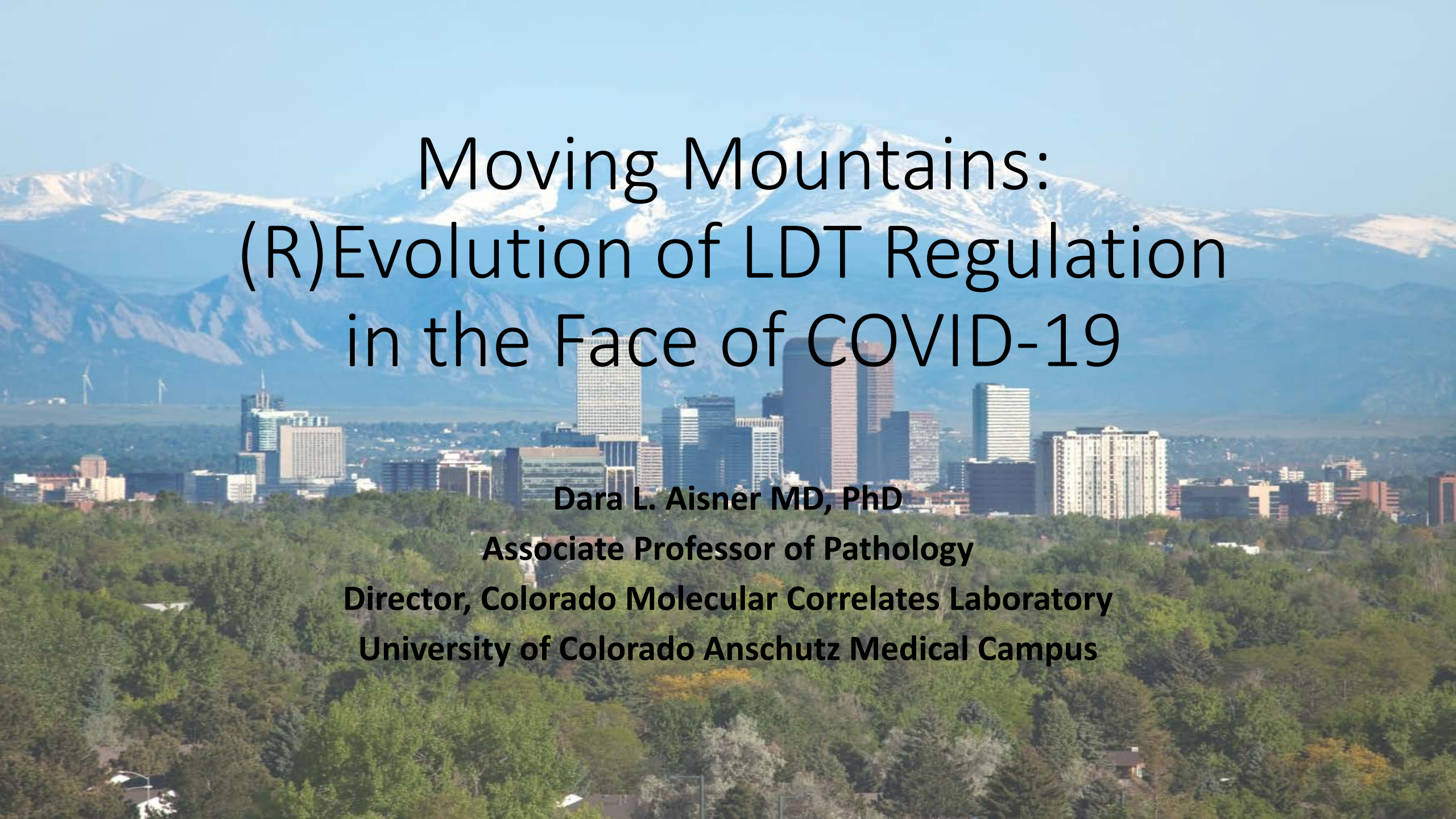


Moving Mountains: (R)Evolution of LDT Regulation in the Face of COVID-19

Dara L. Aisner MD, PhD

Associate Professor of Pathology

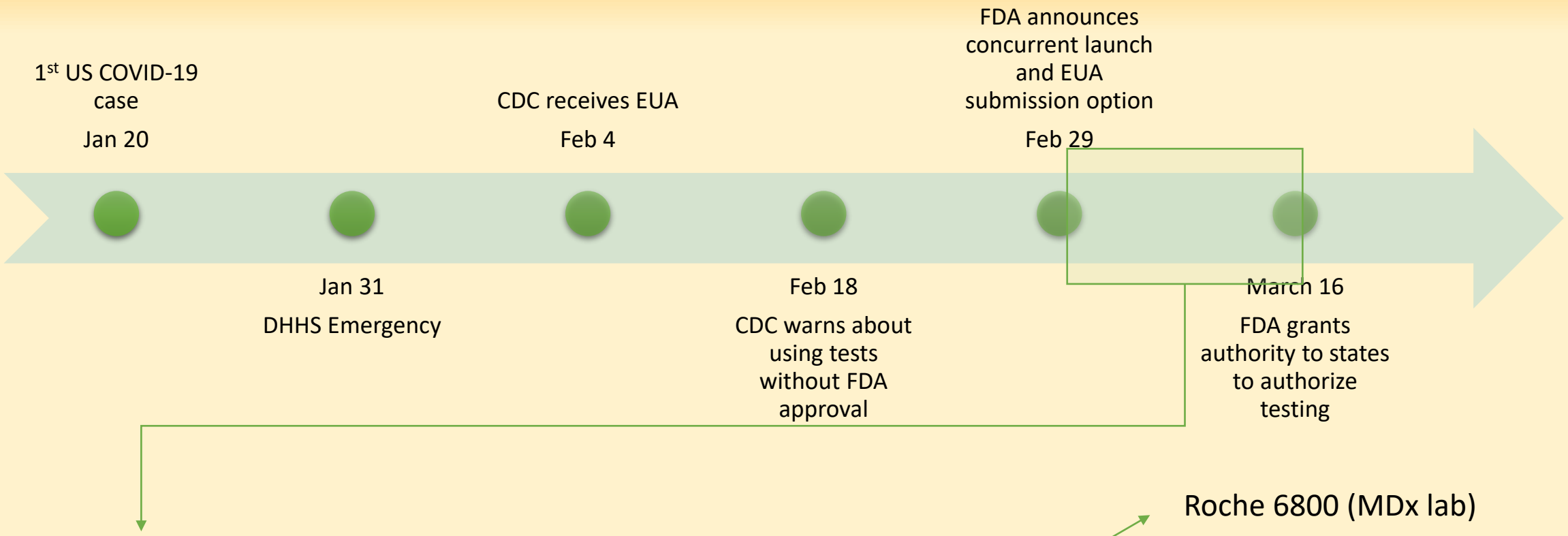
Director, Colorado Molecular Correlates Laboratory

University of Colorado Anschutz Medical Campus

Quick Background

- The experience I'm discussing today is an amalgam of multiple separate CLIA laboratories
  - Main UC Health Hospital Laboratory Molecular Virology (MDx)
  - Satellite laboratories at regional in-network hospitals
  - Colorado Center for Personalized Medicine (CCPM) Biobank Laboratory
  - Colorado Molecular Correlates Laboratory (CMOCO)
- We came together to maximize capacity and throughput during the pandemic
- While I will highlight some frustrations we experienced, we are very grateful to the CDC and FDA for their leadership through the pandemic
- Opinions are mine

Dr. E. Ashwood
Director, UCH Clinical Laboratories
Vice Chair, Department of Pathology



March 10-14
It becomes apparent we will not get adequate supply for testing with existing in-lab platforms

Working group to identify opportunities using existing infrastructure

Identified 4 platforms for possible implementation

- Roche 6800 (MDx lab)
- Cepheid (MDx lab)
- LDT RT-PCR (MDx lab)
- Thermo Fisher (CCPM/CMOCO)

Assay Considerations for Rapid Deployment

- Supply chain assurances led us to prioritize Thermo Fisher (TF)
- BUT…we did not have an on-label (EUA IFU) real-time PCR instrument

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Real-time PCR instrument and equipment	
Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument (used with SDS Software v1.4.1)	4406984 (with laptop computer) 4406985 (with tower computer)

- In our labs: ABI QS5 (x2), ABI QS7, ABI 7500
- No matter what, moving to an unapproved instrument meant we would be bringing up an LDT, requiring a full validation
