PROOF OF CONCEPT: NOVEL PLASMA SEPARATION CARDS FOR HCV/HIV QUANTITATION

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Introduction

A simple, efficient and precise method for blood collection via finger prick, which removes cells and stores plasma at ambient temperature, is needed globally. Current collection methods require electricity driven processing, temperature control, and packaging. Dried blood/plasma spots on filter paper offer an alternative to shipping worldwide but with limited success as reduced assay precision/accuracy are linked to filter paper. Herein we describe functionality/performance of a novel prototype plasma separation card (PSC) composed of a primary separation membrane and secondary plasma collection pad (ViveBio LLC, Alpharetta, GA). PSCs (See Figure 1) separate cellular components via an asymmetric membrane while allowing plasma to be collected and stored at ambient temperature pending quantitative and qualitative analysis.

Methods

To evaluate candidate separation materials, varying volumes of whole blood (WB) were pipetted drop wise (as if finger prick) onto 3 different membranes of varying size, each using an oversized absorbent plasma collection pad (ViveBio LLC, Alpharetta GA). Collection pads were inspected and weighed pre/post WB addition to evaluate percent plasma recovered, hemolysis and time of separation.

The top performing materials were used to generate rapid prototype cards and evaluated for functionality to effectively separate cellular components (i.e., PBMCs) from plasma components. WB (100µl) from previously characterized donors (HLA B*5701 status) was pipetted onto cards and stored overnight. The next day membranes and pads were removed and incubated in 1,000µl SPEX buffer (Roche Diagnostics) on a Thermomixer for 10 min (56ºC, 1,000 RPM). DNA was extracted, quantitated using a NanoDrop and analyzed using bioMONTR Labs' rtPCR assay for detection of the HLA B*5701.

Prototype cards were evaluated for functionality in an HIV viral load assay. HXB2 spiked WB (75µl or 100µl) was pipetted onto cards and stored overnight. The next day pads were removed and incubated in 600µl diH2O on a shaker (20 min @ 150 rpm). 500µl was analyzed by Roche COBAS TaqMan HIV-1 v2.0 for use with High Pure System.

Methods (cont'd)

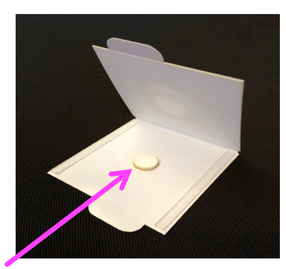
To evaluate alternate recovery methods, HXB2 spiked WB (100µl) was pipetted onto cards and stored overnight. The next day pads were removed, added directly to prepared lysis/binder buffer in the wells of the lysis rack (Roche High Pure Extraction system) or added to tubes containing 1,000µl SPEX buffer (Roche Diagnostics). The tubes containing SPEX buffer were incubated on a Thermomixer for 10 min (56ºC, 1,000). 500µl was analyzed by Roche COBAS TaqMan HIV-1 v2.0 for use with High Pure System.

HCV infectious WB was diluted in normal WB to yield 3 levels. Each level was pipetted onto cards in duplicate (100µl). Cards were stored for 14 days at RT. Collection pads were removed and incubated in 1,000µl SPEX buffer on a Thermomixer for 10 min (56ºC, 1,000 RPM). 500µl was analyzed by Roche COBAS TaqMan HCV v2.0 for use with High Pure System.

For plasma separation membranes #1 and #3, the sample passed through the membrane within 4-15 minutes (min) and plasma was captured on plasma collection pad (~26%- 63% of expected volume). Membrane #2 allowed whole blood to pass through and was disregarded from further testing (See Table 1).

Figure 1. Prototype Plasma Separation Card

Blood is added to the entry port



Plasma Passes through to Collection Pad

Results

Table 1. Materials Evaluation

Separation Material	Dia. (mm)	Dry Weight (g)	Vol. Applied (uL)	Time (min)	Wet Weight (g)	Captured (uL)	% Capture	Hemolysis? (Y/N)
Membrane 1	14	0.048	75	5	0.08	32	43%	Y
	20	--	100	--	--	--	--	Y
Membrane 2	14	0.047	75	4	0.085	38	51%	N
	18	0.047	125	6	0.105	58	46%	N
	20	0.01	75	5	0.019	9	12%	N
	20	0.05	100	5	0.075	25	25%	N
Membrane 3	14	0.046	75	15	0.093	47	63%	Y
	20	0.048	125	5	0.08	32	26%	Y

Membranes and pads were removed from PSCs used to process whole blood from previously characterized donors (HLA B*5701 status) and after rehydration, DNA was extracted. As expected, DNA was not recovered from the pads indicating that all cellular components were captured by the primary membrane. Results of DNA quantitation and HLA status are provided in Table 2.

