Rapid Plasma Separation Device for POC Viral Load Testing: A Proof-of-concept

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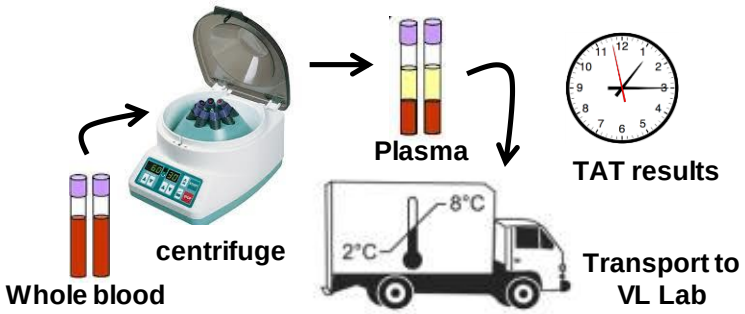
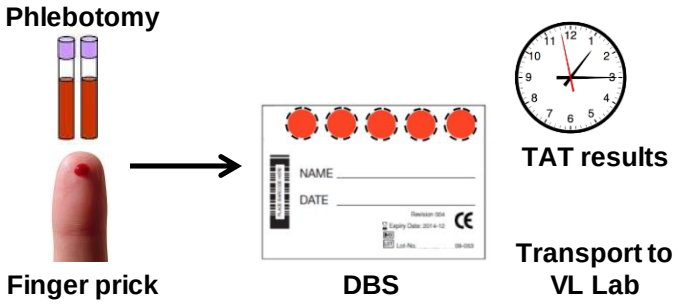
Challenges with Centralized VL Testing

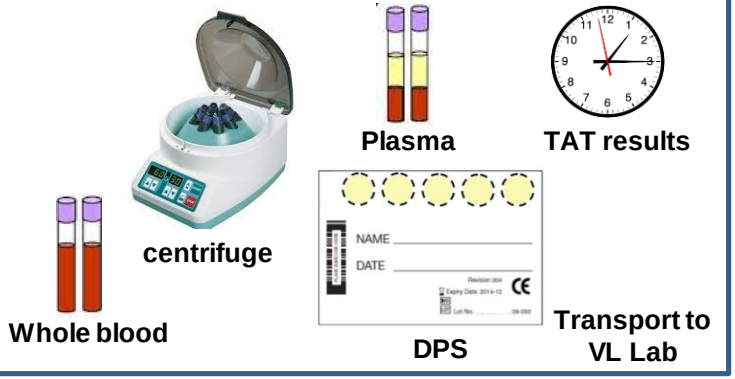
Plasma is the gold standard for VL testing. However, sample transport constraints and cold chain limit testing access in resource-limited settings.



Using dried blood spots (**DBS**) is a practical alternative to plasma, but DBS gives less accurate results than plasma and varies according to the VL platform.



Using dried plasma spots (**DPS**) is not feasible at the point of collection due to the need of electrical centrifuges for blood separation and further sample manipulation.

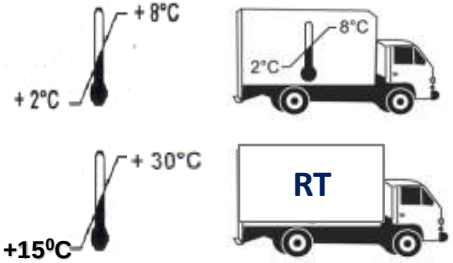


Alternatives to Obtain/Transport Plasma

- **Blood sedimentation**
  (Mwebaza 2013)

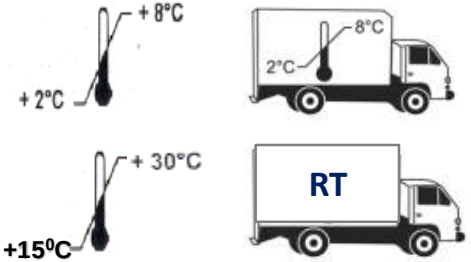
Plasma
DPS

Storage Transport

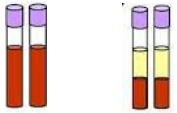


- **Manual centrifugation**

Plasma
DPS



- **Blood/Plasma stabilizers**
  (Kwon 2014)



- **Rapid Plasma separators**
  (Yang 2012, Homsy 2012, Liu 2013)

Plasma →  POC VL

DPS →  → Lab VL

Rapid Plasma Separator

We investigated a prototype rapid plasma separation device manufactured in India.