  - Even if scientifically, we knew we could account for the instrument differences

Rapid Validation of TF TaqPath

- Once we knew we were validating as an LDT, we made a few other small changes to better accommodate workflow
- The lab:
  - Completed a full validation of Thermo test with modifications
  - In 8 days
  - Using 1 QS7 and 1 QS5 instrument
  - With 2 FTE + 15 hrs per day of staffing
  - Wrote a full EUA application

Stephen Wicks, PhD

Kristy Crooks, PhD, FACMG

Shortly Thereafter…

C.0	20 April 2020	• Removed 100-reaction kit. • Added a catalog number for the KingFisher™ Deep-Well 96 Plate. • Updated the catalog number for the Compact Digital Microplate Shaker. • Added catalog number for the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit and removed catalog numbers for individual components of the kit. • Added the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit. • Added an option to extract RNA with 200 µL of sample. • Added Applied Biosystems™ 7500 Real-Time PCR Instrument and Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instruments. • Added Applied Biosystems™ COVID-19 Interpretive Software v1.2 and Applied Biosystems™ COVID-19 Interpretive Software v2.0. • Removed Applied Biosystems™ COVID-19 Interpretive Software v1.0 and Applied Biosystems™ and COVID-19 Interpretive Software v1.1. • Added specific instructions to vortex and centrifuge the reaction plate for RT-PCR ("Prepare the RT-PCR reactions (400-µL sample input, 96-well reaction plate, COVID-19 assay only)" on page 40 and "Prepare the RT-PCR reactions (200-µL sample input, 96-well reaction plate, COVID-19 assay only)" on page 36). • Specified that retesting must be done with the original sample ("Interpretation of the results" on page 106). • Reorganized the content to perform RT-PCR based on the real-time PCR instrument. • Added "Interfering substances" on page 115. • Added information to customer and technical support (page 124).

