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ICVC Charitable Trust, Buckinghamshire

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Evaluation of a dry plasma matrix transport device for genotyping of HIV-1, HBV and HCV and quantification of HIV-1 VL to provide an economic approach for real-time clinical care in resource-limited settings

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Dry Plasma Transport -Academic Research Collaborators

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- Eilidh Macrae -
- Rob Lloyd Jr. – Research Think Tank, Buford, GA, USA
- Rodney Mathis –
- David Burns –
- John Cooper
- Richardo Diaz – Federal University of Sao Paulo, Brazil
- Zahava Grossman – Sheba Medical Centre, Tel Hashoma, Israel
- Mark Holodniy – Veterans Affairs Medical Centre, Palo Alto, USA
- Rami Kantor – Brown University, Providence, USA
- David Back – Department Pharmacology, Liverpool University, UK

Introduction

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- In the developed world optimal HIV/AIDS patient care has established VL, CD4, resistance and other measures to support ART
- Such tests in R-LS require transport of frozen plasma which is prohibitively expensive.
- The concept of dry blood and dry plasma transport has been apparent for more than 25 years
- Our early work with MRC and ICH involved 'Guthry Card' with 50ul blood spots for serology to monitor prevalence of HIV-1 infection
- Later work (WHO) uses a similar low volume (50-100ul) blood on filter paper for transport and perform molecular resistance with limited success (low volume, sub-optimal rescue rate, challenging technology)
- Recently, a cellulose acetate matrix has been described to transport 1ml of dry plasma that may be used to evaluate molecular, serological, biochemical and immunological markers from distance clinical sites
- Here we present an evaluation of the clinical utility of dry plasma versus paired frozen plasma for accurate genotypic characterization of HIV-1 resistance, HIV-1 VL, HBV resistance and HCV genotype.

V-ST (Vivebio Inc)

Methods

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- Anonymous paired dry and frozen plasma from multiple clinical sites were analysed in these studies, collected at one site and posted to a second site. (method next slide)

- HIV-1 resistance by TRUGENE HIV-1 Genotyping of RT/Pr and interpretation by GENELIBRARIAN (n=150)
- HIV-1 VL by HIV-1 bDNA VERSANT assay, version 3 (n=299)
- HBV resistance by INNO-LIPA HBV DR kit (n=20)
- HCV clinical genotype by TRUGENE HCV 5'NC kit (n=3)

Methodology for Dry Plasma Transport using the Matrix Device (V-ST)

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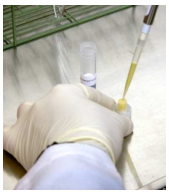
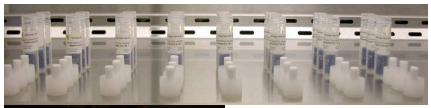
Clinical samples centrifuged or allowed to sediment overnight

Load 1ml plasma onto matrix





Dry in class 2 hood 'DryDock' or drying box

Post using regular Mail as non-infectious Dry blood plasma

Rehydrate on arrival by adding1.175ml buffer and compressing

HIV-1 Resistance RT/Pr
HIV-1 Resistance Int.
HIV-1 Resistance Env.
HIV-1 Viral Load:
- **Amplicor Monitor**
- **bDNA**

HIV-1 Tropism
HIV-1 Subtyping
HBV Resistance
HCV Genotyping
HIV-1 Serology
HIV-1 TDM

Results
HIV-1 Genotypic resistance

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- 150 consecutive dried plasma and frozen plasma pairs were posted to the laboratory (4-5 days), or transported on dry ice (Fedex), respectively, in batches 25
- 137 of 150 (92.3%) pairs provided sequences
- The 13 failures had low VL (<500c/ml) or were on therapy, 2 cases plasma only did not amplify)

- Sequence Similarity in RT/Pr of dried plasma versus frozen plasma
- At the nucleotide level - >98% (NS)
- At the codon (aa) level - >99% (NS)
- No discordance at clinically significant resistance sites (IAS-USA)

- Relative cost (25 samples):frozen plasma $520 v dry plasma $10 (1.9%)

Results: HIV-1 VL (bDNA)

