TYPES OF HIV TESTS AND TESTING ALGORITHMS USED IN SURVEILLANCE AND DIAGNOSTICS

Oktavija Đaković Rode
University Hospital for Infectious Diseases
"Dr. Fran Mihaljević", Zagreb

What is HIV Testing?

- HIV Testing is the process of collecting a specimen (blood, oral fluid, urine) to be tested for the presence of HIV antibodies (or antigen).
  - Can be done confidentially (using the persons name as an identifier) or anonymously (using a unique identifier or code).
  - Can be done standard or rapid

CDC, Atlanta

CDC recommends routine, voluntary HIV screening for adults, September 2006

- *Branson BM et al. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings.* ***MMWR Recomm Rep 2006 Sep 22; 55:1-17***
- New recommendations designed to increase early diagnosis of HIV infection as a pathway to improved treatment and prevention.
- The new recommendations address HIV screening in **health care settings** only, and do not apply to non-clinical settings such as community centers or outreach programs.
- "**Screening** is a basic public health tool used to identify unrecognized health conditions so treatment can be offered before symptoms develop and, for communicable diseases, so interventions can be implemented to reduce the likelihood of continued transmission."

CDC recommends routine, voluntary HIV screening for adults, September 2006

- **HIV infection meets all generally accepted criteria that justify screening:**

1. It is a serious health condition that can be diagnosed while still asymptomatic
2. HIV can be diagnosed by reliable, inexpensive, and noninvasive screening
3. Infected patients may gain years of life if treatment is started before symptoms develop
4. Screening costs are reasonable compared with the anticipated benefits

CDC recommendations for routine, voluntary HIV screening for adults, 2006

1. **HIV screening for all patients of risk**: recommended for everyone age 13 to 64 in healthcare settings, regardless of risk, with an opt-out rather than opt-in approach
2. **Voluntary, "opt-out" approach**: HIV testing must be voluntary and undertaken only with the patient's knowledge; HIV testing is part of routine care and patients have the opportunity to decline testing, but before they should be provided basic information about HIV and the meanings of positive and negative results, and should have opportunity to ask questions
3. **Simplified testing procedure** – pre-test counseling and separate, written consent for HIV testing should no longer be required. Consent for HIV testing can be incorporated into general consent for medical care.
4. **Enhanced screening for pregnant women**: The guidelines for HIV testing of pregnant women were revised from 2001 recommendations. Repeat screening in the third trimester is now recommended not only for women at high risk for HIV but also for women in areas with high HIV prevalence. A rapid test should be used during labor for all women whose HIV status remains unknown at the time of delivery.

WHO SHOULD BE OFFERED HIV TESTING?

CDC recommends routine, voluntary HIV screening for adults, 2006

1. HIV testing should be offered as a part of **routine clinical care in all healthcare** settings for patients between 13 and 64 years.
2. **Annual HIV testing for high-risk patients**, including injection drug users and their sexual partners, persons who trade sex for money or drugs, partners of HIV-infected persons, or persons with more than 1 sexual partner since their last HIV test.
3. HIV testing should be considered a **routine part of healthcare screening**. Patients would be informed that HIV testing will be performed unless they decline, and there should be no special consent for HIV testing. Pregnant women are of particular priority for screening, and clinicians should explore the reasoning of pregnant women who decline screening.
4. HIV testing should be performed in **pregnant women** as early as possible in the pregnancy. A second test may be performed, ideally as less than 36 weeks' gestation, among high-risk women.

WHO SHOULD BE OFFERED HIV TESTING?

CDC recommends routine, voluntary HIV screening for adults, 2006

5. If a woman with unknown HIV status presents ***labor***, a **rapid HIV test** should be performed, and appropriate treatment should be administered for positive test result. A confirmatory test result is not necessary prior to initiating treatment in this situation.
6. **Rapid HIV testing** may also be beneficial for population for whom reporting of results may be difficult. All states require that **positive cases of HIV and AIDS be reported to local health officials**, and clinicians should make patients with positive results aware that they may be contacted by these officials.
7. States vary with regard to the consent and confidentiality or screening for HIV in **adolescents**. Parents should be involved in the testing process if possible, but their participation is not required in some states for HIV testing and reporting.
8. Many individuals at high risk for HIV infection are not part of the regular patient population in the US healthcare system. Using **community-based interventions** to reach into social networks and promote screening may improve rates of HIV detection among these populations.

