COVID-19 Lab Update
Impact of Dropping Prevalence on Diagnostic Accuracy

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Communicable Disease Division
Case

- The staff at a long term care facility are tested every other week
- Staff and resident vaccination rate is high (>85%)
- A nurse tests positive for SARS-CoV-2 by PCR
- They are vaccinated and have no known COVID-19 contacts or symptoms
- The facility hasn’t had a case in months
Possible reasons for a positive result

• The nurse is infected and a true positive
  • They could be Asymptomatic or Presymptomatic even if vaccinated
Vaccination and Diagnostic Testing

• It is still possible, although rare, to get COVID-19 after being fully vaccinated

• Vaccines are effective at preventing severe disease and death

• Current vaccines will not cause positive molecular or antigen results

Possible reasons for a positive result

• The nurse is infected and a true positive
  • They could be Asymptomatic or Presymptomatic
  • They could still shedding from a recent infection (long haulers)
Diagnostic Tests and Viral Load

Weeks

Amount of Virus

Symptoms

Virus

Antigen

Infectious

PCR

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Possible reasons for a positive result

• The nurse is infected and a true positive
  • They could be Asymptomatic or Presymptomatic
  • They could still shedding from a recent infection (long haulers)

• The result was a false positive
  • Human error
  • Mechanical error
  • Random error
What should you do if you suspect a false positive?

- Notify the lab
  - They can do an internal evaluation for a problem
    - Look for trends in reported false positives
    - Look for evidence of a specimen mix-up
    - Evaluate runs for mechanical failures
    - Check quality control metrics for signs of errors
    - Re-test the sample if they still have it (1-7 days)
  - If a problem is identified they can amend the report turning the result negative (very rare)

- Re-test the patient
  - While additional molecular testing can support a positive diagnosis a negative result cannot erase the first positive
  - Two negative molecular tests, collected at least 24 hours apart, can release someone from isolation. But, they remain a recorded case and initiate the outbreak protocols.
Causes of false results

Causes of false negatives

Collection
- Insufficient collection
- Label switched with another patient
- Sample gets too hot or old and degrades

Transport
- Sample gets too hot or old and degrades

Testing
- Sample gets too hot or old and degrades
- Switched with another patient
- Instrument failure

Reporting
- Data entry error

Causes of false positives

Collection
- Switched with another patient
- Sample contaminated

Transport
- Sample contaminated

Testing
- Instrument failure
- Switched with another patient

Reporting
- Data entry error

Error can be intrinsic to the test itself

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It's it easy to tell positive and negative apart?

<table>
<thead>
<tr>
<th>Test Results</th>
<th>No Disease</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>
Biology Means Diversity

![Graph showing frequency of test results for No Disease and Disease.]
Finding the Gray Zone

- **Control Test**
  - Negative
  - Gray zone
  - Positive

<table>
<thead>
<tr>
<th>Disease</th>
<th>No Disease</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>250</td>
<td>700</td>
<td>1,150</td>
</tr>
<tr>
<td>1,600</td>
<td>2,050</td>
<td>2,500</td>
</tr>
<tr>
<td>25,000</td>
<td>250,000</td>
<td>250,000</td>
</tr>
</tbody>
</table>

Test Results

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10

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Back to Basics

<table>
<thead>
<tr>
<th>Test Results</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7</td>
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<td>5</td>
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<td>10</td>
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<td>2</td>
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<td>6</td>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
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<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**Sensitivity** - How good is the test at detecting positives?

