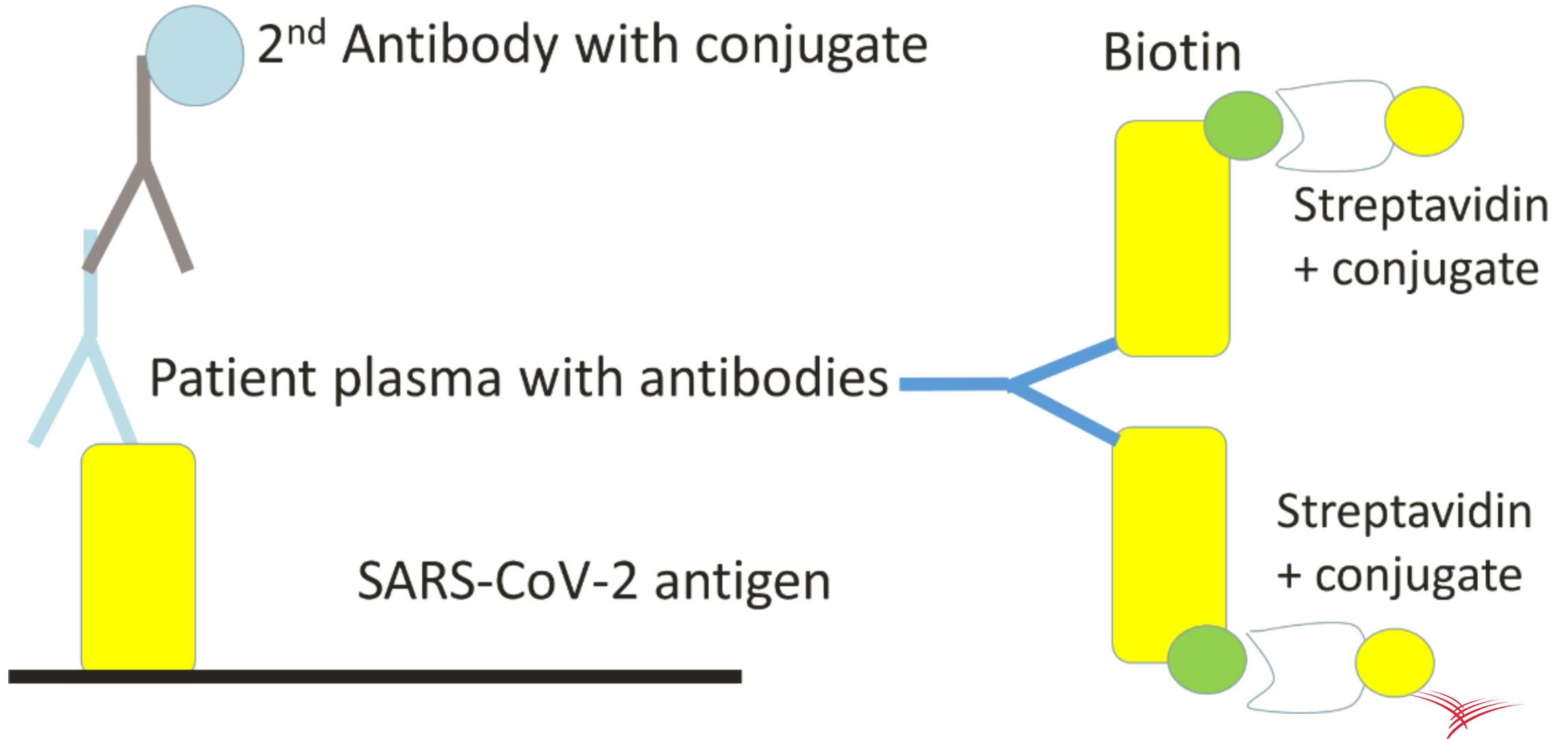# Variations in design of serological SARS-CoV-2 assays

Envelope protein

Membrane protein

Spike protein

Nucleocapsid protein
Enclosing RNA

Lipid membrane

Spike Protein
- S1
- S2
- Receptor Binding Domain

Nucleocapsid

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How do SARS-CoV-2 serological assays generally work

2nd Antibody with conjugate

Patient plasma with antibodies

SARS-CoV-2 antigen

How do SARS-CoV-2serological assays generally work

2nd Antibody with conjugate

Patient plasma with antibodies

SARS-CoV-2 antigen

Biotin

Streptavidin + conjugate

Streptavidin + conjugate

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Types of assays:

ELISA

CLIA

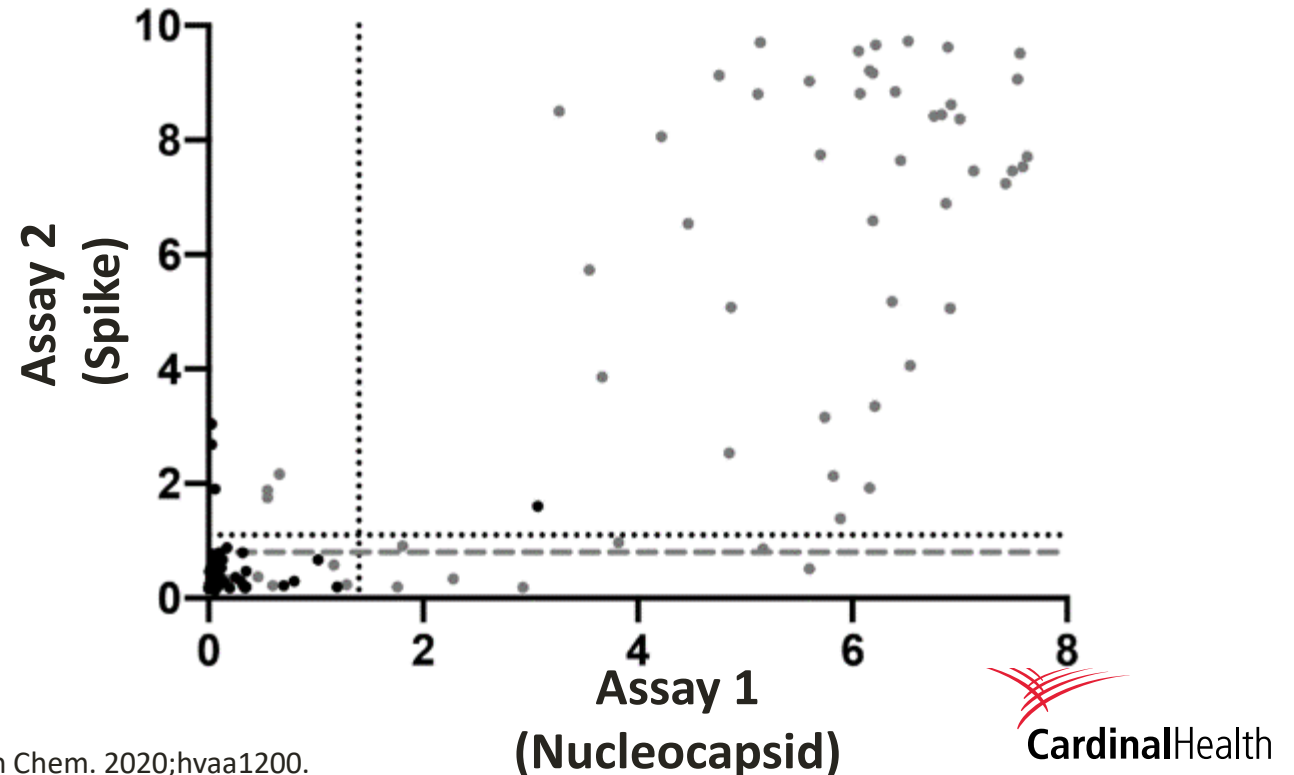## Lateral Flow

CardinalHealth
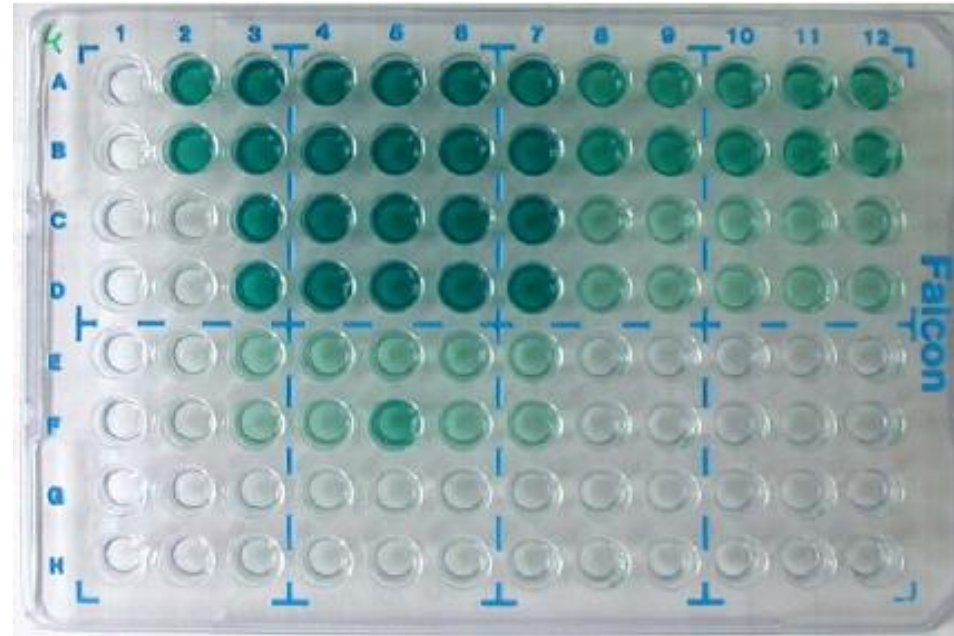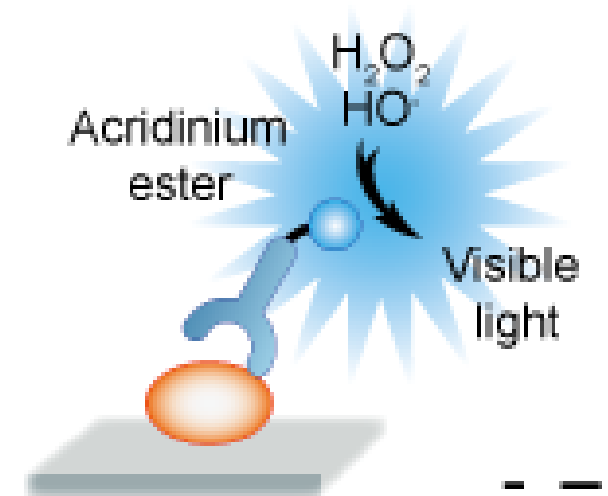
Validating new SARS-CoV-2 serological assays

