COVID 19 Testing, Surveillance, and Diagnosis:
Notes from a Disease Detective

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UCSF Osher Mini Medical School
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Disclosures

• Abbott Diagnostics (research support for pathogen discovery)
• Mammoth Biosciences, Inc. (scientific advisory board)
Ongoing Studies and Aims

- CRISPR-Cas based diagnostic test for SARS-CoV-2
  - Faster, cheaper, portable, and scalable while retaining accuracy
  - >10,000 tests a day (?)
  - Point-of-care and “at-home” testing

- Real-time genomic surveillance of SARS-CoV-2 evolution and spread in California
  - to support public health contact tracing and containment efforts
  - to better understand how the virus is spreading in California
  - to track "signature" mutations in the virus over time

- Accelerating serologic and host response based diagnostics for SARS-CoV-2
  - clinical validation data from UCSF patients to support widespread deployment of antibody testing to the U.S. (collaboration with Abbott Laboratories)
  - Identification of host response biomarkers to diagnose SARS-CoV-2 infection at all stages of the disease and predict clinical severity and outcomes
SARS-CoV-2 Testing
Direct Comparison of SARS-CoV-2 Analytical Limits of Detection across Seven Molecular Assays

Fung, et al., 2020, Journal of Clinical Microbiology
Isothermal Methods are better for POC Testing than PCR

<table>
<thead>
<tr>
<th></th>
<th>PCR</th>
<th>LAMP/RPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requires temperature cycling</td>
<td>Requires temperature cycling</td>
<td>Isothermal: 60 – 65 °C</td>
</tr>
<tr>
<td>Slow: Typically &gt;1h</td>
<td>Slow: Typically &gt;1h</td>
<td>Rapid: Typically 10 - 30min</td>
</tr>
<tr>
<td>Yield: ~0.2 mg</td>
<td>Yield: ~0.2 mg</td>
<td>Yield: ~10-20 mg</td>
</tr>
<tr>
<td>Not amenable to visual detection</td>
<td>Not amenable to visual detection</td>
<td>Visual detection.</td>
</tr>
<tr>
<td>Sensitive to inhibitors</td>
<td>Sensitive to inhibitors</td>
<td>Tolerant to inhibitors (can bypass RNA extraction)</td>
</tr>
</tbody>
</table>
This Year’s Nobel Prize in Chemistry Honors a Revolution

With Crispr, two scientists turned a curiosity of nature into an invention that will transform the human race.

By Walter Isaacson
Mr. Isaacson is the author of the forthcoming “The Code Breaker: Jennifer Doudna, Gene Editing, and the Future of the Human Race.”

Oct. 7, 2020
CRISPR-Based Diagnostics

(modified from Chiu, 2018, Cell Host & Microbe, 23(6):702-704)
CRISPR–Cas12-based detection of SARS-CoV-2

An outbreak of betacoronavirus severe acute respiratory syndrome (SARS-CoV-2) began in Wuhan, China in December 2019. COVID-19, the disease associated with SARS-CoV-2 infection, rapidly spread to produce a global pandemic. We report development of a rapid (<40 min), easy-to-implement and accurate CRISPR-Cas12-based lateral flow assay for detection of SARS-CoV-2 from respiratory swab RNA extracts. We validated our method using convivted reference samples and clinical samples from patients in the United States, including 36 patients with COVID-19 infection and 42 patients with other viral respiratory infections. Our CRISPR-based DETECTR assay provides a visual and faster alternative to the US Centers for Disease Control and Prevention SARS-CoV-2 real-time RT-PCR assay, with 95% positive predictive agreement and 100% negative predictive agreement. COVID-19 in the United States, on 28 February 2020, the US Food and Drug Administration (FDA) permitted individual clinically licensed laboratories to report the results of in-house-developed SARS-CoV-2 diagnostic assays while awaiting results of an EUA submission for approval.

Here we report the development and initial validation of a CRISPR-Cas12-based assay for detection of SARS-CoV-2 from extracted patient sample RNA, called SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR). This assay performs simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT-LAMP) for RNA extracted from nasopharyngeal or oropharyngeal swabs in universal transport medium (UTM), followed by Cas12 detection of predefined coronavirus sequences, after which cleavage of a reporter molecule confirms detection of the virus. We first designed

Figure 5: Publication in *Nature Biotechnology* by University of California, San Francisco (PI: Chiu) and Mammoth Biosciences on development and validation of a CRISPR-Cas12 based DETECTR assay for SARS-CoV-2 detection.
Accelerated Timeline for Development of CRISPR SARS-CoV-2 Test

(Broughton, et al., 2020, Nature Biotechnology, DOI: 10.1038/s41587-020-0513-4)
High Specificity of SARS-CoV-2 CRISPR Probes

![Graph showing raw fluorescence (AU) for different gRNA samples including N-gene gRNA #1, N-gene gRNA #1 (related species variant), N-gene gRNA #2, N - SARS-CoV-2, N - SARS-CoV, and N - bat-SL-CoVZC45.]