**Specificity** - How good is the test at distinguishing true positives from false positives?

**Accuracy** - How good the test is overall at giving a correct diagnosis
### Calculating Test Performance

#### Understanding the Chart

<table>
<thead>
<tr>
<th>Truth</th>
<th>Patients with Disease</th>
<th>Patients without Disease</th>
<th>Total test results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Results</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Positive Test</strong></td>
<td>True positive</td>
<td>False positive</td>
<td>Total positive tests</td>
</tr>
<tr>
<td><strong>Negative Test</strong></td>
<td>False negative</td>
<td>True negative</td>
<td>Total negative tests</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>Total people with disease</td>
<td>Total people without disease</td>
<td>Total People tested</td>
</tr>
</tbody>
</table>

- **Sensitivity**: (% of people with disease that have a positive test)
- **Specificity**: (% of people without disease that have a negative test)

**Prevalence** = % of people tested that have disease
## Calculating Test Performance

### What you need to know
- Prevalence of the disease in the people you are testing
- The sensitivity and specificity of your test

### The Table

<table>
<thead>
<tr>
<th></th>
<th>Patients with Disease</th>
<th>Patients without Disease</th>
<th>Total test results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive Test</strong></td>
<td>A</td>
<td>B</td>
<td>A+B</td>
</tr>
<tr>
<td></td>
<td>% sensitivity x E</td>
<td>(1-% specificity) x F</td>
<td></td>
</tr>
<tr>
<td><strong>Negative Test</strong></td>
<td>C</td>
<td>D</td>
<td>C+D</td>
</tr>
<tr>
<td></td>
<td>(1-% sensitivity) x E</td>
<td>% specificity x F</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>E</td>
<td>F</td>
<td># of people tested</td>
</tr>
<tr>
<td></td>
<td>% prevalence x G</td>
<td>(1-% prevalence) x G</td>
<td></td>
</tr>
</tbody>
</table>

### Calculations

- **PPV** = \( \frac{A}{(A+B)} \times 100 \)
- **NPV** = \( \frac{D}{(C+D)} \times 100 \)
Example 1: Testing a facility using a PCR test during an outbreak

<table>
<thead>
<tr>
<th></th>
<th>Patients with Disease</th>
<th>Patients without Disease</th>
<th>All Patients</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive Test</strong></td>
<td>147</td>
<td>4</td>
<td>151</td>
<td>97%</td>
</tr>
<tr>
<td><strong>Negative Test</strong></td>
<td>3</td>
<td>846</td>
<td>849</td>
<td>NPV</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>150</td>
<td>850</td>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

- **Sensitivity**: 99.5%
- **Specificity**: 99.5%
- **False positive**: 4
- **False negative**: 3
- **Prevalence**: 15%

**Calculations**

- PPV (Positive Predictive Value) = \( \frac{147}{151} \times 100\% = 97\% \)
- NPV (Negative Predictive Value) = \( \frac{846}{849} \times 100\% = 99\% \)
Example 2: Routine testing by PCR of vaccinated employees who wear masks and distance

<table>
<thead>
<tr>
<th></th>
<th>Patients with Disease</th>
<th>Patients without Disease</th>
<th>All Patients</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive Test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>5</td>
<td>9.9</td>
<td>49%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>False positive</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Negative Test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>990</td>
<td>990.1</td>
<td></td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>False negative</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>1000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

98% Sensitivity
99.5% Specificity
0.5% Prevalence

A test is only as good as the population tested
Prevalence has Dropped

7-day percent positive by test, total tests by day

https://www.dhs.wisconsin.gov/covid-19/data.htm
How Dropping Prevalence Impacts PPV

*Based on PCR with 99.5% specificity and antigen with 97% specificity.
How can we maintain confidence in testing?

Two-tier testing strategy

Pro tip: If you use a 2 tiered testing system the first test should be the most sensitive, the second should be highly specific to produce the greatest accuracy.
Testing Strategy for Lower Accuracy Tests
(most point of care tests)

Symptomatic?