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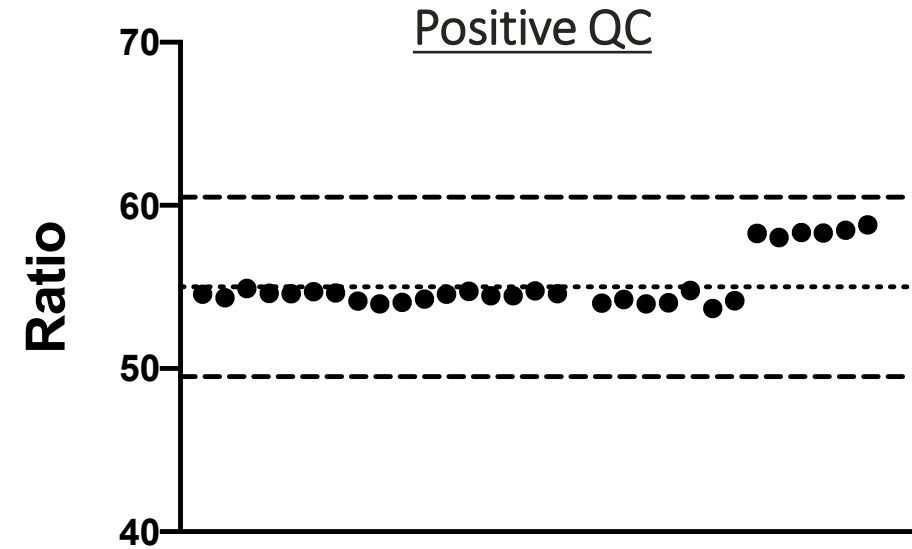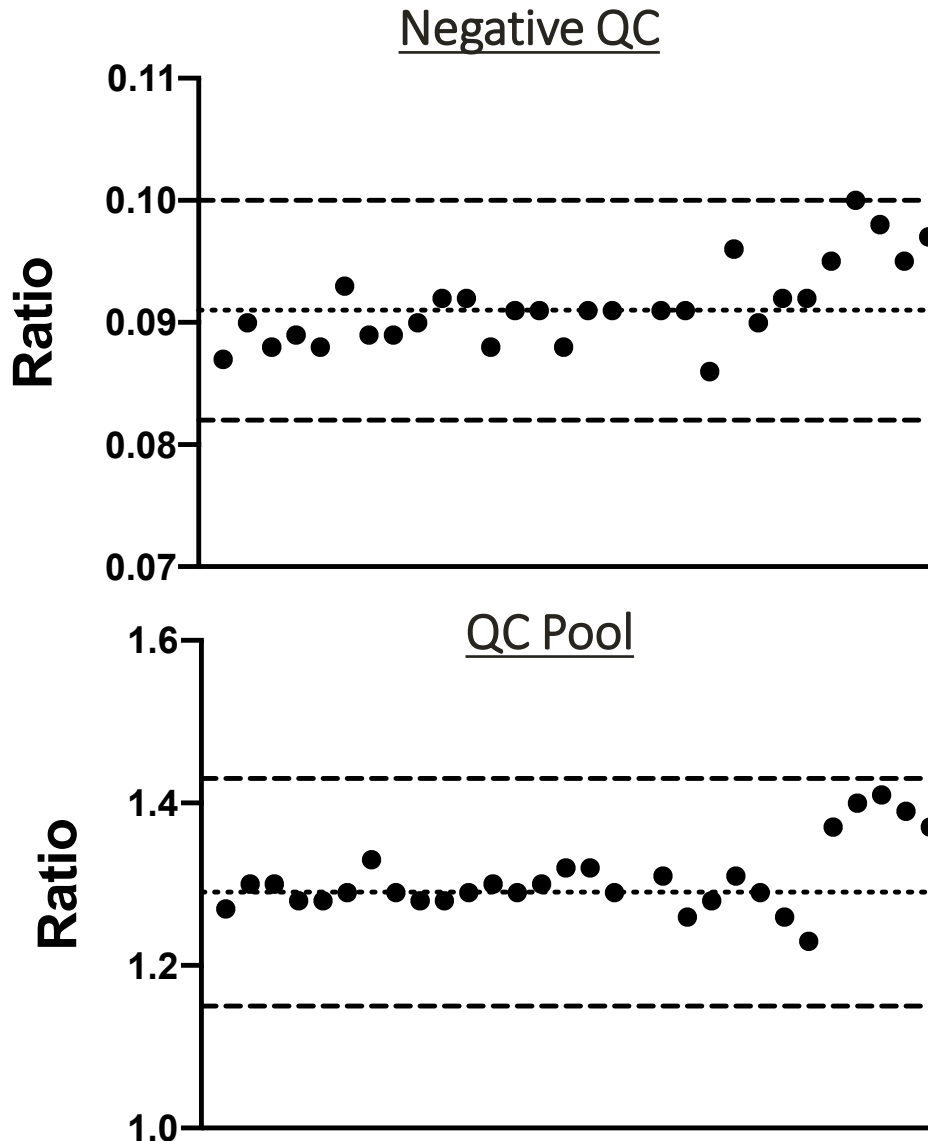2. Precision
3. Interferences
4. Accuracy

CLINICAL AND
LABORATORY
STANDARDS
INSTITUTE®

January 2008

EP12-A2

User Protocol for Evaluation of Qualitative
Test Performance; Approved
Guideline—Second Edition

COLLEGE of AMERICAN
PATHOLOGISTS

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1) Analytic measuring range

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

Tang MS *et al.* Clin Chem. 2020;hvaa1200.

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2) Precision

Negative QC

Positive QC

QC Pool
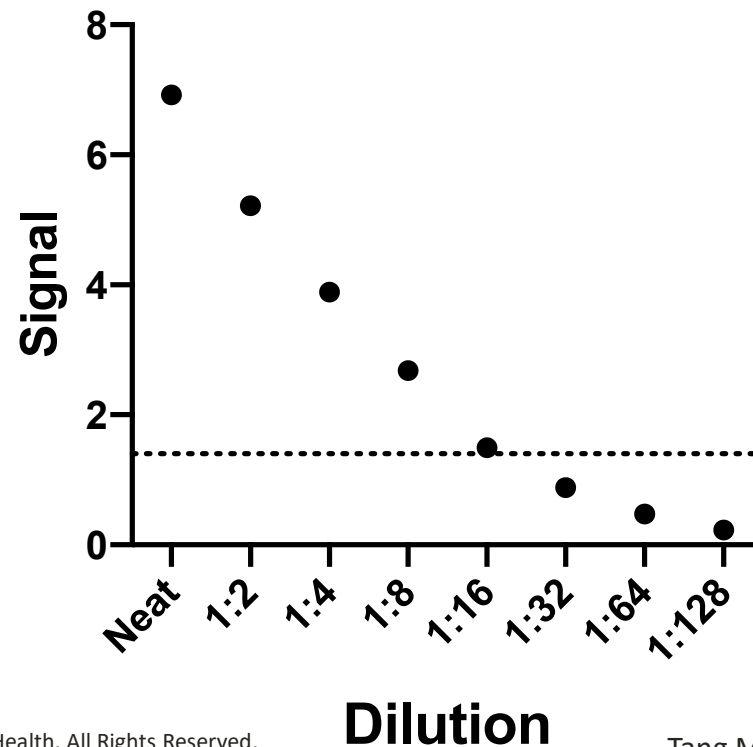
- Test intra-assay and inter-assay

- Sources of imprecision
    - Timing, temperature, reagent etc

- Ideally test at or near the cutoff

   Tang MS *et al.* Clin Chem. 2020;hvaa1200.

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3) Interferences

Hemolysis Index	Result	% of Original
7	1.69	100.0%
47	1.70	100.6%
81	1.70	100.9%
150	1.69	100.3%
284	1.70	100.6%
563	1.68	99.4%
1089	1.70	100.6%

- Effects of other compounds that impact measurement of analyte

- Ie. hemoglobin, triglycerides, bilirubin

- Perform near the cutoff for positive

- Labs may use data from manufacturers

Tang MS *et al.* Clin Chem. 2020;hvaa1200.

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4) Accuracy

- Extent to which a method compares to a reference method
- Ideally RT-PCR confirmed SARS-CoV-2 infection
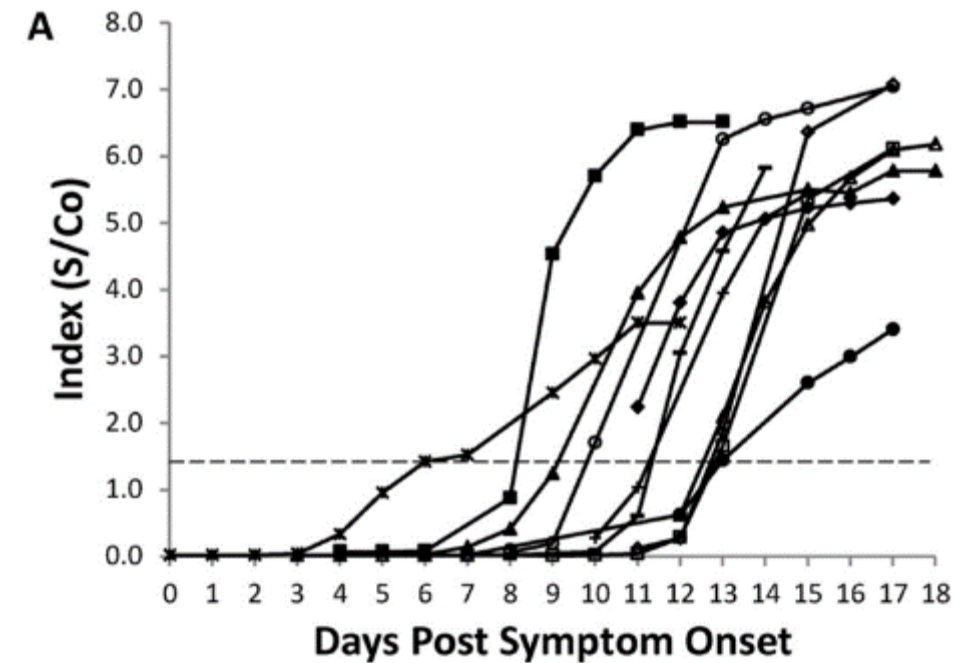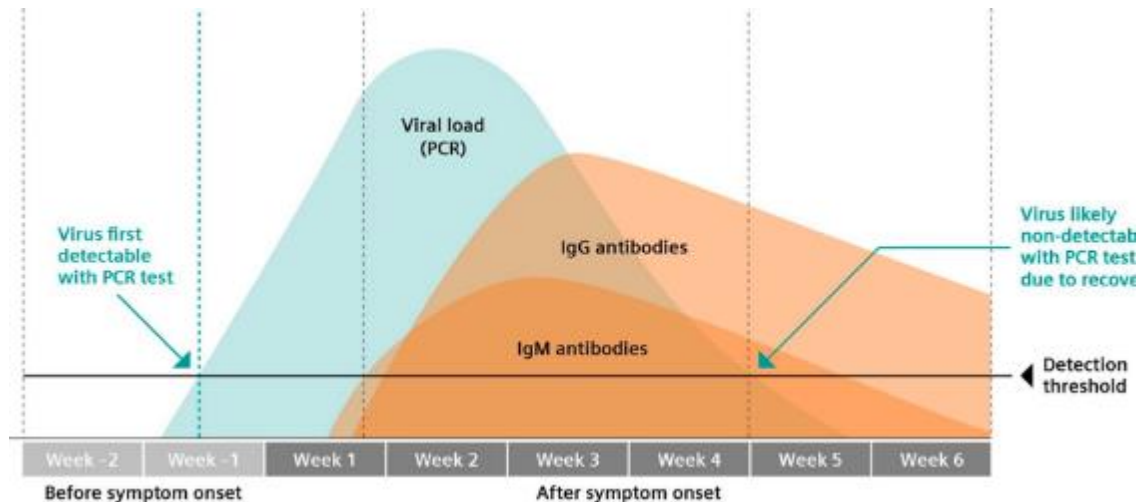
Theel ES *et al.* JCM. 2020;58:e01243-20.

