Coronavirus-Disease 19 (COVID-19) & SARS-CoV-2 Testing

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Outline

- ## COVID-19

  - Clinical presentation

  - SARS-CoV-2 virus basics

  - Numbers, trends & population immunity

- ## SARS-CoV-2 & Testing

  - Test types & interpretation

- ## Take Home Points

Timeline of a Pandemic

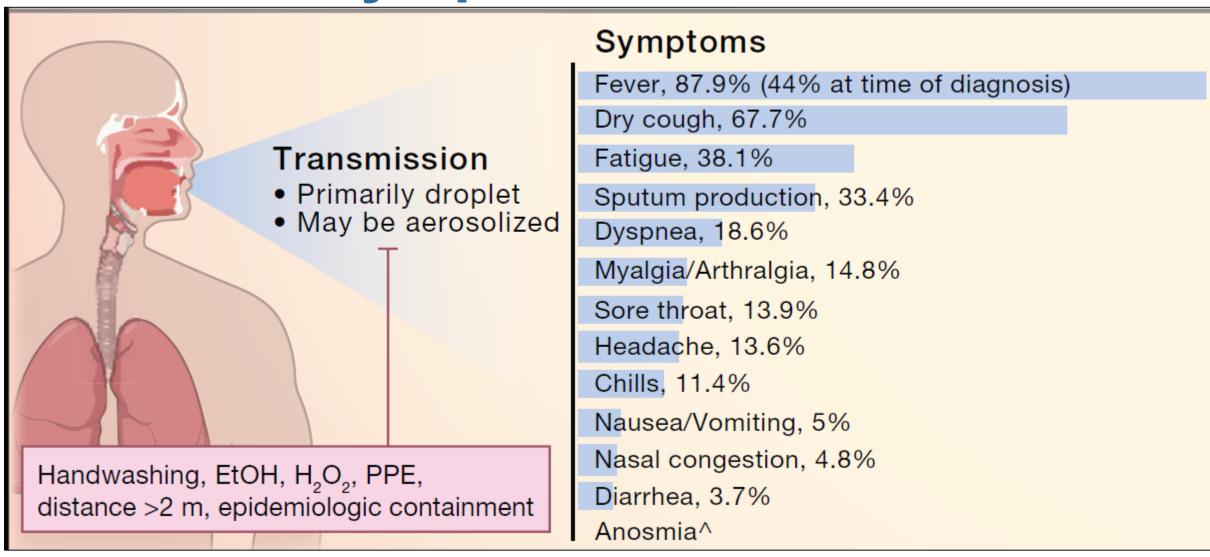| Dec 2019	Jan 2020	Feb 2020	Mar 2020	April 2020	May 2020
Cluster of pneumonia cases in Wuhan, China. Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) identified.	Travelers returning from Wuhan to the US treated & self-isolated at home for 2 weeks. First community spread in US between a traveler & spouse.	First COVID-19 related death in the US in a person with community transmission. Additional deaths in WA state in nursing home in Seattle suburb & Seattle hospital.	WHO declared COVID-19 a pandemic. Restrictions established on state & local levels in US. Coronavirus Aid, Relief, & Economic Security Act signed into law.	SARS-CoV-2 testing in the US increased dramatically to ~199 thousand tests/day. Coordinated research for vaccine & treatments.	Out of 14 million completed tests, 1.77 million confirmed cases & ~103 thousand deaths. *NEJM* report of remdesivir for COVID-19 treatment.

COVID-19 Symptoms

Transmission
- Primarily droplet
- May be aerosolized

Handwashing, EtOH, H_2O_2, PPE, distance >2 m, epidemiologic containment

Symptoms

Fever, 87.9% (44% at time of diagnosis)

Dry cough, 67.7%

Fatigue, 38.1%

Sputum production, 33.4%

Dyspnea, 18.6%

Myalgia/Arthralgia, 14.8%

Sore throat, 13.9%

Headache, 13.6%

Chills, 11.4%

Nausea/Vomiting, 5%

Nasal congestion, 4.8%

Diarrhea, 3.7%

Anosmia^

Oberfeld B, et al. SanpShot: COVID-19. *Cell* (2020)
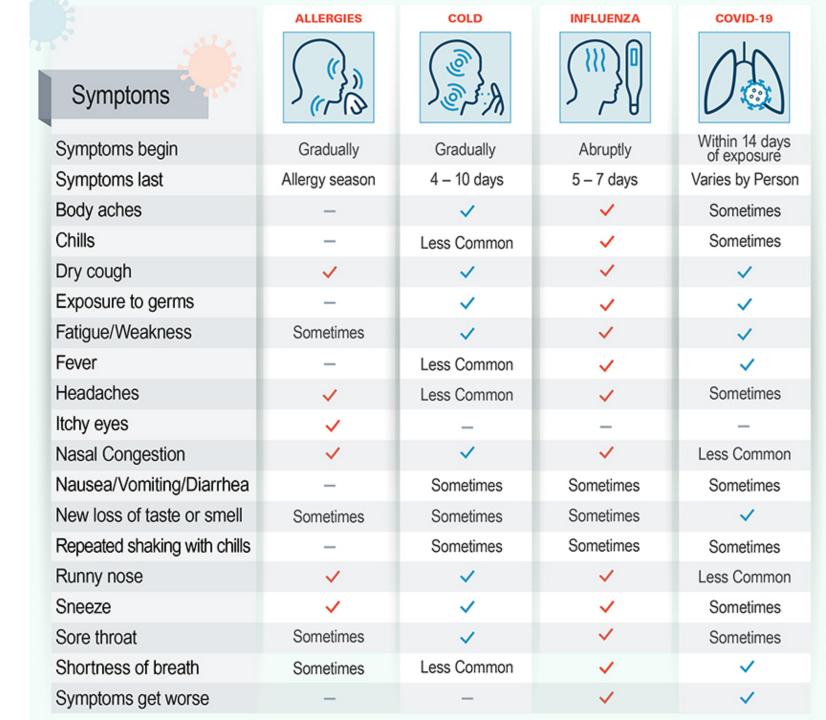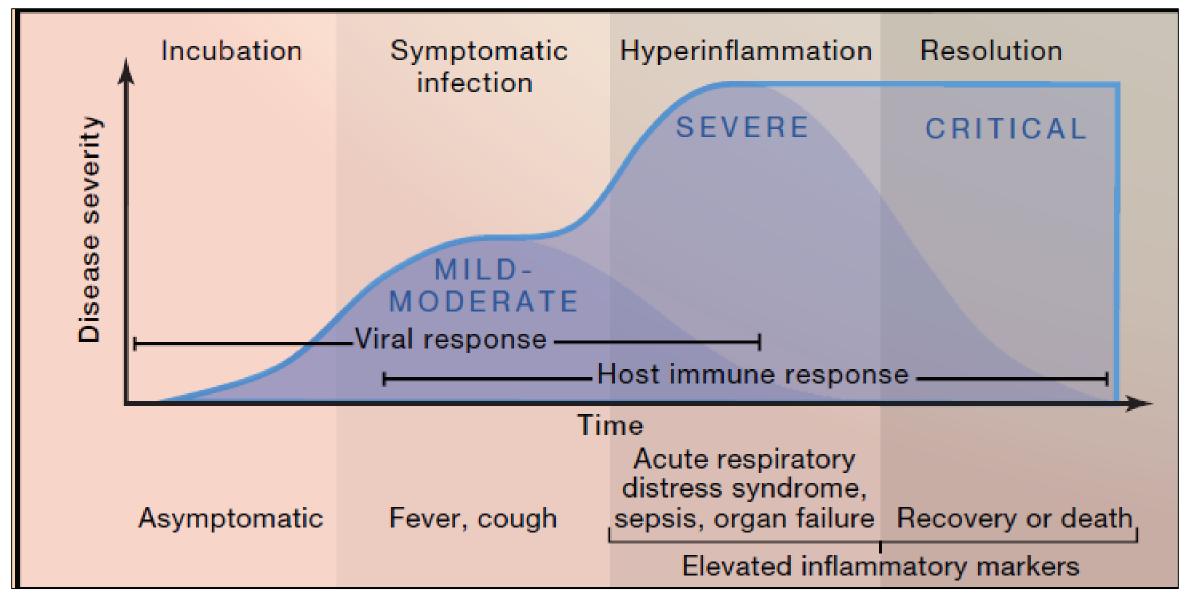
Which Is It?

