Diagnosing HIV Infection

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Disclosures

None
Objectives

1. Outline the CDC recommendations for HIV testing.
2. Recognize the signs and symptoms of Acute HIV infection (AHI).
3. Discuss the diagnostic algorithm for HIV infection.
Clinical Progression of HIV Infection

- Primary Infection
- Acute HIV syndrome
- Wide dissemination of virus
- Seeding of lymphoid organs
- Clinical Latency
- Opportunistic Diseases
- Constitutional Symptoms
- Death

CD4+ T Lymphocyte Count (cells/mm³)

Weeks

Years

HIV RNA Copies per ml Plasma

10^7

10^6

10^5

10^4

10^3

10^2

0

100

200

300

400

500

600

700

800

900

1000

1100

1200
Diagnosing HIV Infection

Clinical signs of HIV infection

- Seborrheic dermatitis
- Folliculitis
- Molluscum contagiosum
- Recurrent HSV
- Dermatomal VZV infection
- Onychomycosis
Diagnosing HIV Infection

Clinical signs of HIV infection

- Thrush, angular cheilitis
- OHL
- Recurrent mouth ulcerations
- Periodontitis, necrotizing ulcerative gingivitis
Diagnosing HIV Infection

Clinical signs of HIV infection
- Persistent generalized adenopathy
- Chronic diarrhea
- Weight loss
- Recurrent upper respiratory infections
- Bacterial pneumonia
- Pulmonary TB

Laboratory abnormalities
- Cytopenias
Diagnosing HIV Infection

- Opportunistic Infections
- Extra-pulmonary TB
- HIV encephalopathy
- AIDS Associated cancers
  - KS
  - Primary CNS lymphoma (PCNSL)
  - B-cell non-Hodgkin’s lymphoma
  - Invasive cervical cancer
Antivirals: Decreasing HIV RNA; Restoring CD4 Cell Counts

Adapted from Cohen CJ. J Med Care Pharmacy 2006. 12(7) 6-11
CDC Recommendations for HIV Screening

Patients in all health-care settings
• HIV screening is recommended for patients in all health-care settings after the patient is notified that testing will be performed unless the patient declines (opt-out screening).
• Persons at high risk for HIV infection should be screened for HIV at least annually.
• Separate written consent for HIV testing should not be required; general consent for medical care should be considered sufficient to encompass consent for HIV testing.
• Prevention counseling should not be required with HIV diagnostic testing or as part of HIV screening programs in health-care settings.
CDC Recommendations for HIV Screening

Pregnant Women

• HIV screening should be included in the routine panel of prenatal screening tests for all pregnant women.
• HIV screening is recommended after the patient is notified that testing will be performed unless the patient declines (opt-out screening).
• Separate written consent for HIV testing should not be required; general consent for medical care should be considered sufficient to encompass consent for HIV testing.
• Repeat screening in the third trimester is recommended in certain jurisdictions with elevated rates of HIV infection among pregnant women.
HIV and Texas Law

- Minors have the right to consent
- Separate written consent for HIV not required.
- Confidentiality and HIPPA requirements do not prevent providers from reporting HIV to public health agencies.
- Persons receiving a positive HIV test result should have face-to-face counseling and linkage to available medical and social support.
Reporting Requirements in Texas

- AHI-within one working day
- All non-acute cases of HIV/AIDS (13 years or older)
- Pediatric cases (children ages 12 or younger)
- Infants perinatally exposed to HIV
Recommendations for HIV Screening of Gay, Bisexual, and Other Men Who Have Sex with Men — United States, 2017

Elizabeth A. DiNenno, PhD; Joseph Prejean, PhD; Kathleen Irwin, MD; Kevin P. Delaney, PhD; Kristina Bowles, MPH; Tricia Martin, MPH; Amrita Tailor, MPH; Gema Dumitru, MD; Mary M. Mullins, MLS; Angela B. Hutchinson, PhD; Amy Lansky, PhD
HIV Diagnostic Tests

Image used courtesy of Dr. Tan from http://www.drtanandpartners.com/the-different-generations-of-elisa-2/
Why Do We Need Improved HIV Assays

• Recent indications for the clinical benefits from antiretroviral treatment (ART) of all persons with HIV infection, including those with acute infection.

• Demonstration that the majority of HIV-2 infections detected by available HIV antibody immunoassays are misclassified as HIV-1 by the HIV-1 Western blot.
The Evolution of HIV Diagnostic Tests

Images used courtesy of Dr. Tan from http://www.drtanandpartners.com/the-different-generations-of-elisa-2/
Third Generation ELISA Test

Image used courtesy of Dr. Tan from http://www.drtanandpartners.com/the-different-generations-of-elisa-2/
Why Do We Need Improved HIV Assays

- Evidence that relying on Western blot or indirect immunofluorescence assay (IFA) for confirmation of reactive initial immunoassay results can produce false-negative or indeterminate results early in the course of HIV infection.

