SARS-CoV-2 Diagnostic Testing

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Conflicts of Interest

- None relevant to this topic

Overview

- Brief review of SARS-CoV-2 virology

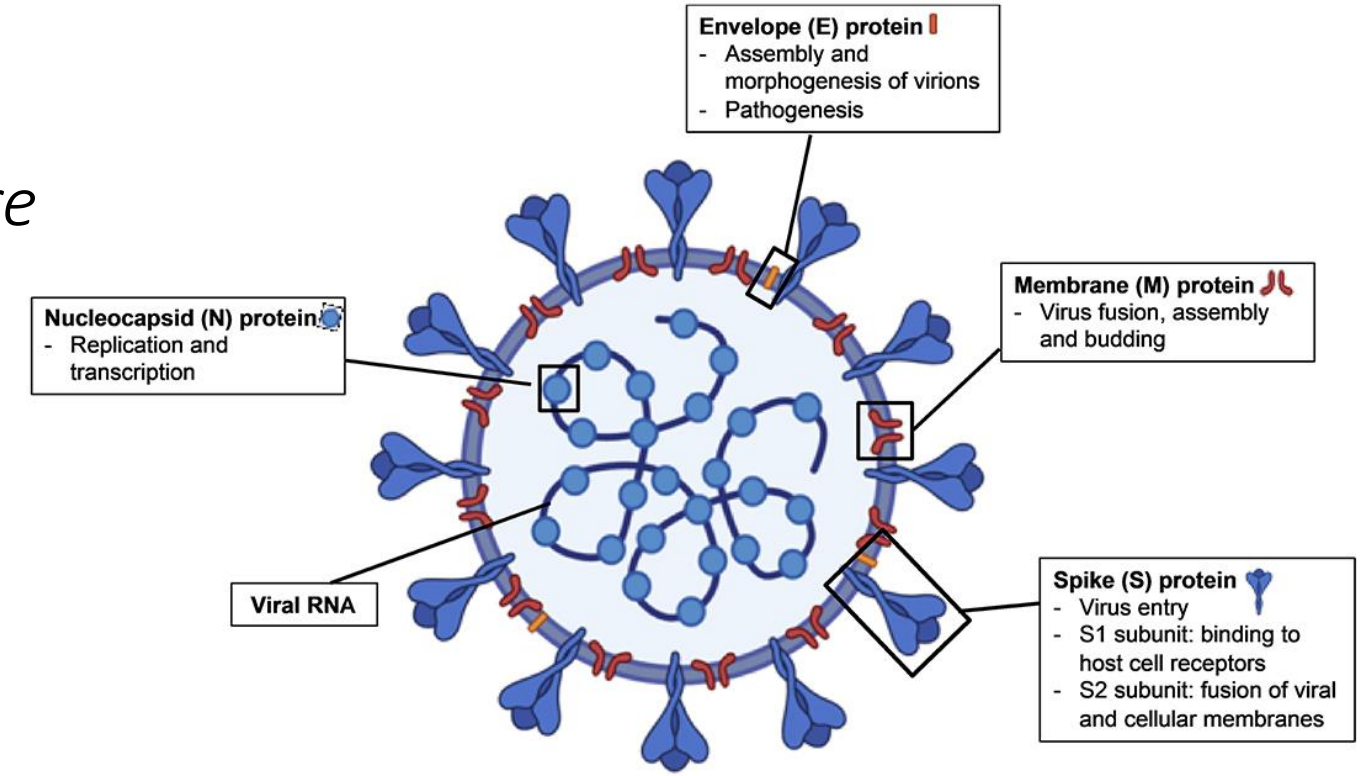
- Review diagnostic methods and test performance
  - Molecular diagnostics
  - Antigen testing
  - Serology

