

Evaluation of SampleTanker[®], a Resource-limited-Setting Friendly Sample Collection Device for HIV-1 Drug Resistance Genotyping Analysis

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Background

- It is estimated that 4 million of AIDS patients were on antiretroviral therapy (ART) in resource-limited settings (RLS) at the end of 2008. To maintain the efficacy of the first line regimens and mitigate the development of HIV drug resistance (DR), HIV DR monitoring is critical.
- Plasma and serum specimens are the gold standard for HIV DR genotyping and they require frozen conditions for storage and shipment, which is expensive and a barrier for implementing HIV DR in RLS. Thus, innovative, easy to use, and requiring no-cold chain condition methodologies for specimen collection, storage and transportation are needed for HIV DR monitoring.
- SampleTanker[®], a novel dried sample collection and transportation device (Research Think Tank, Alpharetta, GA) has been studied as alternative specimen type for viral load and DR genotyping. In this study, we evaluated SampleTanker[®] as a potential blood collection device for HIV DR genotyping.

Methods (1)

Specimen collection and storage:

- 58 Matched blood and plasma specimens collected from ART patients in Honduras were used to prepare SampleTankers (STs) and DPS/DBS.
 - 1mL of plasma or blood was loaded into a SampleTanker matrix and air-dried in a biosafety cabinet overnight and then were capped tightly.
 - 100 µl blood or 50 µl plasma were spotted on each circle of Whatman 903 card.
- Samples except frozen plasma (gold standard, stored at -70°C after collection) were stored at RT for 2 and 4 weeks and then frozen at -70°C before testing.

Methods (2)

Elution of SampleTankers

- Blood/plasma was eluted from SampleTankers using Sample Recovery Kit following the manufacturer's protocol.

PCR, sequencing and genotyping

- The partial *pol* gene encompassing protease and RT regions was amplified using a broadly sensitive in-house genotyping assay.
- Sequences were edited and DR mutations were analyzed by Stanford Drug Resistance Database. Phylogenetic analyses were performed using BioEdit and MEGA 4 software.



SampleTanker Collection Device

Results (1)

- Median viral load (VL) of 58 patients was 2,377 copies/ml of plasma ranged from 449 to 51,274 copies/ml. 15 patients had VL between 400-1,000 and 43 had VL >1,000 copies/ml.
- Identical primary DR mutations were identified from all sample collection devices.
- Pairwise distance analysis revealed <2% sequence diversity comparing to gold standard plasma sequences and all patients were infected with B subtype viruses.

Results (2)

Genotyping efficiency (%) of blood/plasma sample collection devices comparing with gold standard plasmas

Viral Load (copies/ml)	400-1,000 (n=15)				>1,000 (n=43)			
	Dried Plasma		Whole Blood		Dried Plasma		Whole Blood	
	ST	DPS	ST	DBS	ST	DPS	ST	DBS
No. Samples tested	13	13	15	15	40	40	43	43
Genotyping rate (%) 2 weeks at RT	46	31	73	53	48	38	63	79
Genotyping rate (%) 4 weeks at RT	31	23	26	50	50	35	54	61

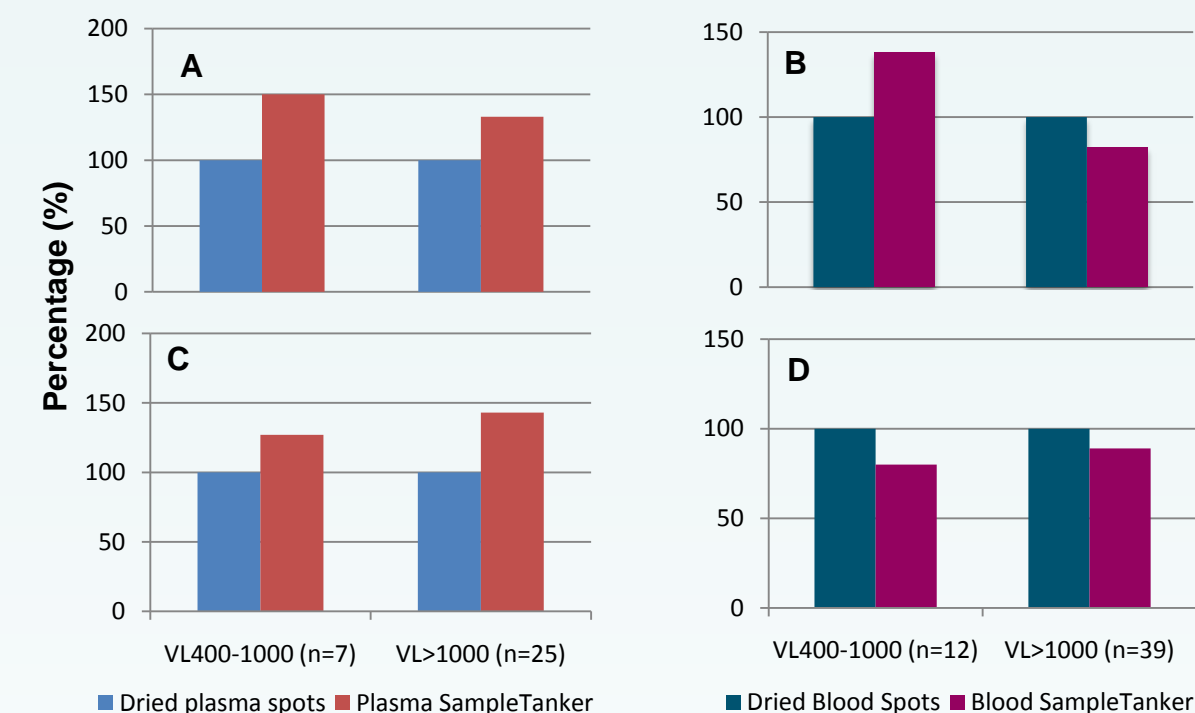
ST: SampleTanker, DPS: Dried plasma spots, DBS: Dried blood spots

