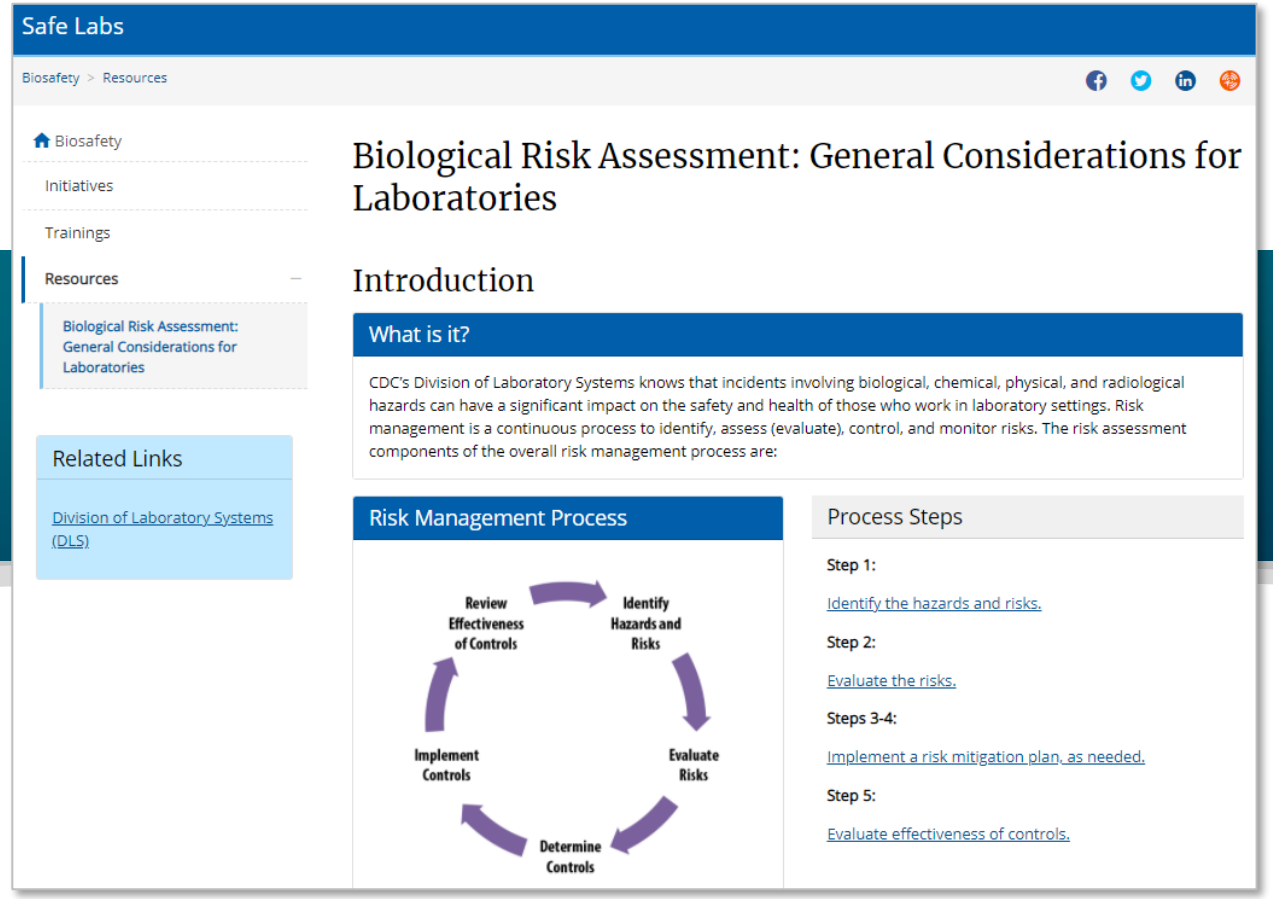
Clinical Laboratory COVID-19 Response Call

Monday, April 5, 2021 at 3:00 PM EDT

- **Welcome**
  - Nancy Anderson, Division of Laboratory Systems, CDC
- **Emergence of SARS-CoV-2 Variants and Impact on IVD Testing**
  - Richard Creager, NIH RADx Variant Task Force
- **Saliva as a Sample Type for SARS-CoV-2 Detection**
  - Anne Wyllie, Yale University
- **FDA Update**
  - Tim Stenzel, U.S. Food and Drug Administration (FDA)

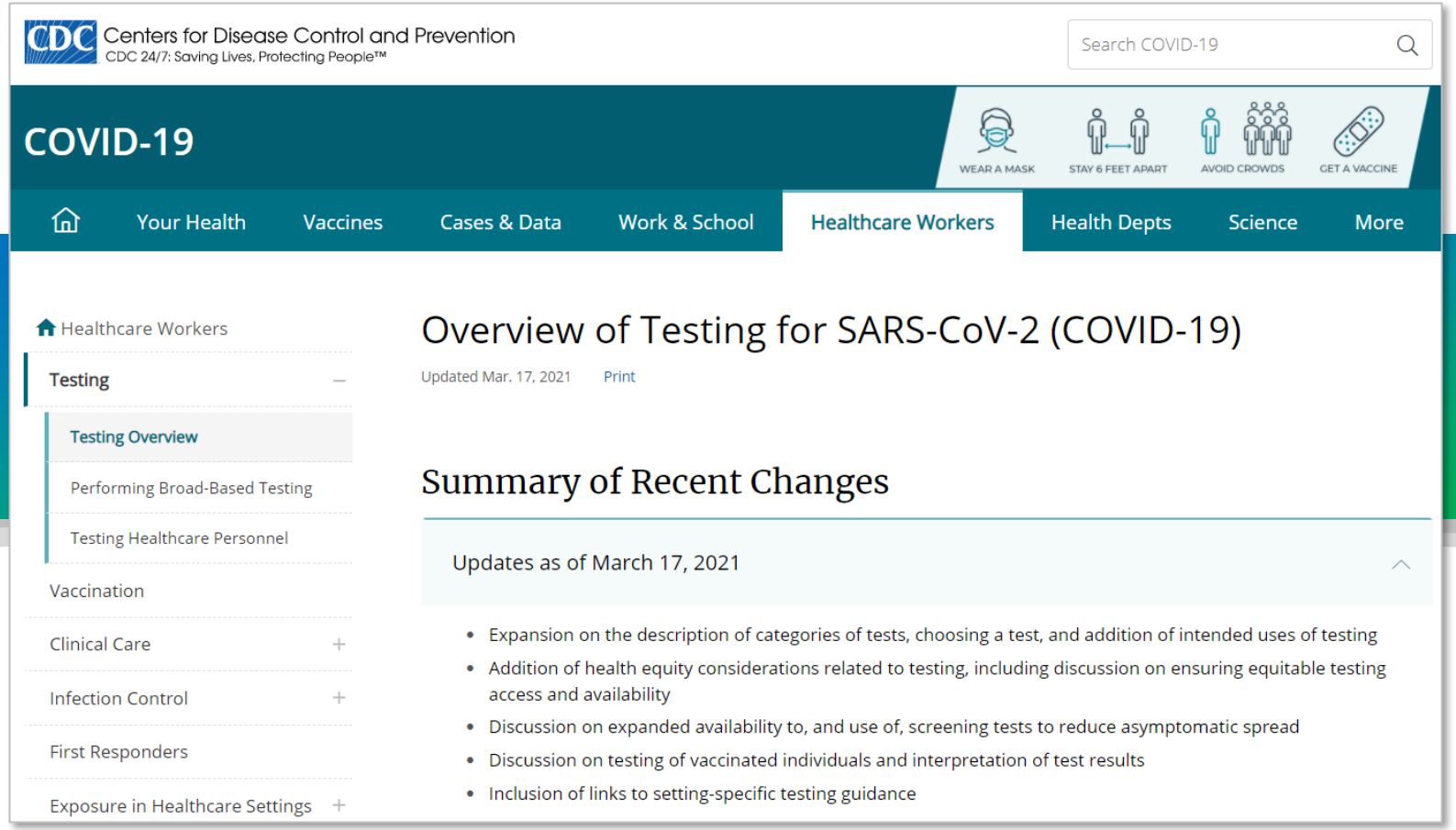
Biological Risk Assessment Webpage

https://www.cdc.gov/safelabs/resources-tools/bio-risk-assessment.html



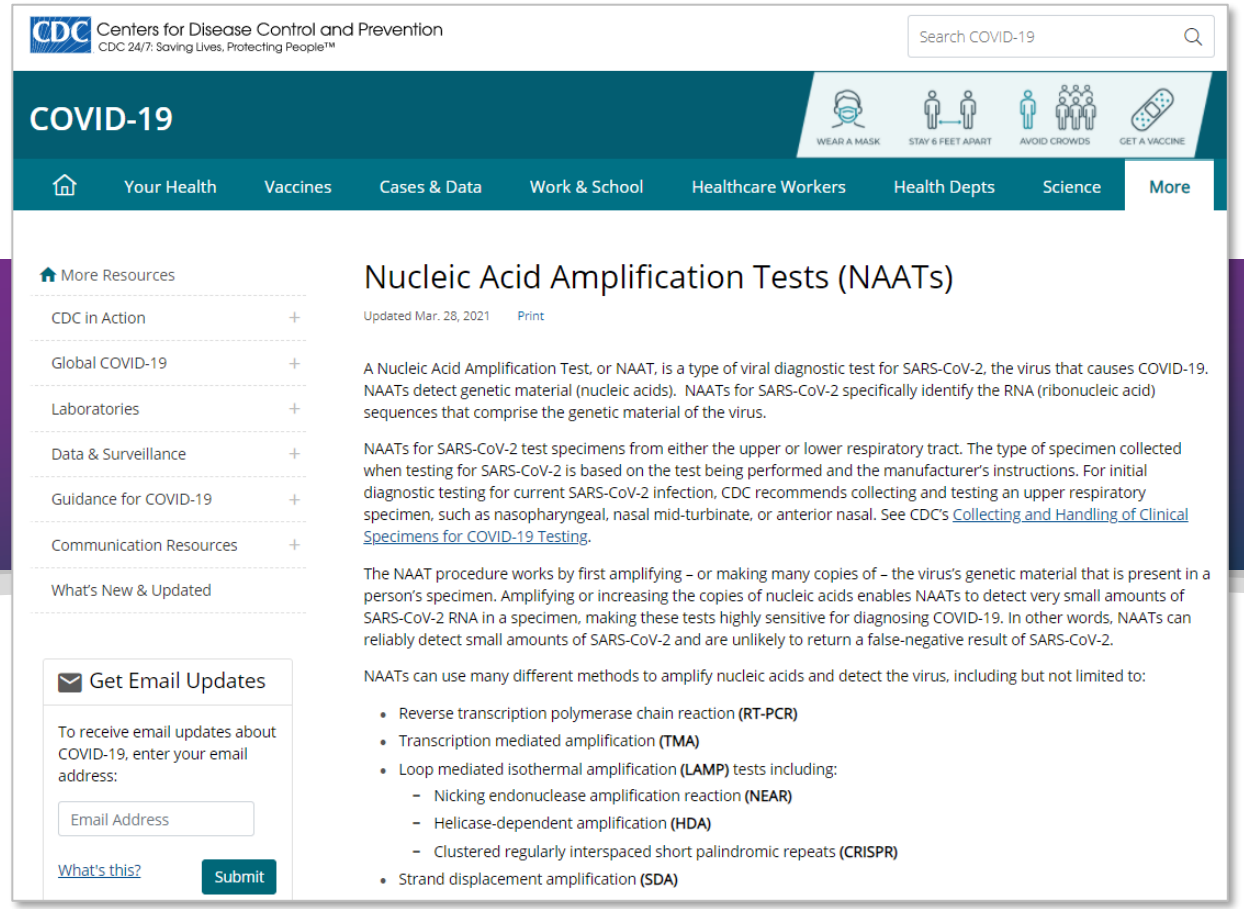
Testing Overview Update

https://www.cdc.gov/coronavirus/2019-ncov/hcp/testing-overview.html



Nucleic Acid Amplification Tests (NAATs) Webpage

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



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

Search COVID-19

COVID-19

WEAR A MASK | STAY 6 FEET APART | AVOID CROWDS | GET A VACCINE

Your Health | Vaccines | Cases & Data | Work & School | Healthcare Workers | Health Depts | Science | More

More Resources

- CDC in Action
- Global COVID-19
- Laboratories
- Data & Surveillance
- Guidance for COVID-19
- Communication Resources
- What's New & Updated

Get Email Updates

To receive email updates about COVID-19, enter your email address:

Email Address

What's this? Submit

Nucleic Acid Amplification Tests (NAATs)

Updated Mar. 28, 2021 Print

A Nucleic Acid Amplification Test, or NAAT, is a type of viral diagnostic test for SARS-CoV-2, the virus that causes COVID-19. NAATs detect genetic material (nucleic acids). NAATs for SARS-CoV-2 specifically identify the RNA (ribonucleic acid) sequences that comprise the genetic material of the virus.