From: [redacted]
Sent: Monday, April 27, 2020 1:44 PM
To: [redacted]
Cc: [redacted]
Subject: EUA200320

As indicated in our "Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency", you may use your test without a new or amended EUA where your test is validated using a bridging study to an EUA-authorized test. One way to bridge to a new component is to establish equivalent performance such as an LoD study with your new modified component (i.e., different thermocycler), and a comparison to the published EUA assay LoD. We recommend testing at least 3-fold serial dilutions (e.g., 0.3x LoD, 1x LoD, etc.) of SARS-CoV-2 viral materials (e.g., whole genomic viral RNA or inactivated virus, etc.) in pooled respiratory sample matrix in triplicate. You have confirmed the LoD of the ThermoFisher TaqPath Assay in your laboratory.

?

B. FDA Notification

Following completion of assay validation, laboratories should notify FDA (e.g., e-mail to CDRH-EUA-Templates@fda.hhs.gov) that their assay has been validated. This notification should include the name of the laboratory, name of the lab director, address, and contact person in this email. FDA will acknowledge receipt of this notification via auto-reply. As noted above, FDA recommends that laboratories submit a completed EUA request within 15 business days of the initial communication to FDA that the assay has been successfully validated.[4]

Kudos to the FDA for implementing this important change!

We Speak Different Languages!

- Laboratorians did not clearly understand that EUA submission was not required at that time

Back to the FDA EUA email:

Based on my review of your data, it does not appear that you require an EUA for the modifications to the ThermoFisher TaqPath Assay. You have the option therefore or withdrawing your EUA request and continuing to test clinical samples and run this assay in your laboratory.

Based on this, we withdrew our EUA application

More on this later…

In the Meantime…EUA #2

- Brought up a separate LDT for alternate specimens (e.g., tracheal aspirates)
- Assigned EUA reviewer was unfamiliar with instruments and reagents
- Very slow responses from reviewer
  - During this time, requirements changed, and we were asked to perform new validation experiments to meet new criteria
  - And acquire (and cross-test) specimens that were virtually impossible to obtain
- More communication delays…
- FDA global announcement that LDT review not required – all communication ceased

Variability of Review

- What a laboratory/director is asked to do will depend highly on the reviewer
- Expertise & opinions of reviewers are variable
- An immensely large staff of reviewers would be required to keep up with LDTs

Greatpeopleinside.com

EUA #3

- In October, with rising case volume, we extensively validated and adopted pooling
- EUA submitted based on prior validation (with withdrawn EUA)
  - Numerous emails about low priority status

Dear Dr. Ashwood:

Thank you for your patience regarding your EUA submission. We have completed the initial review and please find attached our feedback.

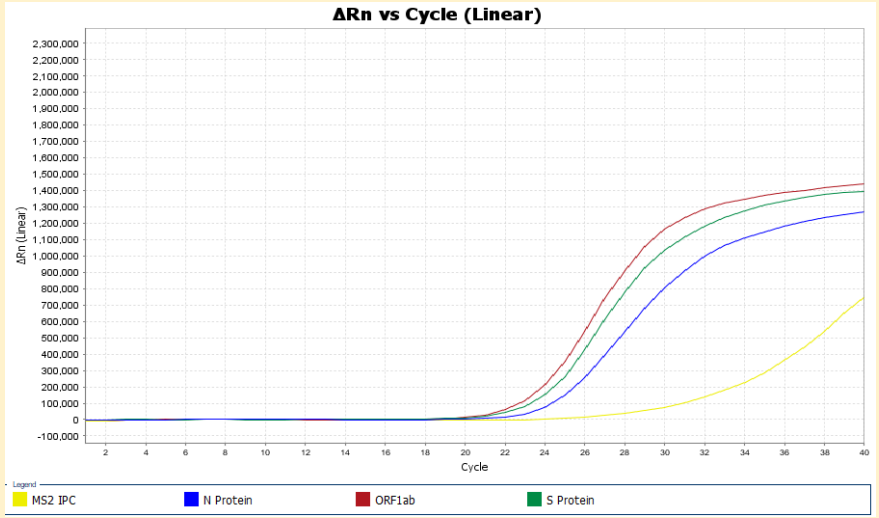
In your submission, you request to use a modified protocol of the TaqPath COVID-19 Combo Kit for in vitro detection of SARS-CoV-2 in symptomatic and asymptomatic populations using pooled samples. Please note that, since your RNA extraction method is currently not authorized for the TaqPath kit according to its IFU, and the assay protocol was modified, the TaqPath COVID-19 Combo kit is considered an unauthorized assay that requires validation in the context of your claimed intended use. However, adequate analytical and clinical validation of this modified assay was not provided. To adequately validate your modified version of the TaqPath COVID-19 combo kit, please provide data from validation studies per my comments in the attached file. Once the requested studies are completed, please update your original template with changes tracked and send back to me.

