Update on Alternatives to Viral Load Testing

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Review of papers and presentations since Jan 2005

- Real time PCR
- P24 antigen
- Cavidi RT VL and phenotype
- Dried blood spots
- Dipstick technology

Real time PCR - DNA

- Luo, et al, CDC, Clin Micro April 2005
- Describe a RT PCR using dried blood spots (903 paper)
- Closed system, used UNG to minimize contamination
- Appears sensitive to 10 copies of DNA/test
- Includes an internal control
- In this MS, only 103 specimens from adults with subtype B and 56 seronegatives were assayed
- No data on other subtypes given, though stated that the assay works with other clades
- Has the assay actually been transferred to a RLS?
- Home brew primers and probes
- Would work best in a centralized lab setting

Real Time PCR - RNA

- Rouet, et al., J Clin Micro, June 2005
- Have set up RT-PCR testing in Abidjan
- Closed system
- Tested 806 individual specimens from adults and kids
- Limit of quantitation ~ 300 cp/ml
- High throughput, very reproducible, ~$12/test
- 97.9% sensitive cfd to bDNA, correlation r=0.90
- 98% sensitive cfd to Roche RNA, r=0.86
- External standard curve, no internal standard
- Home brew primers and probes
- Equipment very expensive to buy ($30,000-40,000) and maintain
- Best for centralized testing labs, not peripheral labs

RealT*ime* PCR

- Abstracts # 663 and 665 (Abbott)
- New probe designed to tolerate mismatches due to genetic diversity of HIV
- Linear dynamic range 40-10 million cp/ml
- All subtypes detected
- 100% specific
- Combined with automated sample prep system and tested in Brazil
- 89/91 specimens detected – 2 negatives also neg in PCR
- $$$$$$$

UP24 ag for infant diagnosis

- Zijenah, et al., JAIDS, Aug 2005
- Tested 164 infants from Zimbabwe (subtype C)
- Used the kit lysis buffer
- Compared to Roche DNA PCR
- $10/test
- These results are similar to others

Parameter	0-18 mo	0-6 mo	7-18 mo
Sens	96.7%	98.1%	89.5%
Spec	96.1%	96.9%	91.1%

UP24 for infant diagnosis

- De Baets, et al, Clin Diag Lab Immun Jan 2005
- Specimens from children in DRC (many subtypes)
- UP24 tests performed in Belgium
- Sensitivity 92.3%, specificity 100% when performed on liquid plasma (n=150)
- Sensitivity 100%, specificity 100% when performed on dried plasma spots (n=87), unspecified S&S paper

Abbott combo Ab/Ag assay

- Vallar et al #372
- P24 antigen test of 226 plasmas from Cameroon, neg for Ab by standard Abbott EIA
- 2 p24 Ag reactive also + for HIV RNA
- Could be useful for identifying cases of acute infection or for diagnosing infants

UP24 and Dried Blood Spots

- Patton, et al., Clin Vacc Immunol Jan 2006 (JoBurg)
- Adapted Up24 assay to work with dried blood spots
- Whatman #1 paper
- Washed prepared DBS with external viral lysis buffer (10 RT), then with kit lysis buffer O/N at 4C. The rest of the assay followed package insert
- Very reproducible
- Sensitivity 98.8%, specificity 100% (n=141), compared to Roche RNA, DNA or NASBA RNA
- Correlation between plasma VL and DBS p24ag- r=0.79
- All specimens initially tested within 6 weeks of draw. AT 12 weeks had lost sensitivity.

Up24 antigen and Cavidi RT VL

- Lombart, et al., AIDS, 2005
- 84 samples from 70 subjects from Burkino Faso
- Cavidi V1.0 was compared to Roche RNA, v1.5
- Very reproducible
- In follow-up of serial samples, RT assay gave more concordant results to RNA, than did Up24

Assay	VL - 1.7-4 N=11	VL- 4.1-4.8 n=15	VL - 4.9-6.5 N=58	Spearman correlation
RT colorimetric	0 (0%)	14 (93%)	58 (100%)	
RT Fluorimetric	8 (73%)	15 (100%)	58 (100%)	0.85
Up24	3 (37%)	12 (80%)	50 (86%)	0.39