- Allergies
- Cold
- Influenza
- COVID-19

https://www.nationaljewish.org/conditions/health-information/health-infographics/allergies-cold-flu-or-covid-19-virus
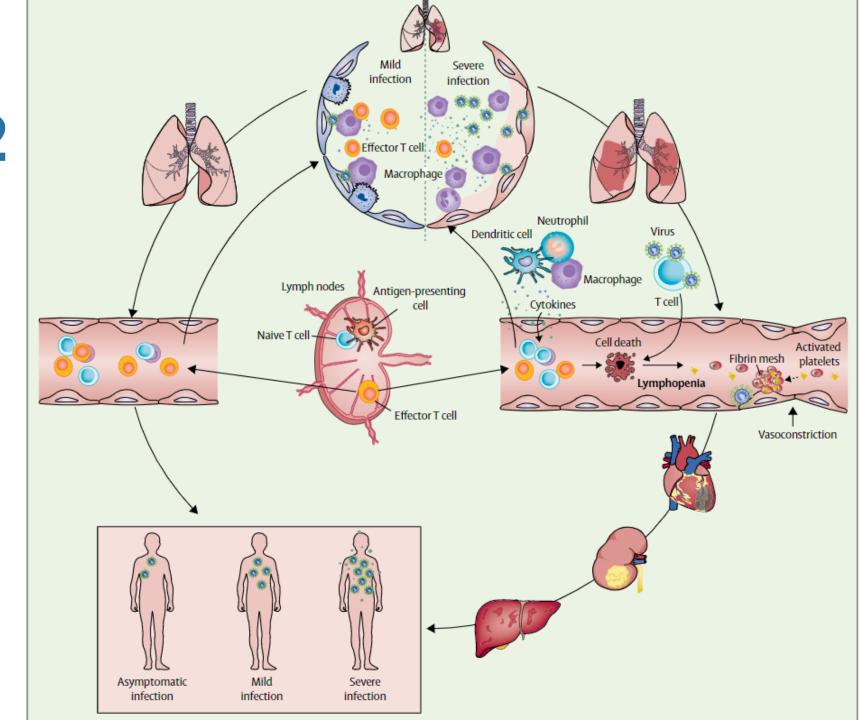
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Symptoms	ALLERGIES	COLD	INFLUENZA	COVID-19
Symptoms begin	Gradually	Gradually	Abruptly	Within 14 days of exposure
Symptoms last	Allergy season	4 – 10 days	5 – 7 days	Varies by Person
Body aches	–	✓	✓	Sometimes
Chills	–	Less Common	✓	Sometimes
Dry cough	✓	✓	✓	✓
Exposure to germs	–	✓	✓	✓
Fatigue/Weakness	Sometimes	✓	✓	✓
Fever	–	Less Common	✓	✓
Headaches	✓	Less Common	✓	Sometimes
Itchy eyes	✓	–	–	–
Nasal Congestion	✓	✓	✓	Less Common
Nausea/Vomiting/Diarrhea	–	Sometimes	Sometimes	Sometimes
New loss of taste or smell	Sometimes	Sometimes	Sometimes	✓
Repeated shaking with chills	–	Sometimes	Sometimes	Sometimes
Runny nose	✓	✓	✓	Less Common
Sneeze	✓	✓	✓	Sometimes
Sore throat	Sometimes	✓	✓	Sometimes
Shortness of breath	Sometimes	Less Common	✓	✓
Symptoms get worse	–	–	✓	✓

COVID-19 Clinical Course

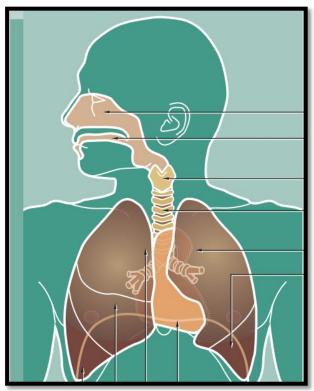Oberfeld B, et al. SanpShot: COVID-19. *Cell* (2020)
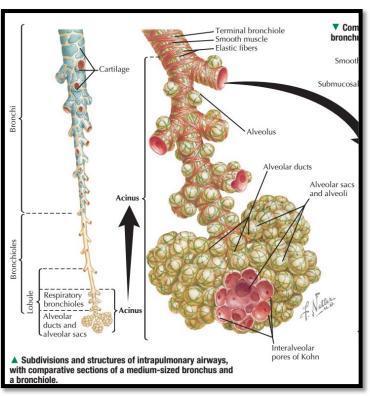
SARS-CoV-2 Infection & COVID-19

