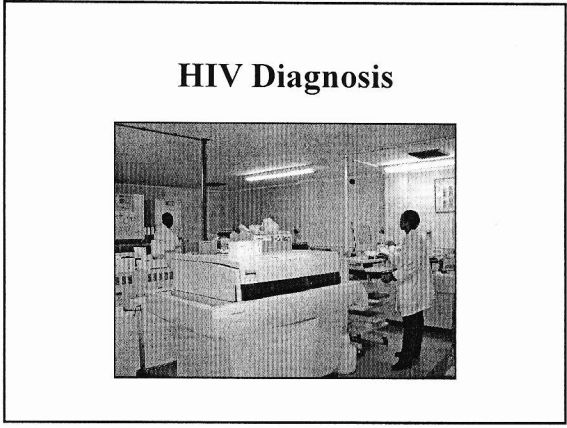
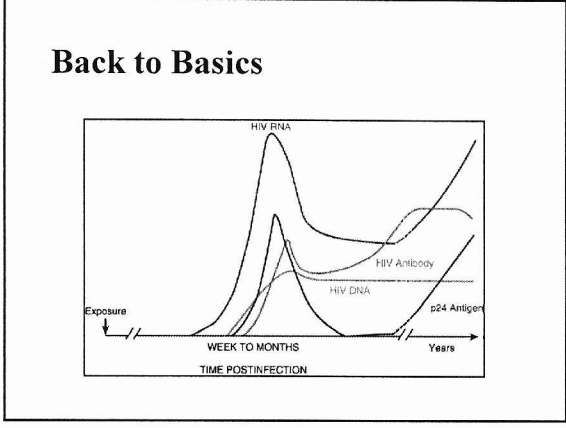
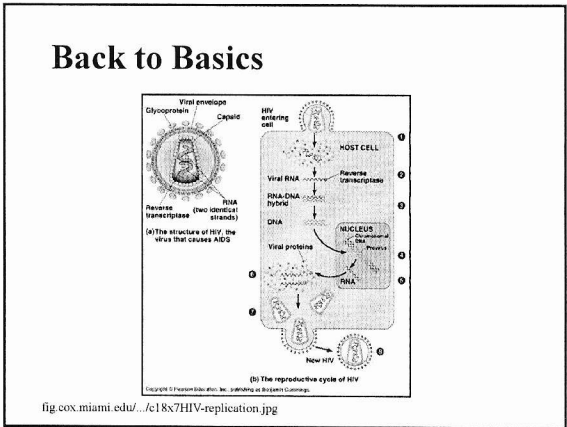
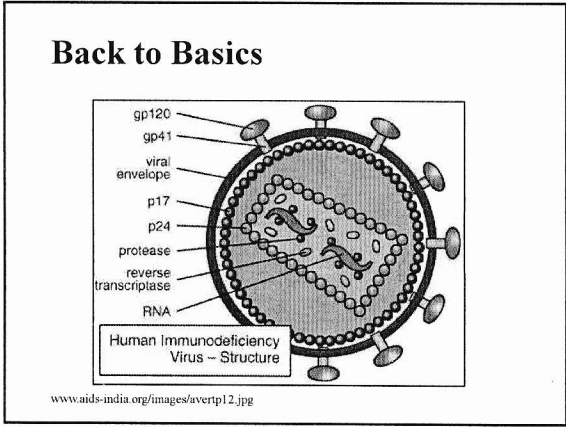


Ins and Outs of Laboratory Testing in HIV

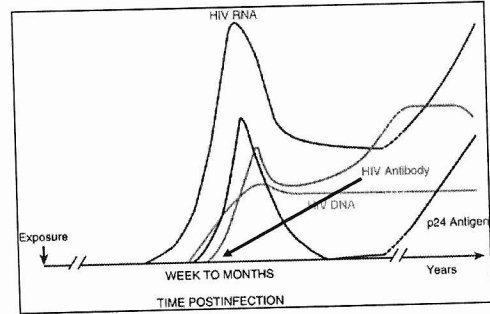
- ### Overview
- HIV diagnosis
 - Adults
 - Infants
 - HIV viral load
 - CD4+ count
 - Genotyping and Phenotyping