- **N - SARS-CoV-2**: CACAATTTCGCCCCAGCGTCTGAGGTCTCCTC
- **N - SARS-CoV**: cacaattttgctccaatgtgcctgtgcctctcttcttgg
- **N - bat-SL-CoVZC45**: cacaattttgctccaatgtgcctgtgcctctctttgg

**PAM**

- **N-gene #1 gRNA**: Compatible with CDC-N2 amplicon, used in final assay
- **N-gene #2 gRNA**: Compatible with WHO N-Sarbeco amplicon
A CRISPR Based Test for SARS-CoV-2

(Broughton, et al., 2020, Nature Biotechnology, DOI: 10.1038/s41587-020-0513-4)
Collaboration with Mammoth Biosciences, Inc.

**LoD:** 20 copies/mL
**Sensitivity:** 38/40 = 95%
**Specificity:** 100%

**UCSF FDA EUA obtained July 10th, 2020**

**FDA Grants EUA for UCSF, Broad Institute, BioSewoom Coronavirus Tests**

Jul 10, 2020 | staff reporter

NEW YORK — The US Food and Drug Administration this week granted separate Emergency Use Authorizations for a CRISPR-based SARS-CoV-2 test developed by the University of California, San Francisco and Mammoth Biosciences, and two PCR-based tests from the Broad Institute and BioSewoom.

The SARS-CoV-2 RNA DETECTR Assay from UCSF and Mammoth uses primer sets and CRISPR guide RNAs specific for the detection of the SARS-CoV-2 N gene. It performs reverse transcription and isothermal amplification, followed by Cas-12-mediated cleavage of the amplified product to confirm detection of the virus.

Collaboration with Mammoth Biosciences, Inc.
Assay Optimization to Accelerate Throughput

5-min lysis and 95°C heat inactivation

Multiplex targets

384-well plates

(Servellita, et al., 2020, manuscript in preparation)
• Collect samples. The assay works with both respiratory swab (nasal, nasopharyngeal, oropharyngeal) or saliva samples.

• Aliquot samples directly into a 96-well plate pre-loaded with the reagents. This step can be automated with robotics. No pre-processing is required!

• Incubate the plate in a real-time PCR machine for 30 minutes to read the fluorescent signal and report results.
Workflow

One-pot
High-throughput
Compatible with robots
One-Pot LAMP / CRISPR Assay with the Sensitivity of qRT-PCR

- **qRT-PCR**
- **Real-time Quantitative Readout**
  - *Cyto9 dye*
- **End-point Qualitative Readout**
  - *FAM dye, CRISPR*
Assay Works with Saliva as well as Swab Samples
<table>
<thead>
<tr>
<th>Category</th>
<th>Feature</th>
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<tbody>
<tr>
<td>Accuracy</td>
<td>Comparable to RT-qPCR at 4 viral copies (genome equivalents) per reaction</td>
</tr>
<tr>
<td>Cost</td>
<td>Inexpensive at about $10 per test</td>
</tr>
<tr>
<td>Ease of Use</td>
<td>Simple and automatable laboratory workflow</td>
</tr>
<tr>
<td>Frequency</td>
<td>&gt;90 samples / run with the use of 96-well plates with external controls</td>
</tr>
<tr>
<td>Performance</td>
<td>“One pot” solution; shows better tolerance to inhibitors than RT-PCR</td>
</tr>
<tr>
<td>Speed</td>
<td>Returns reportable results in approximately 30 minutes</td>
</tr>
</tbody>
</table>
Digital Droplet PCR Accurately Quantifies SARS-CoV-2 Viral Load From Crude Lysate without Nucleic Acid Purification

Vasudevan, et al., 2020, Scientific Reports, in press
POC-ready Workflow for ddLAMP SARS-Cov-2 detection

30 min INCUBATION in 65 °C

Courtesy of the Abate Lab
COVID ddLAMP specificity

 Courtesy of the Abate Lab
COVID ddLAMP sensitivity

EVO

NT-control  Sample 6, 10^-6  Sample 5, 10^-5  Sample 4, 10^-4  Sample 3, 10^-3  Sample 2, 10^-2

iPhone

NT-control  Sample 6, 10^-6  Sample 5, 10^-5  Sample 4, 10^-4  Sample 3, 10^-3  Sample 2, 10^-2

