Development, Validation and Interpretation of Testing for SARS-CoV-2

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Outline

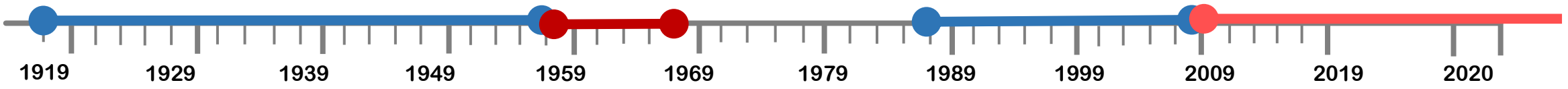
- Pandemics: lessons from the past
- The virus and its challenges
- Viral infection, symptoms, shedding and transmission
- Tests and their comparative performance
- Viral evolution and the role of sequencing

1918
>50 million deaths
(Spanish Flu)

1957
1-2 million deaths
(Asian flu)

2009
363,000 deaths
(Swine flu)

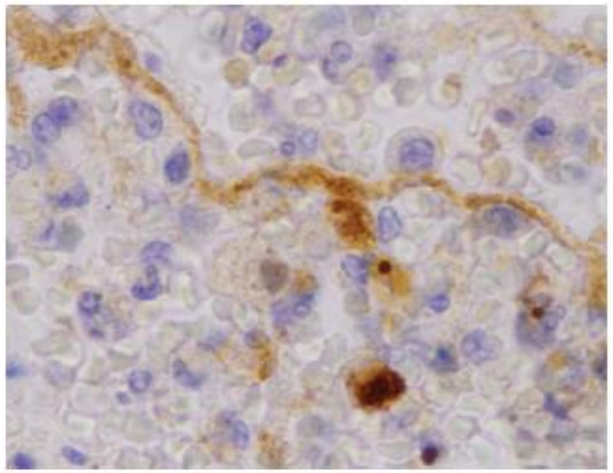
2020 (current)
427,630 (World)
115,271 (US)
(COVID-19)



H1N1 H2N2 H3N2 H1N1

Adapter from Taubenberger et al.

Frozen tissue
Multiple viral and bacterial sequences
Reconstruction of 1918 virus
Test in many animal models



Highest incidence in crowded populations
Virtually all deaths were due to secondary bacterial pneumonia
High mortality in young adults due to higher case incidence

COVID-19 is a different virus
Mitigation is similar process

Taubenberger *et al*., *Sci. Transl. Med.* **11**, (2019)

WHO Global Flu Strategy 2019-2030
(Prevent. Control. Prepare.)

Announced March 2019

World Health Organization

Pillars of Flu Preparedness

(national-community-health care system-physician)

Viral Surveillance and Risk Assessment

Early disease recognition, Diagnostics and Drugs

Vaccines

Infrastructure Preparedness

“non-pharmaceutical”

Non-pharmaceutical Recommendations

- **During a Pandemic: Limit the Spread of Germs** and Prevent Infection

- **Avoid close contact** with people who are sick.
- When you are sick, **keep your distance** from others to protect them from getting sick too.
- **Cover your mouth and nose** with a tissue when coughing or sneezing. It may prevent those around you from getting sick.
- **Washing your hands** often will help protect you from germs.
- **Avoid touching your eyes, nose or mouth**.
- **Practice other good health habits**. Get plenty of sleep, be physically active, manage your stress, drink plenty of fluids, and eat nutritious food.

- **Before a Pandemic:**

- **Store a two week supply of water and food**.
- **Periodically check your regular prescription drugs** to ensure a continuous supply in your home.
- **Have any nonprescription drugs and other health supplies on hand**, including pain relievers, stomach remedies, cough and cold medicines, fluids with electrolytes, and vitamins.
- **Get copies and maintain electronic versions of health records** from doctors, hospitals, pharmacies and other sources and store them, for personal reference. Get help accessing electronic help records.
- **Talk with family members and loved ones** about how they would be cared for if they got sick, or what will be needed to care for them in your home.

https://www.ready.gov/pandemic

Guidelines to Prevent Pandemic Influenza through Non-pharmaceutical Interventions (NPIs) and Community Engagement

MMWR / April 21, 2017 / Vol. 66 / No. 1

Social Distancing

CDC recommendations

Social distancing measures: Even though the evidence base for the effectiveness of some of these measures is limited, CDC might recommend the simultaneous use of multiple social distancing measures to help reduce the spread of influenza in community settings (e.g., schools, workplaces, and mass gatherings) during severe, very severe, or extreme influenza pandemics while minimizing the secondary consequences of the measures. Social distancing measures include the following:

- Increasing the distance to at least 3 feet (*98*) between persons when possible might reduce person-to person transmission. This applies to apparently healthy persons without symptoms. In the event of a very severe or extreme pandemic, this recommended minimal distance between people might be increased.
- Persons in community settings who show symptoms consistent with influenza and who might be infected with (probable) pandemic influenza should be separated from well persons as soon as practical, be sent home, and practice voluntary home isolation.

Use of Face Masks

CDC recommendations

Use of face masks by ill persons: CDC might recommend the use of face masks by ill persons as a source control measure during severe, very severe, or extreme influenza pandemics when crowded community settings cannot be avoided (e.g., when adults and children with influenza symptoms seek medical attention) or when ill persons are in close contact with others (e.g., when symptomatic persons share common spaces with other household members or symptomatic postpartum women care for and nurse their infants). Some evidence indicates that face mask use by ill persons might protect others from infection.

Use of face masks by well persons: CDC does not routinely recommend the use of face masks by well persons in the home or other community settings as a means of avoiding infection during influenza pandemics except under special, high-risk circumstances (https://www.cdc.gov/flu/professionals/infectioncontrol/maskguidance.htm). For example, during a severe pandemic, pregnant women and other persons at high risk for influenza complications might use face masks if unable to avoid crowded settings, especially if no pandemic vaccine is available. In addition, persons caring for ill family members at home (e.g., a parent of a child exhibiting influenza symptoms) might use face masks to avoid infection when in close contact with a patient, just as health care personnel wear masks in health care settings.

Environmental cleaning

CDC recommendations

Environmental surface cleaning measures: CDC recommends environmental surface cleaning measures in all settings, including homes, schools, and workplaces, to remove influenza viruses from frequently touched surfaces and objects. Use of these measures might help prevent transmission of various infectious agents, including seasonal and pandemic influenza (https://www.cdc.gov/nonpharmaceutical-interventions/environmental/index.html; https://www.cdc.gov/oralhealth/infectioncontrol/questions/cleaning-disinfecting-environmental-surfaces.html).

Additional guidance is available from CDC for health care facilities (https://www.cdc.gov/hicpac/pdf/guidelines/eic_in_HCF_03.pdf), schools (https://www.cdc.gov/flu/school/cleaning.htm), and airline, travel, and transportation industries (https://www.cdc.gov/flu/pandemic-resources/archived/transportation-planning.html).

School Closure

CDC recommendations

School closures and dismissals: CDC might recommend the use of preemptive, coordinated school closures and dismissals during severe, very severe, or extreme influenza pandemics. This recommendation is in accord with the conclusions of the U.S. Community Preventive Services Task Force (https://www.thecommunityguide.org/findings/emergency-preparedness-and-response-school-dismissals-reduce-transmission-pandemic-influenza), which makes the following recommendations:

- The task force recommends preemptive, coordinated school dismissals during a severe influenza pandemic.
- The task force found insufficient evidence to recommend for or against preemptive, coordinated school dismissals during a mild or moderate influenza pandemic. In these instances, jurisdictions should make decisions that balance local benefits and potential harms.

Absent from WHO and CDC Pandemic Plans:

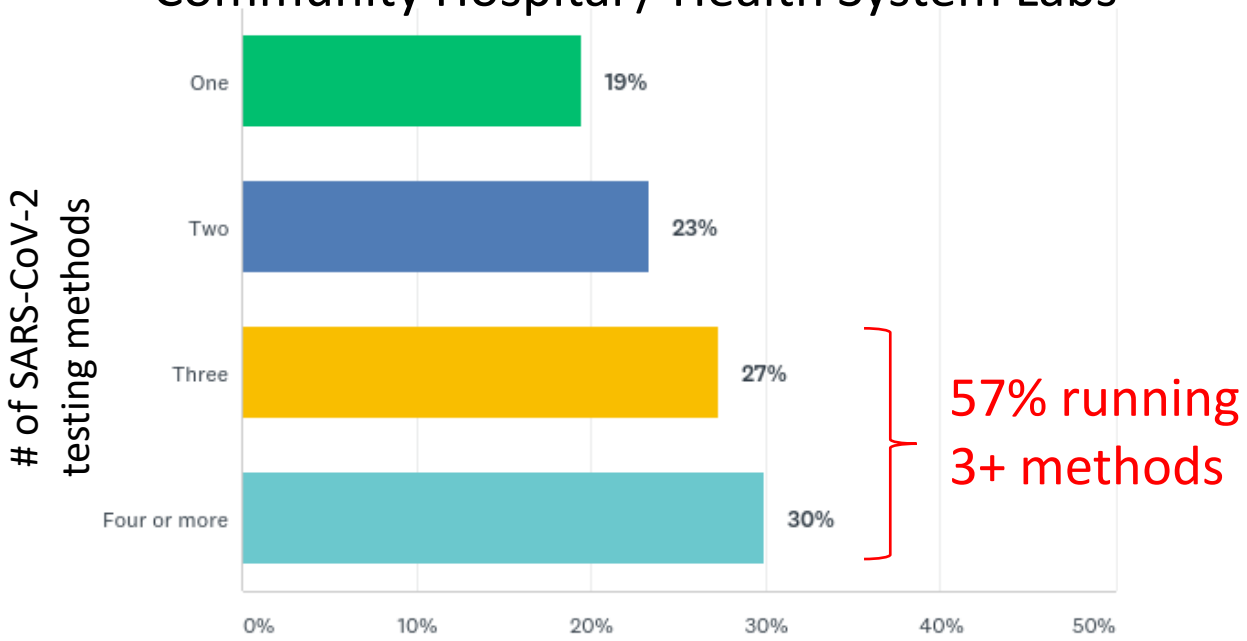
How to get ready for high capacity, rapid and sensitive testing

resulting in …

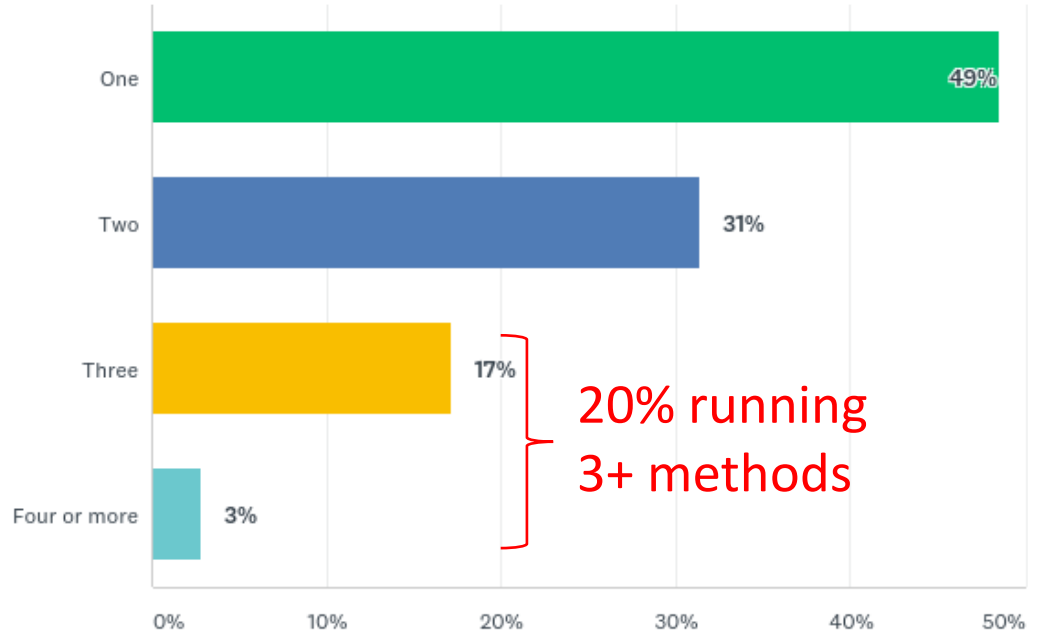
Use: whatever you have
Add: whatever you can get
Experience:
extreme shortfalls in supply chain tests and collection reagents

Due to supply shortages and uncertainties, laboratories are deploying multiple testing methodologies

Academic Medical Center & Community Hospital / Health System Labs



Commercial Reference Labs



Why did you choose this SARS-CoV-2 testing method?

"Whatever reagents were able to receive" "Independent supply chain" "Limited kit availability"

"**Supply chain issues are a major hurdle** currently, which is preventing us from moving forward with this as a primary instrument."

"We use the [company name's] extraction reagents and they are hard to get and the shortage affects our 24 other LDTs."

"We are concerned with this test and have it as a back-up for increased capacity if it needs to be deployed. The supply chain for this test has been very un-reliable."

"Next door (Virology Lab) is offering COVID19 testing on three platforms to minimize the risk of inventory shortage."

AMP Association for Molecular Pathology

All US-based labs: top 10 primary testing methods

SARS-CoV-2 Molecular Testing Methods	Primary* (n=112)	Secondary (n=88)	Tertiary (n=59)
Laboratory developed testing procedure (LDP / LDT) with EUA submission	21%	8%	5%
Roche Molecular Systems cobas SARS-CoV-2	17%	5%	0%
Abbott Molecular RealTime SARS-CoV-2 assay	16%	6%	7%
Cepheid Xpert Xpress SARS-CoV-2 test	8%	19%	25%
Hologic Panther Fusion SARS-CoV-2	6%	1%	0%
Quidel Corporation Lyra SARS-CoV-2 Assay	5%	2%	2%
Thermo Fisher Scientific TaqPath COVID-19 Combo Kit	5%	3%	7%
CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel	4%	9%	7%
DiaSorin Molecular Simplexa COVID-19 Direct assay	4%	13%	9%
Abbott Diagnostics ID NOW COVID-19	3%	2%	9%

** Data sorted by the primary testing method from largest to smallest*

Performance of COVID tests done in a condensed timeframe

Gaps in analysis of tests due to urgency for testing

Rapidly assembled quantified validation and control materials

not carefully standardized to each other.

