Development and evaluation of a simple, filter paper-based method of plasma separation and collection to enhance HIV monitoring in resource-limited settings (RLS)

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Medical Research. Practical Action.

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Background

- Viral load (VL) testing is the standard method for anti- retroviral therapy (ART) monitoring
- However, currently available VL technologies are complex and expensive
- Therefore, restricted to central laboratories in RLS
- WHO recommends the use of Dried Blood Spots (DBS) in RLS (2013 WHO ART guidelines)
- However, DBS suffers from low specificity
- Over quantification due to non-plasma nucleic acids contamination from white blood cells

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Limited Utility of Dried-Blood- and Plasma Spot-Based Screening for Antiretroviral Treatment Failure with Cobas Ampliprep/TaqMan HIV-1 Version 2.0

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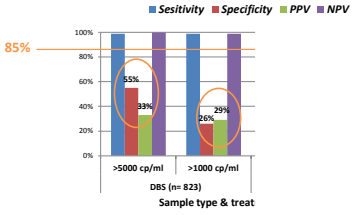

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DBS → Over quantification → Low - Specificity - PPV → - Unnecessary targeted VL testing - Premature regimen switching

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DPS → **Excellent Performance** (Plasma Vs DPS: P> 0.2) → **Complex specimen processing** - phlebotomy & - centrifugation

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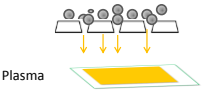
AIMS

- To **develop** filter paper- based method of plasma separation and collection from whole blood
- To **evaluate** prototype plasma separation device for quantification of HIV VL

Methodology

RBC: Agglutination

WBC : Size-exclusion



Cell filtration

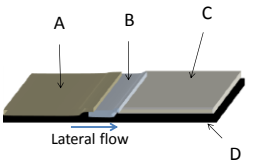
Membranes assembly

A = RBC separator
(Glass fiber + anti GYPA)

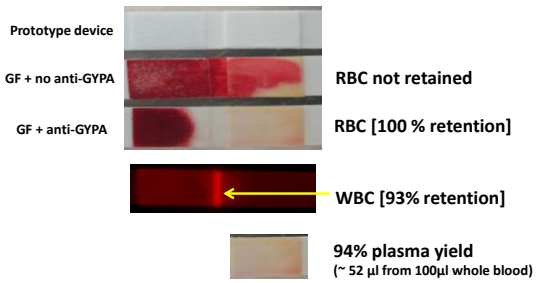
B = WBC separator
(nitrocellulose membrane)

C = Plasma receiver/collector
(Whatman 903)

D = Backing plastic card

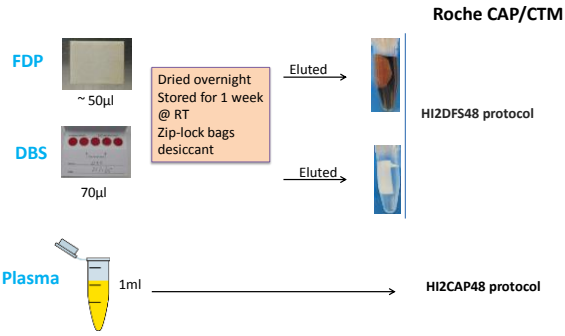


Efficient RBCs and WBCs filtration achieved

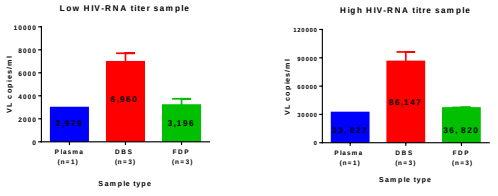


REFERRED = Filtered & dried plasma (FDP)

HIV-RNA recovery from Filtered & Dried Plasma (FDP)



FDP: Comparable HIV RNA recovery to plasma
DBS: Over-quantified by over 2 folds



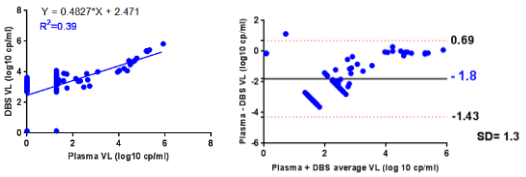
- DBS: Co-amplifyies of proviral DNA & cell-associated RNA

Prospective evaluation study

Participants: HIV patients
Setting : Alfred HIV Clinic
Sample size: 200 patients
Design: Comparative evaluation of FDP Versus:
- **Plasma**
- **DBS**
- **FVE** (Free virus elusion), Roche new method

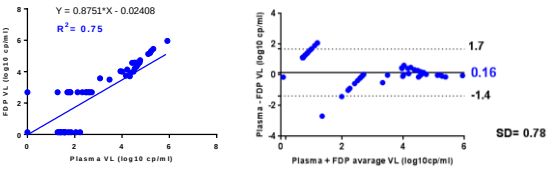
DBS: Weak correlation & concordance

DBS (n=100)



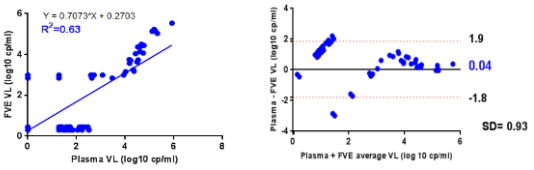
FDP shows good correlation & concordance

FDP (n=191)



FVE shows good concordance

FVE (n = 191)



Performance to diagnose ART failure at 1000 copies/ml cut off

Sample type	Sensitivity (CI)	Specificity (CI)	Positive predictive value (CI)	Negative predictive value(CI)
DBS (n=100)	100 (78- 100) %	46 (35-57)%	27 (14- 37)%	100 (91 -100)%
FDP (n=191)	100 (85 -100)%	100 (98 -100)%	100(85 – 100)%	100 (98 -100)%
FVE (n= 191)	87(66 -97)%	100 (98 -100)%	100 (83 -100)%	98 (95 -99%)

Conclusion

FDP

- Effectively removes blood cell components (RBCs & WBCs)
- ~ 94% plasma yield is achieved
- Avoids proviral DNA and cellular RNA co-amplification with plasma HIV RNA
- Efficiently diagnosed ARV treatment failure at 1000 cps/ml cut-off
- Useful and simple tool for resource limited settings
- Can be used for other assays that need cell-free plasma
- **Further studies in field condition using finger prick blood samples**
- **Further optimization for manufacturing**

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