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Successful International Ambient Patient Plasma Transport of HIV-1 Clade A, B and C Using SampleTanker™, a Novel Dried Transportation Matrix

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BACKGROUND

Viral-load and drug-resistance testing are routinely used to monitor patients infected with Human Immunodeficiency Virus Type 1 (HIV-1).[1] Reference laboratories and clinical trial studies require shipment to testing facilities under controlled 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.[2-8] However, filter paper has limitations, such as sample volume size. We have previously shown the utility of the SampleTanker™ Sample Preparation and Recovery Kit *Beta* (36)*[§] (Research Think Tank, Inc., Alpharetta, GA) to be an inexpensive dried transportation alternative.[9] Here we describe a retrospective analysis of archived HIV-1 subtype A, B and C plasma shipped from Israel to the USA under ambient conditions using SampleTanker.

METHODS

HIV-1 positive plasma specimens (n=72), with draw dates ranging from November 2002 - August 2004, were selected randomly from a population of frozen archived specimens previously genotyped by the TRUGENE™ HIV-1 Genotyping Kit (Bayer HealthCare Tarrytown, NY). Plasma HIV-1 RNA was quantified real-time, using standard Amplicor HIV-1 Monitor® v1.5 assay (Roche Diagnostics, Indianapolis, IN), NucliSens™ HIV-1 QT Assay or NucliSens EasyQ® HIV-1 assay (bioMerieux, Durham, NC), prior to archiving and subsequent SampleTanker transportation. Real-time viral load values ranged from 3,700 to >1,000,000 copies.

Specimen volumes from 600 to 1000 µL of thawed plasma were each added to the SampleTanker matrix, air-dried, packaged and shipped ambient from Israel to Atlanta, Georgia, USA using standard first class mail. The SampleTanker specimens were split into two separate shipment batches each containing 36 specimens. Upon receipt, each SampleTanker Specimens was re-suspended and recovered by adding 1.175 mL reconstitution buffer according to SampleTanker Sample Preparation and Recovery Kit *Beta* (36) protocol. Because plasma input volumes varied (**Table 1**), each specimen under the standard SampleTanker 1 mL input volume was adjusted after recovery for this study. This was accomplished by centrifugation and removing appropriate volume of clarified supernatant to match original plasma input volume. Initially, a volume of 140 µL of reconstituted plasma was directly extracted for HIV-1 viral RNA using the standard protocol from the QIAamp® Viral RNA Mini Kit (QIAGEN, Valencia, CA). Secondarily, a modified ultra-centrifugation extraction of the remaining reconstituted plasma was used on specimens that failed to produce a genotype with the original 140 µL extraction. SampleTanker specimens were run in real-time.

Genotype analysis was performed using the TRUGENE HIV-1 Genotyping Kit and HIV-1 GeneTanker™[§] Complete Assay (Research Think Tank, Inc.). Gag/pol mutations and clade determination were accomplished using the expanded assay regions of the GeneTanker™ assay sequences. Resistance Associated Mutations (RAMs) and polymorphic fingerprint similarity analysis, at the nucleotide and amino acid levels, were compared from the paired original TRUGENE plasma genotype and SampleTanker (TRUGENE & GeneTanker) genotypes for each specimen. Polymorphic fingerprints were accomplished by the alignment of the specimen sequence to the standard reference wild-type LAI-1 sequence contained in the TRUGENE GeneLibrarian™. GeneLibrarian polymorphic fingerprints were directly exported to a novel sequence comparator tool, MuTanker™[§] (Research Think Tank, Inc), for statistical analysis. MuTanker independently compared the IAS relative RAM sites (total of 165 bases or 55 amino acids) and the overall sequence similarity (total nucleotide length of 925 bases or 308 amino acids) for all paired sequences. Although the HIV-1 GeneTanker genotype sequencing and analysis encompassed expanded *gag/pol* regions as compared to TRUGENE, only the 925 complimentary bases of the TRUGENE GeneLibrarian were utilized for this comparative analysis.

RESULTS

Two separate SampleTanker boxes containing 36 dried samples each were shipped from Israel by standard first class mail arriving at the Alpharetta, GA, USA testing facility (mean 19 days) with a combined cost of $14.20 USD. Prospective genotypes were compared to matched retrospective SampleTanker dried specimens. All 72 SampleTanker specimens arrived intact in the shipping packaging provided in the SampleTanker kit.

