

HIV: Rethinking the diagnosis

Nicolette du Plessis
Paediatric Infectious Diseases
nicolette.duplessis@up.ac.za



**IT'S TIME
FOR A
RADICAL
RETHINK**



Key messages

3 ages to consider when making an HIV diagnosis

3 types of tests to consider in HIV diagnostics

Newborn

<18 month old

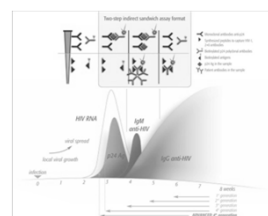
>18 month old



Rapid antigen test

HIV ELISA

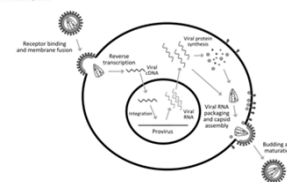
HIV PCR(s)



Laboratory Diagnosis of HIV infection

ELISA

- 1) First generation - whole viral lysates
- 2) Second generation - recombinant antigen
- 3) Third generation - synthetic peptide
- 4) Fourth generation - antigen + antibody (Simultaneous detection of HIV antigen and antibody) - HIV duo





3 AGES

Newborn



If they are perinatally HIV-infected

Need to be treated by week 7 of life (CHER study)

Mortality 20% by week 13

Accurate, earlier infant testing will be beneficial

Newborn



More likely to acquire HIV during the intra-uterine (*72hr rule*) vs intrapartum period

40

:

60



•
•

75

:

25

Newborn



We have successfully decreased HIV acquisition through maternal and infant drug pressure by PMTCT programmes...

Transmission rate 2%

BUT those that are infected also has the drug pressure that can delay the diagnosis

Newborn



Birth
2-4wks post NVP/AZT
Universal testing
Targeted testing



Newborn

Low birth weight <2.5kg
Premature infants
Infants of mothers who were on TB treatment for active TB at any point during pregnancy
Infants born to mothers with VL>1000copies/ μ L
Infants of mothers who were on ART <4 weeks
Infants of mothers who were unbooked or diagnosed HIV-positive in labour or shortly after delivery
Breastfed infant of a newly diagnosed HIV positive breastfeeding mother
Infants who are symptomatic at birth



<18 month old



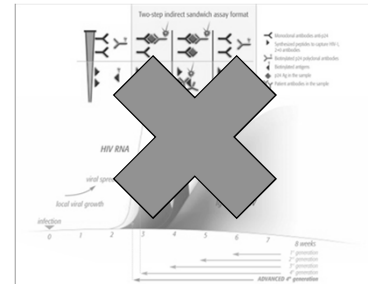
Clearance of maternal anti-HIV antibodies in uninfected infants (seroreversion) can take up to 18 months

Standard serological tests

Rapid antigen tests

HIV ELISA

cannot diagnose HIV infection in this age group



>18 months

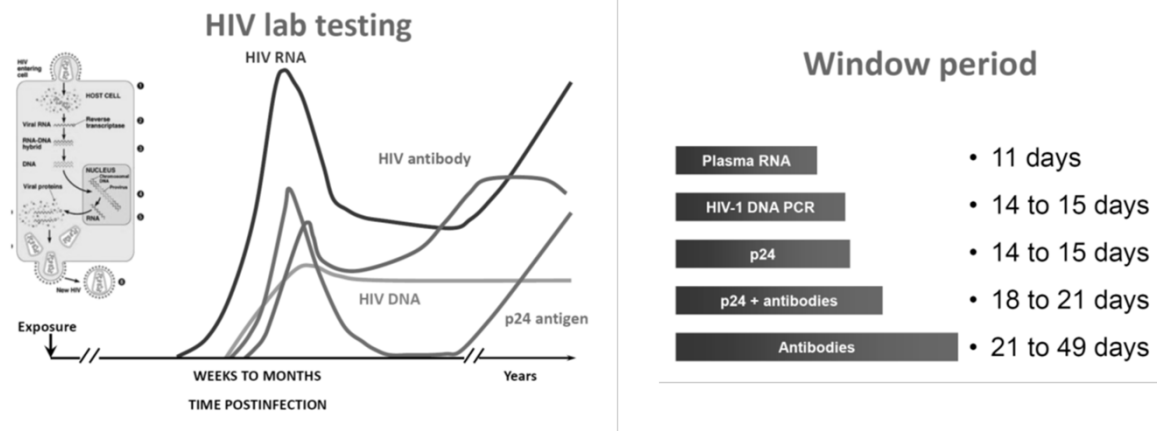


Rapid HIV antibody test or HIV ELISA

Same test used to diagnose or exclude HIV infection in adults

If negative → no clinical features of HIV and breastfeeding stopped >6 weeks → HIV uninfected

