Responding to Global Viral Pandemics, Both New and (Very) Old

Keith R. Jerome, MD PhD

University of Washington

Fred Hutchinson Cancer Research Center

June, 2021

UW Medicine
LABORATORY MEDICINE
VIROLOGY

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Responding to a new global viral pandemic

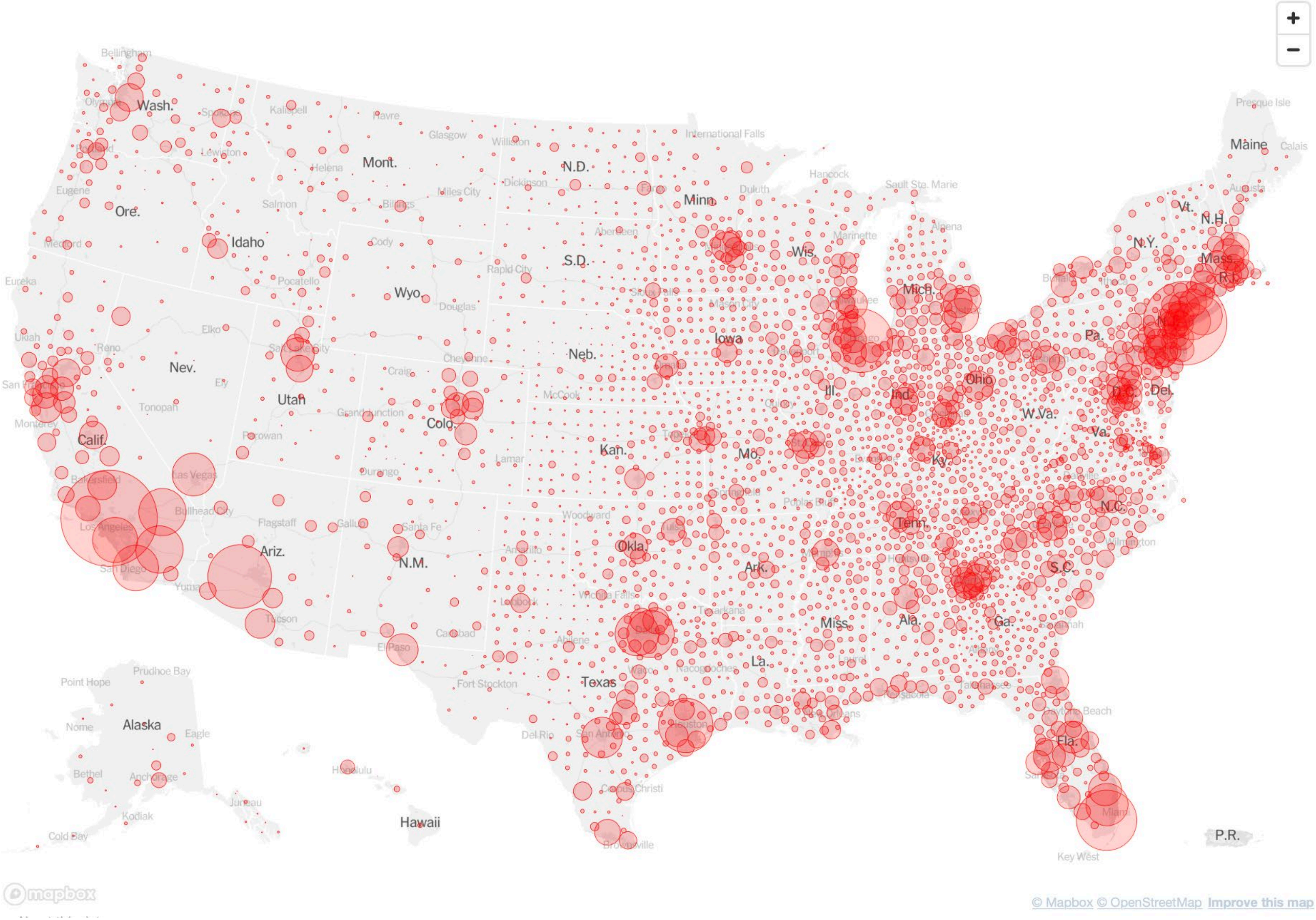
China pneumonia outbreak: Mystery virus probed in Wuhan

3 January 2020

The outbreak has occurred in the city of Wuhan

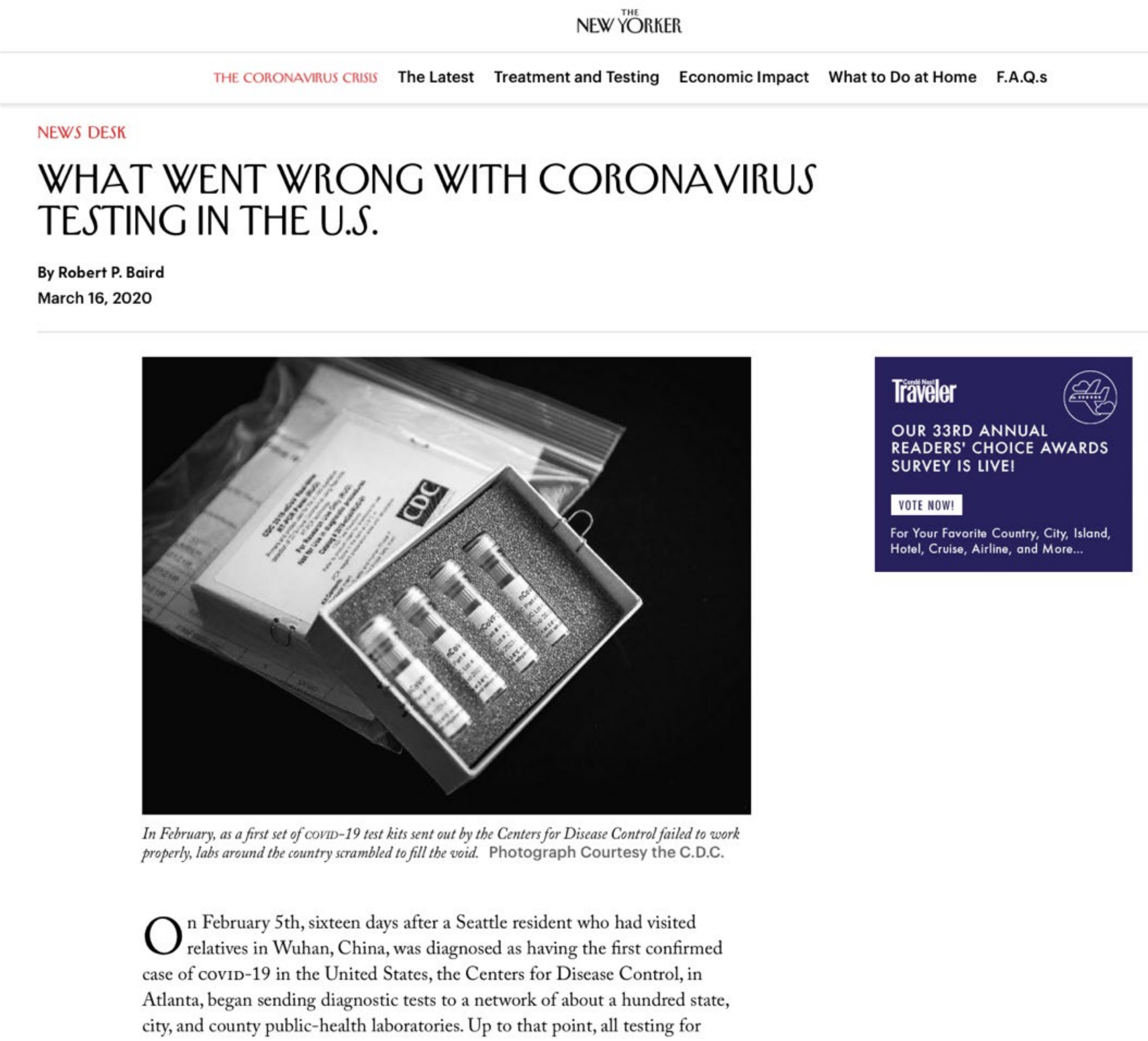
Chinese authorities have launched an investigation into a mysterious viral pneumonia which has infected dozens of people in the central city of Wuhan.

US distribution of cases



NY Times, May 4, 2021

Regulatory hurdles prevented early SARS-CoV-2 testing in the US



THE NEW YORKER

NEWS DESK

WHAT WENT WRONG WITH CORONAVIRUS TESTING IN THE U.S.

By Robert P. Baird
March 16, 2020

In February, as a first set of COVID*-19 test kits sent out by the Centers for Disease Control failed to work properly, labs around the country scrambled to fill the void.* Photograph Courtesy the C.D.C.

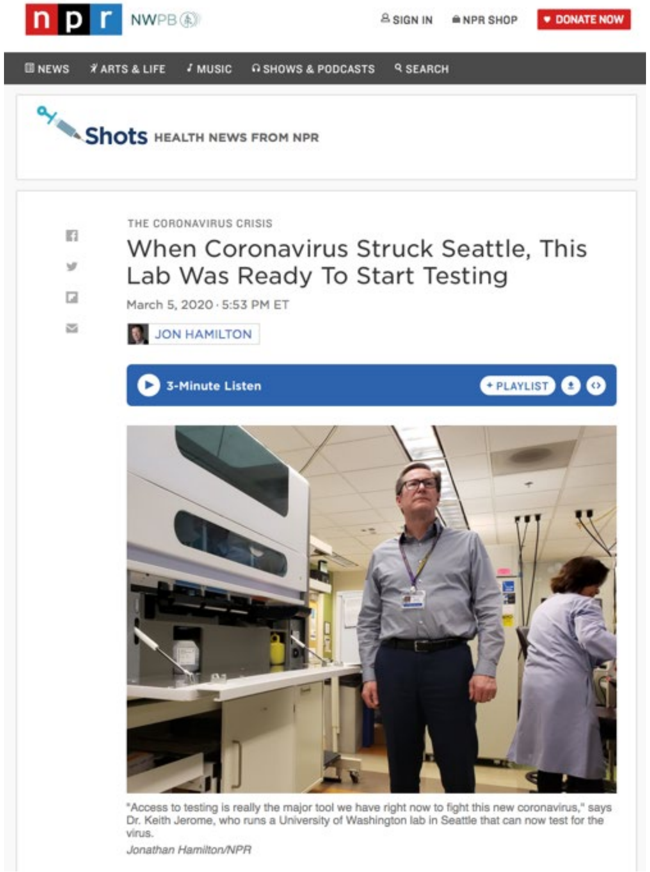
On February 5th, sixteen days after a Seattle resident who had visited relatives in Wuhan, China, was diagnosed as having the first confirmed case of COVID-19 in the United States, the Centers for Disease Control, in Atlanta, began sending diagnostic tests to a network of about a hundred state, city, and county public-health laboratories. Up to that point, all testing for

UW Virology was the first academic lab in the US to offer testing for SARS-CoV-2

UW Medicine gets green light to test for coronavirus

March 4, 2020 at 5:30 pm | *Updated March 5, 2020 at 12:35 am*

1 of 3 | *A medical lab scientist at UW Medicine in Seattle shows a collected nasal swab sample from Washington to be tested for the novel... (Ken Lambert / The Seattle Times)* **More**



THE CORONAVIRUS CRISIS

When Coronavirus Struck Seattle, This Lab Was Ready To Start Testing

March 5, 2020 · 5:53 PM ET

JON HAMILTON

"Access to testing is really the major tool we have right now to fight this new coronavirus," says Dr. Keith Jerome, who runs a University of Washington lab in Seattle that can now test for the virus.

