# Laboratory Serology for SARS-CoV-2

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# DISCLOSURES

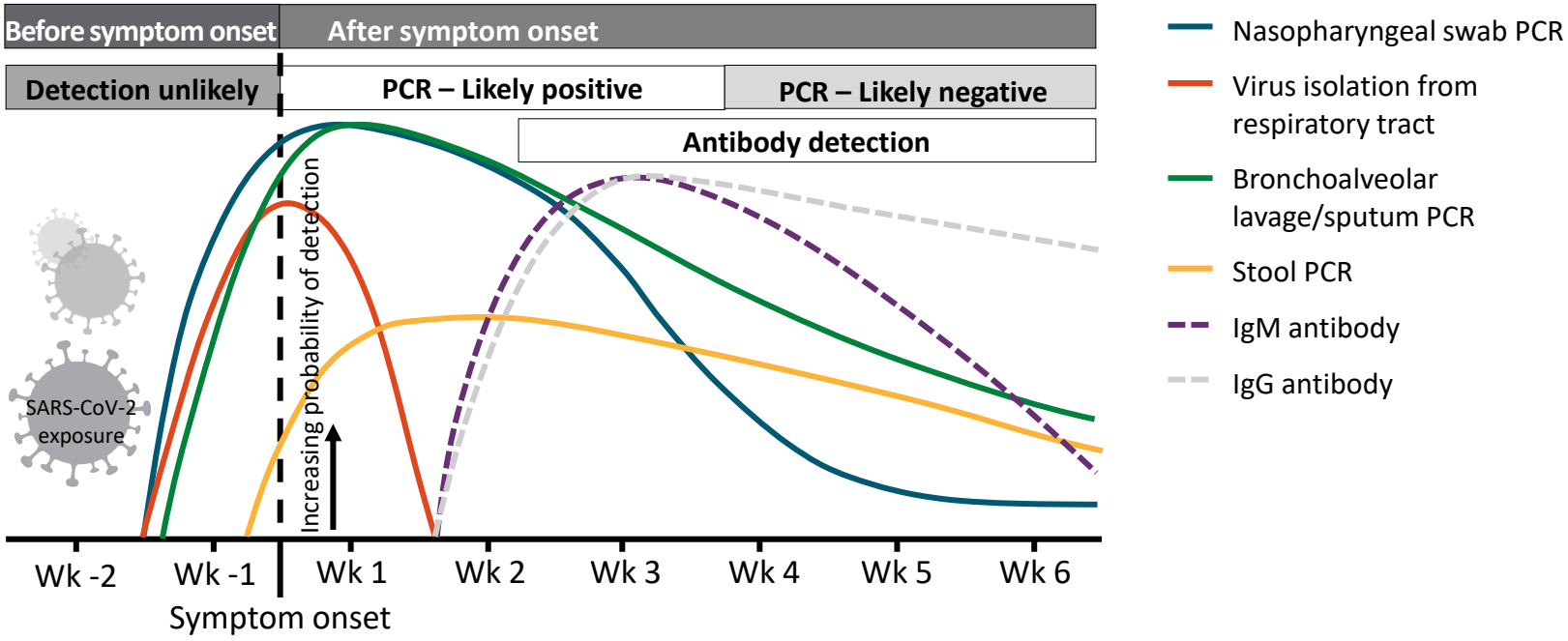
**P Slev**

None

# OBJECTIVES

1. Describe what is known about antibody response to SARS-CoV-2 infection
2. Understand the role of neutralizing antibodies in SARS-CoV-2 infection
3. Understand the advantages and limitations of current serology assays for SARS-CoV-2
4. Describe serology testing as related to vaccines

# Temporal Considerations for Diagnosis



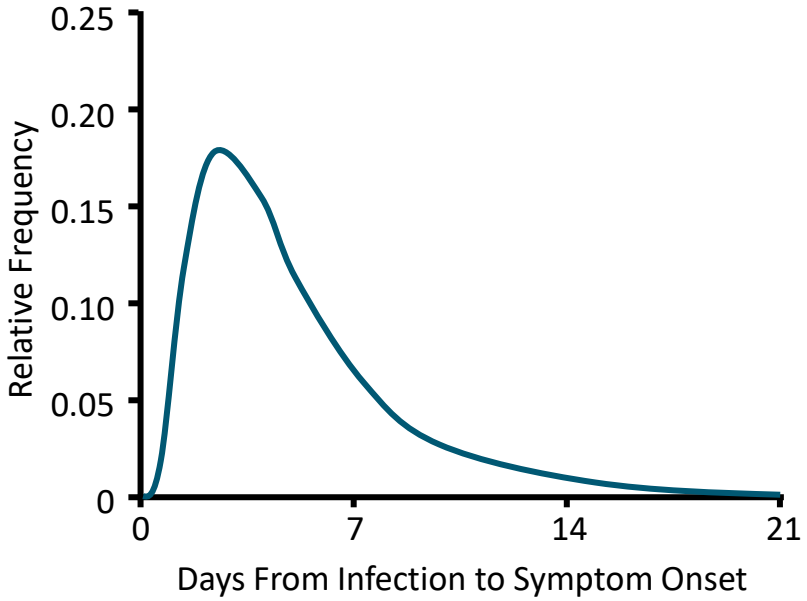



slide credit: clinicaloptions.com

# COVID-19 Incubation: Infection to Illness Onset

- Among 10 confirmed NCIP cases in Wuhan, Hubei province, China[1]
  - Mean incubation: 5.2 days (95% CI: 4.1-7.0)
- Among 181 confirmed SARS-CoV-2 infections occurring outside of Hubei province[2]
  - Median incubation: 5.1 days (95% CI: 4.5-5.8)
  - Symptom onset by Day 11.5 of infection in 97.5% of persons

Estimated Incubation Period Distribution[1]