Table 2. Whole Blood Input on PSCs. Summary of DNA Extraction and HLA B*5701 rtPCR Results when Rehydrated with SPEX Buffer.

Sample ID	Amount of WB used (uL)	PSC Tested	Volume SPEX used for Recovery (uL)	DNA recovered (ng/ul) Corrected for Volume	HLA B*5701 Result
WB Control	500	N/A	N/A	145	Negative
PSC Rep 1	100	membrane	1,000	60	Negative
	N/A	pad	1,000	0	Insufficient template quantity
PCS Rep 2	100	membrane	1,000	58	Negative
	N/A	pad	1,000	0	Insufficient template quantity
PSC Rep 3	100	membrane	1,000	112	Negative
	N/A	pad	1,000	0	Insufficient template quantity
WB Control	500	N/A	N/A	95.7	Positive
PSC Rep 1	100	membrane	1,000	37	Positive
	N/A	pad	1,000	0	Insufficient template quantity
PSC Rep 2	100	membrane	1,000	53	Positive
	N/A	pad	1,000	0	Insufficient template quantity
PSC Rep 3	100	membrane	1,000	43	Positive
	N/A	pad	1,000	0	Insufficient template quantity

The size of the membrane (18mm versus 22mm) and the amount of blood loaded impacted the amount of HIV-1 RNA recovered from the pad. Excluding outliers, with 75µl whole blood (18mm card), the mean viral load was 3.09 c/mL (± 0.28 SD). With 100µl whole blood the mean viral load was 3.92 c/mL (± 0.33) or 3.61 c/mL (± 0.18 SD), for the 18mm and 20mm cards respectively (data not shown).

The use of SPEX buffer to re-hydrate the pad and incubation in a Thermomixer (56ºC, 1,000 RPM) improved recovery of HIV-1 (compared to water or HP lysis/binding buffer). With 100µl whole blood loaded onto the 20mm PSC, the mean viral load was 4.04 c/mL (± 0.15) (See Table 3).

Results (cont'd)

Table 3. Whole Blood Input on PSCs. Summary of HIV-1 Viral Load Results when Rehydrated with SPEX Buffer or HP Lysis/Binding Buffer.

Amount WB	Recovery Buffer Used	Result (LOG c/mL)	Mean (LOG c/mL)	STD DEV
HXB2 Control (frozen plasma)		4.77		
100uL	SPEX (1mL)	4.11	4.04	0.15
100uL	SPEX (1mL)	3.87		
100uL	SPEX (1mL)	4.14		
100uL	Roche HP Lysis/Binding (1 mL)	3.37	3.57	0.36
100uL	Roche HP Lysis/Binding (1 mL)	3.36		
100uL	Roche HP Lysis/Binding (1 mL)	3.98		

HCV RNA was recovered from the plasma collection pad and quantitated, with mean viral load for each level of 4.70, 3.11 and 2.38 LOG IU/mL (See Table 4).

Table 4. Whole Blood Input on PSCs. Summary of HCV Viral Load Results when Rehydrated with SPEX Buffer.

Level	Target (IU/mL)	Target (LOG IU/mL)	Storage	Replicate	HCV RNA (IU/mL)
Frozen Plasma = 6.90					
Volume Loaded					100 uL
1	7,920,000	6.90	14 Days	1	4.63
				2	4.77
				MEAN	4.70
3	316,978	5.50		1	3.10
				2	3.12
				MEAN	3.11
4	63,360	4.80		1	2.20
				2	2.56
				MEAN	2.38

Conclusions

- Plasma Separation Cards (PSCs):
  - collect, separate, and store blood components,
  - eliminate the need for electricity driven processes or cold chain storage,
  - effectively separate cellular components (i.e., PBMCs) in whole blood from cell free plasma,
  - can be used with downstream DNA & RNA molecular based assays (quantitative and qualitative).
- PSCs can offer global solutions and increase accuracy/reproducibility of healthcare services.
- Additional Studies are needed for further validation of this device and to evaluate the composition of plasma collected on the pad compared to plasma separated from whole blood via traditional centrifugation process.

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