Emergence of SARS-CoV-2 and the Laboratory Response to the COVID-19 Pandemic

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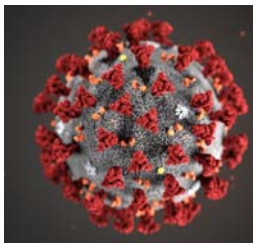
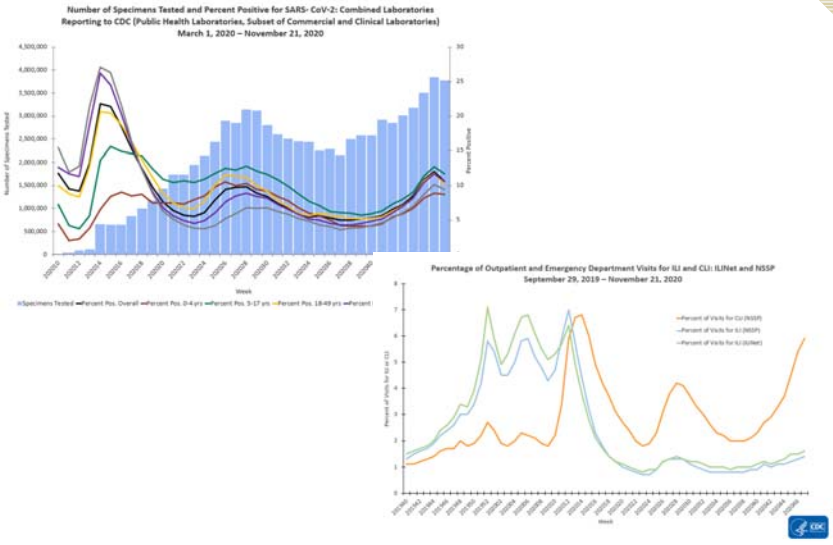


Outline

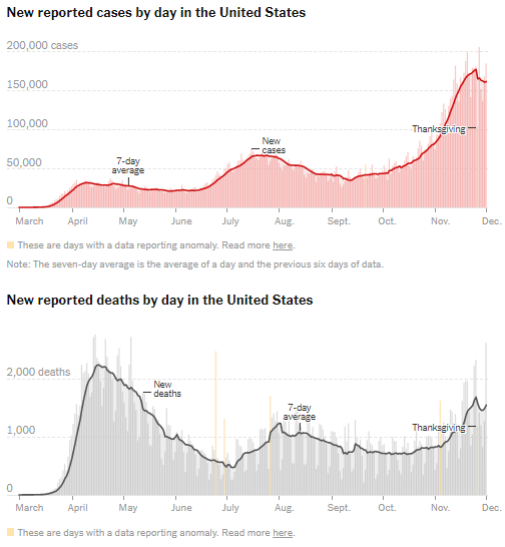
- Useful data dashboards and other resources
- Early days of the pandemic
  - Lab methods used to discover SARS-CoV-2
- Building and maintaining molecular diagnostic capacity
- Serology testing and sero-surveys
- Antigen tests

COVIDView



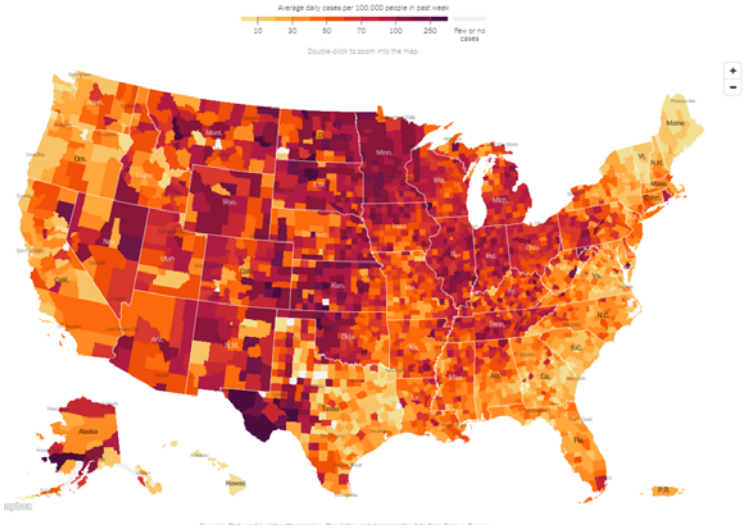
https://www.cdc.gov/coronavirus/2019-ncov/covid-data/covidview/index.html

NY Times website



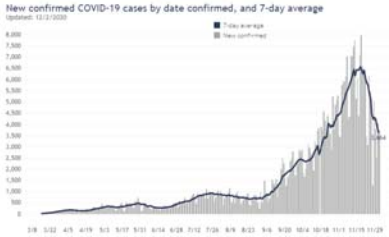
https://www.nytimes.com/interactive/2020/us/coronavirus-us-cases.html