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Basic lab statistics: sensitivity and specificity

	No Infection	**Infection**
Antibody (-)	True neg.	False neg.
Antibody (+)	False pos.	True pos.

$$\text{sensitivity} = \frac{\text{\# true positives}}{\text{\# true positives} + \text{\# false negatives}}$$

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Basic lab statistics: sensitivity and specificity

	No Infection	Infection
Antibody (-)	True neg. **98**	False neg. **10**
Antibody (+)	False pos. **2**	True pos. **90**

$$\text{sensitivity} = \frac{90}{90+10} = 90\%$$

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Basic lab statistics: sensitivity and specificity

	No Infection	**Infection**
Antibody (-)	True neg.	False neg.
Antibody (+)	False pos.	True pos.

$$\text{Specificity} = \frac{\text{\# true negatives}}{\text{\# true negatives} + \text{\# false positives}}$$

CardinalHealth

Basic lab statistics: sensitivity and specificity

	No Infection	**Infection**
Antibody (-)	True neg. **98**	False neg. **10**
Antibody (+)	False pos. **2**	True pos. **90**

$$\text{Specificity} = \frac{98}{98+2} = 98\%$$

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Sample of sensitivity and specificity analysis

Pos	0	0	0	2
Total	50	9	14	80

Ratio plot with categories: Pre-2019, Other Resp., Other Int., Symp. PCR-
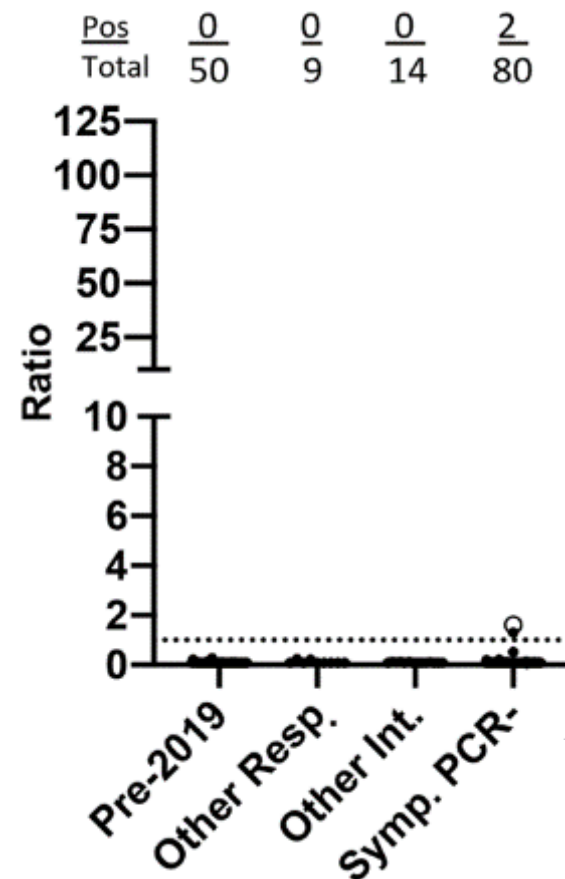
Specificity: 98.69% (95.63-99.84)

Specificity (neg/total)

	Healthy donors	Cross-reactivity panel
Assay 1	100% (149/149)	99% (104/105)
Assay 2	100% (149/149)	99% (104/105)
Assay 3	99.3% (148/149)	96.2% (101/105)

Theel ES *et al.* JCM. 2020;58:e01243-20.
Tang MS. Clin Chem. 2020;hvaa1200.

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Sample of sensitivity and specificity analysis
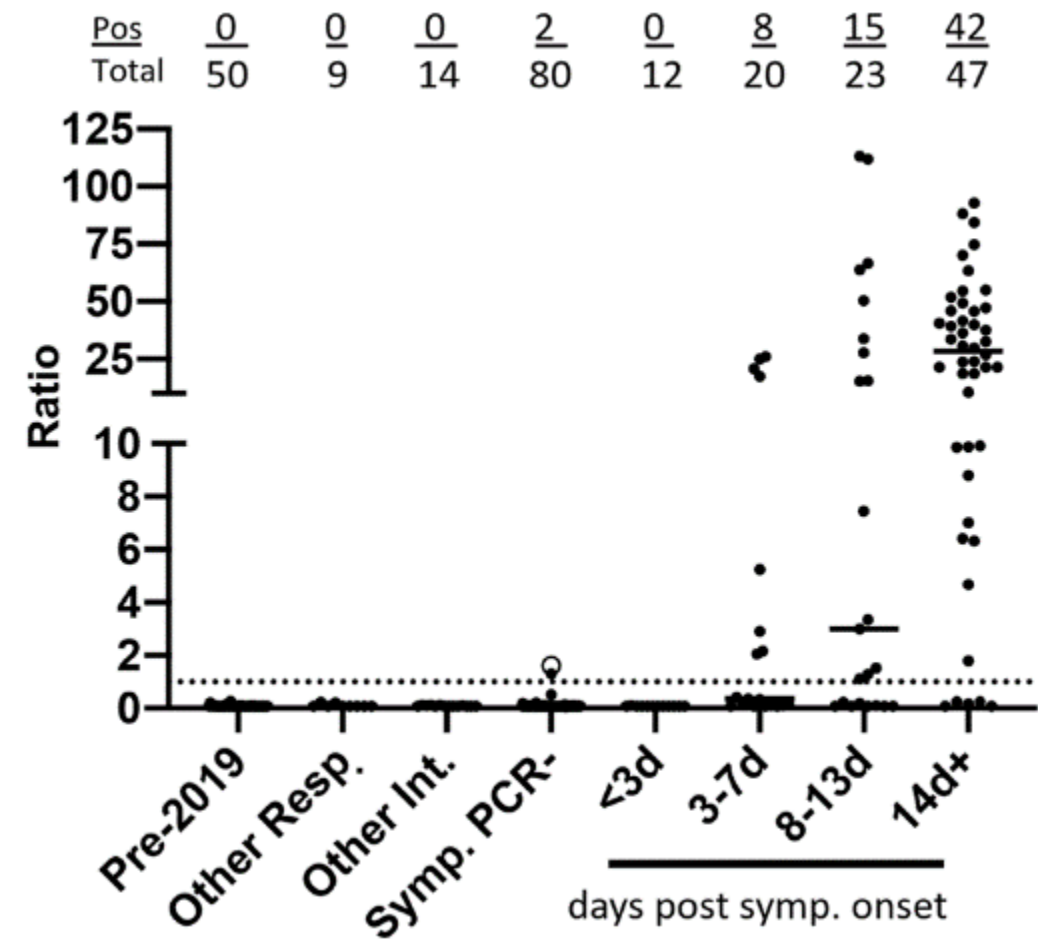
Specificity: 98.69% (95.63-99.84)
Sensitivity: <3d: 0.0%   (0.00-26.47)
          3-7d: 40.0% (19.12-63.95)
          8-13d: 65.22% (42.73-83.62)
          14d+: 89.36% (76.90-96.45)

Tang MS *et al.* Clin Chem. 2020;hvaa1200.

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Sensitivity will vary based on how it is calculated

Expected low sensitivity early in disease
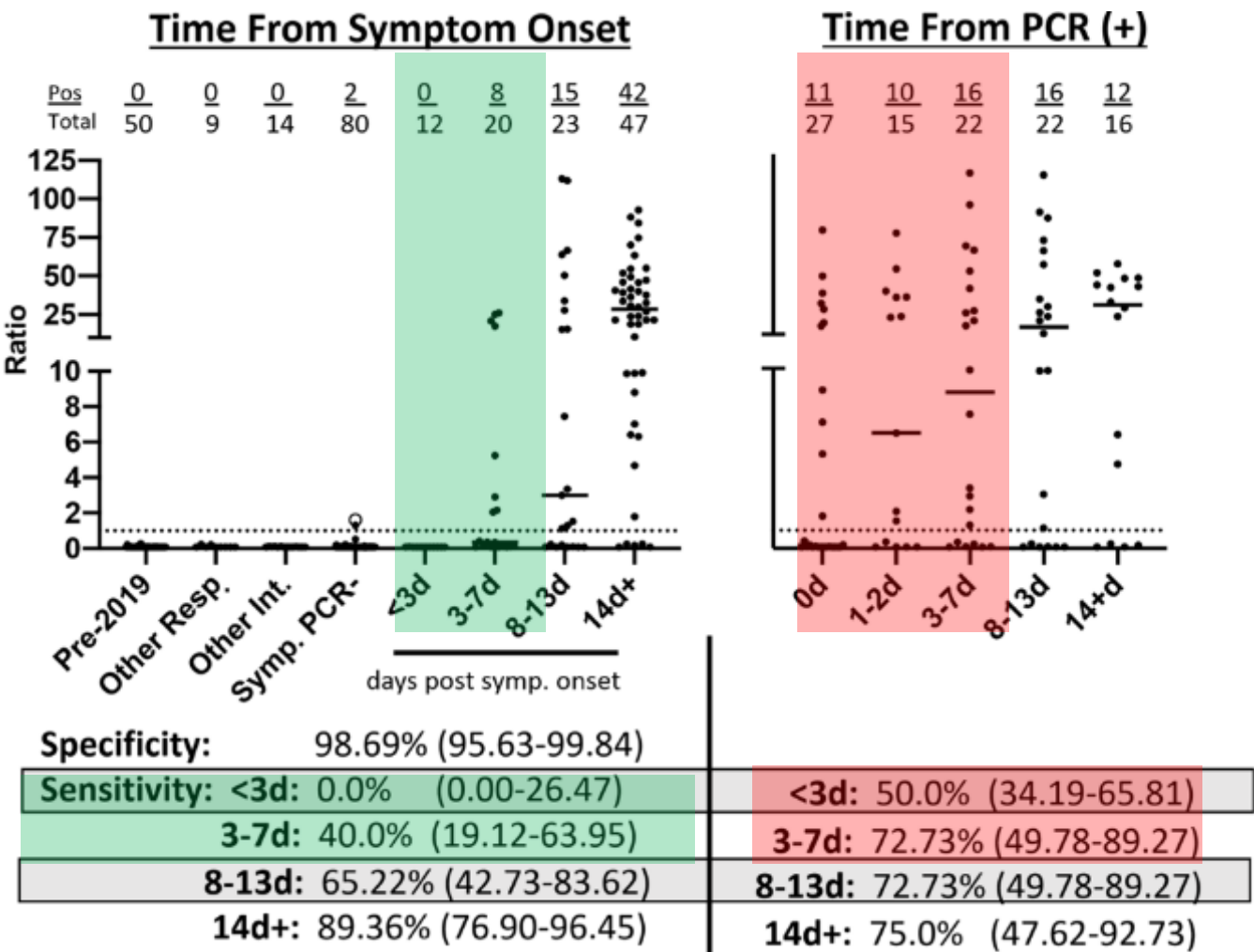
Overestimated sensitivity early in disease

 Tang MS, *et al.* Clin Chem. 2020;hvaa120. doi:10.1093/clinchem/hvaa120

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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

AACC | Better health through laboratory medicine.

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Proposed Utilities for SARS-CoV-2 serology:

1) Diagnosis

2) Identifying Convalescent plasma donors

3) Population screening

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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

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Diagnosis is attractive, because testing supplies are scarce

Percentage of Respondent Laboratories
Unable to Obtain COVID-19 Testing Supplies

Low Sensitivity <7d from symptom onset
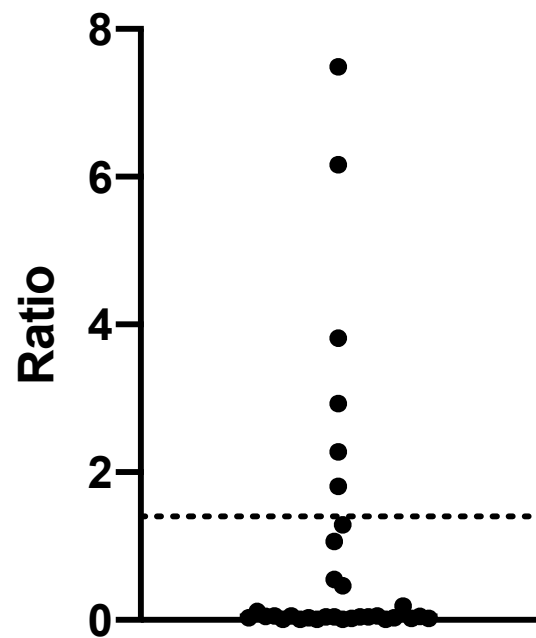
Assay 1 — 18%

Assay 2 — 25%

Assay 3 — 9%

Sensitivity = <7d
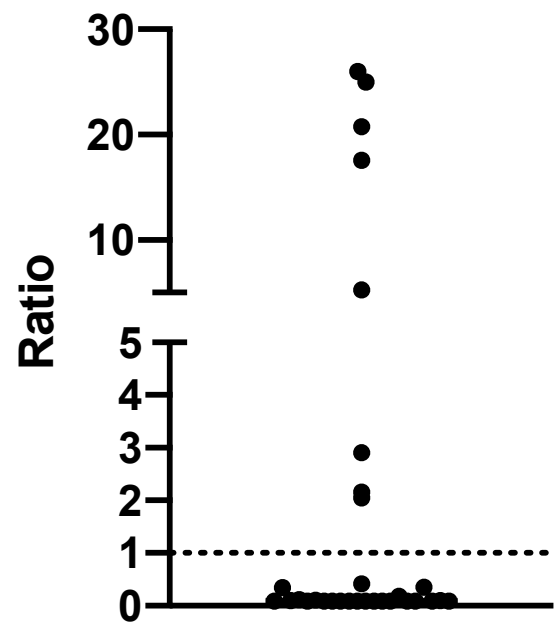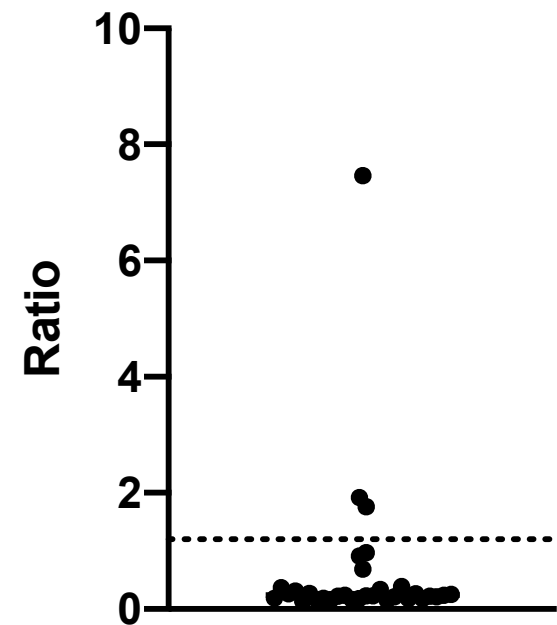
Serology should not be used for acute diagnosis!

