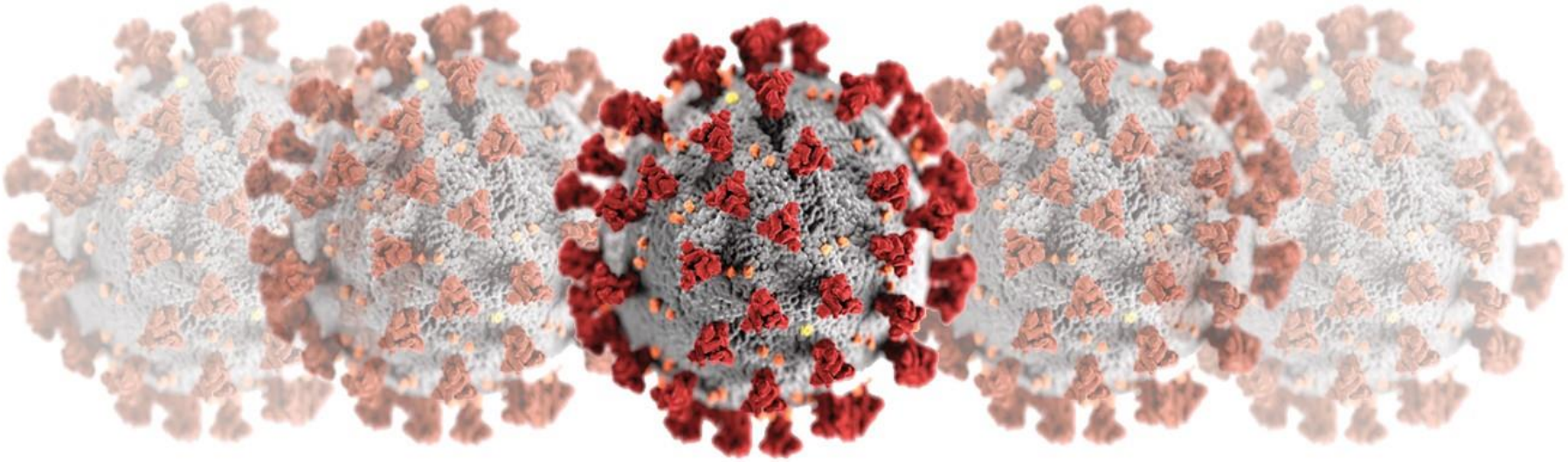
# The Serology of COVID-19:

## The Immune Response, Diagnostics and Potential Therapies



Lynne Sopchak, Ph.D.
Bio2Device Group
April 14, 2020

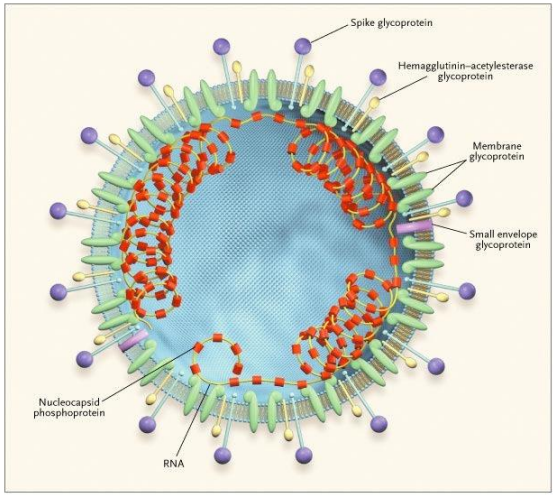
# Outline

- Coronaviruses and history of outbreaks
- Infection
- Immune Response
- Diagnostics
  - Antibody testing
  - Virus testing
- Treatments
  - Passive Immunity
    - Convalescent Plasma
    - Therapeutic Antibodies

# Key Questions

- Why do some experience mild symptoms while for others it is life treating?
  - Viral and host factors affect virulence
    - Viral variants?
    - Prior Coronavirus exposure or vaccination history (i.e. BCG)
    - Human Leukocyte Antigen (HLA) associations
    - Receptor ACE2 /co-receptor TMPRRS2 polymorphisms?
    - Male Predominance --Androgen regulation of TMPRSS2?
- When can we get back to work and normal activities?
  - Understanding SAR-Cov2 biology and the immune response to it
  - Development of diagnostics and treatments/vaccines
- How can I/we help?
  - Shelter-in-place, Be kind to yourself and those around you.
  - If you have been exposed, donation of convalescent serum

# Coronaviruses




- More than 500 unknown coronaviruses in bats; and may be as many 1,000s more
- 7 infect humans; 4 coronavirus HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43 have low pathogenicity, cause mild respiratory symptoms similar to a common cold.

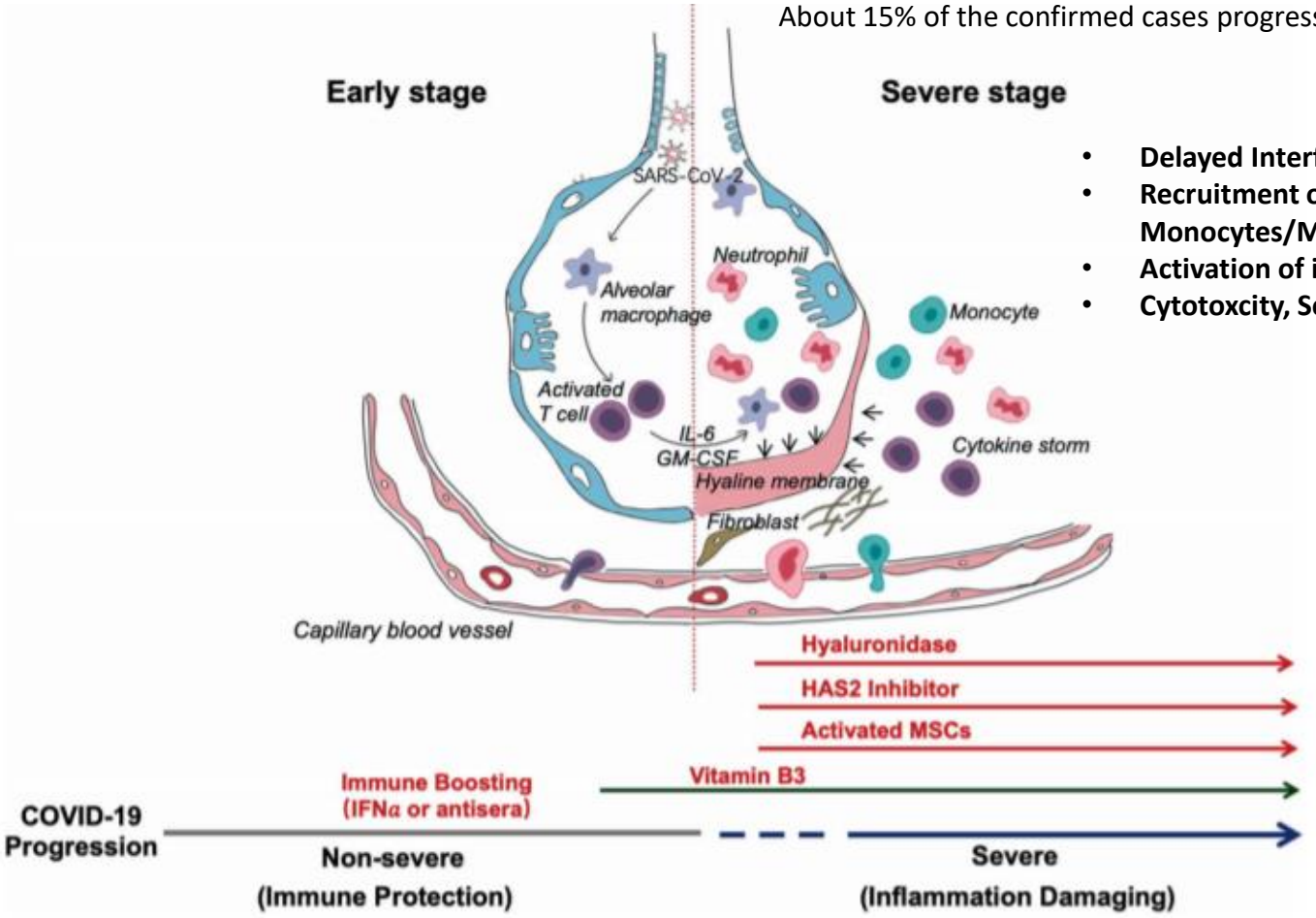
- The transmission of more virulent Coronavirus has occurred 3 time in the last 20years.

| | Year | Intermediate host | Cases | Deaths | Death rate |
|---|---|---|---|---|---|
| **SARS** | 2003 | Bats/civert cats | 8,098 | 774 | 9.6% |
| **MERS** | 2012 | Bats/camels | 2521 | 866 | 34% |
| **COV19** | 2019 | Bats/? | 1,930353 | 119985 | ?% |

