Development, Validation and Interpretation of Testing for SARS-CoV-2

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Outline

• Pandemics: lessons from the past
• The virus and its challenges
• Viral infection, symptoms, shedding and transmission
• Tests and their comparative performance
• Viral evolution and the role of sequencing
1918 >50 million deaths (Spanish Flu)

1957 1-2 million deaths (Asian flu)

2009 363,000 deaths (Swine flu)

2020 (current) 427,630 (World) 115,271 (US) (COVID-19)

Frozen tissue
Multiple viral and bacterial sequences
Reconstruction of 1918 virus
Test in many animal models

Highest incidence in crowded populations
Virtually all deaths were due to secondary bacterial pneumonia
High mortality in young adults due to higher case incidence

COVID-19 is a different virus
Mitigation is similar process

WHO Global Flu Strategy 2019-2030
(Prevent. Control. Prepare.)
Announced March 2019

**Pillars of Flu Preparedness**
(national-community-health care system-physician)

Viral Surveillance and Risk Assessment
Early disease recognition, Diagnostics and Drugs
Vaccines

Infrastructure Preparedness
“non-pharmaceutical”
Non-pharmaceutical Recommendations

• During a Pandemic: Limit the Spread of Germs and Prevent Infection
  - Avoid close contact with people who are sick.
  - When you are sick, keep your distance from others to protect them from getting sick too.
  - Cover your mouth and nose with a tissue when coughing or sneezing. It may prevent those around you from getting sick.
  - Washing your hands often will help protect you from germs.
  - Avoid touching your eyes, nose or mouth.
  - Practice other good health habits. Get plenty of sleep, be physically active, manage your stress, drink plenty of fluids, and eat nutritious food.

• Before a Pandemic:
  - Store a two week supply of water and food.
  - Periodically check your regular prescription drugs to ensure a continuous supply in your home.
  - Have any nonprescription drugs and other health supplies on hand, including pain relievers, stomach remedies, cough and cold medicines, fluids with electrolytes, and vitamins.
  - Get copies and maintain electronic versions of health records from doctors, hospitals, pharmacies and other sources and store them, for personal reference. Get help accessing electronic health records.
  - Talk with family members and loved ones about how they would be cared for if they got sick, or what will be needed to care for them in your home.

https://www.ready.gov/pandemic
Guidelines to Prevent Pandemic Influenza through Non-pharmaceutical Interventions (NPIs) and Community Engagement

**Social Distancing**

- **CDC recommendations**
  - Social distancing measures: Even though the evidence base for the effectiveness of some of these measures is limited, CDC might recommend the simultaneous use of multiple social distancing measures to help reduce the spread of influenza in community settings (e.g., schools, workplaces, and mass gatherings) during severe, very severe, or extreme influenza pandemics while minimizing the secondary consequences of the measures. Social distancing measures include the following:
    - Increasing the distance to at least 3 feet (98) between persons when possible might reduce person-to-person transmission. This applies to apparently healthy persons without symptoms in the event of a very severe or extreme pandemic, this recommended minimal distance between people might be increased.
    - Persons in community settings who show symptoms consistent with influenza and who might be infected with (possibly) pandemic influenza should be separated from well persons as soon as practical, be sent home, and practice voluntary home isolation.

**Use of Face Masks**

- **CDC recommendations**
  - Use of face masks by ill persons: CDC might recommend the use of face masks by ill persons as a source control measure during severe, very severe, or extreme influenza pandemics when crowded community settings cannot be avoided (e.g., when adults and children with influenza symptoms seek medical attention) or when ill persons are in close contact with others (e.g., when symptomatic individuals share common spaces with other household members or symptomatic postpartum women care for and nurse their infants). Some evidence indicates that face mask use by ill persons might protect others from infection.

**Environmental Cleaning**

- **CDC recommendations**
  - Environmental surface cleaning measures: CDC recommends environmental surface cleaning measures in all settings, including homes, schools, and workplaces, to remove influenza viruses from frequently touched surfaces and objects. Use of these measures might help prevent transmission of various infectious agents, including seasonal and pandemic influenza (https://www.cdc.gov/nonpharmaceutical-interventions/environmental/index.html; https://www.cdc.gov/oralhealth/infectioncontrol/questions/cleaning-disinfecting-environmental-surfaces.html).