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."

Utility in symptomatic patients outside of PCR window

Serology helpful for dx?
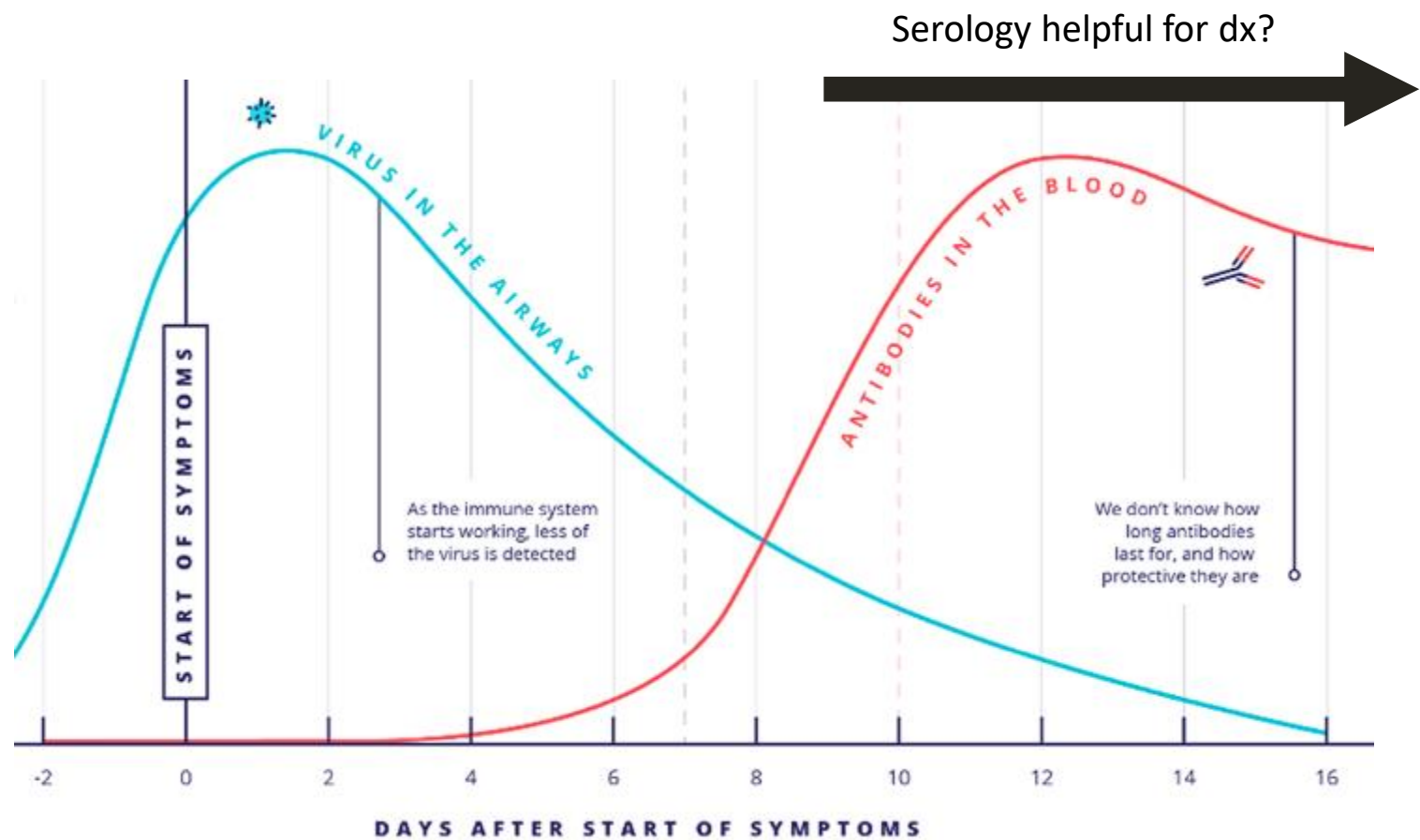
IDSA

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

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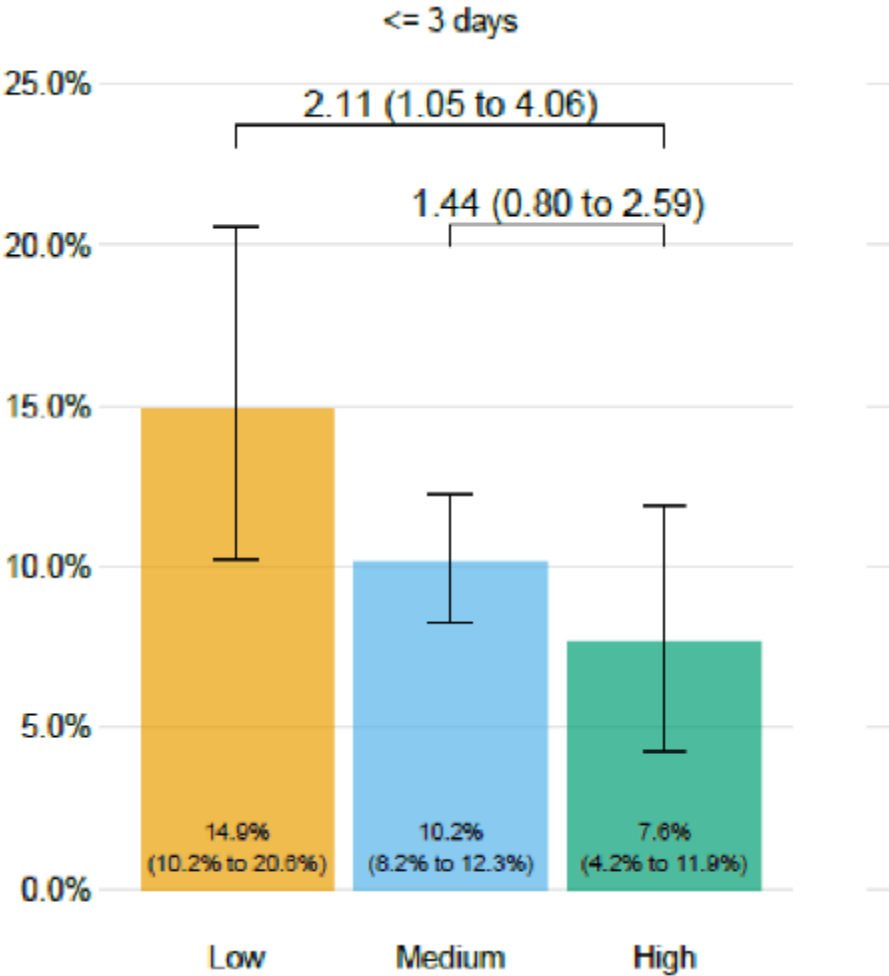# Validation will depend on clinical use

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
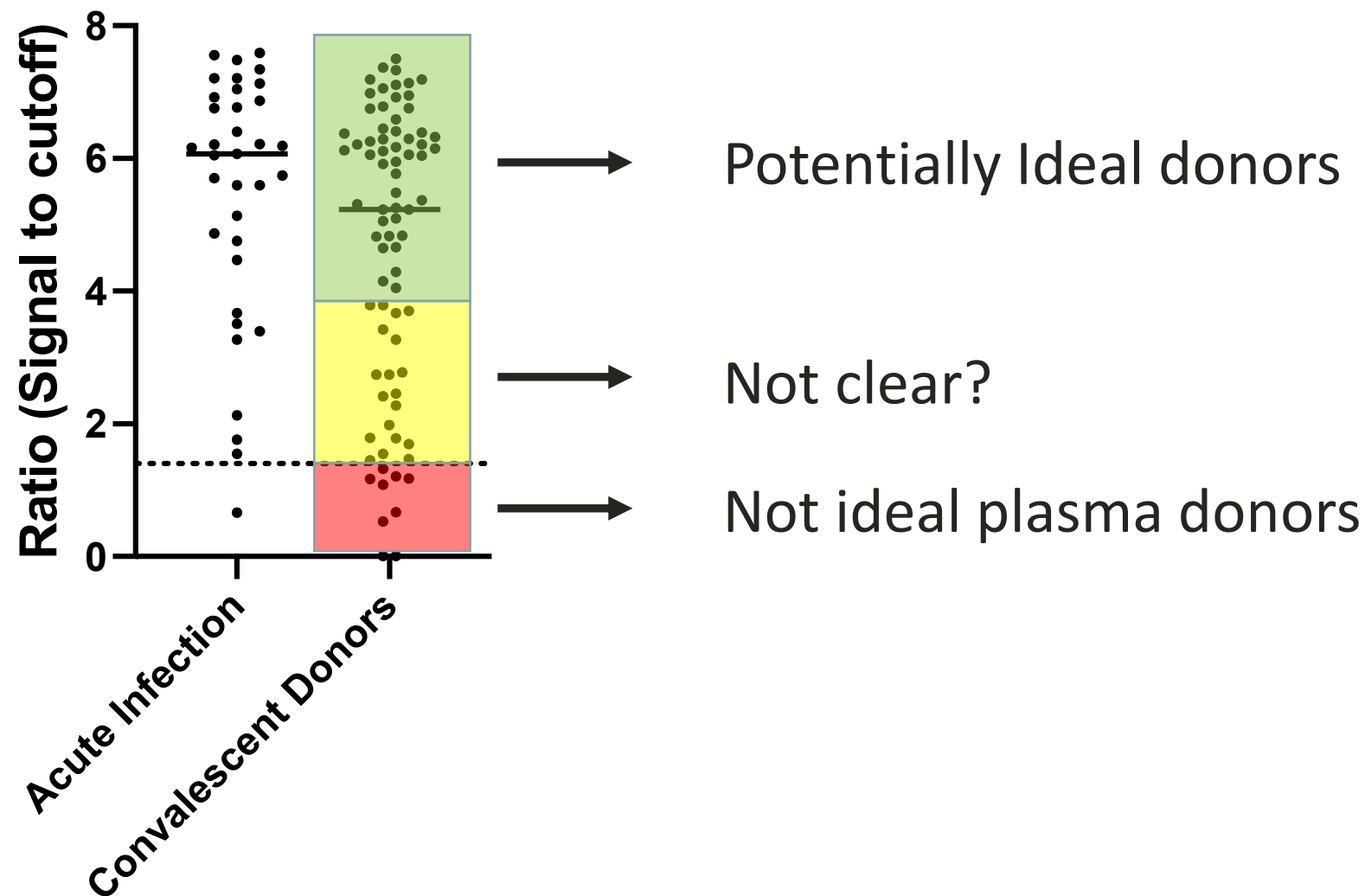
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Use of serology to identify convalescent plasma donors

Joyner MJ. medrxiv 2020. https://pubmed.ncbi.nlm.nih.gov/32817978/

Use of serology to identify convalescent plasma donors

→ Potentially Ideal donors

→ Not clear?

→ Not ideal plasma donors

Ratio (Signal to cutoff)

Acute Infection

Convalescent Donors

     Farnsworth Lab internal Data

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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**

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Positive predictive value, it's a matter of the stats!

	• **No Infection**	**Infection**
Antibody (-)	True neg. **760,000**	False neg. **2,00 0**
Antibody (+)	False pos. **40,000**	True pos. **198,000**

$$\textbf{Specificity} = \frac{TN}{TN + FP} \quad \frac{760,000}{800,000} = \textbf{95\%}$$

$$\textbf{Sensitivity} = \frac{TP}{TP + FN} \quad \frac{198,000}{200,000} = \textbf{99\%}$$

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Positive predictive value, it's a matter of the stats!