# Progression of COVID-19 infection

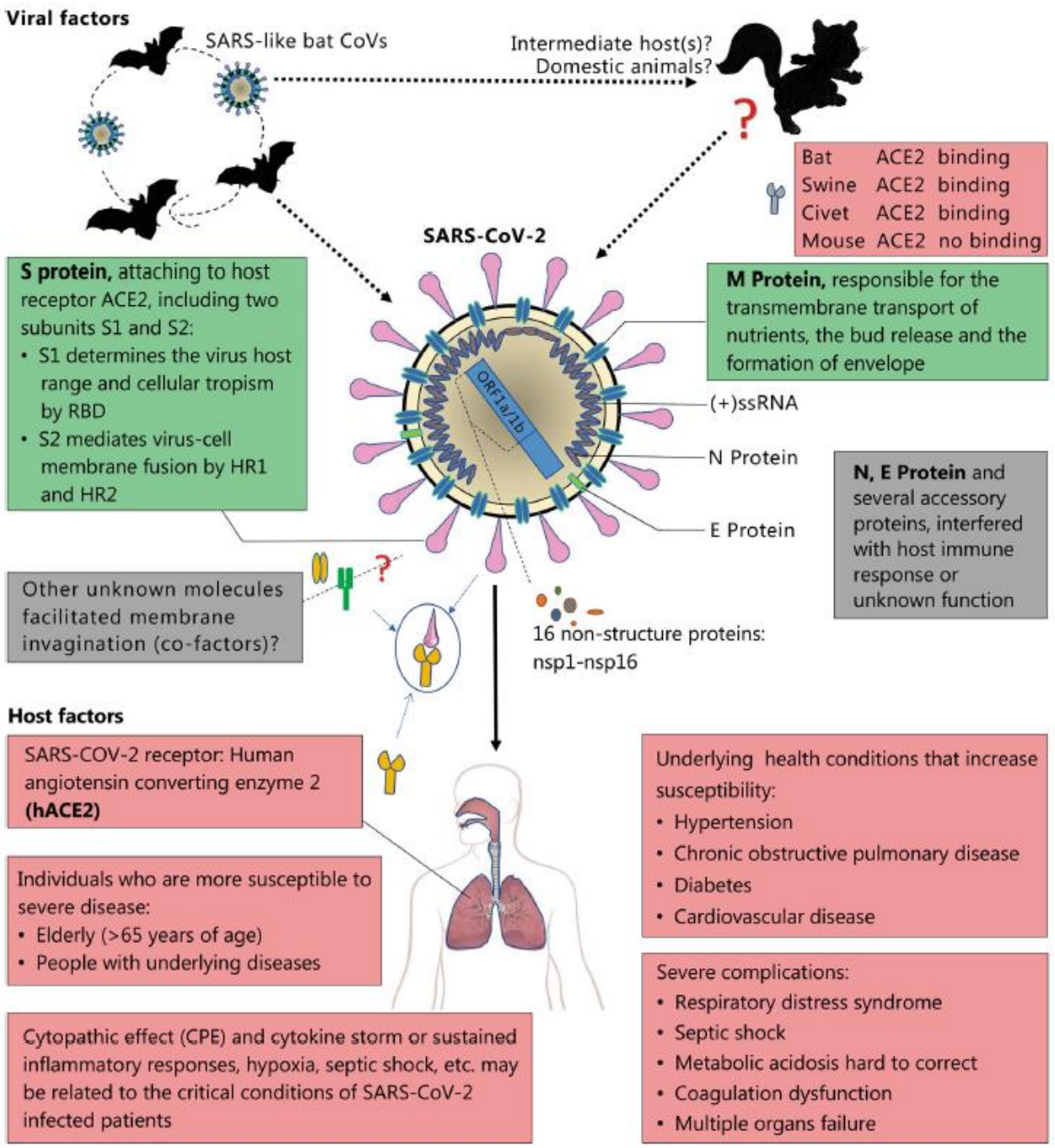
## Potential Adjuvant Interventions

About 15% of the confirmed cases progress to the severe phase




- **Delayed Interferon response**
- **Recruitment of inflammatory Monocytes/Macrophages**
- **Activation of innate immune response**
- **Cytotoxcity, Sepsis, Cytokine Storm**

https://www.nature.com/articles/s41418-020-0530-3.pdf



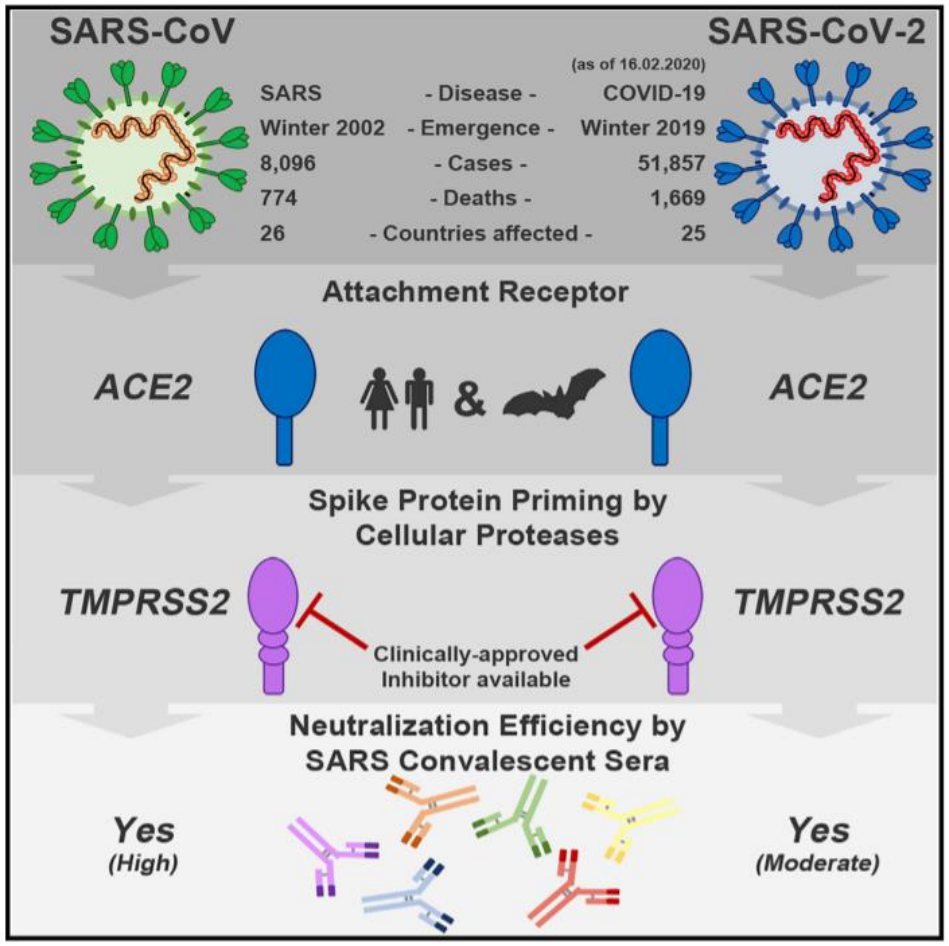

https://doi.org/10.1186/s40779-020-00240-0

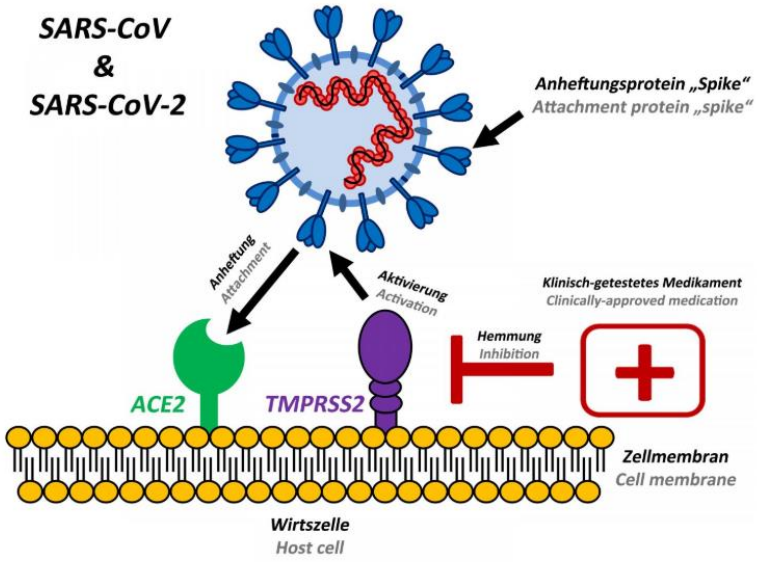
# Viral and Host Factors

Viral factors
- Viral variants?