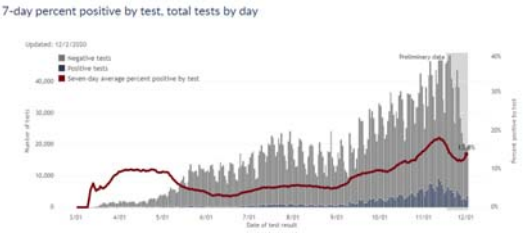
NY Times website



https://www.nytimes.com/interactive/2020/us/coronavirus-us-cases.html

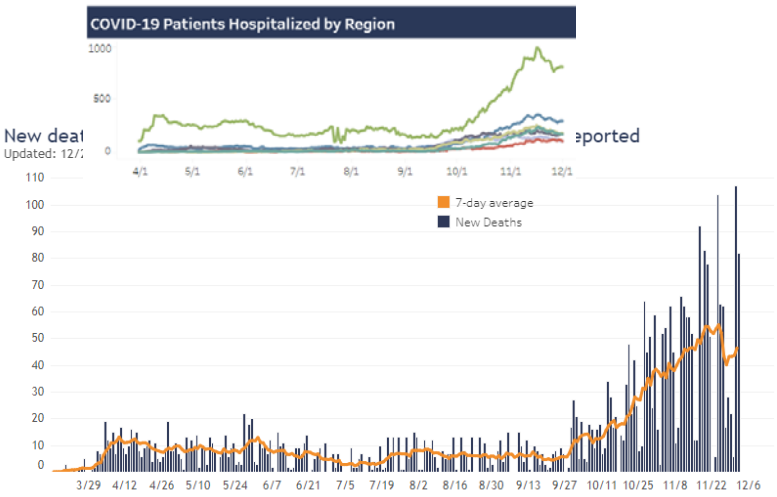
WI DHS





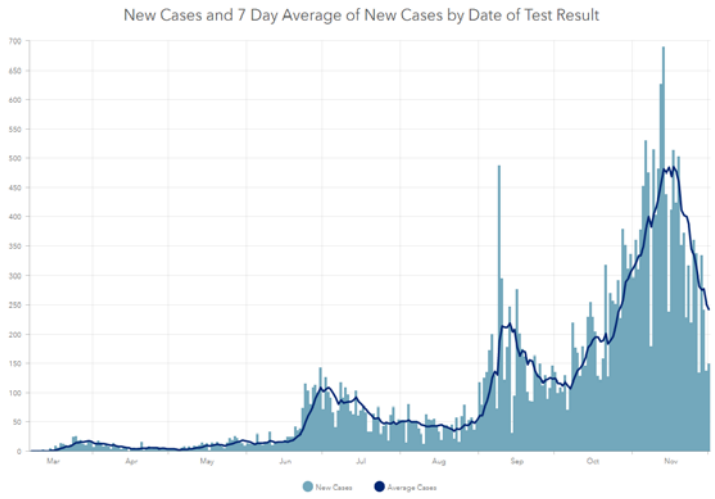
https://www.dhs.wisconsin.gov/covid-19/data.htm

WI DHS



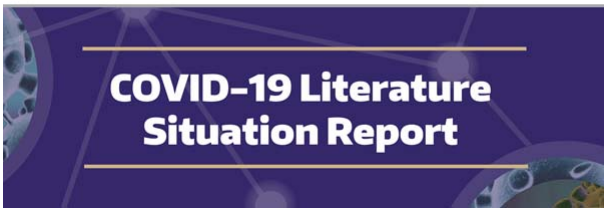
https://www.dhs.wisconsin.gov/covid-19/data.htm

Public Health Madison Dane Co.



https://publichealthmdc.com/coronavirus/data

Univ. of Wash. lit review



https://globalhealth.washington.edu/subscribe

Ct Values

APHL
www.aphl.org

https://www.aphl.org/programs/preparedness/Crisis-Management/Documents/APHL-COVID19-Ct-Values.pdf

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™
LABORATORIES

Frequently Asked Questions about Coronavirus (COVID-19) for Laboratories

https://www.cdc.gov/coronavirus/2019-ncov/lab/faqs.html

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Early Days of the Pandemic: Case study

- 71 yr old male
- Visits clinic with fever and cough
  - Clinician suspects influenza, but test is negative
  - Patient leaves to home care
- 2 days later presents to Emergency Department
  - Still has fever and cough, now difficulty breathing
  - Abnormal chest x-ray
  - Admitted to the hospital
  - All infectious disease tests are negative
  - Infectious Disease physician consulted

Case, continued

- ID doc has seen 3 other similar patients recently
  - All over 70, with underlying conditions
  - Present with pneumonia, but all tests negative
- What does the ID doc do?
  - Consult colleagues
  - Contact public health
    - pneumonia of unknown etiology

ProMED
INTERNATIONAL SOCIETY FOR INFECTIOUS DISEASES

Published Date: 2019-12-30 23:59:00
Subject: PRO/AH/EDR> Undiagnosed pneumonia - China (HU): RFI

Patients with unknown cause of pneumonia in Wuhan have been isolated from multiple hospitals
---
Whether or not it is SARS has not yet been clarified, and citizens need not panic.

On [31 Dec 2019], various hospitals in Wuhan held an emergency symposium on the topic of the treatment of patients with pneumonia of unknown cause in some medical institutions.

