Development and Evaluation of an Affordable HIV Viral Load Assay for use in Resource-Limited Settings

Abraham Alabi
Viral Diseases Programme
MRC Laboratories, Banjul, Gambia

EDCTP 4th Annual Forum
Ouagadougou, Burkina Faso

Presentation Outline:

- Introduction
- Assay Development
- Assay Validation
- Assay Implementation:
  - Projects
  - Capacity Building
- Conclusions
- Future Prospects
- Acknowledgement

Background

Increased resources in recent years mean more people in Resource-Poor Settings now have access to ART

Challenges

- High Cost of Rx Monitoring
- Inadequacy of trained health Personnel
- Lack of Infrastructure

Results

Inadequate Immunological & Virological Monitoring of patients on ART

Direct virological monitoring is achieved by measuring HIV viral load; however, there are limitations of commercial viral load assays:

- High cost
- Inadequate infrastructure & expertise
- Subtype variations
- Both HIV-1 & HIV-2 prevalent in West Africa and there is no commercial VL assay for HIV-2

Therefore, robust & cheap VL assays are needed to monitor viral control in clinical trials/intervention programmes

ASSAY DEVELOPMENT

- Received funding from EDCTP in 2004 and Project commenced in February 2005
- Developed a colorimetric format of an RT-PCR Assay for quantifying HIV RNA in human plasma

Principle:

The Assay is a quantitative reverse-transcribed PCR of the long terminal repeat (LTR) sequence of HIV in which test samples are quantified by comparison with a standard curve

Basic Procedure of Assay:

- Extraction of RNA from Patient's plasma
- Reverse transcription of RNA to cDNA
- PCR with specific HIV LTR primers
- Detection of DNA product by ELONA

Unique features of the Assay:

- Inclusion of internal calibrator to compensate for RNA loss, inhibition, RT-PCR & makes assay competitive
- Simple Technique
- Use of common Lab Equipment
- Affordable

Assay Validation (1)

Table 1. Differences in RNA copies/ml as determined by our HIV-1 colorimetric assay versus NIBSC expected values

Sample	colorimetric	NIBSC	Log10 Diff.
PWS-1	1055	1270	- 0.08
PWS-2	18467	12700	0.16
97/656	32676	35000	- 0.03
PWS-3	100	175	- 0.24

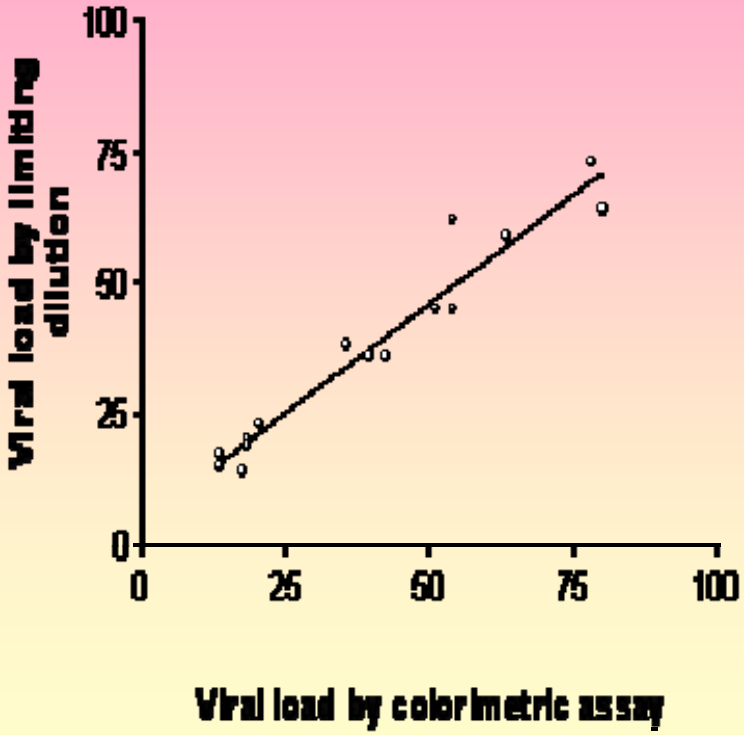
Mean Log Difference = **0.13**

Assay Validation (2)