Universal control materials late to arrive and sometimes not well standardized

Requirements for EUA validation of LOD

30 positive and 30 negative clinical specimens

Dilution series of 3 replicates per concentration with inactivated virus on actual patient specimen matrix

Confirmation at the final concentration of 20 replications with 19/20 required to claim that concentration as LOD

EUA Bridging studies

Altered application of COVID test, now discontinued

Highly accurate comparisons of COVID-19 test sensitivities not available for all tests

(https://www.fda.gov/media/135659/download)

Company	Test	LOD	EUA date
Becton, Dickinson & Company	BioGX SARS-CoV-2 Reagents for BD MAX System	40 copies/mL	4/2/20
Abbott	Abbott RealTime SARS-CoV-2 assay	100 copies/mL	3/18/20
Abbott	ID NOW COVID-19	125 copies/mL	3/27/20
Quest Diagnostics	Quest SARS-CoV-2 rRT-PCR	136 copies/mL	3/17/20
Cepheid	Xpert Xpress SARS-CoV-2 test (lab test)	250 copies/mL	3/20/20
Cepheid	Xpert Xpress SARS-CoV-2 test (point of care test)	250 copies/mL	3/20/20
bioMerieux	BioFire COVID-19 Test	330 copies/mL	3/23/20
Qiagen	QIAstat-Dx Respiratory SARS-CoV-2 Panel	500 copies/mL	3/30/20
DiaSorin	Simplexa COVID-19 Direct assay	500 copies/mL	3/19/20
Quidel	Lyra SARS-CoV-2 Assay	800 copies/mL*	3/17/20
Ipsum	COV-19 IDx Assay	850 copies/mL*	4/2/20
CDC	CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)	3,160 copies/mL; 1,000 copies/mL*	2/4/20
Co-Diagnostics	Logix Smart Coronavirus Disease 2019 (COVID-19) Kit	4,290 copies/mL	4/3/20
Luminex	NxTAG CoV Extended Panel Assay	5,000 copies/mL	3/27/20
LabCorp	COVID-19 RT-PCR Test	6,250 copies/mL*	3/16/20
Luminex	ARIES SARS-CoV-2 Assay	75,000 copies/mL	4/3/20
GenMark	ePlex SARS-CoV-2 Test	100,000 copies/mL	3/19/20
PerkinElmer	PerkinElmer New Coronavirus Nucleic Acid Detection Kit	3 copies/ reaction	3/24/20
Gnomegen	Gnomegen COVID-19 RT-Digital PCR Detection Kit	8 copies/ reaction	4/6/20
Thermo Fisher	TaqPath COVID-19 Combo Kit	10 copies/ reaction	3/13/20
New York State Department of Public Health	New York SARS-CoV2 Real-time Revers Transcriptase (RT)-PCR Diagnostic Panel	25 copies/ reaction	2/29/20
Mesa Biotech	Accula SARS-CoV-2 Test	200 copies/ reaction	3/23/20
Roche	Cobas SARS-CoV2	0.009 TCID50/ mL	3/12/20
Hologic	Panther Fusion SARS-CoV-2	0.01 TCID 50/mL	3/16/20

Sample Collection: An unexpected? supply chain pinch point

Critical Supply Shortage:
Swabs
Collection Tubes
Media

"We have a locked-down commitment"

"There's be a change of plan"

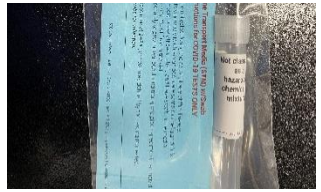
"You can't say exactly why"

"No future commitment"

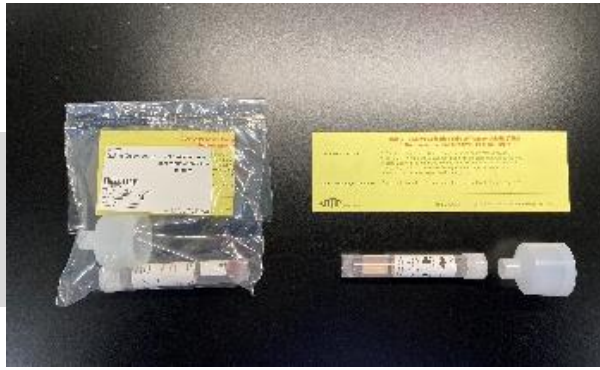
ARUP Solution:

Build your own using generic and widely available reagents (tubes, swabs, media components)

Collection swab shortage
Investigation of 3D printing swabs (based on Norwell Health study)



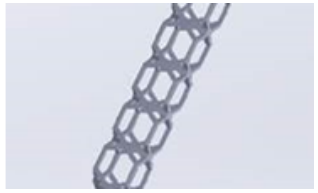
ARUP Transport Media™
Ambient temperature, universal transport media for infectious diseases



Swab supply line secured

Early April 2020

Worldwide test collection kit shortage
ARUP initiates collection kit conservation project



Large scale collection kit production
Utilized saline as media

Automated collection kit production
High volume packaging equipment

From Lincoln Hirayama

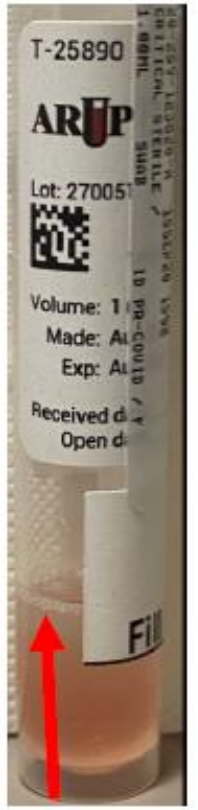
COVID-19 Saliva Collection

- High ‘invalid’ rate at first - 15%!
- Viscosity/higher order protein complexes possibly interfering
- All saliva samples are freeze-thawed → invalid rate now <1%
- Correct amount very important

Acceptable Collection: Bring saliva volume to the “**Fill Line**.”

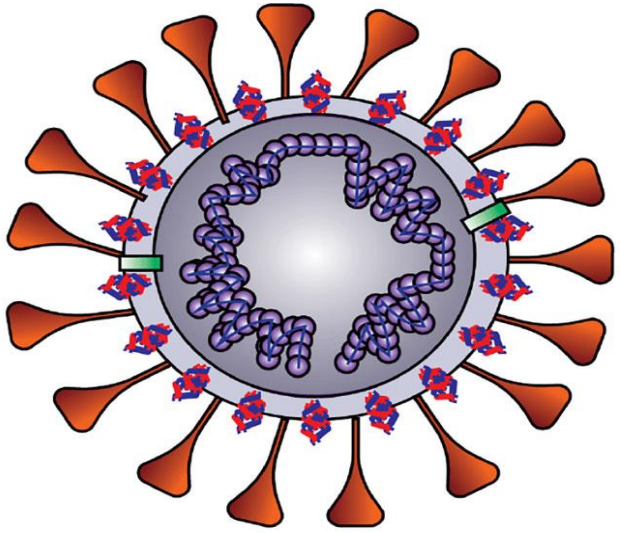
Ignore the bubbles or froth above the liquid level

Unacceptable Collection: An **under filled** saliva collection can generate poor results such as “**False Negatives**”



Unacceptable Collection: Even a small amount of overfilled saliva can create too much viscosity in the sample and generate “**In-valid**” results

Courtesy Ben Kukull



SARS-CoV-2

Spike (S) glycoprotein

Membrane (M) protein

Nucleocapsid (N) protein

Envelope (E) glycoprotein

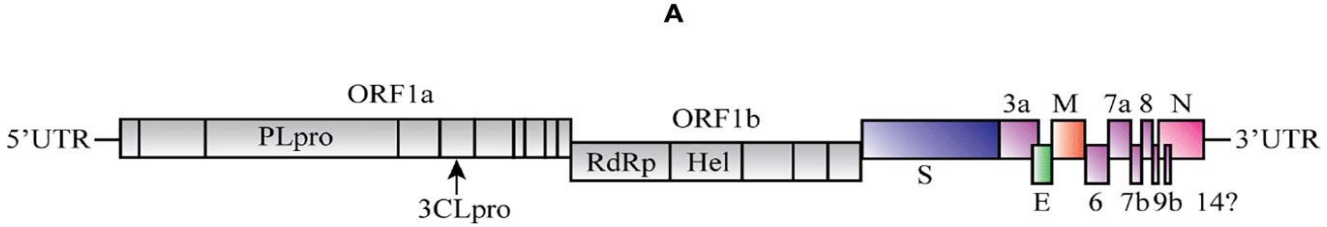
Viral entry:
S1 binding host receptors
S2 fusion viral and cell membranes

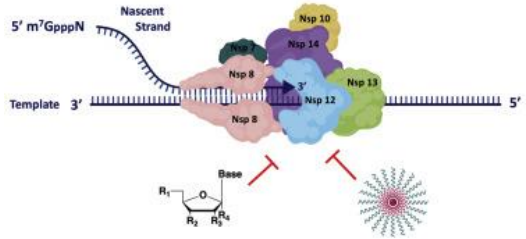
Viral fusion, assembly, budding

Replication and transcription

Assembly, morphogenesis, pathogenesis

Antigen & Antibody Tests





Viral Genome:
Proofreading function (unlike Flu)
Genomes vary by less than 10/nucleotides/3K
Multiple highly conserved targets for NAT tests
ORFab, RdRp, S, E and N genes

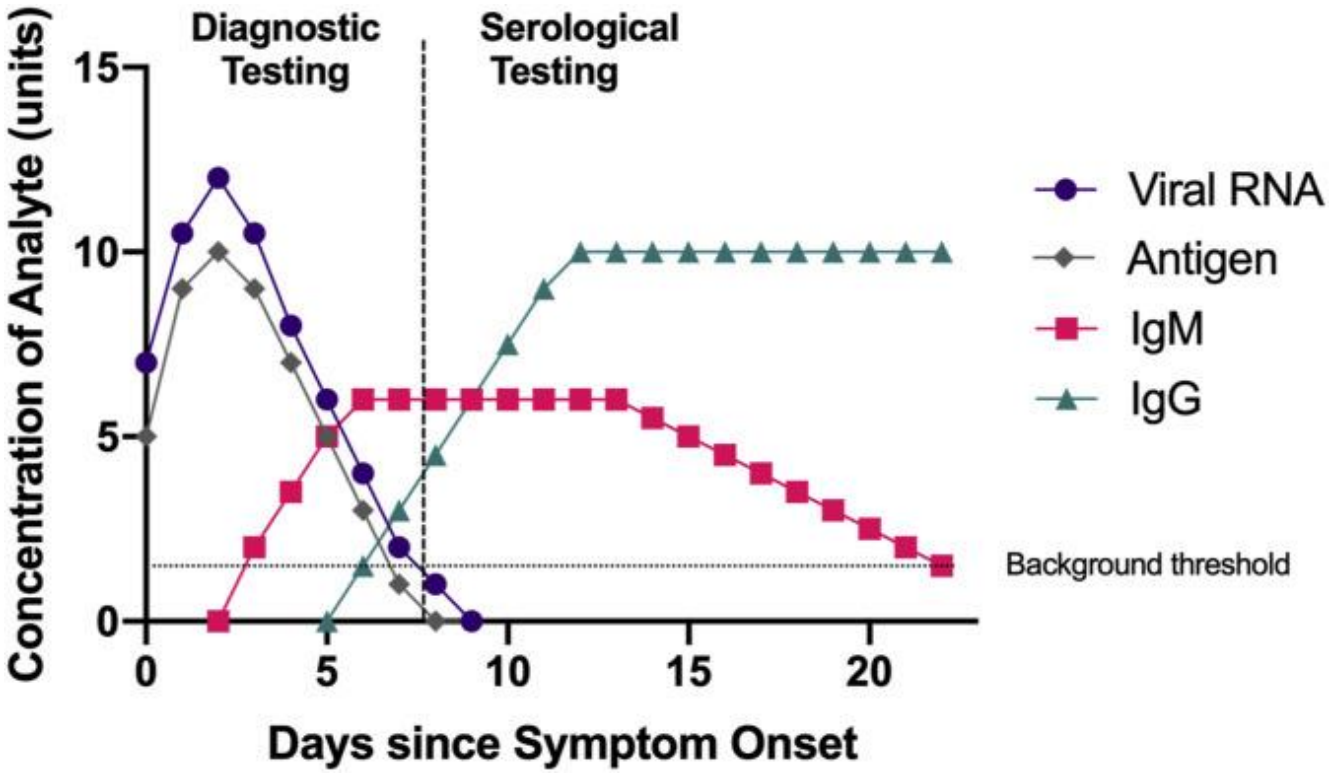
Islam et al. Natureresearch Aug 2020 | https://doi.org/10.1038/s41598-020-70812-6

COVID-19 A Respiratory and Vascular Infection

- **Respiratory failure** – Acute respiratory distress syndrome (ARDS)
- **Secondary infections** -respiratory infections and bacteremia
- **Inflammatory complications -**exuberant inflammatory response, with persistent fevers, elevated inflammatory markers, elevated pro-inflammatory cytokines
- **Cardiac and cardiovascular complications** – Other complications arrhythmias, acute cardiac injury, and shock
- **Thromboembolic complications** –Pulmonary embolism and acute stroke
- **Neurologic complications** -Encephalopathy (common), stroke, movement disorders, motor and sensory deficits, ataxia, and seizures

CDC extended symptoms: https://www.cdc.gov/mmwr/volumes/69/wr/mm6930e1.htm

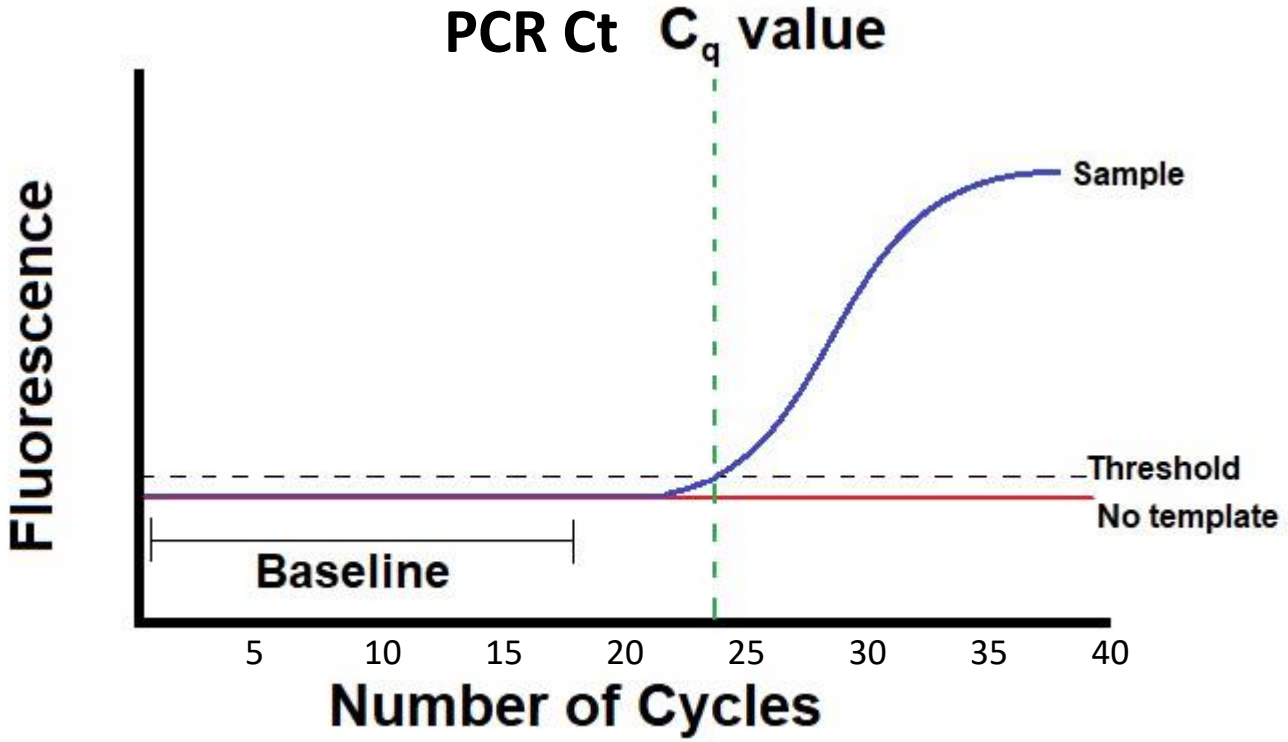
COVID-19 biomarkers for testing

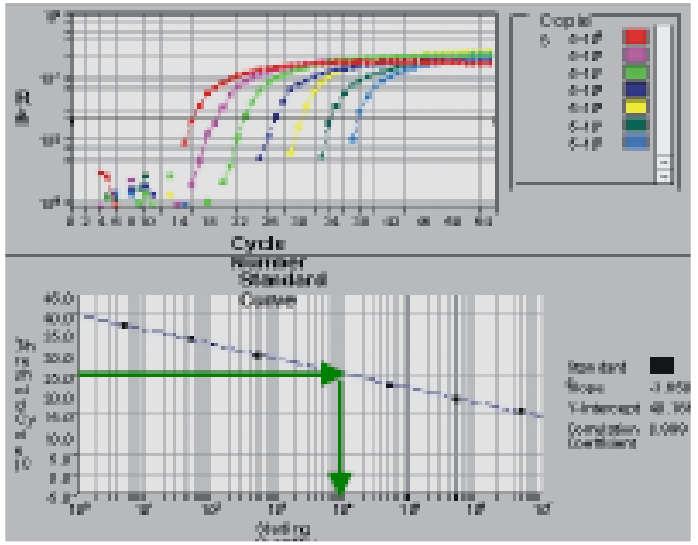


https://www.nist.gov/featured-stories/measuring-sensitivity-covid-tests-new-material-nist

Diagnostic Tests

- Antigen
  - BinaxNow
  - DiaSorin
- NAT high throughput & sensitive
  - Roche PCR (closed box)
  - Hologic fusion PCR (closed box)
  - Hologic Panther TMA RLU signal (closed box)
  - ThermoFisher PCR (open platform)
- NAT POC low throughput-rapid
  - Abbott
  - Cephiad
  - Biofire
- Differentiating clinical vs analytical sensitivity is critical!



