COVID-19 testing

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I CANNOT CONTROL
(So, I can LET GO of these things.)

IF OTHERS FOLLOW THE RULES OF SOCIAL DISTANCING

THE AMOUNT OF TOILET PAPER AT THE STORE

I CAN CONTROL
(So, I will focus on these things.)

MY POSITIVE ATTITUDE

TURNING OFF THE NEWS

FINDING FUN THINGS TO DO AT HOME

THE ACTIONS OF OTHERS

HOW I FOLLOW CDC RECOMMENDATIONS

HOW LONG THIS WILL LAST

MY OWN SOCIAL DISTANCING

LIMITING MY SOCIAL MEDIA

MY KINDNESS & GRACE

OTHER PEOPLE'S MOTIVES

HOW OTHERS REACT

Clipart: Carrie Stephens Art
TheCounselingTeacher.com
Disclaimers etc.

• I am not an Infectious Disease expert
• I do not have a PhD in Microbiology
• Much of this data is derived from the preprint server medRxiv.org (Cold Spring Harbor Laboratory, British Medical Journal and Yale) in addition to published sources from pubmed.org
• I have no financial disclosures.

• Phone call listeners
  *8 to mute
  *6 to unmute
Test evaluation

- PCR testing: specimen type and timing
- IgM and IgG serologic response
- Point of Care serologic testing

Overarching questions
1. Who are you testing?
2. When are you testing?
3. What are you testing?
A 2019-nCoV diagnosis is confirmed if the suspected cases also have one of the following etiological or serological evidence.

1. Positive result in real-time fluorescence RT-PCR detection of novel coronavirus nucleic acid;
2. The sequence of the virus is highly homologues to that of 2019-nCoV.
3. Specific IgM and IgG antibodies against 2019-nCoV test positive in the serum; IgG antibodies specific to 2019-nCoV test positive after previous negative results, or increased by more than 4 times in the recovery phase compared to the acute phase.
Clinical sample type for RT-PCR

• Possible sample types from the literature:
  • Nasopharyngeal or nasal wash
  • Nasal
  • Oropharyngeal
  • Saliva
  • Sputum
  • Bronchoscopy-obtained
  • Blood
  • Stool/anal swab

• PCR testing: Samples undergo successive cycles of amplification of transcript. The transcript targets are labeled with a fluorescent label.

• Cycle threshold (Ct) = number of cycles required for the sample’s fluorescent signal to exceed the background level. A high Ct value means there is less transcript in the sample because it requires more cycles to be seen above background.
Detection of SARS-CoV-2 in Different Types of Clinical Specimens
- \(N = 205\) patients, all positive in at least 1 specimen
- Different collection times for multiple samples

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**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>31.4 (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></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></td>
</tr>
</tbody>
</table>

Abbreviation: ND, no data.
Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-CoV-2) infected pneumonia (COVID-19) preprint Lin et al.

32.7% difference

Table 2. Comparison of qRT-PCR results between throat swabs and sputum specimens

<table>
<thead>
<tr>
<th>No. (%) of sputum specimens result</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>19 (36.5%)</td>
<td>4 (7.7%)</td>
</tr>
<tr>
<td>Negative</td>
<td>21 (40.4%)</td>
<td>8 (15.4%)</td>
</tr>
</tbody>
</table>

$P$ value = 0.001 by McNemar test.

$N = 52$ patients clinically suspected of infection
Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody response during infection by SARS-CoV-2: an observational study. To et al, Lancet 2020

- N=23 patients with 173 samples
- Early morning saliva specimen (e.g. clearing throat)

- N = 67 patients; serial testing of nasopharyngeal swab, sputum and stool.
- In 46 patients, at the time their NP swabs were negative, 60.9% still had positive sputum and 30.4% were positive in stool.
- NP swabs shows fluctuating positive and negative results in 40.3% of patients compared to 3.3% of sputum samples.
<table>
<thead>
<tr>
<th>Time to negative</th>
<th>Median</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal swab</td>
<td>12 days</td>
<td>16.2 days</td>
</tr>
<tr>
<td>Sputum</td>
<td>19 days</td>
<td>22 days</td>
</tr>
</tbody>
</table>

**Nasopharyngeal swab**

- **A**: All patients
- **B**: All patients

**Sputum**

- **A**: All patients
- **B**: All patients
<table>
<thead>
<tr>
<th>Time to negative PCR</th>
<th>Median = 18 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean = 22 days</td>
</tr>
</tbody>
</table>
Patients with clinically more severe disease had a higher viral burden.
21 days after symptom onset, 62.5% of patients had positive sputum compared to 22.5% positive NP swab. N = 40.
Clinical characteristics of the recovered COVID-19 patients with re-detectable positive RNA tests. Preprint An et al.