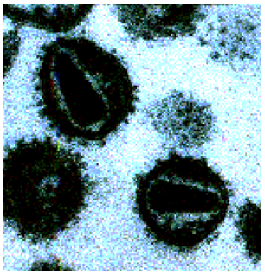
SEROLOGICAL DIAGNOSTIC

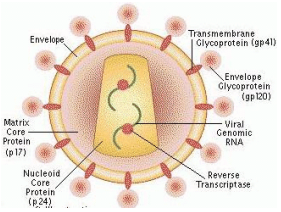
- Detection of virus specific antibody
- Virus antibodies (particularly IgG) usually remain at a detectable level for many years after infection
- Antibody level in the blood is a reflection of the body's past experience or exposure to an antigen

SEROLOGIC TESTING APPLIED TO DETERMINATION OF

- **ACUTE INFECTION**
  - detection of rising titres of antibody between acute and convalescent stages of infection, or detection of IgM in primary infection
- **IMMUNE STATUS** of individuals
- **PREVALENCE** of antibodies in a population

Human Immunodeficiency Virus (HIV)

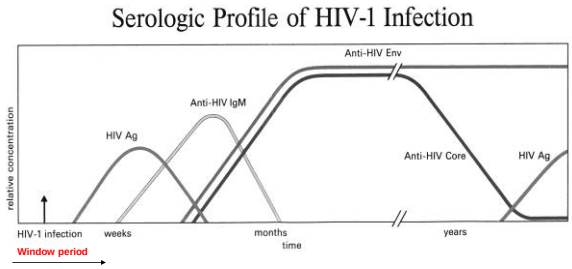




HIV

- HIV antigens and antibodies appear and are detectable at different stages of the seroconversion and infection
- Risk of transmitting viral infection is linked to the window period, which takes place after infection and before the serologic markers detection

Immune response and evolution of antibodies



CHALLENGES OF HIV TESTING

- Sensitivity→ Early diagnostic (window period)
- Specificity→ Cross reactivity
- Detection of HIV-1 and HIV-2 and discrimination between the two viruses
- Easy to perform, low cost

==> One test can not fulfill these requirements
Need to perform a combination of HIV tests for screening and confirmation

SEROLOGICAL METHODS

CLINICAL TECHNIQUES

- Complement fixation tests (CFT)
- Haemaglutination inhibition tests
- Imunfluorescence techniques (IF)
- Neutralization tests
- Counter-immunoelectrophoresis

NEWER TECHNIQUES

- Radioimmunoassay (RIA)
- Enzyme-linked immunosorbent assay (ELISA)
- Particle agglutination
- Western blot (WB)
- Recombinant immunoblot (RIBA), Line immunoassay (LIA)

The clinical laboratory improvement amendments of 1988 (CLIA)

- **CLIA** classifies tests according to their complexity
- **CLIA waiver** = tests must use direct, unprocessed specimens (whole blood, oral fluid) and be easy to perform with a negligible chance of error
- Waived tests can be performed by persons without formal laboratory training outside traditional laboratories.
- In order to purchase CLIA-waived rapid HIV-tests, a facility must **register as a laboratory with the CLIA program** and adhere to the manufacturer's instructions for performing tests.

COMPLEXITY OF HIV TESTS

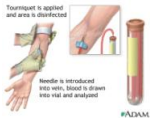
- Level 1 : No additionnal equipment and little or no laboratory experience needed
- Level 2 : Reagent preparation or a multistep process is required; Centrifugation or optimal equipment
- Level 3 : Specific skills such as diluting are required
- Level 4 : Equipment and trained laboratory technician are required

BODY FLUIDS USED FOR HIV TESTING

TECHNOLOGIES

ELISA	Rapid tests	Western Blot
Serum	Serum	Serum
Plasma	Plasma	Plasma
Dried Blood Spots	Whole blood	Dried Blood Spots
Oral Fluids	Oral Fluids	Oral Fluids
Urine		

SPECIMEN FOR SEROLOGICAL DIAGNOSTIC



HUMAN SERUM

- **Blood** should be collected aseptically by venipuncture, allowed to clot, and serum separated from clot after centrifugation
- As a general rule, the volume of blood drawn should equal 2-1/2 times the amount of serum / plasma required
  - For example, to obtain 4 mL serum or plasma, draw at least 10 mL blood

QUALITY SAMPLE

Hyperlipemic, hemolyzed, heat-inactivated samples as well as samples containing particulate matter or exhibiting obvious microbial contamination may cause erroneous results!

