Review of Viral Load Technologies

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Model for HIV Assays in Resource-Poor Settings

- **Reference Center**
  - Viral load
    - Expensive
    - Complex technology
    - Gold standard

- **Provincial or district level**
  - P24/Reverse transcriptase?
    - Lower cost
    - Less complex technology

- **Primary care or rural setting**
  - Ship samples (DBS or fixatives)
    - Least resource intensive
    - Least complex
Commercially Available Viral Load – HIV RNA

- *Roche Monitor, 1.5 – RT-PCR
- *bioMerieux NucliSens-isothermal NASBA
- *Bayer Versant - bDNA
- bioMerieux EasyQ – molecular beacon
- Primagen Retina Rainbow – molecular beacon

* FDA approved
Pros and Cons of HIV RNA Assays

**Advantages**
- High Throughput
- Well validated
- 3 are FDA approved
- Clinician familiarity
- Most subtypes
- Manufacturers QA reagents
- Work with DBS
- Possible reduced price through large volume purchase

**Disadvantages**
- Expensive equipment
- Expensive reagents
- Technologically complex
- Equipment maintenance
Other Assays

- Real time PCR
- P24 antigen
- Cavidi RT
- Flow
- Point of Care –
  - Dipstick
  - Nanoparticles
  - Chip technology
  - Shipping specimens
### Pros and Cons of Real Time PCR Assays

<table>
<thead>
<tr>
<th><strong>Advantages</strong></th>
<th><strong>Disadvantages</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>• Reagents inexpensive compared to commercially available kits</td>
<td>• Very expensive equipment</td>
</tr>
<tr>
<td>• Can be very sensitive (Palmer)</td>
<td>• Home brew assays, so variability in reagents and no manufacturer’s QA</td>
</tr>
<tr>
<td>• Can be very specific (Rouzioux)</td>
<td>• May need different primers and probes for different subtypes</td>
</tr>
<tr>
<td>• Assays for additional pathogens can be developed</td>
<td>• Royalty charge for use of Taq</td>
</tr>
<tr>
<td></td>
<td>• Reproducibility</td>
</tr>
<tr>
<td></td>
<td>• Technologically complex</td>
</tr>
<tr>
<td></td>
<td>• Prone to contamination</td>
</tr>
<tr>
<td></td>
<td>• Clinical validation yet to be done</td>
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</tbody>
</table>
HD24 Ag Assay
Specimen preparation

- Dilute 50 ul of plasma with 250 ul of detergent-containing lysis buffer
- Mix and incubate 10 min at room temp
- Heat for 5 min at 100C in dry heat block
- Cool to room temperature
Up24 Antigen Assay

- Capture antibody
- Antigen 2 hr with shaking RT
- Detector antibody-biotin 1 hr, 37
- Biotinyl-Tyramide 15 min, RT
- Streptavidin-HRP 15 min, 37
- Streptavidin-HRP 15 min, RT
- OPD 30 min, RT
- Capture antibody
Heat Dissociated p24 Antigen

- Assay works very well to diagnose infants (Sutthent, 2003; Sherman, 2004; Nouhin, Bangkok; DeBaets, 2005; Respess CROI)
- New buffer described by Dr. J. Schupbach (JAIDS, 2003) increases sensitivity of the assay (Jennings, ICAAC, 2003; Fiscus, CROI 2004)
- In general studies using the kit buffer have performed less favorably (Bonard, 2003; Prado, 2004) compared to those using the Schupbach buffer (Ribas, 2003; Schupbach, 2003; Stevens, in press)
## HDp24 for Infant Diagnosis

<table>
<thead>
<tr>
<th>Author</th>
<th>N</th>
<th>Sens</th>
<th>Spec</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sutthent (Thailand)</td>
<td>142</td>
<td>100%</td>
<td>100%</td>
<td>A/E, B</td>
</tr>
<tr>
<td>Sherman (So Africa)</td>
<td>203</td>
<td>98%</td>
<td>98.5%</td>
<td>C</td>
</tr>
<tr>
<td>Nouhin (Cambodia)</td>
<td>167</td>
<td>91.1%</td>
<td>99.2%</td>
<td>A/E</td>
</tr>
<tr>
<td>DeBaets (DRC)</td>
<td>150</td>
<td>92.3%</td>
<td>100%</td>
<td>multiple</td>
</tr>
<tr>
<td>DeBaets (DRC)</td>
<td>87</td>
<td>100%</td>
<td>100%</td>
<td>multiple</td>
</tr>
<tr>
<td>Respess (US)</td>
<td>749</td>
<td>95.8%</td>
<td>98.7%</td>
<td>B</td>
</tr>
</tbody>
</table>
### Performance of p24 and Up24 for Detection of Acute HIV