For analysis, log viral load quartiles were established: Quartile-1 from 1,000 to 10,000 (n=9), Quartile-2 from 10,000 to 100,000, (n=37), Quartile-3 from 100,000 to 1,000,000 (n=21) and Quartile-4 at >1,000,000 (n=5) copies/mL (**Table 1**). Overall, 59 of 72 SampleTanker genotypes (81.9%) were obtained using both the TRUGENE HIV-1 Genotyping Kit and the HIV-1 GeneTanker genotyping kit at the 140 uL volume extraction. An additional 5 samples were successfully genotyped for an overall positive result of 88.9% when the remaining volume of reconstituted SampleTanker plasma specimens were ultra-centrifuged prior to extraction. For Quartile-1, five of nine SampleTanker specimens were genotyped at the standard 140 uL extraction (55.6%). The number of successful test increased to 77.8% when two additional genotypes were obtained from the ultra-centrifugation step. For Quartile-2, twenty-nine of thirty-seven specimens were genotyped at the 140 uL extraction (78.4%) volume. This number increased to 83.8% when two additional genotypes were obtained from the ultra-centrifugation step. For Quartile-3, twenty of twenty-one SampleTanker specimens were genotyped at the 140 uL extraction (95.2%) volume. This number increased to 100% when one additional genotype was obtained from the addition of the ultra-centrifugation step. For Quartile 4, five of five genotypes (100%) were obtained for the SampleTanker specimens at the standard 140 µL extraction volume. SampleTanker sequencing results were directly compared to the single real-time TRUGENE reporting result obtained from a laboratory in Israel. The pre-edited TRUGENE real-time sequences were used as the "reference sequences" for direct comparison. SampleTanker sequences were obtained first pass without repeating the assay or they were defined as No Sequence. Stringent criterion for the genotype results were used in this analysis including nucleotide for nucleotide comparisons and heterozygous base-calling. Sixty-one of the sixty-four SampleTanker specimens successfully genotyped had mean similarity scores of >99% and >98% concordance at the nucleic acid and amino acid level for RAMs and polymporphic fingerprints, respectively. These values were obtained using both TRUGENE HIV-1 Genotyping Kit and the HIV-1 GeneTanker genotyping assay (results not shown). Three of the 64 SampleTanker specimens (<5%) were <98% in concordance. When comparing SampleTanker results, the genotype concordances were ≥99% at the nucleotide level, ≥98% at the amino acid level and 100% at the reported RAMs between the TRUGENE and GeneTanker assays. A two codon insertion was detected for a sample at codon 69 of the reverse transcriptase in the original and the SampleTanker paired specimen using both the TRUGENE HIV-1 Genotyping Kit and the HIV-1 GeneTanker genotyping kit.

Table 1: Genotype similarity analysis of SampleTanker specimens.

