Diagnosing HIV: Old school, New school

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No disclosures
Objectives

1. Review the updated HIV testing guidelines and HIV testing algorithm

2. Understand the difference between HIV testing modalities

3. Review clinical cases for practical application of HIV testing algorithm
Case 1

- 28 yo female 24 weeks pregnant presents for prenatal care, HIV testing is done
  - p24 antigen positive

- What now?
  - Reassure patient that test is negative
  - Check HIV viral load
  - Check IFA
  - Start antiretroviral therapy
HIV diagnosis

- 50,000 cases diagnosed annually between 2008-2010

- 83 million adults between 18-64 have been tested for HIV as of 2009

- As of 2011, about 240,000 people in the US do not know they are infected with HIV
For every 100 people living with HIV

- 80 are aware of their infection
- 62 are linked to HIV care
- 41 stay in HIV care
- 36 get antiretroviral therapy
- 28 have a very low amount of virus in their body

http://www.cdc.gov/vitalsigns/HIVtesting/index.html
HIV-1 diagnosis

- Acute HIV-1 infection
  - Rate of transmission is 26 times as high in people with acute infection compared to those with established infection.
  - Accounts for 10-50% of all new transmissions especially in people with multiple sexual partners.

- Goal: Faster turn-around time in testing.
Benefits of HIV testing

- Early diagnosis, linkage to care and initiation of ART:
  - Reduces viremia, decreases rate of viral mutation, lowers viral set point and viral reservoir
  - Preserves immune function and slows progression of disease
  - Decreases severity of disease
  - Reduces HIV transmission

http://stacks.cdc.gov/view/cdc/23447
Testing guidelines

- Initial testing guidelines developed in 1989 by the CDC and the Association of Public Health Laboratories (APHL)
  - HIV-1 antibody assay, confirmed by:
    - Western Blot or
    - HIV-1 indirect immunofluorescence assay

http://stacks.cdc.gov/view/cdc/23447
Testing guidelines

- Updated in 1992 to include:
  - Testing for HIV-1 and HIV-2 antibodies if:
    - Patient demographics suggest HIV-2
    - HIV-1 antibodies negative but clinical suspicion of disease OR
    - HIV-1 Western Blot is indeterminate or negative

http://stacks.cdc.gov/view/cdc/23447
Testing guidelines

- 2004: CDC recommended confirmatory testing of all rapid HIV screening tests with HIV-1 Western Blot or HIV-1 IFA

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HIV-2 diagnosis

- No FDA approved test for confirming presence of HIV-2

- 3rd and 4th generation immunoassays detect HIV-2
  - WB does not detect HIV-2 accurately

- Unknown how long it takes for HIV-2 antibodies to develop

http://stacks.cdc.gov/view/cdc/23447
How to diagnose HIV-1

- Antibody immunoassays
  - ELISA
  - Rapid tests

- Western Blot
  - no longer in guidelines

- Immunofluorescence assay
  - No longer in guidelines

- Nucleic acid testing-new to the guidelines

http://webinars.aphl.org/session-handouts.php?id=14611
Old School

- First and second generation immunoassays
  - Only detect IgG
  - Variable sensitivity in early infection

- HIV-1 Western Blot
  - Misses acute infection
  - Mis-classifies HIV-2 as HIV-1 (cross reactivity)
  - Requires NAT to verify infection in indeterminate samples
  - Requires additional testing to rule out HIV-2

http://stacks.cdc.gov/view/cdc/23447
Limitations of previous modalities

- Misses acute infection
- Western Blot and IFA can produce false negative or indeterminate results
- Western Blot can mis-identify HIV-2 as HIV-1
- Guidelines updated 6/2014
HIV testing in the US

