

**KITSO AIDS Training Program**

Lecture 3:

**Laboratory Diagnostics in HIV/AIDS Care**

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**Laboratory Tests in HIV/AIDS Care:**

- HIV diagnosis:
  - Adults and children > 18 months: ELISA and rapid tests
  - Children and infants < 18 months: DNA PCR test
- Monitoring viral response to HAART: Viral load
- Monitoring immune system for HAART eligibility and immune response to HAART: CD4 cell count
- Monitoring drug toxicity: chemistry, hematology
- ARV resistance assay (Lecture 8)

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**Detection of HIV Infection**

- Antibody methods: measurement of indirect markers (antibodies) produced by the body's immune system in response to HIV infection
  - **ELISA**: Enzyme Linked Immunosorbent Assay (the laboratory technique used)
  - Rapid tests
  - Western Blot
- DNA PCR tests (PCR=polymerase chain reaction, i.e., the laboratory technique used): direct measure of cellular proviral DNA
- RNA PCR (viral load): too expensive for mass screening
- p24 antigen (used to screen blood donations): the inner viral protein coat of the virus
- HIV culture (only used for research)

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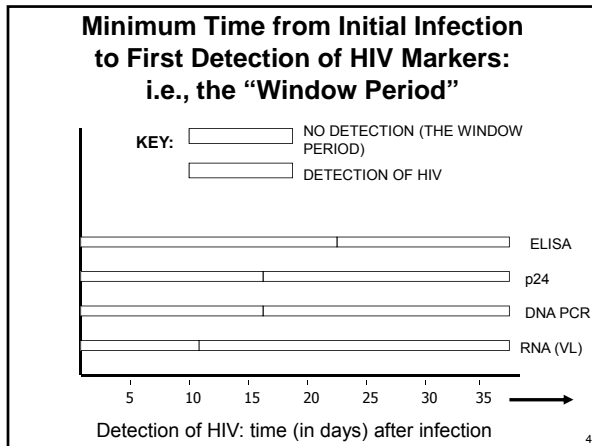
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**The “Window Period”**

- Period of time between initial infection and the first reliable detection of HIV by a lab test. Although testing HIV negative, the person is highly infectious during the window period.
- Window periods vary by test (see prior slide) and by each individual’s immune system genetics—from 2-3 weeks or rarely 6 months or more.
- The majority of infected individuals are positive by ELISA, antigen and/or DNA/RNA tests by 6-8 weeks after infection, with many having sero-converted by 3-4 weeks after infection.
- A patient who has a negative rapid test, and who has had recent high-risk exposure (unsafe sex, STI or STI exposure) should return for repeat testing in 3 months. Safe sex must be reviewed in detail with the patient.

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**HIV Diagnosis in Adults**

- In adults, diagnosis of HIV infection is best determined by the detection of antibodies (markers), which the body produces in response to the virus.
- Antibodies are specialized proteins produced by plasma cells (derived from B-lymphocytes) to fight various pathogens by blocking or neutralizing them. Antibodies formed against HIV are specific for particular proteins unique to HIV. There are many types of anti-HIV antibodies made in response to HIV infection.

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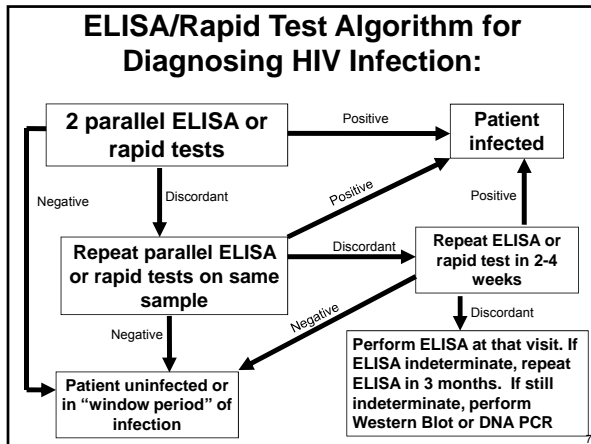
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**HIV Testing:  
Discordant Rapid Test Results**

- Discordant parallel rapid results (one is positive and the other is negative) must be retested *at that visit*.
  - If still discordant in the repeat testing step, the result is considered indeterminate, and repeat rapid testing in 2-4 weeks is necessary.
  - If repeat rapid test 2-4 weeks later is still discordant, perform ELISA test at that visit.
  - If the ELISA remains indeterminate, repeat ELISA should be done after 3 months. If at 3 months the result is still indeterminate, DNA PCR testing or Western Blot is required.