Host Factors
- HLA-associations?
- ACE2 or TMPSSR2 polymorphisms?
- Immune Status

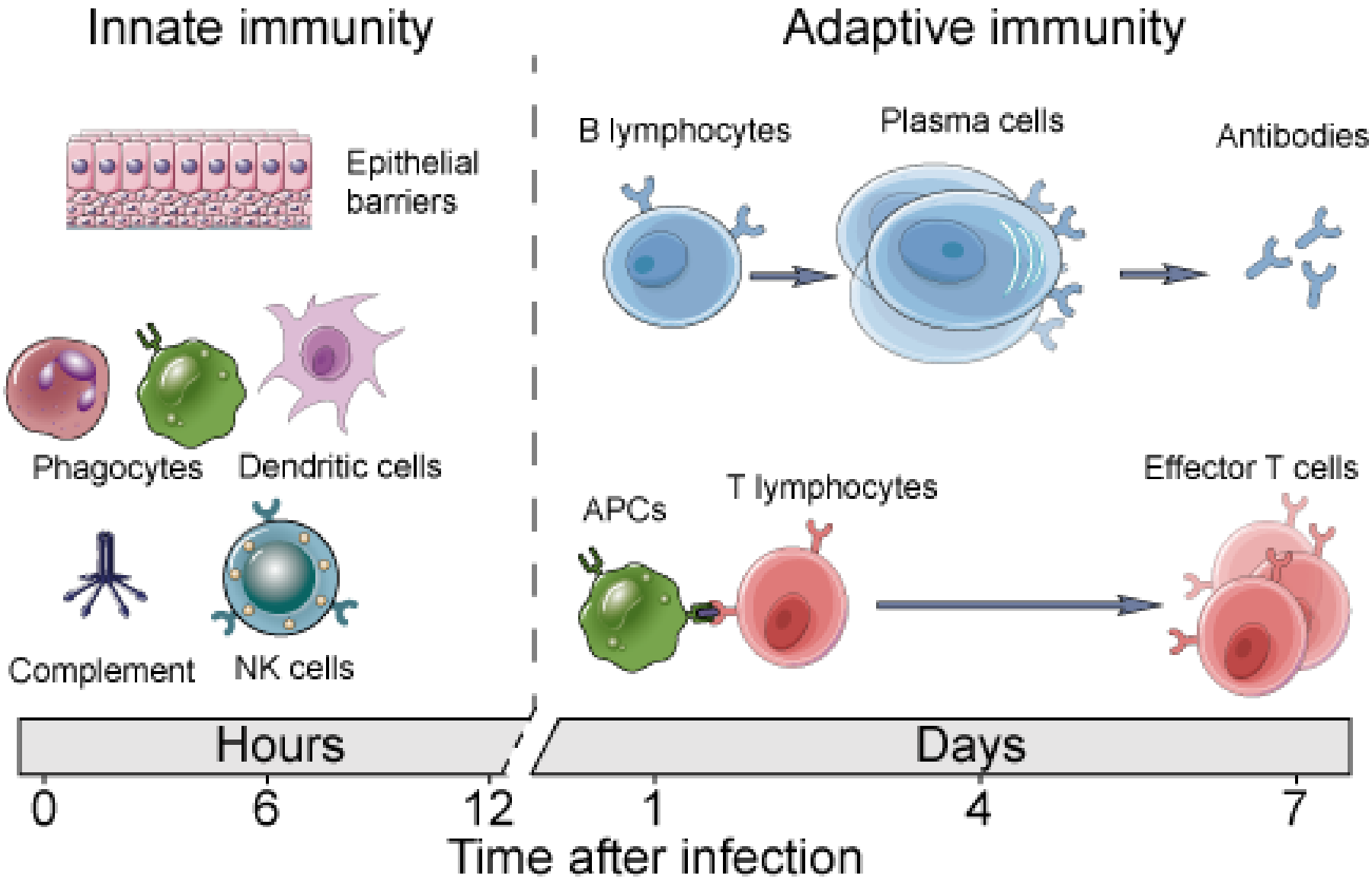
# SARS viral entry



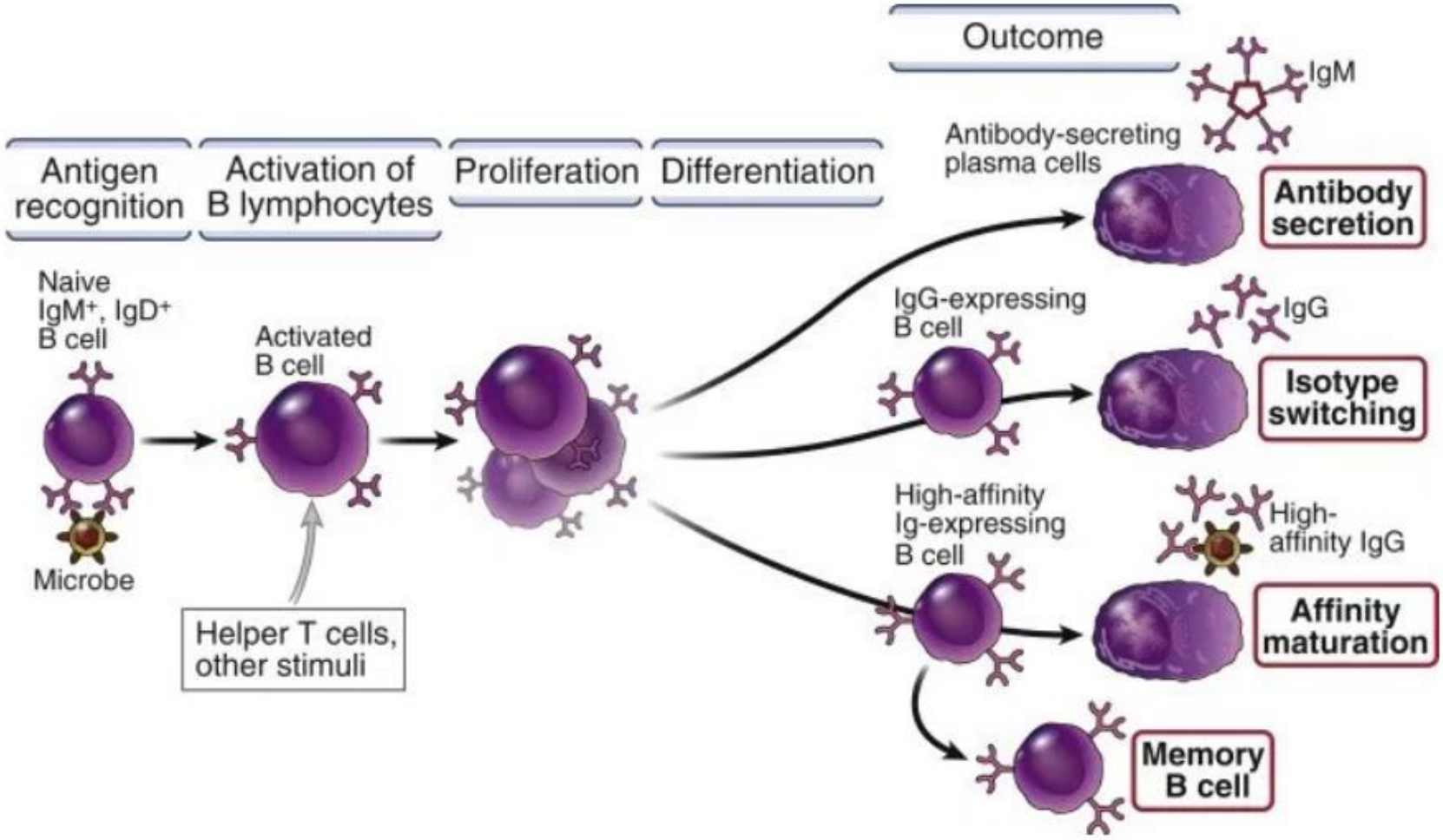




Clinical Trials
TMPRSS2 inhibitor: Camostat
Viral inhibitors: Remdesivir, Favipiravir
Immunomodulator: Lambda
Convalescent Plasma

https://doi.org/10.1016/j.cell.2020.02.052


Innate immunity
Adaptive immunity
Epithelial barriers
B lymphocytes
Plasma cells
Antibodies
Phagocytes
Dendritic cells
APCs
T lymphocytes
Effector T cells
Complement
NK cells
Hours
Days
0
6
12
1
4
7
Time after infection