Jonathan Hamilton/NPR

Seattle flattened the curve

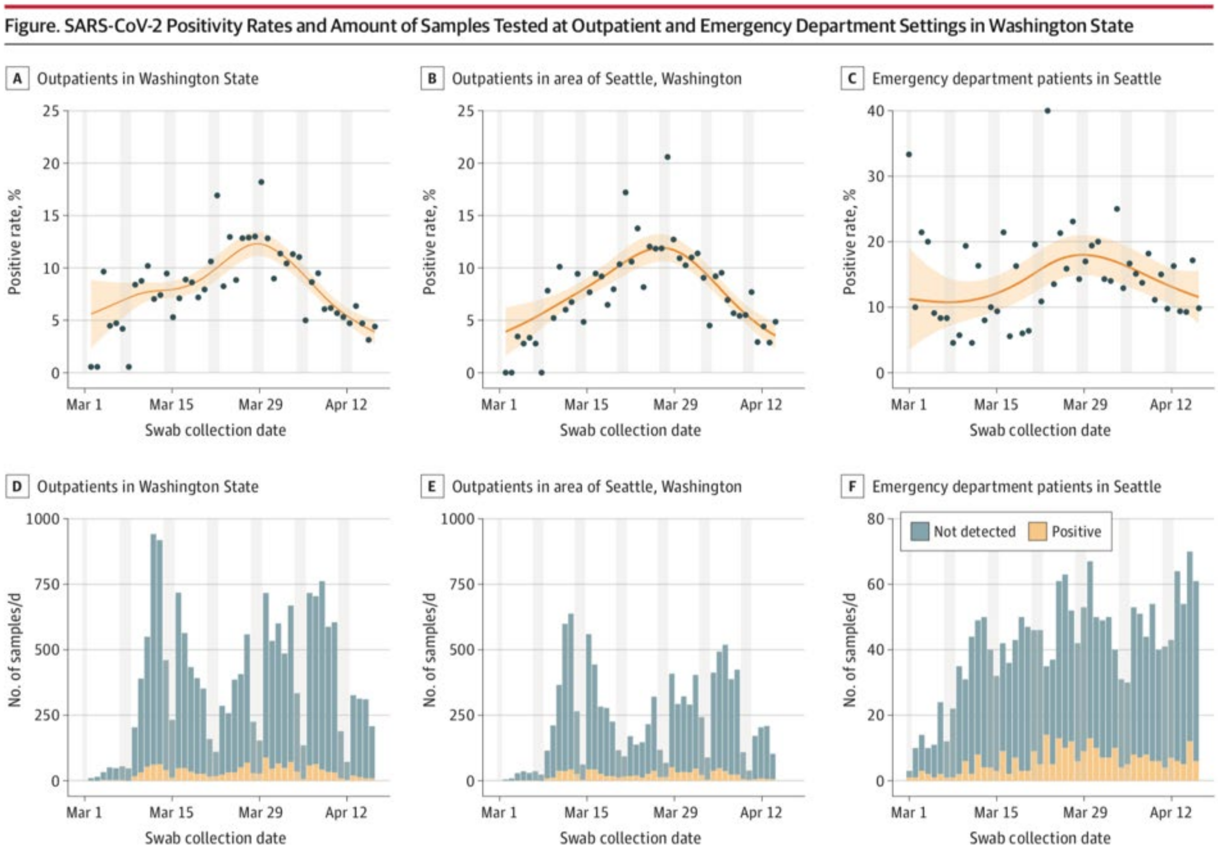


Figure. SARS-CoV-2 Positivity Rates and Amount of Samples Tested at Outpatient and Emergency Department Settings in Washington State

A Outpatients in Washington State

B Outpatients in area of Seattle, Washington

C Emergency department patients in Seattle

D Outpatients in Washington State

E Outpatients in area of Seattle, Washington

F Emergency department patients in Seattle

Research Letter ONLINE FIRST FREE

May 8, 2020

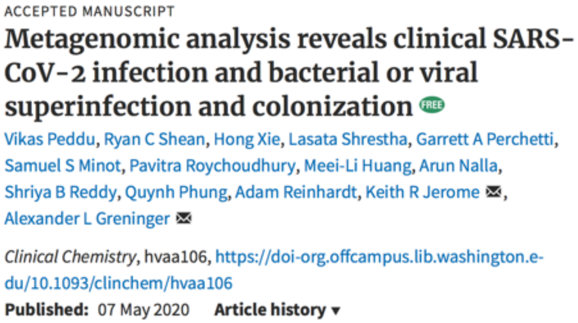
Changes in SARS-CoV-2 Positivity Rate in Outpatients in Seattle and Washington State, March 1-April 16, 2020

April Kaur Randhawa, PhD[1]; Leigh H. Fisher, PhD[1]; Alexander L. Greninger, MD, PhD[2]; Shuying Sue Li, PhD[1]; Jessica Andriesen, PhD[1]; Lawrence Corey, MD[1]; Keith R. Jerome, MD, PhD[2]

› Author Affiliations | Article Information

JAMA. Published online May 8, 2020. doi:10.1001/jama.2020.8097

UW sequencing capability would have allowed discovery of SARS-CoV-2



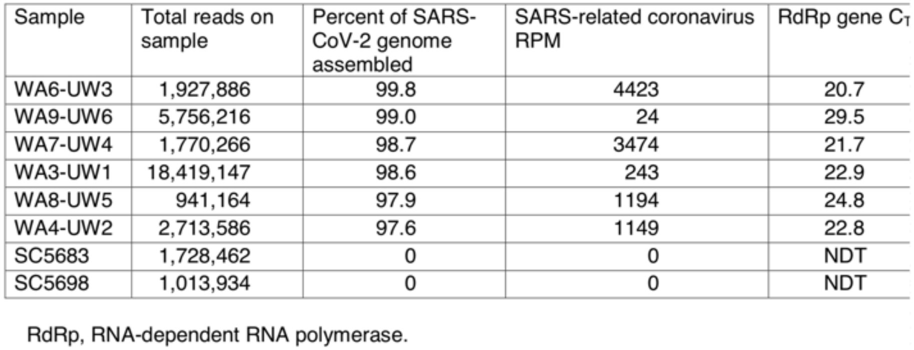
ACCEPTED MANUSCRIPT

Metagenomic analysis reveals clinical SARS-CoV-2 infection and bacterial or viral superinfection and colonization FREE

Vikas Peddu, Ryan C Shean, Hong Xie, Lasata Shrestha, Garrett A Perchetti, Samuel S Minot, Pavitra Roychoudhury, Meei-Li Huang, Arun Nalla, Shriya B Reddy, Quynh Phung, Adam Reinhardt, Keith R Jerome ✉, Alexander L Greninger ✉

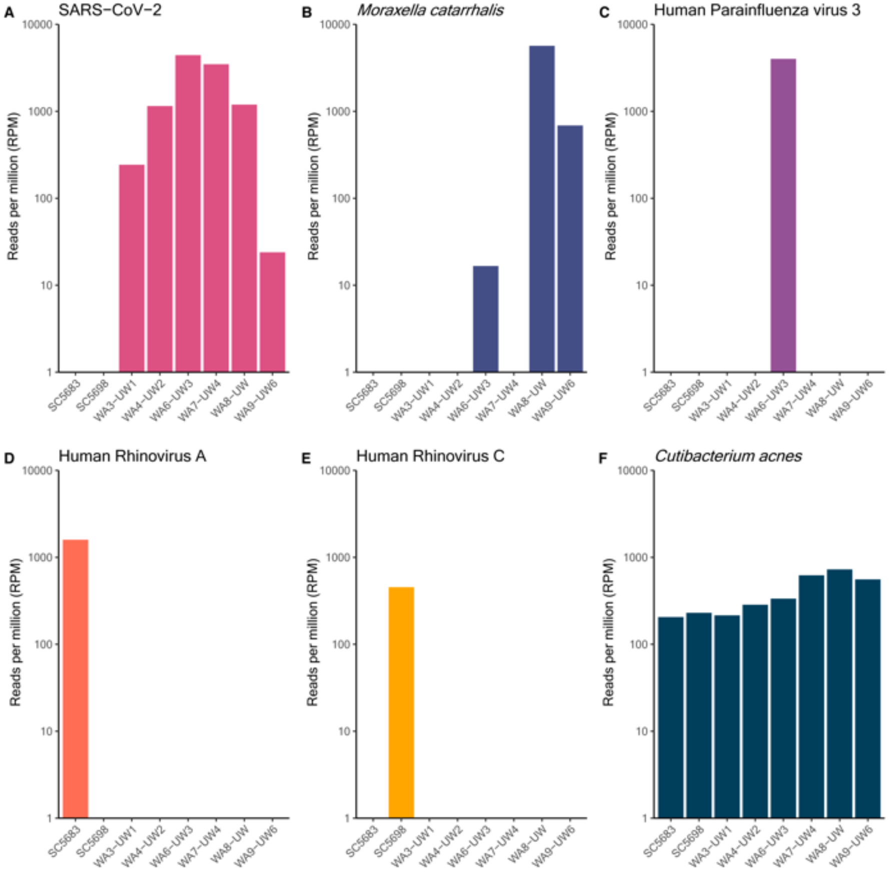
Clinical Chemistry, hvaa106, https://doi-org.offcampus.lib.washington.e-du/10.1093/clinchem/hvaa106

Published: 07 May 2020 **Article history ▾**



Sample	Total reads on sample	Percent of SARS-CoV-2 genome assembled	SARS-related coronavirus RPM	RdRp gene C_T
WA6-UW3	1,927,886	99.8	4423	20.7
WA9-UW6	5,756,216	99.0	24	29.5
WA7-UW4	1,770,266	98.7	3474	21.7
WA3-UW1	18,419,147	98.6	243	22.9
WA8-UW5	941,164	97.9	1194	24.8
WA4-UW2	2,713,586	97.6	1149	22.8
SC5683	1,728,462	0	0	NDT
SC5698	1,013,934	0	0	NDT

RdRp, RNA-dependent RNA polymerase.



Sequencing provides understanding of COVID-19 spread

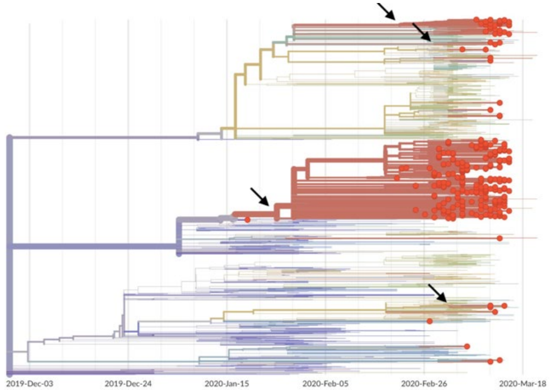
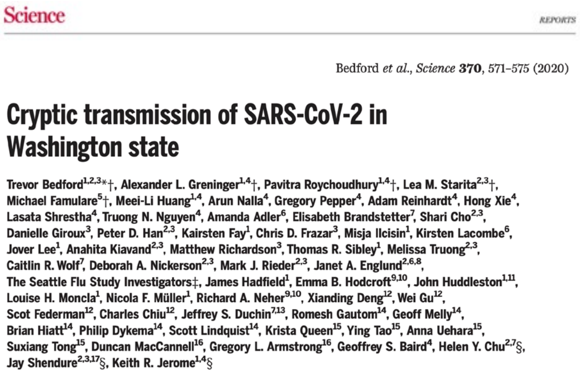


Figure 2. Phylogeny of 346 SARS-CoV-2 viruses collected from Washington State (red circles) on a background of globally collected viruses. Clustering of related viruses indicates community transmission after an introduction event. In addition to the estimated January introduction of the large outbreak clade we see later introduction events (marked by arrows) resulting in smaller community outbreaks.