**School Closure**

- **CDC recommendations**
  - School closures and dismissals: CDC might recommend the use of preemptive, coordinated school closures and dismissals during severe, very severe, or extreme influenza pandemics. This recommendation is in accord with the conclusions of the U.S. Community Preventive Services Task Force (https://www.thecommunityguide.org/findings/emergency-preparedness-and-response-school-dismissals-reduce-transmission-pandemic-influenza), which makes the following recommendations:
    - The task force recommends preemptive, coordinated school dismissals during a severe influenza pandemic.
    - The task force found insufficient evidence to recommend for or against preemptive, coordinated school dismissals during a mild or moderate influenza pandemic. In these instances, jurisdictions should make decisions that balance local benefits and potential harms.
Absent from WHO and CDC Pandemic Plans:

How to get ready for high capacity, rapid and sensitive testing

resulting in ...

**Use:** whatever you have

**Add:** whatever you can get

**Experience:**

extreme shortfalls in supply chain tests and collection reagents
Due to supply shortages and uncertainties, laboratories are deploying multiple testing methodologies.

Why did you choose this SARS-CoV-2 testing method?

“Whatever reagents were able to receive”  “Independent supply chain”  “Limited kit availability”

“Supply chain issues are a major hurdle currently, which is preventing us from moving forward with this as a primary instrument.”

“We use the [company name’s] extraction reagents and they are hard to get and the shortage affects our 24 other LDTs.”

“We are concerned with this test and have it as a back-up for increased capacity if it needs to be deployed. The supply chain for this test has been very un-reliable.”

“Next door (Virology Lab) is offering COVID19 testing on three platforms to minimize the risk of inventory shortage.”
## All US-based labs: top 10 primary testing methods

<table>
<thead>
<tr>
<th>SARS-CoV-2 Molecular Testing Methods</th>
<th>Primary* (n=112)</th>
<th>Secondary (n=88)</th>
<th>Tertiary (n=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory developed testing procedure (LDP / LDT) with EUA submission</td>
<td>21%</td>
<td>8%</td>
<td>5%</td>
</tr>
<tr>
<td>Roche Molecular Systems cobas SARS-CoV-2</td>
<td>17%</td>
<td>5%</td>
<td>0%</td>
</tr>
<tr>
<td>Abbott Molecular RealTime SARS-CoV-2 assay</td>
<td>16%</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>Cepheid Xpert Xpress SARS-CoV-2 test</td>
<td>8%</td>
<td>19%</td>
<td>25%</td>
</tr>
<tr>
<td>Hologic Panther Fusion SARS-CoV-2</td>
<td>6%</td>
<td>1%</td>
<td>0%</td>
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<tr>
<td>Quidel Corporation Lyra SARS-CoV-2 Assay</td>
<td>5%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>Thermo Fisher Scientific TaqPath COVID-19 Combo Kit</td>
<td>5%</td>
<td>3%</td>
<td>7%</td>
</tr>
<tr>
<td>CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel</td>
<td>4%</td>
<td>9%</td>
<td>7%</td>
</tr>
<tr>
<td>DiaSorin Molecular Simplexa COVID-19 Direct assay</td>
<td>4%</td>
<td>13%</td>
<td>9%</td>
</tr>
<tr>
<td>Abbott Diagnostics ID NOW COVID-19</td>
<td>3%</td>
<td>2%</td>
<td>9%</td>
</tr>
</tbody>
</table>

* Data sorted by the primary testing method from largest to smallest

Performance of COVID tests done in a condensed timeframe

Gaps in analysis of tests due to urgency for testing
Rapidly assembled quantified validation and control materials not carefully standardized to each other.
Universal control materials late to arrive and sometimes not well standardized

Requirements for EUA validation of LOD

30 positive and 30 negative clinical specimens
Dilution series of 3 replicates per concentration with inactivated virus on actual patient specimen matrix
Confirmation at the final concentration of 20 replications with 19/20 required to claim that concentration as LOD