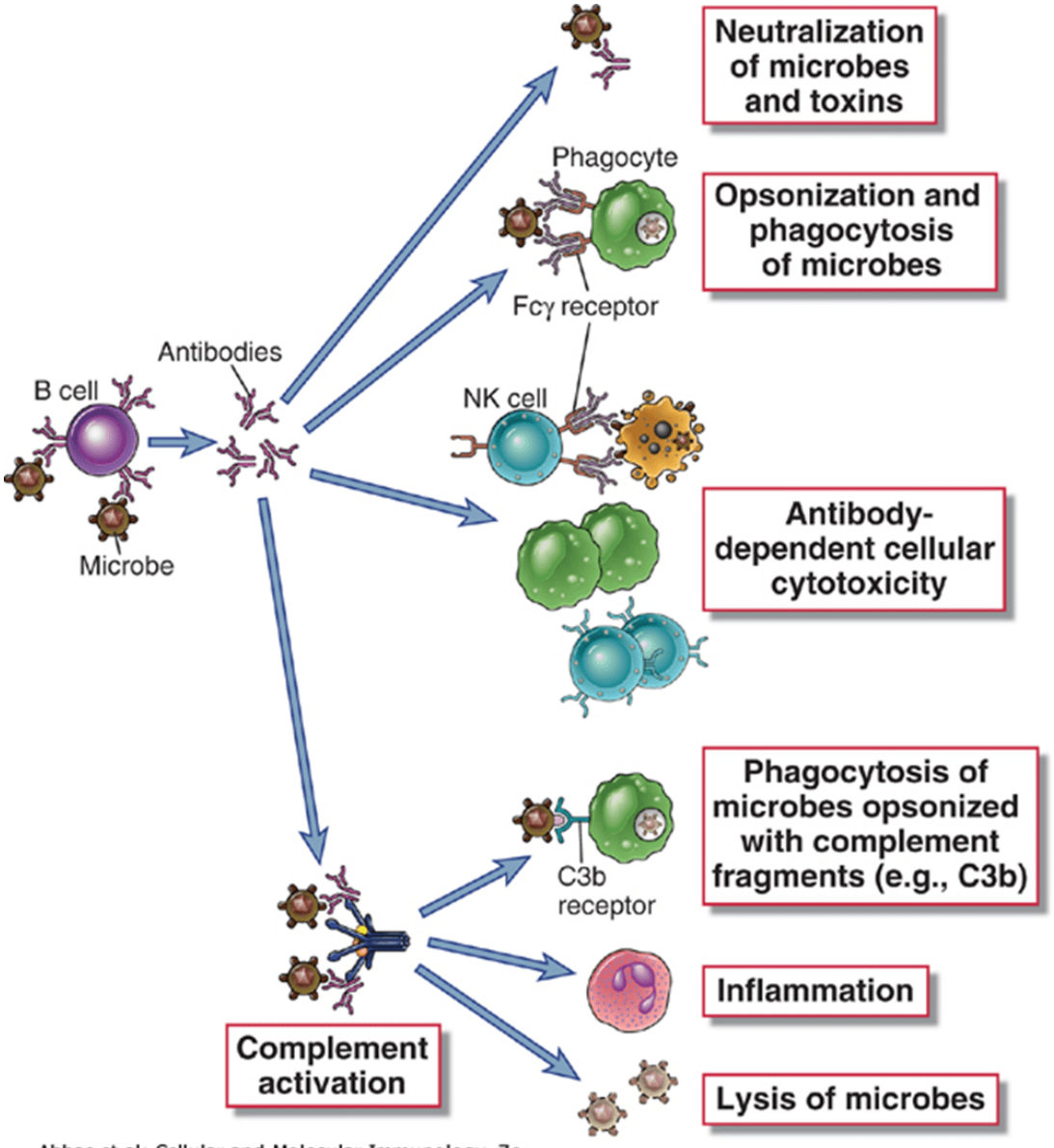
# Maturation of the B cell Response



# How do Antibodies Protect Us?

- **Neutralization**
  - Blocks viral entry
- **Opsonization**
  - Coat the viral particles and aid in phagocytosis/engulfment by immune cells
- **Antibody Dependent Cytotoxicity**
  - Bind to cells expressing viral proteins and facilitate T cytotoxic cells in killing of infected cells
- **Complement Cascade Activation**
  - A cascade of proteins than enhance the immune response through promoting opsonization, cell lysis, chemotaxis and activate cells that mediate inflammation

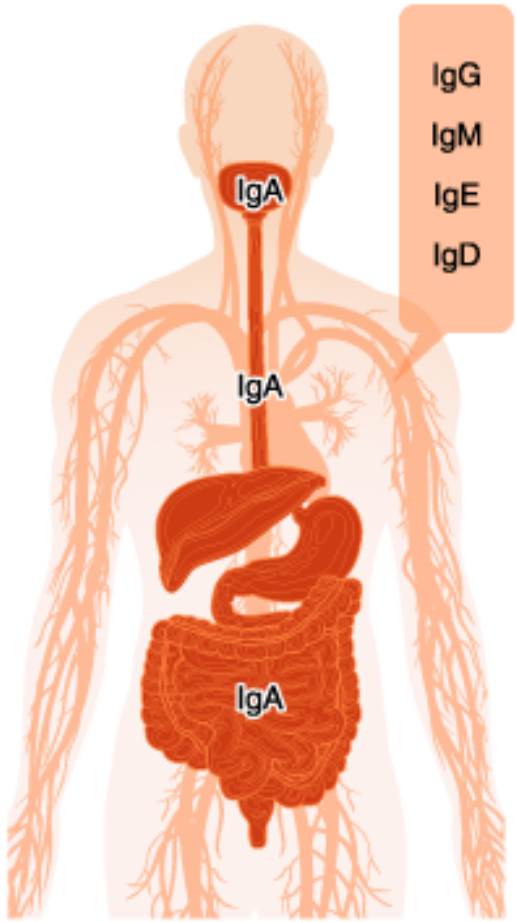
# How do Antibodies Protect Us?



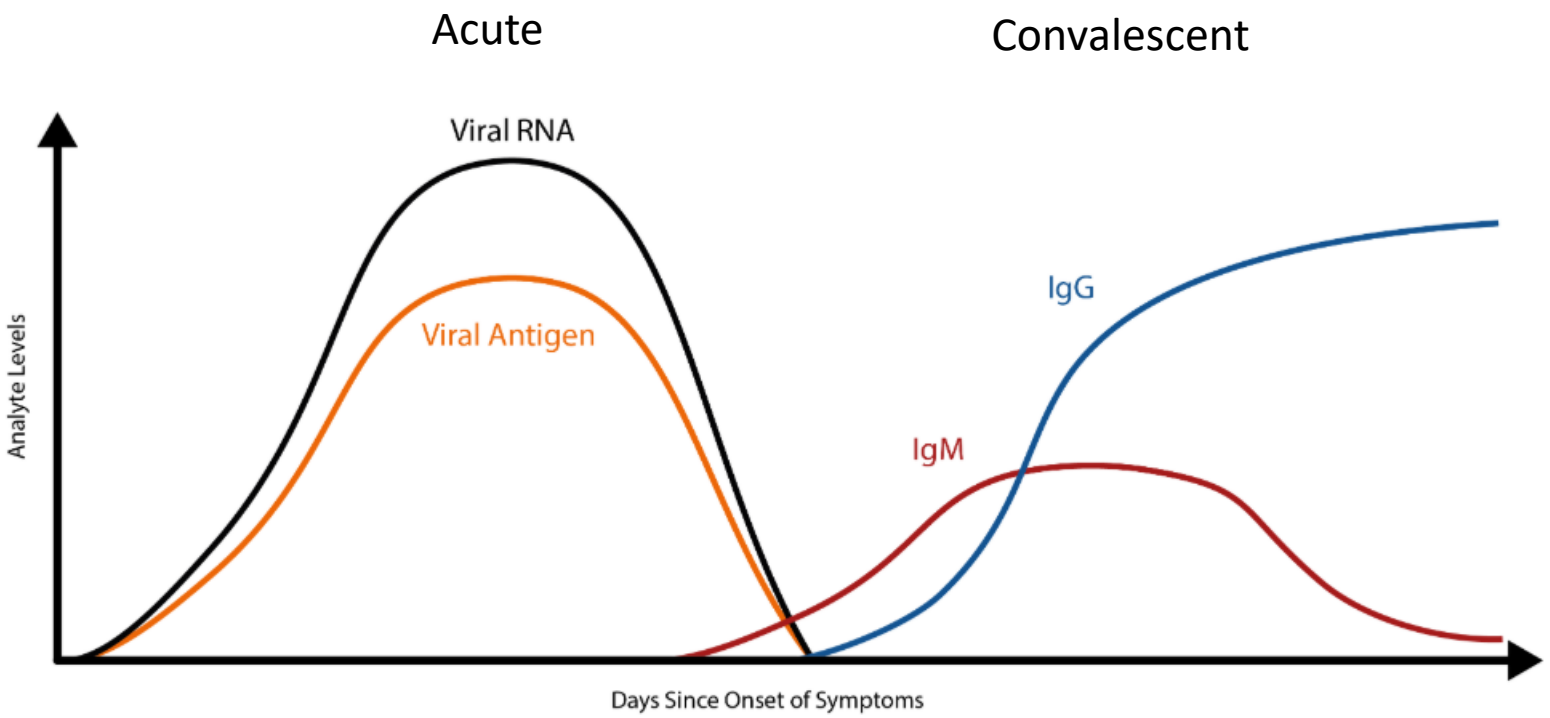
## Types and characteristics of antibodies

| | | |
|---|---|---|
| IgG |  | • Highest opsonization and neutralization activities.<br>• Classified into four subclasses (IgG1, IgG2, IgG3, and IgG4). |
| IgM |  | • Produced first upon antigen invasion. Increases transiently. |
| IgA |  | • Expressed in mucosal tissues. Forms dimers after secretion. |
| IgD |  | • Unknown function. |
| IgE |  | • Involved in allergy. |