- Recognition that risk of HIV transmission from persons with acute and early infection is much higher than that from persons with established infection.
HIV RNA (plasma)

HIV p24 Ag

HIV Ab

Eclipse Period

Acute Infection

Recent Infection

Longstanding Infection

Viral Detection

IgM Antibody Detection

IgG Antibody Western Blot

Seroconversion window

Modified image courtesy of http://dx.doi.org/10.15620/cdc.23447
Early Events in HIV Infection

The Evolution of HIV Diagnostic Tests

Images used courtesy of Dr. Tan from http://www.drtanandpartners.com/the-different-generations-of-elisa-2/
McMichael AJ et al Nature Reviews Immunology 10, 11-23 (January 2010)
Fourth Generation Tests

Created in the late 1990’s, approved in US in 2010. Reduced the test-negative window to ~2 weeks. Gave single result without differentiating between HIV p24 or Ab.
Serologic screening methods

- 4th generation HIV – 1/2 antigen/antibody combination immunoassay
  - Detects presence of HIV-1 p24 antigen
  - Detects antibodies to HIV-1 (group M and group O) and antibodies to HIV-2
  - Test systems include Abbott Architect HIV Ag/Ab Combo chemiluminescence assay and BioRad HIV Combo Ag/Ab EIA
  - Package inserts state sensitivities of 100% and specificities of 99.77 – 99.87%

- Provides diagnosis at day 15 – 16 of HIV infection
- Low prevalence of HIV infections in U.S. (0.5%); PPV = 50%
- Must use differentiation assays for reactive samples
Whom to Test

All patients with signs, symptoms or laboratory findings consistent with HIV disease.

All patients with symptoms consistent with AHI

All individuals with potential exposure to HIV

All pregnant women

All adults between ages 13-75

Repeat yearly testing in individuals at high risk
Case #1

26 year old Caucasian man presented to the ER September 2017 C/O nausea, abdominal pain, diarrhea (5-6 BM/day),

PE: notable for:
- fever 102°F,
- tachycardia,
- thrush

Labs:
- elevated LFT’s (237 AST, 196 ALT, Alk phos:207);
- reactive lymphs, normal Lymphocyte %
- Normal platelet count
- HBV and HAV immune, neg HCV serology
Case #1

Information from OSH

- WBC 1.90, HGB 13.4, HC 39.7, platelet count of 89
- AST: 161, ALT: 167
- Positive HIV test in August 2017
Case #1

At PMH HIV test results

HIV Ag/Ab : Reactive
Geenius HIV 1/2 Ab : HIV Negative
HIV-1 RNA VL : >10,000,000 copies/mL
HIV-1 RNA VL LOG : >7.00 log copies/mL
CD4 cell count : 65 cells/mm³/ 20.4 %
Case #2

22 year old Hispanic man

Presented to Parkland ED on 9/18/17:
- 4-5 episodes of vomiting and diarrhea
- Generalized weakness and subjective fever and chills.

Last HIV screen was one week before and was negative.

No abnormal findings on exam
Case #2

HIV Ag/Ab : Reactive
Geenius HIV 1/2 Ab: HIV Negative
HIV-1 RNA VL : 1,559,918 copies/ml
HIV-1 RNA VL LOG : 6.19
CD4 T CELL count : 383 cells/mm³/23.1 %
CDC algorithm for use with a fourth-generation HIV antibody/antigen screening test.

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 antibodies detected
(+), 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

HIV-1 (-)
HIV-2 (+)
HIV-2 (−)
HIV-2 antibodies detected

HIV-1 (-) or indeterminate

HIV-2 (-)

HIV Ag/Ab Interp

Collected: 09/18/17 19:20
Resulting lab: PARKLAND LAB
Reference range: Nonreactive
Value: Reactive (Abnormal)
Comment: Presumptive evidence of HIV-1 or HIV-2 infection. HIV Differentiation test will be performed.

*Additional information available - comment

Results

Result Information

Flag: Abnormal Status: Final result (Collected: 9/18/2017 19:20) Provider Status: Ordered
PHHS Lab Report

Geenius HIV 1/2 Ab

Collected: 09/18/17 1920
Resulting lab: PARKLAND LAB
Value: HIV Negative
Comment: THIS IS NOT A CONFIRMATORY TEST

If negative or indeterminate, we recommend HIV-1 RNA Qualitative TMA.
Requires a separate order in Epic.

