# INA RESPOND Webinar: Laboratory Diagnosis of COVD-19



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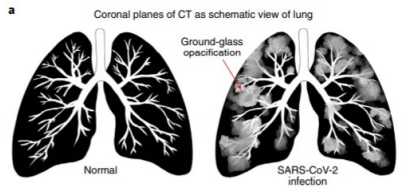
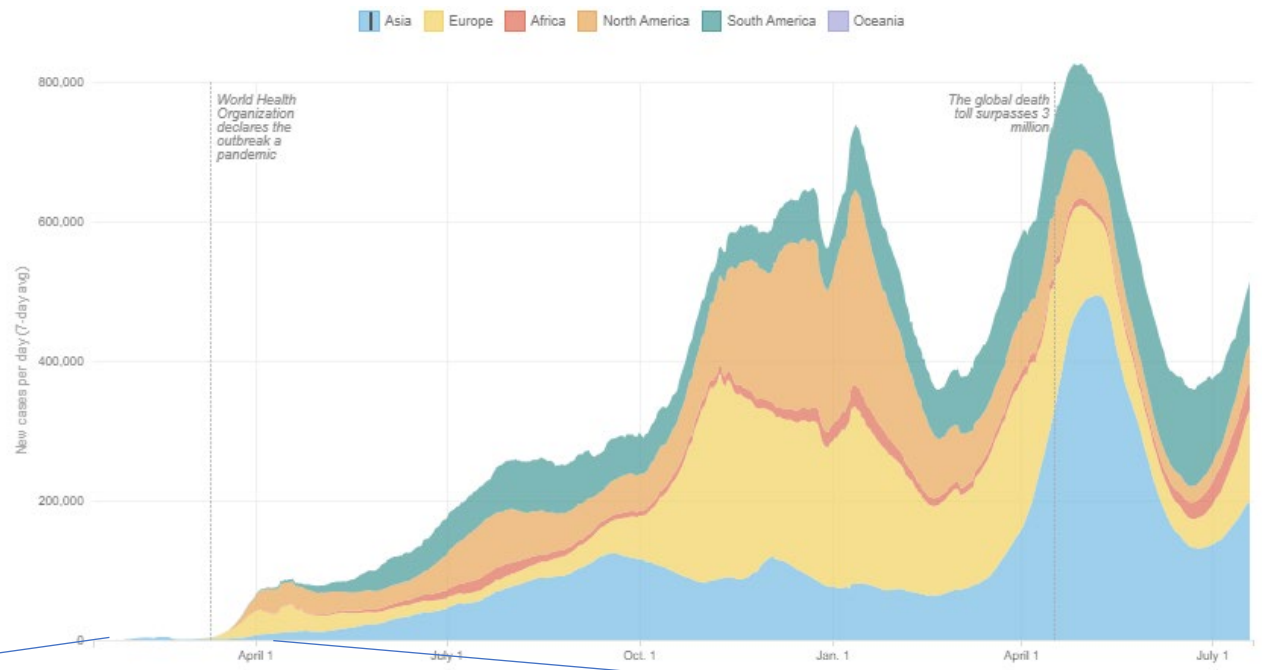
**Collaborative Clinical Research Branch**

**Division of Clinical Research**

**National Institute of Allergy and Infectious Diseases**

# Evolution of diagnostic testing during the COVID-19 pandemic




| 10. Jan 2020 | 16. Jan 2020 | 11. Mar 2020 | 4. April 2020 |
|---|---|---|---|
| SARS-CoV-2 genome sequence published (Wuhan strain) | 1st RT-PCR tests within China | WHO declares pandemic | 1st serological test US FDA EUA |

**Today**

FINDDx:
1126 molecular & immunoassays

FDA EUA:
252 molecular tests
30 antigen tests
85 serology & adaptive immune response tests

Kevadiya, B.D., et al., https://doi.org/10.1038/s41563-020-00906-z;;
https://bioprocessintl.com/analytical/diagnostics/trends-in-development-of-covid-19-diagnostic-tests/;
https://www.mdpi.com/2075-4418/11/2/182/htm#B3-diagnostics-11-00182

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# How do you choose which diagnostic test to use?

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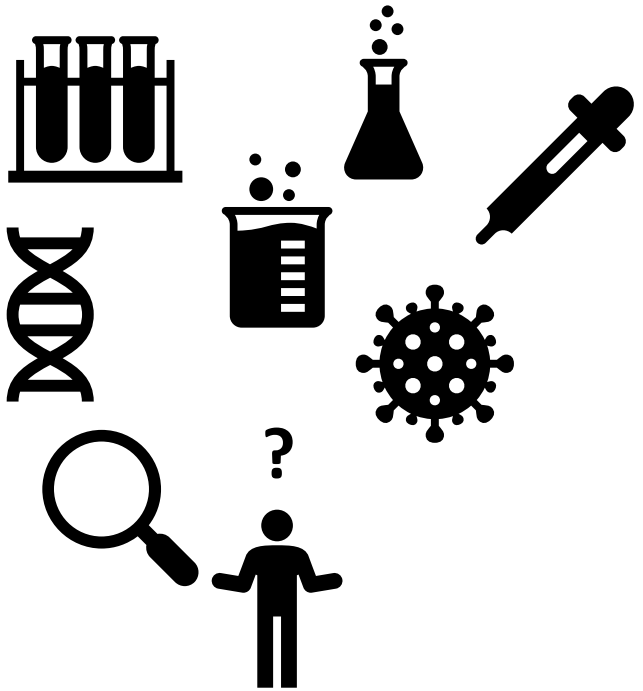
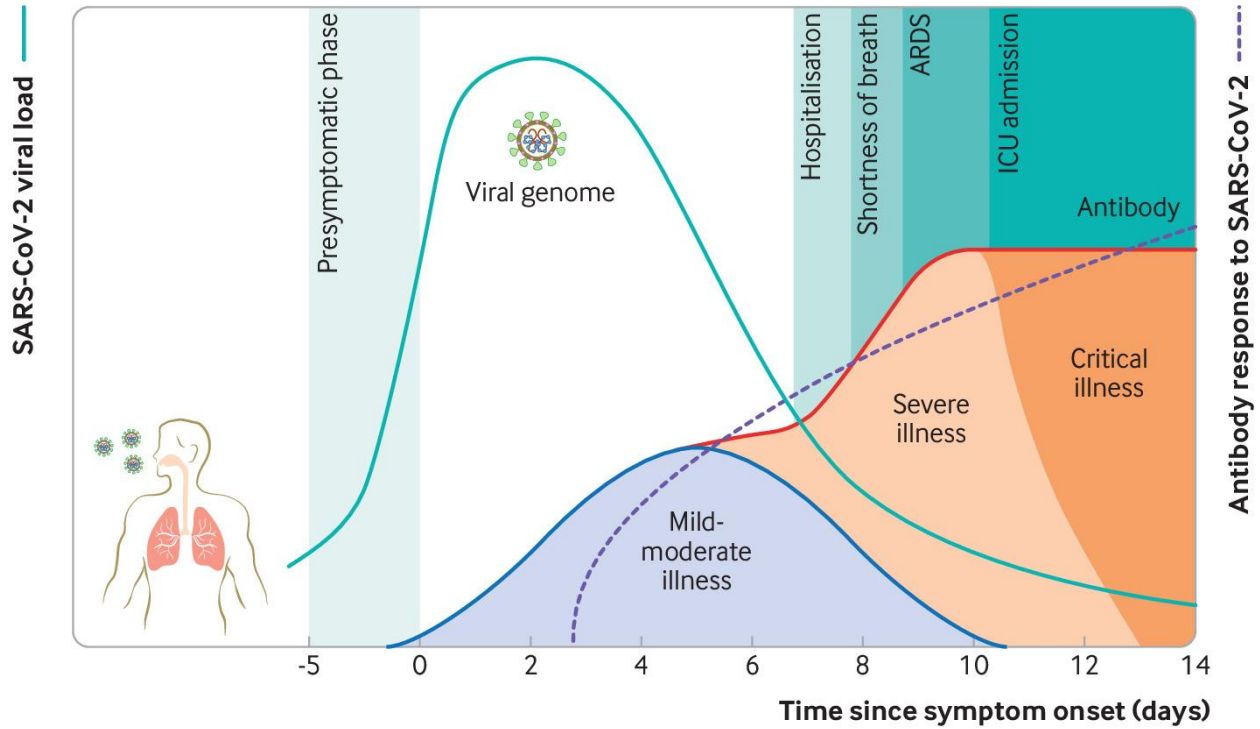
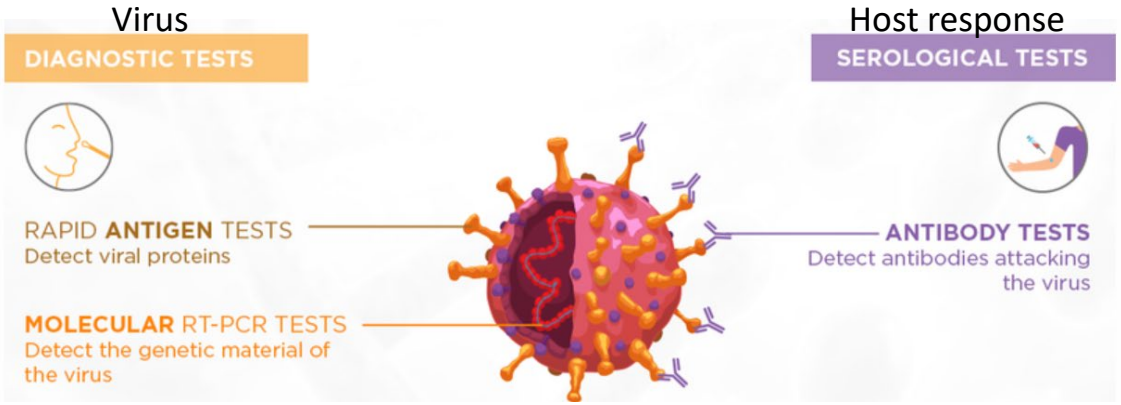



**Depends on several factors**

Kevadiya, B.D., et al., https://doi.org/10.1038/s41563-020-00906-z;;
https://bioprocessintl.com/analytical/diagnostics/trends-in-development-of-covid-19-diagnostic-tests/;
https://www.mdpi.com/2075-4418/11/2/182/htm#B3-diagnostics-11-00182

