HIV Infection

Laboratory diagnosis and monitoring 2006

Philip Cunningham
NSW State Reference Laboratory for HIV/AIDS
St Vincent’s Hospital Sydney
HIV laboratory tests

✓ **diagnosis** of infection
   *acute, recent, established or late stage disease*

✓ **monitoring** of ARV therapies
   *immunological and virological markers*
   *pharmacology*
   *toxicities*
   *surrogate prognostic markers*
   *host factors*

✓ **diagnosis of opportunistic infections**
‘typical’ primary HIV-1 infection

- HIV-1 p24 antigen
- HIV proviral DNA
- HIV antibodies
- HIV viral load

Symptoms:
- 1° infection
- ‘window’ period
- Limit of detection

Time following infection:
- 0 1 2 3 4 5 6 / 2 4 6 8 10 weeks years

NIPH/NCHADS Phnom Penh
Kingdom of Cambodia 2006
HIV Testing
Virus Specific Antibodies

- Screening test – ELISA, EIA, particle agglutination
- Reference (confirmatory) testing – 2nd EIA, western blot
- Supplemental testing – resolve discordant tests
- Serum rapid tests
- Surveillance testing – monitoring incidence

- Testing strategy dictated by population
- Sensitivity vs specificity
ELISA – Enzyme Linked Immunosorbent Assay

- detect HIV-1 and HIV-2 responses
- HIV specific antibody and HIV antigen may be detected in ‘4th’ generation tests
- sensitive, specific and reproducible
- false reactions possible – but low
- suitable for large number of tests – automated
- Medicare listed November 2005

- Screening widely available
- Confirmation in reference laboratories
Antibody/antigen combo

- HIV-1/2 Ag/Ab combo assays detect both antibodies (HIV-1+2) and viral antigen (HIV p24) in single test
- Result in 60 minutes
- Reduction in window period by 3-5 days
  - Acute infection detection without indication
  - Reports of increases in cases identified
- Differences in limit of detection of Ag between brands
  - (140 - <25 pg/mL)
- Issues associated with introduction
  - Strategies and confirmatory algorithms
  - Cost and legal
HIV Ab/Ag Combo strategy

HIV-1/2 Ab/Ag combo (x1)

Non reactive (Ag/Ab)
- Negative

Reactive (Ab/Ag)
- Non-reactive (Ab/Ag) (x2)
  - Negative
- Repeat-reactive (Ab/Ag) (x2)
  - Genescreen (Ab only) (x2)
  - HIV-1 western blot (Ab)
    - Positive
    - Negative/Indeterminate
  - Additional Tests
    - HIV-1 p24 antigen
    - DNA PCR
    - HIV-2
      - Negative
      - Indeterminate
Serology of primary HIV-1 infection

- Antibody responses to immunogenic proteins occur in typical series - diagnostic
- All viral proteins are detected – increasing in intensity
Effect of antiretroviral therapy

- Antigenic stimulus is reduced

- Envelope responses preserved – high antigenic diversity to glycoprotein - less redundancy

- Responses to gag discrete structural proteins are lost - redundancy

- Don’t reach positive status – underreporting
Advanced HIV infection

- Low level antibody test reactivity
- Indeterminate profile – pattern typical of late stage
- Retain glycoprotein reactivity
- Late presenters

helpful

- CD4 lymphocyte count
- HIV proviral DNA PCR
- HIV viral load
DNA PCR
RNA PCR
p24 Ag
3rd gen ELISA
1st gen ELISA
Detuned ELISA

1wk  2wk  3wk  2mo  6mo  1yr  2yr  3yr  +8yr

acute  established  late
Window period

- Antibodies appear within 3-4 weeks

- Antibody testing strategies have limitations – 100% people seroconvert within 12 weeks

- More sensitive testing strategies including direct detection of virus (NAT) and 3rd/4th generation immunoassays will reduce window period to 6 weeks

- HAART therapy during primary infection = delayed antibody response
HIV Testing
Direct Detection of Virus

- **p24 antigen detection** – serology
  - p24 only assays – qualitative and quantitative
  - p24 in combination with antibody
  - Serum

- **Virus isolation - culture**

- **Nucleic acid detection - (NAT)**

  *HIV DNA or RNA?*

  **DNA** qualitative – proviral (cellular)
  resolution of inconclusive serology
diagnosis in infants - maternal antibodies

  **RNA** quantitative – serial viral load
  drug resistance monitoring
  subtyping
Pooled NAT testing in a blood donor setting

