The Pros and Cons of Rapid Infectious Disease Testing

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Director of Scientific Affairs
Objectives:

- Analyze the immunological reactions that enable lateral flow tests to work
- Review potential issues with rapid testing
- Discuss the pros and cons of testing for specific disease states
Infectious Disease in the US

1970: William Stewart, the Surgeon General of the United States declared the U.S. was “ready to close the book on infectious disease as a major health threat”; modern antibiotics, vaccination, and sanitation methods had done the job.

1995: Infectious disease had again become the third leading cause of death, and its incidence is still growing!
One of the top 7 issues that threatens the human race

Global Drivers

- Changed C/N cycles and rising atmospheric GHG concentration
- Increasing antibiotic resistance
- Increasing connectivity (economic, social, ecological)
- Rising human numbers and urbanization
- Increasing per capita resource use
- Nuclear proliferation
- International terrorism

Unwanted Outcomes

CLIMATE

ECOSYSTEM

HUMAN HEALTH

ECONOMIC

Source adapted from: Science, Vol 325, September 2009
Available at: http://www.sciencemag.org/content/325/5948.cover-expansion
Defining Immunological Testing

- **Antigen**: the part of a molecule that an antibody binds to
- **Antibody**: a molecule the body makes to bind to an antigen
Multiple Types Of Antibodies

- **IgM** is first antibody to respond
  - characterizes a recent infection
- **IgG** is second antibody to respond
  - Used for primary and secondary infection

Polyclonal vs. Monoclonal
Serological Response To Infection

- Antibody concentration

![Graph showing IgM and IgG antibody concentrations over time.](image)
# Types Of Immunological Tests

<table>
<thead>
<tr>
<th>Method</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex agglutination</td>
<td>Examples include infectious mononucleosis testing</td>
</tr>
<tr>
<td>EIA or ELISA (Enzyme immunoassay or enzyme-linked immunosorbent assay)</td>
<td>Examples include EBV, <em>Mycoplasma pneumoniae</em>, and Lyme disease</td>
</tr>
<tr>
<td>Multiplexing</td>
<td>Chip technology&lt;br&gt;Bead technology, such as viral panel</td>
</tr>
<tr>
<td>Lateral flow</td>
<td>Examples include pregnancy, <em>Strep A</em>, HIV, and influenza.</td>
</tr>
</tbody>
</table>
Lateral Flow Types

- **Direct antigen**
  - Pregnancy, Strep A, and Influenza

- **Serological**
  - HIV

- **Competitive**
  - Drugs-of-abuse
Direct Antigen Detection

Visualizing Antibody

Antigen

Capture Antibody

Nitrocellulose
Serology Detection

- Looking for a person’s antibody response to disease (the blue antibody)
Drug Screen Test
Competitive Lateral Flow
How the DOA Test Works – T Line formation

- Any colloidal gold labeled mouse anti-drug antibody particle not already saturated by drug molecules can adhere to the immobilized drug conjugate striped at the test line region.

- A colored line will form as a result of the antibody-antigen binding. The red to pink color line formation at the test line is actually the visualization of the colloidal gold-antibody conjugates.

- This visualization of a colored line at the test (T) line region indicates a negative test result.
Negative Result & Interpretation

• If the drug concentration in the urine specimen tested is below the cut-off concentration, sufficient labeled antibodies bind and produce a colored line in the test line region.

• The test line for a negative test may be different in color intensity depending on the amount of the labeled antibody bound to T line.
# Issues With Antibody Based Reactions

<table>
<thead>
<tr>
<th>Issue</th>
<th>Description</th>
</tr>
</thead>
</table>
| Heterophile antibodies, such as HAMA       | • Can cause false results  
• Use HAMA blocker |
| (human anti-mouse antibodies)              |                                                                             |
| Rheumatoid factors                         | • Autoantibodies in clinical sample, usually IgM that can bind to IgG antibodies |
| Hook effect                                | • Analyte is in high concentrations capture and detector antibodies are saturated  
• Creates False negatives |
| Antigen break-down                         | • If antigen denatures, antibodies may not detect  
• In case of hCG, the α and β subunits can detach |
| Interfering substances                     | • Dietary hCG                                                               |
User Issues

Clinical sample acquisition not correct

- Not the right sample, such as throat swab for influenza.
- Could be improper, such as saliva or cheek for Strep A or a poor NP swab with influenza
  - Saliva may cause false positives. Cheek is inadequate and may be a false negative.
- Could be improper storage:
  - Time too long
  - Buffer incorrect
  - Temperature incorrect
User Issues

