HIV- Lab Diagnosis and Monitoring

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11/14/00. Clinical Pathology/Lab Medicine

HIV testing
- Who?
- Laboratory tests
  - HIV antibody
    - EIA
    - Rapid antibody screening
    - Western Blots
    - Immunofluorescence
  - HIV or viral components
    - PCR or branched DNA
    - HIV culture
    - RT-PCR

Serology: General Principles
- Look for viral antigens or anti-viral antibodies
- A four fold or greater rise in titer between two serum specimens provides a positive diagnosis.
- Paired sera, the first taken as early as possible in the illness and the second later

Who is tested?
- All newborns - NY State newborn screening program
- Voluntary testing for all pregnant women
  - AZT decreases transmission rates from 25% to 8%
- Individuals at risk
- Not to donate Blood to find out HIV status

Serological profile in HIV infection

Consent and Confidentiality
- Informed consent for all except newborns, pre and post-test counseling
- Confidential testing
- Anonymous testing

Serology Methods
- Anti-HIV antibody
  - ELISA/EIA
  - Western Blots
  - Rapid antibody screening
  - Immunofluorescence
- HIV antigen
  - p24 antigen
HIV- Lab Diagnosis and Monitoring

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HIV Antibody Screening Tests-1

• **ELISA/EIA**
  – HIV antigens - from virus or recombinant proteins or synthetic peptides are immobilized on microtitre plates
  – Incubate test serum. Wash
  – Enzyme-labeled antibody specific for hu-IgG. Wash.
  – Substrate changes color

Second generation Rapid HIV tests

• Recently approved by the FDA
• Require little or no equipment
• Serum/plasma/whole blood - finger stick
• Detect HIV -1 and 2
• Results in 2 to 5 min. Needs confirmation
• Sensitivity and specificity same as EIA
• WHO strategy for combining 2 or more rapid tests to confirm a diagnosis

HIV Antibody Screening

• Test performed in duplicate
  – Both positive - proceed to confirmatory tests
  – Both negative- report as negative
  – Discordant results- do a third test

• Sensitivity and specificity exceeds 99%

Four FDA-approved Rapid HIV Tests

- Oraquick Advance
- Unigold Recombigen
- Reveal G2
- Multispot

Rapid HIV Tests

OraQuick Advance HIV-1/2

• CLIA-waived for finger stick, whole blood, oral fluid; moderate complexity with plasma
• Store at room temperature
• Screens for HIV-1 and 2
• Results in 20 minutes
Obtain finger stick specimen…

Insert loop into vial and stir

Collect oral fluid specimens by swabbing gums with test device.
Gloves optional; waste not biohazardous

Insert device; test develops in 20 minutes

Positive
Reactive
Control

Positive
HIV-1/2

Read results in 20 – 40 minutes

Remember the tradeoffs…

• Good News: More HIV-positive people receive their test results.

• Bad News: Some people will receive a false-positive result before confirmatory testing.
**Interpreting Rapid Test Results**

For a laboratory test:

**Sensitivity**: Probability test = positive if patient = positive

**Specificity**: Probability test = negative if patient = negative

**Predictive value**:  
- Probability patient = positive if test = positive  
- Probability patient = negative if test = negative

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**Example: Test 1,000 persons**  
**Test Specificity = 99.6% (4/1000)**

HIV prevalence = 10%

True positive: 100  False positive: 4

Positive predictive value: 100/104 = 96%

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**Example: Test 1,000 persons**  
**Test Specificity = 99.6% (4/1000)**

HIV prevalence = 0.4%

True positive: 4  False positive: 4

Positive predictive value: 4/8 = 50%

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**Positive Predictive Value of a Single Test**

Depends on Specificity & Varies with Prevalence

<table>
<thead>
<tr>
<th>HIV Prevalence</th>
<th>OraQuick</th>
<th>Reveal</th>
<th>Uni-Gold</th>
<th>Single EIA</th>
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<tbody>
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<td>50%</td>
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<td>25%</td>
<td>33%</td>
</tr>
</tbody>
</table>

**Test Specificity**

- OraQuick: 99.9%
- Reveal: 99.8%
- Uni-Gold: 99.1%
- Single EIA: 97%
- OraSure: 95%
- Uni-Gold: 87%
- Single EIA: 77%
- OraSure: 63%
- Uni-Gold: 50%
- Single EIA: 25%
- OraSure: 25%
- Uni-Gold: 10%
- Single EIA: 10%

**Additional Resources**

General and technical information (updated frequently):

[www.cdc.gov/hiv/rapid_testing](http://www.cdc.gov/hiv/rapid_testing)

**After the screen.....**

All require confirmatory testing

Follow-up testing for persons with negative or indeterminate confirmatory test results, with a blood specimen collected 4 weeks after the initial reactive rapid test result.

