HIV Testing

Conventional laboratory tests and some new developments

Molecular Diagnosis

NSW State Reference Laboratory for HIV
Centre for Applied Medical Research
St Vincent's Hospital Sydney

St Vincent's Hospital

This presentation

- Refresher on conventional diagnostic testing for HIV
- New developments with *'Point of Care'* rapid tests
- Molecular testing for HIV diagnosis and management
- Some applications

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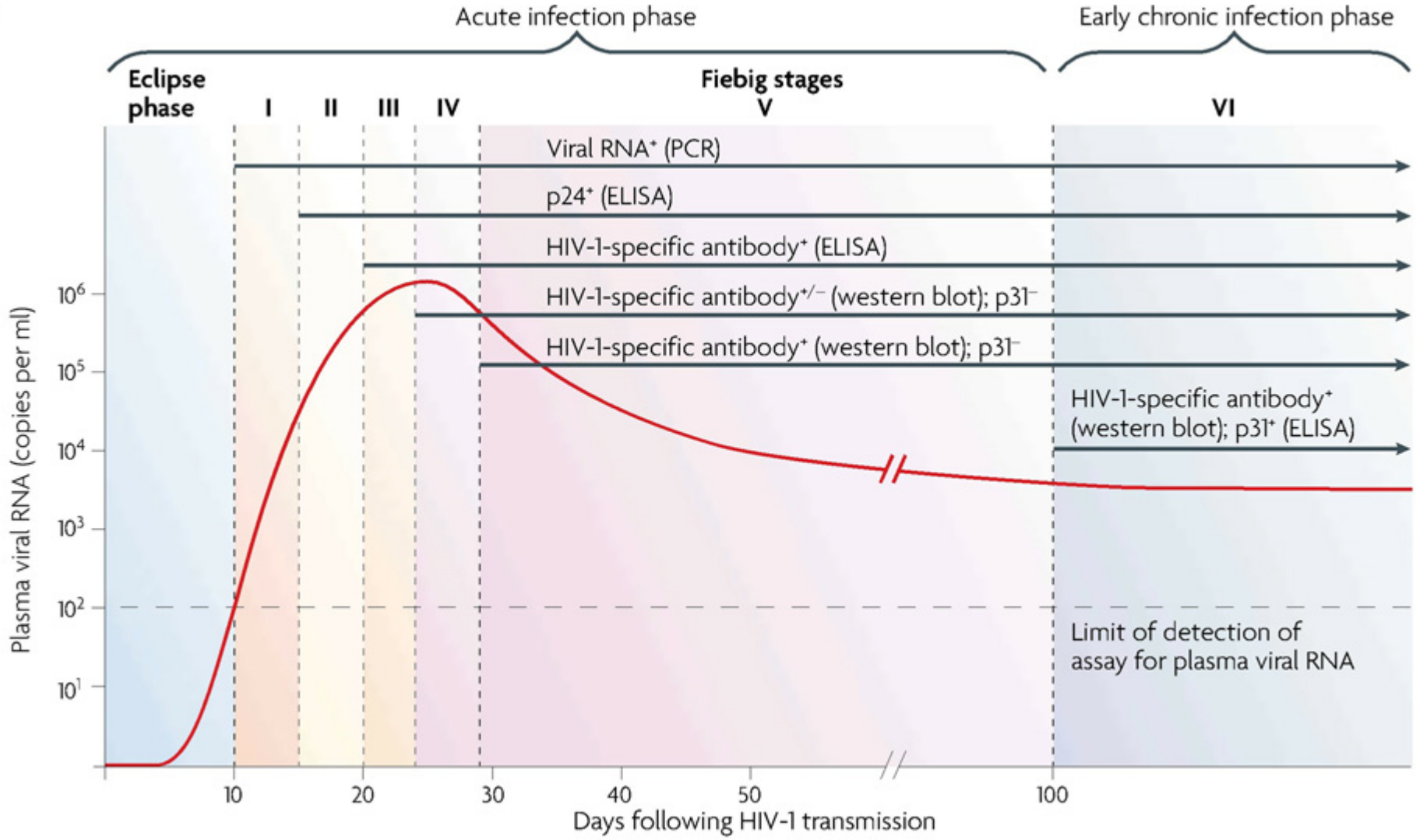
History of HIV testing in Australia

- HIV testing introduced 1985 in Australia and first to have safe blood supply, with blood bank screening also introduced in 1985
- 1987 HIV Testing was described in the 1st National HIV/AIDS Strategy
- Free, confidential testing backbone of HIV prevention
- Seroconversion illness characterised in Australia & targeted lab testing became the norm
- There is emerging concern that HIV testing rates may be dropping



McMichael AJ etal Nature Rev Immuno 2010

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Table 1. HIV testing assays and their "window periods."

HIV test	Assay method	"Window period" estimates, weeks[a]	"Window period" reduction, days[b]
First-generation EIA	Viral particles used to bind patient HIV Ab, detected by marker conjugated to anti-human Ab	~6	…
Second-generation EIA	Same as first-generation EIA except uses purified HIV Ag or recombinant virus	~4–6	10
Third-generation EIA	"Antigen sandwich": synthetic peptide used to bind patient HIV Ab followed by marker conjugated to additional HIV Ag; able to detect IgM	~3–4	6
Fourth-generation EIA	Uses third-generation EIA methodology plus monoclonal Ab to p24 Ag to detect patient p24 Ag	~2	5
Pooled HIV NAT	First combines multiple individual samples into one common pool, then uses PCR or other amplification techniques to detect patient viral nucleic acids	<1–2	3
Individual HIV NAT	As above, except that samples are tested individually rather than diluted by pooling	<1–2	3

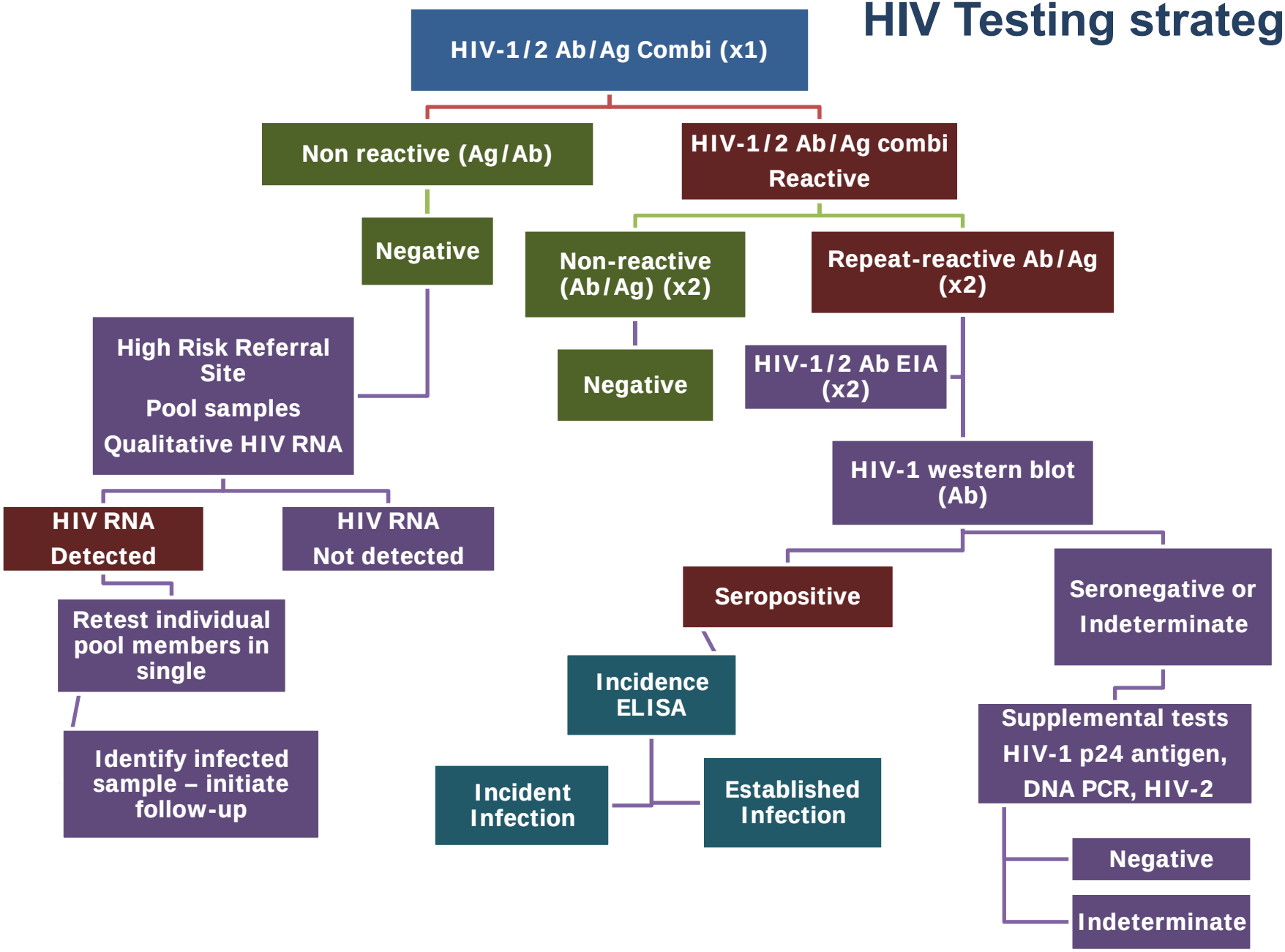
NOTE. Data are from [5, 6, 16, 19]. Ab, antibody; Ag, Antigen; HIV NAT, HIV nucleic acid testing.

[a] "Window periods" listed are averages. For example, although second generation EIAs will detect HIV infection in nearly all individuals within 6 weeks of HIV acquisition, in a study of occupationally exposed health care workers, 5% of subjects did not have seroconversion until at least 6 months following the exposure [20].

[b] Compared with an immediate less sensitive assay, the "window period" for pooled HIV NAT is, on average, 3 days shorter than the "window period" for a fourth-generation EIA.