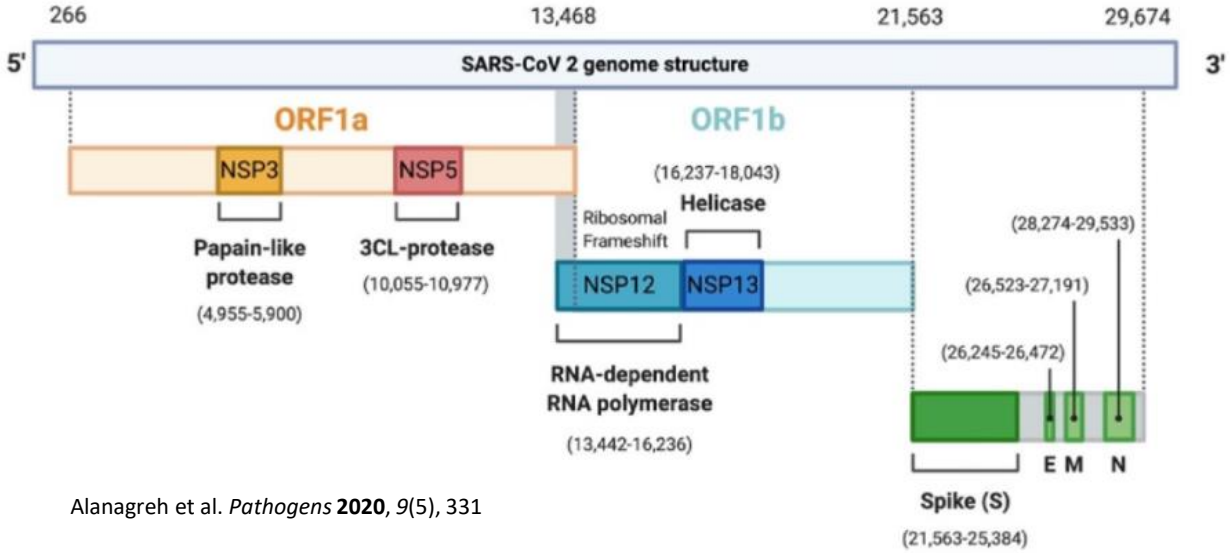
- Highlight testing recommendations

SARS-CoV-2

Protein and genome structure

- Enveloped β-coronavirus
  - ss +RNA
- Diagnostic targets
  - Nucleic acid amplification
    - ORFab, RdRp, S, E and/or N genes
  - Antigen
    - S or N protein
  - Antibody
    - S and/or N proteins





Alanagreh et al. *Pathogens* **2020**, *9*(5), 331

Nucleic acid amplification testing (NAAT)

Analytical test performance

- RNA detection is the diagnostic standard to confirm infection

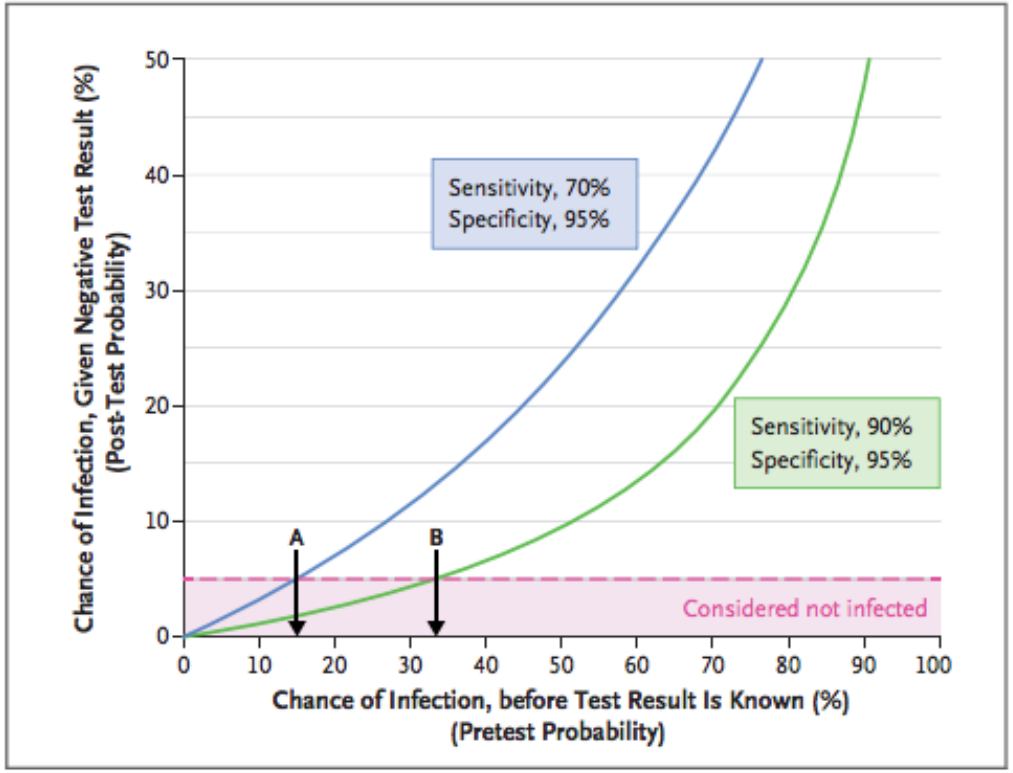
- > 180 FDA emergency use authorization (EUA) molecular tests
  - Various methodologies – isothermal NAAT, PCR, TMA
  - Analytical test characteristics are variable
    - ✓ FDA limit of detection comparison NAAT detectable units (NDU)/mL
      - Swabs in transport media 180 – 1800 NDU/mL
      - Dry swabs 60,000 – 540,000 NDU/mL
      - Saliva 600 – 180,000 NDU/mL
    - ✓ Specificity is high > 95%

Nucleic acid amplification testing (NAAT)

Clinical test performance

- Clinical performance difficult to establish in the absence of a gold-standard
  - ✓ 70% sensitivity range vs. clinical composite
  - ✓ 94% sensitivity vs. NAAT test comparison