EUA Bridging studies
Altered application of COVID test, now discontinued

Highly accurate comparisons of COVID-19 test sensitivities not available for all tests
(https://www.fda.gov/media/135659/download)
<table>
<thead>
<tr>
<th>Company</th>
<th>Test</th>
<th>LOD</th>
<th>EUA date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Becton, Dickinson &amp; Company</td>
<td>BioGX SARS-CoV-2 Reagents for BD MAX System</td>
<td>40 copies/mL</td>
<td>4/2/20</td>
</tr>
<tr>
<td>Abbott</td>
<td>Abbott RealTime SARS-CoV-2 assay</td>
<td>100 copies/mL</td>
<td>3/18/20</td>
</tr>
<tr>
<td>Abbott</td>
<td>ID NOW COVID-19</td>
<td>125 copies/mL</td>
<td>3/27/20</td>
</tr>
<tr>
<td>Quest Diagnostics</td>
<td>Quest SARS-CoV-2 rRT-PCR</td>
<td>136 copies/mL</td>
<td>3/17/20</td>
</tr>
<tr>
<td>Cepheid</td>
<td>Xpert Xpress SARS-CoV-2 test (lab test)</td>
<td>250 copies/mL</td>
<td>3/20/20</td>
</tr>
<tr>
<td>Cepheid</td>
<td>Xpert Xpress SARS-CoV-2 test (point of care test)</td>
<td>250 copies/mL</td>
<td>3/20/20</td>
</tr>
<tr>
<td>bioMerieux</td>
<td>BioFire COVID-19 Test</td>
<td>330 copies/mL</td>
<td>3/23/20</td>
</tr>
<tr>
<td>Qiagen</td>
<td>QIAstat-Dx Respiratory SARS-CoV-2 Panel</td>
<td>500 copies/mL</td>
<td>3/30/20</td>
</tr>
<tr>
<td>DiaSorin</td>
<td>Simplexa COVID-19 Direct assay</td>
<td>500 copies/mL</td>
<td>3/19/20</td>
</tr>
<tr>
<td>Quidel</td>
<td>Lyra SARS-CoV-2 Assay</td>
<td>800 copies/mL*</td>
<td>3/17/20</td>
</tr>
<tr>
<td>Ipsum</td>
<td>COV-19 IDx Assay</td>
<td>850 copies/mL*</td>
<td>4/2/20</td>
</tr>
<tr>
<td>CDC</td>
<td>CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)</td>
<td>3,160 copies/mL; 1,000 copies/mL*</td>
<td>2/4/20</td>
</tr>
<tr>
<td>Co-Diagnostics</td>
<td>Logix Smart Coronavirus Disease 2019 (COVID-19) Kit</td>
<td>4,290 copies/mL</td>
<td>4/3/20</td>
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<tr>
<td>Luminex</td>
<td>NxTAG CoV Extended Panel Assay</td>
<td>5,000 copies/mL</td>
<td>3/27/20</td>
</tr>
<tr>
<td>LabCorp</td>
<td>COVID-19 RT-PCR Test</td>
<td>6,250 copies/mL*</td>
<td>3/16/20</td>
</tr>
<tr>
<td>Luminex</td>
<td>ARIES SARS-CoV-2 Assay</td>
<td>75,000 copies/mL</td>
<td>4/3/20</td>
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<tr>
<td>GenMark</td>
<td>ePlex SARS-CoV-2 Test</td>
<td>100,000 copies/mL</td>
<td>3/19/20</td>
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<tr>
<td>PerkinElmer</td>
<td>PerkinElmer New Coronavirus Nucleic Acid Detection Kit</td>
<td>3 copies/reaction</td>
<td>3/24/20</td>
</tr>
<tr>
<td>Gnomegen</td>
<td>Gnomegen COVID-19 RT-Digital PCR Detection Kit</td>
<td>8 copies/reaction</td>
<td>4/6/20</td>
</tr>
<tr>
<td>Thermo Fisher</td>
<td>TaqPath COVID-19 Combo Kit</td>
<td>10 copies/reaction</td>
<td>3/13/20</td>
</tr>
<tr>
<td>New York State Department of Public Health</td>
<td>New York SARS-CoV2 Real-time Revers Transcriptase (RT)-PCR Diagnostic Panel</td>
<td>25 copies/reaction</td>
<td>2/29/20</td>
</tr>
<tr>
<td>Mesa Biotech</td>
<td>Accula SARS-CoV-2 Test</td>
<td>200 copies/reaction</td>
<td>3/23/20</td>
</tr>
<tr>
<td>Roche</td>
<td>Cobas SARS-CoV2</td>
<td>0.009 TCID 50/mL</td>
<td>3/12/20</td>
</tr>
<tr>
<td>Hologic</td>
<td>Panther Fusion SARS-CoV-2</td>
<td>0.01 TCID 50/mL</td>
<td>3/16/20</td>
</tr>
</tbody>
</table>
Sample Collection: An unexpected supply chain pinch point

