Iowa Adopts New HIV Testing Algorithm

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Objectives

At the completion of this webinar, participants will:

• Be familiar with the difference between HIV antibody, antigen, and RNA
• Have a basic understanding of the new HIV testing algorithm that will be used at the State Lab after 11/6/2012
• Understand the new “diagnostic window of detection” and possible test results for serum samples that are tested at the State Lab after 11/6/2012
• Be familiar with the advantages and disadvantages of rapid vs. conventional HIV testing
• Timeframe and processes for submitting specimens to the lab.
Terms

• HIV Testing Algorithm
  – Sequence in which different diagnostic tests are used to arrive at a definitive diagnosis

• Conventional HIV Test Algorithm
  – Blood (serum) sample taken from client via venipuncture; sample sent to laboratory for testing
  – Screening and supplemental tests performed at lab as necessary
  – Result sent back to provider for delivery to client

• Rapid HIV Test Algorithm
  – Blood or oral fluid sample taken from client via finger stick or oral swab; screening test performed onsite
  – Non-reactive result can be shared with client in less than 1 hour
  – Reactive result requires confirmation with conventional test as described above
HIV Testing Processes
Then and Now
HIV Antibody Testing

- Antibodies are proteins produced by the immune system to neutralize infections or malignant cells.

- Most people develop detectable HIV antibodies 2-8 weeks after infection (average 25 days).

- Current HIV testing algorithm used at the State Lab:
  - EIA screen (3rd Generation)
  - Confirmed by Western Blot (WB)
1989: CDC recommended two-test algorithm for HIV diagnosis

**T1: HIV-1 EIA**

- Non-reactive: Report as HIV Neg.

**T2: Western blot (WB) or immunofluorescence assay (IFA)**

- Reactive:
  - Positive: Report as HIV-1 Pos.
  - Indeterminate: Report as Indeterminate

- Non-reactive: Report as HIV Neg.
HIV Testing has changed over time

- 1985: Blood Banks
- 1987: Patients with STD’s
- 1989: Current HIV Testing algorithm
- 1993: Inpatients where HIV >1%
- 1995: All pregnant women
- 2001: More public and private health care settings
- 2006: Voluntary screening in all health care settings
HIV Progression and Detectable Response

Days since infection

Infection

1st and 2nd Gen

3rd Gen

HIV Antibody

Slide courtesy of Bernie Branson
HIV Progression and Immune Response

- Acute syndrome, Virus dissemination
- Clinical Latency
- Opportunistic Infections

CD4 count (cells/μl)
- Weeks
- 0 3 6 9 12

Virus Load
- 0 250 500 750 1000

Virus RNA (copies/ml)
- Death
- 10^3 10^4 10^5 10^6
Progression of HIV Viral Markers

- **P24 antigen**
- **HIV RNA**
- **Antibodies**

0 7 14 21 28 35 42 49 56 63 70

**Viral and antibody levels**

**Time after infection (days)**

**Acute**

**Seroconversion** → **Established**

**IgM**

**IgG**
p24 Antigen

• An antigen is a virus, part of a virus, or a foreign body that triggers the production of antibodies in the body

• p24 is the antigen on HIV-1 that most commonly provokes an antibody response

• First marker of HIV-1 infection

• Can be detected at 2 weeks from infection
HIV Progression and Detectable Response

Days since infection

p24 Antigen

HIV Antibody

Infection

4th Gen

Slide courtesy of Bernie Branson
4th Generation Ag/Ab Test

• 2 FDA-approved kits available
  – ARCHITECT HIV Ag/Ab Combo (Abbott)
  – GS HIV Ag/Ab Combo EIA (Bio-Rad)
• Detects HIV-1 p24 Ag, HIV-1 and HIV-2 antibodies
• Reactive result:
  – Doesn’t distinguish between Ag and Ab
  – Preliminary positive
  – Supplemental testing required
Why do we need new HIV testing strategies/algorithms?

- Laboratory algorithm established by CDC and APHL (ASTPHLD) in the late 1980’s
  - Over 20 years later remains largely unchanged
- More is known about the disease
  - HIV-1 and HIV-2
  - Window Period
- Evolving technology
  - Tests recently approved by FDA are not included
  - Availability of rapid tests
  - Increased sensitivity of screening assays
  - Western blot and IFA now less sensitive than some screening assays which they are intended to “confirm”
Why do we need new HIV testing strategies/algorithms?