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity p24</td>
<td>0.750</td>
<td>(0.476, 0.927)</td>
</tr>
<tr>
<td>Sensitivity Up24</td>
<td>0.842</td>
<td>(0.604, 0.966)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.995</td>
<td>(0.988, 0.999)</td>
</tr>
<tr>
<td>LR+ p24</td>
<td>161.8</td>
<td>(58.5, 447.8)</td>
</tr>
<tr>
<td>LR+ Up24</td>
<td>181.7</td>
<td>(67.0, 492.3)</td>
</tr>
<tr>
<td>LR-</td>
<td>0.25</td>
<td>(0.11, 0.59)</td>
</tr>
</tbody>
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Patient Monitoring

Dates

Log10 values

11/13/1998
1/13/1999
3/13/1999
5/13/1999
7/13/1999
9/13/1999
11/13/1999
1/13/2000
3/13/2000
5/13/2000
7/13/2000
9/13/2000
11/13/2000
1/13/2001
3/13/2001

vi
p24
Pros and Cons of Heat Dissociated p24 Antigen

- **Advantages**
  - Equipment generally available
  - Less technologically complex
  - High throughput
  - Less prone to contamination
  - Excellent for infant diagnosis
  - Appears to work well in diagnosing acute HIV infection
  - Very reproducible

- **Disadvantages**
  - Doesn’t measure virion-associated molecule, so sometimes get different results than RNA
  - Works best with non-kit buffer, so has similar QA problems to other “home-brew” assays
  - Usually not as sensitive as most of the other assays
  - Need more data on other subtypes and clinical validation
Cavidi ExaVir Assay (RT)

- Newer version of assay much more sensitive (Jennings, unpublished; Greengrass, in press; Shao, Bangkok)
- Being evaluated as an alternative to VL testing (Stevens, in press; Greengrass, in press)
- Phenotype assay – Simon, Bangkok; Ntsala, Bangkok
RT Viral Load Assay: Virion separation procedure
RT Viral Load Assay: RT Activity Determination
Virologic Results

- In Malawi we have tested 126 samples from 40 subjects
- Correlation coefficient for log10 HIV RNA and log10 RT is 0.89
Australian cohort (Greengrass et al, In Press)

Comparison of viral load monitoring using Cavidi ExaVir, Bayer bDNA and Roche Amplicor in HIV patients on ARVs from the Australian cohort, Melbourne, Australia
## Pros and Cons of the ExaVir Assay

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Should work on all subtypes</td>
<td>• Long assay (3 days)</td>
</tr>
<tr>
<td>• Inexpensive equipment</td>
<td>• Phenotype assay only for NNRTIs and T analog NRTIs</td>
</tr>
<tr>
<td>• Sensitive to about 500 cp/ml</td>
<td>• Nearly as expensive as RNA assays if you can get a large volume discount</td>
</tr>
<tr>
<td>• Phenotype from same RT prep</td>
<td></td>
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<tr>
<td>• Less prone to contamination than PCR assays</td>
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Point of Care Tests

- Dipstick – Helen Lee
- Nanoparticles for p24 Ag detection (Kim, Wolinsky)
- Niel Constantine
- Chip Technology – Bill Rodriguez, others
- Shipping specimens
  - Dried blood spots
  - Sample tanker – stabilizes dried plasma - Rob Lloyd
Obstacles to Implementation

- Infrastructure
- Guidelines and consensus protocols
- Lack of trained laboratory personnel
- Training tools
- Monitoring and evaluation tools
- Supply management
- Data management
- Quality assurance (Proficiency testing)
Conclusions

- Commercially available viral load assays are becoming less expensive, but are still technologically complex and best suited for large reference labs.
- Real time PCR assays, though less expensive for reagents, suffer from high equipment costs and lack of QA of reagents.
- HD P24 antigen seems very suitable for infant diagnosis, and much less expensive than NAT.
Conclusions (2)

- Alternative assays for viral load (p24, RT, etc) may be useful in provincial labs, but:
  - Are in a state of flux
  - P24 may not strictly correlate with HIV RNA VL
  - P24 assay gives best results with an external lysis buffer
  - P24 and RT assays need more clinical validation, especially with the latest versions
Conclusions (3)

- Primary care or rural settings for the moment will have to ship samples to a reference laboratory.
- Point of care testing may be available in the next few years, but results will have to carefully QA’d and costs may make it better to ship samples to a reference lab with high throughput, QA, and negotiated kit prices.
Conclusions (4)

- There are many obstacles to introducing laboratory assays in general to labs in resource poor settings.
- Many groups large and small are trying to tackle the problems, many of which are country specific.
- Collaboration by the large groups for a global strategy, and by all players in a given country for the local plan, will reduce confusion and avoid waste of time and effort.
Are VL Assays Necessary for Starting and Monitoring ART?

- Starting ART – Probably not necessary – CD4 is probably adequate

- Monitoring ART for an individual
  - Early to assess efficacy
  - Later to determine failure and time to switch
  - But it’s hard to make comparisons unless you have the baseline value

- Important for public health considerations