NEGATIVE PREDICTIVE VALUE

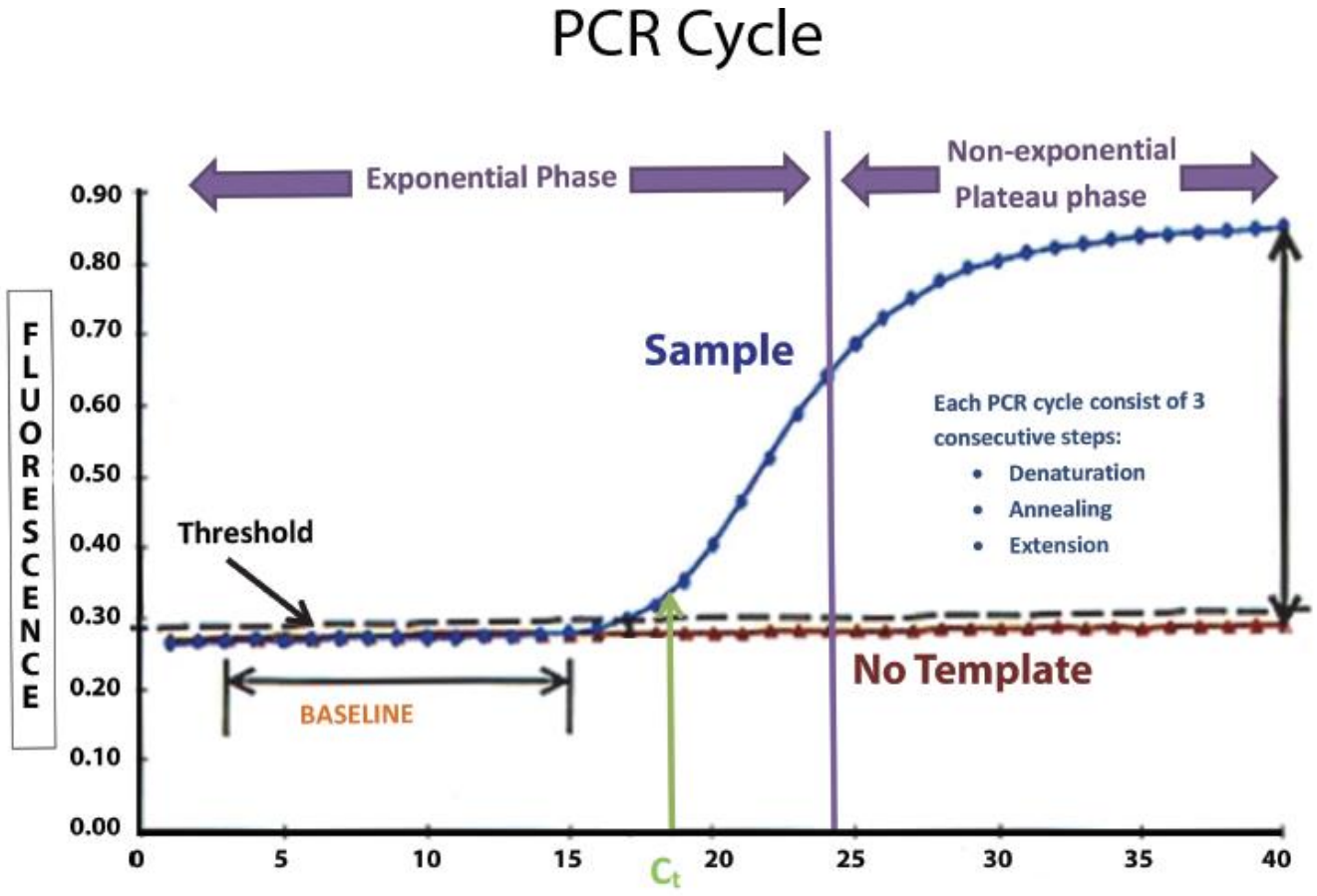


Kucirka et al. Annals Internal Med 2020; https://doi.org/10.7326/M20-1495; Sultan et al. Gastroenterology 2020; PMID: 32735862

Woloshin et al. NEJM 2020; 383(6): e38

Laboratory-based PCR Platforms

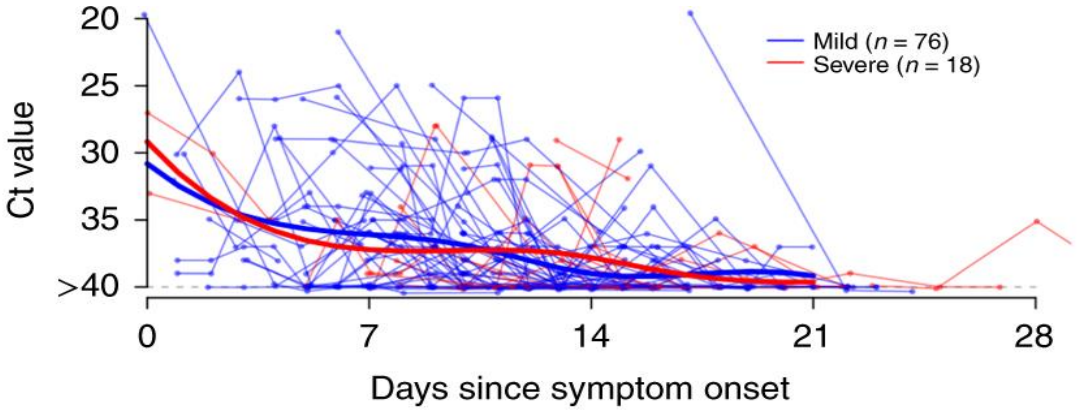
Crossing thresholds (Ct values)