Comparative analysis of DR mutations in the RT gene associated with resistance to NRTIs and NNRTIs

Patient ID	NRTI			NNRTI		
	Plasma	Blood SampleTanker	Dried Blood Spots	Plasma	Blood SampleTanker	Dried Blood Spots
HN001	D67N, Y115F, M184V, K219Q	D67N, Y115F, M184V, K219Q	D67DN, K219R , Y115F, M184V, K219Q	K103N, G190S	K103N, G190S	K103N, G190S
HN013	M41L, D67N, K70R, M184V, T215Y, K219E	M41L, D67N, K70R, M184V, T215Y, K219E	M41L, D67N, K70R, M184V, T215Y, K219E	K101E, G190S	K101E, G190S	K101E, G190S
HN020	M184V	M184MV	M184MV	Y181C	Y181CY	K101R , K181C
HN032	D67N, F77L, Y115F, F116Y, Q151M, M184V, K219E	D67N, F77L, Y115F, F116Y, Q151M, M184V, K219E	D67N, F77L, Y115F, F116Y, Q151M, M184V, K219E	L100I, K103N	L100I, K103N	L100I, K103N
HN034	M41L, D67N, T69D, M184V, L210W, T215Y, K219N	M41L, D67N, T69D, M184V, L210W, T215Y, K219N	M41L, D67N, T69D, M184V, L210W, T215Y, K219N	G190A	G190A	G190A
HN048	M184V	M184V	M184V	K103N	K103N	K103N
HN063	M184V	M184V	M184V	K101H, Y188L	K101H, Y188L	K101HQ, Y188Y , Y188L
HN071	D67N, K70R, L74I, V118IV, M184V, T215F, K219E	D67N, K70R, L74I, V118IV, M184V, T215F, K219E	D67N, K70R, L74I, V118IV, M184V, T215F, K219E	none	none	Y181C
HN080	L74V	L74V	L74V	none	none	none
HN081	D67N, K70R, V75T, M184V, K219Q	D67N, K70R, V75T, M184V, K219Q	D67N, K70R, V75T, M184V, K219Q	Y181C	Y181C	Y181C
HN086	M184V	M184V	M184V	K103R, V106A, V179D	K103R, V106A, V179D	K103R, V106A, V179D
HN097	D67N, K70R, L74V, M184V, T215F, K219E	D67N, K70R, L74V, M184V, T215F, K219E	D67DN, K70KR, L74ILV, M184MV, T215F, K219E , K219EK	A98AG, K103N, V108I, M230L	A98AG, K103N, V108I, M230L	A98AG, K103N, V108I, M230L
HN100	T69N, M184V, K219EK	T69N, K219R , M184V, K219EK	T69N, K70KR, M184V, K219EK	K103N, Y181C, H221HY	K103N, Y181C, H221HY	K103N, Y181C, H221HY
HN101	none	none	none	K103N, V106I	K103N, V106I	K103KN, V106I IV
HN103	M184V	M184V	M184MV	K103N, K238KN	K103N	K103N, K238IV
HN104	none	K181R	none	K103N	K103N	K103N
HN106	T215A	T215A	T215A, K181R	K103N	K103N	K103N
HN117	V118I, M184V	V118I	L74I , V118I, M184MV	G190S	none	G190G S

Mutations different from plasma are shown in red color

Comparative analysis of DPS and DBS (as 100%) with plasma SampleTanker and blood Sample Tanker, panel A, B: stored for 2 weeks at RT, panel C, D: stored for 4 weeks at RT



Conclusions

- Genotyping rate was higher when samples were stored 2 week than 4 week at RT with VL between 400-1000 copies/ml, but less difference was notified with patients had VL >1000 copies/ml.
- Whole blood stored as either DBS or sampleTanker had higher genotyping rates than plasma stored as DPS or dried plasma SampleTankers.
- High concordant drug resistance mutations were revealed among frozen plasma (gold standard), SampleTanker, DBS and DPS specimens.
- Our data indicates SampleTanker[®] can preserve the integrity of dried samples at RT at least as effective as whatman 903 cards for HIV DR genotyping.
- Since SampleTanker can hold larger volume of plasma/blood and has simpler packaging and shipping process, it may be another promising dried sample collection device for HIV DR genotyping.