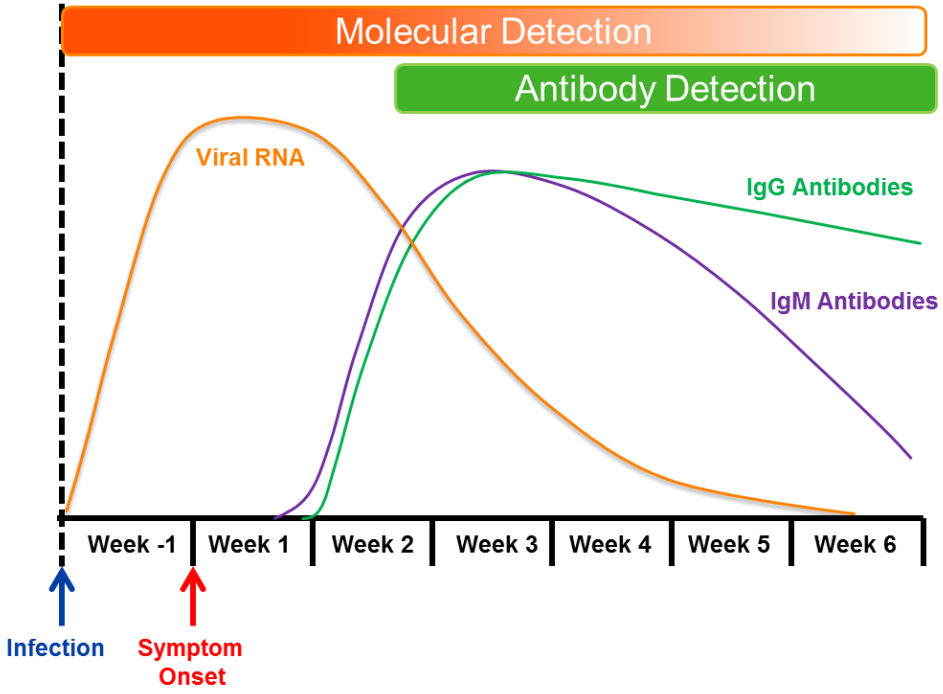

1. Li. NEJM. 2020;382:1199. 2. Lauer. Ann Intern Med. 2020;172:577.

# Timing of Antibody Development

**New virus = no pre-existing antibodies or immunity**

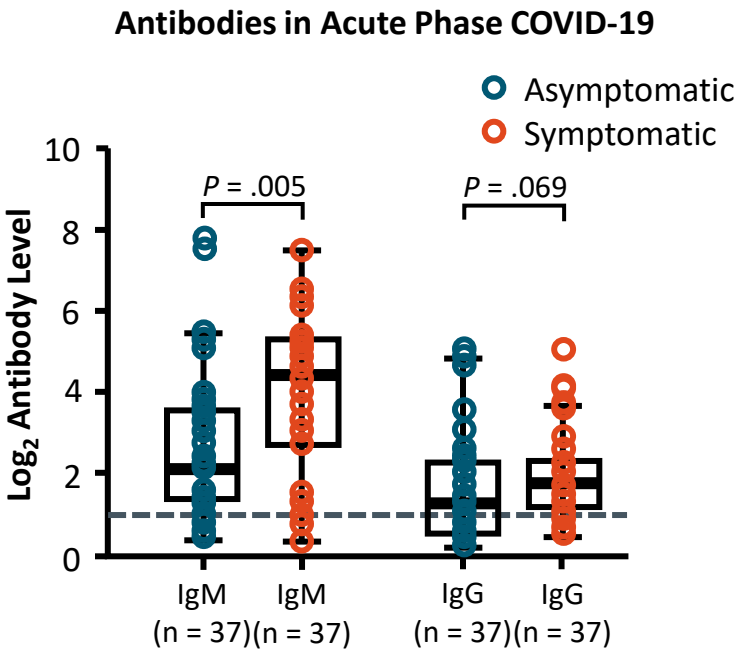
**We are *still* learning about our immune response to SARS-CoV-2**

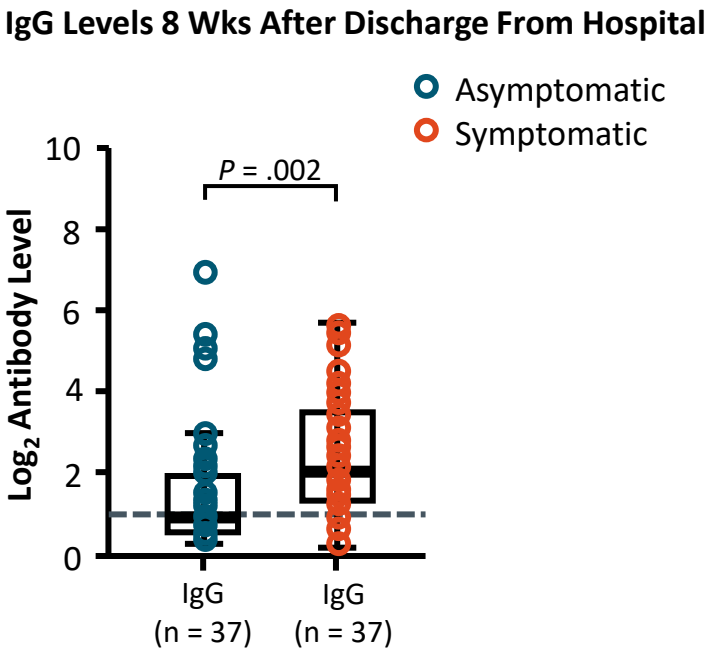
- Many develop Abs ~1-2 weeks after symptoms
- >95% of patients are Ab positive after 2 weeks
- IgM and IgG develop almost simultaneously
- IgM declines more rapidly than IgG, may only detectable for a few weeks post onset
- IgG remains positive for weeks to months post onset



# Q1 – Does antibody response vary with disease severity?

# Antibody Response in COVID-19: Acute Phase vs Early-Convalescent Phase



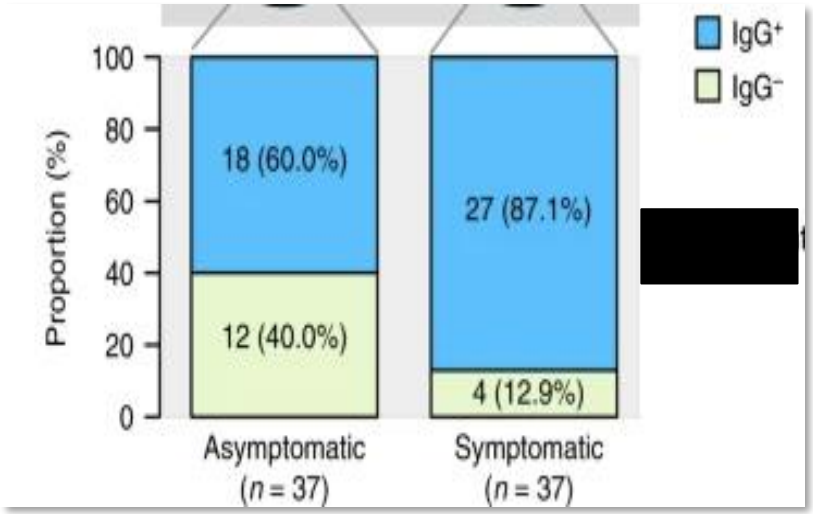




Long. Nat Med. 2020;26:1200.