- 262 patients diagnosed with COVID-19 were discharged, continued isolated for 14 days, and then an additional 2 weeks with weekly follow-up.
  - Discharge criteria included improving symptoms and 2 negative RT-PCR tests
- 38 patients were re-detected on follow up (14.5%) from anal or respiratory samples.
  - Original disease state
    - Moderate (N=27): On re-admission, patients had cough and chest tightness, no fever
    - Mild (N=11): On re-admission, 1 patient had a cough, no one had fever.
  - Patients with originally mild disease were generally younger.
  - Adding negative anal swab as a discharge criteria did not affect rate of patients re-testing as positive.
  - 21 close contacts of these patients were followed and did not have symptoms or positive testing for SARS-CoV-2
Association between clinical, laboratory and CT characteristics and RT-PCR results in the follow-up of COVID-19 patients. Preprint Fu et al.

- N=52 patients who were discharged after COVID-19 diagnosis with 2 consecutive negative PCR tests
- After median post-discharge days 13 (9 to 17 days), 7 patients re-admitted with positive PCR (13.5%) on 2 week follow-up testing
  - 2 had progressive CT chest findings
  - Did not clarify testing sample/site.
Asymptomatic carriers

• Most asymptomatic carrier reports in the literature are found by screening close contacts.
  • Morbidity and Mortality Weekly report cluster of King County nursing home:
    • Testing of residents: 30% positive
    • Of positive patients, 57% were asymptomatic.
    • Within 7 days of testing, 10 of the 13 patients had developed symptoms
    • N=51021 total positive patients
    • 50 patients asymptomatic (0.098%)
  • Close familial contacts: around 5%

• CDC: “Virologic studies have also detected SARS-CoV-2 with RT-PCR low cycle thresholds, indicating larger quantities of viral RNA, and cultured viable virus among persons with asymptomatic and pre-symptomatic SARS-CoV-2 infection.19,24,26,33 The exact degree of SARS-CoV-2 viral RNA shedding that confers risk of transmission is not yet clear.”
Summary PCR

- The timing and site of RT-PCR collection affects the sensitivity of testing.
  - Around day 8-10 after symptoms begin, the sensitivity of nasopharyngeal vs. lower respiratory tract specimens begins to diverge.

- There can be persistent shedding of virus in respiratory and anal specimens.
  - There can be re-detection in a subset of patients (around 14%) by upper respiratory specimens after negative testing.
  - Unclear infectious potential of these patients with recurrent positives or persistently positive sputum or stool specimens.

- Tests that have FDA Emergency Use Authorization can only use the sample types specified by the vendor; e.g. only nasopharyngeal swabs. Other sample types have to be sent to a reference lab that has validated other specimen types.
SARS-CoV-2 antigens for antibody generation

• There are 4 different coronavirus structural proteins:
  • Spike protein with receptor binding domain (S), (RBD)
  • Envelope protein (E)
  • Membrane protein (M)
  • Nucleocapsid protein (N)
• S1 protein RBD show more specificity for SARS-CoV-2 compared to other protein sequences (MERS-CoV, SARS-CoV [2003])
  • SARS-CoV-2 N protein homology is 90% compared to SARS-CoV.

