



Poster 45

# Comparison of Whole Blood, Plasma, and PBMC HIV-1 Resistance Genotyping and Matched Dried Samples Using SampleTanker™ a Novel Dried Transportation Matrix

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## BACKGROUND

Cooperative studies worldwide are addressing the use of whole blood for biomarker analysis in developing countries.<sup>1</sup> We have previously shown the utility of whole blood genotyping and SampleTanker\* (Research Think Tank, Inc.), an inexpensive transportation system for HIV-1 genotypic analysis of dried plasma.<sup>2,3</sup> Herein, we describe the utility of dried whole blood and peripheral blood mononuclear cells (PBMC) using SampleTanker for HIV-1 genotypic analysis.

## METHODS

Twenty treated HIV-1 infected patients with viral loads ranging from 3,000 to 250,000 copies/mL were selected for genotypic analysis. Blood kits containing four VACUTAINER™ EDTA (4 mL each) and one VACUTAINER CPT (8 mL) blood collection tubes (Becton Dickinson) were drawn and inverted several times after collection to mix preservatives completely. Plasma was isolated from three of the four blood collection tubes following the VACUTAINER EDTA product insert methodology. Processed plasma was frozen at minus-70°C until tested. One of the VACUTAINER EDTA blood collection tubes remained unprocessed to represent the whole blood specimen. For each sample, PBMC from a single CPT tubes were counted and separated into two 0.5 mL aliquots each containing 1 x 10<sup>6</sup> to 5 x 10<sup>6</sup> cells. Plasma specimens were tested for viral load using the Roche Amplicor HIV-1 Monitor™ version 1.5 or COBAS Amplicor HIV-1 Monitor version 1.5 Standard methodologies (Roche Molecular Systems).

Paired samples of plasma and SampleTanker plasma, whole blood and SampleTanker whole blood, and PBMC were extracted using the NucliSens® MiniMAG System (bioMérieux) and QIAamp Viral RNA Mini Kit or QIAamp DNA Blood Mini Kit (Qiagen). For the plasma and SampleTanker plasma extractions, 1.0 mL of sample and a standardized elution volume of 60 µL was used with both the NucliSens MiniMAG System and QIAamp Viral RNA Mini Kit. For the whole blood and SampleTanker whole blood (total nucleic acid) extractions, 100 µL was used for the NucliSens MiniMAG System and 200 µL for the QIAamp DNA Blood Kit. To normalize the nucleic acid concentration for each extraction methodology, an elution volume equivalent to the input extraction volume was used. For the PBMC aliquots, the matched samples were centrifuged and resuspended in a standardized 200 µL volume of PBS prior to extraction. The entire 200 µL sample was then extracted and eluted in 200 µL of elution buffer.

The extracted plasma (RNA), whole blood total nucleic acid (viral RNA and DNA) and PBMC (DNA) were genotyped using the TRUGENE HIV-1 Genotyping Assay (Bayer HealthCare). It is important to note that when genotyping HIV-1 from whole blood, both RNA and DNA (proviral and episomal) viral species are potentially amplified and sequenced simultaneously<sup>2</sup>.

Sequences derived from plasma virus were used as the reference for directly comparing sequences from whole blood and dried SampleTanker whole blood specimens. In a secondary analysis, plasma and PBMC derived genotypes were merged and compared as the reference sequence for analysis. Sequences derived from whole blood were directly compared to either of the references using the automated MuTanker™ Comparator software (Research Think Tank, Inc.).

## RESULTS

A total of 200 genotypic assays were performed in this comparative study. Successful genotypes were obtained from 100% (20 of 20) of the plasma, 95% (19 of 20) of the SampleTanker plasma, 95% (19 of 20) of the whole blood, 60% (12 of 20) of the SampleTanker whole blood and 45% (9 of 20) of the PBMC matched specimens using the NucliSens MiniMAG Extraction System (Tables 1 & 2). For the matched samples

Table 1. HIV-1 Specimen Summary.

SAMPLE	RNA Copies/mL	PBMC cells x 10 <sup>6</sup>	SAMPLE TYPE	MiniMAG	QIAamp	SAMPLE	RNA Copies/mL	PBMC cells x 10 <sup>6</sup>	SAMPLE TYPE	MiniMAG	QIAamp
MT1-AA1-R0001	3,070	4.27	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-PS1-R0001	40,219	3.27	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-DM2-R0002	3,950	5.16	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-OM1-R0001	41,800	2.5	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-SD1-R0006	4,120	1.12	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-GL1-R0004	47,400	4.67	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-JE1-R0001	13,900	3.85	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-MB1-R0004	69,100	3.66	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-RM2-R0002	17,178	5	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-PR1-R0002	72,700	4.1	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-JK1-R0007	21,900	3.85	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-GD1-R0005	87,921	1.58	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-MM1-R0001	24,846	5.2	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-MM2-R0001	92,508	4.33	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-TC2-R0002	28,368	4.65	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-HB1-R0003	99,524	5.2	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-WH1-R0003	31,800	1.89	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-EH1-R0002	182,105	5.2	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	
MT1-AI1-R0001	39,296	5	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	failed failed failed failed failed	MT1-DK1-R0002	258,239	4.14	PL ST-PL WB ST-WB PBMC	failed failed failed failed failed	

Table 2. HIV-1 Specimen Legend and Success Rates.