NAATs for SARS-CoV-2 test specimens from either the upper or lower respiratory tract. The type of specimen collected when testing for SARS-CoV-2 is based on the test being performed and the manufacturer's instructions. For initial diagnostic testing for current SARS-CoV-2 infection, CDC recommends collecting and testing an upper respiratory specimen, such as nasopharyngeal, nasal mid-turbinate, or anterior nasal. See CDC's Collecting and Handling of Clinical Specimens for COVID-19 Testing.

The NAAT procedure works by first amplifying – or making many copies of – the virus's genetic material that is present in a person's specimen. Amplifying or increasing the copies of nucleic acids enables NAATs to detect very small amounts of SARS-CoV-2 RNA in a specimen, making these tests highly sensitive for diagnosing COVID-19. In other words, NAATs can reliably detect small amounts of SARS-CoV-2 and are unlikely to return a false-negative result of SARS-CoV-2.

NAATs can use many different methods to amplify nucleic acids and detect the virus, including but not limited to:

- Reverse transcription polymerase chain reaction **(RT-PCR)**
- Transcription mediated amplification **(TMA)**
- Loop mediated isothermal amplification **(LAMP)** tests including:
  - Nicking endonuclease amplification reaction **(NEAR)**
  - Helicase-dependent amplification **(HDA)**
  - Clustered regularly interspaced short palindromic repeats **(CRISPR)**
- Strand displacement amplification **(SDA)**

CLIA SARS-CoV-2 Variant Testing FAQ

https://www.cms.gov/files/document/clia-sars-cov-2-variant.pdf

Revised on March 19, 2021

CLIA SARS-CoV-2 Variant Testing Frequently Asked Question
Date: 3/19/2021

Does a facility that performs surveillance testing to identify SARS- CoV-2 genetic variants need a CLIA certificate?

CMS is temporarily exercising enforcement discretion under CLIA for SARS-CoV-2 genetic variant testing on identified specimens in which patient-specific results are reported to State or local Public Health Departments. As defined by Centers for Disease Control and Prevention (CDC), public health surveillance testing for SARS-CoV-2 is intended to monitor community- or population-level outbreaks of disease, or to characterize the incidence and prevalence of disease. Public health surveillance testing is performed on de-identified specimens, and thus results are not linked to individuals. Public health surveillance testing cannot be used for individual decision-making. See CDC's Testing Strategies for SARS-CoV-2 (Frequently Asked Questions about Coronavirus (COVID-19) for Laboratories).

Generally, surveillance testing using sequencing technology to identify SARS-CoV-2 genetic variants can be performed in a facility that is NOT CLIA certified, provided that patient-specific results are **not** reported to (1) the individual who was tested or (2) their health care provider. If at any time a facility intends to perform testing on identified specimens and report a patient-specific SARS-CoV-2 genetic variant test result to the individual who was tested or to their health care provider, the facility must comply with CLIA and is thereby required to obtain the appropriate CLIA certificate in accordance with 42 CFR Part 493, laboratory requirements.

Upcoming CLIAC Meeting

CLIAC Spring 2021

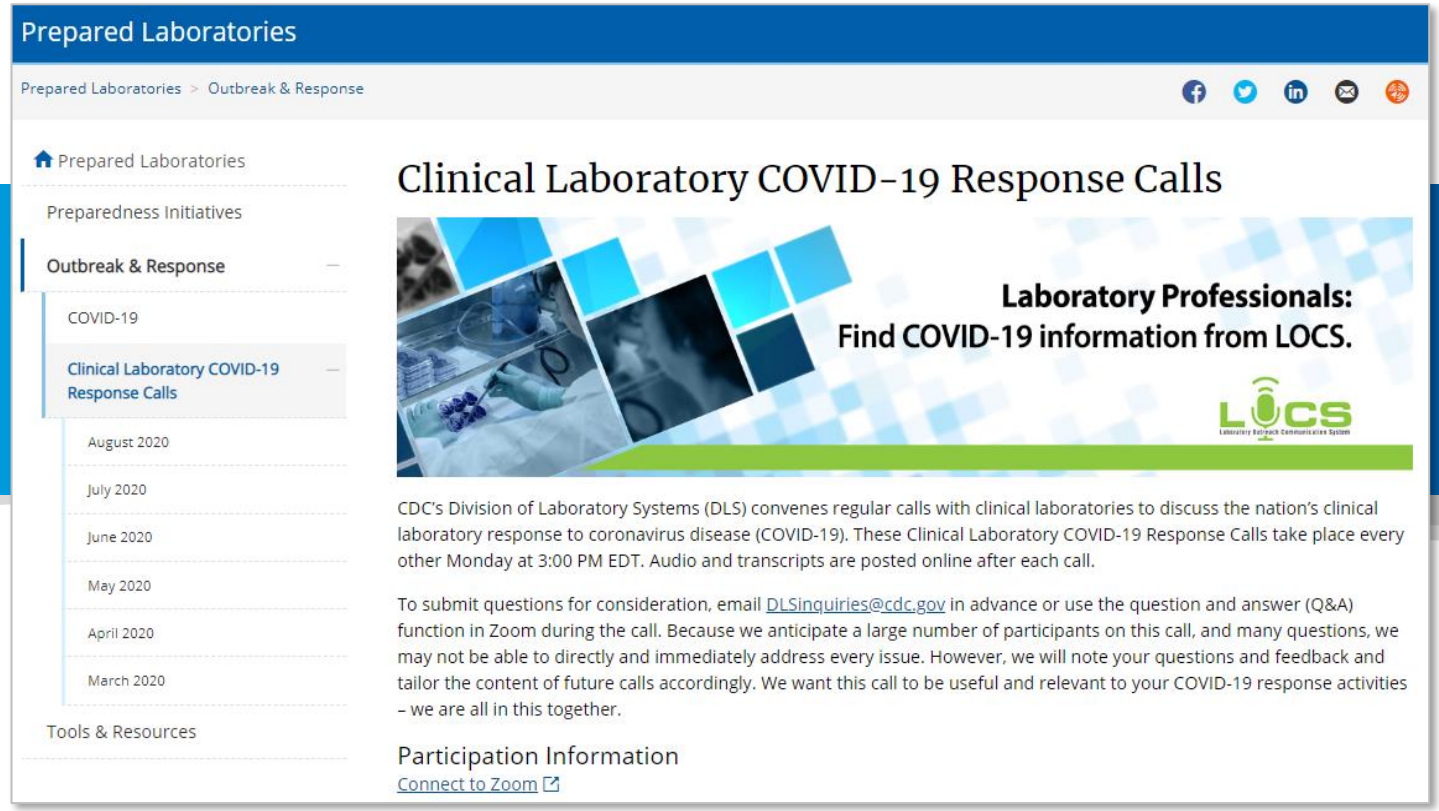
April 14 – 15, 11:00 AM – 6:00 PM, EDT (Virtual Meeting)

- See www.cdc.gov/cliac for meeting information
- Contact CLIAC@cdc.gov to submit written public comments or to present a public comment orally during the meeting

CDC Preparedness Portal

https://www.cdc.gov/csels/dls/preparedlabs/covid-19-clinical-calls.html

Find CLCR call information, transcripts, and audio recordings on the CDC Preparedness Portal



Schedule for Clinical Laboratory COVID-19 Response Calls

The next call will be on **Monday, April 19** from

3:00 PM to 4:00 PM EDT

We Want to Hear from You!

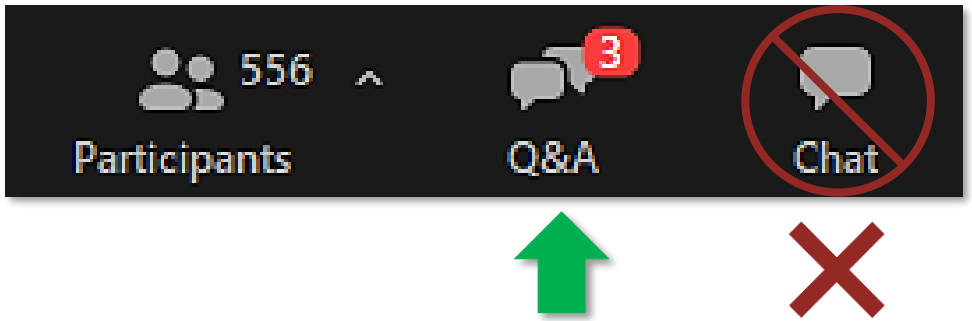
Training and Workforce Development

Questions about education and training?

Contact LabTrainingNeeds@cdc.gov

How to Ask a Question

- **Using the Zoom Webinar System**
  - Click the **Q&A** button in the Zoom webinar system
  - Type your question in the **Q&A** box and submit it
  - Please do not submit a question using the chat button



- For media questions, please contact CDC Media Relations at media@cdc.gov
- If you are a patient, please direct any questions to your healthcare provider

Slide decks may contain presentation material from panelists who are not affiliated with CDC. Presentation content from external panelists may not necessarily reflect CDC's official position on the topic(s) covered.

Emergence of SARS-CoV-2 Variants and Impact on IVD Testing

RADx Variant Task Force
Richard S. Creager, PhD

Acknowledgements

RADX

John Blackwood
Mia Cirrincione
Richard Creager
Dale Gort
Emily Kennedy
Eric Lai
D'lynne Plummer
Thomas Pribyl
Adam Samuta
Megan Shaw
Brian Walsh

Emory University

Leda Bassit
Filipp Frank
Morgan Greanleaf
Wilbur Lam
Cangyuan Li
Eric Ortlund
Anuradha Rao
Raymond Schinazi
Allie Suessmith
Julie Sullivan
Thomas Vanderford

University of Washington

Alex Greninger

Federal Agency Collaborators

BARDA
CDC
DOD
DOE
FDA
NIH

Overview: RADx Variant Task Force

Established

January 2021

The RADx Variant Task Force is a cross-discipline and cross-organization group of scientists and industry leaders with expertise in virology and diagnostic testing.

Objective

Rapidly analyze whether the performance of any of RADx's portfolio of diagnostic tests were affected by the mutations in the new variants.

