Dried Blood Spots and Other Sample Types for HIVDR Testing

DEBORAH PERSAUD, MD

PROFESSOR OF PEDIATRICS

JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

& BLOOMBERG SCHOOL OF PUBLIC HEALTH

JOHNS HOPKINS MEDICINE SCHOOL OF MEDICINE

CENTER FOR AIDS RESEARCH JOHNS HOPKINS UNIVERSITY

Focus

SESSION ORGANIZER

Practical

Operational

Policy

PROGRAM ORGANIZERS

Lessons from pMTCT

Seeding of HIV reservoirs with resistant virus

Next generation sequencing methods

Practical

TOPIC 1

Sample Types: Practicality

Liquid

Plasma

Dried

Whole Blood

Dried Blood Spots (Advantages)

Easy to collect

Easy to store

No pre-processing

RNA and DNA preserved for years with proper storage

Affords analysis on small blood volumes (50-250µL)

Cold chain not necessary for short-term storage (<2 weeks)

Noninfectious after drying

Easily shipped-nonhazardous material by regular mail or courier services

Dried Blood Spots (Disadvantages)

Reduced Sensitivity (12x-lower)

- Limited by blood volume (50-75 µL per spot); generally no more than 2 spots to maximize efficiency and minimize interfering substances
- Nucleic acid degradation under poor storage conditions

Specificity

- contribution of cell-associated nucleic acid
  - DNA and RNA at lower viral loads

Accuracy

- Concordance with plasma results

Operational

TOPIC 2

WHO/HIVResNet Global HIVDR Prevention and Assessment Strategy

Dried blood spots for HIV-1 Drug Resistance and Viral Load Testing: A Review of Current Knowledge and WHO Efforts for Global HIV Drug Resistance Surveillance

Silvia Bertagnolio[1], Neil T. Parkin[2], Michael Jordan[1,3], James Brooks[4] and J. Gerardo García-Lerma[5]

[1]World Health Organization, Geneva, Switzerland; [2]Data First Consulting, Inc., Menlo Park, CA, USA; [3]Tufts University School of Medicine, Boston, USA; [4]National HIV and Retrovirology Laboratories, Public Health Agency of Canada, Ottawa, Ontario, Canada; [5]Laboratory Branch, Division of HIV/AIDS Prevention, Centers for Disease Control, Atlanta, GA, USA

50 representatives from different countries with HIVDR, laboratory, clinical, epidemiologic expertise

Role: Advise WHO on development of standardized tools, methodologies and training to evaluate emergence and transmission of HIVDR worldwide

Assist with development of early warning indicators to alert public health action related to HIV DR and ART programs

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WHO!MANUAL!FOR!HIV!DRUG!RESISTANCE!TESTING!

USING!DRIED!BLOOD!SPOT!SPECIMENS!!

JANUARY 2010

Table 1. Overview of published studies investigating optimal DBS storage conditions

Storage conditions tested				
Study	**Time**	**Temperature/Humidity**	**Desiccant**	**Outcomes**
Garcia-Lerma[15]	1 to 16 weeks	37°C/high humidity, -20˚C	Yes	DBS stable at 37°C only for 1-2 weeks. -20°C recommended for long-term storage. -20°C superior for short and long term.
Buckton[4]	3 months	-20°C, 4°C, 20°C		HIV DNA PCR only. No observed degradation in HIV DNA during 3 month study period.
Bertagnolio[3]	3 months	37°C/85% humidity	Yes	Good amplification rate (90%)
McNulty[8]	6 years 5 years 2-3 years	-30°C Ambient temperature and -70˚C -20°C		Complete degradation at ambient temperature; stable at -30°C and -70°C; -20°C recommended for long-term storage
Nelson[14]	3 to 6 years	Ambient temperature	Yes	Moderately successful amplification rate (69%); 1 log drop in viral load.

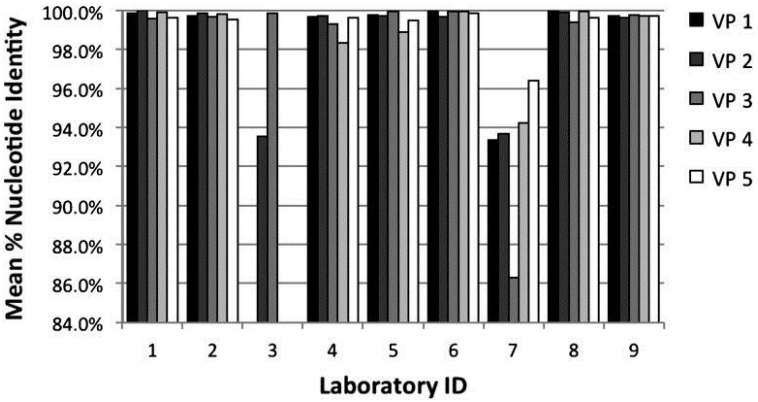
Table 3. Overview of alternative published HIV DBS genotyping methods

