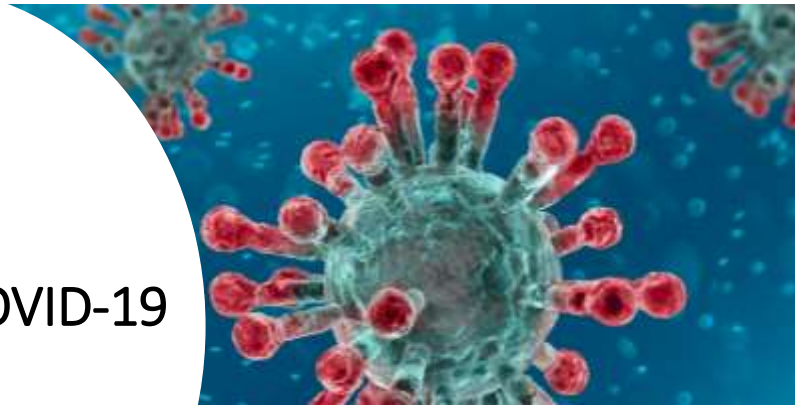


# Diagnostics for COVID-19

November 6, 2020
Yukari C. Manabe, MD
Division of ID, Department of Medicine
Johns Hopkins University School of Medicine

1

# Disclosures

Dr. Manabe receives salary support from NIH funded Rapid Acceleration of Diagnostics (RADx) program (U54EB007958-12, 3U54HL143541-02S2) and has received tests from Quanterix, Becton-Dickinson, and Hologic for research-related purposes. She also receives funding support to Johns Hopkins University from miDiagnostics.

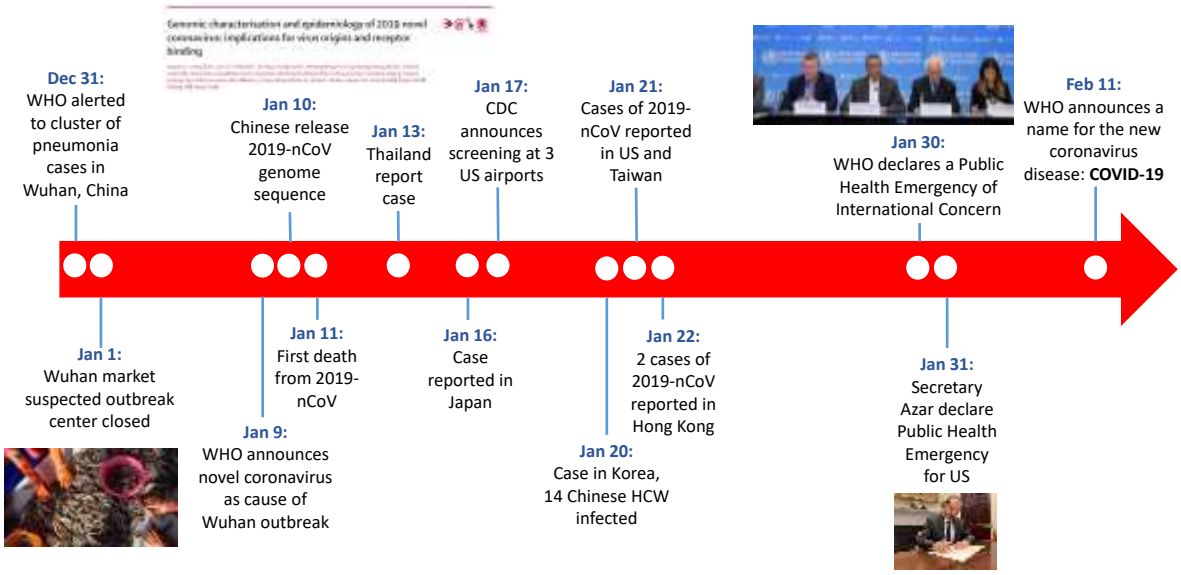
2

# Objectives

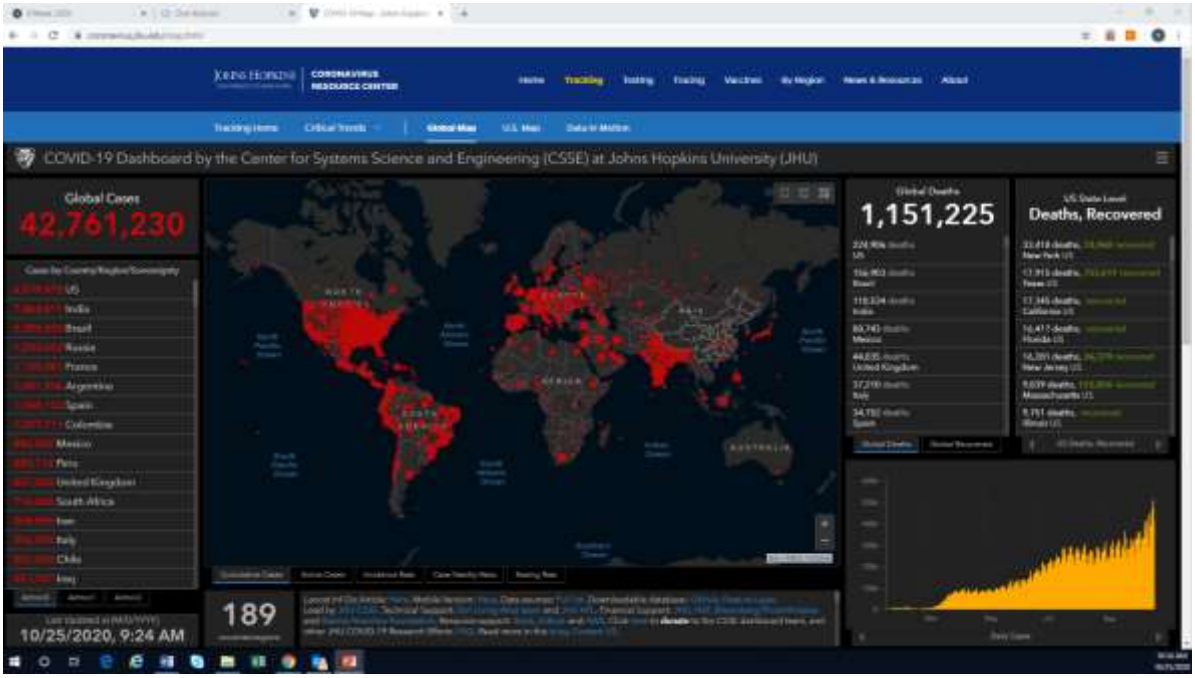
- Understand the natural course of COVID-19 infection
- Describe the performance characteristics of diagnostic tests that directly detect SARS-CoV-2 (molecular and antigen tests)
- Understand when to use COVID-19 antibody tests and how to interpret them

3

# Timeline of Early Events with 2019-nCoV




4




https://coronavirus.jhu.edu/map.html

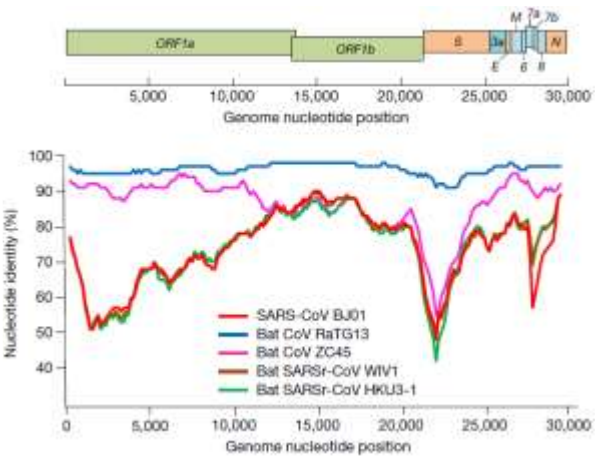
5

Article | Open Access | Published: 03 February 2020

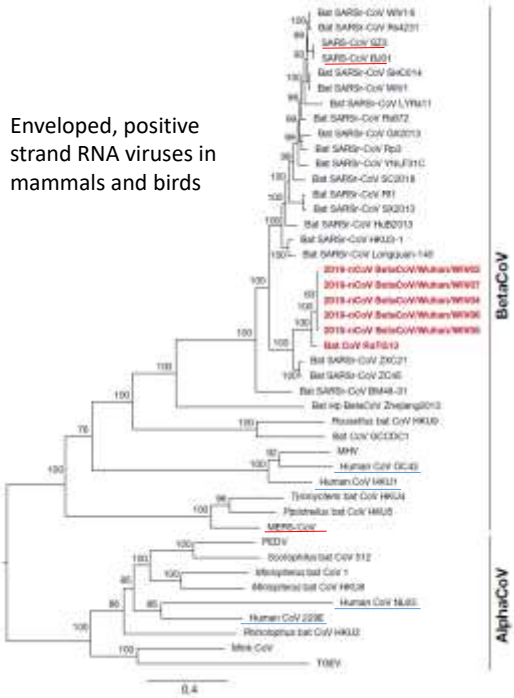
# A pneumonia outbreak associated with a new coronavirus of probable bat origin

