Novel monitoring technologies for HIV viral load and genotyping

TREAT Asia Network
Junior Investigator Meeting

Yam W.C., FRCPath(UK), PhD
Department of Microbiology
Queen Mary Hospital
The University of Hong Kong
HIV patient care

• Patient needs life-long treatment (HAART)
• Requires longitudinal monitoring
  – CD4+ cell count
  – HIV-1 Viral load
  – Drug resistance development
HIV-1
Viral load Testing
HIV Viral load (VL)

- No. of HIV copies in host (plasma)
- VL ↑ → clinical failure
- Effective HAART can suppress VL to undetectable level (<75 copies/mL)
- VL is measured by Real-time Nucleic Acid Amplification Assays

- FDA approved kits
  1. Roche Cobas TaqMan HIV-1 Test
  2. Abbott Real-time HIV-1 Amplification Kit
  3. bioMerieux NucleiSens HIV-1 QT
Evolution of Roche Diagnostics Platforms (RMD)
Roche COBAS® TaqMan® HIV-1 Monitoring Kit

- Single tube RT-PCR
- Measure HIV-1 p24 antigen
- TaqMan® probes
Abbott Real-time HIV-1 Amplification Kit

- Fully automated viral RNA extraction by Abbott m2000
- Sample volume: 0.2 - 1.0mL plasma
- Single tube RT-PCR in 96-well plate
- Primer targeting HIV-1 \textit{pol} gene
- TaqMan® probes
- Lowest Quantitative Limit
  - 40 copies/mL
- Dynamic range
  - 40 - 1 \times 10^7 copies/mL
bioMerieux NucliSens® HIV-1 QT assay

- Automated RNA extraction by easyMAG (magnetic beads)
- Sample volume: 0.2 - 1.0mL
- Use 8 wells strips (total 96 wells)
- NASBA technology
  - RNA amplification
- 2 sets of primers to target 2 different viral genomic regions
- Dynamic range
  - 176- 3.46 x 10^6 copies
<table>
<thead>
<tr>
<th></th>
<th>Roche</th>
<th>Abbott</th>
<th>bioMerieux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (mL)</td>
<td>0.5</td>
<td>0.2 -1.0</td>
<td>0.2 -1.0</td>
</tr>
<tr>
<td>LDL (copies/mL)</td>
<td>40</td>
<td>40</td>
<td>176</td>
</tr>
<tr>
<td>Dynamic range (copies/mL)</td>
<td>40 - 10^7</td>
<td>40 - 10^7</td>
<td>176 - 3.46 X10^6</td>
</tr>
</tbody>
</table>
HIV-1 Genotyping Resistance Test
Antiretroviral drugs
Genomic structure of HIV-1

(Adapted from Los Alamos HIV database - http://www.hiv.lanl.gov/)
## Genotyping resistance test (GRT)

<table>
<thead>
<tr>
<th></th>
<th>Commercial Kits</th>
<th>In-house system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pros</strong></td>
<td>Quality validated (FDA, CE)</td>
<td>Low reagent cost (~US$60-80/sample)</td>
</tr>
<tr>
<td></td>
<td>Simple to use</td>
<td>Easy to modify for targeting new antiretroviral drugs</td>
</tr>
<tr>
<td><strong>Cons</strong></td>
<td>High reagent cost (~US$200/sample)</td>
<td>Usually not fully validated (need External QA Program)</td>
</tr>
<tr>
<td></td>
<td>Currently available for PI, NRTI and NNRTI monitoring only</td>
<td>Cannot be modified for new drugs</td>
</tr>
<tr>
<td></td>
<td>Cannot be modified for new drugs</td>
<td>Technically demanding</td>
</tr>
</tbody>
</table>
HIV-1 GRT Protocol

RNA Extraction

Plasma to viral RNA

Reverse transcription & amplification

RT-PCR & nested PCR

DNA Sequencing

Cycle Sequencing

Turn around time: 5 working days
HKU In-house GRT (for PIs & RTIs)

ViroSeq

HKU In-house
Sequencing electropherogram

M184M → M184V
ATG → GTG

K103K → K103N
AAA → AAC

Sequence electropherogram
## Antiretroviral Drug Resistance Report

**Patient:** BCP M89-0698U  
**D.O.B.:** March 22, 2005  
**Date Drawn:** February 21, 2005  
**Date Reported:** March 22, 2005

### Nucleoside Reverse Transcriptase Inhibitors

<table>
<thead>
<tr>
<th>Evidence of Resistance</th>
<th>K103N</th>
<th>Y181C</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
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<tr>
<td>High</td>
<td></td>
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</tbody>
</table>