Cavidi RT VL

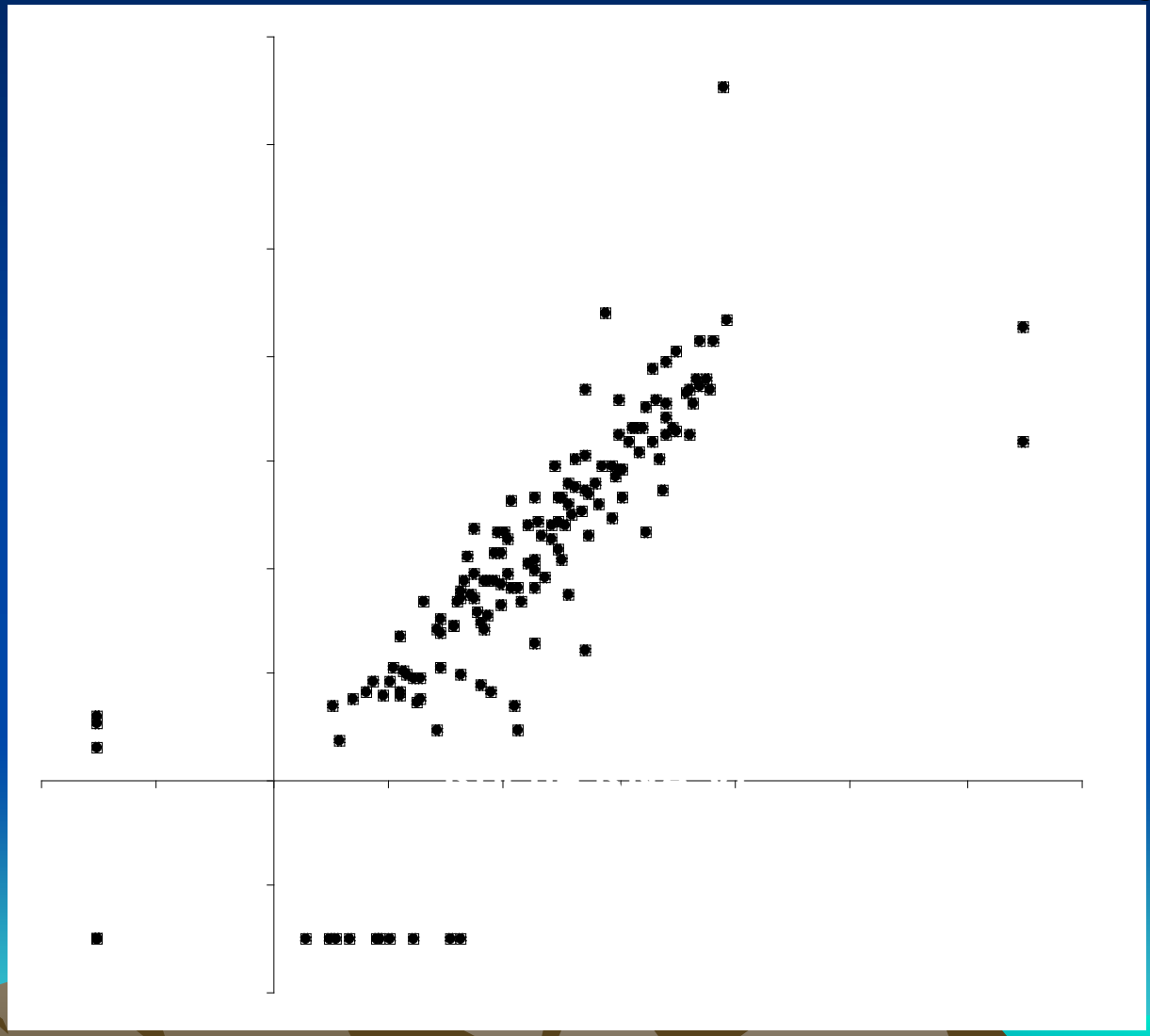
- Seyoum, et al, J Med Virol 2006
- 178 samples from 26 subjects
- Assays conducted in Addis Ababa
- Only used 0.2 ml plasma and used version 1.0
- R=0.65 compared to the NucliSens assay

Cavidi RT VL

- Sivapalasingham et al, J Clin Micro Aug 2005
- Used version 2.0, compared to Roche RNA, v1.5
- Specimens from NYC (29) and Cameroon (21)
- Limit of detection ~2000 cp/ml
- Overall concordance with Roche was 76%
- Cavidi false negatives all had VL <3000 cp/ml and were all from NY
- Correlation, r=0.869
- All testing was done in NY
- $28.13/test, 3 days, 1 ml plasma