Critical Supply Shortage:
- Swabs
- Collection Tubes
- Media

ARUP Solution:
Build your own using generic and widely available reagents (tubes, swabs, media components)

“We have a locked-down commitment”
“There’s be a change of plan”
“You can’t say exactly why”
“No future commitment”

Collection swab shortage
Investigation of 3D printing swabs (based on Norwell Health study)

ARUP Transport Media™
Ambient temperature, universal transport media for infectious diseases

Swab supply line secured
Early April 2020

Worldwide test collection kit shortage
ARUP initiates collection kit conservation project

Large scale collection kit production
Utilized saline as media

Automated collection kit production
High volume packaging equipment

From Lincoln Hirayama
COVID-19 Saliva Collection

- High ‘invalid’ rate at first - 15%!
- Viscosity/higher order protein complexes possibly interfering
- All saliva samples are freeze-thawed → invalid rate now <1%
- Correct amount very important

Courtesy Ben Kukull
**Viral entry:**
- S1 binding host receptors
- S2 fusion viral and cell membranes

**Viral fusion, assembly, budding**

**Replication and transcription**

**Assembly, morphogenesis, pathogenesis**

**Viral Genome:**
- Proofreading function (unlike Flu)
- Genomes vary by less than 10/nucleotides/3K
- Multiple highly conserved targets for NAT tests
  - ORFab, RdRp, S, E and N genes

COVID-19  A Respiratory and Vascular Infection

- **Respiratory failure** – Acute respiratory distress syndrome (ARDS)
- **Secondary infections** - respiratory infections and bacteremia
- **Inflammatory complications** - exuberant inflammatory response, with persistent fevers, elevated inflammatory markers, elevated pro-inflammatory cytokines
- **Cardiac and cardiovascular complications** – Other complications arrhythmias, acute cardiac injury, and shock
- **Thromboembolic complications** – Pulmonary embolism and acute stroke
- **Neurologic complications** - Encephalopathy (common), stroke, movement disorders, motor and sensory deficits, ataxia, and seizures

CDC extended symptoms:  [https://www.cdc.gov/mmwr/volumes/69/wr/mm6930e1.htm](https://www.cdc.gov/mmwr/volumes/69/wr/mm6930e1.htm)
COVID-19 biomarkers for testing

Diagnostic Tests

- Antigen
  - BinaxNow
  - DiaSorin

- NAT high throughput & sensitive
  - Roche PCR (closed box)
  - Hologic fusion PCR (closed box)
  - Hologic Panther TMA RLU signal (closed box)
  - ThermoFisher PCR (open platform)

- NAT POC low throughput-rapid
  - Abbott
  - Cepheid
  - Biofire

- Differentiating clinical vs analytical sensitivity is critical!

Crossing thresholds (Cts) provide a good approximation of viral concentration in a liquid sample.

Cts do not accurately measure viral burden in host.

Cts vary among different instruments!

