Evaluating the Quality and Reliability of Covid-19 Diagnostic Tests

Naveen Agarwal, Ph.D.
Email: Naveen.Agarwal@ExeedQM.com
Website: https://www.ExeedQM.com

ASQ South Atlantic Region
May 21st, 2020
29 states report more than 10,000 cases of COVID-19.

This map shows COVID-19 cases and deaths reported by U.S. states, the District of Columbia, and other U.S.-affiliated jurisdictions. Hover over the map to see the number of cases and deaths reported in each jurisdiction. To go to a jurisdiction's health department website, click on the jurisdiction on the map.

Source: cdc.gov, data as of 21 May 2020
Current Models Project a Lot of Uncertainty

“For estimated infections, we start with death estimates, then work backward, using infection fatality ratios to estimate infections based on deaths”.

Source: Institute for Health Metrics and Evaluation
Doubts and Concerns Persist About Testing

AdventHealth says 25,000 of its COVID-19 test results are 'unreliable'

ORLANDO, Fla. - AdventHealth says the results of more than 25,000 coronavirus tests performed by the health system in Central Florida are ... 1 day ago

Coronavirus (COVID-19) Update: FDA Informs Public About Possible Accuracy Concerns with Abbott ID NOW Point-of-Care Test

Dozens of coronavirus antibody tests on the market were never vetted by the FDA, leading to accuracy concerns

Sources: Various News Outlets
Topics for Today

- Overview of Different Types of Testing
- Focus on Antibody Testing
  - ELISA and Lateral Flow Techniques
  - Understanding Accuracy
  - Understanding “Real World” Performance
  - Current Performance Levels of FDA Authorized Tests
- Testing in Perspective
  - Individual vs. Public Health Considerations
- Discussion
  - How can Quality Professionals help?
  - Q&A
3 Types of Tests for SARS-CoV2

**RT-PCR Tests**
- Detect Viral RNA
- High Sensitivity & Specificity
- Slow, Limited Point of Care

**Antigen Tests**
- Detect Viral Proteins (S, M or N)
- Medium Sensitivity & Specificity
- Rapid Results, Point of Care

**Antibody Tests**
- Detect antibodies
- Medium Sensitivity & Specificity
- Rapid Results, Point of Care

For illustration only, no affiliation with specific tests.
When You Test Matters

Source: “Interpreting Diagnostic Tests for SARS-CoV-2”, JAMA, 6 May 2020
A Quick Overview of Two Techniques

1 ELISA – Enzyme Linked ImmunoSorbent Assays

Microtiter plate coated with SARS-CoV2 recombinant binding domain protein (RBD)

Load diluted sample

Wash and add secondary anti-human IgG HRP

Add substrate
Color change => positive

Note: There are many more steps involved and a second confirmatory, more quantitative test is done for presumed positive samples
A Quick Overview of Two Techniques

2. Lateral Flow Immunoassay

- **Lateral Flow Immunoassay**
  - Source: Cellex q Rapid Test IFU
  - Only C -> negative
  - C and M -> IgM Positive
  - C and G -> IgG Positive
  - C, M and G -> IgM and IgG Positive

![Lateral Flow Immunoassay Diagram](source_image)
More than 100 Tests Authorized by the FDA

- RT-PCR Tests: 92
- Antibody Tests: 12
- Antigen Tests: 1

Source: FDA Emergency Use Authorizations
Count as of May 20, 2020, RT-PCR count includes Lab Developed Tests (LDT)
Understanding Test Accuracy

- **Infected**
  - True Positive
  - False Positive

- **Uninfected**
  - False Negative
  - True Negative

- **Has Antibodies**
- **Does Not Have Antibodies**
Estimating Test Accuracy – Lab Validation

<table>
<thead>
<tr>
<th></th>
<th>Infected</th>
<th>Uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has Antibodies</td>
<td>TP 27</td>
<td>FP 4</td>
</tr>
<tr>
<td>Does Not Have Antibodies</td>
<td>FN 3</td>
<td>TN 76</td>
</tr>
</tbody>
</table>

**Diagnostic Sensitivity, $DS_n$**
- $27/30 = 90\%$
- $95\%$ CI = $73.5\% - 97.9\%$

**Diagnostic Specificity, $DS_p$**
- $76/80 = 95\%$
- $95\%$ CI = $87.7\% - 98.6\%$

For illustration only; based on recently announced FDA expectations for IgM/IgG serology tests.
What Does This Mean in the Real World?