Public health's response

- Public health epidemiologists interview patients to find risk factors
  - Seafood/live animal market a common exposure
- Laboratorians work to figure out etiology
  - One approach; cell culture to grow viruses

Cell culture/Virus culture

- Cell lines grown in plastic flasks
- Cells fed with media on top of them
- Add specimen to cells, wait for CPE
  - Virus growth kills cells, and dead vs. live cells can be seen in microscope
- Try manydifferent cell types
  - Certain viruses only grow in certain cell types
- Virus culture used to be a common diagnostic method for viruses; now it's primarily used for research (and finding novel viruses)

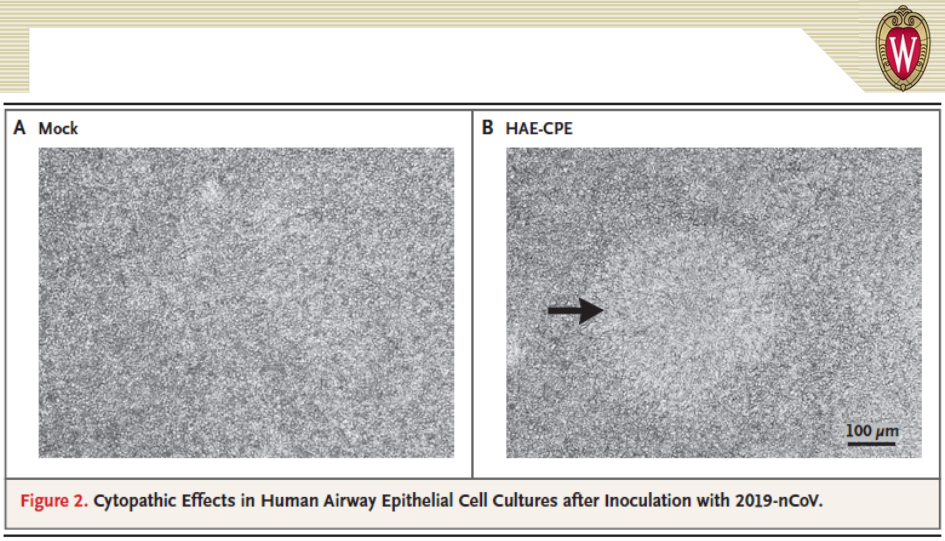


Figure 2. Cytopathic Effects in Human Airway Epithelial Cell Cultures after Inoculation with 2019-nCoV.

- Air-liquid interface cell cultures
- One virus particle first, and CPE spread outward with virus spread

What next?

- Something is causing cell death: how to figure out what it is?
- Most diagnostic tests are very specific, and only look for a single pathogen
- Unbiased pathogen detection:
  - Next-generation sequencing

ProMED
INTERNATIONAL SOCIETY
FOR INFECTIOUS DISEASES

Published Date: 2020-01-05 18:15:37
Subject: PRO/AH/EDR> Undiagnosed pneumonia - China (HU) (03): updates, SARS, MERS ruled out, WHO, RFI

ProMED
INTERNATIONAL SOCIETY
FOR INFECTIOUS DISEASES

Published Date: 2020-01-08 23:19:25
Subject: PRO/AH/EDR> Undiagnosed pneumonia - China (HU) (07): official confirmation of novel coronavirus
Archive Number: 20200108.6878869

How did they know it was a new coronavirus?

ProMED
INTERNATIONAL SOCIETY
FOR INFECTIOUS DISEASES

Published Date: 2020-01-11 12:25:54
Subject: PRO/AH/EDR> Undiagnosed pneumonia - China (HU) (10): genome available, Hong Kong surveill.

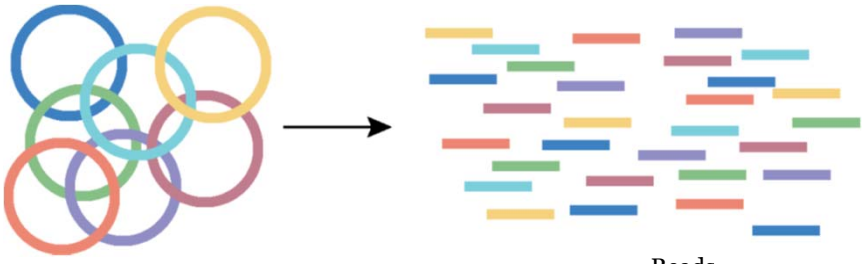
- Next-generation sequencing
  - Massively parallel sequencing


https://www.illumina.com

Subsets of Next-generation sequencing

- Whole Genome Sequencing (WGS) from a bacterial isolate
- Targeted amplicon
  - one gene or region of interest
- Metagenomics
  - sequence everything that's in a sample

Metagenomics



Bacterial genomes,
Human DNA,
Human RNA,
Virus DNA/RNA

Reads

Metagenomics

- Millions of reads per specimen
- For each read, use huge databases to determine:
  - Is it a human sequence?
    - If Y, discard
  - Is it a known bacterial/viral/plant/other sequence?
    - If Y, discard
  - What else is left?
    - Reads that don't match any known sequences
    - Reads that match sequences only ok
      - Not exact matches, but close to other coronaviruses
    - From these reads, can we reconstruct the genome?