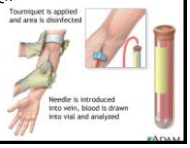
SAMPLE COLLECTION: supplies, collection procedure, storage & shipment

A. SUPPLIES

1. 5 to 7 ml red top or serum separator blood collection tubes
2. Venipuncture supplies
3. 2 mL non-glass serum storage tube

B. COLLECTION PROCEDURES

1. Use of universal precautions is recommended when collecting any biological specimen
2. Properly label a blood collection tube with patient ID and collection date
3. Using acceptable venipuncture technique, collect 5-7 ml whole blood
4. Allow a minimum of 15 minutes to allow clot form
5. Centrifuge sample at ~ 3000 rpm for 15 minutes to separate serum from clot
   - this can also be accomplished by storing the whole blood sample, in an upright position, overnight in the refrigerator (2-6^{0}C)
6. Properly label a 2 mL plastic storage tube with:
   a. Complete patient ID
   b. Serum collection date
7. Transfer 1-2 mL of serum to the storage tube



C. SAMPLE STORAGE

- Serum samples can be stored in the refrigerator at **2-6^{0}C** for up to one week
- Serum samples can be stored frozen at **-20^{0}C** or lower
- For longer storage sera should be **aliquot** because repeatedly frozen and thawed samples may produce erroneous results
- Performance is not affected by sample that have undergone up to 3-4 freeze-thaw cycles

D. SAMPLE PACKING AND SHIPMENT

Transportation of hazardous materials
(materials known or suspected to contain biological agents)

- Diagnostic samples are shipped as "Dangerous goods"
  - They do not need to be shipped as "Infectious agent". Dangerous goods and dry ice shipping regulations must be followed for any diagnostic sample
- Refer to the following web pages for regulated shipping instructions:
  - http://www.cdc.gov/od/ohs/biosfty/shipregs.htm
- Before packing, ensure that the submission form is filled out complete and legible
- Ship serum with a minimum of 5 pounds dry ice or with cold packs
- Use extra dry ice or extra frozen cold packs for Friday, weekend or holiday shipments
- Pack carefully to avoid sample breakage and leaks
- Keep paperwork dry and separate from specimens

PACKAGING OF DIAGNOSTIC SPECIMENS

- *Such material must be packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation*
- This should be interpreted to mean that the contents should not leak to the outside of the shipping container, even if there should be leakage of the primary container(s) during transit, unless the package is severely damaged, e.g. like being run over by a transport vehicle

PLEASE DO NOT!

1. Ship serum in glass
2. Freeze or ship frozen whole blood samples for serum antibody testing
3. Ship samples with incomplete labelling
4. Label tubes with unnecessary (and confusing) information such as investigation's name, study numbers, cage numbers, etc.
5. Allow samples to freeze-thaw before shipping

Optimal storage and shipping can be very expensive and is not practical in many countries!

- **DRIED WHOLE BLOOD SPOTS (DBS)**
- **DRIED SERUM SPOTS (DSS)**
- **DRIED PLASMA SPOTS (DPS)**

DRIED WHOLE BLOOD SPOTS (DBS)
DRIED SERUM SPOTS (DSS)
DRIED PLASMA SPOTS (DPS)

- use in HIV serological and nucleic acid testing
- well-documented & validated
- simple, robust, inexpensive
- surveillance, diagnostic, clinical care management
- two approved EIA tests (Bio-Rad & bioMerieux)

DRIED BLOOD SPOTS
DRIED SERUM/PLASMA SPOTS

- Blood spots dried on filter paper (Guthrie card)
- Filter paper for the collection of whole-blood sample:
  - Scheicher & Schuell (S&S) Grade 903 paper
  - Whatman no. 1 filter paper

DBS STORAGE

- Stored refrigerated (2-8^{0}C), or at room temperature (15-30^{0}C) for 90 days – bioMerieux
- For long-term storage, specimens should be frozen at -20^{0}C or colder at <50% humidity. Although specimens exposed to humidity >= 50% and elevated temperature (37^{0}C) for 14 days did not exhibit a detectable loss in reactivity, it is not recommended routine storage of DBS –bioMerieux
- "Current data indicate that DSS are stable for >3 months at 37^0 C, >5 months at 25^{0}C, 4^{0}C and -20^{0}C. Additional stability studies are ongoing" - *Bharat Parekh, CDC, Atlanta*
- "DBS stable for over 4 years when kept dry" - *Page-Shafer Kimberly, University of California San Francisco, Center for AIDS Prevention Studies*

DBS SHIPMENT

- DBS can be transported by mail or other carrier **with no reasonable expectations of occupational exposure** to blood or other infectious material.
- DBS should be placed in a sealer container, such as a heavy-duty zippered bag with a desiccant.
  - Ideally extra-strong, tear-proof, air-permeable, waterresistant envelopes
- Exposure to a greater than 50% relative humidity may adversely affect stability of the specimen.