15 HIV-2 positive plasma samples

Quantified by LDA &
by Colorimetric assay

Figure 2. Relationship between RNA copies/ml (x10^3) as determined by our HIV-2 colorimetric assay and limiting dilution analysis (LDA)

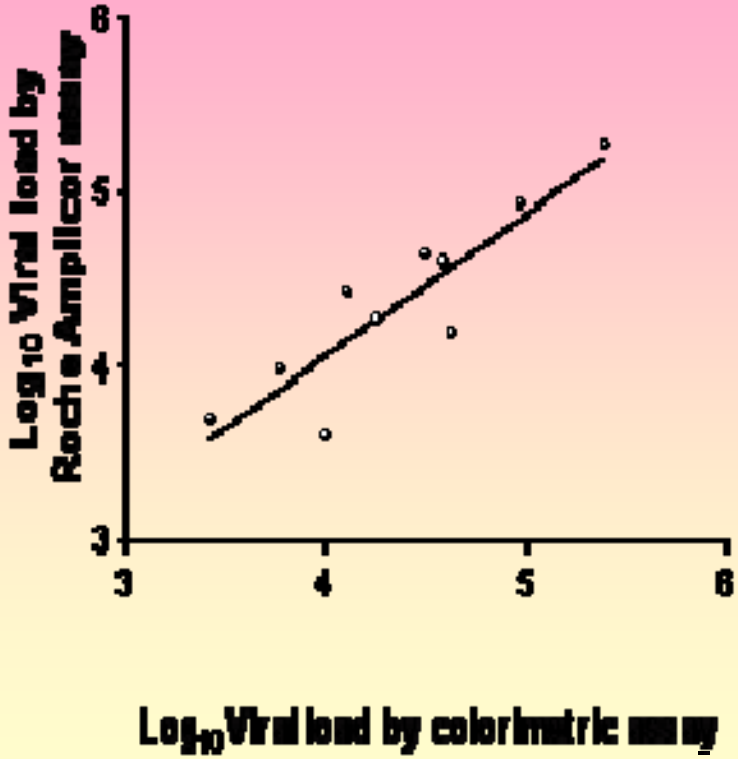


Assay Validation (3)

10 HIV-1 positive plasma samples

Quantified by Roche & by Colorimetric assay

Figure 3. Relationship between RNA copies/ml as determined by our HIV-1 colorimetric assay and Roche amplicor assay (vs 1.5)



Assay Validation (4)

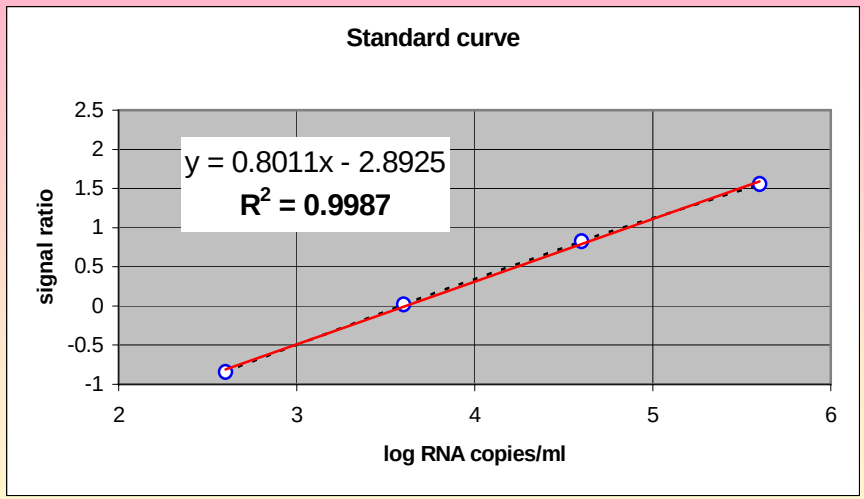
Table 2. Specificity of HIV-1 Assay

Sample	Sample ID	samples type	copies/ml
1	Neg. ctrl		<100
2	Pos. ctrl		5310
3	N004007	HTLV+ve, HIV-ve	<100
4	N004008	HTLV+ve, HIV-ve	<100
5	N004072	HTLV+ve, HIV-ve	<100
6	N027158	HIV2+ve, HIV-1-ve	<100
7	N027160	HIV2+ve, HIV-1-ve	<100
8	N027180	HIV2+ve, HIV-1-ve	<100
9	N027238	HIV2+ve, HIV-1-ve	<100
10	MVA-190	Hep.B+ve, HIV-ve	<100
11	MVA-197	Hep.B+ve, HIV-ve	<100
12	MVA-373	Hep.B+ve, HIV-ve	<100