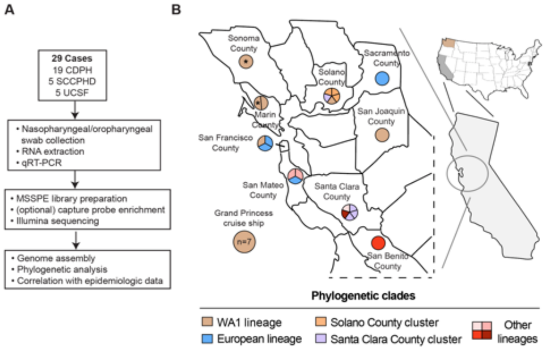


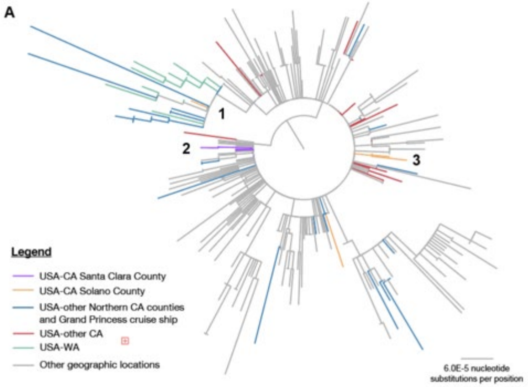
Science REPORTS

Bedford *et al*., *Science* **370**, 571–575 (2020)

Cryptic transmission of SARS-CoV-2 in Washington state

Trevor Bedford[1,2,3]*†, Alexander L. Greninger[1,4]†, Pavitra Roychoudhury[1,4]†, Lea M. Starita[2,3]†, Michael Famulare[5]†, Meei-Li Huang[1,4], Arun Nalla[4], Gregory Pepper[4], Adam Reinhardt[4], Hong Xie[4], Lasata Shrestha[4], Truong N. Nguyen[4], Amanda Adler[6], Elisabeth Brandstetter[7], Shari Cho[2,3], Danielle Giroux[3], Peter D. Han[2,3], Kairsten Fay[1], Chris D. Frazar[3], Misja Ilcisin[1], Kirsten Lacombe[6], Jover Lee[1], Anahita Kiavand[2,3], Matthew Richardson[3], Thomas R. Sibley[1], Melissa Truong[2,3], Caitlin R. Wolf[7], Deborah A. Nickerson[2,3], Mark J. Rieder[2,3], Janet A. Englund[2,6,8], The Seattle Flu Study Investigators‡, James Hadfield[1], Emma B. Hodcroft[9,10], John Huddleston[1,11], Louise H. Moncla[1], Nicola F. Müller[1], Richard A. Neher[9,10], Xianding Deng[12], Wei Gu[12], Scot Federman[12], Charles Chiu[12], Jeffrey S. Duchin[7,13], Romesh Gautom[14], Geoff Melly[14], Brian Hiatt[14], Philip Dykema[14], Scott Lindquist[14], Krista Queen[15], Ying Tao[15], Anna Uehara[15], Suxiang Tong[15], Duncan MacCannell[16], Gregory L. Armstrong[16], Geoffrey S. Baird[4], Helen Y. Chu[2,7]§, Jay Shendure[2,3,17]§, Keith R. Jerome[1,4]§



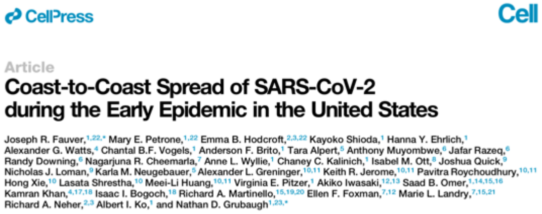


Science REPORTS

Cite as: X. Deng *et al*., *Science*
10.1126/science.abb9263 (2020).

Genomic surveillance reveals multiple introductions of SARS-CoV-2 into Northern California

Xianding Deng[1,2]*, Wei Gu[1,2]*, Scot Federman[1,2]*, Louis du Plessis[3]*, Oliver G. Pybus[3], Nuno Faria[3], Candace Wang[1,2], Guixia Yu[1,2], Brian Bushnell[4], Chao-Yang Pan[5], Hugo Guevara[5], Alicia Sotomayor-Gonzalez[1,2], Kelsey Zorn[6], Allan Gopez[1], Venice Servellita[1], Elaine Hsu[1], Steve Miller[1], Trevor Bedford[7,8], Alexander L. Greninger[7,9], Pavitra Roychoudhury[7,9], Lea M. Starita[8,10], Michael Famulare[11], Helen Y. Chu[9,12], Jay Shendure[8,9,13], Keith R. Jerome[7,9], Catie Anderson[14], Karthik Gangavarapu[14], Mark Zeller[14], Emily Spencer[14], Kristian G. Andersen[14], Duncan MacCannell[15], Clinton R. Paden[15], Yan Li[15], Jing Zhang[15], Suxiang Tong[15], Gregory Armstrong[15], Scott Morrow[16], Matthew Willis[17], Bela T. Matyas[18], Sundari Mase[19], Olivia Kasirye[20], Maggie Park[21], Godfred Masinde[22], Curtis Chan[22], Alexander T. Yu[5], Shua J. Chai[5,15], Elsa Villarino[23], Brandon Bonin[23], Debra A. Wadford[5], Charles Y. Chiu[1,2,24]†



CellPress Cell

Article

Coast-to-Coast Spread of SARS-CoV-2 during the Early Epidemic in the United States

Joseph R. Fauver,[1,22,*] Mary E. Petrone,[1,22] Emma B. Hodcroft,[2,3,22] Kayoko Shioda,[1] Hanna Y. Ehrlich,[1] Alexander G. Watts,[4] Chantal B.F. Vogels,[1] Anderson F. Brito,[1] Tara Alpert,[5] Anthony Muyombwe,[6] Jafar Razeq,[6] Randy Downing,[6] Nagarjuna R. Cheemarla,[7] Anne L. Wyllie,[1] Chaney C. Kalinich,[1] Isabel M. Ott,[8] Joshua Quick,[9] Nicholas J. Loman,[9] Karla M. Neugebauer,[5] Alexander L. Greninger,[10,11] Keith R. Jerome,[10,11] Pavitra Roychoudhury,[10,11] Hong Xie,[10] Lasata Shrestha,[10] Meei-Li Huang,[10,11] Virginia E. Pitzer,[1] Akiko Iwasaki,[12,13] Saad B. Omer,[1,14,15,16] Kamran Khan,[4,17,18] Isaac I. Bogoch,[18] Richard A. Martinello,[15,19,20] Ellen F. Foxman,[7,12] Marie L. Landry,[7,15,21] Richard A. Neher,[2,3] Albert I. Ko,[1] and Nathan D. Grubaugh[1,23,*]

Early experience with hospitalized patients

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Covid-19 in Critically Ill Patients in the Seattle Region — Case Series

Pavan K. Bhatraju, M.D., Bijan J. Ghassemieh, M.D., Michelle Nichols, M.D.,
Richard Kim, M.D., Keith R. Jerome, M.D., Arun K. Nalla, Ph.D.,
Alexander L. Greninger, M.D., Sudhakar Pipavath, M.D., Mark M. Wurfel, M.D., Ph.D.,
Laura Evans, M.D., Patricia M. Kritek, M.D., T. Eoin West, M.D., M.P.H.,
Andrew Luks, M.D., Anthony Gerbino, M.D., Chris R. Dale, M.D.,
Jason D. Goldman, M.D., Shane O'Mahony, M.D.,
and Carmen Mikacenic, M.D.

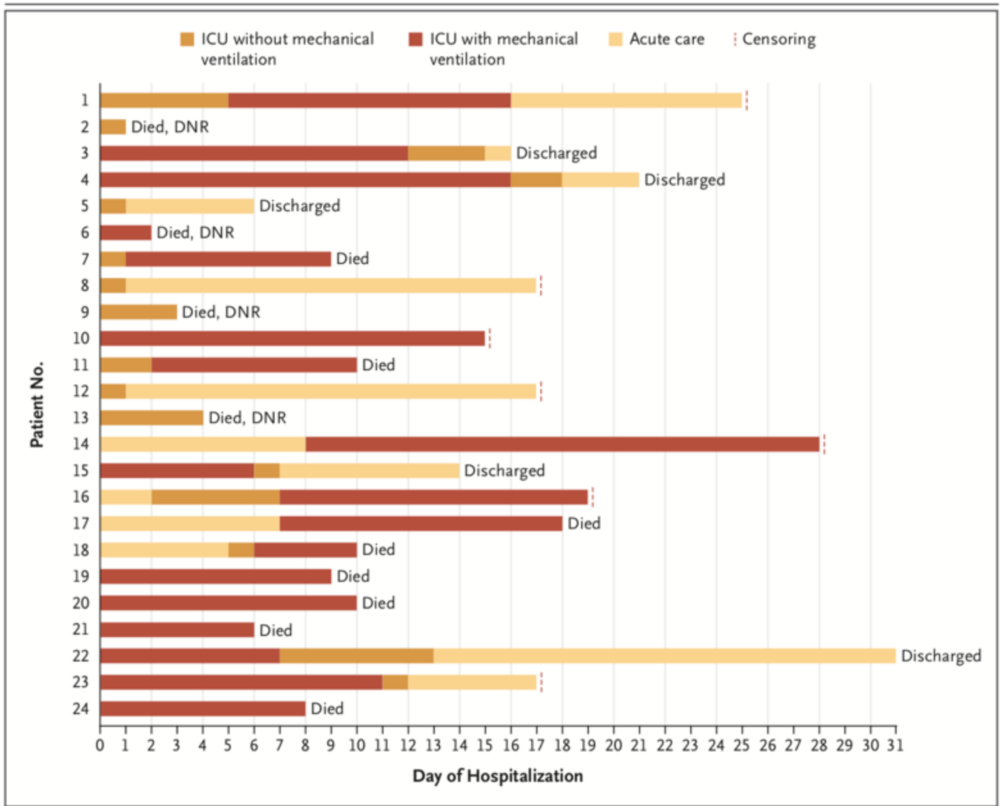
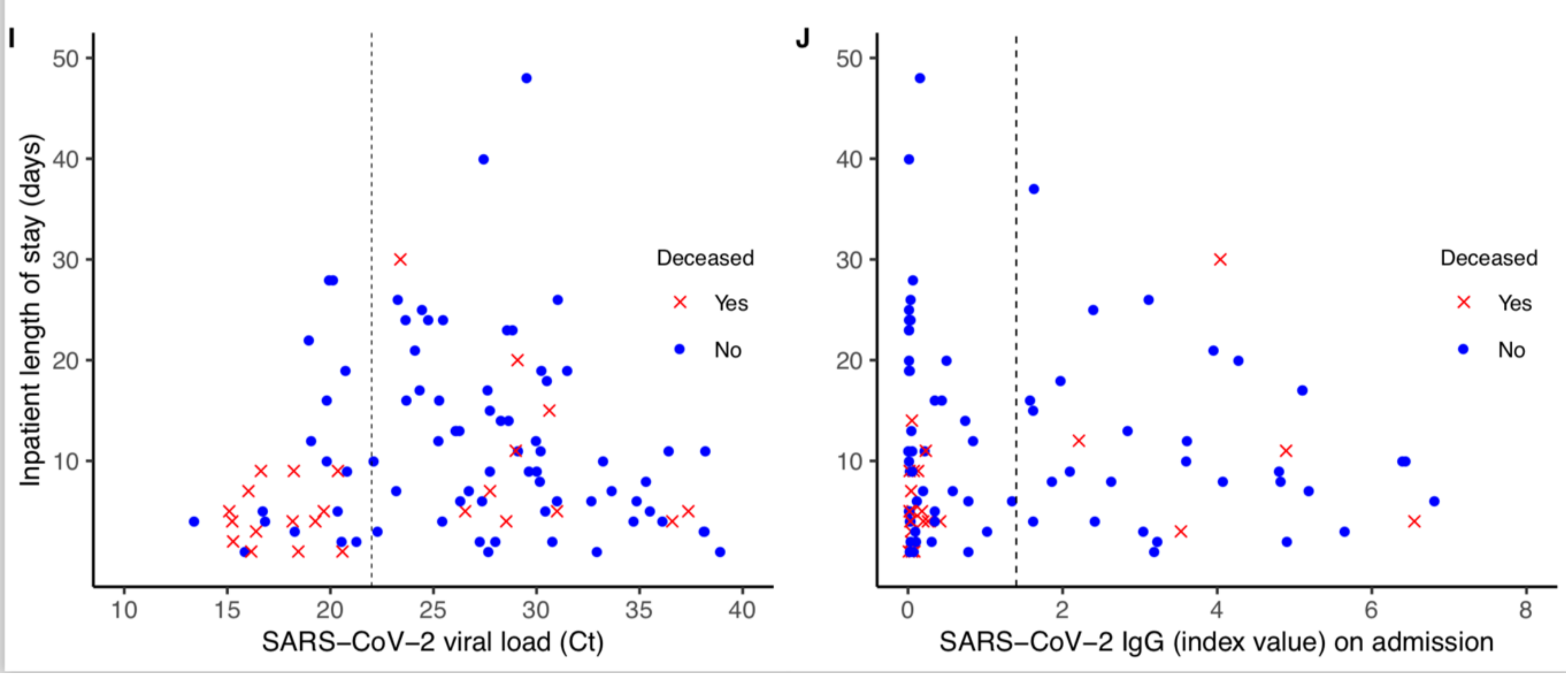


Figure 2. Outcomes for Individual Patients Included in the Case Series.