Study	Genotyping method(s)	Amplicon size	Storage conditions	Sample characteristics	Number of samples tested	Viral load of tested samples (copies/mL)	Amplification success rate*	Sequence concordance vs. plasma†
Masciotra 2007[7]	Viroseq	1.8 kb	-20°C, 18 to 26 weeks	Mostly treatment experienced, subtype B	60	78 to 676,694 (median: 9135)	Overall: 83% VL>2000: 100% VL <2000: 54%	98.8%
Youngpairoj 2008[11]	Viroseq or in-house nested RT-PCR	1.8 kb or 1 kb	4°C, 1 year	Treatment experienced, subtype B	40	518 to 676,694 (median: 13,680)	Viroseq: 57.5% In-house: 95%	94.5% (drug resistance mutations, DBS/in house vs. plasma/ViroSeq)
McNulty 2007[8]	In-house nested RT-PCR	1 kb	-20°C, 2-3 years	Untreated, subtypes from Cameroon, subtypes A, CRF02	40	665 to 645,256 (median: 23,715)	Overall: 92% VL>10,000: 100% VL <10,000: 73%	98.5%
Ziemniak 2006[12]	In-house nested RT-PCR	RT: 663 bp	Ambient, 0-5 months	Treated and untreated patients from the US, subtype B	9	<50 to 94,600 (median: 17,792)	Overall: 94% VL≥193: 100%	Not assessed
Bertagnolio[3]	In house nested RT-PCR	RT: 700 bp	37°C, 85% humidity, 3 months	Untreated subjects from Mexico, subtype B	103	Not all tested	90.1% either PR or RT region; 78.2% for both regions	99.9% (in samples with resistance mutations)
Hallack[6]	Trugene	1.3 kb	-20°C	Treated and untreated patients from the US, subtype B	33	1178 to 414,212 (median: 11,666)	Overall: 78.8% VL >6000: 90.5% VL <6000: 58.3%	99.3%
Garrido[5]	In-house nested RT-PCR: RT and gp41fragments	RT: 726 bp	4°C, *no desiccant*	Treated patients from Angola; many subtypes	77	1000 to 850,000	RT: 30% gp41: 43%	Not assessed
Steegen[10]	In-house nested RT-PCR	PR: 458 bp RT: 646 bp	-20°C	Treated and untreated patients from Kenya; subtypes A, C, D, CRF16	29	55 to >100,000	96.6% either PR or RT region; 89.7% for both regions; VL > 100: 100%	Not assessed
Buckton[4]	In-house nested RT-PCR	PR: 758 bp RT: 805 bp	-20°C	Clinic patients from the UK; subtypes A, B, C, CRF02	12	80 to 115,300 (median 10,950)	PR: 83% RT: 100%	Not assessed

Note: it is likely that the quality of field-collected DBS is substantial inferior to that of lab-collected DBS (which are often used in comparison studies) and especially plasma, with respect to amplification success rates

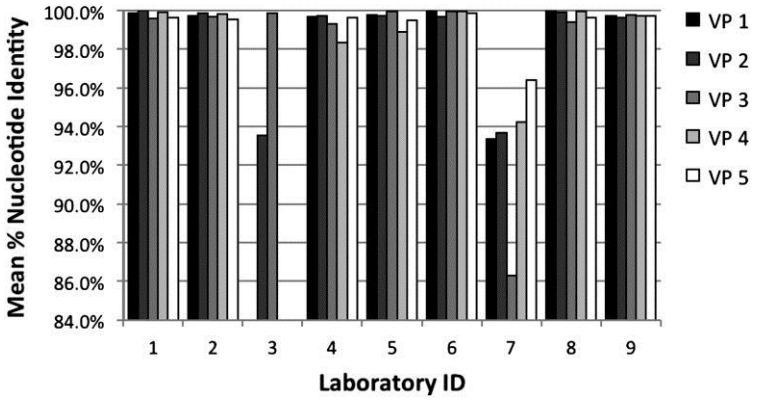
† mean nucleotide sequence identity, unless otherwise noted

In-House Genotyping Performance on DBS in the Global WHO Laboratory Network



Sequence Reproducibility

Mean sequence identity 96.7-100%



Sequence Accuracy

Mean percentage identity 98.4-100%

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Virological Methods 136 (2006) 238–247
Journal of Virological Methods
www.elsevier.com/locate/jviromet