• Evolving technology
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Diagnostic Window of Detection

- The time from infection to detection
- Varies depending on the test used
## Windows of Detection

<table>
<thead>
<tr>
<th>Test</th>
<th>Window of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th Gen:</td>
<td>2 weeks</td>
</tr>
<tr>
<td>• Conventional</td>
<td></td>
</tr>
<tr>
<td>3rd Gen:</td>
<td>2-8 weeks (avg. 25 days)</td>
</tr>
<tr>
<td>• Conventional</td>
<td></td>
</tr>
<tr>
<td>• Rapid HIV Test</td>
<td></td>
</tr>
</tbody>
</table>
Acute HIV Infection

- The risk of transmitting HIV to others is high during acute infection. Therefore, **risk reduction measures are especially important** during this time.

- Initiating antiretroviral treatment during acute HIV infection may:
  - reduce the HIV viral setpoint and preserve key immune response functions that may slow disease progression
  - reduce the likelihood of transmission to others.

- These advantages may be outweighed by practical concerns about an individual patient's ability or readiness to take multiple medications.

- Decisions about treatment are individualized. However, with acute infections, **initiating care with an Infectious Disease clinician is crucial and very time-sensitive**.
Relative Sensitivity of Tests

**FIGURE 2.** Reactivity of FDA-approved assays for HIV-1 compared with Western blot.

From: Branson, JAIDS, 2010, 55 (S2): S102-S105
4\textsuperscript{th} Generation HIV Ag/Ab EIA Test

• Combined antigen/antibody test

• Can detect...
  – p24 antigen
  – HIV-1 antibodies
  – HIV-2 antibodies

• But...cannot tell them apart
What are we looking for from these new testing strategies?

- Resolution of indeterminates
- Ability to confirm HIV-2 infections
- Increased detection of acute infection
- Use of assays as screening or confirmatory/ supplemental tests and as part of multi-test algorithms
The New (Conventional) HIV Testing Algorithm: Get to Know It!
Rapid Tests Performed in the Field follow by the new Algorithm

A: HIV-1/HIV-2 Ag/Ab Immunoassay

A+: Repeat A in duplicate

A(- -): Negative for HIV-1 and HIV-2 Ab and HIV-1 p24 ag

A1( + + or + -):

B: HIV-1/HIV-2 Ab Differentiation Immunoassay

B: HIV-1 (+) HIV-2 (-)

Positive for HIV-1 Ab

B: HIV-1 (-) HIV-2 (+)

Positive for HIV-2 Ab

B: HIV-1 (+) HIV-2 (+)

Positive for HIV Ab

B: HIV-1 (-)/HIV-2 (-) or inconclusive

C: Individual HIV-1 NAT

C+: Positive for HIV-1 RNA

C-: Negative for HIV-1 RNA

* A could be an IgM sensitive antibody immunoassay if the Ag/Ab combination immunoassay is not available.
† Repeating A+ is assay dependent.
‡ Refer to care and follow up testing.
§ HIV positive; further testing required to rule out dual infection
¶ Acute HIV-1 infection.
# Consider HIV-2 DNA testing if clinically indicated.
** If early acute infection is suspected, NAT can be performed.
New HIV Testing Algorithm

Step 1

- 4th Gen EIA (antigen/antibody)
  - reactive
  - -
Multispot HIV Ab Test

- Supplemental test
  - used after a reactive 4th Gen EIA
- Replaces WB
  - More sensitive and specific than WB
  - Faster and less expensive than WB
- Will differentiate HIV-1 and HIV-2
New HIV Testing Algorithm

Step 2

What if you get a non-reactive result from the Multispot antibody test?
Nucleic Acid Amplification Test for HIV-1 RNA

• Supplemental test
  – Used after a reactive EIA and a non-reactive Multispot

• Highly sensitive test which can detect the presence of viral RNA

• HIV-1 RNA/NAAT testing can detect acute HIV-1 infection
New HIV Testing Algorithm

Step 3

4th Gen EIA (antigen/antibody)

reactive

- 

Multispot (antibody)

HIV 1+
HIV 2 +
HIV 1 and 2 -

NAAT (HIV-1 RNA)