Sequence Assembly

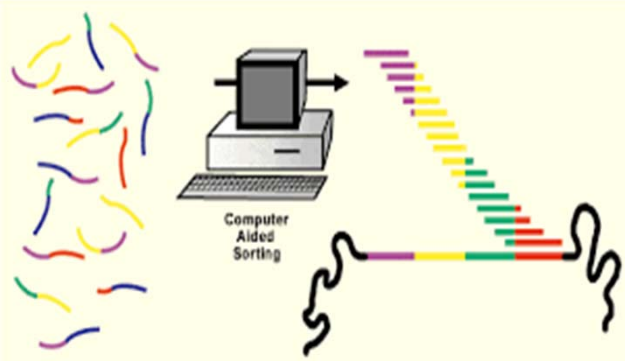


Fig 2: Short fragments of DNA sequence are ordered by overlapping data to recreate the whole genome sequence

- Overlapping data to reconstruct the entire viral genome (>29,000bp)!

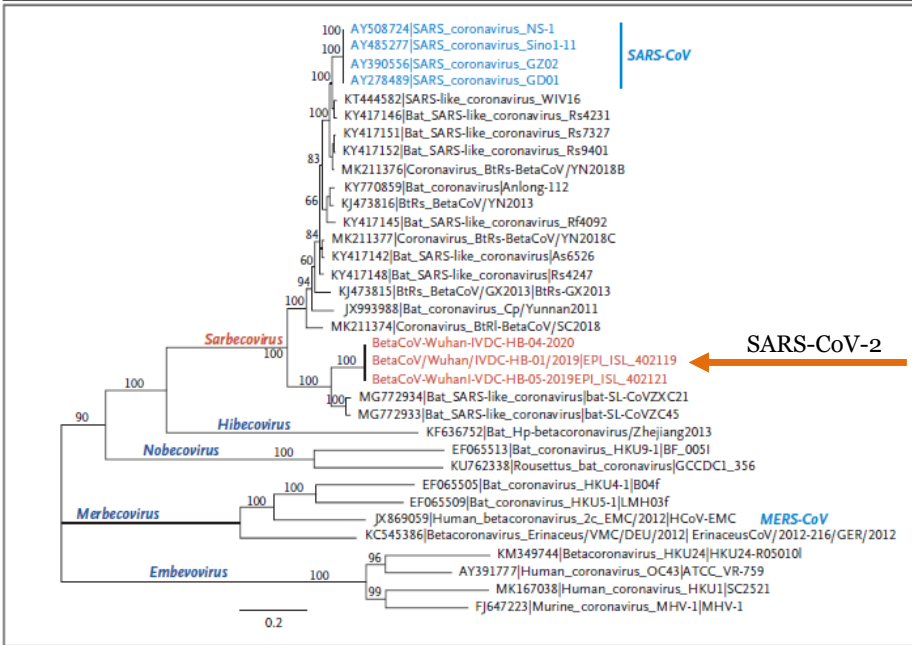


Figure 4. Phylogenetic Analysis of 2019-nCoV and Other Betacoronavirus Genomes in the Orthocoronavirinae Subfamily.

Why is the genome so powerful?

- Compare to other CoV to guess where it originated (bats?)
- Compare multiple SARS-CoV-2 sequences (NextStrain)
- The first step to develop diagnostics
  - Too expensive and slow to do next-generation sequencing on everything
  - Compare to other CoV to look for unique sections in SARS-CoV-2
  - Develop real-time PCR for diagnosis

Real-time PCR

- Diagnostic workhorse
  - Virology, bacteriology, TB, etc, etc, etc
- Two main steps
  - Nucleic acid (DNA, RNA) extraction
  - Nucleic acid amplification
- Sensitive, specific, and fast

Case 1: December 15, 2019

- 71 yr old male
- Visits clinic with fever and cough
  - Clinician suspects influenza, but test is negative
  - While SARS-CoV-2 is being discovered in the lab (weeks for discovery and characterization), patient transmits virus to many others

Case 2: January 25, 2020

- 71 yr old male
- Visits clinic with fever and cough
  - Clinician suspects influenza, but test is negative
  - Clinician also suspects novel coronavirus
    - Immediately isolates patient (in hospital room or at home)
    - Sends specimen to lab
  - Real-time PCR test is positive the next day
  - Few to no exposure to other people = stops transmission

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Steps in laboratory testing for emerging pathogens

	Approx. daily capacity (nationwide)
▪ CDC develops a test	350
▪ CDC rolls out the test to public health labs ▪ WSLH ▪ Milwaukee City Lab	15,000
▪ Large clinical labs bring on testing ▪ Wisconsin labs ▪ National reference labs	>300,000
▪ Commercial manufacturers get approved tests ▪ Small and medium-sized labs can test	>500,000

Overview of SARS-CoV-2 PCR at WSLH

- Officially started testing on March 2nd
- All-of-division response to meet the exploding demand
- Issues with supply chain threatened to shut down testing
- Strategy for testing diversification (validated 6 different extraction platforms in two weeks) allowed us to source multiple supply lines. Luckily, we never went down.
- Tested over 75,000 specimens at WSLH to date
- Continuing other mission critical work, new hires

