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STABILITY OF HIV-1 VIRAL LOAD FROM DRIED PLASMA USING SampleTanker®, A NOVEL DRIED PLASMA TRANSPORTATION MATRIX

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BACKGROUND

We have previously shown the utility of SampleTanker® as an inexpensive transportation system for Human Immunodeficiency Virus Type 1 (HIV-1) using virologic assays from dried plasma specimens. Herein we describe the precision, and 8 week stability of SampleTanker® as a transportation method for shipping specimens at various temperatures and for subsequent HIV-1 quantitative viral load testing.

This study presents an evaluation and direct comparison of frozen plasma to a novel dried ambient transportation matrix in combination with 2 different commercial kits for analysis of HIV viral load. The study also compares the utility of the method in relation to long term shipping or storage at 3 temperature ranges.

METHODS

Approximately 300 HIV-1 patient plasma specimens were pooled and ten 0.5 mL replicate aliquots were taken for quantitative characterization using the COBAS® Amplicor® HIV-1 Monitor version 1.5 standard assay (Roche Molecular Systems). The mean from the ten replicates was calculated and a dilution schema developed targeting a high (350,000 copies/mL) and low (3,500 copies/mL) copy number virus panel using Normal Human Plasma (NHP). The virus panels were spot checked for accuracy using replicate viral load testing.

Five 1 mL replicate aliquots each were created from the diluted specimen panels representing the high and low control frozen reference standards. Each individual specimen was immediately frozen at -80° C and stored until day 2 experimental testing was performed. 1 mL of the experimental plasma at the high and low determinant points was directly applied to 5 replicate SampleTanker® units to determine short term stability at the following temperature conditions: room temperature (23°C), 37°C (incubator) and -80°C and the following termination points: Day 2, 7, 14, 21, 28, and 56. In order to dry specimens completely, SampleTanker® units were placed at the rear vent of an externally venting class II A/B3 biological safety cabinet or placed in a specialized SampleTanker® drying instrument, driDOC™. The hood or driDOC™ instrument were left running overnight. The interior hood humidity reading was 32-35% and the room humidity containing driDOC™ was 44-47%.

HIV-1 viral load was determined using COBAS® Amplicor® HIV-1 Monitor version 1.5 standard method and for a small subset study a Real-Time quantitative method was used, Abbott RealTime HIV-1 Assay and the m2000 System (Abbott Molecular). Significance was analyzed using Student t-test.

*The SampleTanker® technology is a registered trademark and is covered by U.S. patent (U.S.S.N. 60/561,037) and by international patents.

Table 1: Frozen Plasma Replicate Results Using COBAS® Amplicor® Monitor version 1.5

FROZEN PLASMA CONTROLS Day 2 -80° C					
Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀
R-1	380,000	5.58	R-1	2,490	3.40
R-2	338,000	5.53	R-2	3,000	3.48
R-3	458,000	5.66	R-3	4,060	3.61
R-4	307,000	5.49	R-4	4,630	3.67
R-5	431,000	5.63	R-5	2,800	3.45
mean	382,800	5.58	mean	3,396	3.53
SD	62,727	4.80	SD	908	2.96

Table 4: Frozen Plasma and SampleTanker® Comparisons Using Real-Time m2000 System.

FROZEN PLASMA CONTROLS Day 2 -80° C						SampleTanker® 23° C Day 2					
Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀
R-1	263,027	5.42	R-1	3,419	3.53	R-1	175,637	5.24	R-1	1,625	3.21
R-2	259,328	5.41	R-2	2,503	3.40	R-2	109,290	5.04	R-2	1,568	3.20
R-3	351,885	5.55	R-3	3,230	3.51	R-3	142,006	5.16	R-3	1,756	3.24
R-4	290,454	5.46	R-4	2,905	3.46	R-4	136,096	5.13	R-4	1,546	3.19
R-5	282,338	5.45	R-5	1,277	3.11	R-5	162,582	5.28	R-5	1,524	3.18
mean	289,366	5.48	mean	2,667	3.43	mean	151,116	5.18	mean	1,694	3.21
SD	37,183	4.57	SD	851	2.93	SD	33,095	4.52	SD	93	1.97

Table 2: Frozen Plasma and SampleTanker Comparisons Using COBAS® Amplicor® Monitor version 1.5 (Standard Method).