- 4 generations of assays to test for HIV:
  - 1st: Western Blot, IFA
  - 2nd: HIV-1 EIA, 6 rapid HIV Ab tests
  - 3rd: HIV-1/2 immunoassay and HIV1/2 chemiluminescent immunoassays
  - 4th: Same as 3rd gen plus one rapid test that uses separate indicators for HIV-1/2 antigen and antibodies

http://stacks.cdc.gov/view/cdc/23447
Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations

http://stacks.cdc.gov/view/cdc/23447
New generation of HIV testing

- 3rd generation tests:
  - Detect IgM and IgG Ab
  - Antibodies in the person’s serum bind to antigens on assay substrate and to antigens on indicator molecules
  - Antigens are synthetic and recombinant peptides
  - Allows use of lower serum dilutions
  - Increased sensitivity in early seroconversion

http://stacks.cdc.gov/view/cdc/23447
4th Generation tests

- Same as 3rd generation tests but also includes monoclonal antibodies to detect p24 antigen

- Allows for detection of HIV-1 prior to seroconversion

- Does not distinguish between antibody and antigen reactivity

http://stacks.cdc.gov/view/cdc/23447
P24 antigen

- Detected by 4\textsuperscript{th} generation assays 4-10 days after detection of HIV-1 RNA

- Rise in p24 is transient because it binds to HIV antibodies and forms immune complexes
Dx Step 1

- Initial screening
  - 4th generation test: tests for HIV-1/2 IgM and IgG, p24 (specific for HIV-1)

- Presence of detectable HIV Ab varies between 2 weeks and 6 months
  - Cannot rely solely on 4th generation test
Dx Step 2

- If initial test is reactive, 2\textsuperscript{nd} test is done to differentiate HIV-1 from HIV-2
  - Checks for HIV-1/2 IgG only

- If antibody differentiation assay is indeterminate or non-reactive, HIV-1 nucleic acid testing is done
Case 1

- 28 yo female 24 weeks pregnant presents for prenatal care, HIV screening is done
  - p24 antigen positive

- What now?
  - Reassure patient that test is negative
  - Check HIV viral load
  - Check IFA
  - Start ART
Case 1

- Check HIV-1/2 differentiation assay
Case 1

- HIV-1/2 differentiation assay is indeterminate
- Check HIV-1 NAT
Case 2

- 46 yo male gets a rapid HIV test and the result is positive

- What next?
  - Reassure him that it’s a false positive
  - Check antigen/antibody combination immunoassay (4th generation test)
  - Check Western Blot
  - Check viral load
Case 2

- Initial antigen/antibody combination immunoassay:
  - positive

- HIV-1/2 antibody differentiation assay:
  - negative

- What now?

- Check NAT
Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations

HIV-1/2 antigen/antibody combination immunoassay

(+) ↓
(-) ↓

Negative for HIV-1 and HIV-2 antibodies and p24 Ag

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+) ↓ HIV-1 (-) ↓ HIV-1 (+) ↓ HIV-1 (-) or indeterminate
HIV-2 (-) ↓ HIV-2 (+) ↓ HIV-2 (+) ↓ HIV-2 (-)

HIV-1 antibodies detected ↓ HIV-2 antibodies detected ↓ HIV antibodies detected ↓ HIV-1 NAT

(+) indicates reactive test result
(-) indicates nonreactive test result
NAT: nucleic acid test

HIV-1 NAT (+) ↓ HIV-1 NAT (-)
Acute HIV-1 infection ↓ Negative for HIV-1
Case 2

- NAT is positive
- What kind of HIV infection is this?
Case 2

- Acute HIV infection

- HIV-1/2 differentiation immunoassay only detects IgG
Case 3

- 22 yo sexually active female from Nicaragua presents for Pap smear
- HIV screen is positive
- HIV-1/2 antibody differentiation is indeterminate
Case 3

- HIV-1 NAT is negative

You should:
- Start ART
- Check HIV viral load
- Repeat the test
- Reassure her that this is a false positive
HIV-1/2 antigen/antibody combination immunoassay