8 fold variability in CTs in 26 lab survey
Interpreting-Results-of-Diagnostic-Tests
Rhodes et al. CID 2020
Utility PCR Crossing Threshold Analysis/reporting

- Predict rising tide of infections at a given phase of pandemic
- Understand dynamics of viral shedding
- Release patients with still detectable virus from quarantine
- Indicator disease severity and likelihood of death
- Stratification of patient risk
- Crossing thresholds vary with sample type.
Analytic vs Clinical Sensitivity and Specificity

• Traditional test performance validation:
  • Clinical or contrived specimens
  • Comparison to reference test

• Sensitivity:
  • % positivity patients with disease
  • No absolute reference for disease status
  • Reference material lacking
  • COVID EUA allows establishing agreement with results positive material from symptomatic patients or contrived material
  • Swabs and saliva miss infected material

• Specificity:
  • High and reliable for NAT tests

Corman et al. Euro Surveill. 2020;25

Woloshin et al. NEJM 383;6 Aug 2020
Testing:

No immediate value after exposure

~40% false negatives day of symptom onset

Optimal testing time 1-3 days post symptoms ~Day 8

Probability negative PCR (truly infected)

Probability infected (PCR test negative)

Negative RR-PCR results should not do not rule out infection especially in cases with high clinical suspicion
COVID-19 Viral Dynamics and Shedding

Mild vs Severe

> 65 vs <65

He et. Nature Medicine | VOL 26 | 672 MAY 2020 | 672–675 |

Lee et al. JAMA Internal Medicine November 2020 Volume 180, Number 11

Symptomatic vs Asymptomatic

Rhee et al. Duration of SARS-CoV-2 Infectivity cid 2020
Approaches to test comparisons and power to predict % positives and % missed

• Traditional FDA requirements
  • Rigorous LOD
  • Large population studies
  • Examples: HIV, HCV, HPV, Flu

• Comparative testing of samples of different viral concentration identified by PCR crossing thresholds (CTs).
  • Across the spectrum viral concentration
  • Within the spectrum of low viral concentration

• Prediction based on established test LOD and relative CTs, +/- confirmatory testing
Comparative Assay Testing of a Sampled Population

Comparison of Two High-Throughput RTPCR Systems for the Detection of COVID-19

Kraner et al. JCM May 2020

Characteristics of discordant specimens

### Hologic Panther Fusion (n=5257)

<table>
<thead>
<tr>
<th>Cobas 6800 SARS-CoV2 RT-PCR Result</th>
<th>Panther Fusion SARS-CoV-2 RT-PCR Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target 1 Ct Value</td>
<td>Target 2 Ct Value</td>
</tr>
<tr>
<td>35.26</td>
<td>37.69</td>
</tr>
<tr>
<td>N/A</td>
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<td>35.37</td>
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<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### Roche cobas SARS-CoV-2 Assay (n=511)

Distribution CTs Hologic and Roche

(~ 5% Positive Symptomatic Utah Population)

Berry et al. JCM.00743-20
Comparative testing within a spectrum of low viral concentrations

Selectively Test Samples In “Low Range”

Advantage:
- test many low positives
- more accurate predictions

Calculate percentage of samples “likely” missed based on comparative CTs and knowledge of LODs

Calculate expected CT based on Anchor CT and LOD of Less Sensitive Test
Use of CTs to determine numbers of low positives in a population and predict negatives among different assays

Conclusions

Predicted relative sensitivity assumes tests have been accurately determined Limits of Detection (LOD)

Arnaout et al. bioRxiv preprint doi: https://doi.org/10.1101/2020.06.02.131144
Prediction based on established test LOD and relative CTs with Confirmation

Hologic Panther Fusion (n=5257)

Calculate percentage of samples “likely” missed based on comparative CTs of several tests and knowledge of LODs with confirmation by retesting low positive samples identified by high sensitivity test.
“False Negatives”
1.4% of Roche Detected Population
13.5% = 7 samples missed

CDC assay results for 52 low positive Roche samples

<table>
<thead>
<tr>
<th>CDC Assay Result</th>
<th>Roche Ct</th>
<th>Positive</th>
<th>Negative</th>
<th>Inconclusive</th>
<th>Row Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>31-31.9</td>
<td>100% (18)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100% (18)</td>
</tr>
<tr>
<td>32-32.9</td>
<td>85.0% (17)</td>
<td>10.0% (2)</td>
<td>5.0% (1)</td>
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<td>100% (20)</td>
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<tr>
<td>33-33.9</td>
<td>61.5% (8)</td>
<td>30.8% (4)</td>
<td>7.7% (1)</td>
<td>-</td>
<td>100% (13)</td>
</tr>
<tr>
<td>≥34</td>
<td>-</td>
<td>100% (1)</td>
<td>-</td>
<td>-</td>
<td>100% (1)</td>
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<tr>
<td>Grand Total</td>
<td>82.7% (43)</td>
<td>13.5% (7)</td>
<td>3.8% (2)</td>
<td>-</td>
<td>100% (52)</td>
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</table>