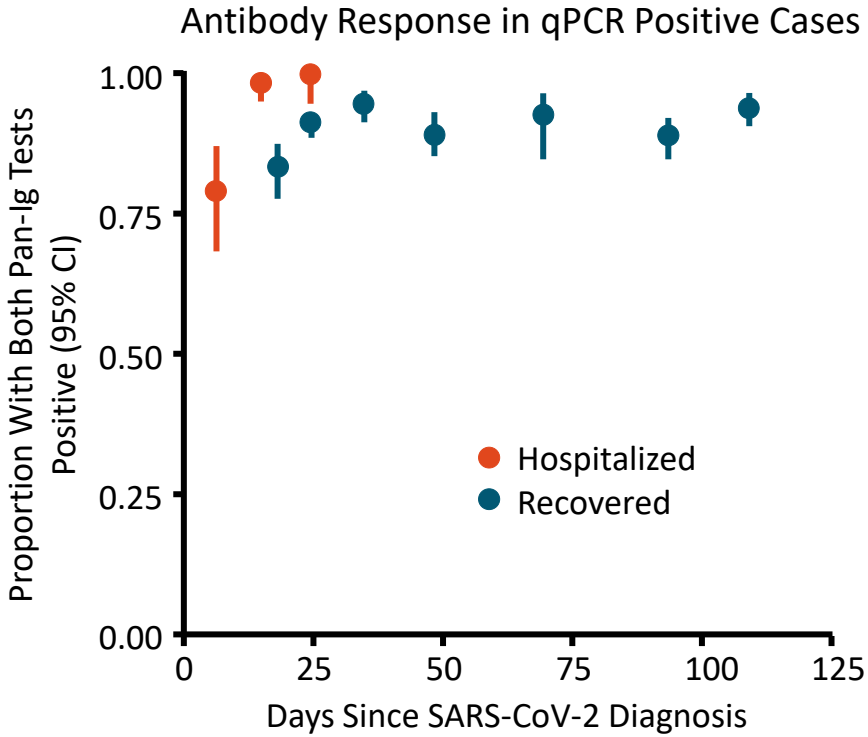
# Q2 – What is the longevity of antibodies post natural infection?

# Antibody Kinetics

- Following acute infection, antibodies *naturally* decline overtime for most viral infections
- Long QX *et.al* study by 8 weeks:
  - ~40% of asymptomatic vs ~13% of symptomatic patients are seronegative



# Antibody Response to SARS-CoV-2 in Iceland



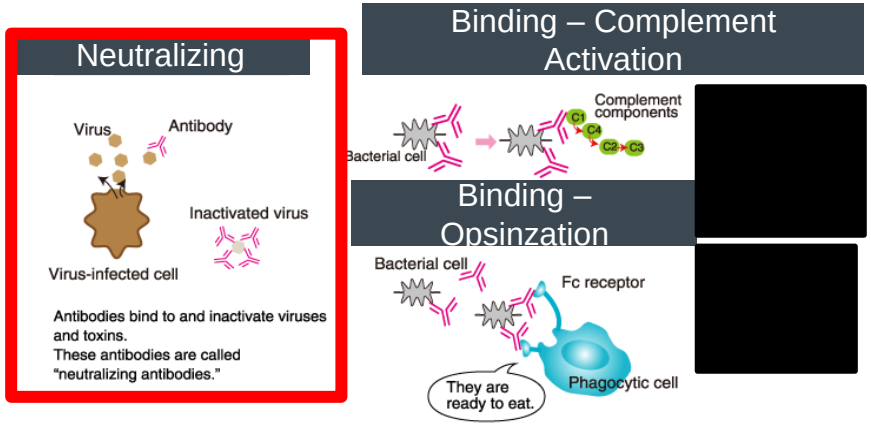

*Anti-N and anti-S1-RBD tests
1797 recovered COVID-19 pts tested over 4 months
Total antibodies increased over first 2 months and then plateau

Gudbjartsson. NEJM. 2020;382:2302.

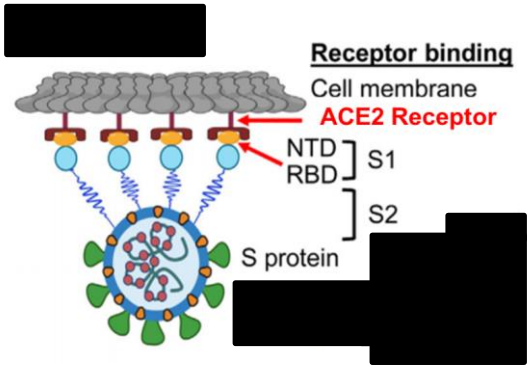
# Q3 – What about protective immunity and neutralizing antibodies?

# Role of Neutralizing Antibodies in Protective Immunity

- Antibodies can be *binding* or *neutralizing*
  - Binding Abs
    - Indicate robustness of immune response
    - Anti-viral activity through interaction with other components of immune system
    - No information about functionality
  - Neutralizing Abs (nAbs)
    - *Independently* block viral binding/entry
    - Key markers of protective immunity
    - Functionality 'level' of immune response
- nAbs target epitopes of the Spike protein
  - Contains RBD for binding host cell ACE2 *and* fusion of viral/cellular membranes
  - NAbs primarily target RBD
- Commercial tests <u>do not</u> distinguish b/w nAbs and non-nAbs




https://www.mblbio.com/bio/g/support/method/antibody-role.html

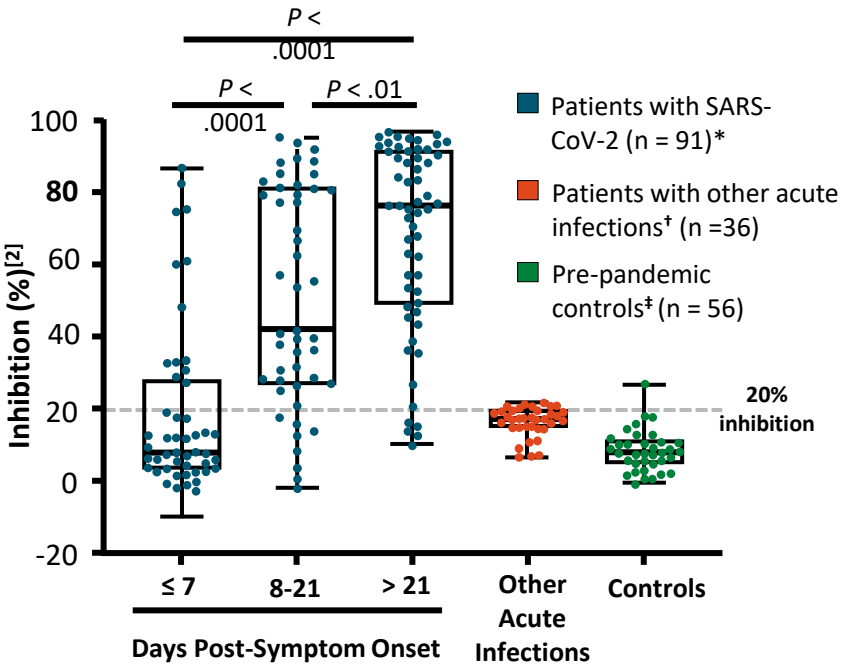



Jiang S, *et. al.* Trends Immuno. DOI:https://doi.org/10.1016/j.it.2020.03.007

slide credit - Dr. Theel

# Measuring Virus Neutralization Without High Containment Facilities

- Neutralization can be measured multiple ways
- Classical methods use live or pseudotyped virus and determine the serum dilution that inhibits virus growth[1,2]
- The surrogate viral neutralization test (sVNT) is a simpler method that assesses binding to ACE2[1,2]