Do-not-resuscitate (DNR) designates orders that were in place before hospital admission. As of March 23, 2020, a total of 12 patients (50%) had died. Six patients who had received mechanical ventilation had been extubated and three patients remained intubated. Five patients had been discharged from the hospital. All the patients had at least 14 days of follow up. Dashed red lines indicate censoring of data.

Viral load at presentation is an important prognostic factor



EDITOR'S CHOICE

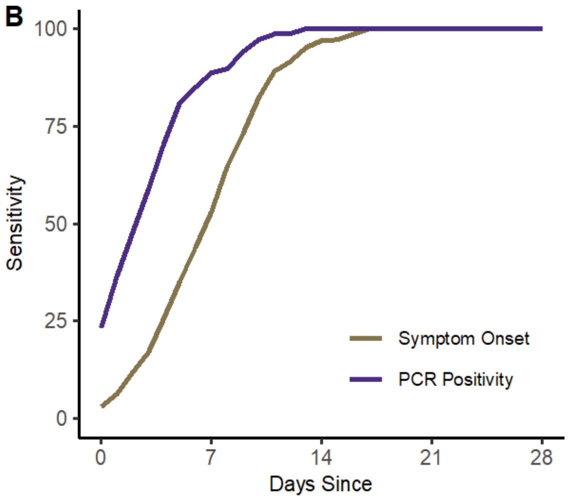
SARS-CoV-2 Viral Load on Admission Is Associated With 30-Day Mortality

Andrew Bryan, Susan L Fink, Meghan A Gattuso, Gregory Pepper, Anu Chaudhary, Mark H Wener, Chihiro Morishima, Keith R Jerome, Patrick C Mathias, Alexander L Greninger

Open Forum Infectious Diseases, Volume 7, Issue 12, December 2020, ofaa535, https://doi-org.offcampus.lib.washington.edu/10.1093/ofid/ofaa535

Antibody (serologic) tests for COVID-19

- Testing for antibody gives a historic record of infection status
- Population-based studies of SARS-CoV-2 seroprevalence
- Inform public health policy/recommendations
- In very select circumstances, as an adjunct to primary diagnosis
- Support of research into immunity and vaccines



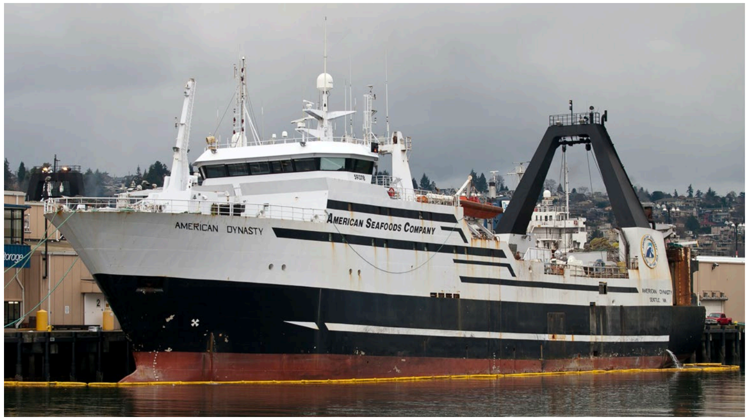
Immunoassays

Performance Characteristics of the Abbott Architect SARS-CoV-2 IgG Assay and Seroprevalence in Boise, Idaho

Andrew Bryan, Gregory Pepper, Mark H. Wener, Susan L. Fink, Chihiro Morishima, Anu Chaudhary, Keith R. Jerome, Patrick C. Mathias, Alexander L. Greninger

DOI: 10.1128/JCM.00941-20

Serologic assays – prediction of protective immunity



NY Times

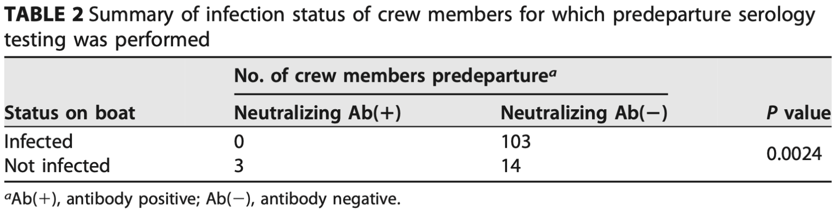
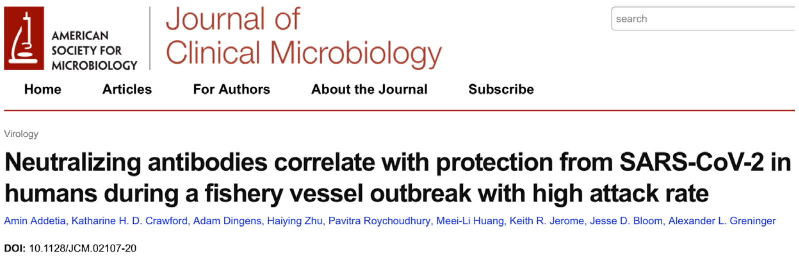


TABLE 2 Summary of infection status of crew members for which predeparture serology testing was performed

	No. of crew members predeparture[a]		
Status on boat	**Neutralizing Ab(+)**	**Neutralizing Ab(−)**	***P* value**
Infected	0	103	0.0024
Not infected	3	14	

[a] Ab(+), antibody positive; Ab(−), antibody negative.



Journal of Clinical Microbiology

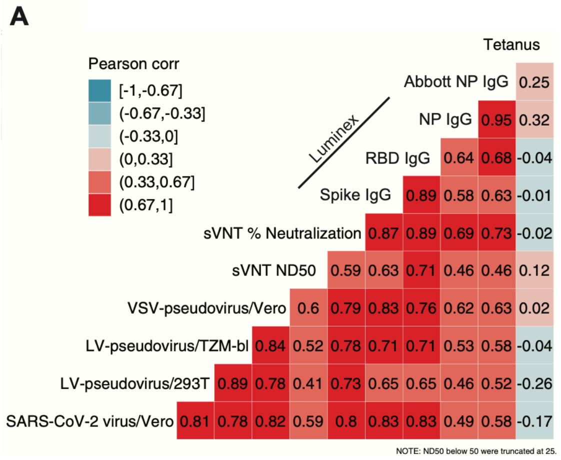
Virology

Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with high attack rate

Amin Addetia, Katharine H. D. Crawford, Adam Dingens, Haiying Zhu, Pavitra Roychoudhury, Meei-Li Huang, Keith R. Jerome, Jesse D. Bloom, Alexander L. Greninger

DOI: 10.1128/JCM.02107-20

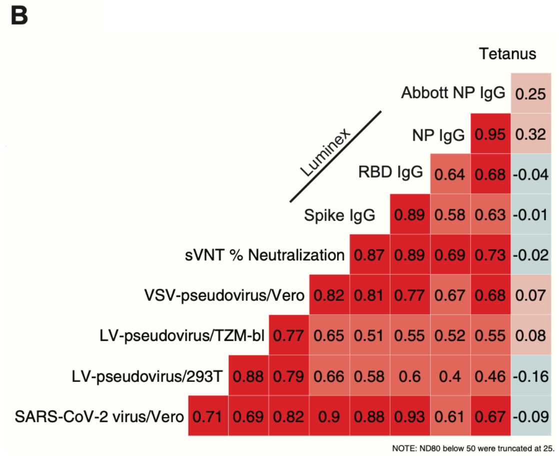
A



Tetanus										
Abbott NP IgG										0.25
NP IgG									0.95	0.32
RBD IgG								0.64	0.68	-0.04
Spike IgG							0.89	0.58	0.63	-0.01
sVNT % Neutralization						0.87	0.89	0.69	0.73	-0.02
sVNT ND50					0.59	0.63	0.71	0.46	0.46	0.12
VSV-pseudovirus/Vero				0.6	0.79	0.83	0.76	0.62	0.63	0.02
LV-pseudovirus/TZM-bl			0.84	0.52	0.78	0.71	0.71	0.53	0.58	-0.04
LV-pseudovirus/293T		0.89	0.78	0.41	0.73	0.65	0.65	0.46	0.52	-0.26
SARS-CoV-2 virus/Vero	0.81	0.78	0.82	0.59	0.8	0.83	0.83	0.49	0.58	-0.17

NOTE: ND50 below 50 were truncated at 25.

B



Tetanus									
Abbott NP IgG									0.25
NP IgG								0.95	0.32
RBD IgG							0.64	0.68	-0.04
Spike IgG						0.89	0.58	0.63	-0.01
sVNT % Neutralization					0.87	0.89	0.69	0.73	-0.02
VSV-pseudovirus/Vero				0.82	0.81	0.77	0.67	0.68	0.07
LV-pseudovirus/TZM-bl			0.77	0.65	0.51	0.55	0.52	0.55	0.08
LV-pseudovirus/293T		0.88	0.79	0.66	0.58	0.6	0.4	0.46	-0.16
SARS-CoV-2 virus/Vero	0.71	0.69	0.82	0.9	0.88	0.93	0.61	0.67	-0.09

NOTE: ND80 below 50 were truncated at 25.

