HIV Monitoring Technologies for Resource-Limited Settings

Review of Viral Load Technologies

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Model for HIV Assays in Resource-Poor Settings

- Reference Center>>>
- Provincial or district level >>>>>
- Primary care or rural setting>>>

- Viral load
  - Expensive
  - Complex technology
  - Gold standard
- P24/Reverse transcriptase?
  - Lower cost
  - Less complex technology
- Ship samples (DBS or fixatives)
  - Least resource intensive
  - Least complex


NC PROJECT


DON'T KEEP FOOD-STUFFS AND
DRINKS IN THIS FRIDGE
CHONDE TISAIKE ZAKUDYA
KAPENA ZAKUMWA M'KATIMU

Steps to Validation and Technology Transfer

- Performance characteristics
  - Sensitivity
  - Specificity
  - Precision
  - Reproducibility
  - Linearity
- Clinical validation
  - Diagnosis
  - Clinical monitoring
  - Progression of disease
- Costs (Equipment, reagents, personnel)
- Technology transfer
- Proficiency testing
- Dissemination/Acceptance

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

- NASBA – WePpB 2059; ThPeB 7045
- Versant – MoPeB 3140; MoPeC 3419
- bioMerieux Easy Q – McLernon, CROI, 2004; MoPeB 3123; MoPeB 3145
- Retina Rainbow – WePpB2064; WePeE6864
- Abbreviated Roche assay – MoPeB 3093

Pros and Cons of HIV RNA Assays

- **Advantages**
  - High Throughput
  - Well validated
  - 3 are FDA approved
  - Clinician familiarity
  - Most (all) 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
- Point of Care –
  - Dipstick
  - Chip technology
  - Shipping specimens

Real Time PCR

- Several recent papers (Palmer, et al 2003; Gibellini, et al., 2004)
- Real-time immuno-PCR (Barletta, et al 2004; MoPeB 3170)
- Several posters here – MoPeB 3114; MoPeB 3115; MoPeB 3116; MoPeB 3143; MoPeB 3145; MoPeB 3162; MoPeB 3167)

Pros and Cons of Real Time PCR Assays

- **Advantages**
  - Reagents inexpensive compared to commercially available kits
  - Can be very sensitive (Palmer to 1 cp/ml, using 7 ml of plasma)
- **Disadvantages**
  - Very expensive equipment costs
  - Home brew assays, so variability in reagents and no manufacturer's QA
  - Reproducibility
  - Technologically complex
  - Prone to contamination
  - Clinical validation yet to be done

Heat Dissociated p24 Antigen

- Assay works very well to diagnose infants (Sutthent, 2003; Sherman, 2004; Fiscus, unpublished; MoPeB 3112; WePpB 2057)
- 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)

Heat Dissociated p24 Antigen

- Other sources of p24 kits with heat stable epitopes are Zeptometrix and Innogenetics
- Posters – MoPeB 3144; MoPeB 3168; TuPeA 4357; TuPpB 2036)

Pros and Cons of Heat Dissociated p24 Antigen

- **Advantages**
  - Equipment generally available
  - Less technologically complex
  - High through put
  - Less prone to contamination
  - Excellent for infant diagnosis
  - Very reproducible
- **Disadvantages**
  - Doesn't measure virion-associated molecule, so often get different results than RNA
  - Works best with non-kit buffer, therefore, has similar QA problems to other "home-brew" assays
  - Usually not as sensitive as most of the other assays
  - Limited dynamic range
  - Need more data on other subtypes and clinical validation
  - Probably as expensive as RNA assays if you can get a large volume discount

Cavidi ExaVir Assay (RT)

- Newer version of assay much more sensitive (Jennings, unpublished; Crowe, unpublished; MoPeB 3171)
- Being evaluated as an alternative to VL testing (Stevens, in press; TuPpB 2037)
- Phenotype assay – MoPeB3155; WePeB5733

Pros and Cons of the ExaVir Assay

- **Advantages**
  - Should work on all subtypes
  - Inexpensive equipment
  - Sensitive to at least 400 cp/ml
  - Phenotype from same RT prep
  - Less prone to contamination than PCR assays
- **Disadvantages**
  - Very long assay (3 days)
  - Tedious extraction process
  - Phenotype assay only for NNRTIs and T analog NRTIs
  - Probably as expensive as RNA assays if you can get a large volume discount

Point of Care Tests

- Dipstick – Helen Lee
- Chip Technology – Bill Rodriguez, others
- Shipping specimens
  - Dried blood spots
  - Sample tanker – stabilizes dried plasma
  - Tempus RNA stability tube
  - Transfix

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 suitable for infant diagnosis, and much less expensive than NAT

Conclusions (2)

- Alternative assays for viral load (p24 and RT) 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 a home-brew 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

Issues to consider when choosing the viral load assay?

- Performance of assay – dynamic range, specificity, reproducibility, subtype specificity
- Cost of the assay, and infrastructure
- Cost and availability of the personnel
- Specimen shipment and storage
- Specimen volume
- Quality control, Contamination control, Internal controls
- Availability of automation steps
- Turn around time (less important)