# Status in the course of infection, what you are trying to detect, & the reason



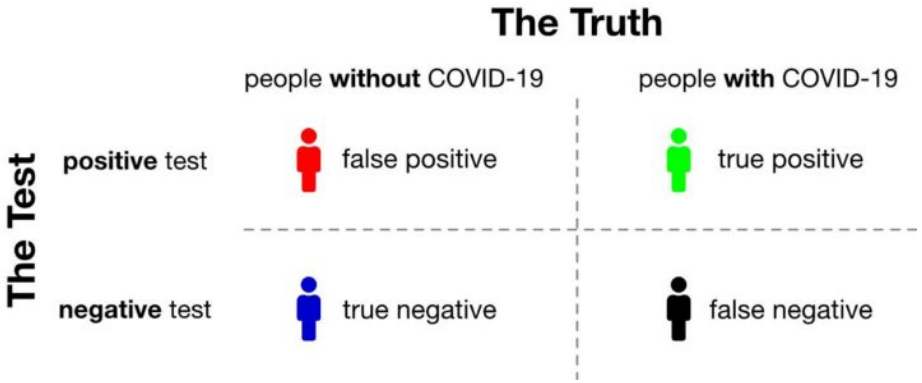

## What is the reason for the testing?

- Clinical decision making
- Public health purposes
- Research purposes

Cevik, M., et al., https://doi.org/10.1136/bmj.m3862; https://nipdlabs.com.cy/articles/blog/what-is-the-difference-between-rapid-antigen-tests-molecular-rt-pcr-tests-and-antibody-tests/

# Performance

## Test performance




Sensitivity: ability to accurately identify those infected

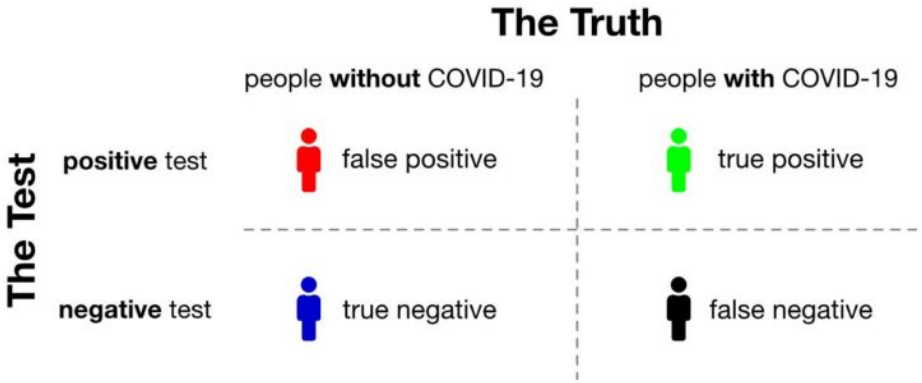
Specificity: ability to accurately identify those *not* infected

These are calculated base on a gold standard and are most important for the laboratory team

https://www.statnews.com/2020/03/31/covid-19-overcoming-testing-challenges/

# Performance

## Test performance

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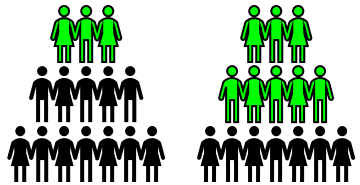
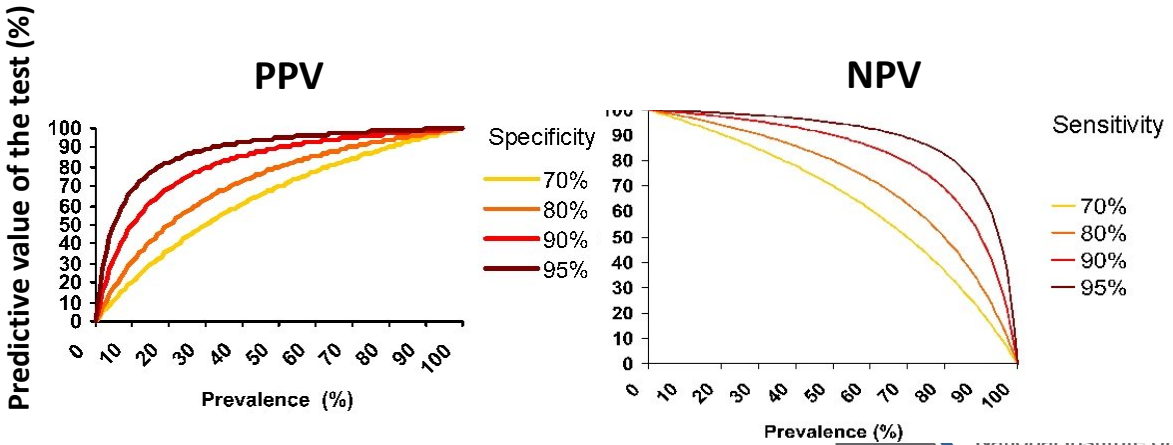
## Predictive Value



Positive predicative value (PPV): probability of being infected with a positive test

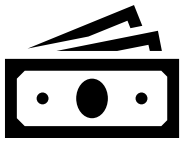
Negative predictive value (NPV): probability of not being infected with a negative test

Most important for clinicians and individuals being tested

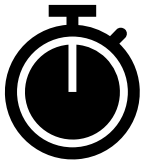


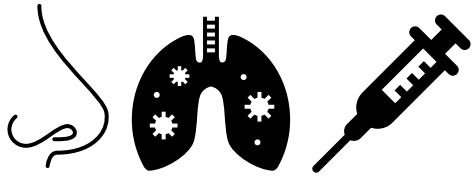

https://www.statnews.com/2020/03/31/covid-19-overcoming-testing-challenges/; WHO. Laboratory Training for Field Epidemiologists

# Other considerations

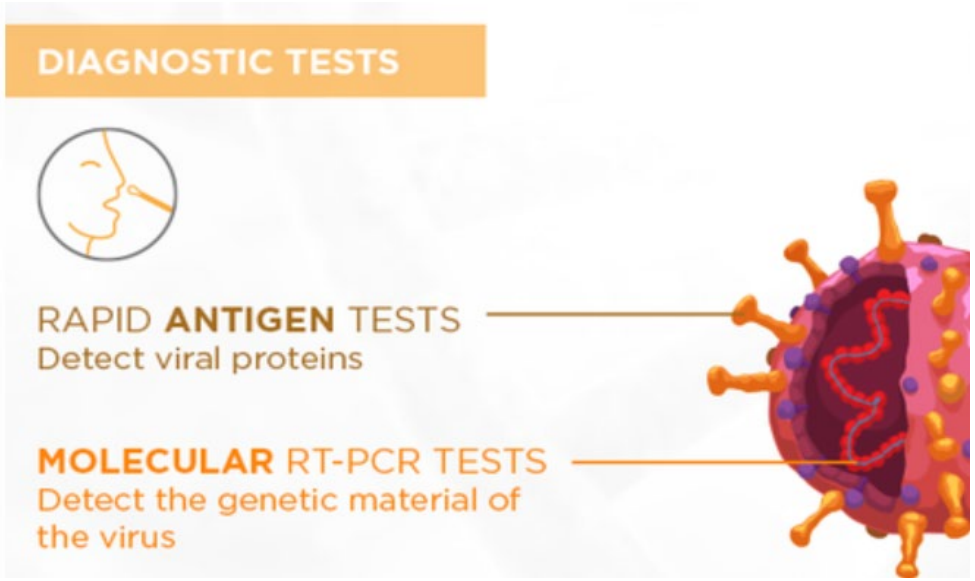
**Cost** 

**Complexity** 

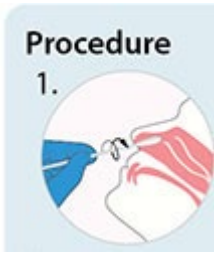
**Time** 

**Specimen type & collection** 

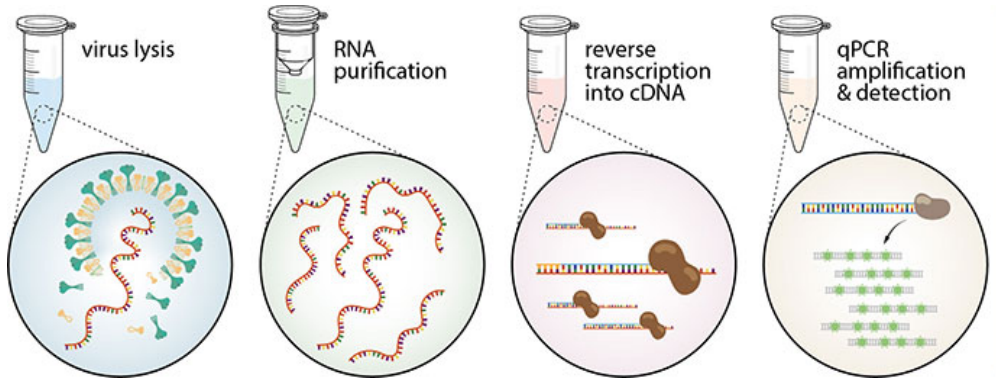
# Detection of the virus: molecular or antigen test?



# Molecular testing versus antigen testing: technology

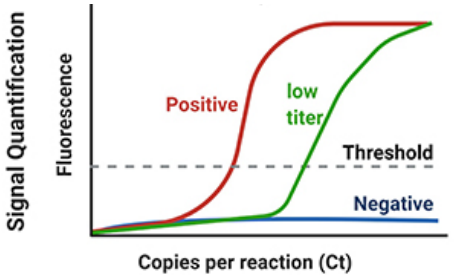



**Molecular Testing**

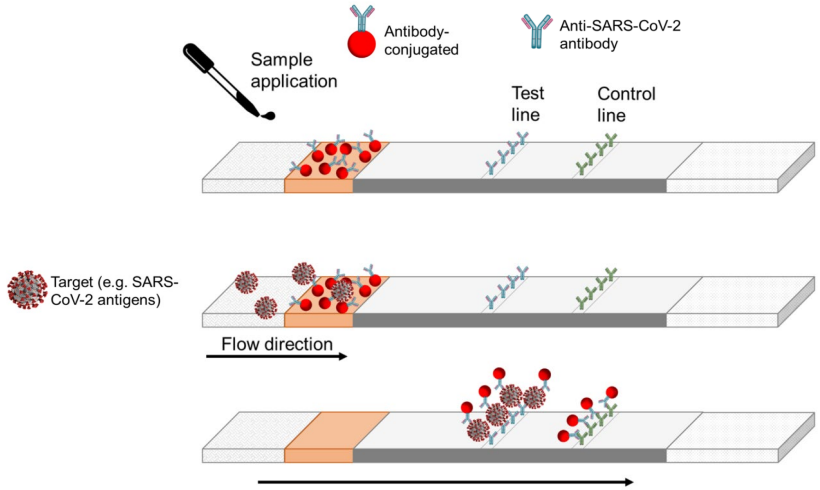


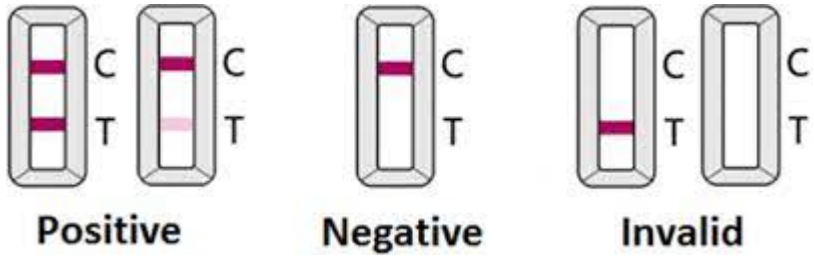

**Results**

Positive Negative




**Antigen Testing**



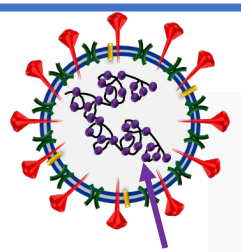

https://www.enzolifesciences.com/science-center/technotes/2020/july/how-do-coronavirus-disease-covid-19-tests-work?/; http://newsite.glasgowkelvin.ac.uk/winter-break-covid-19-student-testing-programme/; https://www.lateralflows.com/lateral-flow-assays/; Habali, Z., et al., https://doi.org/10.3389/fbioe.2020.605702