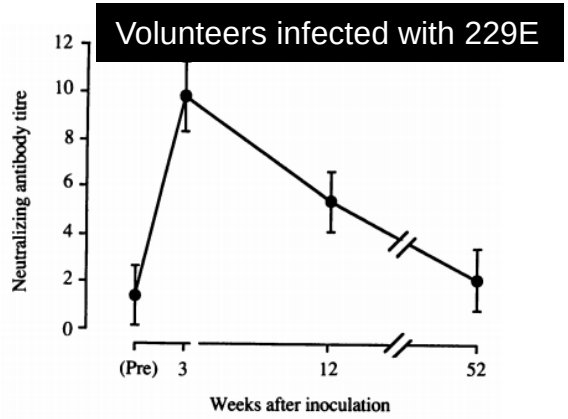


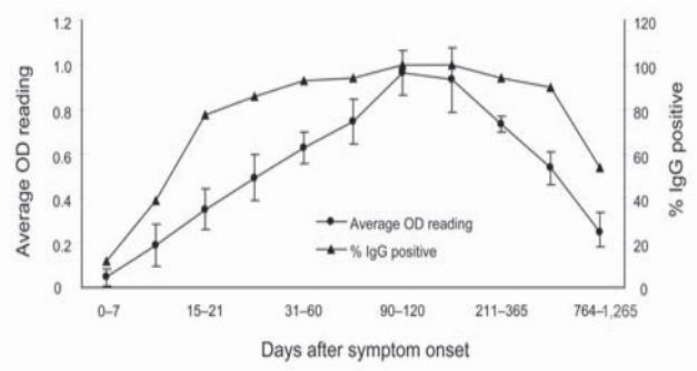

*Included 71 mild cases, 17 moderate cases, and 3 severe cases. †Included patients with seasonal coronavirus infections or other acute infections (e.g. dengue, CMV, or EBV). ‡Serum from a random cohort of patients in Australia obtained in 2018.

1. Tan. Nat Biotechnol. 2020;38:1073. 2. Bond. J Infect Dis. 2020;222:1280.

# What do we know about nAbs and immunity to other CoVs?

- **Common CoVs (volunteer studies):**
  - IgG peaks ~2 wks post infection and decline over ~ 1 year
  - Re-challenge at 1 yr:
    - 66% shed virus, *none* developed colds
  - Protective antibody levels thought to drop off at ~2 yrs
- **SARS:**
  - Abs max out ~3-5 months post infection
  - Decline to undetectable by 3-6 yrs
- **MERS:**
  - Neutralizing antibodies remain detectable for at least ~3 yrs
- **The unknown:**
  - What level of nAbs are protective and for how long?




slide credit - Dr. Theel


Wu LP, *et. al. EID*. 2007;13(10):1562-1564
Tang F, *et. al. J Immuno*. 2011;186(12);7264-7268
Bao L, *et. al.* bioRxiv:https://doi.org/10.1101/2020.03.13.990226

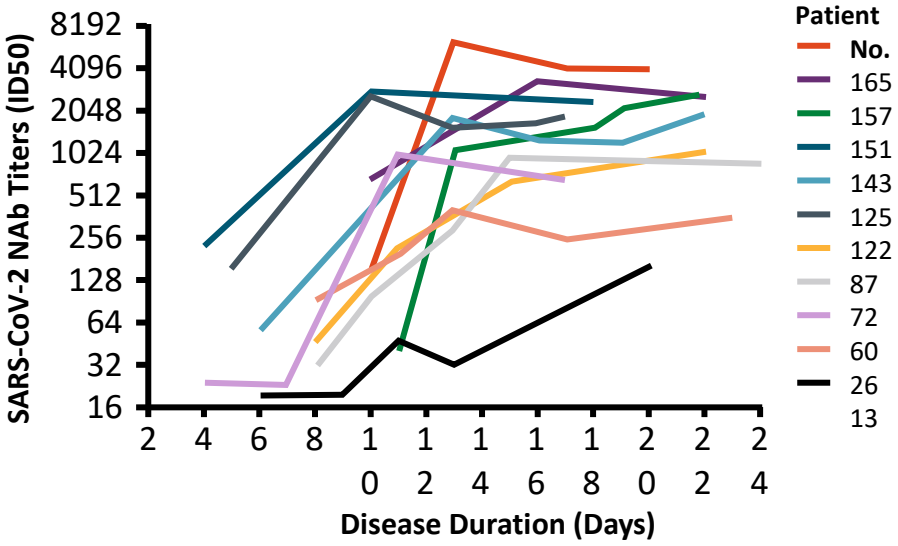
# Neutralizing Antibodies to SARS-CoV-2

- Plasma collected from recovered patients with COVID-19 who had mild symptoms (N = 175)
- Neutralizing antibody titers* varied

| NAb Titers | Value (N = 175) |
|---|---|
| Titer range, ID50 | < 40 to 21,567 |
| Patients with undetectable level, % | 6 |
| Patients with detectable level, % | |
| ▪ ID50: < 500 (very low) | 30 |
| ▪ ID50: 500-999 (medium low) | 17 |
| ▪ ID50: 1000-2500 (medium high) | 39 |
| ▪ ID50: > 2500 (high) | 14 |

*Assessed via pseudotyped, lentiviral vector-based assay.

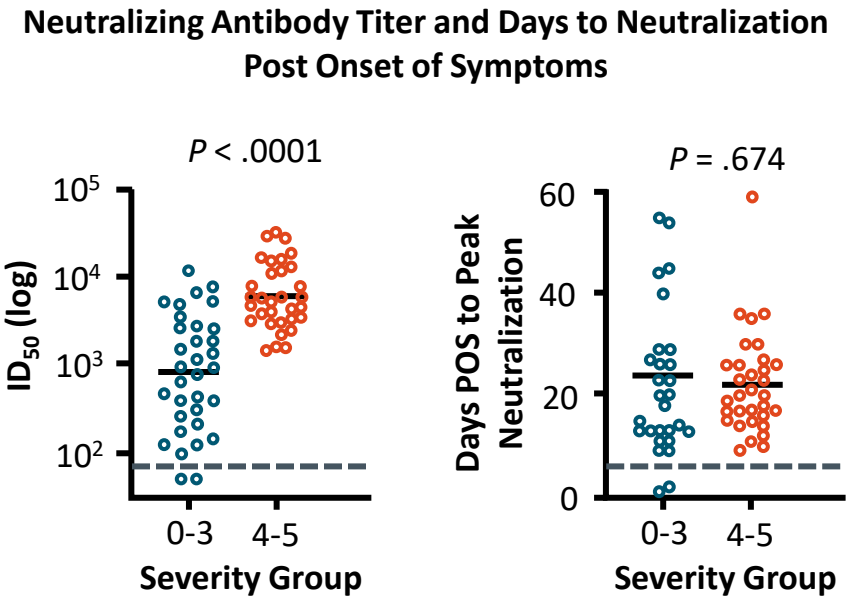
- Neutralizing and spike-binding antibodies emerged concurrently between 10-15 days following disease onset




Wu. JAMA Intern Med. 2020;189:10.