Fig. 4 Validity of an Assay Run

Raw data

	1	2	3	4
A	5643	38547		
B	5403	38099		
C	19498	19119		
D	21014	19518		
E	30318	4397	12567	21136
F	29937	4549	13015	20496
G	33587	894.1	24.6	43999
H	37554	1067	34.7	17928

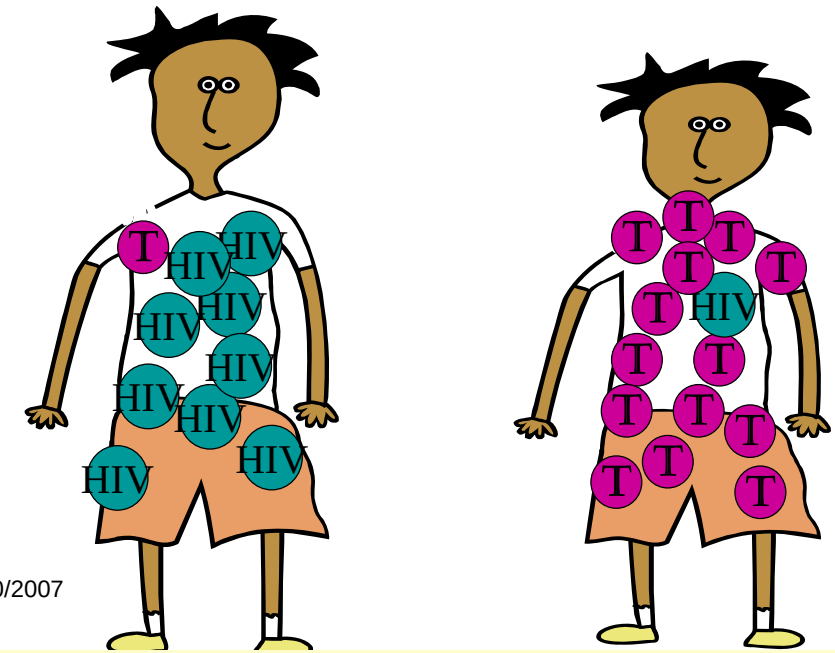


Sample	Sample ID	Sample date	samples type	copies/ml
1				<100
2				2223

Assay Implementation

How do we know the medications are working

Before Rx On Rx



22/10/2007

APPLICATIONS OF OUR HIV VIRAL LOAD ASSAY (1)

1. HIV Pathogenesis & Transmission:
    - •High VL correlates with higher Transmission rates (O'Donovan et al., 2000)
    - •High baseline VL correlates with poor prognosis (Alabi et al., 2003)
    - •VL Dynamics in HIV-1 & HIV-2 Dual Infections
2. Treatment Monitoring:
    - •Initiation & modification of antiviral treatment
    - •Efficacy of Rx Regimens
    - •Early recognition of resistance development

APPLICATIONS OF OUR HIV VIRAL LOAD ASSAY (2)

HAART in Gambia

- HAART became available in The Gambia in Oct 2004 through the Global Fund for AIDS,TB and Malaria.
- The MRC is one of the main centres in Gambia where these drugs are available.
- Other centres are RVTH and HOC

APPLICATIONS OF OUR HIV VIRAL LOAD ASSAY (3)

Drug combinations in our GUM

HIV-1:

AZT or D4T+3TC+NVP

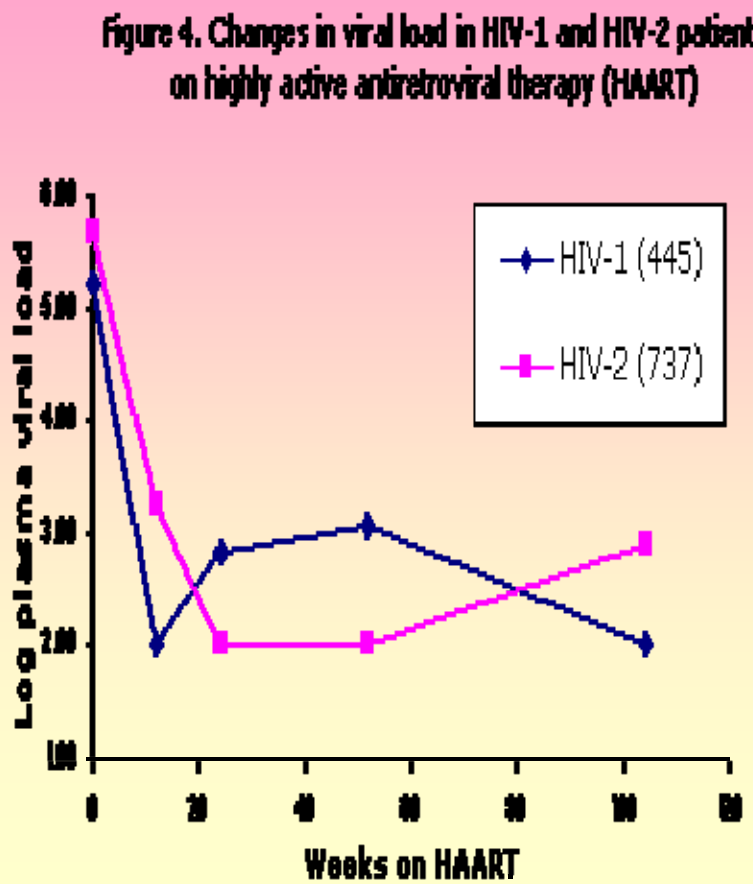
HIV-2 or HIV-Dual:

AZT or D4T+3TC+Kaletra(LPV/Rit)

APPLICATION OF OUR HIV VIRAL LOAD ASSAY (4)