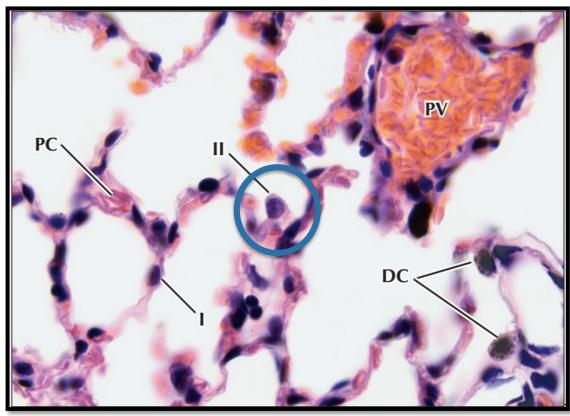Li H, et al. SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet* (2020)

Normal Respiratory Tree & Lung Air Sacs

Young B, Woodford P, O'Dowd G. *Wheater's Functional Histology: A Text and Colour.* 6th Ed. Elsevier: Philadelphia, PA, 2014
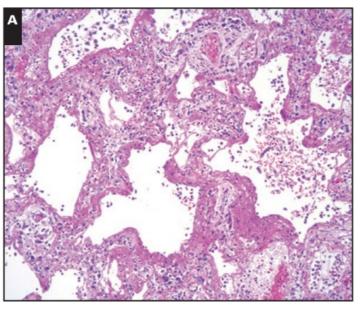
Ovalle WK, Nahirney PC. *Netter's Essential Histology, 2nd Ed.* Elsevier: Philadelphia, PA, 2013.

Spectrum of COVID-19 Lung Disease

Diffuse Alveolar Damage - Acute

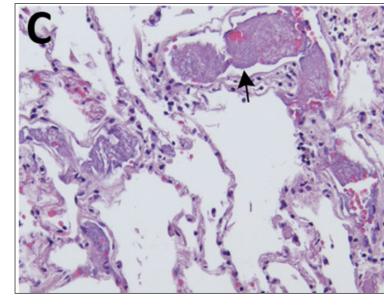Sekulic M, et al. *Am J Clin Pathol* (2020)
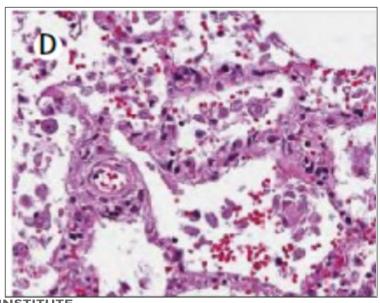

Pulmonary Microthrombi

Martines RB, et al. *Emerging Infectious Disease* (2020)

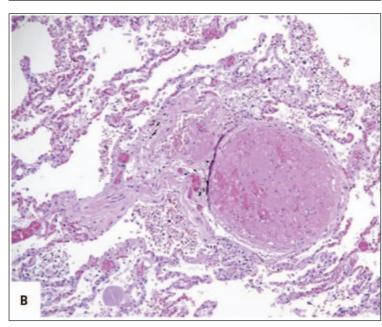Perivascular Lymphoid Infiltrate

Fox SE, et al. *The Lancet* (2020)
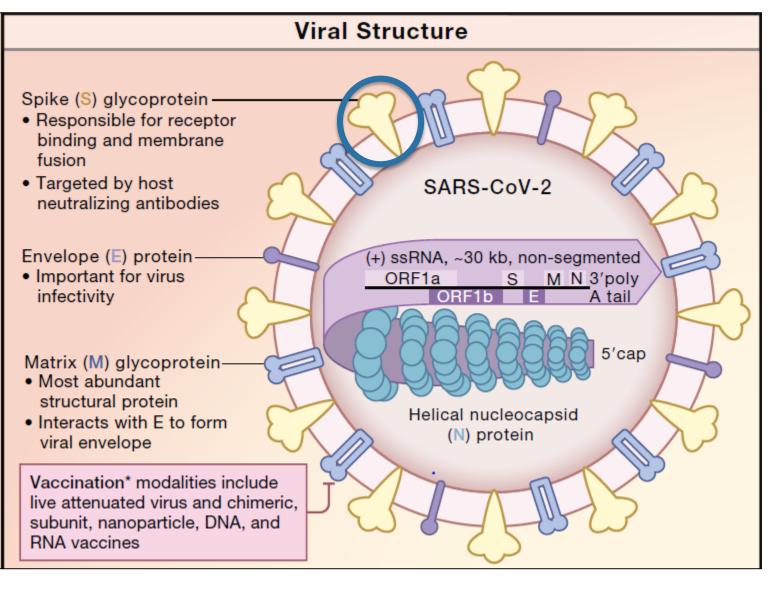

Pulmonary Artery Thrombosis

Lax SF, et al. *Annals of Internal Medicine* (2020)

SARS-CoV-2

Viral Structure

SARS-CoV-2

Spike (**S**) glycoprotein
- Responsible for receptor binding and membrane fusion
- Targeted by host neutralizing antibodies

Envelope (**E**) protein
- Important for virus infectivity

Matrix (**M**) glycoprotein
- Most abundant structural protein
- Interacts with E to form viral envelope

Vaccination* modalities include live attenuated virus and chimeric, subunit, nanoparticle, DNA, and RNA vaccines

