HIV Testing Paradigms

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Objectives

- HIV 1 and HIV 2
- Standard HIV testing
- Viral load testing
- Rapid HIV testing
- Acute HIV diagnosis
- Pooled HIV testing
- Conclusions
AIDS at 25

SPECIAL REPORT

AMERICA:
The New Faces of HIV

THE WORLD:
25 Million Dead And Counting

Jennifer Jako, 33, has been living with AIDS for nine years. She is seven months pregnant.
Relationship of HIV Groups, Subtypes

HIV

HIV-1

Group O
Subtype A
Subtype B
CRF02_AG

Group N
Subtype C

Group M
Subtype D
Subtype E*
Subtype F
Subtype G
Subtype H
Subtype J
Subtype K

HIV-2

Subtype A

Subtype B

Other minor subtypes

*More accurately, CRF01_AE

CDC
Modes of HIV-1 Sequence Diversification. The crosses signify copying errors made during reverse transcription, while red and blue proviruses represent phylogenetically distinct virus strains.
HIV-1: Non-M Group Diagnosis

- Group O HIV-1
  - All US-licensed tests in the 1990s failed to detect at least 1 HIV-1 group O infection in a panel of 7 (Lancet 1994; 344:1333)

- Group N HIV-1
  - Inconsistently reactive in most commercial HIV-EIAs

CDC
Group O Prevalence, Africa, Mid 1990s

CDC
HIV-2 in the United States

- 137 cases reported to date in the US HIV/AIDS surveillance system
- Many cases likely identified, but not in the surveillance system
- New York City
  - 159 HIV-2 positive *specimens* from 7/88 to 2/00
  - Beatrice et al, 13th Int’l AIDS Conf

CDC
HIV-2 Diagnosis

- HIV-2
  - 60-91% are cross reactive with HIV-1 EIA
- Group M, non-B
  - Essentially 100% detection with commercially available HIV-1 EIA
  - Case report of A/G/J recombinant from Benin with serologic dx failures

CDC
FIGURE 1. Centers for Disease Control and Prevention testing algorithm for use with combination HIV-1/HIV-2 enzyme immunoassays (EIAs)

HIV-1/HIV-2 EIA

Repeatedly Reactive

HIV-1 Western Blot

Positive

Repeat as HIV positive

Negative

Indeterminate

HIV-2 EIA

Repeatedly Reactive

HIV-2 Supplemental Test (e.g., Western Blot)

Positive

Indeterminate
Seronegative HIV Infections: A by-product of stimulated surveillance?

- Since first report of group O HIV infection in the US, increased requests for group O diagnostics
- Identified a group of 8 patients with HIV-1 subtype B infections who are persistently antibody EIA seronegative
Seronegative HIV Infections

- Case definition:
  - infection with HIV (PCR or viral culture positive)
  - at least two negative antibody screening test results from independent laboratories on two different specimens collected on different dates
  - the presence of at least one AIDS-defining opportunistic illness at the time the samples were collected
Seronegative HIV/AIDS

Persistently negative HIV-1 antibody enzyme immunoassay screening results for patients with HIV-1 infection and AIDS: serologic, clinical, and virologic results


Objective: To describe persons with HIV infection and AIDS but with persistently negative HIV antibody enzyme immunoassay (EIA) results.

Design: Surveillance for persons meeting a case definition for HIV-1-seronegative AIDS.
## Clinical Results -- Seronegative AIDS

<table>
<thead>
<tr>
<th>Case</th>
<th>CD4 Range</th>
<th># Negative Tests</th>
<th>Viral Load</th>
<th>Other + Ab Titers</th>
<th>IgG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>96</td>
<td>9</td>
<td>337,000</td>
<td>HbsAb</td>
<td>2900</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>4</td>
<td>ND</td>
<td>None</td>
<td>1310</td>
</tr>
<tr>
<td>3</td>
<td>0-11</td>
<td>7</td>
<td>773,000</td>
<td>CMV, HSV-1</td>
<td>773</td>
</tr>
<tr>
<td>4</td>
<td>8-18</td>
<td>6</td>
<td>ND</td>
<td>None</td>
<td>1490</td>
</tr>
<tr>
<td>5</td>
<td>2-3</td>
<td>3</td>
<td>254,000</td>
<td>CMV, EBV, HSV-2, B-19</td>
<td>2060</td>
</tr>
<tr>
<td>6</td>
<td>1-30</td>
<td>12</td>
<td>167,000</td>
<td>HbsAB, CMV</td>
<td>2250</td>
</tr>
<tr>
<td>7</td>
<td>69-129</td>
<td>39</td>
<td>ND</td>
<td>CMV, EBV</td>
<td>1000</td>
</tr>
<tr>
<td>8</td>
<td>180-230</td>
<td>6</td>
<td>199,000</td>
<td>HbsAb</td>
<td>1050</td>
</tr>
</tbody>
</table>

CDC
Viral Load Measurements

RT-PCR
NASBA
bDNA
DNA testing
Significance - Monitoring

Historically, failure to detect RNA, or falsely low RNA determinations with RT-PCR (version 1.0), and NASBA (subtype G)

Current versions of commercially available products are significantly improved.