Improper procedure

- Extraction step for Strep A. If the user doesn’t wait, the antigen won’t be properly exposed and so sensitivity will suffer.
- Mixing caps, touching reagent bottle to sample... 
- Read time
  - Too short – sometimes, people can look quickly and the front of gold can look like a positive.
  - Too long – The PI rules. Anything beyond read time is not acceptable.
Lateral Flow

Advantages

• Fast, can triage with it & ACTIONABLE RESULTS to direct treatment
• Easy to use
• Can be CLIA-waived

Disadvantages

• Cumbersome to do large volume testing
• Testing multiple analytes at the same time is limited
• Often, not at sensitive as gold standards or molecular*
Statistics!
Definitions

• Sensitivity
  – Analytical Sensitivity
    • The smallest value that can be distinguished from zero (minimal detectable concentration [MDC] or Limit of Detection [LOD]).
    • For qualitative products, this is accomplished by variable tests.
  – Clinical Sensitivity
    • The percentage of the total number of true positives (disease state) reported as positive by the assay.
Definitions

• Specificity
  – Analytical Specificity
    • The ability of the assay to detect the analyte of interest without detecting related compounds.
  – Clinical Specificity
    • The percentage of the total number of true negatives (disease free) reported as negative by the assay.
Definitions

• Predictive Values
  – Positive Predictive Value
    • The percentage of the time that an assay positive result is a true positive.
    • *If we say it’s positive, you can bet it’s positive - Rule In!*
  – Negative Predictive Value
    • The percentage of the time that an assay negative result is a true negative.
    • *If we say it’s negative, you can bet it’s negative - Rule Out!*

<table>
<thead>
<tr>
<th>Actual</th>
<th>Assayed</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positives</td>
<td>Total Positives</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>True Negatives</td>
<td>Total Negatives</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

Sensitivity = True +ve Detected/Actual True +ve = 10/10 = 100.0%
Specificity = True -ve Detected/Actual True -ve = 9/10 = 90.0%
PPV = True +ve Detected/Total # of Assay +ve = 10/11 = 90.9%
NPV = True -ve Detected/Total # of Assay -ve = 9/9 = 100.0%
Sensitivity vs Specificity vs PPV vs NPV

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

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

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

**NPV:** Probability patient=negative if test=negative
• Flu is seasonal. Prevalence of the disease is different in June than in January.
• This will impact the perceived performance of the test

Test 1,000 persons

Test Specificity = 99.6% (4/1000)

Prevalence = 10%

True positive: 100  False positive: 4

Positive predictive value: 100/104 = 96%
Test 1,000 persons

Test Specificity = 99.6% (4/1000) Prevalence = 10%
True positive: 100 False positive: 4
Positive predictive value: 100/104 = 96%

Prevalence = 0.4%

True positive: 4 False positive: 4
Positive predictive value: 4/8 = 50%

www.cdc.gov/hiv/rapid_testing
Concordance

- Commonly assessed with a truth table
- These data can be used to establish sensitivity, specificity, PPV, NPV, etc.
- Also commonly used to compare different assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Diagnosis positive</th>
<th>Diagnosis negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>True positive</td>
<td>False positive</td>
</tr>
<tr>
<td>negative</td>
<td>False negative</td>
<td>True negative</td>
</tr>
</tbody>
</table>
### Truth Table and Formulas

- **Sensitivity**
  \[
  TP / (TP + FN) \times 100
  \]
- **Specificity**
  \[
  TN / (TN + FP) \times 100
  \]
- **PPV**
  \[
  TP / (TP + FP) \times 100
  \]
- **NPV**
  \[
  TN / (TN + FN) \times 100
  \]

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<td>True negative</td>
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Specific Lateral Flow Examples
Influenza & RSV
What are the US cases like each year?

5 - 20% of the population gets the flu every year.

More than 200,000 people each year are hospitalized from flu-related complications.

About 36,000 people die each year due to flu.
Aren’t you supposed to build immunity to influenza?

| The problem with influenza, like the common cold, is that there are many different strains. |
| That is also why the performance of rapid tests are different every year! |
Influenza

Pros

• Excellent specificity
  • Good positive predictive value
• Important in triaging patients
• Cost-effective

Cons

• Variable sensitivity
  • Negatives may need to be backed up with PCR or culture
• Important to take the right sample
• Important to take sample at the right time
RSV

Pros

• High specificity
• Moderately high sensitivity
• Help with triaging
• Isolation in premature baby wards

Cons

• Negatives may need to be backed up with culture
• Sensitivity not good in geriatric population
Legionella and S. pneumoniae
Pneumonia
Current Number of Pneumonia Cases (US)

Overall
- 37 million ambulatory care visits per year for acute respiratory infections (physician and ER visits combined)