### Nonnucleoside Reverse Transcriptase Inhibitors

<table>
<thead>
<tr>
<th>Evidence of Resistance</th>
<th>V118I</th>
<th>T215Y</th>
<th>T208V</th>
<th>L210W</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Low</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>High</td>
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</tbody>
</table>

### Protease Inhibitors

<table>
<thead>
<tr>
<th>Evidence of Resistance</th>
<th>K103N</th>
<th>Y181C</th>
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<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
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<tr>
<td>High</td>
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</table>

*Note: at least one mutation shown has not been fully validated.  
**Note: at least one mutation shown has not been clinically validated  
***For at least one mutation, both notes above apply (SEE FOOTNOTE 3 FOR MUTATION SPECIFIC INFORMATION).
### HIVdb: Genotypic Resistance Interpretation Algorithm

**SeqID:** KB0279-602  **Date:** 24-Jul-2008

#### Summary Data
- Sequence includes PR: codons 1 - 99
- Sequence includes RT: codons 1 - 411
- There are no insertions or deletions
- This sequence is from the closest reference isolate:

  1. **1. PR:** B (92.3%)
  2. **2. RT:** B (95.5%)

#### Nucleoside RTIs and Non-Nucleoside RTIs

<table>
<thead>
<tr>
<th>NRTIs</th>
<th>PI</th>
<th>NNRTIs</th>
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<tbody>
<tr>
<td>50%</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>90%</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>95%</td>
<td>1</td>
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#### Drug Resistance Interpretation

**RT**
- **None**

**PR Other Mutations:**
- **L10FY**, **K20IT**, **M36I**, **A71AV**, **V77I**, **I93L**
- **E36D**, **I62V**, **I64V**

**Protease Mutations**
- **E44A**
- **D67N**
- **M184V**
- **K70R**
- **A71AV**
- **V77I**
- **L90M**
- **I93L**
- **E36D**, **I62V**, **I64V**

**NRTIs and NNRTIs Resistance Interpretation**
- **D4T**
- **NVP**
- **EFV**
- **K219E**
- **T215Y**

**Drug Resistance Scoring**

<table>
<thead>
<tr>
<th>ATV</th>
<th>DDC</th>
<th>TDF</th>
<th>3TC</th>
<th>ABC</th>
<th>AZT</th>
<th>D4T</th>
<th>DDI</th>
<th>FTC</th>
<th>TDF</th>
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HKU IH GRT (for PIs, RTIs)

1\textsuperscript{st} PCR Product (~2100 bp)

2\textsuperscript{nd} PCR Product (~1800bp)
New HKU IH GRT (for PIs, RTIs, INIs)

1st PCR Product (~3500 bp)

2nd PCR Product (~1800bp)

2nd PCR Product (~1200bp)
HKU IH GRT

• Applicable to subtype B and most non-B or recombinant strains

• VL sensitivity: \(~500\) copies/mL

• Cover \(>95\%\) of routine GRT samples in HK
  – Backup by Commercial Kit

References:
- JCV 2006(35)
- JCV 2007(39)
- JCV 2009 (in press)
- HIV Medicine 2009 (in press)
External Quality Assurance Programme

Quality Control for Molecular Diagnostics (QCMD)

European QC programme for HIV Drug Resistance
West of Scotland Science Park

Treat Asia External Quality Assessment Scheme (TAQAS)

QC programme for HIV Drug Resistance in Asia
National Serology Reference Laboratory, Australia
HIV-1 Epidemiology Monitoring

• Use *pol* gene sequences for genotyping and phylogenetic analysis
  – REGA HIV-1 Genotyping Tool (BioAfrica)
  – NCBI Viral Genotyping Tool (NCBI)

• Use bioinformatics computer software to analysis transmission of HIV-1
  – MUSCLE, BioEdit, **PAUP* 4.0**, BEAST

• Compile phylogenetic results with the clinical epidemiological background of patients
REGA HIV-1 Genotyping Tool

http://www.bioafrica.net/rega-genotype/html/subtypinghiv.html

- Correctly genotype non-B sequences
- Web-based and FREE
NCBI Viral Genotyping Tool

Transmission of HIV-1 in Hong Kong

Summary

• HIV-1 viral load (VL) and genotyping resistance test (GRT):
  – essential for HIV-1 patient care

• Lowest detection limit (VL): 40 copies/mL

• New generation of GRT:
  – new ARV drugs eg. INI, CCR5 antagonist

• GRT can be used for HIV-1 subtyping

• The pol sequences generated in GRT can also be used for epidemiological investigation
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