HIV-2: No cross reactivity; SIV?

Group O:

- bDNA underquantifies, Monitor v1.5 does not detect; Nuclisens reports detection of group O and group N
- Experimental methods reported (Plantier 2003, Ernest 2001, deMendoza 2001)
Significance - Treatment

- Group O isolates generally insensitive to non-nucleoside reverse transcriptase inhibitors
- In vitro testing indicates that Group M, subtype D isolates show a tendency to lower susceptibility to nucleoside analogues, NNRTIs, and PIs
- CRF01_AE and CRF02_AG
  - Some strains with reduced susceptibility to abacavir, NNRTIs, and atazanavir

CDC
Significance - Treatment

HIV-2:

Non-nucleoside reverse transcriptase inhibitors generally ineffective

PIs bind HIV-2 protease 10-100 times less strongly than HIV-1; clinical significance unclear

Insensitive to enfuvirtide

CDC
Rapid HIV Testing
HIV–1/2 Rapid Tests

- Fingerstick or oral swab
- CLIA-waived
- Screening test only
- NOT for known HIV-infected on HAART!
- NOT for follow-up on PEP
<table>
<thead>
<tr>
<th>Test</th>
<th>Specimen Type</th>
<th>CLIA Category</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Manufacturer</th>
<th>Approved for HIV-2 Detection</th>
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</thead>
<tbody>
<tr>
<td>OraQuick Advance Rapid HIV-1/2 Antibody Test</td>
<td>Whole blood (finger stick or venipuncture)</td>
<td>Waived</td>
<td>99.6% (98.5-99.9)</td>
<td>100% (99.7-100)</td>
<td>OraSure Technologies</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Oral fluid</td>
<td>Waived</td>
<td>99.3% (98.4-99.7)</td>
<td>99.8% (99.6-99.9)</td>
<td><a href="http://www.orasure.com">www.orasure.com</a></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>Moderate complexity</td>
<td>99.6% (98.9-99.8)</td>
<td>99.9% (99.6-99.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uni-Gold Recombigen HIV</td>
<td>Whole blood (finger stick or venipuncture)</td>
<td>Waived</td>
<td>100% (99.5-100)</td>
<td>99.7% (99.0-100)</td>
<td>Trinity Biotech</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Serum/plasma</td>
<td>Moderate complexity</td>
<td>100% (99.5-100)</td>
<td>99.8% (99.3-100)</td>
<td><a href="http://www.unigoldhiv.com">www.unigoldhiv.com</a></td>
<td></td>
</tr>
<tr>
<td>Reveal G2</td>
<td>Serum</td>
<td>Moderate complexity</td>
<td>99.8% (99.2-100)</td>
<td>99.1% (98.8-99.4)</td>
<td>MedMira</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>Moderate complexity</td>
<td>99.8% (99.0-100)</td>
<td>98.6% (98.4-98.8)</td>
<td><a href="http://www.medmira.com">www.medmira.com</a></td>
<td></td>
</tr>
<tr>
<td>MultiSpot HIV-1/HIV-2</td>
<td>Serum/plasma</td>
<td>Moderate complexity</td>
<td>100% (99.9-100)</td>
<td>99.9% (99.8-100)</td>
<td>BioRad Laboratories</td>
<td>Yes, differentiates HIV-1 from HIV-2</td>
</tr>
<tr>
<td></td>
<td>HIV-2</td>
<td>Moderate complexity</td>
<td>100% (99.7-100)</td>
<td></td>
<td><a href="http://www.biorad.com">www.biorad.com</a></td>
<td></td>
</tr>
</tbody>
</table>

OraQuick Reactive Results

Reactive (Positive) Result:

f) Lines appear in both the control (C) and test (T) areas.

g) One line may appear lighter or less consistent than the other.

h) Even a very faint test (T) band should be read as reactive.
60-second Rapid Test

- INSTI™ HIV-1/HIV-2 kit
  - Work ongoing for HIV-2
- Equivalent to Health Canada approved test, Abbott AxSym GO, in sensitivity, specificity, & early Ab detection
- High concordance for finger-stick blood, whole blood, plasma, serum

Fonseca K et al. British Columbia Centre for Disease Control
Rapid Test Implementation

- Office setting
- Post-exposure testing of source patients
- Obstetrical units
- Emergency rooms
- Other point-of-care sites
Acute HIV Infection
Primary HIV Infection

Slide compliments of Dr. Neal Gregory
Chatham, NY
What is the earliest an HIV Ab test can detect HIV?