*Additional information available - comment

Results

Result Information
Status: Final result (Collected: 9/18/2017 19:20)  Provider Status: Ordered
HIV Diagnostic Testing Algorithm

HIV-1/2 Antigen/Antibody Combination Immunoassay

Repeatedly Reactive:
- Geenius HIV-1/HIV-2 Differentiation Test

Nonreactive: HIV-1 antigen and HIV-1/HIV-2 antibodies were not detected.

- If recent HIV exposure is suspected, redraw and repeat testing or consider testing for HIV-1 RNA Qualitative TMA.

HIV-1 POSITIVE
- Recommend HIV-1 RNA Qualitative TMA.

HIV NEGATIVE
- HIV-1 INDETERMINATE

HIV-2 INDETERMINATE
- HIV-1 INDETERMINATE

HIV-2 POSITIVE
- WITH HIV-1 CROSS-REACTIVITY

HIV-1 RNA Qualitative TMA

HIV POSITIVE UNTYPABLE (Undifferentiated)
- Recommend HIV-1 RNA Qualitative TMA
- AND HIV-2 DNA/RNA Qualitative RT-PCR to assess for possible HIV-1 / HIV-2 dual infection.

HIV-2 INDETERMINATE
- Recommend HIV-1 RNA Qualitative TMA
- AND HIV-2 DNA/RNA Qualitative RT-PCR

Direction in orange boxes: Requires separate order(s) in Epic by ordering provider.

Created: K. Gantt 11/9/2016;
PHHS ver. 1/23/2017
Evolution of HIV Diagnostic Screening tests

1ST GENERATION
- Not recommended for HIV screening
- First available in 1985
- Detect IgG Abs to HIV-1 whole viral lysate

2ND GENERATION
- Not recommended for laboratory-based HIV screening
- Detect IgG Abs to HIV-1 and HIV-2 using synthetic peptides
- Improved specificity

3RD GENERATION
- Alternative diagnostic screening tests when 4th-generation testing is unavailable
- Detect both IgG and IgM Abs to HIV-1 and HIV-2
- Improved sensitivity

4TH GENERATION
- Recommended as the first step in the CDC/APHL diagnostic testing algorithm
- Detect both IgG and IgM Abs to HIV-1 and HIV-2 plus HIV-1 p24 Ag
- Can detect HIV-1 infection earlier in the acute phase
- Maximized specificity and sensitivity
The New Algorithm

2012 recommendations and a new algorithm for diagnostic HIV testing:

◦ To diagnose people earlier;

◦ To better and more accurately distinguish HIV-1 from HIV-2; and

◦ To get results back to people sooner.
Reasons for False-Positive HIV Screening Test Results

• Increased sensitivity of assays, leading to reduced specificity

• Technical errors

• Presence of HIV Abs in recipients of HIV-1 trial vaccines

• Other rare possibilities:
  
  • Hypergammaglobulinemia/Abs reactive to cellular components

  • Influenza vaccination may cause cross-reactivity with HIV Ab assays. The time course for such cross-reactivity remains uncertain.
Reasons for False-Negative HIV Screening Test Results

• Individual is in the eclipse period before detection of Ag or HIV RNA is possible.

• Individual is in acute phase of infection (before seroconversion) but is screened using a less sensitive method that detects Abs only.

• Individual is in the early stage of seroconversion but is screened using a less sensitive method that does not detect early (IgM) Abs.

• Technical errors
Reasons for False-Negative HIV Screening Test Results

• Other rare possibilities:

• Delayed Ab synthesis in infants and persons receiving PEP or PrEP or who have concurrent acute hepatitis C infection.
• Diminished immune response in individuals receiving intensive or long-term immunosuppressive therapy.
• Congenital or drug-induced hypogammaglobulinemia or agammaglobulinemia
• Insufficient host Ab response (i.e., advanced HIV disease)
• Unavailability of Abs due to the formation of Ag-Ab complexes
HIV-1/HIV-2 Differentiation Assay

FDA approved, March 2013

Serum Control

HIV-1 Recombinant gp41

HIV-2 Peptide gp36

HIV-1 Peptide gp41

Multispot HIV-1/HIV-2

Image courtesy of the CDC from https://www.hiv.uw.edu/go/screening-diagnosis/diagnostic-testing/core-concept/all
HIV-1/HIV-2 Differentiation Assays

FDA approved, March 2013
Serum Control
HIV-1 Recombinant gp41
Product Withdrawal
July 29, 2016
HIV-2 Peptide gp36
HIV-1 Peptide gp41
Multispot HIV-1/HIV-2

FDA approved, Oct. 2014
Geenius HIV-1/HIV-2

Images courtesy of the CDC from https://www.hiv.uw.edu/go/screening-diagnosis/diagnostic-testing/core-concept/all
Geenius™ HIV-1/HIV-2 Lines