Stekler J CID 2007

HIV Testing strategy

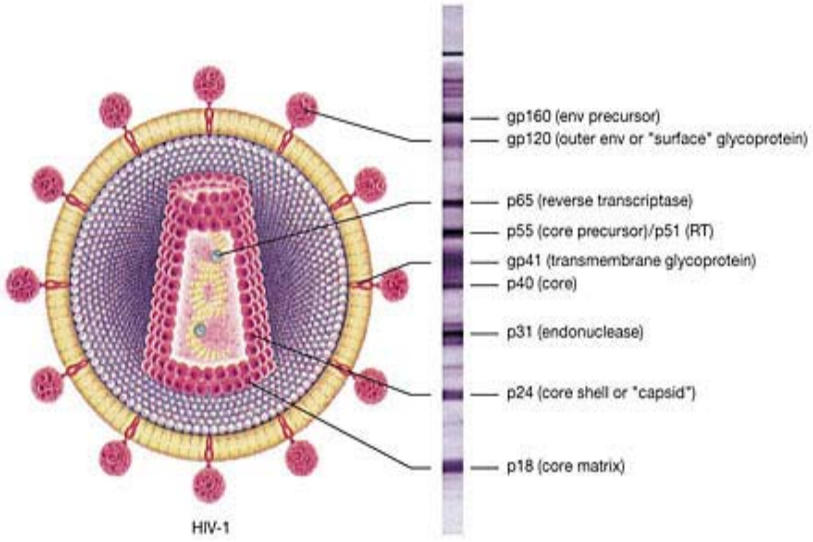


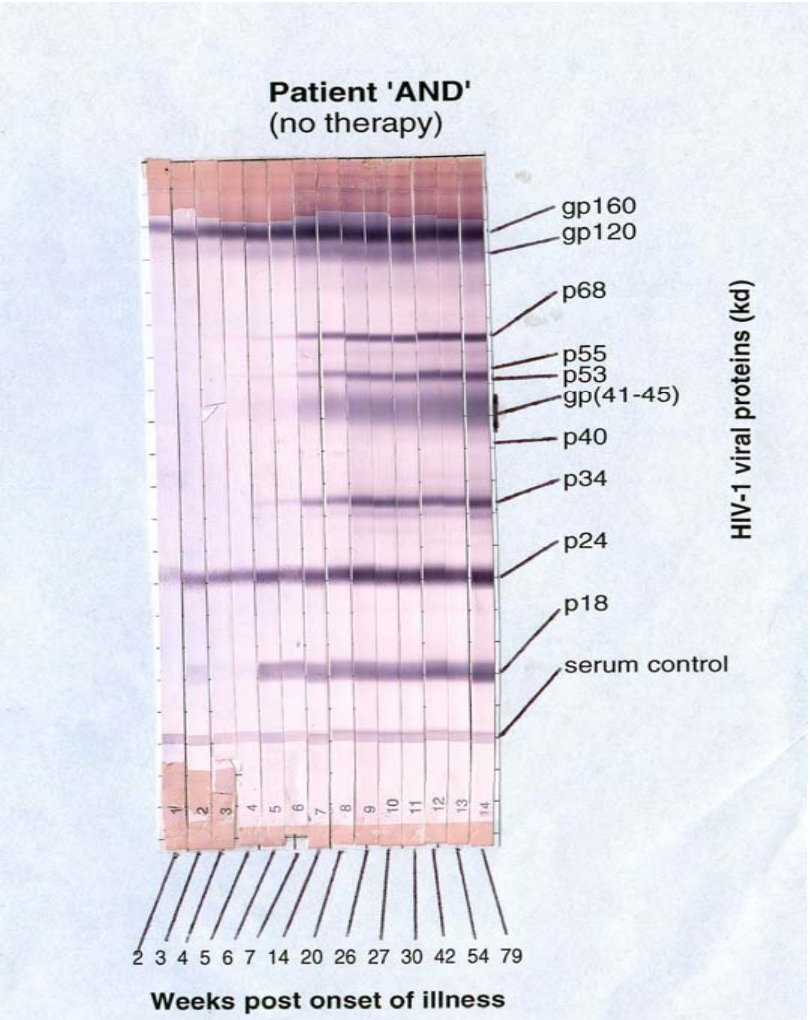
Courtesy Philip Cunningham, St Vincents Hospital Sydney

Serology of primary HIV-1 infection

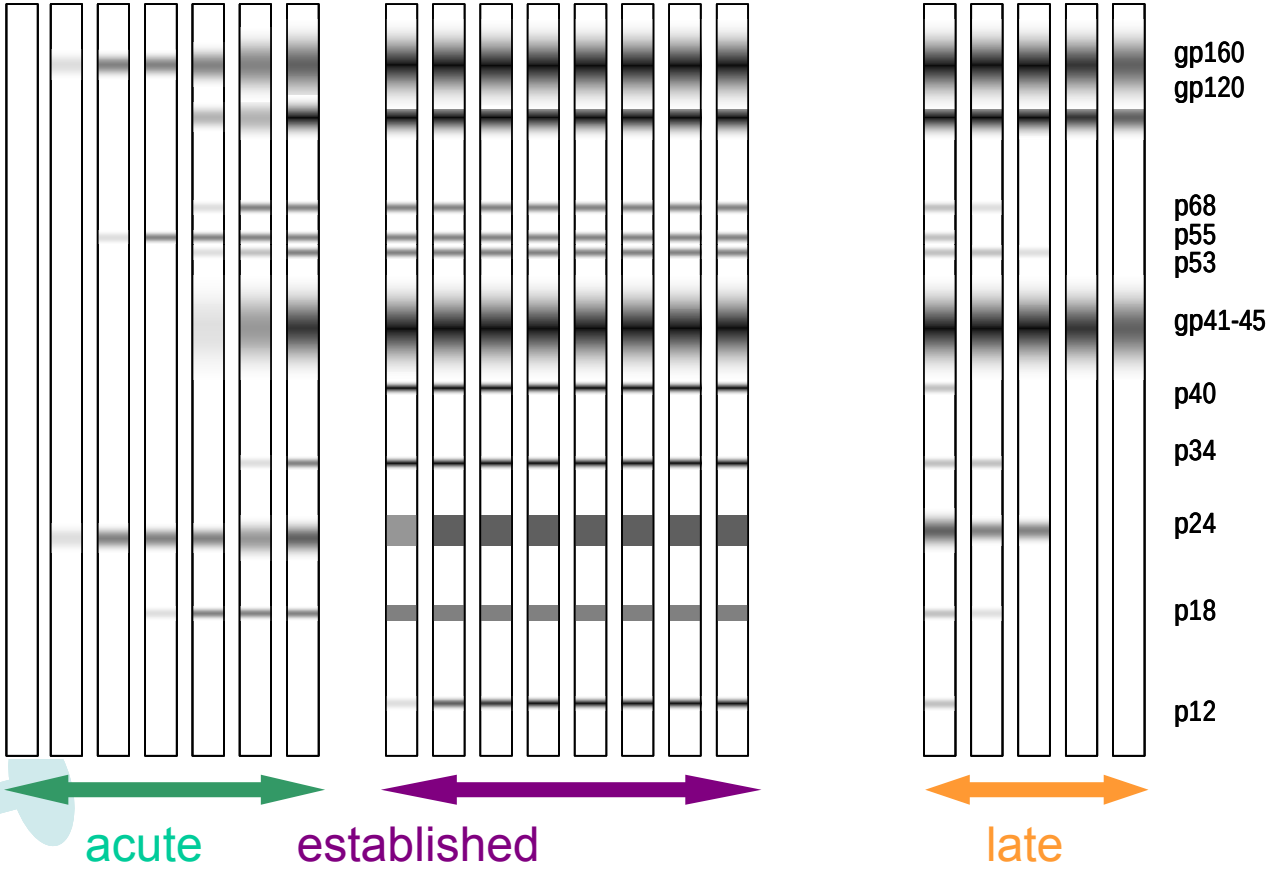
(Western Blot)

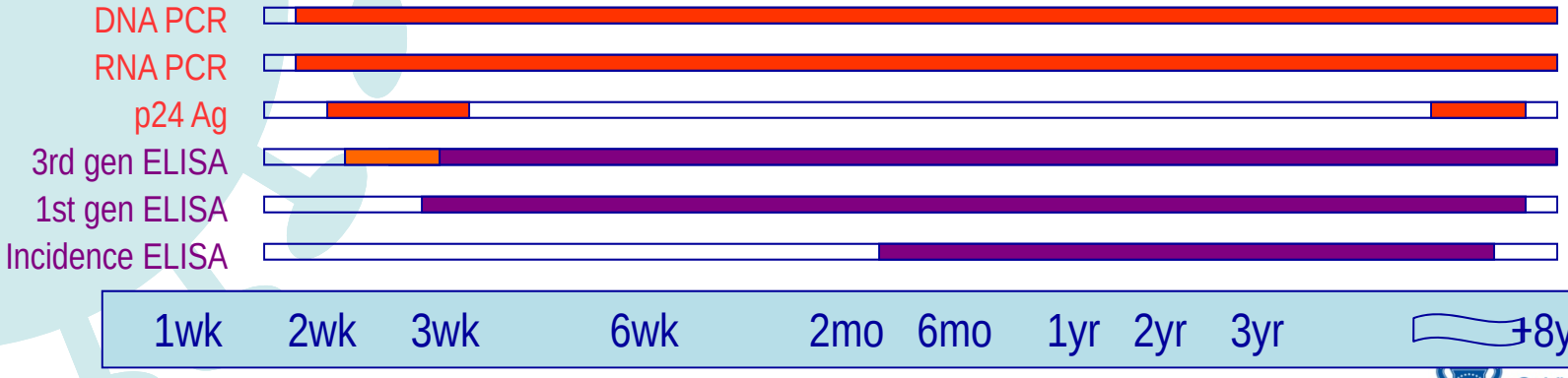
- Antibody tests may be negative or low level (indeterminate)
- Antibodies to viral proteins are incrementally detected – increasing in intensity over time (diagnostic)





Courtesy Philip Cunningham, St Vincents Hospital Sydney





Courtesy Philip Cunningham, St Vincents Hospital Sydney

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Western blot criteria

NEGATIVE: No reactivity to any viral specific bands

POSITIVE: Reactivity to at least one (1) envelope glycoprotein (gp41-45, gp120 or gp160), and at least three (3) viral specific bands of the *HIV-1 gag* or *pol gene product* series

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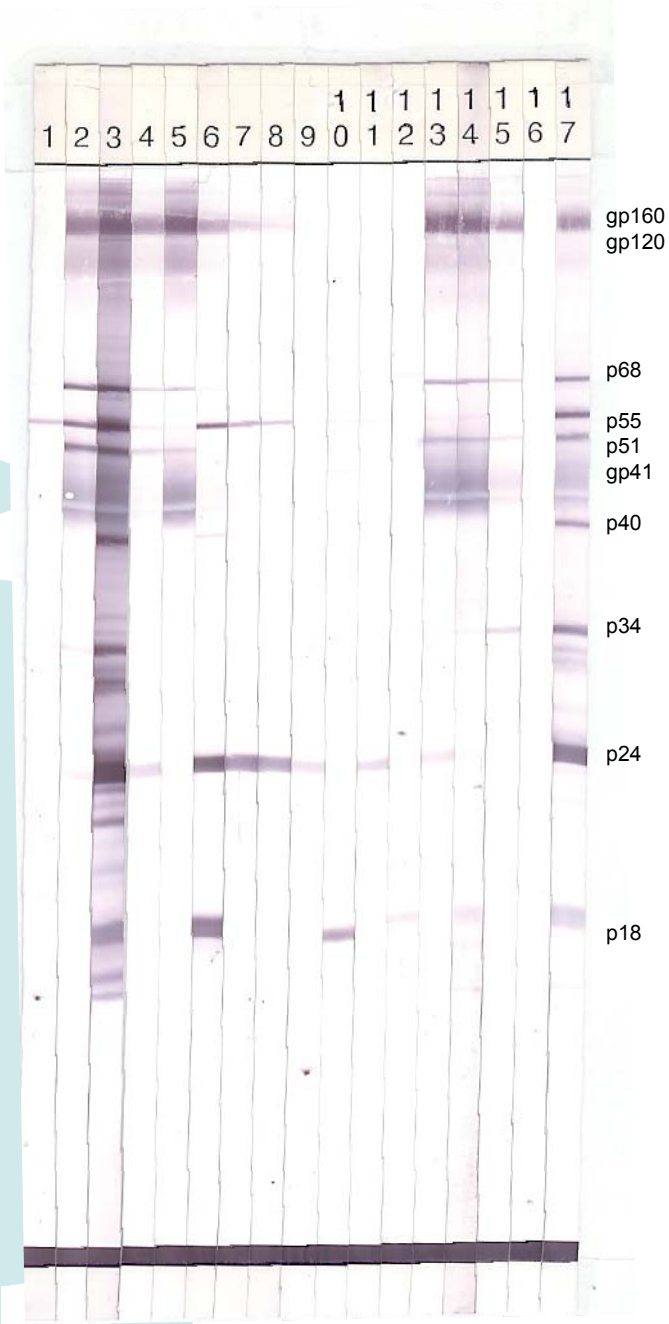
Western blot reactivity interpretation