DBS - Collection

Good !

Bad !

National HIV & Retrovirology Labs
Public Health Agency of Canada
Ottawa, Ontario

Calypte® HIV-1 BED Incidence EIA
(IgG-Capture HIV-EIA)

Enzyme Immunoassay for
Population Estimates of HIV-1 Incidence

Cat. No. 98003 192 tests
Two Components

Refrigerator Kit (98005) - Store at 2-8°C
and
Freezer Pack (98004) - Store at or below -20°C

For Research Use Only

Do Not Use for Diagnostic Purposes

For HIV-1 Population Incidence Estimation Only

STARHS = Serologic Testing Algorithm for Recent HIV Seroconversions

CALYPTE BIOMEDICAL CORPORATION

Manufactured in the USA by:
Calypte Biomedical Corporation
1500 E. Gude Drive, Suite A
Rockville, Maryland 20850

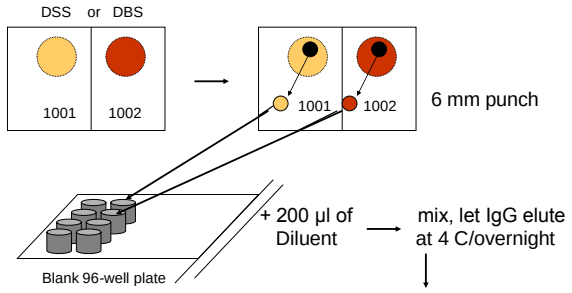
Within the USA only Tel: 1-877-CALYPTE

Use of DBS and DSS: Optimized procedure for BED-CEIA (*Calypte*)

- Preparation : 50 µl of serum or 100 µl blood on SS# 903
- Dry overnight in the hood, store in sealed bag with desiccant
- Using 6mm puncher, make one punch into a 96-well blank plate
- Add 200 µl specimen diluent, mix and incubate overnight at 4-8^{0}C for IgG elution
- Next day, add 50 µl of specimen diluent to the BED assay plate. Transfer 50 µl of eluent to the BED plate, mix and perform the assay as described starting with specimen incubation.
- Use serum spot controls and CAL for calculating OD-n

Bharat Parekh, CDC, Atlanta, GA

Schematic of the procedure



Bharat Parekh, CDC, Atlanta, GA

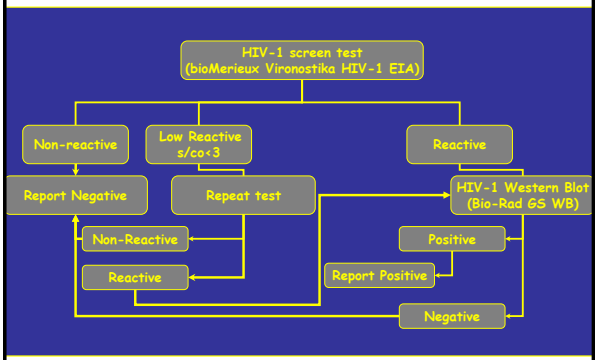
Dried Blood Spots (DBS)
(antibody elution-GSrLAV EIA)

1. **1/4" disk is punched into an uncoated plate.**
2. **200uL of specimen diluent (normal bovine serum / 0.1% Proclin added and mixed well.**
3. **eluted o/n at 4^0 C.**
4. **next day samples are brought to RT and mixed well.**
5. **Mix 40uLsample into 60uL sp.diluent into coated plate.**

   Rest of assay as per norm.

National HIV & Retrovirology Labs
Public Health Agency of Canada
Ottawa, Ontario

HIV testing Algorithm - DBS



National HIV & Retrovirology Labs, Public Health Agency of Canada, Ottawa, Ontario

DBS

- DBS are economical, easy to collect, transport and store.
- Their ease of use and versatility make DBS an ideal tool for large scale surveillance studies, both domestically and abroad!