## Distribution in the body



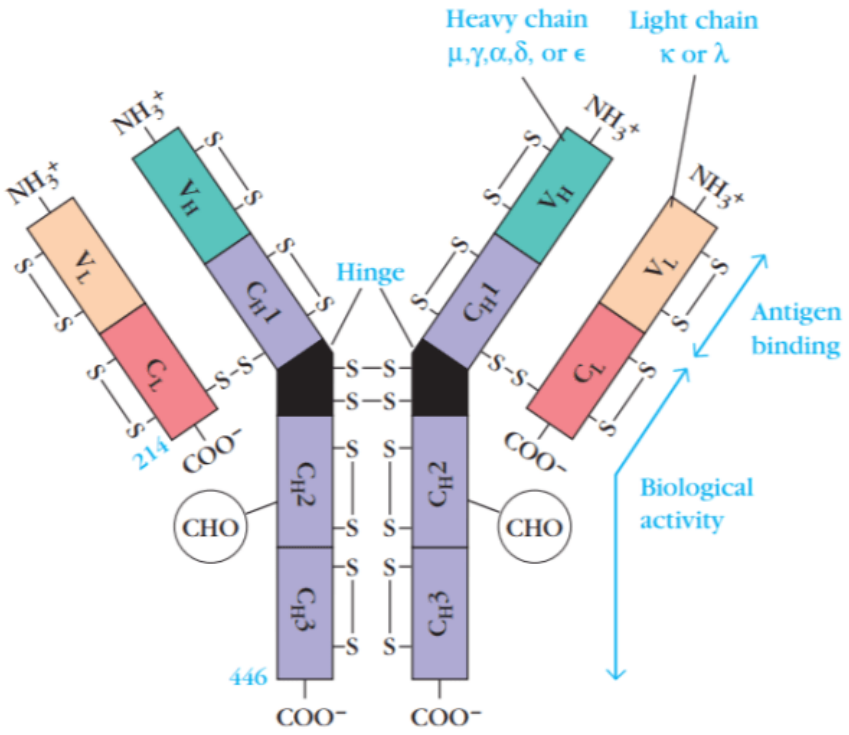
# Expected Time Course of COVID-19 Infection




https://thenativeantigencompany.com/why-we-need-antigen-and-antibody-tests-for-covid-19/

# Antibodies

Immune response, Diagnostic tools, Therapeutics




- An Indicator of a productive immune response
  - IgM & IgG titers
  - Neutralizing Ab = protection
- Diagnostic Tools in Immunoassays
  - Antigen binding site provides unique specificity
- Therapeutics
  - Passive Immunotherapies
    - Convalescent Plasma
    - Monoclonal Antibodies
  - Active Immunotherapies
    - Vaccines

# Emergency Use Authorizations (EUAs) for the Development of Viral and Serologic Testing

- Emergency Use Authorizations (EUAs)
  - EUA allows you to sell your product right away, then you send them your data, and if they don't like it, you will have to take your product back
  - FDA approval still required for commercialization
- Multi-effort Task Force has been formed to evaluate new tests
  - BARDA, FDA, NIH, DOD, OSTP, CDC
  - Standardized panel of serum 30-40 (+), 75 (–) samples for test evaluation within the next 3 weeks.
- Clinical Laboratory COVID-19 Response Weekly Calls-Every Monday at 12pm PST/3pmEST

https://www.cdc.gov/safelabs/resources-tools/covid-19-weekly-clinical-calls.html

Coronavirus
COVID-19

# PRIORITIES FOR TESTING PATIENTS WITH SUSPECTED COVID-19 INFECTION

## COVID-19 Symptoms: Fever, Cough, and Shortness of Breath

### PRIORITY 1

**Ensures optimal care options for all hospitalized patients, lessen the risk of healthcare-associated infections, and maintain the integrity of the U.S. healthcare system**

- Hospitalized patients
- Healthcare facility workers with symptoms

1

2

### PRIORITY 2

**Ensures those at highest risk of complication of infection are rapidly identified and appropriately triaged**

- Patients in long-term care facilities with symptoms
- Patients 65 years of age and older with symptoms
- Patients with underlying conditions with symptoms
- First responders with symptoms

### PRIORITY 3

**As resources allow, test individuals in the surrounding community of rapidly increasing hospital cases to decrease community spread, and ensure health of essential workers**

- Critical infrastructure workers with symptoms
- Individuals who do not meet any of the above categories with symptoms
- Healthcare facility workers and first responders
- Individuals with mild symptoms in communities experiencing high numbers of COVID-19 hospitalizations

3

NON-PRIORITY

### NON-PRIORITY

- Individuals without symptoms

For more information visit: coronavirus.gov

# Fast, Portable Tests Come Online to Curb Coronavirus Pandemic

| Antibody Detection | Virus Detection |
|---|---|
| Cellex | PCR-Based |
| Biolidics | Luminex |
| Biomedomics, Becton Dickinson | Abbott |
| Guangzhou Wondfo Biotech | Cepheid |
| Innovita Biological Technology, Scanwell Health | CRISPR-Based |
| Jiangsu Medomics Medical Technologies | Mammoth Bioscience |
| Pharmact | Sherlock Biosciences, Cepheid |
| Snibe Diagnostic | Caspr Biotech |
| Zhejiang Orient Gene Biotech, Aytu Bioscience | Antigen Detection |
| Biomerica | Sona Nanotech, GE Healthcare Life Sciences |
| Sugentech | |
| Xiamen AmonMed Biotechnology | |
| Predictive Laboratories | |
| Ortho Clinical Diagnostics | |

https://www.nature.com/articles/d41587-020-00010-2 , http://www.centerforhealthsecurity.org/resources/COVID-19/serology/Serology-based-tests-for-COVID-19.html

# Assay Development

## Consideration & Challenges

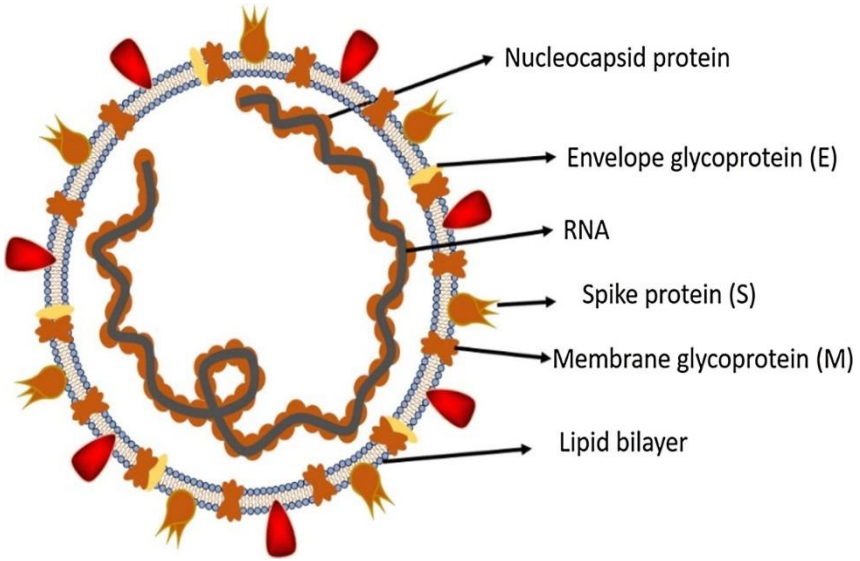
## Creating a sensitive and specific ImmunoAssay

- Choosing an antigen is critical
  - Unique, the spike most divergent but nucleocapsid is the most abundant
  - Does not cross react with other coronaviruses
- Selecting secondary antibody reagents
  - https://rockland-inc.com/choosing-a-secondary-antibody.aspx
- Picking the appropriate platform
  - Rapid diagnostic test, ELISA or Neutralizing Antibody test

www.thelancet.com vol395 1101-1102 4/4/20

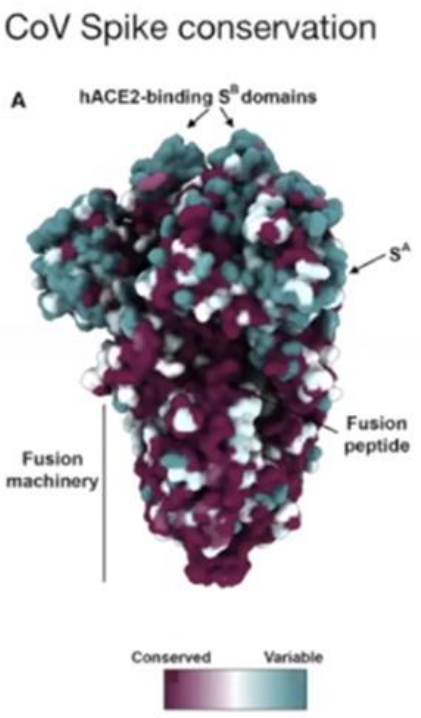
# Assay Development
## Antigen selection is critical