(+) ssRNA, ~30 kb, non-segmented

ORF1a   S   M N 3'poly
ORF1b   E   A tail

5'cap

Helical nucleocapsid (**N**) protein

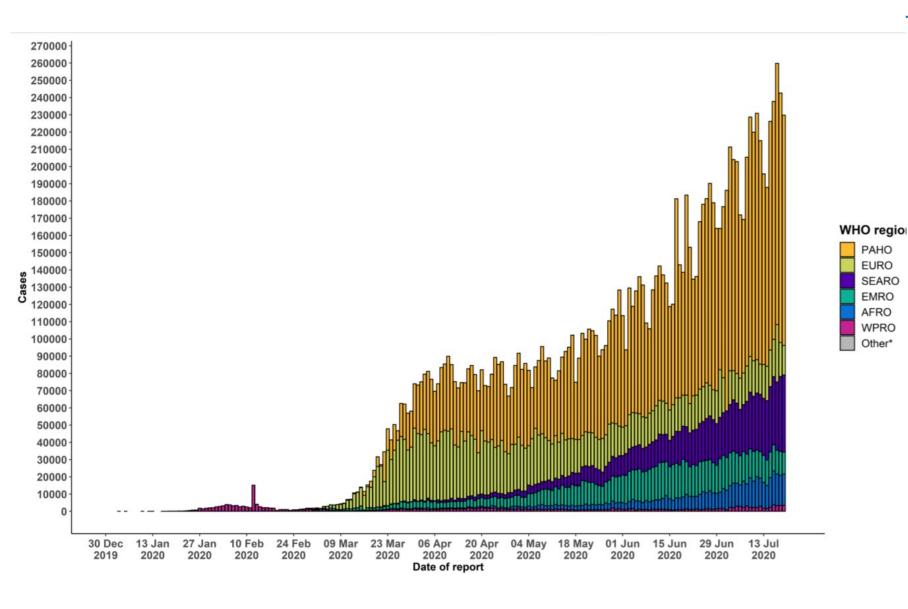<u>S</u>evere <u>A</u>cute <u>R</u>espiratory <u>S</u>yndrome <u>Co</u>rona<u>v</u>irus-<u>2</u>

Adapted from Oberfeld B, et al. SanpShot: COVID-19. *Cell* (2020)

Presentations of Coronaviruses

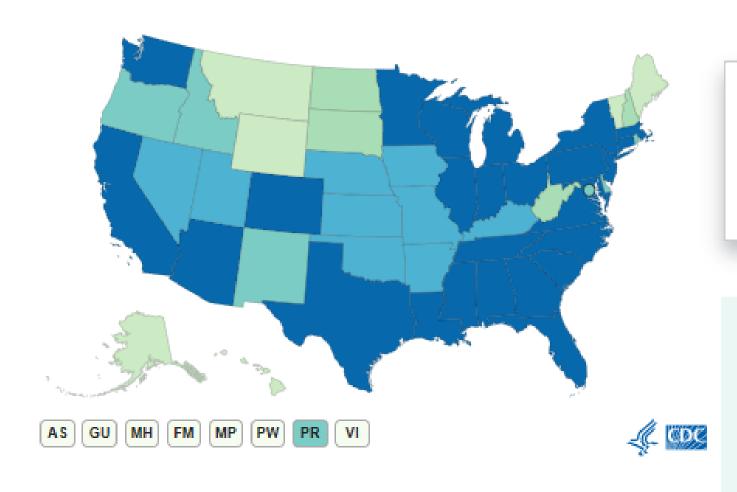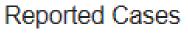| Parameter	Common Cold	SARS	MERS	COVID-19
First Identified	1966	2002	2012	2019
Initial Location	Global	China	Saudi Arabia	China
Clinical Severity	Mild	Severe, can be fatal	Severe, can be fatal	Severe, can be fatal
Viruses	4 known	SARS-CoV	MERS-CoV	SARS-CoV-2
Known Outbreaks	N/A	2002-2003	2012	2019-2020
Reservoir & Intermediary Hosts	Bats, Rodents, Birds	Bats, Civet Cats	Bats, Camels	Bats

WHO Global Reports

- 14,348,858 cases confirmed
- 603,691 deaths

(as of 7/20/2020)

https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200720-covid-19-sitrep-182.pdf?sfvrsn=60aabc5c_2

Accessed 7/21/2020

U.S. COVID-19 Cases

Reported Cases

- 0 to 1,000
- 1,001 to 5,000
- 5,001 to 10,000
- 10,001 to 20,000
- 20,001 to 40,000
- 40,001 or more

AS | GU | MH | FM | MP | PW | PR | VI

TOTAL CASES
3,761,362
63,201 New Cases*

TOTAL DEATHS
140,157
498 New Deaths*

*Compared to yesterday's data   About the Data

U.S. COVID-19 Cases & Race/Ethnicity

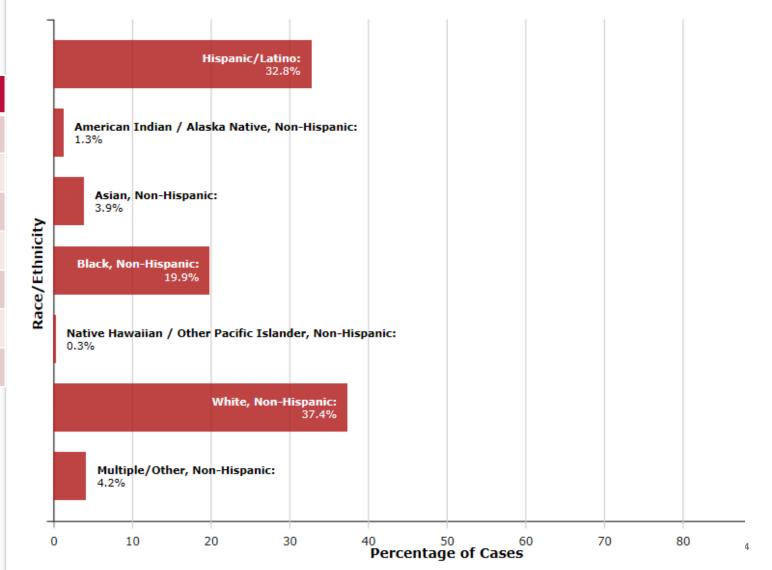| Race/Ethnicity	% Pop.
Hispanic/Latinx	18.3
American Indian/Alaskan Native	1.3
Asian, Non-Hispanic	5.9
Black, Non-Hispanic	13.4
Native Hawaiian/Pacific Islander	0.2
White, Non-Hispanic	60.4
Multiple, Other Non-Hispanic	2.7

https://www.cdc.gov/covid-data-tracker/index.html#demographics

https://www.census.gov/quickfacts/fact/table/US/PST045219

Accessed 7/21/2020

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Cases by Race/Ethnicity:
Data from 2,451,286 cases. Race/Ethnicity was available for 1,377,305 (56%) cases.