Laboratory Diagnosis of HIV

- Serology is the usual method for diagnosing HIV infection.
- Serological tests can be divided into **screening** and **confirmatory** assays.
- Screening assays should be as sensitive whereas confirmatory assays should be as specific as possible.

SEROLOGICAL DIAGNOSTIC

- **Screening assays** - **EIA**s are the most frequently used screening assays. The sensitivity and specificity of the presently available commercial systems now approaches 100% but false positive and negative reactions occur
- **Confirmatory assays** - **Western blot (WB)** is regarded as the gold standard for serological diagnosis. However, its sensitivity is lower than screening EIAs. **Line immunoassays (LIA)** incorporate various HIV antigens on nitrocellulose strips. The interpretation of results is similar to WB. It is more sensitive and specific.

CURRENT HIV TECHNOLOGIES

- Detection of antibodies
- Screening tests
  - Enzyme immunosorbent assays (EIAs)
  - Simple/rapid immuno-diagnostics assays
- Confirmatory or supplemental tests
  - Western blot (WB)
  - Line immunoassays (LIAs)
- Alternatives to confirmatory tests
  - Repetitive EIA or rapid assays

HIV Screening tests
EIAs (Enzyme Immuno Assays)

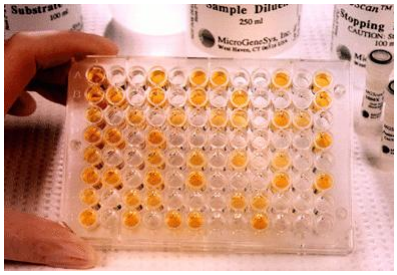
ELISAs (Enzyme Linked Immunosorbent Assays)

- Four immunologic principles
  - Indirect
  - Competition
  - Sandwich
  - Immuno-capture
- Suitable for large numbers of specimens

Evolution of HIV ELISAs

- First generation
  Ag : Purified lysates of HIV
  Poor sensitivity and specificity
- Second generation
  Ag : HIV-recombinant proteins and/ or peptides
  Detection of HIV-1 and HIV-2
  Poor sensitivity, improved specificity
- Third generation
  Ag : HIV-recombinant proteins and/ or peptides
  Detection of IgM and Ig G, improved sensitivity
  Detection of HIV group O
- Fourth generation
  Capacity to detect P24 Ag and antibodies

ELISA for HIV antibody



Microplate ELISA for HIV antibody: coloured wells indicate reactivity

Ag-Ab EIA

- In order to reduce the window period time of HIV infection and laboratory detection, a new generation screening EIA had been developed
- HIV Ag-Ab test allows the simultaneous detection of anti-HIV-1 (M&O groups) and anti-HIV-2 antibodies, and antigens in human serum or plasma
- A significantly higher sensitivity than previous assays
- **Genscreen Ultra HIV Ag-Ab BioRad**
- **The diagnostic window is reduced by about 2.7-2.8 days**

Genscreen Ultra HIV Ag-Ab BioRad

- Solid phase is coated with:
  - Monoclonal Ab against p24 HIV-1 Ag
  - Purified Ag: gp160 recombinant protein, a synthetic peptide mimicking a totally artificial (i.e. encoded by no existing virus) HIV-1 group O-specific epitope and a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein

- **Sensitivity 100%**
- **Specificity**
  - **Blood donors 99.95%**
  - **Clinical samples 99.75%**
  - **Different pathologies (not linked to HIV) 98.72%**
    - pregnant women, RF, autoimmune diseases, chronic renal failure, dialysis, other viral or bacterial diseases (HAV, HBV, HCV, Rubella, Toxoplasmosis, Mumps, Measels, CMV, HSV, EBV, VZV, HTLV1, Malarial, Flu vaccinated patients)

Genscreen Ultra HIV Ag-Ab BioRad LIMITS OF THE TEST

- Very low titre of HIV antigen or antibodies may not be detectable during the first stage of the infection, consequently a negative result indicates that the tested sample does not contain detectable HIV antigen or anti-HIV antibodies. However, such a result does not prelude the possibility of exposure to an HIV-1 / HIV-2 infection.
- The variability of HIV-1 (group M and O) and HIV-2 allows the possibility of false negative reactions. No known test method can offer complete assurance that HIV virus is absent.
- Highly sensitive ELISA may produce false positive results.
- To verify the specificity of the reaction, every positive result should be **confirmed** with an appropriate method (western blot).