Courtesy of the Abate Lab
COVID ddLAMP sensitivity - EVO vs. iPhone-base readout

Courtesy of the Abate Lab
SARS-CoV-2 Genomic Sequencing
Genomic surveillance reveals multiple introductions of SARS-CoV-2 into Northern California

Epidemic in Northern California

Genome sequencing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreaks is valuable for tracing the sources and perhaps for drawing lessons about preventing future outbreaks. Genomic analysis by Deng et al. revealed that Northern California experienced a complex series of introductions of the virus, deriving not only from state-to-state transmission but also from international travel by air and ship. The study highlights the importance of being able to rapidly test and trace contacts of positive cases to enable swift control.

Science, this issue p. 582
Genomic Surveillance Reveals Multiple Introductions of SARS-CoV-2 into Northern California

Deng, et al., 2020, Science
Metagenomic Sequencing with Spiked Primer Enrichment (MSSPE)

Genomic Study Design and Sampling of Northern California Counties

(Deng, et al., 2020, Science)
(Deng, et al., 2020, Science)
January 19th, 2020

First Case of 2019 Novel Coronavirus in the United States


Fremont Bulletin

Coronavirus: 103 Grand Princess passengers test positive for coronavirus, amid questions over quarantine

Two passengers who traveled on the Grand Princess cruise ship have died, and at least 103 have been confirmed positive for the new ...

Coast-to-coast spread of SARS-CoV-2 during the early epidemic in the United States


Cryptic transmission of SARS-CoV-2 in Washington State

Trevor Bedford, Alexander S. Greninger, Paivi Roychoudhury, Les M. Starita, Michael Famulare, Mei-Li Huang, Arun Nall, Gregory Pepper, Adam Reinhardt, Hong Xie, Lisa S. Shrestha, Truong N. Nguyen, Amanda Adler, Elisabeth Brandtstrøm, Shari Cho, Danielle Giroux, Peter D. Han, Kiansten Fay, Chris D. Frazier, Maya B. Ricci, Kirsten Louchene, Javer Lee, Anahita Kavard, Matthew Richardson, Thomas C. Slep, Melissa Truong, Caitlin R. Wolf, Deborah A. Nickerson, Mark J. Rieder, Janet E. Anglund, the Seattle Flu Study Investigators, James Huffman, Emma B. Hodcroft, John Huddleston, Louise H. Ponsa, Nicolas F. Müller, Richard A. Neher, Xianding Deng, Wei Gu, Scott Federman, Charles Chiu, Jeff Duchi, Ramesh Gautam, Geoff M. Moly, Brian Hant, Philip Dykema, Scott Lindquist, Krisa Queen, Ying Tao, Anns Ushara, Sivagami Yong, Duncan MacCannell, Gregory L. Armstrong, Geoffrey S. Baird, Helen Y. Chu, Jay Shendure, Keith R. Jerome

doi: https://doi.org/10.1101/2020.04.02.20051417

(Deng, et al., 2020, Science)
C.D.C. Confirms First Possible Community Transmission of Coronavirus in U.S.

A case in California may be the first infection without a known link to travel abroad.
Early Dynamics of SARS-CoV-2 Spread in Santa Clara Revealed by Genomic Epidemiology

Korber, et al., 2020, Cell

(Villarino, et al., 2020, manuscript In preparation; collaboration with Santa Clara Department of Public Health)
Santa Clara County Outbreak

(Villarino, et al., 2020, manuscript In preparation)
Coronavirus Death in California Came Weeks Before First Known U.S. Death

The earliest U.S. deaths publicly attributed to the virus had been on Feb. 26, when two people died in the Seattle area. Santa Clara County said an autopsy showed a Feb. 6 death was also related.

(Villarino, et al., 2020, manuscript In preparation)
Sixteen Strains Introduced into Santa Clara County from January 27 to March 21

(Villarino, et al., 2020, manuscript In preparation)
Fate of Circulating Santa Clara County Strains

(Villarino, et al., 2020, manuscript In preparation)
Fate of Circulating Santa Clara County Strains

(Villarino, et al., 2020, manuscript In preparation)
Associating Early COVID-19 Cases in San Francisco with Domestic and International Travel

Timely Intervention and Control of a Novel Coronavirus (COVID-19) Outbreak at a Large Long-Term Care Facility — San Francisco, California, 2020
(Laguna Honda Hospital Outbreak)

Kamarkar, et al., 2020, in preparation; collaboration with California Department of Public Health

(Kamarkar, et al., 2020, manuscript In preparation; collaboration with California Department of Public Health)
Explosive Growth of the D614G Strain in San Bernardino, Southern California, Mar-Apr 2020

(Deng, et al., 2020, manuscript in preparation)
SPHERES (Sequencing for Public Health Emergency Response, Epidemiology and Surveillance) Consortium

21 State Public Health Departments
17 County Public Health Departments
CDC
NCBI
APHL
and…

(courtesy of Duncan MacCannell, CDC)
Unprecedented participation and support from academia, nonprofits, and the private sector

A growing and incomplete list...

