HIV- Lab Diagnosis and Monitoring

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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

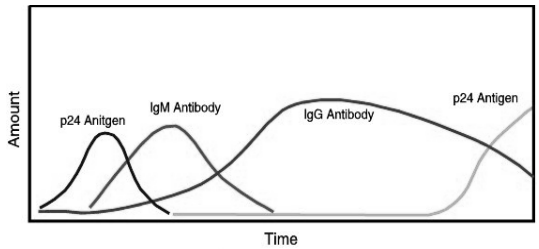
Consent and Confidentiality

- 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



From Van de Peere P, Lepage P, and Simonon A, "Biological Markers Associated With Prolonged Survival in African Children Maternally Infected By the Human Immunodeficiency Virus Type 1," *AIDS Res Hum Retroviruses*, 1992, 8(4):435, with permission.

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

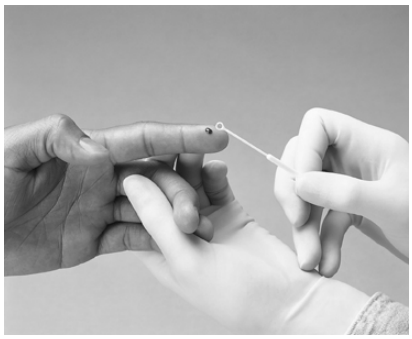
Unigold Recombigen

Reveal G2

Multispot

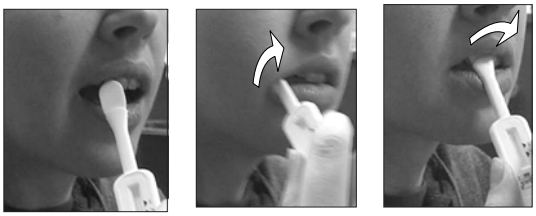
OraQuick Advance HIV-1/2

- 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…

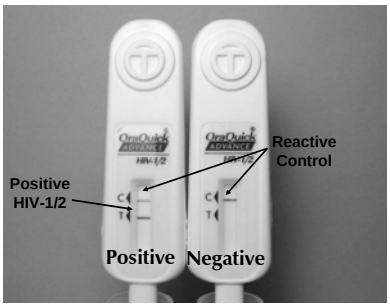
Insert loop into vial and stir



Collect oral fluid specimens by swabbing gums with test device.

Gloves optional; waste not biohazardous

Insert device; test develops in 20 minutes



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%

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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

	Predictive Value, Positive Test			
HIV Prevalence	**OraQuick**	**Reveal**	**Uni-Gold**	**Single EIA**
10%	99%	92%	97%	98%
5%	98%	85%	95%	96%
2%	95%	69%	87%	91%
1%	91%	53%	77%	83%
0.5%	83%	36%	63%	71%
0.3%	75%	25%	50%	60%
0.1%	50%	10%	25%	33%
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

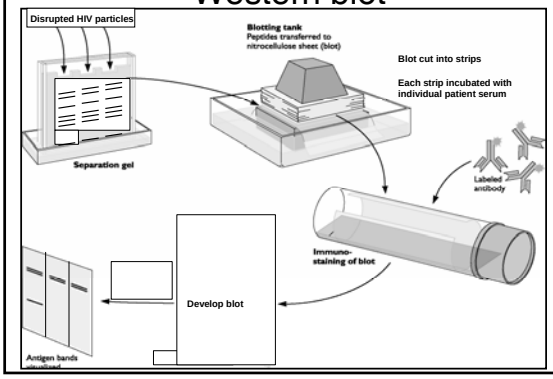
Follow-up testing for persons with negative or indeterminate confirmatory test results, with a blood specimen collected 4 weeks after the initial reactive rapid test result.

WHO strategy for combining 2 or more rapid tests to confirm a diagnosis

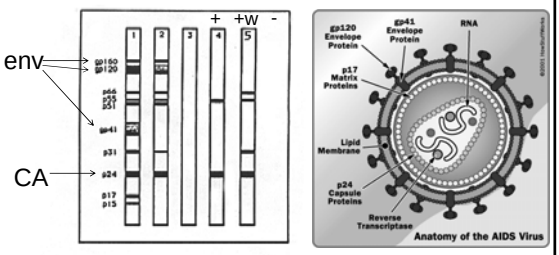
Confirmatory testing of positive HIV screens

- Western Blots
- Immunofluorescence

Western blot



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 seroreverted 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…