HIV INFECTION
LABORATORY DIAGNOSIS

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THE 2010-2014 HIV/STI STRATEGIC PLAN

• The goal of this strategic plan is to reduce the number of new HIV/STI infections and enhance the quality and length of life of persons infected with and affected by HIV/STI

• = Early diagnosis
HIV
HIV TRANSMISSION

• Sexually
  – Heterosexual
  – MSM
  – Sexual toys

• Blood
  – Transfusion
  – Accident

• Mother to child transmission*
  – Pregnancy
  – Intra-Partum
  – Post Partum
LABORATORY DIAGNOSIS

Immuno Assay (IA) TEST
• Antigen detection (P24)
  – Rapid Test
• Antibodies detection
  – Screen
  – Confirmation
• PCR (Molecular diagnosis – Viral Load)
• CD4 count
HIV / AIDS: WHO

HIV infection:
A case of HIV infection is defined as an individual with HIV infection irrespective of clinical stage (including severe or stage 4 clinical disease, also known as AIDS) confirmed by laboratory criteria according to country definitions and requirements.
Laboratory Diagnosis: WHO

- Adults and children 18 months or older HIV infection is diagnosed based on:

  positive HIV antibody testing (rapid or laboratory-based enzyme immunoassay). This is confirmed by a second HIV antibody test (rapid or laboratory-based enzyme immunoassay) relying on different antigens or of different operating characteristics;

  and/or; positive virological test for HIV or its components (HIV-RNA or HIV-DNA or ultrasensitive HIV p24 antigen) confirmed by a second virological test obtained from a separate determination.
Children younger than 18 months:
HIV infection is diagnosed based on positive virological test for HIV or its components (HIV-RNA or HIV-DNA or ultrasensitive HIV p24 antigen) confirmed by a second virological test obtained from a separate determination taken more than four weeks after birth.

Positive HIV antibody testing is not recommended for definitive or confirmatory diagnosis of HIV infection in children until 18 months of age.
Typical evolution of key viral and serological markers during the first weeks following infection with HIV-1 (schematic diagram).

Viral markers: RNA, Ribonucleic acid; DNA, Desoxyribonucleic acid; Ag, Antigen. Immunological markers: IgM/IgG, Immunoglobulin M/G antibodies.

Tests that distinguish recently acquired HIV-1 infection from those that are long-standing take advantage of these events.
Rapid test use

- Clinical studies have demonstrated that the sensitivity\(^2\) and the specificity\(^3\) of rapid HIV tests are comparable to those of EIAs often used for screening. The negative predictive value\(^4\) of a screening test is high at the HIV prevalence observed in most U.S. testing settings (CDC, 1998). Therefore, a client with a negative rapid HIV test result can be told he or she is not infected. However, because HIV antibodies take time to develop, retesting should be recommended to persons with a recent possible exposure (sexual contact or needle sharing within 3 months). As with any screening test, the positive predictive value of a reactive rapid HIV test may be low in populations with low prevalence (see Appendix). Because some reactive test results may be false-positive, every reactive rapid test must be confirmed by a supplemental test (either Western blot or immunofluorescence assay [IFA]). (CDC, 1989).
ELISA TEST

• Semi-Quantitative
  – Index
• Cut-Off
  – Negative
  – Gray Zone
  – Positive
• Automatized machine
• Print report
Architec i 1000

- Daily control
  - Positive
  - Negative
- Calculation by Software
- Optical Density (OD) calculation
- Cut-Off calculation
Cut-Off and Gray Zone

Graph showing the Cut-Off, CO + 10%, and CO - 10% levels.
GS HIV-1 Western Blot

- gp160 (env precursor)
- gp120 (outer env or "surface" glycoprotein)
- p65 (reverse transcriptase)
- p55 (core precursor)/p51 (RT)
- gp41 (transmembrane glycoprotein)
- p40 (core)
- p31 (endonuclease)
- p24 (core shell or "capsid")
- p18 (core matrix)
Positive Predictive Value of HIV Tests in Populations with Differing HIV Prevalence
Example: Testing 1,000 Persons HIV +