24 x negative results

Re-test ALL Individual samples
## Residual risk and NAT

<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>1 in 1,250,000</td>
<td>1 in 4,800,000</td>
</tr>
<tr>
<td>HCV</td>
<td>1 in 230,000</td>
<td>1 in 3,100,000</td>
</tr>
<tr>
<td>HBV</td>
<td>1 in 150,000</td>
<td>1 in 1,000,000</td>
</tr>
</tbody>
</table>
High risk screening

- Re-test ALL Individual samples
- 6 x negative results

- Utility in high risk collection centres and high case load primary care centres
- Ampliscreen HIV test v1.5
- HIV Ag-Ab combo test negative samples pooled and NAT tested every 3 days

Cohen M, Busch M et al. South Carolina and San Fransisco

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Kingdom of Cambodia 2006
POC testing – rapid tests

- **Use of rapid HIV testing strategies**
  - High sensitivity >99%
  - High specificity >99%
  - Good reproducibility †
  - Unprocessed sample type – capillary blood
  - Little laboratory equipment required
  - No need for constant water / electricity supply
  - Few steps – rapid to perform
  - Visual interpretation
  - Storage at room temperature
## Predictive values for rapid HIV tests

<table>
<thead>
<tr>
<th>HIV prevalence</th>
<th>0.1%</th>
<th>1%</th>
<th>5%</th>
<th>10%</th>
<th>30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPV single test</td>
<td>100%</td>
<td>100%</td>
<td>99.9%</td>
<td>99.9%</td>
<td>99.6%</td>
</tr>
<tr>
<td>PPV single test</td>
<td>9%</td>
<td>50%</td>
<td>84%</td>
<td>92%</td>
<td>98%</td>
</tr>
<tr>
<td>PPV two tests</td>
<td>91%</td>
<td>99%</td>
<td>99.8%</td>
<td>99.9%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Assuming 99% sensitivity and 99% specificity

**WHO/CDC rapid testing guidelines 2004**
Rapid testing strategy

Blood Sample

Test 1

Negative

Result given

Reactive

Test 2

Negative

Result given

Positive result

Retest to confirm or Result given

Discordant

Retest after 6 weeks

Referral lab

WHO/CDC rapid testing guidelines 2004
POC testing issues

- Regulatory / licensing issues
- Environmental factors
- Training
- Test counseling
- Performance characteristics
- Cost
- Utility in certain settings
Monitoring established infections and treatment
Monitoring effect of ARV Therapy

- **viral load**
  - Test of choice
  - when to start therapy
  - when to change therapy
  - relative benefit of different regimens

- **CD4+ lymphocyte count**

- **clinical examination**

- **drug resistance testing**
Monitoring effect of ARV Therapy

- goals of therapy
- viral load BLD (50 or 400 copies)
  - also clinical benefit
  - survival
  - VL < 5000 copies
- CD4 > 300-500
- prevent resistance
Quantitative Viral Load Assays

- **Widely available commercial tests include:**
  - Roche Amplicor HIV-1 Monitor® (PCR)
  - Bayer Quantiplex HIV-RNA (bDNA)
  - Nuclisens nucleic acid sequence base amplification (NASBA)

- **Emerging new tests**
  - Real time systems – advantages – extended analytical range
  - EasyQ - Biomerieux
  - TaqMan - Roche
  - RealTime - Abbott
## HIV Viral Load Tests

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Principle</th>
<th>Results</th>
<th>Availability</th>
<th>Analytical range</th>
</tr>
</thead>
</table>
| Roche        | RT-PCR *(gag)* (COBAS HIV MONITOR v1.5) | copies/mL (6 hours to result) | Widely | <50 – 100,000  
<400 – 750,000 |
| Bayer        | HIV Branched DNA 3.0 *(bDNA)* *(pol)* | copies./mL (results 2x less than Roche) (36 hours to result) | NSW, Vic | <50 – 800,000 |
| Biomerieux   | HIV-1 QT NASBA *(gag)* | Copies/mL (6 hours to result) | NSW | <400 – 1,000,000  
<80 – 500,000 |
| Roche        | Real time *(Taqman)* *(gag)* | Copies/mL (4-6 hours to result) | New (no sites) | <40 – 10,000,000 |
| Biomerieux   | EasyQ HIV-1 real time TMA *(gag)* | Copies/mL & IU/mL (4-5 hours to results) | New (no sites) | <40 – 10,000,000 |
| Abbott       | Celera Realtime PCR m2000 *(pol integrase)* | copies/mL | Evaluation | <40 – 10,000,000 |
| Artus        | Realtime PCR – Rotorgene | Copies/mL | Evaluation | <40 – 10,000,000 |
Interpretation of VL Results