SAMPLE COLLECTION TYPE	MiniMAG Methodology	QIAamp Methodology
Plasma (PL)	100% (20 of 20)	80% (16 of 20)
SampleTanker Plasma (ST-PL)	95% (19 of 20)	85% (17 of 20)
Whole Blood (WB)	95% (19 of 20)	95% (19 of 20)
SampleTanker Whole Blood (ST-WB)	60% (12 of 20)	75% (15 of 20)
Peripheral Blood Mononuclear Cells (PBMC)	45% (9 of 20)	85% (17 of 20)

Figure 1. Example MuTanker Report.

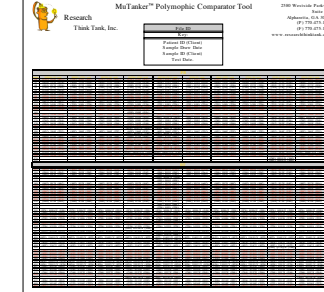


Table 3. Paired HIV-1 Specimens Percent Similarity Scores.

SAMPLE	% Similarity PL vs ST-PL MiniMAG and Qiagen				% Similarity PL vs WB MiniMAG and Qiagen				% Similarity PL vs ST-WB MiniMAG and Qiagen			
	RAM		Polymorphic Fingerprint		RAM		Polymorphic Fingerprint		RAM		Polymorphic Fingerprint	
	NA (n=165)	AA (n=55)	NA (n=925)	AA (n=308)	NA (n=165)	AA (n=55)	NA (n=925)	AA (n=308)	NA (n=165)	AA (n=55)	NA (n=925)	AA (n=308)
MT1-AA1-R0001	99.4/NA	100/NA	99.9/NA	100/NA	100/99.7	100/100	99.6/99.8	99.8/99.8	NA	NA	NA	NA
MT1-JM2-R0002	98.8/98.6	97.3/98.2	98.5/97.6	95.4/94.8	93.6/95.6	88.2/90.9	99.5/97.4	94.2/96.4	99.1/99.7	97.3/100	98.5/98.3	97.2/97.6
MT1-JD1-R0006	97.0/NA	96.4/NA	98.1/NA	99.4/NA	99.7/NA	100/NA	99.5/NA	99.4/NA	NA	NA	NA	NA
MT1-JE1-R0001	100/100	100/100	99.8/99.7	100/100	100/100	100/100	99.4/99.7	100/100	99.4/99.7	100/100	99.0/99.2	100/100
MT1-RM2-R0002	99.7/99.4	100/99.1	99.6/99.7	99.5/99.4	99.7/99.7	100/100	99.4/99.7	98.9/99.4	NA/99.7	NA/100	NA/98.7	NA/98.6
MT1-JK1-R0007	99.7/100	99.1/100	99.9/99.8	99.8/99.8	99.4/99.7	98.2/99.1	99.6/99.9	99.4/99.7	98.8/99.7	97.3/99.1	99.3/99.5	98.9/99.4
MT1-MM1-R0001	100/100	100/100	99.9/99.6	100/100	99.7/100	100/100	98.5/99.8	100/100	NA/100	NA/100	NA/99.6	NA/100
MT1-TC2-R0002	100/100	100/100	100/100	100/100	100/100	100/100	99.9/99.9	100/100	100/100	100/100	99.9/99.9	100/100
MT1-WH1-R0003	100/99.4	100/99.1	99.9/99.4	100/99.4	100/100	100/100	99.9/99.9	100/100	99.7/99.4	99.1/99.1	99.5/99.4	99.7/99.4
MT1-AI1-R0001	NA	NA	NA	NA	100/100	100/100	99.7/99.6	100/99.7	NA	NA	NA	NA
MT1-PS1-R0001	99.4/NA	100/NA	99.2/NA	98.5/NA	NA	NA	NA	NA	NA	NA	NA	NA
MT1-OM1-R0001	99.7/99.7	100/100	99.6/99.7	100/99.8	100/100	100/100	99.1/99.8	100/99.7	99.4/98.5	99.1/96.3	99.2/99.2	99.7/98.7
MT1-GL1-R0004	100/99.7	100/100	99.7/99.7	100/100	99.4/100	100/100	99.5/99.7	100/100	99.1/99.4	98.2/99.1	97.8/99.5	95.6/99.8
MT1-MB1-R0004	98.8/100	98.2/100	99.2/99.5	99.7/100	98.8/99.7	100/100	97.8/99.7	96.6/100	NA/92.7	NA/87.3	NA/97.0	NA/97.1
MT1-PR1-R0002	99.1/100	100/100	99.8/99.7	100/100	100/99.7	100/100	99.9/99.6	100/100	99.1/98.5	100/100	99.6/99.2	100/100
MT1-WH1-R0003	100/99.4	100/99.1	99.9/99.4	100/99.4	99.7/100	100/100	99.7/99.7	100/100	99.1/99.4	98.6/98.6	99.6/99.0	98.8/98.3
MT1-MM2-R0001	100/99.7	100/99.1	100/99.7	100/99.7	NA	NA	NA	NA	NA	NA	NA	NA
MT1-HB1-R0003	100/100	100/100	100/100	100/100	100/100	100/100	100/100	100/100	99.7/100	100/100	99.7/99.8	99.4/99.8
MT1-EH1-R0002	100/100	100/100	99.9/99.9	99.8/100	99.7/100	100/100	99.5/99.9	99.4/100	99.1/99.7	97.3/99.1	99.4/99.7	99.4/99.8
MT1-DK1-R0002	100/100	100/100	100/99.9	100/100	100/100	100/100	100/100	100/100	99.7/100	100/100	100/100	100/100
Overall	98.6/99.8	99.5/99.7	99.6/99.6	99.6/99.6	99.4/99.7	99.2/99.4	99.5/99.7	99.3/99.7	99.4/99.1	98.9/98.6	99.3/99.2	99.1/99.1

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

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