SARS-CoV-2 Variants: Nucleic Acid Amplification (NAAT) and Antigen Testing

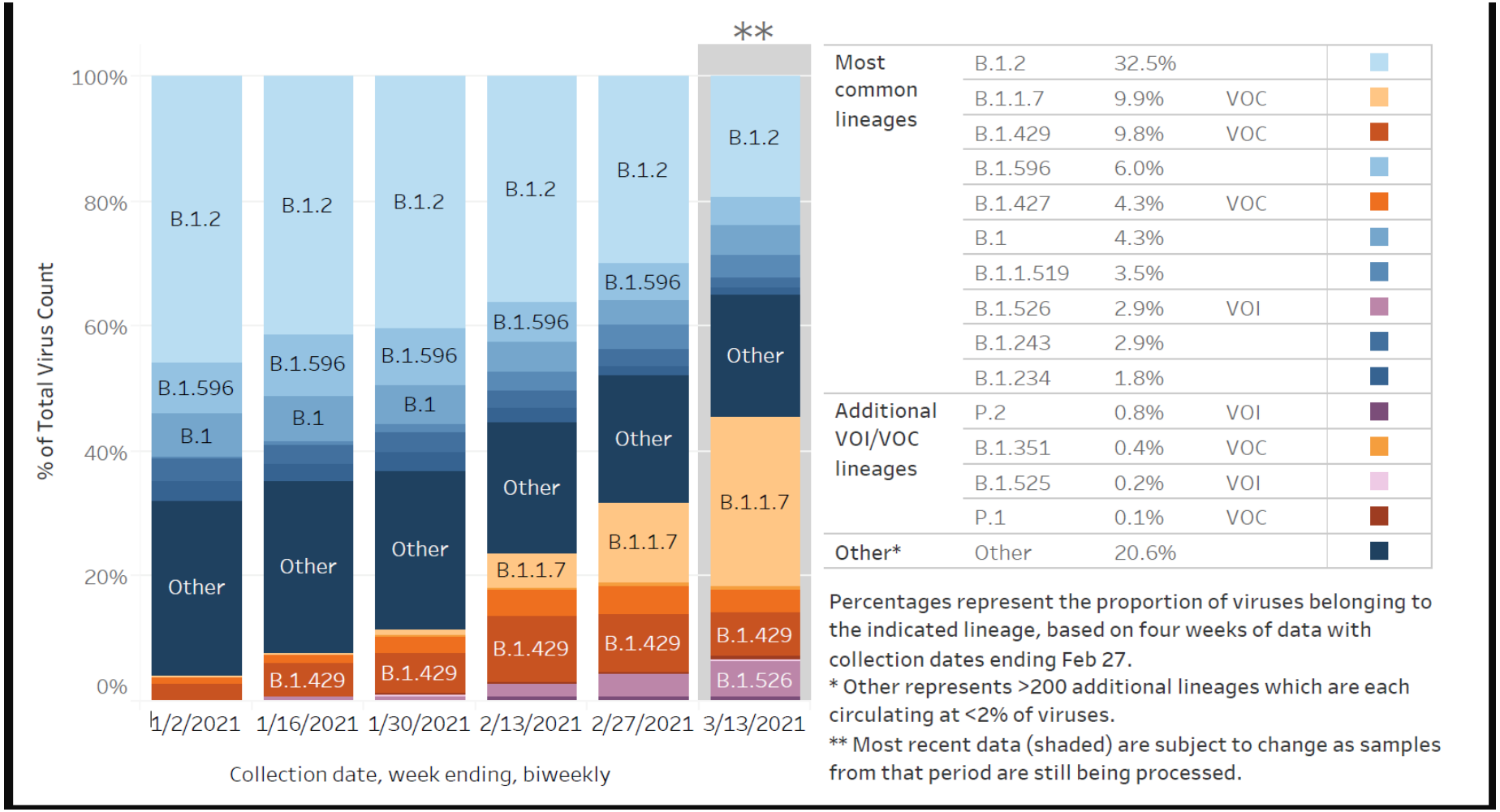
- Employs arrangement of primers and/or probes (or antibodies) to recognize and bind to specific sequences or protein in the virus
- Viral variants of interest arise through changes in their nucleotide sequences and amino acids
- Nucleotide changes impacts primer/probe ability to bind/amplify changed sequence
- Amino acid substitutions or deletions may change conformation structure or folding of the viral protein (epitope)

Change due to variant	Impact on Test
No binding	May lead to false negative
Reduced binding	May lead to decreased sensitivity (limit of detection)

in silico analysis and lab testing are required

- Used to model the impact of the mutation may primer/probe binding
- Lab testing is needed to confirm the *in silico* analysis

SARS-CoV-2 Variants Circulating in the United States

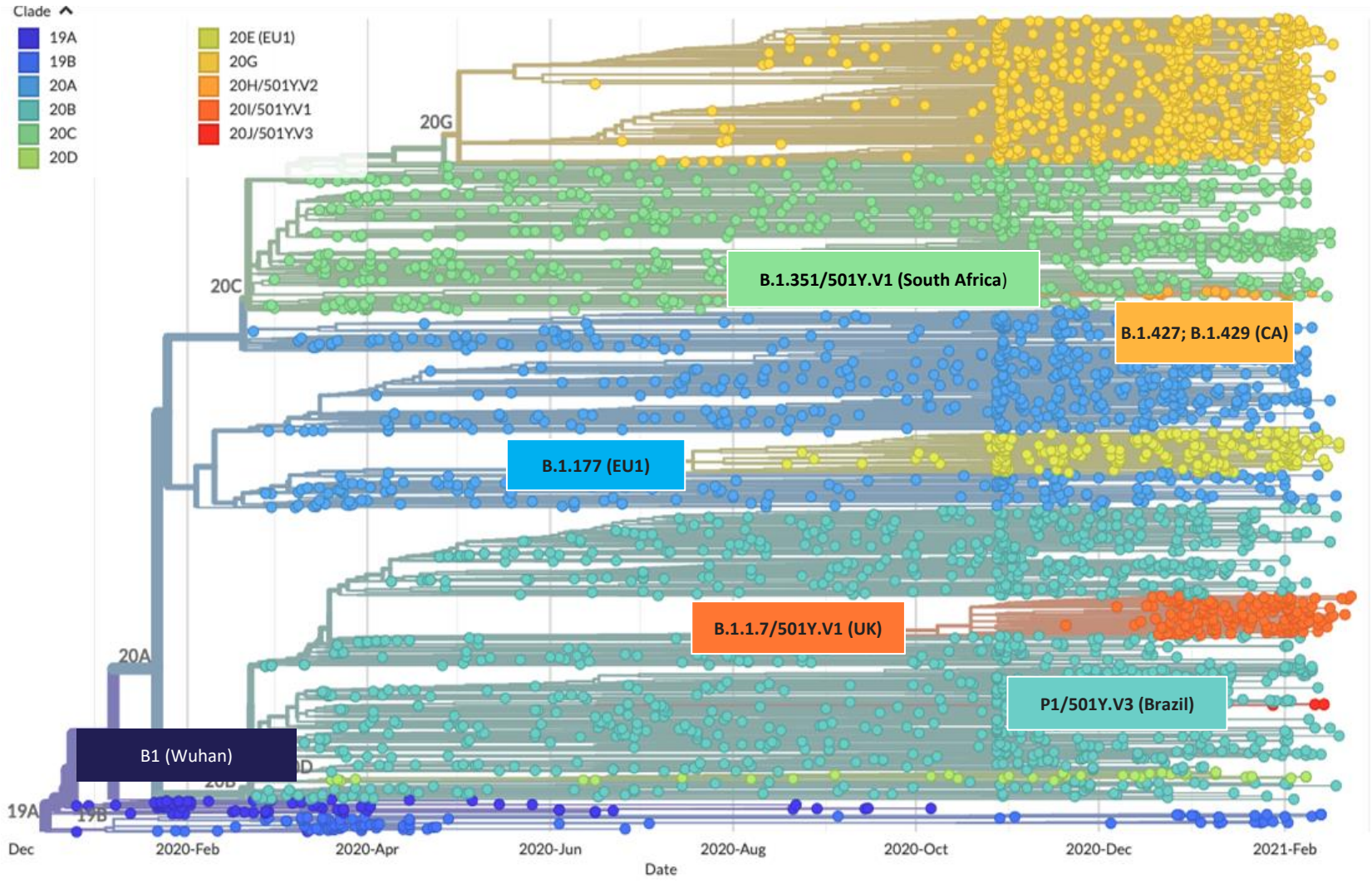


Most common lineages	B.1.2	32.5%		
	B.1.1.7	9.9%	VOC	
	B.1.429	9.8%	VOC	
	B.1.596	6.0%		
	B.1.427	4.3%	VOC	
	B.1	4.3%		
	B.1.1.519	3.5%		
	B.1.526	2.9%	VOI	
	B.1.243	2.9%		
	B.1.234	1.8%		
Additional VOI/VOC lineages	P.2	0.8%	VOI	
	B.1.351	0.4%	VOC	
	B.1.525	0.2%	VOI	
	P.1	0.1%	VOC	
Other*	Other	20.6%		

Percentages represent the proportion of viruses belonging to the indicated lineage, based on four weeks of data with collection dates ending Feb 27.
* Other represents >200 additional lineages which are each circulating at <2% of viruses.
** Most recent data (shaded) are subject to change as samples from that period are still being processed.

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

SARS-CoV-2 Variant Timeline: North America



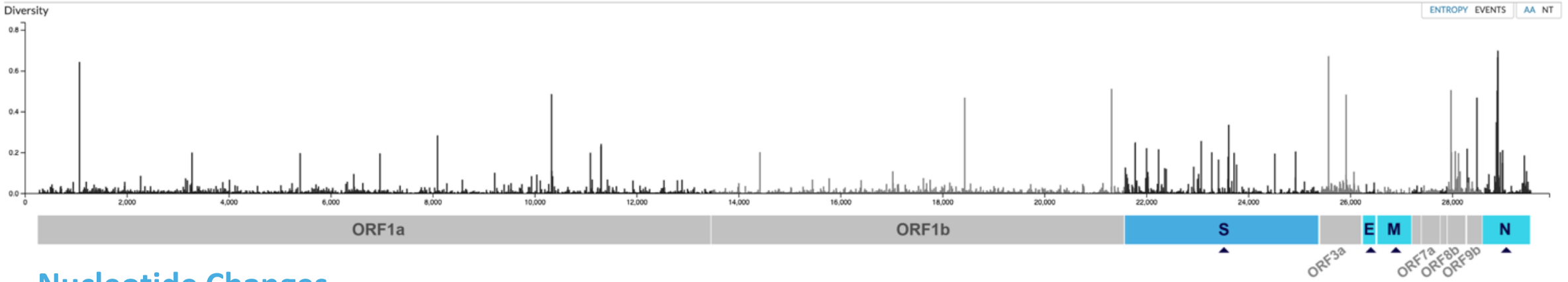
Key takeaways:

- Graphic depicts Dec 2019 to February 2021
- "Variants" are really families of related mutations
- B117 is not “one thing” but literally thousands of distinct sequences
- Over 800,000 unique sequences in GISAID

