Evaluating HIV Test Performance

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Performance Characteristics

- **Sensitivity**
  - The ability of the test to identify correctly those who have the disease

- **Specificity**
  - The ability of the test to identify correctly those who do not have the disease
Determining Sensitivity & Specificity

- Must know the correct disease status to calculate
- **Gold standard** is the best test available
- Classic: Use a 2 x 2 table to compare performance of the new test to the gold standard test
Test Parameters

DISEASE

+  
  TRUE POSITIVE  FALSE POSITIVE

  a  b

  c  d

  FALSE NEGATIVE  TRUE NEGATIVE

TEST

Positive Predictive Value
\[ \frac{a}{a+b} \]

Negative Predictive Value
\[ \frac{d}{c+d} \]

Sensitivity
\[ \frac{a}{a+c} \]

Specificity
\[ \frac{d}{b+d} \]
Interpreting HIV Test Results

For a laboratory test:

**Sensitivity**: Probability test=positive if patient=positive

**Specificity**: Probability test=negative if patient=negative

For a person:

**Predictive value**:

Positive: Probability patient=positive if test=positive

Negative: Probability patient=negative if test=negative
Positive or negative?

Cutoff

100% Sensitivity

100% Specificity

Frequency

100% negative predictive value

PATIENTS WITHOUT DISEASE

100% positive predictive value

PATIENTS WITH DISEASE

Test Results

PATIENTS WITHOUT DISEASE

PATIENTS WITH DISEASE

100% negative predictive value

100% positive predictive value
Where to Set the Cutoff?

- If the penalty for missing a case is high (e.g., the disease is fatal and treatment exists, or disease spreads easily):
  - Use a cutoff with high sensitivity
  - Maximize true positives

- Higher sensitivity = lower specificity
  - Increases false-positives
PATIENTS WITHOUT DISEASE

PATIENTS WITH DISEASE

100% negative predictive value

100% positive predictive value

100% Sensitivity

100% Specificity

Test Results

Frequency

Cutoff
Example:  Test 10,000 persons
         Test Specificity = 99.8%  (2/1000)

HIV prevalence = 3%

True positive:  300  False positive:  20

Positive predictive value:  $\frac{300}{320} = 94\%$
Example: Test 10,000 persons

Test Specificity = 99.8% (2/1000)

HIV prevalence = 3%
True positive: 300 False positive: 20
Positive predictive value: 300/320 = 94%

Acute HIV prevalence = 0.02%
True positive: 2 False positive: 20
Positive predictive value: 2/22 = 9%
Positive Predictive Value: Newborn Screening

- 3.7 million infants each year, screened, twice

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cases</th>
<th>Incidence</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKU</td>
<td>289</td>
<td>1:13,050</td>
<td>2.65%</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>54</td>
<td>1:62,800</td>
<td>0.57%</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1203</td>
<td>1:3,300</td>
<td>1.77%</td>
</tr>
<tr>
<td>Adrenal Hyperplasia</td>
<td>51</td>
<td>1:25,100</td>
<td>0.53%</td>
</tr>
</tbody>
</table>

-Arch Pediatr Adolesc Med, 2000
Age-Specific Breast Cancer Incidence Rates U.S., All Races (SEER 1984-88)

Rates per 100,000 Population of the Specified Five-year Age Group

Source: SEER Program.
## Results of First Screening Mammography by Age Group — UCSF Mobile Mammography Program

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Cancer Detected</th>
<th>No Cancer Detected</th>
<th>Total Abnormal</th>
<th>Positive Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–39</td>
<td>9</td>
<td>273</td>
<td>282</td>
<td>3%</td>
</tr>
<tr>
<td>40–49</td>
<td>26</td>
<td>571</td>
<td>597</td>
<td>4%</td>
</tr>
<tr>
<td>50–59</td>
<td>30</td>
<td>297</td>
<td>327</td>
<td>9%</td>
</tr>
<tr>
<td>60–69</td>
<td>46</td>
<td>230</td>
<td>276</td>
<td>17%</td>
</tr>
<tr>
<td>70</td>
<td>26</td>
<td>108</td>
<td>134</td>
<td>19%</td>
</tr>
</tbody>
</table>
**PPV of First Screening Mammography by Age and Family History of Breast Cancer**

