MAYO CLINIC

DEPARTMENT OF LABORATORY MEDICINE AND PATHOLOGY

# COVID-19 Testing

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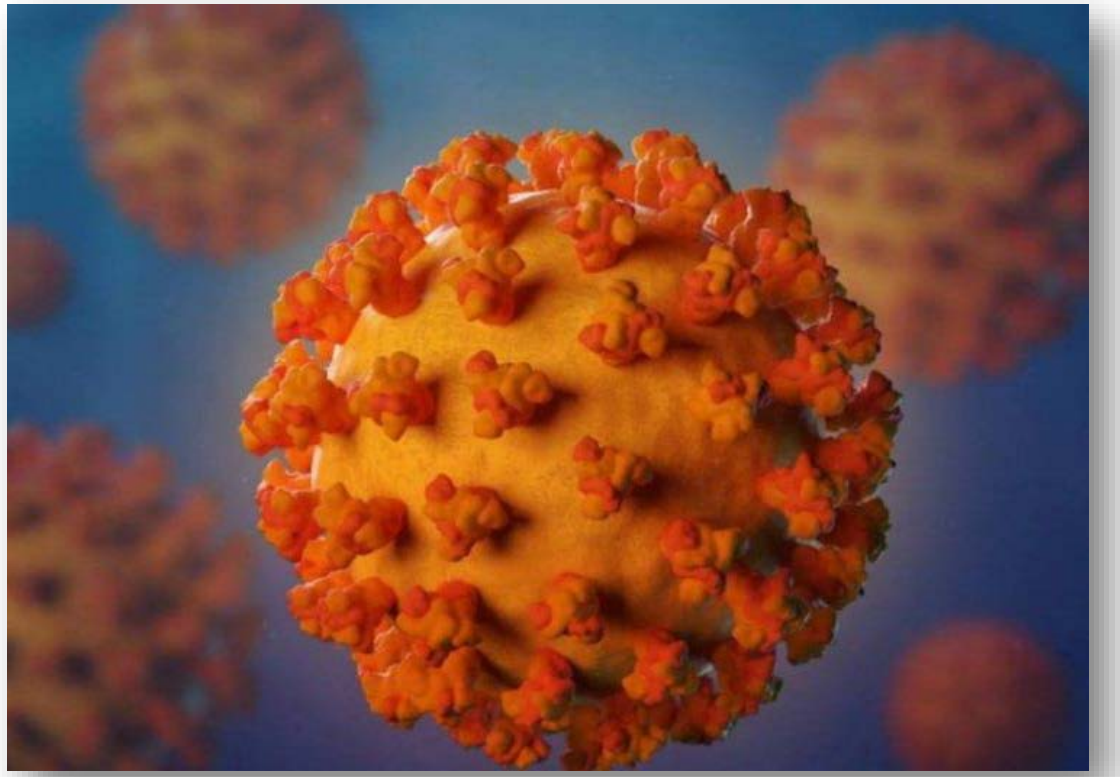
# Coronaviruses: From the Common Cold to Global Contagion

Common human coronaviruses:

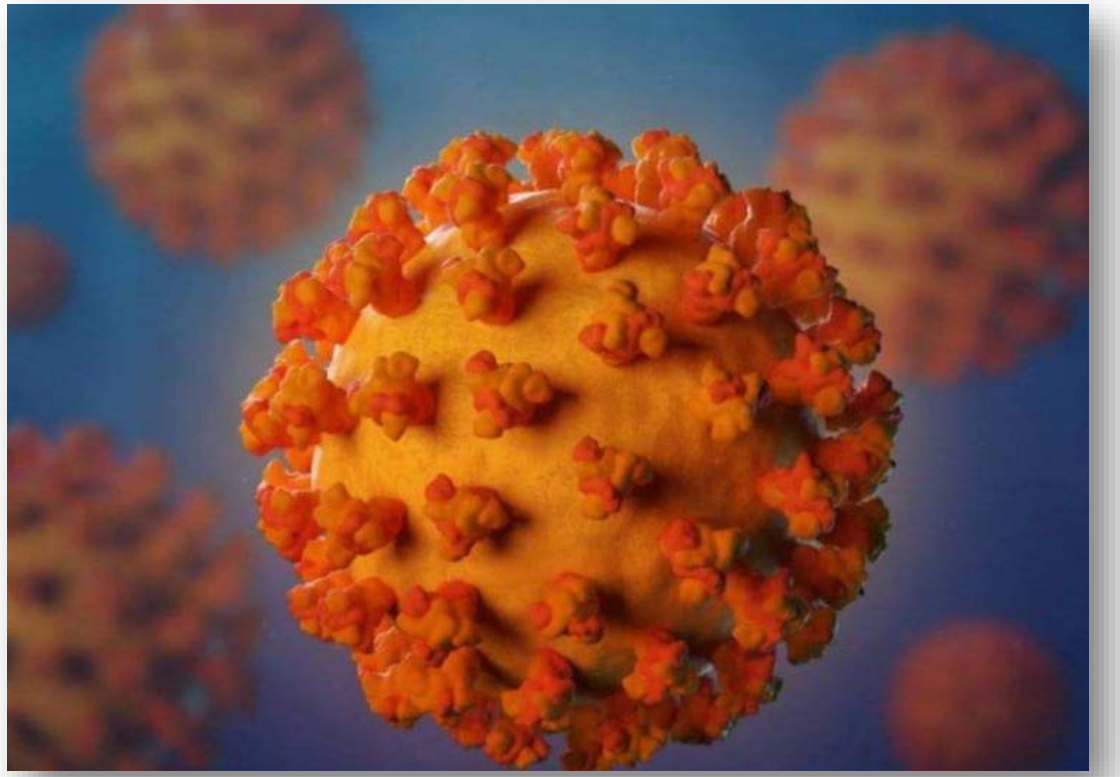
- HCoV-OC43
- HCoV-NL63
- HCoV-229E
- HCoV-HKU1



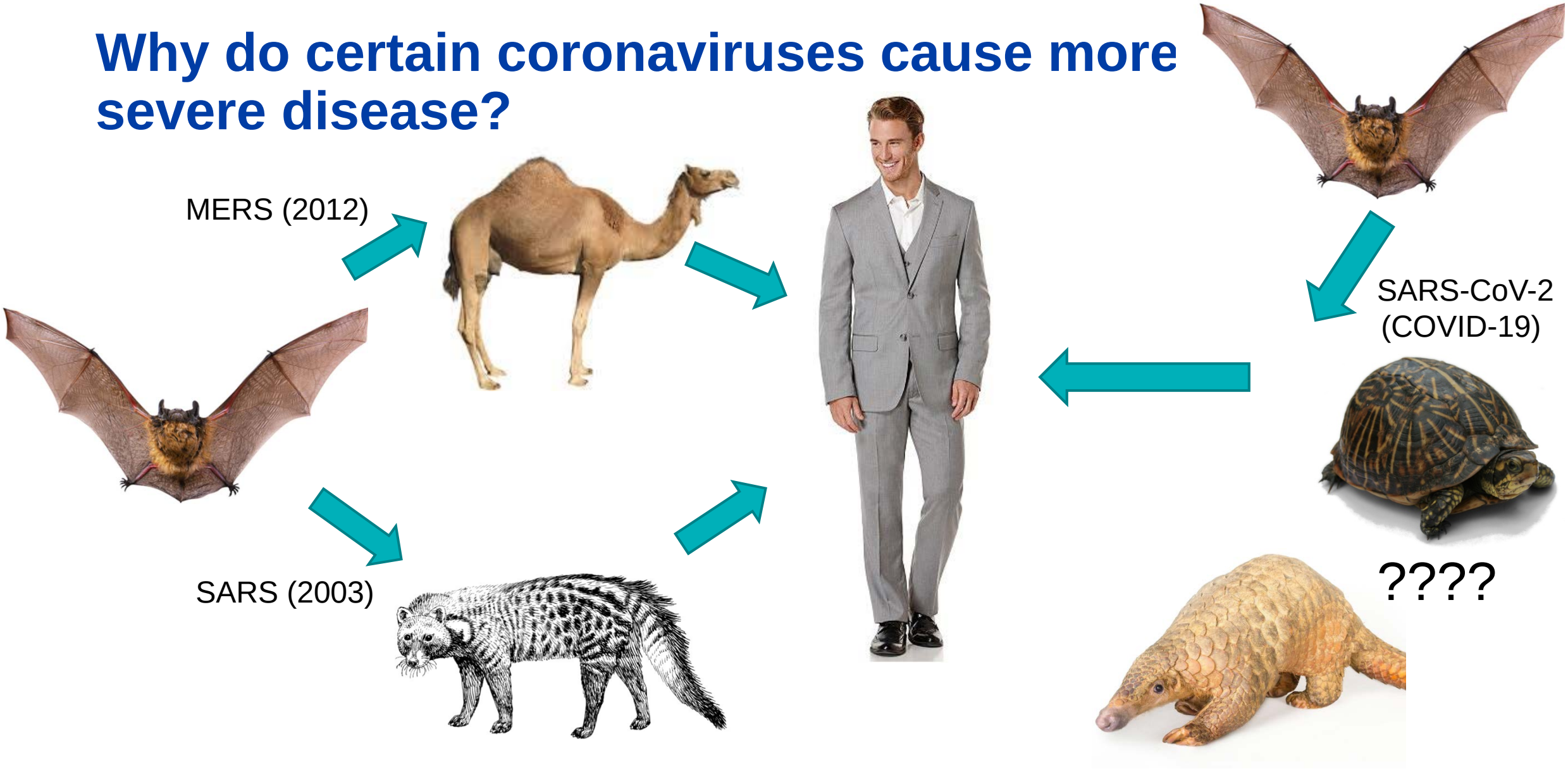
# Coronaviruses: From the Common Cold to Global Contagion