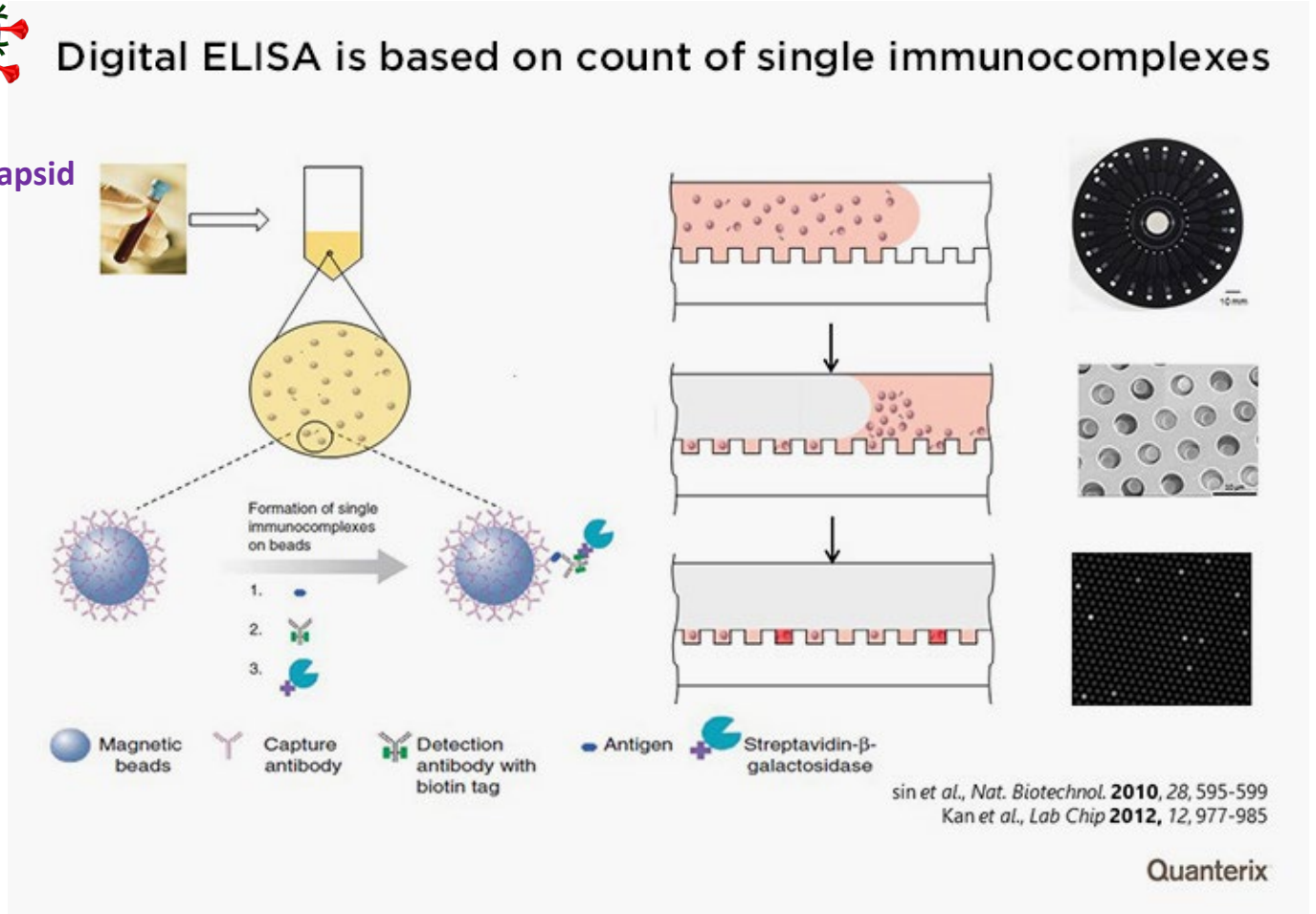
# Molecular testing versus antigen testing

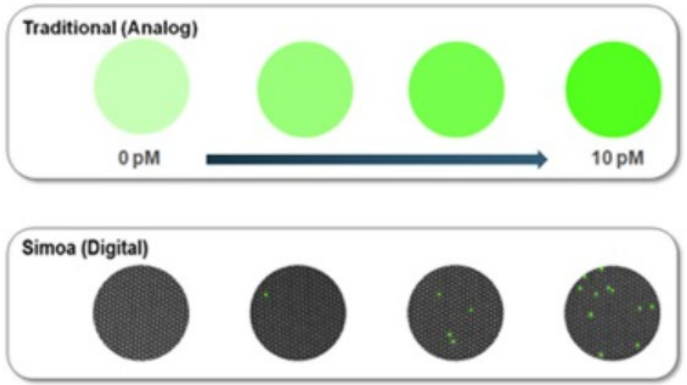
| | Molecular Test | Antigen Test |
|---|---|---|
| Other names | Nucleic acid amplification test (NAAT), RT-PCR | Rapid diagnostic test |
| Detects | Viral genome (SARS-CoV-2 RNA: one or multiple genes)<br>Amplification step | Viral antigen (Nucleocapsid or Spike)<br>No amplification step |
| Specimen type | Respiratory swab, throat swab, saliva, wastewater | Nasal or throat swab |
| Advantages | Gold standard, highly accurate (only needs to be run once)<br>Low limit of detection (“only need a little bit”) | Rapid results, low complexity, low cost<br><br>High limit of detection |
| Limitations | Can have long turnaround time, requires special equipment<br>Higher cost | Lower sensitivity compared to molecular tests (especially when asymptomatic/pre-symptomatic) |

# New technology: quantitative antigen testing with very low limit of detection

- Quanterix Simoa
  - Single Molecule Array (Simoa)
  - Detects nucleocapsid
  - Plasma, serum, VTM (swab), saliva, DBS
  - Limit of quantitation (LoQ) = 3 pg/mL
    - ~1,000+ more sensitive than traditional ELISA antigen assays



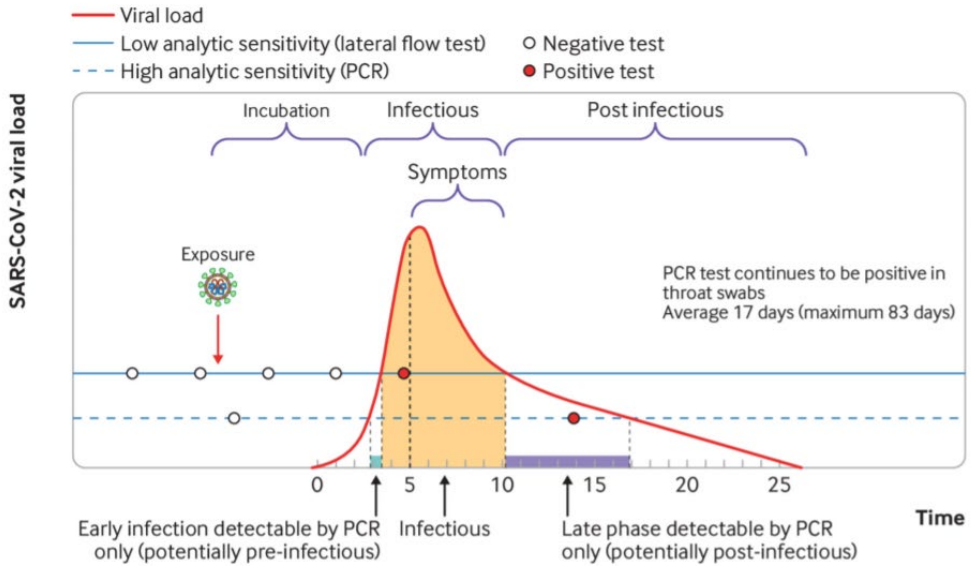



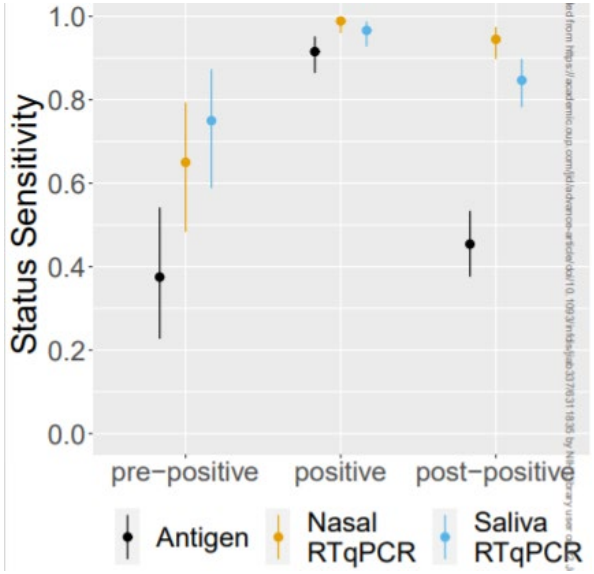




Has FDA EUA but is complex and upfront cost of the machine is expensive

Use is advantageous as a research test

# Test choice: detection of the virus during the course of infection




Crozier A., https://www.bmj.com/content/372/bmj.n208; Avanzato, A.N. et al., https://www.cell.com/cell/pdf/S0092-8674(20)31456-2.pdf; Cento, V. et al., https://www.journalofinfection.com/article/S0163-4453(20)30405-9/pdf; D'Andres, A. et al., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7279900/; Tiwari, T. et al., http://dx.doi.org/10.1136/bcr-2020-241087; Pekosz, A., et al., https://doi.org/10.1093/cid/ciaa1706; Pickering, S., et al.;., https://doi.org/10.1016/S2666-5247(21)00143-9 ; Huang, C.G, et al., ***https://doi.org/10.1128/JCM.01068-20;*** Crozier A., https://www.bmj.com/content/372/bmj.n208; McKay, S.L. et al., https://www.acpjournals.org/doi/pdf/10.7326/M21-0422