Dual path platform
Add 5μL serum/plasma or
15μL whole blood to specimen well
Add 5 drops buffer to buffer well
Insert test cassette in reader for automated interpretation
<table>
<thead>
<tr>
<th>HIV-1 RESULT</th>
<th>HIV-2 RESULT</th>
<th>ASSAY INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>HIV NEGATIVE</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Negative</td>
<td>HIV-1 INDETERMINATE(^a)</td>
</tr>
<tr>
<td>Negative</td>
<td>Indeterminate</td>
<td>HIV-2 INDETERMINATE(^b)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>HIV INDETERMINATE(^c)</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Positive</td>
<td>Indeterminate</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Positive</td>
<td>HIV-2 POSITIVE with HIV-1 cross-reactivity: Antibody to HIV-2 confirmed in the sample. HIV-1 positivity (with only one HIV-1 envelope band, gp160 or gp41), is due to cross-reactivity and precludes confirmation of HIV-1(^*). *Note: Differentiation features managed by proprietary algorithm.</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>HIV POSITIVE Un typable (undifferentiated): Antibodies to HIV-1 and HIV-2 confirmed in the sample. This may occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1, or may be due to co-infection with both HIV-1 and HIV-2 (rare)(^*). *Note: Differentiation features managed by proprietary algorithm.</td>
</tr>
</tbody>
</table>

\(^a\) HIV-1 band(s) detected but did not meet the criteria for HIV-1 Positive  
\(^b\) HIV-2 band(s) detected but did not meet the criteria for HIV-2 Positive  
\(^c\) HIV band(s) detected but did not meet the criteria for HIV-1 Positive or HIV-2 Positive
Predictive Values for the Geenius and Multispot

Fig. 4. Positive and negative predictive values for the Geenius and Multispot assays in populations with prevalence from 0% to 5%. Inset for populations of 0–60% prevalence.

Acute HIV Infection
Signs and Symptoms

- Fever (75%)
- Fatigue (68%)
- Pharyngitis (40%)
- Arthralgia, myalgias (49%)
- Rash (48%)
- Diarrhea (27%)
- Neurologic symptoms (24%)
- Weight loss (25%)
- Oral/genital ulcerations (10-20%)
AHI-Laboratory Findings

Lymphopenia
Atypical lymphocytosis
Thrombocytopenia may occur
Elevated transaminases
Low CD4 cell count
HIV Ab usually negative
HIV Ab/Ag test (80% sensitive in symptomatic AHI)
HIV viral load generally very high (over 100K/1 million copies/ml)
AHI-Differential Diagnosis

Epstein-Barr virus infection
CMV
Viral hepatitis
Enteroviral infection
Secondary syphilis
Toxoplasmosis
Influenza
HSV + Erythema multiforme
AHI-Differential Diagnosis

Drug reaction
Behcet’s disease
Acute SLE
Laboratory Tests

Throat cultures (bacterial and viral respiratory pathogens)
Syphilis test
Hepatitis serology
HCV RNA
EBV VCA IgM/IgG
CMV PCR
Toxoplasma titers
AHI-Management

Order HIV genotype **but do not wait for resistance testing results!**

Initiate ARVT

- PI-based regimen or
- NSTI-based regimen
YOUR HIV TEST RESULTS EXPIRE EVERY TIME YOU HAVE RISKY SEX.

#MyHIVTestingDay

www.cdc.gov/hiv/basics/testing.html
4th generation HIV-1/2 immunoassay

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-2 (+)
HIV-1 antibodies detected HIV-2 antibodies detected HIV antibodies detected

HIV-1 RNA viral load

RNA (+) RNA (-)
Acute HIV-1 infection Negative for HIV-1

June 27, 2014
Fifth Generation Tests

Detects both Ag and Ab

Provides separate results for each analyte

Also provides separate results for HIV-1 and HIV-2 Ab

Differentiation assays will not be needed.

No algorithm using these tests yet!
CLIA-waived Rapid HIV Antibody Tests

Oraquick Advance

DPP HIV 1/2

Chembio Sure Check

INSTI HIV 1/2

Chembio Stat Pak

Uni-Gold Recombigen

CLIA: Clinical Laboratory Improvement Amendments
Chembio SureCheck

DPP HIV 1/2

INSTI HIV 1/2

What’s new?
DPP HIV-1/2

- Finger-stick, oral fluid
- Swab gums 4 times (15 seconds) or 10 µL whole blood
- Read time 10-25 min blood 40 min oral fluid
INSTI HIV-1/2

- CLIA-waived for whole blood, finger-stick
- 50 μL specimen volume
- Results <1 minute
- Detects IgM antibodies

Moshgabadi et al, J Clin Virol 2015
Summary

- HIV screening tests keep getting better.
- HIV RNA Viral load will play an increasingly important role in diagnosis.
THANK YOU