By the time we got this response (15.5 weeks later), we were done with pooling anyway (high positivity rate, new high-throughput platform)

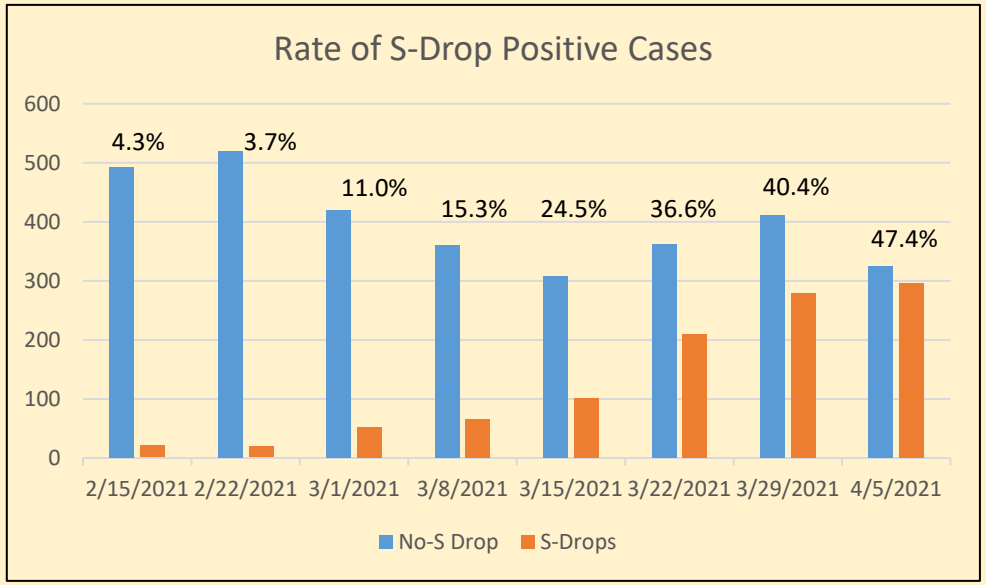
OK, so what *have* we learned from this? What can we learn and change?

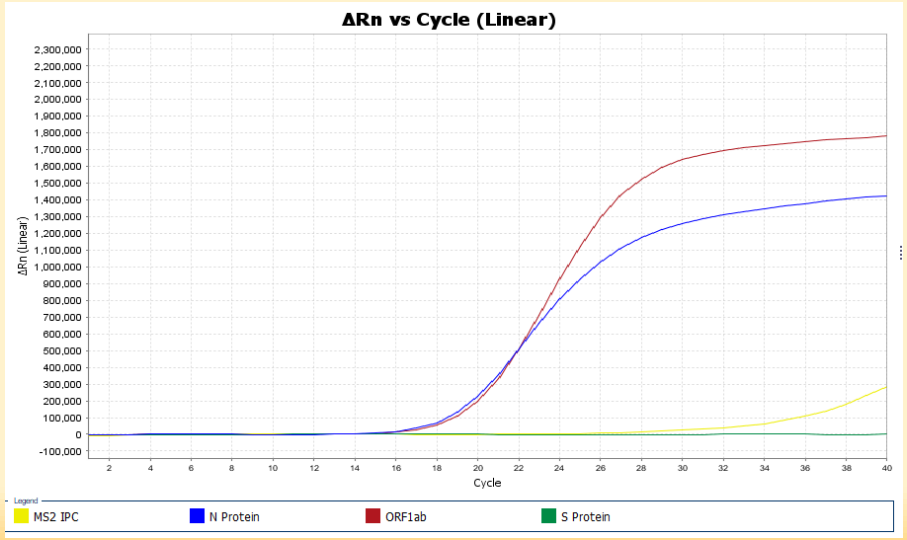
1. Testing performed at a local level, with local expertise, is more nimble than boxed-and-shipped kits
   - Can adapt to supply chain shortages and replacing constituents with bridging studies – not locked in!
   - Can adapt to changes in biology and science
   - In-depth knowledge of the assay inherently mitigates risk

Changes in Biology and Science



Typical SARS-CoV-2 positive results





S-drop pattern = probable B.1.1.7

OK, so what *have* we learned from this? What can we learn and change?