Community-Acquired Pneumonia (CAP)
- Each year 2 - 3 million cases of CAP result in ~ 10 million physician visits & 500,000 hospitalizations in the US
- Average mortality is 10-25% in hospitalized patients with CAP

Hospital Acquired Pneumonia
- Standard definition: onset of symptoms occurs approx 3 days after admission
- 250,000 - 350,000 cases of nosocomial pneumonia per year
- 25 - 50% mortality rate
Pneumonia Rapid Testing – *Legionella* and *S. pneumoniae*

**Pros**

- Urinary antigen is easily acquired vs. sputum
- Sputum needs to be qualified
- *Legionella* is a notoriously difficult to culture
- Same day results can lead to directed therapy
- Recommended by IDSA guidelines

**Cons**

- Negatives should be backed up by culture
- Other pathogens can cause pneumonia
Strep A
Rapid Testing for *Strep A*

**Specificity is high so no additional work need be done with a positive result**

**Sensitivity is not as high**

- Back up children with culture confirmation
- IDSA guidelines say that negatives on adults don’t need to be confirmed due to lower *Strep A* rate
Infectious Mononucleosis
Epstein-Barr Virus
Infectious Mononucleosis

Average age for IM is under 2 or 14-16

Rapid tests are built on heterophile response
  • Does not last like traditional IgG
  • Studies suggest heterophile tests effective in 80-85% of population
  • Positive tests usually don’t have confirmatory testing

Can confirm with EBV specific serological tests
HIV
Role for Rapid HIV Tests

- Increase receipt of test results
- Increase identification of HIV-infected pregnant women so they can receive effective prophylaxis
- Increase feasibility of testing in acute-care settings with same-day results
- Increase number of venues where testing can be offered to high-risk persons
Awareness of Serostatus Among People with HIV and Estimates of Transmission

- ~25% Unaware of Infection
- ~75% Aware of Infection

Account for:

- ~54 - 70% of New Infections
- ~30 - 46% of New Infections

People Living with HIV/AIDS: 1,039,000-1,185,000
New Sexual Infections Each Year: ~32,000

Marks, et al, AIDS 2006
HIV Infection & Laboratory Markers

HIV Rapid Test Pros & Cons

Pros

• Excellent sensitivity and specificity
• Can detect infection earlier than gold standard
• Positive results have been shown to reduce risk factors

Cons

• Still not perfect
• Viral RNA does detect earlier
Enteric Testing

**C. difficile**
- Can put on correct antibiotic
- Can put in/take out of isolation

**Shigatoxin**
- Discontinue antibiotics

**Giardia/Cryptosporidium**
- Administer correct antibiotics
Evaluate severity and duration
Obtain history and physical examination\(^1\text{-}^5\)
Treat dehydration
Report suspected outbreaks\(^6\)
Check all that apply: \(^7\)

A. Community acquired or traveler's diarrhea
(esp. if accompanied by significant fever or blood in stool)

- Culture or test for:
  - Salmonella
  - Shigella
  - Campylobacter
  - *E. coli O157:H7* (if blood in stool also test for Shiga toxin and refer isolates if toxin pos.)
  - *C. difficile toxins A ± B* (if antibiotics or chemotherapy taken in recent weeks)

Consider quinolone for suspected shigellosis in adults (fever, inflammation); macrolide for suspected resistant *Campylobacter*; avoid antimotility or certain antimicrobial drugs if suspected STEC (afibrile, bloody diarrhea)\(^8\)

B. Nosocomial diarrhea
(onset after >3 d in hospital)

- Test for *C. difficile toxins A ± B*
  (in suspect nosocomial outbreaks, in patients with bloody stools, and in infants, also add tests in panel A)

- Discontinue antimicrobials if possible; consider metronidazole if illness worsens or persists

C. Persistent diarrhea >7d
(esp. if immunocompromised)

- Consider parasites\(^9\)
  - *Giardia*
  - *Cryptosporidium*
  - *Cyclospora*
  - *Isospora belli*

- + Inflammatory screen\(^7\)

  - If HIV pos., add:
    - *Microsporidia* (Gram-chromotrope)
    - *M. avium complex* + panel A

Treat per results of tests
ED Scenario

Person comes in with respiratory symptoms
- Is it viral or bacterial?
- If bacterial, can you narrow therapy?

Treat with broad spectrum antibiotic like fluoroquinolone

C. difficile O27 resistant to fluoroquinolone
- Length of stay ↑
- Cost of treating patient ↑
- Hospital reputation ↓
Discussion

Rapid assays play a significant part in diagnoses for health care

Antibiotic therapy can be better directed with specific diagnosis so as to reduce resistance rates
QUESTIONS?