WELCOME to the

Learning Together About COVID-19

Session will start in less than 15 minutes
Conflict of Interest Disclosure Statement

Dr. Martin and Dr. Talbot have no conflicts of interest.
Testing Regulatory Framework: Normal Times

1) adopt IVDs
2) create Lab-Developed Tests

Clinical Laboratory Improvement Act (CLIA)

Academic & Commercial Laboratories

Commercial In Vitro Diagnostic Tests (IVD)
Testing Regulatory Framework: Public Health Emergency

1) adopt IVDs
2) create Lab-Developed Tests

Commercial In Vitro Diagnostic Tests (IVD), Emergency Use Authorization

Clinical Laboratory Improvement Act (CLIA)

Academic & Commercial Laboratories
Testing for SARS-CoV-2
Tests for Viral RNA to Detect Active Cases

- CDC real time RT-PCR
  - 24 Jan: publicly posted assay protocol
  - 4 Feb Emergency Use Authorization (EUA)
  - 10 Feb: CDC sent reagents to partners
    - Recalled
  - 28 Feb EUA allows use
- 2 March NH PHL
- ~5 hours per run
- 29 samples per run
Available Testing

EUA as of 4/12/20: 32 Commercial Assays
10 Academic/Reference Laboratories

- Roche Molecular Systems
- Quest
- Hologic, Inc
- Abbott Molecular
- Diasorin
- LabCorp
- BGI Genomics
- BioFire
- Mesa Biotech
- NeuMoDx
- Qiagen
- Gnomegen
- Luminex
- NeuMoDx
- DiaCarta
- Gnomegen
- ScienCell Research Laboratories
- Ipsun Dx
- BD Luminex Corp

Timeline:
- 2/4
- 2/11
- 2/18
- 2/25
- 3/3
- 3/10
- 3/17
- 3/24
- 3/31
- 4/7
- 4/14

Project ECHO
Dartmouth-Hitchcock
Near-Patient Rapid Molecular Tests

• Xpert® Xpress SARS-CoV-2 Test (cartridge) for use on GeneXpert Dx platform (Cepheid)
  • EUA 24 March
  • Results in 45 minutes
  • 4 samples at a time

• ID NOW COVID-19® test for use in tabletop ID NOW analyzer (Abbott Diagnostics)
  • EUA 27 March
  • Results in ≤ 15 minutes
  • 1 sample at a time
Limiting Factors for Laboratory Adoption

• Which commercial platforms are already in lab?
• Can new instruments be obtained from commercial manufacturers?
  • Significant capital investment, shortages
• Can reagents be obtained from commercial manufacturers?
• Are collection kits available?
  • Flocked swabs
  • Viral transport media
Challenges in Every Step of Pathway

• Clinicians permitted to test
  • CDC, then State

• Clinicians willing to test

• PPE and site available for safe testing

• Specimen collection materials
  • Flocked swabs
  • Viral transport media

• Turnaround time

Statista, accessed April 12, 2020
Data on Presence of RNA in Disease Course

- Single patient in Korea
- Viral load kinetics


Dartmouth-Hitchcock
Data on Presence of RNA in Disease Course

- 80 infected patients
- Median viral load
- Throat and sputum samples
- Collected at different stages after disease onset

Who To Test: Acute Disease

Do any of the following criteria apply?

The individual:
• is a healthcare provider
• exposed others in a healthcare or long-term care setting
• had contact to large numbers of people who may need public health intervention

Hospitalized with fever or respiratory illness?

Test, because these individuals may fuel community COVID-19 transmission or pose a risk to vulnerable populations.

Test, because diagnosis of COVID-19 may affect patient management and informs use of limited airborne infection isolation and PPE supplies.

The decision to test should be based on person’s signs/symptoms, patient vulnerability (e.g. comorbidities, advanced age), risk of exposing others, and ability to self-isolate. Patients with mild illness, who are not in need of medical care, can self-isolate at home and monitor for symptom progression. Consider testing if symptoms worsen. Patients who are not tested but asked to self-isolate should remain home until:

- At least 7 days have passed since symptoms first appeared, and
- At least 72 hours (3 days) have passed since recovery – which is defined as resolution of fever without the use of fever-reducing medications and improvement in respiratory symptoms.
Serology Testing

• Antibodies are made around 9-11 days post-infection
• Not immediately useful for diagnosing acute illness
• Proposed clinical uses:
  • Identify immunity in asymptomatic patients/health care workers
  • Identify convalescent plasma donors
  • Understand role of asymptomatic persons in epidemic
RNA Detected

Symptom Onset

IgG-positive

Is this protective immunity?

