



Poster #
 XIII International HIV Drug Resistance Workshop
 Tenerife Sur-Costa Adeje,
 Canary Islands, Spain
 June 8-12, 2004

Comparison of Viral Load and Resistance Genotyping Between Frozen Plasma and a Novel Dried Plasma Transportation Medium (SampleTanker™) on Treated Patient Samples.

R.M. Lloyd JR¹, D.A. Burns¹, A. Thompson¹, J.T. Huong¹, R.L. Mathis¹, M. Holodniy², B. Yen-Lieberman³, W. Armstrong³, A. Taagee³ and P.M. Feorino¹
¹Research Think Tank, Inc., Alpharetta, GA, ²Veterans Affairs Medical Center, Palo Alto, CA and ³Cleveland Clinic Foundation, Cleveland, OH, USA.

2580 Westside Parkway, Suite 450
 Alpharetta, GA 30004-7426, USA
 Phone: 770-475-1185 Fax: 770-457-6652
 www.researchthinktank.com



BACKGROUND

HIV-1 viral load and genotypic drug resistance testing are useful in monitoring infection and treatment strategies.¹ Both viral load and resistance testing have strong correlation with response or lack of response to antiviral therapy.² Reference laboratories and clinical trial sponsors require patient plasma to be shipped to testing facilities under frozen conditions, which is expensive and cumbersome. Dried blood spots on filter paper have shown promise as a method of sample collection for CD4+ counts, serology, PCR tests and quantitative assays.³⁻⁹ Although filter paper has potential as a collection medium, inhibition and sensitivity remain questionable. This study evaluates a novel dried sample collection medium, SampleTanker Beta (Research Think Tank, Inc., Alpharetta, GA.), a non-paper-based matrix, as a potential alternative to frozen plasma and filter paper methods for the storage and transportation of virus-infected plasma specimens. This study will present initial viral load (HIV) and genotyping data (HCV and HIV) obtained from SampleTanker specimens after being stored dry at ambient temperatures for 1 and/or 7 days.

METHODS

Viral load, RNA extraction, and genotyping: HIV-1 viral loads were determined using either the Standard or UltraSensitive AMPLICOR HIV-1 MONITOR[®] Test v1.5 (Roche Diagnostics, Indianapolis, IN), VERSANT[®] HIV-1 RNA 3.0 Assay (bDNA) (Bayer Healthcare, Tarrytown, NY) or NucliSens[®] HIV-1 QT Assay (bioMérieux, Durham, NC). Total viral RNA for all samples used in genotyping were extracted using the QIAamp[®] Viral RNA Mini Kit (Qiagen, Valencia, CA). HIV-1 genotype was determined using either or both the TRUGENE[®] HIV-1 Genotyping Kit (Bayer Healthcare) and the HIV-1 GeneTanker Genotyping Complete Assay (Research Think Tank, Inc.). The HCV genotype was determined using the TRUGENE HCV 5'NC Genotyping Kit (Bayer Healthcare). All sequencing, data processing and reporting were performed using the OpenGene[®] DNA Sequencing System (Bayer Healthcare).



Figure 1: SampleTanker.

SampleTanker (Beta version) general methodology: The SampleTanker matrix (Figure 1) has a maximum capacity of 1 mL. A 1 mL volume of plasma was added to each matrix, allowed to air-dry in a biosafety cabinet for 4-5 hours, then packaged in the SampleTanker tube and stored or shipped at ambient temperature. Dried sample matrices were re-hydrated with the appropriate volume of Reconstitution Buffer to recover 1mL of reconstituted plasma.

Amplificor viral load intra-assay reproducibility using SampleTanker: Three matrices were prepared for each of six randomly selected HIV-1 positive plasma samples (N=18). The matrix specimens were reconstituted and recovered on day 7 post preparation and were used to examine the intra-assay viral load reproducibility of the UltraSensitive AMPLICOR viral load assay.