Peng Zhou, Xing-Lou Yang, [...] Zheng-Li Shi

*Nature* **579**, 270–273(2020) | Cite this article



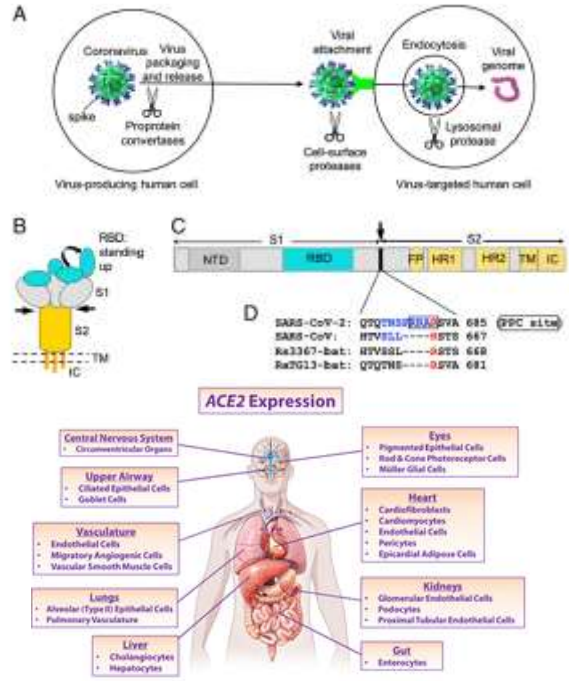

Enveloped, positive strand RNA viruses in mammals and birds




6

# Cell Entry of SARS-CoV-2

- SARS-CoV-2 spike protein preactivated by proprotein convertase (PPC) furin
- Proteolytically activated by human proteases (TMPRSS2)
- Binds to human ACE2 receptor
  - ACE2 – lungs (type 2 pneumocytes), heart , blood vessels, kidney, liver, GI tract, epithelial cells
  - Angiotensin II receptor which then leads to break down of angiotensin II, controls blood pressure and blocks organ damage
  - Alternate angiotensin II type 1 receptor or lack of ACE2 (in mice) leads to lung injury inflammation and leaky vessels.
  - More ACE2 receptors in hypertension (and in those on ACE inhibitors and ARBs), diabetes, coronary heart disease.

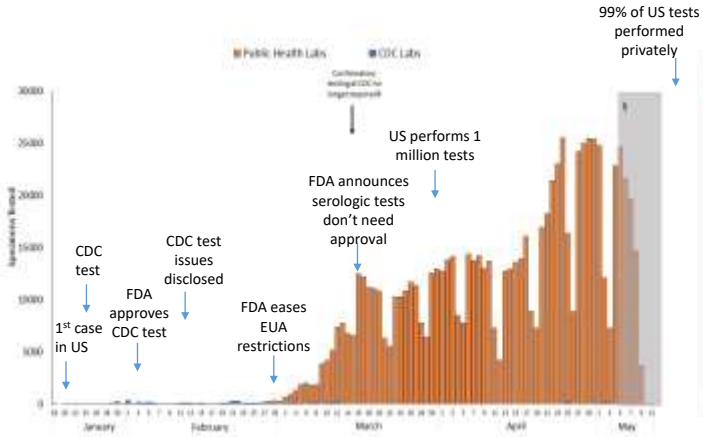


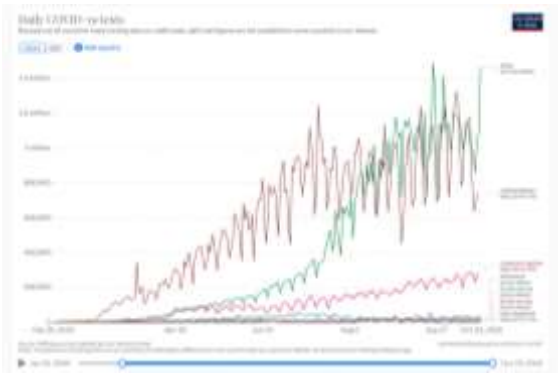

Shang J et al. PNAS. www.pnas.org/cgi/doi/10.1073/pnas.2003138117
M Gheblawi. Circulation Research. 126 (10), p1456-1474.

7

# Private Sector Testing Outstrips Public Sector Testing

(As of May 11, CDC labs tested 6,275, US Public Health Labs tested 818, 682)



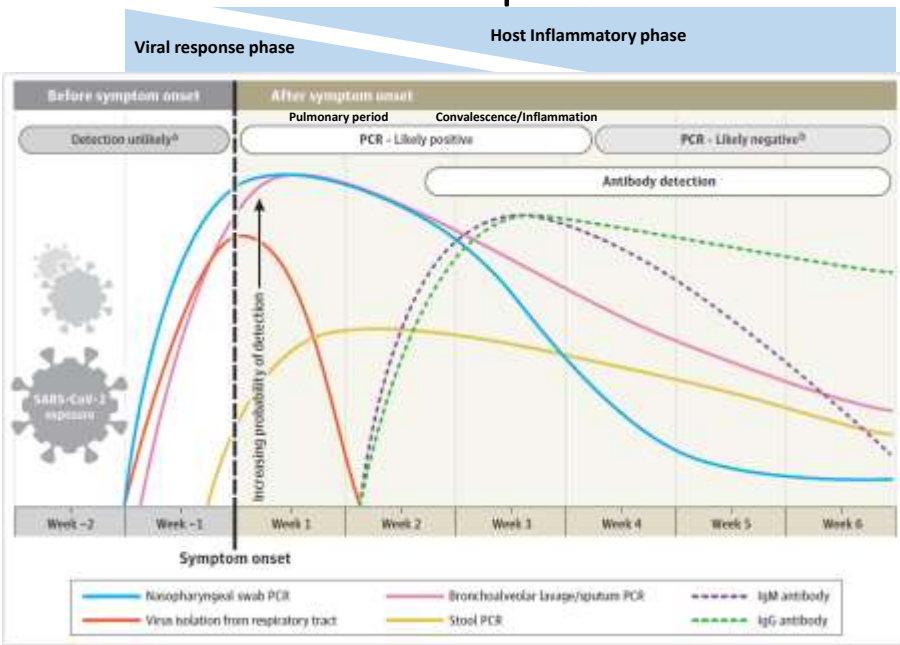



**4.3 million tests per day needed to suppress new infections…**

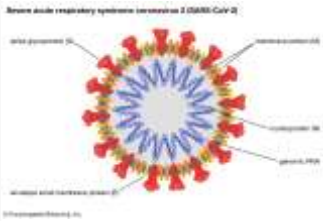
https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/previous-testing-in-us.html
https://ourworldindata.org/grapher/full-list-covid-19-tests-per-day
Brown TS, Walensky RP JAMA; https://globalepidemics.org/july-6-2020-state-testing-targets/

