

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

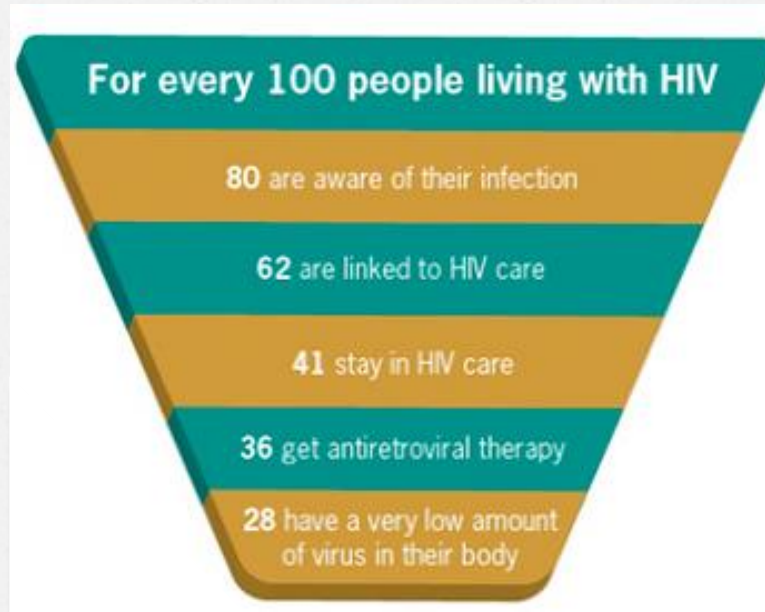
- 28 yo female 24 weeks pregnant presents for prenatal care, HIV testing is done
 - p24 antigen positive
- What now?
 - Reassure patient that test is negative
 - Check HIV viral load
 - Check IFA
 - Start antiretroviral therapy

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

Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations

<http://stacks.cdc.gov/view/cdc/23447>



<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

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

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

Testing guidelines

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

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

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

Old School

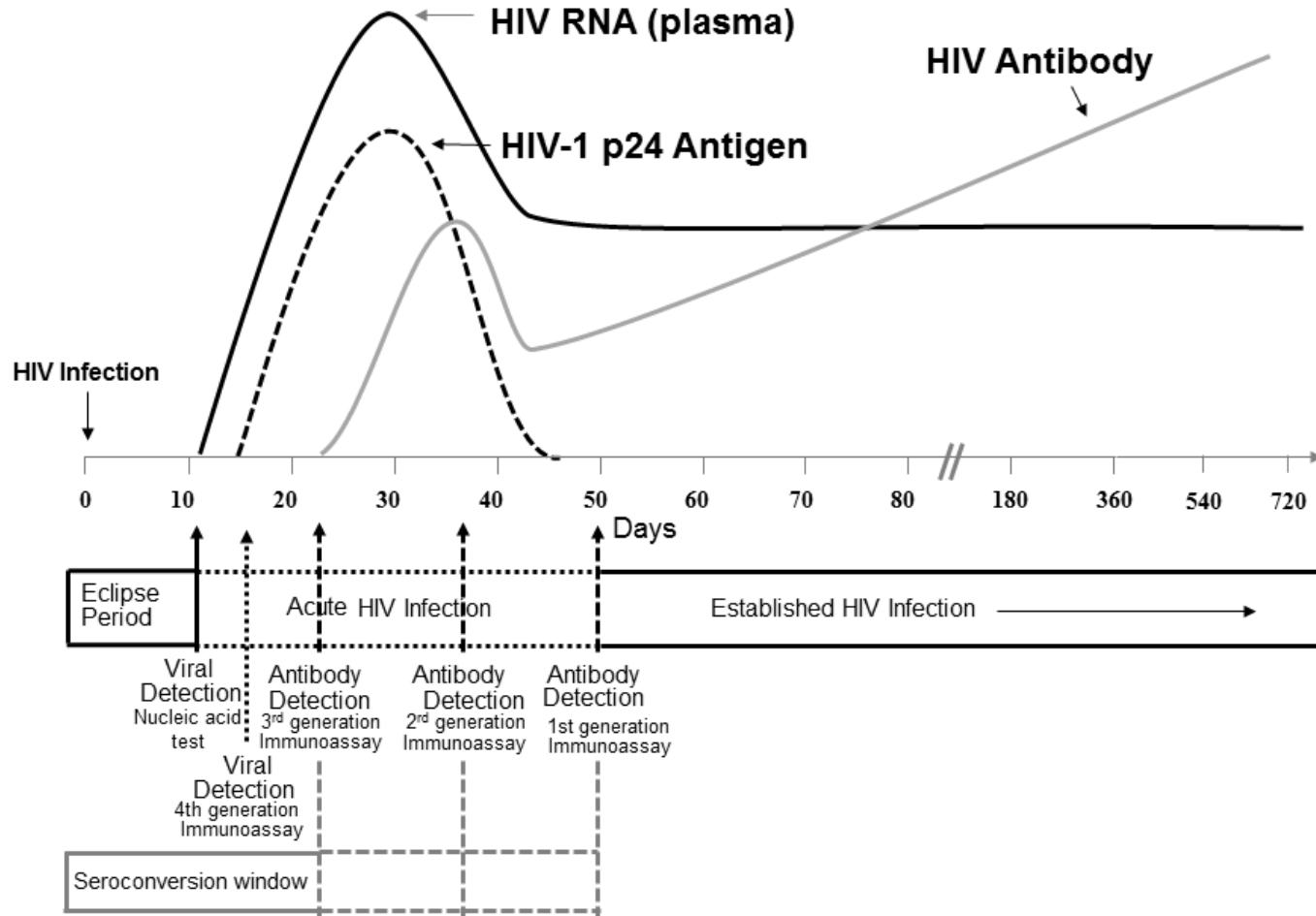
- 1st and 2nd generation immunoassays
 - Only detect IgG
 - Variable sensitivity in early infection
- HIV-1 Western Blot
 - Misses acute infection
 - Mis-classifies HIV-2 as HIV-1 (cross reactivity)
 - Requires NAT to verify infection in indeterminate samples
 - Requires additional testing to rule out HIV-2