	• **No Infection**	**Infection**
Antibody (-)	True neg. **760,000**	False neg. **2,00 0**
Antibody (+)	False pos. **40,000**	True pos. **198,000**

Positive predictive value = $\dfrac{\text{\# true positive}}{\text{\# true positive + \# false positives}}$
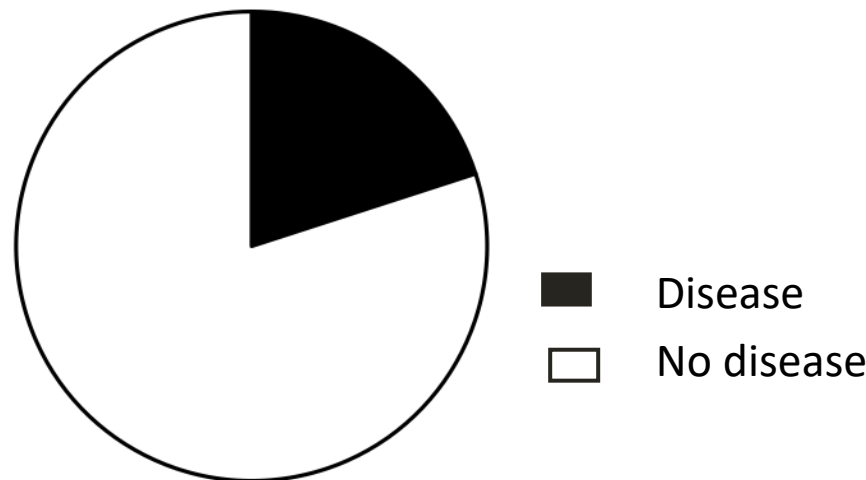
CardinalHealth

Positive predictive value, it's a matter of the stats!

	No Infection	Infection
Antibody (-)	True neg. **760,000**	False neg. **2,00 0**
Antibody (+)	False pos. **40,000**	True pos. **198,000**

Positive predictive value = $\dfrac{198{,}000}{198{,}00 + 40{,}000}$ **= 83.2%**

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Positive predictive value is impacted by prevalence

Prevalence 20%

■ Disease
☐ No disease

200,000 Cases

Sensitivity 99%
Population 1,000,000

Specificity	TP	FP	PPV
99.5%	198,000	4,000	98.0%
95%	198,000	40,000	83.2%

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Positive predictive value is impacted by prevalence
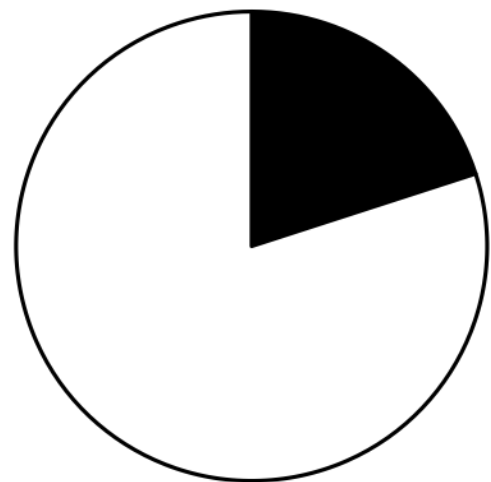
Prevalence 20%

Prevalence 2%
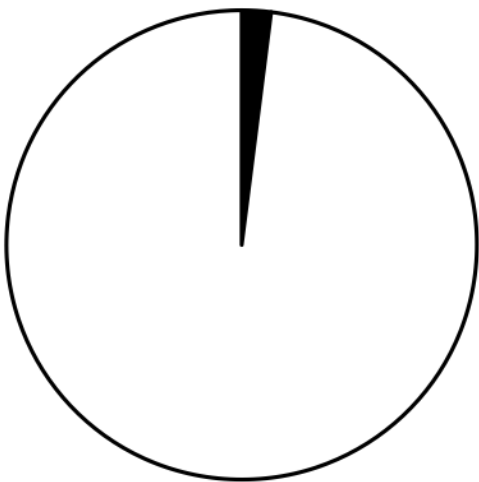
■ Disease
□ No disease

200,000 Cases

20,000 Cases

Sensitivity 99%
Population 1,000,000

Specificity	TP	FP	PPV
99.5%	198,000	4,000	98.0%
95%	198,000	40,000	83.2%

Specificity	TP	FP	PPV
99.5%	19,800	4,000	83.2%
95%	19,800	40,000	33.3%

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Prevalence will vary by location!

Missouri Prevalence

2.0%

0.2%

0.2% prevalence

Specificity	TP	FP	PPV
99.5%	1,980	4,980	39.8%
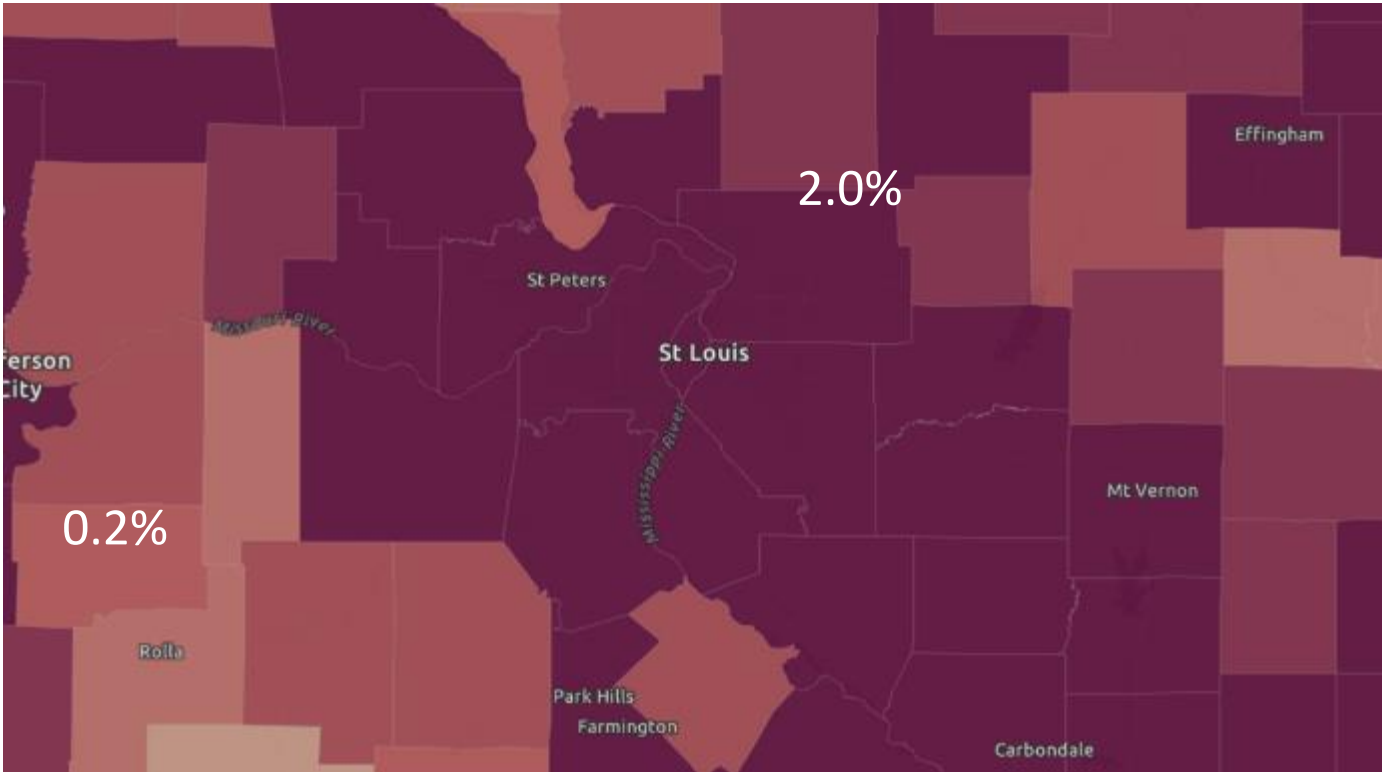
Assumes sensitivity 99%, population of 1,000,0000

https://coronavirus.jhu.edu/map.html

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Confidence in your specificity matters!

COVID-19 Antibody Seroprevalence in Santa Clara County, California

Eran Bendavid[1], Bianca Mulaney[2], Neeraj Sood[3], Soleil Shah[2], Emilia Ling[2], Rebecca Bromley-Dulfano[2], Cara Lai[2], Zoe Weissberg[2], Rodrigo Saavedra-Walker[4], Jim Tedrow[5], Dona Tversky[6], Andrew Bogan[7], Thomas Kupiec[8], Daniel Eichner[9], Ribhav Gupta[10], John P.A. Ioannidis[1,10], Jay Bhattacharya[1]

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%

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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"

Prevalence of COVID-19 in the population	PPV for one test (SE=90%, SP=99.8%)
2%	90.2%
5%	95.9%
10%	98.0%
30%	99.5%

Prevalence of COVID-19 in the population	PPV for one test (SE=90%, SP=95%)	PPV for two orthogonal tests (SE=90%, SP=95%)
2%	26.9%	86.9%
5%	48.6%	94.5%
10%	66.7%	97.3%
30%	88.5%	99.3%

https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html#table1

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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.

✓ Accept   ✕ Cancel

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How physicians are using serology at our hospital?

Ordering Patterns over Thirty Days of Testing

Time From Symptom Onset	N (%)
<3 days	18 (3%)
3-7 days	21 (4%)
8-13 days	8 (1%)
>14 days	423 (76%)
Never symptomatic	87 (16%)
Total	**557**

Mostly outpatients seeing PCPs

~20%
~10%
~70%

- Prevalence Studies
- In-patient
- Out-patient

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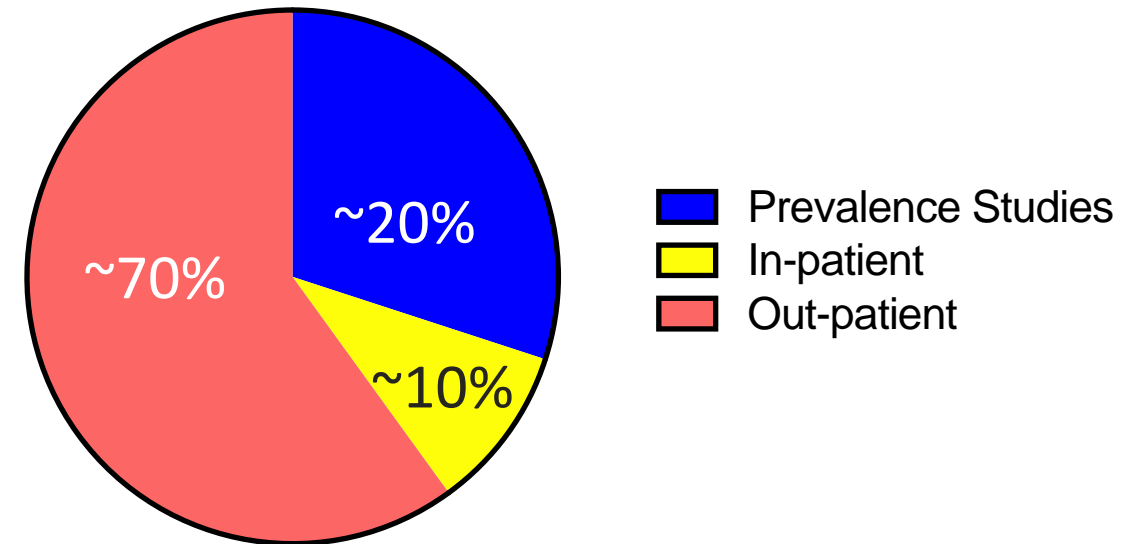
I have antibodies to SARS-CoV-2, am I immune?