8

# Viral Load and Host Responses



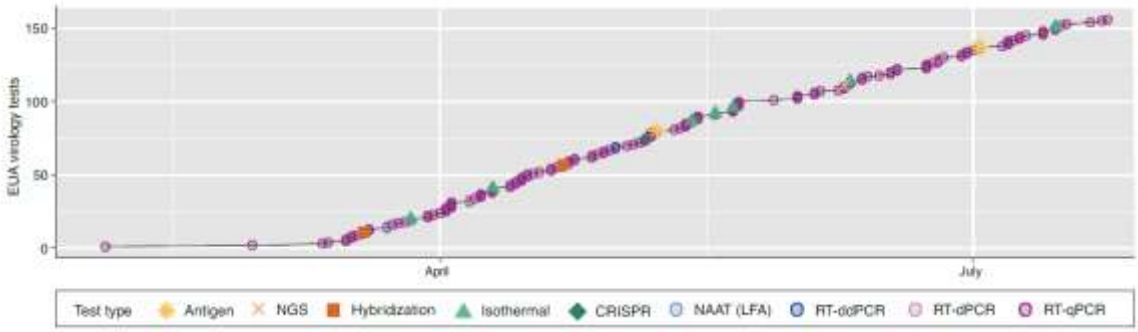

**Incubation**: ~5 days (2-14)
**Symptoms**: ~12 days (8-16)
**Seroconversion**: 5-10 days after symptoms



Sethuraman, Jeremiah, Ryo JAMA

9

# FDA Emergency Use Authorization Direct Detection of Virus



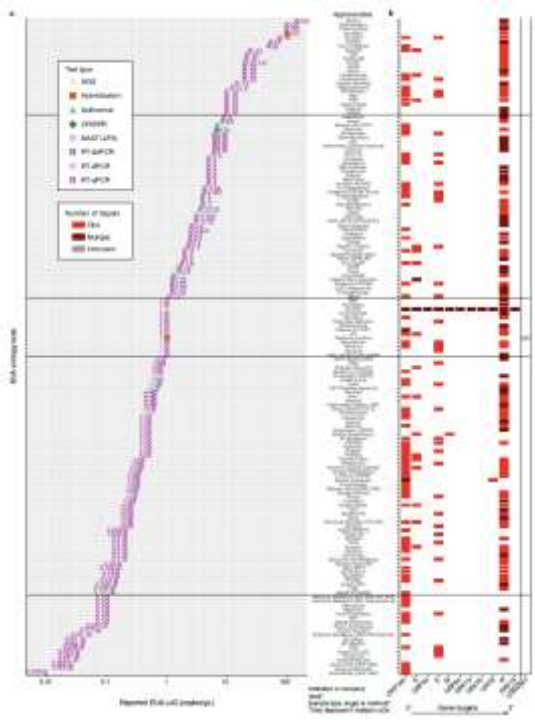

- >200 tests have authorization since this paper was published

MacKay MJ Nature Biotech 38:1021-7
https://www.resiliencehealth.com/tests.html

10

# Are all automated platforms of similar sensitivity?

- More than 3 log range in sensitivity
- Input – direct swab vs viral transport medium
- Pre-extraction followed by RT-PCR tends to be more sensitive than integrated automated platforms
- False negatives occur even with the most sensitive platforms (timing of testing after symptoms, sample type, test used)



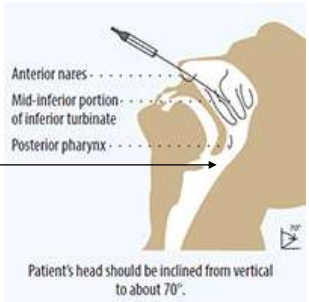
https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-publishes-comparative-performance-data-covid-19-molecular-diagnostic
MacKay MJ Nature Biotech 38:1021-7

11

# Sample Types

- Nasal, nasopharyngeal, mid-turbinate: $10^6$-$10^9$ copies/swab
- Throat (oropharyngeal): $10^4$-$10^8$ copies/swab
- Sputum: $10^6$-$10^{11}$ copies/ml
- Stool: $10^4$-$10^8$ copies/g
- Blood: <5% positive
- Urine: not detectable

Oropharyngeal

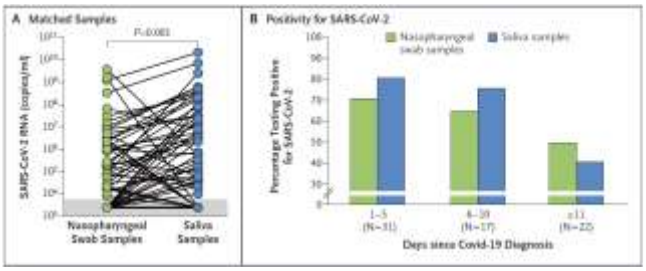



Weissleder R et al. Science Transl Med DOI: 10.1126/scitranslmed.abc1931

12

# Sample types: NP, mid-turbinate, anterior nares, saliva, drool, oral fluid, sputum….

- NP swabs are becoming less attractive
- Variability occurs with sample type, timing during illness, quality of sample
  - Passive drool – similar range of cycle thresholds to NP swab, 2 positive salivary results on days when NP or Bronchoalveolar swab negative (Azzi)
  - Saliva – "coughed up by clearing the throat" (To)
  - Spit – morning specimen, 70 inpatients (97 NP, 97 saliva) (Wyllie)



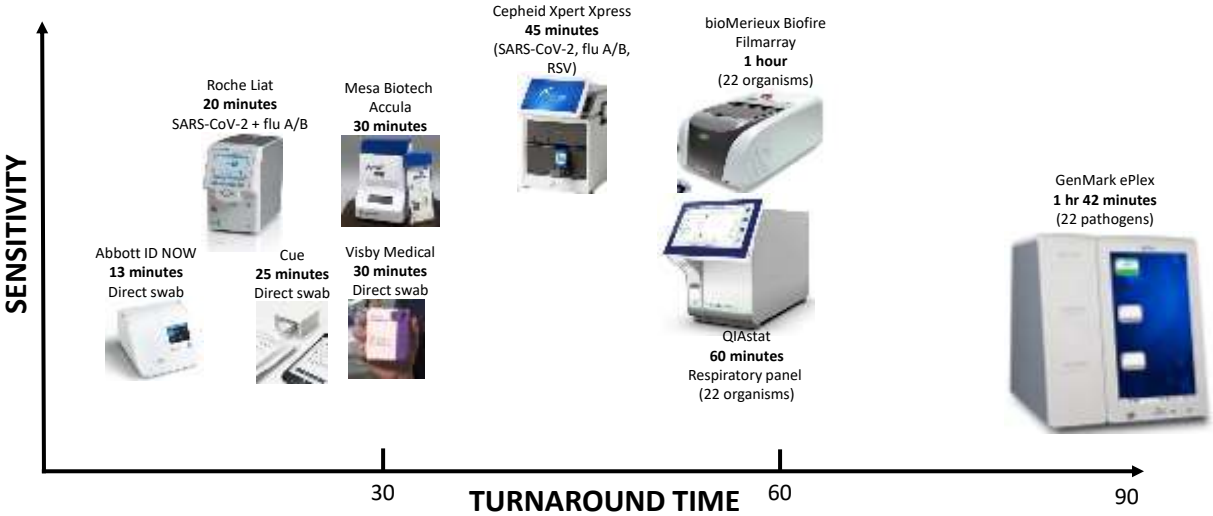

- Higher RNA loads in saliva 5.58 (95% CI: 5.09-6.07) vs NP 4.93 log copies/ml (95%CI:4.53-5.33)
- Higher proportion of saliva positives up to 10 days after diagnostics (saliva 81% vs NP 71%)
- Also sensitive in asymptomatic HCW screening

Azzi L. *J Infect* https://doi.org/10.1016/j.jinf.2020.04.005
To KKW *Lancet Infect Dis* https://doi.org/10.1016/S1473-3099(20)30196-1
Wyllie A. NEJM 2020