# Neutralizing Antibody Response in SARS-CoV-2 Infection

- 269 sequential serum samples collected at 2 London Hospitals from 65 patients diagnosed with SARS-CoV-2 by RT-PCR
- Persons with more severe disease had a greater magnitude of neutralizing antibody response
  - Days to peak neutralization did not differ by disease severity

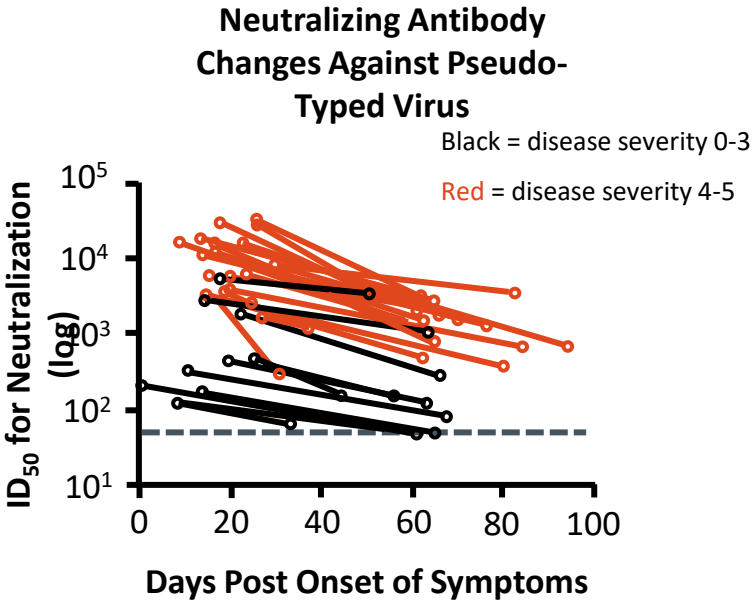



Seow. Nat Microbiol. 2020;[Epub].

Slide credit: clinicaloptions.com

# Kinetics of Neutralizing Antibody Reponses in SARS-CoV-2 Infection

- Average time to *detectable* neutralization = 14.3 days POS (range: 3-59)
- Average time to *peak* neutralization = 23.1 days POS (range: 1-66)
- Patients with low neutralizing Ab response ($ID_{50}$ 100-300) return to baseline or undetectable at approximately 50 days
- Patients with robust neutralizing Ab responses maintain titers > 1000 even after initial decline

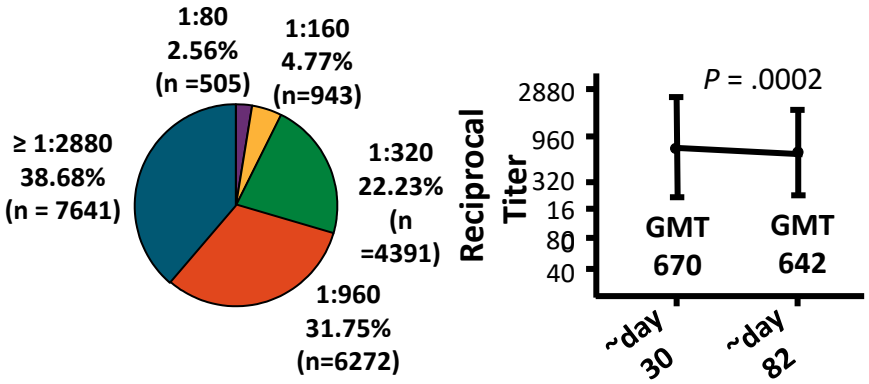



Seow. Nat Microbiol. 2020;[Epub].

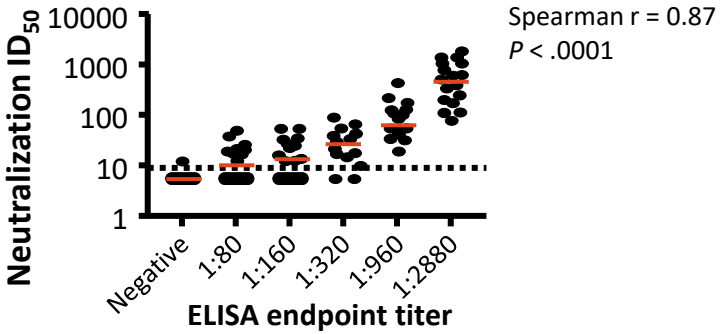
# Stability of Antibody Response Following COVID-19 Recovery

- Antibody responses were assessed in individuals screened at Mount Sinai Health System in NYC (N = 19,752)
  - Screened patients either had confirmed SARS-CoV-2 infection by RT-PCR or suspected disease
  - Additional samples collected through voluntary employee screening
  - < 5% of cases required emergency department evaluation or hospitalization
  - 121 individuals donated blood samples at 2 intervals (~ 30 days post symptom onset and a mean of 82 days post symptom onset)

**Binding IgG Antibodies to Spike Protein**




**Neutralization* by ELISA Titer**




Wajnberg. Science. December 4, 2020.

*Microneutralization assay.