1	*Indeterminate group 1 – likely biological false positive*	p55
2	*Positive – advanced HIV infection, late stage (seroreversion)*	gp160, gp120, p68, p55, p51, gp41
3	*Positive - established HIV infection*	gp160, gp120, p68, p55, p51, gp41, p40, p34, p24, p18
4	*Positive – recent HIV infection – seroconversion from group 4 indeterminate to positive profile*	gp160, gp120, p68, p55, p51, p24
5	*Indeterminate group 4 – advanced HIV infection, late stage (seroreversion)*	gp160, gp120, p68, gp41
6	*Positive – recent seroconversion in acute HIV infection*	gp160, p55, p24, p18
7	*Indeterminate group 4 – acute HIV infection*	gp160, p55, p24
8	*Indeterminate group 4 – acute HIV infection*	gp160, p55, p24
9	*Indeterminate group 3 – either acute infection or non specific reactivity, require supplemental test results or HIV DNA nucleic acid*	p24
10	*Indeterminate group 2 – probable non-specific reactivity*	p18
11	*Indeterminate group 3 - either acute infection or non specific reactivity, require supplemental test results or HIV DNA nucleic acid*	p24
12	*Indeterminate group 2 – probable non-specific reactivity*	p18
13	*Positive – advanced HIV infection , late stage(seroreversion)*	gp160, gp120, p68, p55, p51, gp41, p24
14	*Positive – advanced HIV infection , late stage (seroreversion)*	gp160, gp120, p68, p55, p51, gp41, p18
15	*Positive – advanced HIV infection, late stage (seroreversion)*	gp160, p68, p51, gp41, p34
16	*Negative (negative control)*	no viral bands
17	*Positive – established HIV infection (positive control)*	gp160, gp120, p68, p55, p51, gp41, p40, p34, p24, p18

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Primary HIV Infection: *Why are we so interested?*

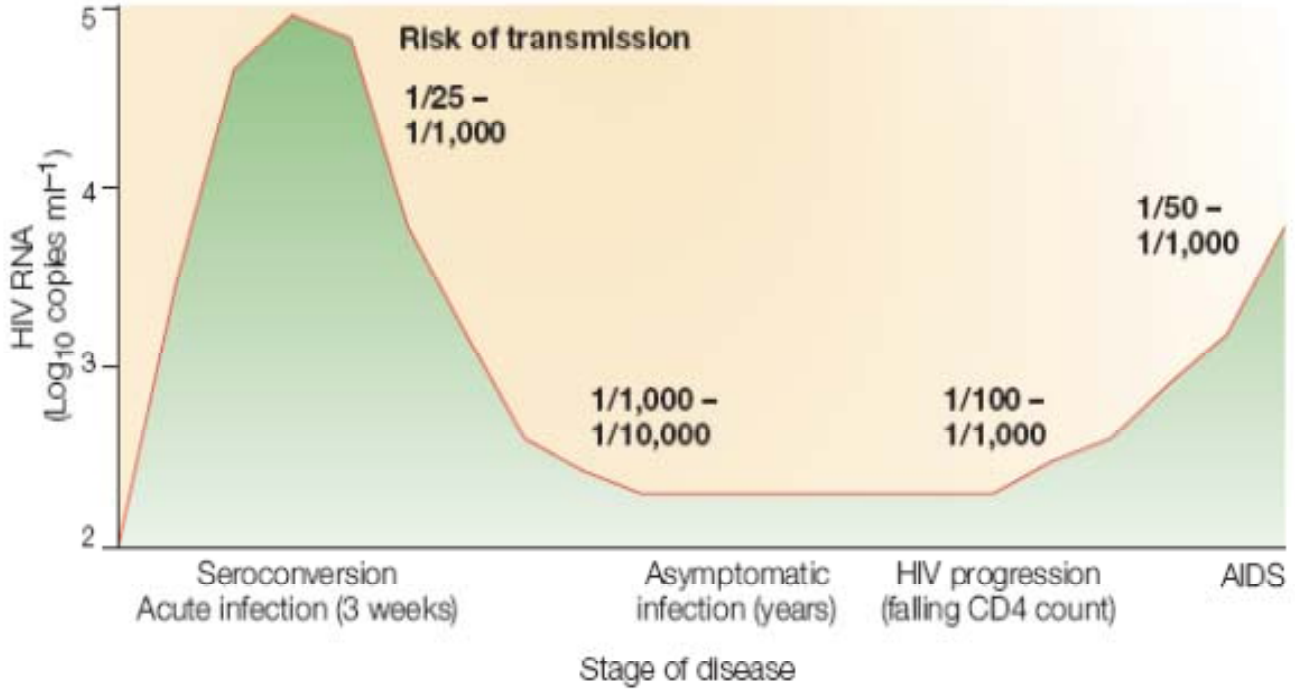

Figure 1 | **The changing viral load during the different stages of disease and the effects of viral load on the probability of sexual transmission of HIV.**

Galvin S, Cohen M Nat Rev Micro 2004

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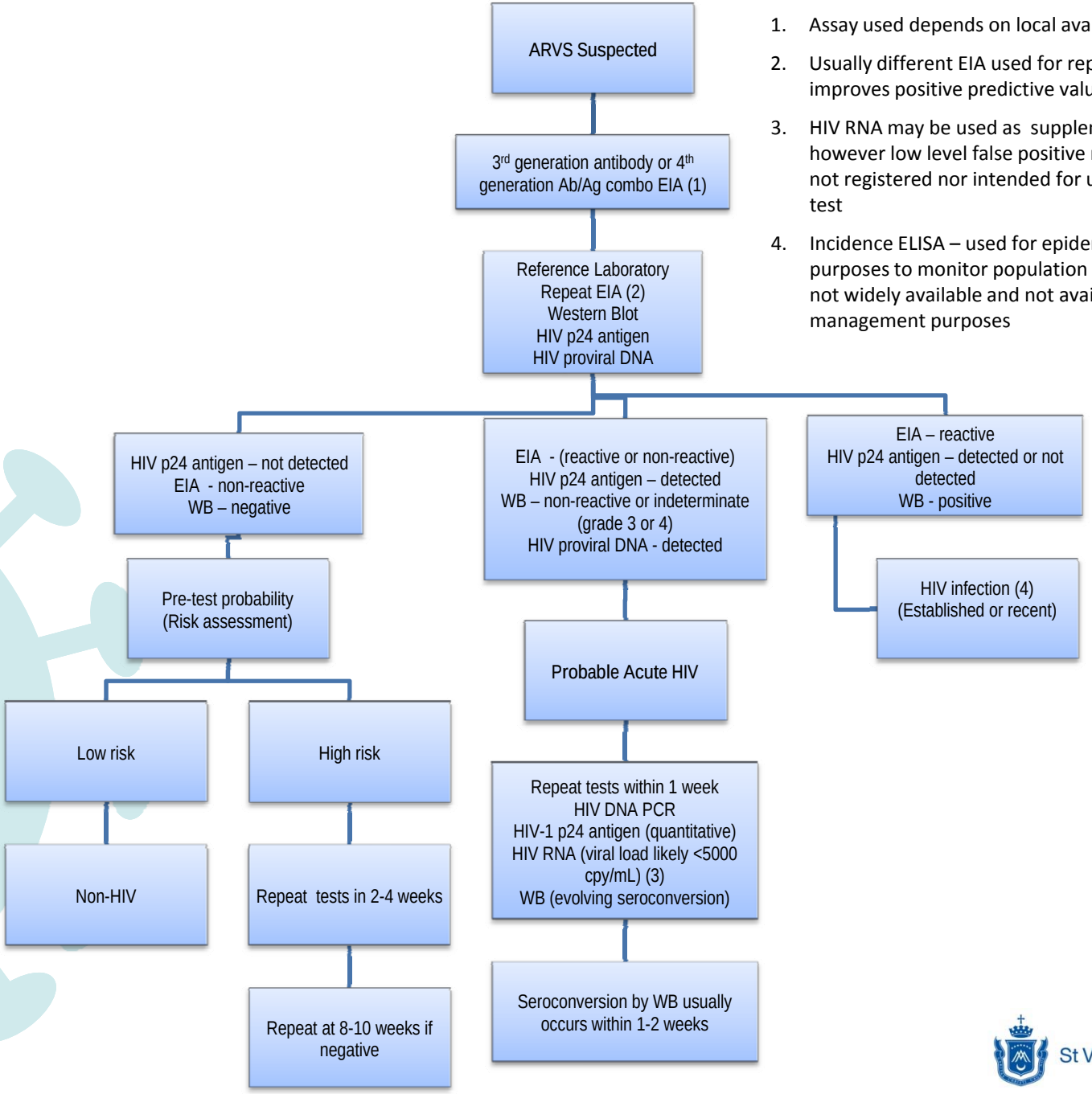

1. Assay used depends on local availability
2. Usually different EIA used for repeat testing – improves positive predictive value
3. HIV RNA may be used as supplementary test however low level false positive results do occur – not registered nor intended for use as diagnostic test
4. Incidence ELISA – used for epidemiological purposes to monitor population incidence rates– not widely available and not available for clinical management purposes

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HIV Testing Policy

- The first testing policy was introduced in 1998 it was written by a committee and endorsed by the Commonwealth
- Subsequently updated in 2006, but only issued as a PDF document and available via websites

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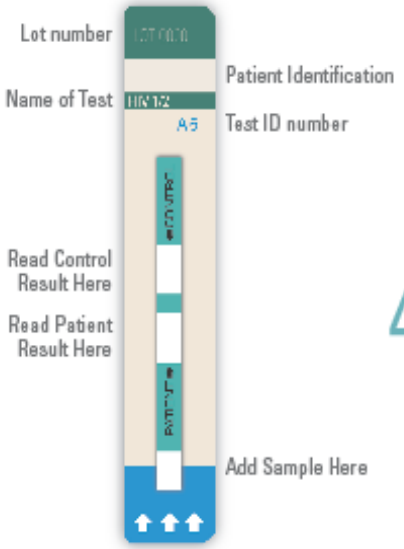
Point of Care Testing

- Short incubation tests
- Test which can be taken to the person being tested, rather than the sample having to be transported to another location
- Can be done using:
  - Capillary blood dropped onto a sample well which is soaked with a reagent or into a chamber to which reagent is added
  - Oral fluid swabbed from the gum line

Determine™ HIV-1/2

Whole Blood Procedure

(Refer to package inserts for assay procedures) (Refer to the other side for Serum/Plasma procedure)

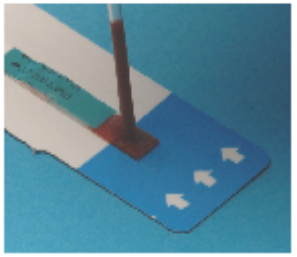


1 Remove tests

Note: Removal of the test units should start from the right side of the test card to preserve the lot number which appears on the left side of the card.