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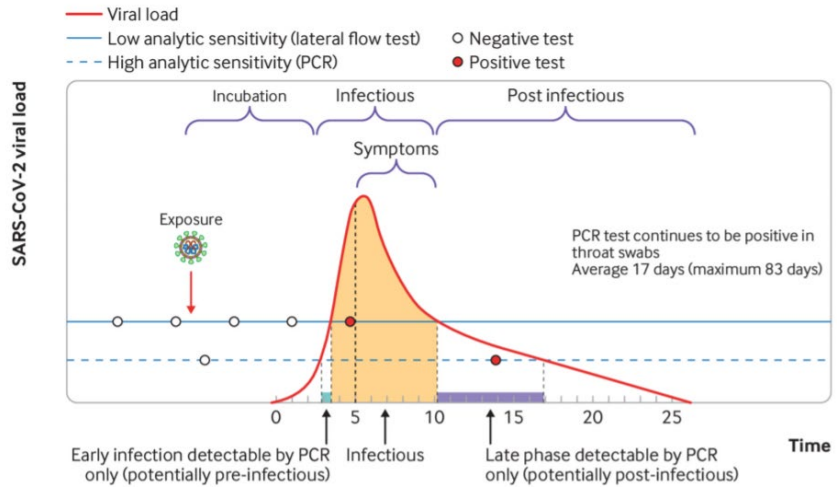


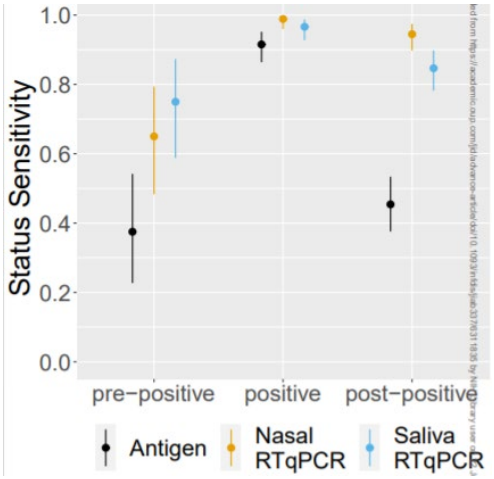


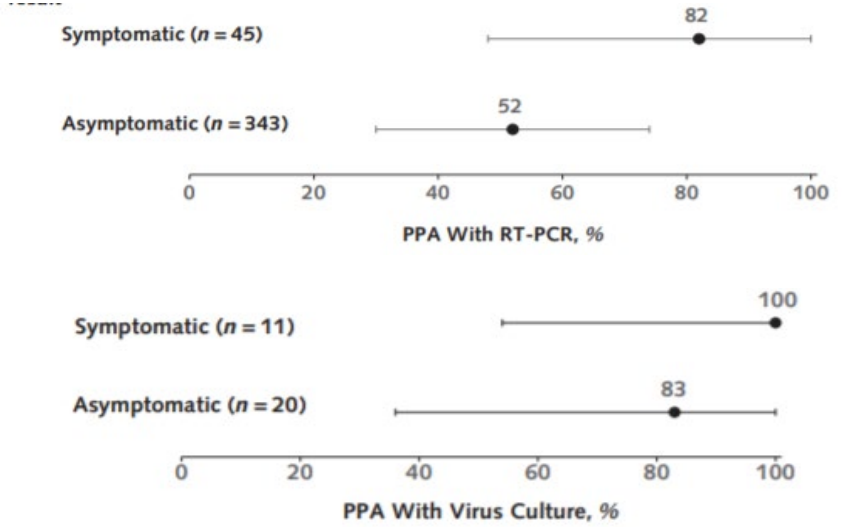


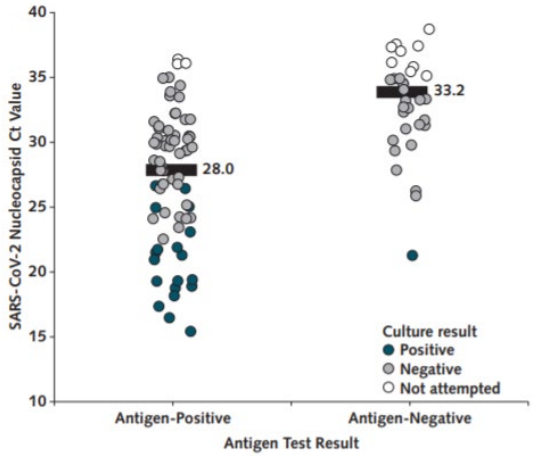


Crozier A., https://www.bmj.com/content/372/bmj.n208; Avanzato, A.N. et al., https://www.cell.com/cell/pdf/S0092-8674(20)31456-2.pdf; Cento, V. et al., https://www.journalofinfection.com/article/S0163-4453(20)30405-9/pdf; D'Andres, A. et al., https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7279900/; Tiwari, T. et al., http://dx.doi.org/10.1136/bcr-2020-241087; Pekosz, A., et al., https://doi.org/10.1093/cid/ciaa1706; Pickering, S., et al.;., https://doi.org/10.1016/S2666-5247(21)00143-9 ; Huang, C.G, et al., ***https://doi.org/10.1128/JCM.01068-20;*** Crozier A., https://www.bmj.com/content/372/bmj.n208; McKay, S.L. et al., https://www.acpjournals.org/doi/pdf/10.7326/M21-0422

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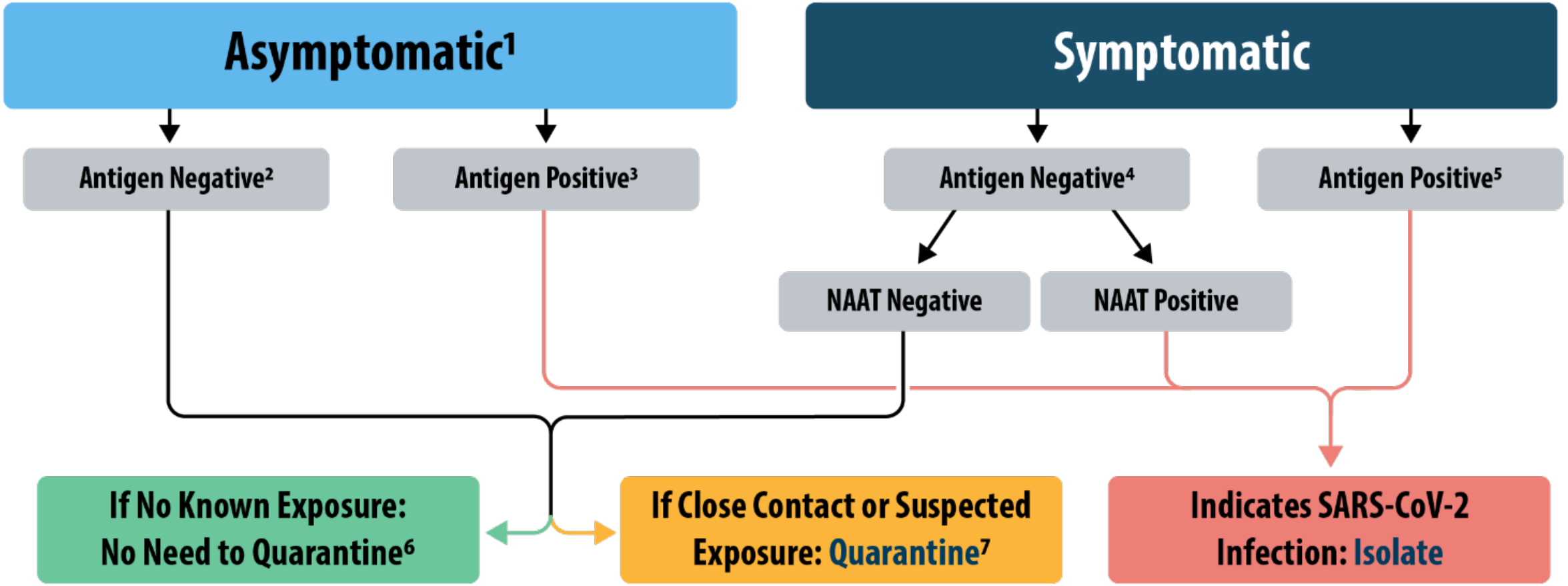
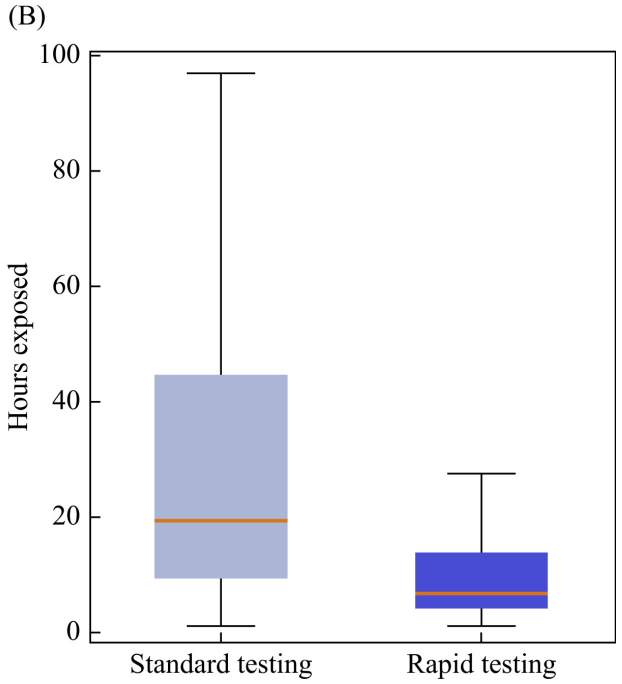
# Testing algorithms in community settings using antigen tests