Nucleocapsid protein is most abundant




Shereen et al 2020 https://doi.org/10.1016/j.jare.2020.03.005

Spike protein is the most divergent




Walls et al., 2020 https://doi.org/10.1016/j.cell.2020.02.058

# Antibody Assay Platforms

| Type of test | Time to results | What it tells us | What it cannot tell us |
|---|---|---|---|
| Rapid diagnostic test (RDT) | 10-30 minutes | The presence or absence (qualitative) of antibodies against the virus present in patient serum. | The quantifiable amount of antibodies in the patient serum, or if these antibodies are able to protect against future infection |
| Enzyme linked immunosorbent assay (ELISA) | 1-5 hours | The presence or absence (quantitative) of antibodies against the virus present in patient serum. | If the antibodies are able to protect against future infection. |
| Neutralization assay | 3-5 days | The presence of active antibodies in patient serum that are able to inhibit virus growth ex vivo, in a cell culture system. Indicates if the patient is protected against future infection. | It may miss antibodies to viral proteins that are not involved in replication. |

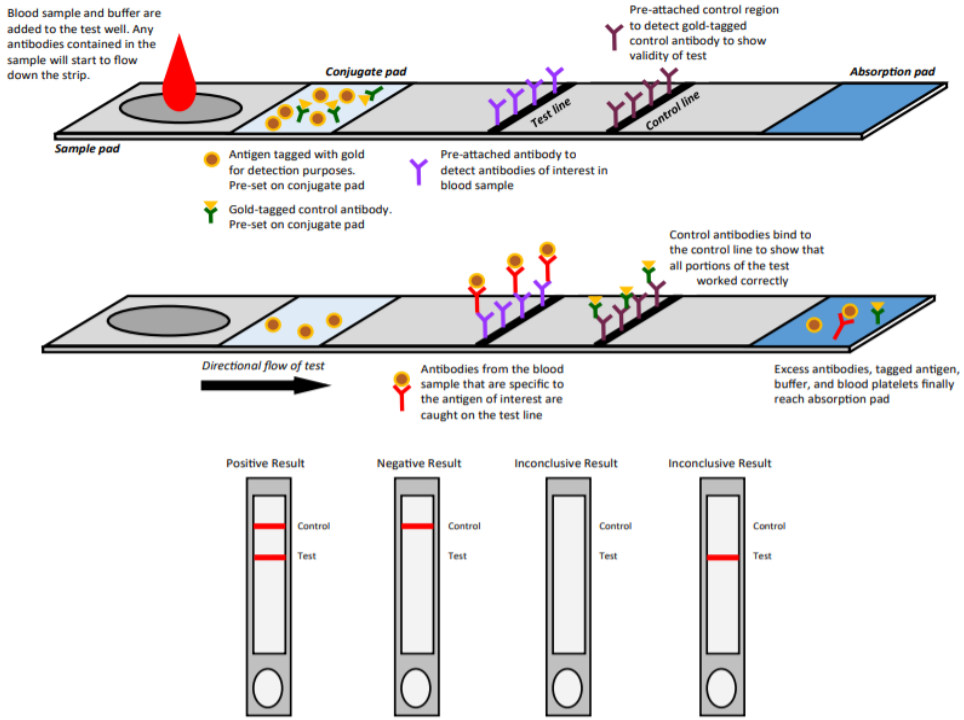
http://www.centerforhealthsecurity.org/resources/COVID-19/serology/Serology-based-tests-for-COVID-19.html

# Cellex: First FDA Approved Rapid Diagnostic Test

## EUA Approval for Diagnostics Use in the US

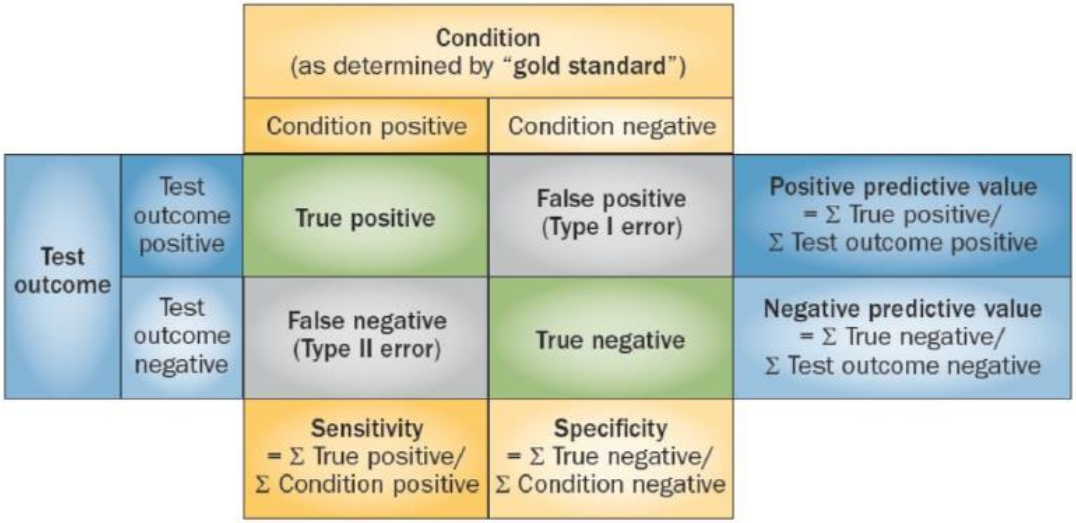

- Detects IgM and IgG to the nucleocapsid protein of SARS-CoV-2.
- Sensitivity is 93.8% and specificity is 95.6%
- Tested at 2 Chinese hospitals in a total of 128 COVID19 positive patients, and 250 COVID19 negative patients as detected by RT-qPCR.

Lateral Flow Chromatographic Immunoassay




http://www.centerforhealthsecurity.org/resources/COVID-19/serology/Serology-based-tests-for-COVID-19.html

# Sensitivity, Specificity, Prevalence and Predictive Value




The mathematical relationship between the predictive value of a biomarker, sensitivity, specificity and prevalence is defined by Bayes Theorem, which mathematically can be reduced to the following equations:

**PPV** = (sensitivity)(prevalence)/
(sensitivity)(prevalence)
+ (1 – specificity)(1 – prevalence)

**NPV** = (specificity)(1 – prevalence)/
(specificity)(1 – prevalence)
+ (1 – sensitivity)(prevalence)

Predictive value (probability that the patient actually has the disease) is typically more important to a doctor & patient than sensitivity and specificity of the test

Lo, Kaplan and Kirk *Nature Reviews Nephrology* 10, 215 (2014).

# Impact of Prevalence on Predictive Value

**Assuming you have a test with 95% sensitivity and 95% specificity**

| Disease Prevalence in the Intended Test Population | Probability of having the Disease if you have a Positive Result |
|---|---|
| 0.1% | 1.9% |
| 1% | 16% |
| 10% | 68% |
| 20% | 83% |
| 50% | 95% |

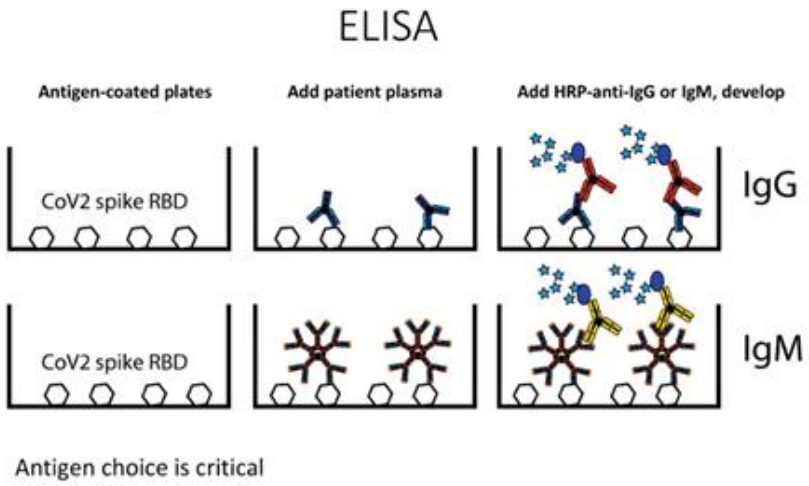
- If prevalence is very low even if sensitivity and specificity are high, test results will have high false positive rate
- The Context of use determines the PPV or NPV