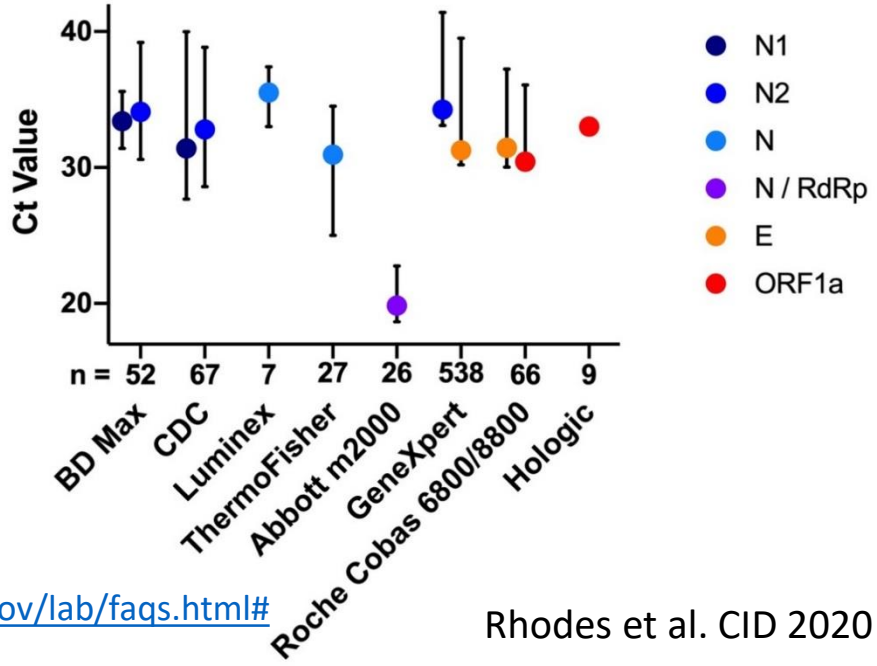


CTs for same viral concentration differ among tests!

Crossing thresholds (Cts) provide a good approximation of viral concentration in a liquid sample

Cts do not accurately measure viral burden in host

Cts vary among different instruments!



8 fold variability in CTs in 26 lab survey

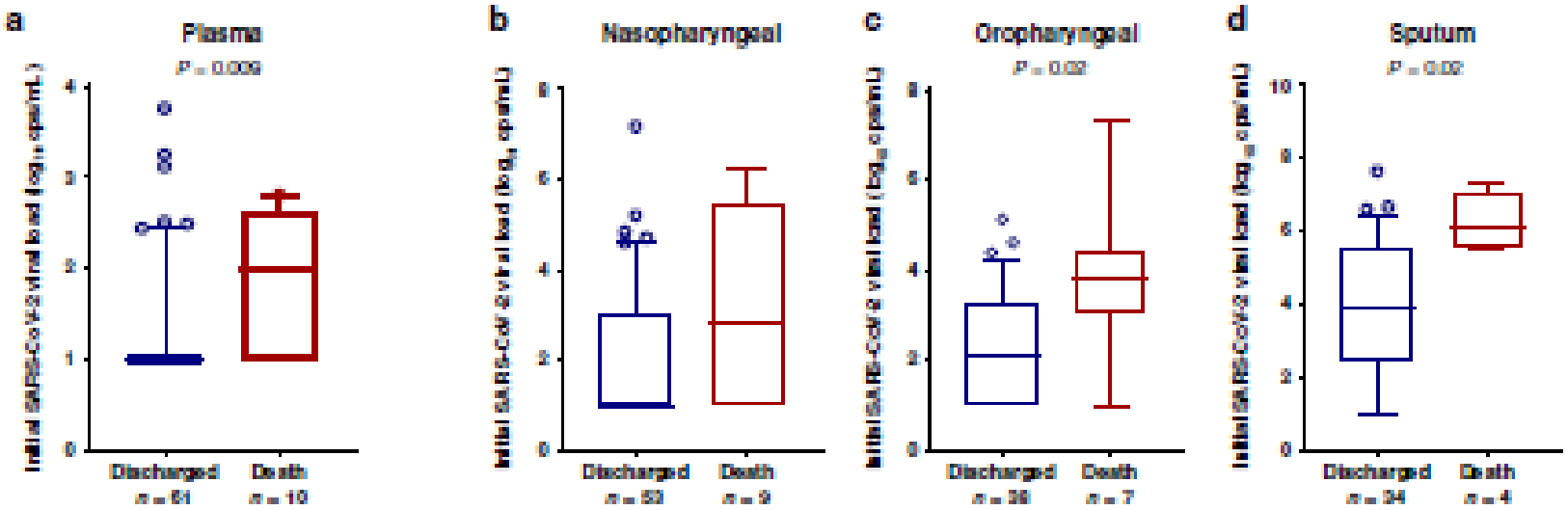
https://www.publichealthontario.ca/-/media

https://www.cdc.gov/coronavirus/2019-ncov/lab/faqs.html#Interpreting-Results-of-Diagnostic-Tests

Rhodes et al. CID 2020

Utility PCR Crossing Threshold Analysis/reporting

- Predict rising tide of infections at a given phase of pandemic
- Understand dynamics of viral shedding
- Release patients with still detectable virus from quarantine
- Indicator disease severity and likelihood of death
- Stratification of patient risk
- Crossing thresholds vary with sample type.

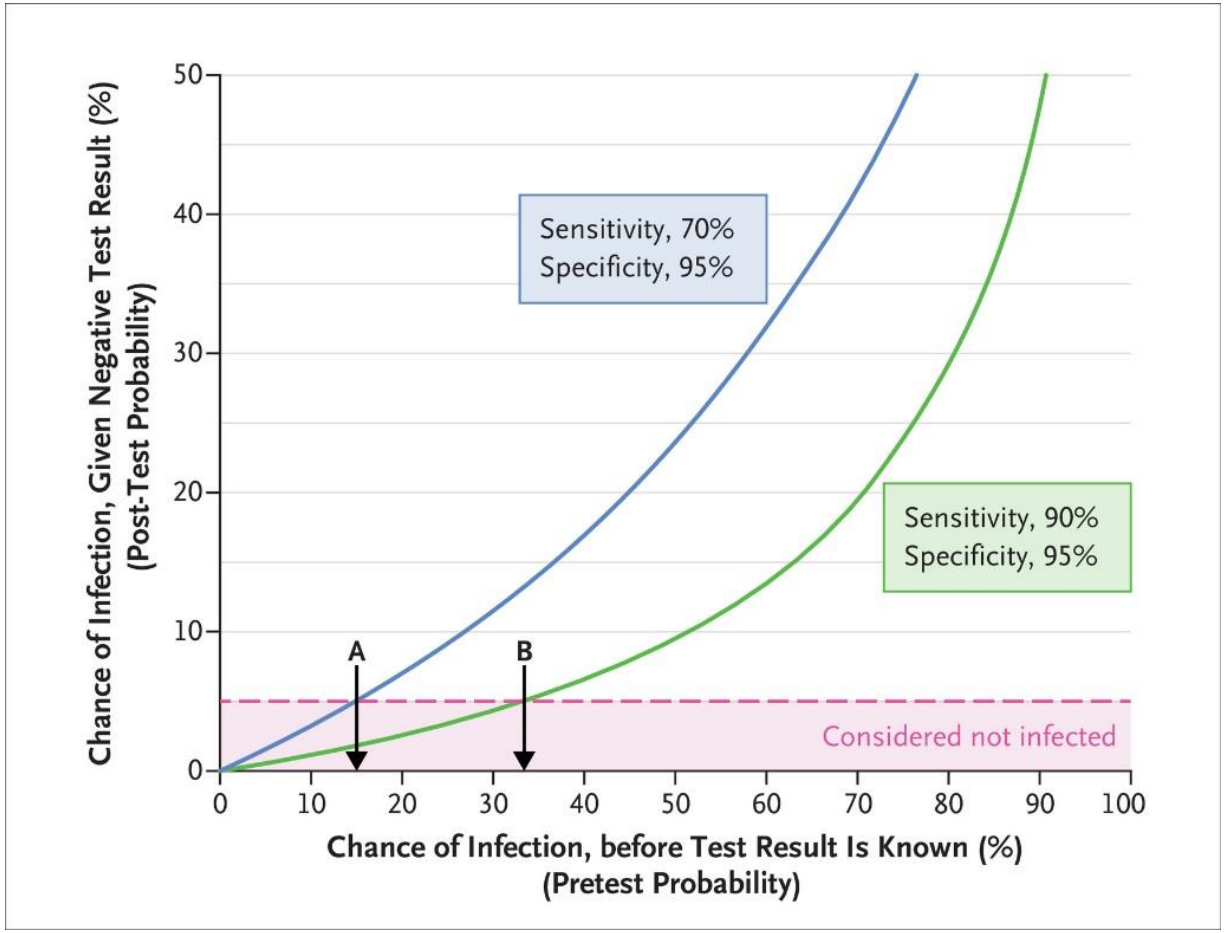


NATURE COMMUNICATIONS | (2020) 11:5493 |

Bryan et al. Open Forum Infect Dis. 2020 Nov 3;7(12)

Analytic vs Clinical Sensitivity and Specificity

- Traditional test performance validation:
  - Clinical or contrived specimens
  - Comparison to reference test
- Sensitivity:
  - % positivity patients with disease
  - No absolute reference for disease status
  - Reference material lacking
  - COVID EUA allows establishing agreement with results positive material from symptomatic patients or contrived material
  - Swabs and saliva miss infected material
- Specificity:
  - High and reliable for NAT tests



Corman et al. Euro Surveill. 2020;25

Woloshin et al. NEJM 383;6 Aug 2020

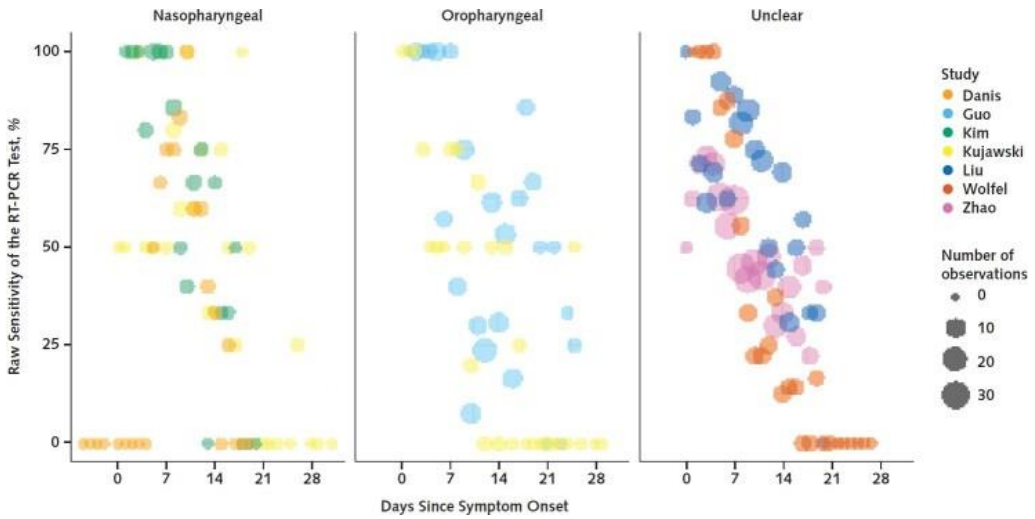
Testing:

No immediate value after exposure

~40 % false negatives day of symptom onset

Optimal testing time 1-3 days post symptoms
~Day 8

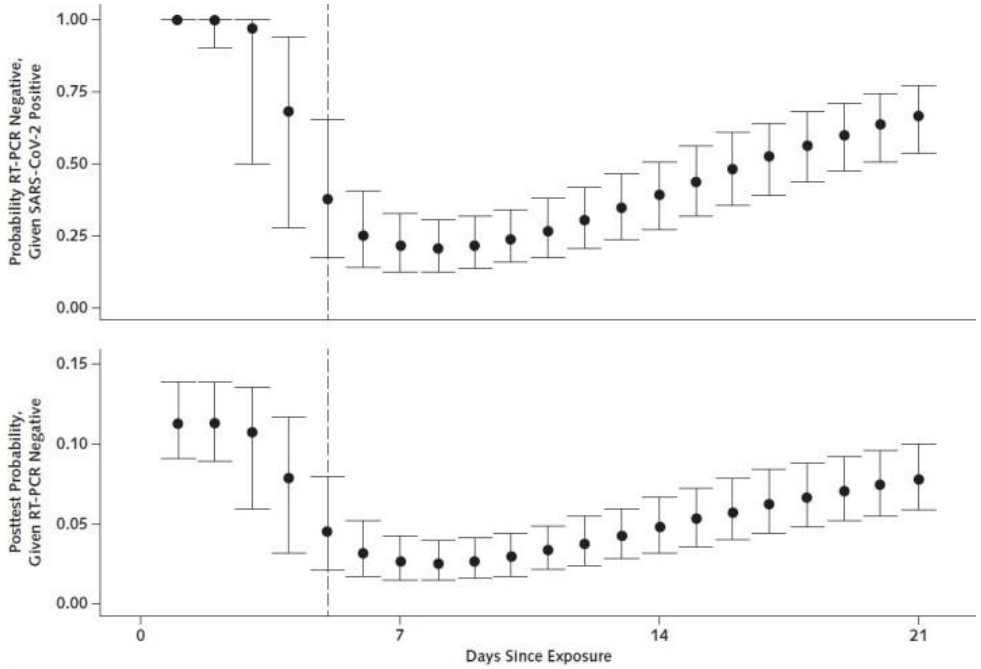
Negative RR-PCR results should not do not rule out infection especially in cases with high clinical suspicion



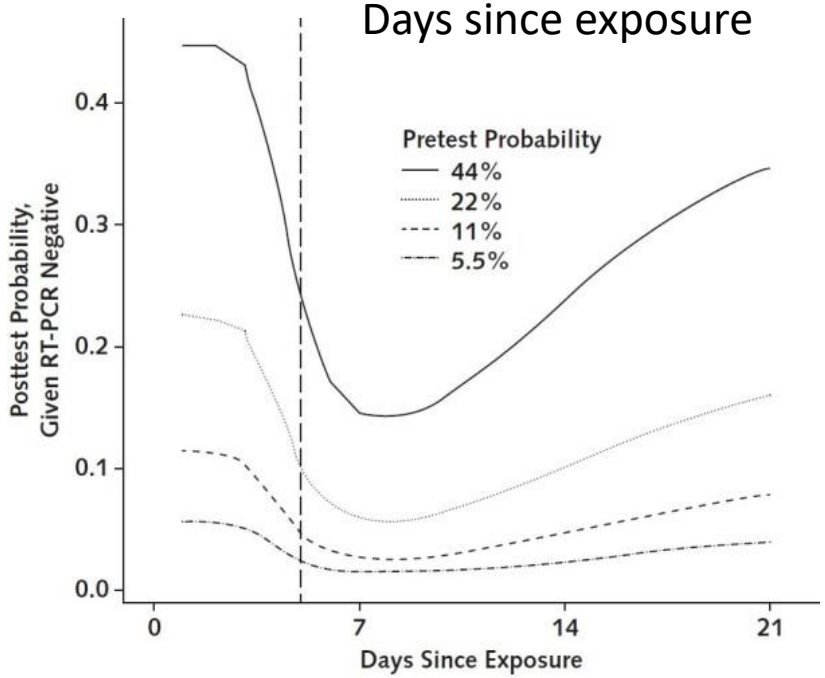
Kucirka et al. *Ann Intern Med.* doi:10.7326/M20-1495

Probability negative PCR (truly infected)

Probability infected (PCR test negative)