https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html#table1

# Targeted rapid molecular testing when resources are limited

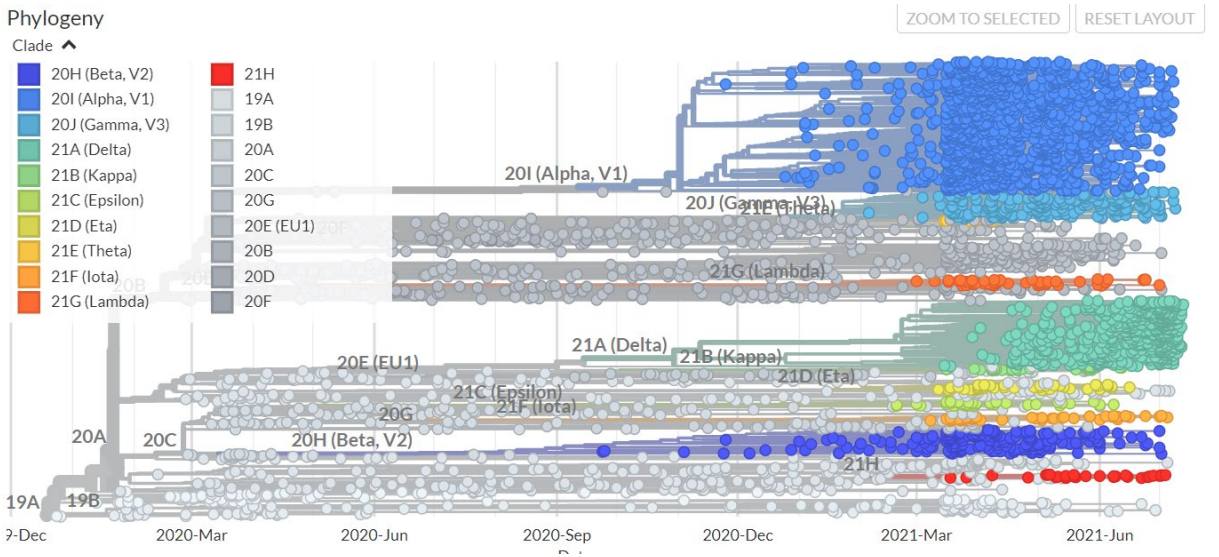
- Johns Hopkins Hospital Emergency Department (Maryland, USA)
  - March-June 2020
  - In the ED: SARS-CoV-2 suspected cases cohorted with confirmed cases while waiting for test results
  - Median turnaround time 7.8 hours (IQR: 3.71–11.68) on standard NAAT
  - Implement rapid NAAT (GeneXpert)
    - TAT: 1.90 hour (IQR: 1.40–2.82)
  - Reduced median time for exposure (negative cases): 65.6% reduction (12.6 hours)
    - Increased treatment capacity
    - Saved PPE for HCW

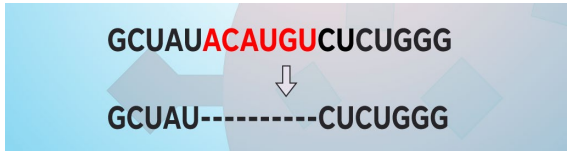


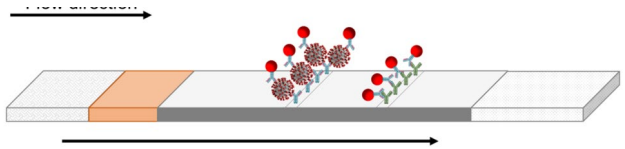

Hinson, J.S., et al., https://doi.org/10.1016/j.jhin.2020.09.035

# Variants impact on diagnostics

- Most diagnostics developed based on the Wuhan sequence
- Global spread of variants: reason for concern about false negatives
  - Monitoring impact on diagnostics (USG, FINDDx, PATH)
- Molecular tests:
  - Vulnerable to mutations
  - Tests with multiple targets may retain sensitivity
- Antigen assays:
  - Most target the nucleocapsid (fewer mutations)
  - Recognize a protein (mutations may impact level of detection but retain sensitivity)



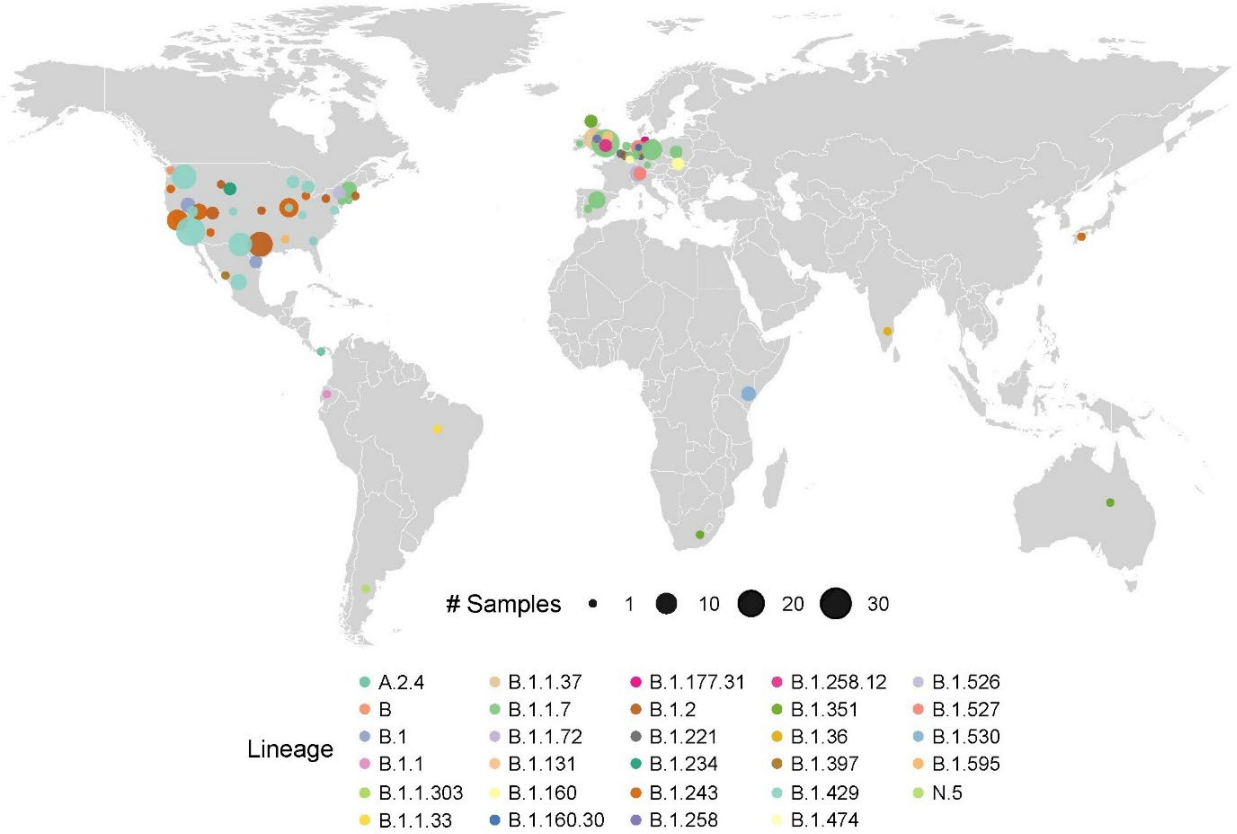



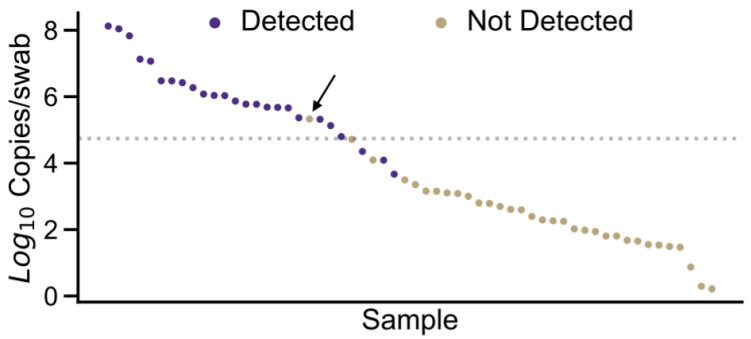



https://nextstrain.org/ncov/gisaid/global
https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-viral-mutations-impact-covid-19-tests;
https://www.finddx.org/wp-content/uploads/2021/03/COVID-variants-report-FINAL-12MAR2021.pdf; https://www.path.org/programs/diagnostics/covid-dashboard-implications-variants-covid-19-molecular-test-detection/

# Although antigen assays are less susceptible to impact by variants…

- Quidel Sofia SARS Antigen FIA
- Nucleocapsid: D399N (found in multiple lineages)

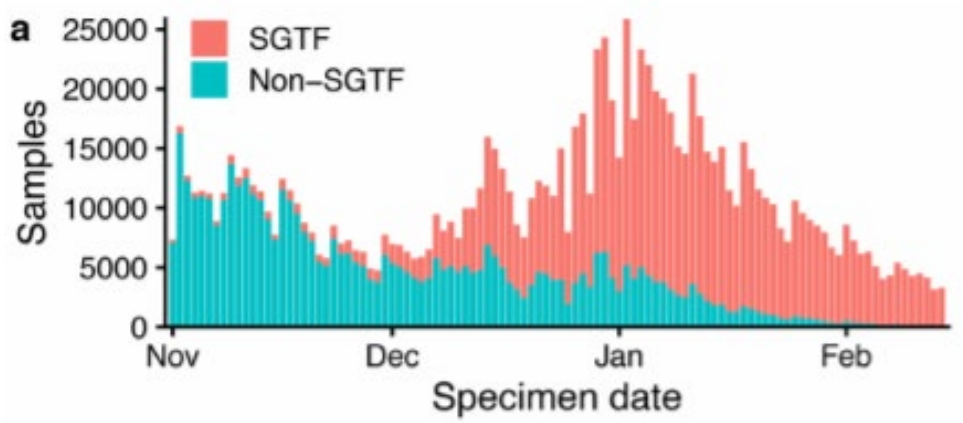



Bourassa, L. et al., https://www.medrxiv.org/content/10.1101/2021.05.05.21256527v1.full.pdf

# S gene target failure: screen for B.1.1.7 (alpha)

- B.1.1.7 (alpha): Δ69/70 Spike
- TaqPath assay
  - RT-PCR with targets in spike, nucleocapsid, ORF1a
  - Deletion causes “S gene target failure” (SGTF)
  - Retains analytic sensitivity



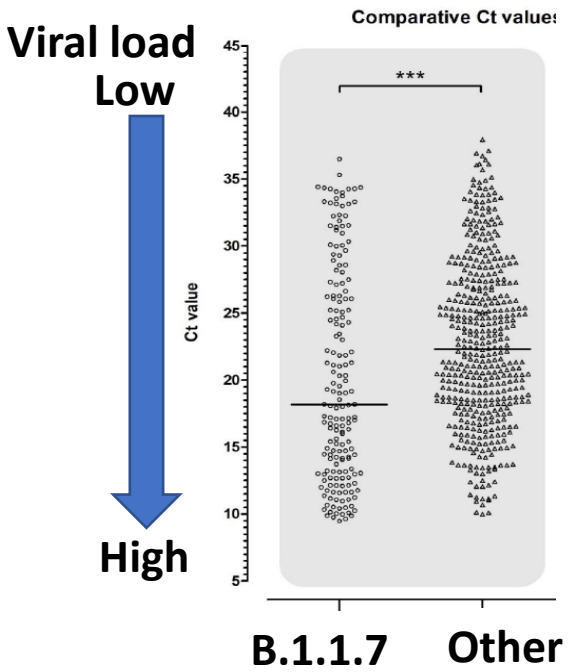

- Used to monitor for B.1.1.7 early:
  - Toronto, Portugal, USA, UK

- Caution when using an assay like this to screen for a variant, mutation can occur in other lineages
  - Couple with sequencing