HIV-1 vl undetectable after 12 wks on HAART

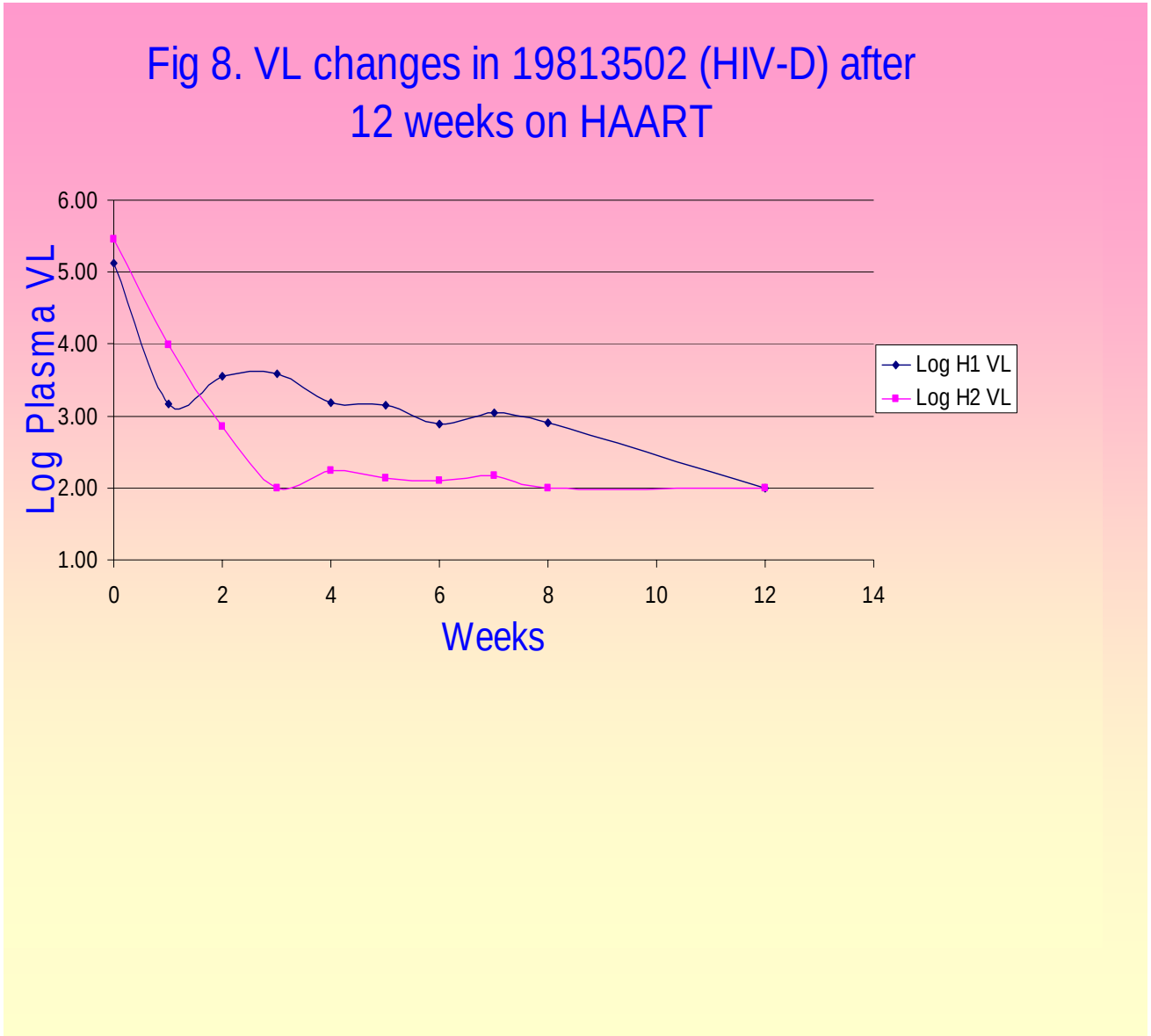
HIV-2 vl undetectable after 24 wks on HAART



APPLICATION OF OUR HIV VIRAL LOAD ASSAY (5)

HIV-1 vl undetectable after 12 wks on HAART

HIV-2 vl undetectable after 3 wks on HAART



Capacity Building/Training

1. WEST AFRICA SUB-REGIONAL TRAINING ON HIV VIRAL LOAD ASSAY, SEPTEMBER 18-22, 2006, MRC LABORATORIES, BANJUL, GAMBIA

2. SOUTHERN AFRICA REGIONAL TRAINING ON HIV VIRAL LOAD ASSAY, SEPTEMBER 24-28, 2007, KENYA MEDICAL RESEARCH INSTITUTE (KEMRI), NAIROBI, KENYA

PARTICIPANTS AT VIRAL LOAD TRAINING, KEMRI, NAIROBI

Existing & Proposed Networks

North-South:

- A Collaboration on HIV-2 infection in Europe (ACHIeV$_2$E)
  - a multisite project to evaluate various HIV-2 assays in Europe (coordinator: Bernard Antoine, Bordeaux, France)

South-South:

- Collaboration with Gambia's National AIDS Programme
- Scientists/Institutions that participated in sub-regional Training Workshop, September 18-22, 2006
- Scientists/Institutions that participated in Regional Training Workshop, September 24-28, 2007