Weeks Post-infection

1 2 3 4 5 6 7 8 9 10 11 12
Neutralizing Antibodies: Plaque Reduction Neutralization Test (PRNT)
Antibody Response to SARS-CoV-1

623 recovered SARS-CoV-1 patients
Antibody Response to SARS-CoV-2

Wolfel et al. Nature March 2020 DOI: 10.1038/s41586-020-2196-x
Serology Testing

• FDA EUA not needed to market
• Over 70 commercial manufacturers available: truly the Wild West
  • Enzyme immunoassay format
  • Many products developed with very few patient samples
• Validation for individual laboratories: complex
  • Must obtain specimens from convalescent patients
  • Assessing cross-reactivity
Serologic Tests: qSARS-CoV-2 IgG/IgM Rapid Test (Cellex, Inc)

• First serological test to receive EUA: 3 April
• Limited to labs certified to perform moderate and high complexity tests
• Negative result does not preclude SARS-CoV-2 infection and should not be used as sole basis for patient management decisions
• Sensitivity early after infection is unknown, and false positives for IgG and IgM antibodies may occur due to cross-reactivity
CDC’s Application of Serology Test

• CDC is using qSARS-CoV-2 IgG/IgM Rapid Test (Cellex, Inc.) to conduct a population serosurvey of persons never diagnosed with COVID-19

• A separate, large nationwide serosurvey of blood donors sampled monthly from March to October 2020 is also underway
Tests for SARS-CoV-2/COVID-19 and potential uses.

Tests for SARS-CoV-2/COVID-19 and Potential Uses

**Type of Test**
- Nucleic acid amplification test for viral RNA
  - Nasopharyngeal swab, oropharyngeal swab, sputum, bronchoalveolar lavage fluid, others

**Measure**
- Current infection with SARS-CoV-2

**Value**
- Inform individual of infection status so they can anticipate course of illness and take action to prevent transmission
- Inform patient management and actions needed to prevent transmission
- Inform actions needed to prevent transmission

**Beneficiary**
- Individual
- Healthcare or long-term care facility
- Public health

**Type of Test**
- Antibody detection

**Measure**
- Past exposure to SARS-CoV-2

**Value**
- Detect susceptible individuals (antibody negative) and those previously infected
- Identify individuals with neutralizing antibodies
- Facilitate contact tracing and surveillance

**Beneficiary**
- Identify those potentially immune to SARS-CoV-2 (if tests can detect protective immunity, individuals could be returned to work)
- Healthcare facilities: Experimental therapy
- Public health

References


How Good Are These Tests?

- **Analytical Sensitivity:** The ability of a test to detect when present in clinical specimen
- **Clinical Sensitivity:** The ability of a test to identify a person’s overall infection status
  - Anatomic location of specimen
  - Method/quality of collection
  - Specimen transport
  - Burden of virus
  - Disease severity
  - Timing of specimen within disease course
How Good Are These Tests?

1070 specimens (various sources) collected from 205 inpatients

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

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

Abbreviation: ND, no data.
How Good Are These Tests?

-20 hospitalized patients

Figure. Severe Acute Respiratory Syndrome Coronavirus 2 Distribution and Shedding Patterns Among 20 Hospitalized Patients
How Good Are These Tests?

• No test is perfect
• PCR testing is the most sensitive and specific method we have of detecting active infection
• “Standard-of-care” testing for other respiratory viruses
• You must use the test specimen validated by YOUR testing pipeline
  • Almost all assays with EUA from FDA are approved for NP swabs
  • Large reference labs (Quest, LabCorps) have validated many specimen types
How Good Are These Tests?

• Analytical Sensitivity & Specificity
• CDC Assay:
  • Reliably detects virus at concentrations as low as $10^{0.5}$ copies/microliter
    • (3160 copies per mL of transport media)
  • Does not cross-react with SARS-CoV-1, MERS, seasonal coronaviruses, other respiratory pathogens, normal microbiota
NP Swab vs. OP Swab

• NP swab is thought to perform slightly better than OP swab
  • Recommended by CDC
  • Published data are scant and not peer-reviewed
NP Swab vs. OP Swab

Aggregated Ct values of 17 symptomatic patients
NP Swab vs. OP Swab

[Graph showing the comparison between Throat swabs and Nasopharyngeal swab in terms of LOG10 RNA copies/swab over days post symptom onset.]
Serology Testing: Positive Predictive Value

• Cellex Test
  • “Sensitivity = 93.8%”
  • “Specificity = 95.6%”

• Positive Predictive Value of test depends on disease prevalence!
  • If 1% prevalence, 1 in 6 positive tests are true positives