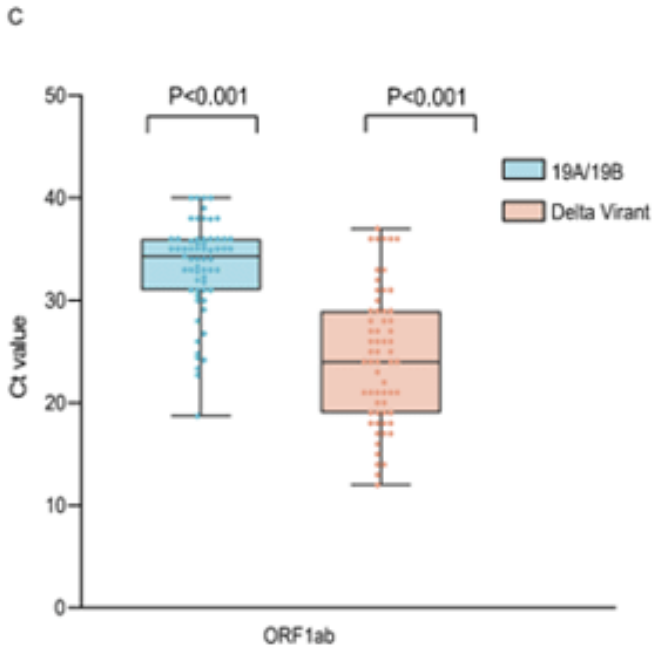
Davies et al., https://www.nature.com/articles/s41586-021-03426-1_reference.pdf; Toronto: https://jamanetwork.com/journals/jama/fullarticle/2778599; Portugal: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2021.26.10.2100130; USA: https://www.cdc.gov/mmwr/volumes/70/wr/mm7003e2.htm; UK: https://www.nature.com/articles/s41586-021-03426-1; https://virological.org/t/detection-of-non-b-1-1-7-spike-69-70-sequences-b-1-375-in-the-united-states/587

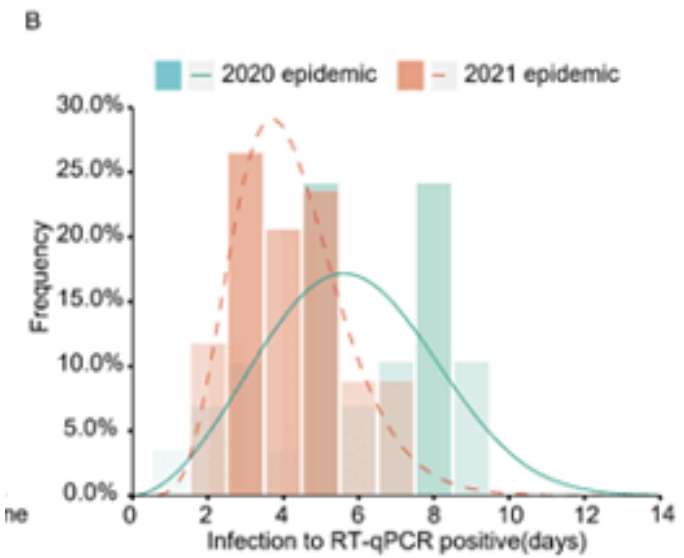
# Variants: monitoring trends in Ct values B.1.1.7 (alpha) & B.1.617.2 (delta)

## B.1.1.7 (alpha)




## B.1.617.2 (delta)



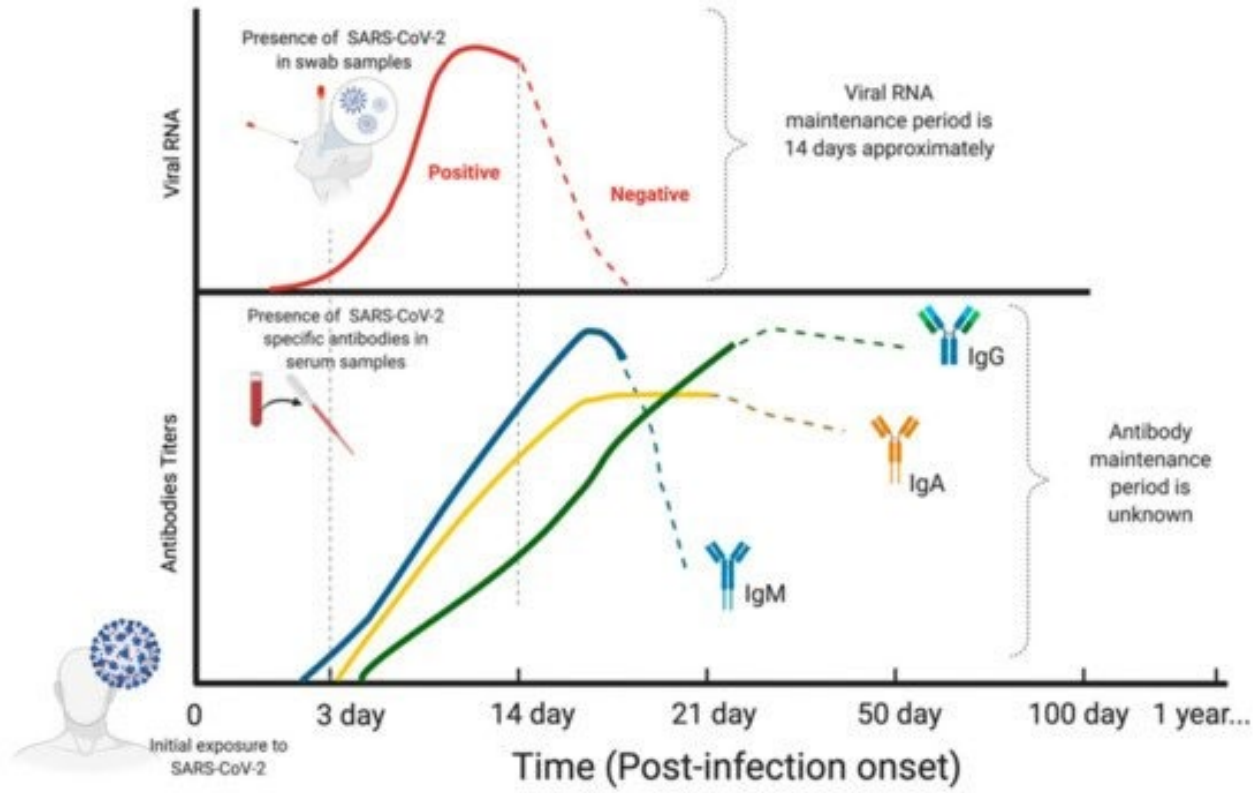




Estimated viral load ~1,000 times higher than 19A/19B

Kidd, et al. The Journal of Infectious Diseases, jiab082; Ratcliff, et al. medRxiv 2021.02.24.21251989; Borges et al., https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2021.26.10.2100130; Li, B. et al., https://virological.org/t/viral-infection-and-transmission-in-a-large-well-traced-outbreak-caused-by-the-delta-sars-cov-2-variant/724

# Serological tests

- Convalescent or post-vaccination




Guevara-Hoyer, A. et al. https://www.mdpi.com/2075-4418/11/4/678; Machado et al., https://doi.org/10.3390/v13010040; Lu, Y. et al., https://onlinelibrary.wiley.com/doi/10.1111/sji.13088

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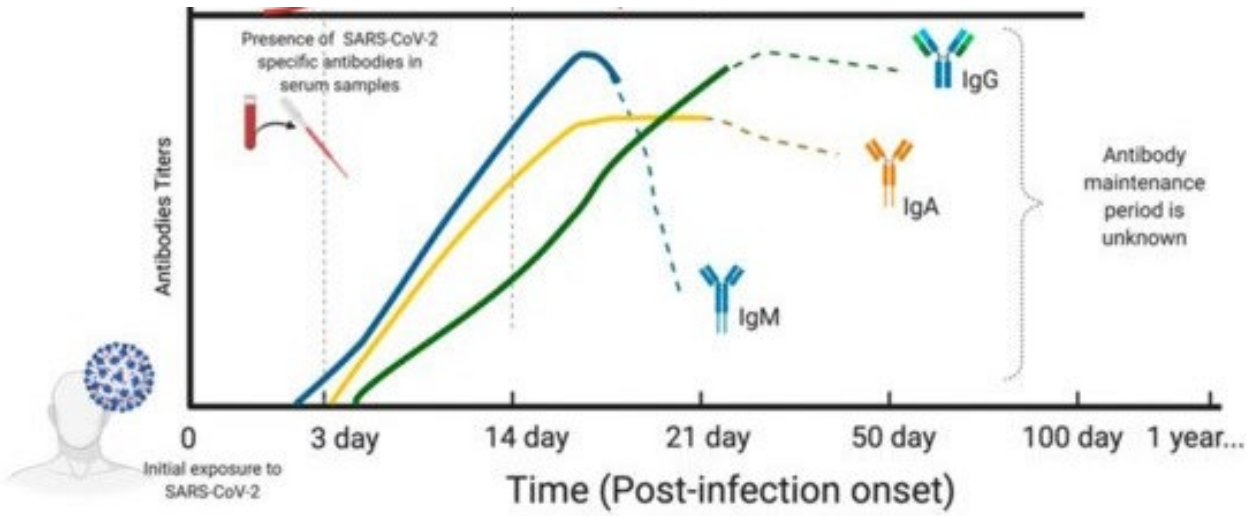
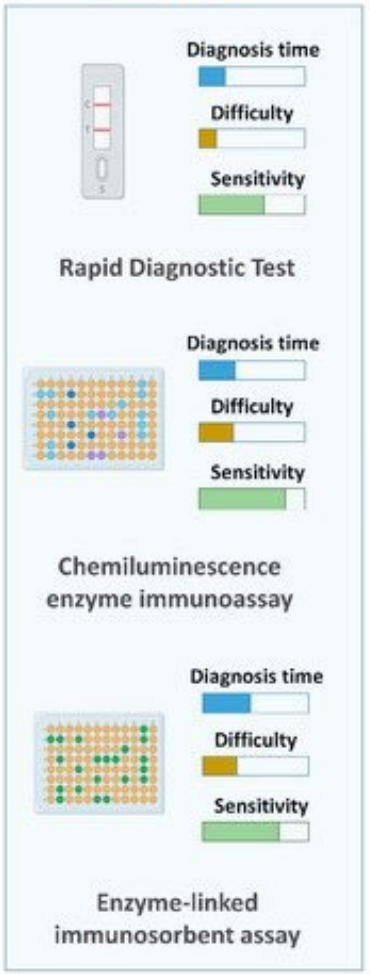
# Serological tests

