

The role of HIV testing in controlling the HIV epidemic



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Deputy Director

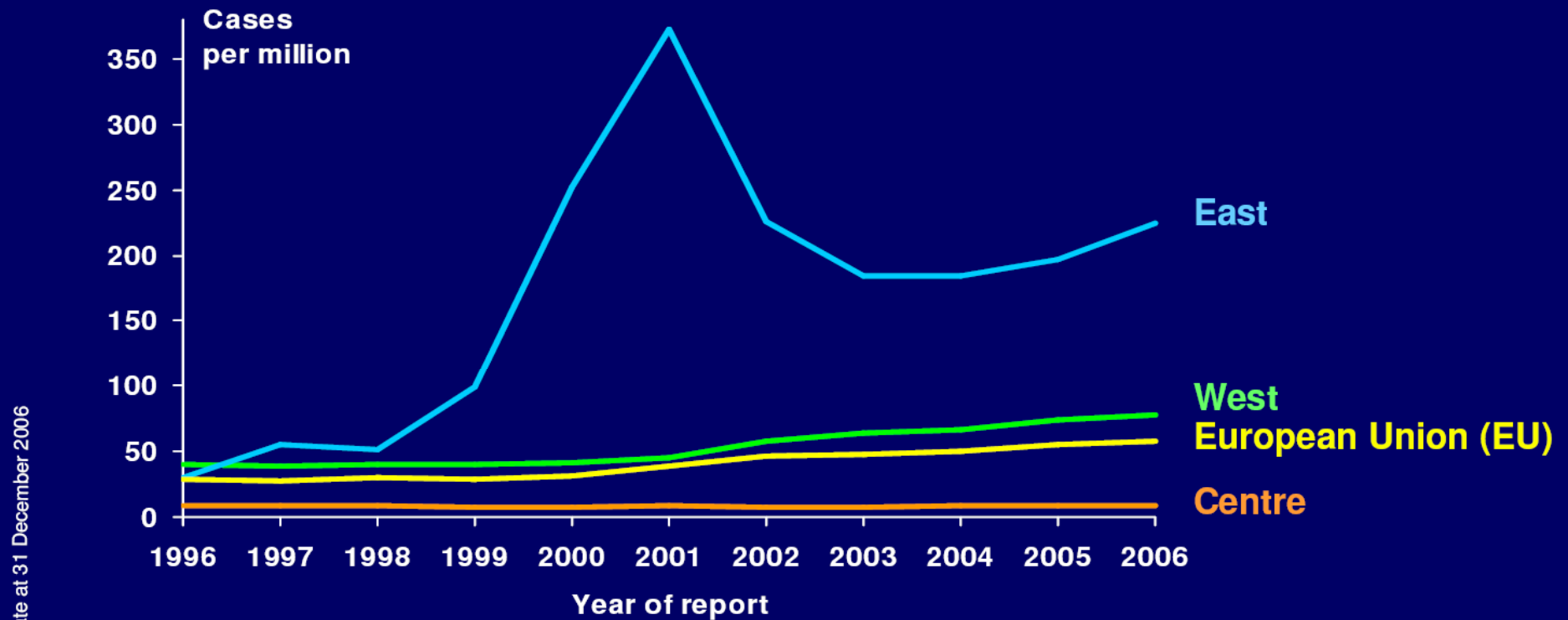
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Health Protection Agency Centre for Infections
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University of London

- **Background epidemiology**
- **Why HIV testing is a key intervention**
- **Early detection of primary HIV infection**
- **Confirmatory testing**
- **Unusual cases**

Key signposts from HIV epidemiology

Newly diagnosed HIV infection rate (/10⁶) in Europe (1996-2006)

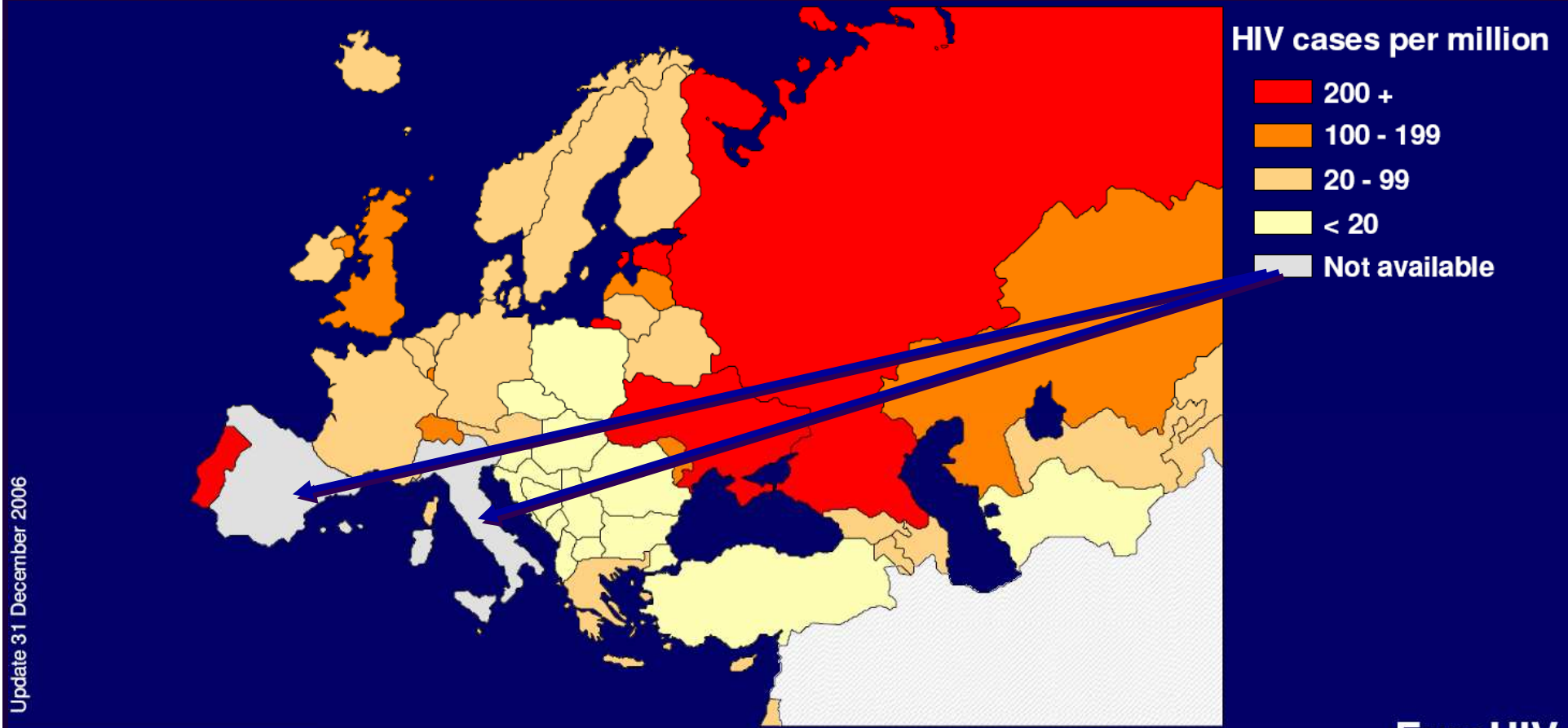


Update at 31 December 2006

EuroHIV

* Countries excluded (data not available for the whole period): West: Andorra, Austria (EU), France (EU), Greece (EU), Italy (EU), Malta (EU), Monaco, Netherlands (EU), Norway, Portugal (EU), Spain (EU); East: Uzbekistan

Newly diagnosed HIV infection rate (/10⁶) in Europe (2006)

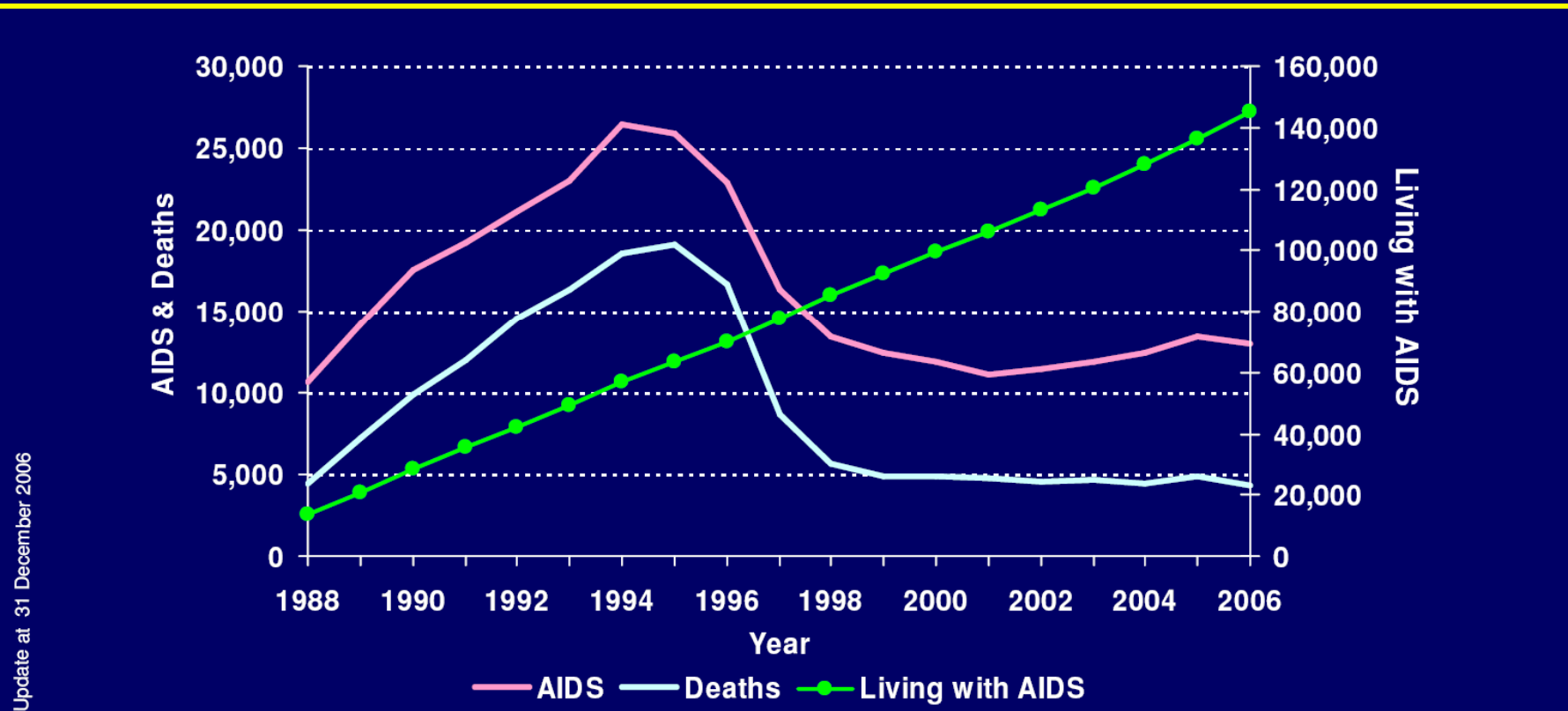


EuroHIV

The increasing burden of HIV care (1988 – 2006)



AIDS cases, deaths, and persons living with AIDS, by year, 1988-2006, WHO European Region*



EuroHIV

Data adjusted for reporting delays

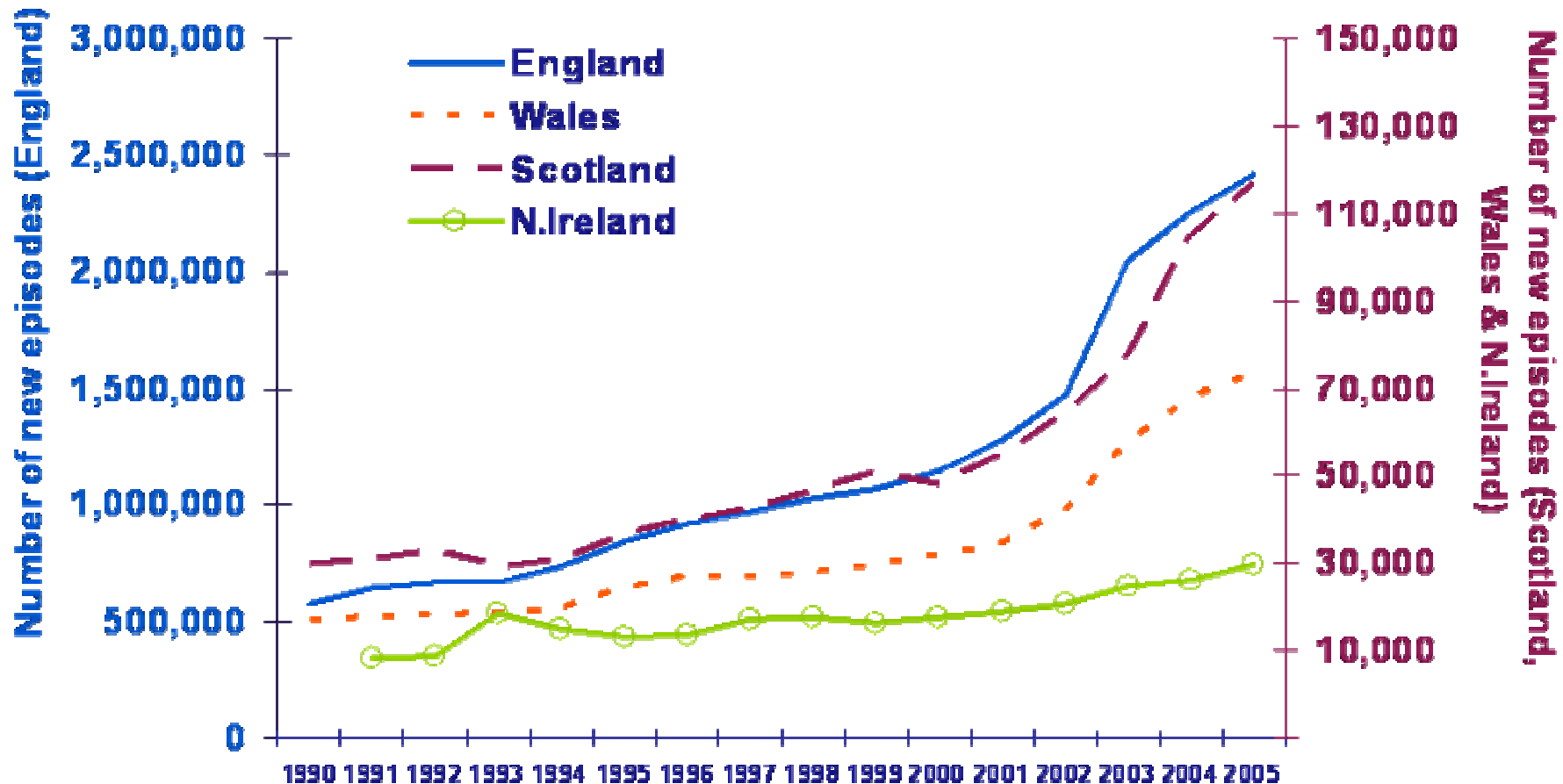
* Excluding Andorra, Azerbaijan, Monaco, Netherlands, Uzbekistan: data not available for the whole period

Why does the HIV epidemic continue to grow, more or less unabated?

Why does the HIV epidemic continue to grow at pace?

- increasing risk behaviour

1.

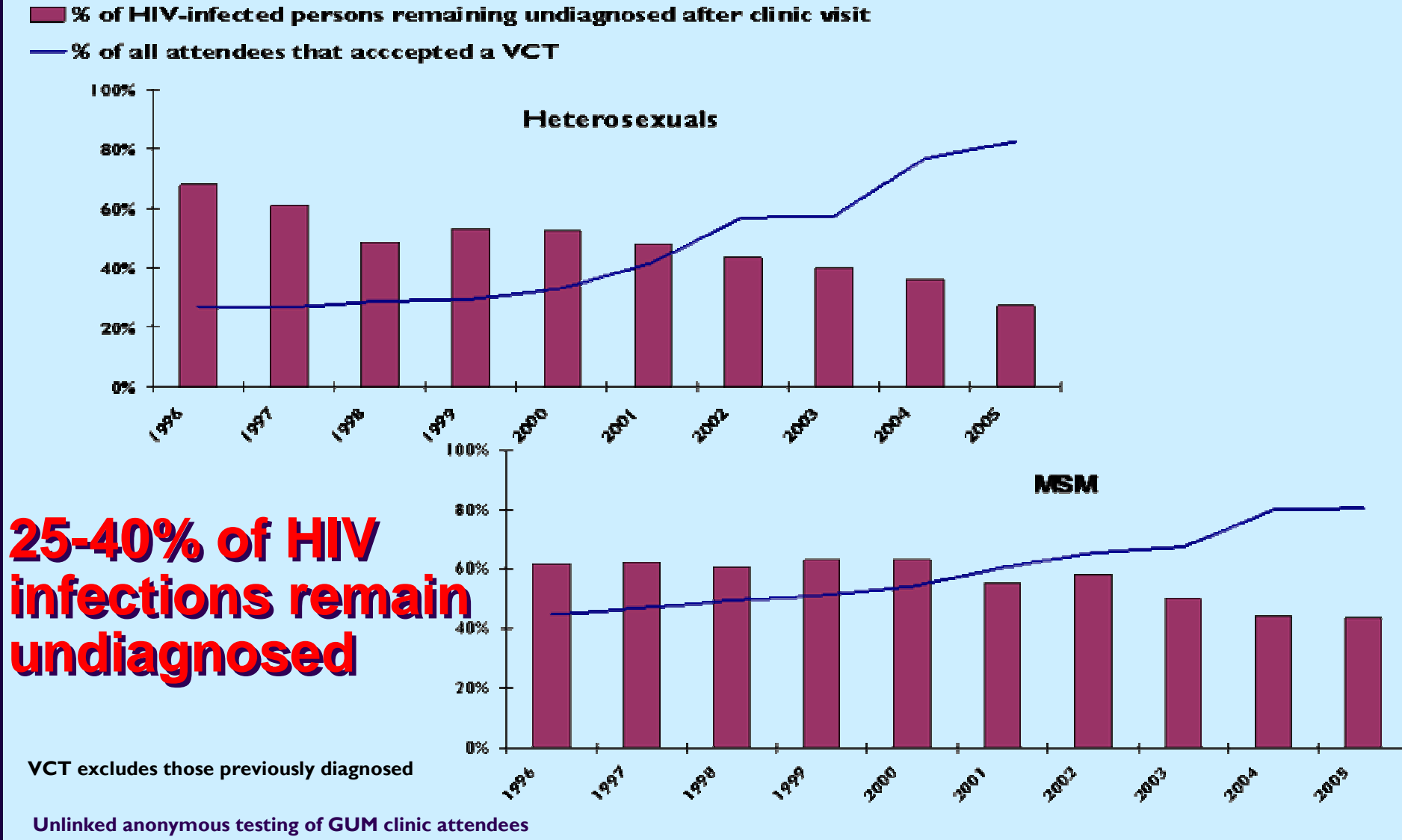


* Data are unavailable for Northern Ireland in 1990
Data source: KC60 statutory returns and ISD(D)5 data.

Why does the HIV epidemic continue to grow at pace?

- increased VCT uptake, but

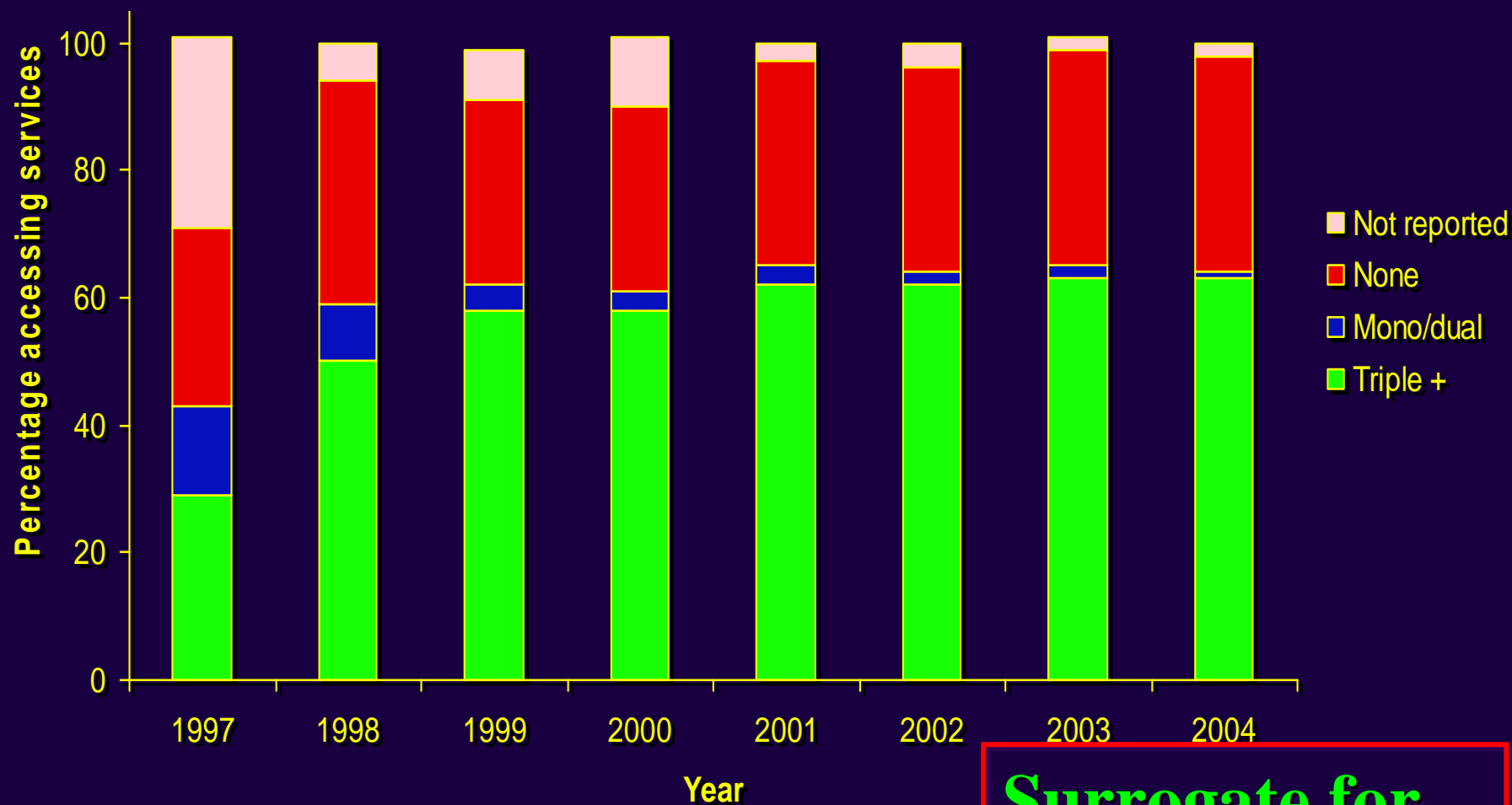
2.



Why does the HIV epidemic continue to grow at pace?

~60% of infections require HAART....

3.



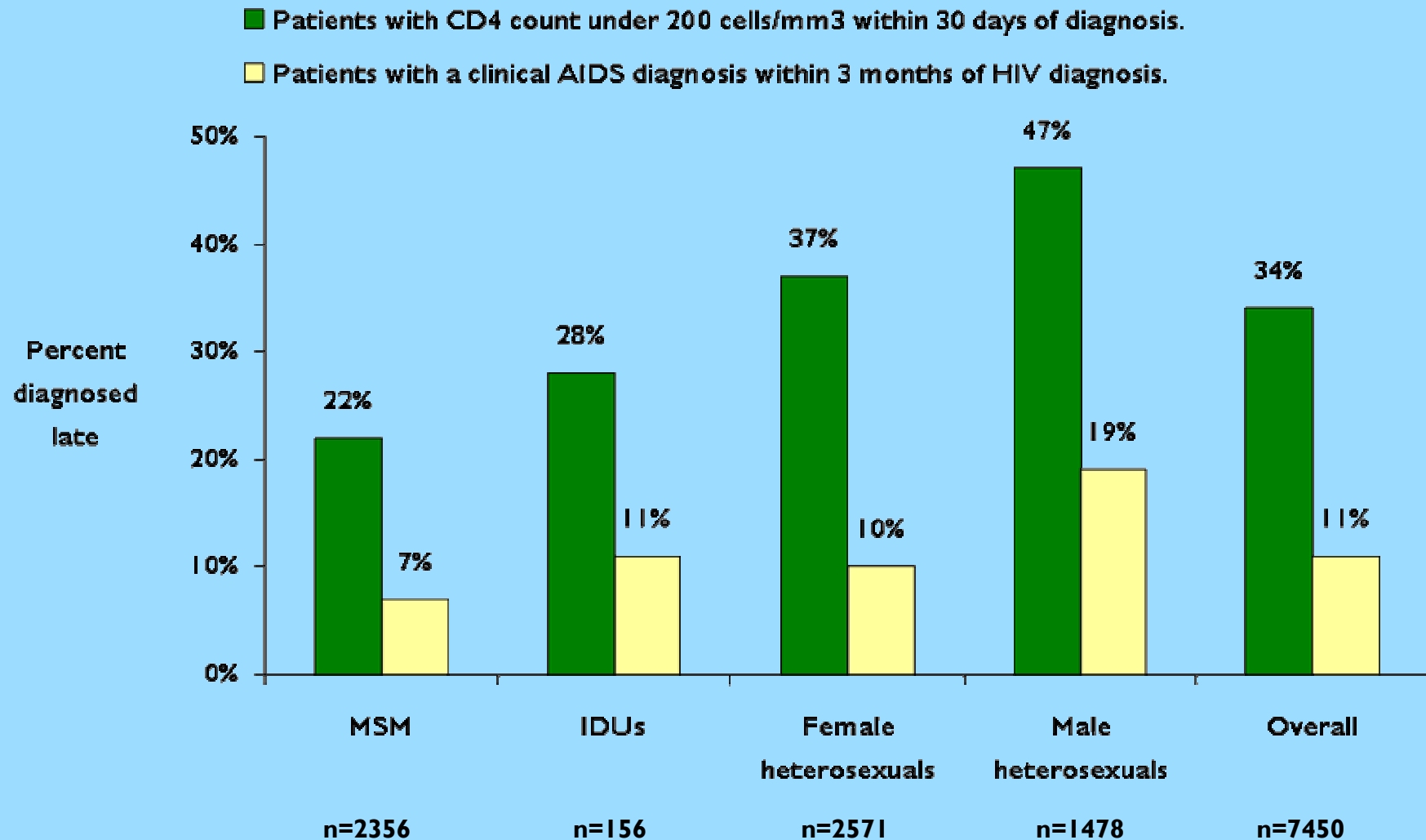
Data source: SOPHID and CD4 Monitoring, United Kingdom 2004

Surrogate for high infectivity

Why does the HIV epidemic continue to grow at pace?

HIV infection is often diagnosed late

4.



Reports of HIV/AIDS diagnosis and CD4 Surveillance

Summary of drivers of on-going HIV transmission



- **High risk behaviour continues at high rates**
- **High rate of STIs**
- **~30% HIV undiagnosed**
- **20-50% late diagnoses**
- **Up to 60% undiagnosed highly infectious**

Increased HIV testing is an essential intervention



- **No vaccine!**
- **Behavioural change**
- **Others, eg. male circumcision, microbicides?**
- **Post- sexual exposure prophylaxis**
- **Therapy**
- **Prevention of mother-to-child transmission**

- ✓ **Effective interventions need diagnosis of HIV**
- ✓ **Improved access to HIV testing is essential**

The Value of early HIV diagnosis



NO tests

1st gen Tests
blood safety,
epidemiology;
no therapy;
stigma;
'death sentence'

Better tests
blood safety,
epidemiology;
mono therapy

3rd gen tests
dual therapy
Role of STIs in
transmission

HAART
Treatable disease;
Reduced infectivity'
?reduced incidence?
'clear' personal and PH
benefits of testing

4th gen tests – earlier diagnosis
PEP to contacts
Rebound effect
Increased risk-taking;
Treatment failure & resistance
→ Increased incidence
→ Erosion of PH benefit

1980

1985

1990

1995

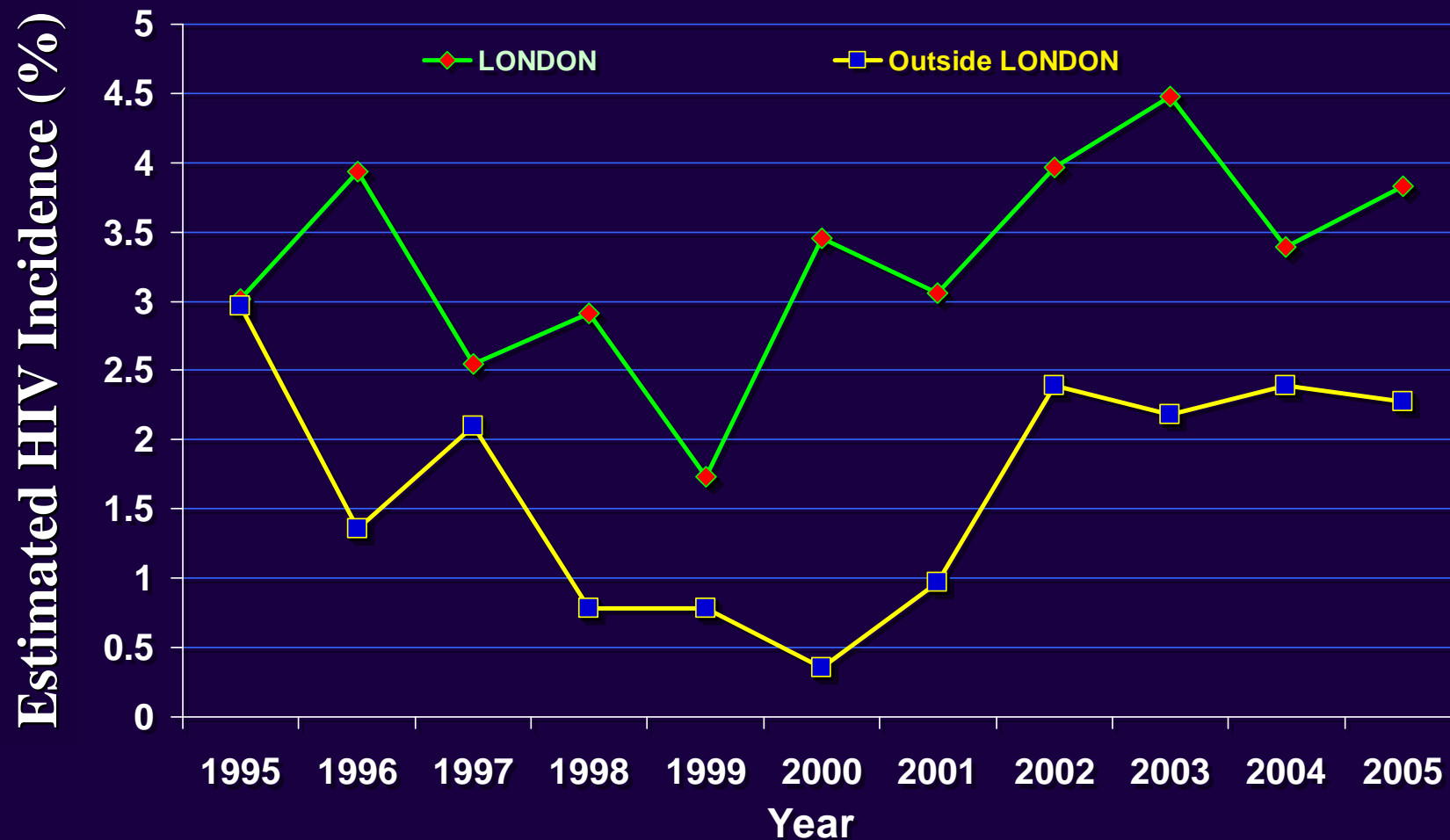
2000

2005

HIV Incidence in MSM attending STI clinics: **BY REGION**



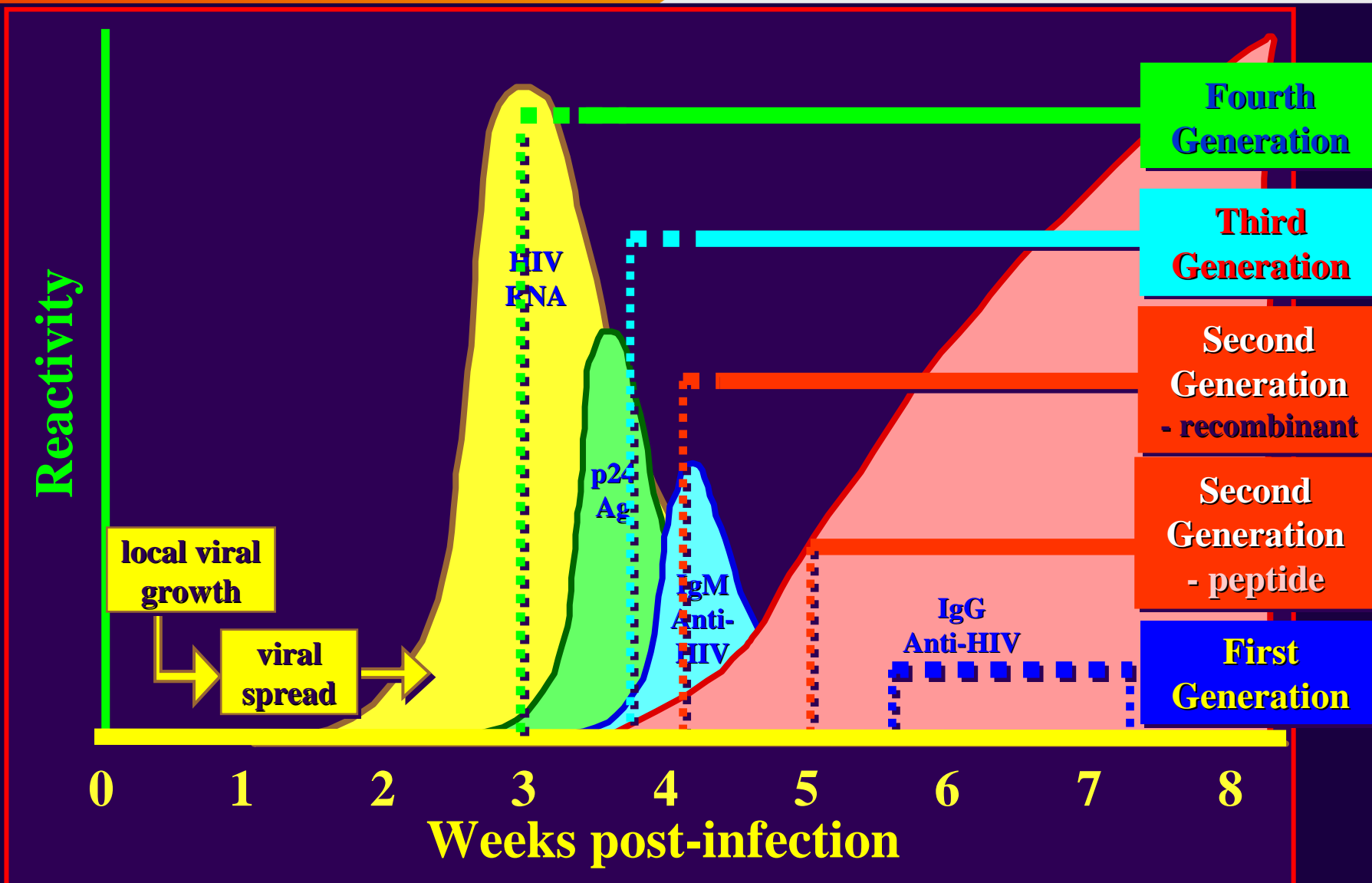
Trend in HIV incidence in MSM attending STI clinics



Early Detection of HIV Infection

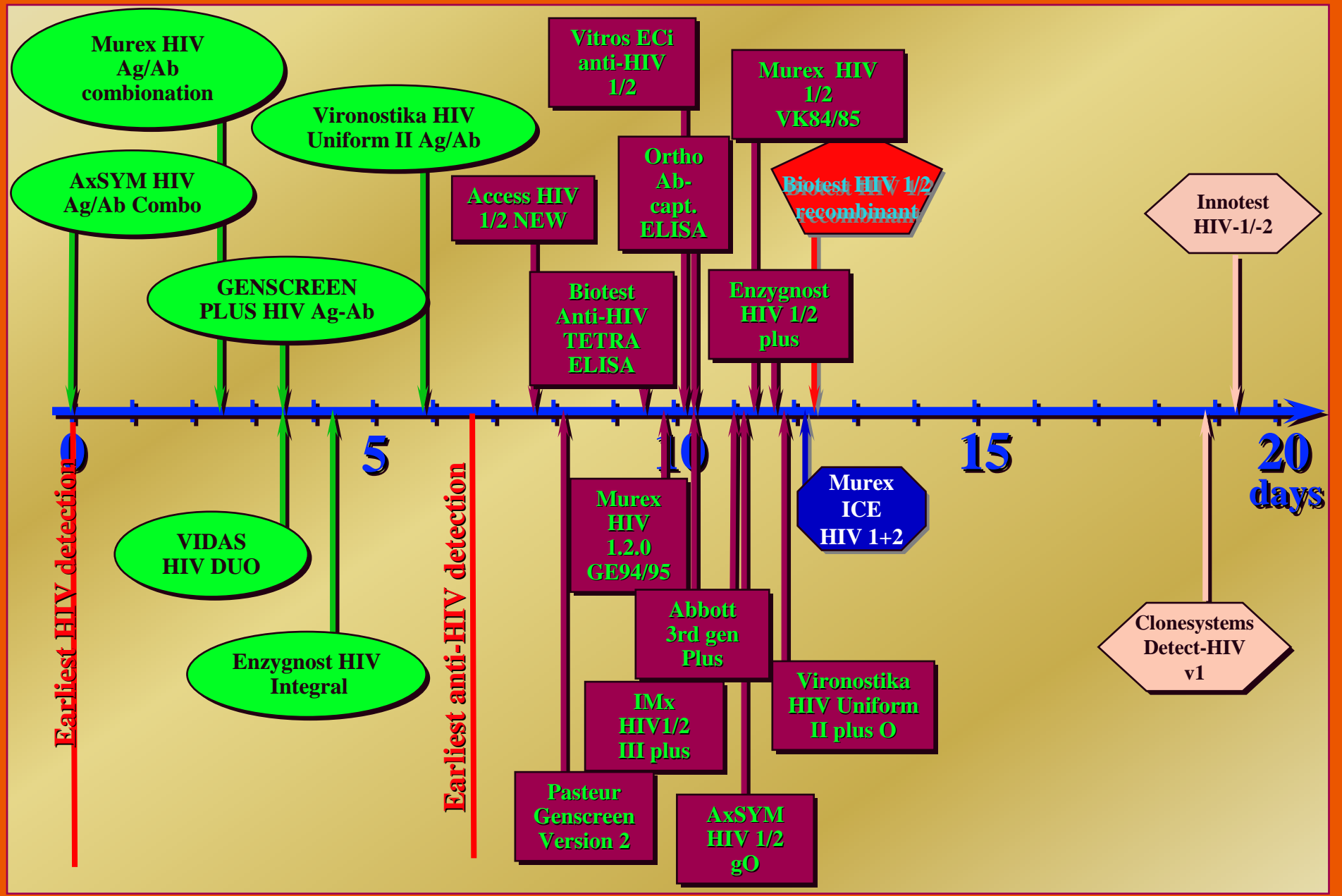
**a key aspect of control
& prevention**

Development of Markers of Primary HIV Infection & HIV Screening Test Performance



Understanding Applied to Serological Detection of Primary HIV Infection

(35 Seroconversion Panels)



PCR Investigation of HIV serology negatives

(*Pilcher et al, NEJM 2005*)



109,250 eligible serum specimens



Screened **NEGATIVES** by PCR in pools of 90 sera

(9 pools of 10)



23 confirmed HIV RNA positive

2 HIV RNA false positives

583 confirmed new anti-HIV positive



107 recent HIV by detuned test

Outcomes:

Increased detection of recent HIV by ~20%

Increased new diagnoses by ~4%

Costs:

Per specimens processed \$3.63

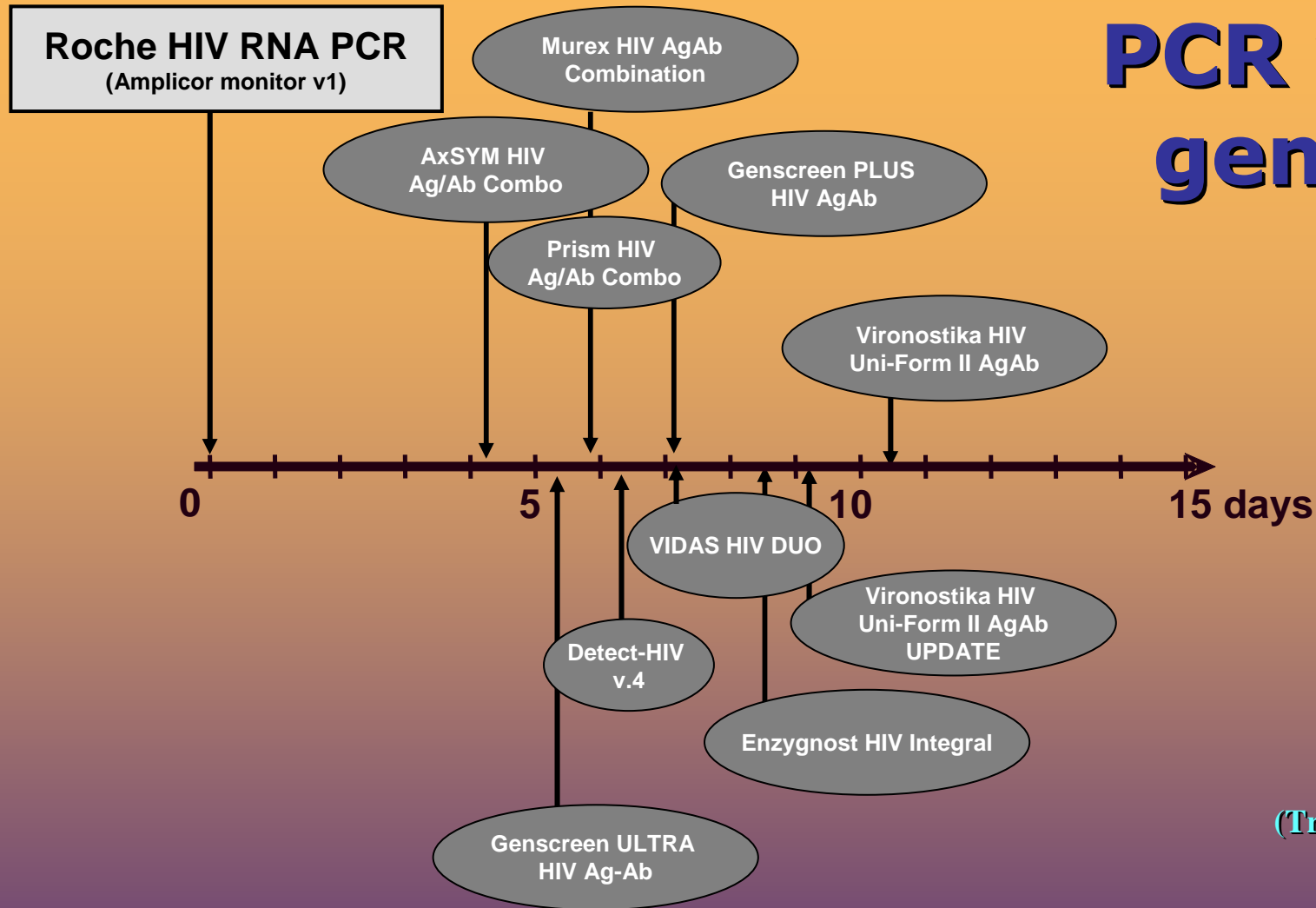
Per additional diagnosis \$17,515

↓ ↓ cost-effective in EU

Long-term benefits ?

Public health benefits ?

PCR vs 4th gen EIAs



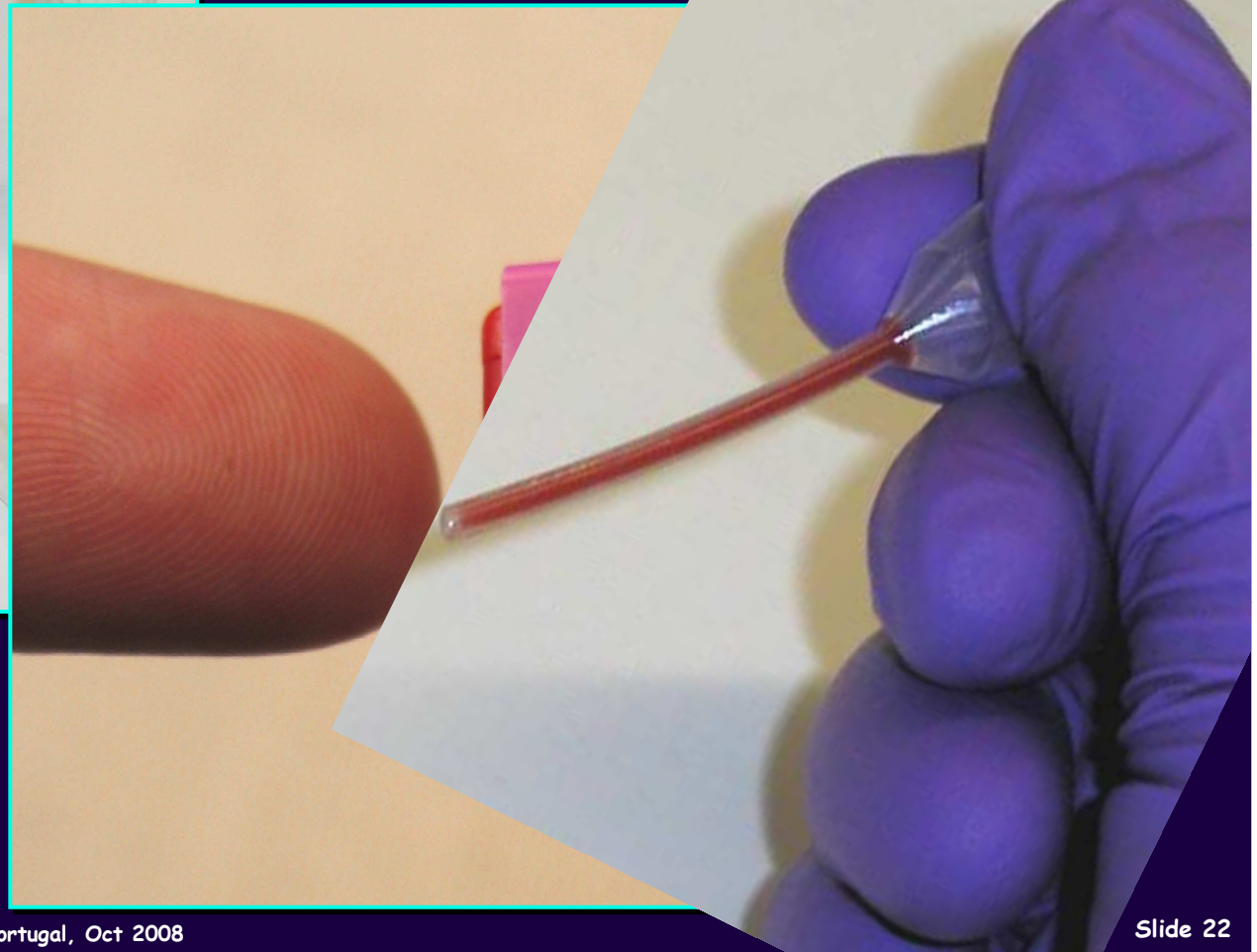
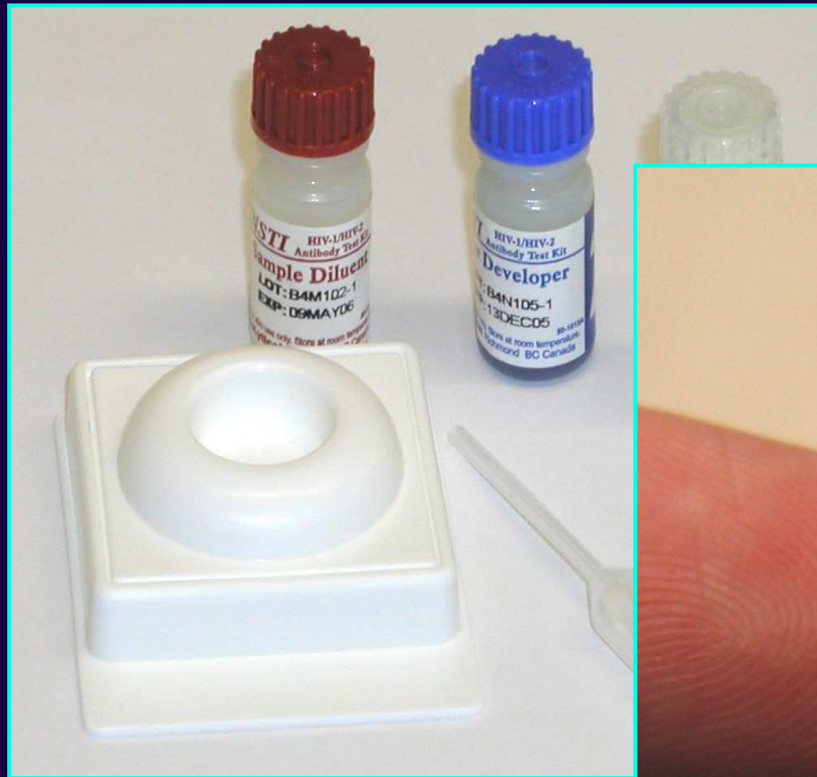
Perry KR, et al
(Transfusion Medicine,
2008)

Based on data generated by testing 10 seroconversion panels in each of the HIV kits shown.

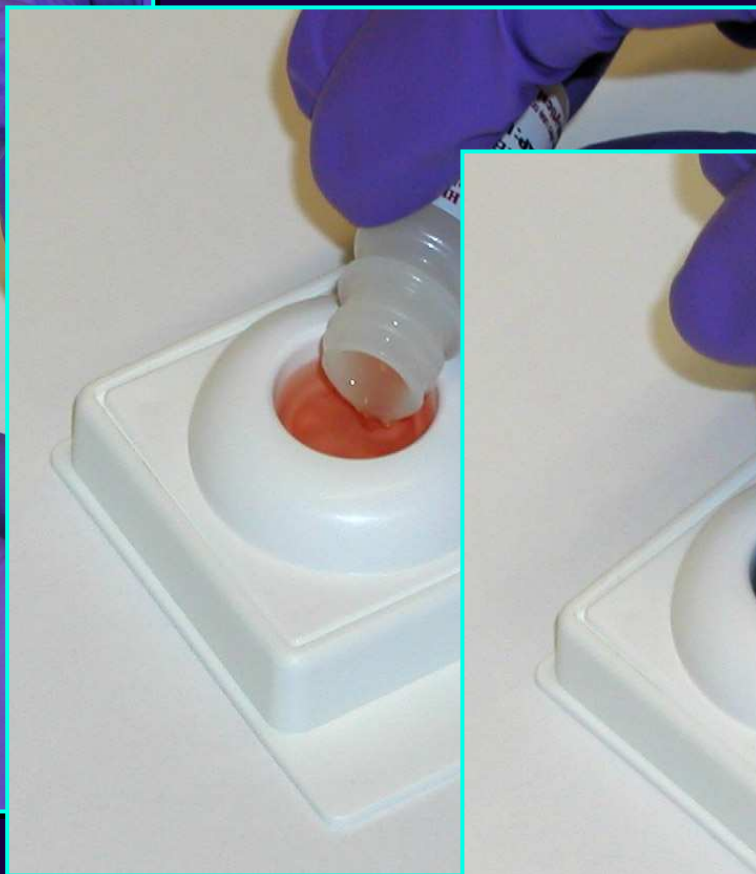
○ = Combined Ag/Ab assay; □ = Roche Amplacor monitor v1 (83088); BBI data

Point-of-Care Testing

Point-of-Care Test (POCT): Materials



Point-of-Care Test (POCT): Processing Test..... 1



Add Developer

Point-of-Care Test (POCT): Processing test..... 2



Quality System is **ESSENTIAL**, but challenging

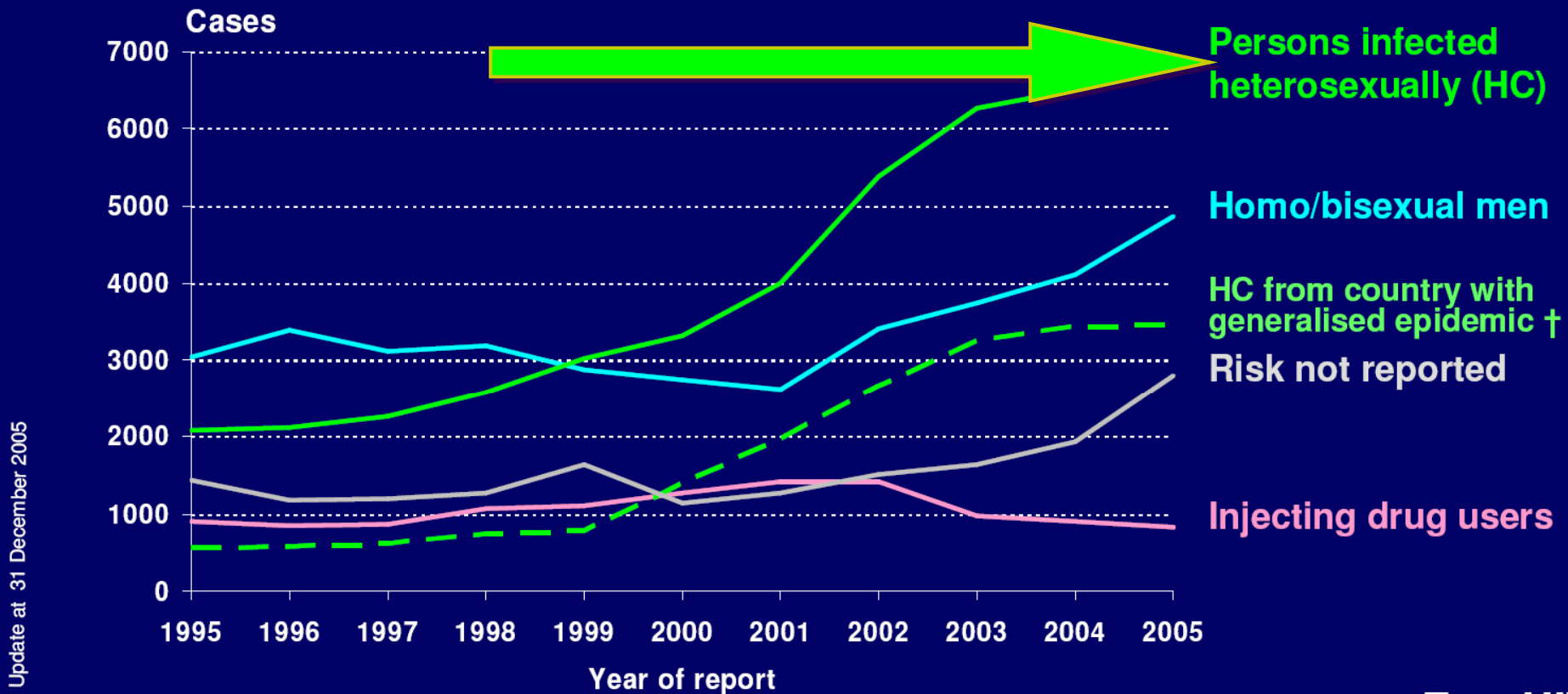


- **Standard operating procedures**
 - **Performing the Test**
 - **Interpreting the result**
 - **Counselling**
 - **Giving results**
 - **Confirmatory procedures**
 - **Referral of client/ patient**
- **Test selection (quality manufacturer)**
- **Training**
- **Records**
- **Batch acceptance testing**
- **Quality control**
 - **Kit controls**
 - **External controls**
- **Performance assessment**

What about 'over-the-counter' home HIV tests?

Mother-to-Child Transmission (MTCT) of HIV

Within the EU the greatest number of new HIV diagnoses are among heterosexuals.....



EuroHIV

* Countries with data available for the whole period: Belgium, Cyprus, Czech Republic, Denmark, Finland, Germany, Greece, Hungary, Ireland, Latvia, Lithuania, Luxembourg, Poland, Slovakia, Slovenia, Sweden, United Kingdom.
 † Countries excluded (data not available for the whole period: Ireland, Malta, Poland, Slovenia)

When does MTCT of HIV occur ?



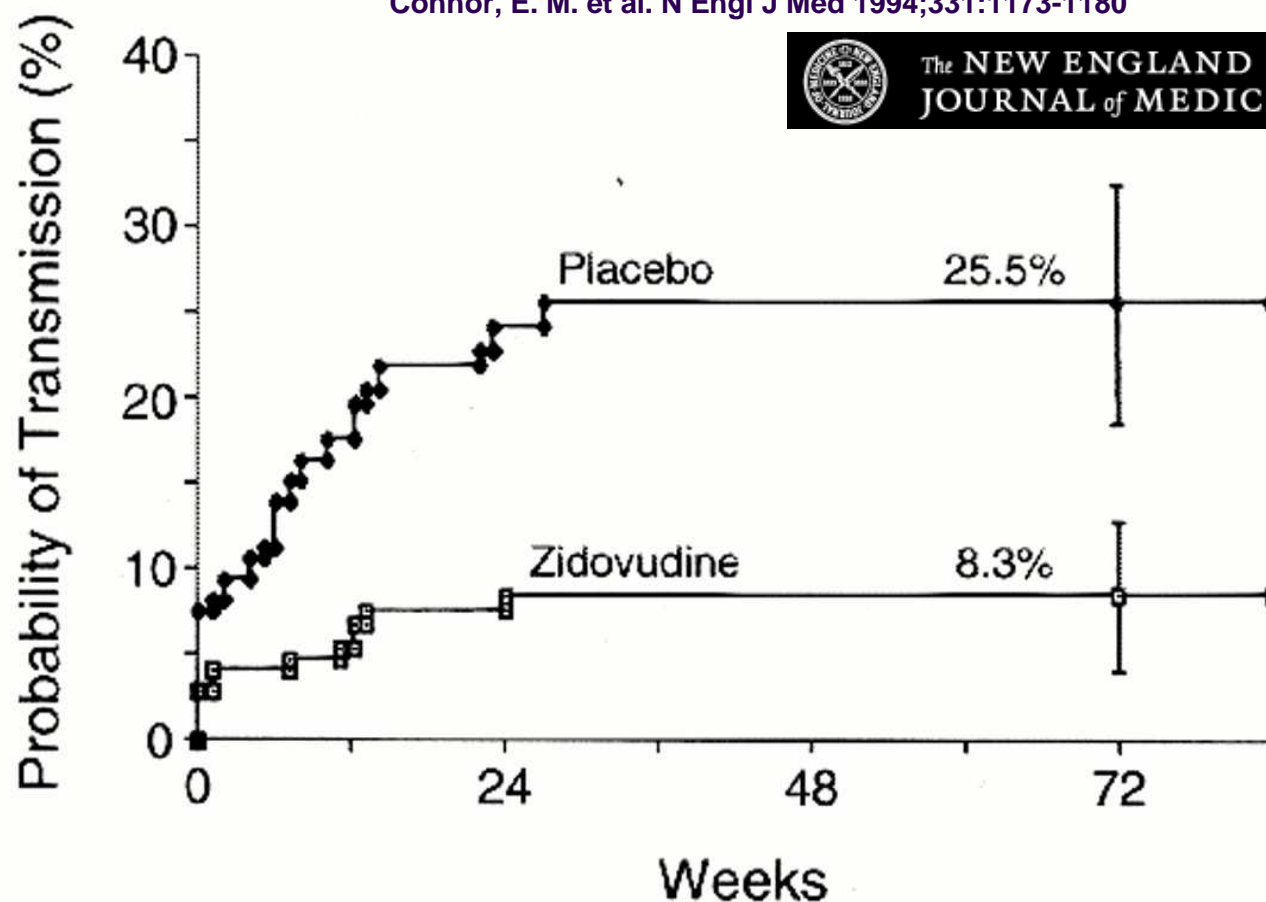
Without interventions 30-50% transmission

- ***in utero*** **5-10%**
- **Peri-natally** **~ 40%**
- **Post-natally** **~ 50%**

Interrupting HIV MTCT by ART: proof of concept - ACTG 076 (2)



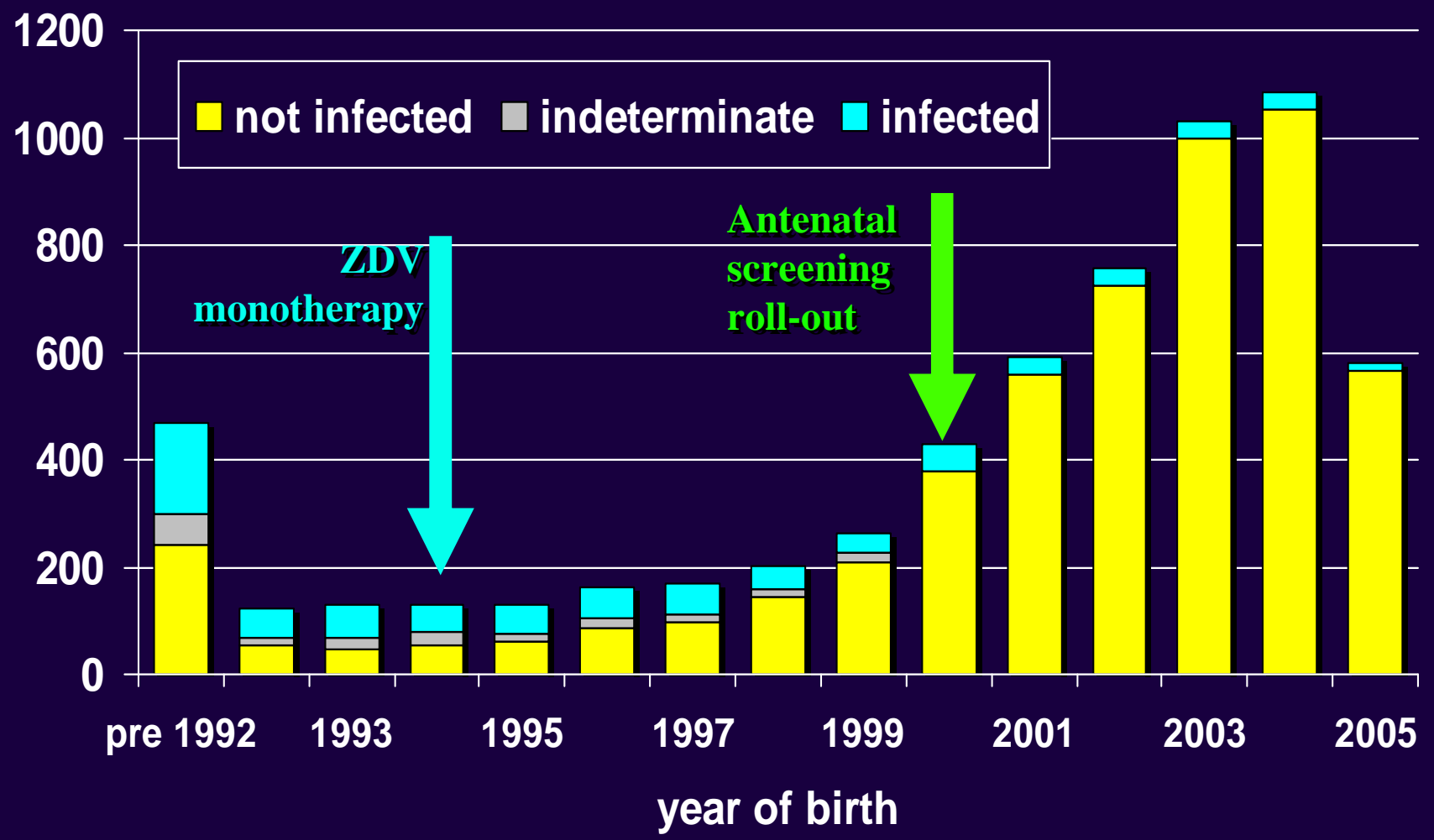
Connor, E. M. et al. N Engl J Med 1994;331:1173-1180



Placebo	183	84	42	37
Zidovudine	180	105	51	43

Children born in UK & Ireland to HIV infected women

reported by end of September 2005, likely infection status



Getting the correct result: **Confirmatory Testing**

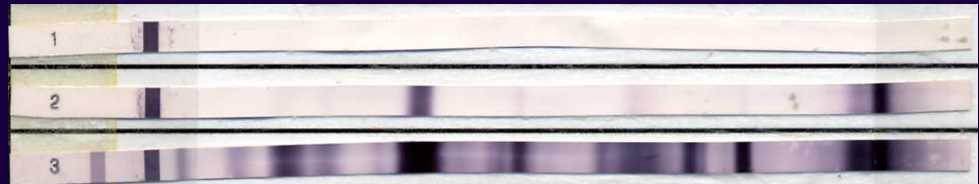
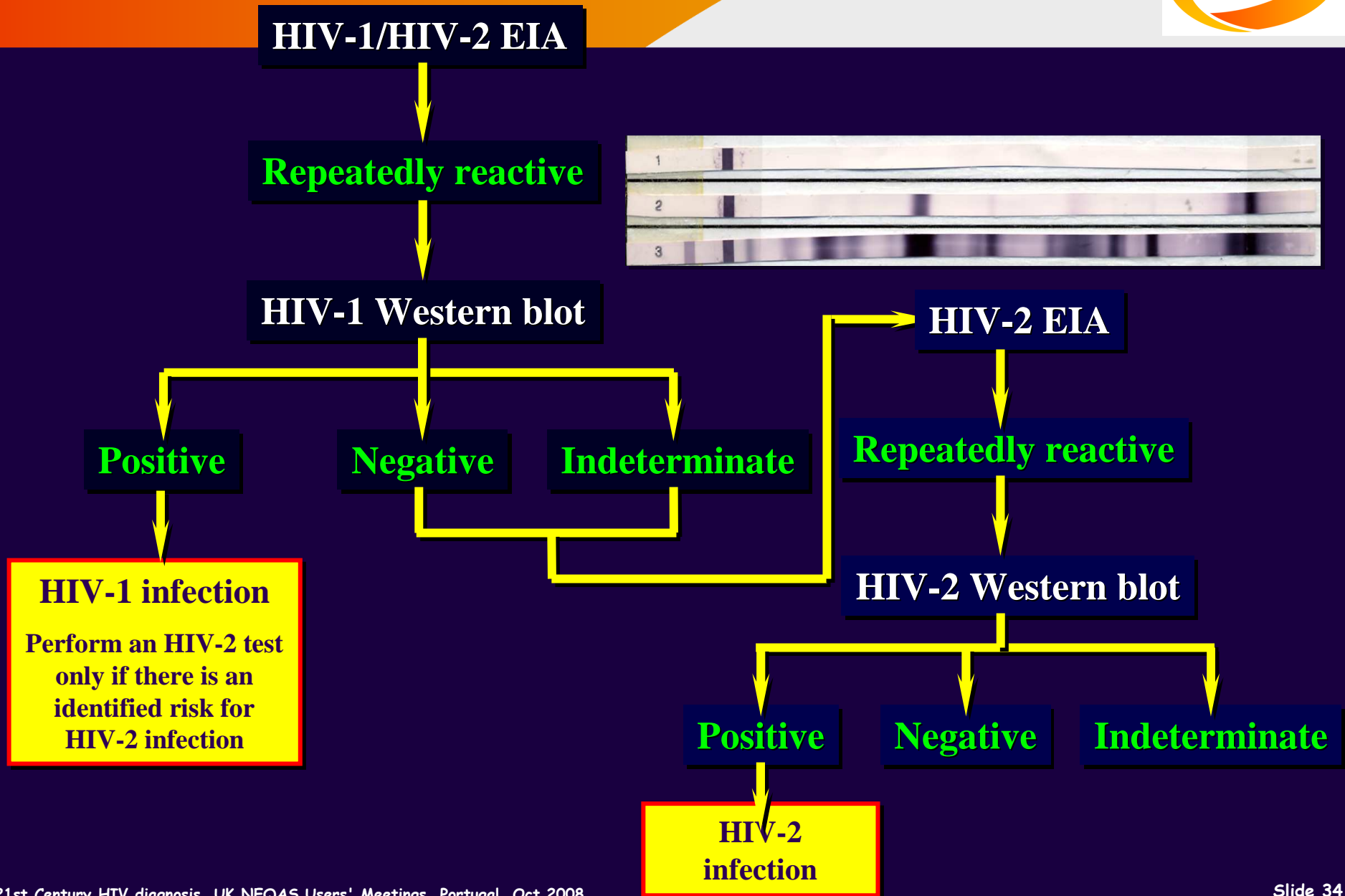
Confirmatory testing: Aims



- **PPV → 1.00**
- **NPV → 1.00**
- **Distinguish HIV-1 from HIV-2**
- **Minimal indeterminate results**
- **Identify laboratory errors, e.g. x-contamination**
- **Simple & rapid**
- **Cost-effective**

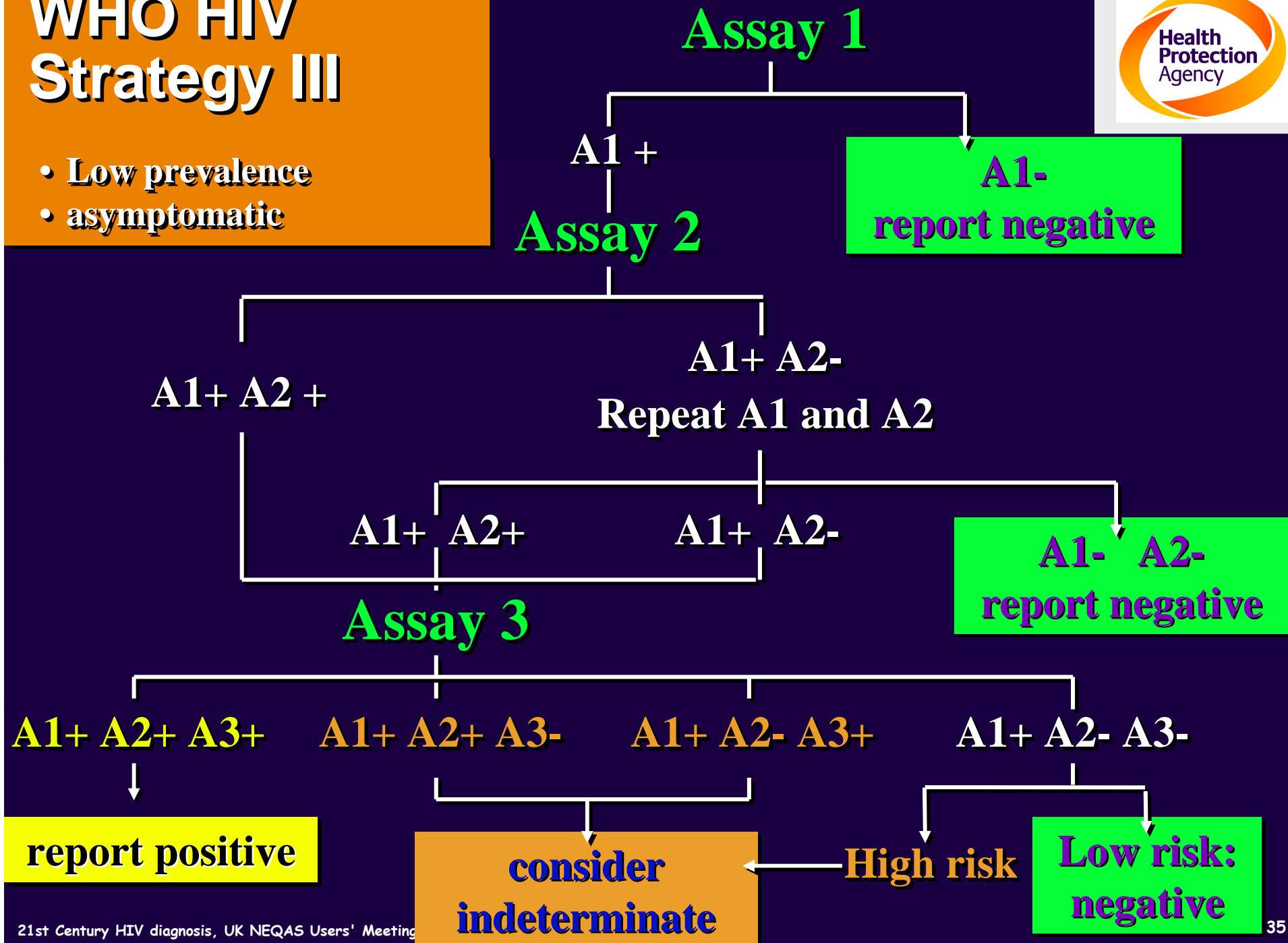
'Conventional' HIV Confirmatory Strategy

ASTPHLD circa 1995



WHO HIV Strategy III

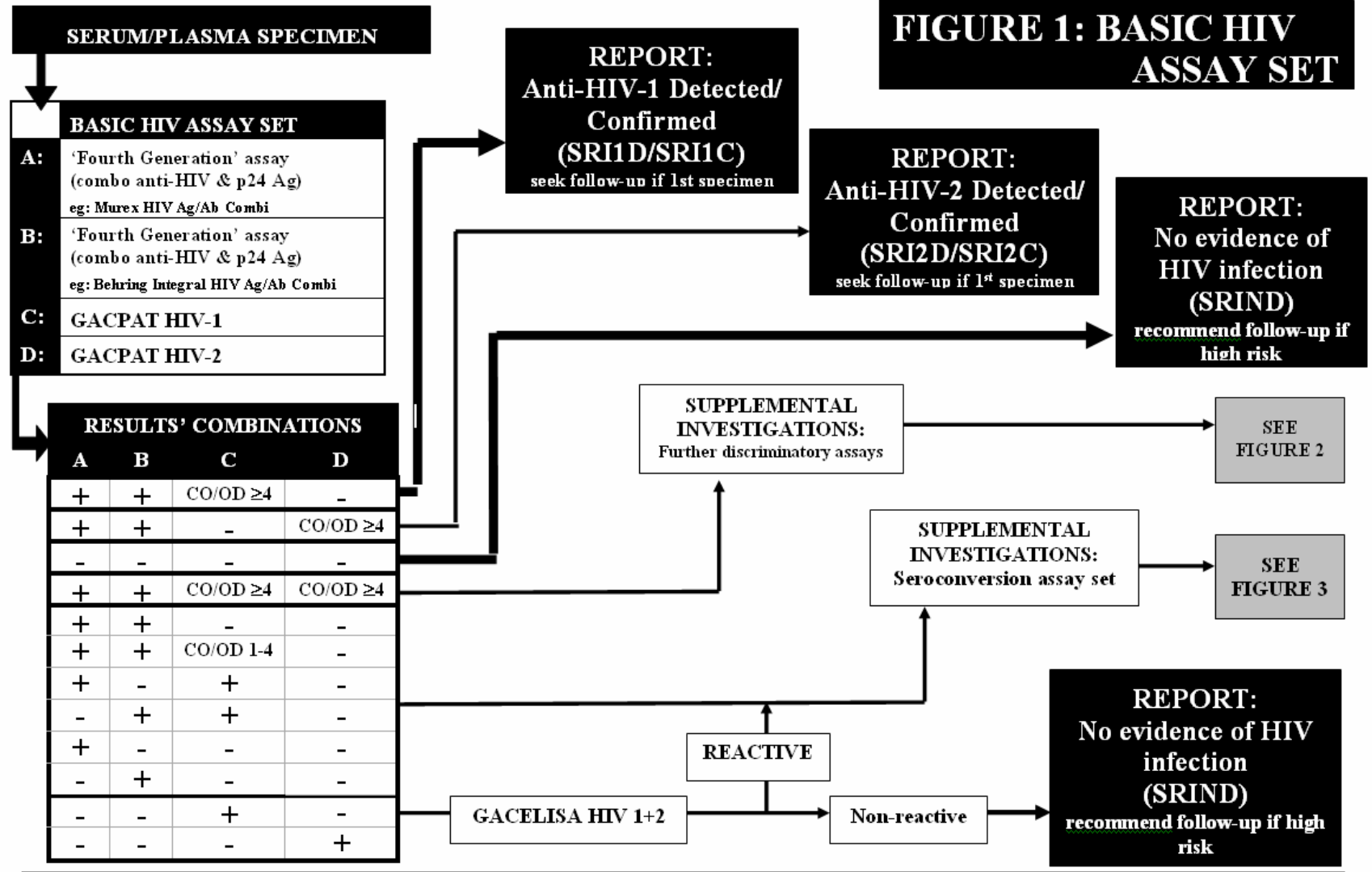
- Low prevalence
- asymptomatic



HPA Virus Reference Dept modular approach to HIV confirmation: Basic Assay Set



FIGURE 1: BASIC HIV ASSAY SET



PPVs for 2 or 3 different tests in series to confirm a diagnosis

Assay sens 99%; spec 98%



<i>Prevalence</i>	<i>1 test</i>	<i>2 tests</i>	<i>3 tests</i>
0.09%	4.3%	68.8%	<u>99.1%</u>
0.5%	19.9%	92.5%	<u>99.8%</u>
2.0%	50.3%	98.0%	<u>99.96%</u>
5.0%	72.3%	<u>99.2%</u>	<u>99.98%</u>
10.0%	91.9%	<u>99.7%</u>	<u>99.99%</u>
30.0%	95.5%	<u>99.9%</u>	<u>100%</u>

assumes false results are unrelated

But its not that simple !!!



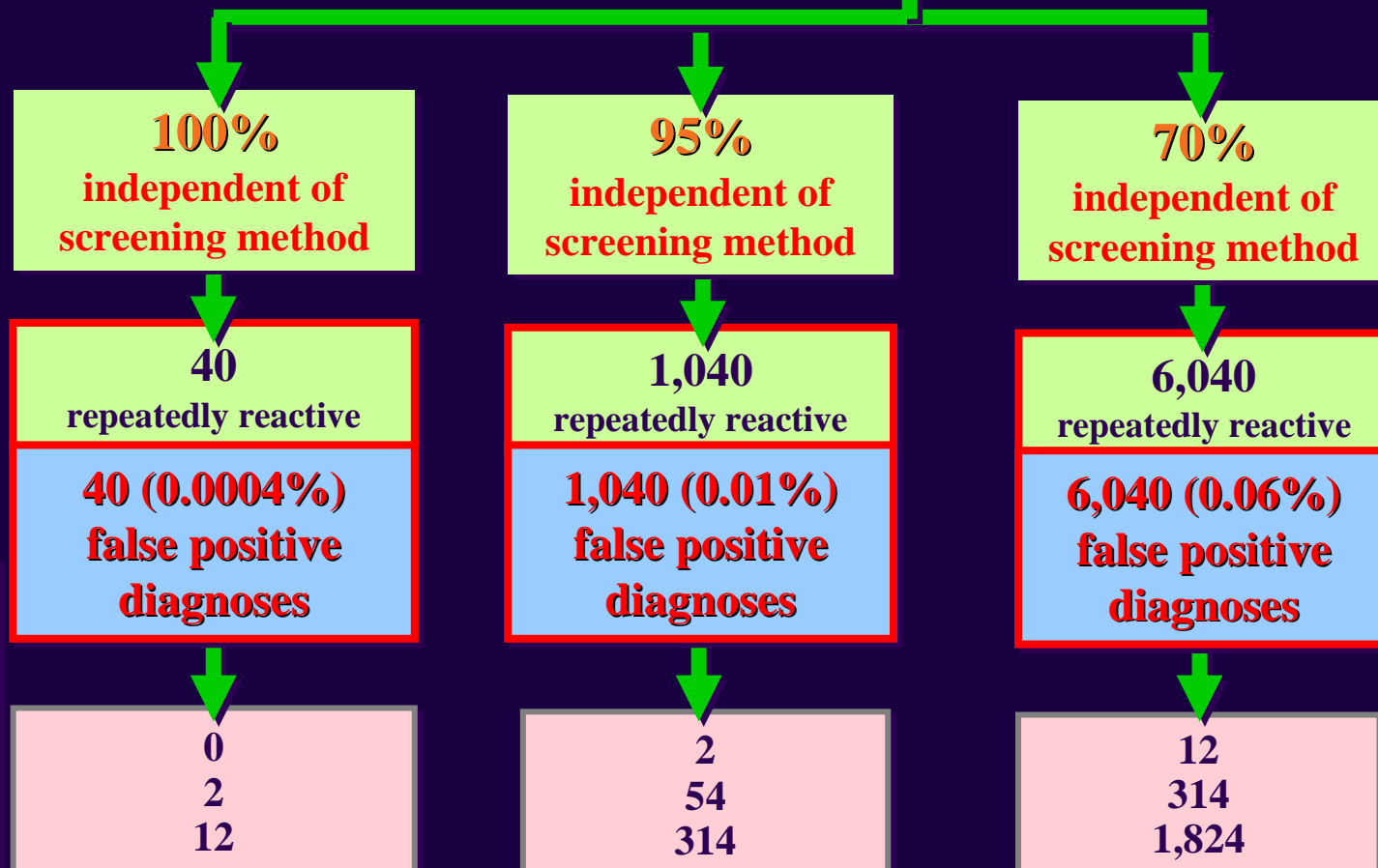
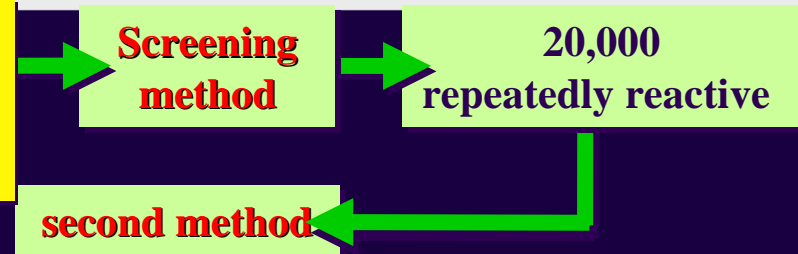
➤ Application of Assay 1.....

- **Highly selected population for Assay 2**
- **Impact on Assay 2 performance:**
 - **Sensitivity**
 - **Specificity (shared false +ve)**
- **This effect should be taken into account**
- **Influence of Assay 1 and 2 on Assay 3 ?**

Impact of the particular choice of each test in an algorithm



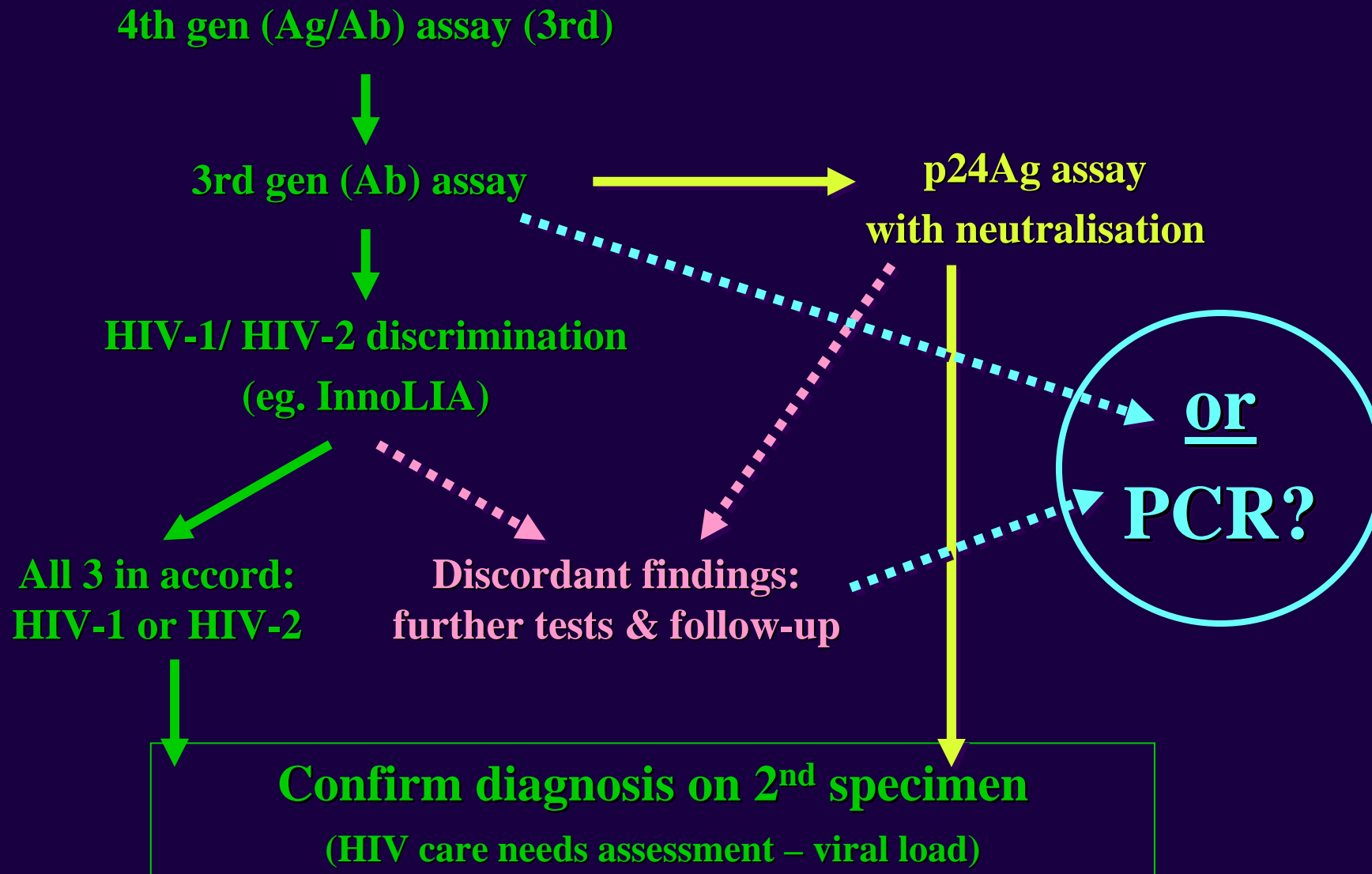
- Test HIV-negative population..... 10,000,000
- First (screening) method repeat reactive rate..... 0.2%
- Second method repeat reactive rate..... 0.2%



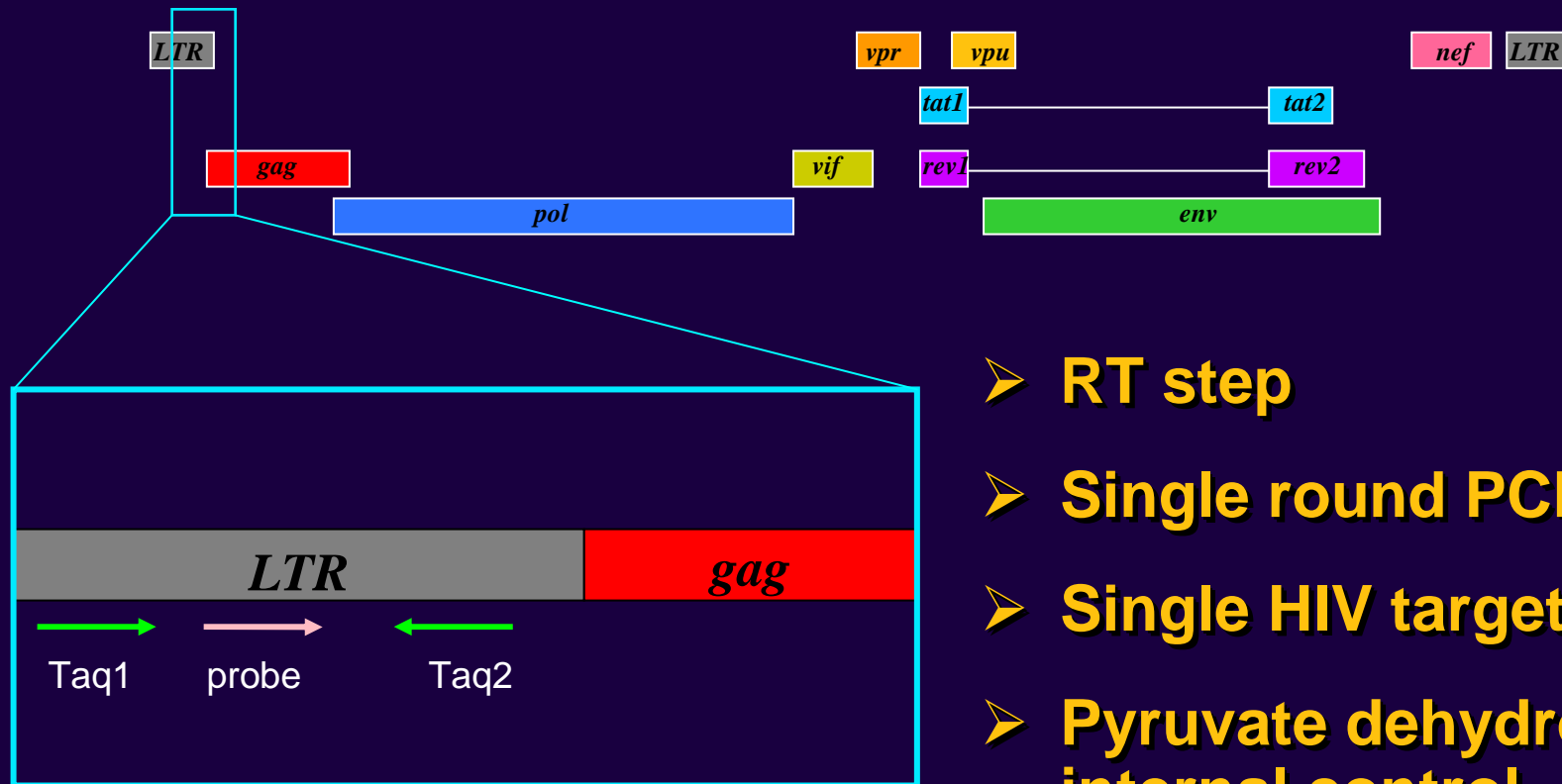
Third method
RR rate 0.2%
Independence:

100%
95%
70%

Simplified Minimum Generic Confirmatory Algorithm (UK Regional Labs): fundamentally a 3 test strategy

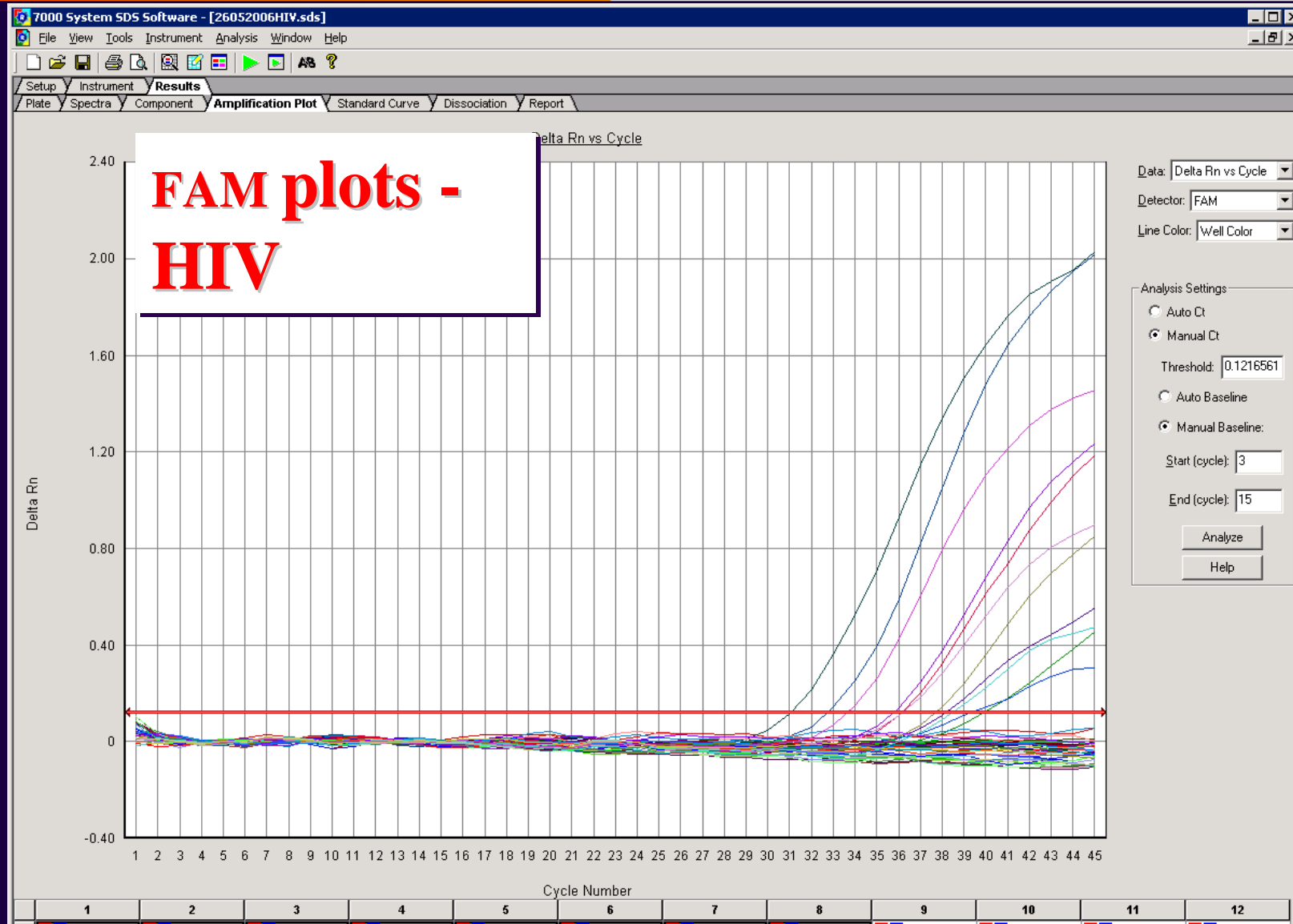


ABI Prism 7000 PCR for HIV-1 RNA or proviral DNA (UCL)

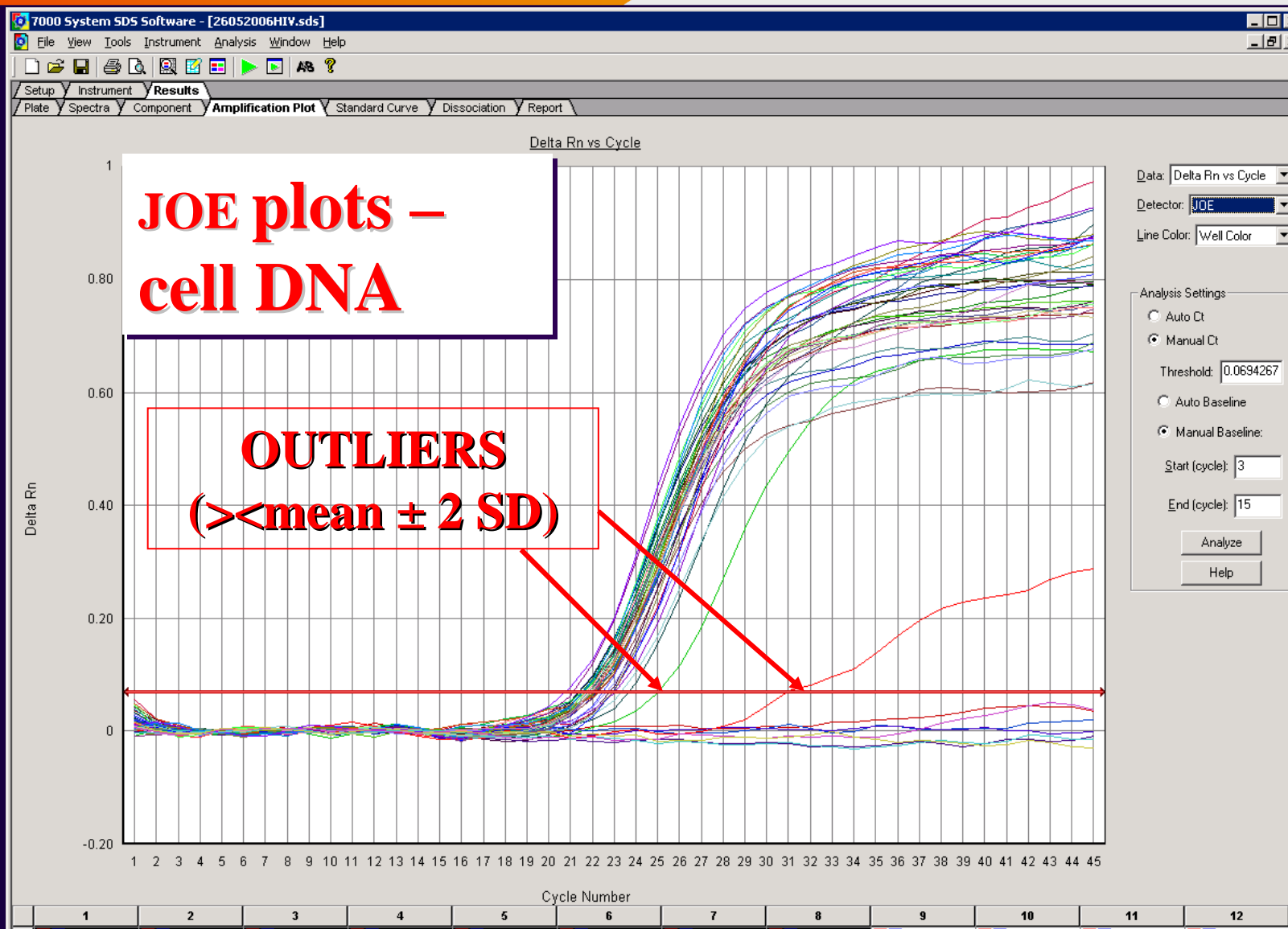


- RT step
- Single round PCR
- Single HIV target (5'LTR)
- Pyruvate dehydrogenase internal control
- LDL <20Geq in 10⁶ cells
- Benefits of real-time

HIV-1 specific target plots



Internal control plots (PDH)



3 unusual case studies.....

Example Difficult Diagnosis: History

Case 1



- **Healthy female patient**
- **Age 45yrs**
- **Changing sexual partner**
- **No evidence of recent risky behaviour/exposure**
- **Immigrant from West Africa**
- **Occasional return visits**
- **Mixed, generally weak, reactions in local screening tests**
- **'Low risk' needlestick injury in October 1998**

Example Difficult Diagnosis: Western blots

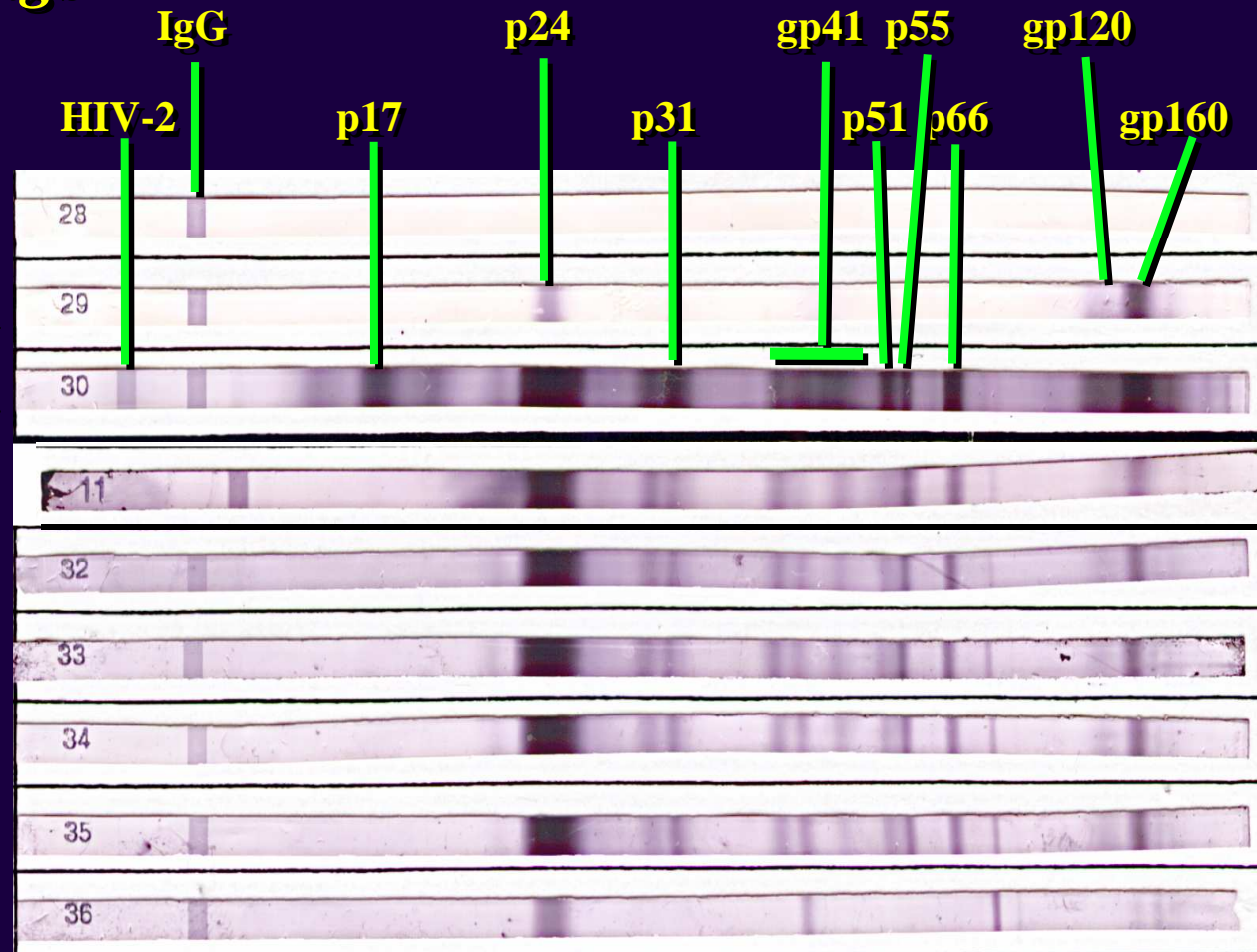
Case 1



Western blot findings

Negative control
Weak Pos. control
Strong Pos. control

6. March '00
5. December
4. December
3. October
2. September
1. January '99



Serum

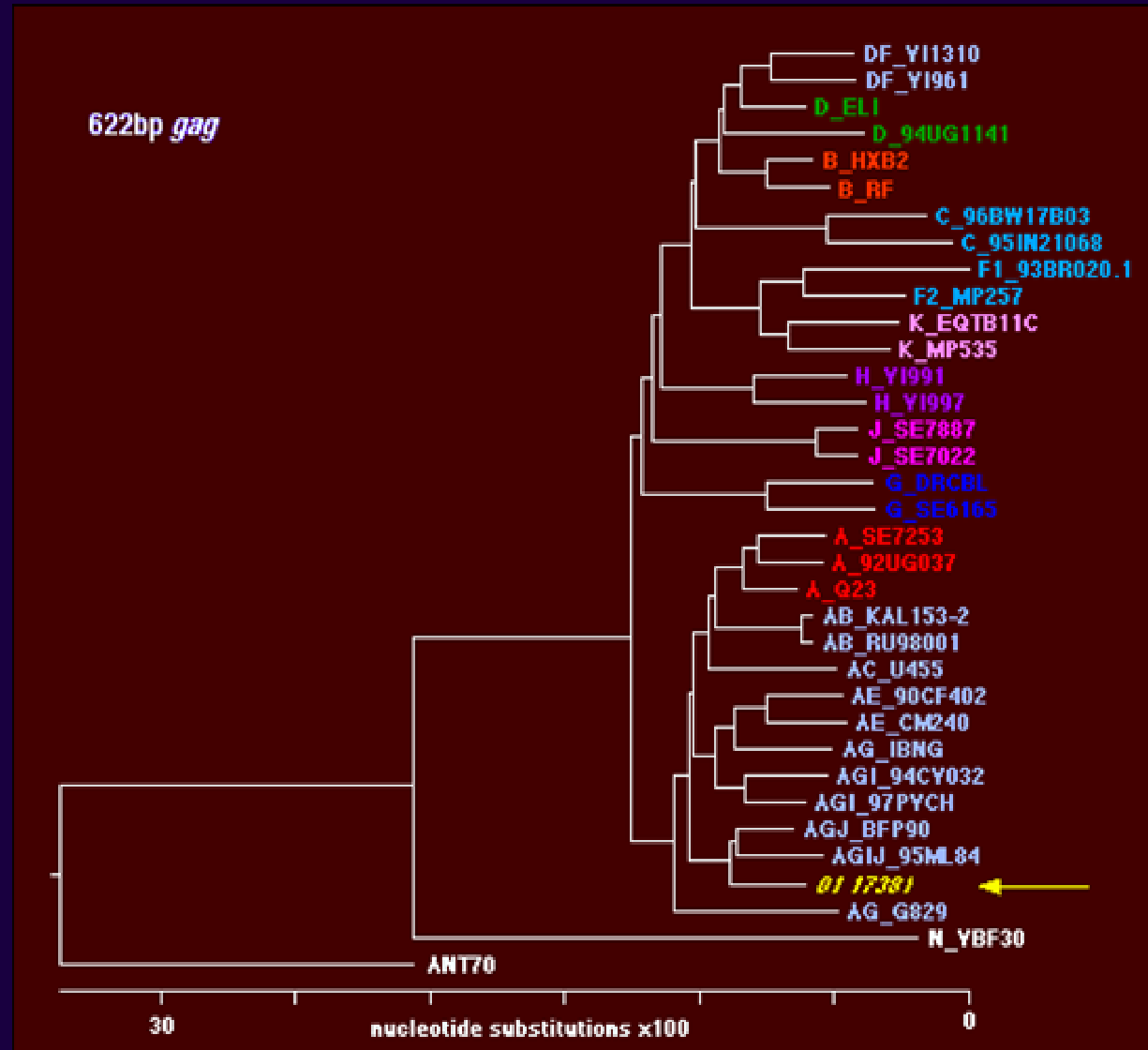
- *RT-PCR on several specimens negative*
- *'Standard' primers*
- *'Highly divergent' primers eg. HIV-1 O; SIV*

WBC pellet

- *Proviral DNA yielded gag & integrase products*
- *Sequence analysis*
- *Phylogenetic Tree*

Example Difficult Case: Phylogenetic Tree

Case 1



Amino Acid Alignment Reveals STOP Codons in p24

Case 1



```

Majority      VQNAQGQMVHQALSSPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKI
                80          90          100         110         120         130
A_SE7253      VQNAQGQMVHQSLSPRTLNAWVKVIEEKAFSPEVIPVFSALSEGATPQDLNNMMLNIVGGHQAAMQMLKI
AB_RU98001    VQNAQGQMTHQSMSSPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNNMMLNIVGGHQAAMQMLKI
AE_CM240      VQNAQGQMAHQPLSPRTLNAWVKVVEEKGFNPEVIPMFSALSEGATPQDLNNMMLNIVGGHQAAMQMLKE
AG_IBNG       VQNAKGQMTHQSMSSPRTLNAWVKVIEEKGFSPPEVIPMFSALSEGATPQDLNNMMLNIVGGHQAAMQMLKI
AGI_97PVCH   VQNAQGQMVHQAMSSPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNNMMLNIVGGHQAAMQMLKI
AGJ_BFP90    VQNAQGQMVHQAMSSPRTLNAWVKVIEDKAFSPEVIPMFTALSEGATPQDLNNMMLNIVGGHQAAMQMLKI
B_HXB2       VQNIQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKE
C_95IN21068  VQNLQGQMVHQAISPRTLNAWVKVIEEKAFSPEVIPMFTALSEGATPQDLNTMLNTVGGHQAAMQMLKI
D_ELI        VQNLQGQMVHQAISPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKE
F1_93BR020.1 VQNLQGQMVHQSLSPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKI
F2_MP257     LQNLQGQMVHQSLSPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKI
G_DRCBL      VQNAQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFTALSEGATPQDLNTMLNTVGGHQAAMQMLKE
H_VI997      VQNAQGQMVHQPISHRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNNAMLNTVGGHQAAMQMLKI
J_SE7022     VQNLQGQPVHQALSSPRTLNAWVKVIEEKAFSPEVIPMFSALSEGATPQDLNTMLNTIGGHQAAMQMLKI
K_MP535      VQNLQGQMVHQALSSPRTLNAWVKVIEEKAFSPEVIPMFTALSEGATPQDLNTMLNTVGGHQAAMQMLKI
01_17381     VQNAQGQMVHQAISPRTLNAWVKVIEEKAFSPEVIPMFTALSEGATPQDLNNMMLNIVGGHQAAMQMLKI
    
```

```

Majority      INEEAAEWDRLHPVHAGPIPPGQMREPRGSDIAGTTSTLQEQIGWMTSNPPIPVGEIYKRWIIP
                150         160         170         180         190         200
A_SE7253      INEEAAEWDRLHPPAHAGPVAPGQMREPRGSDIAGTTSTLQEQIGWMTGNPPIPVGDIYKRWIIP
AB_RU98001    INEEAAEWDRLHPAQAGPFPPPGQMREPRGSDIAGTTSTLQEQIGWMTSNPPIPVGDIYKRWIIP
AE_CM240      INEEPAEWDRVHPVHAGPIPPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
AG_IBNG       INEEAAEWDRVHPVHAGPIPPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
AGI_97PVCH   INEEAAEWDRAHPVHAGPIPPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
AGJ_BFP90    INEEAAEWDRVHPVHAGPIPPGQIREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
B_HXB2       INEEAAEWDRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
C_95IN21068  INEEAAEWDRLHPVVPAGPIAPGQLREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
D_ELI        INEEAAEWDRLHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
F1_93BR020.1 INEEAAEWDRLHPTQAGPIPPGQIREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
F2_MP257     INEEAAEWDRLHPVHAGPIPPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
G_DRCBL      INDEAAEWDRLHPQAGPIAPGQIRDPTGSDIAGATSTLQEQIGWMTNPPIPVGDIYKRWIIP
H_VI997      INEEAAEWDRLHPVHAGPIPPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGDIYKRWIIP
J_SE7022     INEEAAEWDRVHPVHAGPVAPGQVREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKRWIIP
K_MP535      INDEAAEWDRLHPVHAGPIPPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKRWIIP
01_17381     INEEAAEWDRTHPVHAGPIQPGQMREPRGSDIAGTTSTLQEQIGWMTNPPIPVGEIYKRWIIP
    
```

Conclusion:
 Probably infected in west Africa with a replication deficient virus



- **March 2005: MSM presented with multiple penile ulcers & generalised macular rash;**
- **Recent partner had infectious syphilis;**
- **UAI with 3 casual partners in past 3/12;**
- **HIV & syphilis tests in 2002 NEGATIVE**

- **Syphilis serology POSITIVE;**
- **AxSym HIV Ag/Ab & VIDAS HIV DUO both POSITIVE**
- **HIV RNA BDL**

A case with persisting incomplete HIV profile....

Case 2



Test method	March 2005
Anti-HIV 1 /2 + p24ag (Integral Ag/Ab EIA)	3.9
Anti-HIV 1 /2 + p24ag (Abbott Murex)	18.2
Anti-HIV1 (GACPAT)	0.26
Anti-HIV2 (GACPAT)	0.17
Anti-HIV 1/2 IgG (in-house GACELISA)	1.9
Anti-HIV IgM	0.46
Anti-HIV IgA	2.5
HIV p24 antigen	0.60
Western blot	HIV-1
HIV-1 RNA	--
HIV-1 proviral DNA	--

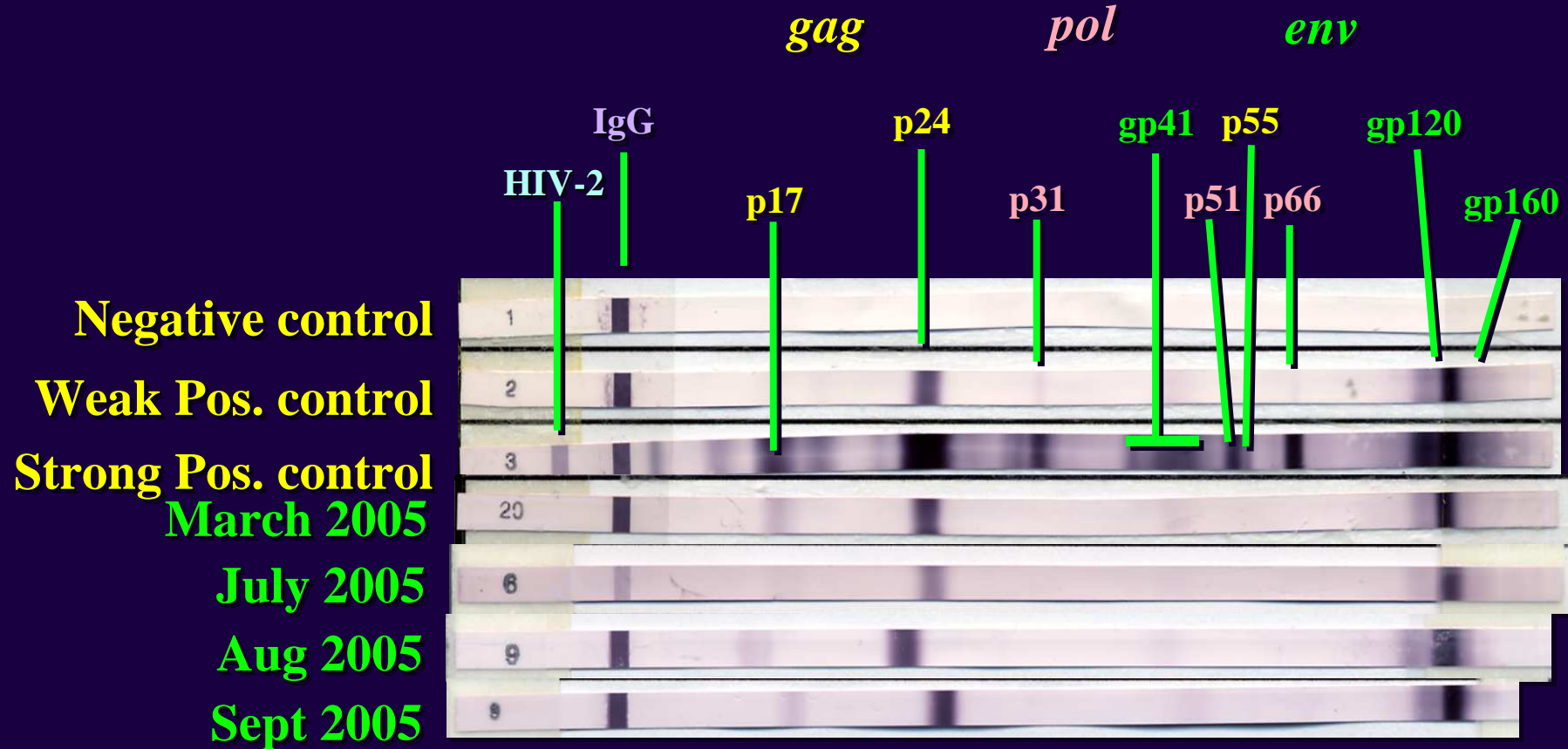
¹ Abbott m2000rt quantitative HIV RNA PCR;

² Real time in-house qualitative PCR directed at HIV-1 LTR region (total nucleic acid);

³ Block-based in-house qualitative multiplex PCR directed at HIV-1 *gag* & *int* regions.

Western blot profiles: No change to April 2007

Case 2



- Was this a recent HIV infection?
- Did HIV infection occur at all?
- Was HIV cleared?

The case of the 'self-curing' patient.....

1.

Case 3



2001

Apr02

25Jul02

15Aug02

20Aug02

23Aug02

UAI

Multiple partners

Possible HIV SCVN illness

**Routine HIV test
NEG**

Regular partner HIV positive

**Routine HIV test
NEG**

**Vironostika wk+ve;
IMx -ve**

**Vironostika wk+ve;
IMx -ve**

**Vironostika +++;
IMx wk+ve;**

**Vironostika +++;
IMx wk+ve**

InnoLIA HIV-1

VL 147c/ml

**VRD anti-HIV & p24Ag
NEG**

VRD

'possible SCVN'

(Feb 2004)

The case of the 'self-curing' patient.....

3.

Case 3



- Subsequent 'regular' viral loads..... BDL
- June 2003 repeat HIV serology.... **NEGATIVE!!**
- Further tests on.....
 - Dec 03; Mar 04; July 04; Oct 04; Nov 05
 - **ALL NEGATIVE** for any HIV markers sought, including HIV RNA & HIV pDNA (Oct 04)
 - **CD4 counts normal**

So, was he infected with HIV?

Concluding Remarks - Overall



- **HIV Testing is an essential tool in control of HIV epidemic**
- **Increased access to testing needed**
 - **Structured eg. antenatal; STI clinics**
 - **Opportunistic, eg. GP; A&E**
 - **Targeted?**
 - **Blanket?**
 - **Non-medical/ out-of-hours settings (POCT)**
- **Confirming HIV infection generally straightforward**
 - **Defined algorithm/ validated outcomes**
 - **Confirm diagnosis on a second specimen**
 - **Occasional difficult diagnoses – refer to specialist HIV laboratory**

Acknowledgements



Background Epidemiology

A Complex Picture: HIV & other Sexually Transmitted Infections in the United Kingdom: 2006 (HPA Annual Report)

Colleagues in HIV & STI Department, Cfl

EuroHIV Network

HIV Reference

Jenny Tosswill, Katrina Barlow

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HIV kit performance

Keith Perry & Kit Evaluation Unit and KEG, NBS

Recent HIV Infections/ HIV incidence

Gary Murphy, Elaine McKinney, Caterina Hill

Martin Fisher, David Pao (Brighton & Sussex University Hospital)

MTCT

Jenny Tosswill

Dr Pat Tookey, Institute of Child Health; Hermioe Lyall, St Mary's Hospital, London

Miscellaneous:

All those involved in seeing and treating the patients, the collection and testing of specimens and referring to Cfl for reference testing

Thank you for your attention