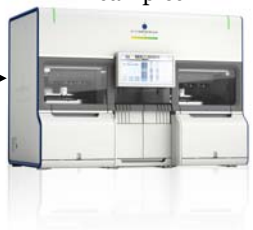
Testing Advancements at WSLH: RNA extraction

EZ1 - 14 samples

Spin column – low volume

Magna Pure LC - 24 samples

eMAG - 48 samples



QIAcube HT - 96 samples

KingFisher Flex - 96 samples

Testing Advancements at WSLH: PCR

CDC 2019-nCoV
- 29 samples/plate

→

ThermoFisher TaqPath
- 94 samples/plate

+

CDC FluSC2 multiplex
- 94 samples/plate

Current Testing

KingFisher Flex + ABI 7500 Fast Dx

x3  +  x11

Hologic Panther-
300-500/day

x2

Building Capacity Statewide – Wisconsin Clinical Laboratory Network

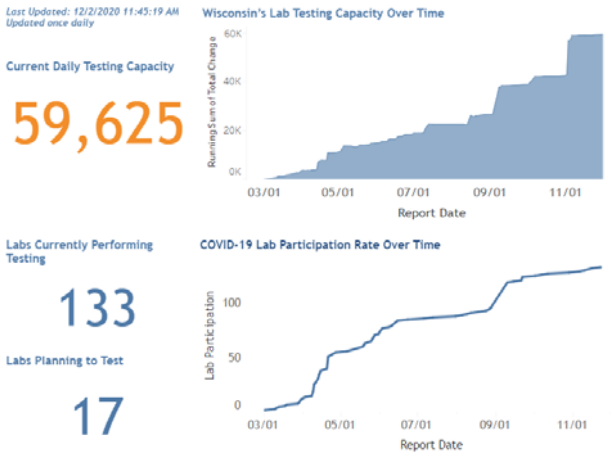
Thanks for all that you have done!!

How WSLH has assisted:

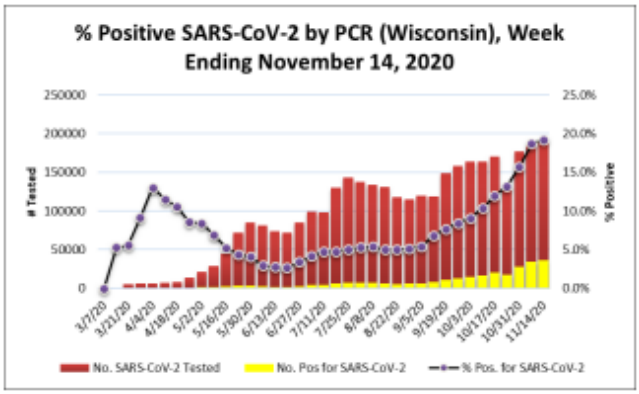
- >100 Validation Panels
- ~20 Webinars
- >30 Lab Messages
- Maintained listserv
- Technical Consultants

Leveraged and strengthened the WCLN through SARS-CoV-2 pandemic response

Statewide Estimated Daily Test Capacity

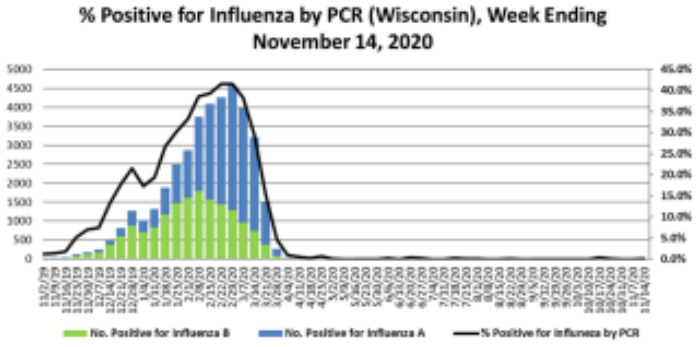


Testing Increase in Wisconsin



WSLH, Wisconsin Laboratory Surveillance Report dated Nov 24.

Influenza virtually absent



WSLH, Wisconsin Laboratory Surveillance Report

Week Ending November 14, 2020*

Resp. Pathogen PCR	# Tested	% Positive
SARS-CoV-2	189,764	19.2↑
Rhinovirus/ Entero-virus	846	6.0 ↓
Influenza	6,823	<1
RSV	1,073	0
Human metapneu-movirus	910	0
Adenovirus	40	0
Parainfluenza	902	0
Seasonal corona-viruses	40	0
B. pertussis	226	<1

Maintaining PCR Capacity

- Continuous supply chain and logistics issues
  - Swabs
  - Transport media
  - Extraction reagents
  - N95s
  - Gloves
  - Plastic pipet tips