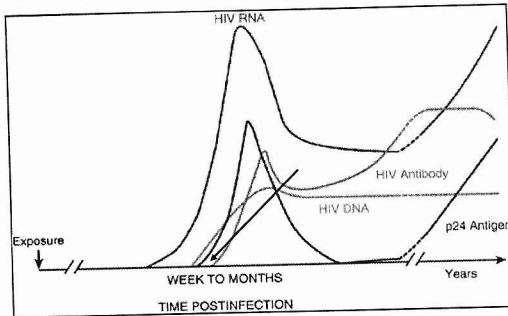
HIV Diagnosis

- Based on detection of HIV-specific antibodies
 - Found in virtually 100% of HIV infected patients
- If positive
 - 2 tests per specimen
 - 2 specimens

HIV Diagnosis



4th Generation Combination Assay



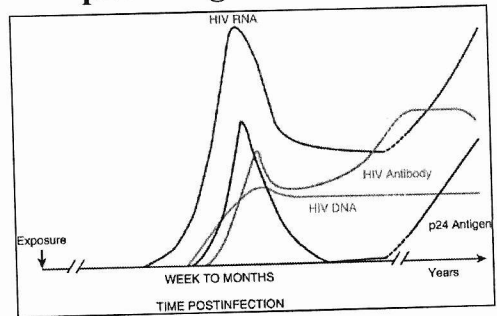
4th Generation Combination Assay

- Advantages
 - Decreases window-period to 18 days
 - Detects infection during late stages of disease when antibodies may be low
 - Almost 100% sensitive, ± 99.6% specific

4th Generation Combination Assay

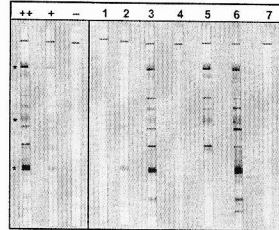
- Disadvantages
 - Window-period
 - Occasional low positives occur (< 1%)
 - Early seroconversion (16%)
 - False positive (70%)
 - Can usually be solved with repeat testing after 7 days
- If repeat low positive results, may proceed to molecular testing (HIV DNA PCR)

p24 Antigen Detection

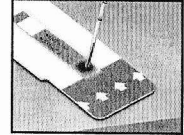


Western Blot

- May be used for confirmation
- Differentiates b/t HIV-1 and HIV-2
- Relatively expensive and labour intensive
- May give indeterminate results



Rapid HIV Test



- “Bed-side testing” method
 - Useful if result is needed quickly
 - Helps to minimise “unclaimed” test results
- Detects HIV-specific antibodies
- Sensitivity > 99%
- Specificity 98-99%
- Result must be confirmed

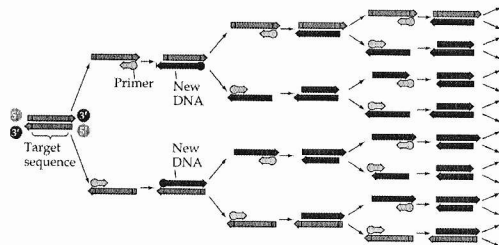
HIV Diagnosis - <18 months

- Maternal HIV-specific antibodies cross the placenta
- May be detectable up to 18 months of age
- Detection of HIV-specific antibodies in a child <18 months old ≠ HIV infection
- Must detect the virus itself

HIV Diagnosis - <18 months

- p24 antigen detection
 - If positive indicates infection
 - If negative, does not exclude infection
- HIV DNA PCR

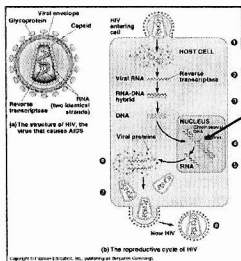
HIV DNA PCR



HIV DNA PCR



HIV DNA PCR



- Detects HIV provirus in leukocytes

HIV DNA PCR

- Confirm positive results with a second test
- If positive in first week of life = infected in utero
- Testing at 6 weeks should pick up most of those infected at birth
 - If negative, repeat after 3 months of age
- If ongoing exposure, repeat testing 6 weeks after last exposure

HIV DNA PCR

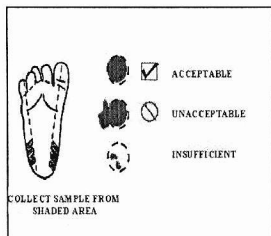
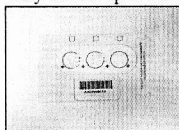
- Sensitivity 95%
- Specificity 98%
- Advantages
 - If positive, indicates infection
 - Best option available for diagnosis of HIV infection in infants