Versant viral load intra-assay reproducibility using SampleTanker: Six matrices were prepared for each of three serial dilutions from an HIV-1 positive sample (N=18). The matrix specimens were reconstituted and recovered on day 1 post preparation and were used to examine the intra-assay viral load reproducibility of the Versant viral load assay.

NucliSens viral load intra-assay reproducibility using SampleTanker: Five matrices were prepared for each of three randomly selected HIV-1 positive plasma samples (N=15). The matrix specimens were reconstituted and recovered on day 3.5 post preparation and were used to examine the intra-assay viral load reproducibility of the NucliSens viral load assay.

HIV-1 genotyping stability: Three archived HIV-1 positive plasma samples with previously determined viral-loads were randomly selected to prepare SampleTanker matrices for genotype stability testing. Two identical sets of matrices were prepared for each sample following the method described above. The dry packaged matrices were stored at ambient temperature for up to 7 days. Reconstituted plasma for each set of matrices was recovered on either day 1 or day 7, respectively. For each sample the entire recovered volume was extracted. Genotyping was performed on all samples using the TRUGENE HIV-1 genotyping kit, following the manufacturer's protocol. The matrix genotypes were then compared with previously determined frozen plasma-derived genotypes.

HIV-1/HCV co-infection: For each of three HIV-1/HCV co-infected plasma samples, a single matrix and frozen plasma aliquot were prepared by an external laboratory and shipped overnight to Research Think Tank. The plasma aliquots were shipped on dry ice, while the SampleTanker specimens were shipped separately at ambient temperature. Upon receipt, the matrix specimens were stored at ambient temperature until reconstituted for testing on day 7 post preparation. The corresponding frozen plasma were thawed for testing in parallel on day 7. All samples were assayed for viral load in duplicate using the Standard AMPLICOR viral load assay. A 140 µL volume of each sample was extracted for total RNA (HIV-1 and HCV), then genotyped using the TRUGENE HIV-1, HIV-1 GeneTanker Complete and HCV TRUGENE 5'NC kits, following manufacturer's protocol.

Phenotyping: RNA extracted from matched SampleTanker and frozen plasma HIV-1/HCV co-infected specimens were submitted for PhenoScrip[®] (VIRalliance, Paris, France) phenotyping analysis.

RESULTS

SampleTanker Characteristics: The SampleTanker consisted of an absorbent fibrous matrix for the preparation of dried plasma specimens and a desiccant storage/transportation tube. The matrix yielded an approximate recovered plasma volume of 1.035 +/-0.03mL. SampleTanker specimens were dried in a biosafety cabinet, at ambient temperature, for a minimum of 4.5 hours prior to packaging into storage tube. The storage tubes contained desiccant to maintain a dry environment for the specimen during storage and/or transportation.

Table 1: A. Standard Roche viral load for 9 randomly selected specimens. B. Bayer bDNA HIV-1 viral load for 12 randomly selected specimens.

Sample	Description	RNA Concentration	RNA Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL Variance
1	Plasma	21,592	7.33	7.33	0.46
1	SampleTanker	7,975	3.93	3.93	0.38
2	Plasma	544,265	5.74	5.74	0.38
2	SampleTanker	331,294	5.52	5.52	0.38
3	Plasma	23,174	4.32	4.32	0.38
3	SampleTanker	20,220	4.30	4.30	0.38
4	Plasma	4,388	3.63	3.63	0.38
4	SampleTanker	2,423	3.40	3.40	0.38
5	Plasma	564,780	6.02	6.02	0.38
5	SampleTanker	138,432	5.13	5.13	0.38
6	Plasma	11,260	4.04	4.04	0.38
6	SampleTanker	6,532	3.81	3.81	0.38
7	Plasma	807,320	6.28	6.28	0.38
7	SampleTanker	4,729	3.68	3.68	0.38
8	Plasma	564,780	6.02	6.02	0.38
8	SampleTanker	1,022	3.52	3.52	0.38