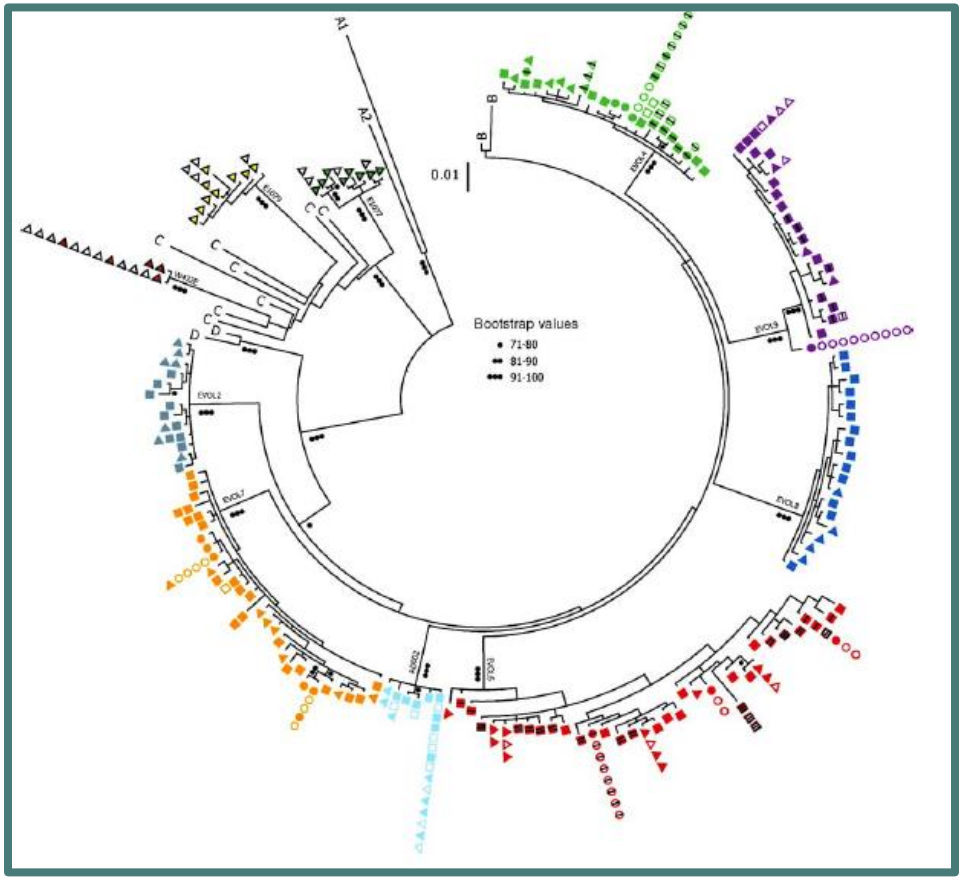
A sensitive genotyping assay for detection of drug resistance mutations in reverse transcriptase of HIV-1 subtypes B and C in samples stored as dried blood spots or frozen RNA extracts

Carrie Ziemniak [a], Allison George-Agwu [a], William J. Moss [b], Stuart C. Ray [c], Deborah Persaud [a,*]

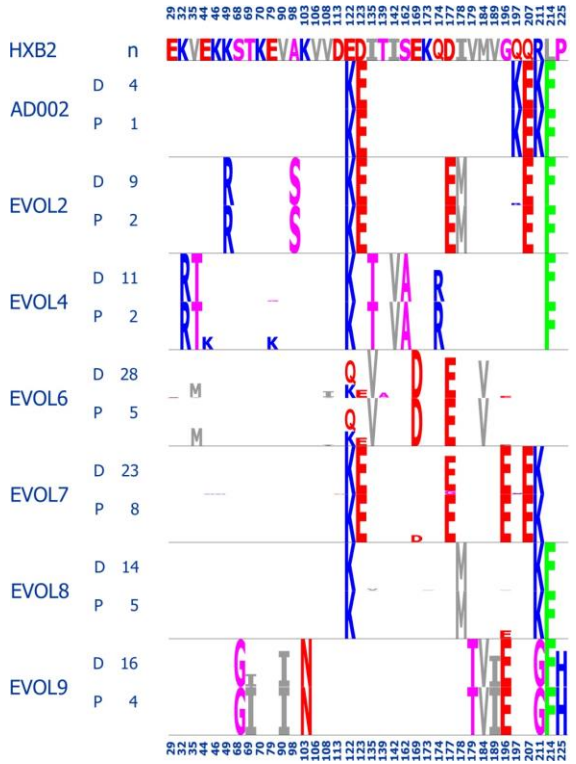
[a] *Department of Pediatrics, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Park Building, Baltimore, MD 21287, United States*
[b] *Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States*
[c] *Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, United States*

Received 3 March 2006; received in revised form 16 May 2006; accepted 25 May 2006
Available online 7 July 2006

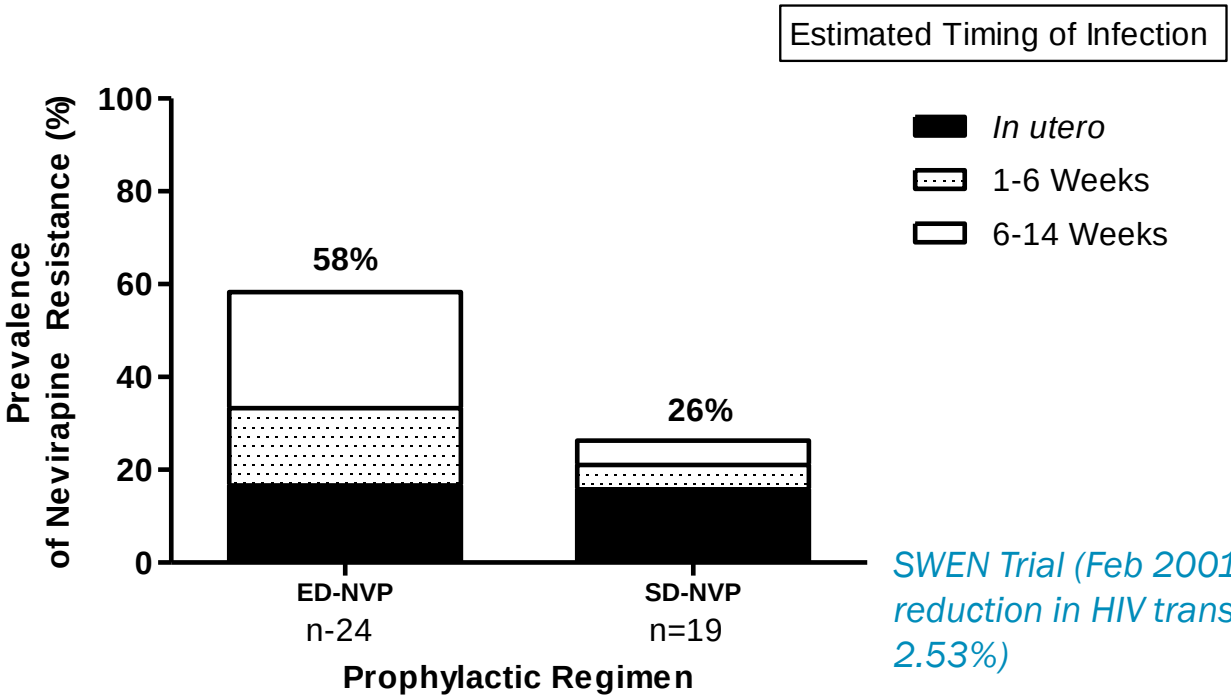
Concordance between Plasma and DBS Genotypes