HIV DNA PCR

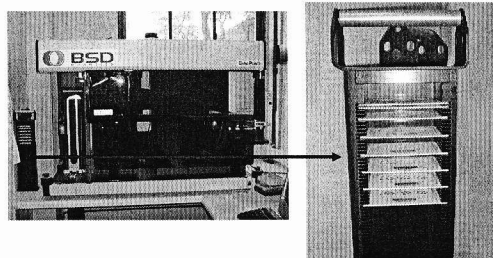
- Disadvantages
 - Only detects HIV-1
 - Primer mismatch, esp. with non-B subtypes
 - Low copy number
 - False negatives
 - False positives due to contamination
 - Relatively expensive

HIV DNA PCR

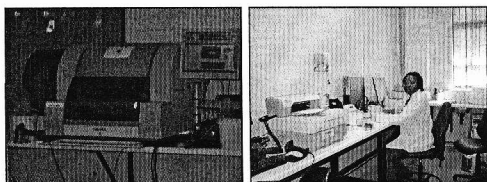
- Specimens
 - EDTA blood (minimum of 200µl)
 - Dry blood spot card



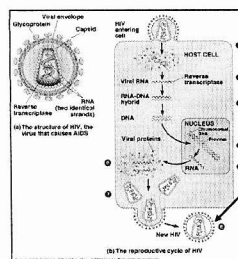
HIV DNA PCR



HIV Viral Load



HIV Viral Load



- Detects and quantifies HIV-1 RNA levels in plasma

HIV Viral Load

- Used as
 - Prognostic indicator
 - A guide when to start therapy
 - An indicator of response to therapy
 - An indicator of the development of resistance

HIV Viral Load

- Number of different methods used
 - Abbott RealTime (PCR based)
 - Reference range 40 – 10 000 000 copies/ml
 - Roche Amplicor (PCR based)
 - Reference range 40 – 750 000 copies/ml
 - Quantiplex (bdNA)
 - Reference range 50 – 500 000 copies/ml
 - Nuclisens (NASBA based)
 - Reference range 25 – 3 000 000 copies/ml

HIV Viral Load

- Ideal to use the same machine and same laboratory for follow-up of a patient
- Result given as
 - number of RNA copies/ml
 - log value
- Log value provides a smaller number that is easier to work with
 - e.g. 100 = 2 log
 - 1000 = 3 log
 - 10000 = 4 log

HIV Viral Load

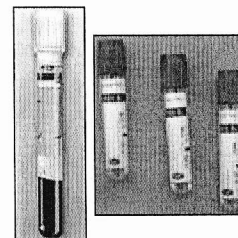
- **LDL** – Lower than the Detectable Limit of the assay used
- Possible reasons for an LDL result:
 - Patient is on therapy
 - Patient is not HIV positive
 - Patient is infected with HIV-2 or a strain of HIV-1 which is not detected by the assay

HIV Viral Load

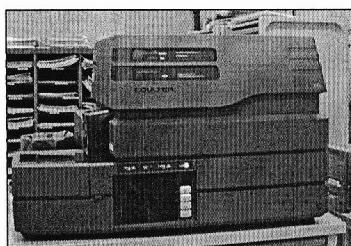
- Limitations
 - Risk of contamination → low false positive results
 - Under-detection of certain strains
- Should not be used as a diagnostic test

HIV Viral Load

- Requirements
 - EDTA specimen
 - PPT specimen
 - Test can be run on as little as 200µl of plasma, but results are optimal with 1ml of plasma



CD4+ Count



- Performed on a flow cytometer

CD4+ Count

- Uses
 - Measure of disease progression and severity (staging)
 - Assists with determining risk for certain opportunistic infections and neoplasms
 - Guides treatment decisions
 - Indication of response to treatment