Viral load and kinetics

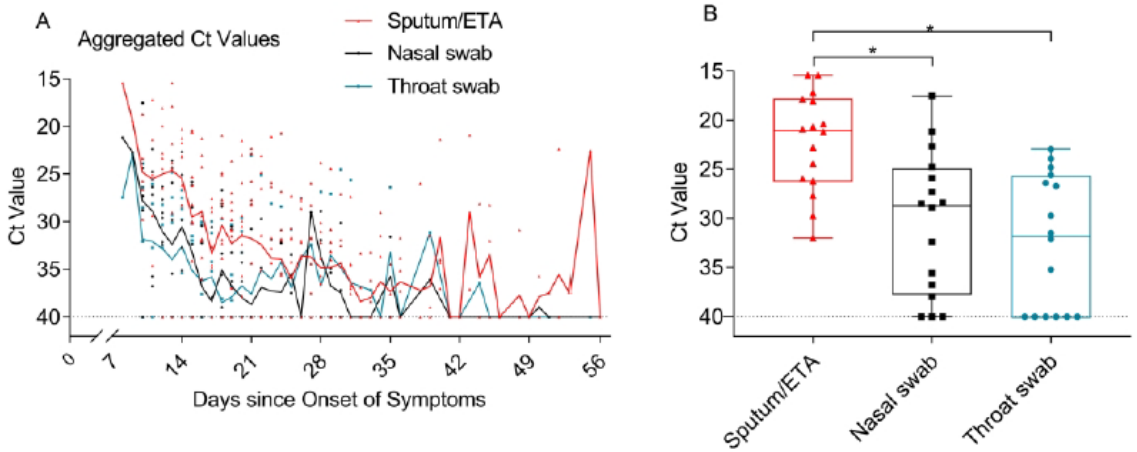
Importance of time post-symptom onset, host and specimen type

Temporal dynamics of virus shedding throat swabs



He *et al.* *Nat Med* 2020 **26,** 672–675 (2020)

Temporal dynamics upper versus lower respiratory tract shedding



Huang et al. *Am J Resp Crit Care Med* 2020; 201(11): 1435-1438

- Duration of viral shedding in upper tract 17 days, maximum 83 days Cevik et al. Lancet Microbe 2020 DOI:https://doi.org/10.1016/S2666-5247(20)30172-5
  - typically longer in critically ill, immunocompromised and lower respiratory tract samples

Zhou et al. Lancet. 2020; **395**: 1054-1062; Tong-Zeng et al .JCM 2020; **https://doi.org/10.1002/jmv.26280;**

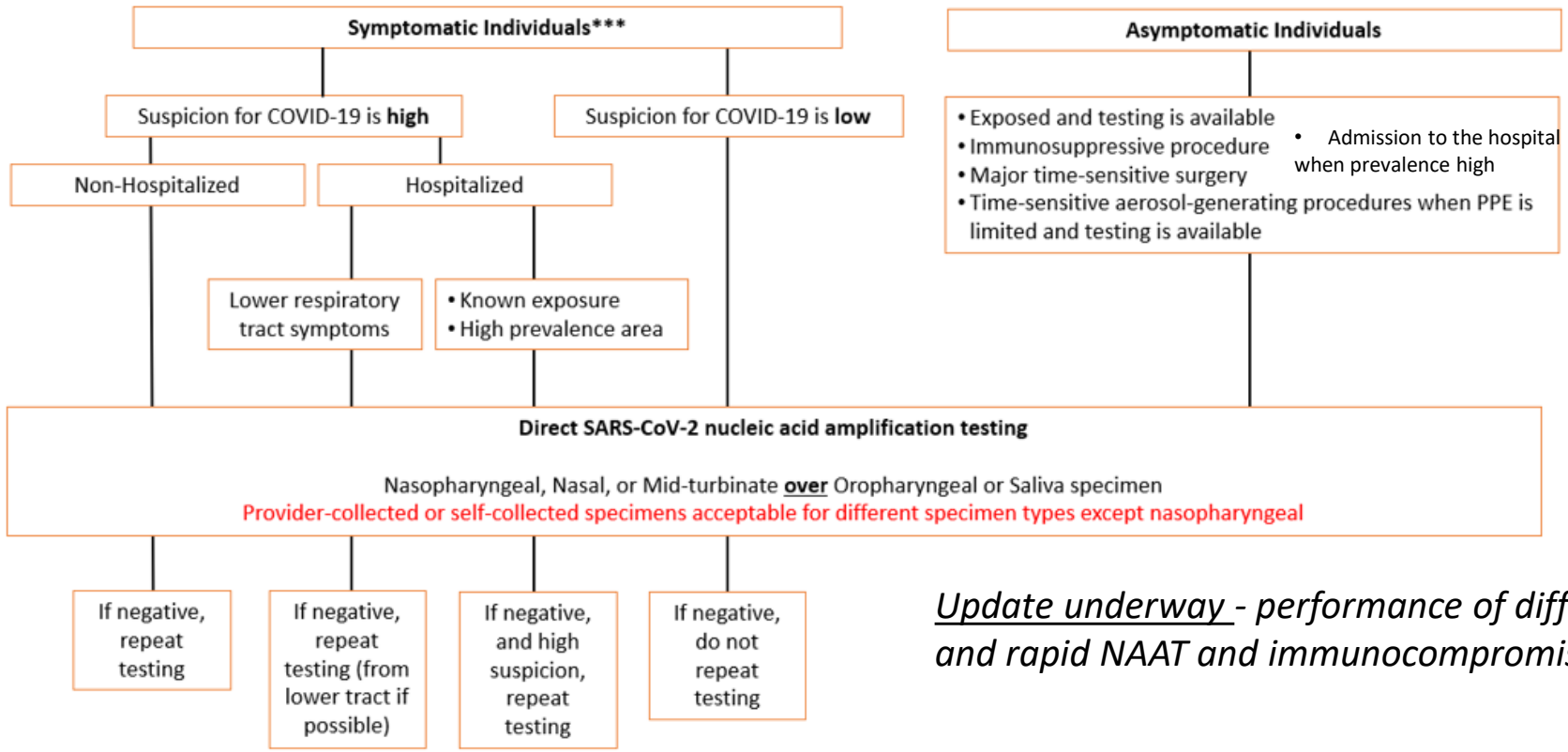
- Virus loads in asymptomatic individuals roughly equivalent to symptomatic patients