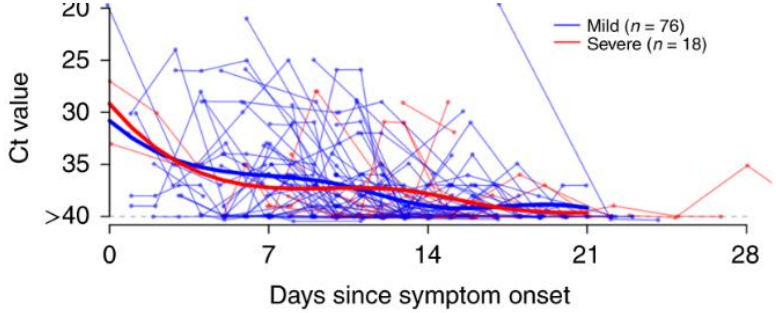


Days since exposure

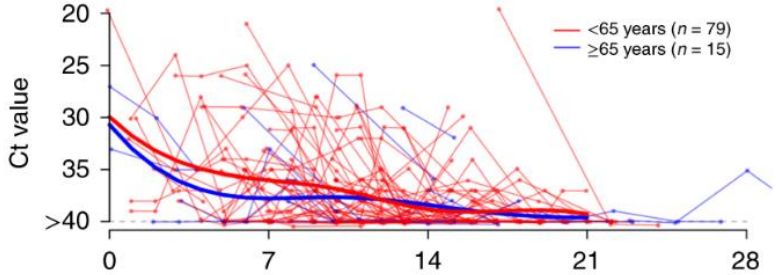


COVID-19 Viral Dynamics and Shedding

Mild vs Severe

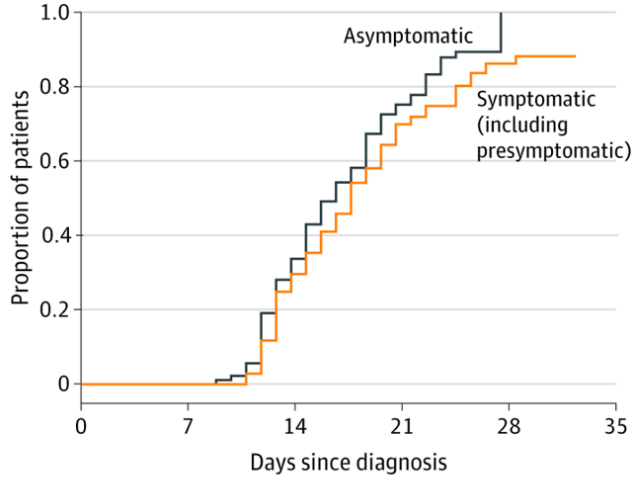


> 65 vs <65



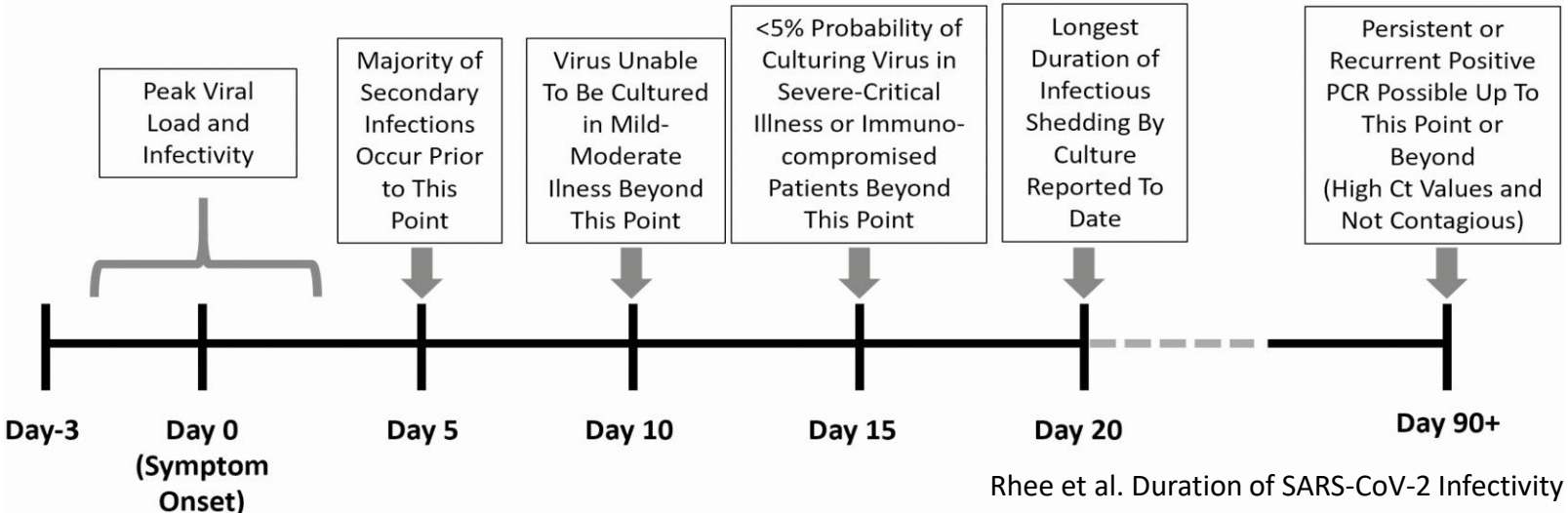
He et. Nature Medicine | VOL 26 | **672** MAY 2020 | 672–675 |

Symptomatic vs Asymptomatic



No. at risk						
Asymptomatic	89	89	59	19	0	0
Symptomatic (including presymptomatic)	213	213	149	49	6	0

Lee et al. JAMA Internal Medicine November 2020 Volume 180, Number 11



Rhee et al. Duration of SARS-CoV-2 Infectivity cid 2020

Approaches to test comparisons and power to predict % positives and % missed

- Traditional FDA requirements
  - Rigorous LOD
  - Large population studies
  - Examples: HIV, HCV HPV, Flu
- Comparative testing of samples of different viral concentration identified by PCR crossing thresholds (CTs).
  - Across the spectrum viral concentration
  - Within the spectrum of low viral concentration
- Prediction based on established test LOD and relative CTs, +/- confirmatory testing

Comparative Assay Testing of a Sampled Population

Comparison of Two High-Throughput RTPCR Systems for the Detection of COVID-19

Kraner et al. JCM May 2020

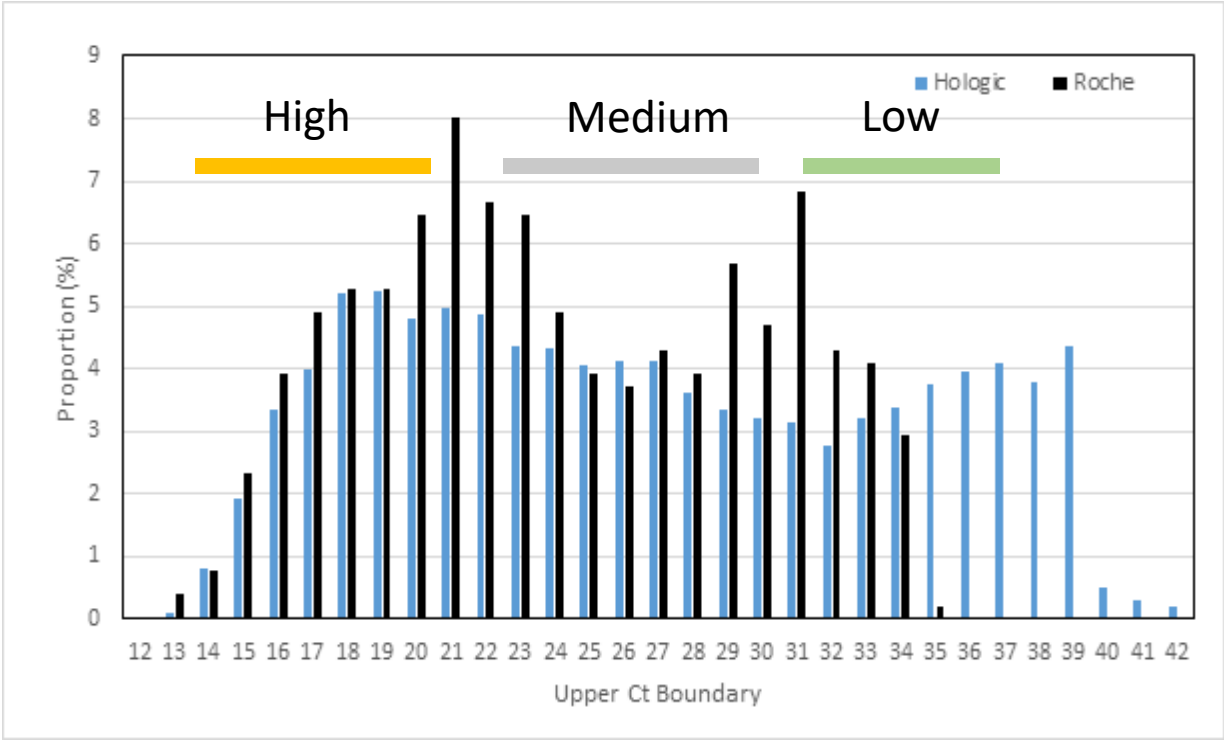
Characteristics of discordant specimens

cobas 6800 SARS-CoV02 RT-PCR Result	
Target 1 Ct Value	**Target 2 Ct Value**
35.26	37.69
N/A	N/A
N/A	N/A
N/A	N/A
N/A	N/A
N/A	N/A
N/A	38.26 [c]
N/A	N/A
35.37	N/A
N/A	37.64 c
35.84	38.05
N/A	N/A
N/A	N/A
36.13	N/A

Panther Fusion SARS-CoV-2 RT-PCR Result
CT Value
N/A
36.5
38.1
38.1
36.9
N/A
38
N/A
N/A
N/A
N/A
N/A
38
38.1
N/A

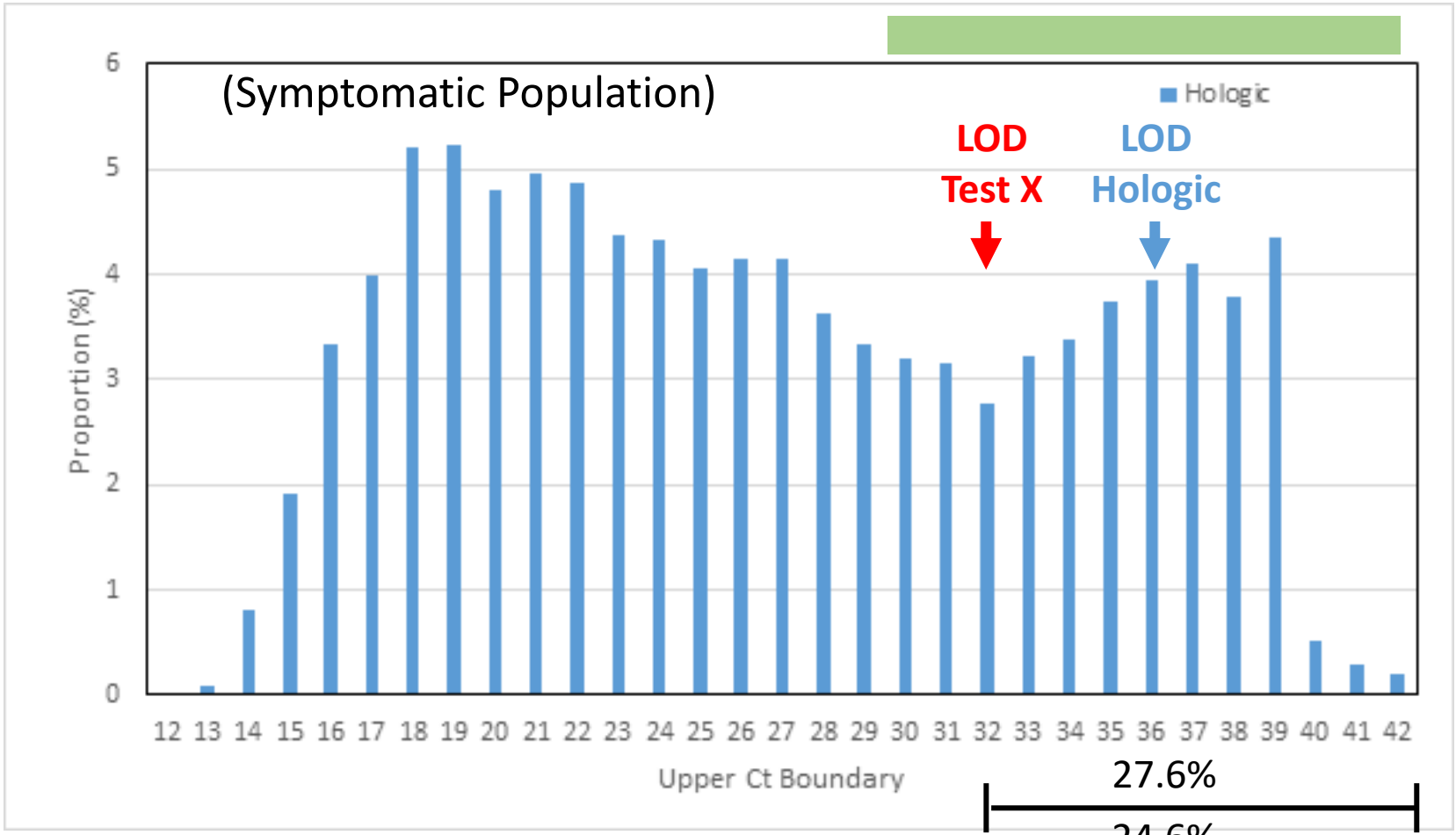
Berry et al. JCM.00743-20

Distribution CTs Hologic and Roche
(~ 5% Positive Symptomatic Utah Population)



Hologic Panther Fusion (n=5257) Roche cobas SARS-CoV-2 Assay (n=511)

Comparative testing within a spectrum of low viral concentrations



Hologic Panther Fusion (n=5257)

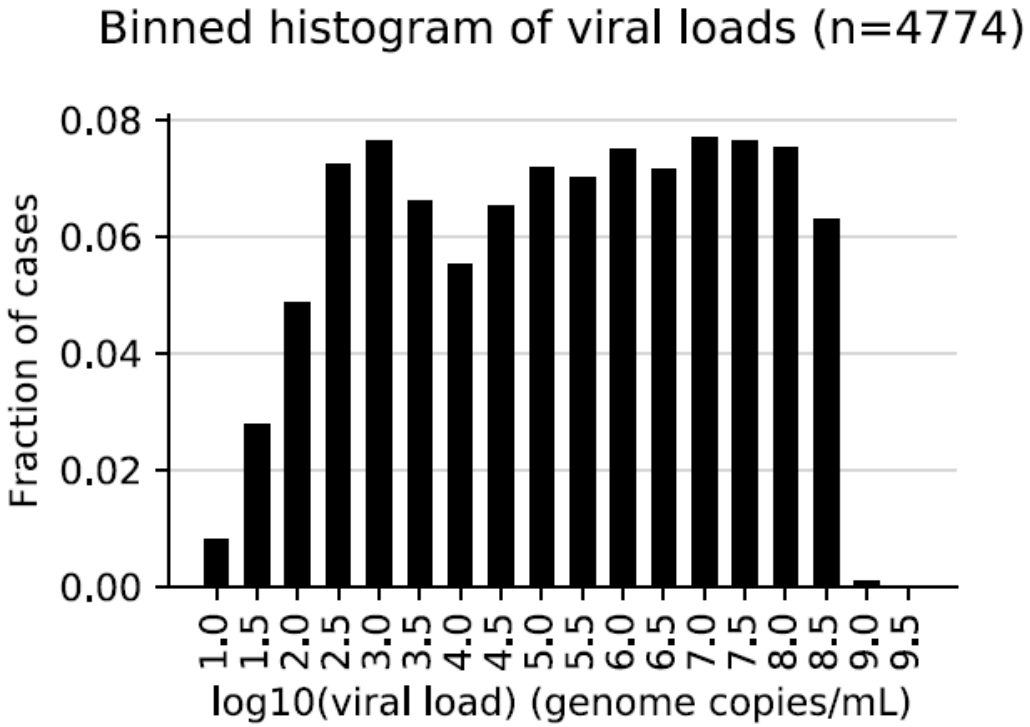
Selectively Test Samples
In “Low Range”