Yes
- COVID-19 antigen test
  - Probable case: Isolate patient - Start contact tracing
  - Second test for COVID-19
    - Positive: Confirmed case: Isolate patient - Start contact tracing
    - Negative: Not a case - Consider if additional testing is warranted (flu or other respiratory viruses)

No
- Close contact?
  - Yes
    - Quarantine
  - No
    - COVID-19 antigen test
      - Probable case: Isolate patient - Start contact tracing
      - Second test for COVID-19
        - Positive: Confirmed Case - Continue to isolate
        - Negative: Not a case: Can leave isolation if second test was within 48 hours of the first

Note: Confirmatory testing should be performed as soon as possible and within 48 hours. PCR as the second test is highly preferred for the greatest accuracy. Antigen tests can only result in probable cases, not confirmed cases.

DHS Guidance- HAN #17 [https://content.govdelivery.com/accounts/WIDHS/bulletins/2a24fd9](https://content.govdelivery.com/accounts/WIDHS/bulletins/2a24fd9)
Testing strategy for lower accuracy tests

- Symptoms
  - Positive: Retest (PCR if available)
  - Negative: No Retest
- No Symptoms
  - Positive: No Retest
  - Negative: No Retest
- Exposure
  - Positive: Quarantine 14 days
  - Negative: Wait 3-5 days before you test

For more information on COVID-19 testing in Wisconsin, visit: www.dhs.wisconsin.gov/testing

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What the right amount of testing?

• Depends on the goal
  • Identify all cases
  • Monitor for new variants
  • Understand disease prevalence

• Aim to have 5-10% of tests be positive
  • Too high and you might be missing positives
  • Too low and positives will be more likely to be false
Adjusting Prevalence

• Prevalence is about who you are testing which can be changed by targeting different patient populations

• Ways to increase prevalence (too many false positives)
  • Only test people at higher risk of disease
    • Congregate settings
    • Outbreak investigations
    • Close contacts
    • People with symptoms of COVID-19

• Ways to decrease prevalence (missing too many cases)
  • Test more people
  • Test the same people more often
How Dropping Prevalence Impacts PPV

- **Goldilocks Zone**
- **Danger Zone**

*Based on PCR with 99.5% specificity and antigen with 97% specificity.*
Example 2: Routine testing by PCR of vaccinated employees who wear masks and distance.

<table>
<thead>
<tr>
<th>Test Result</th>
<th>Patients with Disease</th>
<th>Patients without Disease</th>
<th>All Patients</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Test</td>
<td>49</td>
<td>5</td>
<td>54</td>
<td>99.5%</td>
<td>99%</td>
</tr>
<tr>
<td>Negative Test</td>
<td>1</td>
<td>945</td>
<td>946</td>
<td>99%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>950</td>
<td>1000</td>
<td>98%</td>
<td>99.5%</td>
</tr>
</tbody>
</table>

5% Prevalence

Sensitivity: 98%
Specificity: 99.5%
PPV: 91%
NPV: 99%
Example 3: Routine testing by antigen test of employees who wear masks and distance using two-tier testing

<table>
<thead>
<tr>
<th>Test #1 (antigen)</th>
<th>Test #2 (PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients with Disease</strong></td>
<td><strong>Patients with Disease</strong></td>
</tr>
<tr>
<td><strong>Positive Test</strong></td>
<td>19.7</td>
</tr>
<tr>
<td><strong>Negative Test</strong></td>
<td>0.3</td>
</tr>
<tr>
<td><strong>False positive</strong></td>
<td></td>
</tr>
<tr>
<td><strong>False negative</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>All Patients</strong></th>
<th><strong>Positive Test</strong></th>
<th><strong>Negative Test</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPV</strong></td>
<td>98%</td>
<td>99.5%</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>99.9%</td>
<td>98.3%</td>
</tr>
<tr>
<td><strong>Prevalence</strong></td>
<td>2%</td>
<td>45.6%</td>
</tr>
</tbody>
</table>

98.7% Sensitivity 97.6% Specificity

98% Sensitivity 99.5% Specificity
Summary

• No test is perfect, errors will happen
• The error rate goes up as prevalence goes down
• Prevalence in WI has dropped a lot
• Strategically altering the prevalence in the people you test allows for greater accuracy