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



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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

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

P24 antigen

- Detected by 4th 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

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

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, 2nd 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

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 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 (+)
HIV-2 (-)

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

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

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

HIV-1 antibodies detected

HIV-2 antibodies detected

HIV antibodies detected

HIV-1 NAT

HIV-1 NAT (+)
Acute HIV-1 infection

HIV-1 NAT (-)
Negative for HIV-1

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

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

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

Case 6

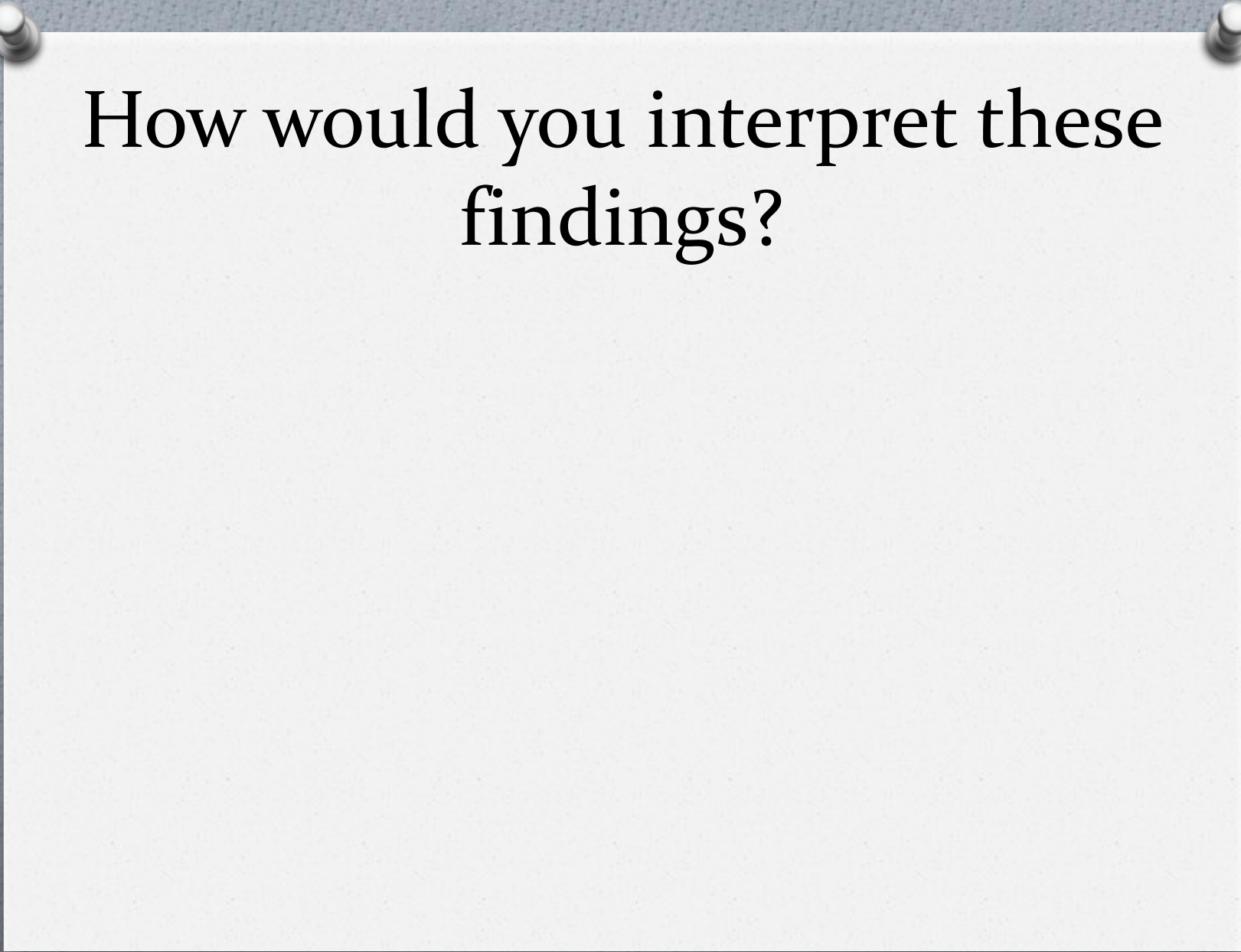
- 50 year old Nigerian male has a positive HIV antibody screen and gets an indeterminate Western blot
- An HIV-1 NAT is checked
- HIV-1 NAT is negative
 - Next step?

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

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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

Multiplex 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

HIV-1 peptide: turns purple if gp41 envelope glycoprotein is present


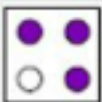


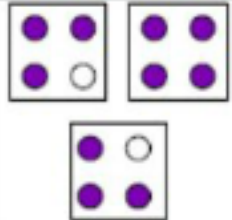
HIV-2: turns purple if gp36 envelope glycoprotein is present

