New Technologies in Molecular Diagnosis: HIV

NSW State Reference Laboratory for HIV/AIDS & Molecular Diagnostic Medicine Laboratory, SydPath

St Vincent’s Hospital Sydney
Trends in molecular diagnostics

- Detection of target genes of interest
- Quantification

**Infectious diseases**
- HIV
- Hepatitis C & B
- TB / MAC
- Cytomegalovirus
- Herpes simplex
- Varicella zoster
- CT/GC
- HPV

- Profiling mutations associated with disease outcome

- Hepatitis C genotype
- HIV drug resistance genotype
- Host genetic factors
- Thrombophilia
- CyP450 – drug metabolism
- HLA type
Key developments

Technology

- Uptake in diagnostic arena
- Alternative methods to PCR – SDA, TMA, LCR, NASBA, bDNA
- Availability of Analyte Specific Reagents (ASR)
- Trend to real time or kinetic formats
- Automation
- Contamination and inhibitor control
What is driving commercial NAT platform development

- Reduce sources of error
- Reduce tedious processes
- Improve time to result
- Improved analytical range
- Improve limit of detection
- Improve specificity
Pre amplification - extraction

- Volumetric errors amplified
- Tedious – manual, repetitive
- Specimen integrity
Post amplification & detection

Endpoint detection

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

Comparison of Amplification Methods

HIV, HBV, HCV, CMV
Standard curve
Amplify signal of label – no amplicon issues
Overnight
High throughput
Limited extraction
Kinetic / real time product detection
Monitoring in Real time

Agarose Gel Blotting  FRET
Real time PCR
Real Time PCR with 5’ Nuclease Assay

Product detection during amplification

Fluorescence Emission
Quenched

Fluorescence Emission Detected
### 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 | <40 – 10,000,000 |
| Biomerieux   | EasyQ HIV-1 real time TMA *(gag)* | Copies/mL & IU/mL  
(4-5 hours to results) | New | <40 – 10,000,000 |
| Abbott       | Celera Realtime PCR m2000 *(pol integrase)* | copies/mL | New /  
evaluation | <40 – 10,000,000 |
| Artus        | Realtime PCR – Rotorgene | Copies/mL | Evaluation | <40 – 10,000,000 |
Selected Applications

- Primary HIV diagnosis & enhanced surveillance
- Infant HIV diagnosis
- HIV treatment & progression monitoring
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
    - acute infection (pre-seroconversion)
    - RNA quantitative – monitoring / serial viral load
    - drug resistance monitoring
    - subtyping – treatment and surveillance
Minipool NAT testing in blood donors

4 mini pools of 6 samples each
4 mini pools combined and tested
(total = 24 samples in single test)
Minipool NAT testing in blood donors

Individual retesting of each minipool members to identify the positive sample
Enhanced surveillance of acute primary HIV infection

Eligible samples referred from high case load primary care practices screened NEGATIVE by standard diagnostic serology tests

Re-test ALL Mini pool members to identify the positive sample

Mini pools of 6 samples each
HIV drug resistance testing
Breakthrough of resistance

Viral Load

- Drug-susceptible Virus
- Drug-resistant Virus

- Not taking medicines properly
- Taking medicines properly
DNA sequencing
### Mutations in the Protease Gene Associated with Reduced Susceptibility to Protease Inhibitors

#### Protease Inhibitors

<table>
<thead>
<tr>
<th>Multi-Protease Inhibitor Resistance (accumulation of mutations)</th>
<th>L</th>
<th>M</th>
<th>I</th>
<th>V</th>
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<th>Lopinavir/Ritonavir&lt;sup&gt;14,15&lt;/sup&gt;</th>
<th>L</th>
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#### Mutations

- **Amino Acid, Wild-Type**
- **Primary (protease only)**
- **Secondary (protease only)**
- **Amino Acid, Substitution**
- **Vertical Pink Lines Indicate NAMs**
- **Selected In Vitro**
- **Insertion**

See Footnotes 6 and 15.

