Molecular Methods in Diagnosis of HIV Infection

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Traditional algorithm for HIV testing

A HIV Immunoassay

A+ Repeat A in duplicate

A++ or A+++

A HIV-1 WB or HIV-1 IFA

B Positive

Positive for HIV-1 antibodies

B Negative

Negative for HIV-1 antibodies

B Indeterminate

Inconclusive for HIV-1 antibodies; request redraw in 2-4 weeks; requires medical follow-up for further evaluation and testing

A- Negative for HIV-1 antibodies
Traditional algorithm for HIV testing

Molecular detection of HIV in the following situations

To determine HIV status during window period

Detection of HIV infection in newborns

Indeterminate Western Blot results

To monitor viral load level in HIV infected individuals during anti-retroviral therapy
Challenges with the Diagnostic Algorithms

studies suggest that many as 50% of HIV transmissions occur during the **acute and early stage** of the illness

- **High viral load** (much greater than 10 million viral copies/mm3)

- **Risky behaviors during this period** (unaware of his/her HIV status)

- **The nonspecific symptoms of acute infection** (unrecognized as an indication of HIV infection)
Challenges with the Diagnostic Algorithms

Antibody tests do not detect infection in ~10% of infected persons at highest risk of transmission.

Western blot (WB) confirmation is less sensitive during early infection than many widely used screening tests.

WB and Immunofluorescent Assay (IFA) supplemental assays cannot differentiate HIV-1 from HIV-2 infections.
Challenges with the Diagnostic Algorithms

Detect acute HIV infections

Differentiate HIV-1 from HIV-2

Eliminate indeterminate and inconclusive results whenever possible

Get timely results to facilitate initiation of care
The changing landscape of HIV diagnostics
The New Algorithm

The first step:
Ag/Ab combination immunoassay

The second step:
HIV-1/HIV-2 discriminatory assay

The third step:
Nucleic Acid Amplification Tests (NAATs)
New Diagnostic Algorithms for Detecting HIV Infection

A new algorithm for HIV screening, as proposed by CDC/ APHL, and adopted by CLSI (Figure adapted from Styer et al, 2011)
HIV diagnosis in Adults and Children >18 months

Those who are suspect to acute HIV infection:

• persons with a recent sexual or parenteral exposure with a known HIV-infected partner
• unsafe sexual practices with other men
• needle-sharing
• persons with a newly diagnosed sexually transmitted infection
• persons with aseptic meningitis
• Pregnant or breastfeeding patients
A new algorithm for HIV screening, as proposed by CDC/APHL, and adopted by CLSI (Figure adapted from Styer et al, 2011)
HIV testing for acute infection

A qualitative HIV RNA test is the approved method for such a diagnosis.

Quantitative RNA test may serve this purpose when a qualitative RNA test is not available in a given setting.

HIV RNA levels tend to be very high in acute infection. Low-level positive PCR results (<5000 copies/mL) are often not diagnostic of acute HIV infection and should be repeated to exclude a false-positive result.
Diagnosis of acute HIV infection in pregnancy

An HIV serologic screening test in conjunction with a plasma HIV RNA assay

The plasma RNA test should be performed even if the serologic screening test is negative

Detection of HIV RNA or antigen in the absence of HIV antibody should be repeated with a new specimen immediately to confirm the presence of HIV RNA

To exclude a false-positive result, HIV serologic testing should be repeated 2 to 3 weeks after diagnosis by HIV RNA testing to confirm infection

Initiation of ART is strongly recommended for pregnant women
Tests for HIV Infection Status in Infants <18 months

HIV nucleic acid detection: Highly sensitive and specific test for early detection of HIV infection in infants

HIV-1 DNA PCR: detects the integrated DNA form of the virus and should be used only for the detection of infection in infants born to mothers infected with HIV-1

All initial positive DNA PCRs should be confirmed with a second PCR test on a separate specimen

HIV-RNA-PCR: qualitative or quantitative (in plasma)
Quantitative assays are referred to as viral load (VL) assays and are primarily reserved for prognosis and monitoring treatment response.

These important advances have led to VL assays with broader dynamic range and coverage for rare HIV genotypes, and **analytical sensitivity ranges as low as 20 to 50 copies/mL**, depending on the assay.
Viral Load Assays

Several different HIV viral load tests have been developed:

COBAS AmpliPrep/COBAS TaqMan HIV-1 Test

Versant/Quantiplex HIV-1 RNA, or bDNA (Chiron/Siemens)

NucliSens HIV-1 QT, or NASBA (bioMérieux)

Abbott Real-Time HIV-1
Drug Resistance Tests

At **baseline**, regardless of whether ARV therapy is being initiated

in ARV therapy-naïve patients **before initiation of ARV**

therapy (genotypic testing)

Resistance testing should be performed promptly in cases of virologic **failure or incomplete viral suppression**

while receiving ARV therapy (genotypic and/or phenotypic testing)
Genotyping

A genotypic assay provides an indirect measure of drug resistance because it is based on detection of the mutations known to be associated with resistance.

Two direct sequencing-based methods have been approved by the FDA:
- the TruGene HIV-1 Genotyping assay (Siemens)
- the ViroSeq HIV-1 Genotyping System (Celera Diagnostics)
A phenotypic assay provides a **direct measure of drug resistance**

The currently available phenotypic assays use **recombinant DNA methods** to measure the ability of a patient’s virus to grow in the presence of a drug.

Two companies, Tibotec-Virco (Antivirogram) and Monogram-Biosciences (PhenoSense), offer phenotypic resistance testing.
Co-Receptor Tropism Assay

- Co-receptor tropism analysis determines the type of cellular co-receptor (either CCR5 or CXCR4) that an HIV-infected individual’s dominant viral population uses to gain access to host cells.

- The majority of acutely or recently infected individuals, including perinatally infected children, have a CCR5-utilizing virus.

- The drugs that target the CCR5 co-receptor, such as maraviroc, will likely be effective in these patients.

- The CCR5-tropic virus predominates early in HIV infection, whereas CXCR4-tropic virus is often present in late-stage disease.
HLA-B 5701 testing

• HLA-B 5701 testing should be performed before initiating abacavir-based therapy

• Individuals with human leukocyte antigen (HLA)-B* 5701, HLA-DR7, and HLA-DQ3 have a genetic predisposition to development of abacavir hypersensitivity