Coronaviruses associated with severe disease:

- SARS (2002-2003)
- MERS (2012)
- SARS-CoV-2 (2019-2020)



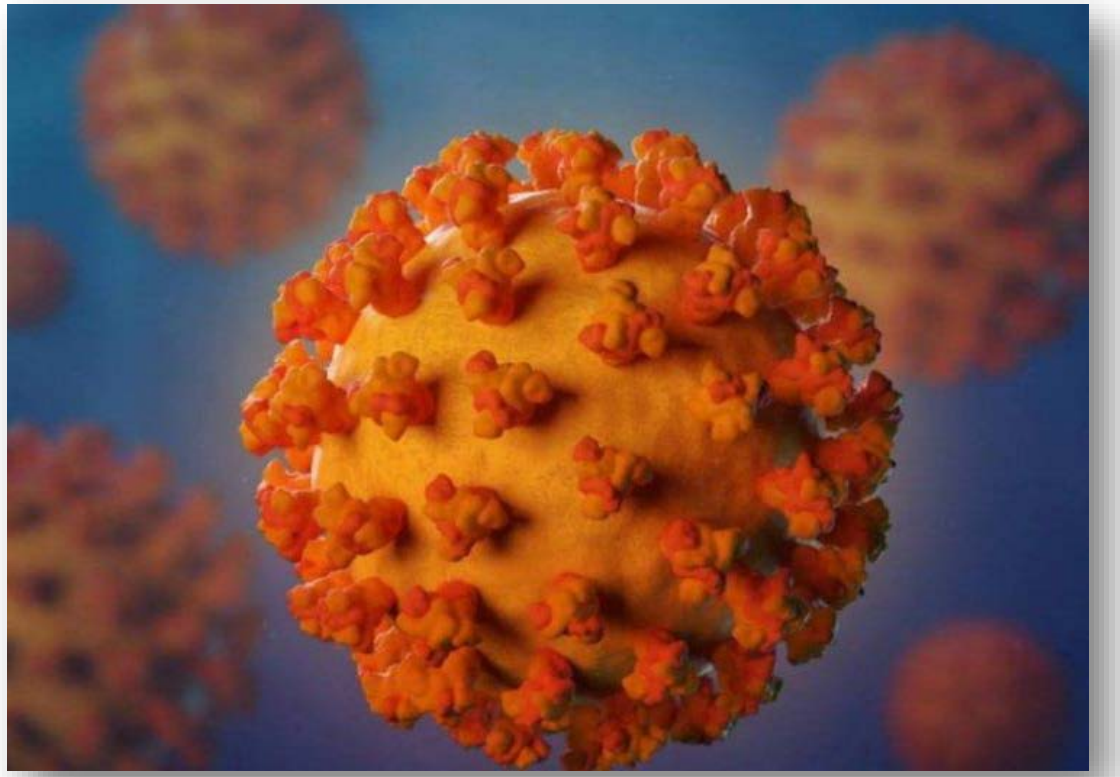
# Why do certain coronaviruses cause more severe disease?