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

Multispot Results and Interpretation

Appearance	Interpretation of result	Next step
	Nonreactive	HIV-1 NAT (RNA)
	Positive for HIV-1 antibodies	Initiate care
	Indeterminate for HIV-1 antibodies	HIV-1 NAT (RNA)
	Positive for HIV-2 antibodies	Initiate care
	Positive for HIV antibodies (Undifferentiated)	Initiate care; Consider NAT to rule out or confirm dual infection

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

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

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

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

Home testing: FDA approved

Anti-HIV Specimen Collection Devices, Testing Services, and Home Test Kits							
Tradename	Infectious Agent	Format	Specimen	Use	Manufacturer	Approval Date	STN
Home Access HIV-1 Test System	HIV-1	Dried Blood Spot Collection Device	Dried Blood Spot	In Vitro Diagnostic: Self-use by people who wish to obtain anonymous HIV testing	Home Access Health Corp., Hoffman Estates, IL	7/22/1996	BP950002
OraSure HIV-1 Oral Specimen Collection Device	HIV-1	Oral Specimen Collection Device	Oral Fluid	For Use with HIV diagnostic assays that have been approved for use with this device.	OraSure Technologies Bethlehem, PA	12/23/1994	BP910001
OraQuick In-Home HIV Test	HIV-1, HIV-2	Immunoassay	Oral fluid	Over-the-counter (OTC) diagnostic home-use test. A positive result is preliminary and follow-up confirmatory testing is needed.	OraSure Technologies Bethlehem, PA	07/03/2012	BP120001

http://www.fda.gov/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/BloodDonorScreening/InfectiousDisease/ucm080466.htm#anti_HIV_Collection_Testing_Home_Use_Kits