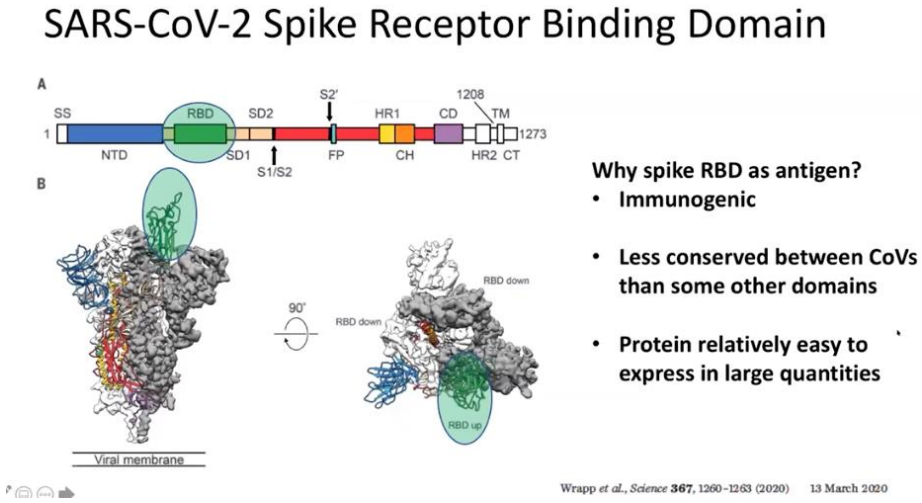
**Predictive Value is not intrinsic to the test. It depends on the prevalence of disease**

# Antibody Testing is Multi-Purpose

- Contact tracing weeks or longer after a suspected infection in an individual.
- Verify that vaccines are working as intended during clinical trials
- Determine how many asymptomatic cases have occurred in a population to help inform public policy makers

# Stanford Rolls Out Antibody Testing




On April 3rd & 4th, >2500 samples from volunteers for a study to examine the antibodies in the blood from the population representative to gauge the percentage of residents that have been exposed to SAR-Cov-2

500 tests/day with a 2-3day turnaround

https://www.youtube.com/watch?time_continue=1&v=Xm76adKULY4&feature=emb_logo

# Antibody Testing used in Conjunction with Viral Testing to investigate SARS-CoV-2 Infection & the Immune Response

## Interpretation

**IgM neg, IgG neg**:

- No evidence for prior exposure to SARS-CoV-2, but cannot exclude active infection.
- Negative IgM and IgG expected in the initial days after infection.
- Some patients infected by CoV-2 have negative IgG for more than two weeks post-onset of symptoms.
- Correlation with NP swab PCR recommended to assess for active infection.

**IgM pos, IgG neg**:

- Exposure to SARS-CoV-2, and could indicate early infection.
- Correlation of the results with clinical findings and NP swab PCR recommended to assess for active infection.

**IgM neg, IgG pos**:

- Exposure to SARS-CoV-2. This result does not exclude active infection.
- Correlation with clinical findings and NP swab PCR recommended to assess for active infection.
- May have protective IgG immunity to the virus, but the requirements for immunity are not yet known.

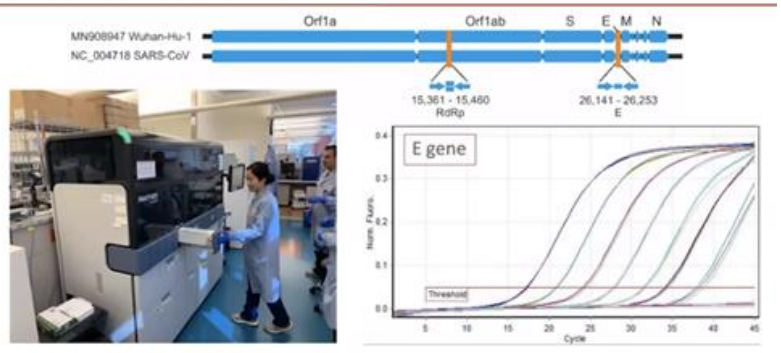
**IgM pos, IgG pos**:

- Exposure to SARS-CoV-2. This result does not exclude active infection.
- Correlation with clinical findings and NP swab PCR recommended to assess for active infection.
- May have protective IgG immunity to the virus, but the requirements for immunity are not yet known.

https://www.youtube.com/watch?time_continue=1&v=Xm76adKULY4&feature=emb_logo

# Stanford Ramps Viral Testing

## SARS-CoV-2 (COVID-19) rRT-PCR




LABSARSCOV2
Current Capacity >2000 tests per day
Turnaround Time <24 hours (mean = 9 hours)

**Stanford Laboratory-developed test & Hologic Panther Fusion® Assays**

## Cepheid GeneXpert SARS-CoV-2 Assay

Led by
Jason Kurzer, MD


LAB**STAT**COV2
Turnaround Time ~90 min

## Clinical Pathology: Facilitating Collaborative COVID-19 Research

- Bonnie Maldonado, Andra Blomkans
  - Serial SARS-CoV-2 Shedding and Viral Load During Validation of Nasal Swab Self-collection
- Kari Nadeau, Neera Ahuja
  - A Multicenter, Adaptive, Randomized Blinded Controlled Trial of the Safety and Efficacy of Investigational Therapeutics for the Treatment of COVID-19
  - A Phase 3, Randomized, Placebo-Controlled Study of Lenzilumab in Hospitalized Patients with COVID-19 Pneumonia
- Euan Ashley, Carlos Bustamante, Manuel Rivas, Vicki Parikh
  - Genomic epidemiology of SARS-Cov-2 and host genetics in COVID-19
- Mike Snyder
  - Determining pathogenicity of various variants and strains of COVID-19
- Ami Bhatt
  - Investigation of possible oral-fecal transmission of SARS-CoV-2 in the Bay Area
- Jason Andrews
  - Genomic epidemiologic investigation of SARS-CoV-2 transmission
- Taia Wang
  - Serologic Response to SARS-CoV-2
- Aruna Subramaniam, Phil Grant, Shanthi Kappagoda
  - Remdesevir for hospitalized adults with COVID-19
- Julie Parsonnet, Upi Singh
  - A Phase 2 Randomized, Open Label Study of a Single Dose of Peginterferon Lambda-1a Compared with Placebo in Outpatients with Mild COVID-19

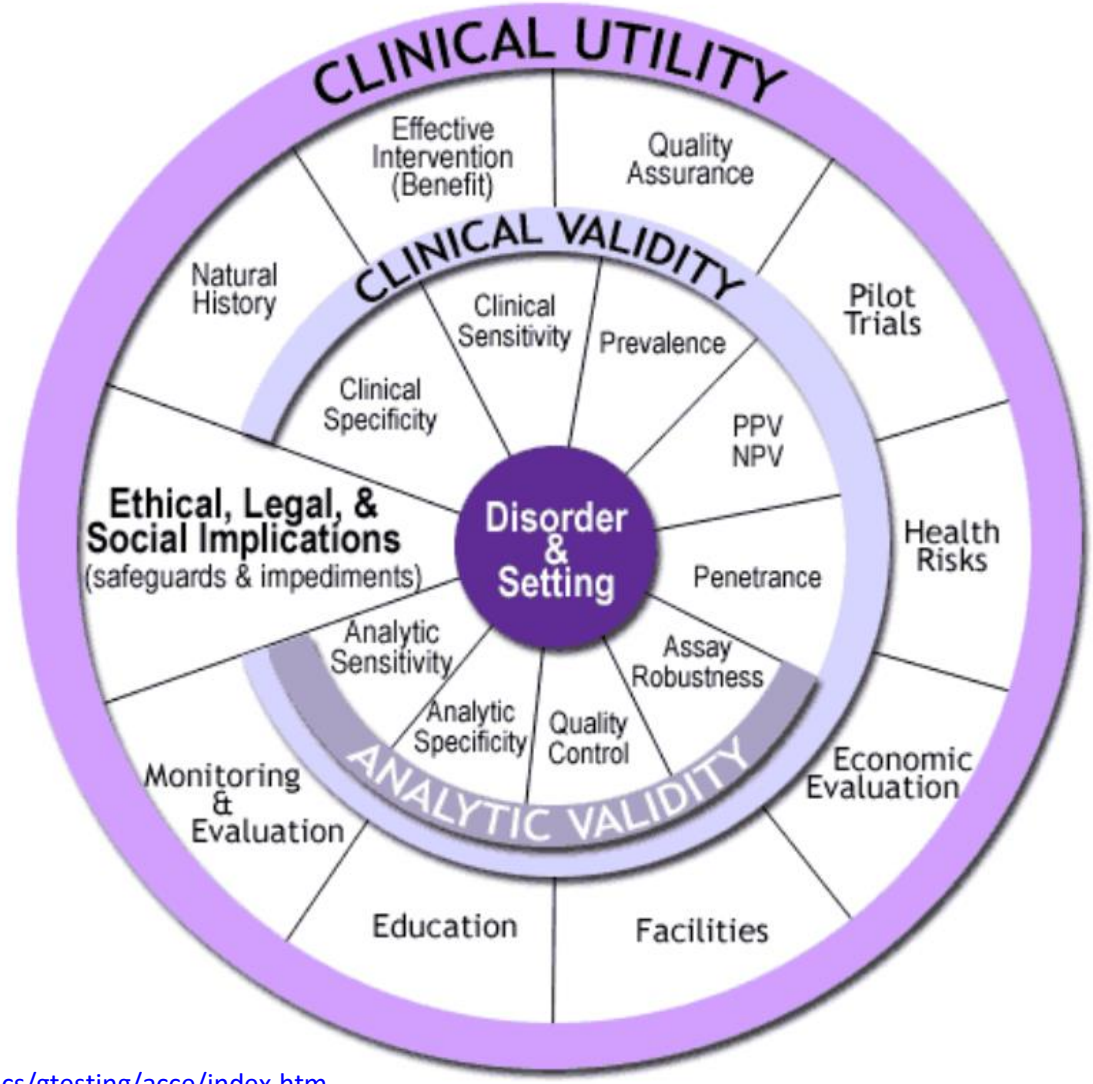
## Indications for Rapid SARS-CoV-2 rRT-PCR