663 bp HIV RT



Genotyping from DBS to Assess Clinical Trial Outcomes (Ethiopian Infants)

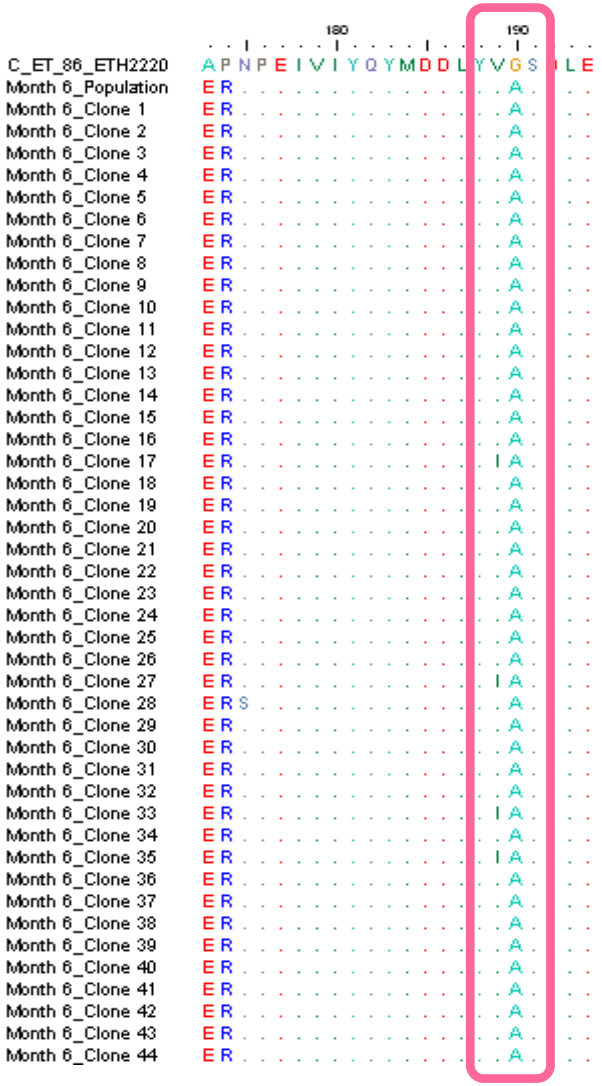


SWEN Trial (Feb 2001-March 2007); 46% reduction in HIV transmission (5.27% vs. 2.53%)

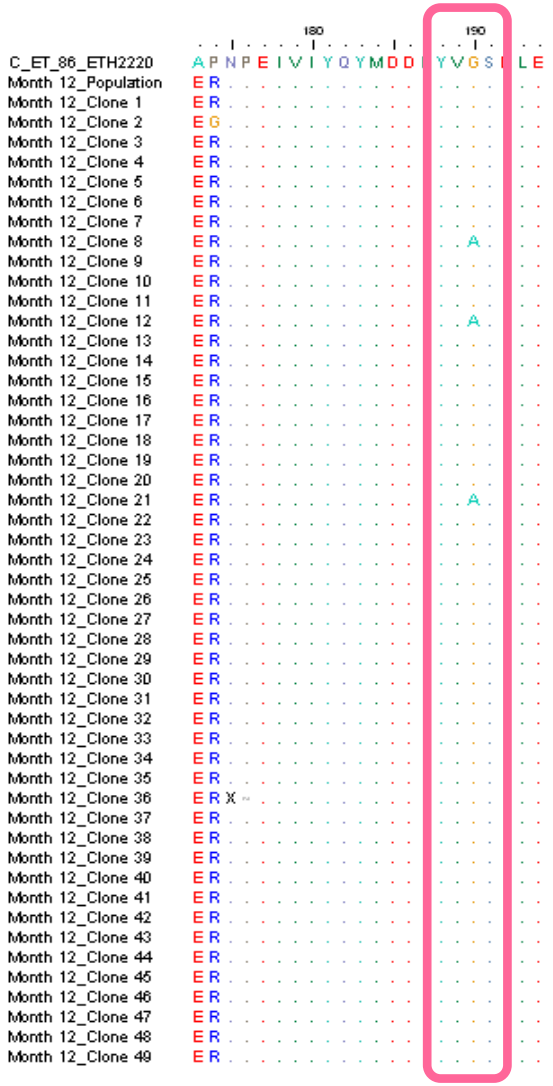
Genotyping successful from 93% of 46 infants with DBS collected at age 6 months
Stored for a median of 3.2 years (IQR 1.6-4.1 yrs)
Relevant clinical findings: Higher prevalence of nevirapine resistance at 6 months in ED-NVP compared with SD-NVP exposed infants
56% still with NVP resistance detectable at one year