- Standard testing detects HIV about 2 weeks after symptoms, or 3-4 weeks after infection.

- 4th generation HIV test
  - Combines Ab with p24Ag detection
  - Moves the diagnosis up to about 2 weeks from time of infection
Diagnosing Acute HIV

- HIV RNA PCR testing (viral load)
  - Detectable about 5 days after infection

- HIV antibody testing done concomitantly

- HIV antibody test repeated in 2–4 weeks, to document seroconversion
HIV-1 RNA Qualitative Assay

- FDA-approved in U.S in October, 2006 for the diagnosis of primary HIV-1 infection
  - Sensitivity similar to current VL assays
- Previously used to screen blood and plasma donors
- APTIMA HIV-1 RNA Qualitative assay
  - Gen-Probe Incorporated
Sensitive-Less-Sensitive Assay

- Determines recent infection: surveillance
- Detuned assays
  - STARHS – Serological Testing Algorithm for Recent HIV Seroconversion
  - Detects HIV Ab that has formed within last 120 days
  - Takes advantage of lower Ab titer early in HIV
  - Uses a lower sensitivity assay (higher Ab cutoff)
Recent HIV Infection?

- Negative detuned assay
  - Recent infection

- Positive detuned assay
  - Infection occurred more than 4 - 6 months ago
BED Capture Enzyme ImmunoAssay

- Updated version
- Branched peptide that includes gp41 sequences from HIV-1 subtypes B, E & D
- Detects a gradual increase in the proportion of HIV-1-specific IgG in total IgG for 2 years after seroconversion
- Extends seroconversion incidence period to 6 months

Pooled HIV Nucleic Acid Testing
Pooling Schema

Individual specimens

Pool of 10
Pooling Schema

Individual specimens
N=100

Pools of 10
Pooling Schema

Individual specimens
N=100

Pools of 10

Master pool
Resolution Testing

Individual testing on 10 specimens

Pools of 10 screened

Master pools screened

A   B   C   D  E
Algorithm of the Procedures of the North Carolina Department of Health and Human Services for HIV Testing, Notification, and Surveillance

Nucleic Acid Amplification Testing (NAAT)

- Tested pools of 90 patient samples using NucliSens HIV-1 QL assay (bioMerieux)
  - Sensitivity to 75 copies/ml
- Pool of 90 specimens should detect at least one specimen of 90 with > 6750 copies/ml of HIV-1 RNA.

Flow Chart of Study Population

110,890 Persons requested voluntary counseling and testing

- 1427 Had insufficient data
- 213 Were previously HIV-positive

109,250 Successfully tested: constituted population at risk

- 583 Had enzyme immunoassay and Western blot tests showing established HIV infection
- 107 Had less-sensitive enzyme immunoassay test showing recent infection

108,667 Had negative or indeterminate results on enzyme immunoassay or Western blot tests; RNA screened

- 108,642 Had negative results on RNA screening; HIV-negative
- 25 Were RNA-positive

23 Were confirmed to have acute HIV infection

2 Had repeated enzyme immunoassay and RNA screening through wk 12: RNA false positive; HIV-negative

Acute HIV-1 Infections

- 2 of 25 were false positive NAATs
- Of the 23 acutely infected
  - 1 patient pregnant
  - 30% had symptoms of primary HIV
  - 26% developed symptoms later
  - Median VL 258,000 c/ml (2,609 – 5 million)
- PPV of combined Ab and NAA algorithm was 0.997 (95% CI, 0.988 - >0.999)

Frequency of Newly Diagnosed HIV Infections in North Carolina, November 1, 2002, through October 31, 2003, According to Type of Testing Site and Stage of Disease
CDC Calls for Routine HIV Testing

- September, 2006: CDC recommends testing of all people between the ages of 13 and 64 for HIV in the U.S.

- Estimates that 25% of the people living with HIV in the U.S. are unaware they are infected
  - Estimate that this group accounts for up to two-thirds of HIV transmission
Summary

- Incredible variation exists in HIV subtypes
- Clinical vigilance is the best way to identify important variants, from a clinical or public health perspective
- Rapid testing should help more people get tested: point-of-care
- Vigilance for acute HIV also critical
Special Thanks

- Patrick Sullivan, DVM
  - Centers for Disease Control,
    - Atlanta, Georgia USA