Classic serologic teaching

Serologic tests for SARS-CoV-19 overview

- IgG vs IgM in SARS-CoV-19:
  - IgM and IgG does not appear in all patients.
  - Antibody response may be low in patients with mild symptoms and may be absent in immunocompromised patients (verbal communication, Dr. V. Luzzi Prov. Oregon Core Lab)
  - IgG and IgM may appear concurrently or IgG may appear before IgM

- Different manufacturers of antibody proteins and different methods of antibody measurement.
Serology characteristics of SARS Co-V-2 infection since exposure and post symptom onset. Preprint Lou et. al

• Study population:
  • Positive PCR from deep sputum sample N=80, 45 of whom had a known day 0 documented exposure
  • Negative (healthy) N=300

• Immunoassays
  • ELISA (enzyme-linked immunosorbent assays), CMIA (chemiluminescence), LFIA (colloidal gold lateral flow)
  • RNA testing of confirmed patients performed by deep sputum sampling
Table 3. Performance of different detections in different periods post onset

<table>
<thead>
<tr>
<th>Days after onset</th>
<th>No. Patients</th>
<th>RNA* n(+)</th>
<th>Sensitivity (%)</th>
<th>ELISA-Ab n(+)</th>
<th>Sensitivity (%)</th>
<th>ELISA-IgM n(+)</th>
<th>Sensitivity (%)</th>
<th>ELISA-IgG n(+)</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7</td>
<td>39</td>
<td>36$</td>
<td>100.0</td>
<td>25</td>
<td>64.1</td>
<td>13</td>
<td>33.3</td>
<td>13</td>
<td>33.3</td>
</tr>
<tr>
<td>8-14</td>
<td>75</td>
<td>64$</td>
<td>90.1</td>
<td>74</td>
<td>98.7</td>
<td>65</td>
<td>86.7</td>
<td>57</td>
<td>76.0</td>
</tr>
<tr>
<td>15-29</td>
<td>60</td>
<td>41$</td>
<td>70.7</td>
<td>60</td>
<td>100.0</td>
<td>58</td>
<td>96.7</td>
<td>56</td>
<td>93.3</td>
</tr>
</tbody>
</table>

* RNA was tested using deep sputum sample.

$ There were 36, 71 and 58 patients had RNA testing during the periods between 0-7, 8-14 and 15-29 days post onset, respectively.

IgM and total antibodies: receptor binding domain antigen; IgG: nucleoprotein
Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody response during infection by SARS-CoV-2: an observational study. To et al, Lancet 2020

- N= 23 patients
- Both IgG and IgM against NP and RBD were tested
- Serologic conversion for anti-RBD was slightly sooner than anti-NP
- However, IgG seroconversion before IgM was seen more often with anti-NP

<table>
<thead>
<tr>
<th>Courses of disease, days*</th>
<th>Patients, no.</th>
<th>Positives, no./total no. (%)</th>
<th>RNA positive in NS, sputum, and/or stool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6/58 (10.3)</td>
<td>2/58 (3.4)</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>19/62 (30.6)</td>
<td>12/62 (19.4)</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>26/61 (42.6)</td>
<td>31/61 (50.8)</td>
</tr>
<tr>
<td>14</td>
<td>61</td>
<td>25/54 (46.3)</td>
<td>32/54 (59.3)</td>
</tr>
<tr>
<td>21</td>
<td>54</td>
<td>20/35 (57.1)</td>
<td>26/35 (74.3)</td>
</tr>
<tr>
<td>28</td>
<td>35</td>
<td>9/22 (40.9)</td>
<td>17/22 (77.3)</td>
</tr>
<tr>
<td>35</td>
<td>22</td>
<td>5/15 (33.3)</td>
<td>13/15 (86.7)</td>
</tr>
<tr>
<td>42</td>
<td>15</td>
<td>0</td>
<td>4/5 (80.0)</td>
</tr>
<tr>
<td>49</td>
<td>5</td>
<td>28/65 (43.1)</td>
<td>45/65 (69.2)</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Days were counted from symptom onset; NS: nasopharyngeal swab.
3 tiers of antibody response seen, with higher titers seen in patients with more severe disease for both IgM and IgG, with similar time of appearance; N=65

- Strong responders: peak >2x cutoff value (31% IgM, 22.2% IgG)
- Weak responders: peak 1 – 2x cutoff value (17.2% IgM, 61.1% IgG)
- Negative responders: peak titer below cutoff value (51.7% IgM, 16.7% IgG)

Weak responders for IgG had a higher viral clearance rate than strong responders; weak antibody response means faster viral clearance.