According to the manufacturer, the device separates plasma from whole blood within 10 minutes, depending of the haematocrit, based on the principle of membrane filtration.

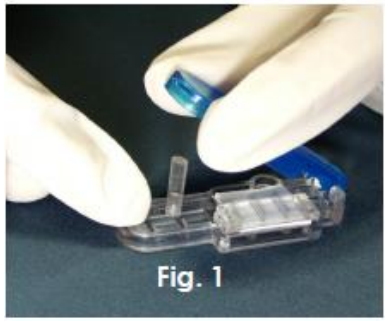


Fig. 1

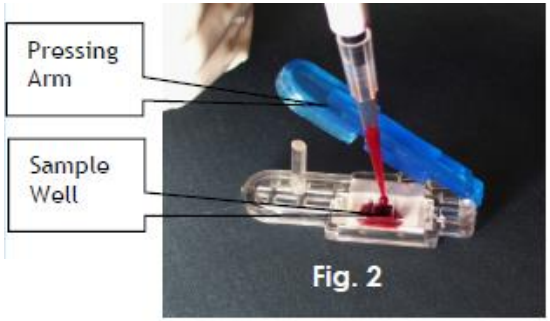


Fig. 2

350 µL blood

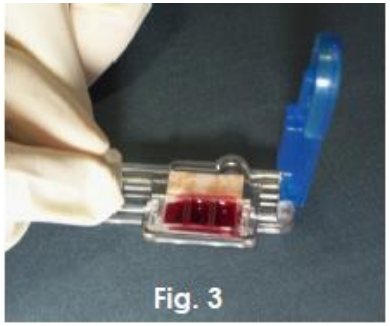


Fig. 3

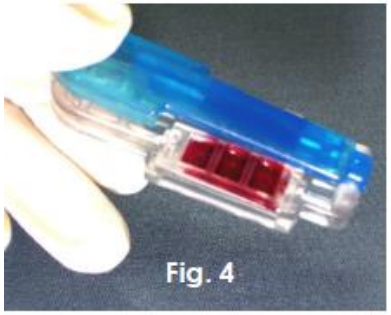


Fig. 4

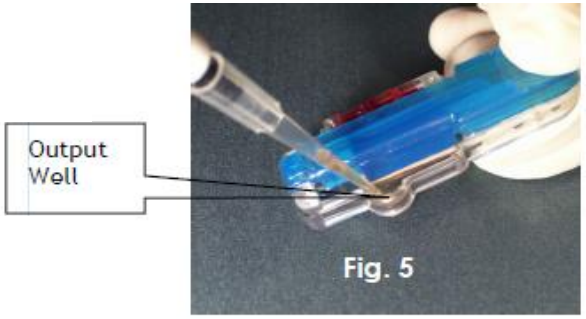


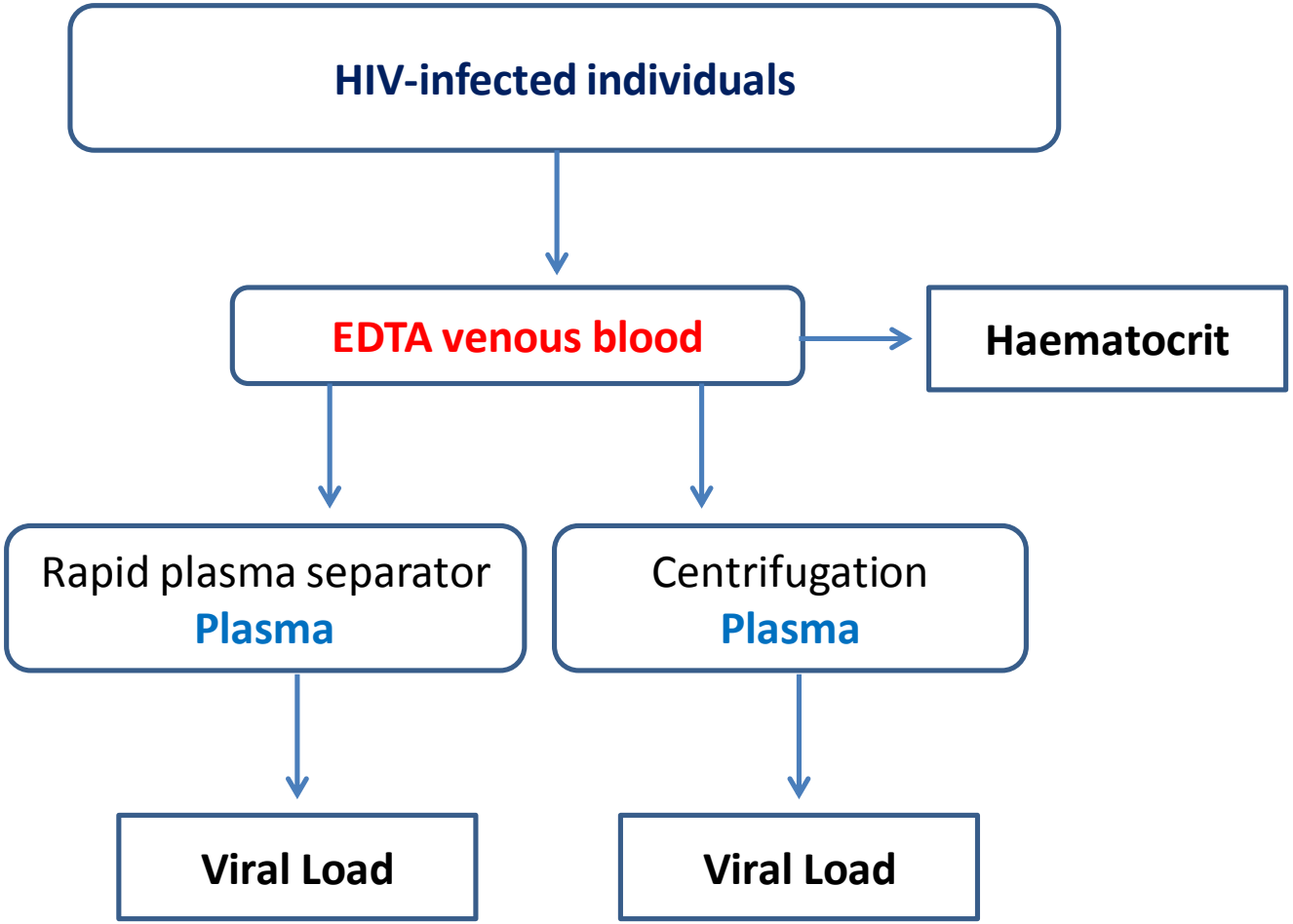
Fig. 5

60 µL to 100 µL plasma

EXPERIMENT DESCRIPTION

- **Study objectives**
  - To assess the ease-of-use of the RPSD
  - To determine the amount of free plasma generated
  - To conduct VL testing on the plasma generated
- **Period:** November 2013
- **Study setting:** National Microbiology Reference Laboratory (NMRL), Harare, Zimbabwe.
- **Sample type:** Left-over EDTA whole blood specimens from HIV-infected individuals.
- **VL assay:** NucleSENS EasyQ v2.0