1. Testing performed at a local level, with local expertise, is more nimble than boxed-and-shipped kits
2. There are numerous facets of testing that are important
   - Safe and effective are obvious priorities
   - However if the testing cannot meet other clinical metrics – turnaround time, analytic sensitivity, specimen type etc. then it isn't functional

Alternate Specimens

Journal of Clinical Virology 128 (2020) 104438

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Short communication

Validation of SARS-CoV-2 detection across multiple specimen types

Garrett A. Perchetti[a], Arun K. Nalla[a], Meei-Li Huang[a], Haiying Zhu[a], Yulun Wei[a], Larry Stensland[a], Michelle A. Loprieno[b], Keith R. Jerome[a,b], Alexander L. Greninger[a,b,*]

[a] Department of Laboratory Medicine, Virology Division, University of Washington, Seattle, WA, United States
[b] Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, Seattle, WA, United States

Diagnostic Microbiology and Infectious Disease 99 (2021) 115228

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

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

Analysis of sputum/tracheal aspirate and nasopharyngeal samples for SARS-CoV-2 detection by laboratory-developed test and Panther Fusion system

Phyu M. Thwe, Ping Ren *

Letters

JAMA May 12, 2020 Volume 323, Number 18 1843

RESEARCH LETTER

Detection of SARS-CoV-2 in Different Types of Clinical Specimens

An epidemic of respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in China and has spread to other countries.[1] Real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) of nasopharyngeal swabs typically has been used to confirm the clinical diagnosis.[2] However, whether the virus can be detected in specimens from other sites, and therefore potentially transmitted in other ways than by respiratory droplets, is unknown.

Table. Detection Results of Clinical Specimens by Real-Time Reverse Transcriptase–Polymerase Chain Reaction

Specimens and values	Bronchoalveolar lavage fluid (n = 15)	Fibrobronchoscope brush biopsy (n = 13)	Sputum (n = 104)	Nasal swabs (n = 8)	Pharyngeal swabs (n = 398)	Feces (n = 153)	Blood (n = 307)	Urine (n = 72)
Positive test result, No. (%)	14 (93)	6 (46)	75 (72)	5 (63)	126 (32)	44 (29)	3 (1)	0
Cycle threshold, mean (SD)	31.1 (3.0)	33.8 (3.9)	31.1 (5.2)	24.3 (8.6)	32.1 (4.2)	31.4 (5.1)	34.6 (0.7)	ND
Range	26.4-36.2	26.9-36.8	18.4-38.8	16.9-38.4	20.8-38.6	22.3-38.4	34.1-35.4	
95% CI	28.9-33.2	29.8-37.9	29.3-33.0	13.7-35.0	31.2-33.1	29.4-33.5	0.0-36.4	

Abbreviation: ND, no data.

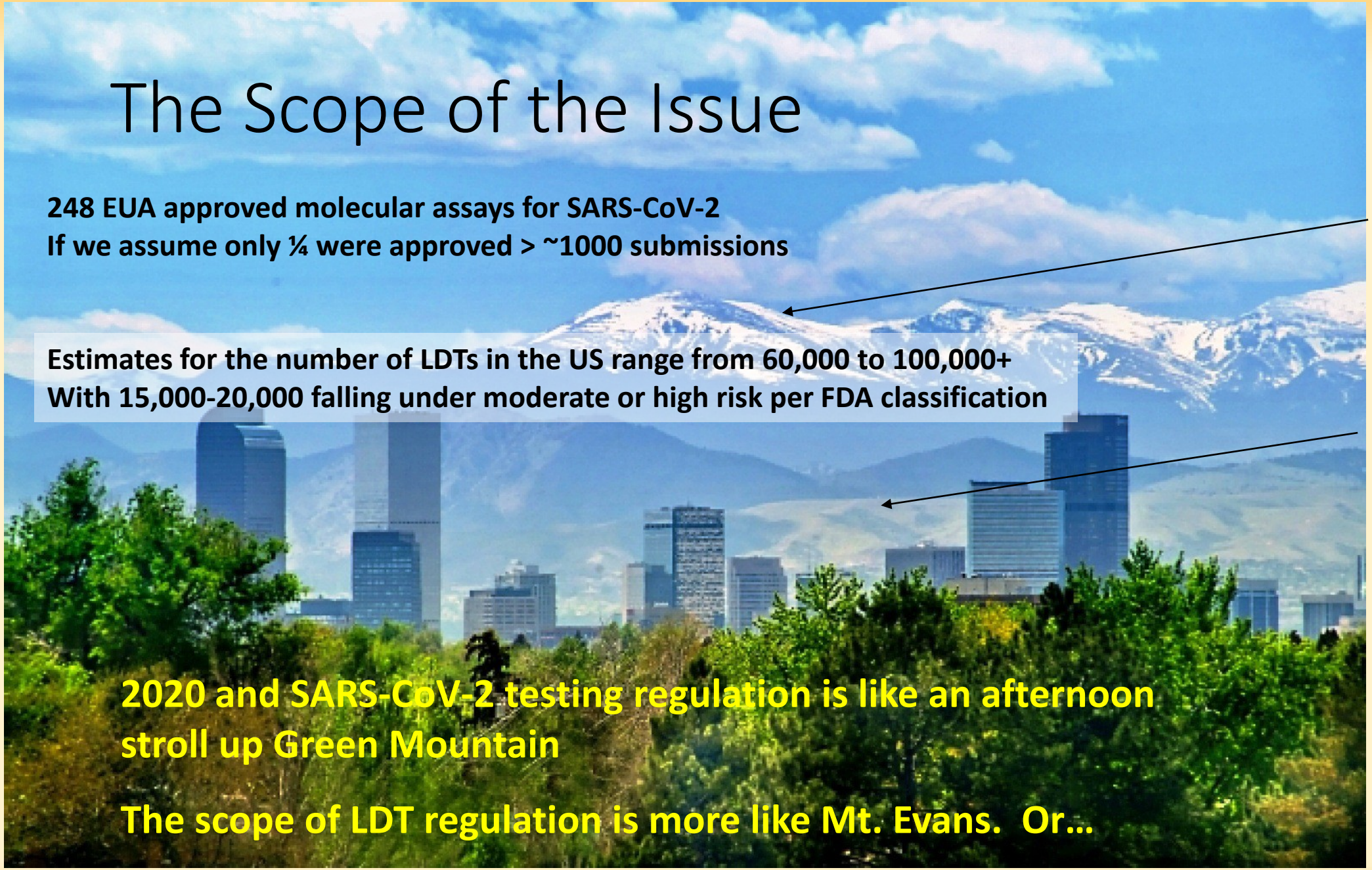
OK, so what *have* we learned from this? What can we learn and change?