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**Discordant HIV Test Results  
 in Pregnant Women**

- Prompt and accurate HIV diagnosis in pregnant women is essential in order to begin indicated referral and treatment for PMTCT, as well as for the mother's health.
- Discordant rapid test results in a pregnant women require *priority ELISA testing at that visit*, with results within two days of testing.
- If the ELISA test is discordant, then repeat ELISA with Western Blot and viral load must be done immediately, with results within two days of testing. If these tests are equivocal or discordant, an HIV Specialist must be consulted at once.

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### Western Blot

- Most commonly used confirmatory test.
- Detects antibodies directed at specific HIV envelope and core proteins.
- Obtain when ELISA/rapid testing is repeatedly indeterminate.

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### Sero-Diagnosis of HIV Infection

- HIV diagnostic tests are *the most accurate, specific, and sensitive tests used in medicine.*
- The ELISA remains the “gold standard” of HIV diagnosis, and is used for mass screening and for blood specimens delivered to the laboratory.
- *In Botswana, rapid tests are diagnostic for HIV infection* when the above algorithm is followed. Indications for rapid testing: remote areas, PMTCT, PEP, VCT, need for quick diagnosis, and when patient is believed at risk for not returning for result

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### Rapid Tests

- Quick, cheap, easy to use.
- Specimens: blood, urine, saliva
- Easy to store. However, *ensure that the reagents are stored properly, and that they have not expired.*
- A laboratory is not required, but interpretation requires trained personnel.
- Combinations of rapid tests are highly sensitive and specific.

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**Sample Required for ELISA**

- Five (5) milliliters of whole blood in plain or purple-top EDTA tubes.
- Samples should be stored in the refrigerator at 4° C and NOT frozen.
- Samples should be sent to the lab within 24 hours.

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**Labeling of Lab Test Tubes  
(All Assays)**

- Tubes should be labeled with:
  - PATIENT IDENTIFICATION NUMBER (ID/Omang)
  - Date of collection

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**Filling out Lab Test Forms  
(All Assays)**

- Fill in patient information:
  - ID / Omang / other specified ID (e.g., birth certificate, passport)
  - Patient initials (as a cross-check for errors)
  - Patient gender (M or F)
  - Patient date of birth (day / month / year)
- Fill in sample information:
  - Date specimen drawn (day/month/year)
  - Time specimen drawn (24 hour clock)
- Site information:
  - Full name and signature or stamp of clinician

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**Diagnosis in Infants**

- Diagnosis of HIV infection by antibody serology in infants under 18 months of age is complicated by the presence of maternal antibodies passed to the infant both in-utero and via breast milk.
  - Most of these antibodies, which disappear by 18 months of life, are protective early in life against respiratory and diarrheal pathogens. HIV antibodies are *markers of maternal* infection only, and do not necessarily indicate HIV infection of the infant, who has a chance of remaining uninfected with HIV up to 60% of the time, even without PMTCT intervention.

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**DNA PCR Assay**

- Because of the presence of maternal HIV antibodies, diagnosis of HIV infection in infants is made by DNA PCR, i.e., detection of cellular proviral DNA.
- PCR, which is based on cellular proviral HIV DNA, provides a qualitative test result: positive or negative. DNA PCR sensitivity increases with age in early infancy: 25-45% in the first week of life, > 90% by 2 weeks, and nearly 100% by 2 months of age.
- DNA PCR in Botswana can be done on either whole blood specimens or dried blood spots (DBS). 17

17

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**Dried Blood Spot (DBS) DNA PCR**

- Blood for DBS can be obtained by a simple skin prick on the infant's heel.
  - Phlebotomy is not necessary
  - Procedure requires only a few drops of blood
- Easy to store and transport
- More rapid turn-around of results is expected.
- DBS is diagnostic for HIV infection.