Lee et al. JAMA Internal Med 2020; Ra et al. Thorax 2020

Clinical Infectious Diseases
IDSA FEATURES

Infectious Diseases Society of America Guidelines on the Diagnosis of Coronavirus Disease 2019

Kimberly E. Hanson,[1] Angela M. Caliendo,[2] Cesar A. Arias,[3] Janet A. Englund,[4] Mark J. Lee,[5] Mark Loeb,[6] Robin Patel,[7] Abdallah El Alayli,[8] Mohamad A. Kalot,[9] Yngve Falck-Ytter,[10] Valery Lavergne,[11] Rebecca L. Morgan,[12] M. Hassan Murad,[13] Shahnaz Sultan,[14] Adarsh Bhimraj,[15] and Reem A. Mustafa[16]



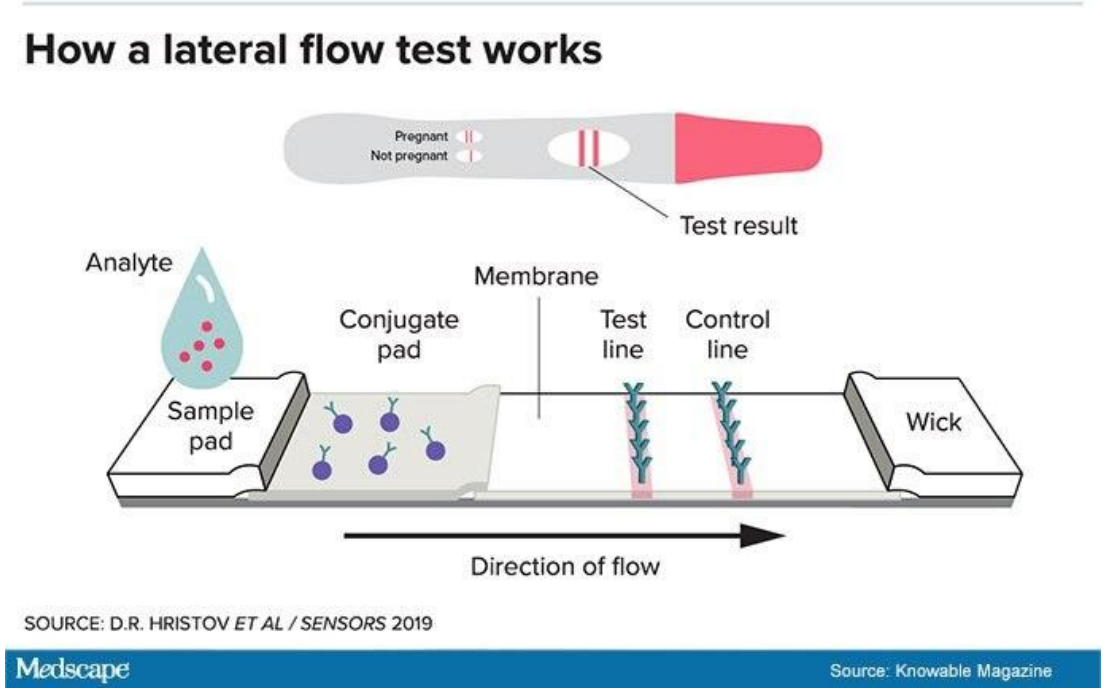
Update underway - performance of different specimen types and rapid NAAT and immunocompromised patients

***Note:**

- Testing should be prioritized for symptomatic patients first.
- When resources are adequate, testing for selected asymptomatic individuals can also be considered.

Antigen Testing

- 7 EUA immunoassays
  - Nasal or NP swab
  - Difficult to compare LODs
- Low complexity and low cost with rapid turn around (~ 15 minutes)
- Less sensitive than NAAT (~ 85% vs. PCR)
  - Best when viral load is highest
    - 1st 7 days of symptoms
    - Ct cut-offs ≤ 25-30 range
    - Less sensitive in children?
    - **High clinical suspicion or medium-high prevalence back up with NAAT!**
- FDA issued an alert about false positive results



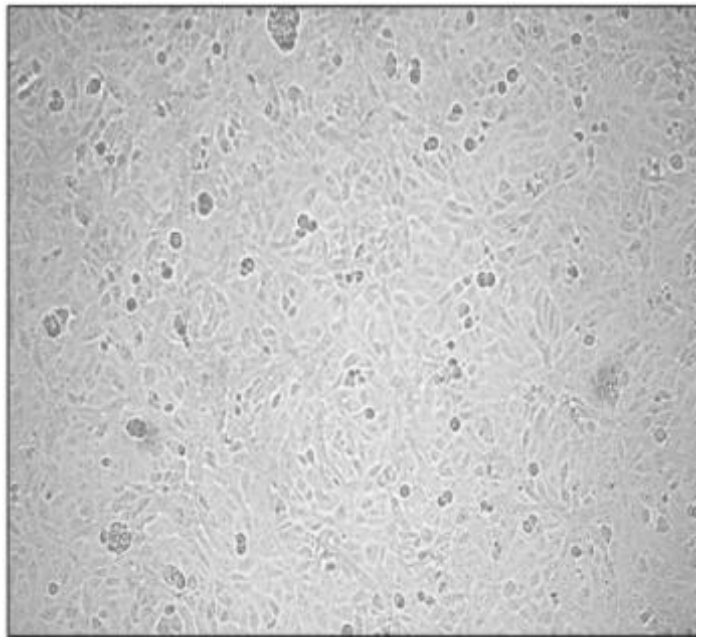
Pollack et al. https://www.mass.gov/info-details/about-covid-19-testing;
Albert et al. medRxiv **2020**: 2020.10.16.20213850
Pilarowski et a. medRxiv **2020**: 2020.11.02.20223891.