CD4+ count	Infectious	Other
>500	Recurrent URTI Bacterial, viral & fungal skin infections Vaginal candidiasis Aseptic meningitis	Persistent generalized lymphadenopathy Minor mucocutaneous manifestations Guillain-Barré syndrome & Bell's palsy Parotidomegaly
200-500	Pulmonary tuberculosis Pneumonia (bacterial) Herpes zoster Oral & oesophageal candidiasis Oral hairy leukoplakia Bacillary angiomatosis	CIN & cervical cancer Idiopathic thrombocytopenic purpura Hodgkin's lymphoma Lymphocytic interstitial pneumonitis Kaposi's sarcoma
50-200	Extrapulmonary tuberculosis <i>Pneumocystis jirovecii</i> pneumonia Cryptococcal meningitis Toxoplasmosis Cryptosporidiosis (chronic) Microsporidiosis Histoplasmosis Chronic herpes simplex ulcers Septicemia (non-typhoidal salmonella) Progressive multifocal leucoencephalopathy	Wasting Anaemia Peripheral neuropathy HIV-associated dementia Non-Hodgkins lymphoma Cardiomyopathy Vacuolar myelopathy
<50	Cytomegalovirus (disseminated) <i>Mycobacterium avium</i> complex	

CD4+ Count

- Specimen
 - EDTA blood
- Limitations:
 - Needs to be measured within 24 hours or 5 days (depending on method used) for accurate result
 - Diurnal variation
 - Affected by concurrent illnesses

Reasons for Low CD4+ Count

- Congenital
 - Aplasia of lymphopoietic cells
 - Severe combined immunodeficiencies
 - Ataxia-telangiectasia
 - Wiscott-Aldrich syndrome
 - Immunodeficiency with thymoma
 - Cartilage-hair hypoplasia
- Idiopathic CD4+ T-lymphocytopenia

Reasons for Low CD4+ Count

- Infections
 - HIV
 - Other viral illnesses e.g. influenza
 - Bacterial infections e.g. TB
 - Parasitic infections e.g. malaria
 - Fungal infections e.g. coccidiomycosis
 - Sepsis and septic shock

Reasons for Low CD4+ Count

- Multi-organ failure
- Trauma inc. burns
- Transfusions
- Malnutrition and dietary deficiency
 - e.g. alcohol abuse
- Autoimmune diseases
 - e.g. SLE, myasthenia gravis
- Aplastic anaemia

Reasons for Low CD4+ Count

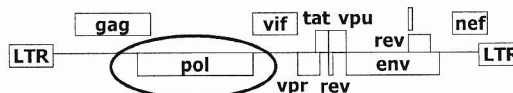
- Protein losing enteropathy
- Immunosuppressive therapy
- Carcinoma and lymphoma
- Over-exercising
- Pregnancy
- Psychological stress
- Normal daily variation

Reasons for Falsely High CD4+ Count

- Splenectomy
 - Look at CD4+ %
- Co-infection with HTLV-1

Genotyping

- To detect mutations which mediate drug resistance
- The reverse transcriptase and protease regions of the pol gene are sequenced



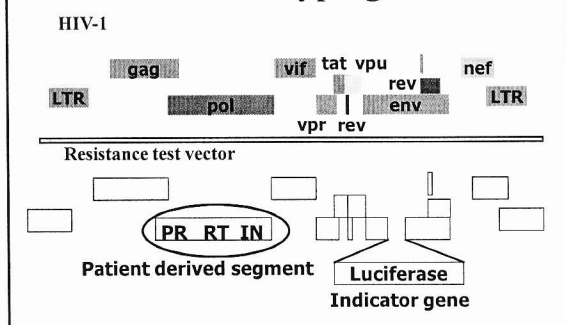
Genotyping

- Sequence that is obtained does not necessarily reflect what is happening in vivo, only a guide
- Programmes are available that assist with the interpretation of results
 - e.g. HIV Drug Resistance Database

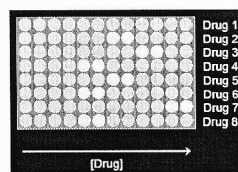
Genotyping

- Recommendations are available as to whom should be tested
 - Failing therapy after ≥ 2 previous regimes
 - Post-exposure prophylaxis if source on treatment
 - Pregnant patients
- Must have a viral load of >5000 copies/ml

Phenotyping



Phenotyping



- Resistance test vector with patient derived segment grown in presence of drug
- If susceptible, virus does not replicate
- If resistant, virus replicates

Phenotyping

- More accurate than genotyping
 - Actual measure of drug susceptibility
- Expensive
- Requires a specialised laboratory

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