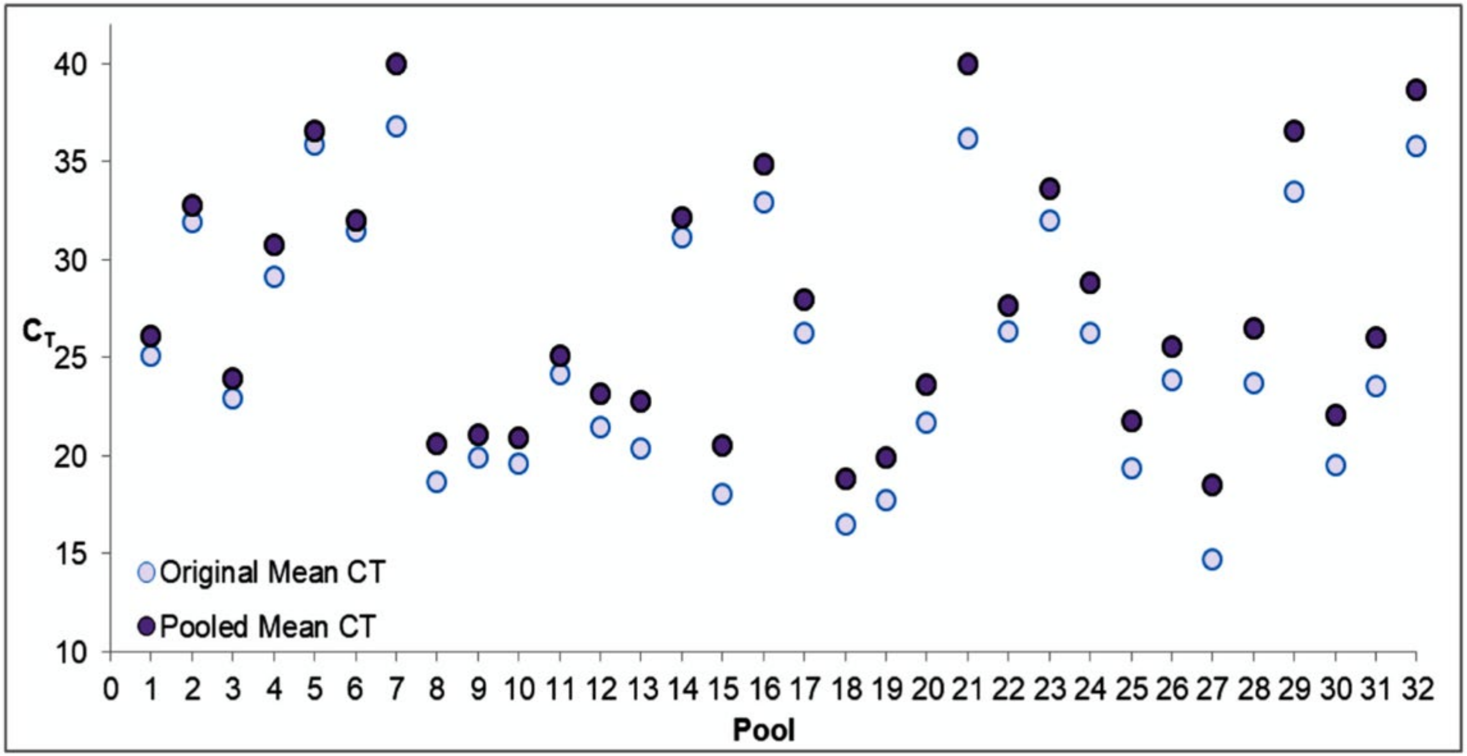
› medRxiv. 2020 Dec 8;2020.12.07.20245431. doi: 10.1101/2020.12.07.20245431. Preprint

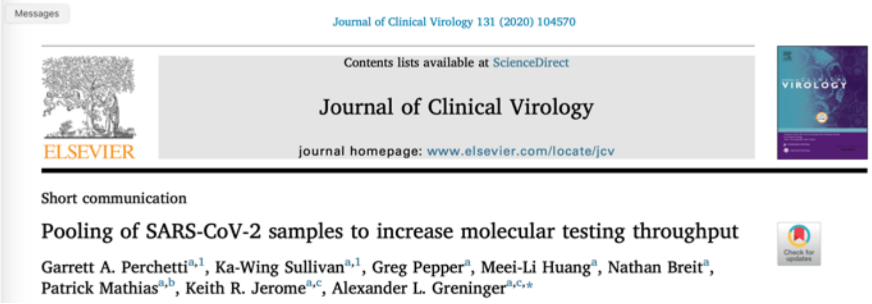
Evaluation of SARS-CoV-2 neutralization assays for antibody monitoring in natural infection and vaccine trials

Anton M Sholukh, Andrew Fiore-Gartland, Emily S Ford, Yixuan Hou, Longping Victor Tse, Florian A Lempp, Hanna Kaiser, Russell Saint Germain, Emily Bossard, Jia Jin Kee, Kurt Diem, Andrew B Stuart, Peter B Rupert, Chance Brock, Matthew Buerger, Margaret K Doll, April Kaur Randhawa, Leonidas Stamatatos, Roland K Strong, Colleen McLaughlin, Keith R Jerome, Ralph S Baric, David Montefiori, Lawrence Corey

PMID: 33330875 PMCID: PMC7743084 DOI: 10.1101/2020.12.07.20245431

Expanding access to COVID testing: sample pooling





Journal of Clinical Virology 131 (2020) 104570

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

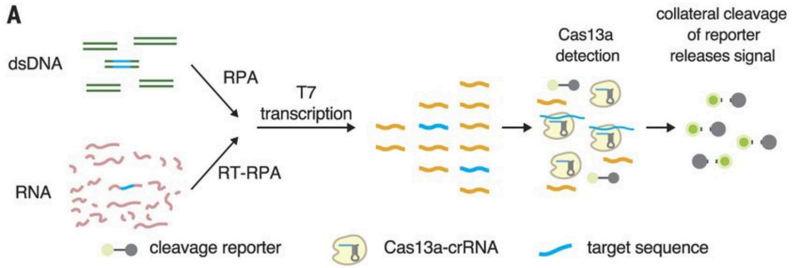
Short communication

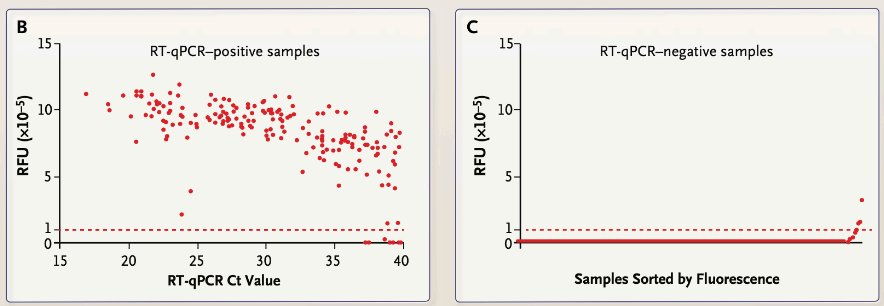
Pooling of SARS-CoV-2 samples to increase molecular testing throughput

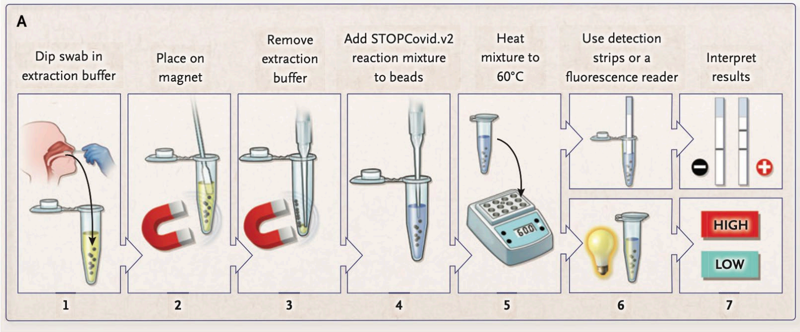
Garrett A. Perchetti[a,1], Ka-Wing Sullivan[a,1], Greg Pepper[a], Meei-Li Huang[a], Nathan Breit[a], Patrick Mathias[a,b], Keith R. Jerome[a,c], Alexander L. Greninger[a,c,*]

Expanding access to COVID testing: CRISPR-based methods

SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing







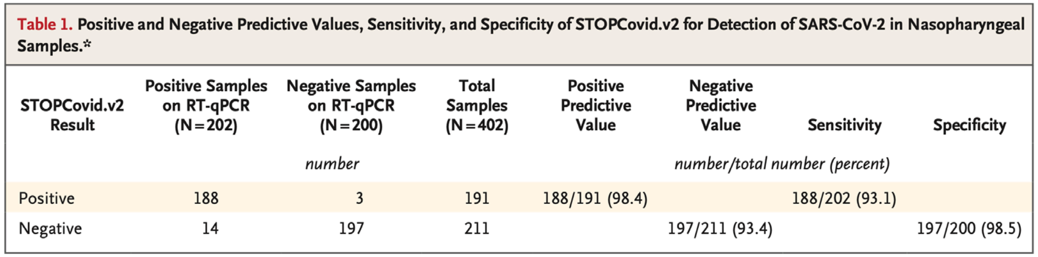


Table 1. Positive and Negative Predictive Values, Sensitivity, and Specificity of STOPCovid.v2 for Detection of SARS-CoV-2 in Nasopharyngeal Samples.*

STOPCovid.v2 Result	Positive Samples on RT-qPCR (N=202)	Negative Samples on RT-qPCR (N=200)	Total Samples (N=402)	Positive Predictive Value	Negative Predictive Value	Sensitivity	Specificity
	number			number/total number (percent)			
Positive	188	3	191	188/191 (98.4)		188/202 (93.1)	
Negative	14	197	211		197/211 (93.4)		197/200 (98.5)

STOP (SHERLOCK Testing in One Pot)-COVID

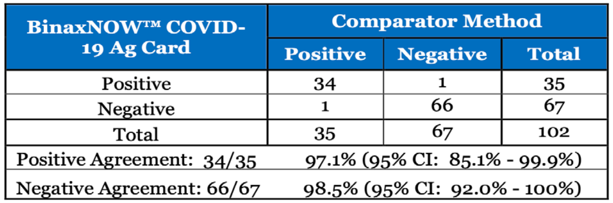
- Loop-mediated isothermal amplification
- Cas12b detection
- 40-70 minutes (fluorescence vs lateral flow)

> N Engl J Med. 2020 Oct 8;383(15):1492-1494. doi: 10.1056/NEJMc2026172. Epub 2020 Sep 16.

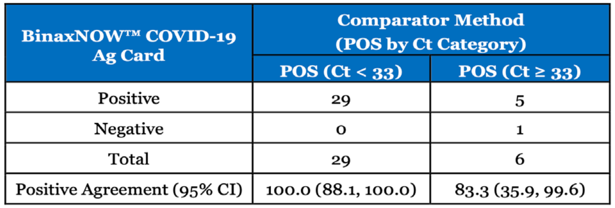
Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing

Julia Joung[1], Alim Ladha[1], Makoto Saito[2], Nam-Gyun Kim[3], Ann E Woolley[4], Michael Segel[2], Robert P J Barretto[5], Amardeep Ranu[6], Rhiannon K Macrae[2], Guilhem Faure[2], Eleonora I Ioannidi[1], Rohan N Krajeski[1], Robert Bruneau[3], Meei-Li W Huang[3], Xu G Yu[7], Jonathan Z Li[4], Bruce D Walker[7], Deborah T Hung[2], Alexander L Greninger[3], Keith R Jerome[8], Jonathan S Gootenberg[9], Omar O Abudayyeh[9], Feng Zhang[10]

Expanding access to COVID testing: antigen testing



BinaxNOW™ COVID-19 Ag Card	Comparator Method		
	Positive	Negative	Total
Positive	34	1	35
Negative	1	66	67
Total	35	67	102
Positive Agreement: 34/35	97.1% (95% CI: 85.1% - 99.9%)		
Negative Agreement: 66/67	98.5% (95% CI: 92.0% - 100%)		



BinaxNOW™ COVID-19 Ag Card	Comparator Method (POS by Ct Category)	
	POS (Ct < 33)	POS (Ct ≥ 33)
Positive	29	5
Negative	0	1
Total	29	6
Positive Agreement (95% CI)	100.0 (88.1, 100.0)	83.3 (35.9, 99.6)