*Not retested – Insufficient RNA remaining

“False Negatives”
1.7% of Hologic Detected Population
6.4% = 9 samples missed

CDC assay results for 140 low positive Hologic samples

<table>
<thead>
<tr>
<th>CDC Assay Result</th>
<th>Hologic Ct</th>
<th>Positive</th>
<th>Negative</th>
<th>Inconclusive</th>
<th>Row Total</th>
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<tr>
<td>32-32.9</td>
<td>94.1% (16)</td>
<td>-</td>
<td>5.9% (1)*</td>
<td>-</td>
<td>100% (17)</td>
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<td>33-33.9</td>
<td>100% (29)</td>
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<td>34-34.9</td>
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<td>100% (15)</td>
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<td>100% (19)</td>
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<td>38-38.9</td>
<td>65.0% (13)</td>
<td>30.0% (6)</td>
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<td>100% (20)</td>
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<td>&gt;39</td>
<td>33.3% (2)</td>
<td>33.3% (2)</td>
<td>33.3% (2)</td>
<td>-</td>
<td>100% (6)</td>
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<tr>
<td>Grand Total</td>
<td>90.7% (127)</td>
<td>6.4% (9)</td>
<td>2.1% (3)</td>
<td>-</td>
<td>100% (140)</td>
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</tbody>
</table>

*Not retested – Insufficient RNA remaining
"False Negatives"
1.7% of Hologic Detected Population
5.7% = 8 samples missed

"False Negatives"
14.6% of Hologic Detected Population
54.3% = 76 samples missed

ThermoFisher assay results for 140 low positive Hologic samples

<table>
<thead>
<tr>
<th>Hologic Ct</th>
<th>Positive</th>
<th>Negative</th>
<th>Inconclusive</th>
<th>Row Total</th>
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<tbody>
<tr>
<td>32-32.9</td>
<td>100% (17)</td>
<td>-</td>
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<td>100% (17)</td>
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<td>33-33.9</td>
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<tr>
<td>34-34.9</td>
<td>100% (21)</td>
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<td>35-35.9</td>
<td>100% (13)</td>
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<td>100% (13)</td>
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<tr>
<td>36-36.9</td>
<td>100% (15)</td>
<td>-</td>
<td>-</td>
<td>100% (15)</td>
</tr>
<tr>
<td>37-37.9</td>
<td>94.7% (18)</td>
<td>5.3% (1)</td>
<td>-</td>
<td>100% (19)</td>
</tr>
<tr>
<td>38-38.9</td>
<td>75.0% (15)</td>
<td>15.0% (3)</td>
<td>10.0% (2)</td>
<td>100% (20)</td>
</tr>
<tr>
<td>&gt;39</td>
<td>66.7% (4)</td>
<td>33.3% (2)</td>
<td>-</td>
<td>100% (6)</td>
</tr>
<tr>
<td>Grand Total</td>
<td>94.3% (132)</td>
<td>4.3% (6)</td>
<td>1.4% (2)</td>
<td>100% (140)</td>
</tr>
</tbody>
</table>