(+) → Negative for HIV-1 and HIV-2 antibodies and p24 Ag

HIV-1/HIV-2 antibody differentiation immunoassay

(-) →

HIV-1 (+) or indeterminate
HIV-2 (-)

HIV-1 (−) or indeterminate
HIV-2 (+)

HIV-1 (+)
HIV-2 (+)

HIV-1 (−)
HIV-2 (−)

HIV-1 antibodies detected
HIV-2 antibodies detected
HIV antibodies detected

HIV-1 NAT

(+) indicates reactive test result
(-) indicates nonreactive test result
NAT: nucleic acid test

HIV-1 NAT (+) Acute HIV-1 infection
HIV-1 NAT (−) Negative for HIV-1

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http://stacks.cdc.gov/view/cdc/23447
Case 3

False positive, reassure patient

- Encourage safe sex practices
- Repeat testing based on exposures
HIV-2

- HIV-2 most common in West Africa
  - 200 cases in the US as of 2009
  - India, North America, Europe
- About 50% of people with HIV-2 have undetectable VL
- HIV-2 NAT is unreliable
- 60-92% HIV-2 cases test positive for HIV-1 on WB
- May need to check proviral DNA to confirm the presence of HIV-2

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Case 4

- 18 year old male gets a negative result on a rapid HIV test
- Does he need any further testing?
Case 4

- If this took place in the 1990’s, he would have required Western Blot or IFA
  - Older rapid tests did not identify acute infection
  - Older rapid tests were not very sensitive

- 2014: no further testing required
Case 5

- 33 year old female has a positive 4th generation test, repeat is also positive

- HIV-1/2 antibody differentiation assay is positive for HIV-1

- HIV-1 NAT is negative
HIV-1/2 antigen/antibody combination immunoassay

(+)  
(-)  
Negative for HIV-1 and HIV-2 antibodies and p24 Ag

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)  
HIV-1 (-)  
HIV-1 (+)  
HIV-1 (-) or indeterminate

HIV-2 (-)  
HIV-2 (+)  
HIV-2 (+)  
HIV-2 (-)

HIV-1 antibodies detected  
HIV-2 antibodies detected  
HIV antibodies detected  
HIV-1 NAT

(+) indicates reactive test result  
(-) indicates nonreactive test result  
NAT: nucleic acid test

HIV-1 NAT (+)  
Acute HIV-1 infection  
HIV-1 NAT (-)  
Negative for HIV-1
Case 5

- False negative HIV-1 NAT

- NAT is negative in 2-4% of people with established infection
  - Elite controllers
  - Already on ART

Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations
http://stacks.cdc.gov/view/cdc/23447
Case 6

- 50 year old Nigerian male has a positive HIV antibody screen and gets an indeterminate Western blot

- An HIV-1 NAT is checked

- HIV-1 NAT is negative
  - Next step?
Case 6

- Check HIV-1/2 antibody differentiation immunoassay:
  - Positive HIV-2
  - HIV-2 NAT is unreliable
  - Proviral HIV-2 DNA is difficult to obtain

- Demographics suggest HIV-2
  - Start ART
Case 7

- 44 yo male with thrush has HIV testing done in ED
  - 4th generation test
    - Positive
  - HIV-1/2 differentiation test
    - Negative
  - VL
    - Over 3 million
How would you interpret these findings?
HIV positive

- 4th generation test
  - Positive
- HIV-1/2 differentiation test
  - Negative
- VL
  - Over 3 million
- False negative differentiation test in patients on treatment
FDA approved tests

- HIV-1/2 antigen/antibody combination immunoassay, 4th generation test
  - Architect HIV Ag/Ab Combo
  - GS HIV Combo Ag/Ab EIA

- HIV-1/2 differentiation assay
  - Multispot HIV-1/HIV-0 Rapid Test

- HIV-1 NAT
  - APTIMA HIV-1 RNA Qualitative Assay
  - Procleix Ultrio

[ FDA approved tests for the Diagnosis of HIV Infection: Updated Recommendations ]
[ http://stacks.cdc.gov/view/cdc/23447 ]
Architect Ag/Ab Combo