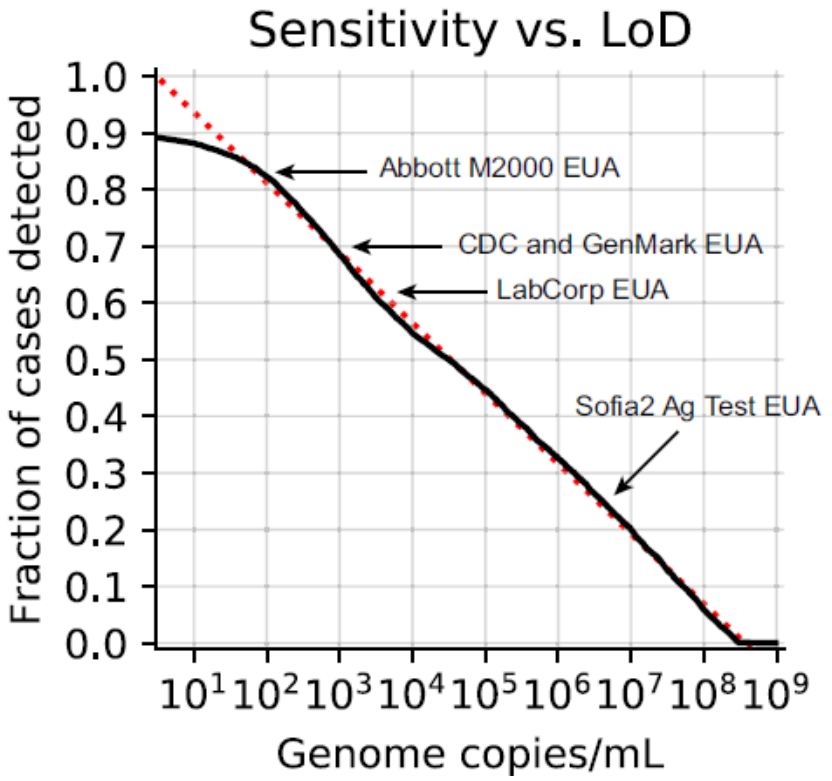
Advantage:
test many low positives
more accurate predictions

Calculate percentage of samples “likely” missed based on comparative CTs and knowledge of LODs

Calculate expected CT based on **Anchor CT** and **LOD of Less Sensitive Test**

Use of CTs to determine numbers of low positives in a population and predict negatives among different assays



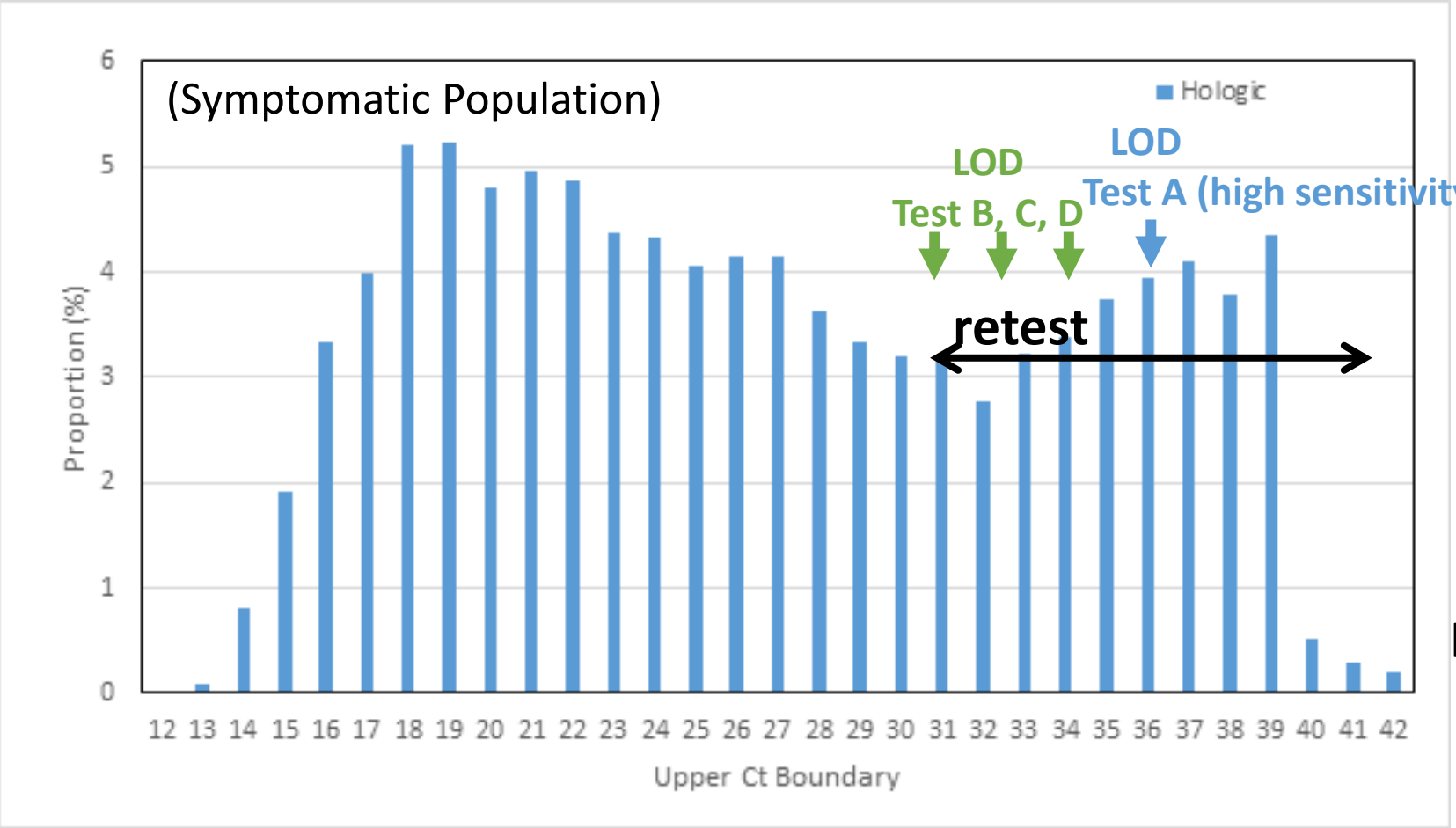


Conclusions

Predicted relative sensitivity assumes tests have been accurately determined Limits of Detection (LOD)

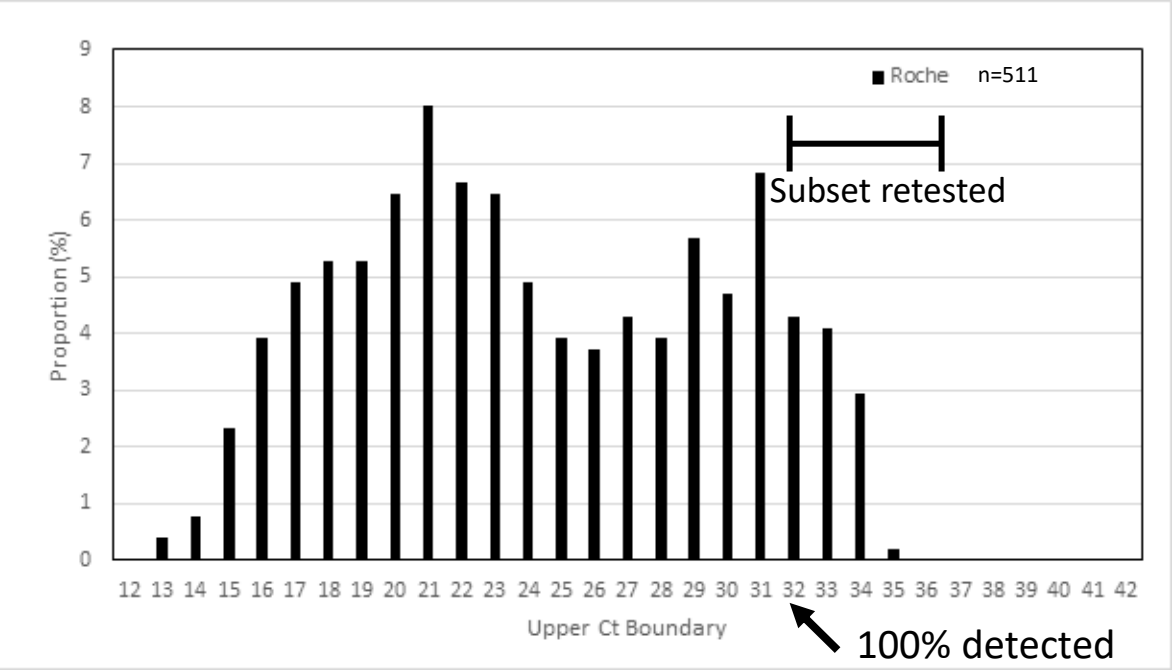
Arnaout et al. bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.131144

Prediction based on established test LOD and relative CTs with Confirmation



Calculate percentage of samples “likely” missed based on comparative CTs of several tests and knowledge of LODs with confirmation by retesting low positive samples Identified by high sensitivity test

Hologic Panther Fusion (n=5257)

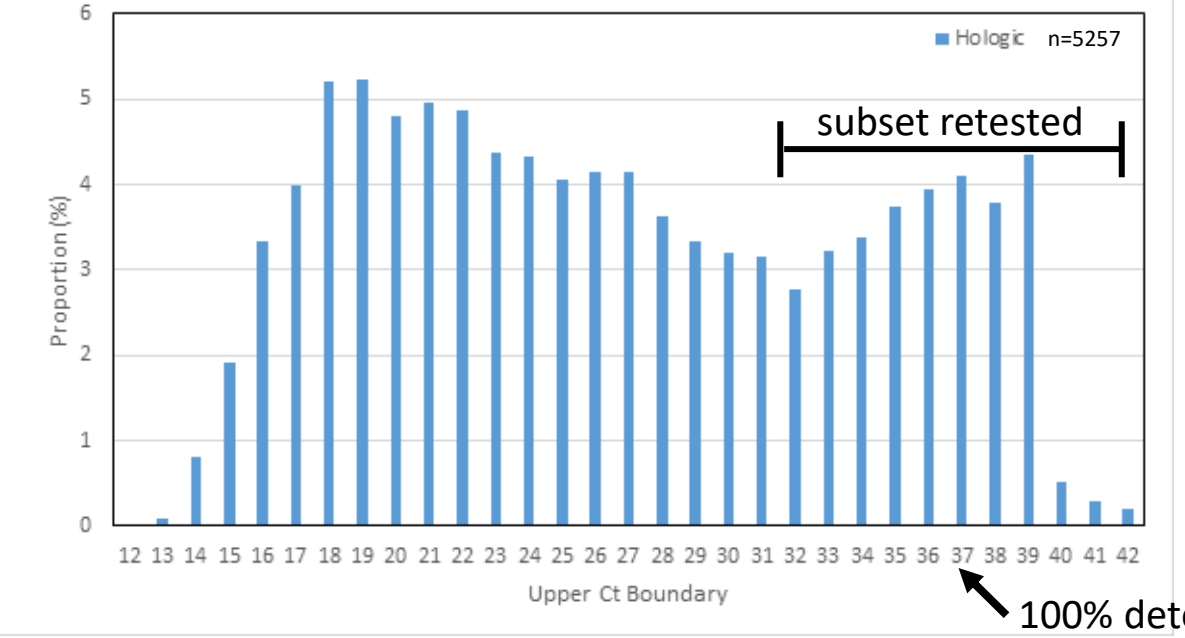


"False Negatives"
1.4% of
Roche Detected
Population

13.5% = 7
samples
missed

CDC assay results for 52 low positive Roche samples

	CDC Assay Result			
Roche Ct	Positive	Negative	Inconclusive	Row Total
31-31.9	100% (18)	-	-	100% (18)
32-32.9	85.0% (17)	10.0% (2)	5.0% (1)	100% (20)
33-33.9	61.5% (8)	30.8% (4)	7.7% (1)	100% (13)
≥34	-	100% (1)	-	100% (1)
Grand Total	82.7% (43)	13.5% (7)	3.8% (2)	100% (52)



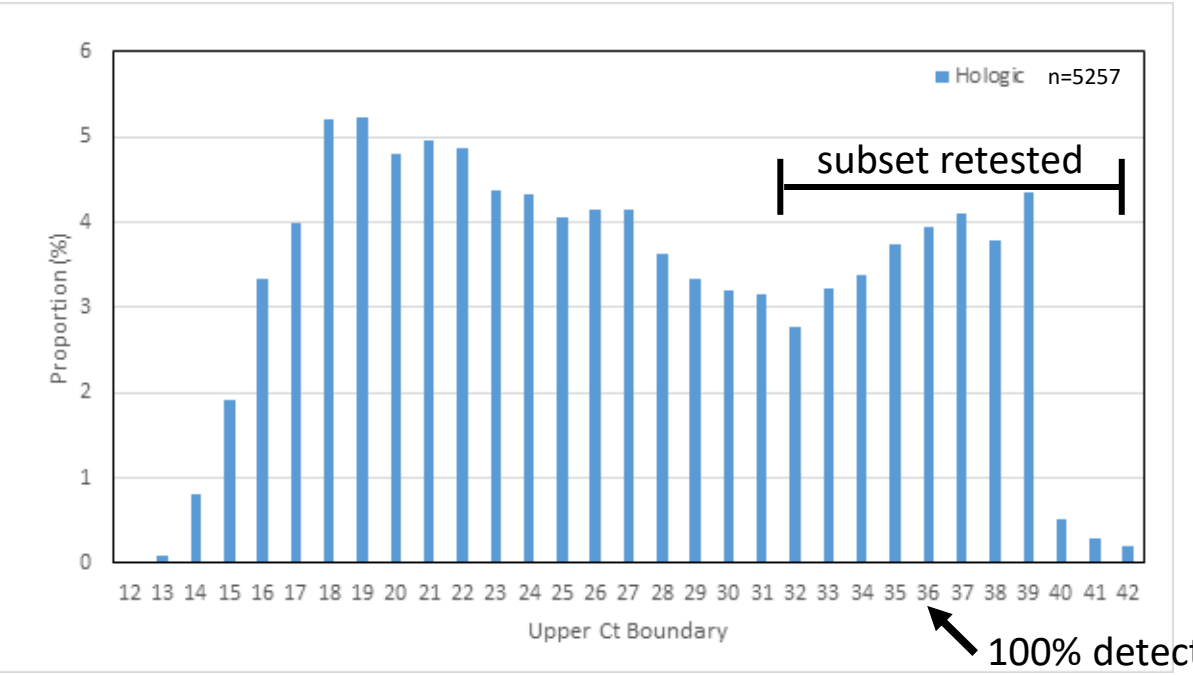
"False Negatives"
1.7% of
Hologic Detected
Population

6.4% = 9
samples
missed

CDC assay results for 140 low positive Hologic samples

	CDC Assay Result			
Hologic Ct	Positive	Negative	Inconclusive	Row Total
32-32.9	94.1% (16)	-	5.9% (1)*	100% (17)
33-33.9	100% (29)	-	-	100% (29)
34-34.9	100% (21)	-	-	100% (21)
35-35.9	100% (13)	-	-	100% (13)
36-36.9	100% (15)	-	-	100% (15)
37-37.9	94.7% (18)	5.3% (1)	-	100% (19)
38-38.9	65.0% (13)	30.0% (6)	5.0% (1)	100% (20)
>39	33.3% (2)	33.3% (2)	33.3% (2)	100% (6)
Grand Total	90.7% (127)	6.4% (9)	2.1% (3)	100% (140)

*Not retested – Insufficient RNA remaining

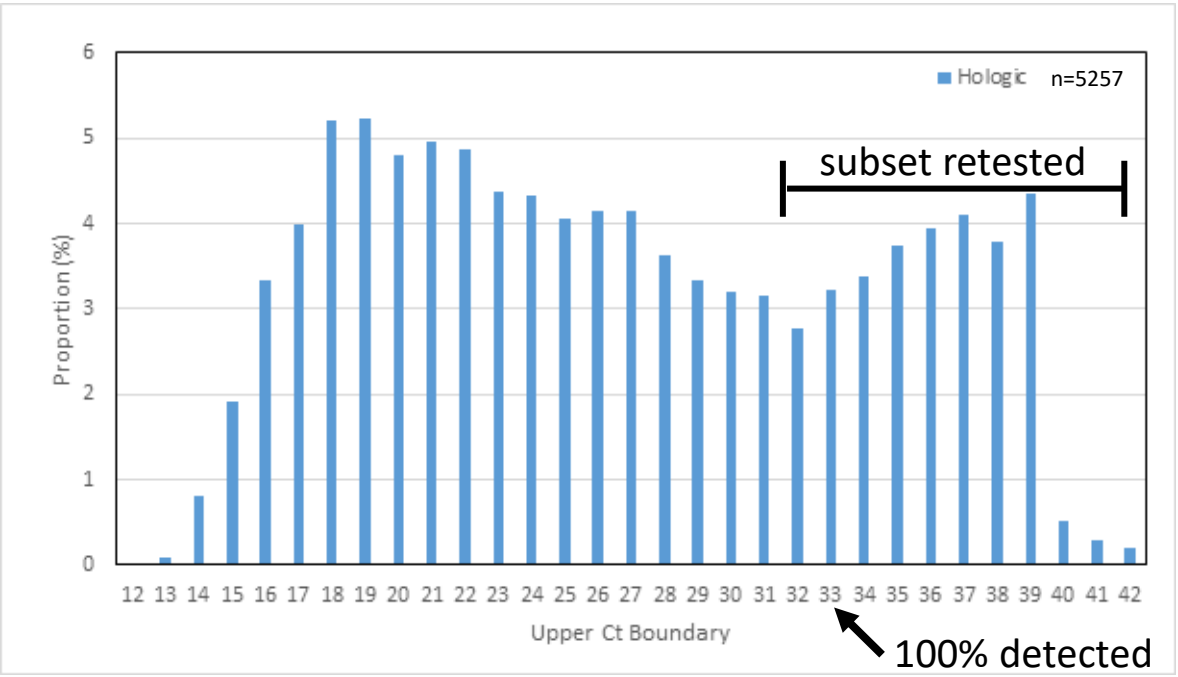


"False Negatives"
1.7% of
Hologic Detected
Population

5.7% = 8
samples
missed

ThermoFisher assay results for 140 low positive Hologic samples

	ThermoFisher Assay Result			
Hologic Ct	Positive	Negative	Inconclusive	Row Total
32-32.9	100% (17)	-	-	100% (17)
33-33.9	100% (29)	-	-	100% (29)
34-34.9	100% (21)	-	-	100% (21)
35-35.9	100% (13)	-	-	100% (13)
36-36.9	100% (15)	-	-	100% (15)
37-37.9	94.7% (18)	5.3% (1)	-	100% (19)
38-38.9	75.0% (15)	15.0% (3)	10.0% (2)	100% (20)
>39	66.7% (4)	33.3% (2)	-	100% (6)
Grand Total	94.3% (132)	4.3% (6)	1.4% (2)	100% (140)