2 Remove cover

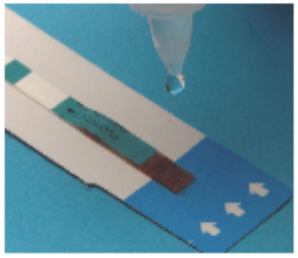
3 Add sample



Add sample (50µl) to sample pad (finger stick or venipuncture)

Wait
1 minute

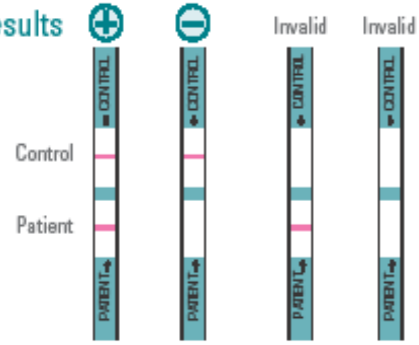
4 Add chase buffer



Add one drop of chase buffer

Wait
15 minutes

5 Read results



determine

detect... diagnose... determine...

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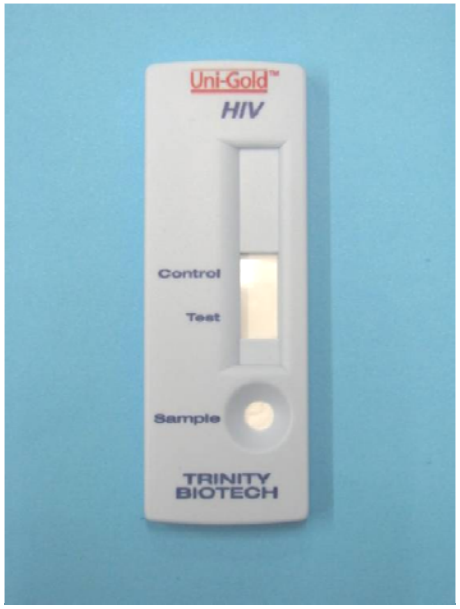
enquiry@determinetest.com

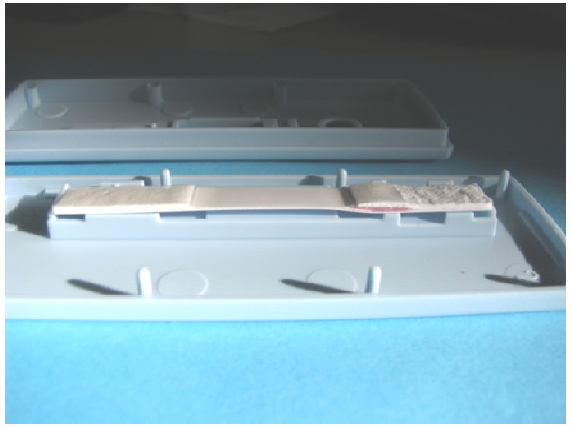
Inverness medical professional diagnostics

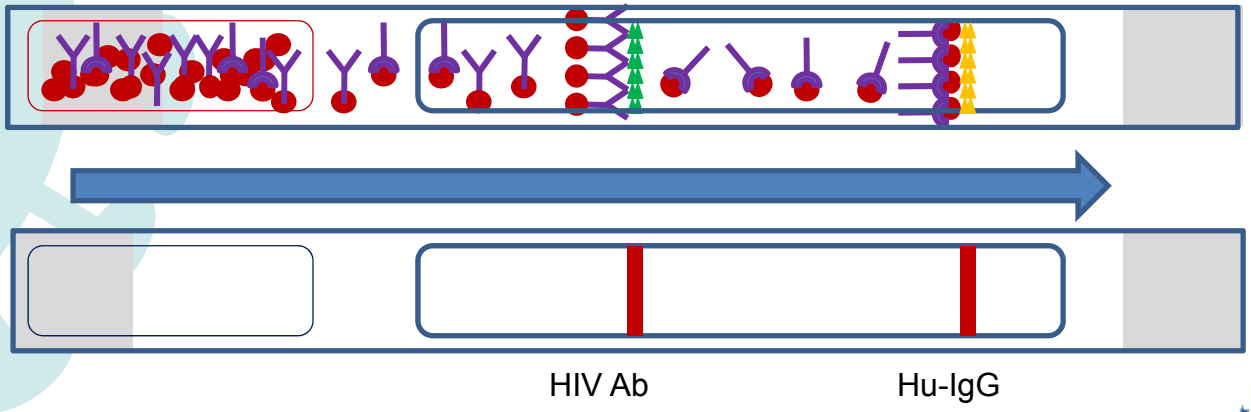
Immunochromatography

- IgG Ab
- α-HIV Ab
- HIV-Ag
- α –hu-IgG
- label

Sample







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MULTISPOT HIV-1/HIV-2
Wash solution 85 ml
Solution de lavage
Solución de lavado
Waschlösung
Soluzione di lavaggio
494048
MULTISPOT HIV-1/HIV-2
Stopping solution 55 ml
Solution d'arrêt
Solución de parada
Stopplösung
Soluzione d'arresto
(0.5% H_2SO_4)
484148-1
MULTISPOT HIV-1/HIV-2
Wash solution 85 ml
Solution de lavage
Solución de lavado
Waschlösung
Soluzione di lavaggio
494048
MULTISPOT HIV1/HIV2
Cartridge • Module
IN VITRO TEST
BIO-RAD
MULTISPOT HIV1/HIV2
Cartridge • Module
IN VITRO TEST
BIO-RAD
MULTISPOT HIV-1/HIV-2
MULTISPOT HIV-1/HIV-2
72269
50 Unitary rapid tests
YME IMMUNOASSAY FOR
DETECTION OF HIV 1 AND HIV 2 ANTIBODIES
ERUM OR PLASMA
IN VITRO DIAGNOSTIC USE ONLY

Advance Awareness™

OraQuick® ADVANCE® Rapid Antibody Test HIV-1/2

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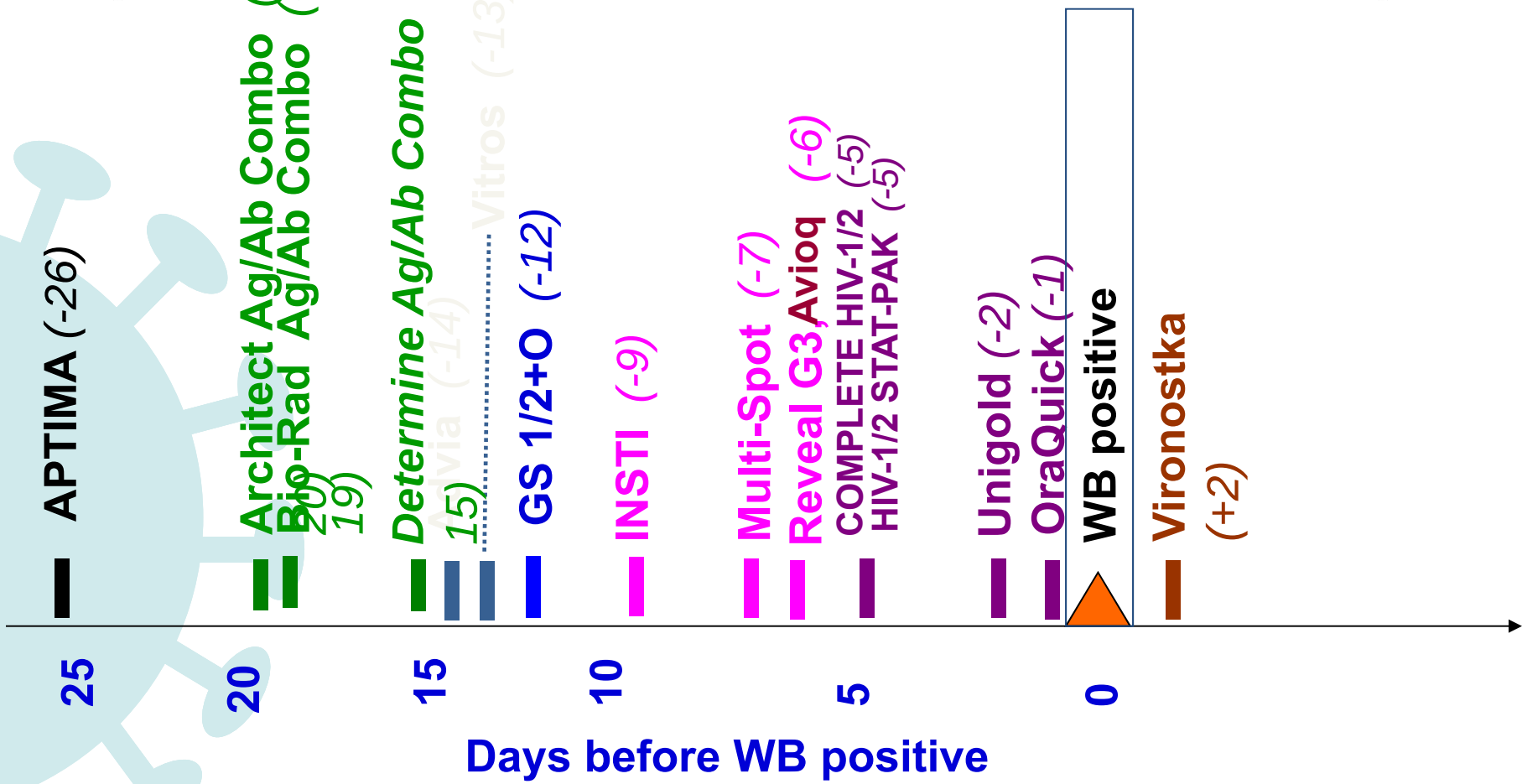
Sequence of Test Positivity Relative to WB

166 specimens, 17 Seroconverters - 50 % Positive Cumulative Frequency



Modified from *Masciotra et al, J Clin Virol 2011* and *Owen et al, J Clin Micro 2008*

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Demand for & on point of care tests

- Increase testing rates ✓
- Increase engagement with care ?
- Increase # people on treatment ✓
- Will failure to identify acute infection cause a problem ?