SWEN Study Team; Lancet 2008; Persaud D et al. ARHR 2011

Preservation of Low-frequency Nevirapine Resistance Mutations in DBS



Month 6
G190 A Clonal Frequency (n=44) = 100%



Month 12
G190 A Clonal Frequency(n=49) = 6.1%

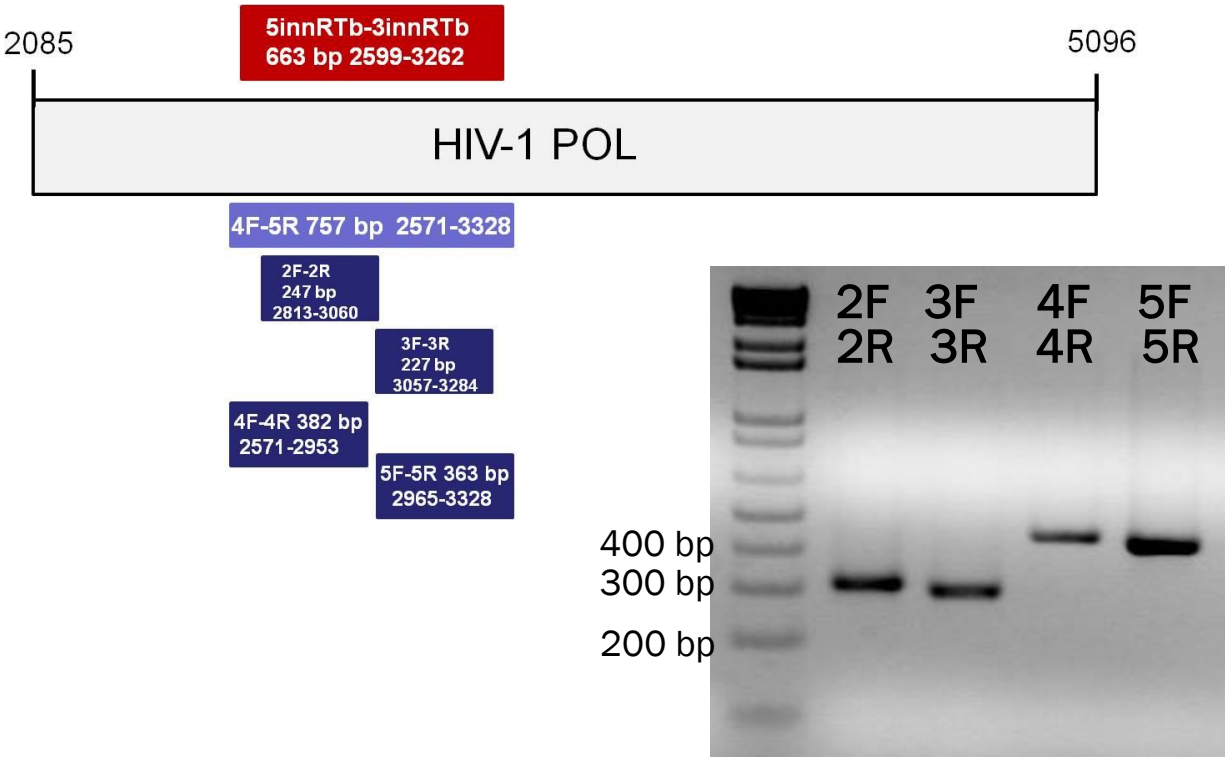
Conclusion

FEASIBILITY OF GENOTYPING FROM DBS COLLECTED DURING A CLINICAL TRIAL IN A RESOURCE-CONSTRAINED SETTING AND DESPITE YEARS OF STORAGE

Clinical Trial#2
IMPAACT P1060

Use of Dried-Blood-Spot Samples and In-House Assays To Identify Antiretroviral Drug Resistance in HIV-Infected Children in Resource-Constrained Settings[▽]

Carrie Ziemniak,[1] Yohannes Mengistu,[2] Andrea Ruff,[3] Ya-Hui Chen,[1] Leila Khaki,[4] Abubaker Bedri,[2] Birgitte B. Simen,[5] Paul Palumbo,[6] Susan H. Eshleman,[4] and Deborah Persaud[1]*



Storage Conditions and Yield of Genotyping Clinical Trial #2 (IMPAACT P1060)

-

	Initial Yield (No. Positive/ No .Tested) [%]	Negative Samples Tested with Shorter Amplicons (No. Positive/ No. Tested) [%]	Overall Yield with complete coverage of HIV-RT (No. Positive/ No. Tested) [%]
Overall	38/49 [78%]	3/11 [27%]	41/49 [84%]
Optimally Stored	29/33 [88%]	1/4 [25%]	30/33* [91%]
Sub-optimally Stored	9/16 [56%]	2/7 [43%]	11/16 [69%]