- Exchanges with clinical labs to keep all labs running

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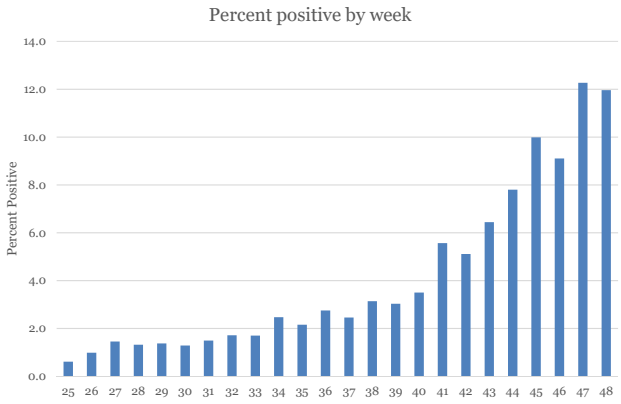
Serology at WSLH – SARS-CoV-2 IgG

show survey of the health of Wisconsin

Red Cross Serology Data

American Red Cross donations from healthy donors that screened reactive for antibodies to SARS-CoV-2 by Ortho total immunoglobulin test



Serology at WSLH – SARS-CoV-2 IgG

Strategies

- ~1000 samples/quarter with SHOW
- Inform specific outbreaks
- Collaborate with CDC/DHS/UW
  - UW-Madison and UW-Oshkosh studies

Abbott Architect

Morbidity and Mortality Weekly Report (*MMWR*)

CDC

COVID-19 Outbreak at an Overnight Summer School Retreat — Wisconsin, July–August 2020

Weekly / October 30, 2020 / 69(43);1600–1604

Ian W. Pray, PhD[1,2]; Suzanne N. Gibbons-Burgener, DVM, PhD[1]; Avi Z. Rosenberg, MD, PhD[3]; Devlin Cole, MD[1,4]; Shmuel Borenstein[5]; Allen Bateman, PhD[6]; Eric Pevzner, PhD[7]; Ryan P. Westergaard, MD, PhD[1,4] (View author affiliations)

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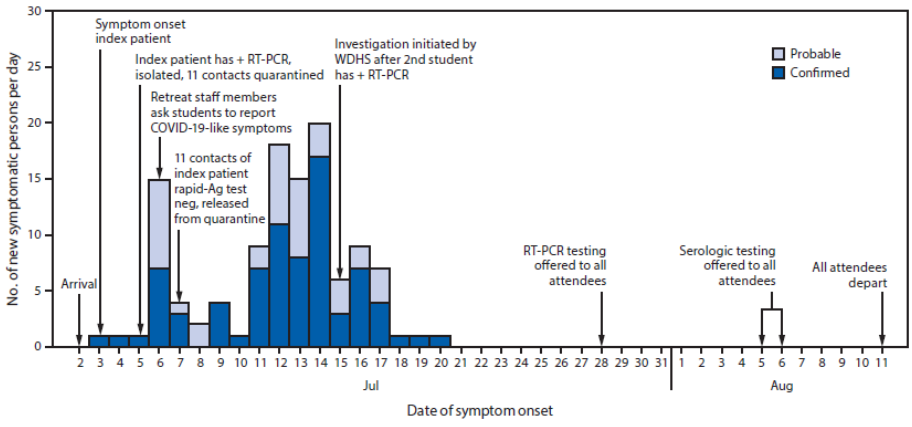
- July-August, 152 high school boys/counselors/staff from 21 states had a staff retreat in Wisconsin
- Prior to arrival, all attendees had either:
  - Positive serologic test OR
  - Negative PCR within 7 days
- After arrival, no physical distancing or masking
- Many students symptomatic 2 weeks after arrival; two PCR positive for SARS-CoV-2
- PCR testing of everyone: 78 (51%) PCR positive
- In addition, 38 (25%) met clinical criteria for COVID-19.
- End of retreat, IgG conducted at WSLH: 118 (78%) IgG positive

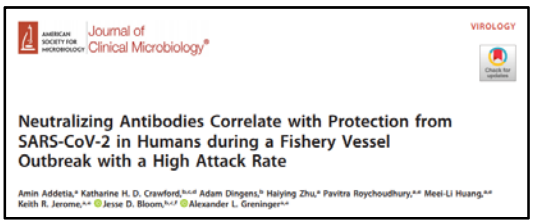
FIGURE. Dates of symptom onset of confirmed (n = 78) and probable (n = 38) COVID-19 cases at an overnight summer school retreat — Wisconsin, July 2–August 11, 2020



- WGS showed highly related virus strains
- Large outbreak likely from a single student; emphasizes mitigation measures need to be maintained throughout retreats/camps

- 24 (16%) attendees provided documentation of a positive serology results before the retreat. All 24 received negative RT-PCR results. Six (25%) experienced mild symptoms at the retreat but were not classified as having confirmed or probable COVID-19.
- Excluding the 24 attendees with previous positive serologic results, the COVID-19 attack rate on the remaining susceptible population was 91% (116 of 128).