"False Negatives"
14.6% of
Hologic Detected
Population

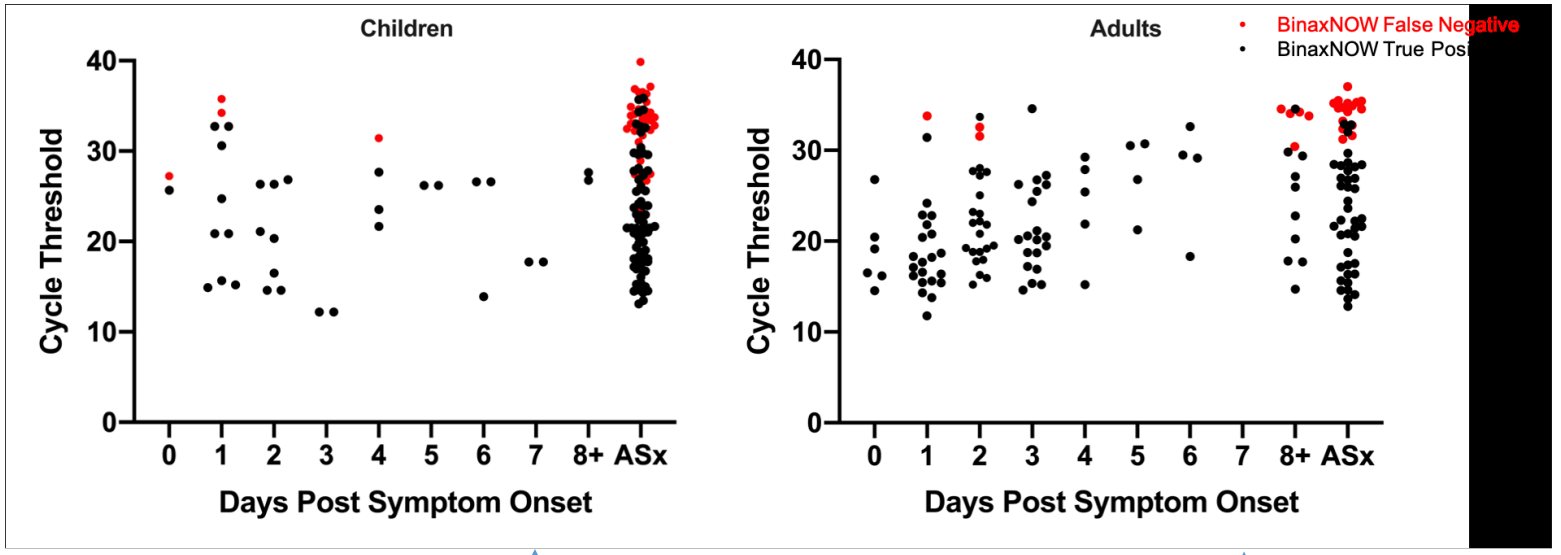
54.3% = 76
samples
missed

Quidel assay results for 140 low positive Hologic samples

	Quidel Assay Result			
Hologic Ct	Positive	Negative	Invalid	Row Total
32-32.9	100% (17)	-	-	100% (17)
33-33.9	89.7% (26)	6.9% (2)	3.4% (1)	100% (29)
34-34.9	38.1% (8)	57.1% (12)	4.8% (1)	100% (21)
35-35.9	23.1% (3)	61.5% (8)	15.4% (2)	100% (13)
36-36.9	13.3% (2)	73.3% (11)	13.3% (2)	100% (15)
37-37.9	-	100% (19)	-	100% (19)
38-38.9	-	95.0% (19)	5.0% (1)	100% (20)
>39	-	83.3% (5)	16.7% (1)	100% (6)
Grand Total	40.0% (56)	54.3% (76)	5.7% (8)	100% (140)

Assessing Sensitivity COVID-19 Antigen Test (BinaxNOW)

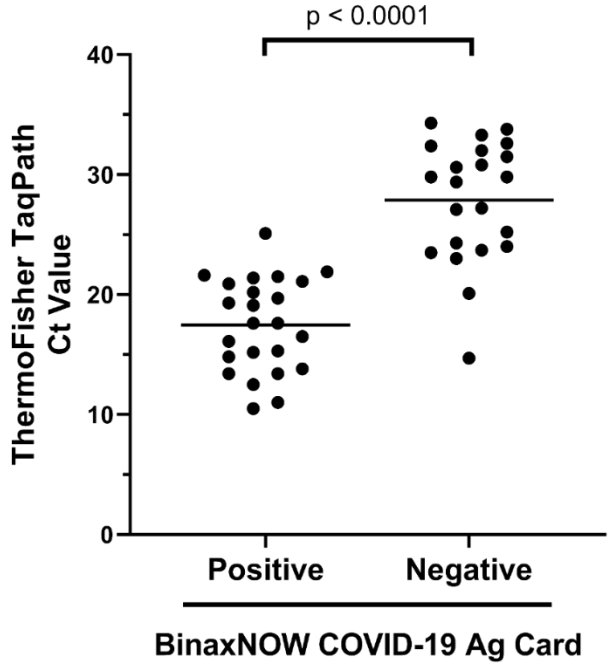
Symptomatic Population
1380 Adults 928 Children



Sensitivity 95%
Specificity 100%

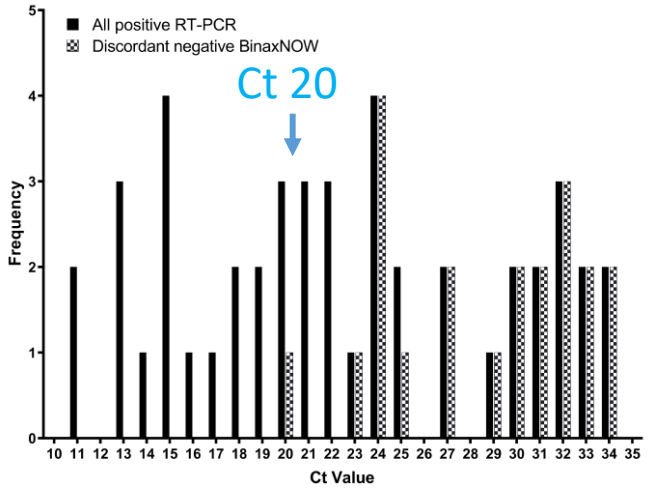
Sensitivity 84.6%
Specificity 100%

Asymptomatic Population
2,645
College Students



All group sensitivity & Crossing Threshold
99.3% Ct <25
95.8% Ct <30
81.2% Ct <35.

Co-collected Nasal Swabs
46 (1.7 %) NAT positive
24 (0.9%) Antigen positive
53.3 % sensitive, 100% specific



Pollock et al. https://doi.org/10.1101/2021.01.09.21249499;

Perchetti et al. **J Clin Microbiol doi:10.1128/JCM.02880-20**

Okoye/Barker/Pearson et al. Accepted JCM Jan 2021

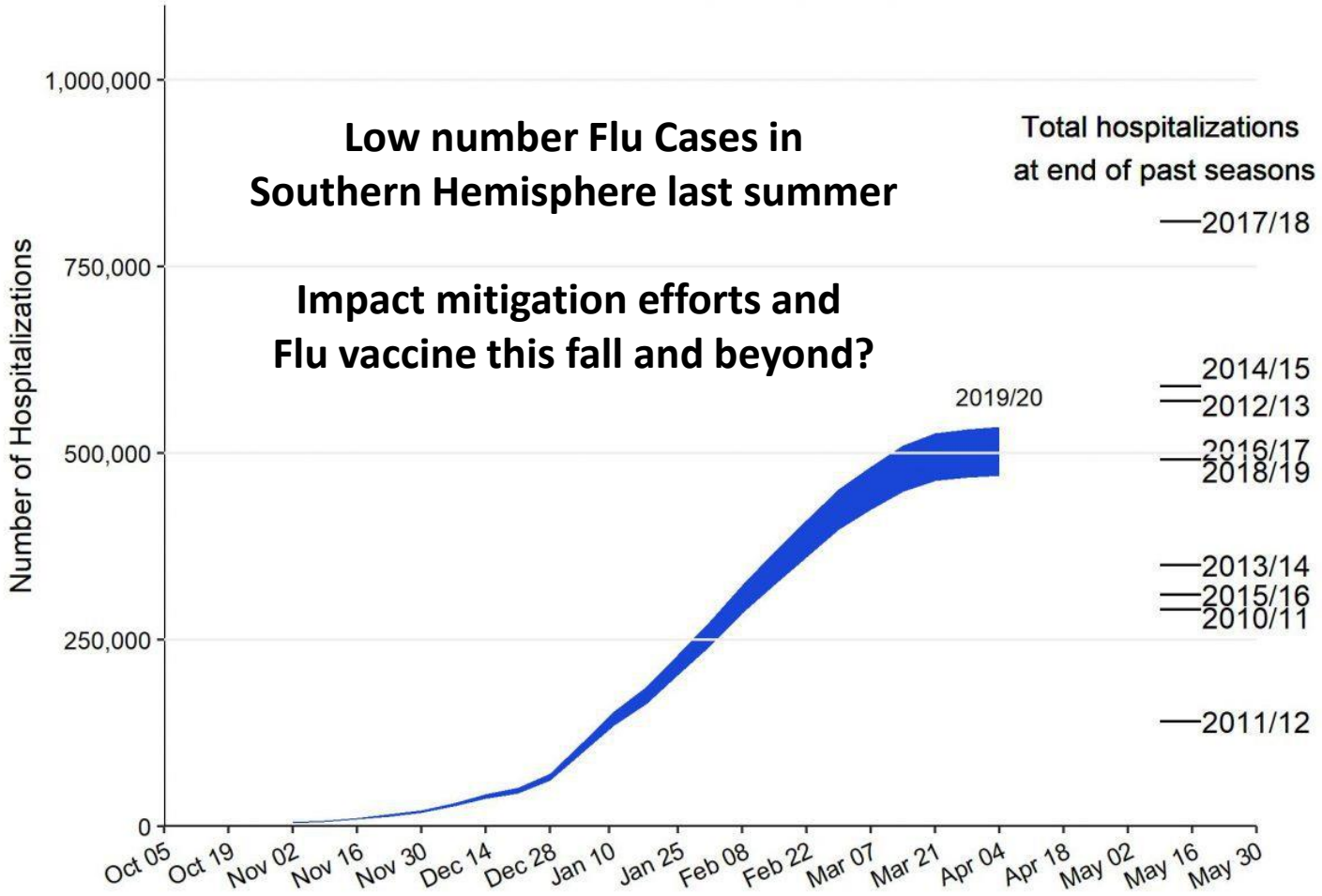
Co-Circulation COVID and Other Respiratory Viruses (Flu A, Flu B, RSV AB 2020-21?

39,000,000 – 56,000,000
flu **illnesses**

18,000,000 – 26,000,000
flu **medical visits**

410,000 – 740,000
flu **hospitalizations**

24,000 – 62,000
flu **deaths**

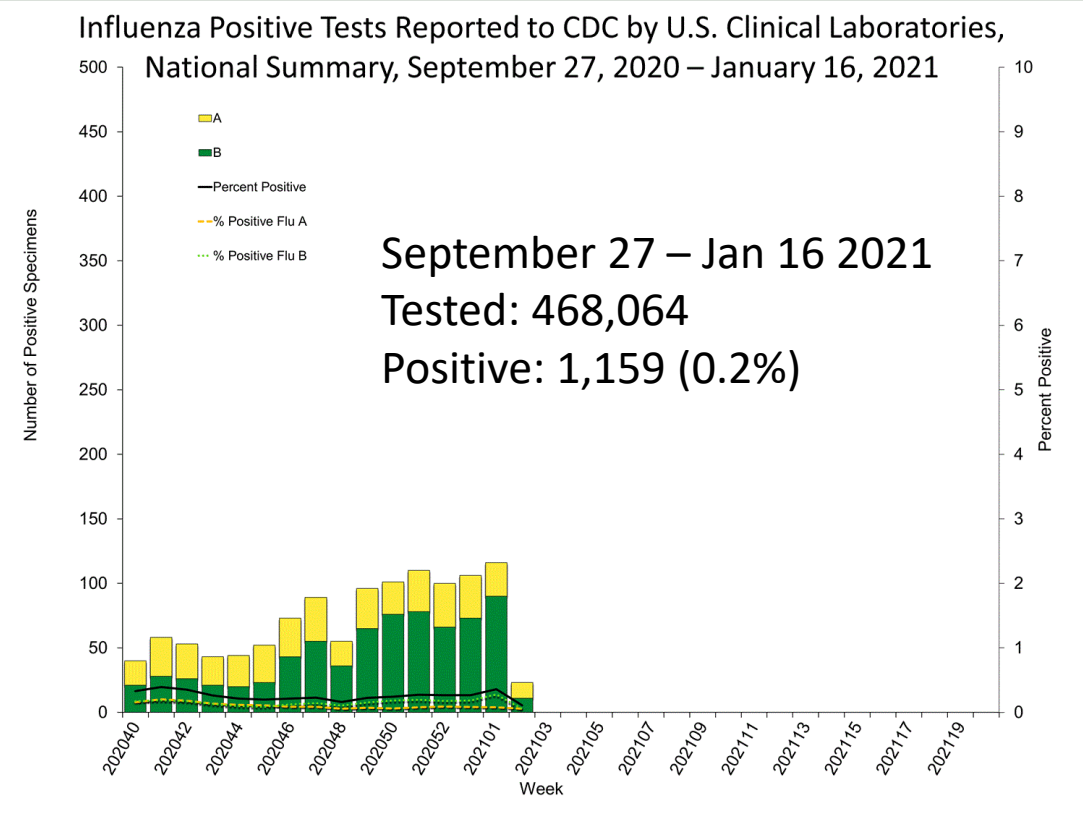


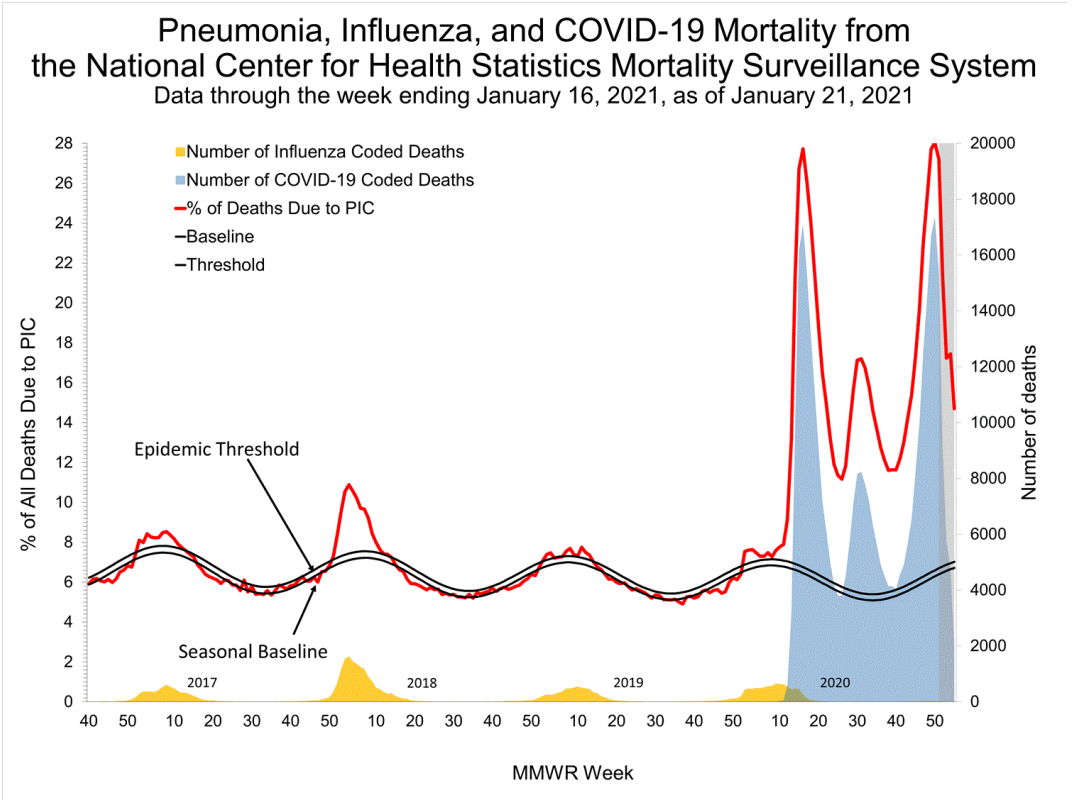
https://www.cdc.gov/flu/about/burden/preliminary-in-season-estimates.htm

COVID/Flu Twindemic?