1. Testing performed at a local level, with local expertise, is more nimble than boxed-and-shipped kits
2. There are numerous facets of testing that are important
3. Timelines to deploy assays are critical
   - Premarket review of LDTs: the scope will mean major delays as a way of doing business
   - The very first full (*de novo*) approval for a SARS-CoV-2 assay came **372 days** after the pandemic was declared

The Scope of the Issue

248 EUA approved molecular assays for SARS-CoV-2
If we assume only ¼ were approved > ~1000 submissions

Estimates for the number of LDTs in the US range from 60,000 to 100,000+
With 15,000-20,000 falling under moderate or high risk per FDA classification



Mt. Evans
Elevation: 14,265’

Green Mountain
Elevation: 6857’

2020 and SARS-CoV-2 testing regulation is like an afternoon stroll up Green Mountain

The scope of LDT regulation is more like Mt. Evans. Or…


Mt. Denali
Elevation: 20,320
For reference:
Mt. Carpe
Elevation 12,550

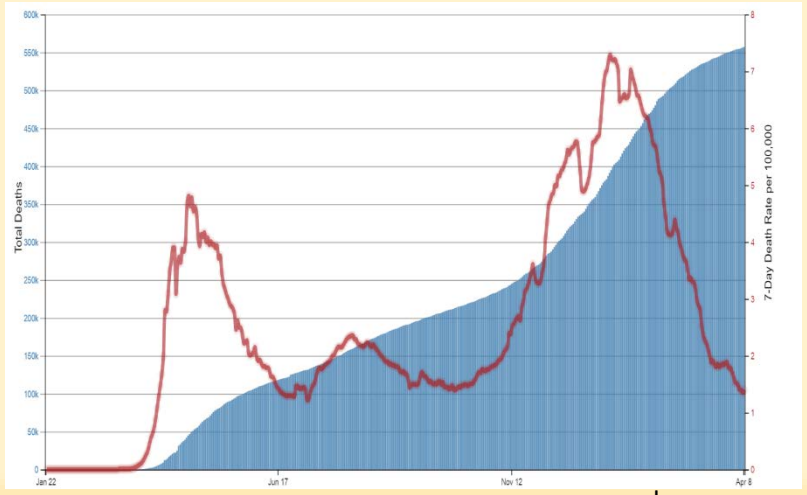
Cancer is a PANDEMIC

We should think about testing for cancer treatment with the same urgency

Per Capita Mortality for SARS-CoV-2 in the US

170.1 per 100,000

Over a span of ~16 months

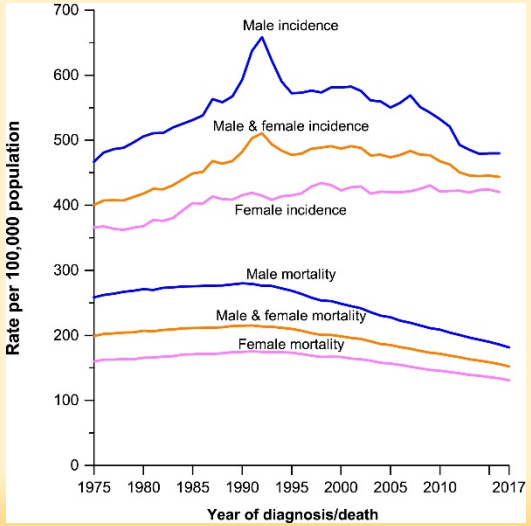


cdc.gov

Per Capita Mortality for Cancer in the US

158.3 per 100,000

Over a span of 40+ years



Siegel et al, 2020

Issues in Oncology Testing
(They're Similar!)