+ 

-
HIV Progression and Detectable Response

- HIV-1 RNA (plasma)
- HIV p24 Antigen
- HIV Antibody

Days since infection

Infection

Slide courtesy of Bernie Branson
### New HIV Testing Algorithm Results

<table>
<thead>
<tr>
<th>Lab Report</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative.</strong> HIV-1 p24 antigen, HIV-1 and HIV-2 antibodies not detected.</td>
<td>If client did not have risk in the two weeks before the test or since, the client does not have HIV.</td>
</tr>
<tr>
<td><strong>Positive.</strong> HIV-1 antibodies detected.</td>
<td>The client has HIV-1.</td>
</tr>
<tr>
<td><strong>Positive.</strong> HIV-2 antibodies detected.</td>
<td>The client has HIV-2.</td>
</tr>
<tr>
<td><strong>Positive.</strong> A reactive HIV antigen/antibody test and a positive HIV-1 RNA test indicate acute HIV-1 infection.</td>
<td>The client has HIV-1 and the test result indicates that s/he was recently infected (likely 2-8 weeks before taking the test).</td>
</tr>
<tr>
<td><strong>Negative.</strong> HIV antibodies not detected. No detectable HIV-1 RNA. HIV-2 infection cannot be excluded.**</td>
<td>The client does not have HIV-1. The client should be retested in two weeks to rule out possibility of acute HIV-2.</td>
</tr>
</tbody>
</table>
Point of Care Tests in Iowa

OraQuick ADVANCE

If Positive and blood not drawn
SHL performs Oral Fluid Western Blot

If Positive and blood drawn

Clearview Complete HIV 1/2

If Positive
SHL performs Antigen/Antibody follows algorithm
What if the Multispot is Negative?

• SHL will send the serum to Florida’s State Public Health Lab for NAAT testing.
  – If NAAT is positive, the patient is HIV positive
  – If NAAT is negative, the patient is HIV negative
HIV Testing Instructions

Submission Requirements:
• Serum, Plasma and Oral Fluids are accepted for HIV antibody testing.
• Acceptable specimens for HIV Ag/Ab Combo testing include serum and plasma ONLY.
• Label tube with patient’s name or unique identifier, date of birth (DOB), and the date of collection.
• **UNLABELED SPECIMENS WILL NOT BE TESTED.**
• A completed HIV Test Request Form must accompany specimen.

Specimen Collection and Handling:
• Blood samples must be collected in a red stopper, serum separator (SST), or a tube without anticoagulants. When possible samples should be centrifuged to separate serum from cells.
• Following collection serum samples may be stored at room temperature for 3 days or 2-8°C for 7 days.
• 1 ml of serum is the minimum recommended volume for submission.
• Oral fluid samples must be collected in an OraSure collection device. See device for collection instructions.
• OraSure specimens may be stored from 4-37°C for a maximum of 21 days from the time of collection, including the time for shipping and testing.
• Label specimen, wrap the collection tube in absorbent material, and place into a biohazard bag.
HIV Testing Instructions (continued)

Complete Test Request Form
• Complete a Test Request Form which includes the following information:
  – Two unique identifiers including the patients name if an Iowa test site, other states can use unique identification number.
  – Must indicate patient’s date of birth.
  – Specimen type and date of collection.
  – Test requested marked clearly.
  – Previous reactive test method (if applicable).
  – Clinician and return address of the submitter.
  – ID on specimen collection container must match the ID on the Test Request Form.

Shipping Instructions
• Include completed HIV Test Request Form in outside pocket of biohazard bag.
• Roll up the bag and place in mailer.
• Seal mailer with a S-coded self-adhesive wrapper provided with SHL kit or equivalent.
• Ship at ambient temperature as soon as possible via first class mail. DO NOT USE WET ICE.
• Ship multiple specimens in packaging compliant with USPS or IATA regulations.
• If delays in transport are anticipated, refrigerate specimens until shipment.

Contact Information
• For test request forms and kits call 319-335-4379.
• Test request forms may be obtained online at http://www.shl.uiowa.edu/testmenu/clinicaltestmenu.xml
• Any questions should be directed to Serology @ 319-335-4275.
Also Remember

• Test must be received in 3 days by SHL or refrigerate for up to 7 days
Iowa Guidelines

• Use Clearview Rapid
• Architect Ag/Ab Test is performed at SHL and confirms positive Clearview Rapid
For More Information

Proposed HIV Test Algorithms:
http://www.hivtestingconferencearchive.org/hivtesting2010/
Click on “HIV Testing Algorithms: A Status Report”

CDC HIV testing resources:
http://www.cdc.gov/hiv/topics/testing/index.htm
http://www.cdc.gov/dls/waivedtests

APHL resources:
http://www.aphl.org/aphlprograms/infectious/hiv/Pages/default.aspx

NASTAD resources:
http://www.nastad.org/resources.aspx?searchkey=hiv%20prevention
Questions?