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
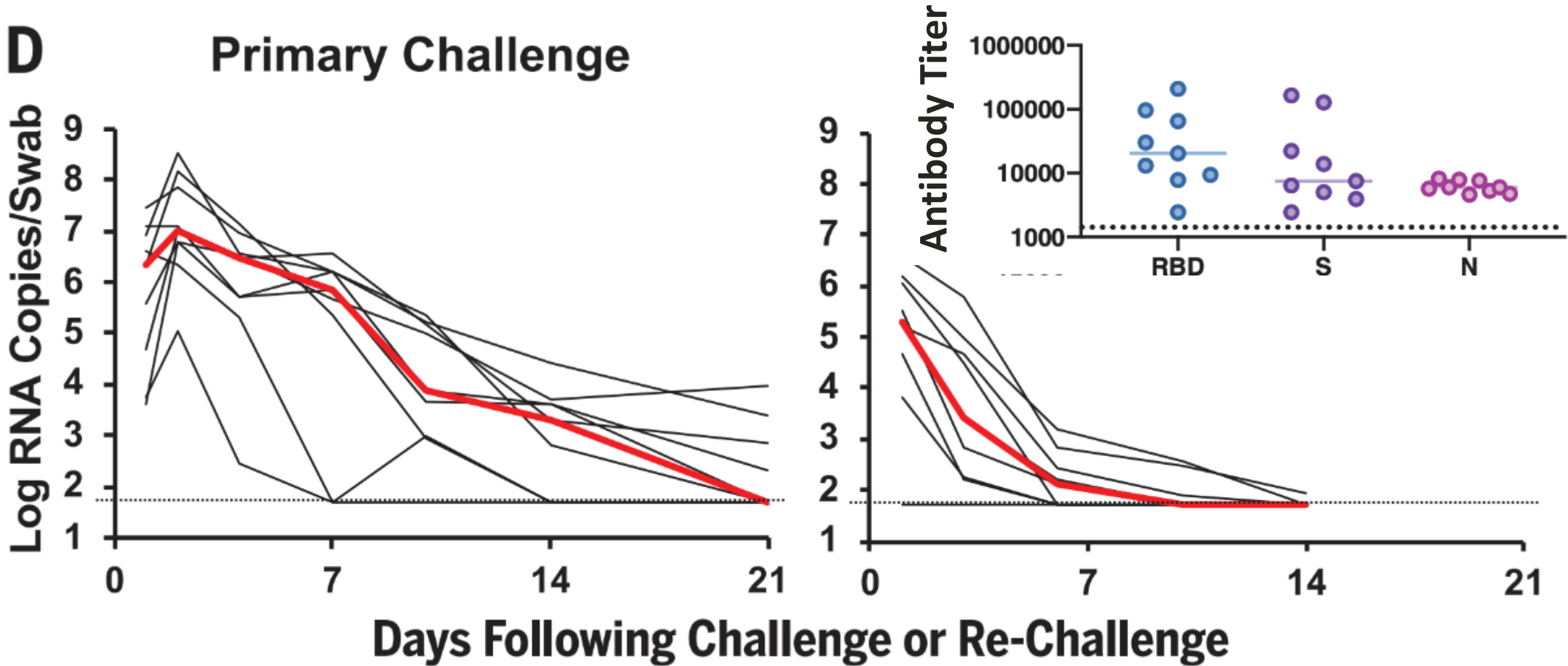
Chandrashekar A, *et. al. Science*. 2020. DOI: 10.1126/science.abc4776

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Durability of antibody response is undefined
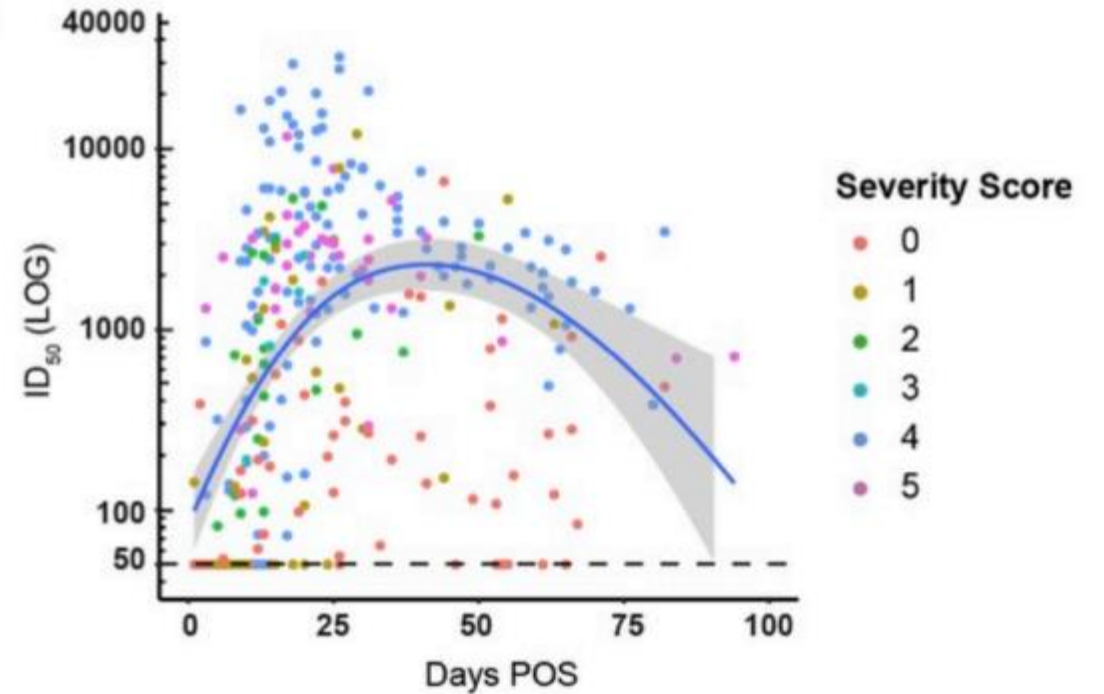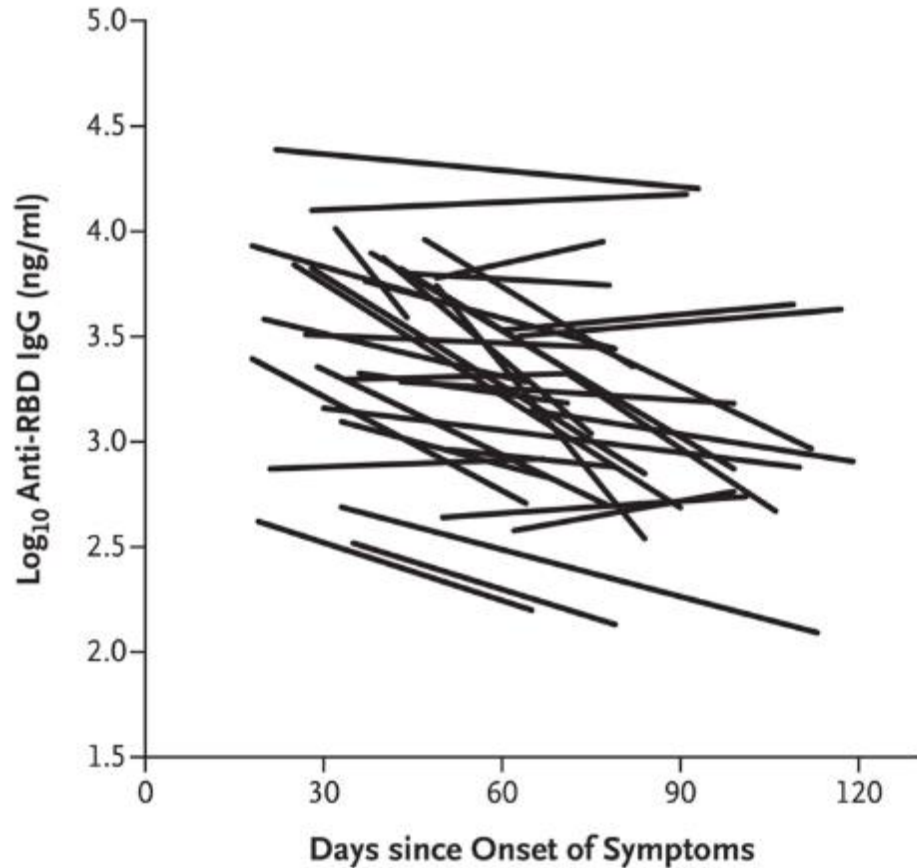
But clearly decreases with time

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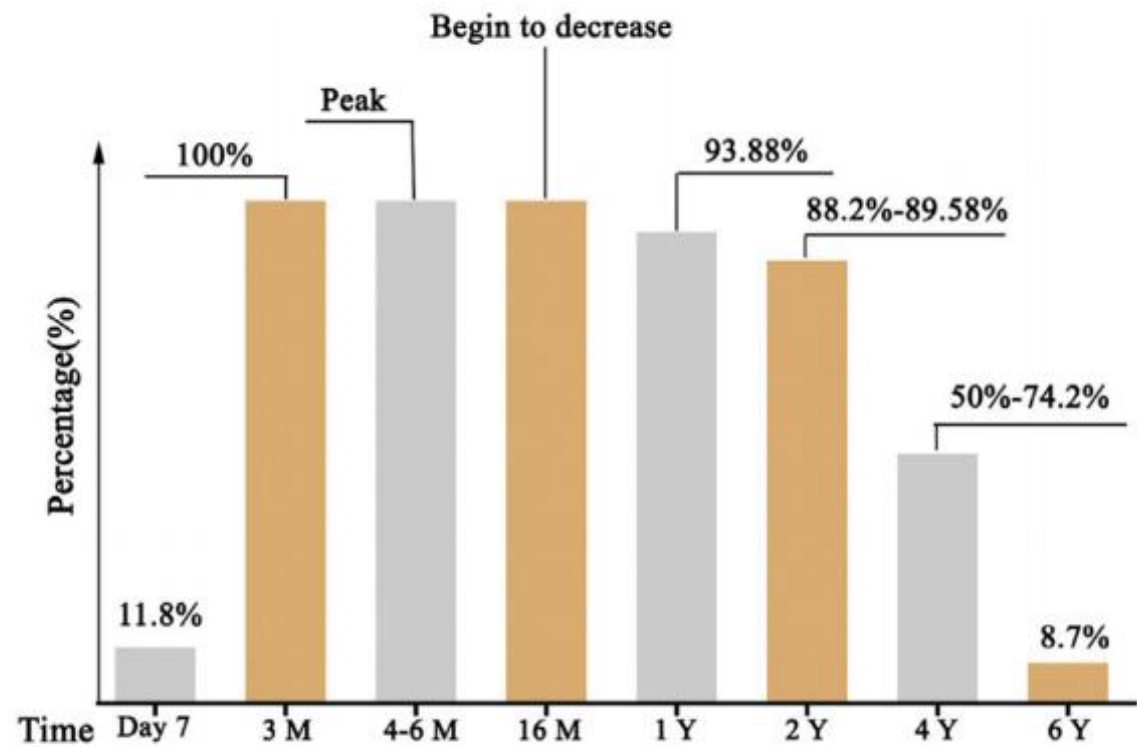
Using SARS-CoV as a surrogate for SARS-CoV-2 antibodies?