Detection of HIV p24 Ag

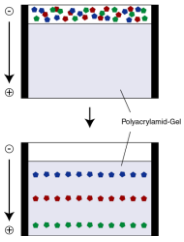
- **Blood bank safety (Primary infections)**
  - Standard ELISAs
  - Ag assays
  - Ag/Ab assays
- **Diagnosis of pediatric HIV-1 infections**
  - Signal-amplification-boosted ELISA
  - Heat-denatured plasma

CONFIRMATORY TESTS

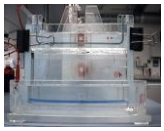
- **Western Blot (WB)**
  Purified antigenes from lysates of HIV on nitrocellulose bands
- **Line immuno assays (LIA)**
  HIV synthetic peptides on bands

WESTERN BLOT

Polyacrylamid Gelelectrophoresis (PAGE)

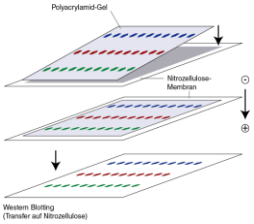


The single antigens of the antigen mix are electrophoretic separated by their molecular weight. Small antigens move fast, big antigens move slow.

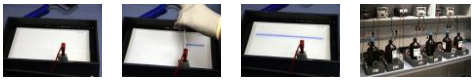


WESTERN BLOT

Western Blotting



The electrophoretically separated antigens are transferred from the polyacrylamide gel to a nitrocellulosis filter membrane



WESTERN BLOT

Principle of the Assay

A test strip loaded with native or recombinant antigens is incubated with diluted samples

Incubation while horizontal shaking

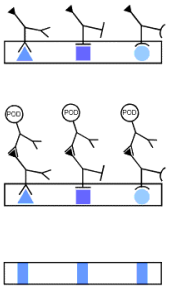
Sucking and washing steps

Chromogen conjugated antibodies (IgG, IgM or IgA) are added

Incubation while horizontal shaking

Sucking and washing steps

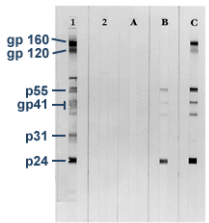
5 – 10 minutes after addition of the substrate solution, insoluble colour bands develop at the sites on the test strips occupied by antibodies



WESTERN BLOT (WB) for HIV antibody

HIV-1 Western Blot

- Lane1: Positive Control
- Lane 2: Negative Control
- Sample A: Negative
- Sample B: Indeterminate
- Sample C: Positive



Line immunoassays (LIA) for HIV antibody

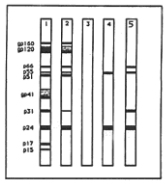


Figure:

Examples of reactions by an HIV-1 Western blot:

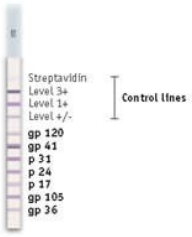
1. Positive control (strong)
2. Positive control (weak)
3. Negative control
4. Indeterminate profile
5. Indeterminate profile (highly suggestive)

- Important antibodies are those against the envelope glycoproteins gp120, gp160, gp41, gp105, gp36
- p24 antibody is usually present but may be absent in the later stages of HIV infection

HIV confirmation
INNO-LIA HIV I/II Score, Innogenetics

- A line immunoassay (LIA) to confirm the presence of antibodies against HIV-1, including group O, and HIV-2 in human serum or plasma
- Differentiate between HIV-1 and HIV-2
- In contrast with WB technique the Ag are fixed as fine lines on the membrane, avoiding difficult-to-control processes such as electrophoresis and blotting, and resulting in a highly reproducible assay

INNO-LIA HIV I/II Score



Positive for HIV-1 Ab:
•One HIV-1 Ag (sgp 120 or 41 gp) positive(≥1+): max reactivity of ± is allowed of one HIV-2 line (sgp 105 or gp 36)
•Both HIV-1 Ag (sgp 120 & gp 41) positive (≥1+): max reactivity of 1+ is allowed on one HIV-2 line (sgp 105 or gp 36)

Positive for HIV-2 Ab:
•One HIV-2 Ag (sgp 105 or gp36) positive(≥1+): max reactivity of ± is allowed on one HIV-1 line (sgp 120 or gp 41)
•Both HIV-2 Ag (sgp 105 & gp 36) positive (≥1+): max reactivity of 1+ is allowed on one HIV-1 line (sgp 120 or gp 41)

