COVID-19 Diagnostic Testing

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Disclosures

RMH is past CSO of Accelerate Diagnostics, which is the sole distributor of a recently-EUA authorized IgG/IgM SARS-CoV-2 test.
1. Complicated processes and paperwork (aka, denial and dysfunction)
2. Assume you have the illness, even if you test negative
3. Your test is positive... but should it be?
4. Spit tests
5. Pool tests
“What we’re not so good at is figuring out all the forms and working with the bureaucracy of the federal government.”

- Alex Greninger, UWashington
What is EUA, why does it apply to COVID-19 testing?

The EUA authorities allow FDA to help strengthen the nation’s public health protections threats by facilitating the availability and use of medical countermeasures initiatives (MCMs).

Under section 564 of the FD&C Act, the FDA Commissioner may authorize the use of unapproved medical products, or unapproved uses of approved medical products, after the HHS Secretary has made a declaration of emergency justifying authorization of emergency use, to diagnose, treat, or prevent serious or life-threatening disease or conditions caused by threats.
A tiny bit of recent history

Jan 31: HHS determines public health emergency

Feb 4: FDA issues EUA for CDC’s test for testing in CDC-qualified labs

Feb 14: many problems with CDC assay

Feb 18: CDC warns labs against testing without EUA

Feb 29: FDA “does not object” to the use of tests while pursuing EUA

May 11: FDA “does not object” if a lab modifies an EUA-authorized test without getting an EUA

May 11: FDA recommends developers obtain and use actual patient specimens

July 18: First EUA for sample pooling

July 24: First EUA for screening

Very limited testing
If you want to test for COVID-19, you need to send us your data first.
CDC Assay: 2019-nCoV

- Target: N1 and N2
  - Different regions of nucleocapsid gene
  - Amplified individually (not multiplexed)
- Internal control: human RNase P gene
- 2 extraction options
- Single mastermix supplier
- Run on an ABI 7500Fast Dx instrument

- July 22: CDC expands extraction platforms, master mixes
Thermo Fisher ABI 7500 Fast Real-Time PCR

SKU: BA8768768767
CONDITION: Used
AVAILABILITY: Usually Ships in 1 to 3 business days
SHIPPING: $550.00 (Fixed Shipping Cost)
WARRANTY: 1 year replacement Warranty
RETURNS: 30 Days Money Back Guarantee

$85,000.00

Write a Review
What if I need to change the CDC’s assay?

February – May 2020: amend your EUA
Often requires negotiation with FDA... something clinical labs “not so good at”.

May – present day:
Change of specimen type?
• No need to notify FDA if the specimen was previously authorized for another test of the same technology, provided the lab validates the specimen type

• Change to components?
  • No need to notify FDA if a bridging study was done
    • Parallel testing with new and original components
    • “would like to see validation data informally via email”
Interpreting the results of the CDC assay

<table>
<thead>
<tr>
<th>N1</th>
<th>N2</th>
<th>RP</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>Positive</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>Inconclusive</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Negative</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Invalid</td>
</tr>
</tbody>
</table>

+ interpretive only if detected at <40 cycles

“My patient keeps getting inconclusive test results.”

Most likely reason: near LOD of the assay

Targets

One test is probably not going to cut it...

- February 4: CDC EUA
- March 14: Roche EUA
- March 16: Hologic EUA
- March 21: Cepheid EUA
- March 30: 20 EUAs
Fast forward to Oct 8, 2020

**Individual EUAs for Molecular Diagnostic Tests for SARS-CoV-2**

This table includes information about authorized SARS-CoV-2 molecular diagnostic tests. These EUAs have been issued for each individual test with certain conditions of authorization required of the manufacturer and authorized laboratories.