Conclusions

Cheap high through-put assay developed

Lots of successes

Marketable product

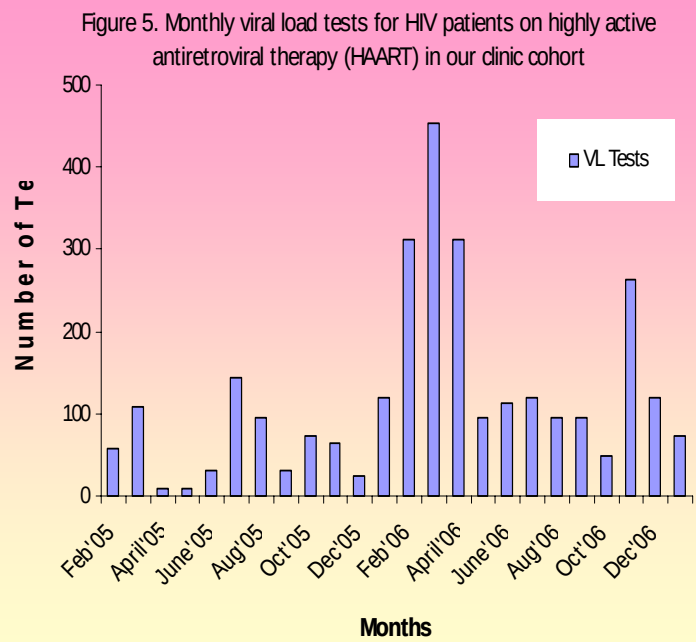
Low cost compared to other assays

User friendly assay

FUTURE PROSPECTS

* Conduct a multi-centre evaluation
* Evaluate assay for HIV subtypes
* Possibly commercialise assay

APPLICATION OF OUR HIV VIRAL LOAD ASSAY (5)



Acknowledgement

MRC, Banjul

Clayton Onyango

Modou Camara

Pa Saidou Chaw

Sam MacConkey

Steve Kaye

Hilton Whittle

Sarah Rowland-Jones

Tumani Corrah

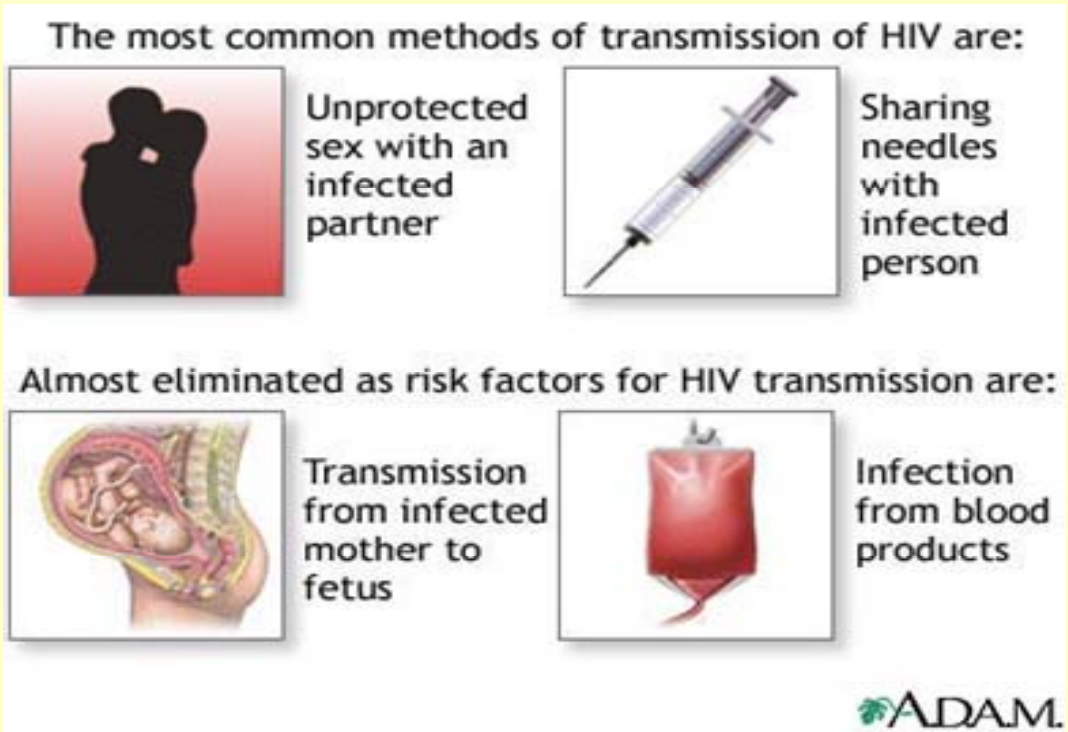
NIBSC, London

Neil Berry

Harry Holmes

EDCTP

HIV TRANSMISSION



Every 14 seconds a person between 15 and 24 years old is infected with HIV virus, accounting for half all new cases of the disease - U.N. Population Fund 2006