<table>
<thead>
<tr>
<th>Days after onset</th>
<th>n</th>
<th>RNA n(+)</th>
<th>Sensitivity (%)&lt;brihave 95% CI</th>
<th>Ab n(+)</th>
<th>Sensitivity (%)&lt;brihave 95% CI</th>
<th>IgM n(+)</th>
<th>Sensitivity (%)&lt;brihave 95% CI</th>
<th>IgG n(+)</th>
<th>Sensitivity (%)&lt;brihave 95% CI</th>
<th>RNA+Ab n(+)</th>
<th>Sensitivity (%)&lt;brihave 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>173</td>
<td>112&lt;sup&gt;$&lt;/sup&gt;</td>
<td>67.1 (59.4, 74.1)</td>
<td>161</td>
<td>93.1 (88.2, 96.4)</td>
<td>143</td>
<td>82.7 (76.2, 88)</td>
<td>112</td>
<td>64.7 (57.1, 71.8)</td>
<td>172</td>
<td>99.4 (96.8, 100.0)</td>
</tr>
<tr>
<td>1-7</td>
<td>94</td>
<td>58&lt;sup&gt;$&lt;/sup&gt;</td>
<td>66.7 (55.7, 76.4)</td>
<td>36</td>
<td>38.3 (28.5, 48.9)</td>
<td>27</td>
<td>28.7 (19.9, 39.0)</td>
<td>18</td>
<td>19.1 (11.8, 28.6)</td>
<td>74</td>
<td>78.7 (69.1, 86.5)</td>
</tr>
<tr>
<td>8-14</td>
<td>135</td>
<td>67&lt;sup&gt;$&lt;/sup&gt;</td>
<td>54.0 (44.8, 63.0)</td>
<td>121</td>
<td>89.6 (83.2, 94.2)</td>
<td>99</td>
<td>73.3 (65.0, 80.6)</td>
<td>73</td>
<td>54.1 (45.3, 62.7)</td>
<td>131</td>
<td>97.0 (92.6, 99.2)</td>
</tr>
<tr>
<td>15-39</td>
<td>90</td>
<td>25&lt;sup&gt;$&lt;/sup&gt;</td>
<td>45.5 (32.0, 59.5)</td>
<td>90</td>
<td>100.0 (96.0, 100.0)</td>
<td>83*</td>
<td>94.3 (87.2, 98.1)</td>
<td>71&lt;sup&gt;#&lt;/sup&gt;</td>
<td>79.8 (69.9, 87.6)</td>
<td>90</td>
<td>100.0 (96.0, 100.0)</td>
</tr>
</tbody>
</table>

* Two patients missed IgM tests due to inadequate plasma samples. <sup>$</sup> One patient missed IgG tests due to inadequate plasma samples. <sup>$</sup> There were 7, 11 and 35 patients had not been performed RNA testing during the 1-7 onset day, 8-14 onset day and 15-39 onset day, respectively.
• "Respiratory tract" RNA specimen
• Total antibody and IgM against RBD protein and IgG against nucleoprotein

<table>
<thead>
<tr>
<th>Positive (%)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>&gt;21 Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>50.0</td>
<td>62.5</td>
<td>71.4</td>
<td>73.3</td>
<td>71.4</td>
<td>62.9</td>
<td>62.2</td>
<td>44.7</td>
<td>41.9</td>
<td>46.4</td>
<td>42.9</td>
<td>47.8</td>
<td>30.0</td>
<td>33.3</td>
<td>40.0</td>
<td>27.3</td>
<td>45.5</td>
<td>22.2</td>
<td>50.0</td>
<td>40.0</td>
<td>48.0</td>
</tr>
<tr>
<td>Ab</td>
<td>50.0</td>
<td>25.0</td>
<td>17.7</td>
<td>29.4</td>
<td>27.3</td>
<td>36.8</td>
<td>41.4</td>
<td>57.1</td>
<td>68.2</td>
<td>69.7</td>
<td>83.3</td>
<td>95.0</td>
<td>92.0</td>
<td>91.3</td>
<td>100.0</td>
<td>100.0</td>
<td>87.5</td>
<td>94.1</td>
<td>95.2</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>IgM</td>
<td>50.0</td>
<td>25.0</td>
<td>17.7</td>
<td>11.8</td>
<td>27.3</td>
<td>24.3</td>
<td>37.9</td>
<td>40.5</td>
<td>34.1</td>
<td>64.5</td>
<td>70.0</td>
<td>76.9</td>
<td>83.3</td>
<td>80.0</td>
<td>85.7</td>
<td>91.3</td>
<td>79.2</td>
<td>73.3</td>
<td>90.0</td>
<td>88.9</td>
<td>66.7</td>
</tr>
<tr>
<td>IgG</td>
<td>50.0</td>
<td>12.5</td>
<td>5.9</td>
<td>0</td>
<td>18.2</td>
<td>18.9</td>
<td>25.0</td>
<td>23.8</td>
<td>30.2</td>
<td>36.7</td>
<td>56.7</td>
<td>57.9</td>
<td>48.0</td>
<td>42.9</td>
<td>68.2</td>
<td>82.6</td>
<td>66.7</td>
<td>56.3</td>
<td>80.0</td>
<td>77.8</td>
<td>66.7</td>
</tr>
</tbody>
</table>