Cavidi RT VL and Phenotype

R=0.92



Fiscus CROI 2006

EFV exposure, genotypes and Cavidi Phenotype

Sample	EFV use	Cavidi phenotype results	Genotype Results							
			L100I (n=1)	V108I (n=1)	K103N (n=10)	V106M (n=0)	Y181C (n=3)	Y188L (n=2)	G190A (n=2)	G190S (n=1)
988	Current	Resistant			■					
5002	Current	Resistant			■					
5286	Current	Resistant					■			■
879	Past	Resistant			■					
1522	Past	Resistant			■					
2166	Past	Resistant			■					
3398	Past	Resistant			■					
5061	Past	Resistant			■					
6694	Past	Resistant		■	■					
1817	Past	Resistant						■		
7218	Past	Resistant						■		
66	Past	Resistant							■	
1511	Past	Resistant					■			
1892	Past	Resistant					■			
5605	Past	Resistant							■	
2056	Never	Resistant			■					
6023	Never	Resistant	■		■					
601	Current	Sensitive								
665	Past	Sensitive								
1592	Past	Sensitive								
4864	Past	Sensitive								
368	Never	Sensitive								
1125	Never	Sensitive								
[illegible]	Never	Sensitive								
[illegible]21	Never	Sensitive								
7253	Never	Sensitive								

Dried Blood Spots

- Sherman, et al., JAIDS April 2005
  - DBS at 6 wk – Whatman #1 paper, 9-19mo storage at room temp with no desicant, 288 specimens
  - Easy extraction procedure
  - Roche HIV DNA, v 1.5
  - 100% sensitivity; 99.6% specificity
- Uttayamakul, et al. J Virol Methods 2005
  - 100 sero-, 109 sero+
  - DNA PCR Whatman 94% sens 100% spec
    Isocode 89.4% sens 100% spec
  - RNA NucliSens QL 89.7% sens 97.5% spec
  - RNA NS QT – DBS cfd Plasma – r=0.817
- Lou – RT PCR
- Patton – p24 antigen and DBS

DBS at CROI 2006

- Garcia-Lerma # 666 - used for surveillance of drug resistance in Cameroon (stored at -20 2-3 yr) and VQA panels – one stored at -20, one at -70 and one at RT
- 5/6 DBS stored at -20 and -70 could be sequenced; 0/3 stored at RT
- 34/37 (92%) of Cameroonian DBS amplified
- Proviral DNA contributed significantly, but were usually concordant with plasma sequence

DPS to Monitor Resistance

- Dachraoui Poster #549
- 20ul DPS from Tunisian subjects
- Stored at RT with desicant, mailed to France within 5-10 days, then stored at -80
- 67-77% of PR, RT, and gp41 sequenced successfully
- 87-100% sensitivity with VL >10,000
- Less successful with lower VL

Infant Diagnosis - #715

- Creek et al
- DBS collection and testing in Botswana 6-17 weeks
- 61 DBS – 100% concordance with Roche DNA in validation study
- Only 2% of 822 DBS rejected, results TAT ~8 days

Point of Care Testing - Dipstick

- Dineva, et al., J Clin Micro Aug 2005
- Multiplex dipstick for detecting HBV, HCV and HIV nucleic acids
- Extraction – High Pure kit (Roche)
- Amplification – Taqman realtime RT-PCR
- Detection – dipstick – 15 min
- Detection limits – 50IU HBV DNA, 125 IU HCV RNA, 500 IU HIV RNA

Conclusions (1)

- The field is moving forward rapidly
- RT PCR – equipment still expensive with maintenance issues, largely homebrew, best suited for centralized lab setting
- P24 antigen has been successfully used for infant diagnosis in many settings, but still struggling for acceptance compared to NAT
- Cavidi VL and phenotype assay very promising, but need more data

Conclusions (2)

- DBS (and DPS) are gathering wide acceptance for diagnosis and resistance surveillance, but room temperature storage is proving to be a problem for proteins and DNA. Will probably need to maintain the cold chain which may reduce utility. RNA seems to have more stability at ambient temperature
- POC testing, such as Helen Lee's dipstick, will continue to be the Holy Grail, but need considerable more work
- Infant diagnosis and monitoring at peripheral sites may have to rely on detection or quantitation of RNA through the use of DBS or RNAlater