## Q1 - Does antibody response vary with disease severity?

Severity of SARS-CoV-2 infection does affect magnitude of antibody response

## Q2 - What is the longevity of antibodies post natural infection?

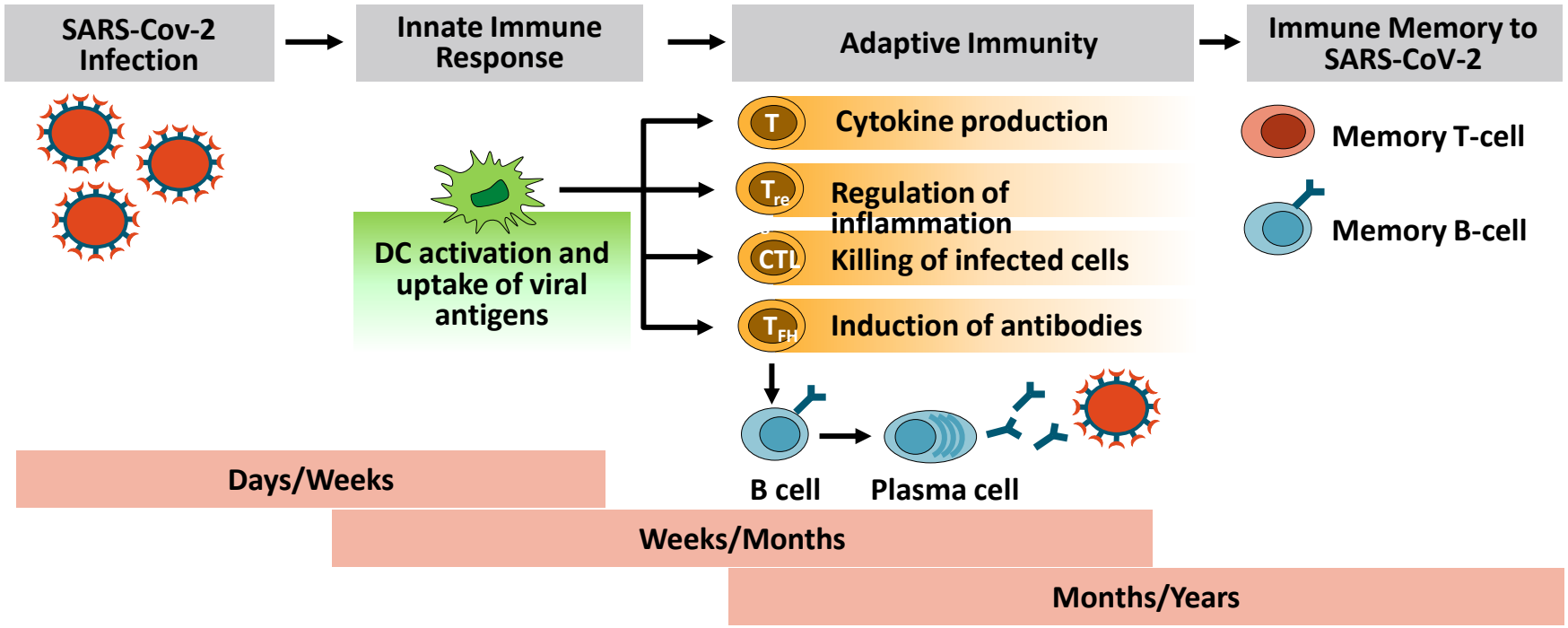
IgG antibodies can be detected several months post-infection (8 months)

IgG antibodies do decline over months

## Q3 - What about neutralizing antibodies?

Symptomatic individuals generally develop nAb titer but it is highly variable

# Potential Immune Correlates of Protection to SARS-CoV-2 Infection




Cox. Nat Rev Immunol. 2020;20:581.

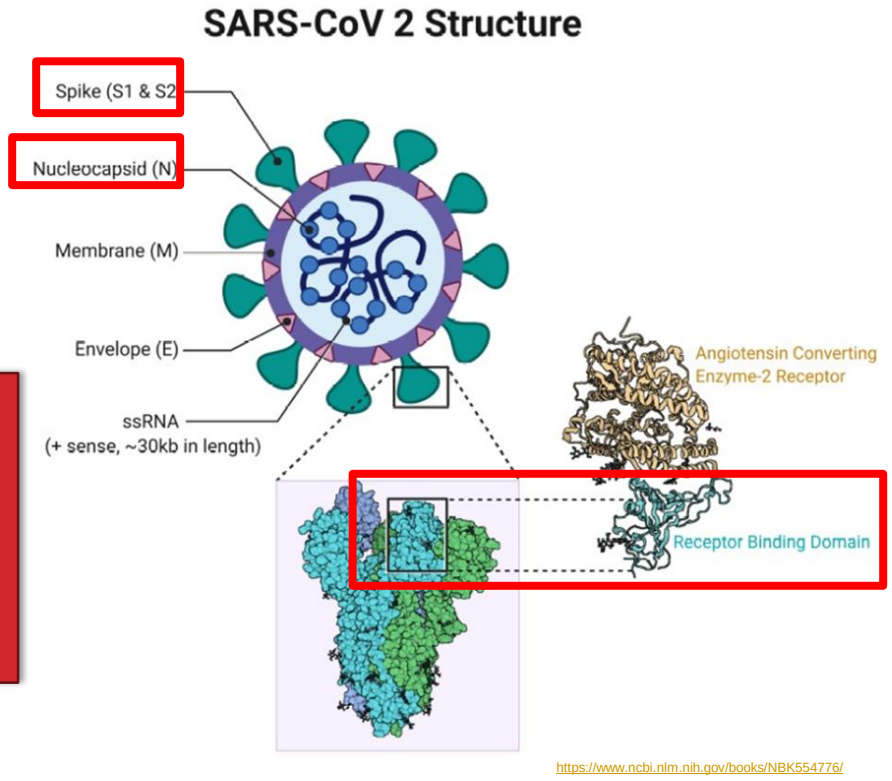
# Summary: Duration of T-Cell Reponses to SARS-CoV-2

- 100% of individuals with prior severe COVID-19 and 87% with prior mild COVID-19 demonstrated SARS-CoV-2–specific memory T-cell responses in convalescent phase (25-58 days after disease onset)[1]
- SARS-CoV-2–specific CD4+ and CD8+ T-cell recall responses were present in 41% of seronegative individuals, including individuals in the convalescent phase with a history of mild COVID-19 (3/31), exposed family members (9/28), and healthy individuals (5/31)[1]
- In another study, spike-specific memory CD4+ and CD8+ T-cells were maintained ~ 3 mos following symptom onset in patients with mild SARS-CoV-2 (N = 15)[2]

1. Sekine. Cell. 2020;[Epub]. 2. Rodda. MedRxiv. 2020;[Preprint]. Note: this study has not been peer reviewed.

# Different SARS-CoV-2 Antibody Test Designs

- **Format**
  Lateral Flow Assays (LFAs)
  Enzyme Linked Immunosorbent Assays
  Chemiluminescent Immunoassays
- **Specimen Type**
  Serum, plasma
  Finger stick/venous whole blood (LFAs)
- **CDC COVID-19 Guidelines (May 23, 2020):**
  - No advantage testing for IgG, IgM & IgG or Total
  - Testing for IgA *not recommended!*
- **SARS-CoV-2 Protein Used in the test**
  Spike protein – Subunit 1 and/or 2 (S1/S2)
  Receptor Binding domain (RBD)
  Nucleocapsid



# SARS-CoV-2 Antibody Tests

| Type of Test*[1] | Time to Results[1] | Sensitivity[1] | Specificity[1] | What It Tells Us[1] | What It Cannot Tell Us[1] | Approved for Diagnostic Use[1,2] |
|---|---|---|---|---|---|---|
| Rapid diagnostic test (RDT) | 10-30 mins | 87.9% to 99.0% | 95.6% to 100% | Presence of antiviral antibodies (qualitative) | Antibody titer, neutralizing activity | US (FDA EUA), EU, China, Australia |
| Enzyme-linked immunosorbent assay (ELISA) | 2-5 hrs | 13.9% (0-10 days) to 100% (≥ 21 days) | 99% to 100% | Presence and level of antiviral antibodies (quantitative) | Neutralizing activity | US (FDA EUA), Australia |
| Neutralization assay | 3-5 days | 90% | Not stated | Presence of antibodies that can inhibit virus growth (ex vivo) | May miss antibodies to viral proteins not involved in replication | Singapore |
| Chemiluminescent immunoassay | 1-2 hrs | 65.5% (0-6 days) to 100% (≥ 14 days) | 93.0% to 99.8% | Presence and level of antiviral antibodies (quantitative) | Neutralizing activity | US (FDA EUA) |

*Some additional tests have been approved that do not fit these categories or are proprietary.