Quidel assay results for 140 low positive Hologic samples

<table>
<thead>
<tr>
<th>Hologic Ct</th>
<th>Positive</th>
<th>Negative</th>
<th>Invalid</th>
<th>Row Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>32-32.9</td>
<td>100% (17)</td>
<td>-</td>
<td>-</td>
<td>100% (17)</td>
</tr>
<tr>
<td>33-33.9</td>
<td>89.7% (26)</td>
<td>6.9% (2)</td>
<td>3.4% (1)</td>
<td>100% (29)</td>
</tr>
<tr>
<td>34-34.9</td>
<td>38.1% (8)</td>
<td>57.1% (12)</td>
<td>4.8% (1)</td>
<td>100% (21)</td>
</tr>
<tr>
<td>35-35.9</td>
<td>23.1% (3)</td>
<td>61.5% (8)</td>
<td>15.4% (2)</td>
<td>100% (13)</td>
</tr>
<tr>
<td>36-36.9</td>
<td>13.3% (2)</td>
<td>73.3% (11)</td>
<td>13.3% (2)</td>
<td>100% (15)</td>
</tr>
<tr>
<td>37-37.9</td>
<td>-</td>
<td>100% (19)</td>
<td>-</td>
<td>100% (19)</td>
</tr>
<tr>
<td>38-38.9</td>
<td>-</td>
<td>95.0% (19)</td>
<td>5.0% (1)</td>
<td>100% (20)</td>
</tr>
<tr>
<td>&gt;39</td>
<td>-</td>
<td>83.3% (5)</td>
<td>16.7% (1)</td>
<td>100% (6)</td>
</tr>
<tr>
<td>Grand Total</td>
<td>40.0% (56)</td>
<td>54.3% (76)</td>
<td>5.7% (8)</td>
<td>100% (140)</td>
</tr>
</tbody>
</table>
Assessing Sensitivity COVID-19 Antigen Test (BinaxNOW)

**Symptomatic Population**
- 1380 Adults 928 Children

**Asymptomatic Population**
- 2,645 College Students

**Results**
- Co-collected Nasal Swabs: 46 (1.7%) NAT positive, 24 (0.9%) Antigen positive
- Sensitivity 95%
- Specificity 100%
- Sensitivity 84.6%
- Specificity 100%

All group sensitivity & Crossing Threshold:
- 99.3% Ct <25
- 95.8% Ct <30
- 81.2% Ct <35.

Pollock et al. https://doi.org/10.1101/2021.01.09.21249499;

Okoye/Barker/Pearson et al. Accepted JCM Jan 2021
Co-Circulation COVID and Other Respiratory Viruses
(Flu A, Flu B, RSV AB 2020-21?)

39,000,000 – 56,000,000 flu illnesses

18,000,000 – 26,000,000 flu medical visits

410,000 – 740,000 flu hospitalizations

24,000 – 62,000 flu deaths

Low number Flu Cases in Southern Hemisphere last summer

Impact mitigation efforts and Flu vaccine this fall and beyond?

https://www.cdc.gov/flu/about/burden/preliminary-in-season-estimates.htm
COVID/Flu Twindemic?

• Issues
  • Shared symptoms
  • Co-infections more lethal?
  • Competition testing resource
  • Availability of high throughput Co-tests

• Commitment to a specific % Co-test reagents
• Adapt to COVID testing process
• Deal with unused reagents in case of low Flu season

Influenza Positive Tests Reported to CDC by U.S. Clinical Laboratories, National Summary, September 27, 2020 – January 16, 2021

September 27 – Jan 16 2021
Tested: 468,064
Positive: 1,159 (0.2%)
“Because some of the symptoms of flu and COVID-19 are similar, it may be hard to tell the difference between them based on symptoms alone, and testing may be needed to help confirm a diagnosis.”

Co-Testing

- Attractive to symptomatic patient
- Detect and differentiate COVID, Flu, RSV
  For diagnosis, treatment, tracking
  (provides a “final” diagnosis)
- Potential to improve operational efficiency and cost for patients, labs, and public health

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Influenza</th>
<th>COVID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever/Chills</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Cough</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Sore throat</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Body aches</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Headache</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fatigue</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Vomiting/diarrhea</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Loss of taste or smell</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
RSV: Disease Burden and Impacted Populations

Each year in U.S.:
- 2.1 million outpatient visits among children younger than 5 years old
- 57,527 hospitalizations among children younger than 5 years old
- 177,000 hospitalizations among adults older than 65 years
- 14,000 deaths among adults older than 65 years

"RSV infection is an important illness in elderly and high-risk adults, with a disease burden similar to that of non-pandemic influenza A in a population in which the prevalence of vaccination for influenza is high” Falsey et al.