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HIV Testing
Direct Detection of Virus

- **Virus isolation – PBMC co-culture**
  - Insensitive
  - Requires containment laboratory (PC3)
  - ~ 3-6 weeks culture
  - Detect with p24 antigen or RT activity in culture supernate

- **HIV-1 p24 antigen**
  - Simple serology – serum - no additional sample required
  - Sensitivity about 30 copies HIV RNA (10pg)
  - Now incorporated in 4th generation screening tests
  - Limited use during acute and ? Advanced or late infection

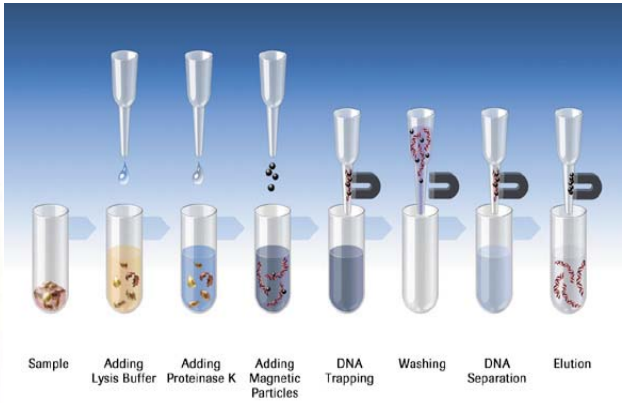
- **Nucleic acid detection - (NAT)**
  - Qualitative tests more sensitive and specific – blood/tissue screening
  - Most products available are quantitive viral load tests – intended use is NOT diagnostic
  - Circulating cell free RNA or integrated cellular DNA ??
  - Dedicated samples required
  - Laboratory facilities

NAT tests for HIV

DNA	RNA	RNA
Qualitative	Quantitative (viral load)	Qualitative
<10 copies	<50 copies	<10 copies
Highly specific	Specificity issues at low range	Highly specific
HIV supplemental diagnostic test	HIV monitoring test	Blood and tissue donor screening
•*Resolve indeterminate serology* •*Acute infection diagnosis (pre-seroconversion)* •*Early infant diagnosis*	•*Treatment response* •*Prognostic marker* •*Clinical decision point*	•*Detection of HIV in pooled or single blood/tissue donors*
Integrated and unintegrated cellular DNA	Extracellular RNA (free virion)	Extracellular RNA (free virion)
Whole blood Dried Blood Spot (DBS)	Plasma DBS	Plasma Cadaveric blood

Pre amplification - extraction

- Volumetric errors amplified
- Tedious – manual, repetitive
- Specimen integrity



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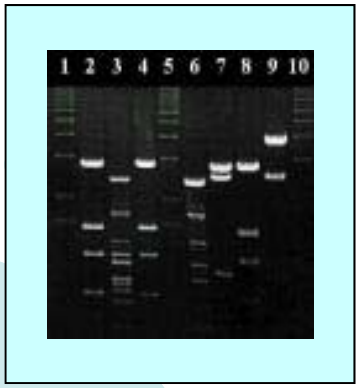
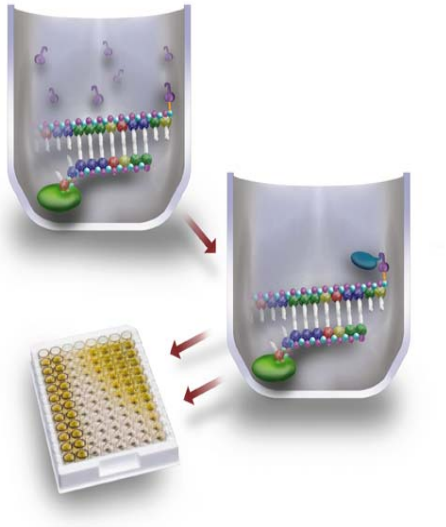
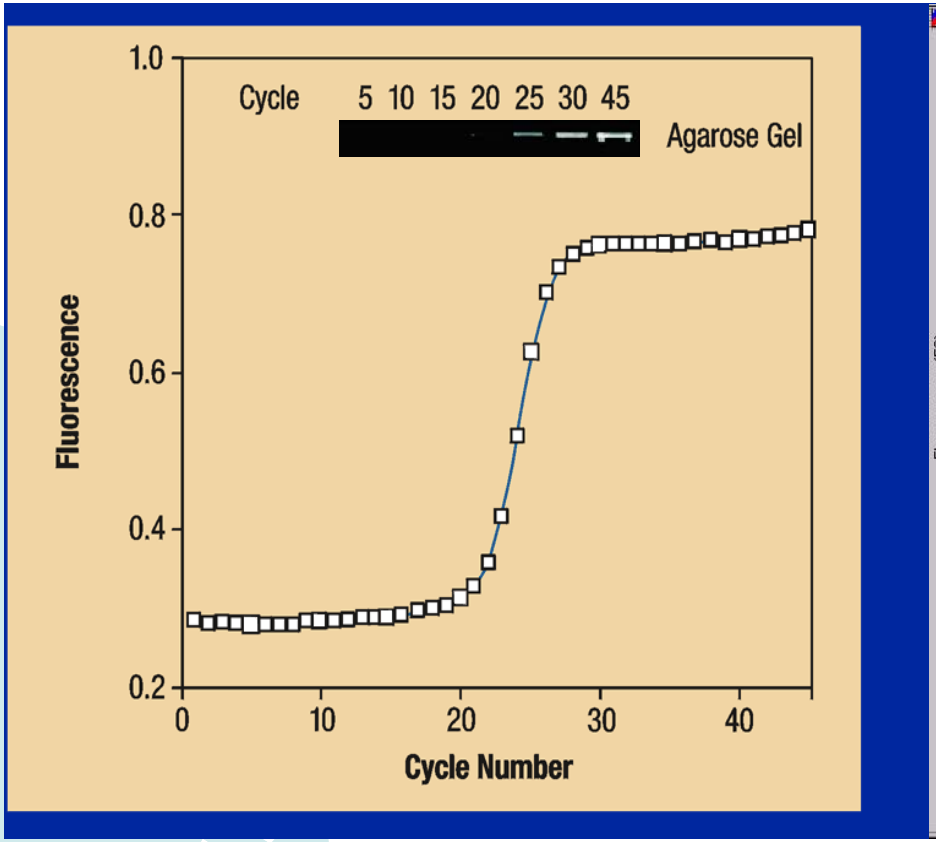
Post amplification & detection
Endpoint detection



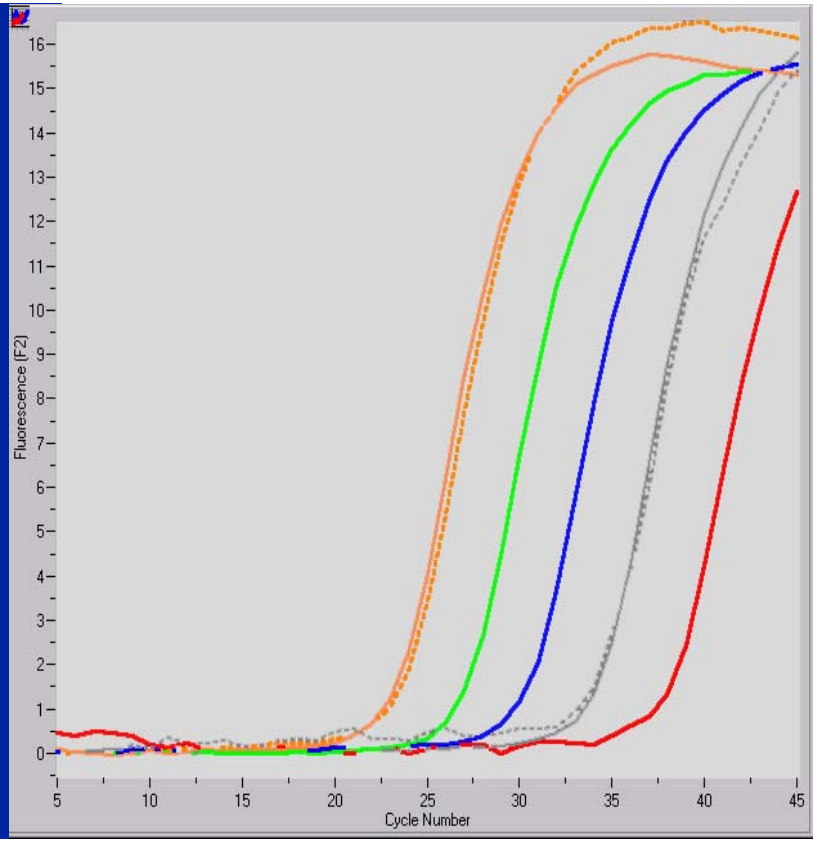


- Volumetric error
- Fragment size vs. probe hybridisation
- Time to result
- Automation calibration issues
- Result calculations

Monitoring in Real time



Agarose Gel Blotting



FRET

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Real time PCR

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QIAGEN

Roche
Diagnostics

the New m2000
RealTime PCR System

Abbott
A Promise for Life

SIEMENS

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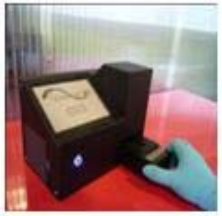
Point-of-Care Viral Load and EID Technologies in the Pipeline*

Liat



WAVE 80
EOSCAPE

Micronics

ALL

Alere

NWGHF EID

SAMBA VL

SAMBA EID

NWGHF VL

GeneXpert

Biohelix

2012

2013

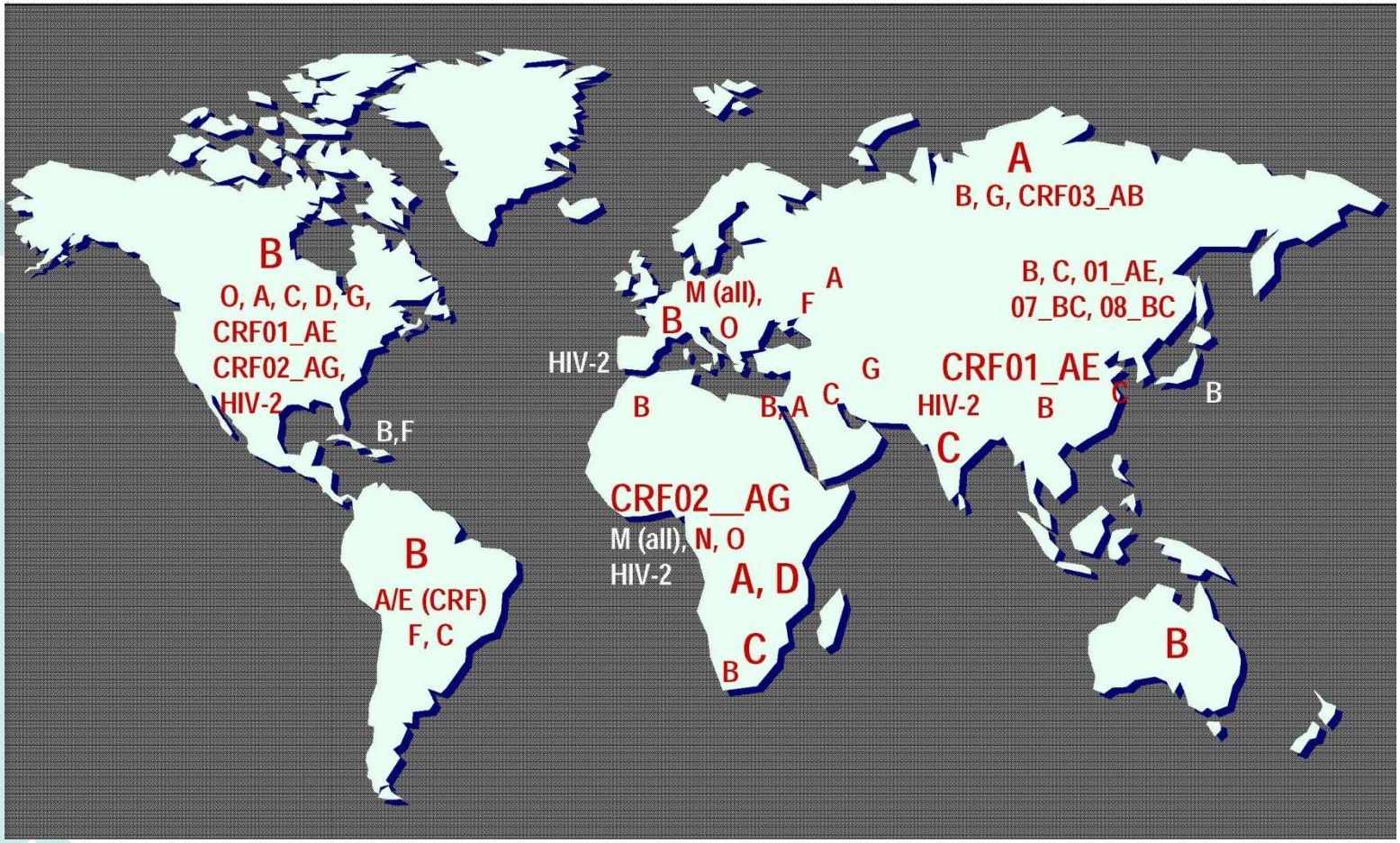
2014

2015

*Estimated; timeline and sequence may change.