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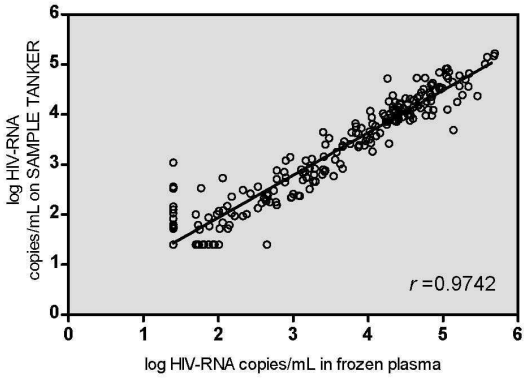
Cross Sectional VL Evaluation

n=299 samples
<50 c/ml = 99
<4 log c/ml = 100
<6 log c/ml = 100

Dried for 12 hours, stored RT
And rehydrated 4 days later

Overall correlation r=0.974
(p<0.0001)

Of 99 <50 – 12 >50 from DP
(mean of 12 = 128 c/ml)
Of 200 >50 – 12<50 from DP
(mean of 12 = 47 c/ml)



Results: Genotyping with HBV INNO-LiPA-DR

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- *n=20 clinical samples, 120 assays*
- *0.5ml loaded onto matrix and dried or frozen -70C*
- *Stored for 2 days and tested as pairs*
- *DNA extracted on Nuclisens MiniMAG extraction system*
- *Nested PCR amplicons loaded onto auto-LiPA for reverse hybridization reaction, detection and analysis*

- Plasma reverse hybridization 20/20 (100%) – DR
- Dry plasma 17/20 (85%) – DR
- Reflex 2nd testing on failures 18/20 (90%) – DR
- Overall, average success rate for 3 INNO-LiPA – DR, Pre-core and genotyping assays were plasma versus dry plasma: 100% v 93.3%.
- Overall data provided highly concordant results

Results: HCV Genotyping

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- Three HIV-1/HCV co-infected patients underwent HCV genotyping using TRUGENE HCV 5'NC genotyping kit
- Frozen plasma was transported on dry ice and the dried plasma samples were posted using CDC-guidelines for dried blood shipment.
- Results were compared using automated reporting of HCV 5'NC on the OpenGene system
- Sequence similarity for dried plasma v frozen plasma at nucleotide level 99.4%:99.4% and at amino acid level 99.7%:99.7%

Sample	VL(log c/ml)	Plasma	Dried Plasma
1.	2.57	1a	1a
2.	4.01	2b	2b
3.	4.53	3*	3a *subtype unresolved

- It was noted signal to noise and peak signal intensity were greater in dried plasma versus frozen plasma

Serology and Biochemistry

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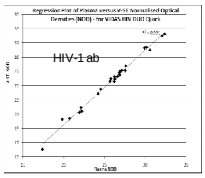
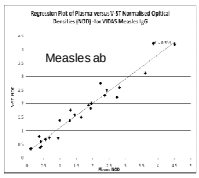
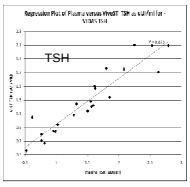
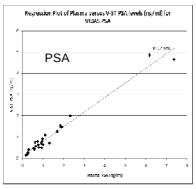
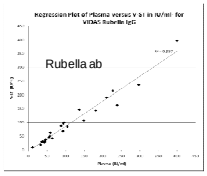
Mini-VIDAS Analyser (Biomerieux)
25 plasma/dry plasma pairs per tablet – highly signifcant correlation

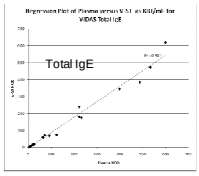


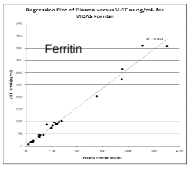


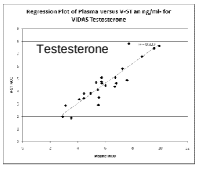












Conclusions

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- This study showed highly significant correlations between plasma and dry plasma results for all these clinically relevant molecular assays.
- Further data suggests it may be equally significant for serology, biochemistry and TDM.
- It is a highly flexible tool for transporting and storing dry plasma with no temperature constraints, at very low cost, from resource-limited settings.
- In combination with modern electronic communications it offers economic opportunities for distant clinical care for adults and children, and for support of clinical trials.
- The authors believe it provides the opportunity for a paradigm shift in relation to real-time clinical care of those in resource-limited settings.