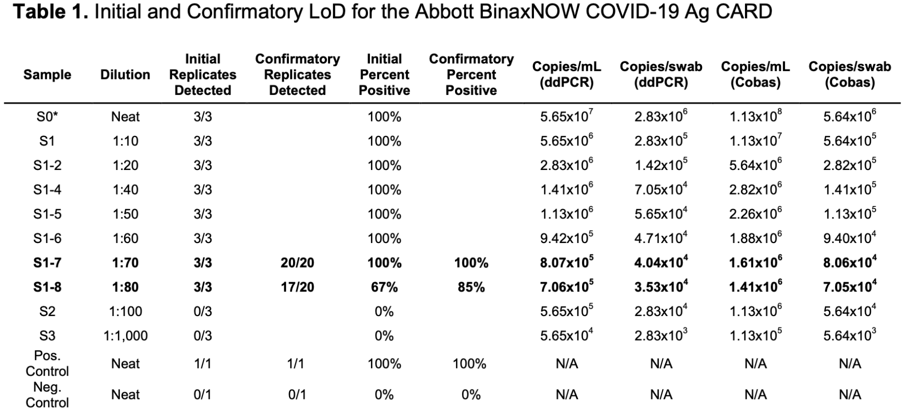


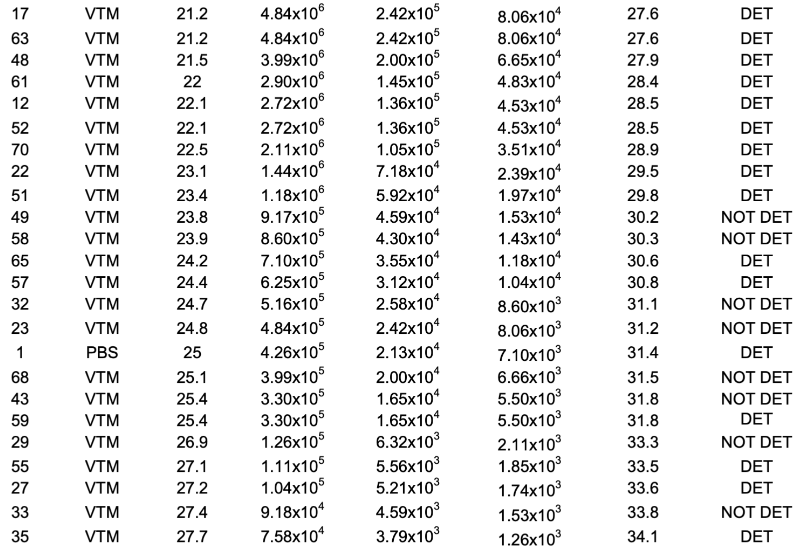
Table 1. Initial and Confirmatory LoD for the Abbott BinaxNOW COVID-19 Ag CARD

Sample	Dilution	Initial Replicates Detected	Confirmatory Replicates Detected	Initial Percent Positive	Confirmatory Percent Positive	Copies/mL (ddPCR)	Copies/swab (ddPCR)	Copies/mL (Cobas)	Copies/swab (Cobas)
S0*	Neat	3/3		100%		$5.65x10^{7}$	$2.83x10^{6}$	$1.13x10^{8}$	$5.64x10^{6}$
S1	1:10	3/3		100%		$5.65x10^{6}$	$2.83x10^{5}$	$1.13x10^{7}$	$5.64x10^{5}$
S1-2	1:20	3/3		100%		$2.83x10^{6}$	$1.42x10^{5}$	$5.64x10^{6}$	$2.82x10^{5}$
S1-4	1:40	3/3		100%		$1.41x10^{6}$	$7.05x10^{4}$	$2.82x10^{6}$	$1.41x10^{5}$
S1-5	1:50	3/3		100%		$1.13x10^{6}$	$5.65x10^{4}$	$2.26x10^{6}$	$1.13x10^{5}$
S1-6	1:60	3/3		100%		$9.42x10^{5}$	$4.71x10^{4}$	$1.88x10^{6}$	$9.40x10^{4}$
S1-7	**1:70**	**3/3**	**20/20**	**100%**	**100%**	**$8.07x10^{5}$**	**$4.04x10^{4}$**	**$1.61x10^{6}$**	**$8.06x10^{4}$**
S1-8	**1:80**	**3/3**	**17/20**	**67%**	**85%**	**$7.06x10^{5}$**	**$3.53x10^{4}$**	**$1.41x10^{6}$**	**$7.05x10^{4}$**
S2	1:100	0/3		0%		$5.65x10^{5}$	$2.83x10^{4}$	$1.13x10^{6}$	$5.64x10^{4}$
S3	1:1,000	0/3		0%		$5.65x10^{4}$	$2.83x10^{3}$	$1.13x10^{5}$	$5.64x10^{3}$
Pos. Control	Neat	1/1	1/1	100%	100%	N/A	N/A	N/A	N/A
Neg. Control	Neat	0/1	0/1	0%	0%	N/A	N/A	N/A	N/A

> J Clin Microbiol. 2020 Dec 11;JCM.02880-20. doi: 10.1128/JCM.02880-20. Online ahead of print.

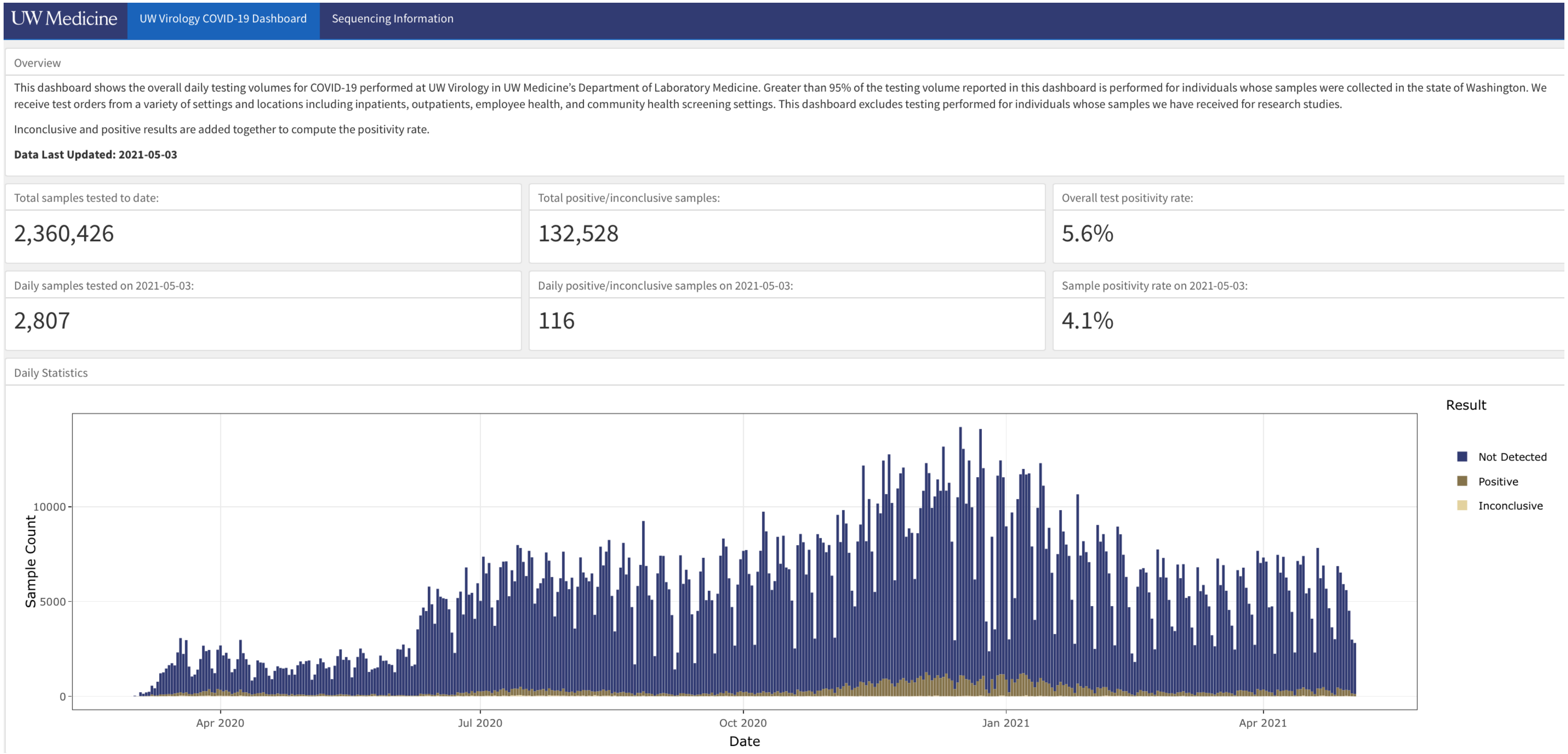
Analytical Sensitivity of the Abbott BinaxNOW COVID-19 Ag CARD

Garrett A Perchetti [1], Meei-Li Huang [1], Margaret G Mills [1], Keith R Jerome [1] [2], Alexander L Greninger [3] [2]



17	VTM	21.2	$4.84x10^{6}$	$2.42x10^{5}$	$8.06x10^{4}$	27.6	DET
63	VTM	21.2	$4.84x10^{6}$	$2.42x10^{5}$	$8.06x10^{4}$	27.6	DET
48	VTM	21.5	$3.99x10^{6}$	$2.00x10^{5}$	$6.65x10^{4}$	27.9	DET
61	VTM	22	$2.90x10^{6}$	$1.45x10^{5}$	$4.83x10^{4}$	28.4	DET
12	VTM	22.1	$2.72x10^{6}$	$1.36x10^{5}$	$4.53x10^{4}$	28.5	DET
52	VTM	22.1	$2.72x10^{6}$	$1.36x10^{5}$	$4.53x10^{4}$	28.5	DET
70	VTM	22.5	$2.11x10^{6}$	$1.05x10^{5}$	$3.51x10^{4}$	28.9	DET
22	VTM	23.1	$1.44x10^{6}$	$7.18x10^{4}$	$2.39x10^{4}$	29.5	DET
51	VTM	23.4	$1.18x10^{6}$	$5.92x10^{4}$	$1.97x10^{4}$	29.8	DET
49	VTM	23.8	$9.17x10^{5}$	$4.59x10^{4}$	$1.53x10^{4}$	30.2	NOT DET
58	VTM	23.9	$8.60x10^{5}$	$4.30x10^{4}$	$1.43x10^{4}$	30.3	NOT DET
65	VTM	24.2	$7.10x10^{5}$	$3.55x10^{4}$	$1.18x10^{4}$	30.6	DET
57	VTM	24.4	$6.25x10^{5}$	$3.12x10^{4}$	$1.04x10^{4}$	30.8	DET
32	VTM	24.7	$5.16x10^{5}$	$2.58x10^{4}$	$8.60x10^{3}$	31.1	NOT DET
23	VTM	24.8	$4.84x10^{5}$	$2.42x10^{4}$	$8.06x10^{3}$	31.2	NOT DET
1	PBS	25	$4.26x10^{5}$	$2.13x10^{4}$	$7.10x10^{3}$	31.4	DET
68	VTM	25.1	$3.99x10^{5}$	$2.00x10^{4}$	$6.66x10^{3}$	31.5	NOT DET
43	VTM	25.4	$3.30x10^{5}$	$1.65x10^{4}$	$5.50x10^{3}$	31.8	NOT DET
59	VTM	25.4	$3.30x10^{5}$	$1.65x10^{4}$	$5.50x10^{3}$	31.8	DET
29	VTM	26.9	$1.26x10^{5}$	$6.32x10^{3}$	$2.11x10^{3}$	33.3	NOT DET
55	VTM	27.1	$1.11x10^{5}$	$5.56x10^{3}$	$1.85x10^{3}$	33.5	DET
27	VTM	27.2	$1.04x10^{5}$	$5.21x10^{3}$	$1.74x10^{3}$	33.6	DET
33	VTM	27.4	$9.18x10^{4}$	$4.59x10^{3}$	$1.53x10^{3}$	33.8	NOT DET
35	VTM	27.7	$7.58x10^{4}$	$3.79x10^{3}$	$1.26x10^{3}$	34.1	DET
38	VTM	27.7	$7.58x10^{4}$	$3.79x10^{3}$	$1.26x10^{3}$	34.1	DET

We’re not done with SARS-CoV-2



Overview

This dashboard shows the overall daily testing volumes for COVID-19 performed at UW Virology in UW Medicine’s Department of Laboratory Medicine. Greater than 95% of the testing volume reported in this dashboard is performed for individuals whose samples were collected in the state of Washington. We receive test orders from a variety of settings and locations including inpatients, outpatients, employee health, and community health screening settings. This dashboard excludes testing performed for individuals whose samples we have received for research studies.

Inconclusive and positive results are added together to compute the positivity rate.