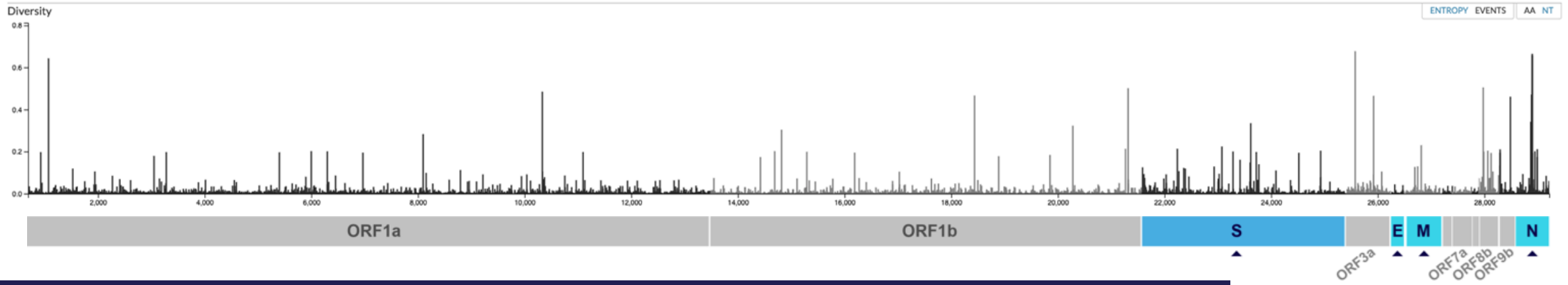
GISAID Nextstrain

SARS-CoV-2: Mutations Across the Viral Genome: North America

Amino Acid Changes



Nucleotide Changes



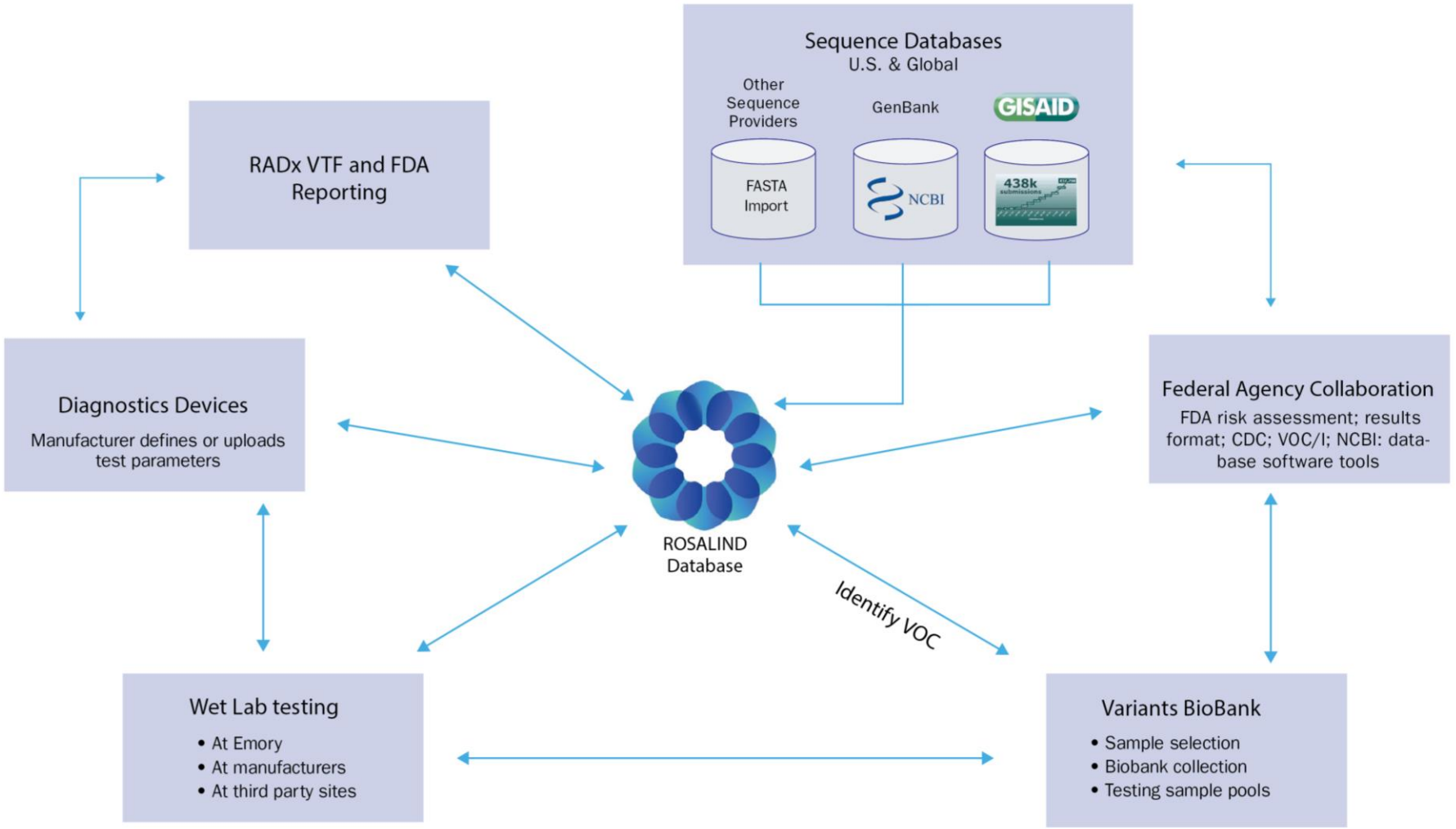
Key takeaway:

Every gene is impacted, not just spike protein

Big spikes are occurring for a reason - evolutionary advantage, biological pressure

GISAID Nextstrain

RADx VTF Components



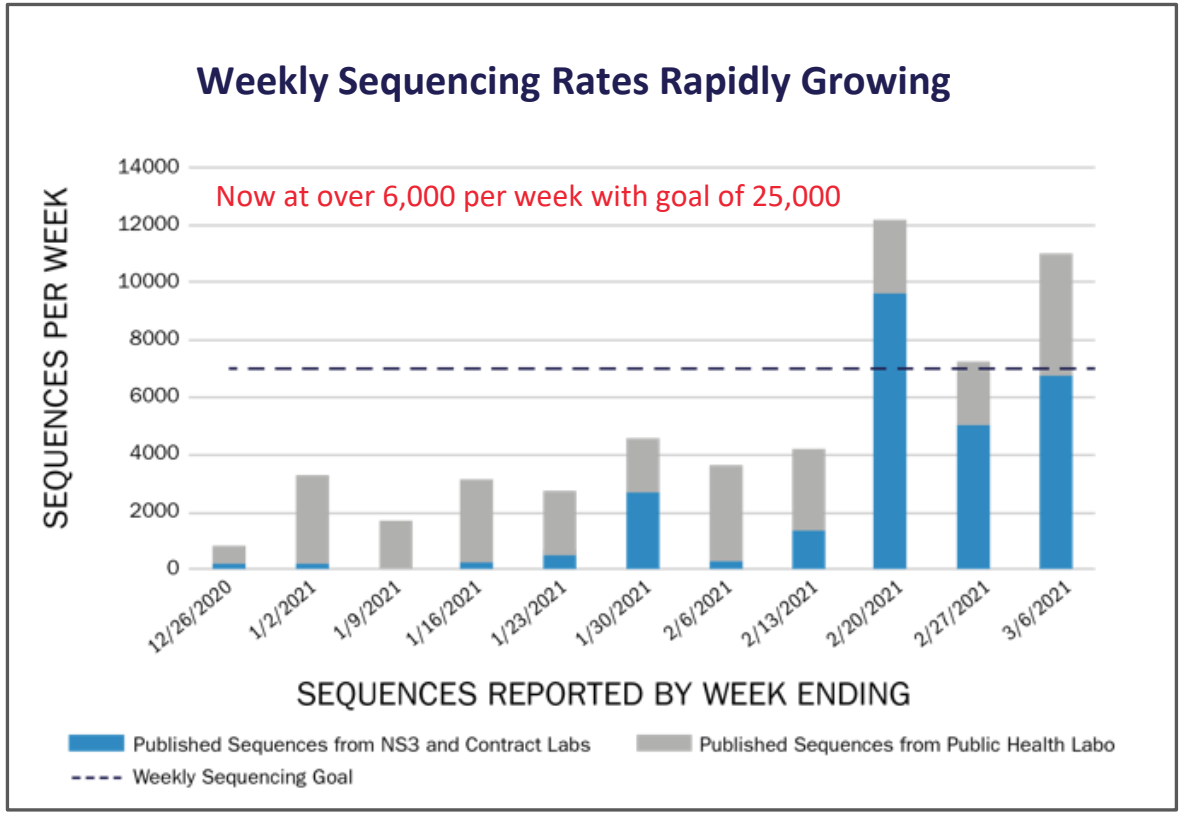
CDC SARS-CoV-2 Surveillance System

CDC Leads the National SARS-CoV-2 Surveillance System (NS3)

- Partnering with commercial diagnostic laboratories
- Collaborating with universities
- Supporting state, territorial, local and tribal health department

Goals

- Detect ability to spread more quickly
- Detect ability to cause milder or more severe disease
- Detect ability to evade detection by specific diagnostic tests
- Detect decreased susceptibility to therapeutics that employ monoclonal antibodies
- Detect ability to evade natural or vaccine-induced immunity



CDC Continues to Lead and Build Ongoing Field Surveillance Engine; RADx is partnering with the labs to acquire and build a variant biobank

RADx VTF: Progress

Bioinformatics

- In collaboration with the FDA and NIH, a custom bioinformatics platform was developed for the detection of variants of concern and to evaluate the effect of the variants on diagnostics assays by both informatics approaches and integration of wet lab results .
- Pulls information from comprehensive global and US-specific datasets including the GISAID, NCBI GenBank and Emory variant biobank
- Results reported to RADx and individual teams if potential issues are detected

Variants Sample Procurement and BioBank at Emory University

- Sourcing variant samples from CDC funded sequencing laboratories across the country
- Procured >800 samples covering > 12 mutations including variants B.1.2, B.1.1.7, B1.351, P1, P2, B.1.427, B.1.429, B.1.525 and B.1.526.

Lab Testing with Variants

- Testing of RADx portfolio with variant sample pools (five serial diluted concentrations) begins in April.
- Test results upload to Rosalind platform for reporting and optimization of analysis pipelines

Bioinformatics Analysis Pipeline and Results

INCIDENT ANALYSIS COVERAGE MAP

Rapidly assess test coverage & issues

Comprehensive analysis for each diagnostic test with summary by region

Highlights the new and ongoing variants, based on most recent data and frequency history

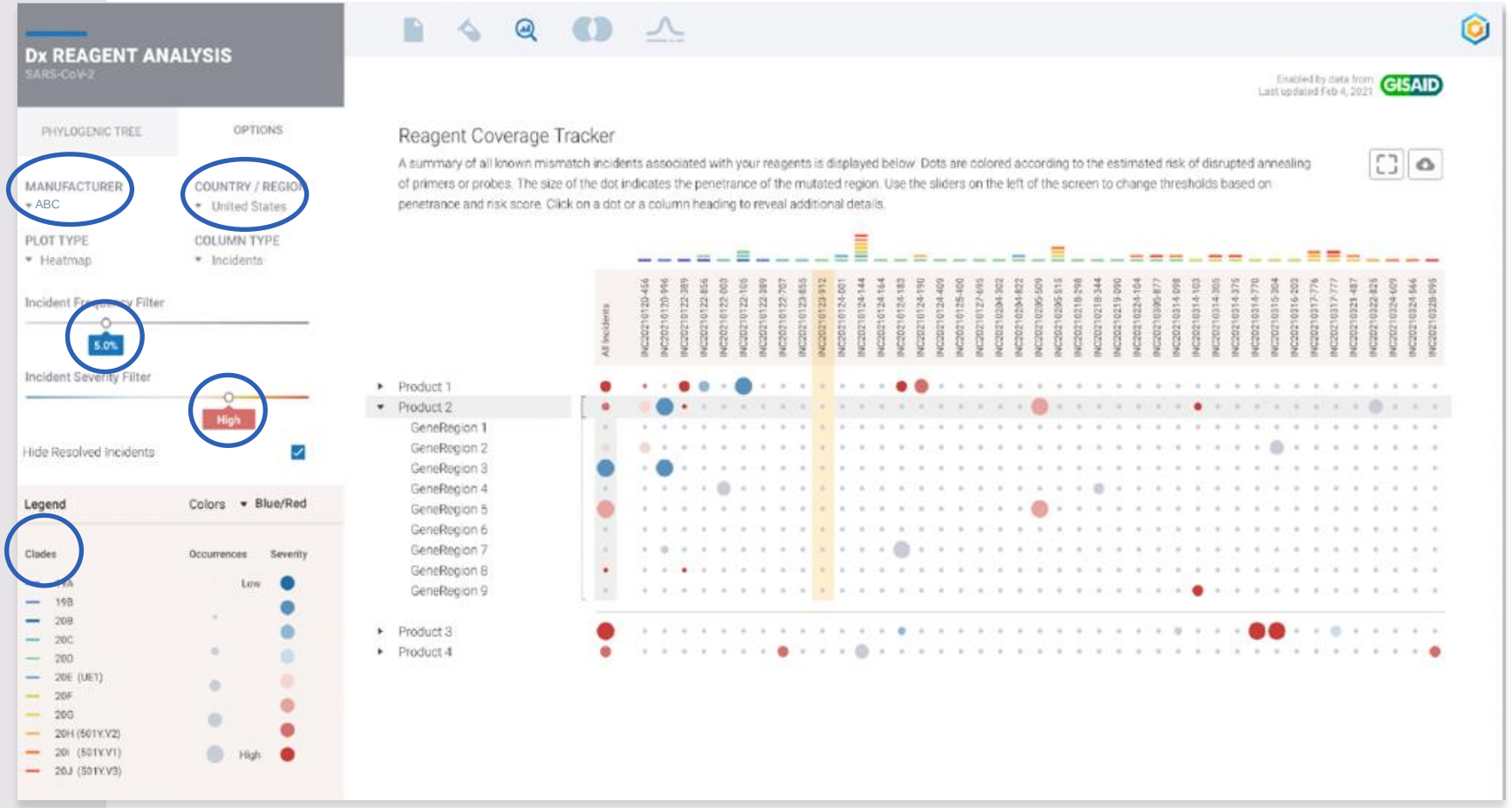
RADx & agencies have option to view each manufacturer

Each manufacturer sees private results

Geographic selection shows emerging strains/clades/mutations

Phylogenic tree shows evolution between strains/clades

Example Bioinformatics Output



Summary

SARS-CoV-2 continuing to mutate

- The virus is rapidly mutating in the U.S.
- New variants of concern are appearing in multiple states
- Impacting testing, vaccines and public health