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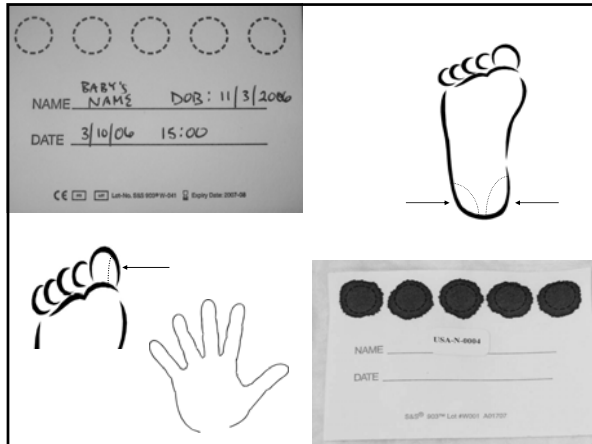
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### Pediatric HIV Testing

- HIV-exposed babies should have DNA PCR testing, ideally at 6 weeks of age, with immediate follow-up DNA PCR to confirm a positive result. **Infants whose first DNA PCR is positive should immediately be referred for HAART initiation**, without waiting for the confirmatory DNA PCR.
  - Infants seen between 4 and 6 weeks of age may have DNA PCR testing at that time, in order not to miss testing them at 6 weeks.

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### Pediatric HIV Testing (2)

- Breastfed babies who test HIV negative at 6 weeks of age should have HIV testing 6 weeks after cessation of breastfeeding, the type of HIV test depending upon the age of the child at testing.
- Babies not breastfed, and whose 6 week DNA PCR is negative, should have an ELISA or rapid test at age 18 months, to allow for undisclosed breastfeeding or rare false negative DNA PCR results.

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## Monitoring HIV Disease Progression

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- ### HIV Disease Progression
- HIV progression, as well as response to therapy, can be monitored by:
    - Clinical markers:
      - HIV/AIDS-related conditions
      - HIV/AIDS-related mortality
    - Laboratory markers:
      - Viral load
      - CD4 cell count/%

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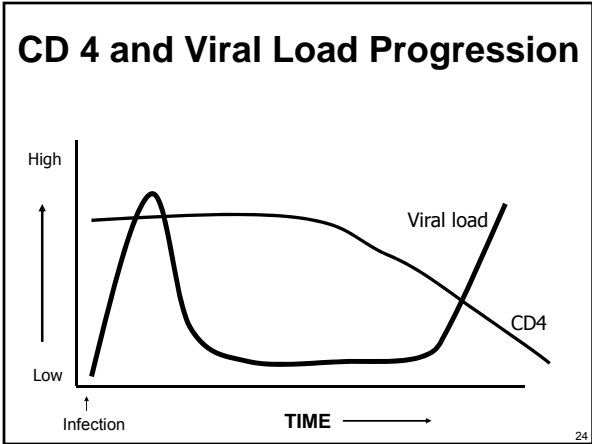
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**Viral Load**

- The number of virus particles per ml of blood, obtained by quantifying HIV RNA, which is *an indicator of viral replication*.
- With the standard assay used in Botswana, 400 to 750,000 HIV copies per ml of blood can be detected.
- **Baseline viral load must NO LONGER BE DONE prior to HAART initiation!**
  - This change will facilitate prompt review of returning viral load results, which will always be on patients on HAART.

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**Viral Load (2)**

- Monitor viral load at 3 and 6 months post-HAART initiation for all age ranges.
- If the 6 month post-initiation viral load is < 400 copies/mL, monitor viral load **every 6 months** thereafter **for adults**.
- All patients < 20 years old who are on HAART must have viral loads **every 3 months**, because of the high treatment failure rates in pediatric and adolescent patients.

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**Sample Collection for Viral Load**

- Collect blood in 5-7 ml EDTA anticoagulant tube (purple top) for adults, 3ml tube for infants.
- Tube should be mixed well by inverting slowly 5-10 times IMMEDIATELY after collection to prevent clotting.
- Store in the 4° C refrigerator while awaiting transportation to lab.
- Sample should be transported to lab within 4-6 hours of collection in cool box with ice pack at 4° C.