<table>
<thead>
<tr>
<th>Date EUA Issued or Last Updated</th>
<th>Entity</th>
<th>Diagnostic (Most Recent Letter of Authorization) and Date EUA Original Issue</th>
<th>Attributes</th>
<th>Authorized Setting(s)</th>
<th>Authorization Documents</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/02/2020</td>
<td>Inbiis International, Inc</td>
<td>SMART DETECT SARS-CoV-2 RT-PCR KIT 04/07/2020</td>
<td>Real-time RT-PCR</td>
<td>H</td>
<td>HCP, Patients, IFU</td>
</tr>
<tr>
<td>10/02/2020</td>
<td>Cuur Diagnostics</td>
<td>Cuur Diagnostics SARS-CoV-2 Molecular Assay 08/24/2020</td>
<td>Real-time RT-PCR</td>
<td>H</td>
<td>HCP, Patients, EUA Summary</td>
</tr>
<tr>
<td>10/02/2020</td>
<td>Detectchem Inc.</td>
<td>MOBILE DETECT BMCC19 (MD-BMCC19) Test Kit 09/01/2020</td>
<td>RT-LAMP</td>
<td>H, M</td>
<td>HCP, Patients, IFU</td>
</tr>
<tr>
<td>10/02/2020</td>
<td>BioFire Diagnostics, LLC</td>
<td>BioFire Respiratory Panel 2.1 EZ (BPZ2.1 EZ) 10/02/2020</td>
<td>RT, Nested multiplex PCR, Multi analyte</td>
<td>H, M, W</td>
<td>HCP, Patients, IFU</td>
</tr>
</tbody>
</table>

The FDA has authorized ~170 EUA COVID-19 Molecular tests.

**Many flavors:**
- RT-PCR
- RT-LAMP
- Nested multiplex PCR
- TMA
- qSTAR

FDA views these as the same technology for EUA.

**FDA Will No Longer Review EUA Submissions for SARS-CoV-2 Lab-Developed Tests**

Oct 07, 2020 | Madeleine Johnson

NEW YORK – The US Food and Drug Administration announced on Wednesday it will no longer review SARS-CoV-2 Emergency Use Authorization submissions for lab-developed tests.

In the latest of its weekly virtual town hall sessions, the agency asserted that the change is consistent with the recent statement by the US Department of Health and Human Services and will also help FDA prioritize the review process during the pandemic.

On the call, Timothy Stenzel, director of the Office of In Vitro Diagnostics and Radiological Health at FDA’s Center for Devices and Radiological Health, summarized a statement from the agency’s FAQ page and provided additional comments.
Table 14: LoD determination

<table>
<thead>
<tr>
<th>Viral Copies in Sample (cp/μL VTM)</th>
<th>Total Positive (ORF1ab)</th>
<th>Total Positive (N)</th>
<th>Total Positive (RNaseP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.0</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>10.8</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>7.2</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>6.3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>5.4</td>
<td>2/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>*4.5</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>3.6</td>
<td>1/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>2.7</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>1.8</td>
<td>1/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>**0.9</td>
<td>1/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>0.0</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Positive Control</td>
<td>3/3</td>
<td>3/3</td>
<td>N/A</td>
</tr>
<tr>
<td>Negative Control</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

*Estimated LoD of ORF1ab target (4.5 cp/ul VTM)
**Estimated LoD of N target (0.9 cp/ul VTM)

Table 17: Wet testing of potential cross reactive organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC Cat. Number</th>
<th>Concentration</th>
<th>ORF1ab</th>
<th>N</th>
<th>RNaseP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human coronavirus 229E</td>
<td>ATCC®-VR-740D</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Human coronavirus OC43</td>
<td>ATCC®-VR-1558D</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Human coronavirus HKU1</td>
<td>ATCC®-VR-3262SD</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Human coronavirus NL63</td>
<td>ATCC®-3263SD</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Influenza A</td>
<td>VR-95DQ</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Influenza B</td>
<td>VR-1885DQ</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>ATCC®-VR-1580DQ</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC®-27853D-5</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Staphylococcus epidermis</td>
<td>ATCC®-122280D-5</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>ATCC®-10231D-5</td>
<td>1x10^6 copies/mL</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
</tr>
</tbody>
</table>