Overall Mean Variance between Frozen Plasma and SampleTanker = 0.25

Sample	Description	RNA Concentration	RNA Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL Variance
11	Plasma	18,227	4.25	4.25	0.47
11	SampleTanker	4,805	3.69	3.69	0.37
12	Plasma	80,228	4.89	4.89	0.47
12	SampleTanker	16,555	4.22	4.22	0.47
13	Plasma	<75	NA	NA	NA
13	SampleTanker	<75	NA	NA	NA
14	Plasma	<75	NA	NA	NA
14	SampleTanker	<75	NA	NA	NA
15	Plasma	<75	NA	NA	NA
15	SampleTanker	<75	NA	NA	NA
16	Plasma	698,600	5.88	5.88	0.31
16	SampleTanker	228,160	5.22	5.22	0.31
17	Plasma	8,255	4.01	4.01	0.31
17	SampleTanker	7,882	3.93	3.93	0.31
18	Plasma	12,209	4.09	4.09	0.33
18	SampleTanker	12,209	4.09	4.09	0.33
19	Plasma	376	2.58	2.58	0.74
19	SampleTanker	821,941	6.91	6.91	0.34
20	Plasma	20,849	4.93	4.93	0.40
20	SampleTanker	11,793	4.05	4.05	0.48

Overall Mean Variance between Frozen Plasma and SampleTanker = 0.51

Table 4: bioMérieux NucliSens HIV-1 QT Assay reproducibility using SampleTanker.

Sample	Description	RNA Concentration	RNA Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL Variance
21	SampleTanker	870	2.94	2.94	0.48
SampleTanker	840	2.94	2.94	2.94	0.48
SampleTanker	520	2.72	2.63	2.63	0.58
SampleTanker	980	2.77	2.77	2.77	0.58
SampleTanker	810	2.91	2.91	2.91	0.58
32	SampleTanker	16,700	4.22	4.22	0.24
SampleTanker	21,362	4.29	4.29	4.29	0.24
SampleTanker	16,580	4.28	4.28	4.28	0.24
SampleTanker	16,390	4.29	4.29	4.29	0.24
SampleTanker	22,375	4.32	4.32	4.32	0.24
33	SampleTanker	130,000	5.11	5.11	0.19
SampleTanker	110,500	5.04	5.04	5.04	0.19
SampleTanker	95,000	4.98	4.98	4.98	0.19
SampleTanker	120,000	5.08	5.08	5.08	0.19
SampleTanker	138,000	5.14	5.14	5.14	0.19

SampleTanker Overall Mean Variance = 0.20

Table 5: HIV-1 anti-retroviral resistance-associated mutations using the TruGene HIV-1 kit for three frozen plasma and SampleTanker isolates genotyped at day 1 and day 7.

Sample	Description	RNA Concentration	RNA Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL Variance
34	Frozen Plasma	100,000	5.20	5.20	0.15
34	SampleTanker day 1	100,000	5.20	5.20	0.15
34	SampleTanker day 7	100,000	5.20	5.20	0.15
35	Frozen Plasma	64,700	4.81	4.81	0.15
35	SampleTanker day 1	64,700	4.81	4.81	0.15
35	SampleTanker day 7	64,700	4.81	4.81	0.15
36	Frozen Plasma	24,792	4.39	4.39	0.15
36	SampleTanker day 1	24,792	4.39	4.39	0.15
36	SampleTanker day 7	24,792	4.39	4.39	0.15

Key:
 PR = protease
 RT = reverse transcriptase
 RED = Drug resistance associated mutations
 GREEN = Mutations at non-drug resistance sites
 Note: SampleTanker Genotypes were obtained from RNA extracted from 8 1 mL volume of reconstituted plasma. Viral load values were determined from frozen plasma.

Table 2: Roche UltraSensitive HIV-1 viral load reproducibility using SampleTanker.