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### Sample Collection for Viral Load (2)

- If sample cannot reach testing lab within 6 hours, viral load sample can be collected in a PPT tube.
- Local lab should centrifuge PPT tube (whitish top) samples to separate plasma, and then ship to testing lab within 24 hours.

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### Viral Load Results

- Turn-around time should be 2 weeks.
- “Undetectable” result indicates viral load below 400 copies/ml.
- Recent study in Botswana:
  - median viral load in asymptomatic patients: 36,000 copies/ml.
  - median viral load in AIDS patients: 296,000 copies/ml.
- Potential 0.2-0.3 log (1.5-fold) inherent variability in viral load assay, due to biologic variation, which is not clinically significant.
- A significant change in viral load is  $\geq 0.5$  log ( $\geq 3$ -fold change).
- Any acute infection, e.g., TB, pneumonia, viral infections, may transiently increase viral load.

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### CD4 Cell Count CD4 %

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**CD4 Cell Count/%**

- CD4 cell count measures the number of CD4 cells per microliter ( $\mu\text{L}$ , cubic millimeter) of blood. The CD4% measures the % of total lymphocytes that are CD4 cells.
- The CD4 count/% is a measure of the degree of immune deficiency and the stage of HIV disease progression.
- The CD4 count/% is an important test for HAART eligibility, and for monitoring the recovery of the immune system under treatment.
  - Obtain CD4 count/% at baseline, and then at 3 and 6 months post-HAART initiation, and then **every 6 months** thereafter for all age groups.

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**Sample Collection for CD4 Count/%**

- Three (3) ml of whole blood in EDTA anticoagulant (purple top tube).
- Tube should be mixed well by inverting slowly 5-10 times IMMEDIATELY after collection to prevent clotting.
- Sample should be transported to the lab within 24 hours.
- Store and transport sample at room temperature: 20-30 °C (cool box without ice pack).

32

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**CD4 Cell Count/%**

- Turn-around time should be 72 hours.
- Measure CD4 cell count/%:
  - As an eligibility screen for therapy
  - At baseline, 3 and 6 months post-HAART initiation, and then **every 6 months** thereafter for all age groups.
  - Monitoring adult patients not yet on HAART:
    - If CD4 count > 400 cells/ $\mu\text{L}$ : every 6 months
    - If CD4 between 250 and 400 cells/ $\mu\text{L}$ : every 3 months
    - Monitoring frequency may be increased if indicated by clinical condition, e.g., WHO stage 2 condition.
  - Pediatric patients not yet on HAART require every 3 month visits for clinical evaluation and CD4%.

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### Interpretation of CD4 Counts

- Use absolute/total CD4 cell count in adults.
- Use CD4% and absolute CD4 count in infants and children.
- Ignore CD8 count and CD4:CD8 ratio.
- There can be a 25-30 % variability in CD4 cell count, which may be due to natural biologic variation, and is not of clinical significance. When uncertain whether or not a variation in CD4 cell count is significant, refer to the CD4%, which normally does not change more than 2 percentage points with natural biologic variation.
- Acute infections, e.g., TB, viral URI, pneumonia, can lower the CD4 count, as CD4 cells are consumed fighting the acute infection.

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### Priority Viral Loads and CD4 Counts

- All viral loads on patients under 20 years of age
- Repeat viral load to confirm virologic failure when viral load is detectable after previously having been undetectable on HAART
- Viral load performed *prior* to switching ARV(s) for toxicity, side effects, or avoidance of d4T and/or d4T/ddI, *if there is no fully suppressed viral load done within the prior 6 months*
- Follow-up 6 week viral load to confirm continued virologic suppression after switching from a prior adult or pediatric regimen containing d4T and/or ddI to new regimens under the 2008 guidelines

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### Priority Viral Loads and CD4 Counts (2)

- Follow-up viral load after restarting/continuing HAART after completed interventions for treatment failure due to severe non-adherence, drug interactions, inappropriate ARV dose, and severe gastroenteritis
- Follow-up 6 week viral load after ARV switch for side effects or toxicity, when there had previously been full virologic suppression prior to the switch

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### Priority Viral Loads and CD4 Counts (3)

- CD4 count must be given priority status for any pregnant woman who is not yet on HAART, in order to determine her eligibility for HAART.
- Clinicians should label laboratory forms as “priority,” and note the reason for this designation.
- When there are delays in returning viral loads and/or CD4 counts, clinicians should contact the laboratory directly for information about the length of delay, and not reorder lab tests unnecessarily (and thereby only lengthen the delays).