We must remain vigilant and collect real time field data

- Surveillance is critical… and then need to be proactive
- Continue to find new ways to rapidly accelerate development, or adjustment, of tests, therapeutics and vaccines

RADx model greatly expedites speed to address new and emerging potential SARS-CoV-2 testing issues

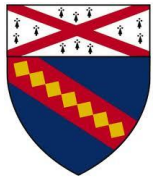
- Need to continuously monitor the field and rapidly innovate as the scientific evidence dictates

Saliva as a sample type for SARS-CoV-2 detection

Anne L. Wyllie, PhD

Associate Research Scientist

Epidemiology of Microbial Diseases



Yale SCHOOL OF PUBLIC HEALTH

Saliva as a sample type for COVID-19 inpatients

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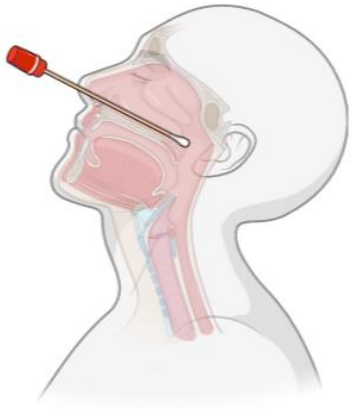
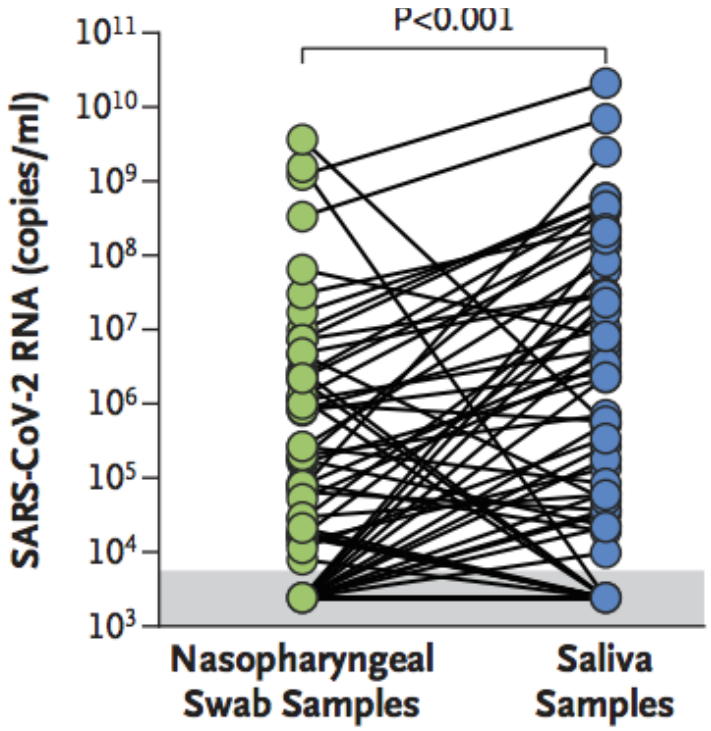


- Difficult
- Uncomfortable
- Risky



Saliva as a sample type to aid testing challenges

Wyllie et al, NEJM. 2020

Saliva tests must be designed for saliva

Yale

- **Saliva is the collection of all oral fluid**
  - Not sputum or mucus
  - No sniffing or coughing (also risk of respiratory droplets)
  - If not properly collected it can be difficult to work with

- **Saliva is not a traditional diagnostic sample type**
  - Methods for swabs don't necessarily work on saliva:
    - From 54 RT-qPCR-based saliva vs. swab comparison studies:
      - 69% found saliva to have greater or similar (≤10% difference) sensitivity
      - Saliva detected an additional 10% of positive cases (NP swab negative)

Discrepancies in comparison studies due to poor samples or methods?

Tan et al. The Lancet Respiratory Medicine. In press.

Good quality saliva samples CAN be collected

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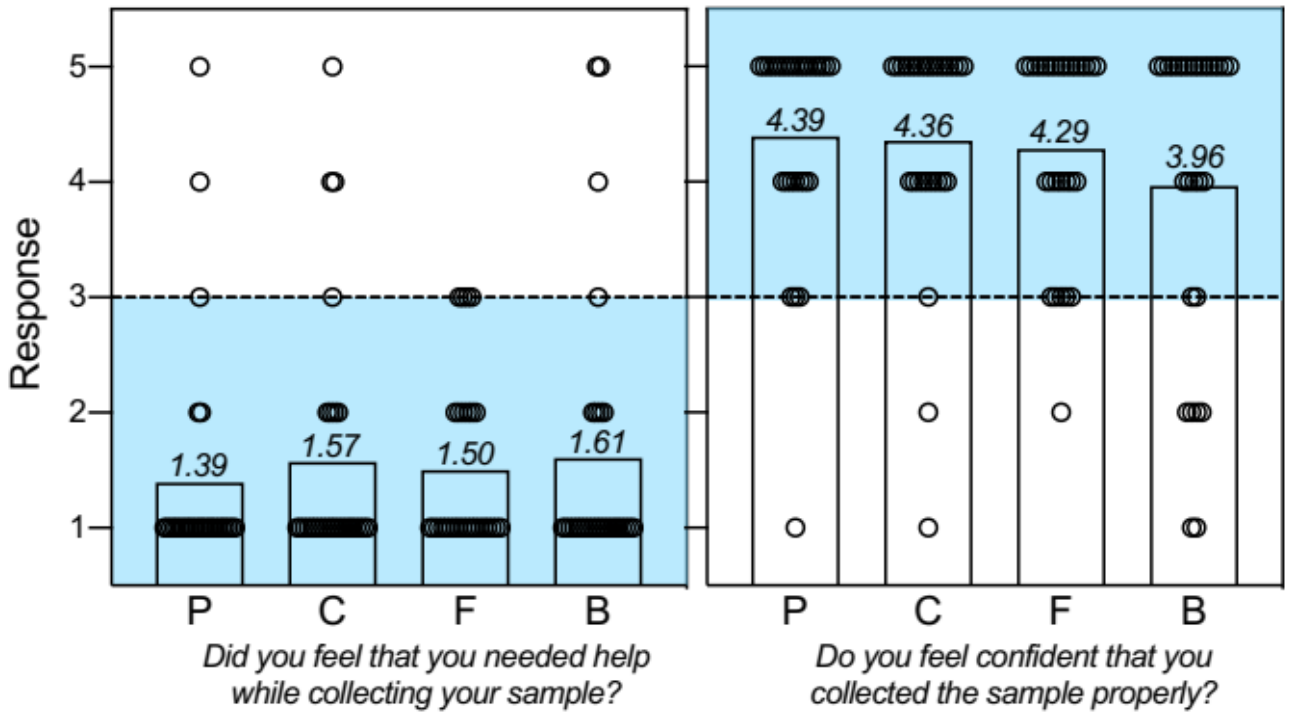
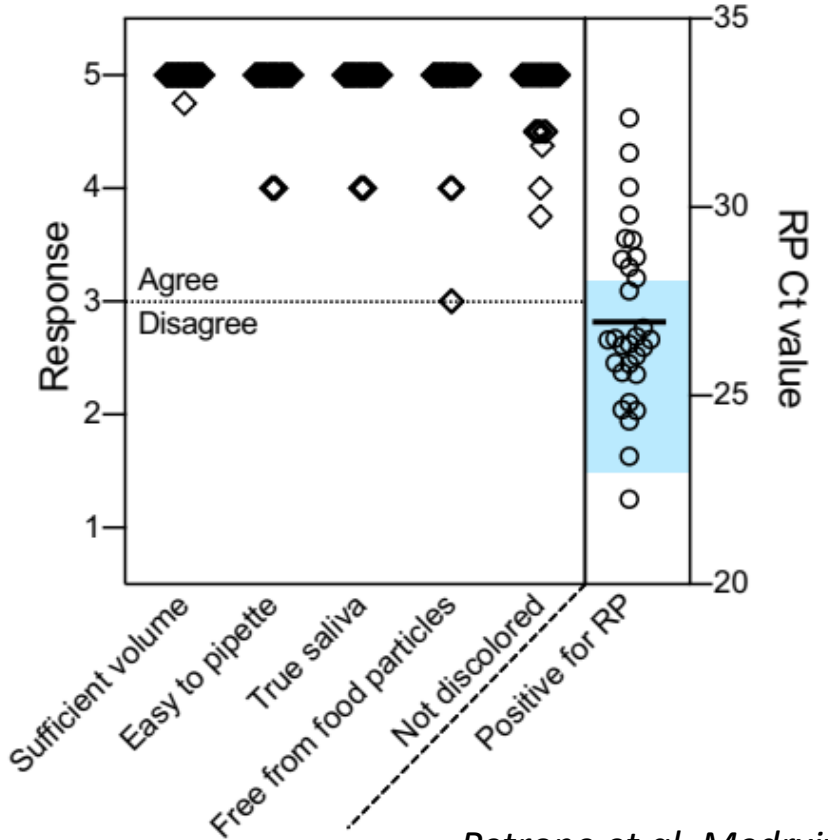
- Be patient, think about your favorite food, don't sniff or cough, don't 'spit'
- **30 study participants, 4 devices each, "unobserved" collection**

Participant feedback



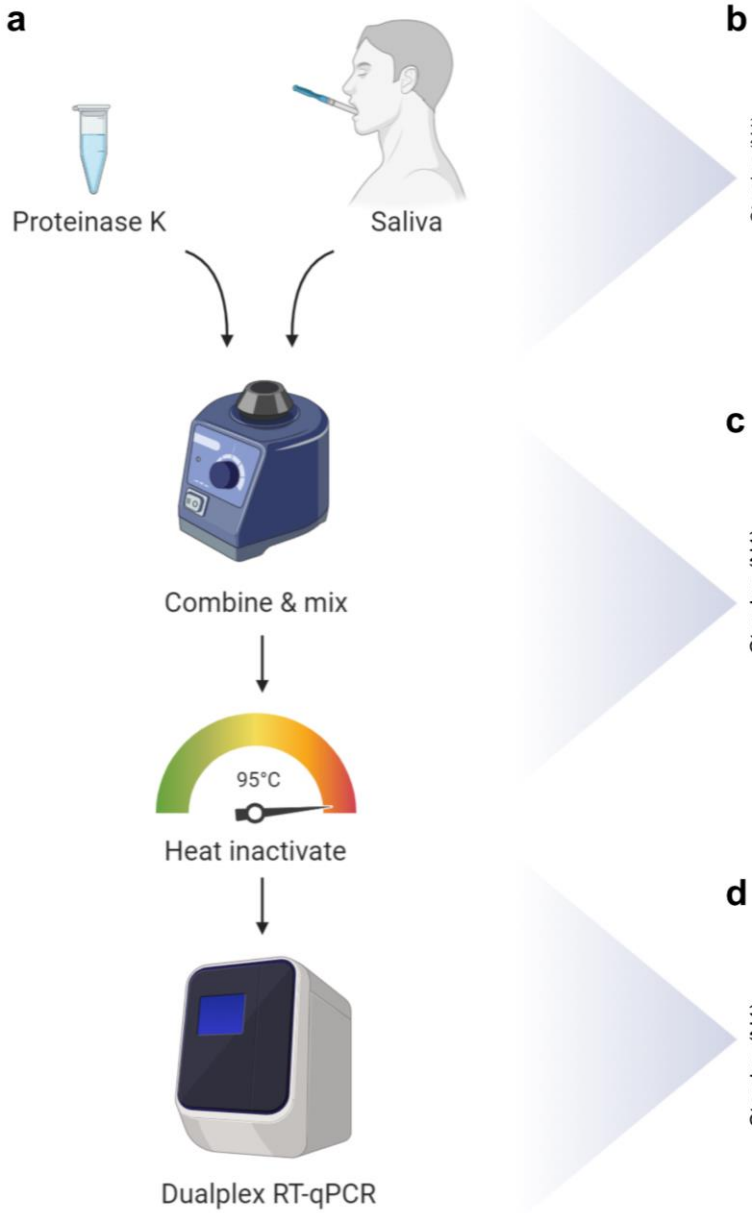
Lab feedback

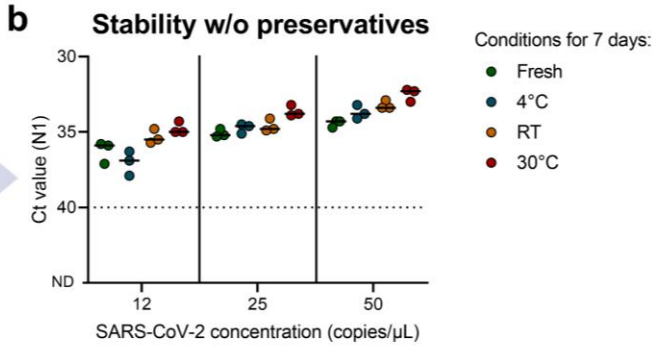


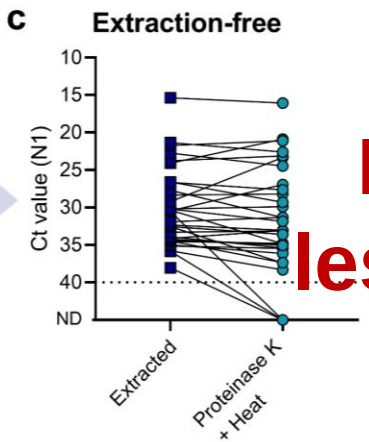
Petrone et al. Medrxiv. 2021

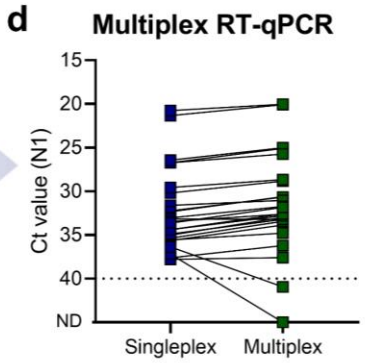
SalivaDirect™: extraction-free and dualplex RT-qPCR