All Age Groups

Hispanic/Latino:
32.8%

American Indian / Alaska Native, Non-Hispanic:
1.3%

Asian, Non-Hispanic:
3.9%

Black, Non-Hispanic:
19.9%

Native Hawaiian / Other Pacific Islander, Non-Hispanic:
0.3%

White, Non-Hispanic:
37.4%

Multiple/Other, Non-Hispanic:
4.2%

Race/Ethnicity

Percentage of Cases

U.S. COVID-19 Deaths & Race/Ethnicity

Race/Ethnicity	% Pop.
Hispanic/Latinx	18.3
American Indian/Alaskan Native	1.3
Asian, Non-Hispanic	5.9
Black, Non-Hispanic	13.4
Native Hawaiian/Pacific Islander	0.2
White, Non-Hispanic	60.4
Multiple, Other Non-Hispanic	2.7

https://www.cdc.gov/covid-data-tracker/index.html#demographics

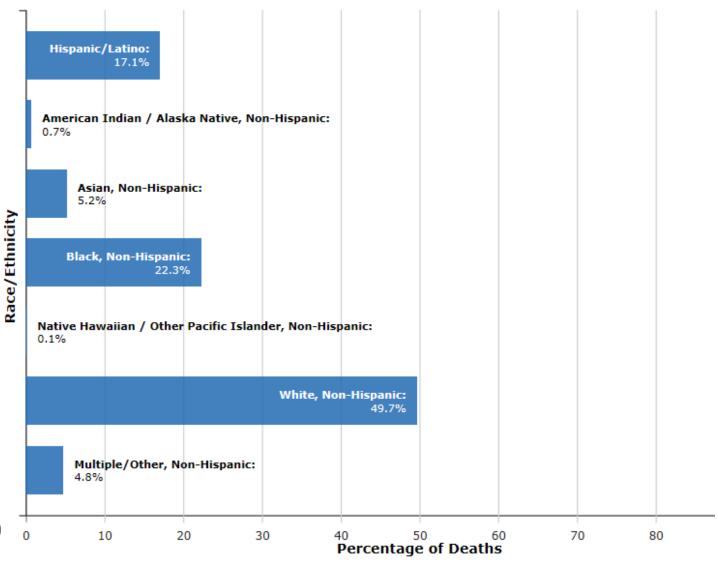https://www.census.gov/quickfacts/fact/table/US/PST045219

Accessed 7/21/2020

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Deaths by Race/Ethnicity:
Data from 109,577 deaths. Race/Ethnicity was available for 91,906 (83%) deaths.

All Age Groups ▾

Hispanic/Latino:
17.1%

American Indian / Alaska Native, Non-Hispanic:
0.7%

Asian, Non-Hispanic:
5.2%

Black, Non-Hispanic:
22.3%

Native Hawaiian / Other Pacific Islander, Non-Hispanic:
0.1%

White, Non-Hispanic:
49.7%

Multiple/Other, Non-Hispanic:
4.8%

Race/Ethnicity

Percentage of Deaths

U.S. COVID-19 Trends

Daily infections and testing ⓘ

Estimated infections | Confirmed infections | Tests

Scenario ⓘ [Projection ✕] [Easing ✕] [Masks ✕]

Today

- - Current projection   - - Mandates easing   - - Universal Masks

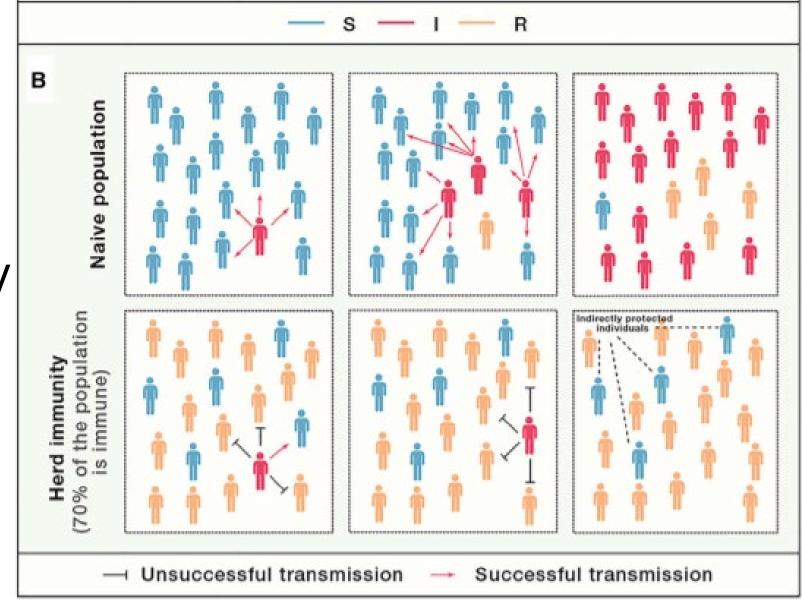Shaded areas indicate 95% uncertainty interval. ⓘ

https://covid19.healthdata.org/united-states-of-america
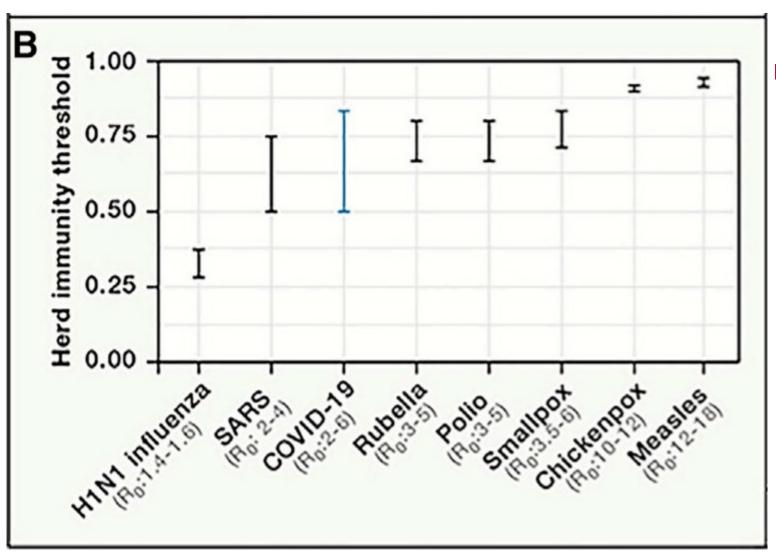
Accessed 7/21/2020

Herd Immunity

- Protection of vulnerable populations through widespread immunity

- Routes to Achieving
  - Native immunity through exposure
  - Widespread vaccination

Randolph HE & Barreiro LB. Herd Immunity: Understanding COVID-19 *Cell* (2020)

Comparative Herd Immunity Thresholds

- When the proportion of susceptible people in a population is below the threshold for transmission

Randolph HE & Barreiro LB. Herd Immunity: Understanding COVID-19 *Cell* (2020)