SampleTanker® 37° C Day 2				SampleTanker® 37° C DAY 7				SampleTanker® 37° C Day 14				SampleTanker® 37° C Day 24				SampleTanker® 37° C Day 56													
Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀	Rep No.	VALUE (copies/ml)	LOG ₁₀									
R-1	181,000	5.18	R-1	1,160	3.06	R-1	92,400	4.97	R-1	1,060	3.03	R-1	301,000	5.00	R-1	680	2.83	R-1	87,800	4.94	R-1	1,100	3.04	R-1	52,400	4.72	R-1	<400	
R-2	198,000	5.01	R-2	1,000	3.00	R-2	298,000	5.48	R-2	1,130	3.05	R-2	125,000	5.10	R-2	888	2.95	R-2	87,000	4.93	R-2	607	2.78	R-2	62,300	4.73	R-2	<400	
R-3	125,000	5.10	R-3	791	2.90	R-3	121,000	5.08	R-3	1,790	3.25	R-3	77,000	4.89	R-3	803	2.90	R-3	119,000	5.08	R-3	1,600	3.20	R-3	39,200	4.59	R-3	627	2.80
R-4	176,000	5.25	R-4	872	2.94	R-4	133,000	5.12	R-4	886	2.99	R-4	72,300	4.86	R-4	948	2.98	R-4	105,000	5.02	R-4	918	2.96	R-4	48,100	4.68	R-4	1,040	3.02
R-5	119,000	5.08	R-5	1,150	3.06	R-5	99,800	4.79	R-5	1,090	3.03	R-5	115,000	5.04	R-5	1,200	3.09	R-5	73,300	4.87	R-5	936	2.97	R-5	47,000	4.62	R-5	637	2.80
mean	135,800	5.13	mean	993	3.00	mean	138,800	5.14	mean	1,197	3.08	mean	97,050	4.99	mean	944	2.97	mean	96,480	4.98	mean	1,032	3.01	mean	48,320	4.66	mean	768	2.89
SD	37,472	4.44	SD	182	2.21	SD	88,086	4.94	SD	238	2.93	SD	22,243	4.38	SD	194	2.29	SD	17,288	4.24	SD	364	2.96	SD	9,282	3.97	SD	238	2.37

Table 3: Summary of SampleTanker® Comparisons to Frozen Plasma Using COBAS® Amplicor® Monitor version 1.5 Assay.

Summary	DAY 2	DAY 7	DAY 14	DAY 24	DAY 56
37° C High Panel log ₁₀ variance	-0.45	-0.44	-0.60	-0.60	-0.90
37° C Low Panel log ₁₀ variance	-0.53	-0.45	-0.56	-0.52	-0.65
22° C High Panel log ₁₀ variance	-0.38	-0.40	-0.44	-0.46	-0.45
22° C Low Panel log ₁₀ variance	-0.29	-0.43	-0.39	-0.42	-0.42
-85° C High Panel log ₁₀ variance	-0.49	-0.24	-0.32	-0.32	-0.24
-85° C Low Panel log ₁₀ variance	-0.23	-0.26	-0.18	-0.32	-0.26

Figure 1: driDOC™ SampleTanker® Drying Instrument (Technology Think Tank Inc.)



RESULTS

Overall intra-assay mean variance among 5 dried replicates across all time points and temperatures was < 0.20 log₁₀ copies/mL (P = NS). Compared to baseline frozen plasma, there was a mean reduction in viral load of 0.38-0.4 and 0.23-0.53 log₁₀ copies/mL at the high and low copy samples respectively at day 2 depending on the temperature of storage (all comparisons, P < 0.05). However, when day 2 SampleTanker® was compared to day 56 SampleTanker®, there was no significant difference in HIV log copy number out to 56 days of storage at 23°C or -80°C, and out to 28 days at 37°C, but there was a further significant decline in viral load for samples held at 37°C for 56 days (P < .002).

Real-Time viral load analysis comparing Day 2 SampleTanker® stored at 23° C to frozen plasma had a mean reduction in viral load of 0.28 and 0.22 log₁₀ copies/mL at the high and low copy samples.

CONCLUSIONS

- Viral load results from dried samples using SampleTanker® were highly reproducible.
- SampleTanker® demonstrated marked stability in conditions that simulate sample transport.
- SampleTanker® stability was independent of viral load assay used.
- HIV-1 viral load assays using dried plasma yielded somewhat lower values compared to frozen plasma but results were generally within the accepted assay variation for replicate samples.
- These data on stability and reproducibility suggest that SampleTanker® has promise for use in HIV clinical trials and patient management.

REFERENCES

1. Lloyd Jr RM, Burns DA, Thompson A, Huang JT, Mathis RL, Holodniy M, Yen-Lieberman B, Armstrong W, Taegle A and Feorino PM. Comparison of Viral Load and Resistance Genotyping Between Frozen Plasma and a Novel Dried Plasma Transportation Medium (SampleTanker™) on Treated Patient Samples. Poster 121. XIII International HIV Drug Resistance Workshop Tenerife Sur-Costa Adeje, Canary Islands, Spain, June 8-12, 2004.
2. Lloyd RM Jr, Holodniy M, Burns DA, Huang JT, McClernon DR, Levy I, Lorber M, Pollack S, Riesenberg K and Grossman Z. 2005. Successful International Ambient Patient Plasma Transport of HIV-1 Clade A, B and C Using SampleTanker™, a Novel Dried Transportation Matrix. The 12th Conference on Retroviruses and Opportunistic Infections. Boston, MA, USA. Feb 26-25, 2005.

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