- Detects p24 Ag, HIV-1 (group M and O), and HIV-2 Ab
  - Uses 5 recombinant proteins and 2 synthetic peptides derived from HIV-1/2 native sequence transmembrane proteins

- Does not distinguish between Ab and p24 Ag

Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations
http://stacks.cdc.gov/view/cdc/23447
Multispot differentiation assay

- Differentiates between HIV-1 and HIV-2 Ab in serum or plasma
- Gene sequences are similar between HIV-1 and HIV-2, envelope proteins are type specific
- Results available in about 30 minutes

*Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations*
http://stacks.cdc.gov/view/cdc/23447
HIV-1 peptide: turns purple if gp41 envelope glycoprotein is present

HIV-2: turns purple if gp36 envelope glycoprotein is present

Recombinant HIV-1: gp41 glycoprotein expressed in E.coli
Indeterminate results

- Reactivity to synthetic gp41 peptide or the recombinant gp41 protein, but not both OR
- Detects Ab for both HIV-1 and HIV-2
- Lab follows dilution protocol, repeats multispot and if still undifferentiated, considered positive
  - Consider NAT
  - Consider dual infection

http://webinars.aphl.org/session-handouts.php?id=14611
## Multispot Results and Interpretation

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Interpretation of result</th>
<th>Next step</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Nonreactive" /></td>
<td>Nonreactive</td>
<td>HIV-1 NAT (RNA)</td>
</tr>
<tr>
<td><img src="image" alt="Positive for HIV-1 antibodies" /></td>
<td>Positive for HIV-1 antibodies</td>
<td>Initiate care</td>
</tr>
<tr>
<td><img src="image" alt="Indeterminate for HIV-1 antibodies" /></td>
<td>Indeterminate for HIV-1 antibodies</td>
<td>HIV-1 NAT (RNA)</td>
</tr>
<tr>
<td><img src="image" alt="Positive for HIV-2 antibodies" /></td>
<td>Positive for HIV-2 antibodies</td>
<td>Initiate care</td>
</tr>
<tr>
<td><img src="image" alt="Positive for HIV antibodies (Undifferentiated)" /></td>
<td>Positive for HIV antibodies (Undifferentiated)</td>
<td>Initiate care; Consider NAT to rule out or confirm dual infection</td>
</tr>
</tbody>
</table>
Sensitivity/specificity

- 4th gen: Specificity of 99.5%-100%
- Differentiation assay: 99-99.9%
- NAT: 99.6-99.9%

- 4th gen: Reactive in 62-83% of specimens negative by Western Blot but positive by NAT
NAT: nucleic acid test

- APTIMA
  - FDA approved for aiding in diagnosis of HIV
  - Qualitative assay
  - Results available in 1-2 days

- HIV RNA (viral load)
  - Not approved for diagnosis, only for monitoring
  - Quantitative assay

http://webinars.aphl.org/session-handouts.php?id=14611
NAT

- Labs can send specimens to APHL approved sites, state or commercial labs for NAT testing
- Important step in the algorithm
  - Helps detect acute infection
  - Helps detect false positive 4th generation test
  - Reduces number of indeterminate tests
  - VL cannot be used in lieu of NAT but can supplement clinical diagnosis

http://webinars.aphl.org/session-handouts.php?id=14611
Home HIV testing

- Not part of the algorithm in the CDC guidelines
  - Decreased sensitivity for detecting acute infection compared to lab based 4th generation test
  - 4th generation IA detects acute infection, home tests detect IgG

_Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations_

http://stacks.cdc.gov/view/cdc/23447
## Anti-HIV Specimen Collection Devices, Testing Services, and Home Test Kits