Table 17: Contrived clinical sample evaluation

<table>
<thead>
<tr>
<th>Sample Concentration</th>
<th>Number of samples</th>
<th>Number Detected</th>
<th>% Agreement (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x LoD</td>
<td>5</td>
<td>5</td>
<td>100% (NA*)</td>
</tr>
<tr>
<td>3x LoD</td>
<td>5</td>
<td>5</td>
<td>100% (NA*)</td>
</tr>
<tr>
<td>2x LoD</td>
<td>20</td>
<td>20</td>
<td>100% (83.9% - 100%)</td>
</tr>
<tr>
<td>Negative specimens (NS)</td>
<td>30</td>
<td>0</td>
<td>100% (88.6% - 100%)</td>
</tr>
</tbody>
</table>

NA*, confidence intervals not calculated for sample sizes of 5 or less

Interfering substances? “we do not anticipate interference”
Other examples of clinical performance studies for EUA tests

<table>
<thead>
<tr>
<th>Assay</th>
<th>Contrived (pos/neg)</th>
<th>Real (pos/neg)</th>
<th>Additional Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hologic</td>
<td>139/217</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cepheid</td>
<td>0</td>
<td>45/45</td>
<td></td>
</tr>
<tr>
<td>Abbott ID NOW</td>
<td>30/30</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Roche cobas</td>
<td>50/100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>CDC</td>
<td>0</td>
<td>13/104</td>
<td>Also tested ~2700 PUI</td>
</tr>
<tr>
<td>Cedars-Sinai</td>
<td>0</td>
<td>35/35</td>
<td></td>
</tr>
</tbody>
</table>

Seeding occurs at the assay’s LOD – i.e., 1x, 2x, 5x LOD, defined by manufacturer
Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)

Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff

What exactly is required for EUA today?*

- You must describe the intended use:
  - Technology
  - Presumptive/definitive detection
  - Specimen type
  - Where tests is limited to, who can run it
  - What instruments you’ll use
  - Controls

- Information on manufacturing (where, who, how much), distribution

- New instruments: safety checks, IT checks etc

*most recent FDA guidance, July 28, 2020
What exactly is required for EUA?

• LOD
  • Test inactivated virus spiked into clinical matrix (or RNA)
  • 2-3 dilution series, 3 replicates at each -> confirm with 20 replicates
  • “use most challenging matrix” (i.e., NP)

• Inclusivity
  • In silico, 100% detectable

• Cross-reactivity
  • FDA defines panels; in silico ok if organism “difficult to obtain”
  • Expect <80% homology – but can be justified

• Interference – no need to check unless a new RNA extraction method
## Clinical evaluation

<table>
<thead>
<tr>
<th>Minimum number of + specimens</th>
<th>30 &quot;natural&quot; specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum number of – specimens</td>
<td>30 from any of:</td>
</tr>
<tr>
<td></td>
<td>- Individuals suspected of COVID-19</td>
</tr>
<tr>
<td></td>
<td>- Archived from patients with respiratory infection</td>
</tr>
<tr>
<td></td>
<td>- Collected prior to COVID-19 pandemic</td>
</tr>
<tr>
<td>Comparator method</td>
<td>Compare to another EUA RT-PCR</td>
</tr>
<tr>
<td></td>
<td>Ideally, one that has shown high sensitivity with FDA panel</td>
</tr>
<tr>
<td>Acceptance criteria</td>
<td>95% PPA and NPA</td>
</tr>
</tbody>
</table>

Time to review – depends.
As of October 7th:
Prioritize EUA for point of care tests, home collection tests, at-home tests, tests that reduce reliance on certain types of tests supplies, and high-throughput widely distributed tests
Some LDTs are in a 3+ month review
174 Authorized EUAs for SARS-CoV-2: How do they compare?

Why is the LOD so different?

- Assay target
- Variation on primers, probes, technology
- Extraction method, PCR efficiency
- Etc, etc, etc

- This is **analytical sensitivity** – clinical sensitivity also includes pre-test factors
Commercial assay targets
<table>
<thead>
<tr>
<th>Institute</th>
<th>Target</th>
<th>Frequency of mutations in published genomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>China CDC</td>
<td>N</td>
<td>12.7%</td>
</tr>
<tr>
<td></td>
<td>ORF1</td>
<td>0.2 – 0.4%</td>
</tr>
<tr>
<td>Charite</td>
<td>E</td>
<td>0.4%</td>
</tr>
<tr>
<td></td>
<td>RdRp</td>
<td>99.8%</td>
</tr>
<tr>
<td>HKU</td>
<td>N</td>
<td>0.5%</td>
</tr>
<tr>
<td>US CDC</td>
<td>N1</td>
<td>0.2-0.4%</td>
</tr>
<tr>
<td></td>
<td>N2</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>N3</td>
<td>3.9%**</td>
</tr>
</tbody>
</table>