- Issues
  - Shared symptoms
  - Co-infections more lethal?
  - Competition testing resource
  - Availability of high throughput Co-tests

- Commitment to a specific % Co-test reagents
- Adapt to COVID testing process
- Deal with unused reagents in case of low Flu season





Centers for Disease Control and Prevention

"Because some of the symptoms of flu and COVID-19 are similar, **it may be hard to tell the difference between them based on symptoms alone, and testing may be needed to help confirm a diagnosis."**

Co-Testing

Attractive to symptomatic patient

Detect and differentiate COVID, Flu, RSV
For diagnosis, treatment, tracking
(provides a "final" diagnosis)

Potential to improve operational efficiency and cost for patients, labs, and public health

Symptom	Influenza	COVID
Fever/Chills	✓	✓
Cough	✓	✓
Sore throat	✓	✓
Nasal congestion	✓	✓
Body aches	✓	✓
Headache	✓	✓
Fatigue	✓	✓
Vomiting/diarrhea	✓	✓
Loss of taste or smell		✓
Shortness of breath		✓

RSV: Disease Burden and Impacted Populations

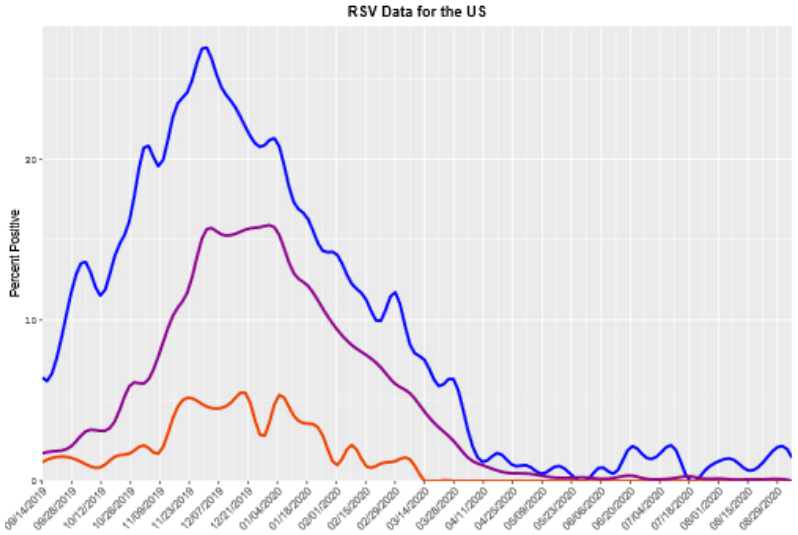
Each year in U.S. :

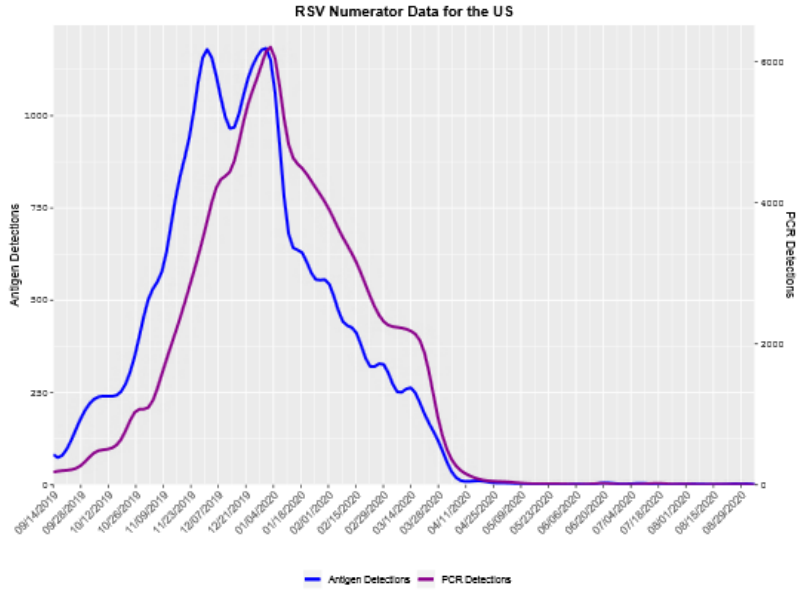
- 2.1 million outpatient visits among **children younger than 5 years old**[1]
- 57,527 hospitalizations among children younger than **5 years old**[1]
- 177,000 hospitalizations among adults older than **65 years**[2]
- 14,000 deaths among adults older than **65 years**[2]

"RSV infection is an important illness in elderly and high-risk adults, with a disease burden similar to that of non-pandemic influenza A in a population in which the prevalence of vaccination for influenza is high"
Falsey et al.

Testing for RSV is appropriate for adults over 65 as well as children as well as transplant patients of any age

RSV in a COVID/Flu not indicated in uncompromised population 5-65





Hall CB et al. New Engl J Med. 2009;360(6):588-98 The burden of respiratory syncytial virus infection in young children

Falsey AR et al. *New Engl J Med*. 2005;352(17):1749-59 Respiratory syncytial virus infection in elderly and high-risk adults

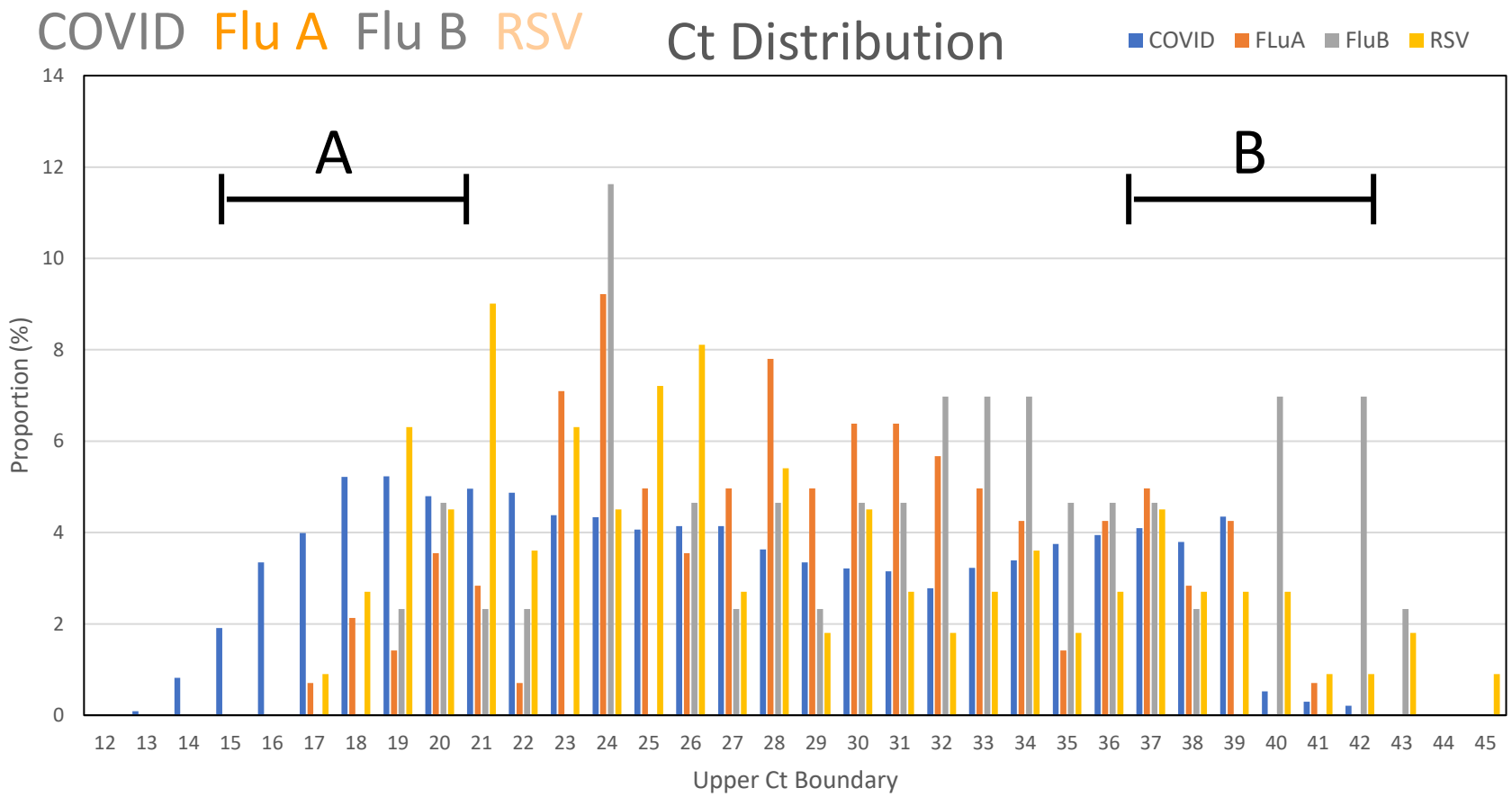
RSV Co-infections in Hospitalized Young Children

From: American Academy of Pediatrics
https://www.aappublications.org/news/2020/06/10/coronavirusbronchiolitis061020

Researchers studied 1,880 children hospitalized with bronchiolitis from two multicenter cohorts — a group under 2 years from 2007-'10 and a group under 1 year from 2011-'14. Children were tested for 18 viruses, including four endemic coronaviruses (CoV), which are not the newest CoV identified (SARS-CoV-2).

Roughly 12% of the children had a coronavirus. **Of those, 85% also had another infection, most commonly respiratory syncytial virus (RSV),** according to "Severe Coronavirus Bronchiolitis in the Pre-COVID-19 Era,"
(Mansbach JM, et al. *Pediatrics*. June 10, 2020 https://doi.org/10.1542/peds.2020-1267).

What is the impact of Population A viruses on Population B detectability for Co-Positive Samples?



Data: Panther fusion

Co-testing Analytic Challenges (single well, high throughput assays)

- **Optimize tests for high sensitivity and specificity for multiple viral targets**
- **Single well multiplex Co-detection**
  - 4 targets: COVID, IC, Flu A, Flu B
  - 5 targets: COVID, IC, Flu A, Flu B, RSV (AB)
  - 6 targets: COVID, IC, Flu A, Flu B, RSV (AB), host target

* • **Issue: Fluorescence bleed through <u>Impacts Test Specificity</u>**
- **Current Limitation:**
  - Max out at 5-6 targets
  - Multi-well or extract once amplify many
- **Current Fluor Limitations:**
  - Max out at 5-6 targets
  - Alternative: Multi-well or extract once amplify many

* • **Co-positive samples: Potential for suppression of signal of minor viral population by major population?**
- **<u>Impacts Test Spensitivity</u>**

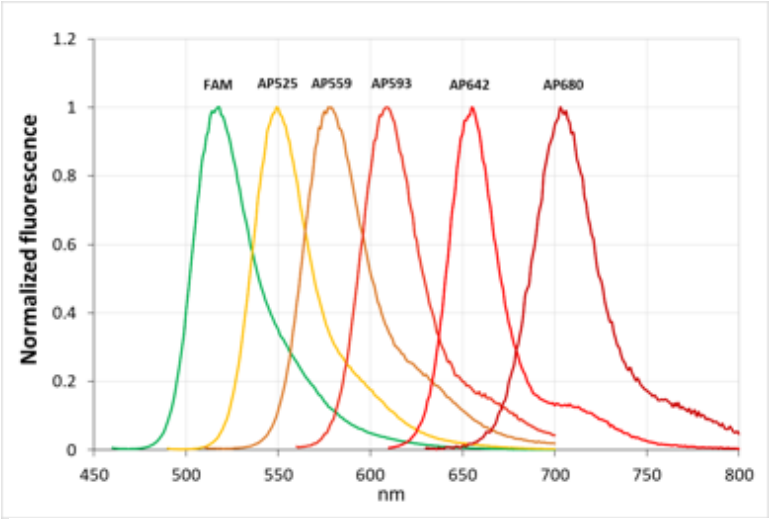


Image courtesy Walt Mahoney Elitec Group

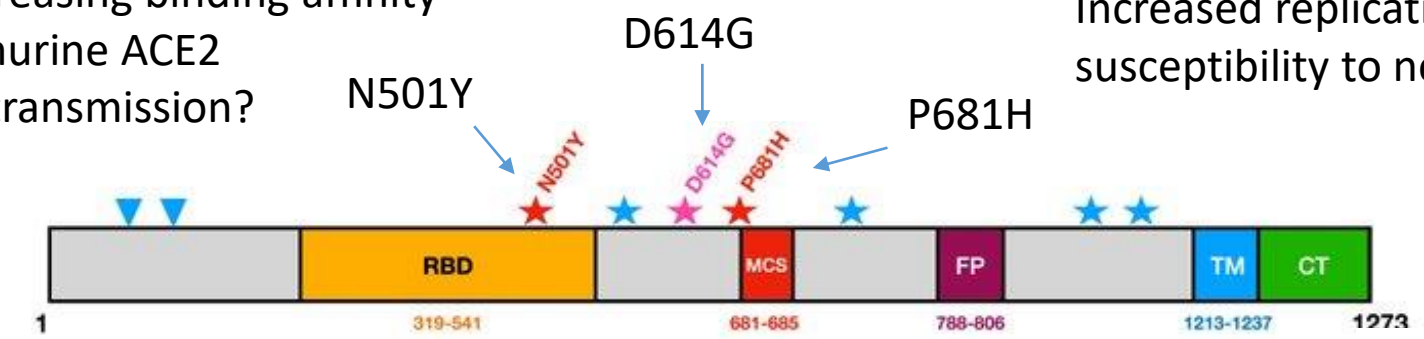
ARUP study:Roche, Hologic, and Chromacode assays are resilient to bleed through and suppression!