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### Monitoring for ARV Toxicity

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### Chemistry and Hematology

- Before start of HAART: full blood count, full chemistry (including AST/ALT, glucose, urea, creatinine), RPR, total cholesterol and triglycerides
- Monitoring labs post-HAART initiation:
  - FBC:
    - If on AZT-based HAART: at 1, 3, and 12 months, ***then annually only***, and as clinically indicated
    - If not on AZT-based HAART: ***annually only***, and as clinically indicated
  - AST/ALT:
    - If on NVP-based HAART: at 2, 4, and 12 weeks, thereafter ***only as clinically indicated***
    - If on EFV-based HAART: at 4 and 12 weeks, thereafter ***only as clinically indicated***
    - If on PI-based HAART: only as clinically indicated
  - Glucose and total cholesterol/triglycerides annually ***only if on PI-based HAART***

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### Creatinine Clearance for TDF-Based HAART

- Renal function must be monitored for all tenofovir- (TDF) containing HAART.
- Blood urea and creatinine are not accurate measures of renal function, and can still be in normal range in spite of significant renal impairment.
- Creatinine clearance must be calculated at baseline, at 3 months post-initiation, and thereafter every 6 months for all patients on TDF. The following equations should be used to calculate creatinine clearance using serum creatinine ( $\mu$ moles/liter), weight (kgs), and age in years:
  - Males:  $1.22 \times [(140 - \text{age in yrs}) \times \text{wt (kg)}] / [\text{serum creat.}]$
  - Females:  $1.037 \times [(140 - \text{age in yrs}) \times \text{wt (kg)}] / [\text{serum creat.}]$

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### Samples Required for Chemistry and Hematology Tests

Tests	Type of tube	Volume required	Comments
FBC	EDTA (purple top)	3 ml	Mix gently by inverting 5-10 times
AST/ALT	Plain/no additive (red top)	3-5 ml	Do not mix: allow time to clot before testing
Creatinine			
Amylase			
Proteins, CO <sub>2</sub>			
CPK, TBil			
Lipase			
Cholesterol			
Triglycerides	Plain (red)	3 ml	Fasting sample/clotted
HDL/LDL			
Lactate	Heparin tube on ice	3 ml	Mix gently by inverting 5-10 times
Glucose	Sodium fluoride (Grey Top)	2 ml	Mix gently by inverting 5-10 times

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### Other Tests

- Syphilis serology (5 ml plain red-top tube).
  - Perform at baseline and thereafter as clinically indicated. Positive results require treatment and indicated follow-up.
- Hepatitis B antigen (5 ml plain red-top tube).
  - Not a baseline test. Obtain only when HBV "flare" is suspected after stopping TDF/FTC (or 3TC).
- The sedimentation rate (ESR) is nonspecific and of no clinical value. It should not be done.

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**Summary:**  
**Lab Testing in 2008 Guidelines**

- **Baseline viral load must not be done.**
- Viral load should be done at 3 and 6 months after HAART initiation. Once viral load is < 400 copies/mL at 6 months after HAART initiation, it should be measured every 6 months for adults and every 3 months for pediatric and adolescent patients (under 20 years of age).
- CD4 count/%: monitor only every 6 months for all ages, once 3 and 6 month post-initiation monitoring has passed.

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**Lab Testing in 2008 Guidelines (2)**

- Frequency of FBC and ALT/AST monitoring has been decreased, and is determined by the specific HAART regimen (see prior slides). **CLINICIANS MUST NOT ROUTINELY ORDER EVERY THREE MONTH FBC, AST/ALT, CD4%, AND VIRAL LOAD.**
- Failure to follow the new laboratory testing schedule jeopardizes the financial viability of the nation's HIV/AIDS programs.

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