Positive for HIV antibodies (untypable):
•Different combination as the ones described above

HIV confirmation
INNO-LIA HIV I/II Score, Innogenetics

- All HIV strains detectable with one strip:
  - HIV-1, HIV-2, HIV-1 group O
- Recombinant proteins and synthetic peptides from HIV-1 and HIV-2, and a synthetic peptide from HIV-1 group O are coated as discrete lines on a nylon strip with plastic backing
- **Specificity**
  - Blood donors **96.7%**
  - Clinical samples **96.1%**
  - Different pathologies (not linked to HIV) **94.4%**
- **Sensitivity 100%**

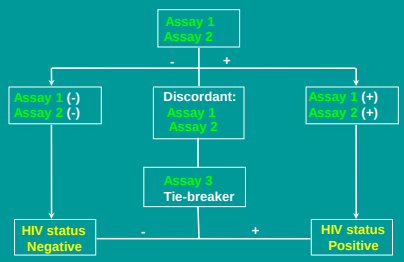
HIV TESTING STRATEGIES

- **DEFINITION**
  Combination of tests
  - First test: Highly sensitive
  - Second test: Highly specific

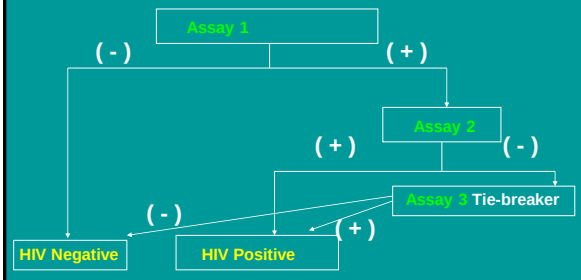
- **Parallel testing algorithm**
  - Samples are tested simultaneously by two different tests

- **Serial testing algorithm**
  - Samples tested by a first test
    - Negative samples are not retested
    - Positive samples are retested by one or
    - several tests upon results

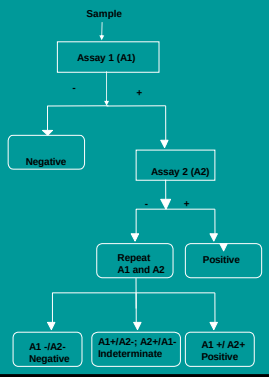
HIV Parallel testing algorithm



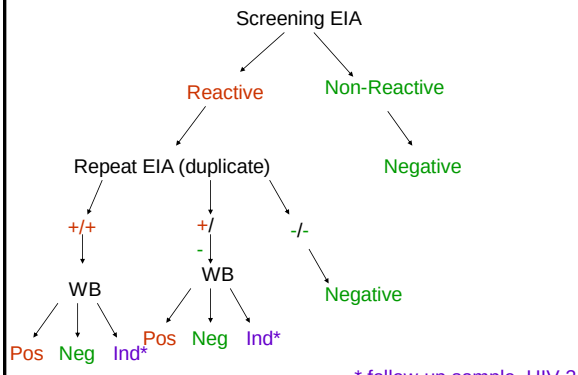
HIV Serial testing algorithm



WHO/UNAIDS HIV testing strategy II



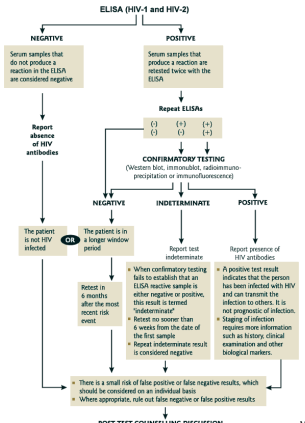
Current Diagnostic Algorithm



CDC

* follow-up sample, HIV-2

Interpretation of HIV testing



www.cic.gc.ca

OTHER DIAGNOSTIC ASSAYS

- It normally takes 4-6 weeks before HIV-antibody appears following exposure
- A diagnosis of HIV infection may be made earlier by the detection of HIV antigen, pro-DNA, and RNA
- However, there are very few circumstances when this is justified, e.g. diagnosis of HIV infection in babies born to HIV-infected mothers

RAPID ANTIBODY TESTS

- Simple to use and require little or no specialized equipment
- Possible to provide test results at the time the test is done

- *Greenwald et al. A rapid review of rapid HIV antibody tests. Curr Inf Dis Rep 2006, 8:125-131*

- Approximately 40-50% of HIV-patients are diagnosed with AIDS within 1 year of first testing HIV-positive
- The CDC estimates that in 2000 31% of patients who tested HIV-positive at public sector testing site did not return to receive their results
- HIV testing opportunities can be expanded to medical and nonmedical settings
- "Advancing HIV Prevention: New Strategies for a Changing Epidemic" (AHP)
- The importance of using rapid tests to facilitate access to early diagnosis in high prevalence areas, for high-risk individuals, and for women during labor and delivery who have not previously been tested and in nontraditional testing settings.