Viral Culture

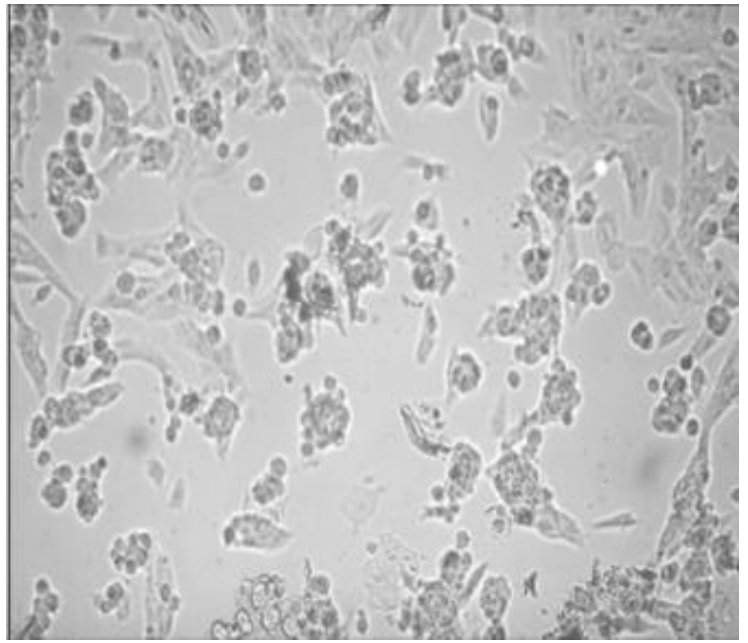
- BSL3 pathogen
- Likely grows in routine clinical virology cell lines (R-mix, RhMK) without CPE
- Vero E6 cells display highest titers and CPE

Leland and Ginocchio **DOI:** 10.1128/CMR.00002-06

Uninfected VERO cells



SARS CoV cytopathic effect (CPE) 24 hrs



Vicenzi et al. EID 2004; 10(3)

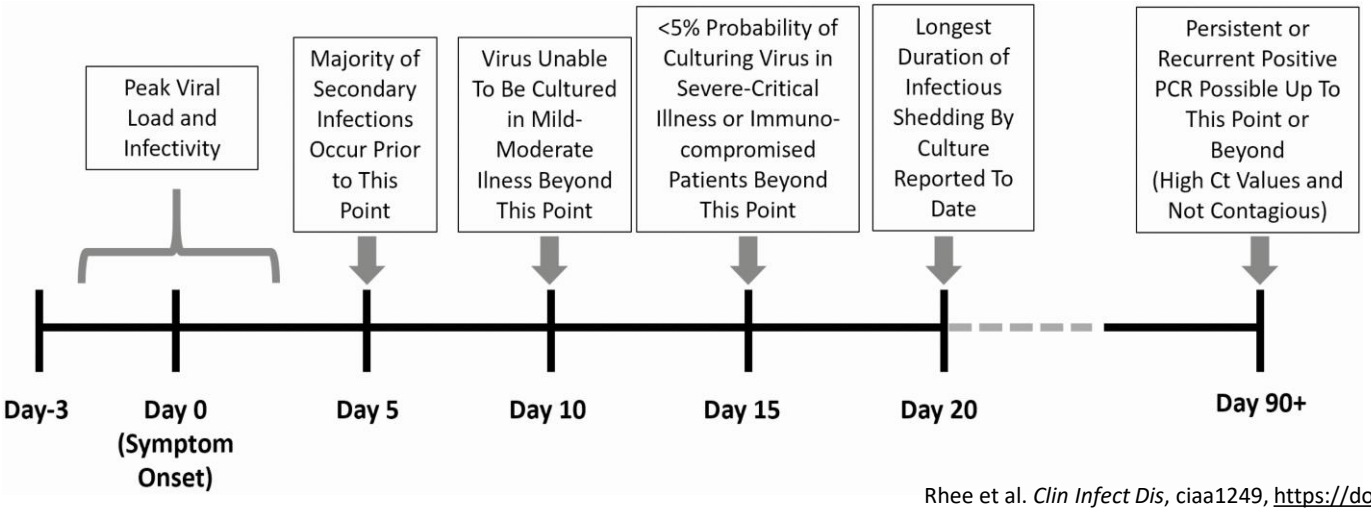
The RNA or Antigen detection vs. culture

- Animal models – culture positivity correlates with transmissibility
  - No direct human studies
- Detectable RNA does not equal culture positivity or infectiousness
  - Envelope lysis and/or virus particle aggregation prevents subsequent infection, but does not eliminate nucleic acid, which degrades slowly over time
  - RNA fragments associated with intracellular vesicles protected from degradation
- Antigen detection may correlate better than PCR with culture