Sample	Description	RNA Concentration	RNA Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL Variance
22	SampleTanker	3,861	3.59	3.59	0.21
SampleTanker	4,085	3.61	3.61	3.61	0.21
SampleTanker	14,940	4.17	4.17	4.17	0.09
SampleTanker	9,214	3.97	3.97	3.97	0.09
SampleTanker	9,214	3.97	3.97	3.97	0.09
24	SampleTanker	19,981	4.30	4.30	0.53
SampleTanker	19,981	4.30	4.30	4.30	0.53
SampleTanker	25,195	4.40	4.40	4.40	0.53
SampleTanker	37,387	4.57	4.57	4.57	0.53
26	SampleTanker	1,275	3.11	3.11	0.55
SampleTanker	1,837	3.26	3.26	3.26	0.55
SampleTanker	1,550	3.25	3.25	3.25	0.55
27	SampleTanker	1,837	3.30	3.30	0.55
SampleTanker	991	2.98	2.98	2.98	0.55
SampleTanker	2,274	3.11	3.11	3.11	0.55

SampleTanker Overall Mean Variance = 0.55

Table 3: Bayer bDNA HIV-1 viral load reproducibility using SampleTanker.

Sample	Description	RNA Concentration	RNA Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL	Mean Log ₁₀ IU/mL Variance
28	SampleTanker	6140	4.04	4.04	0.03
SampleTanker	34,285	4.54	4.54	4.54	0.03
SampleTanker	35,380	4.56	4.56	4.56	0.03
SampleTanker	37,791	4.58	4.58	4.58	0.03
SampleTanker	30,269	4.60	4.60	4.60	0.03
SampleTanker	40,205	4.63	4.63	4.63	0.03
29	SampleTanker	6,430	3.74	3.74	0.04
SampleTanker	5,206	3.72	3.72	3.72	0.04
SampleTanker	6,160	3.71	3.71	3.71	0.04
SampleTanker	6,142	3.70	3.70	3.70	0.04
SampleTanker	6,811	3.80	3.80	3.80	0.04
SampleTanker	5,905	3.77	3.77	3.77	0.04
30	SampleTanker	709	2.86	2.86	0.07
SampleTanker	710	2.86	2.86	2.86	0.07
SampleTanker	417	2.83	2.83	2.83	0.07
SampleTanker	568	2.78	2.78	2.78	0.07
SampleTanker	476	2.80	2.80	2.80	0.07

SampleTanker Overall Mean Variance = 0.25

Viral load assays: The mean log₁₀ difference between matched frozen and SampleTanker dried plasma specimens using the standard AMPLICOR HIV-1 and VERSANT HIV-1 assay was 0.36 and 0.51, respectively (Table 1A and B). Intra-assay quantitative reproducibility experiments for SampleTanker indicated an overall Log₁₀ mean variance of 0.05 for the UltraSensitive AMPLICOR HIV-1 assay at day 7 (Table 2), 0.05 for the VERSANT HIV-1 assay at day 1 (Table 3) and 0.06 for the NucliSens HIV-1 QT assay at day 3.5 (Table 4). Viral load values obtained from SampleTanker specimens were consistently lower than those obtained from matched frozen plasma specimens.

Genotyping assays: The sequence quality generated between matched SampleTanker and frozen plasma specimens was comparable. However, in several sequences SampleTanker specimens exhibited an increase in sequence quality (data not shown). Mutation profiles obtained using either the TRUGENE HIV-1 and/or HIV-1 GeneTanker genotyping kits exhibited a high degree of concordance between matched SampleTanker and frozen plasma specimens (Table 5 and 6). This concordance was consistent regardless of HIV-1 viral-load, storage time or shipping conditions prior to genotype testing. Among matched co-infected SampleTanker and frozen plasma specimens, there was a 100% concordance at the genotype level for HCV using the TRUGENE HCV 5'NC kit. While samples 37 and 39 were in agreement at the subtype level, HCV subtype was unable to be determined for the sample 38 plasma specimens.

Phenotyping: Phenotype results were successfully obtained for all matched HIV-1/HCV co-infected SampleTanker and frozen plasma specimens (data not shown).