- Flexibility is essential
  - Specimen type
  - Adapting to new clinical information
  - Adding targets (NGS assays)
  - Complicated by ever-evolving therapeutic targets
  - Etc!
- Boxed and shipped is just different from an assay developed in-house
  - Boxed and shipped is great for some circumstances
  - But an experienced professional can mitigate risk when they know all the elements of an assay

Some Possible Solutions

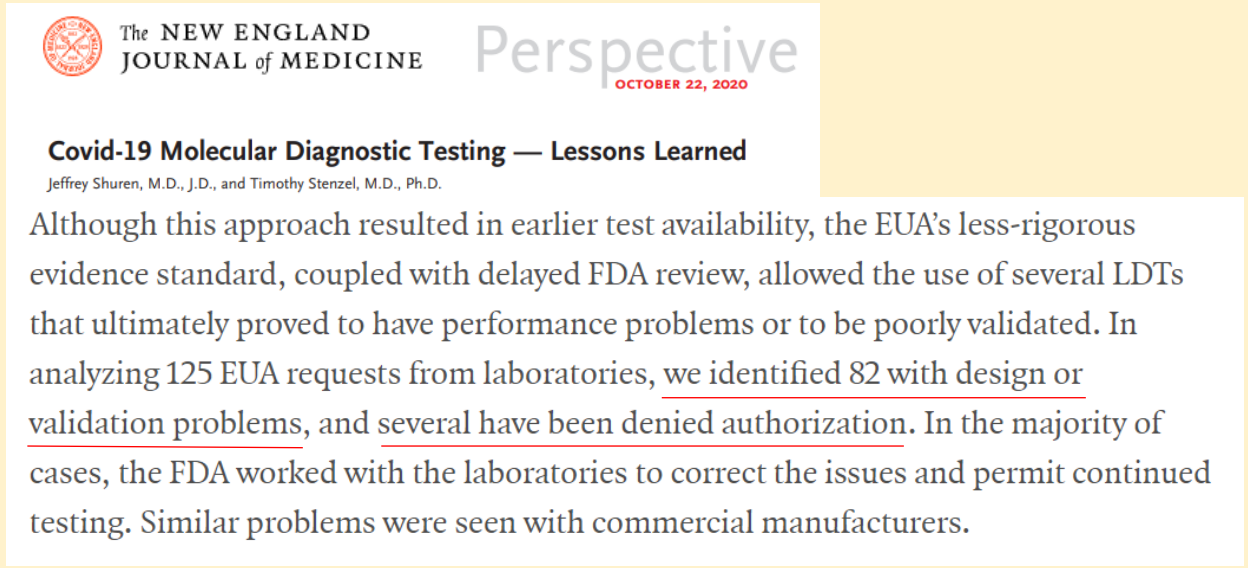
1. Consider the standards

   What level of assurance does an EUA-level review provide?

   Is it sufficient?

   Could this be the new bar (as opposed to level of evidence for PMA/510K)?

   Would this apply to all assays or only locally deployed assays?



The NEW ENGLAND JOURNAL of MEDICINE

Perspective

OCTOBER 22, 2020

Covid-19 Molecular Diagnostic Testing — Lessons Learned

Jeffrey Shuren, M.D., J.D., and Timothy Stenzel, M.D., Ph.D.

Although this approach resulted in earlier test availability, the EUA's less-rigorous evidence standard, coupled with delayed FDA review, allowed the use of several LDTs that ultimately proved to have performance problems or to be poorly validated. In analyzing 125 EUA requests from laboratories, we identified 82 with design or validation problems, and several have been denied authorization. In the majority of cases, the FDA worked with the laboratories to correct the issues and permit continued testing. Similar problems were seen with commercial manufacturers.

- How many of these issues were major?
- How often were assay conditions/ performance characteristics changed?
- Were standards to mitigate risk for boxed-and-shipped kits being applied to LDTs?
- Can the equivalent be achieved with teaching laboratorians what you would like to see?
- Does the high eventual approval rate suggest other (less difficult) solutions?
- Is there room for dialogue about standards for LDTs being different (from boxed-and-shipped kits)?