Pekosz et al. medRxiv **2020**: 2020.10.02.20205708

Duration of culture positivity and infectivity

Evidence base for isolation recommendations



Rhee et al. *Clin Infect Dis*, ciaa1249, https://doi.org/10.1093/cid/ciaa1249

- Rare replication competent virus > 9 days after symptom onset (longest reported 20 days)
  - majority of transmission events within 5 days of symptom onset
- Culture negative when Ct > 35 (**Ct values across platforms not directly comparable**)
- Current recommendation - wait 10 to 20 days after symptom onset to discontinue transmission-based precautions
  - Note of caution - relapsing infection (and culture positivity) reported in severely immunocompromised after 20 days

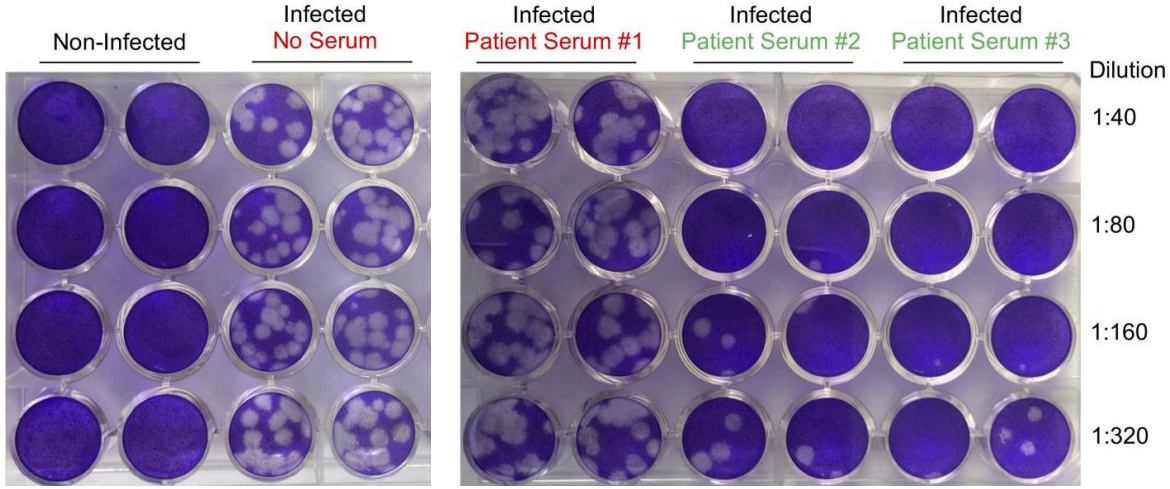
Anti-SARS-CoV-2 antibody testing

- Types of antibodies
  - Binding antibodies (Abs)
    - IgM, IgG, IgA and total
  - Neutralizing antibodies (nAbs)
    - Block cell infection (S protein)

- 56 EUA tests detect anti-S and/or N Abs
  - Do not differentiate binding vs. neutralizing
  - Variable test performance across assays

- Neutralization assays research/reference lab
  - Plaque reduction assays with natural or pseudovirus
  - Variable nAb titer correlation with commercial EUA tests

Plaque Reduction Neutralization Testing



JCI Insight. 2020;5(22):e143213. https://doi.org/10.1172/jci.insight.143213

Tang et al. Clin Chem 2020; Bonelli JCM 2020; 24;58(9):e01224-20; Suhandynata et al Clin Chem 2020

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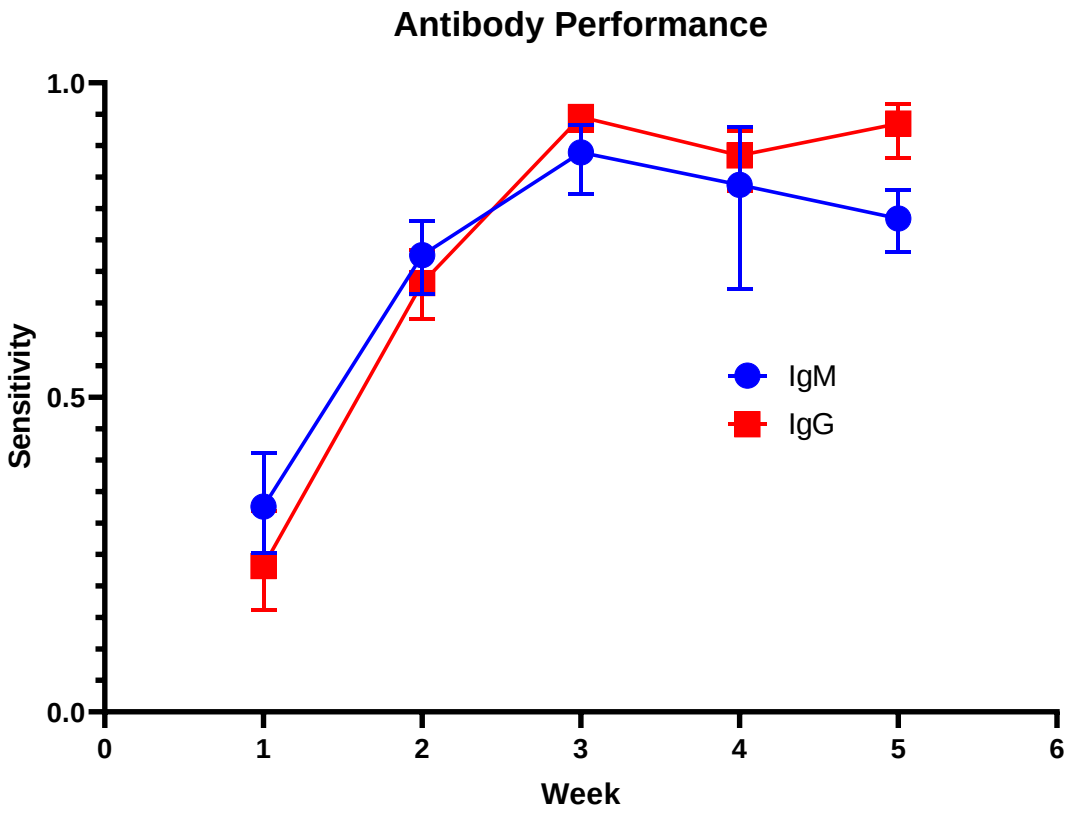