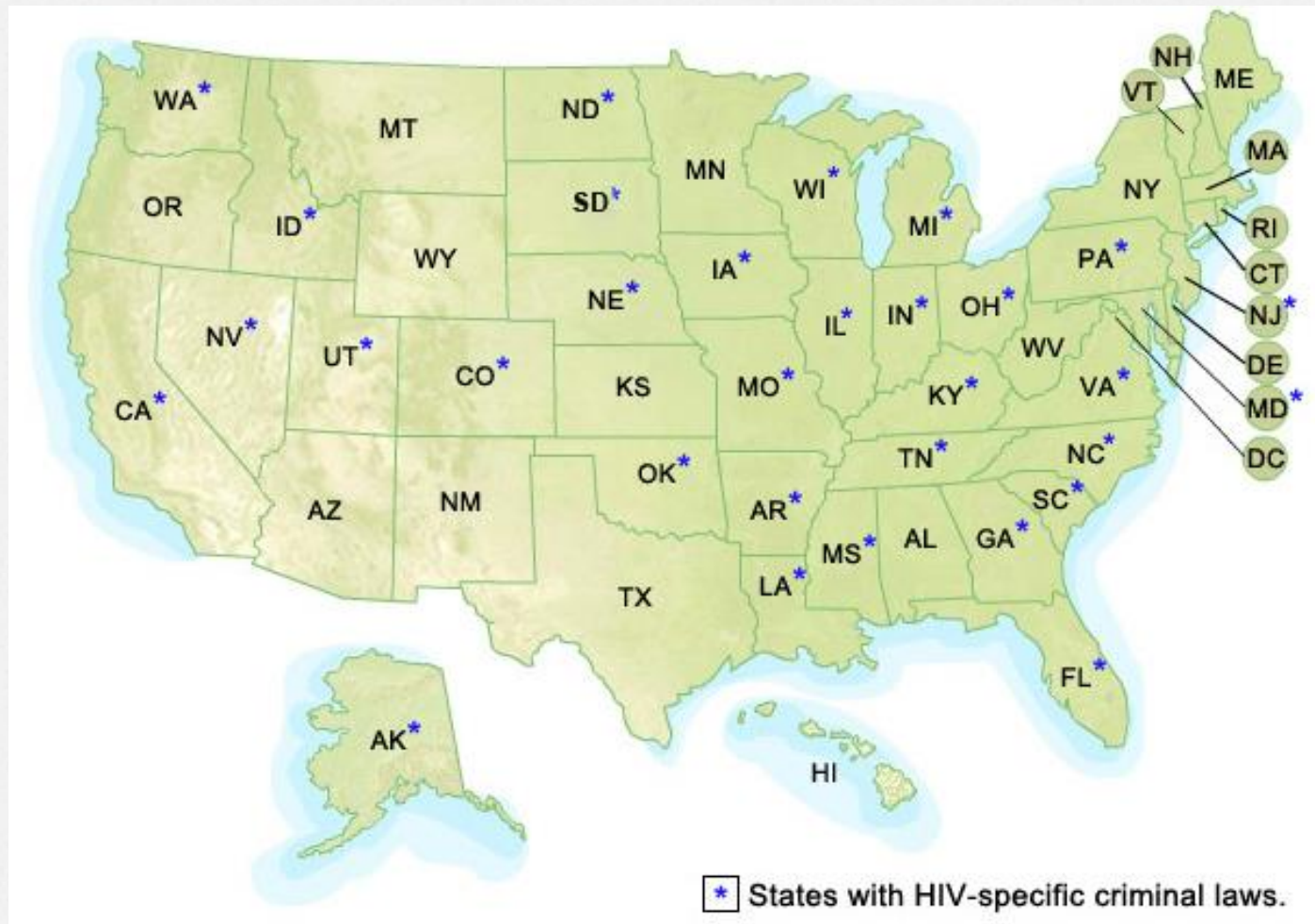


Positive Rapid Test

- 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

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**



<http://www.cdc.gov/hiv/policies/law/states/index.html>

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

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

For more information

Texas

HIV TESTING LAWS	
Requirements	Statute Citation
Informed Consent	TEX. HEALTH & SAFETY CODE ANN. § 81.105 
	TEX. HEALTH & SAFETY CODE ANN. § 81.106 
Counseling	TEX. HEALTH & SAFETY CODE ANN. § 81.109 
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

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