Overview of Infant HIV Diagnosis Based on Detection and Quantification

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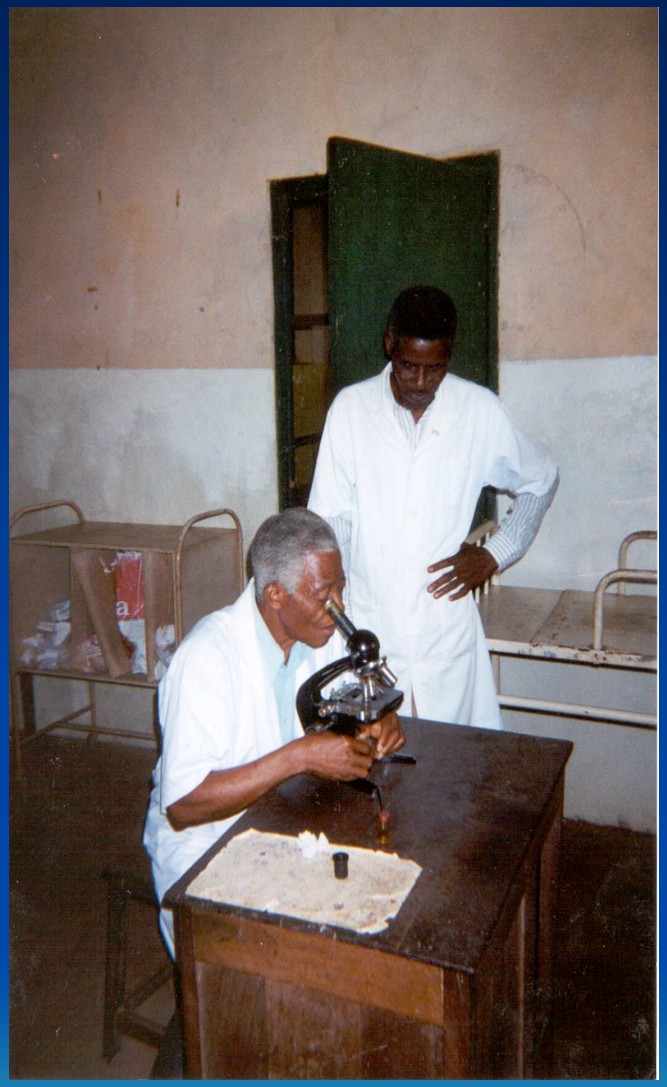
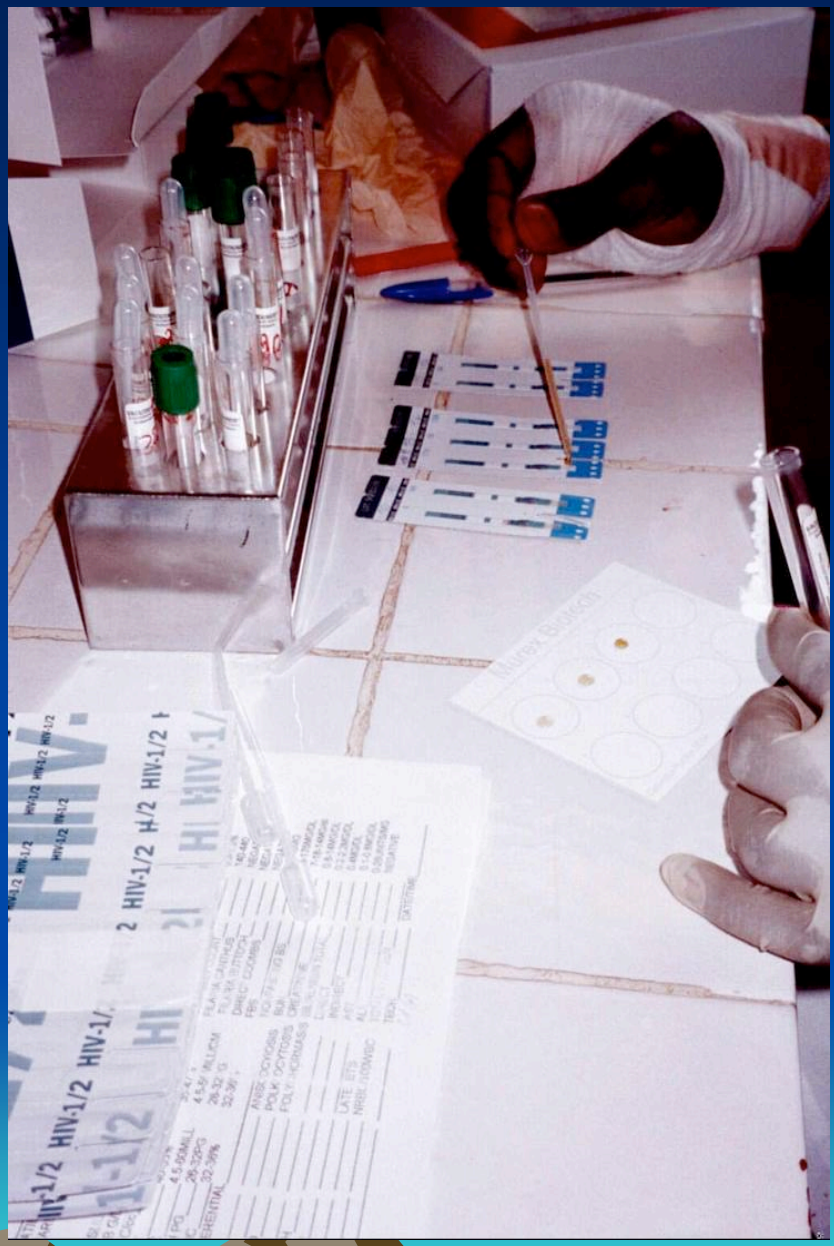
Model for HIV Assays in Resource-Poor Settings