COVID-19 vs. Influenza

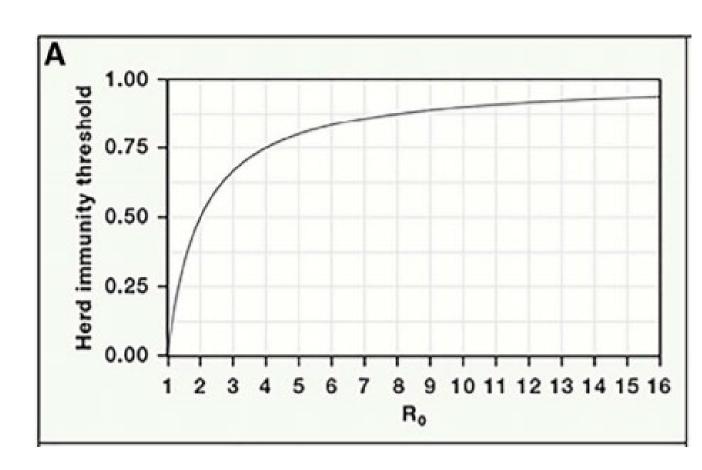| Parameter	Influenza	COVID-19
Presentation	Respiratory Disease	Respiratory Disease Multiorgan System Failure
Transmission	Contact, Respiratory Droplets, Fomites	Contact, Respiratory Droplets, Fomites
Incubation Period	2 days (1 to 4 days)	4 to 5 days
Serial Interval	3 days	5 to 6 days
Reproductive Number	0.9 to 2.1	2 to 2.5
Children	Drivers of transmission	Clinical attack rates low
Severe Disease	Varies annually & by age	15% require oxygen 5% require ventilation
Crude Mortality Ratio	0.1%	1-4%

https://www.hopkinsmedicine.org/health/conditions-and-diseases/coronavirus/coronavirus-disease-2019-vs-the-flu
https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200306-sitrep-46-covid-19.pdf?sfvrsn=96b04adf_4

Oberfeld B, et al. SnapShot: COVID-19. *Cell* (2020).

Determinants of Reaching Herd Immunity

R_0: Basic Reproductive #

Average # of secondary infections from a single infectious individual in a completely susceptible population

Estimated SARS-CoV-2 R_0: 2.2 to 5.7

Randolph HE & Barreiro LB. Herd Immunity: Understanding COVID-19 *Cell* (2020)

Testing Considerations

- What test should be conducted?

- Who should be tested?

- What specimen should be collected?

- How are test results interpreted?

- What are the recommended actions by test type & result?

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USA
48,603,115
TESTS REPORTED
CDC | Updated: Jul 20 2020 5:45PM

USA
4,500,438
POSITIVE TESTS
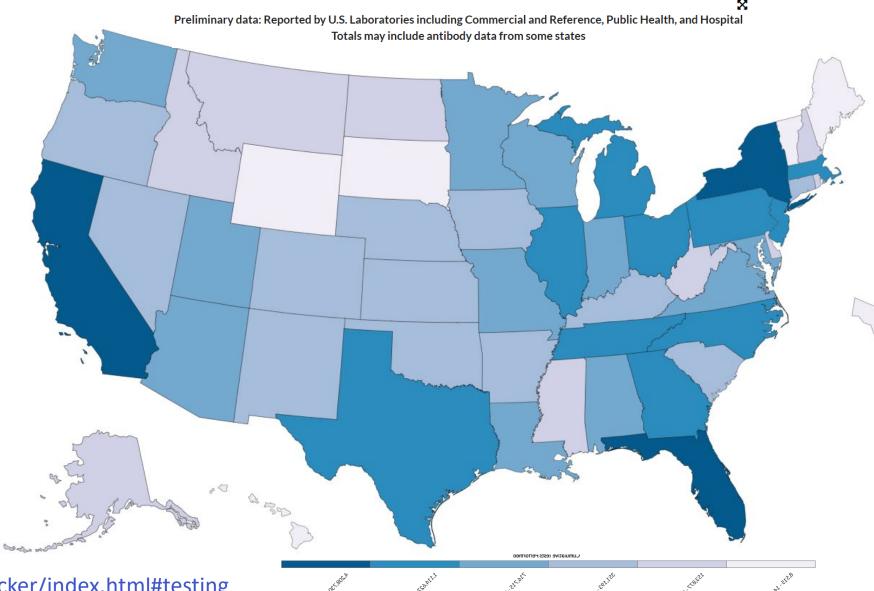CDC | Updated: Jul 20 2020 5:45PM

USA
9%
OVERALL % POSITIVE
CDC | Updated: Jul 20 2020 5:45PM

United States Laboratory Testing
Commercial and Reference, Public Health, and Hospital Laboratories

Preliminary data: Reported by U.S. Laboratories including Commercial and Reference, Public Health, and Hospital
Totals may include antibody data from some states

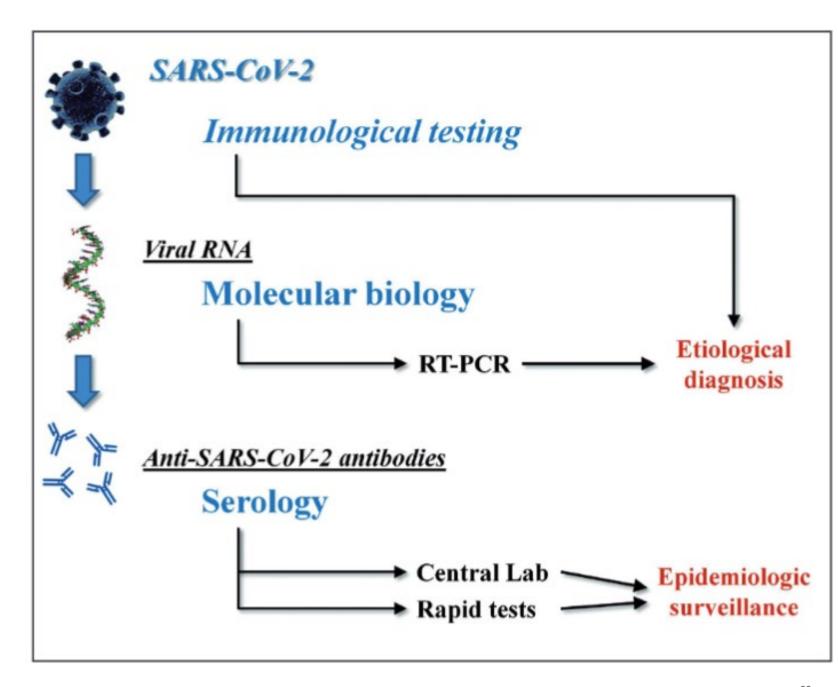https://www.cdc.gov/covid-data-tracker/index.html#testing