If positive → confirm → second rapid test / ELISA



3 Test types

HIV rapid antigen test

3rd generation Rapid test: Anti-HIV IgG antibodies

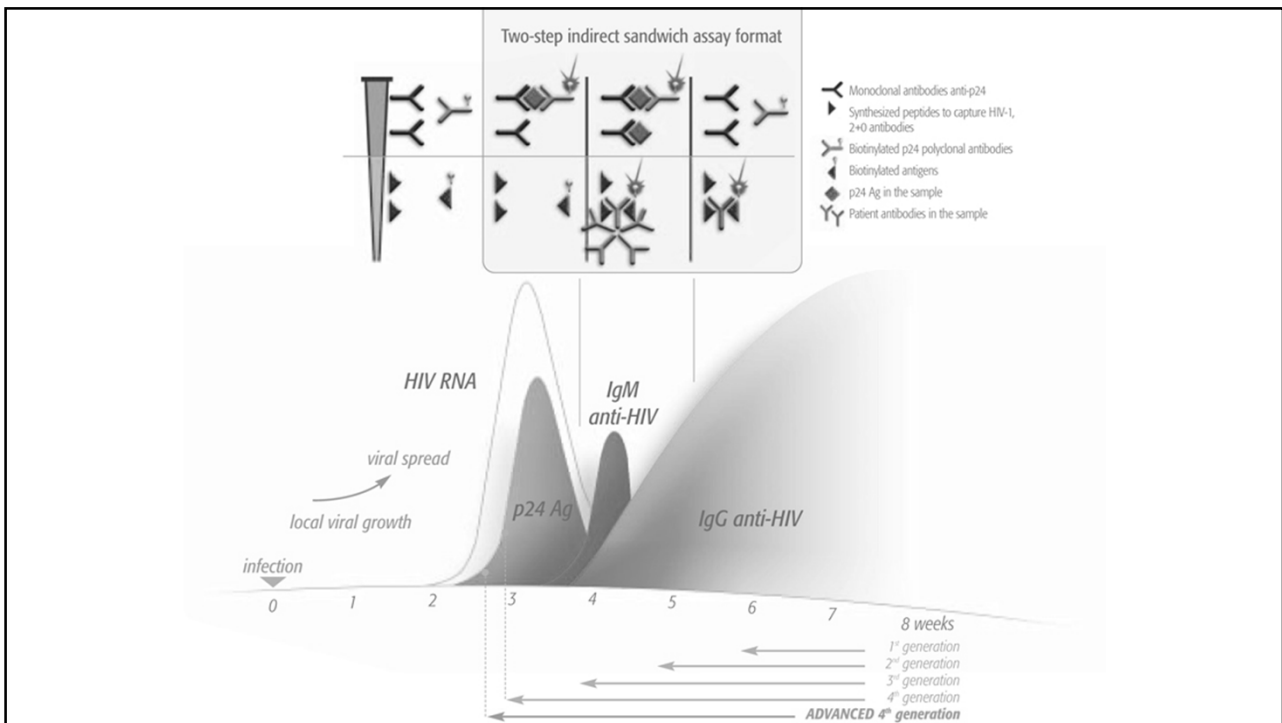
In most of our clinics

4th generation Rapid test: Anti-HIV IgM + IgG antibodies with a p24 component

In some laboratories

Recent data: p24 component not accurate in SA setting

Interpret as a serological test only



HIV ELISA

4th generation test

HIV-1 group M (major) and O (outlier)

Subtypes and CRF (circulating recombinant forms)

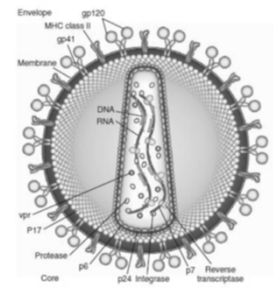
HIV-2

Anti-HIV IgG

Anti-HIV IgM

p24 Ag (viral capsid)

Does not differentiate...