Testing for RSV is appropriate for adults over 65 as well as children as well as transplant patients of any age

RSV in a COVID/Flu not indicated in uncompromised population 5-65

RSV Co-infections in Hospitalized Young Children

From: American Academy of Pediatrics
https://www.aappublications.org/news/2020/06/10/coronavirusbronchiolitis061020

Researchers studied 1,880 children hospitalized with bronchiolitis from two multicenter cohorts — a group under 2 years from 2007-'10 and a group under 1 year from 2011-'14. Children were tested for 18 viruses, including four endemic coronaviruses (CoV), which are not the newest CoV identified (SARS-CoV-2).

What is the impact of Population A viruses on Population B detectability for Co-Positive Samples?

![Ct Distribution Diagram]

Data: Panther fusion
Co-testing Analytic Challenges (single well, high throughput assays)

- Optimize tests for high sensitivity and specificity for multiple viral targets
- Single well multiplex Co-detection
  - 4 targets: COVID, IC, Flu A, Flu B
  - 5 targets: COVID, IC, Flu A, Flu B, RSV (AB)
  - 6 targets: COVID, IC, Flu A, Flu B, RSV (AB), host target

• **Issue:** Fluorescence bleed through impacts test specificity
  - Current Limitation:
    - Max out at 5-6 targets
    - Multi-well or extract once amplify many
  - Current Fluor Limitations:
    - Max out at 5-6 targets
    - Alternative: Multi-well or extract once amplify many

• Co-positive samples: Potential for suppression of signal of minor viral population by major population?
  - Impacts test spensitivity

ARUP study: Roche, Hologic, and Chromacode assays are resilient to bleed through and suppression!
COVID-19 Mutations and Their Consequences

One of six key contact residues within the receptor-binding domain (RBD) identified as increasing binding affinity to human and murine ACE2. Impact on viral transmission?

- N501Y: Increase infectivity with greater viral replication in lung and airway tissue.
- D614G
- P681H: Adjacent to the furin cleavage site. Increased replication and decreased susceptibility to neutralization.

Nature | Vol 585 | 10 September 2020 | Corrected 16 September

Translational Genomics Research Institute
COVID-19 Mutations and Timeline

• D614G variant
  • Emerged in late Jan. or early Feb. 2020
  • Replaced the initial SARS-CoV-2 strain identified in China
  • Increased infectivity and transmission

• “Denmark Mink” variant
  • Emerged mink farm sector June 2020
  • Variant “cluster 5” Nov 5 in 12 human cases with new mutations.
  • Worry of reduced viral neutralization and vaccine effectiveness not confirmed

• VOC 202012/01, lineage B.1.1.7, “UK” variant
  • Reported Kent England Sept 2020
  • Spread rapidly to be dominant English strain
  • Clear capacity to spread more quickly
  • Multiple mutations including D614G, P681H and deletion 69-70.

• 501Y.V2 lineage B.351 variant “South Africa”
  • Reported Dec 2020 with combination of mutations
  • Worry more rapid spreading, vaccine resistant
Identification and Tracking “UK” Variant: Screen for S Target Drop Outs

Normal S Target Amplification

S Target Drop Out

Deletion under S Target Probe Leads to S drop out

Wyoming UK Variant Identified by ARUP S drop out screening and sequencing

Issues “UK” Variant screening by S Target Drop Out Identification

• Most tests don’t signal S target drop out.
  • Will current tests be modified to avoid drop out?

• S target drop out requires confirmatory identification by sequencing
  • Low prevalence population presents high burden for sequencing
  • U.S. COVID sequencing capacity is lacking (compare to UK)

• Solutions
  • Expand sequencing capacities (federal, academic, commercial)
  • Engage labs with high “sequence to diagnosis” capacities (Ginkgo model)
  • Validate Multiplex mutation detection assays

>5,000 Positive Samples screened for S drop out
~ 150 drop outs identified and sequenced
Jan 8-15
4 UK variants identified
Unmet Needs in Testing

- Super NAT Tests
- Home sample collection conveniently and safely linked to testing
- Affordable and scalable sequencing
- Better informatics for communicating with test populations
ARUP, University of Utah, and Utah State Teams