Geographic distribution of HIV

Subtype B: represents ~10% of HIV-1 infections worldwide



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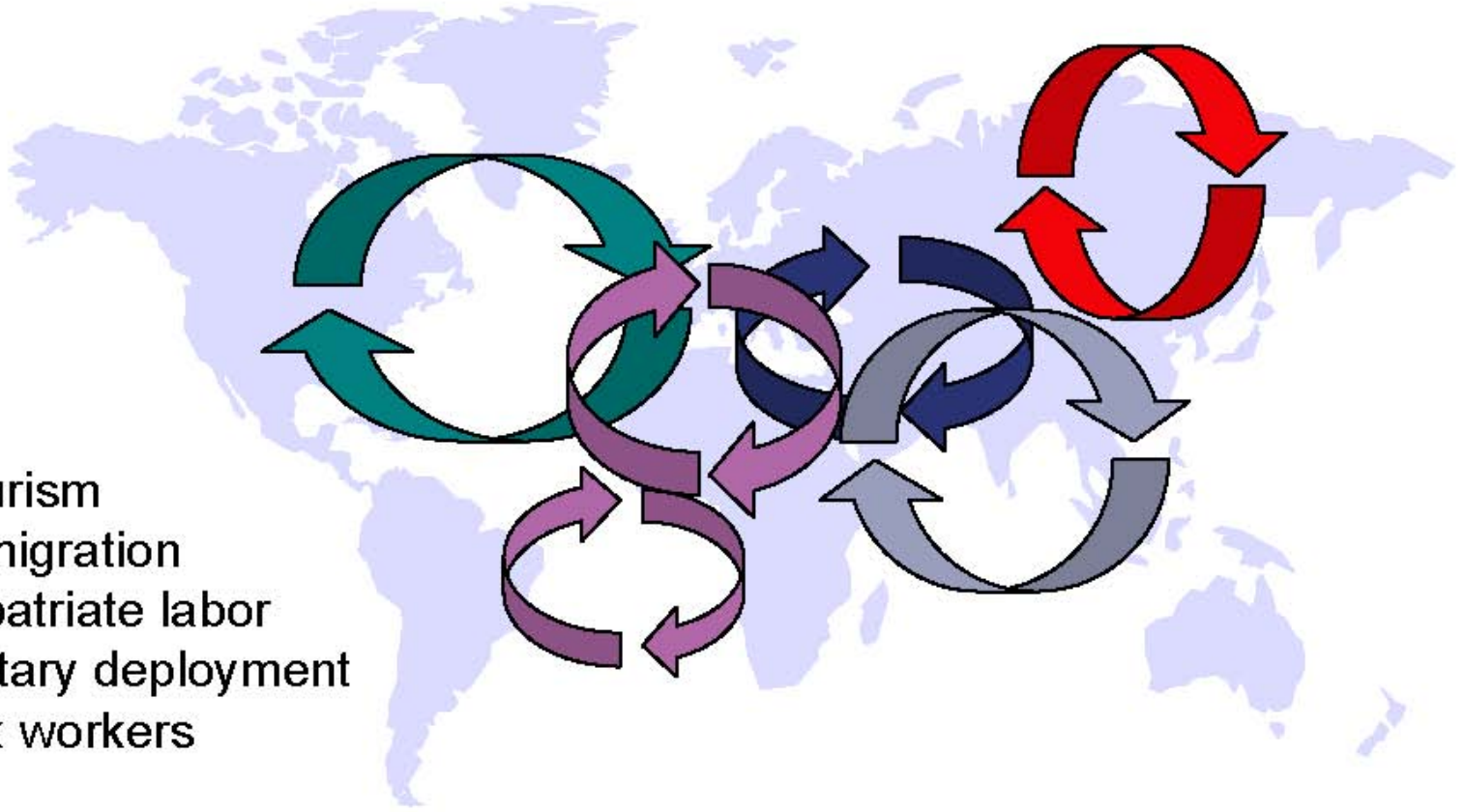
Increasing Spread of HIV Diversity