- Emergency Department PUIs prior to admission
- Intensive Care & Inpatient PUIs
- Patients prior to discharge to congregate setting (e.g. SNF, jail, etc.)
- Symptomatic patients undergoing active chemo- or radiation therapy
- Patients prior to transplant (e.g. solid organ, bone marrow, stem cell, etc.)
- Pre-op patients prior to high-risk surgery
- Organ donors prior to harvest
- Symptomatic patients in labor
- Patient selection for COVID clinical trials

https://www.youtube.com/watch?time_continue=1&v=Xm76adKULY4&feature=emb_logo

# Assay Validation

## Analytical Validity, Clinical Validity & Clinical Utility




https://www.cdc.gov/genomics/gtesting/acce/index.htm

# Immunotherapies

- **Convalescent Plasma**

  **Donations**

  - Stanford Blood Center https://stanfordbloodcenter.org/convalescent-plasma-from-recovered-covid-19-patients/
  - Vitalant https://vitalant.org/COVIDFree
  - COVID-19 Convalescent Plasma Project (CCPP19) Leadership Group https://ccpp19.org/
    - Physicians and scientists from 57 institutions in 46 states who have self-organized for the purpose of investigating the use of convalescent plasma in the current COVID-19 pandemic
- **Therapeutic Neutralizing Antibodies**
  - Distributed Bio, AstraZeneca, Vir Biotechnology, Sanguine Biosciences
- **Vaccines**
  - Moderna

# Unanswered Questions

What will seroconversion look like for SAR-Cov-2?

How long will immunity last?  Will it be protective?

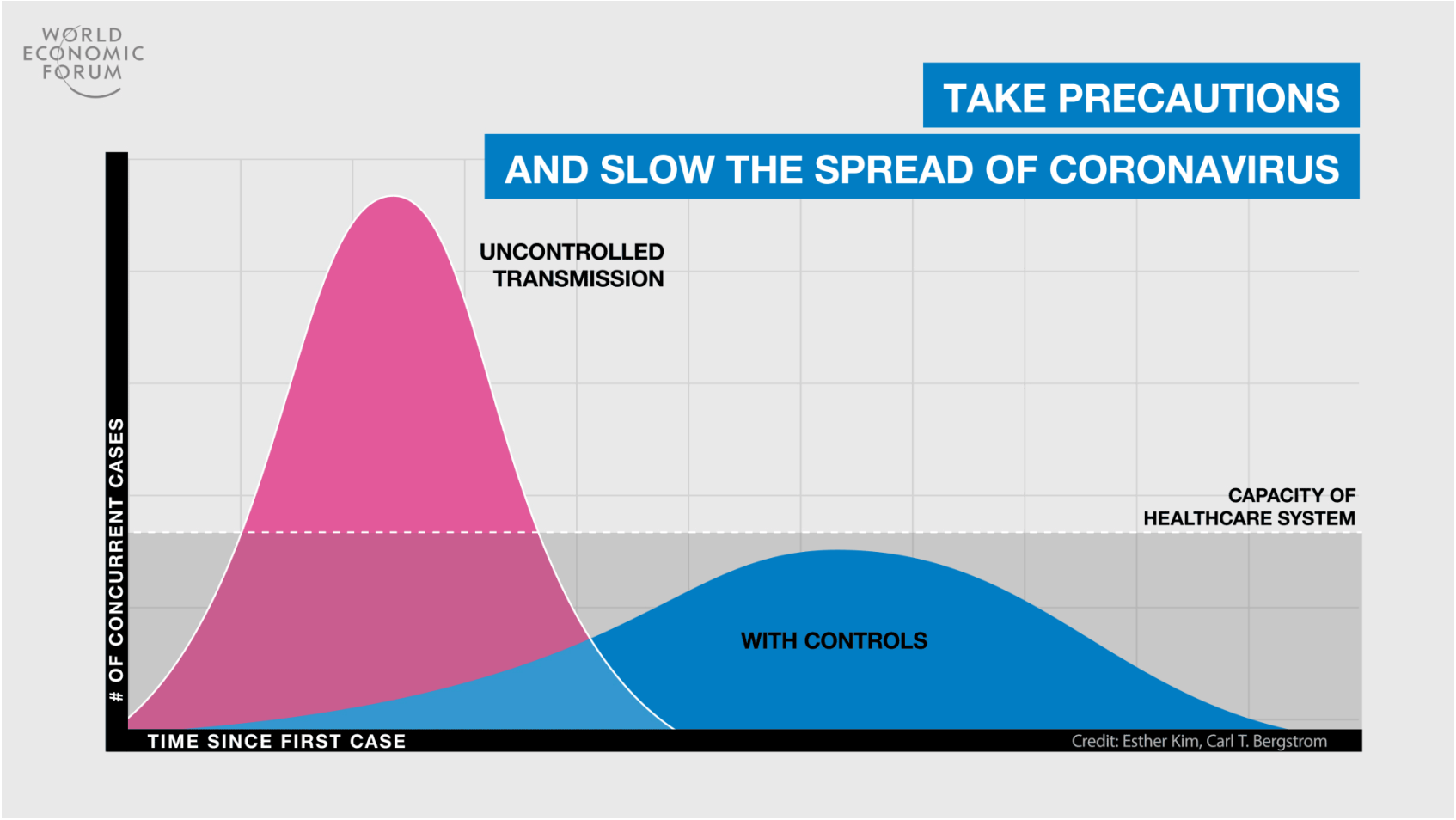
Do people who had mild or no symptoms gain the same protection?

Will the virus mutate to evade the immune response?

How quickly will wide spread testing give us a better understanding of this disease?

Which treatments (repurpose drugs, convalescent plasma, therapeutic neutralizing antibodies or vaccines) will decrease mortality in the seriously ill and provide safety to the broader population?

# Thank you for patience
# & doing you part to flatten the curve



# How do I distinguish between COVID-19 and other respiratory illnesses?

| Symptom | COVID-19 | Common Cold | Flu | Allergy |
|---|---|---|---|---|
| Fever | Common | Rare | Common | Occasional |
| Dry Cough | Common | Mild | Common | Occasional |
| Shortness of Breath | Common | No | No | Common |
| Headache | Occasional | Rare | Common | Occasional |
| Aches and Pains | Occasional | Common | Common | No |
| Sore Throat | Occasional | Common | Common | No |
| Fatigue | Occasional | Occasional | Common | Occasional |
| Diarrhoea | Common | No | Occasional | No |
| Runny Nose | Rare | Common | Occasional | Common |
| Sneezing | No | Common | No | Common |

# References


**Coronaviruses 101: Focus on Molecular Virology** https://www.youtube.com/watch?v=8_bOhZd6ieM

**Why it's too early to start giving out "immunity passports"**
https://www.technologyreview.com/2020/04/09/998974/immunity-passports-cornavirus-antibody-test-outside/

**TMPRSS2 and COVID-19: Serendipity or opportunity for intervention?** DOI: 10.1158/2159-8290.CD-20-0451

**Why We Need Antigen and Antibody Tests for COVID-19**
https://thenativeantigencompany.com/why-we-need-antigen-and-antibody-tests-for-covid-19/

**Fast, portable tests come online to curb coronavirus pandemic**
https://www.nature.com/articles/d41587-020-00010-2

**Developing antibody tests for SARS-CoV-2** www.thelancet.com vol395 1101-1102 4/4/20

**COVID-19 infection: the perspectives on immune responses** https://www.nature.com/articles/s41418-020-0530-3

**Deployment of convalescent plasma for the prevention and treatment of COVID-19**
https://doi.org/10.1172/JCI138745.