Pooled test characteristics

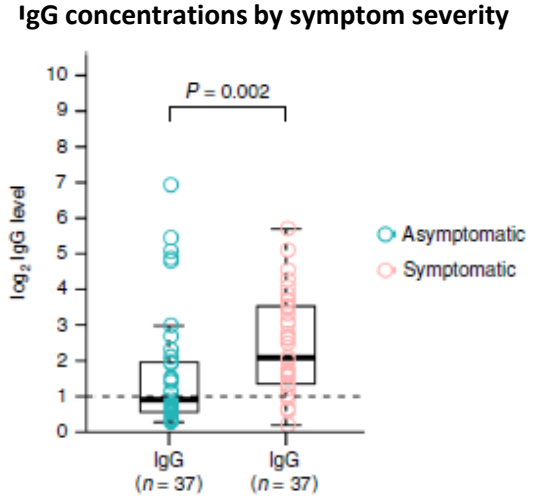
Sensitivity IgM week 2 = 73%
Sensitivity IgG week 4 = 88%
Specificity >97%

No difference by antigen targeted

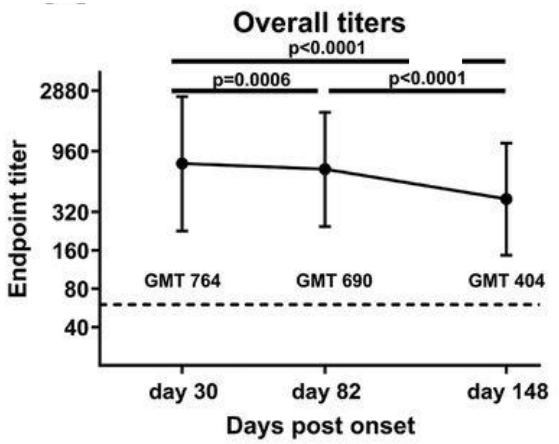
Antibody Testing

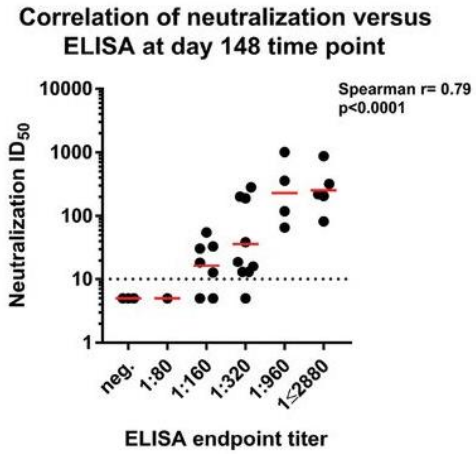
Clinical observations and recommendations

- Duration of the Ab response and protection
  - Animal studies – infection provides at least short-term immunity and anamnestic response
  - Ab titers correlate with severity of illness
  - NYC study anti-S Ab titers relatively stable for a least 5 months (n=121 volunteers)



Long QX et al. Nature Medicine 2020





Wajnberg et al. Science 2020

Antibody Testing

Clinical utility for diagnosis

- Individuals with a history of COVID-19-like illness
  - when clinical suspicion is high and repeated NAAT negative to support diagnosis
  - for defining multisystem inflammatory syndrome in children (MIS-C)

- Titer that correlates with protection **not yet identified**

- Identifying convalescent serum donors

- No role for donor/recipient screening or pre-chemo

Reinfection

	Sex	Age (years)	First infection (Ct)	Second infection (Ct)	Intervening period (days)	Antibody after first infection	Antibody after reinfection
Hong Kong[3]	Male	33	Mild (N/A)	Asymptomatic (27)	142	Negative	IgG+
Nevada, USA[2]	Male	25	Mild (35)	Hospitalised (35)	48	N/A	IgM+ and IgG+
Belgium[4]	Female	51	Mild (26–27)	Milder (33)	93	N/A	IgG+
Ecuador[5]	Male	46	Mild (37)	Worse (N/A)	63	IgM+ and IgG–	IgM+ and IgG+

Data were obtained Sept 14, 2020, for reinfection cases confirmed by viral genome sequences. Ct=cycle threshold. N/A=not available. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

Iwasaki *Lancet infect Dis* 2020; DOI:https://doi.org/10.1016/S1473-3099(20)30783-0

- Repeat testing positive > 90 days after initial test
- Confirmation requires paired whole genome sequencing (WGS)
- Ct <33-35 in second sample or seroconversion is suggestive

Conclusions

- NAAT remains the diagnostic reference test to confirm infection
- Know the tests you laboratory is performing
- Many unanswered diagnostic questions remain
  - Predictors of infectious/replication competent virus
  - Immune correlates of protection
  - Test performance in children and asymptomatic individuals