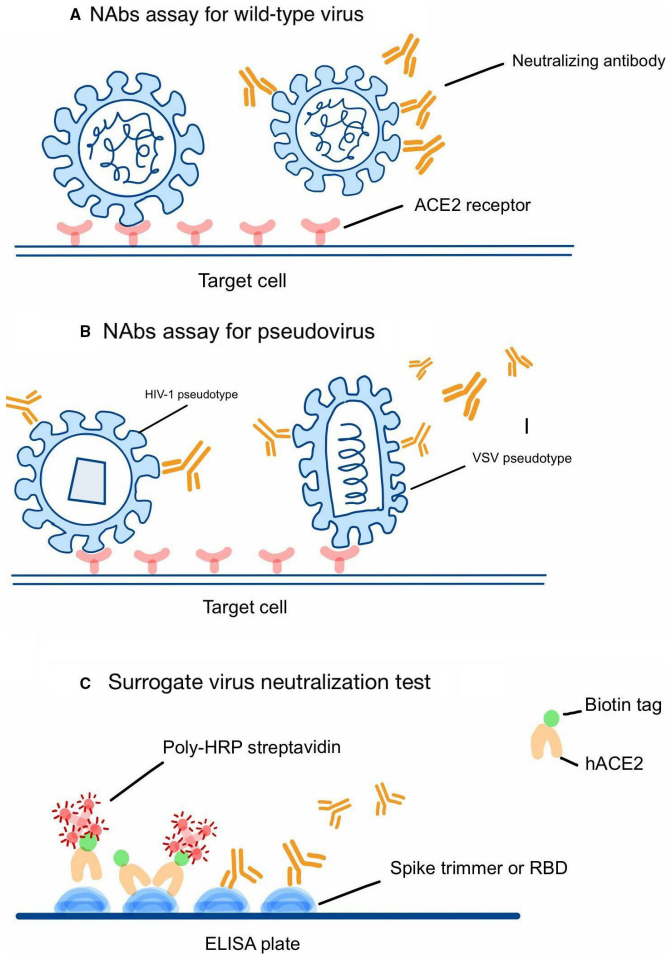



## Positive/negative Titer

### Main types




## Functional (neutralization)




Guevara-Hoyer, A. et al. https://www.mdpi.com/2075-4418/11/4/678; Machado et a.., https://doi.org/10.3390/v13010040; Lu, Y. et al., https://onlinelibrary.wiley.com/doi/10.1111/sji.13088

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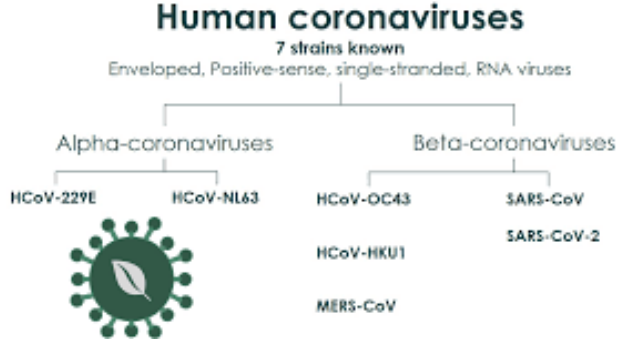
NIH National Institute of Allergy and Infectious Diseases

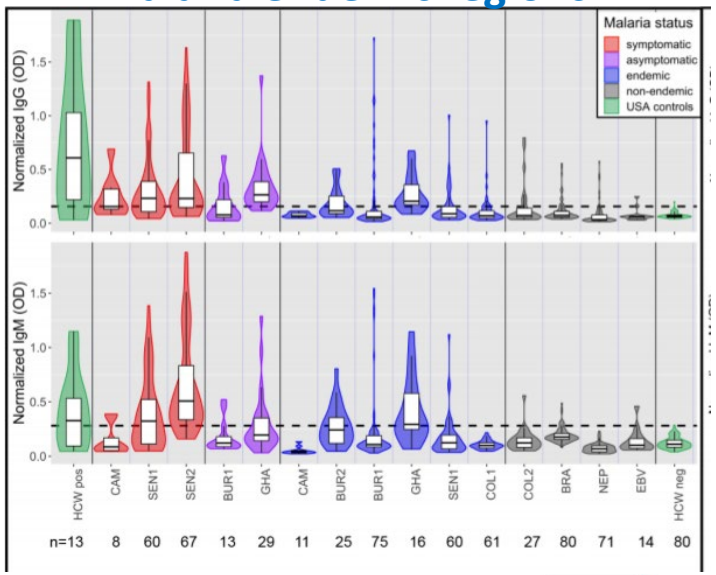
# Serological testing considerations

- Similar to molecular and antigen testing:
  - Cost (turnaround time is not as critical)
  - Complexity and biosafety
    - Neutralization versus ELISA versus lateral flow assay
  - Performance: sensitivity, specificity, PPV, NPV
    - How long following infection or vaccination do you test
    - What does the test measure
      - Antibody type: IgM, IgA, IgG or total antibody
      - Anti-Spike or anti-nucleocapsid
    - Cross-reactivity (specificity)
  - Purpose
    - Do you need a functional assay? A titer? Or a yes/no?

## Cross-reactivity




## Malaria endemic regions



# Serological testing during the pandemic

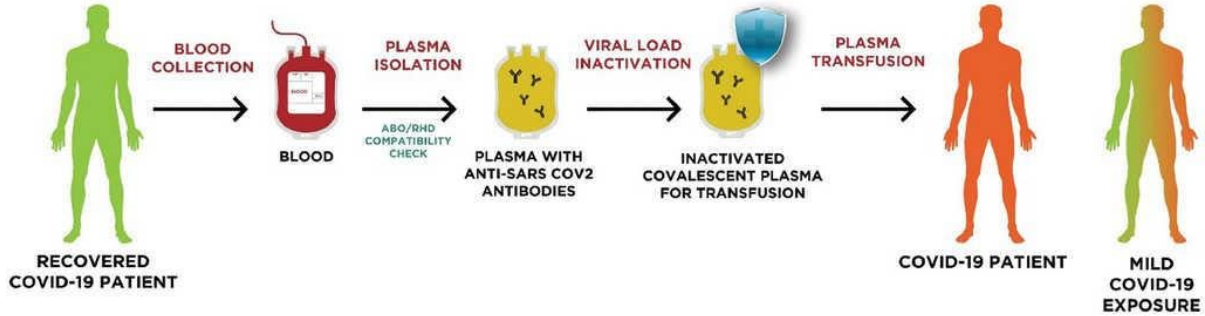
1. Serosurveys
   - Identify proportion of the population potentially infected over time
     - General population (prospective, observational via blood donors)
     - Healthcare workers
     - Methods vary (ELISA and lateral flow mostly)

**Systematic review serosurveys: May 2021**

404 records reporting the seroprevalence of SARS-CoV-2 included in meta-analysis
8 from African region
120 from region of the Americas
19 from Eastern Mediterranean region
194 from European region
19 from South-East Asia region
44 from Western Pacific region

2. Screening convalescent plasma
   - Confirm presence of antibodies
   - Importance of titer

   - Screening of hIVIG in 013 INSIGHT ITAC to standardize product (nAb)

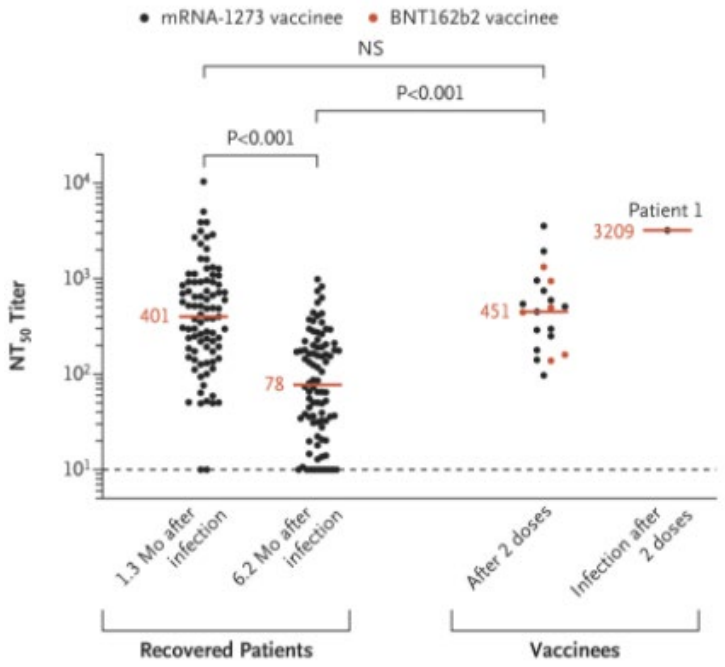


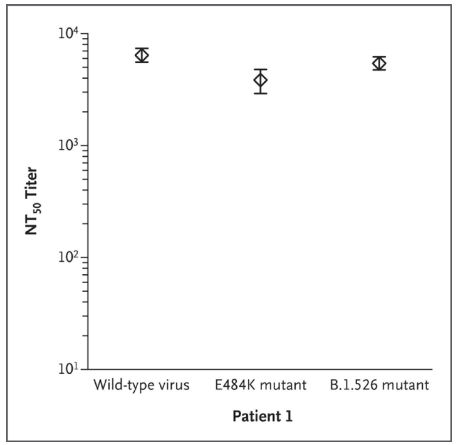

Hematol Transfus Cell Ther. 2021;43:201–11

Reviewed in https://www.thelancet.com/action/showPdf?pii=S2214-109X%2821%2900057-7; Chen et al., https://www.sciencedirect.com/science/article/pii/S2214109X21000267

# Serological testing following vaccination

- Not recommended by the US FDA
- ELISA and lateral flow assays: are there antibodies?
  - Will not tell if a person is protected
- Neutralization and pseudoneutralization assays: are the antibodies functional?
  - Will not tell if a person is 100% protected
- Could be useful at the population level to look at antibody kinetics over time following vaccination
  - Across populations and with different vaccines (InVITE study)