1. Johns Hopkins Center for Health Security. Serology-based tests for COVID-19. https://www.centerforhealthsecurity.org/resources/COVID-19/serology/Serology-based-tests-for-COVID-19.html
2. Australian Therapeutic Goods Administration. https://www.tga.gov.au/covid-19-test-kits-included-artg-legal-supply-Australia

# SARS-CoV-2 Serologic Test Regulations in US: Where We Started

**Initially, the Food and Drug Administration did not require emergency use authorization (EUA) for SARS-CoV-2 serologic tests because:**

- Antibody tests were not meant to be diagnostic
- Intended to be used to answer the question of prevalence
- Intended to limit antibody testing to CLIA-certified high-complexity labs
- Indicated that this policy would be re-visited

# FDA GUIDANCE FOR SARS-COV-2 SEROLOGIC TESTS

**March 2020 – Notify FDA**

**May 4th, 2020 new guidance:**

- Manufacturers *must* submit validation data for EUA w/in 10 days from the date of FDA notification
- FDA has provided specific performance threshold requirements
- Serology EUA template available ( manufacturer, laboratory)
- Umbrella Route – independent evaluation through NIH's National Cancer Institute (NCI)

# SARS-COV-2 Serology Test Performance Thresholds

- Sensitivity
  - 30 samples
  - <7 days, 8-14 days, > 15 days (post-PCR pos)
  - Acceptance criteria – 90% PPA

- Specificity
  - 75 samples
  - Pre-pandemic samples
  - Acceptance criteria – 95% PPA

# Assay Performance Characteristics

https://www.fda.gov/medical-devices/emergency-situations-medical-devices/eua-authorized-serology-test-performance

**Developer:**
**Test:** SARS-CoV-2 IgG
**Technology:** High Throughput CMIA
**Target:** Nucleocapsid

| Antibody | Performance Measure | Estimate of Performance | 95% Confidence Interval |
|---|---|---|---|
| IgG | Sensitivity (PPA) | 100% (88/88) | (95.8%; 100%) |
| IgG | Specificity (NPA) | 99.6% (1066/1070) | (99.0%; 99.9%) |
| IgG | PPV at prevalence = 5% | 93.4% | (84.0%; 97.3%) |
| IgG | NPV at prevalence = 5% | 100% | (99.8%; 100%) |

**Test Facts:**

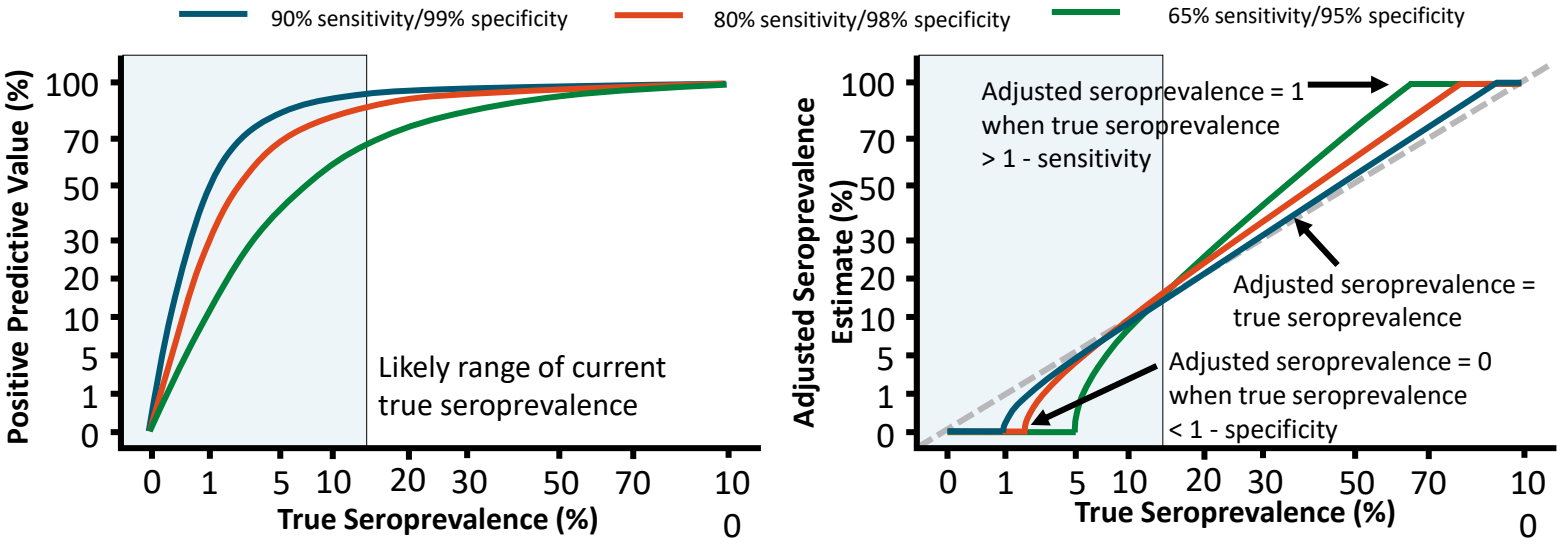
- Information for Healthcare Providers
- Information for Recipients
- Instructions for Use
- CDC's Evaluation Report
- CDC's Evaluation Data

# SARS-CoV-2 Antibody Tests

| Date EUA Issued or Last Updated | Entity | Diagnostic (Most Recent Letter of Authorization) and Date EUA Originally Issued | Attributes[3] | Authorized Setting(s)[1] | Authorization Documents[2] |
|---|---|---|---|---|---|
| 11/06/2020 | GenScript USA Inc. | cPass SARS-CoV-2 Neutralization Antibody Detection Kit 11/06/2020 | Total Neutralizing Antibodies, Blocking ELISA | H | HCP, Recipients, IFU |
| 10/31/2020 | Access Bio, Inc. | CareStart COVID-19 IgM/IgG 07/24/2020 | IgM and IgG, Lateral Flow | H, M | HCP, Recipients, IFU |
| 10/30/2020 | Ortho Clinical Diagnostics, Inc. | VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Reagent Pack 04/14/2020 | Total Antibody, CLIA | H, M | HCP, Recipients, IFU |
| 10/30/2020 | Bio-Rad Laboratories, Inc. | Platelia SARS-CoV-2 Total Ab assay 04/29/2020 | Total Antibody, ELISA | H | HCP, Recipients, IFU |
| 10/30/2020 | Siemens Healthcare Diagnostics Inc. | Dimension Vista SARS-CoV-2 Total antibody assay (COV2T) 06/08/2020 | Total Antibody, CLIA | H, M | HCP, Recipients, IFU |
| 10/30/2020 | Siemens Healthcare Diagnostics Inc. | Dimension EXL SARS-CoV-2 Total antibody assay (CV2T) 06/08/2020 | Total Antibody, CLIA | H, M | HCP, Recipients, IFU |