Less than 65% sensitivity for any 1 test (RNA, IgG, IgM)
Table 3. Serological presence of antibodies against SARS-CoV-2 in patients with undetectable viral RNA at different time since onset of disease.

<table>
<thead>
<tr>
<th>Days after onset</th>
<th>No. of patients</th>
<th>Detectable antibody in plasma, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>with undetectable RNA*</td>
<td>Ab (28.6)</td>
</tr>
<tr>
<td>1-3</td>
<td>7</td>
<td>2 (28.6)</td>
</tr>
<tr>
<td>4-7</td>
<td>28</td>
<td>15 (53.6)</td>
</tr>
<tr>
<td>8-14</td>
<td>57</td>
<td>56 (98.2)</td>
</tr>
<tr>
<td>15-39</td>
<td>30</td>
<td>30 (100)</td>
</tr>
</tbody>
</table>

* RNA was tested using throat/nasal swab sample.

- N = 63 patients
- PCR diagnosis by nasal and/or pharyngeal swabs
- Antibodies to Spike protein and nucleocapsid protein
- N = 27 patients subset seroconversion
  - Synchronous IgG & IgM: 10/27
  - IgM before IgG: 7/27
  - IgM after IgG: 10/27
• A married pair were diagnosed with COVID February 4, 2020.
• 164 people were identified as close contacts (Jan 20 – Feb 6)
• Cohort RT-PCR tested Jan 31-Feb 9
• Cohort serum samples collected March 1

6.1% of close contacts asymptomatic but infected
Comparison of serologic literature

- Serology characteristics of SARS Co-V-2 infection since exposure and post symptom onset. Preprint Lou et al.

1. Day 14 IgG % positive: 50.8%
2. Day 8-14 IgG % positive: 54.1%
3. Day 8-14 IgG % positive 76.4%

Same antibodies tested by the same manufacturer
Comparison of serologic literature: Subset of PCR-positive patients without seroconversion

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>IgM</th>
<th>IgG</th>
<th>Antibody target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livzon Diagnostics</td>
<td>56.9%</td>
<td>30.8%</td>
<td>IgM and IgG: N protein</td>
</tr>
<tr>
<td>Beijing Wantai Biological Pharmacy</td>
<td>7.5%</td>
<td>11.2%</td>
<td>IgM: RBD IgG: N protein</td>
</tr>
<tr>
<td>Beijing Wantai Biological Pharmacy</td>
<td>17.3%</td>
<td>35.3%</td>
<td>IgM: RBD IgG: N protein</td>
</tr>
<tr>
<td>Lab developed</td>
<td>22%</td>
<td>Not reported</td>
<td>IgM and IgG: N protein</td>
</tr>
</tbody>
</table>
Antibody specificity

- Endemic coronaviruses with seasonal variance
  - OC43
  - NL63
  - 229E
  - HKU1
- Requires validation of antibody specificity

• Validating IgM/IgG assay specificity important as COVID-19 surge predictions vary across the United States.

• Later in season, increased endemic coronavirus cases will appear, and a specific antibody is needed to distinguish from SARS-CoV-19.


- Cross reactivity of antibodies to N nucleocapsid protein
- ELISA assay showing reactivity of antibodies to endemic coronavirus and to SARS-CoV-2
FDA statement on serologic testing: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-diagnostic-testing-sars-cov-2: “What serology tests are being offered...for Coronavirus Disease-19?”