<table>
<thead>
<tr>
<th>HIV Prevalence</th>
<th>True positive (number)</th>
<th>False positive (number)</th>
<th>Positive Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>100</td>
<td>2</td>
<td>98%</td>
</tr>
<tr>
<td>5%</td>
<td>50</td>
<td>2</td>
<td>96%</td>
</tr>
<tr>
<td>2%</td>
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<td>2</td>
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<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>0.1%</td>
<td>1</td>
<td>2</td>
<td>33%</td>
</tr>
</tbody>
</table>
Curacao Population

• In 2009 the Curacao population was estimated at **141,766**, consisting of 76,900 women (54%) and 64,866 men (46%)[1]. A significant proportion (30%) of the population consists of young persons aged 10-24 years, at the same time there is an ageing trend in the adult population[2].


Curacao HIV cases

- Two institutions providing confirmatory testing of HIV, the Analytical Diagnostic Centre and the Red Cross Blood Bank. The first case of HIV/AIDS was registered in 1985, and up to 2008 a cumulative number of 1812 HIV positive persons were registered[1]. The largest percentage of these cases (1183/ 65.3%) was diagnosed in Curacao, the most populous of the five islands of the Netherlands Antilles[2]. The cumulative male/female ratio for the Curacao cases was 1.4: 1. Between 1985 and 2007 the mean age at diagnosis for Curacao cases increased from approximately 34 years to approximately 40 years[3].

PPV Screening test in Curacao

- Curacao population: 141766
- HIV positive patient: 1183
- HIV prevalence: 0.8%
- PPV screening test: 80%
- False positive: 20%
HIV Rapid test sensitivity

- FDA has required, as a condition of approval, that the lower estimated test sensitivity and specificity should be 98% or greater.

- HIV Curacao
  - Cases 1183
  - Sensitivity 99%
    - 12 false negative cases (plus cases in “immunological window”)

Fourth-Generation ELISA Simultaneous Detection HIV Antigen / Antibodies

- HIV-1 P-24 Antigen
- HIV-1/2 Antibodies
- Sensitivity 100%
- Specificity 99.5% overall
  99.7% in low risk individuals

Confirmatory test: VIRAL LOAD
Reduction of Diagnostic Window by New Fourth-Generation Human Immunodeficiency Virus Screening Assays†

Bernard Weber,1,2* El Hadji Mbargane Fall,1 Annemarie Berger,2 and Hans Wilhelm Doerr2
Algorithm 1.2 Laboratory diagnosis of HIV infection for adults (protocol of Virus Unit, Department of Health*)

ELISA for antibody to HIV 1 and 2

**Reactive**
- Another ELISA for HIV antibody, different from the first one
- Western blot

**Non-reactive**
- Antibody to HIV 1 and 2 negative

**Indeterminate:** neither positive nor negative

**Positive (US CDC): any 2 of bands p24, gp41 and gp120/160**
- Repeat HIV antibody test in 1 month

**Negative**
- Indeterminate

**Repeat HIV antibody after 6 months if has HIV risk**
- Indeterminate

**HIV infected**

*Modification may be needed by individual laboratory, based on the same principles*
Algorithm 3(A) Laboratory diagnosis of HIV infection for adults
(adapted from protocol of Public Health Laboratory Centre, Centre for Health Protection, Department of Health)

Specimen

Two different HIV antigen and antibody combined ELISA test
(4th generation HIV screening test)

Both assays positive or equivocal

Discrepant result between the two tests

3rd ELISA test

Positive or equivocal

Western Blot

Report as negative

If necessary

- HIV-2 Western Blot
- line immunoblot
- PCR

Final Result
HIV COMBO
in ADC LABORATORY
Antigen/Antibodies Detection
Fourth Generation Test
Typical evolution of key viral and serological markers during the first weeks following infection with HIV-1 (schematic diagram)

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Thank you!