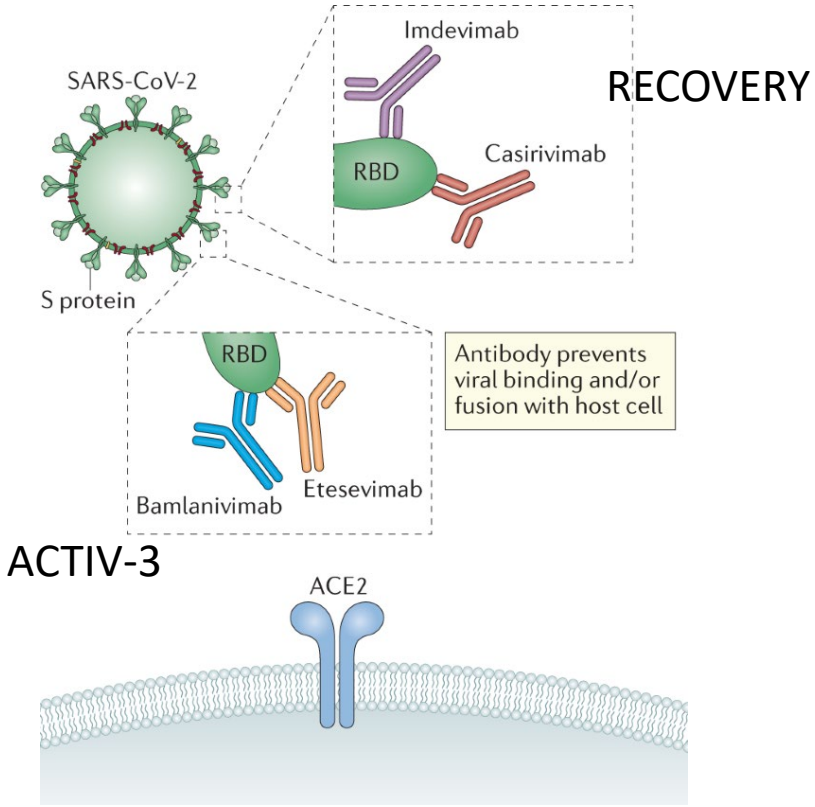



https://www.fda.gov/medical-devices/safety-communications/antibody-testing-not-currently-recommended-assess-immunity-after-covid-19-vaccination-fda-safety;
https://www.nejm.org/doi/full/10.1056/NEJMoa2105000

# Serological testing when treating with monoclonal antibodies?

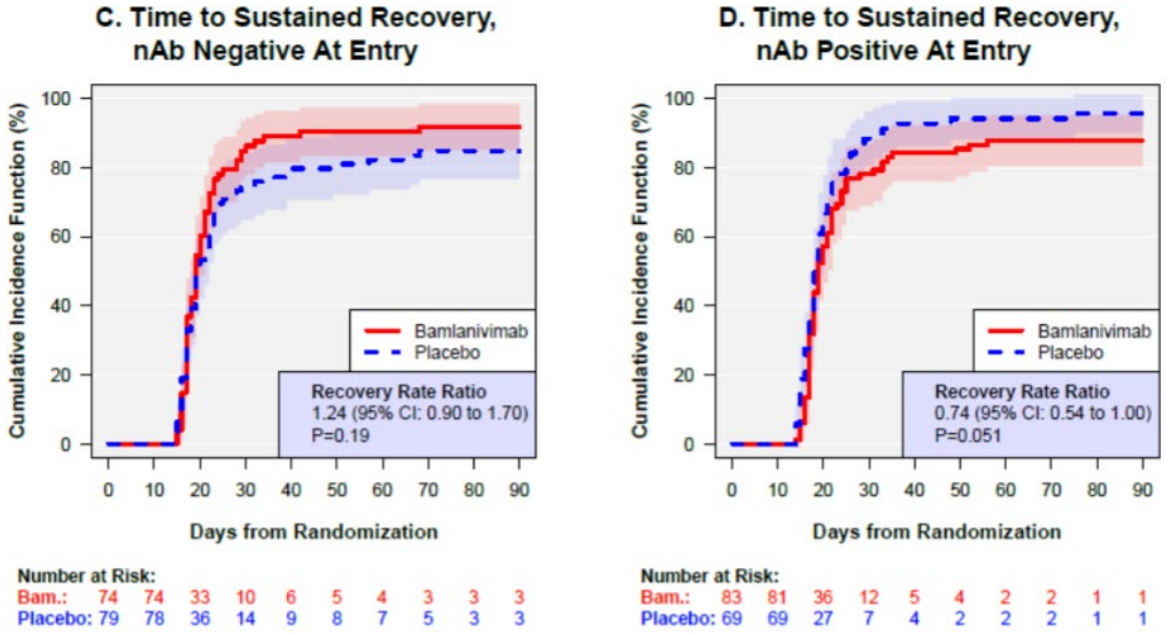
- Results of ACTIV-3 and RECOVERY Trial on monoclonal antibody treatment in hospitalized patients
  - ACTIV-3: Bamlanivimab
    - had FDA EUA for mild to moderate COVID-19
    - Due to recent rise in variants (gamma, delta, and epsilon. Mutations E484K and L452R ) EUA revoked
  - RECOVERY: REGEN-COV mAb cocktail
    - FDA EUA for treatment of mild to moderate COVID-19

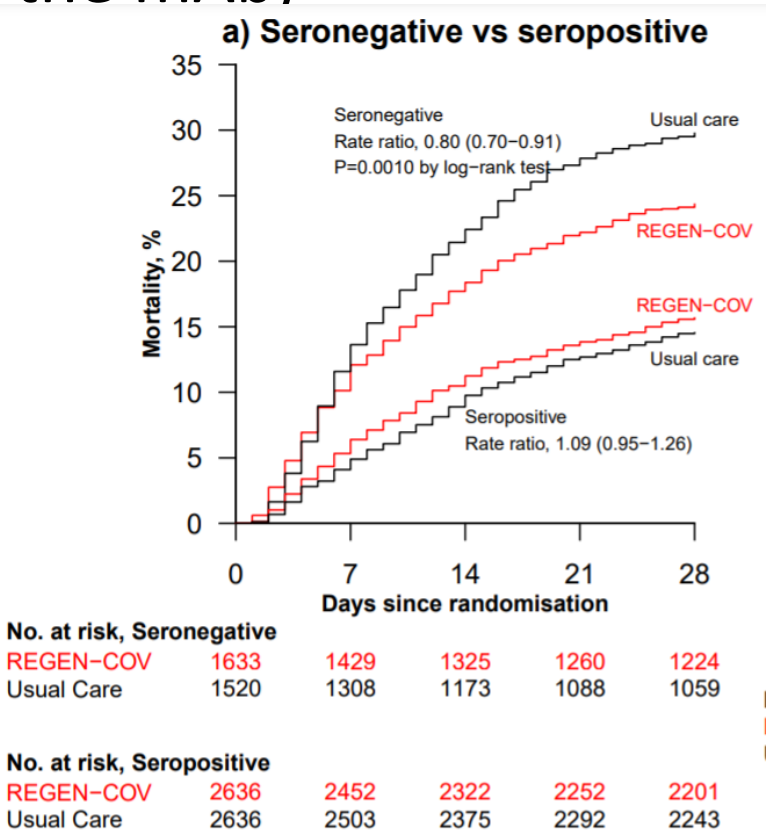



https://www.nature.com/articles/s41577-021-00542-x

# Serological testing when treating with monoclonal antibodies?

- Both trials: null result
- But: benefit in the participants who were seronegative (did not have endogenous antibodies prior to infusion of the mAb)




https://www.medrxiv.org/content/10.1101/2021.07.19.21260559v1.full.pdf;
https://www.medrxiv.org/content/10.1101/2021.06.15.21258542v1.full.pdf

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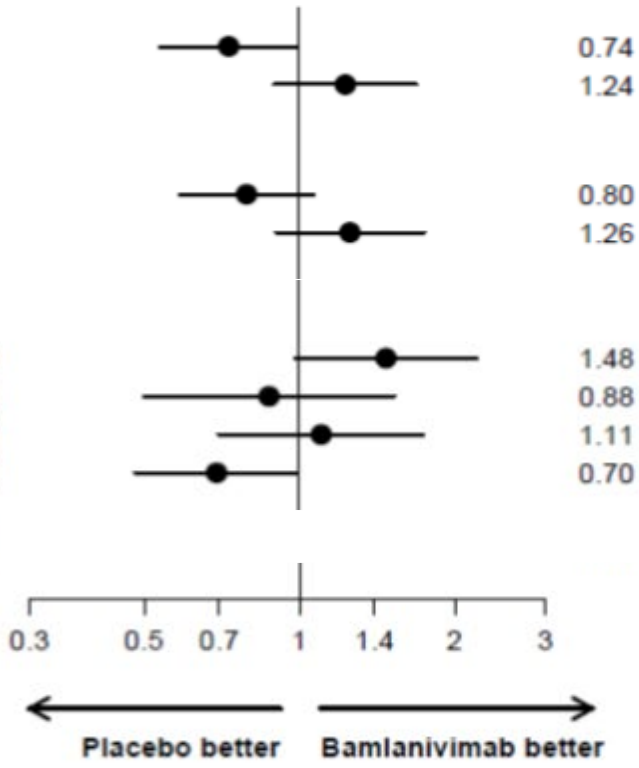
# Serological testing when treating with monoclonal antibodies?

- Combined serological assay (sVNT) with Quanterix N antigen assay: benefit in antigen high group

| Subgroup | Pct. in Subgrp | Bamlanivimab Pts | Bamlanivimab N | Bamlanivimab (%) | Placebo Pts | Placebo N | Placebo (%) | RRR(Bam./Placebo) (95% CI) | RRR | P for Interact. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Neutralizing Ab (nAb)** | | | | | | | | | | 0.018 |
| Positive (nAb+) | 50 | 83 | 72 | (87) | 69 | 66 | (96) | | 0.74 | |
| Negative (nAb−) | 50 | 74 | 67 | (91) | 79 | 67 | (85) | | 1.24 | |
| **Antigen (Ag)** | | | | | | | | | | 0.046 |
| < 1000 pg/mL (low) | 50 | 71 | 65 | (92) | 81 | 78 | (96) | | 0.80 | |
| ≥ 1000 pg/mL (high) | 50 | 86 | 74 | (86) | 67 | 55 | (82) | | 1.26 | |
| **Antigen by nAb** | | | | | | | | | | 0.038 |
| Ag high, nAb− | 33 | 55 | 49 | (89) | 47 | 37 | (79) | | 1.48 | |
| Ag high, nAb+ | 17 | 31 | 25 | (81) | 20 | 18 | (90) | | 0.88 | |
| Ag low, nAb− | 17 | 19 | 18 | (95) | 32 | 30 | (94) | | 1.11 | |
| Ag low, nAb+ | 33 | 52 | 47 | (90) | 49 | 48 | (98) | | 0.70 | |
| **Overall** | 100 | 163 | 144 | (88) | 151 | 136 | (90) | | | |




** Ag low: Antigen <1000 pg/mL;

https://www.medrxiv.org/content/10.1101/2021.06.15.21258542v1.full.pdf

# Summary

- Diagnostics for SARS-CoV-2: incredible development of different assays and technologies for detection of the virus and host immune response
- Choice of assay depends on question being addressed, performance characteristics, community prevalence, cost, turnaround time, complexity, and specimen type
- Testing algorithms and targeted testing can be applied
- Monitoring of the impact of variants on diagnostics is important as the pandemic continues
- Serological assays may have clinical utility

# Thank you!
# Terima kasih!