Green, primer; red, probe. Asterix = one or more mismatches

** removed from CDC assay, 03/15
△382 Virus: Deletion of ORF8

Common finding in 2003 SARS-CoV

- 22% of patients in Singapore, originated in Wuhan
- co-infection with WT

Role of ORF8 unclear: may relate to immune evasion (downregulates MHC-I molecules)

Infection with Delta 382 = lower concentration of proinflammatory cytokines, chemokines, growth factors associated with severe COVID-19

Young et al. 2020. The Lancet. 396:603-611
Sensitivity vs. LOD

**LOD**
- Minimum concentration that can be detected in sample and differentiated from noise
  - Theoretical
  
  We generally understand this

**Clinical Sensitivity**
- Proportion of patients tested with the disease that are detected by the assay

  - Sample
  - Disease stage
  - Age
  - Disease severity
  - Symptoms vs. no

  We don’t understand this
If You Have Coronavirus Symptoms, Assume You Have the Illness, Even if You Test Negative

Just because a coronavirus test says you don’t have the virus doesn’t mean you aren’t infected — or infectious.
The probability of a negative test result if truly positive is approximately 39% within the "window period" following symptom onset. The optimal time to test is between the third and fifth day of symptom onset, as indicated by the graph. This window period is crucial for accurate testing outcomes.
Factors that impact pretest probability:
- exposure
- masking
- symptoms
Woloshin et al. 2020 NEJM 383:e38

![Graph showing the relationship between pre-test probability and post-test probability with different sensitivity and specificity values.](image-url)
Your Coronavirus Test Is Positive. Maybe It Shouldn’t Be.

The usual diagnostic tests may simply be too sensitive and too slow to contain the spread of the virus.
What about specificity?

<table>
<thead>
<tr>
<th>Target Concentration</th>
<th>Number Concordant/Number Tested</th>
<th>% Agreement [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X LOD</td>
<td>20/20</td>
<td>100% [83.9% - 100%]</td>
</tr>
<tr>
<td>5X LOD</td>
<td>10/10</td>
<td>100% [72.3% - 100%]</td>
</tr>
<tr>
<td>Negative</td>
<td>30/30</td>
<td>100% [88.7% - 100%]</td>
</tr>
</tbody>
</table>

ID NOW COVID-19 Test Agreement with the Expected Results by Sample Concentration

Sensitivity 85.7-100%
Specificity 85.7 – 100%
Assume 99% sensitivity
99.9% specificity

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>PPV</th>
<th>NPV</th>
<th>% of results that are FP</th>
<th>Tested per day</th>
<th>Number of True +</th>
<th>Number of False +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening general population</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2%</td>
<td>66.4%</td>
<td>99.9%</td>
<td>34.6%</td>
<td>1MM</td>
<td>1980</td>
<td>1000</td>
</tr>
<tr>
<td>1%</td>
<td>90.1%</td>
<td>99.9%</td>
<td>9.9%</td>
<td>1MM</td>
<td>9900</td>
<td>1200</td>
</tr>
<tr>
<td>Testing symptomatic patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td>99.0%</td>
<td>99.8%</td>
<td>1.0%</td>
<td>800</td>
<td>80</td>
<td>1</td>
</tr>
</tbody>
</table>

~300 tests until positive
~90 tests until positive

If test all population, 0.9 MM positives, ~300K are incorrect
Going back to “optimal timing”

For general population value of a second test is very low

For exposed individuals, repeat 1x may be reasonable

NPV remains high throughout

Assumptions:
- 90% sensitivity
- 99% specificity
- Patients not re-exposed
- Only negative patients retested

Test | FP | TP | FN  
--- | --- | --- | --- 
1   | 100| 180| 20  
2   | 82 | 1800| 20  
3   | 80 | 180| 20  

MMWR 69(20):632-635; Arkansas church event attack rate 38%
How about for patients with risk of exposure after 1\textsuperscript{st} test?
CT Values?