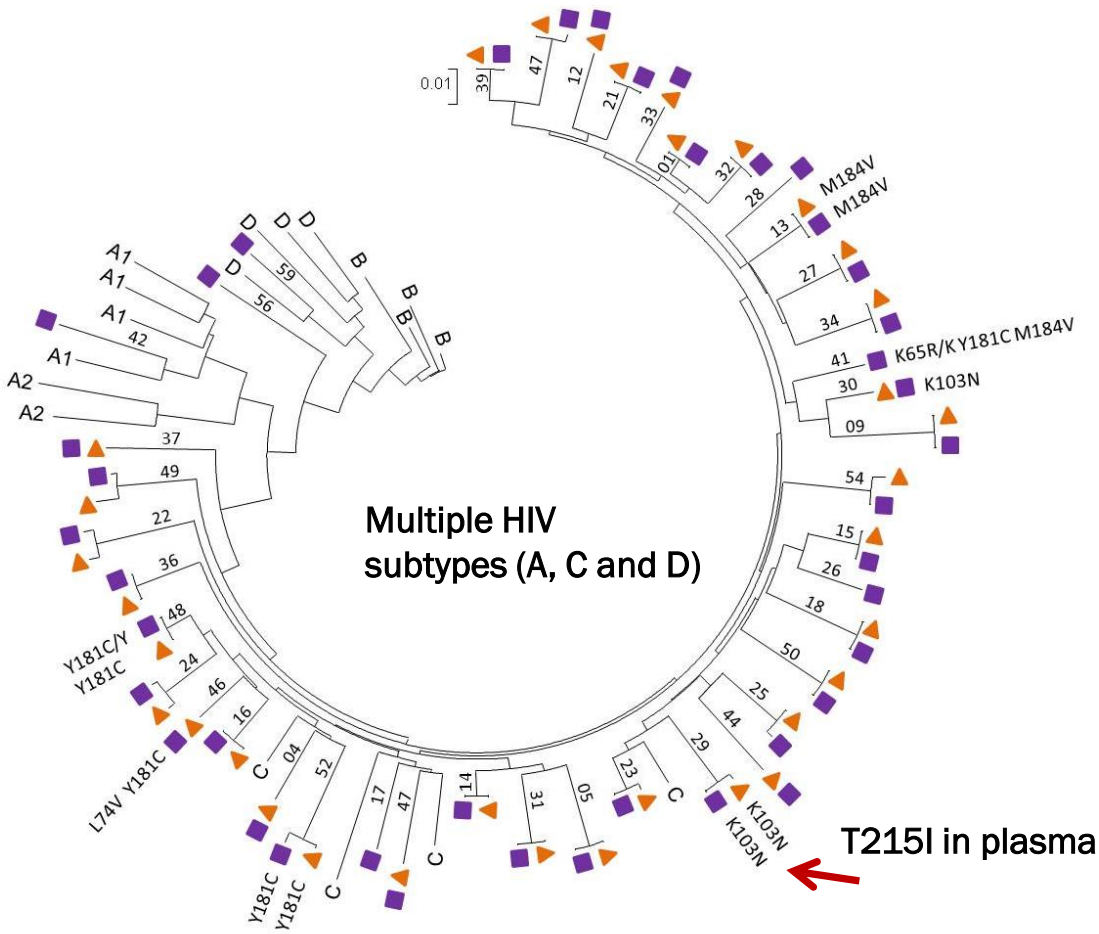
Stored for 0.3 to 1.8 years

Yield increased with optimal storage and with shorter amplicons

Ziemniak C et al. JCM 2011

Clinical Trial#2: IMPAACT P1060

100% concordant with plasma genotypes by Viroseq



Triangle: plasma genotype
Square :DBS genotypes

Conclusion

EQUIVALENCY OF DBS GENOTYPING WITH PLASMA BY POPULATION SEQUENCING

FEASIBLE FOR MONITORING CLINICAL TRIALS AND ART OUTCOMES IN RESOURCE-CONSTRAINED SETTINGS

Next Generation Sequencing Methods

Pyrosequencing Dried Blood Spots Reveals Differences in HIV Drug Resistance between Treatment Naïve and Experienced Patients

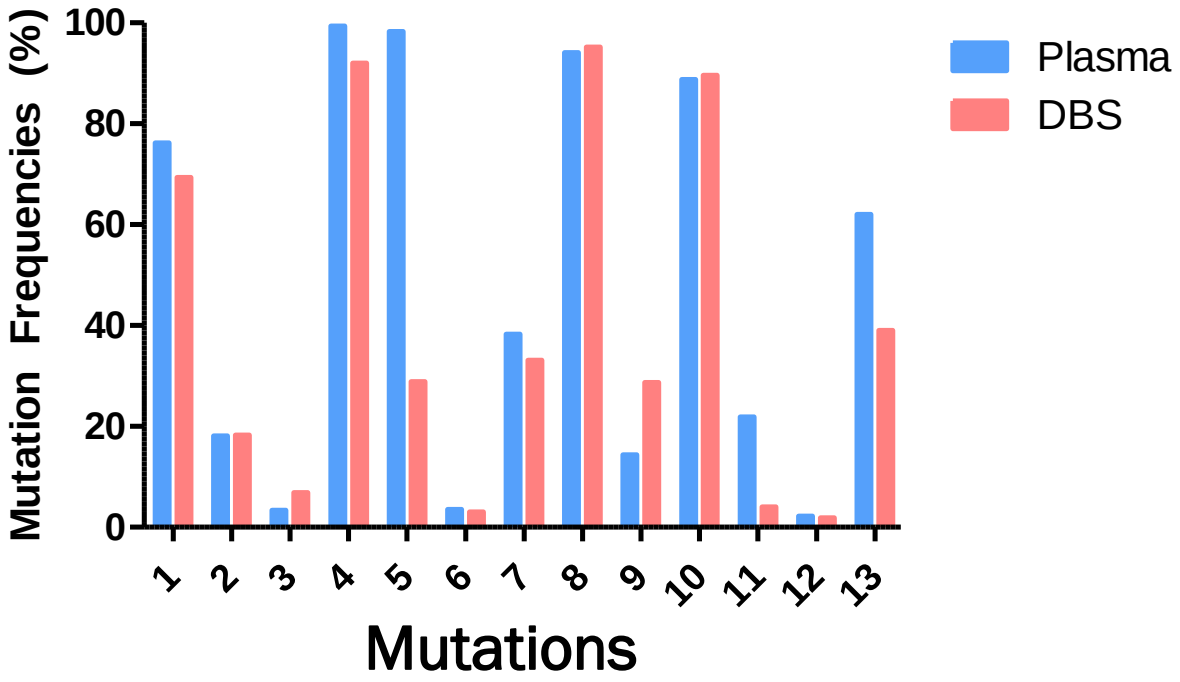
Hezhao Ji[1], Yang Li[1], Binhua Liang[2,3], Richard Pilon[1], Paul MacPherson[4], Michèle Bergeron[1], John Kim[1], Morag Graham[3,5], Gary Van Domselaar[2], Paul Sandstrom[1], James Brooks[1]*