Real-world data of prior infection/antibodies being protective

1. Summer retreat outbreak
2. Longitudinal study at UW
3. 

   - Fishing vessel outbreak with 85% attack rate (104/122)
   - Only 3 had prior neutralizing antibodies; none tested positive or had any symptoms
   - Presence of neutralizing antibodies from prior infection was associated with protection against reinfection

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Antigen tests

- Diagnostic, to identify acute infection
- Identify the virus
- Targets a virus protein
- Sample-to-answer tests
- Rapid (~15 min)
- Less sensitive than PCR

Antigen tests in the news

FOR IMMEDIATE RELEASE
July 14, 2020

Contact: HHS Press Office
202-205-0143
ashmedia@hhs.gov

Trump Administration Announces Initiative for More and Faster COVID-19 Testing in Nursing Homes

- Rapid point-of-care diagnostic test instruments and tests will be distributed to nursing homes in COVID-19 hotspot geographic areas with the United States.
- Scale-up to most (almost all?) nursing homes in the country
- A one-time procurement of devices and tests targeted to facilitate on-site testing among nursing home residents, staff, and visitors.

https://www.hhs.gov/about/news/2020/07/14/trump-administration-announces-initiative-more-faster-covid-19-testing-nursing-homes.html

Antigen tests in the news

Governor Phil Scott
@GovPhilScott

As @healthvermont Commissioner Mark Levine, M.D., reported today — in response to the reports of 65 positive antigen tests from a private facility in Manchester — 1,613 people were tested through various sites in the area last week by the state. All but 5 were negative. 1/3

7:10 PM · Jul 21, 2020 · Twitter for iPhone

4 Retweets and comments **22** Likes

Governor Phil Scott @GovPhilScott · Jul 21
Replying to @GovPhilScott
A PCR test, which is recommended to confirm the antigen result, has be conducted for 52 of the 65 individuals so far.

Of those 52 people, 48 tested negative. Four people tested positive and are considered confirmed COVID-19 cases. 2/3

3 11

Governor Phil Scott @GovPhilScott · Jul 21
The Health Department is working with the Centers for Disease Control and Prevention about the tests and our investigation to help determine why there were so many false-positives at this non-Health Department facility, which used antigen tests. 3/3

Ohio Gov. Mike DeWine tests negative for coronavirus after earlier testing positive

DeWine announced late Thursday that he tested negative for the coronavirus after sharing earlier in the day that he had tested positive.

The governor's initial screening in Cleveland, ahead of his planned meeting with President Trump, was an antigen test. The test he took later in the day was done via the polymerase chain reaction method, which is used more commonly than the screening required before having contact with Trump.

Antigen tests in the news

Nevada Department of Health and Human Services
DIVISION OF PUBLIC AND BEHAVIORAL HEALTH

Technical Bulletin

Date: October 2, 2020
Topic: Discontinue the Use of Antigen Testing in Skilled Nursing Facilities Until Further Notice
Contact: Melissa Peek-Bullock, State Epidemiologist, Office of Public Health Investigations and Epidemiology
To: Health Care Providers and Long-Term Care Facilities

FDA U.S. FOOD & DRUG ADMINISTRATION

Potential for False Positive Results with Antigen Tests for Rapid Detection of SARS-CoV-2 - Letter to Clinical Laboratory Staff and Health Care Providers

Urgent need to evaluate SARS-CoV-2 antigen tests!

Sofia vs. Hologic TMA

JCM Accepted Manuscript Posted Online 25 November 2020
J Clin Microbiol doi:10.1128/JCM.02727-20

1 TITLE: Comparison of Quidel Sofia SARS FIA Test to Hologic Aptima SARS-CoV-2 TMA Test
2 for Diagnosis of COVID-19 in Symptomatic Outpatients
3
4 RUNNING TITLE: Sofia FIA vs. Aptima TMA for Diagnosis of COVID-19
5
6 AUTHORS: Eric T. Beck[1,2,*], Wendy Paar[1,2,3], Lara Fojut[2,4], Jordan Serwe[2,3], and Renee R.
7 Jahnke[2,4]

Sofia vs. Hologic TMA

- 347 symptomatic outpatients
- Nasal swab for Sofia, NP for TMA
- Sensitivity:
  - Symptomatic ≤5d from symptom onset: 82%
  - Symptomatic >5d from symptom onset: 55%
- Specificity: >99% (one false positive)

Sofia vs. PCR, CDC/DHS/UW

Acknowledgement: Ian Pray, EIS officer with CDC/DHS
Disclaimer: these are preliminary findings/recommendations and CDC will be using this and other studies to finalize guidance on Ag testing soon.

FDA Emergency Use Authorization:
- **Symptomatic patients**, within 5-7 days of onset
- Sofia accuracy: 97% sensitive; 100% specific

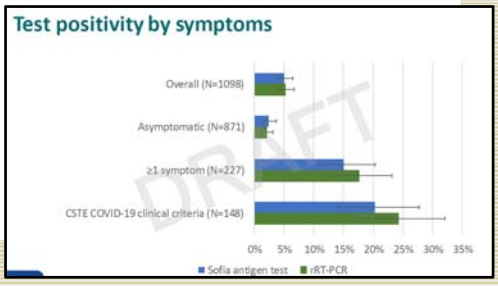
Current widespread use for **asymptomatic screening**
- College campuses
- Nursing homes
- Correctional facilities
- Community testing sites

Asymptomatic screening considered **"Off-label"** by FDA

Sofia vs. PCR, CDC/DHS/UW

Objective: Evaluate the diagnostic performance of the Sofia SARS Antigen Fluorescent Immunoassay (FIA) compared to real time RT-PCR and viral culture in asymptomatic and symptomatic persons on two university campuses