							% Similarity Using TRUGENE HIV-1 Genotyping Kit			
							Resistance Associated Mutations		Polymorphic Fingerprint	
	Sample	Draw Date	Clade	Viral Load	Viral Load Method	Plasma Input Vol(uL)	165 n.a.	55 a.a.	925 n.a.	308 a.a.
Quartile-1	W-33871	27-Jun-04	C	3,700	Amplicor	1000	No Sequence	No Sequence	No Sequence	No Sequence
	W-32368	07-Jan-04	C	3,870	Amplicor	750	99.4%	99.1%	99.2%	99.2%
	W-32092*	07-Jan-04	B	4,000	EasyQ	1,000	99.7%	100.0%	99.6%	99.8%
	W-32297	26-Jan-04	B	4,400	EasyQ	750	100.0%	100.0%	100.0%	100.0%
	W-33362	01-Apr-04	C	4,600	Amplicor	1000	98.2%	97.3%	98.7%	98.1%
	W-34120	28-Jul-04	C	4,970	Amplicor	750	91.8%	81.8%	95.8%	92.5%
	W-28908	NA	C	7,000	NA	1,000	100.0%	100.0%	99.2%	99.0%
	W-32371	02-Feb-04	C	7,860	Amplicor	750	No Sequence	No Sequence	No Sequence	No Sequence
	W-32183	15-Sep-03	C	9,700	Amplicor	1,000	99.6%	100.0%	99.2%	99.5%
Quartile-2	W-32814	11-Mar-04	C	10,700	Amplicor	1000	100.0%	100.0%	99.6%	99.8%
	W-32529	17-Feb-04	B	12,400	Amplicor	1,000	97.0%	96.4%	95.8%	95.1%
	W-29079	24-Mar-03	C	12,800	Amplicor	1,000	No Sequence	No Sequence	No Sequence	No Sequence
	W-28376	22-Jan-03	B	14,000	Nuclisens	1,000	99.7%	100.0%	98.1%	97.4%
	W-29636	19-May-03	AG	14,000	Nuclisens	1,000	No Sequence	No Sequence	No Sequence	No Sequence
	W-33390	19-May-04	B	16,000	EasyQ	1000	99.7%	99.1%	99.2%	99.5%
	W-30182	01-Jul-03	C	17,000	Amplicor	1,000	No Sequence	No Sequence	No Sequence	No Sequence
	W-32931	31-Mar-04	B	17,000	Nuclisens	1000	100.0%	100.0%	99.4%	99.2%
	W-29182	02-Apr-03	B	18,000	Nuclisens	1,000	98.8%	97.3%	99.0%	98.5%
	W-33873	12-Jan-04	C	18,900	Amplicor	600	No Sequence	No Sequence	No Sequence	No Sequence
	W-31147	02-Oct-03	C	19,500	Amplicor	1,000	99.7%	100.0%	99.6%	99.5%
	W-32579	20-Oct-03	C	19,700	Amplicor	600	99.7%	99.1%	99.6%	99.4%
	W-33648	21-Jun-04	B	21,000	EasyQ Sp	1,000	100.0%	100.0%	99.2%	99.1%
	W-29346	NA	C	23,100	NA	1,000	99.7%	99.1%	99.4%	99.5%
	W-33394	11-Feb-04	C	23,700	Amplicor	750	97.9%	100.0%	98.1%	99.2%
	W-32813	08-Mar-04	C	24,000	Amplicor	750	97.0%	97.3%	98.3%	98.2%
	W-32712	24-Feb-04	C	24,800	Amplicor	750	98.8%	98.2%	99.0%	98.5%
	W-28131	26-Dec-02	B	26,000	Amplicor	1,000	94.5%	85.5%	94.9%	93.2%
	W-34064	02-Aug-04	C	27,800	Amplicor	750	98.8%	99.1%	99.6%	99.2%
	W-33277	10-May-04	GH	28,568	Nuclisens	600	99.7%	99.1%	99.5%	99.8%
	W-29222	25-Mar-03	C	30,800	Amplicor	1,000	97.8%	97.6%	98.6%	98.3%
	W-29599	13-May-03	C	34,000	Amplicor	1,000	99.7%	99.1%	99.0%	98.7%
	W-34119	27-Jul-04	C	34,200	Amplicor	600	100.0%	100.0%	99.6%	99.5%
	W-29752	22-May-03	AG	39,500	Amplicor	1,000	No Sequence	No Sequence	No Sequence	No Sequence
	W-28547	05-Mar-03	AG	43,000	Nuclisens	1,000	100.0%	100.0%	99.7%	99.5%
	W-29220	27-Nov-02	B	43,000	Amplicor	1,000	100.0%	100.0%	99.4%	98.7%
	W-32655	24-Feb-04	C	43,400	Amplicor	750	100.0%	100.0%	99.9%	99.8%
	W-32545	23-Feb-04	C	47,000	EasyQ	750	99.4%	100.0%	99.4%	99.5%
	W-31739	17-Nov-03	B	52,200	Amplicor	1,000	100.0%	100.0%	99.1%	98.9%
	W-33872	12-Jul-04	C	57,300	Amplicor	750	99.7%	99.1%	98.9%	98.5%
	W-28218	06-Jan-03	C	61,200	Amplicor	1,000	No Sequence	No Sequence	No Sequence	No Sequence
	W-32185	09-Nov-03	C	65,900	Amplicor	1,000	100.0%	100.0%	99.9%	100.0%
	W-32900	29-Mar-04	B	69,000	EasyQ	1000	99.7%	100.0%	99.2%	99.4%
	W-32095	02-Sep-03	C	72,600	Amplicor	1,000	99.7%	100.0%	99.0%	98.9%
	W-30195	07-Apr-03	B	75,100	Amplicor	1,000	98.8%	100.0%	97.8%	98.1%
	W-29219	01-Apr-03	AE	89,400	Amplicor	1,000	100.0%	100.0%	99.2%	99.7%
	W-33714	28-Jun-04	B	93,000	EasyQ Sp	600	99.4%	100.0%	99.5%	99.8%
Quartile-3	W-32461	29-Dec-03	C	120,000	Amplicor	750	100.0%	100.0%	100.0%	100.0%
	W-30480	28-Jul-03	D	124,000	Amplicor	1,000	97.9%	97.0%	98.9%	98.8%
	W-30070	15-Jun-03	C	125,000	Amplicor	1,000	100.0%	100.0%	99.8%	99.8%
	W-30068	15-Jun-03	C	140,000	Amplicor	1,000	100.0%	100.0%	99.8%	99.7%
	W-30072	17-Jun-03	C	150,000	Amplicor	1,000	99.7%	99.1%	99.7%	99.4%
	W-33677	20-Jun-04	C	160,000	Amplicor	1000	99.7%	99.1%	99.8%	99.7%
	W-29720	13-Apr-03	C	190,000	Amplicor	1,000	99.4%	99.1%	99.0%	98.7%
	W-32508	18-Feb-04	B	210,000	EasyQ Sp	1,000	100.0%	100.0%	99.7%	99.7%
	W-32148	02-Dec-03	B	270,000	Amplicor	1,000	100.0%	100.0%	99.8%	99.5%
	W-29884	09-Jun-03	C	271,000	Amplicor	1,000	98.8%	96.4%	96.7%	95.1%
	W-33395	10-May-04	C	311,000	Nuclisens	1000	99.7%	100.0%	99.5%	99.5%
	W-30391	14-Jul-03	C	333,000	Amplicor	1,000	99.4%	100.0%	98.8%	98.9%
	W-32665	24-Feb-04	C	340,000	Amplicor	750	99.7%	99.1%	99.8%	99.8%
	W-28910	02-Mar-03	B	405,000	Amplicor	1,000	100.0%	100.0%	99.8%	100.0%
	W-28735	01-Jan-03	C	432,000	Amplicor	750	91.2%	82.7%	96.0%	93.7%
	W-34146	09-Aug-04	C	500,000	Amplicor	750	99.4%	100.0%	99.8%	100.0%
	W-30971	11-Sep-03	C	533,000	Amplicor	1,000	99.4%	98.5%	99.1%	98.3%
	W-32583	14-Jan-04	C	603,000	Amplicor	750	98.8%	100.0%	99.4%	99.8%
	W-29522	05-May-03	C	649,000	Amplicor	1,000	97.9%	99.1%	98.5%	98.8%
	W-28733	31-Dec-02	C	700,000	Amplicor	1,000	98.8%	100.0%	99.5%	100.0%
	W-29716	27-Apr-03	B	960,000	Amplicor	1,000	99.7%	99.1%	99.4%	98.2%
Quartile-4	W-31955	16-Dec-03	C	1,960,000	Amplicor	750	100.0%	100.0%	99.8%	99.8%
	W-32460	12-Feb-04	B	2,410,000	Amplicor	1,000	99.4%	99.1%	99.1%	99.0%
	W-29225	06-Apr-03	C	2,430,000	Amplicor	1,000	100.0%	100.0%	99.5%	99.7%
	W-32160	13-Jan-04	C	6,880,000	Amplicor	750	99.7%	99.1%	99.5%	99.7%
	W-32161	13-Jan-04	C	19,300,000	Amplicor	750	100.0%	100.0%	99.6%	99.0%
						Mean =	99.1%	98.6%	99.0%	98.8%