# SARS-CoV-2 (COVID-19): Molecular Testing

Molecular (real-time PCR) tests have generally targeted a combination of the following genes:

- Nucleocapsid (N)
- Open reading frame 1ab (Orf)
- Envelope (E)
- RNA dependent RNA polymerase (RdRp)



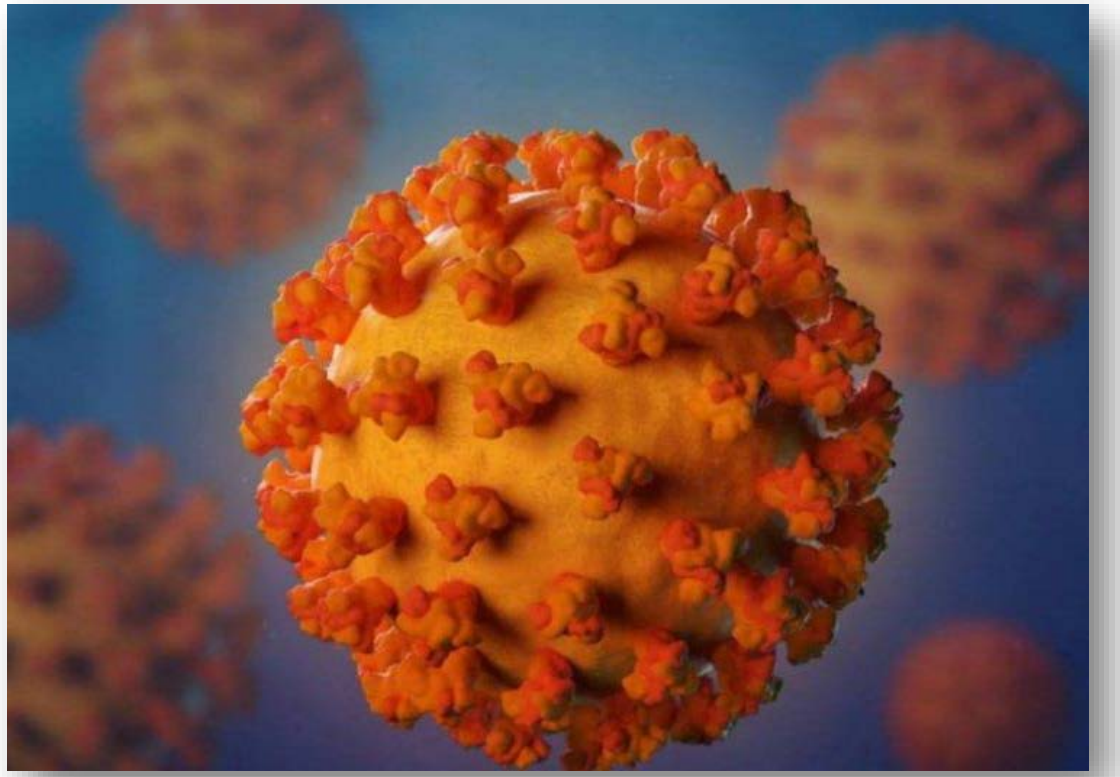
# SARS-CoV-2 (COVID-19): Molecular Testing

Appropriate sample types:

- Nasopharyngeal swab (**preferred**)
- Oropharyngeal (throat) swab

***If*** evidence of LRTI or later in disease course:

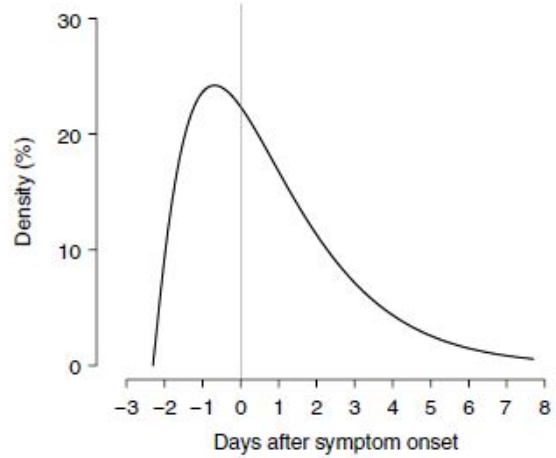
- Sputum
- BAL fluid
- Tracheal secretions



# SARS-CoV-2 (COVID-19): Molecular Detection

*When* is SARS-CoV-2 shed at the highest amount?

- Peak viral shedding ~24 h **prior** to symptom onset
- Detection in upper airway (i.e., NP swab) likely drops after 3-5 days post onset




He, X. Nature Med, 15 Apr 2020

*What is the sensitivity* of the COVID-19 PCR test?