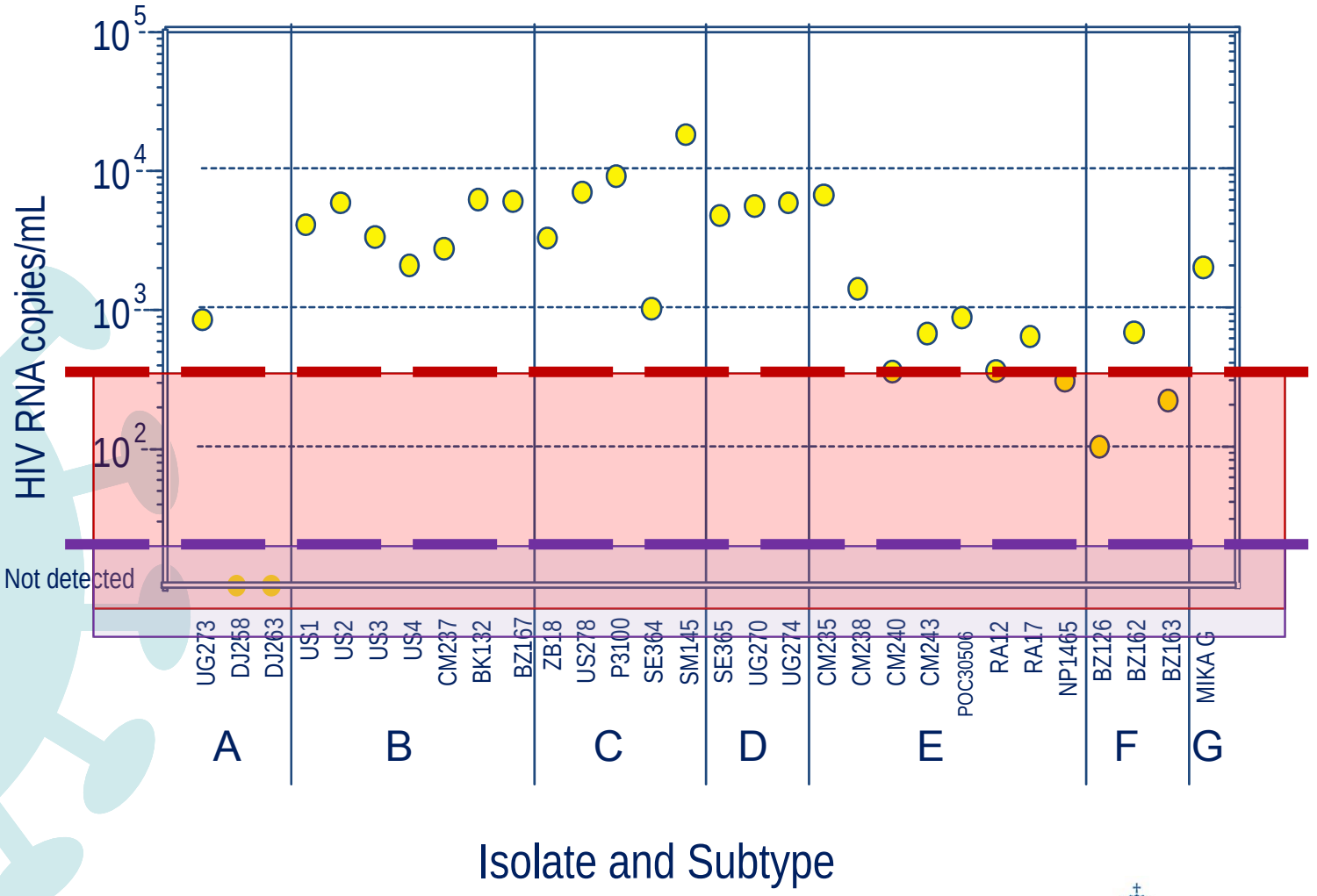


- Tourism
- Immigration
- Expatriate labor
- Military deployment
- Sex workers

Continual redistribution of HIV variants

Courtesy: Dr John Hackett

Qualitative vs quantitative assays



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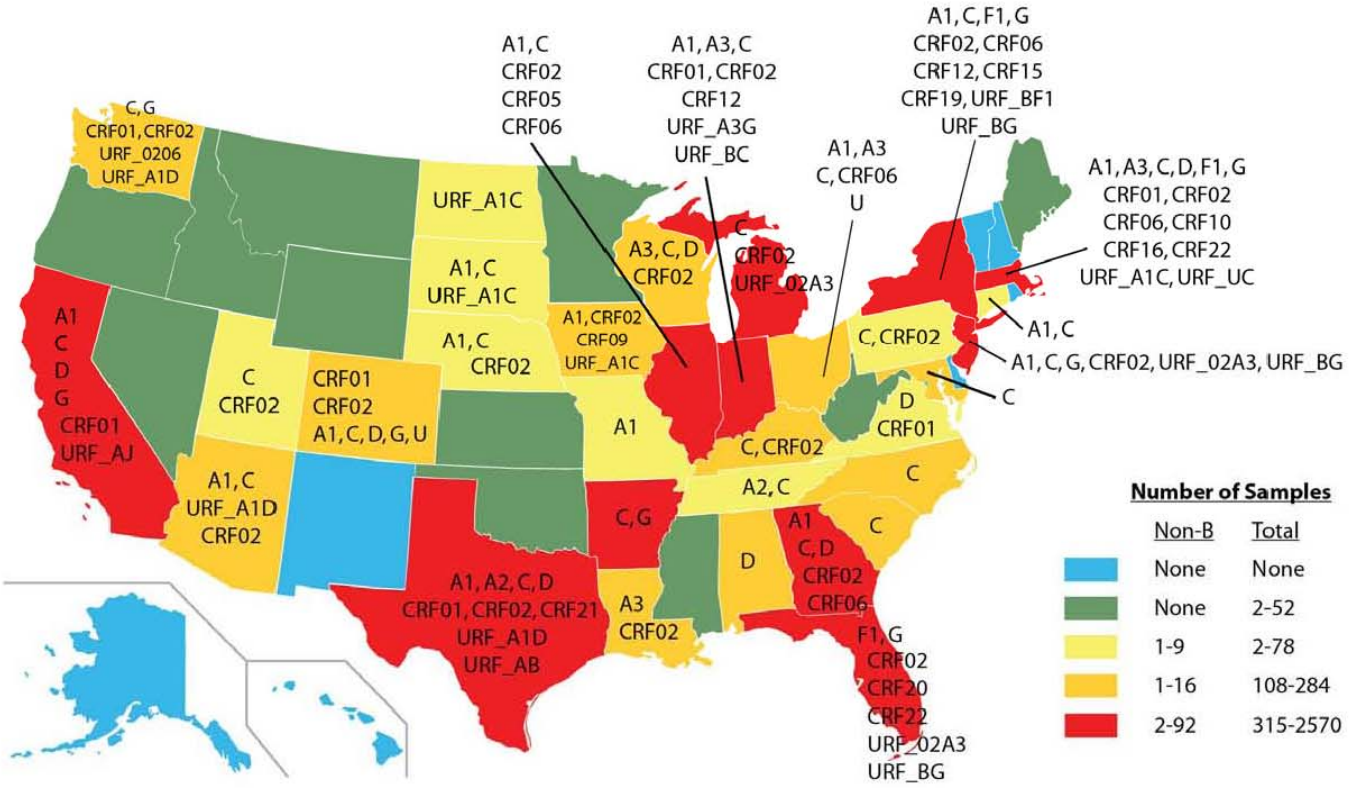
HIV subtype distribution in USA

- *n* = 12,650; 43 states
- 392 non-B strains (3.1%; 32 states)
- 5 subtypes; 13 CRFs; 24 URFs

Pyne *et al* 16th CROI (2009); 292
Data thru Sept 09



Geographic distribution of non-B subtypes. Samples were received from 43 states, 32 of which included non-B subtype samples. The non-B subtypes are listed for each state. The colors indicate the total number of samples received and the number of non-B samples received from each state.

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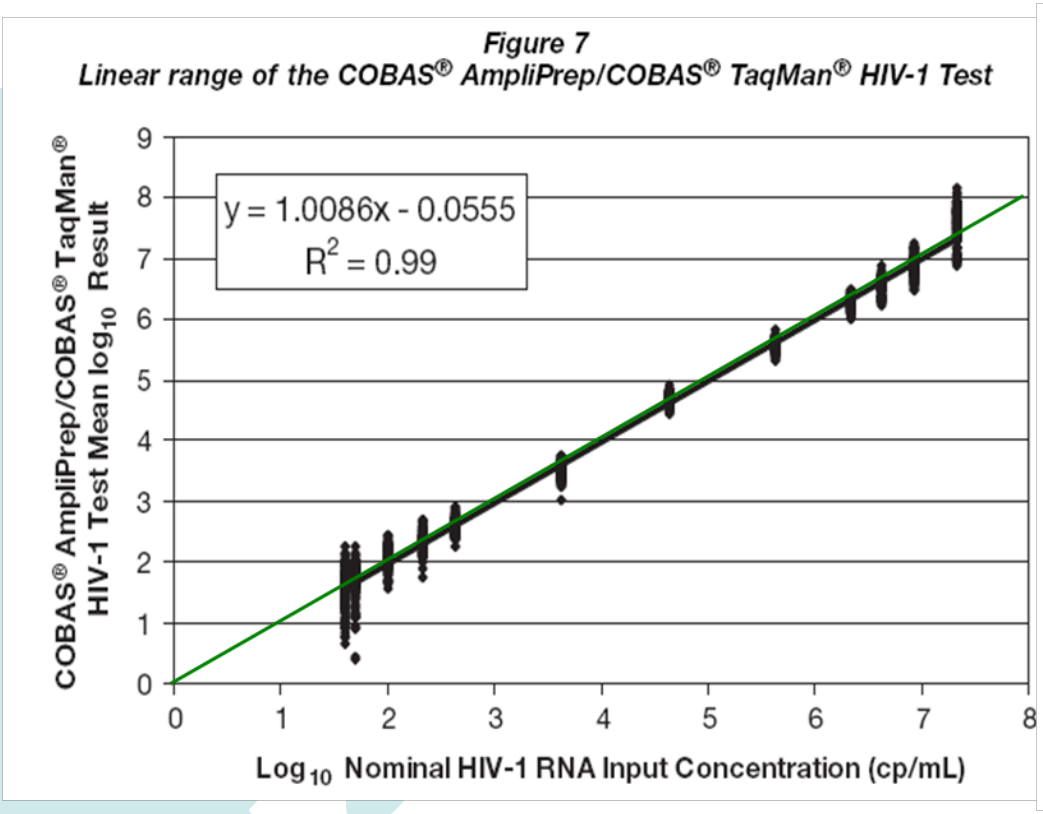
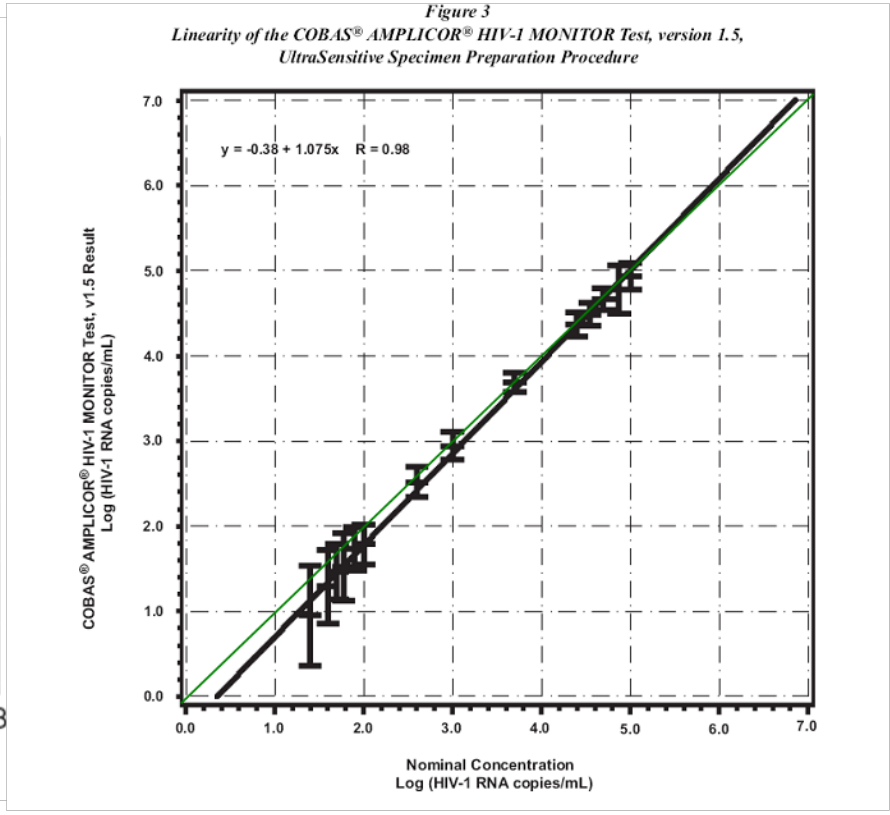
Real time versus end-point detection

COBAS Taqman vs COBAS Amplicor HIV MONITOR

Accuracy at lower limit of detection





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Roche Product Bulletin Sept 2008

Real time versus end-point detection

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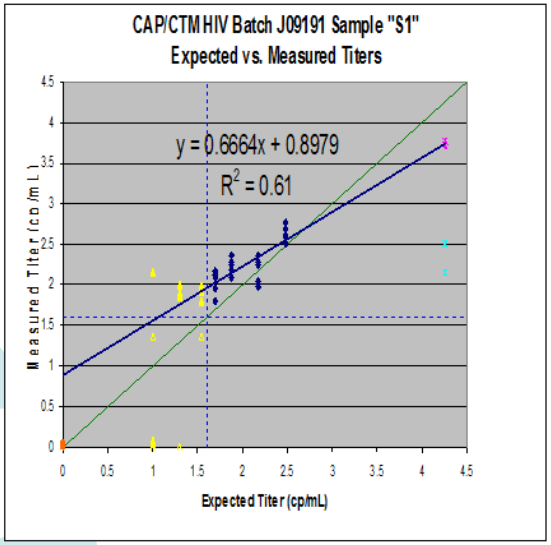

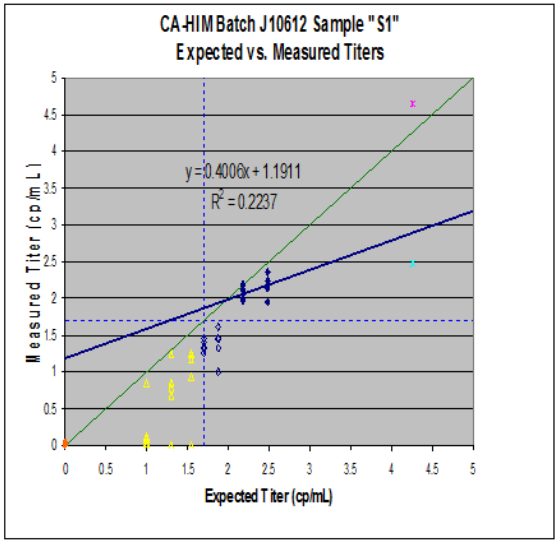

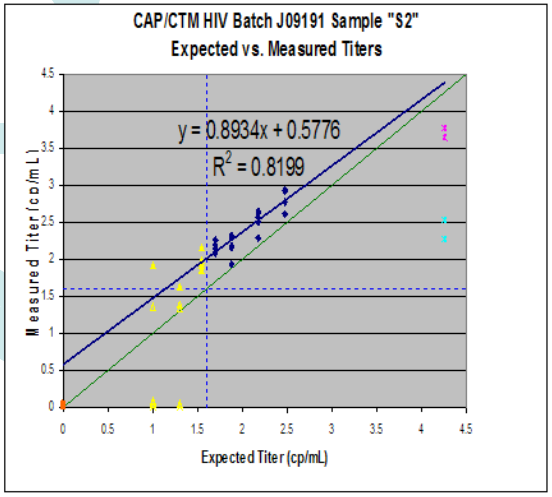

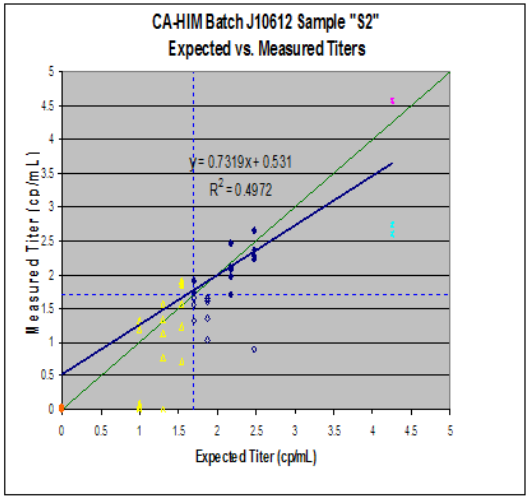

Source: Roche Molecular

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Early infant diagnosis – Qualitative HIV DNA testing

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Diagnostic Testing in Infants

- In infants <18 mos, use virologic assays that directly detect HIV: HIV DNA PCR or HIV RNA (AII)
  - Persistent maternal antibody give false positive antibody results
- Virologic testing of HIV-exposed infant (AII)
  - 14-21 days of age
  - 1-2 months
  - 4-6 months
- Virologic testing at birth should be considered for infants at high risk of infection (BIII)
- HIV antibody test
  - 12-18 months to document seroreversion in HIV-uninfected infants (BIII)
  - Diagnostic test for children ≥18 months (AII)

Criteria for HIV Diagnosis

- 2 positive HIV virologic tests on separate blood samples (regardless of age) (AII)
- Positive HIV antibody test with confirmatory Western blot (or IFA) at age ≥18 months (AII)

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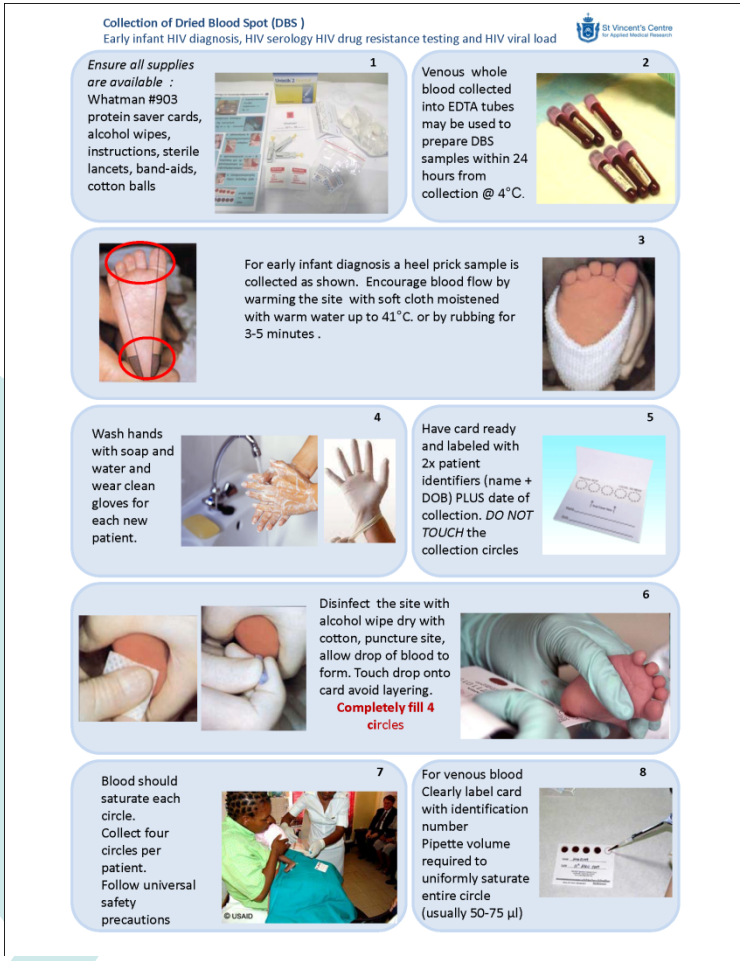
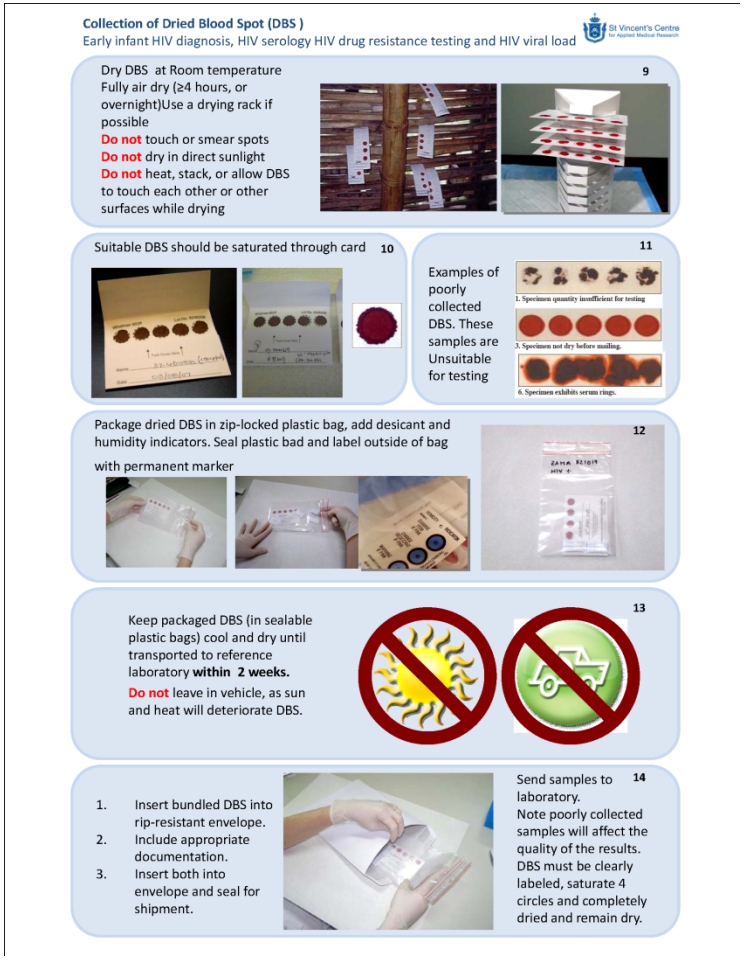
Development of a DBS collection SOP





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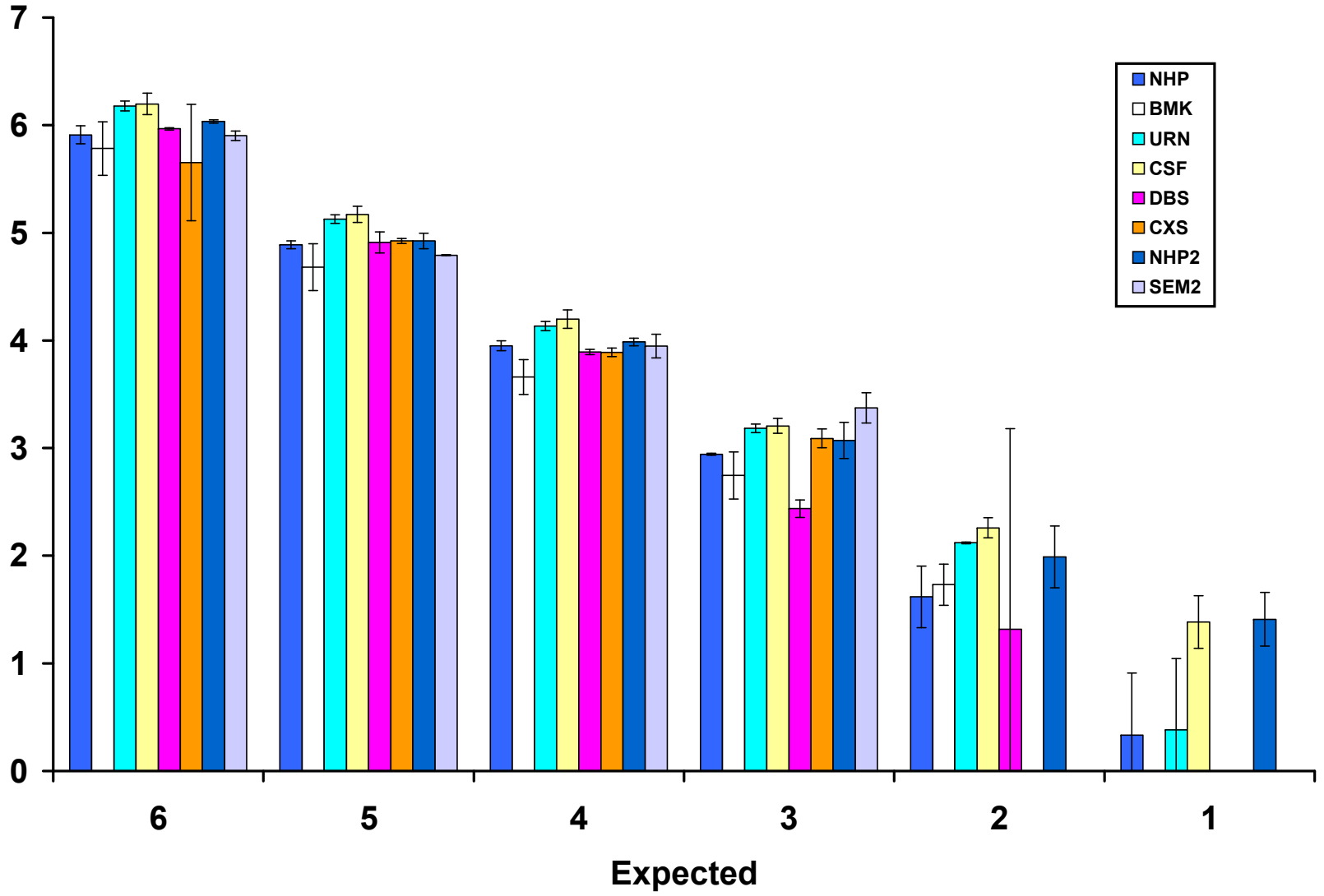
DBS for HIV viral load?



A Loftis, R Kshatriya, K McCall-Culbreath, S Fiscus and J Nelson. IAS 2009

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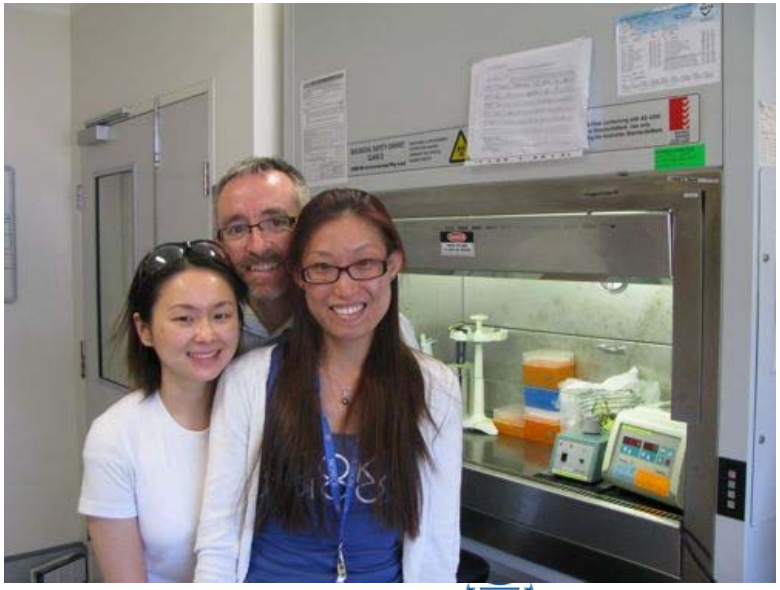

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