Accessed 7/21/2020

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SARS-CoV-2 Test Types & Applications

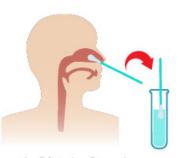Lippi G et al. Current laboratory diagnostics of coronavirus disease 2019 (COVID-19). *Acta Biomed* (2020)

SARS-CoV-2 Test Types*

*There are also viral antigen tests that detect viral proteins

https://asm.org/Articles/2020/April/COVID-19-Testing-FAQs
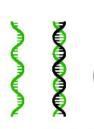

Molecular Tests (Nucleic Acid Detection)
Diagnose active SARS-CoV-2 infections

1. Obtain Specimen: Swab.
2. Extract RNA from specimen and convert to DNA.
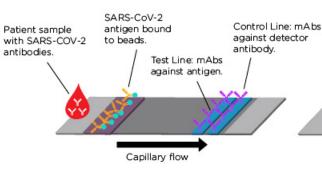3. Amplify by PCR with SARS-CoV-2 specific primers.
4. Interpret results: presence of viral RNA indicates active SARS-CoV-2 infection.

Antibody Tests (Serology)
Detect immune response to SARS-CoV-2 exposure

Patient sample with SARS-COV-2 antibodies.

SARS-CoV-2 antigen bound to beads.

Test Line: mAbs against antigen.

Control Line: mAbs against detector antibody.

Test Line: positive

Control Line: valid test
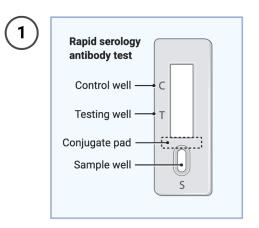
Capillary flow

1. Obtain Specimen: Blood Sample.
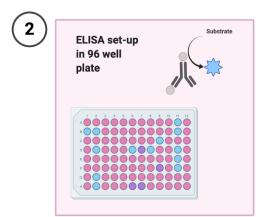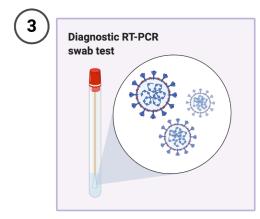2. Expose specimen to SARS-CoV-2 specific antigens.
3. Interpret results: color change indicates previous exposure to SARS-CoV-2.

Comparison of main types of COVID-19 tests

https://covidtestingproject.org/faq.html

	Rapid serology antibody test	ELISA	Diagnostic RT-PCR swab test
Sample input	**Serum** or **plasma** sample (whole blood or finger prick also possible)	**Serum** or **plasma** sample	**Nasopharyngeal** (NP) or **Oropharyngeal** (OP) **swab** sample
Result output	Detection of **IgM/IgG antibodies** via color change of strip in lateral flow assay	Detection of **IgM/IgG** or RBD IgG **antibodies**, via colorimetric assay	Detection of **viral SARS-CoV-2 RNA** via cDNA sequencing
Strengths	**Very low relative cost**, can be conducted at **point-of-care** or at **home**, ease-of-use, **fast results** (5-15 min, highly accurate detection of IgM/IgG **several days after onset**	**Robust detection of seroconversion** status in a laboratory setting, can detect IgM/IgG highly accurately **several days after onset or sooner**	Gold-standard **diagnostic** test, **directly detects virus** presence (sequencing viral nucleic acids), most **accurate results early** in disease presentation
Limitations	Requires rigorous testing of **cross-reactivity** with other immune response, **variation of test specificity & sensitivity** among manufacturers	Requires rigorous testing of **cross-reactivity** with other immune response, requires **laboratory setting**	**Labor intensive**, requires numerous **additional reagents** and **specialized equipment**, can **lose accuracy** after ~5 days since symptom onset, sensitive to **sample collection error**

Prioritization for Viral Testing

Nucleic Acid or Protein Antigen

- Person w/ COVID-19 signs/symptoms
- Healthcare professional w/ even mild signs/symptoms
- All contacts of confirmed case
- Neonate born to mother w/ COVID-19
- Expanded contacts in high-risk settings*
- Asymptomatic individuals in high-risk settings*
- Pre- & Post-admission to facility w/ or w/o symptoms
- Pre- & Post-procedures w/ or w/o symptoms

- <u>High-Risk Settings*</u>
  - Nursing home
  - Psychiatric residential facility
  - Rehabilitation facility
  - Home for people with intellectual disabilities
  - Detention facilities
  - Homeless shelters
  - High-density workplace

Accessed: 6/15/2020

https://www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html 26

Antibody Testing

- Not for diagnosing an acute infection
- In combination with viral testing for patient presenting late in infection
- Not to determine immunity to SARS-CoV-2 <u>at this time</u>
- Patient suspected of post-infectious syndrome (multisystem inflammatory syndrome in children, MIS-C)
- To understand infection transmission dynamics in the population

What Specimen?

Conducting Passages

Upper Respiratory Tract
- Nasal Cavity
- Pharynx
- Larynx

Lower Respiratory Tract
- Trachea
- Primary Bronchi
- Lungs

https://training.seer.cancer.gov/anatomy/respiratory/passages/

Table 2. Biological sources where severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be detected in coronavirus disease 2019 (COVID-19) patients.