- Likely depends on several factors
  - Timing of collection
  - Sample type
  - Quality of sample collected
  - Test

Table. Detection Results of Clinical Specimens by Real-Time Reverse Transcriptase–Polymerase Chain Reaction

| Specimens and values | Bronchoalveolar lavage fluid (n = 15) | Fibrobronchoscope brush biopsy (n = 13) | Sputum (n = 104) | Nasal swabs (n = 8) | Pharyngeal swabs (n = 398) | Feces (n = 153) | Blood (n = 307) | Urine (n = 72) |
|---|---|---|---|---|---|---|---|---|
| Positive test result, No. (%) | 14 (93) | 6 (46) | 75 (72) | 5 (63) | 126 (32) | 44 (29) | 3 (1) | 0 |
| Cycle threshold, mean (SD) | 31.1 (3.0) | 33.8 (3.9) | 31.1 (5.2) | 24.3 (8.6) | 32.1 (4.2) | 31.4 (5.1) | 34.6 (0.7) | ND |
| Range | 26.4-36.2 | 26.9-36.8 | 18.4-38.8 | 16.9-38.4 | 20.8-38.6 | 22.3-38.4 | 34.1-35.4 | |
| 95% CI | 28.9-33.2 | 29.8-37.9 | 29.3-33.0 | 13.7-35.0 | 31.2-33.1 | 29.4-33.5 | 0.0-36.4 | |

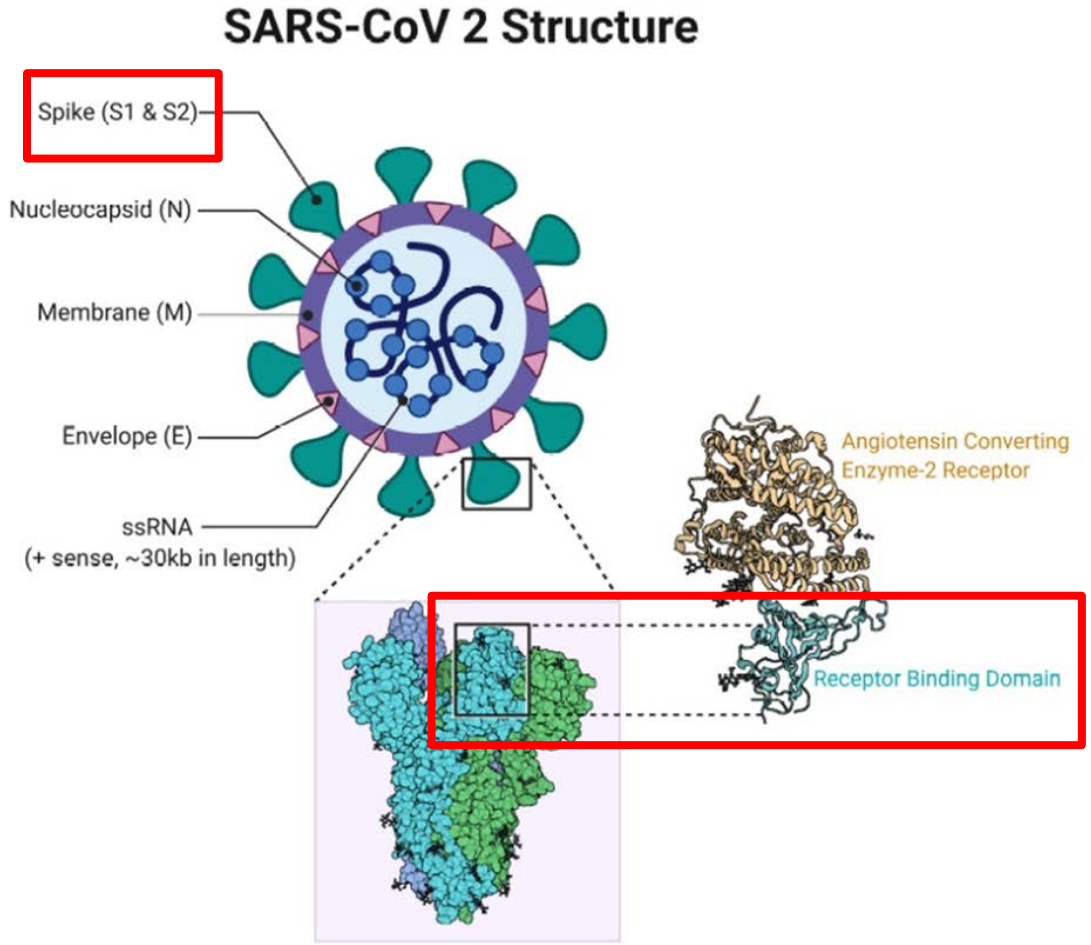
Abbreviation: ND, no data.