13

# Automated Molecular Testing Platforms (March 12-present)

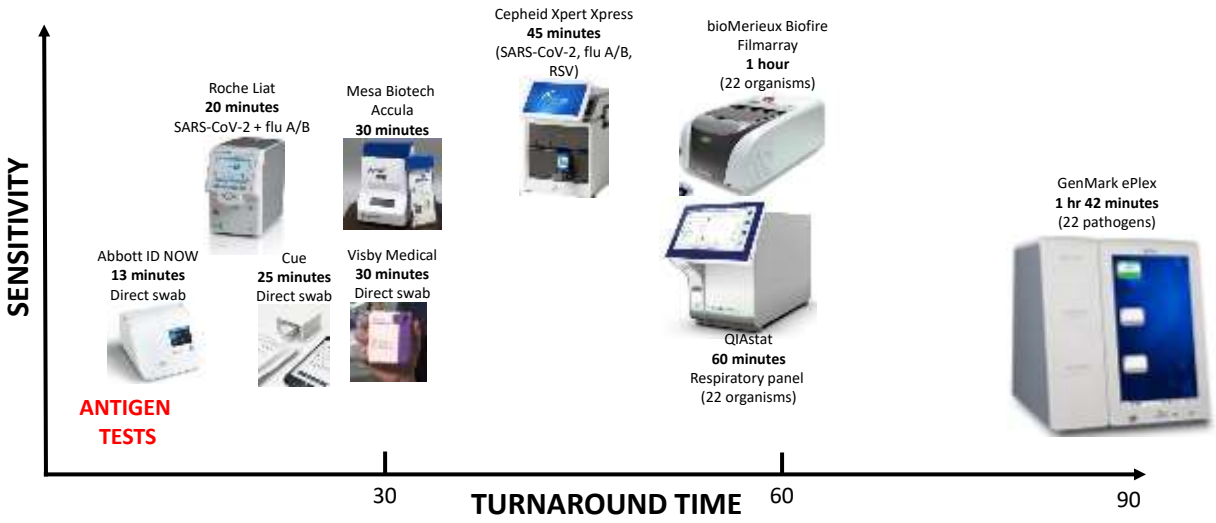
9 modular cartridge based devices




14

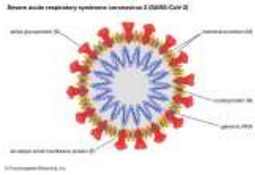
# Automated Molecular Testing Platforms (March 12-present)

9 modular cartridge based devices




15

## Four Emergency Use Authorized Antigen Assays

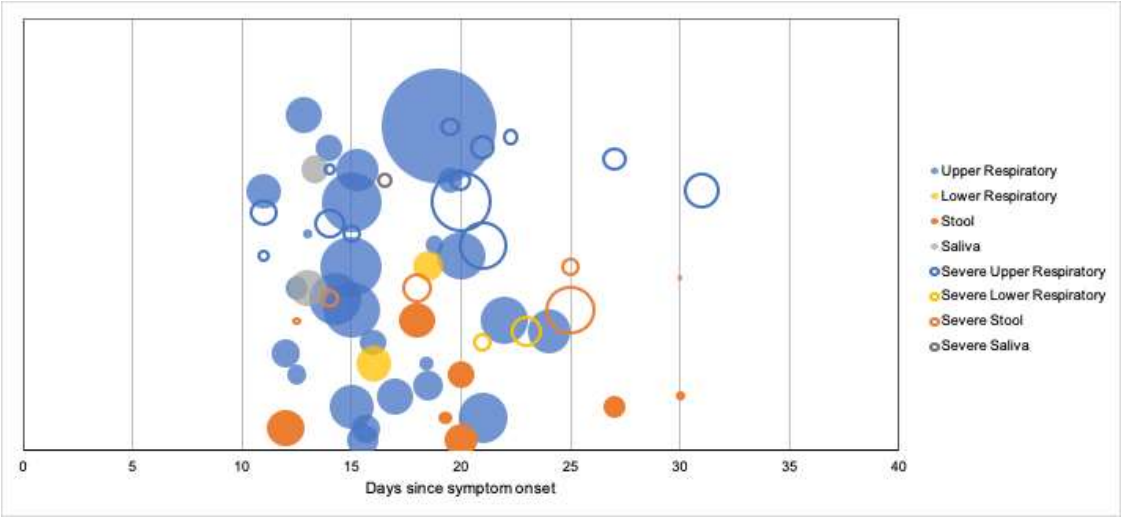


- Lateral flow assay – ~15 minutes
- Qualitative detection of SARS-CoV-2 nucleocapsid protein antigen in nasopharyngeal or nasal swabs directly
- Do not distinguish between SARS-CoV and SARS-CoV-2
- Clinical sensitivity 80%-97% compared to EUA molecular device
- Clinical specificity 96-100% No cross-reactivity with seasonal CoVs

Many more coming, though likely to have similar sensitivities, amenable to lateral flow RDT

16

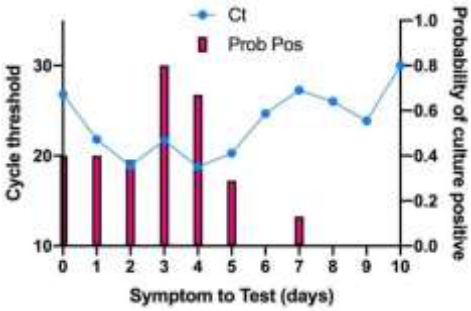
# Duration of RT-PCR positivity




Azamfirei R unpublished

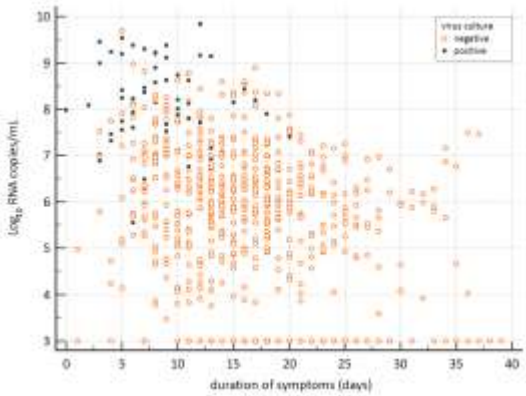
17

# What does a persistently positive RT-PCR test mean?




**Details:**
Manitoba, Canada
90 specimens
NP swabs and ETT specimens
E gene RT-PCR (single target)
Vero cell culture, CPE readout @ 4days

Bullard J, et al. *Clin Infect Dis* 2020 ciaa638

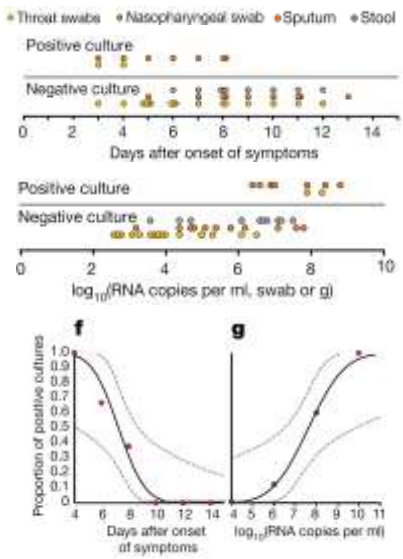



**Details:**
Netherlands, 129 hospitalized COVID patients
23 of 129 (17.8%) infectious viral shedding URT, sputum 690 samples
<5% infectious virus after 15.2 (13.4-17.2) days after symptoms
Neutralizing titer of 1:20 associated with non-infectious