Yale







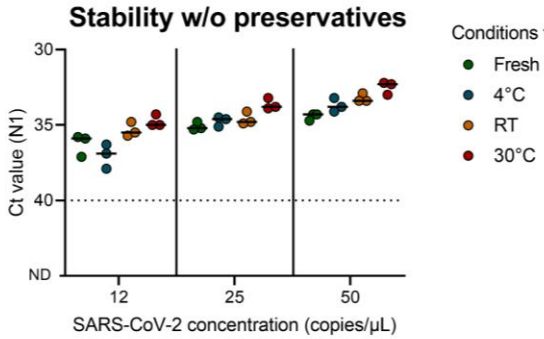


Less invasive, less expensive, less prone to supply chain issues

Vogels et al., 2020. medRxiv

SalivaDirect™: extraction-free and dualplex RT-qPCR

Yale



Conditions for 7 days:
- Fresh
- 4°C
- RT
- 30°C

Saliva is stable without preservatives

No expensive collection device required

Maintains sensitivity w/out RNA extraction

Removes expensive and time-consuming step

Maintains sensitivity with dualplex PCR

Low detection limits and internal control

Vogels et al., 2020. medRxiv

SalivaDirect™: extraction-free and dualplex RT-qPCR

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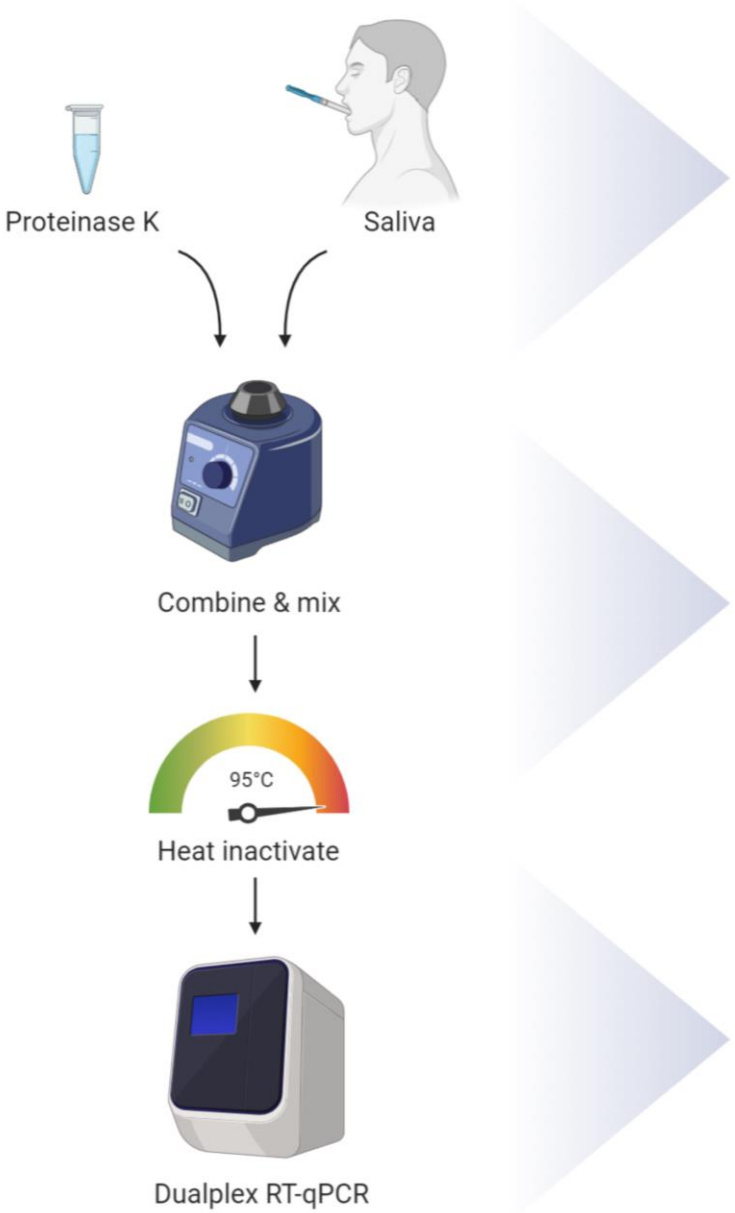

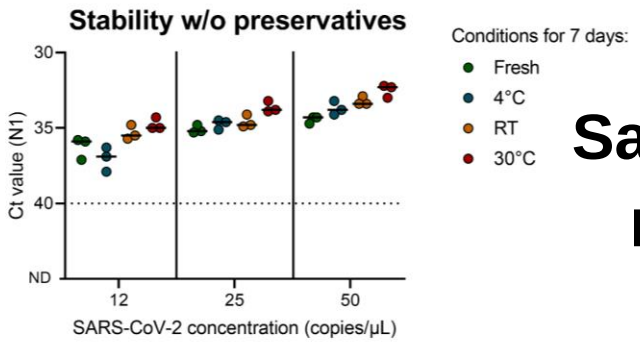

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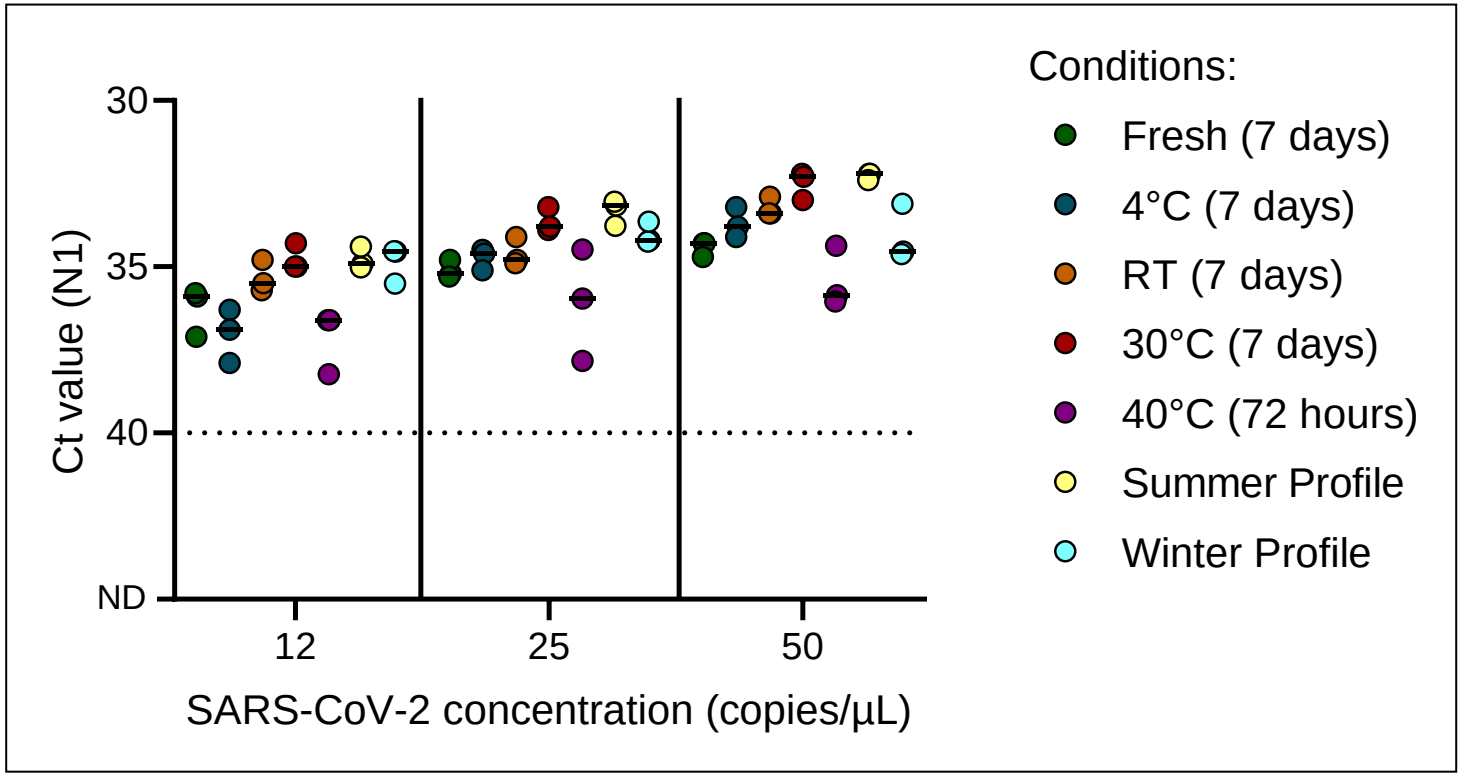

Vogels et al., 2020. medRxiv

SalivaDirect™: extraction-free and dualplex RT-qPCR

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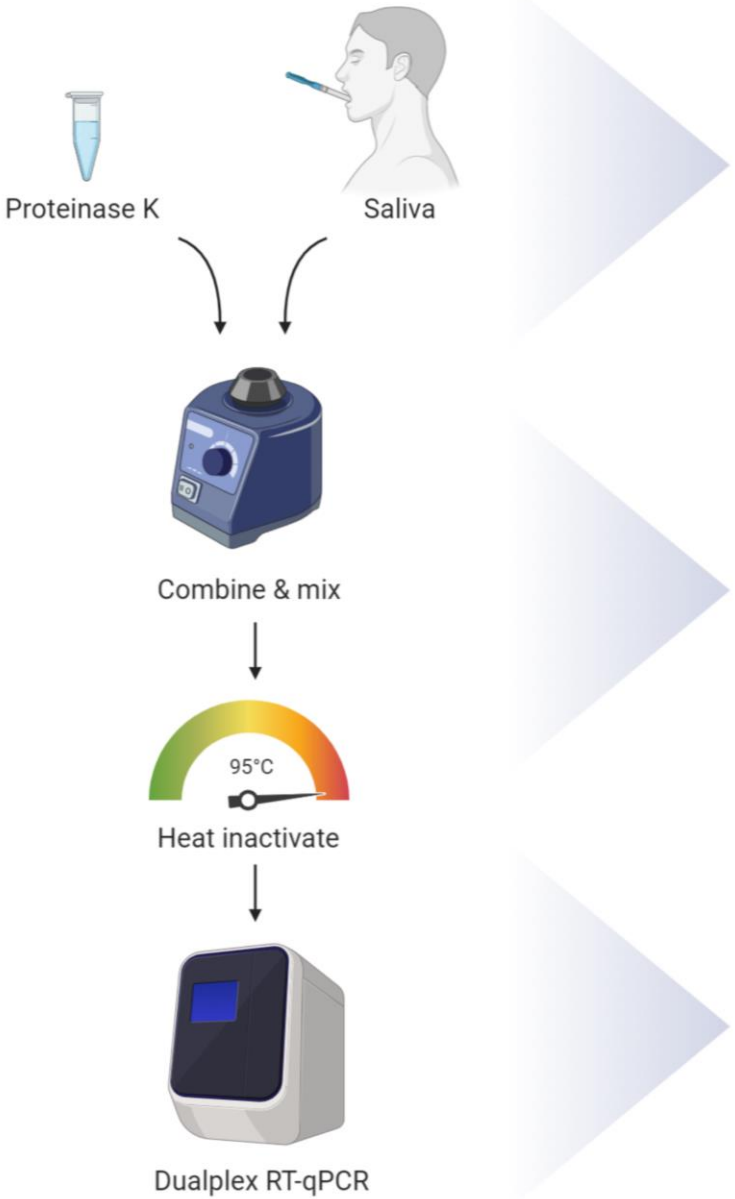