Wang W. JAMA, 5 Mar 2020

# Serologic Testing for IgG Antibodies Against SARS-CoV-2

# COVID-19 Serologic Testing at Mayo Clinic

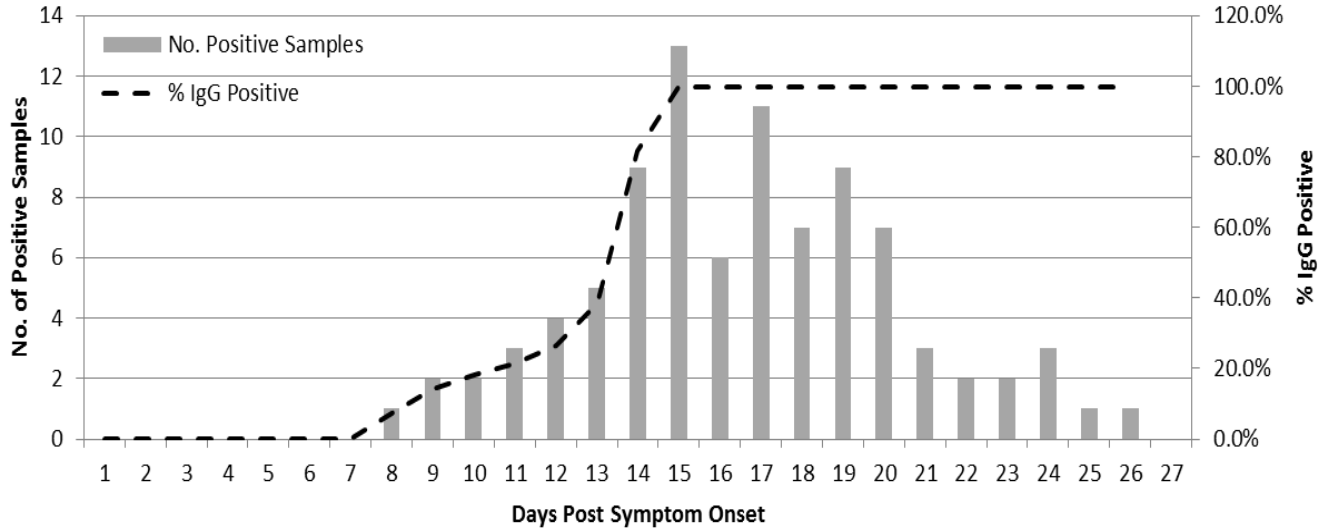
- Goal: Use antibody testing as a marker of prior infection with COVID-19
  - Not an alternative or additional marker of acute/recent infection
  - Focus on IgG-class antibodies to SARS-CoV-2
    - Longer lasting antibody class
    - Associated with neutralizing antibody activity and potential protective immunity
- Currently using the SARS-CoV-2 IgG ELISA from Euroimmun Inc. (Lubeck, Germany)
  - Detects antibodies against the Spike protein