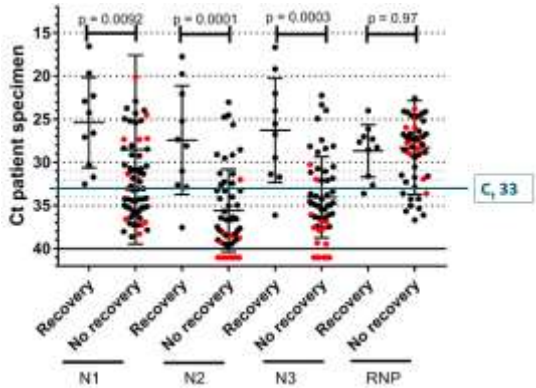
van Kampen JJA Medrxiv

18

# RT-PCR Positive vs Culture Positive




Wölfel R *Nature* 2020 doi: 10.1038/s41586-020-2196-x



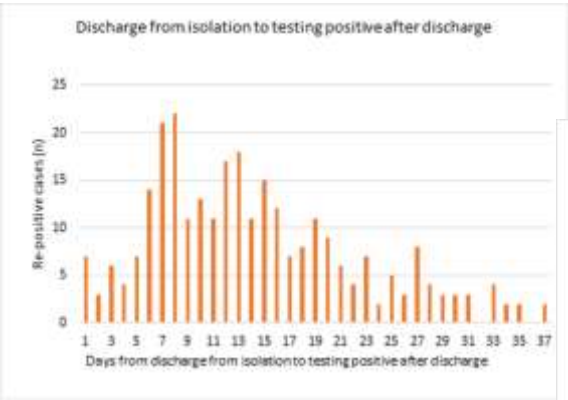

https://www.cdc.gov/coronavirus/2019-ncov/community/strategy-discontinue-isolation.html

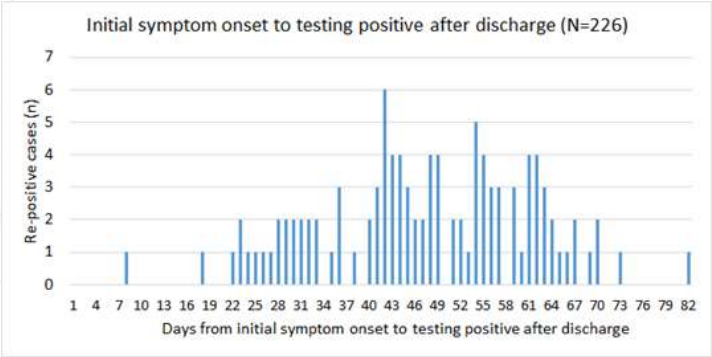
12-15 days virus culture positivity
one case of 18 days after symptoms (RT-PCR positive 63 days)[1]
?Higher viral loads in severe COVID

[1]Liu WD J Infect
Liu Y Lancet Infect Dis; Zheng S BMJ; Lui G J Infect; He X, Nat Med; Xu T Int J Infect Dis

19

# Korean CDC: Findings from investigation and analysis of "re-positive" cases




https://www.cdc.go.kr/board/board.es?mid=a30402000000&bid=0030&act=view&list_no=367267&nPage=1

20

## Korean CDC: Findings from investigation and analysis of "re-positive" cases

***SUMMARY***

- Epidemiological investigation and contact investigation of 285 re-positive cases
- 60% tested as screening, others tested for symptom onset. Of the 284 cases for which symptoms were investigated, 126 (45%) were symptomatic.
- From the 285 re-positive cases, a total of 790 contacts were identified (351=family; 439=others). From the monitoring of contacts, as of now, no case has been found that was newly confirmed from exposure during re-positive period alone.

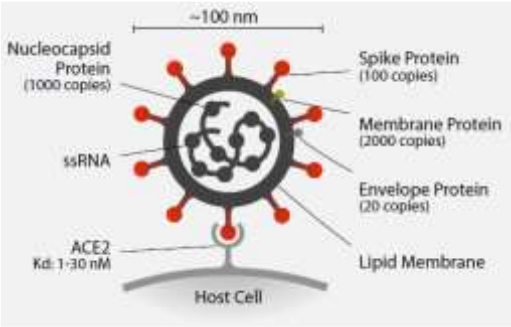
https://www.cdc.go.kr/board/board.es?mid=a30402000000&bid=0030&act=view&list_no=367267&nPage=1

21

# Serological Testing

Validated tests

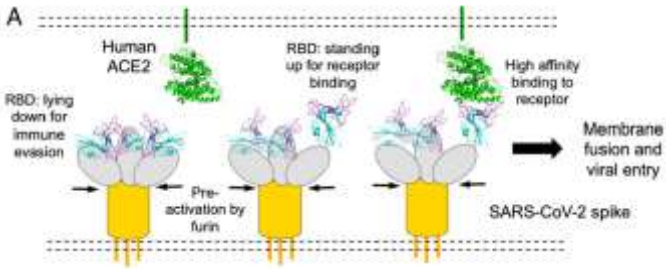
- Detects different antigens (spike 1, spike 2, receptor binding domain, nucleocapsid protein)




**Spike protein**

- full length spike less specific, more sensitive
- RBD – more specific, less sensitive, RBD has to be standing up to be recognized, anti-RBD correlates best with neutralizing immunity

**Nucleocapsid** – Target in automated platforms




Shang J et al. PNAS. www.pnas.org/cgi/doi/10.1073/pnas.2003138117
Wrapp D et al. Science 10.1126/scicence.abb2507 (2020)

22

# Types of Serology Tests

| Type of Test | Rapid Diagnostic Test (RDT) | ELISA | Neutralization Assays | Pseudotyped particle entry inhibitor assay |
|---|---|---|---|---|
| Read-out | Qualitative | Qualitative/ (semi) Quantitative | Quantitative | Quantitative |
| How | Point-of-care, lateral flow test, whole blood, plasma, serum | Lab-based test, Binding of patient antibodies to a fixed viral protein, plasma, serum | Lab-based test, cell culture live virus assay that tests ability of the patient antibodies to confer protective immunity | SARS-CoV-2 spike protein is grafted onto harmless viruses or virus-like particles |
| What | IgM, IgG or antigen specific to SARS-CoV-2 for a single patient | IgM, IgA, IgG specific to SARS-CoV-2 for many patients at once | Neutralizing titer; measure of antibody functionality | Neutralizing titer surrogate |
| Time | 10-30 minutes | 2-5 hours | 3-5 days | |

https://www.centerforhealthsecurity.org/our-work/publications/developing-a-national-strategy-for-serology-antibody-testing-in-the-US

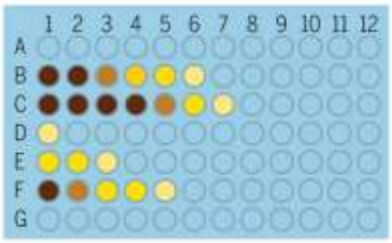
23

Science AAAS

## Quantitative and binary readouts in serology assays

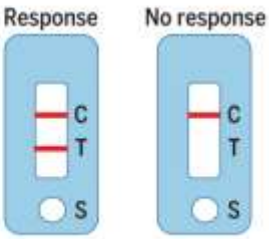
Quantitative and binary serology tests can provide important information about infection.

**Quantitative assays [e.g., enzyme-linked immunosorbent assay (ELISA)]**




| Titers | Protected? |
|---|---|
| Negative | No |
| Titer 1:12,150 | Yes |
| Titer 1:36,450 | Yes |
| Titer 1:50 | No |
| Titer 1:450 | No |
| Titer 1:4050 | Yes |
| Negative | No |

**Assay with binary result (e.g., lateral flow assay)**



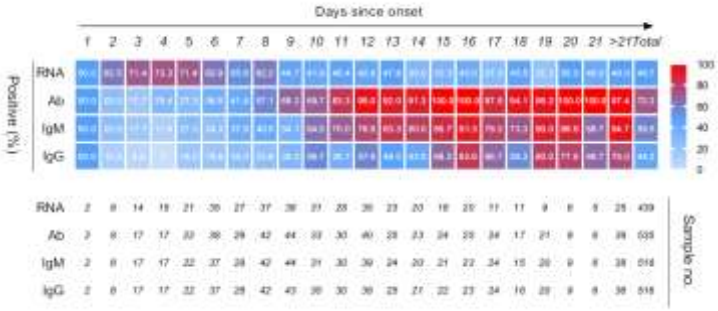

| | Quantitative assays | Assay with binary result |
|---|---|---|
| **Result** | Quantitative titer | Yes or no |
| **Linked to protection?** | A quantitative titer can be linked to protection | A positive result can be loosely associated with protection |
| **Could predict protection duration?** | Yes | No |
| **Scalability** | Moderate | High |
| **Ease of use** | Performed in specialized laboratories | Easy to use, even as point-of-care test |

Florian Krammer, and Viviana Simon Science 2020;368:1060-1061

24

# IDSA Guidelines Serologic Testing

- When SARS-CoV-2 infection requires laboratory confirmation for clinical or epidemiological purposes, IDSA guidelines suggest testing for SARS-CoV-2 IgG or total antibody 3-4 weeks after symptom onset to detect evidence of past SARS-CoV-2 infection
- IDSA guidelines suggest using IgG antibody to provide evidence of COVID-19 infection in symptomatic patients with a high clinical suspicion and repeatedly negative NAAT testing.