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

- NAT (RNA/DNA)
  - 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





On-site realities

Outline

- 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
- $$$$$$$

NC and NYC PACTS Data

Time from Birth	N	Sensitivity	Specificity
0-7 days	114	62.7% 94.7%	99.7 99.0%
8-30 days	180	91.6% 93.8%	98.4 99.1%
31-90 days	368	94.4% 96.1%	97.6% 98.6%
91-180 days	141	91.4% 94.0%	98.4% 98.6%
>180 days	93	97.0% 94.0%	95.4% 99.1%

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.

HDp24 for Infant Diagnosis

Author	N	Buffer	% Sens	% Spec	Subtype
Sutthent	142	BioMer	100	100	A/E, B
Sherman	203	Kit	98	98.5	C
Nouhin	167	Kit?	91.1	99.2	A/E
DeBaets	150	Ext	92.3	100	multiple
DeBaets	87	Ext	100	100	multiple
Zijenah	164	Kit	96.7	96.1	C
Patton	141	Ext	98.8	100	C
Respess	757/482	Kit/Ext	93/98.4	95.6/98.9	B

Cavidi RT VL

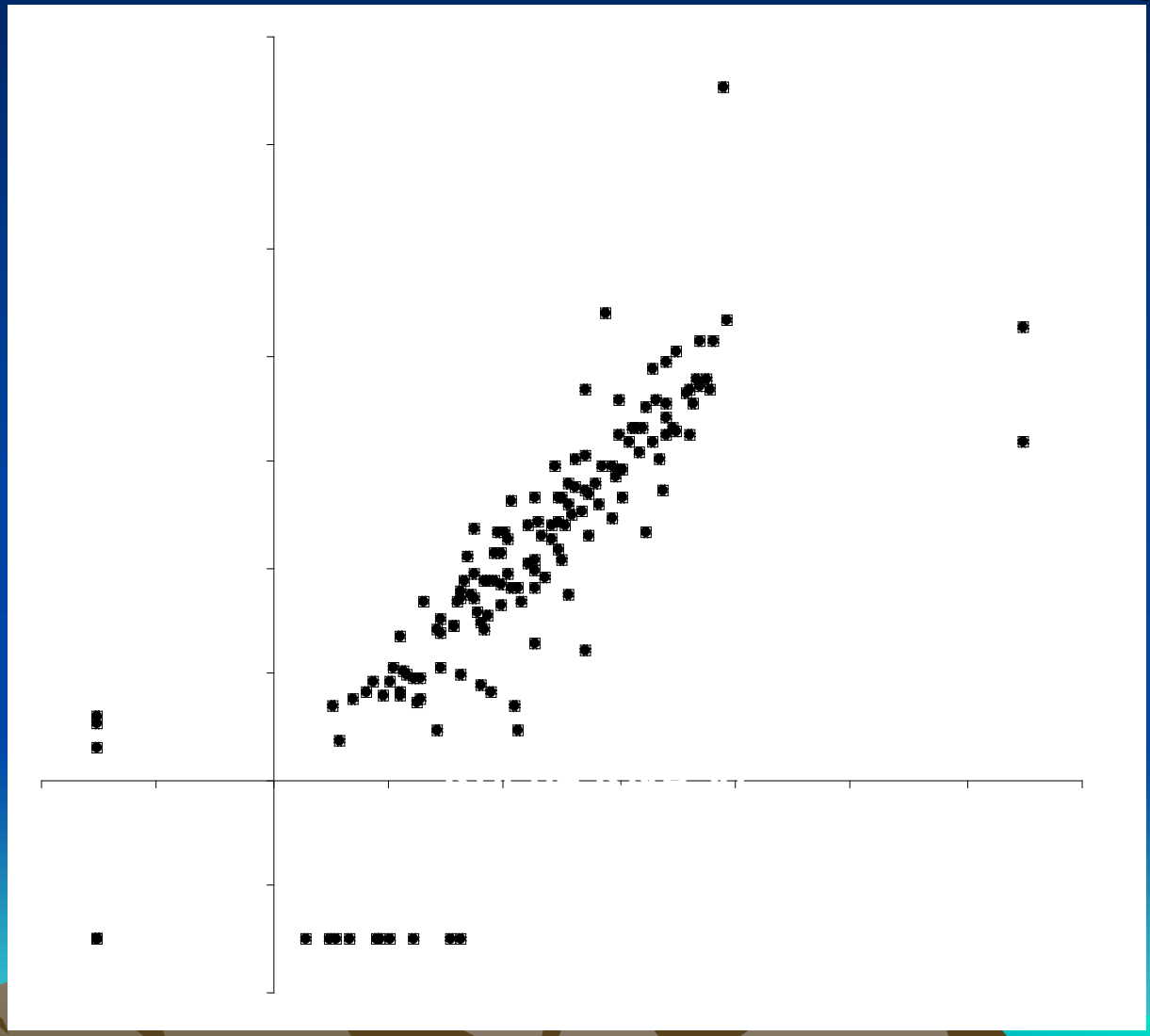
- Seyoum, et al, J Med Virol 2006
- 178 samples from 26 subjects
- Assays conducted in Addis Ababa
- Only used 0.2 ml plasma and used version 1.0
- R=0.65 compared to the NucliSens assay

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

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

Other Things to Consider

- Centralized testing vs decentralized and POC
- Get specimen to lab and result back to site
- Getting the infected child into care
- Training and EQA for decentralized sites
- For VL monitoring – what are the cut-offs?
- Substudies in clinical trials to evaluate assays and cut-offs

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 is still struggling for acceptance compared to NAT. >1500 children tested in PE p24– Sens 91-100%, Spec 95-100%
- Cavidi VL and phenotype assay very promising, but need more data, esp with smaller volumes

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, need considerably 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

Define how early virologic diagnosis can be institutionalized into PH setting

KEY POINTS

- **Early diagnosis is key**
  - **Huge loss to F/U**
  - **50% mortality**
  - **Rapid disease progession**
- **6 week HIV virologic assay (DNA, RNA, Up24, dipstick-POC)**
- **Need support of MOH, clinicians, lab, family**

ACTION ITEMS

- **Continue to develop, evaluate, and validate simpler, cheaper methods for infant diagnosis**
- **Disseminate this info**
- **Improve the DBS methodologies**
- **Develop SOPs and training materials**
- **Train nurses, midwives, clinicians, lab techs**
- **Increase lab capacity including lab techs**
- **External QA program**
- **Integrate into national health care program**
- **Set targets, monitor and evaluate**