Methods:
UW-Oshkosh and UW-Madison
- September 28-October 9
- Nested in existing university testing protocols
  - –Weekly surveillance testing
  - –Quarantine testing
- Symptom questionnaire
- Paired nasal swabs (Sofia and PCR)



Sofia vs. PCR, CDC/DHS/UW

Sensitivity, specificity, PPV, and NPV of Sofia SARS Antigen FIA compared to RT-PCR

Symptomatic (≥1 symptom)			
	RT-PCR		
Antigen	Positive	Negative	Total
Positive	32	2	**34**
Negative	8	185	**193**
Total	**40**	**187**	**227**

- **Sensitivity**: **80.0%** (95% CI 64.4%-90.9%)
- **Specificity**: **98.9%** (95% CI 96.2%-99.9%)
- **PPV**: **94.1%** (95% CI 80.3%-99.3%)
- **NPV**: **95.9%** (95% CI 92.0%-98.2%)

Asymptomatic			
	RT-PCR		
Antigen	Positive	Negative	Total
Positive	7	14	**21**
Negative	10	840	**850**
Total	**17**	**854**	**871**

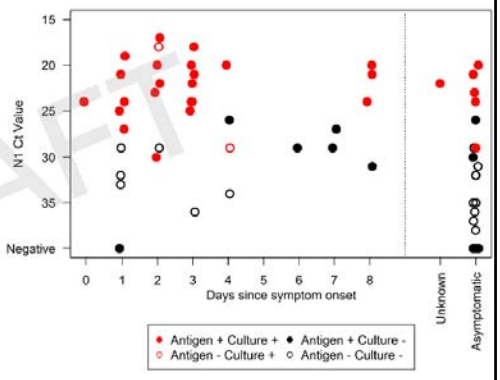
- **Sensitivity**: **41.2%** (95% CI 18.4%-67.1%)
- **Specificity**: **98.4%** (95% CI 97.3%-99.1%)
- **PPV**: **33.3%** (95% CI 14.6%-57.0%)
- **NPV**: **98.4%** (95% CI 97.8%-99.4%)

Sofia vs. PCR, CDC/DHS/UW

Viral culture and Ct values (n = 73)

False Negative Results (antigen - / PCR +)
- Higher Ct values (mean 32.2 vs. 23.5)
- Less likely to be cultured
  - 2 (11%) out of 18 culture positive

False Positive Results (antigen + / PCR -)
- 0 of 16 were culture positive
- No definitive cause identified



Sofia vs. PCR, CDC/DHS/UW

Summary of findings

- Sofia antigen test had **lower sensitivity** (80.0%) and **lower specificity** (98.9%) than reported in EUA data (96.7%; 100%) in symptomatic individuals
- For asymptomatic screening, sensitivity was **41.2%** (7/17) and positive predictive value was **33.3%** (7/21)
- Virus recovery was possible from **2 of 18 false negative** antigen results
- **Confirmatory molecular testing** should be prioritized for:
  - **Negative antigen results in symptomatic persons** when COVID-19 is suspected
  - **Positive antigen results in asymptomatic persons** where pre-test probability is low

BinaxNOW evaluation: another state PHL

- Paired BinaxNOW (nasal swab) vs CDC nCoV PCR (NP swab)
- Sensitivity
  - Symptomatic (within 7 days symptom onset): 81%
  - Symptomatic (many beyond 7 days symptom onset): 44%
  - Asymptomatic: 17%
- Specificity
  - 100%

*unpublished

BD Veritor vs. PCR

JCM Accepted Manuscript Posted Online 6 October 2020
J. Clin. Microbiol. doi:10.1128/JCM.02338-20

1 **Clinical evaluation of BD Veritor SARS-CoV-2 point-of-care test performance compared to**
2 **PCR-based testing and versus the Sofia 2 SARS Antigen point-of-care test.**
3

- Paired Veritor (nasal swab) vs Lyra real-time PCR (NP or OP swab)
- Individuals ≤7 days from symptom onset
- Veritor sensitivity:
  - 76%-88%, depending on how many days since symptom onset
- Specificity: >99%

Antigen test data thus far

- In symptomatic people within 5-7 days of symptom onset:
  - 80% sensitive, 98-99% specific
- In asymptomatic people:
  - Much lower sensitivity compared to PCR
  - Higher proportion of false positives
    - low prevalence population

Recent reasons for hope

PFIZER AND BIONTECH ANNOUNCE VACCINE CANDIDATE AGAINST COVID-19 ACHIEVED SUCCESS IN FIRST INTERIM ANALYSIS FROM PHASE 3 STUDY

Monday, November 09, 2020 - 06:45am

Moderna Announces Primary Efficacy Analysis in Phase 3 COVE Study for Its COVID-19 Vaccine Candidate and Filing Today with U.S. FDA for Emergency Use Authorization

November 30, 2020 at 6:59 AM EST PDF Version

Whole genome sequencing teaser

"What We've Learned About COVID-19 Through Genomics"
Kelsey Florek, PhD MPH
December 10, noon-1pm

Nextstrain.org

Questions?