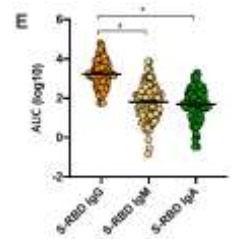

- In pediatric patients with multisystem inflammatory syndrome, IDSA guidelines suggest using both IgG antibody and NAAT to provide evidence of current or past COVID-19 infection.

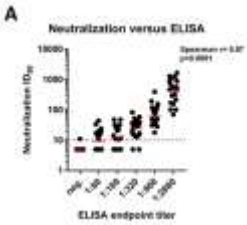
IDSA https://www.idsociety.org/practice-guideline/covid-19-guideline-serology/
Zhao CID 2020

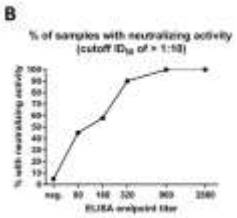
25

# Serologic Immune Responses in Convalescent Patients

- 126 convalescent plasma potential donors ≥28 days after RT-PCR confirmed infection, IgG titers predominated (S1, full length S, S-RBD)[1]
  - 80% with neutralizing titers
  - **S-RBD IgG AUC had highest correlation with neutralizing titer AUC (0.79)**
  - Male sex, older age, hospitalization with COVID-19 associated with increase antibody responses
- 19,860 NYC individuals showed stable titers for 3 months, anti-spike antibody correlate with neutralizing titers
- SARS-CoV-2 nucleoprotein induces more cross reactivity[3]
- Remaining questions: How long does immunity last? What initial titer is necessary? What is the relationship between immune response and protection?[3]



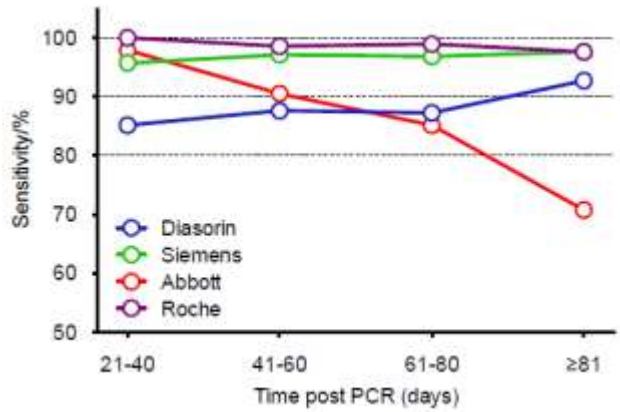




[1]Klein S et al. https://www.medrxiv.org/content/10.1101/2020.06.26.20139063v1
[2]Wajnberg MedRxiv https://www.medrxiv.org/content/10.1101/2020.07.14.20151126v1
[3]Krammer F Simon V Science 2020

26

# Not all serologic assays perform well over time

- Choice of assay for serosurveys is important
- Neutralization titers decrease with time and may not correlate with ELISA assay

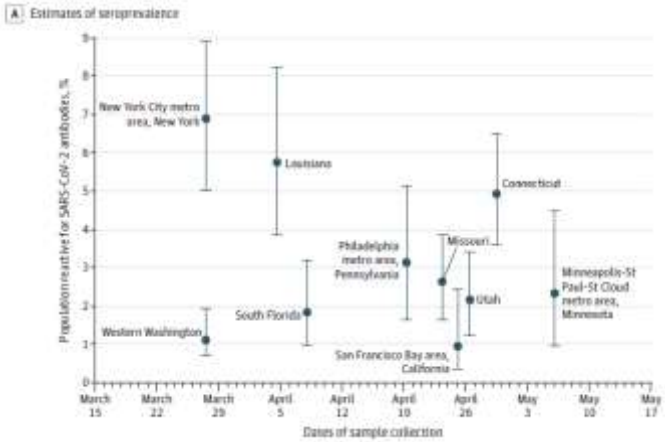



| | | SENSITIVITY (95% confidence intervals) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| day | n | ABBOTT | | DIASORIN | | SIEMENS | | ROCHE | |
| 21-40 | 47 | 97.87 | 88.7-100 | 85.11 | 71.7-93.8 | 95.74 | 85.5-99.5 | 100.00 | 92.5-100.0 |
| 41-60 | 137 | 90.51 | 84.3-94.9 | 87.59 | 80.9-92.6 | 97.08 | 92.7-99.2 | 98.54 | 94.8-99.8 |
| 61-80 | 94 | 85.11 | 76.3-91.6 | 87.23 | 78.8-93.2 | 96.81 | 91.0-99.3 | 98.94 | 94.2-100.0 |
| ≥81 | 41 | 70.73 | 54.5-83.9 | 92.68 | 80.1-98.5 | 97.56 | 87.1-100.0 | 97.56 | 87.1-100.0 |

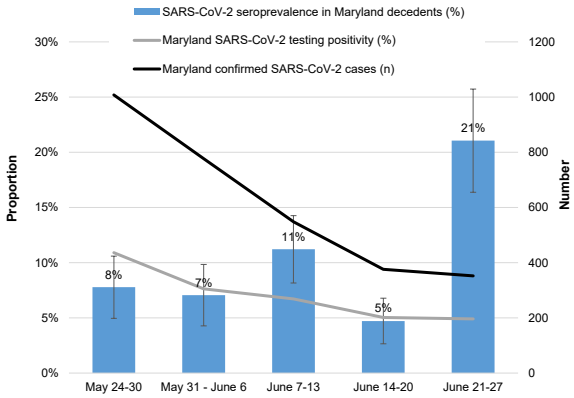
Muesksch Medrxiv https://www.medrxiv.org/content/10.1101/2020.08.05.20169128v1

27

# Serosurveys




Havers FP JAMA Int Med 2020




Althoff, Laeyndecker, Manabe, Li, unpublished

28

# Declare the past, diagnose the present, foretell the future

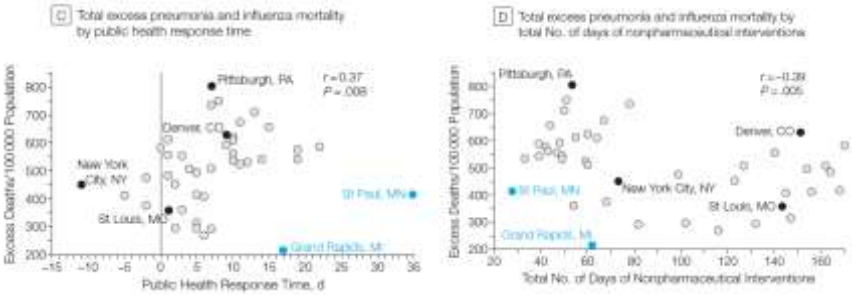
-Hippocrates

29

## Lessons from the 1918 Influenza Epidemic

- Study of weekly pneumonia and influenza mortality data for 43 US cities (1922, 22% of current US population)
- Nonpharmaceutical interventions
  - School Closure
  - Public Gathering Bans
  - Isolation and Quarantine

https://cdn.theatlantic.com/thumbor/a-gfDbQ2NGz-sVwiWMRLVwHbohw=/900x627/media/img/photo/2018/04/the-1918-flu-pandemic-photos-from-a/f01_165-WW-269B-025/original.jpg