RAPID HIV TESTS

CHARACTERISTICS

- Simple
- Provide same day results

Four immunologic principles

Particle agglutination
Immunodot (dipstick)
Immunofiltration (flow-through device)
Immunochromatography (lateral flow)

SUITABLE FOR LOW VOLUMES AND LIMITED RESSOURCES TESTING SITES

RAPID ANTIBODY TESTS

Four rapid HIV tests have been approved by the FDA:

1. OraQuick / OraQuick Advance Rapid HIV 1/2 Antibody test (OraSure Technologies, Inc., Bethlehem, PA)
2. Reveal / Reveal G2 Rapid HIV-1 Antibody test (MedMira, Halifax, Nova Scotia)
3. Uni-Gold Recombigen HIV Test (Trinity BioTech, Bray, Ireland)
4. Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories, Redmond, WA)

RAPID ANTIBODY TESTS

- HIV Ag are affixed to the test strip or membrane
- If HIV Abs are present in the specimen being tested, they bind to the affixed Ag
- The kit's colorimetric reagent binds to these immunoglobulins creating an indicator that is visually detectable.

- Rapid tests are screening tests that require confirmation if reactive!

Requirements for Performing Rapid HIV Tests

- Any organization that performs a rapid HIV test in order to provide results to patients is considered to be a laboratory under the Clinical Laboratory Improvement Amendments of 1988 (CLIA)
- All laboratories must comply with the regulations of the CLIA Program and with any applicable state requirements http://www.cms.gov/clia/
- QA program

EXTERNAL CONTROL FOR RAPID TESTING

- Requiring the periodic of external control (known HIV-positive and -negative specimens):

1. By each new operator prior to performing the test on patients
2. When a new lot of tests kits is used
3. Upon recipient of a new shipment of test kits
4. When the temperature of the storage or testing area falls outside the recommended range
5. At periodic intervals determined by the testing facility, usually based on their volume of testing

SUBJECT INFORMATION SHEETS

- Provided by each manufacturer
- Includes basic information about
  - HIV/AIDS
  - HIV testing
  - how the test works
  - what the test results mean
  - specifies that reactive rapid test results need to be confirmed

COUNSELING AND TESTING

- All patients with reactive results should be counseled on risk-reduction behavior while awaiting the results of confirmatory testing.
- "Your preliminary test results is positive, but we won't know for sure if you are infected with HIV until we get the results from your confirmatory test. In the meantime, you should take precautions to avoid transmitting the virus!
- NEED CLINICAL FOLLOW-UP OF PERSONS WITH DISCORDANT RESULTS →

→ **THE TRUE GOLD STANDARD**

Rapid tests and ELISAs advantages

RAPID TESTS	ELISAs
Flexible	Batch capability good for ≥ 100 specimens at same time
Time to result(< 30 min)	Can be automated
Skilled staff not required	Centralized (QA/QC)
Very easy to interpret results	May be highly sensitive for seroconverters
On site testing	Cost per test less than cost per rapid test
Minimal equipment and reagents required	
Tests can be stored at room temperature	
Complexity 1-3	

Rapid tests and ELISAs disadvantages

RAPID TESTS	ELISAs
Small numbers for each test run	Less flexible(need a minimum numbers for maximum
QA/QC at multiple sites	Time to result(>1.5h)
Some tests less sensitive (for seroconverters)	Complexity 4
May cost more per individual test than EIA	Skilled technician required
Interreader variability may provide inconsistent results (e.g., particle agglutination)	Refrigerated reagents
	Requires sophisticated equipments
	High equipment maintenance

CHANGES FOR THE LABORATORY

- New testing environment:
  - fewer screening performed while Western blot confirmation continues
- Greater role for handling specimens for incidence & resistance
- Gain familiarity with rapid tests
- Participation in rapid tests training as an expanded laboratory activity