	Average Sequence Concordance Rates		
Plasma viral load (c/ml)	DBS vs. Plasma	DBS vs. PBMC	Plasma vs. PBMC
504-50,192	82.9 ± 11.9%	78.9 ± 10.9%	75.3 ± 14.7%
<5000	72%	75.3%	65.7%
≥5000	88.8%	80.9%	80.6%

Plasma/DBS concordance highest at VL≥5000 copies/ml and with no ART exposure

Hezhao J et al. PLOS ONE 2013

Next Generation Sequencing from DBS Clinical Trial #2 (IMPAACT P1060)



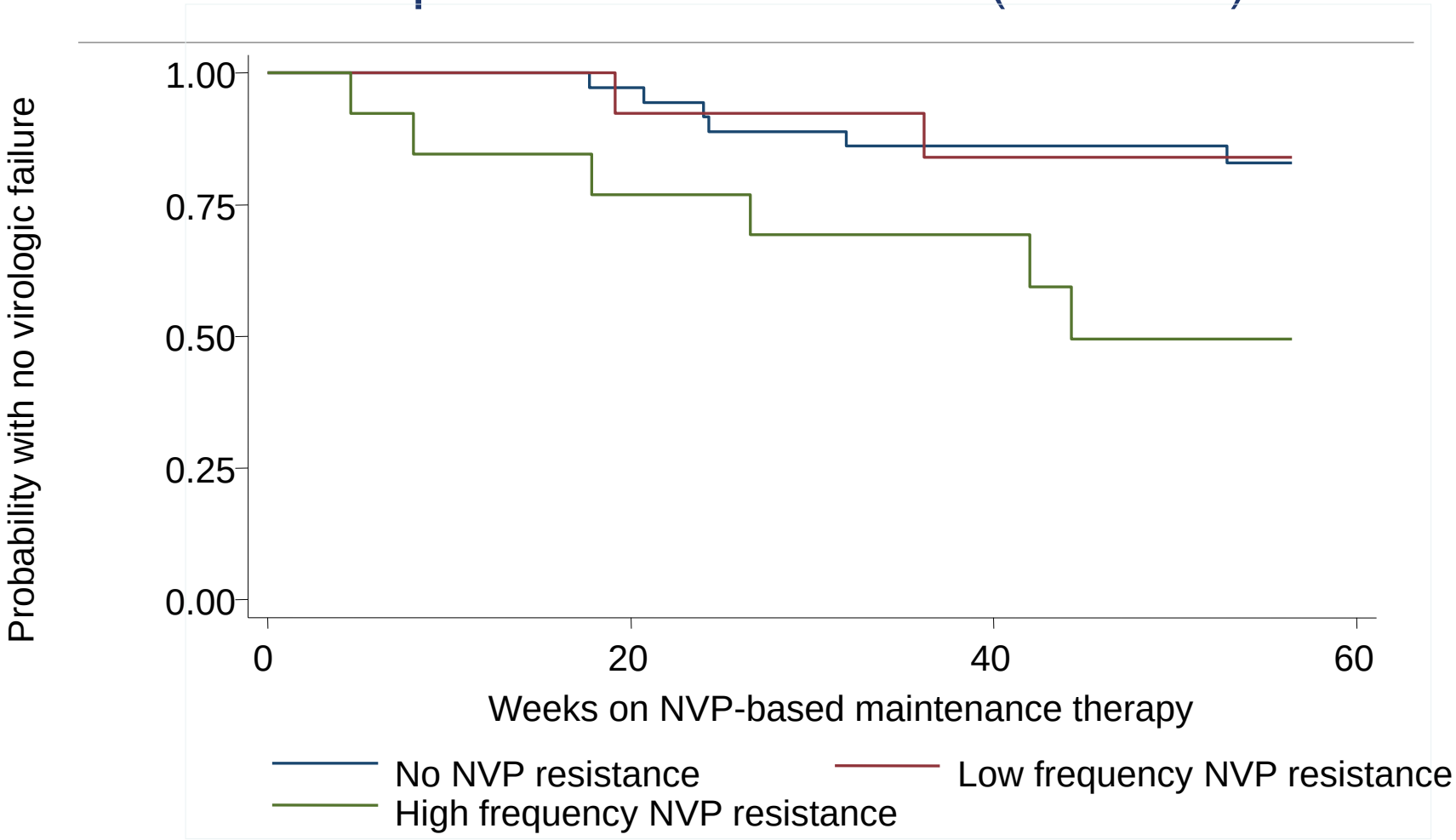
97.1% concordance between DRMs in plasma and DBS