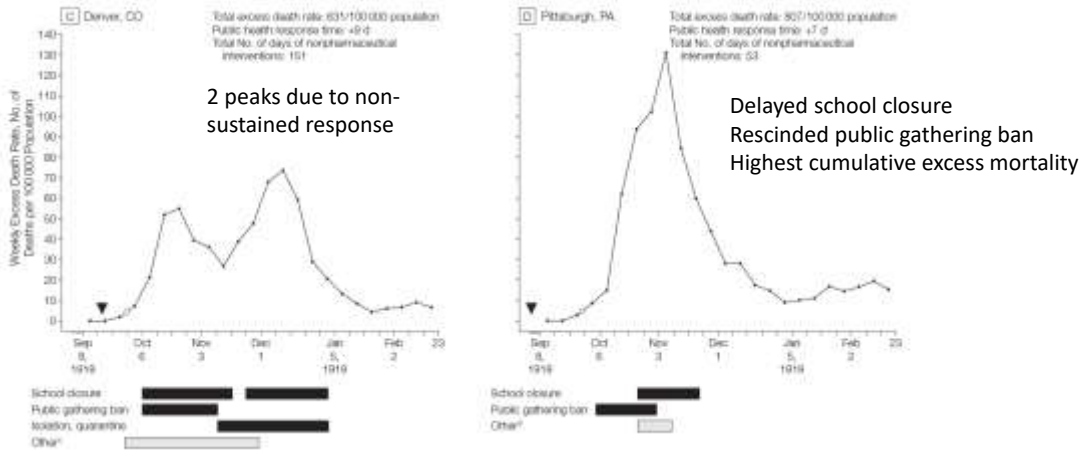


https://inteng-storage.s3.amazonaws.com/img/iea/o2Gz0Mlmwp/sizes/spanish-flu-main_md.jpg

Markle H, et al. JAMA. 2007 Aug 8;298(6):644-54. doi: 10.1001/jama.298.6.644

30

# Lessons from the 1918 Influenza Epidemic




Markle H, et al. JAMA. 2007 Aug 8;298(6):644-54. doi: 10.1001/jama.298.6.644

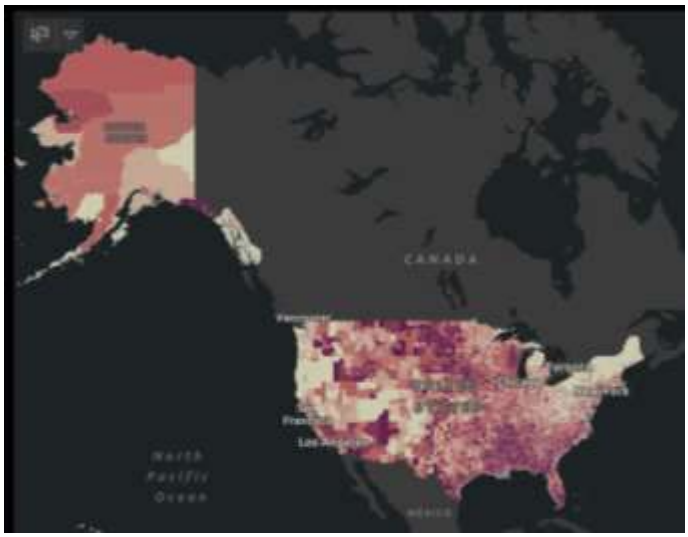
31

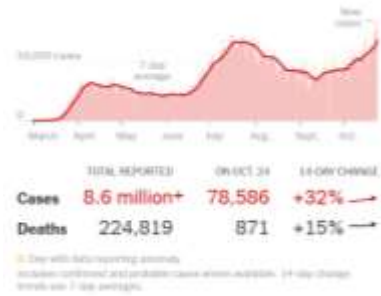
# Lessons from the 1918 Influenza Epidemic

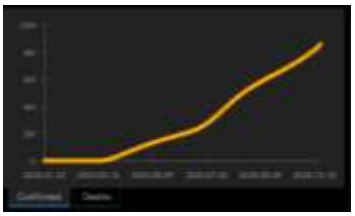
- Sustained implementation of multiple interventions→ peak death rates ~50% lower than those that did not.
- Death rates climbed if interventions were lifted too early.
- "Second waves": inverse correlation of height of first and second peak weekly mortality rates.
  - Cities with low first wave peaks at greater risk of large second wave
  - Low first wave peak cities experiences second wave sooner: 6-9 weeks after the first peak vs 10-14 weeks for cities with higher first peak mortality rates
- No city experienced a second wave with the main non-pharmaceutical battery in place.

Hatchett RJ et al. Proc Natl Acad Sci U S A. 2007 May 1;104(18):7582-7.
Michael Melia and Natasha Chida COVID Rounds Johns Hopkins CCGHE

32



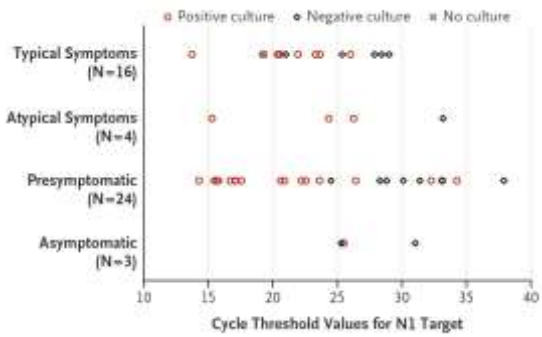
As of October 25, 2020 **8,584,571 Cases**

**224,929 Deaths**

https://coronavirus.jhu.edu/map.html; https://www.nytimes.com/interactive/2020/us/coronavirus-us-cases.html

33

# SARS-CoV-2 Superpower: Asymptomatic Presymptomatic Infection




Epidemiologic evidence

- Incubation periods for presymptomatic primary patients with distinct exposures ranged from 3-11 days
- Presymptomatic primary patients with travel history to an area with active transmission, the time from last exposure to symptom ≥2 - ≥9 days

Virologic Evidence

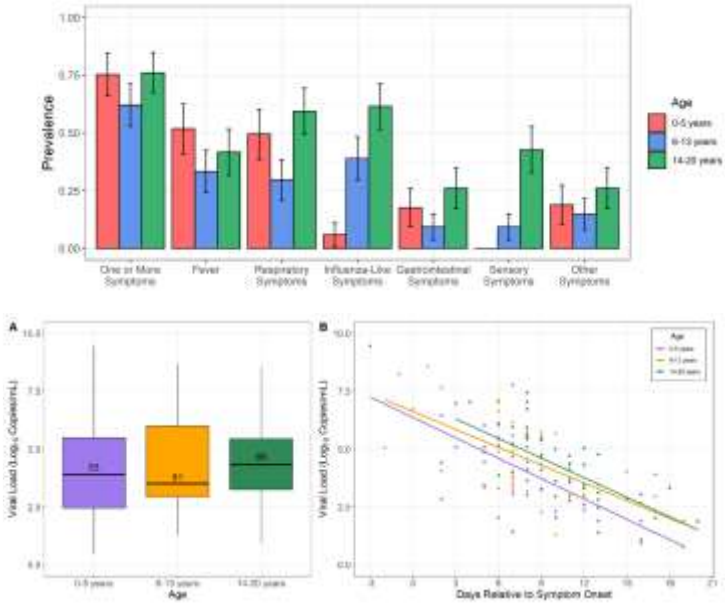
- Mean RT-PCR CT value 24.2 presymptomatic, 27.3 for asymptomatic. Viral culture positive 64% presymptomatic, none in asymptomatic (Arons et al.)
- 2 of 116 asymptomatic passengers from Wuhan to Germany were RT-PCR positive and Caco-2 cell positive

Arons M et al. NEJM DOI: 10.1056/NEJMoa2008457
Furukawa NW https://wwwnc.cdc.gov/eid/article/26/7/20-1595_article#r23
Hoehl S NEJM letter https://www.nejm.org/doi/10.1056/NEJMc2001899#