CONCLUSION

- Intra-assay viral load results using SampleTanker for three FDA approved quantitative assays were highly reproducible.
- Reproducibility results suggests that SampleTanker has promise in clinical trial management.
- Genotype accuracy and reproducibility using SampleTanker are comparable to data published in the TRUGENE product inserts for frozen plasma samples.
- Mutation profiles for the expanded regions of the HIV-1 GeneTanker Genotyping Complete Assay were concordant with those gene regions contained in the TRUGENE HIV-1 Genotyping Assay for all frozen and SampleTanker plasma specimens.
- HCV genotyping was concordant with matched frozen and SampleTanker plasma specimens.
- SampleTanker yielded results in a recombinant HIV-1 phenotyping assay.
- The ability to produce viral load, genotype and recombinant phenotype reports for all SampleTanker specimens suggest its potential utility in other quantitative and qualitative assays.
- Use of the SampleTanker dried plasma technology gives adequate results for quantitative and qualitative clinical specimen testing.
- SampleTanker is a convenient, cost-effective alternative to the storage and shipping of frozen plasma for nucleic acid testing, in turn possibly eliminating the need for batched specimen shipments in real-time trials worldwide.
- Further comparison and time point studies are ongoing and show promise for SampleTanker use in clinical studies.

REFERENCES

- Zhang M and Versalovic J. 2002. HIV update. Diagnostic tests and markers of disease progression and response to therapy. *Am J Clin Pathol.* Dec;118 Suppl:S26-32.
- Yahi N, Tamalet C, Toures C, Tivoli N, Ariasi F, Volot F, Gastaut JA, Gallais H, Moreau J and Fantini J. 1999. Mutation patterns of the reverse transcriptase and protease genes in human immunodeficiency virus type 1-infected patients undergoing combination therapy: survey of 787 sequences. *J Clin Microbiol.* 37(12):1099-106.
- Chaisomchit S, Wichajam R, Chowprecha S and Chareonsirawatana W. 2003. A simple method for extraction and purification of genomic DNA from dried blood spots on filter paper. *Southeast Asian J Trop Med Public Health.* Sep;34(3):641-5.
- Solomon SS, Solomon S, Rodriguez II, McGarvey ST, Ganesh AK, Thyagarajan SP, Mahajan AP and Mayer KH. 2002. Dried blood spots (DBS): a valuable tool for HIV surveillance in developing/tropical countries. *Int J STD AIDS.* Jan;13(1):25-8.
- Wang B, Lu X, Lu Y. 1998. Detection of proviral gene of human immunodeficiency virus type 1 from dried blood spots. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi.* Sep;12(3):281-4.
- Mwaba P, Cassol S, Pilon R, Chintu C, Janes M, Nunn A and Zumia A. 2003. Use of dried whole blood spots to measure CD4+ lymphocyte counts in HIV-1-infected patients. *Lancet.* Nov 1;362(9394):1459-60.
- Brambila D, Jennings C, Aldrovandi G, Bremer J, Comeau AM, Cassol SA, Dickover R, Jackson JB, Pitt J, Sullivan JL, Butcher A, Grosso L, Reichelderfer P and Fiscus SA. 2003. Multicenter evaluation of use of dried blood and plasma spot specimens in quantitative assays for human immunodeficiency virus RNA: measurement, precision, and RNA stability. *J. Clin. Microbiol.* May;41(5):1888-93.
- Cassol S, Gill MJ, Pilon R, Cormier M, Voigt RF, Willoughby B and Forbes J. 1997. Quantification of human immunodeficiency virus type 1 RNA from dried plasma spots collected on filter paper. *J. Clin. Microbiol.* Nov;35(11):2795-801.
- Mwaba P, Cassol S, Nunn A, Pilon R, Chintu C, Janes M and Zumia A. 2003. Whole blood versus plasma spots for measurement of HIV-1 viral load in HIV-infected African patients. *Lancet.* Dec 20;362(9401):2067-8.

*For Research Use Only. Not For Use In Diagnostic Procedures.