Biological source	Detection rate
Bronchoalveolar lavage fluid	>90%
Saliva	~90%
Sputum	~70%
Nasopharyngeal AND oropharyngeal swabs	~70%
Nasal swabs	~60%
Pharyngeal swabs	~30%
Stool	~30%
Throat washing	~30%
Blood	15–30%

Lippi G et al. Current laboratory diagnostics of coronavirus disease 2019 (COVID-19). *Acta Biomed* (2020)

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SARS-CoV-2 RNA & Antibody Detection

Sethuraman N et al. Interpreting Diagnostic Tests for SARS-CoV-2. *NEJM* (2020)

Figure. Estimated Variation Over Time in Diagnostic Tests for Detection of SARS-CoV-2 Infection Relative to Symptom Onset

Before symptom onset

After symptom onset

Detection unlikely[a]

PCR - Likely positive

PCR - Likely negative[b]

Antibody detection

Increasing probability of detection

SARS-CoV-2 exposure

Week −2 | Week −1 | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6

Symptom onset

— Nasopharyngeal swab PCR
— Virus isolation from respiratory tract
— Bronchoalveolar lavage/sputum PCR
— Stool PCR
---- IgM antibody
---- IgG antibody

Test Interpretation

Status of person according to "gold standard"

	Has the condition	Does not have the condition	
Positive	a — True positive	b — False positive	Row entries for determining **positive predictive value**
Negative	c — False negative	d — True negative	Row entries for determining **negative predictive value**

Result from screening test

Column entries for determining **sensitivity**

Column entries for determining **specificity**

Trevethan R. Sensitivity, Specificity, and Predictive Values: Foundations, Pliabilities, and Pitfalls in Research and Practice. *Frontiers in Public Health* (2017)

Test Interpretation

Watson J et al. Interpreting a COVID-19 test result. *BMJ* (2020)
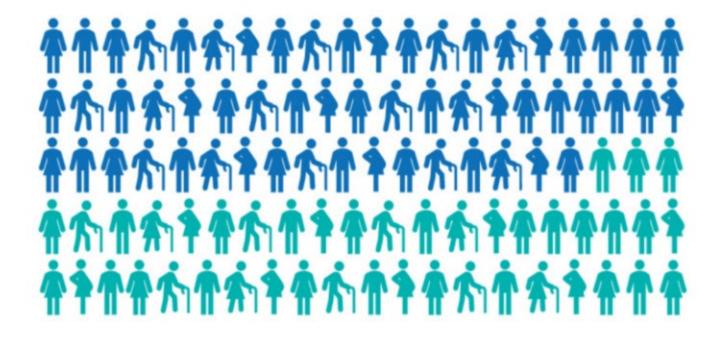
100 people at risk of covid-19
Pre-test probability 80%

80 people have covid-19

20 people do not have covid-19

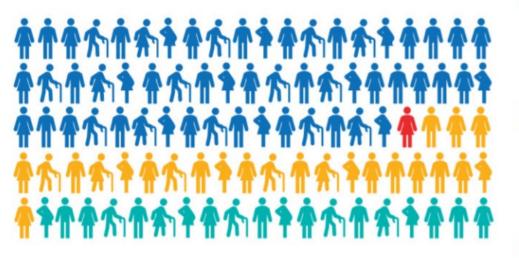They are tested for covid-19 using the RT-PCR test:

Test Interpretation

Watson J et al. Interpreting a COVID-19 test result. *BMJ* (2020)

57 people have a test result suggesting that they have covid-19 (test positive)

43 people have a test result suggesting that they do not have covid-19 (test negative)

But who actually has covid-19?

Test Interpretation

Watson J et al. Interpreting a COVID-19 test result. *BMJ* (2020)

Diagnosis

56 people who test positive have covid-19 ("true positive")

1 person who tests positive does not have covid-19 ("false positive")

24 people who test negative have covid-19 ("false negative")

19 people who test negative do not have covid-19 ("true negative")
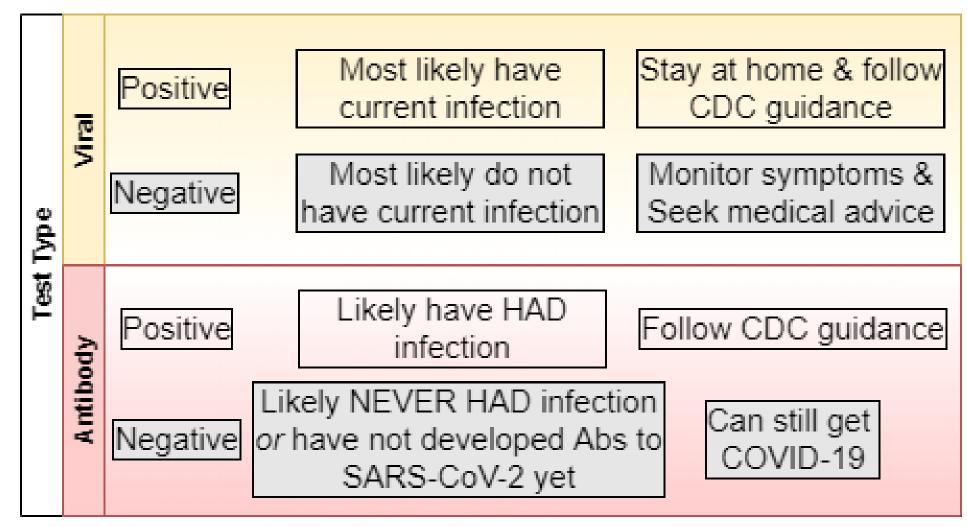
Consequences

Appropriately told to self-isolate

Told they need to self-isolate when they would be safe to go out

Told they do not need to self-isolate and so go out and infect more people

Told they do not need to self-isolate and are safe to go out without infecting more people

Actions Recommended by CDC

Test Type			
Viral	Positive	Most likely have current infection	Stay at home & follow CDC guidance
	Negative	Most likely do not have current infection	Monitor symptoms & Seek medical advice
Antibody	Positive	Likely have HAD infection	Follow CDC guidance
	Negative	Likely NEVER HAD infection *or* have not developed Abs to SARS-CoV-2 yet	Can still get COVID-19

Derived from & Accessed 6/15/2020
https://www.whitehouse.gov/wp-content/uploads/2020/05/Testing-Guidance.pdf

Actions Recommended by CDC

Test Type			
	Antibody & Viral	Viral+/Antibody+	Most likely have current infection	Stay at home & follow CDC guidance
		Viral+/Antibody-	Most likely have current infection	Stay at home & follow CDC guidance
		Viral-/Antibody+	Likely HAD infection & RECOVERED	Take precautions & retest if provider or workplace deems necessary
		Viral-/Antibody-	Likely NEVER HAD infection	Take precautions & retest if provider or workplace deems necessary

Derived from & Accessed 6/15/2020
https://www.whitehouse.gov/wp-content/uploads/2020/05/Testing-Guidance.pdf

Take Home Points

- Understanding test type and interpretation are critical for understanding application to public health planning

- Prior exposure does not always confer long-lasting immunity
  - Potential for reinfection uncertain

- Importance of precautionary measures to slow transmission on a population scale to protect self & those in proximity until effective vaccine available
  - Self-isolation if symptomatic & Social distancing
  - Exercising good hygiene
  - Wearing protective face covering

www.cancer.gov   www.cancer.gov/espanol