34

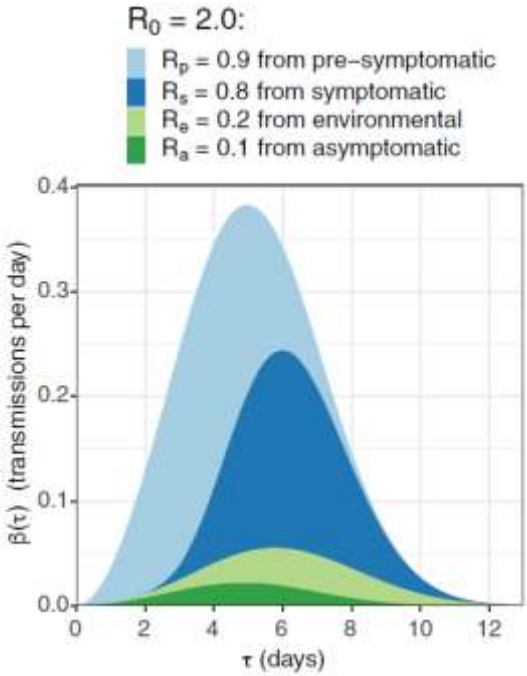
# BRAVE Kids Study

- 382 SARS-CoV-2 exposed children
- 289 (76%) infected
- Associated with Hispanic, h/o asthma, infected sibling contact
- 6-13 years 38% asymptomatic, 30% respiratory symptoms (compared to younger 49% and older age 14-20 59%)
- Adolescents with influenza like illness (61%), GI (26%), sensory symptoms (43%), prolonged illness 7 days (4,12)
- No difference in RT-PCR load based on symptoms




Hurst JH et al. Medrxiv https://doi.org/10.1101/2020.08.18.20166835

35




# Modeling Transmission

- Total $R_o$=2.0
- Presymptomatic $R_o$=0.9
- Symptomatic $R_o$=0.8
- Environmental $R_o$=0.1

**Implications**

- Lower case-fatality rate
- Community interventions to slow transmission –social distance, face masks
- Increase capacity for widespread testing and contact tracing for asymptomatics

Ferretti L Science. 2020;eabb6936

36

# Non-Pharmaceutical Interventions

| | Studies and participants | Relative effect (95% CI) | Anticipated absolute effect (95% CI), eg, chance of viral infection or transmission | | Difference (95% CI) | Certainty* | What happens (standardised GRADE terminology)** |
|---|---|---|---|---|---|---|---|
| | | | Comparison group | Intervention group | | | |
| Physical distance ≥1 m vs <1 m | Nine adjusted studies (n=7782); 29 unadjusted studies (n=10 736) | aOR 0·18 (0·09 to 0·38); unadjusted RR 0·30 (95% CI 0·20 to 0·44) | Shorter distance, 12·8% | Further distance, 2·6% (1·3 to 5·3) | −10·2% (−11·5 to −7·5) | Moderate† | A physical distance of more than 1 m probably results in a large reduction in virus infection; for every 1 m further away in distancing, the relative effect might increase 2·02 times |
| Face mask vs no face mask | Ten adjusted studies (n=2647); 29 unadjusted studies (n=10 170) | aOR 0·15 (0·07 to 0·34); unadjusted RR 0·34 (95% CI 0·26 to 0·45) | No face mask, 17·4% | Face mask, 3·1% (1·5 to 6·7) | −14·3% (−15·9 to −10·7) | Low‡ | Medical or surgical face masks might result in a large reduction in virus infection; N95 respirators might be associated with a larger reduction in risk compared with surgical or similar masks§ |
| Eye protection (faceshield, goggles) vs no eye protection | 13 unadjusted studies (n=3713) | Unadjusted RR 0·34 (0·22 to 0·52)¶ | No eye protection, 16·0% | Eye protection, 5·5% (3·6 to 8·5) | −10·6% (−12·5 to −7·7) | Low‖ | Eye protection might result in a large reduction in virus infection |

Chu DK Lancet 2020 https://doi.org/10.1016/S0140-6736(20)31142-9Get rights and content

37

# COVID-19 Testing: Objectives for testing

**INDIVIDUAL**

- Sensitive – NP molecular test
- Interrupt household transmission
- 24 hour turnaround time
- Different willing-to-pay thresholds

**PUBLIC HEALTH**

- Remove those most likely to have transmissible disease (triage)
- Less sensitive tests may be OK
- Rapid tests
- Inexpensive

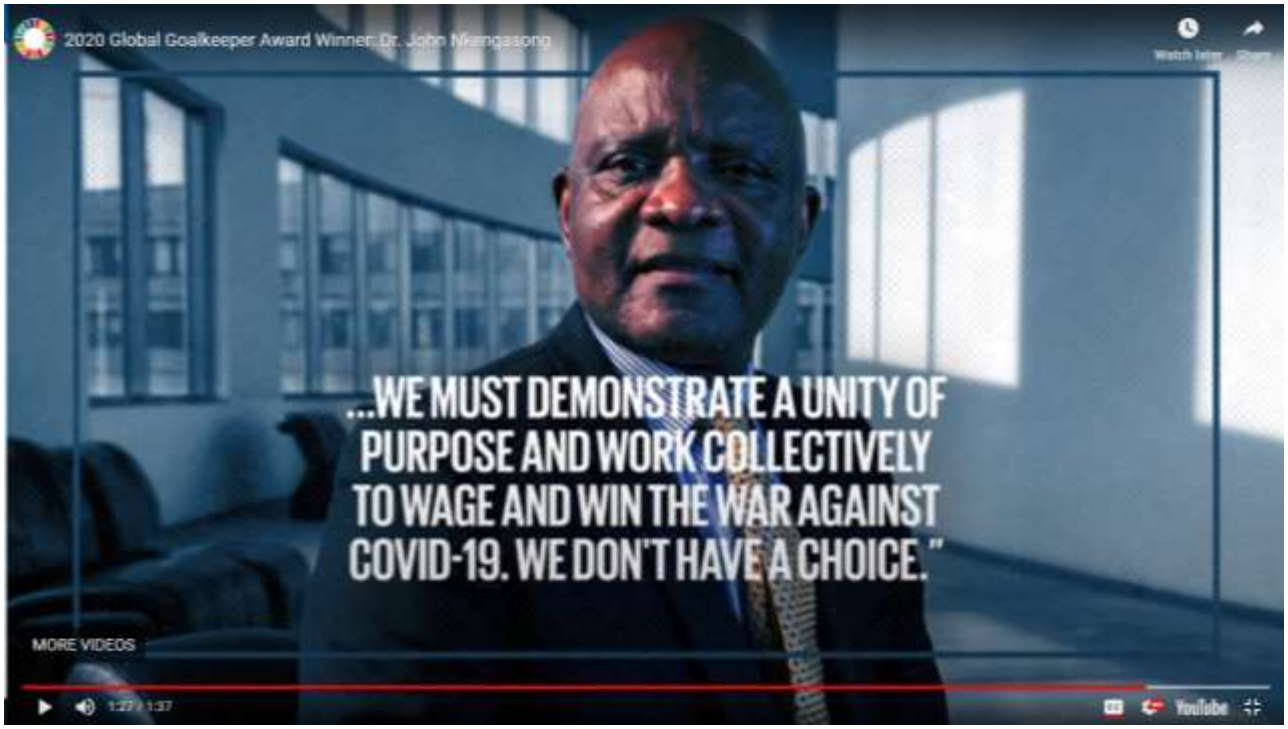
**More data are needed on antigen test performance in asymptomatic people**

38

# Summary

- COVID-19 pandemic →over 43 million cases, >1.1 million deaths worldwide with increasing cases in US and second wave in Europe
- Molecular (RT-PCR) and antigen tests directly detect the virus
- Relationship between RT-PCR positivity, culturable virus, and transmission need more data
- Serologic tests should not be used to diagnose acute disease: only 80% of patients develop neutralizing immunity and the duration of protection is unknown
- Asymptomatic and presymptomatic patients account for a significant proportion of transmitted infection
- Social distancing, masking, and non-pharmaceutical interventions should be adopted

39




40