Some pearls in interpreting the PCR results...

Always correlate clinically

What does indeterminate mean

Longer cycle time

Lower intensity signal

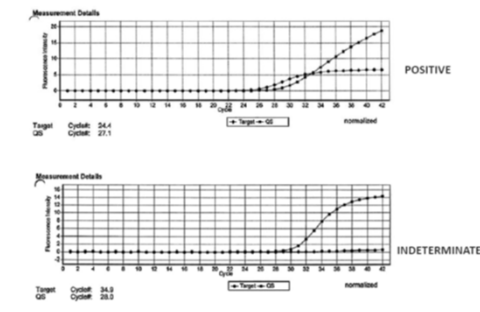
What do you do

Submit new, adequate sample

Repeat on same platform

Then test on another platform

Counsel the patient (NB)



Quantitative PCR testing (HIV viral load)

Diagnostic issue = how many copies...??

Treatment monitoring tool of choice

Useful to confirm an HIV diagnosis

DBS

Plasma

Serum (private labs) – anti-HIV + HIV viral load

Prove exposure
Diagnose infection
Confirm infection
AND CORRELATE CLINICALLY

Bringing it all together...

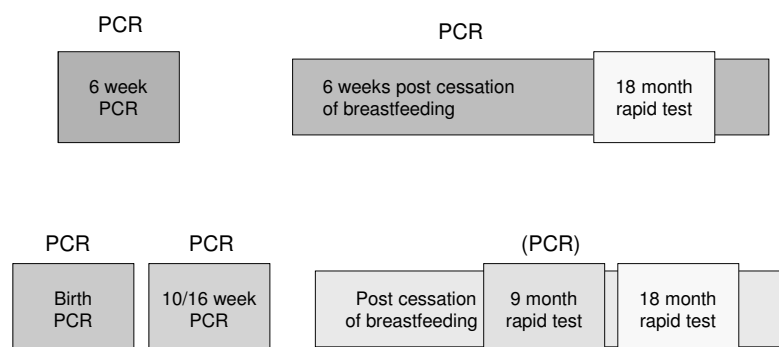
1. Proper counselling
2. Diagnose HIV exposure
3. Diagnose HIV infection in the child
 1. <18 months: HIV PCR
 2. >18 months: HIV Rapid / HIV ELISA
4. Confirm HIV infection in the child
 1. <18 months: 2nd HIV PCR
 2. >18 months: Confirm ELISA / (Rapid)
5. HIV disease management
 1. CD 4 cell count
 2. Symptomatic classification (WHO/CDC)
 3. HIV VL for monitoring treatment response

The future...

Timing of testing

Point-of-care testing

Current vs Ideal Testing Algorithm



9-month testing likely to replace 'post cessation of breastfeeding' testing

3rd PCR where resources available

Slide courtesy prof Gayle Sherman, NHLS

HIV POC platforms

Point-of-care

Rapid

ELISA

HIV DNA PCR

HIV RNA PCR

CD4 cell count

Application in a busy SA clinic...

Application in EID...

Test	Turnaround time/ capacity	Sample needed	Cost test/ instrument*	Power	Environment	Training (dispenser)
SARSA	60 minutes 4 samples per run	200µl plasma or 100 µl blood	180 \$2500 to \$3000	AC or battery	N/A	Minimal
LatM Analyzer	30 to 55 minutes 8 to 75 samples per day (depending on level of detection)	200µl plasma or 10-50 µl of finger stick blood	180	AC or battery	Operating temperature 5 to 35o C (50 to 95o F) Humidity < 90% relative humidity Maximum altitude: N/A (permissible atmospheric pressure: 85 to 1020 hPa)	One hour
Alice	30 to 60 minutes	25 µl finger stick	180	On board rechargeable battery	Operating temperature 5 to 40o C (50 to 104o F) Humidity < 90% relative humidity Maximum altitude: N/A (permissible atmospheric pressure: 85 to 1020 hPa)	Less than 90 minutes