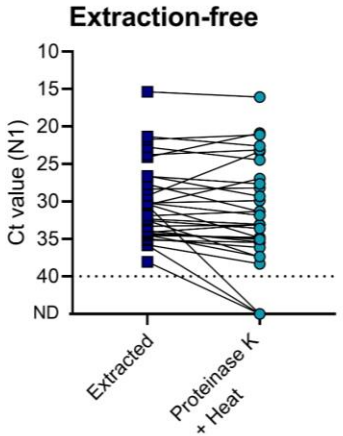

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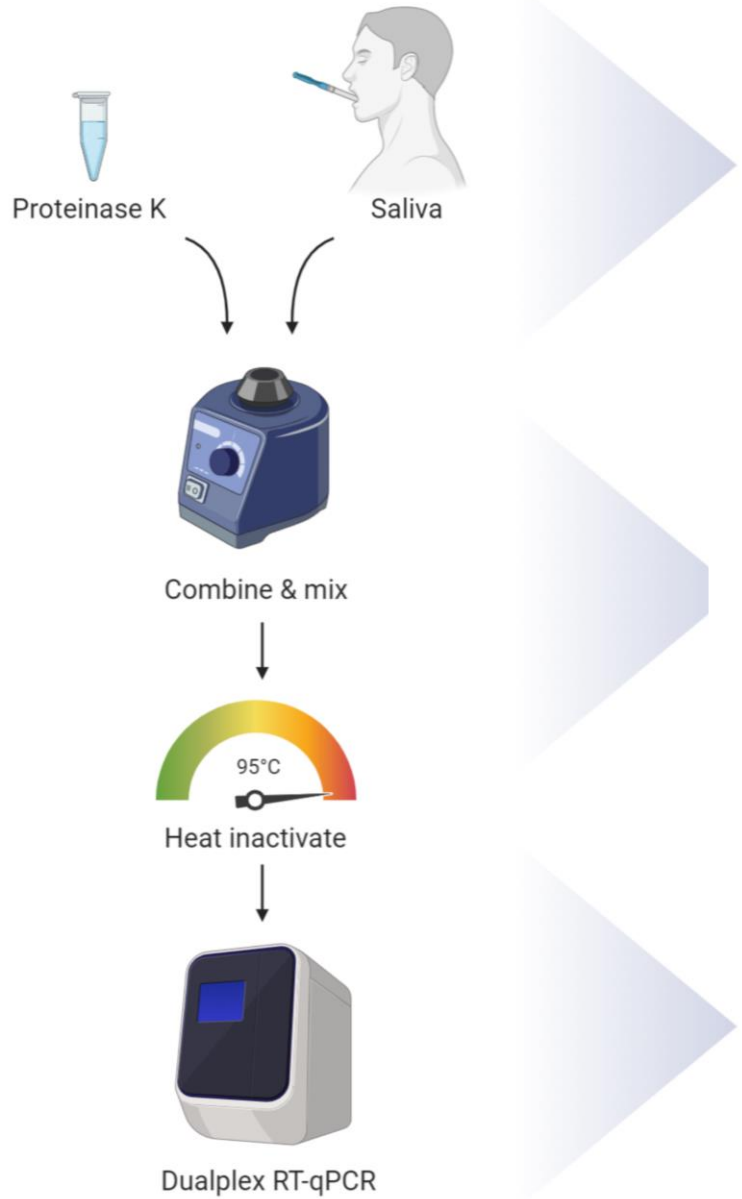

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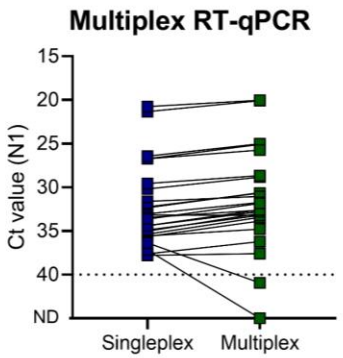


Maintains sensitivity with dualplex PCR

Low detection limits and internal control

Vogels et al., 2020. medRxiv

Validated with multiple vendors (ongoing updates)

Yale

Table 1: Validated reagents and instruments for use with SalivaDirect.

Item	Vendor	Product Name	Catalog number
Proteinase K	ThermoFisher Scientific	MagMAX Viral/Pathogen Proteinase K	A42363
	New England Biolabs	Proteinase K, Molecular Biology Grade	P8107S
	AmericanBio	Proteinase K	AB00925-00100
RT-qPCR kit	New England Biolabs	Luna Universal Probe One-Step RT-qPCR Kit	E3006E
	Bio-Rad	Reliance One-Step Multiplex RT-qPCR Supermix	12010176
	ThermoFisher Scientific	TaqPath 1-Step RT-qPCR Master Mix, GC	A15299
RT-qPCR instrument	Bio-Rad	CFX96 Touch Real-Time PCR Detection System	
	ThermoFisher Scientific	Applied Biosystems 7500 Fast Real-Time PCR System	
	ThermoFisher Scientific	Applied Biosystems 7500 Fast Dx Real-Time PCR System	

SalivaDirect™ costs $1.29-$4.37* in reagents per sample

**based on list prices, standard discounts should bring costs down to ~$1/sample*

A (near) universal RT-qPCR protocol

Yale

Step	Temperature	Time
1	52°C	10 min
2	95°C	2 min
3	95°C	10 sec
4	55°C	30 sec
5	Read plate (Use channels to detect FAM and Cy5 fluorophores)	

Repeat steps 3-5 for 44 cycles.

Meeting high throughput testing needs

Yale

96-well format	NEB Luna (2x)	Bio-Rad Reliance	Thermo TaqPath
Master mix	10 µL	5 µL	5 µL
RT	1 µL	-	-
Primer-probe-water mix	4 µL	4 µL	4 µL
Nuclease-free water	-	6 µL	6 µL
Saliva lysate or control	5 µL	5 µL	5 µL

384-well format	NEB Luna (2x)	NEB Luna 4X	Bio-Rad Reliance	Thermo TaqPath
Master mix	5 µL	2.5 µL	2.5 µL	2.5 µL
RT	0.5 µL	-	-	-
Primer-probe-water mix	2 µL	2 µL	2 µL	2 µL
Nuclease-free water	-	3 µL	3 µL	3 µL
Saliva lysate or control	2.5 µL	2.5 µL	2.5 µL	2.5 µL

Meeting high throughput testing needs

Yale

		Concentration (copies/µL; positive replicates)							
INSTRUMENT	PCR KIT	100	50	25	12	6	3	1.5	0
CFX 384	NEB Luna 2x	3/3	3/3	3/3	3/3	3/3	**3/3**	0/3	0/3
QS 5	NEB Luna 2x	3/3	3/3	3/3	3/3	3/3	**3/3**	0/3	0/3
QS 5	NEB Luna 4x	3/3	3/3	3/3	3/3	**3/3**	2/3	0/3	0/3
QS 5	Bio-rad Reliance	3/3	3/3	3/3	3/3	3/3	**3/3**	1/3	0/3
QS 6	NEB Luna 2x	3/3	3/3	3/3	3/3	3/3	**3/3**	1/3	0/3
QS 7 Pro	NEB Luna 2x	3/3	3/3	3/3	3/3	3/3	**3/3**	1/3	0/3
QS 7 Pro	NEB Luna 4x	3/3	3/3	3/3	3/3	3/3	3/3	**3/3**	0/3
QS 7 Pro	Bio-rad Reliance	3/3	3/3	3/3	3/3	3/3	**3/3**	2/3	0/3
QS 7 Pro	TaqPath One Step	3/3	3/3	3/3	3/3	3/3	**3/3**	1/3	0/3
QS 7 Flex	NEB Luna 2x	3/3	3/3	3/3	3/3	**3/3**	2/3	1/3	0/3
QS 7 Flex	NEB Luna 4x	3/3	3/3	3/3	3/3	3/3	3/3	**3/3**	0/3
QS 12k Flex	NEB Luna 4x	3/3	3/3	3/3	3/3	3/3	**3/3**	1/3	0/3

The protocol continues to evolve

Yale

Workflow 1:
Standard SalivaDirect Protocol

Proteinase K Treatment

↓

Heat inactivation

↓

RT-PCR

Workflow 2:
Heat pre-treatment prior to standard SalivaDirect Protocol

Heat pre-treatment
95°C 30 minutes
65°C 15 minutes

↓

Proteinase K Treatment

↓

Heat inactivation

↓

RT-PCR

Workflow 3:
Heat pre-treatment without Proteinase K/heat inactivation

Heat pre-treatment
95°C for 5 minutes
95°C 30 minutes
65°C 15 minutes

↓

RT-PCR

Safer handling of samples, while improving viscosity of the saliva for easier pipetting

Sensitivity of pre-treatment heat prior to SalivaDirect:

Yale

Without the addition of Proteinase K and heat inactivation step

	Concentration (positive replicates)		
	6 copies/µL	3 copies/µL	1.5 copies/µL
65°C for 15 mins	20/20	**20/20**	18/20
95°C for 5 mins	20/20	**19/20**	18/20
95°C for 30 mins	**20/20**	15/20	14/20

With Proteinase K and heat inactivation step

	Concentration (positive replicates)		
	6 copies/µL	3 copies/µL	1.5 copies/µL
65°C for 15 mins	**20/20**	17/20	15/20
95°C for 30 mins	**20/20**	16/20	19/20

SalivaDirect™ is suitable for large-scale screening

Yale

Table 2. Parallel Testing of Anterior Nares/Oropharyngeal Swabs and Saliva from NBA Players, Staff, and Contractors

		Quest/BioReference	
		AN/OP Swab	
		Positive	Negative
SalivaDirect Saliva	positive	17	2
	negative	2	3,746
	invalid	0	12
Total		19	3,760

Invalid samples = 0.3% (12/3,779). Positive agreement = 89.5% (17/19). Negative agreement = 99.9% (3,746/3,748 valid samples). Overall agreement = 99.9% (3,763/3,767 valid samples).

3,779 paired samples

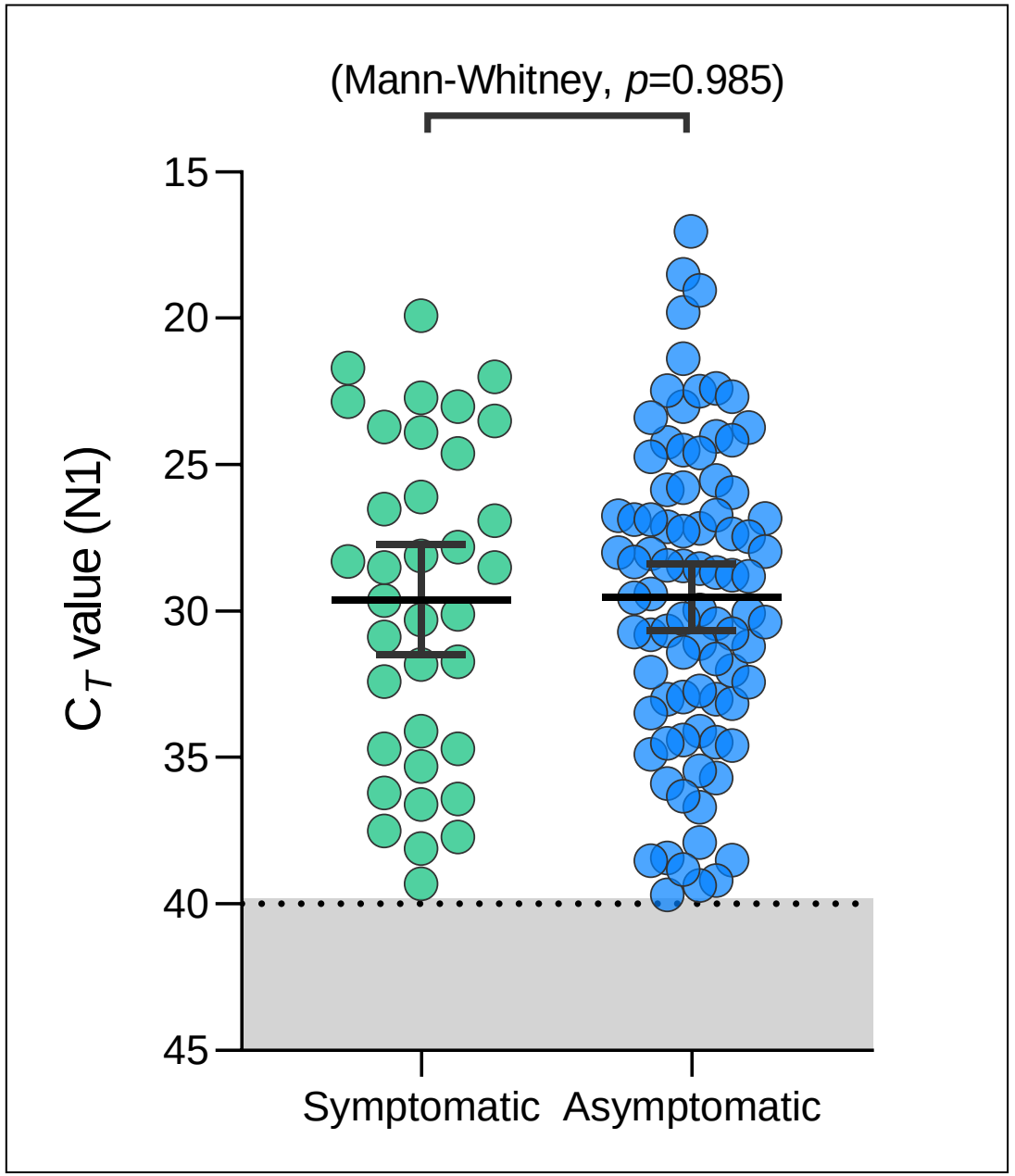
Invalid rate = 0.3%

False positive = 0.03-0.05%

Vogels et al. Med. 2020

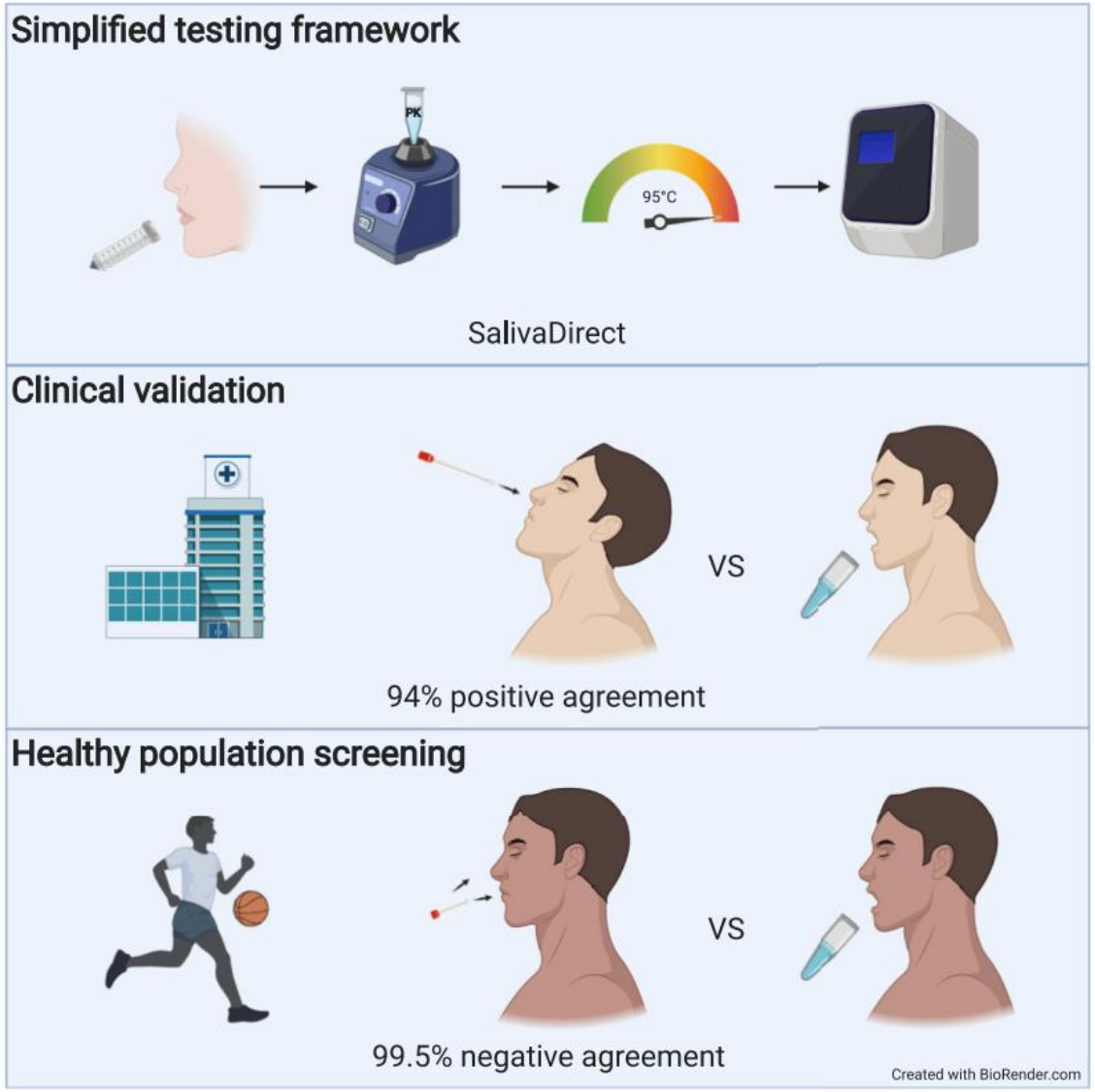
Viral detection in saliva: symptomatic vs. asymptomatic

Yale



SalivaDirect™: an open-source initiative for COVID testing

Yale



Key points:

- Protocol, not a test kit, no commercialization
- We make no profit: no licensing fees, no commission or royalties.
- Authorize CLIA-certified labs
- EUA updates extend to all authorized labs

- ➢ Growing network of testing labs nationwide
- ➢ Reduce testing costs
- ➢ Reduce implementation time to use saliva
- ➢ Increase the number of tests in the community

FDA Update

Tim Stenzel
U.S. Food and Drug Administration (FDA)

U.S. Food and Drug Administration (FDA)

- **COVID-19 Emergency Use Authorization (EUA) Information for Medical Devices**
  https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations
- **COVID-19 In Vitro Diagnostic EUAs**
  https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas
- **COVID-19 Frequently Asked Questions**
  https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/coronavirus-disease-2019-covid-19-frequently-asked-questions
- **COVID-19 Updates**
  https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#2019-ncov
- **FDA Townhall Meetings**
  https://www.fda.gov/medical-devices/workshops-conferences-medical-devices/virtual-town-hall-series-immediately-effect-guidance-coronavirus-covid-19-diagnostic-tests-06032020
- **Independent Evaluations of COVID-19 Serological Tests**
  https://open.fda.gov/apis/device/covid19serology/

FDA U.S. FOOD & DRUG ADMINISTRATION

U.S. Food and Drug Administration (FDA)

- **COVID-19 Diagnostic Development**
  CDRH-EUA-Templates@fda.hhs.gov

- **Spot Shortages of Testing Supplies: 24-Hour Support Available**
  1. Call 1-888-INFO-FDA (1-888-463-6332)
  2. Then press star (*)

- **FDA MedWatch**
  https://www.fda.gov/safety/medwatch-fda-safety-information-and-adverse-event-reporting-program

U.S. FOOD & DRUG ADMINISTRATION

CDC Social Media

https://www.facebook.com/CDC

https://twitter.com/cdcgov

https://www.linkedin.com/company/cdc

Thank You For Your Time!

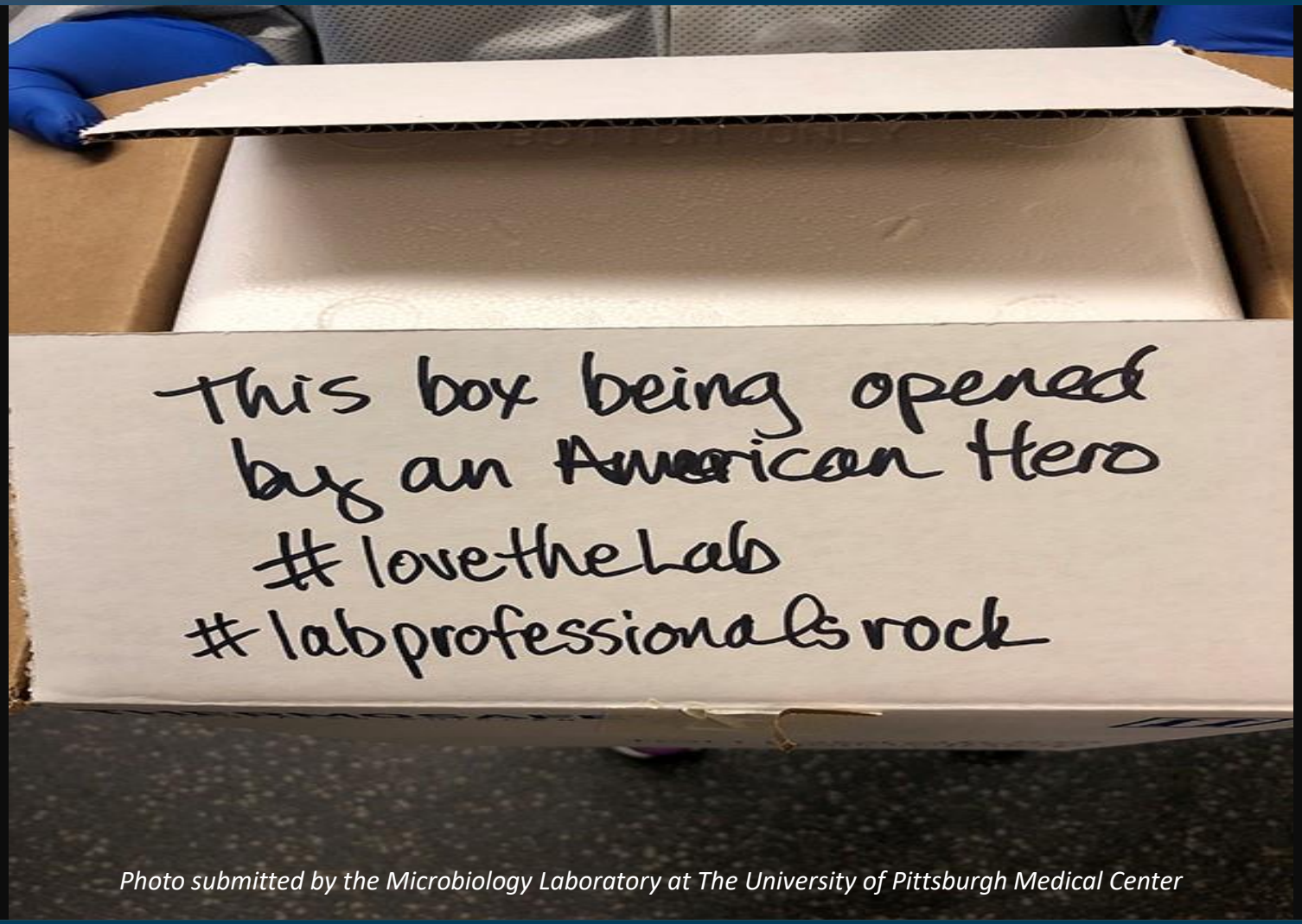


Photo submitted by the Microbiology Laboratory at The University of Pittsburgh Medical Center