Median mutant frequencies DBS 3.4% (IQR 1.6-30.0%) vs. 3.5% (IQR 1.4-38.3%) in plasma

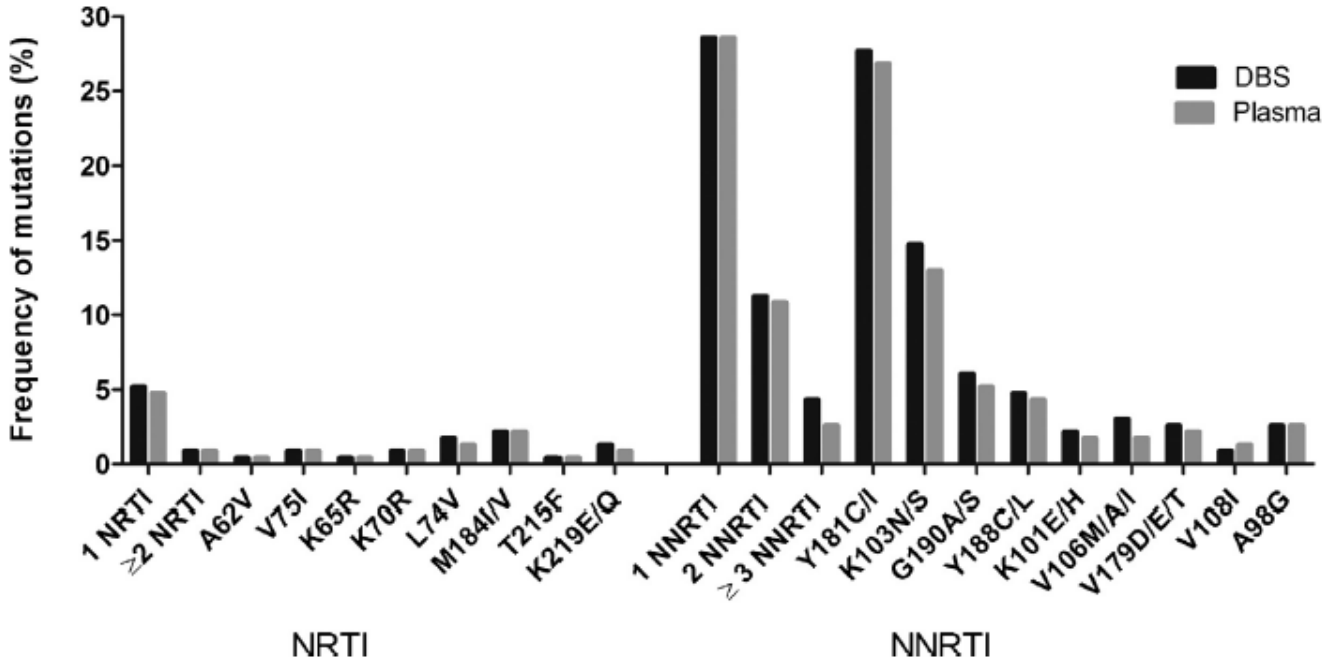
Discordance more likely when present at low frequencies (median 1.5%; IQR 1.2-3.2)

Ziemniak C et al. Abstract P89; IAS Peds 2012

Associations between Nevirapine Resistance Frequencies and Virologic Failure with Nevirapine-based HAART (P1060)



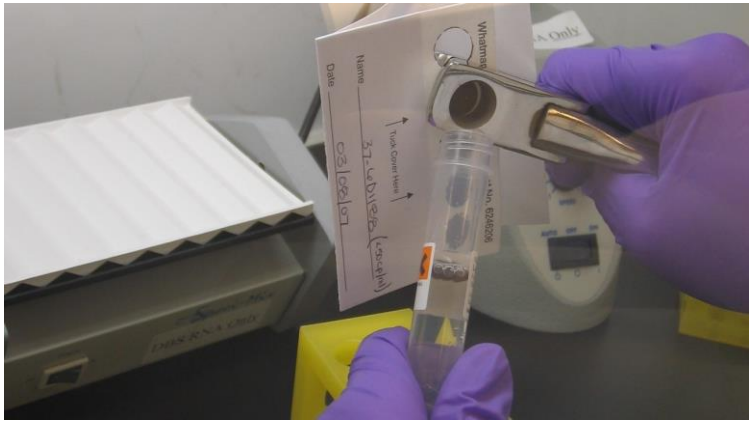
High Sensitivity and Concordance of DBS and Plasma Genotypes



Location	Population (N=238)	Approach	Findings
South Africa (5 sites)	Median age 27 wks [IQR 13-51 wks]	Paired plasma and DBS immediately stored at -80	Yield: 97.9% plasma 98.7% DBS 92% concordance 99.5% mean nucleotide identity

Salimo A et al. J. Virol Methods 2015

DBS facilitates Drug Resistance Monitoring in Resource-Constrained Settings



Macha, Zambia (Photo courtesy of W. Moss, MD, MPH)

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