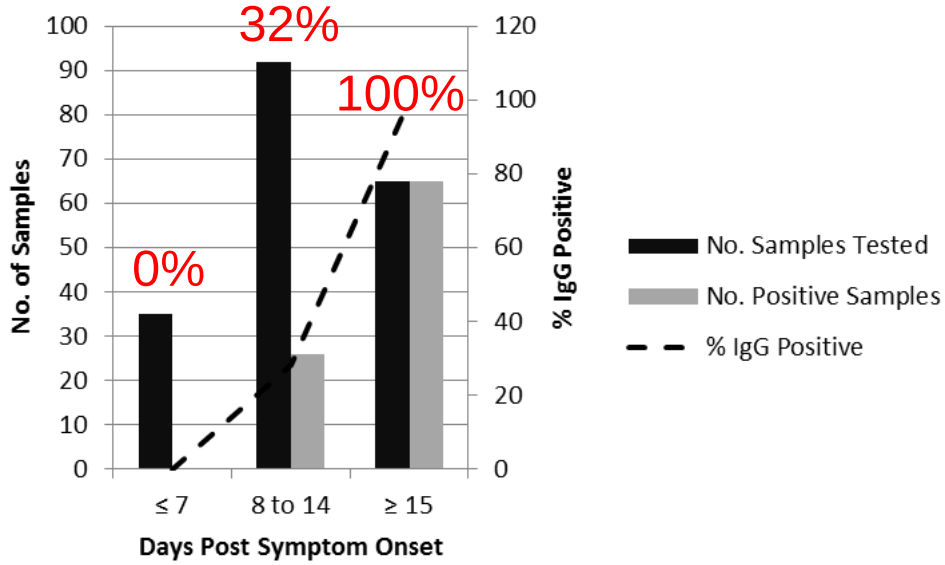



https://www.ncbi.nlm.nih.gov/books/NBK554776/

# Performance Characteristic: Sensitivity

- Sample set:
  - Sera from 33 hospitalized patients with PCR-confirmed COVID-19
  - 25 patients had ≥ 2 serial samples




- Sensitivity has not yet been evaluated in patients with mild or asymptomatic infection

# Performance Characteristic: Specificity

- Sample set:
  - Normal donors: 150 sera from healthy donors collected in 2018 (pre-outbreak)
  - Cross-reactivity panel: 119 sera with antibodies to other pathogens collected in Feb. 2020

| | % Negative | | |
|---|---|---|---|
| | 2018 Normal Donor Sera | Cross-Reactivity Panel | Overall |
| Euroimmun IgG ELISA | 99.3% (149/150) | 95.8% (114/119)* | 97.7%* |

*Cannot rule out COVID-19 infection in 5 samples

- Do antibodies to other coronaviruses (CoVs) cause false positive results by this test?
  - >90% of adults over 50 years are positive for antibodies to all 4 circulating CoVs
  - Based on 2018 healthy donor specificity studies, cross-reactivity of antibodies to other CoVs on the SARS-CoV-2 IgG ELISA appears to be infrequent.
- Specificity is also impacted by prevalence of disease

# SARS-CoV-2 IgG Result Interpretation

- **Positive**:
  - SARS-CoV-2 IgG antibodies are detected
  - Results suggest recent or prior infection with SARS-CoV-2.
  - Protective immunity cannot be inferred based on these results alone.
- **Negative**:
  - No IgG antibodies to SARS-CoV-2 detected
  - Negative results may occur in serum collected too soon following infection, in immunosuppressed individuals, or in some individuals with prior mild illness
  - This test should not be used to exclude active/recent COVID-19
- **Indeterminate**:
  - Consider repeat testing in 5-7 days

# Recommendations for COVID-19 Serologic Test Utilization

- Similar recommendations by ASM, IDSA, ACLA, ASCP and others:
  - Identification of individuals previously infected with COVID-19
    - Epidemiology and seroprevalence studies
    - Facilitate contact tracing
  - Identification of potential convalescent plasma donors
  - Evaluation of immune response to candidate vaccines
  - Aid in COVID-19 PCR-negative patients who present later in disease course
- Serologic testing should NOT be used to:
  - Diagnose acute/recent COVID-19 infection
  - Determine whether or not a patient has protective immunity
  - Guide PPE use

# Tests for SARS-CoV-2/COVID-19 and Potential Uses

| Type of Test | Measure | Value | Beneficiary |
|---|---|---|---|
| **Nucleic acid amplification test for viral RNA** *(nasopharyngeal swab, oropharyngeal swab, sputum, bronchoalveolar lavage fluid, others)* | Current infection with SARS-CoV-2 | • Inform individual of infection status so they can anticipate course of illness and take action to prevent transmission<br>• Inform patient management and actions needed to prevent transmission<br>• Inform actions needed to prevent transmission | • Individual<br>• Healthcare or long-term care facility<br>• Public health |
| **Antibody detection** | Past exposure to SARS-CoV-2 | • Detect susceptible individuals (antibody negative) and those previously infected<br>• Identify individuals with neutralizing antibodies<br>• Facilitate contact tracing and surveillance | • Identify those potentially immune to SARS-CoV-2 (if tests can detect protective immunity, individuals could be returned to work)<br>• Healthcare facilities: Experimental therapy<br>• Public health |

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Patel R, *et. al.* Report from the American Society for Microbiology COVID-19 International Summit. *mBio*. 2020;11(2)