Some Possible Solutions

1. Consider the standards
2. Consider the expertise
   - The academic laboratory community has a wealth of knowledge
     - Mechanism to participate in assay review?
     - Peer review process?
   - Professional certification/credentialing: attach this responsibility/recognition to the *individual*, not the lab (e.g. CLIA license holder)
     - Analogy: DEA licenses for prescribing controlled substances are attached to the individual

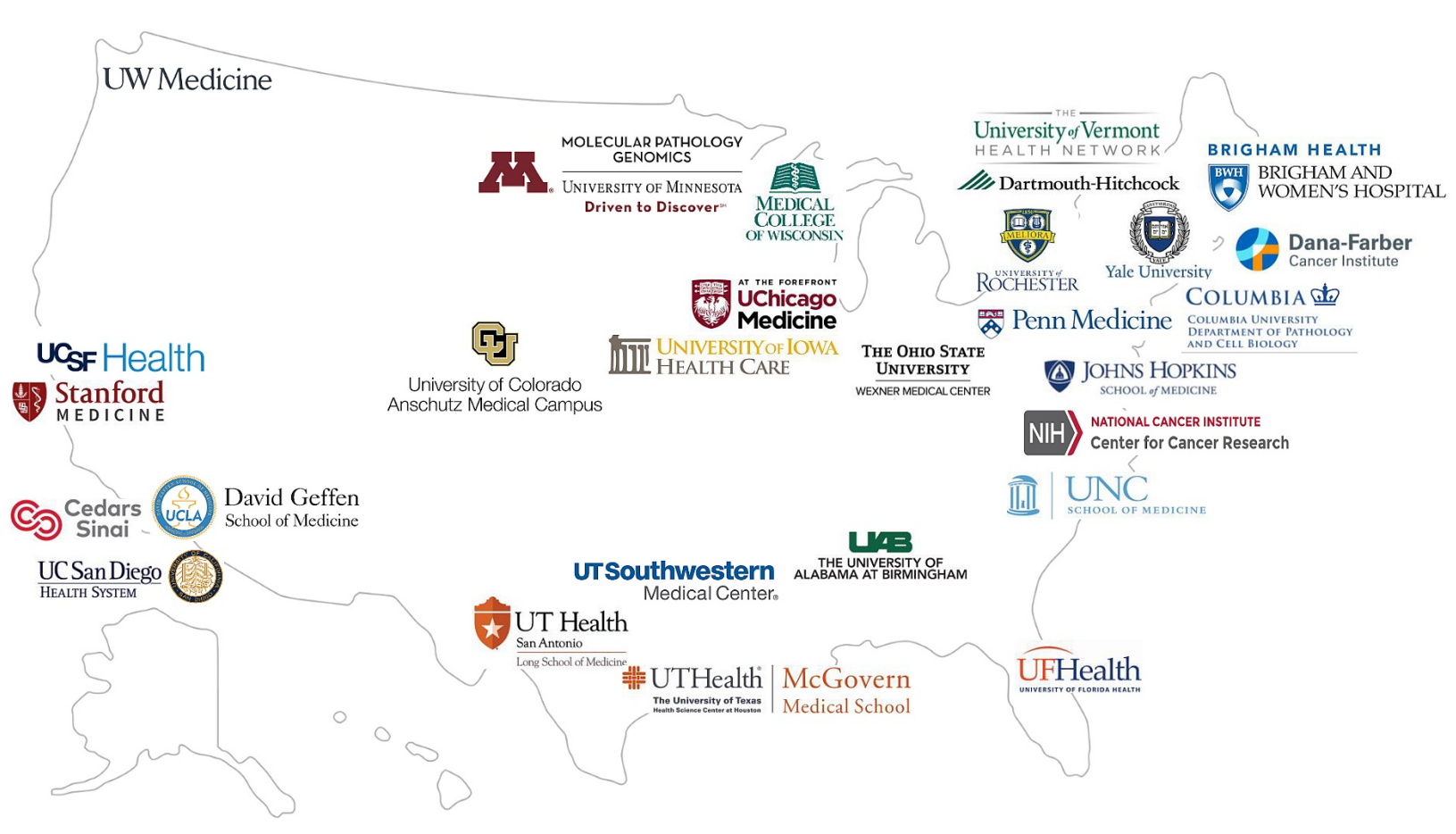
Some Possible Solutions

1. Consider the standards
2. Consider the expertise
3. Consider the motive
   - Commercial laboratories vs. labs with little $ motivation

Some Possible Solutions

1. Consider the standards
2. Consider the expertise
3. Consider the motive
4. Consider existing infrastructure
    - CLIA modernization
        - Acknowledges the value and risk mitigation of professional laboratory practice

The Genomics Organization for Academic Laboratories (GOAL)



- Consortium of 28 academic laboratories
- Shared capture-NGS reagent purchase
- Working towards shared bioinformatics
- Much more!
- = Opportunity to understand and minimize sources of variability

University of Colorado Covid-19 Response Laboratory Team(s)

- University of Colorado School of Medicine Department of Pathology
- UCH MDx lab
- UCH Microbiology Lab
- Colorado Center for Personalized Medicine Biobank
- Colorado Molecular Correlates Laboratory
- Exsera Biolabs
- UCH Command Center
- UCH Infection Control
- UCH EPIC/Beaker
- UCH Send-Outs
- Anschutz Medical Campus Occupational Safety
- Children’s Hospital Colorado
- Colorado Department of Public Health & Environment