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

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
Consent and Confidentiality

• Informed consent for all except newborns, pre and post-test counseling

• Confidential testing
• Anonymous testing

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
Serological profile in HIV infection

Serology Methods

- Anti-HIV antibody
  - ELISA/EIA
  - Western Blots
  - Rapid antibody screening
  - Immunofluorescence

- HIV antigen
  - p24 antigen

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

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%
Rapid HIV Tests

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
Four FDA-approved Rapid HIV Tests

Oraquick Advance

Unigold Recombigen

Reveal G2

Multispot

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
HIV- Lab Diagnosis and Monitoring

Ila R. Singh, M.D., Ph.D.
11/14/00. Clinical Pathology/Lab Medicine

Positive Negative
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

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%
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\%

HIV prevalence = 0.4%
True positive: 4  False positive: 4

Positive predictive value: \(4/8 = 50\%

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>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>99%</td>
<td>92%</td>
<td>97%</td>
<td>98%</td>
</tr>
<tr>
<td>5%</td>
<td>98%</td>
<td>85%</td>
<td>95%</td>
<td>96%</td>
</tr>
<tr>
<td>2%</td>
<td>95%</td>
<td>69%</td>
<td>87%</td>
<td>91%</td>
</tr>
<tr>
<td>1%</td>
<td>91%</td>
<td>53%</td>
<td>77%</td>
<td>83%</td>
</tr>
<tr>
<td>0.5%</td>
<td>83%</td>
<td>36%</td>
<td>63%</td>
<td>71%</td>
</tr>
<tr>
<td>0.3%</td>
<td>75%</td>
<td>25%</td>
<td>50%</td>
<td>60%</td>
</tr>
<tr>
<td>0.1%</td>
<td>50%</td>
<td>10%</td>
<td>25%</td>
<td>33%</td>
</tr>
</tbody>
</table>

Test Specificity
99.9% 99.1% 99.7% 99.8%
Additional Resources

General and technical information (updated frequently):

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

**Western blot**

- Disrupted HIV particles
- Blotting tank, peptides transferred to nitrocellulose sheet (blot)
- Blot cut into strips
- Each strip incubated with individual patient serum
- Labeled antibody
- Immunostaining of blot
- Develop blot
**Western Blot**

 Env = gp160 = gp120 + gp40
 Gag = p66 = p24 + p17 + p10 + p6

**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
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
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

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 18 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
Determining HIV infection status

- **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

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…