METHODOLOGY



RESULTS

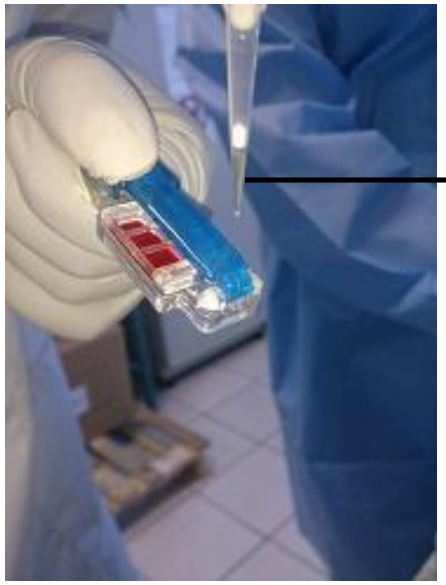
Ease-of-use

→ Device small and easy to manipulate

→ Device easy to dispose of

→ Requires **350 µL** of whole blood

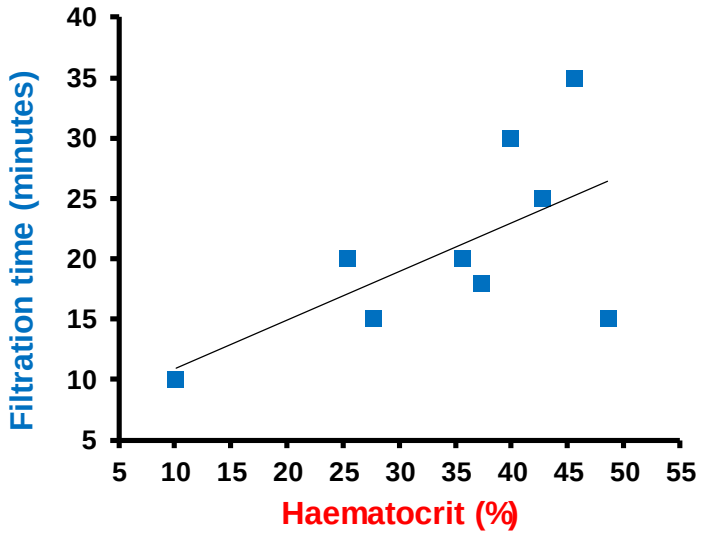
→ Precise pipetting is necessary

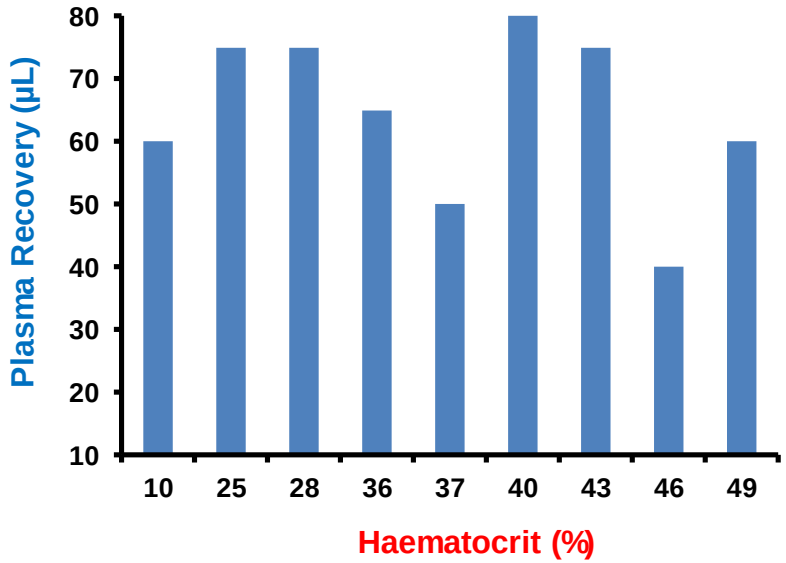


RESULTS

Ten devices were used, of which 9 generated valid results.

- The mean haematocrit was 36% (range: 10 to 49%)
- The mean filtration time was **21 min** (range: 10 to 35 min)
- As expected, filtration time increased with haematocrit (See right)





- The mean plasma recovery was **64.3 µL** (range: 40 to 80 µL)
- Plasma recovery was not associated with haematocrit (See left)

RESULTS

Volume of Plasma (µL)	Filtered Plasma (copies/mL)	Centrifuged Plasma (copies/mL)
60	1,200	1,700
75	15,000	18,000
50	60,000	80,000
75	330,000	390,000
40	TND	TND
65	TND	TND
60	TND	TND
75	22,000	6,300
80	TND	2,600

Conconrdant

Overestimation
Underestimation

CONCLUSIONS

- **Liquid plasma was successfully obtained** by 9 out of 10 devices.
- The novel device **simplifies plasma collection** and could potentially be used in combination with new POC VL technologies that require small amounts of cell-free plasma.
- Although the device was simple to use, **it required EDTA whole blood**, precise pipetting, and the filtration time was longer than claimed by the manufacturer.
- The lack of association between plasma recovery and haematocrit may indicate **poor filter efficiency**.
- This was a small proof of concept study → It is worth doing **further larger studies** to obtain a more reliable definition of the device's performance characteristics.
- The **feasibility of non-laboratory staff using the device** to obtain adequate amounts of cell-free plasma from **finger-stick blood** for VL testing is also worth investigating.

DISCUSSION

- New **POC VL technologies** show differences in **type of technology**, **volume of specimen required** and **TAT to results**.
- Use of **whole blood** with **RT-PCR assays** may compromise **accuracy** of results (**false positives** due to the co-amplification of intracelullar nucleic acids)
- Isothermal amplification assays **(NASBA)** may limit the contribution of proviral DNA from whole blood.
- **POCs with in-built plasma separation components** will facilitate testing with plasma

Product	Type of assay	Blood volume (µL)	Plasma volume (µL)
Liat	RT-PCR	75	150
AlereQ	RT-PCR	25	500
TrueLab	RT-PCR	50	
GeneXpert	RT-PCR	-	1000
SAMBA I	NASBA	-	300
CPA	NASBA	50-100	
Ziva (Cadivi)	ELISA-RT	-	500
Wave80	NASBA	100	100
SAMBA II	NASBA	120	200
NWUGH Savanna	RT-PCR	150	150

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THANK YOU