WHO strategy for combining 2 or more rapid tests to confirm a diagnosis
Confirmatory testing of positive HIV screens

- Western Blots
- Immunofluorescence

Interpretation of Western Blots

- **Positive**, if bands are present at the site of two or more of the following HIV antigens
  - p24 (gag or capsid protein)
  - gp41 (envelope protein)
  - gp120/160 (envelope protein)
- **Negative**, if no viral bands
- **Indeterminate**, if fewer than 2 of the bands
  - HIV-2 infection
  - Early infection

Western blot

Immunofluorescence IFA

- Another FDA approved method for confirming
- Slides with fixed HIV infected cells
- Takes ~90 mins
- Needs fluorescence microscope

HIV DNA PCR Test

- Very sensitive test for detecting specific HIV proviral sequences in PBMCs
- Extract DNA from PBMCs
- Incubate with Taq, dNTPs, specific primers
- 30 - 35 cycles of amplification
- Can detect single provirus from 15,000 PBMCs (100µl newborns, 500µl adults)
- Results in ~48 hrs
Indications for HIV DNA PCR test
- Repeatedly indeterminate Western blots
- Infants born to HIV-positive mothers
- Pregnant women who may have had recent exposure to HIV
- Individuals recently involved in a very high risk exposure (within the last 72 h) who might be considered for post-exposure prevention treatment
- Severe humoral deficiency- end-stage AIDS

Interpretation of HIV PCR test
- Positive result (band of the right size) needs confirmation by second PCR or culture
- Negative results also needs confirmation (CDC - exclusion in newborns, 2 negatives both after 1 mo. and one after 4 mo. of age
- False positives: contamination in lab

Determining HIV infection status
- Under 18 months
  - Infected
    - Meet criteria for AIDS
    - Positive result on 2 separate occasions for either HIV DNA PCR or culture
  - Uninfected
    - Born to HIV positive mothers but serorevert according to tests at 6 and 16 months of age
    - Two negative cultures or PCRs after 1 mo. and at least one test at 4-6 mo.
  - HIV exposed
    - Unknown antibody status
    - Seropositive but under 18 mo. of age

- Over 18 months of age
  - Screening tests
    - If repeated positive - confirm with Western
    - If repeated negative- repeat after window period,
    - If repeated indeterminate- repeat after window period and consider DNA testing

HIV Culture
- PBMCs from patients are co-cultured with mitogen-stimulated normal donor PBMCs
- Culture supernatant is periodically tested for reverse transcriptase
- Specificity and positive predictive value approaching 100% but still needs confirmation by a second culture or PCR
- Positive result in 1-2 weeks, negative in 30 days
- Technically demanding and expensive

Quantitative RT-PCR (Viral load test)
- RT-PCR (Roche)
- Branched DNA (Chiron)
- Nucleic acid sequence-based amplification (Organon Teknika)
- All reliable and reproducible, but use the same test for comparisons
Indications for HIV-1 Plasma RNA measurement

- Use only in HIV-1 antibody positive patients to:
  - Predict prognosis. Combine with CD4 counts to increase predictive value
  - Determine initiation of therapy
  - Measure treatment response
  - Indicate drug failure
  - Assess risk of transmission from mother to fetus
  - Determine prognosis for the infant
- **Not to be used as a screening test**

Resistance testing

- **Genotyping**
  - Sequencing the reverse transcriptase and protease coding regions to look for mutations that signify resistance or cross resistance
- **Phenotyping**
  - Growing pt's virus in the presence of drugs and determining MIC_{50} or MIC_{90}
  - Minority resistant populations not detected
  - None are approved by FDA

Testing Algorithm…