Point of Care	Manufacturer	Sample Type	Sensitivity	Specificity
OrionQuick ADVANCE Rapid HIV-1/2 Antibody Test	Orion Diagnostics	Oral Fluid Whole Blood (fingerstick or venipuncture) Plasma	99.2% 99.8% 99.8%	99.9% 100% 99.9%
Clearview COMPLETE HIV 1/2	Inverness Medical Professional Diagnostics	Whole Blood (fingerstick or venipuncture) Serum & Plasma	99.7% 99.7%	99.9% 99.9%
Clearview HIV 1/2 STAT PAK	Inverness Medical Professional Diagnostics	Whole Blood (fingerstick or venipuncture) Serum & Plasma	99.7% 99.7%	99.9% 99.9%
Reveal Q-3 Rapid HIV-1 Antibody Test	Abbott, Inc.	Serum Plasma	99.8% 99.8%	99.1% 99.1%
Uni-Gold Recombigen HIV	Trinity Biotech	Whole Blood (fingerstick or venipuncture) Serum & Plasma	100% 100%	99.7% 99.9%
Multiple HIV-1/2 Rapid Test	Bio-Rad Laboratories	Serum Plasma	100% 100%	99.9% 99.9%
INSTI HIV-1 Antibody Test*	Abbott Laboratories	Plasma Whole Blood (fingerstick or venipuncture)	99.9% 99.9%	100.0% 100.0%

*None use detect HIV-1 and HIV-2 but may only have FDA approval for HIV-1.
Table 1. FDA-cleared rapid tests in the United States.
This table omits the INSTI information, was published in a Clinical Laboratory News (CLN) article by the author in 2008.

Test	Turnaround time/ capacity	Sample needed	Cost test/ instrument*	Power	Environment	Training (dispenser)
Zymyx C14 counter	10 minutes 40 samples per day	100 µl finger stick blood	\$6-7 \$300	None	180	Less than 30 minutes
Burnet Institute C14 counter	20 minutes 8-10 tests per hour (running cartridges in parallel)	10 µl finger stick blood; can also use venous blood	180	Battery	180	Less than 100 minutes
DaktariIM C14 Counter	8 minutes 40-50 samples per day	20 µl finger stick blood applied to cartridge	\$8 \$800	AC, on board long life rechargeable battery	Temperature 40 to 50	Less than 90 minutes
M Bio C14 system	20 minutes 8-10 tests per hour	10 µl finger stick blood; can also use venous blood	180	Battery	180	Less than 90 minutes

Newborn

<18 month old

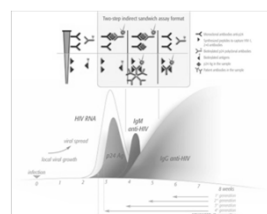
>18 month old



Rapid antigen test

HIV ELISA

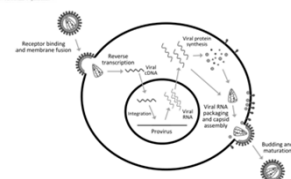
HIV PCR(s)



Laboratory Diagnosis of HIV infection

ELISA

- 1) First generation - whole viral lysates
- 2) Second generation - recombinant antigen
- 3) Third generation - synthetic peptide
- 4) Fourth generation - antigen + antibody (Simultaneous detection of HIV antigen and antibody) - HIV duo



I thank you...

DBS cards

- Dried blood spots have been extensively used for diagnostics and monitoring of HIV infections
 - Antibody testing
 - p24 antigen testing
 - CD4 counts
 - Drug resistance
 - Qualitative HIV-1 PCR
 - Viral load quantification
- The use of DBS has facilitated the early detection of HIV-1 and subsequent antiretroviral intervention for millions of infants worldwide
 - can be collected by heel or finger prick and without the need for venepuncture
 - can be stored at room temperature
 - more easily transported to the testing laboratory
 - less biohazard risk

Heel prick:



Toe prick:

