The role of HIV testing in controlling the HIV epidemic

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Overview

- 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 (/10^6) in Europe (1996-2006)

* 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 (/10^6) in Europe (2006)
The increasing burden of HIV care (1988 – 2006)

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

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

* 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

25-40% of HIV infections remain undiagnosed

VCT excludes those previously diagnosed

Unlinked anonymous testing of GUM clinic attendees
Why does the HIV epidemic continue to grow at pace?

~60% of infections require HAART....

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

- Patients with CD4 count under 200 cells/mm³ within 30 days of diagnosis.
- Patients with a clinical AIDS diagnosis within 3 months of HIV diagnosis.

Reports of HIV/AIDS diagnosis and CD4 Surveillance

- MSM: 22%, 7%
- IDUs: 28%, 11%
- Female heterosexuals: 37%, 10%
- Male heterosexuals: 47%, 19%
- Overall: 34%, 11%

21st Century HIV diagnosis, UK NEQAS Users’ Meetings, Portugal, Oct 2008
Summary of drivers of ongoing 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

- **1980**: NO tests
  - blood safety, epidemiology; no therapy; stigma; ‘death sentence’

- **1985**: 1st gen Tests
  - blood safety, epidemiology; no therapy; stigma; ‘death sentence’

- **1990**: 3rd gen tests
  - dual therapy
  - Role of STIs in transmission

- **1995**: Better tests
  - blood safety, epidemiology; mono therapy

- **2000**: 4th gen tests – earlier diagnosis
  - PEP to contacts
  - Rebound effect
  - Increased risk-taking
  - Treatment failure & resistance
    → Increased incidence
    → Erosion of PH benefit

- **2005**: HAART
  - Treatable disease
  - Reduced infectivity
  - ?reduced incidence?
  - ‘clear’ personal and PH benefits of testing

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21st Century HIV diagnosis, UK NEQAS Users' Meetings, Portugal, Oct 2008
HIV Incidence in MSM attending STI clinics: **BY REGION**

Trend in HIV incidence in MSM attending STI clinics

![Graph showing the trend in HIV incidence in MSM attending STI clinics by region from 1995 to 2005.](image)

- **Estimated HIV Incidence (%)**

**Legend:**
- **LONDON**
- **Outside LONDON**
Early Detection of HIV Infection

a key aspect of control & prevention
Development of Markers of Primary HIV Infection & HIV Screening Test Performance

- HIV RNA
- p24 Ag
- IgM Anti-HIV
- IgG Anti-HIV

First Generation - peptide
Second Generation - recombinant
Third Generation
Fourth Generation

Weeks post-infection:
0 1 2 3 4 5 6 7 8

Reactivity:
local viral growth
viral spread

Reactivity:
0 1 2 3 4 5 6 7 8

First Generation
Second Generation - peptide
Third Generation
Fourth Generation

Reactivity:
local viral growth
viral spread

Reactivity:
0 1 2 3 4 5 6 7 8

First Generation
Second Generation - peptide
Third Generation
Fourth Generation
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 ?
Based on data generated by testing 10 seroconversion panels in each of the HIV kits shown.

- Combined Ag/Ab assay
- Roche Amplicor monitor v1 (83088); BBI data

Perry KR, et al
(Transfusion Medicine, 2008)
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

Following addition of clarifying solution
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.

* 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?

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

Without interventions 30-50% transmission
Interrupting HIV MTCT by ART: proof of concept - ACTG 076 (2)


### Graph:

- **Y-axis:** Probability of Transmission (%)
- **X-axis:** Weeks
- **Data Points:**
  - Placebo: 183, 84, 42, 37
  - Zidovudine: 180, 105, 51, 43

- **Statistics:**
  - Placebo: 25.5%
  - Zidovudine: 8.3%
Children born in UK & Ireland to HIV infected women, reported by end of September 2005, likely infection status

- Not infected
- Indeterminate
- Infected


Pat Tookey, Institute of Child Health
Getting the correct result:
Confirmatory Testing
Confirmatory testing: Aims

- PPV $\rightarrow$ 1.00
- NPV $\rightarrow$ 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

HIV-1/HIV-2 EIA

Repeatedly reactive

HIV-1 Western blot

Positive
Negative
Indeterminate

HIV-1 infection
Perform an HIV-2 test only if there is an identified risk for HIV-2 infection

HIV-2 EIA

Repeatedly reactive

HIV-2 Western blot

Positive
Negative
Indeterminate

HIV-2 infection
WHO HIV Strategy III

- Low prevalence
- Asymptomatic

Assay 1

A1+  
A1-  
Report negative

Assay 2

A1+ A2+  
Repeat A1 and A2

A1+ A2-  

Assay 3

A1+ A2+ A3+  
Report positive

A1+ A2+ A3-  
Consider indeterminate

A1+ A2- A3+  
High risk

A1+ A2- A3-  
Low risk: negative
HPA Virus Reference Dept modular approach to HIV confirmation: Basic Assay Set

- **Basic Assay Set**
  - **A:** 'Fourth Generation' assay
    - (combo anti-HIV & p24 Antigens)
    - eg: Murex HIV Ag/Ab Combi
  - **B:** 'Fourth Generation' assay
    - (combo anti-HIV & p24 Antigens)
    - eg: Behring integral HIV Ag/Ab Combi
  - **C:** GACPAT HIV-1
  - **D:** GACPAT HIV-2

**Results' Combinations**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>CO/OD ≥4</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>CO/OD ≥4</td>
</tr>
<tr>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>CO/OD ≥4</td>
<td>CO/OD ≥4</td>
</tr>
</tbody>
</table>

**Figure 1: Basic HIV Assay Set**

- **Report:** Anti-HIV-1 Detected/Confirmed (SRI1D/SRI1C)
  - seek follow-up if 1st specimen
- **Report:** Anti-HIV-2 Detected/Confirmed (SRI2D/SRI2C)
  - seek follow-up if 1st specimen
- **Report:** No evidence of HIV infection (SRIND)
  - recommend follow-up if high risk

**Supplemental Investigations:**

- Further discriminatory assays
- Seroconversion assay set

**Supplemental Investigations**

- **SOP No. V-5134/04-07**
- **Copy No.**
- **Page 5 of 10**
### PPVs for 2 or 3 different tests in series to confirm a diagnosis

Assay sens 99%; spec 98%

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

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%

- Screening method
- Repeatedly reactive: 20,000

- Second method

- Independent of screening method

- Repeatedly reactive:
  - 100%: 40
  - 95%: 1,040
  - 70%: 6,040

- False positive diagnoses:
  - 100%: 40 (0.0004%)
  - 95%: 1,040 (0.01%)
  - 70%: 6,040 (0.06%)
Simplified Minimum Generic Confirmatory Algorithm (UK Regional Labs): fundamentally a 3 test strategy

- 4th gen (Ag/Ab) assay (3rd)
- 3rd gen (Ab) assay
- HIV-1/ HIV-2 discrimination (eg. InnoLIA)

All 3 in accord: HIV-1 or HIV-2

Discordant findings: further tests & follow-up

Confirm diagnosis on 2nd specimen (HIV care needs assessment – viral load)

or

p24Ag assay with neutralisation

or

PCR?
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 10^6 cells
- Benefits of real-time
HIV-1 specific target plots

FAM plots - HIV
Internal control plots (PDH)

JOE plots – cell DNA

OUTLIERS (><mean ± 2 SD)
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

**Western blot findings**

- **Negative control**
- **Weak Pos. control**
- **Strong Pos. control**

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

- IgG
- HIV-2
- p17
- p24
- gp41
- p55
- gp120
- p31
- p51
- p66
- gp160
- gp120
**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

Majority sequence:

VQNAQGQMHQQALSPRTLNA VKIEEAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQLK

Case 1

Conclusion: Probably infected in west Africa with a replication deficient virus
A case with persisting incomplete HIV profile….

- 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
## Case 2

### A case with persisting incomplete HIV profile....

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

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?

Negative control
Weak Pos. control
Strong Pos. control
March 2005
July 2005
Aug 2005
Sept 2005

HIV-2
p17
p24
p31
gp41
p51
p66
gp120
gp160

IgG
p55
gag
pol
env

p31
p51
p66

March 2005
July 2005
Aug 2005
Sept 2005
The case of the ‘self-curing’ patient…… 1.

| Case 3 |
|-------------|---------------------------------|------------------|------------------|------------------|------------------|
| **2001**    | **Apr02**                       | **25Jul02**      | **15Aug02**      | **20Aug02**      | **23Aug02**      |
| Routine HIV test | UAI                           | Possible HIV SCVN illness |
| Regular partner HIV positive | Multiple partners   | Vironostika wk+ve; IMx -ve |
| Routine HIV test | Vironostika wk+ve; IMx -ve | Vironostika +++; IMx wk+ve; InnoLIA HIV-1 |
| 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...
The case of the ‘self-curing’ patient...... 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

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Colleagues in HIV & STI Department, CfI
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MTCT
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Thank you for your attention