4’th Generation HIV
Antigen/Antibody Assays and
the Move Away from Western
Blot Confirmation

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Topics
1. Brief review of the taxonomy, structure and replication of HIV
2. Transmission of SIV to humans and the appearance of HIV
3. Viral evolution, recombinant viruses, and the early HIV-1 epidemic in Africa and spread of HIV-1 to North America
4. Development of diagnostic tests
5. New recommendations for diagnostic testing
HIV Taxonomy and Structure

• HIV is a member of the genus *Lentivirus* within the family *Retroviridae*
  – Virions contain two identical copies of ssRNA~10Kb in length
  – Codes for 9 genes, including the 3 most important
    • *gag* (structural) p24
    • *env* (envelope) gp160 → gp120 and gp41
    • *pol* (polymerase) RT and integrase

HIV Classification

• HIV viruses classified based on phylogenetic relatedness of nucleotide sequences
• Hierarchical system
  1. Types (50% sequence divergence)
  2. Groups (30-50% sequence divergence)
  3. Subtypes (≤ 20% intra & ≤ 35% inter subtype)
  4. Sub-subtypes
  5. Recombinant forms
Phylogenetic Classification of HIV

Where Did HIV Come From?

- HIV as a zoonosis
- Simian immunodeficiency viruses (SIVs)
  - Found in >40 species of non-human primates
  - Transmission to humans by butchering or consuming of infected animals
  - Evidence
    - Viral genome organization
    - Viral genome sequence similarity
    - Geographic coincidence of infected primates and humans

Figure 2. HIV Classification Scheme
1 Circulating recombinant form
2 Unique recombinant form

*Endemic in West Africa: 68 cases reported to CDC in U.S. from 1996-2006*
Plausible Route of Transmission

Bushmeat Market in West Central Africa


Multiple Transmission Events

Transmission of SIV to Humans

Schematic trees showing multiple zoonotic transmissions of SIVcpz and SIVsm to humans. Branches in black indicate evolution of SIV within its natural host, black arrows indicate points of cross-species transmission and branches in red indicate evolution within humans.

**Error-Prone Replication**

- HIV replicates in an error-prone manner. RT has a high mutation rate ($10^{-4}$ per base per cycle or 1 mutation per genome per cycle)
- HIV has a very high turnover rate (10$^9$ virions produced each day)
- HIV RT is highly recombinogenic
- HIV exists in the host as a swarm of similar but divergent variants called a quasispecies

**HIV-1 Group M Recombinants**

HIV-1 Group M subtypes are: A, B, C, D, E, F, G, H, J, K

Crossover points
**Origin of HIV in Africa**

- Central Africa is the only region in the world where all HIV-1 groups (M, N & O) & all group M subtypes (A-H, J & K) are found
- This diversity suggests central Africa as the epicenter of the HIV epidemic
- Each major lineage (M,N,O) is believed to have arisen through a separate zoonotic infection into humans from chimpanzee SIVs
- Studies have indicated HIV entered the human population sometime from 1900-1930’s

**The emergence of HIV/AIDS in the Americas and Beyond**

1. Haiti appears to have the oldest HIV/AIDS epidemic outside of sub-Saharan Africa
2. Subtype B apparently moved from central Africa (Kinshasa) to Haiti around 1966
3. HIV was possibly brought to Haiti by Haitians returning from the Congo in the middle 1960’s after their independence in 1960
4. A pandemic clade emerged from Haiti into the Americas and beyond in about 1969
5. HIV circulated cryptically in the US for ~12 years prior to the recognition of AIDS in 1981
Global Distribution of HIV-1 Subtypes

Implications of HIV Diversity

- Some subtypes may be associated with:
  - More aggressive disease
  - Transmission differences
    - Homosexual vs heterosexual
    - Mother to child
  - Treatment
    - Most drugs have been designed to treat subtype B
Implications of HIV Diversity

- Impact on diagnostic and treatment monitoring assays
- “Inaccurate Diagnosis of HIV-1 Group M and O Is a Key Challenge for Ongoing Universal Access to Antiretroviral Treatment and HIV Prevention in Cameroon” Aghokeng et al. PLoS ONE 2009
Initial/Screening Antibody Tests for HIV

- Verify that a patient presenting with symptoms is HIV-infected
- Determine HIV status of asymptomatic person who may have been exposed
- Due to window period these tests are unable to identify all HIV infections
- Remain the most important and effective means to determine HIV status

Initial/Screening Antibody Tests for HIV

- Initial tests may produce false positive results
- Initial HIV tests must be used appropriately, and if reactive, be followed by a supplemental to rule out false positive results
HIV Antibody Screening Tests

• First generation
  – Utilized viral Ags from lysates immobilized on a solid phase for Ab capture and detected Ab using an antihuman IgG conjugate
• Second generation
  – Utilized recombinant or synthetic peptide Ag to increase sensitivity/specificity
HIV Antibody Screening Tests