- different viral strains - subtypes
- day to day variability
- inter-laboratory variability
- variability between assays
- inter-current infections
- recent vaccination
- specimen quality - transport laboratory factors
- baseline - 2 x tests - 4 weeks apart
### VLT Assay variability

- **PCR is exponential** – log transform copy numbers
- **0.25 log\(_{10}\)** variation is normal *(between labs same method)*

<table>
<thead>
<tr>
<th>HIV-1 RNA copy</th>
<th>Acceptable difference</th>
</tr>
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<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>50</td>
<td>1.70</td>
</tr>
<tr>
<td>600</td>
<td>2.78</td>
</tr>
<tr>
<td>10,000</td>
<td>4.00</td>
</tr>
<tr>
<td>60,000</td>
<td>4.78</td>
</tr>
<tr>
<td>500,000</td>
<td>5.70</td>
</tr>
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Kingdom of Cambodia 2006*
VLT Assay variability

- PCR is exponential – log transform copy numbers
- $0.20 \log_{(10)}$ variation is normal (within lab)

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<td>500,000</td>
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</table>
Undetectable by the most sensitive test

HIV RNA (copies/mL)

Weeks on Therapy

20 weeks earlier

LLD Standard VLT

LLD Ultrasensitive
Resistance testing
Recommendations for resistance testing

1. Treatment naïve patients with acute or recent infection virologic failure during therapy
2. Therapy failure including suboptimal viral suppression after initiation of ARV
3. Pregnant HIV infected women and paediatric cases with detectable virus load, when therapy change is considered
4. ‘source’ patient when post exposure prophylaxis is considered
Considerations

- **drug naïve patients with chronic infection in**
  who treatment is to be started

- Recommend testing the earliest stored sample if
  suspicion of resistance is high or prevalence of
  resistance in the population exceeds 10%
How is it done?

- **Genotypic resistance**
  - DNA sequencing – mutation detection
  - Interpretation issues - consensus
  - Subtype determination possible
  - $200 - $400

- **Phenotypic resistance**
  - Virus exposed to drug
  - Molecular based methods
  - >$1000
Breakthrough of HIV resistance
DNA sequencing and Virtual Phenotype

- Computer assisted interpretation
- Issues with update in rapidly evolving virus
- Online updates
- In house sequencing advantages
QIAamp Spin Column Procedure
in microfuges  on vacuum manifolds

Sample → Lyse → Bind → Vacuum → Wash → Vacuum → Elute → Pure DNA or RNA

Pure DNA or RNA

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**TRUENE HIV-1 RESISTANCE REPORT Example**

**Sample ID:** GM4-X-034  
**Patient ID:** 2112-45-23769  
**Patient Name:** Doe, John  
**Date Created:** August 12, 2001  
**Physician:** Dr. Tom Johnson  
**Institution:** Mt. Sinai Hospital  
**Report Date:** August 13, 2001, 12:00:59-04:00

**Relevant RT Mutations:** K65R Q151L M184V T215F

### Nucleoside RT Inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistance Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>zidovudine</td>
<td>Resistance</td>
</tr>
<tr>
<td>didanosine</td>
<td>Resistance</td>
</tr>
<tr>
<td>zalcitabine</td>
<td>Resistance</td>
</tr>
<tr>
<td>lamivudine</td>
<td>Resistance</td>
</tr>
<tr>
<td>stavudine</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>abacavir</td>
<td>Resistance</td>
</tr>
<tr>
<td>tenofovir</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>emtricitabine</td>
<td>Possible Resistance</td>
</tr>
</tbody>
</table>

### Non-Nucleoside RT Inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistance Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>nevirapine</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>delavirdine</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>efavirenz</td>
<td>No Evidence of Resistance</td>
</tr>
</tbody>
</table>

### Relevant Protease Mutations: G48V

### Protease Inhibitors

<table>
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<th>Resistance Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>saquinavir</td>
<td>Resistance</td>
</tr>
<tr>
<td>indinavir</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>ritonavir</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>nelfinavir</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>amprenavir</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>atazanavir with ritonavir</td>
<td>No Evidence of Resistance</td>
</tr>
</tbody>
</table>

**Resistant Interpretation**

Based upon an international expert panel interpretation of in vitro phenotypic and in vitro virologic response data available as of February 2001 for correlation of Protease and RT sequences to antiretroviral drug resistance.

*Please refer to comments(s) in Mutation Details sections.*

**Signature:**  
**Date:**  
**Name (Print):**  
**Title:**

**VisiGene Technologies**  
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