# SARS-CoV-2 Serology in Epidemiology

- Serology tests with high but < 100% specificity may lead to false-positive results when used in areas with low incidence
  - Example: A test with 96% specificity and 90% sensitivity used in an area where 5% of population has been infected → 54% of positive results would indicate true infection




Bryant. Sci Immunol. 2020;5:eabc6347.

clinicaloptions.com

# The Issue of Disease Prevalence on Performance of SARS-CoV-2 Antibody Tests

- Positive and negative predictive values are dependent on test performance characteristics *and* disease prevalence
- In regions with low COVID-19 prevalence, the risk of a false positive result by serologic tests, even with excellent specificity, is higher

**CDC recommendation to increase assay PPV:**

1. **Utilize assays with *at least* 99.5% specificity**
2. **Use orthogonal, 2-assay testing algorithm**
3. **Only screen high-risk/high-prevalence regions**

# SARS-CoV-2 Antibody Tests

| | |
|---|---|
| Indications | Past infection/Exposure<br>Surveillance for SARS-CoV-2 infection<br>Screening & identification of convalescent plasma donors (CCP)<br>Multisystem Inflammatory Syndrome in Children (MIS-C)<br>Individuals presenting late in disease course (PCR negative but symptoms consistent with SARS-CoV-2 infections)<br>Potentially evaluate response to vaccine |
| Considerations | False negatives - low sensitivity in the 7 days post symptom onset<br>- in individuals with mild or asymptomatic disease |
| | False positives - due to cross-reactivity with other viruses<br>- in low prevalence populations |

# SARS-CoV-2 Antibody Assays

- Currently: many EUA  authorized commercially available serologic tests for SARS-CoV-2
  - Some did not receive or submit for EUA
  - Some had EUA revoked
- No antibody tests are approved for at-home collection or at-home testing
- assays approved as semi-quant
- CLIA waived rapid tests
- One assay for CCP qualification
- One neutralizing antibody assay

# Vaccines

| Manufacturer | Type | Administration | Immune Response |
|---|---|---|---|
| Pfizer<br>BNT162b2 | Spike mRNA in LNPs | i.m.<br>boost at 21 days | nAb and cellular response |
| Moderna<br>mRNA-1273 | Spike mRNA in LNPs | i.m.<br>boost at 28 days | nAb and Th1 response |
| AstraZeneca<br>ChADOx1/AZD1222 | Inactivated chimpanzee Adv expressing spike | i.m.<br>boost at 28 days | nAb and Th1 response |

# mRNA Vaccines Against SARS-CoV-2

| Vaccine | Description | Phase (Total N) | Case Count, n | Primary Endpoint: Prevention of Symptomatic COVID-19 | Additional Analyses Reported |
|---|---|---|---|---|---|
| BNT162b2 (Pfizer)[1] | Vaccinations on Day 1 and Day 21 in persons ≥ 12 yrs of age with nucleoside-modified mRNA (modRNA) encoding the membrane-bound full-length spike protein | II/III (43,661)* | 170 (final) | 95% 7 days after second dose ($P < .0001$) | ▪ > 94% efficacy in adults > 65 yrs of age<br>▪ 9/10 severe cases occurred in placebo group |
| mRNA-1273 (Moderna)[2-4] | Vaccinations on Day 1 and Day 29 in persons ≥ 18 yrs of age with mRNA encoding a prefusion stabilized spike protein | III (30,000)† | 95 (interim) | 94.5% 14 days after second dose ($P < .0001$) | ▪ 11/11 severe cases occurred in placebo group |

*41,135 had received second dose as of November 13, 2020. 42% of volunteers had diverse ethnic backgrounds; 41% were 56-85 yrs of age.
†Includes more than 7000 persons > 65 yrs of age and more than 5000 < 65 yrs of age with high-risk chronic diseases, such as diabetes, severe obesity, and cardiac disease. 37% of volunteers from racial and ethnic minorities.

1. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-conclude-phase-3-study-covid-19-vaccine. Press release only, not peer reviewed. 2. https://investors.modernatx.com/news-releases/news-release-details/modernas-covid-19-vaccine-candidate-meets-its-primary-efficacy. Press release only, not peer reviewed. 3. https://investors.modernatx.com/news-releases/news-release-details/modernas-covid-19-vaccine-candidate-meets-its-primary-efficacy. Press release only, not peer reviewed. 4. NCT04470427.

# Vaccine Considerations

- Primary endpoint in mRNA vaccine trials was symptomatic illness, therefore viral shedding and transmission potential is not known
- Duration of vaccine immunity unknown
- Long-term safety data is needed for new technology
- Efficacy established in healthy 18 – 65
- No efficacy established in children (<16) and pregnant women
- Efficacy is not established in subpopulations
- SARS-CoV-2 mutations and viral escape immune selection pressure due to mass vaccination is unknown

# Vaccines & Serology

- Vaccines target development of antibodies against spike & RBD because these are the targets for neutralizing antibodies
- Testing with a serology assay that uses a spike target can detect antibodies developed in response to current vaccines
- However, anti-spike antibodies also develop in response to natural infection
- Antibodies against nucleocapsid are only detected in individuals that have been infected
- Currently, there are no recommendations for serology testing as a follow-up to vaccination

# Summary

- Antibody Kinetics

  IgM & IgG  antibodies develop in response to SARS-CoV-2 infection

  Disease severity likely affects magnitude of antibody response

  Antibody declines over time,  3- 4 months

  Antibody decline may not mean lack of protection – memory B-cells present

  Neutralizing antibodies are detectable in the majority of  symptomatic patients but titer and duration vary

- Antibody Testing Utility

  epidemiologic & seroprevalence studies

  supporting diagnosis of COVID-19 and related syndromes (MIS-C)

  vaccine efficacy

  identifying & manufacturing COVID-19 convalescent plasma (CCP)

# Summary

- Antibody Testing – What and When

  Antibody tests are not useful for diagnosis, lack sensitivity

  Useful for assessing exposure if used after at least 14 days post symptom onset (not clear for how long)

  Standalone IgM assays not currently recommended

  IgA assays not currently recommended

  No clear advantage to testing for one antibody class over another, although assays that detect IgM may increase sensitivity for detection of acute cases

  Use an assay with high specificity > 99.5%, orthogonal approach, or only test individuals with high probability of exposure

  No current recommendation for vaccine qualification or follow-up

  Post vaccine testing should be performed 2 weeks post last dose

  Serology testing using a nucleocapsid target can be useful in determining natural infection

# Thank you!