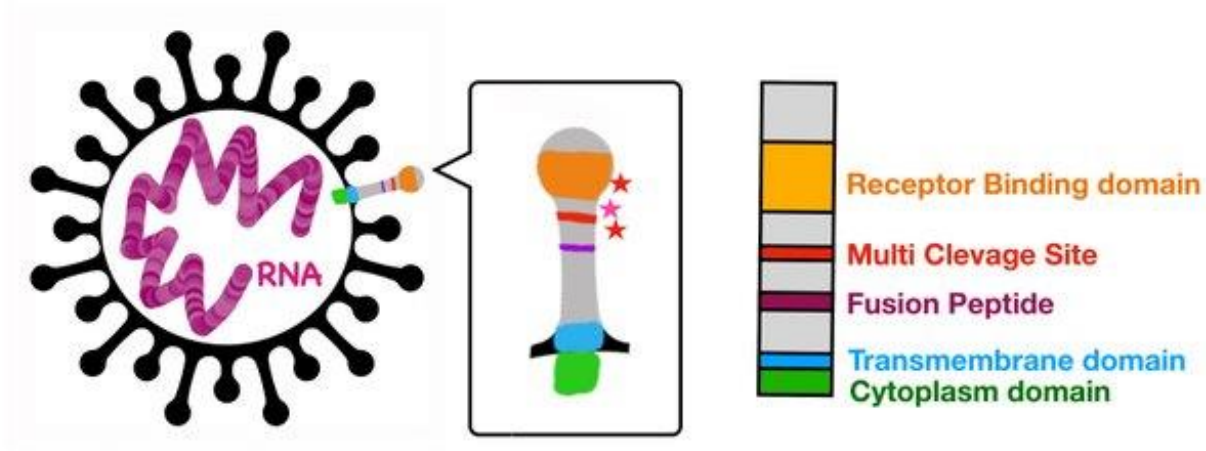
COVID-19 Mutations and Their Consequences

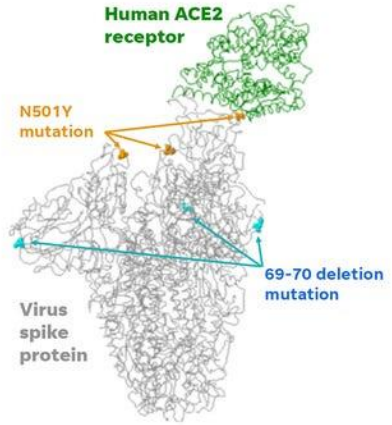
One of six key contact residues
within the receptor-binding domain (RBD)
Identified as increasing binding affinity
to human and murine ACE2
Impact on viral transmission?

Increase infectivity with
greater viral replication
lung and airway tissue

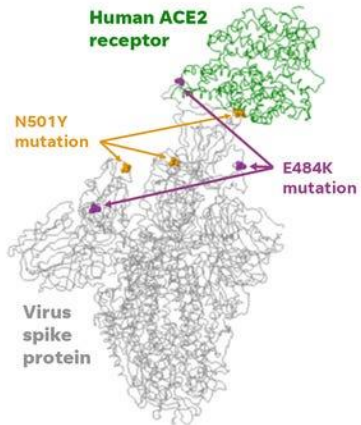
Adjacent to the furin cleavage site.
Increased replication and decreased
susceptibility to neutralization







The U.K. variant



The South Africa variant

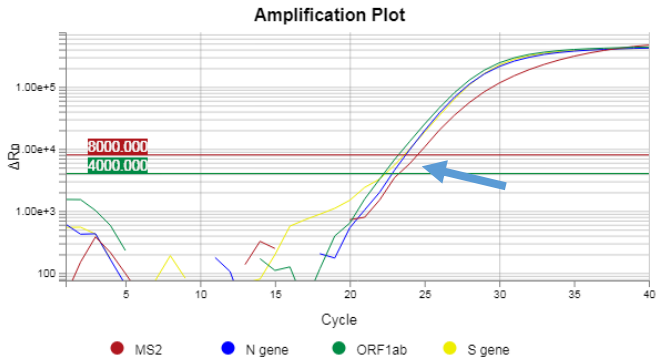
@ceronlab
@raticosdefutbol

Nature | Vol 585 | 10 September 2020 | Corrected 16 September

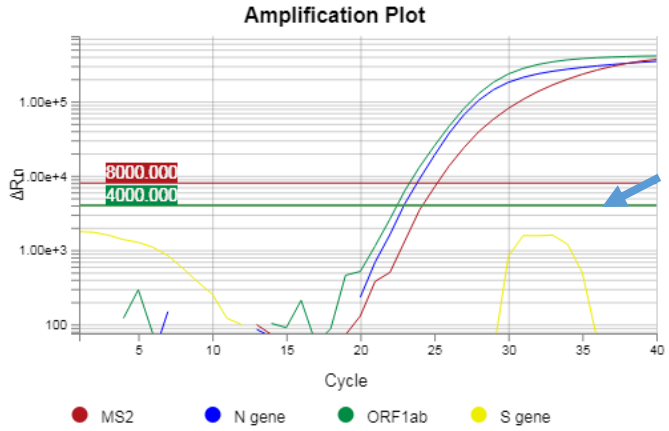
COVID-19 Mutations and Timeline

- D614G variant
  - Emerged in late Jan. or early Feb. 2020
  - Replaced the initial SARS-CoV-2 strain identified in China
  - Increased infectivity and transmission
- “Denmark Mink” variant
  - Emerged mink farm sector June 2020
  - Variant “cluster 5” Nov 5 in 12 human cases with new mutations.
  - Worry of reduced viral neutralization and vaccine effectiveness not confirmed
- VOC 202012/01, lineage B.1.1.7, “UK” variant
  - Reported Kent England Sept 2020
  - Spread rapidly to be dominant English strain
  - Clear capacity to spread more quickly
  - Multiple mutations including D614G, P681H and deletion 69-70.
- 501Y.V2 lineage B.351 variant “South Africa”
  - Reported Dec 2020 with combination of mutations
  - Worry more rapid spreading, vaccine resistant

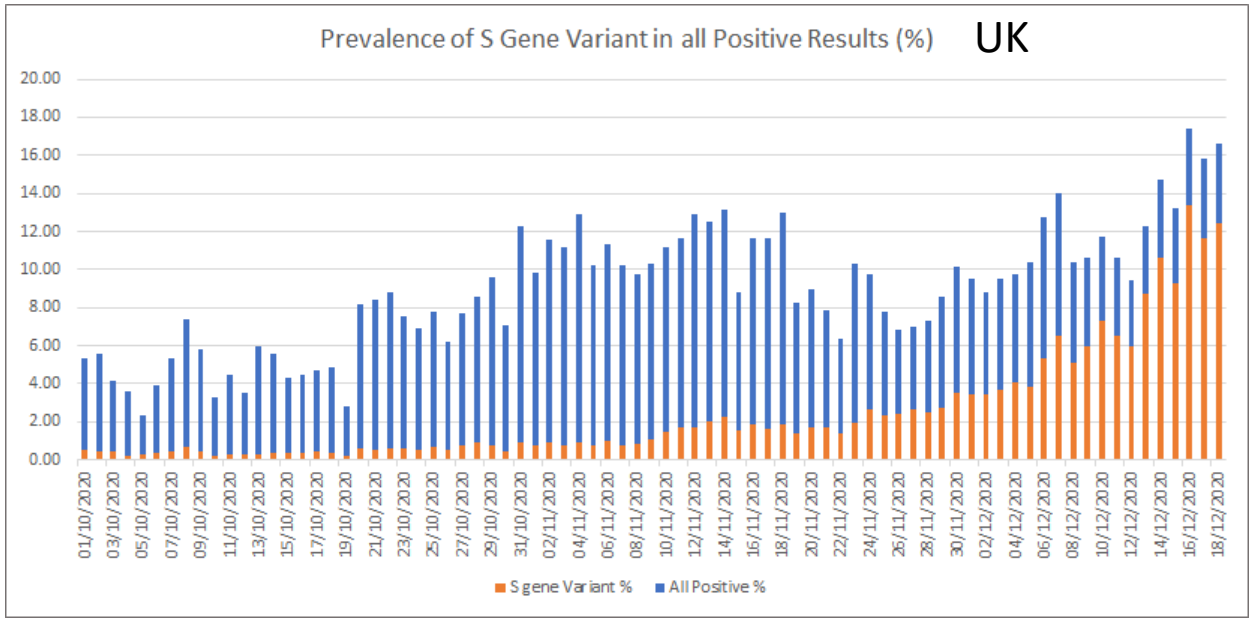
Identification and Tracking "UK" Variant: Screen for S Target Drop Outs

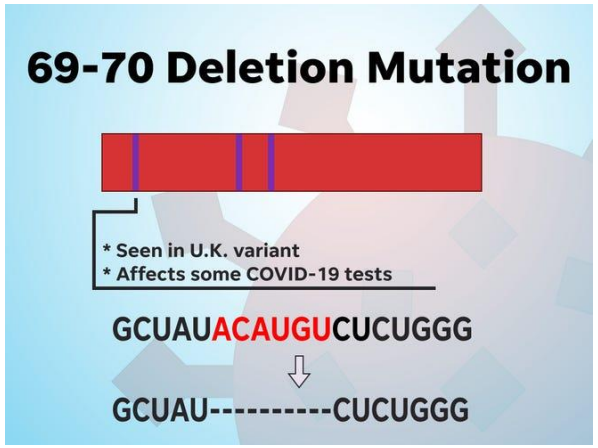


Normal S Target Amplification

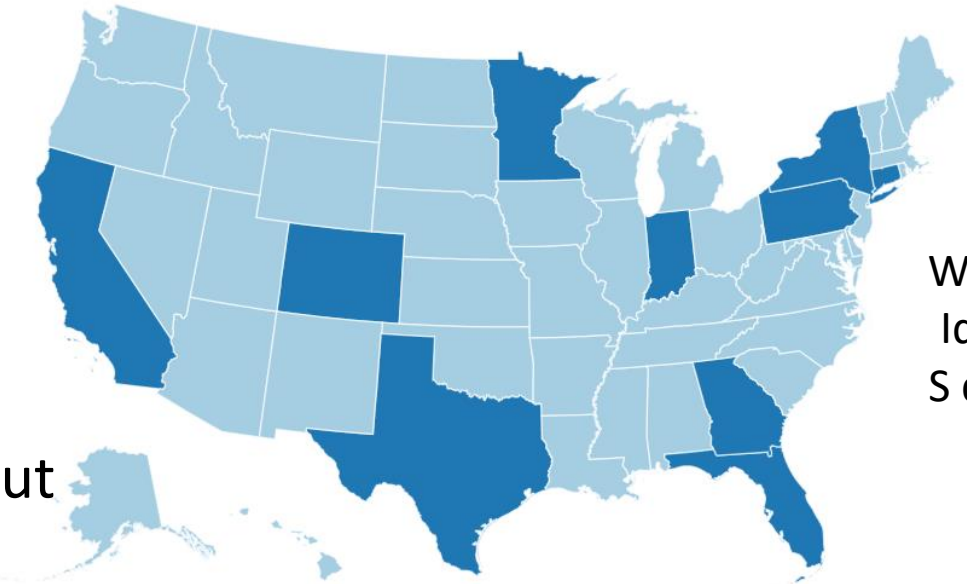


S Target Drop Out





Deletion under S Target Probe Leads to S drop out



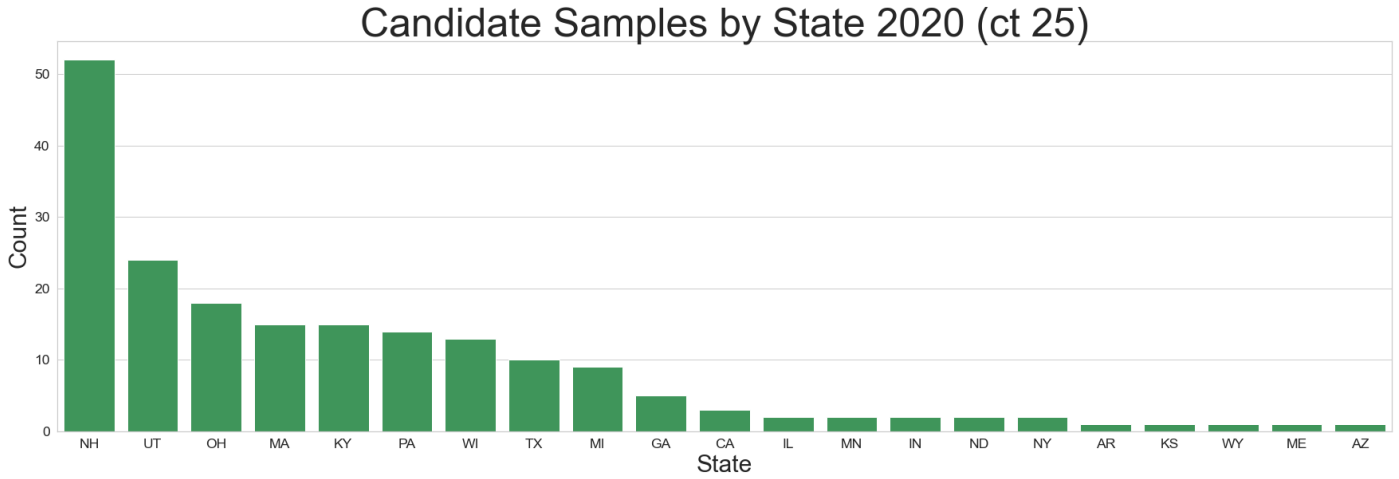
Wyoming UK Variant Identified by ARUP S drop out screening and sequencing

https://www.cdc.gov/coronavirus/2019-ncov/transmission/variant-cases.html

Issues “UK” Variant screening by S Target Drop Out Identification

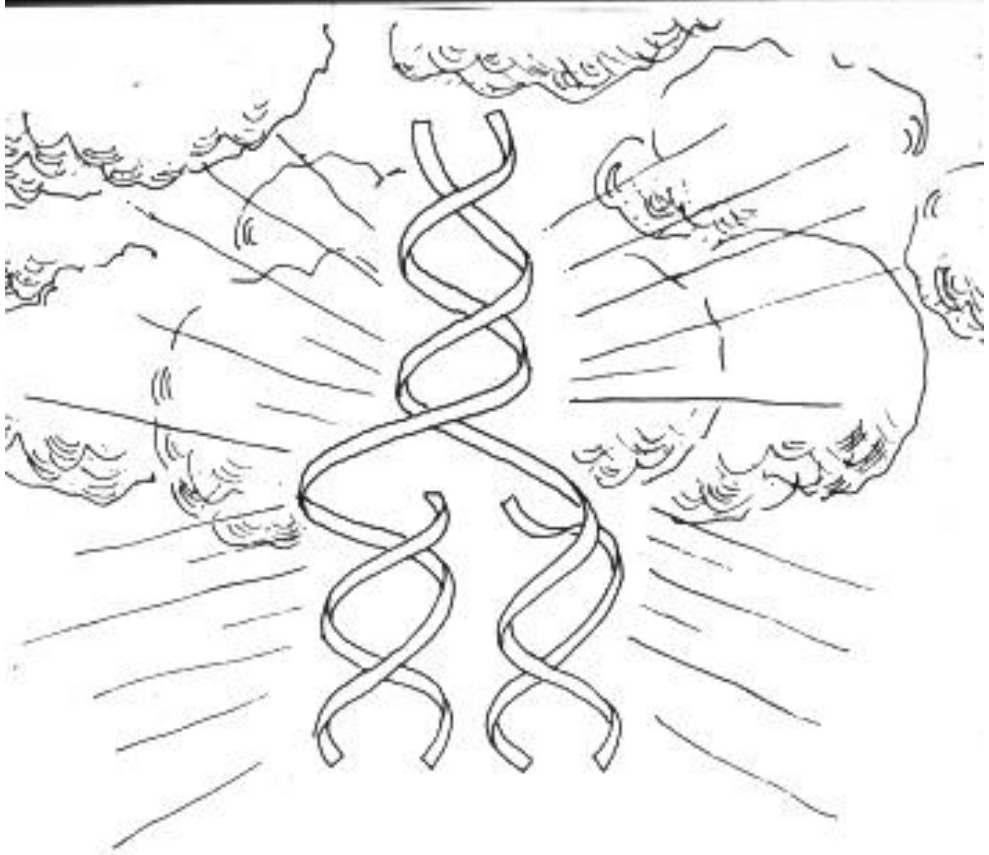
- Most tests don’t signal S target drop out.
  - Will current tests be modified to avoid drop out?
- S target drop out requires confirmatory identification by sequencing
  - Low prevalence population presents high burden for sequencing
  - U.S. COVID sequencing capacity is lacking (compare to UK)
- Solutions
  - Expand sequencing capacities (federal, academic, commercial)
  - Engage labs with high “sequence to diagnosis” capacities (Ginkgo model)
  - Validate Multiplex mutation detection assays

>5,000 Positive Samples screened for S drop out
~ 150 drop outs identified and sequenced
Jan 8-15
4 UK variants identified



Unmet Needs in Testing

- Super NAT Tests
- Home sample collection conveniently and safely linked to testing
- Affordable and scalable sequencing
- Better informatics for communicating with test populations



ARUP, University of Utah, and Utah State Teams