The Evolution of HIV Testing

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Objectives

1. Outline the history of HIV testing.
2. Describe the limitations of the first testing methods that were produced for the detection of HIV.
3. Discuss the need for an updated algorithm.
4. Identify the new objectives in the current algorithm and describe the test methods involved.
Historical Background

First well-documented case dates to 1959 in an African man

HIV first clinically recognized in 1981 (Reported in MMWR and NEJM)

- Unexpected clusters of diseases including Kaposi’s sarcoma and PCP in young homosexual men
- Depletion of CD4 positive helper T-cells

Isolation and description of the virus associated with AIDS in 1983
ELISA TURNS 30

BETTER HIV TESTING BECOMES EVER MORE CRUCIAL AS PREVENTION EFFORT'S INCREASE.

By Ben Page

The U.S. Food and Drug Administration (FDA) has approved a new HIV test that can detect the virus in just 15 minutes. The test, called OraSure, uses a small strip of paper and a drop of blood to detect HIV antibodies, which can be infectious within weeks of exposure. The test is designed to be used in remote areas where traditional HIV testing is not feasible.

The test works by using a small sample of blood to create a landscape of the virus, which can then be detected by a machine. The test has been approved by the FDA and is now available in the U.S. and other countries.

The test is one of several new technologies that are making HIV testing more accessible and effective. Other new technologies include a test that can detect HIV in saliva, which is less invasive and easier to use. The test is also more sensitive and can detect smaller amounts of the virus, which can be important for early detection and treatment.

The test is also being used in clinical trials to test new drugs and vaccines that are being developed to prevent and treat HIV. The drug development pipeline is long and expensive, but the test is helping to accelerate the process by allowing researchers to quickly test new drugs and vaccines in clinical trials.

In conclusion, the new HIV test is a major step forward in the fight against HIV. It is more accessible, easier to use, and more sensitive than traditional testing methods. The test is also helping to accelerate the development of new drugs and vaccines, which could lead to a cure for HIV in the future.
First Generation ELISA Antibody Test
Western Blot

A purified HIV antigen mixture is layered onto a gel slab and electrophoresed. The antigens migrate through the gel, with the higher molecular weight proteins forming bands near the top.

The protein bands are then transferred onto nitrocellulose filter paper, which is cut into strips and incubated with the patient's serum. If the serum contains HIV antibodies, these bind with their corresponding antigen bands.

(In the actual assay, these bands are not visible. They are shown here for illustration purposes.)
Second Generation ELISA Test

- Substrate
- Enzyme
- Anti-HIV Antibody
- Anti-HIV IgG Antibody
- HIV 1 and HIV 2 Antigens
- Substrate color change
HIV-1/HIV-2 EIA
Repeatedly reactive
Western Blot

Positive
HIV positive

Negative
Indeterminate
HIV-2 EIA
Repeatedly reactive
HIV-2 WB

CDC Algorithm for second generation HIV tests. MMWR Recomm Rep 199241(RR-12) 1-9
Third Generation ELISA Test
HIV Infection

- **Primary infection**
- **Acute HIV syndrome**
  - Wide dissemination of virus
  - Seeding of lymphoid organs
- **Clinical latency**
- **Symptoms of AIDS**
  - Opportunistic diseases
  - Constitutional symptoms
  - Death

- **CD4+ Lymphocyte Count (cells/mm³)**
- **HIV RNA Copies per mL plasma**

- **Weeks**

- **Years**
McMichael AJ et al Nature Reviews Immunology 10, 11-23 (January 2010)
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.
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.
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

- 4\textsuperscript{th} 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 confirmatory assays for reactive samples
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.
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 (+) 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

(+ ) 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

Centers for Disease Control and Prevention and Association of Public Health Laboratories. 27 June 2014.
Laboratory testing for the diagnosis of HIV infection: updated recommendations.
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

• 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
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
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 INDETERMINATEa</td>
</tr>
<tr>
<td>Negative</td>
<td>Indeterminate</td>
<td>HIV-2 INDETERMINATEb</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>HIV INDETERMINATEc</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Positive</td>
<td>Indeterminate</td>
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</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</td>
</tr>
</tbody>
</table>

- **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.

- **HIV POSITIVE UNTYPABLE** (untypable): 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.

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*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.

4th generation HIV-1/2 immunoassay

HIV-1/HIV-2 antibody differentiation immunoassay

- HIV-1 (+)
  - HIV-1 antibodies detected
- HIV-2 (-)
  - Negative for HIV-1 and HIV-2 antibodies and p24 Ag
- HIV-1 (-) or indeterminate
  - HIV-2 (-)
  - HIV-1 RNA viral load
    - RNA (+)
      - Acute HIV-1 infection
    - RNA (-)
      - Negative for HIV-1

June 27, 2014
4th generation HIV-1/2 immunoassay

(+)

HIV-1 RNA viral load

VL detectable
HIV-1 infection

Useful clinical information

VL (-)

HIV-1/HIV-2 antibody differentiation assay

HIV-1+ (Viral suppression)

HIV-2+ HIV-2 infection

Negative

(-)

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

What’s new?

INSTI HIV 1/2
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
Evolution of HIV Diagnostic Screening Tests.

**1st Generation**
- Not recommended for HIV screening
- First available in 1985
- Detect IgG Abs to HIV-1 using 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 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
- Maximizes specificity and sensitivity
Summary

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

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.
HIV Testing Recommendations

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.
THANK YOU