Data Last Updated: 2021-05-03

Total samples tested to date:	Total positive/inconclusive samples:	Overall test positivity rate:
2,360,426	132,528	5.6%
Daily samples tested on 2021-05-03:	Daily positive/inconclusive samples on 2021-05-03:	Sample positivity rate on 2021-05-03:
2,807	116	4.1%

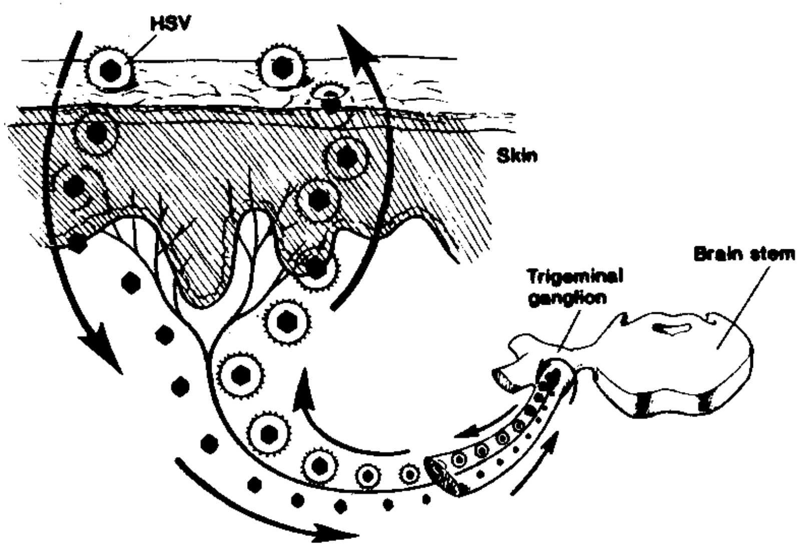
Daily Statistics

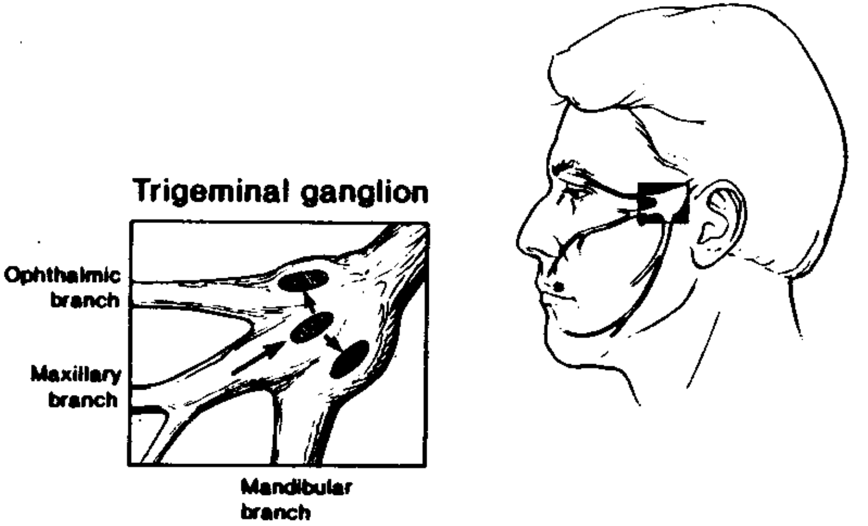
The future of the fight against SARS-CoV-2

- Variants continue to arise and account for an increasing proportion of infections – UW Virology is now sequencing essentially all positives
- Demand for RT-PCR and other types of direct testing likely to continue as school and economic activity resumes, and as additional waves of infection occur
- Demand for serology likely to increase now that data is available that positivity correlates with protection from disease
- Therapeutic pipeline is uncertain (late diagnosis, substantial immunopathological component)
- Vaccines are highly protective, including against variants, but “herd immunity” many prove an elusive goal

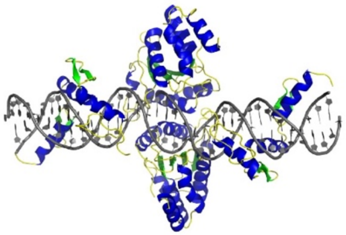
Responding to a (very) old global viral pandemic

- HSV-1 infected our hominid ancestors about 6M years ago (before we diverged from chimps)
- HSV-2 was transferred from ancient chimpanzees to our ancestors (Homo erectus) ~1.2 million years ago
- HSV-1 seroprevalence >50%; HSV-2 ~14% (US)
- Establishes lifelong latency in the trigeminal or dorsal root ganglia
- Recurrent orofacial or anogenital ulceration, encephalitis, keratitis, neonatal disease
- Clinical recurrences range from almost 0 to >12/year
- Current therapies suppress the virus, but do not cure because of latent virus
- Vaccine efforts have been unsuccessful



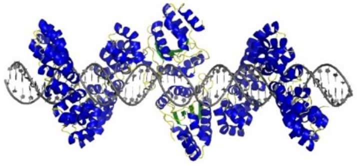


Our toolbox: gene-editing enzymes



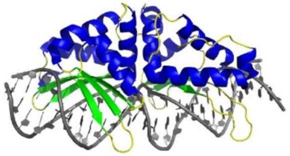
Zinc finger nuclease

2 ORFs
~1.6 kb coding sequence
Leaves 5' overhangs
Relatively simple retargeting
Moderately high specificity
Moderately difficult vectorization/delivery



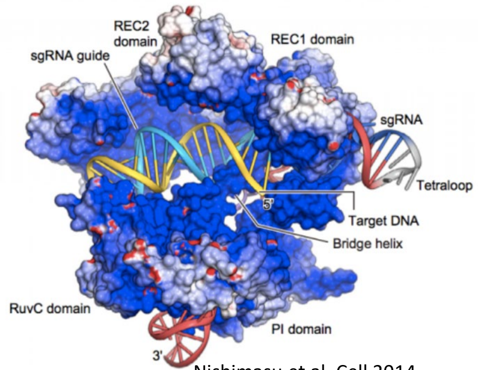
TAL effector nuclease

2 ORFs
~2.7 kb coding sequence
Leaves 5’ overhangs
Very simple retargeting
High specificity
Difficult vectorization/delivery



Meganuclease

1 ORFs
~0.8 kb coding sequence
Leaves 3’ overhangs
More difficult retargeting
High specificity
Easiest vectorization/delivery



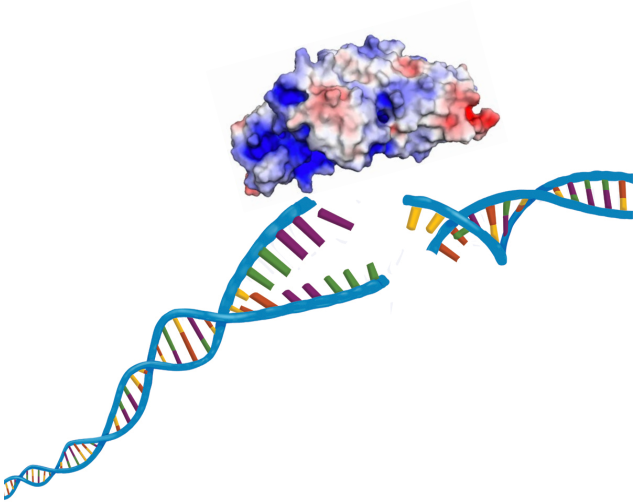
Nishimasu et al, Cell 2014

CRISPR/Cas

1 ORF + guide RNA
>3.3 kb coding sequence
Leaves blunt ends
Trivial retargeting
Specificity controversial
Difficult vectorization

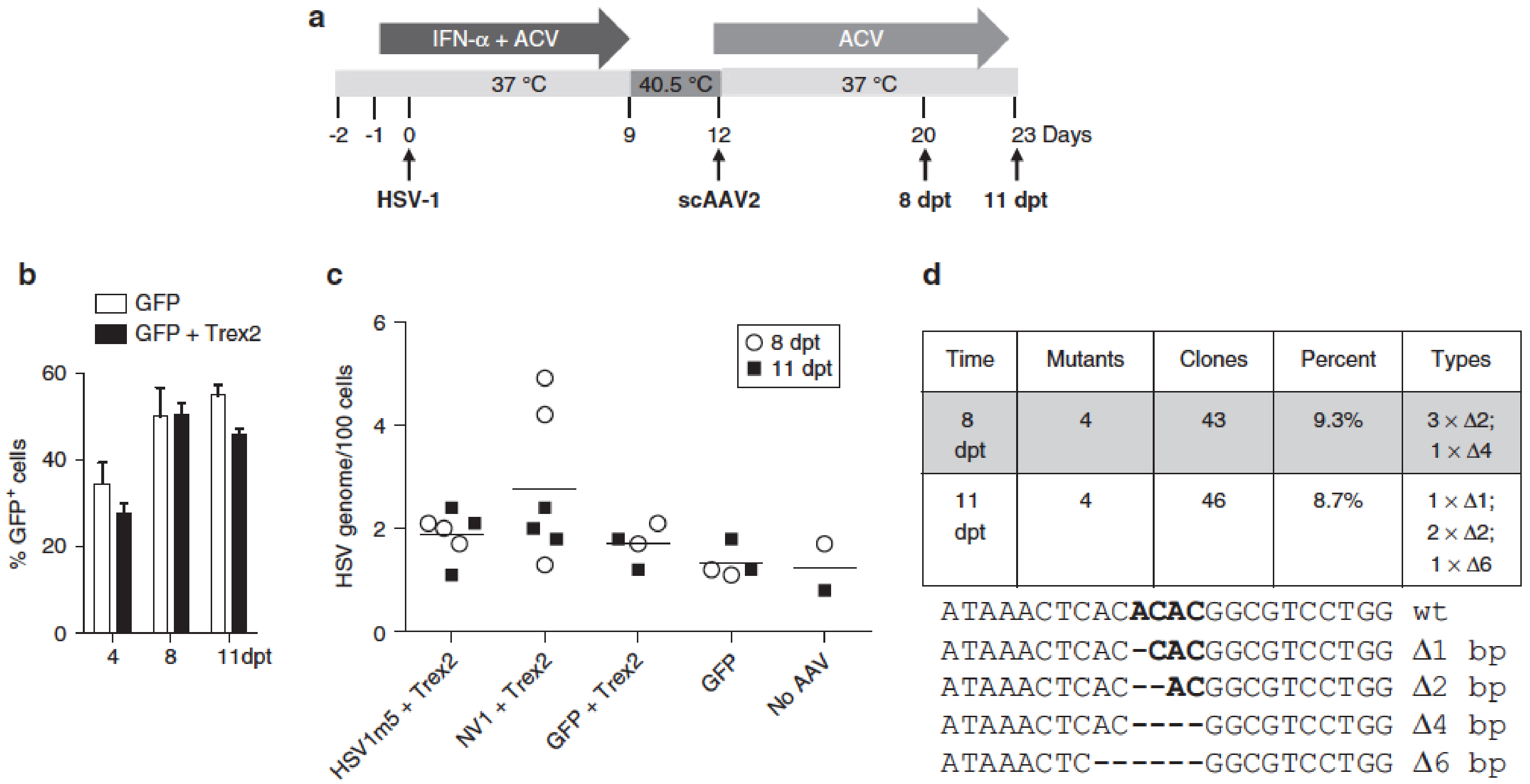
modified from Schiffer et al, J Virol 2012

Our meganucleases target HSV



- Once they enter a cell, our meganucleases search for a very specific sequence in the HSV genome
- If they find it, they cut the DNA there
- Since the HSV sequence is very different from the human genome, human DNA is left alone
- The cell recognizes the break in the HSV genome and repairs it
- This can lead to deletions of part of HSV, knocking out essential viral functions

Meganucleases can edit HSV *in vitro*



Time	Mutants	Clones	Percent	Types
8 dpt	4	43	9.3%	3 × Δ2; 1 × Δ4
11 dpt	4	46	8.7%	1 × Δ1; 2 × Δ2; 1 × Δ6

ATAAACTCAC**ACAC**GGCGTCCTGG wt
ATAAACTCAC-**CAC**GGCGTCCTGG Δ1 bp
ATAAACTCAC--**AC**GGCGTCCTGG Δ2 bp
ATAAACTCAC----GGCGTCCTGG Δ4 bp
ATAAACTC------GGCGTCCTGG Δ6 bp