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Women without a Family History of Breast Cancer</th>
<th>Women with a Family History of Breast Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–39</td>
<td>3%</td>
<td>4%</td>
</tr>
<tr>
<td>40–49</td>
<td>4%</td>
<td>13%</td>
</tr>
<tr>
<td>50–59</td>
<td>9%</td>
<td>22%</td>
</tr>
<tr>
<td>60–69</td>
<td>17%</td>
<td>14%</td>
</tr>
<tr>
<td>70</td>
<td>19%</td>
<td>24%</td>
</tr>
</tbody>
</table>

Positive Predictive Value and **Pre-Test Probability of Disease**
Use of Multiple Tests in Sequence

- After a **positive** screening test, conduct a second test on persons who test positive.
- Decision rule: Must be positive on - both tests - either test

- Positive on **both** tests = reduces false positives
  - Increases specificity, but reduces sensitivity
Net Specificity, Sequential Tests

Net Specificity = Spec₁ + Spec₂ − (Spec₁ x Spec₂)

Example: Spec₁ = .996, Spec₂ = .998

- Spec₁ + Spec₂ = 1.994
- Spec₁ x Spec₂ = .994
- Net Specificity = 1.00
Western blot

- Interpretive Criteria:
  - Any two:
    - p24, gp41, gp120/160
FDA-approved HIV-1/HIV-2 Antibody Differentiation Assay
Geenius™ HIV-1/2 Lines

HIV-1 & HIV-2 Associated Lines

- gp36
- gp41
- gp140
- gp160
- p31*
- p24
- Control Band

HIV-2

HIV-1

* inside the nucleocapsid
<table>
<thead>
<tr>
<th>HIV-1 RESULT</th>
<th>HIV-2 RESULT</th>
<th>ASSAY INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>HIV NEGATIVE</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Negative</td>
<td>HIV-1 INDETERMINATE&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative</td>
<td>Indeterminate</td>
<td>HIV-2 INDETERMINATE&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>HIV INDETERMINATE&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Positive</td>
<td>Indeterminate</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
</tbody>
</table>
| Positive     | Positive     | HIV-2 POSITIVE with HIV-1 cross-reactivity: Antibody to HIV-2 confirmed in the sample. HIV-1 positivity (with only one HIV-1 envelope band, gp160 or gp41), is due to cross-reactivity and precludes confirmation of HIV-1*.  
  *Note: Differentiation features managed by proprietary algorithm. |
| Positive     | Positive     | HIV POSITIVE Untypable (undifferentiated): Antibodies to HIV-1 and HIV-2 confirmed in the sample. This may occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1, or may be due to co-infection with both HIV-1 and HIV-2 (rare)*.  
  *Note: Differentiation features managed by proprietary algorithm. |

<sup>a</sup> HIV-1 band(s) detected but did not meet the criteria for HIV-1 Positive

<sup>b</sup> HIV-2 band(s) detected but did not meet the criteria for HIV-2 Positive

<sup>c</sup> HIV band(s) detected but did not meet the criteria for HIV-1 Positive or HIV-2 Positive
Net Sensitivity, Sequential Tests

- Net Sensitivity = Sens1 x Sens2

- Example: Sens1 = .998, Sens 2 = .998
  - Sens1 x Sens2 = .996
Example: Sequential Rapid Tests

- Rapid Test 1: Sens .993, Spec .998
- Rapid Test 2: Sens .997, Spec .999

- Net **Specificity** = Spec1 + Spec 2 − (Spec1 x Spec2)
  = .998 + .999 - .997 = 1.0

- Net **Sensitivity** = Sens1 x Sens2
  = .993 x .997 = .990
Clinical Sensitivity