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].
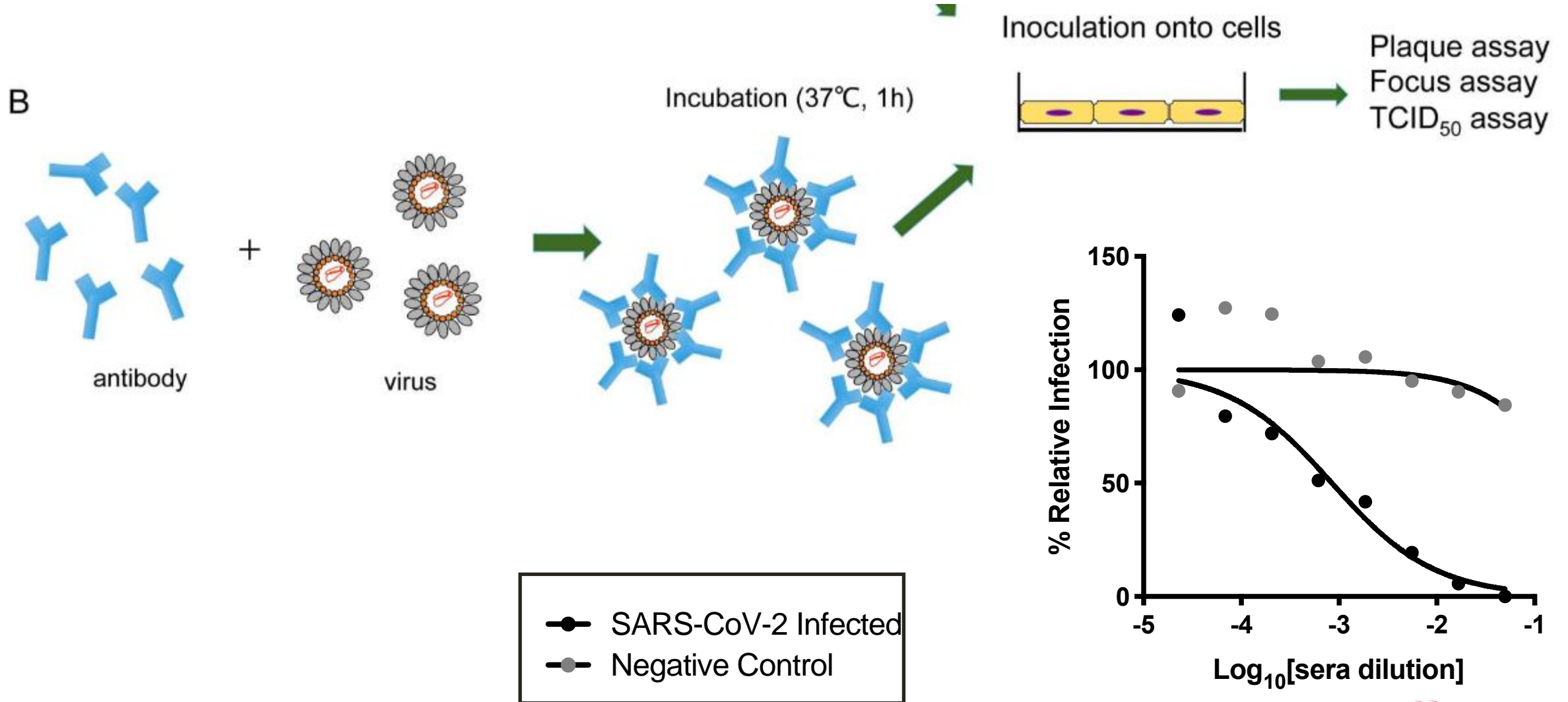
Lin Q et al.,Journal of Microbiology, Immunology and Infection, https://doi.org/10.1016/j.jmii.2020.03.0

Antibodies types and protection

- Antibodies can be *binding* or *neutralizing*

  - Binding (non-neutralizing) Abs

    - Produced at high levels,

    - unable to independently prevent infection

    - Bind and flag pathogen as 'invader'

    - Good markers of prior infection

  - 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

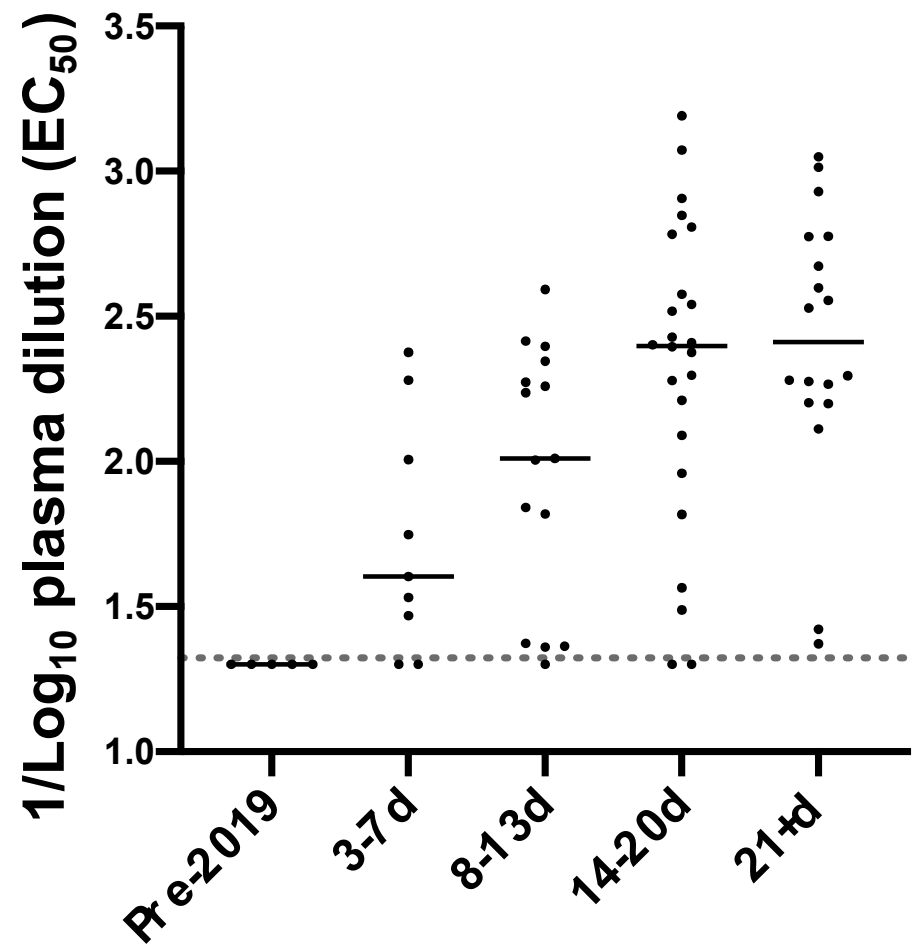- **Commercially available assays <u>do not</u> distinguish NAbs from non-NAbs**

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Assays for neutralizing antibodies are laborious

Inoculation onto cells

Incubation (37°C, 1h)

Plaque assay
Focus assay
$TCID_{50}$ assay

B

antibody + virus

SARS-CoV-2 Infected
Negative Control
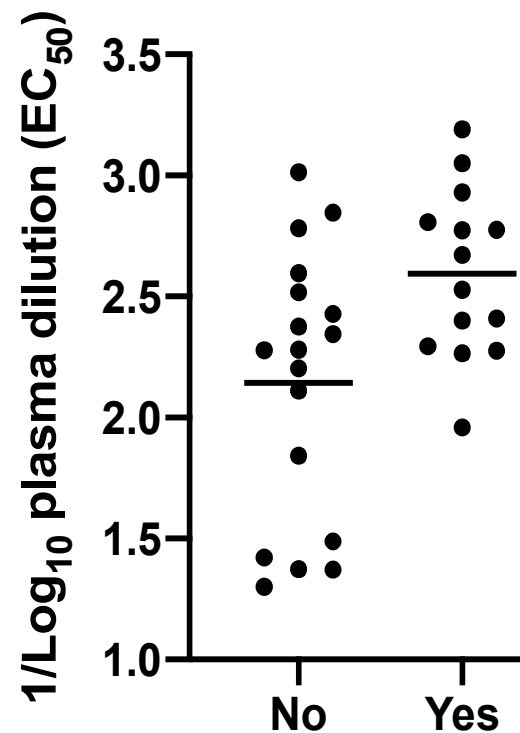
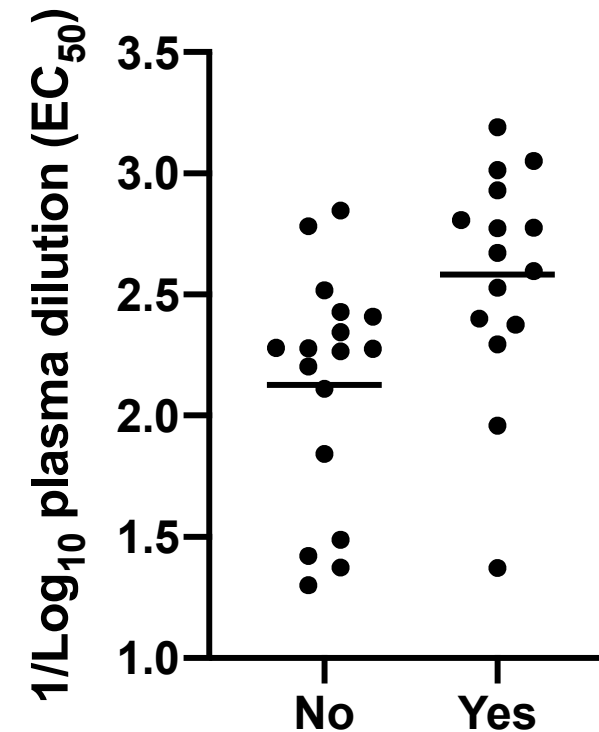% Relative Infection
Log_{10}[sera dilution]

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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

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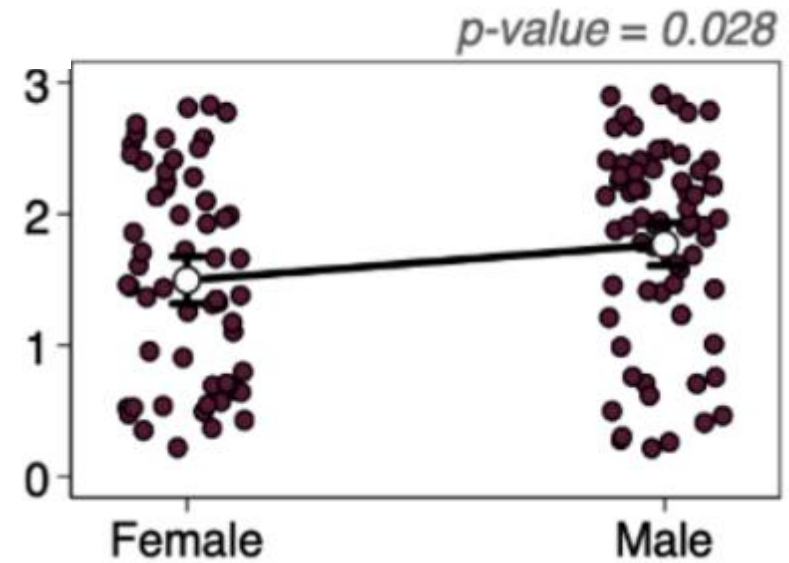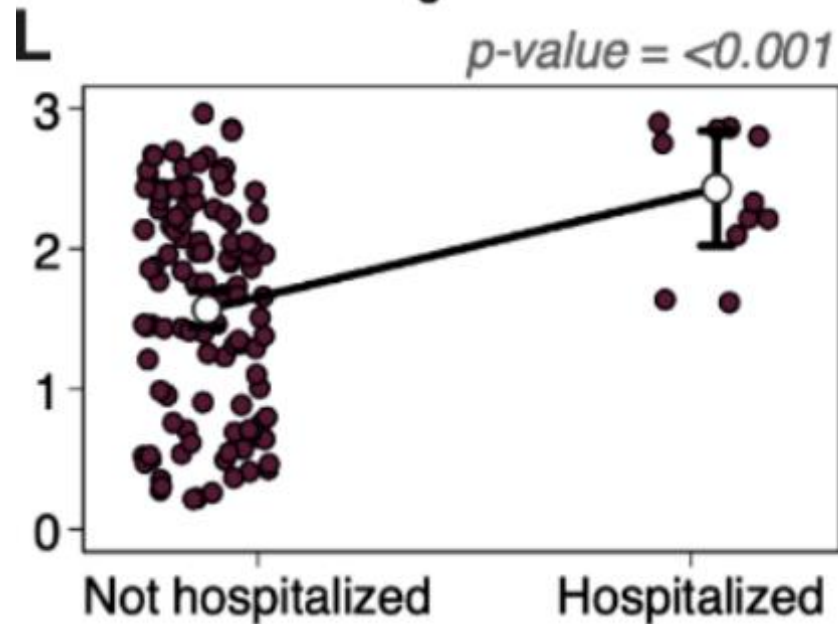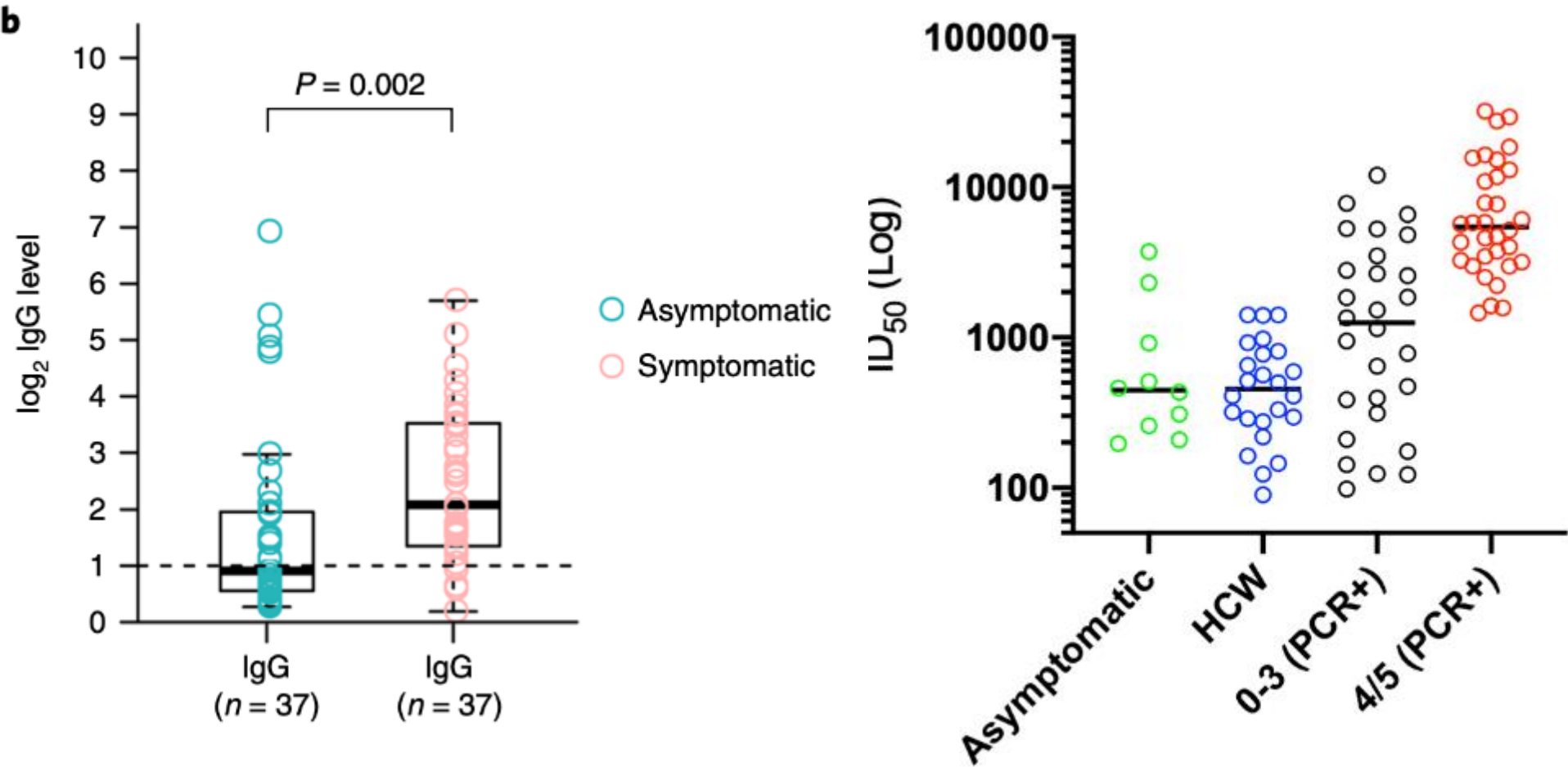
Worse outcomes associated with higher neutralizing titer