See Footnote 14.
TRUGENE HIV-1 RESISTANCE REPORT Example

Sample ID: 094E-X-034
Patient ID: 212-45-23/09
Patient Name: Doe, John
Date Drawn: August 13, 2001
Physician: Dr. John Johnson
Institution: Mt. Sinai Hospital
Report Date: August 19, 2001, 12:00:55-0400

Relevant RT Mutations: K65R Q151L M184V T215F

Drug Class

Nucleoside RT Inhibitors | Resistance Interpretation
-----------------------|-----------------------
zidovudine             | Resistance
didanosine             | Resistance
zalcitabine            | Resistance
lamivudine             | Resistance
 stavudine              | Possible Resistance
abacavir               | Resistance
tenofovir              | Possible Resistance
toscarit               | Possible Resistance

Non-Nucleoside RT inhibitors | Resistance Interpretation
----------------------------|-----------------------
nevirapine              | No Evidence of Resistance
delavirdine             | No Evidence of Resistance
efavirenz               | No Evidence of Resistance

Relevant Protease Mutations: G48V

Protease Inhibitors | Resistance Interpretation
-------------------|-----------------------
sequinsin            | Resistance
indinavir            | No Evidence of Resistance
ritonavir            | No Evidence of Resistance
nefrinavir           | No Evidence of Resistance
amprenavir           | No Evidence of Resistance
lopinavir with ritonavir | No Evidence of Resistance

Resistance interpretation is 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. These include primary and secondary mutations. * (pass) refer to comments in mutation details sections.

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

Guideline™ Rules developed by international expert panel based on interpretation of available in vitro phenotypic and in vitro virologic response data. Utilizes published studies.

Treatment decisions should be made in consideration of all relevant clinical and laboratory findings and the prescribing information of the drugs in question.

The TRUGENE HIV-1 Genotyping Test Resistance Report uses Guideline™ rules developed by an international expert panel.

HIV-1 genotype analysis by DNA sequencing. For In-Vitro Diagnostic Use.
Neonatal HIV diagnosis

- **Serologic assays**
  - Maternal antibodies persist up to 18 months postpartum
  - Antibody tests not helpful in newborn diagnosis
  - Sero-reversion (pos → neg) in serial samples
  - HIV-1 p24 antigen limited value – complexed by Ab

- **Virologic assays**
  - Virus culture from PBMC
  - Maternal HIV-1 RNA in obstetric setting is useful in predicting risk of perinatal transmission
  - HIV DNA and RNA useful in infant
    - Detection in infant is diagnostic for perinatal HIV infection
    - Useful in timing of transmission (in utero, intrapartum, post partum)
    - Monitoring response to therapy in infected infant
Qualitative HIV DNA PCR

- Detects HIV proviral DNA in peripheral blood mononuclear cells
- Most often recommended as preferred virologic test
- Sensitivity varies from 50% in the first month to >96% after 1 month (Zaman MM et al. Clin Infect Dis 2002; 34:417-18)
- Meta analysis of 96 studies using DNA PCR in infants reported 91.6% median sensitivity and 100% median specificity in early diagnosis (Owens DK et al. JAMA 1996;275:1342-48)

- 38% (29-46% 90%CI) were detectable at 48hrs
- 93% (76-97% 09%CI) detectable at 14 days
Positive 48h (likely intrauterine Infection - early)

HIV infection reasonably excluded in non-breast fed infant if negative in 2 or more ≥1 month and ≥4 months

Positive 14d (likely intrapartum Infection - late)

>2 negative HIV Ab tests (<1 month apart)
Loss Ab/ neg DNA = un-infected

Infant still Ab+ at 12mo – retest 15-18mo

HIV RNA/CD4

HIV Ab+ >18mo = HIV infection

Months post partum
HIV laboratory test quiz?

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<th>HIV Ag</th>
<th>HIV RNA</th>
<th>HIV DNA</th>
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Established HIV infection on ARV therapy
Recent primary Infection
BFP, maternal Ab in uninfected infant
Acute infection
Very early acute infection
False positive
HIV RNA viral load