• As stated in Section IV.D of the FDA's Policy for Diagnostic Tests for Coronavirus Disease-2019, the FDA does not intend to object to the development and distribution by commercial manufacturers, or development and use by laboratories, of serology tests to identify antibodies to SARS-CoV-2, where the test has been validated, notification is provided to FDA, and information along the lines of the following is included in the test reports:

• This test has not been reviewed by the FDA.

• Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.

• Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.

• Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
FDA defines SARS-CoV-2 serological diagnostic tests as tests that identify antibodies (e.g. IgM, IgG) to SARS-CoV-2 from clinical specimens. FDA recommends that the following validation studies be conducted for a SARS-CoV-2 serological assay:

- Cross-reactivity/Analytical Specificity
- Class Specificity
- Clinical Agreement Study.

The clinical agreement study is intended to establish the performance characteristics (e.g. sensitivity/PPA, specificity/NPA) of the test. FDA recommends that clinical accuracy should be established on human specimens from patients with microbiologically confirmed COVID-19 infection.”
• Assure Tech (Hangzhou) Co., Ltd.'s COVID-19 IgG/IgM Rapid Test Device
• Autobio Diagnostics' Anti-SARS-CoV-2 Rapid Test
• Beijing Decombio Biotechnology Co., Ltd. Novel Coronavirus IgM/IgG Combo Rapid Test Cassette (Serum/Plasma/Whole blood)
• Beijing Diagreat Biotechnologies Co., Ltd. 2019-nCoV IgG Antibody Determination Kit
• Beijing Diagreat Biotechnologies Co., Ltd. 2019-nCoV IgM Antibody Determination Kit
• BioMedomics, Inc. COVID-19 IgM-IgG Rapid Test
• BNX, Inc. Rapid Response™ COVID-19 IgG/IgM Test Cassette
• Core Technology Co., Ltd. CoreTest COVID-19 IgM/IgG Ab Test
• Coronacide™ COVID-19 IgM/IgG Rapid Test
• Diazyme Laboratories, Inc. Diazyme DZ-LITE SARS-CoV-2 IgG CLIA Kit
• Diazyme Laboratories, Inc. Diazyme DZ-Lite SARS-CoV-2 IgM CLIA Kit
• Guangzhou Wondfo Biotech Co., Ltd. SARS-CoV-2 Antibody Test
• Hangzhou Clongene Biotech Co., Ltd. Clongene COVID-19 IgM/IgG Rapid Test Cassette
• Hangzhou Biotest Biotech's COVID-19 IgG/IgM Rapid Assay Kit
• Hangzhou Testsealabs Biotechnology Co., Ltd. One Step SARS-CoV2 (COVID-19) IgG/IgM Test
• Healgen Scientific, LLC. COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
• INNOVITA (Tangshan) Biological Technology Co., Ltd. 2019-nCoV Ab Test (Colloidal Gold)
• Jiangsu Macro & Micro-Test Med-Tech Co., Ltd. SARS-CoV-2 IgM/IgG Rapid Assay Kit (Colloidal Gold)
• Medical Systems Biotechnology Co., Ltd. Coronavirus Disease 2019 Antibody (IgM/IgG) Combined Test Kit
• Nirmidas Biotech, Inc. COVID-19 (SARS-CoV-2) IgM/IgG Antibody Detection Kit
• Phamatech Inc. COVID19 IgG/IgM Rapid Test
• Promedical COVID-19 Rapid Test (Wondfo SARS-CoV-2 Antibody Test (Lateral Flow Method))
• SD Biosensor STANDARD Q COVID-19 IgM/IgG Duo
• Suzhou Kangheshun Medical Technology Co., Ltd SARS-CoV-2 IgG/IgM Rapid Test Cassette
• Telepoint Medical Services SARS-CoV-2
• United Biomedical, Inc. UBI® SARS-CoV-2 ELISA
• Zhejiang Orient Gene Biotech, Co., Ltd. COVID-19 IgG/IgM Antibody Rapid Test Kit
• Zhengzhou Fortune Bioscience Co., Ltd. COVID-19 IgG Antibody Rapid Test Kit (Colloidal Gold Immunochromatography method)
• Zhengzhou Fortune Bioscience Co., Ltd. COVID-19 IgM Antibody Rapid Test Kit (Colloidal Gold Immunochromatography method)
• Zhengzhou Fortune Bioscience Co., Ltd. COVID-19 Antibody Rapid Test Kit (Colloidal Gold Immunochromatography method)
• Zuhai Encode Medical Engineering Co., Ltd Novel Coronavirus (COVID-19) IgG/IgM Rapid Test Device
• Zuhai Livzon Diagnostics, Inc. Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2) (Colloidal Gold)

Tests listed on FDA website as offering POC serology
Serologic point of care tests

• Most test both IgM and IgG.