<table>
<thead>
<tr>
<th>Group</th>
<th>Number Tested</th>
<th>Non-Reactive</th>
<th>Initially Reactive</th>
<th>Repeatedly Reactive</th>
<th>Initially Reactive</th>
<th>Repeatedly Reactive</th>
<th>Repeatedly Reactive</th>
<th>HIV-1 Western blot Reactive*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>808</td>
<td>0</td>
<td>808</td>
<td>808</td>
<td>808</td>
<td>808</td>
<td>808</td>
<td>808**</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>251</td>
<td>0</td>
<td>251</td>
<td>251</td>
<td>251</td>
<td>251</td>
<td>251</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1059</td>
<td>0 (0.00%)</td>
<td>1059 (100.0%)</td>
<td>1059 (100.0%)</td>
<td>1059 (100.0%)</td>
<td>1059 (100.0%)</td>
<td>1059 (100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Clinical Sensitivity 100% (95% CI 99.72% - 100%)
### Reactivity in High Risk Populations for HIV-1 and HIV-2

<table>
<thead>
<tr>
<th>Results Obtained with Assay</th>
<th>Licensed HIV-1/HIV-2 EIA</th>
<th>Repeatedly Reactive Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number Tested</strong></td>
<td><strong>Non-Reactive</strong></td>
<td><strong>Initially Reactive</strong></td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>554</td>
<td>526 (94.95%)</td>
<td>28 (5.05%)</td>
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</table>
## Seroconversion Sensitivity

<table>
<thead>
<tr>
<th>Panel</th>
<th>Specimen Information</th>
<th>Rapid Test</th>
<th>EIA #1</th>
<th>EIA #2</th>
<th>EIA #3</th>
<th>EIA #4</th>
<th>EIA #5</th>
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<tbody>
<tr>
<td></td>
<td>Relative Day of Bleed</td>
<td>Rapid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1</td>
<td>R</td>
<td>RR</td>
<td>RR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>R</td>
<td>RR</td>
<td>RR</td>
<td>NR</td>
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<td>NR</td>
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<tr>
<td></td>
<td>8</td>
<td>R</td>
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<td>RR</td>
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<tr>
<td></td>
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<td>Q</td>
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<tr>
<td></td>
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<td>R</td>
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<td>RR</td>
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<td>R (M)</td>
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<td>R</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>22</td>
<td>R</td>
<td>RR</td>
<td>RR</td>
<td>RR</td>
<td>RR</td>
<td>RR</td>
</tr>
</tbody>
</table>
Relative Seroconversion Sensitivity

- 26 seroconverters were analyzed with 14 tests
- 17 seroconverters with WB positive used for cumulative frequency analysis
Sequence of Test Positivity Relative to WB (plasma)

50% Cumulative Frequency, Days before WB positive

HIV Infection and Laboratory Markers

- HIV RNA (plasma)
- HIV p24 Ag
- IgM
- IgG
- HIV Antibody

HIV Infection and Laboratory Markers

HIV RNA (plasma)
HIV p24 Ag
IgM
IgG
HIV Antibody

Days

0 10 20 30 40 50 60 70 80 90 100

Last negative
First positive

Infection

What is the Window Period?

HIV Detection

- HIV RNA (plasma)
- HIV Ab
- HIV p24 Ag

Eclipse Period
Acute Infection
Recent Infection
Longstanding Infection

Viral Detection
Antibody Detection 3rd generation EIA
Antibody Detection 2nd generation EIA
Antibody Detection 1st generation EIA

Seroconversion window

Days
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 240 300 360
Days Since NAT Reactivity

Note: For illustration purposes only. Based on preliminary analysis, now superseded
Other Measures of Performance

- For use with multiple observations:
  - Positive likelihood ratio
  - Negative likelihood ratio

- Validity

- Reproducibility

- Reliability
Specificity, NPV, Negative LR of DNA PCR
New York State Department of Health

N=2,093 Children with definitive status
N=7,544 DNA PCR tests

Specificity, NPV, Negative LR over Months

- Specificity remains constant at 1.0
- NPV remains constant at 1.0
- Negative LR decreases over time, with the last false negative occurring at 7 weeks (43-49 days)
Summary

- Sensitivity & Specificity depend on
  - Which “gold standard”
  - Which cutoff
  - Combinations of multiple tests

- Predictive Value depends on
  - Specificity and Prevalence
  - Pre-test probability of disease
Summary

- Tests with good clinical sensitivity differ in their seroconversion sensitivity

- Window periods are getting shorter but are difficult to estimate

- New HIV tests will continue to create new challenges