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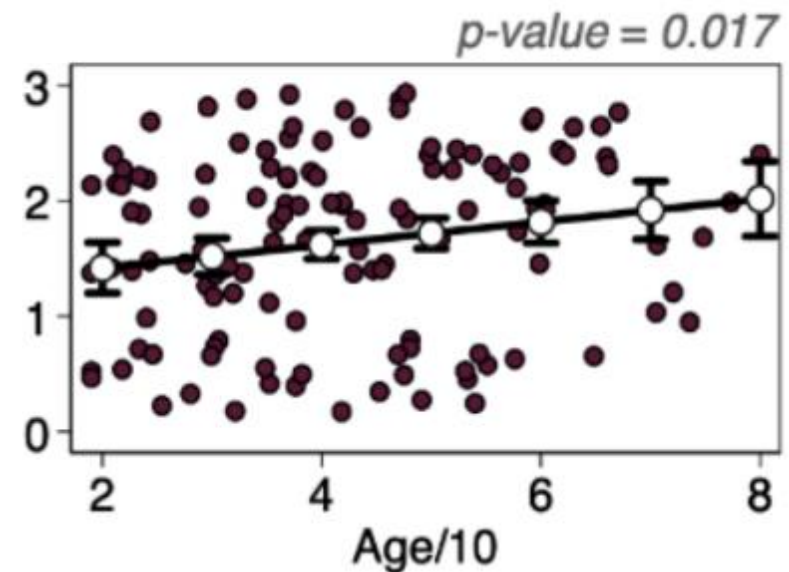
People with less severe disease have reduced antibodies

Long Q. Nature Medicine 2020; doi: 10.1038/s41591-020-0965-6.

Seow J *et al.* medrxIV. 2020.
https://www.medrxiv.org/content/10.1101/2020.07.09.20148429v1.full.pdf

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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

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Poor correlation between serological and neutralizing assays

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

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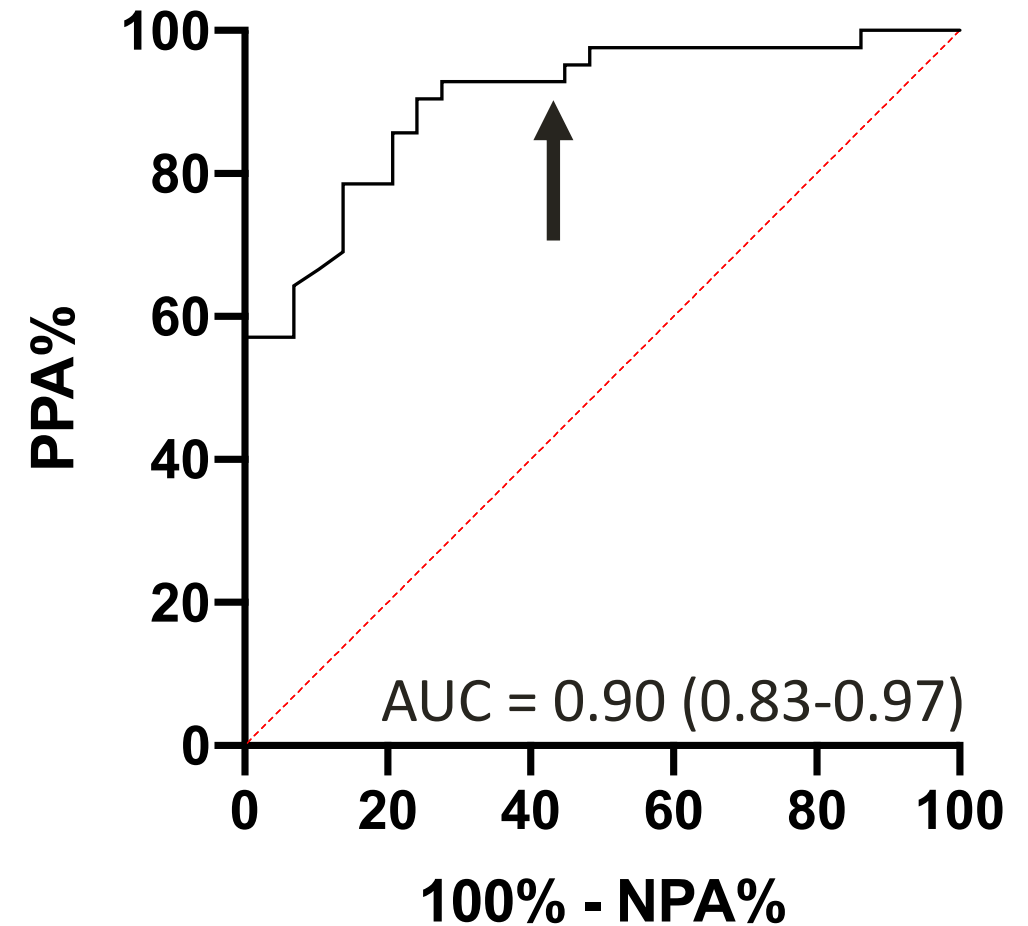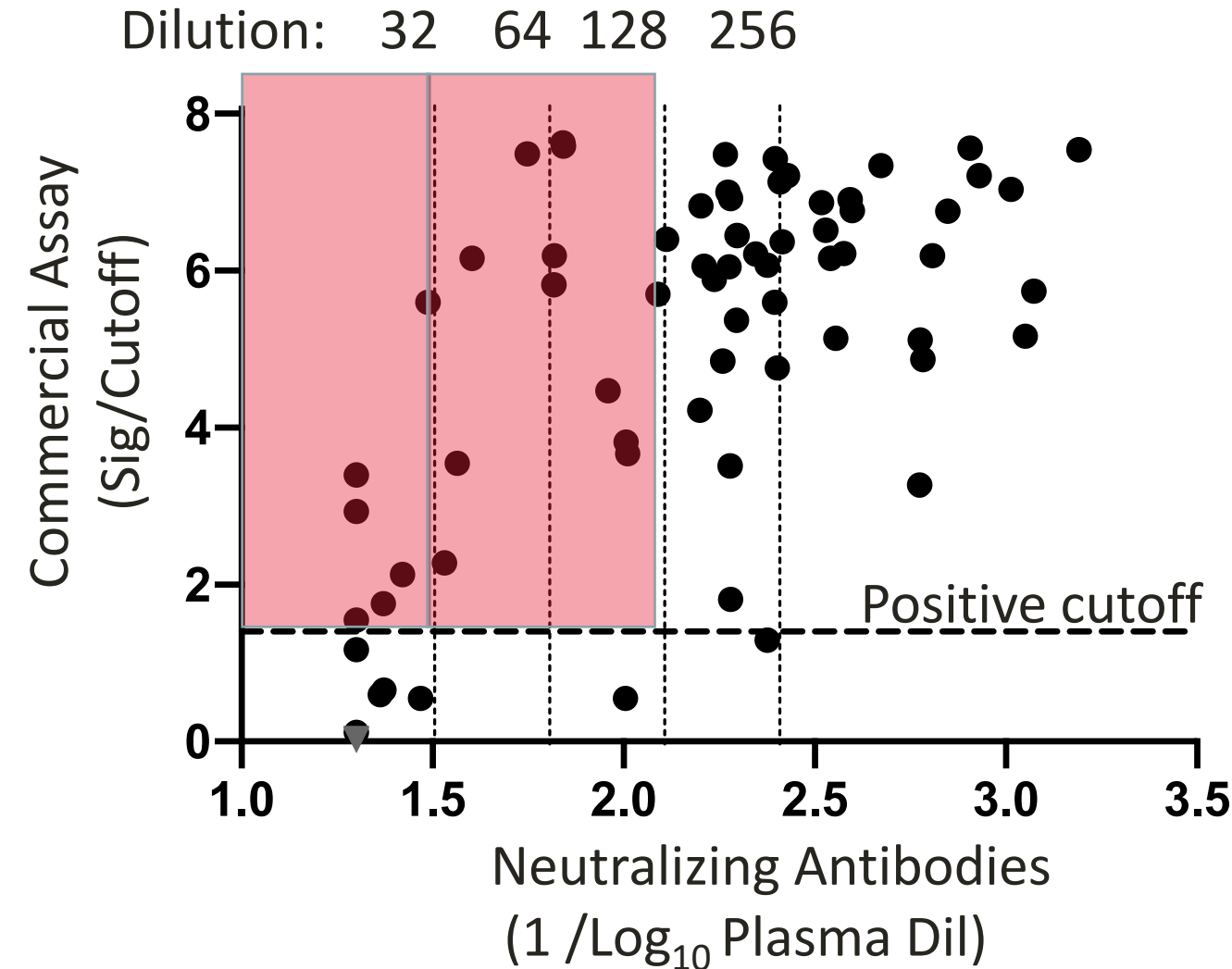
Take home:

- 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

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Questions?

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

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