• In their vendor documentation of kit performance, most (23/25) do not specify the timeline of when the samples were taken in the patient course.

• Sensitivity
  • IgM: 65.1% to 97.9%.
  • IgG: 91.8% to 100%

• Sample types include serum, plasma, whole blood (including fingerstick).
Summary

- Asymptomatic patients with a negative upper respiratory tract PCR test can be detected, as shown by serologic studies (up to 4% of close contacts).

- Serologic technicalities:
  - IgM response can be absent, weak or delayed.
  - IgG response can appear synchronously or precede IgM.
  - IgM serologic testing is technically difficult, and major reference labs are largely considering offering IgG alone.

- A higher IgM and IgG titer seems to correspond with more severe disease; the corollary is that weaker titers are seen in patients with mild disease or patients who are immunocompromised.
  - Any test for detection has to be able to identify low responders.
Summary

• Serologic testing can be used to rule in but not rule out patients, particularly in the early phase of symptoms.

• Serological testing for IgG could be useful as an epidemiologic test.

• Point of care kits that test IgM and IgG may be able to distinguish acute from long past infection, but we do not know how long the IgM level is elevated.
  • Not all patients will produce IgM or IgG
Open questions

• How was the sputum sample collected given most patients have a dry cough?
• How uniform is the titering procedure?
• How long does the IgM or IgG peak last?
• Do the antibodies confer immunity, and if so, for how long?
• How specific are these tests for SARS-CoV-2 compared to other endemic coronaviruses which will rise in the autumn and winter?
Countries reject China pandemic product batches
The Netherlands, Spain and Turkey question quality of face masks and tests

Spain, Europe's worst-hit country after Italy, says coronavirus tests it bought from China are failing to detect positive cases

Business Insider and Financial Times: Shenzhen Bioeasy Biotechnology Company, March 26, 2020
Thank you

- PRMCE Lab staff and leadership team
- Dr. George Diaz
- Dr. Veronica Luzzi
- Infection Prevention and Employee Health (Sarah Wilkerson, Audrey Meier, Kim Fuller)

- Questions or references request? Kirstine.oh@providence.org

• “What complexity testing personnel can perform the Emergency Use Authorization (EUA) COVID-19 tests?

• Almost all currently EUA-authorized tests for COVID-19 are FDA-authorized for use by laboratories that meet the CLIA requirements for either moderate or high complexity testing. Therefore, testing personnel must meet the appropriate moderate or high complexity CLIA testing personnel qualification requirements depending on which EUA-authorized tests are being used by the laboratory.”

• This may not apply with rapid serologic tests, but until the tests are evaluated by FDA, the classification appears to be moderately complex.
## Requirements for CLIA non-waived moderately complex testing

<table>
<thead>
<tr>
<th>Oversight</th>
<th>Personnel</th>
<th>Competency Assessments</th>
<th>Quality Assurance and Quality Control Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLIA license</td>
<td>Lab medical director with 2 years testing experience</td>
<td>Timing: initial (first training + 6 month) + 12 month annually</td>
<td>Defined QC and QA program with daily QC and annual review of QA</td>
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<tr>
<td>Biennial inspections (CAP, DOH, JAHCO, or CMS)</td>
<td>Testing personnel: education transcripts</td>
<td>Contents of assessment (6 elements)</td>
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<tr>
<td></td>
<td></td>
<td>1. Direct observation of testing</td>
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<td>2. Result recording monitoring</td>
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<td>3. Review of QC, Maintenance, PT, worksheets</td>
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<td>4. Direct observation of maintenance checks</td>
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<td>5. Performance testing of known samples (e.g. proficiency testing)</td>
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<td>6. Assessment of problem solving skills</td>
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<tr>
<td></td>
<td>Technical consultant: at least B.S. degree with 2 years of testing experience</td>
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