<table>
<thead>
<tr>
<th>Tradename</th>
<th>Infectious Agent</th>
<th>Format</th>
<th>Specimen</th>
<th>Use</th>
<th>Manufacturer</th>
<th>Approval Date</th>
<th>STN</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraSure HIV-1 Oral Specimen Collection Device</td>
<td>HIV-1</td>
<td>Oral Specimen Collection Device</td>
<td>Oral Fluid</td>
<td>For Use with HIV diagnostic assays that have been approved for use with this device.</td>
<td>OraSure Technologies Bethlehem, PA</td>
<td>12/23/1994</td>
<td>BP910001</td>
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<tr>
<td>OraQuick In: Home HIV Test</td>
<td>HIV-1, HIV-2</td>
<td>Immunoassay</td>
<td>Oral fluid</td>
<td>Over-the-counter (OTC) diagnostic home-use test. A positive result is preliminary and follow-up confirmatory testing is needed.</td>
<td>OraSure Technologies Bethlehem, PA</td>
<td>07/03/2012</td>
<td>BP120001</td>
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[http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm080466.htm#anti_HIV_CollectionTestingHomeUseKits](http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm080466.htm#anti_HIV_CollectionTestingHomeUseKits)
Positive Rapid Test

- Lab that processes sample verifies infection with EIA and Western Blot
  - If EIA/WB negative, false positive rapid test
  - If EIA + but WB indeterminate, likely indicates evolving infection

- Ideally, patient should see provider who would then repeat testing with new algorithm

http://stacks.cdc.gov/view/cdc/23447
Criminal penalties

- People with HIV who knowingly expose others to HIV can be prosecuted, laws and penalties vary from state to state
- 24 states require disclosure of HIV status to sexual partners
- 14 states require disclosure of HIV status to needle sharing partners
- 25 states criminalize behaviors that may increase risk of exposing others to HIV

Mandatory HIV testing

- TX state law allows for mandatory testing if one of the following type of workers has been exposed to HIV:
  - (1) a law enforcement officer;
  - (2) a fire fighter;
  - (3) an emergency medical service employee or paramedic;
  - (4) a correctional officer;
  - (5) an employee, contractor, or volunteer, other than a correctional officer, who performs a service in a correctional facility as defined by Section 1.07, Penal Code, or a secure correctional facility or secure detention facility as defined by Section 51.02, Family Code; or
  - (6) an employee of a juvenile probation department.

Mandatory HIV testing

- Screening of blood, body fluids, tissue, organs or blood products to be used in organ donation
- Residents in mental health facilities only if it would affect their medical or social management
- Sudden or imminent threat to public health
- Failure to abide by TX laws re: mandatory testing is a Class A misdemeanor

http://www.statutes.legis.state.tx.us/Docs/HS/htm/HS.81.htm#81.005
Mandatory HIV testing

- If a person is required to get tested for HIV, they can refuse.
- If they refuse, they can be court ordered to get tested.
- If they continue to refuse, the state’s prosecuting attorney will take them to court.

http://www.statutes.legis.state.tx.us/Docs/HS/htm/HS.81.htm#81.005
For more information

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Statute Citation</th>
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| Informed Consent | TEX. HEALTH & SAFETY CODE ANN. § 81.105
|                | TEX. HEALTH & SAFETY CODE ANN. § 81.106 |
| Counseling    | TEX. HEALTH & SAFETY CODE ANN. § 81.109 |

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LABORATORY HIV REPORTING LAWS

25 Tex. Admin. Code § 97.133

http://www.cdc.gov/hiv/policies/law/states/index.html#Texas
Test all adults!

- Verbal informed consent is required
  - Written consent is not required, can opt out
  - Also applies to pregnant women in TX

- General consent for medical care includes HIV testing

- Prevention counseling should not be required prior to HIV testing

http://www.cdc.gov/hiv/policies/law/states/testing.html
Test all adults!

- Test everyone 13-64 at least once as part of routine medical care
- Test high risk individuals yearly
- Early diagnosis=early care, treatment and prevention
Questions and comments