(courtesy of Duncan MacCannell, CDC)
Novel Host-Based Diagnostics for SARS-CoV-2
Goals of Study

• To identify diagnostic or prognostic biomarkers of COVID-19 from nasal swabs and blood

• To uncover differential genes and pathways involved in COVID-19 pathogenesis

• To develop and validate host response diagnostic and severity assays for COVID-19

• To develop a predictive model for COVID-19 severity based on clinical metadata and host response testing
Cell-free DNA

Tissue

Modified from Sookoian and Pirola, 2017
Circulating Cell-Free DNA Reveals Significant Cell, Tissue, and Organ Specific Injury Associated with COVID-19

Cheng, et al., 2020, under review
Collaboration with Iwjin lab, Cornell University
A diagnostic biosignature for SARS-CoV-2 infection from host response RNA
profiling of respiratory swabs and whole blood

Running Title: Host Response of COVID-19 Patients

Authors: Dianna L. Ng\textsuperscript{1,2}, Andrea C. Granados\textsuperscript{2,3}, Yale A. Santos\textsuperscript{2,3}, Venice Servellita\textsuperscript{2,3}, Gregory M. Goldgof\textsuperscript{2}, Cam Meydan\textsuperscript{4,5,6}, Alicia Sotomayor-Gonzalez\textsuperscript{2,3},
Andrew G. Levine\textsuperscript{2}, Joanna Baloerek\textsuperscript{2}, Lucy M. Han\textsuperscript{1}, Naomi Akiyama\textsuperscript{1}, Kent Truong\textsuperscript{1}, Neil M. Neumann\textsuperscript{1}, David N. Nguyen\textsuperscript{7}, Sagar P. Bapat\textsuperscript{2}, Jing Cheng\textsuperscript{8,9}, Claudia Sanchez-San Martin\textsuperscript{2,3}, Scot Federman\textsuperscript{2,3}, Allan Lopez\textsuperscript{2,3}, Tony Li\textsuperscript{10}, Ray Chan\textsuperscript{2}, Cynthia Chu\textsuperscript{2},
Chao-Yang Pan\textsuperscript{11}, Hugo Guevara\textsuperscript{11}, Debra Wadford\textsuperscript{11}, Steve Miller\textsuperscript{2,3}, Christopher Mason\textsuperscript{4,5,12,13}, Charles Chiu\textsuperscript{2,3,7}

Ng, et al., 2020, under review
Differential Signaling Host Pathways between SARS-CoV-2 and Other Infections

Ng, et al., 2020, under review
Overlapping but Heightened Host Responses in More Severely Ill COVID-19 Patients

Ng, et al., 2020, under review
A Two-Layer Classifier for SARS-CoV-2 Diagnosis from Nasal Swabs

Layer 1 Training
- 110 SARS-CoV-2 Confirmed Samples
- 74 Samples for which no pathogen was identified
- AUC of .992 achieved

Layer 2 Training
- 110 SARS-CoV-2 Confirmed samples
- 93 Other Confirmed ARI samples:
  - 9 seasonal Coronavirus
  - 60 Influenza
  - 6 Metapneumovirus
  - 2 Parainfluenza
  - 2 Adenovirus
  - 6 RSV
  - 8 Rhinovirus
- AUC of .998 achieved
# Performance of the Classifier

## Combined Test Results (Layer 1+2)

<table>
<thead>
<tr>
<th></th>
<th>SARS-CoV-2</th>
<th>NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>4</td>
<td>42</td>
</tr>
</tbody>
</table>

Sensitivity: .7857  
Specificity: .9130  
Accuracy: .8649

*For a sample to be considered *positive* for SARS-CoV-2 it must be classified as such by both layers*
Luminex Combinati AbsoluteQ Multiplex Digital PCR Platform

Prep Sample → Load Plate → Run Digital PCR → View Results

Sample prep identical to qPCR

Fixed microchamber array for robust absolute quantification.

Low sample waste design for best-in-class accuracy and precision.

Standard microplate format for easy handling.

Courtesy of Luminex, Inc.