* RT69 insertion detected.

Table 2: Cost estimate for SampleTanker.

Shipper	Shipping Time	Transportation Type	Dry Ice	Weight (lb/oz)	Est. Cost/package or box (USD)
Israel Postal Service / US Postal Service	standard 1st class (>3 days)	Passenger	NO	0/17.5	$7.10
World Courier (will refill box with dry ice)	2-3 days	Passenger or Cargo	YES	20/0	$800.05
Federal Express (does not refill box with dry ice)	2-3 days	Passenger	YES	20/0	$614.46
		Cargo	YES	20/0	$712.74

CONCLUSIONS

- The SampleTanker dried plasma specimens were stable under ambient conditions when shipped from Israel to the USA using standard parcel services.
- SampleTanker does not require expedited next day transit.
- SampleTanker is a cost-effective alternative to the conventional shipping of frozen or refrigerated samples, with potential utility in cooperative studies or in routine testing of blood collected in the remote places of developing countries (**Table 2**).
- The increased volume capacity of SampleTanker allows for multiple analyte testing.
- The accuracy and reproducibility of the genotype results were comparable to the TRUGENE HIV-1 Genotyping product insert specifications used for FDA approval.
- The TRUGENE HIV-1 Genotyping kit and HIV-1 GeneTanker Complete kit were comparable in genotypic results.
- Concordance of protease and reverse transcriptase sequences between TRUGENE and GeneTanker validate the expanded linked gag/pol and envelope gene regions obtained from the HIV-1 GeneTanker Complete assay (data not shown).
- MuTanker is a quick and useful tool for direct comparisons of genetic sequences, polymorphic fingerprints and sequential isolates.

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

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