Ct value for amplification target (N1, N2, or N3) in the CDC RT-PCR assay. RNP = human RNase P. Red dots indicate specimens with inconclusive RT-PCR amplification according to their corresponding Ct values and culture results.

Data from proficiency testing – same specimen sent to 700 laboratories.

Variability of Ct across platforms shown.
What does it take for a PCR to be “quantitative”? 

- qPCR is a relative method – standardization is challenging
  - Ct depends on many factors, including threshold set, choice of reporter, day-to-day variation in measurement
  - No SARS-CoV-2 standards

- Ct = number of cycles required to produce a constant emission of fluorescence – recorded relative to a defined threshold setting and cycle number at which fluorescence generated crosses the threshold is the reaction Ct.
Hook effect

• competition between amplicon strands and probes = reduced fluorescent signal
  • Single strands of amplicons re-anneal faster than probes with amplicons
• Nuclease activity of polymerase can result in decreasing probe signal
• Optimization: DNA template, probe/MgCl2 concentrations
If target dilute, primers may not land on all template molecules—single Ct value changes—typically occurs above Ct 29.


Subsampling = not all targets get bound by primer. Lower proportion are amplified than present.
Evaluation across country reference assays

![Graph showing efficiency and intercept values for different assays across countries.](image)
COVID-19 Spit Tests Used by NBA Are Now Authorized by FDA

A new saliva-based diagnostic does not require a “brain-tickling” swab, and it can be used with a range of chemical reagents
One should consider alternative methods to nasal screening in patients with known prior skull base defects, history of sinus or skull base surgery, or predisposing conditions to skull base erosion.
Cell infection depends on ACE2 receptor, MPRSS2 protease activity / cathepsin B/L

Infection occurs in ciliated, goblet cells, club cells but not basal cells

Hui et al. 2020. The Lancet Resp Medicine In press
In the microbiology lab...
Saliva

- Virus in saliva:
  - Entry from NP fluids/throat?
  - Replication salivary glands

- Salivary glands early infection site in SARS
Spit vs. swab

- 70 inpatients with co-collected saliva + NP swabs

Less variation in levels of SARS-COV-2 in saliva vs. NP
Also less variability in human RNase P (collection)

Wyllie et al. NEJM 383:1283-6
Asymptomatic healthcare workers (self-collect)
Safer reopening will require millions more Covid-19 tests per day. One solution: ‘pool testing’

By ANDREW JOSEPH @DrewQJoseph / JUNE 26, 2020
Why tests the pool?

• Supply chain

• More tests done with fewer PCR supplies

• Does not address:
  • Workforce
  • Swab availability
  • Time to results
  • Possibly even throughput
### Pooling

<table>
<thead>
<tr>
<th>P, percent of positive subjects in the tested population</th>
<th>( n_{\text{max efficiency}} ) (n corresponding to the maximal efficiency)</th>
<th>Efficiency of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>11</td>
<td>5.11</td>
</tr>
<tr>
<td>10%</td>
<td>4</td>
<td>1.68</td>
</tr>
<tr>
<td>15%</td>
<td>3</td>
<td>1.39</td>
</tr>
<tr>
<td>20%</td>
<td>3</td>
<td>1.22</td>
</tr>
<tr>
<td>25%</td>
<td>3</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Does it make sense?

VUMC:
13% positive among symptomatic
1% positive among asymptomatic

... workflow, pipetting, tracking
Etc, etc, etc
Impact of pooling on Ct values

A

B

Avoiding breaking the pool

Summary

• We are getting to a better place with testing
• PCR assays differ – we know about the LOD, not so much clinical sensitivity
• “Optimal” test window is probably days 3-5 of symptoms
• Commercial tests are qualitative – the CT value is meaningless on its own
• Saliva looks pretty good
• Test the pool, if you have a low prevalence and lots of people who like to pipette
Thank you!

Clinical Microbiology
VUMC