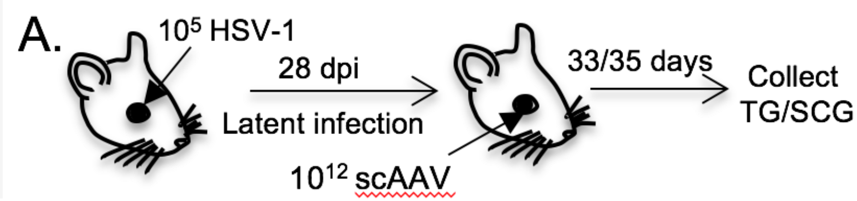
Citation: Molecular Therapy—Nucleic Acids (2014) 3, e146; doi:10.1038/mtna.2013.75

www.nature.com/mtna

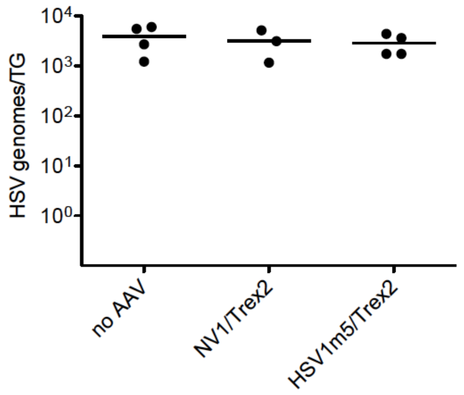
In vitro Inactivation of Latent HSV by Targeted Mutagenesis Using an HSV-specific Homing Endonuclease

Martine Aubert[1], Nicole M Boyle[1], Daniel Stone[1], Laurence Stensland[2], Meei-Li Huang[2], Amalia S Magaret[1,2], Roman Galetto[3], David J Rawlings[4–6], Andrew M Scharenberg[3–6], and Keith R Jerome[1,2]

In vivo mutagenesis of latent HSV



c. HSV genome quantification



g. Next-Gen Sequencing data

Next-Gen sequencing				Other assays	
Mice	**Treatment**	**deletions**	**Total deletion**	**Clonal sequencing**	**T7E1 assay**
1	PBS	Δ1, Δ2	0.04%	N/A	-
5	NV1/Trex2	Δ1	0.02%	N/A	-
8	HSV1m5/ Trex2	Δ1, Δ2, Δ4, Δ8	**2.05%**	**1.1%**	**1.5%**
9	HSV1m5/ Trex2	Δ1, Δ2	0.07%	0%	-
10	HSV1m5/ Trex2	Δ1, Δ2, Δ4 Δ6, Δ8, Δ11, Δ14	**3.92%**	**2.2%**	**1.4%**
11	HSV1m5/ Trex2	Δ1, Δ2, Δ4 Δ5, Δ6	**2.03%**	0%	-


JCI insight

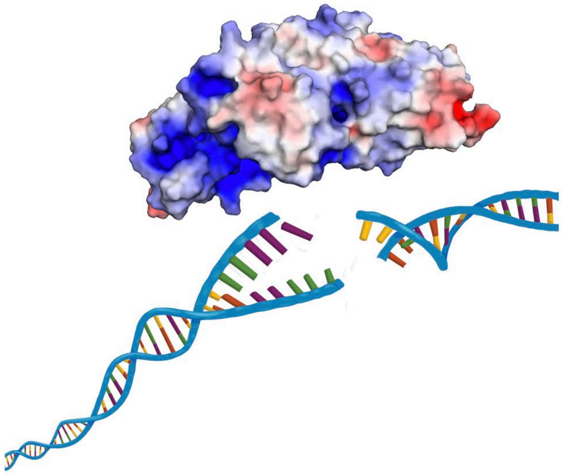
RESEARCH ARTICLE

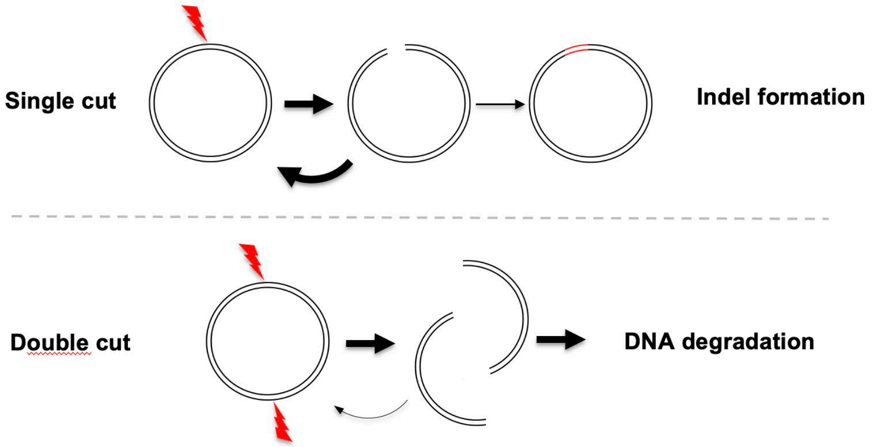
In vivo disruption of latent HSV by designer endonuclease therapy

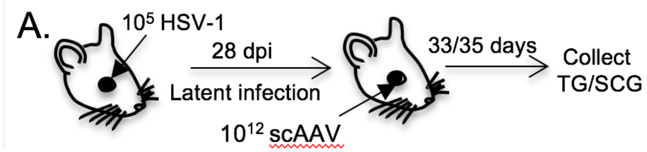
Martine Aubert,[1] Emily A. Madden,[1] Michelle Loprieno,[1] Harshana S. DeSilva Feelixge,[1] Laurence Stensland,[2] Meei-Li Huang,[2] Alexander L. Greninger,[2] Pavitra Roychoudhury,[1] Nixon Niyonzima,[1] Thuy Nguyen,[2] Amalia Magaret,[1,2] Roman Galleto,[3] Daniel Stone,[1] and Keith R. Jerome[1,2]

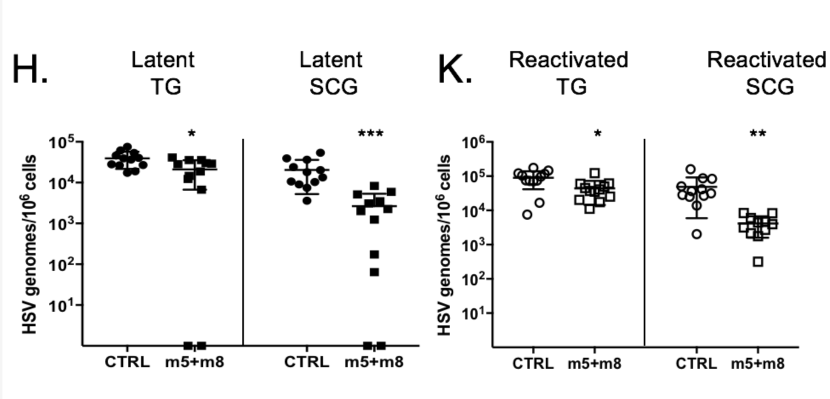
JCI Insight. 2016;1(14):e88468.
doi:10.1172/jci.insight.88468.

Optimized two-MN treatment leads to >90% reduction of HSV genomes in SCG









nature COMMUNICATIONS

ARTICLE

Check for updates

https://doi.org/10.1038/s41467-020-17936-5 OPEN

Gene editing and elimination of latent herpes simplex virus in vivo

Martine Aubert[1], Daniel E. Strongin[2], Pavitra Roychoudhury[2], Michelle A. Loprieno[1], Anoria K. Haick[1], Lindsay M. Klouser[1], Laurence Stensland[2], Meei-Li Huang[2], Negar Makhsous[2], Alexander Tait[2], Harshana S. De Silva Feelixge[1], Roman Galetto[3], Philippe Duchateau[3], Alexander L. Greninger[2], Daniel Stone[1] & Keith R. Jerome[1,2]✉

The future of the fight against HSV

- Increasing the efficacy of the gene editing approach
- More realistic animal models
  - Effect on viral shedding
  - Efficacy against genital infection
- Aiming for human clinical trial in 2023-2024

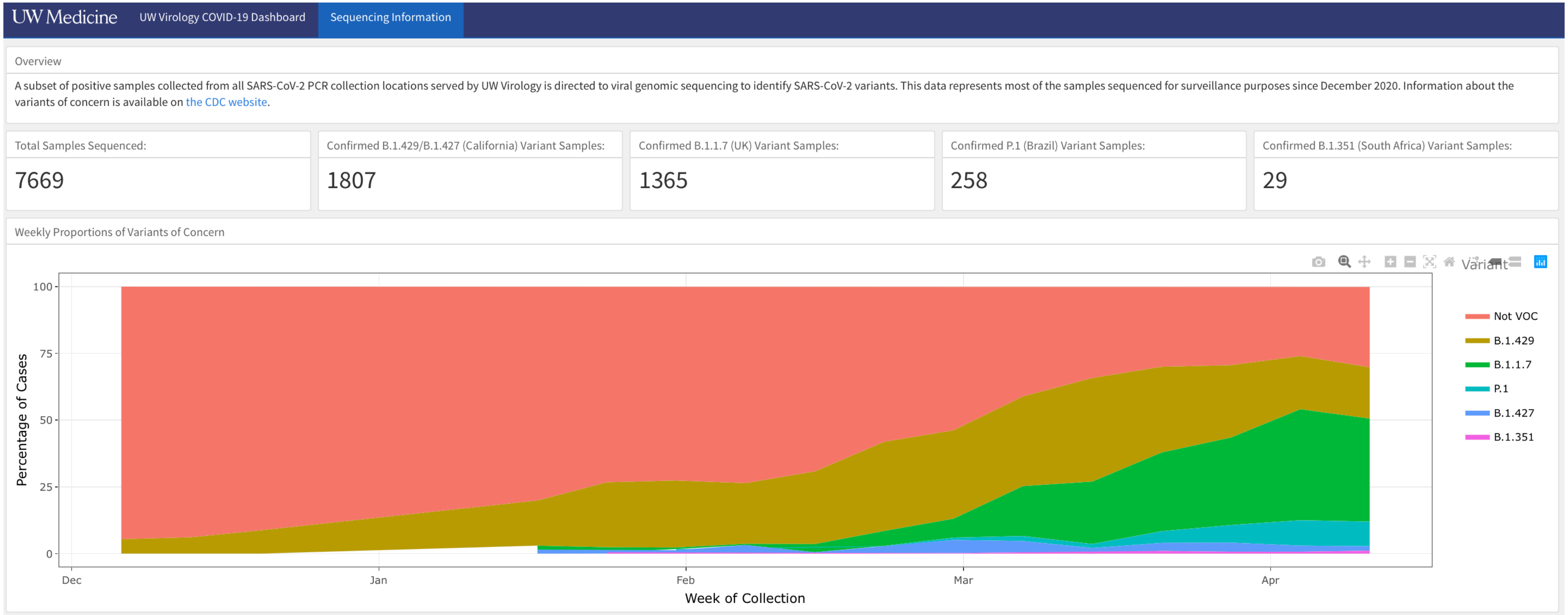
Acknowledgments

UW Virology
Department of Laboratory Medicine and Pathology
UW Medicine
Fred Hutch
many, many collaborators

UW Medicine
LABORATORY MEDICINE
VIROLOGY

FRED HUTCH
CURES START HERE™

UW Virology sequencing – predominant strains



Overview

A subset of positive samples collected from all SARS-CoV-2 PCR collection locations served by UW Virology is directed to viral genomic sequencing to identify SARS-CoV-2 variants. This data represents most of the samples sequenced for surveillance purposes since December 2020. Information about the variants of concern is available on the CDC website.

Total Samples Sequenced:	Confirmed B.1.429/B.1.427 (California) Variant Samples:	Confirmed B.1.1.7 (UK) Variant Samples:	Confirmed P.1 (Brazil) Variant Samples:	Confirmed B.1.351 (South Africa) Variant Samples:
7669	1807	1365	258	29

Weekly Proportions of Variants of Concern