- Third generation (HIV1/2)
  - Utilized Ag sandwich method. Immobilized Ag captures HIV Ab and Ab detected via a second labeled Ag. Allows for detection of both HIV specific IgM and IgG

- Fourth generation (HIV1/2)
  - Combine 3’rd gen Ab detection with HIV p24 Ag detection
    - Retains 3’rd gen test high level of Ab sensitivity plus ability to detection p24.
    - 4-8 day reduction in window period relative to 3’rd gen tests allowing for detection of more than 80% of acute HIV infections otherwise detected only by RNA assays

4’th Generation Tests

- Among high risk groups, those who are Ab negative but have acute infection represent 10% of all positives but 4’th generation tests pick up ~80% of these
4’th Generation Tests

- Currently only 2 FDA-Approved 4’th gen assays
  - Abbott ARCHITECT HIV Ag/Ab Combo
    - Only run on Architect chemistry/immunoassay analyzers
  - Bio-Rad GS HIV Combo Ag/Ab EIA
    - Can be performed manually or on the Bio-Rad Evolis automated microplate system
Previous HIV Testing Algorithm

• The previous HIV serological screening algorithm was originally described in 1989
  – Consisted of an initial screening EIA assay followed by a supplemental test for EIA positive specimens

HIV Supplemental Tests

• Western Blot
• Line Immunoassays
• Indirect Immunofluorescence
• Nucleic Acid Tests
Western Blot

- Electrophorese viral lysate to separate proteins according to size
- Transfer proteins to nitrocellulose sheet
- React sera with nitrocellulose strips containing bound HIV proteins
- Detect with Ab and substrate

HIV Antibody Supplemental Test: Western Blot
Western Blot

• Interpretation
  – Any two of p24, gp41, and gp120/160 must be present for a specimen to be reported positive
  – The most stringent criteria for a negative WB requires that there be no distinct bands, either viral or cellular; other criteria only specify no viral bands
  – An indeterminate result is one that fails to meet the criteria for either a positive or negative result

• Disadvantages
  – Doesn’t detect IgM
  – Indeterminate results further prolong time to answer
  – Can take up to 3 weeks after a POS EIA to produce a positive result
  – Specialty test performed in reference labs
  – 60-70% of HIV2 infections produce a WB result indicating HIV1 infection resulting in an incorrect diagnosis
New HIV Testing Algorithm

- The proposed algorithm
  - Initial 3’rd or 4’th generation HIV 1/2 screening EIA
  - HIV 1/2 differentiation assay for positive screening EIA specimens
  - HIV RNA assay for HIV 1/2 differentiation assay negative results
Interpretation of the Multispot HIV-1/-2 Rapid Test Results

Multispot Test Advantages

- Differentiates HIV1 and HIV2 infection
- A little more sensitive than Western blot
- Has a window period up to 7 days shorter than Western blot
- Less expensive than Western blot
- Easier to interpret than Western blot
- Takes ~ 15 minutes
HIV-1 RNA Tests

- Gen-Probe Aptima HIV-1 RNA Qualitative Kit
  - Only HIV NAAT FDA-approved as a diagnostic test
- Viral Load Assays
  - Not approved as diagnostic assays but have been used following validation in individual laboratories

Current vs Proposed HIV Testing Algorithm

Styer et al., J. Clin Virol 2011
Summary of New Algorithm

3'rd/4'th Generation EIA → (if positive) HIV1/2
Differentiation Assay → (if negative) HIV RNA Assay#

*Note: 3-5% of EIA/Western Blot positive specimens are HIV RNA negative. In these rare cases could perform Western Blot or another supplemental test.

Acute HIV Infection

- The prevalence of HIV antibody negative/HIV RNA positive specimens ranges from 0.5/1000 tested to 4.0/1000 tested in the U.S.
- Acute infection accounts for 5-10% of all cases
Acute Infection

• The risk of transmission of HIV is increased during acute infection
  – Higher levels of viremia
  – Presence of more infectious/transmissible variants
  – Studies have shown that individuals infected for < 6 months account for ~50% of transmissions

Acute Infection and the New Algorithm

• Acute infection will be detected as p24 and/or Ab positive; HIV1/2 differentiation assay negative; RNA positive specimens
  – Consider WB or other supplemental test for cases of Ab/AgPos/RNA Neg where acute infection is still a concern
• The rapidity of this method will allow for faster institution of antiviral treatment and investigation of sexual contacts
Summary

• Due to the manner in which they entered the human population and their high mutation rate, HIV viruses are a very heterogeneous group of viruses.
• This heterogeneity has important implications, including posing problems for diagnostic testing.

Summary

• The screening EIA test for anti-HIV Ab followed by confirmation of positive results with a supplemental test remains the gold-standard for HIV diagnosis.
• A new algorithm has been proposed that eliminates the Western blot and will result in a more accurate result with the ability to more quickly treat acute infections.
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