Say you got a **positive** result and you ask your doctor – “Are you sure I **have** the antibodies to SARS-CoV-2?”

Or, say you got a **negative** result and you ask your doctor – “Are you sure I **don’t have** the antibodies to SARS-CoV-2?”

Note – none of the current antibody tests are authorized for use at home
We Need to Know the Predictive Value of a Test

Positive Predictive Value (PPV)

Given a \textit{positive} result, what is the likelihood that the individual \textit{has} antibodies to SARS-CoV-2?

Negative Predictive Value (NPV)

Given a \textit{negative} result, what is the likelihood that the individual \textit{does not} have antibodies to SARS-CoV-2?

Note – An alternate approach is to use Likelihood Ratios
So, How Well Does a Test Perform in Real Life?

Diagnostic Sensitivity, $DS_n = 90\%$

Diagnostic Specificity, $DS_p = 95\%$

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>49%</td>
<td>99%</td>
</tr>
<tr>
<td>25%</td>
<td>86%</td>
<td>97%</td>
</tr>
</tbody>
</table>
But Wait…Recall the 95% Confidence Interval?

**Diagnostic Sensitivity, $DS_n = 90\%$**
95% CI = 73.5% - 97.9%

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>25%</td>
<td>98%</td>
</tr>
<tr>
<td>25%</td>
<td>77%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Diagnostic Specificity, $DS_p = 95\%$**
95% CI = 87.7% - 98.6%

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>25%</td>
<td>98%</td>
</tr>
<tr>
<td>25%</td>
<td>77%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Should We Increase $D_{Sn}$ or $D_{Sp}$?

**Scenario 1:**

<table>
<thead>
<tr>
<th></th>
<th>$D_{Sn} = 95%$</th>
<th>$D_{Sp} = 95%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% Prevalence</td>
<td>50%</td>
<td>100%</td>
</tr>
<tr>
<td>25% Prevalence</td>
<td>86%</td>
<td>98%</td>
</tr>
<tr>
<td>NPV</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$D_{Sn}$ and $D_{Sp}$ represent sensitivity and specificity, respectively. PPV and NPV represent positive predictive value and negative predictive value, respectively.
### Should We Increase $DS_n$ or $DS_p$?

**Scenario 2:**

<table>
<thead>
<tr>
<th></th>
<th>$DS_n = 90%$</th>
<th>$DS_p = 99%$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPV</strong></td>
<td>83%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>97%</td>
<td>97%</td>
</tr>
</tbody>
</table>

- **5% Prevalence**
- **25% Prevalence**
Lab Performance of FDA Authorized Tests

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Specificity (Negative Agreement)</th>
<th>Sensitivity (Positive Agreement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>100%</td>
<td>95%</td>
</tr>
<tr>
<td>Lateral Flow</td>
<td>85%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Data Source: EUA Authorized Serology Test Performance

Limited Data Availability on Precision

Reported %Coefficient of Variation for an ELISA Antibody Test

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Ratio</th>
<th>%Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.07</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>1.12</td>
<td>16.2</td>
</tr>
<tr>
<td>3</td>
<td>2.37</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>5.23</td>
<td>4</td>
</tr>
</tbody>
</table>

**Negative**

Sample 1: 0.07

Sample 2: 1.12

Sample 3: 2.37

Sample 4: 5.23

**Borderline**

Sample 1: 0.07

Sample 2: 1.12

Sample 3: 2.37

Sample 4: 5.23

**Positive**

Sample 1: 0.07

Sample 2: 1.12

Sample 3: 2.37

Sample 4: 5.23

Data Source: Instruction for Use [https://www.fda.gov/media/137609/download](https://www.fda.gov/media/137609/download)
Expected Real-World Test Performance

