PROOF OF CONCEPT: PLASMA SEPARATION CARDS FOR COLLECTION IN RESOURCE-LIMITED SETTINGS

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McClernon AM, McClernon DR
bioMONTR® Labs, Research Triangle Park, NC

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 offers 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 absorbent wick (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 onto 3 different membranes of varying size, each using an oversized absorbent wick (ViveBio LLC, Alpharetta GA). Wicks were inspected and weighed pre/post whole blood addition to evaluate percent plasma recovered, hemolysis, and time of separation.

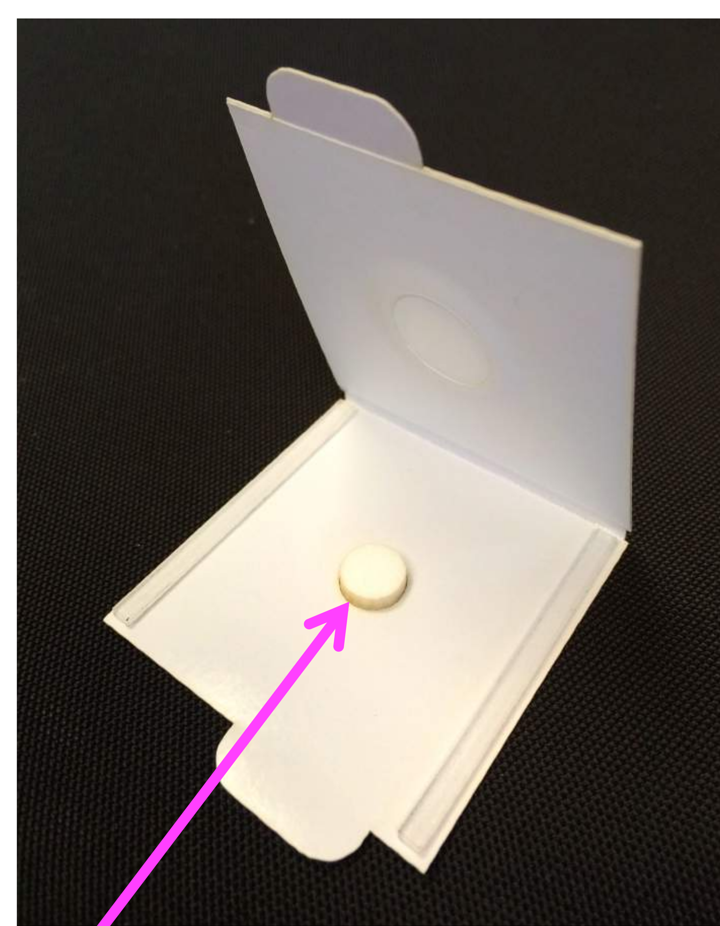
The top performing materials were used to generate rapid prototype cards and then evaluated for functionality in an HIV viral load assay. HXB2 spiked whole blood (75µl or 100µl) was pipetted onto cards and stored overnight. The next day wicks were removed from cards, added to tubes with 600µl diH2O, and incubated on shaker (20 min @ 150 rpm). 500µl was analyzed by Roche COBAS TaqMan HIV-1 v2.0 for use with High Pure System.

Additionally, HXB2 spiked whole blood (100µl) was pipetted onto cards and stored overnight. The next day wicks were removed from cards, 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 transferred to an eppendorf Thermomixer and incubated at 56°C and 1,000 RPM continuous shaking for 10 minutes. 500µl was analyzed by Roche COBAS TaqMan HIV-1 v2.0 for use with High Pure System.

Another set of prototype cards were selected to evaluate functionality for separation of cellular components (i.e., PBMCs) from plasma components. Whole blood from previously characterized donors (HLA B*5701 status) was pipetted onto cards and stored overnight. The next day, membranes and wicks were removed from cards and added to tubes containing 1,000µl SPEX buffer (Roche Diagnostics). The tubes were transferred to an eppendorf Thermomixer and incubated at 56°C and 1,000 RPM continuous shaking for 10 minutes. DNA was extracted from 500µl of each, quantitated using a NanoDrop and analyzed using a rtPCR assay for detection of the HLA B*5701 allele.

Figure 1. Prototype Plasma Separation Card

Blood is added to the entry port



Plasma Passes through to Wick

Results

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

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

The size of the membrane (18mm versus 22mm) and the amount of blood loaded impacted the amount of HIV-1 RNA recovered from the wick. Excluding outliers, with 75µl whole blood (18mm card), the mean viral load was 3.09 (± 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 (See Table 2).

Table 2. Whole Blood Input on PSCs. Summary of HIV-1 Viral Load Results when Rehydrated with Water.

					ALL DATA POINTS		OUTLIERS EXCLUDED	
Card #	Card Design	Volume WB loaded (uL)	Result (c/mL)	Result (LOG c/mL)	Mean (LOG c/mL)	SD	Mean (LOG c/mL)	SD
1	20mm	100	3320	3.52	3.38	0.50	3.61	0.18
2		100	3830	3.58				
3		100	4330	3.64				
4*		100	255	2.41				
5		100	3440	3.54				
6		100	4330	3.64				
7*		100	359	2.56				
8		100	8670	3.94				
9		100	5180	3.71				
10		100	1930	3.29				
11	18mm 1173B	100	15200	4.18	3.92	0.33	3.92	0.33
12		100	3560	3.55				
13		100	10800	4.03				
14		75	956	2.98	3.09	1.52	3.09	0.28
15		75	638	2.80				
16*		75	<34	<1.53				
17		75	788	2.90				
18*		75	<34	<1.53				
19		75	1950	3.29				
20		75	2890	3.46				

*** = Outlier**
Card #4 Matrix was not cut the same as others. Had an outer ring (see picture).
Card #7 May not have been affixed well prior to loading. Top popped up and had to be re-affixed.
Card #16 Blood did not absorb as quickly/completely as noted on other cards.
Card #18 Blood did not absorb as quickly/completely as noted on other cards.

Results (cont'd)

The use of SPEX buffer to re-hydrate the wick and incubation in a Thermomixer (56°C and 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).

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		

Membranes and wicks 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 wicks indicating that all cellular components were captured by the primary membrane. Results of DNA quantitation and HLA status are provided in Table 4.

Table 4. Whole Blood Input on PSCs. Summary of DNA Extraction and HLA B*5701 rtPCR Results when Rehydrated with SPEX Buffer.

Donor	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
Donor 1 (HLA B* 5701 negative)	WB Control	500	N/A	N/A	145	Negative
	PSC Rep 1	100	membrane	1,000	60	Negative
		N/A	wick	1,000	0	Insufficient template quantity
	PCS Rep 2	100	membrane	1,000	58	Negative
		N/A	wick	1,000	0	Insufficient template quantity
	PSC Rep 3	100	membrane	1,000	112	Negative
		N/A	wick	1,000	0	Insufficient template quantity
Donor 2 (HLA B*5701 postiive)	WB Control	500	N/A	N/A	95.7	Positive
	PSC Rep 1	100	membrane	1,000	37	Positive
		N/A	wick	1,000	0	Insufficient template quantity
	PSC Rep 2	100	membrane	1,000	53	Positive
		N/A	wick	1,000	0	Insufficient template quantity
	PSC Rep 3	100	membrane	1,000	43	Positive
		N/A	wick	1,000	0	Insufficient template quantity

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 to evaluate the composition of plasma collected on the wick compared to plasma separated from whole blood via traditional centrifugation process.

bioMONTR® Labs

Send correspondence to:
Anita McClernon
amcclernon@biomontr.com