Positive Predictive Value (PPV)
5% Prevalence

<table>
<thead>
<tr>
<th>Test Manufacturer</th>
<th>Abbott_Alinity</th>
<th>Abbott_Architect</th>
<th>Bio-Rad</th>
<th>Cellex</th>
<th>Chembio</th>
<th>DiaSorin</th>
<th>EUROIMMIN</th>
<th>Mount Sinai</th>
<th>Ortho-IgG</th>
<th>Ortho-Total</th>
<th>Roche</th>
<th>Wadsworth NY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPV (%)</td>
<td>90%</td>
<td>80%</td>
<td>70%</td>
<td>60%</td>
<td>50%</td>
<td>40%</td>
<td>30%</td>
<td>20%</td>
<td>10%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
</tr>
</tbody>
</table>

Negative Predictive Value (PPV)
5% Prevalence

Source: FDA website
FDA Has Now Established Minimum Requirements

Independent Validation of the Covid-19 Serology Test

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Serum/Plasma</th>
<th>Serum/Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Samples</td>
<td>30 Confirmed Positive</td>
<td>80 Confirmed Negative Or Pre-Covid19 10 must be HIV Positive</td>
</tr>
<tr>
<td>Requirement</td>
<td>Combined – 90% Pos. Agreement IgM – 70% Positive Agreement IgG – 90% Positive Agreement</td>
<td>Combined – 95% Neg. Agreement No cross reactivity with HIV</td>
</tr>
</tbody>
</table>

Source: FDA Letter of Authorization [https://www.fda.gov/media/137470/download](https://www.fda.gov/media/137470/download)
Emerging FDA Priorities for Testing

- Saliva based tests
- At-home collection and testing
- Rapid Point-of-Care testing
- More antigen tests
- More options for swabs – 3D printing etc.

Source: FDA Virtual Town Hall Meetings
Let Us Put This in Perspective

Individual Level Concerns

Do I have the virus?

I have some symptoms – is it flu or Covid-19?

I haven't fully recovered, am I safe now?
Public Health Considerations
Balancing Social Distancing with Opening Up

Detecting $T_F$ allows us to tolerate a higher $R_0$ because we can test, trace and isolate.

$R_0$ # Infected by one person
$S_Y$ # Symptomatic
$T_F$ # Tested Positive
$T_R$ Days of Recovery

$$\frac{R_0}{T_R} \leq 1 \quad \text{and} \quad \frac{T_F}{T_R} \leq 1$$

Source: IDSS, MIT Institute for Data, Systems and Society
Large Scale Population Studies on the Way

National Sero-survey, April 2020
- Recruiting 10,000 volunteers nationwide
- At-home blood sample collection or at NIH
- ELISA testing, both IgM and IgG
- ClinicalTrials.Gov Identifier NCT04334954

Multiple Seroprevalence Surveys
- Large Scale: Focusing first on WA, NY and later across the United States
- Community Level: County level sampling
- Special Population: Healthcare workers etc.

Source: NIH, CDC websites
How Quality Professionals can Help

- Ignore sensational headlines
- Clearly understand benefit/risk of any action
- Help develop effective protocols and actionable metrics
- Monitor, control and build confidence
- Be sensitive to individual concerns
In Closing....

- Current Covid-19 pandemic has impacted all aspects of life
- More than 100 tests have been authorized by the FDA for emergency use
- RT-PCR, Antigen and Antibody tests offer different options across the infection lifecycle
- Testing is only one part of the overall strategy
- Quality professionals can make a significant contribution to their organizations and society at large

We are all in this together!
About Exeed™

Portfolio of Innovative Quality Solutions in 4 Broad Areas

- Customer Experience
- Regulatory Compliance
- Risk Management
- Quality Culture

Email: Info@ExeedQM.com
Web: www.ExeedQM.com
Phone: 1-833-MY-EXEED
Free Monthly Industry News

In less than 15 minutes

Sign up now

Sign Up for Our Newsletter: https://www.exeedqm.com/blog-sign-up