

Phenotypic and Genotypic HIV-1 Drug Resistance and Tropism Testing Performed on Plasma Samples Stored in a Dried State

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Background: Transporting frozen HIV⁺ plasma for drug resistance and tropism testing is expensive and logistically difficult from remote study sites. Shipping costs would be dramatically reduced by transporting samples in a dried state at ambient temperature. In addition, the dried samples are no longer infectious, and can be shipped without biohazard restrictions.

Objectives: To evaluate the amplification efficiency of HIV-1 *pol* and *env* genes from plasma stored in a dried state and to determine the concordance of genotypic and phenotypic drug resistance and viral tropism data compared to conventionally frozen samples.

Methods: Plasma was obtained from 25 HIV-infected patients for *pol* amplification and 23 patients for *env* amplification. Viral loads ranged from <50 to 750,000 c/ml. Plasma from each patient was frozen and stored at -80°C and a second aliquot was dried onto a novel, non-paper collection matrix (SampleTanker®, Research Think Tank, Inc., Atlanta, GA) and stored at room temperature for up to seven days. HIV RNA was isolated and *env* and *pol* genes were amplified by RT-PCR. Phenotypic and genotypic drug resistance was determined by the Phenosense GT™ assay and viral tropism was determined by the Trofile™ co-receptor tropism assay.

Results: *Pol* amplification was successful in 24/25 frozen and 23/25 dried samples. *Env* amplification was successful in 20/23 frozen and 21/23 dried samples. Genotypic *pol* data from dried and frozen matched samples had a high degree of concordance with 98.3% sequence identity and only minor differences in relative proportions of nucleotide mixtures. Phenotypic drug susceptibility of frozen and dried samples were within 2 fold for 99% of comparisons. Frozen and dried *env* samples showed 100% concordance in tropism results

Conclusions: The amplification sensitivity of *pol* and *env* was similar in dried versus frozen plasma samples. There was high concordance of genotypic and phenotypic drug resistance results, as well as viral tropism between dried and frozen samples. This study demonstrates the feasibility of using this novel technology for drying down plasma as an effective way to reduce the financial and logistical burdens of shipping samples for drug resistance and viral tropism testing from remote and/or resource-poor study sites.

Fig 1: Plasma is dried onto a matrix and shipped at ambient temperature in a capped vial (SampleTanker®, Research Think Tank, Inc.).

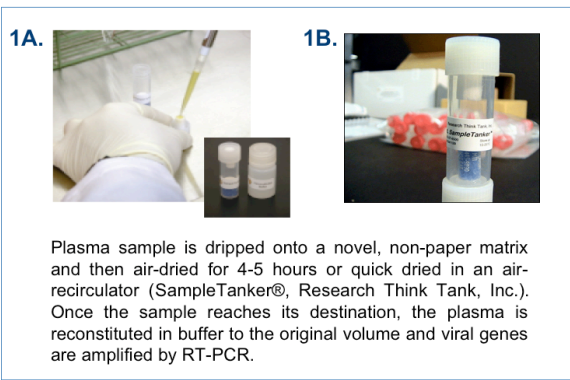


Table 1: Pol RT-PCR amplification was successful in 24/25 frozen and 23/25 dried plasma samples.

Patient ID Number	Viral Load (c/ml)	RT-PCR Band Intensity	
		Frozen Plasma	Dried Plasma
P1*	134	-	-
P2	1,102	++	+
P3	3,304	++	+++
P4	3,492	++	++
P5	6,079	+++	+++
P6	6,960	+++	+++
P7	8,600	+++	+++
P8*	10,353	+++	++
P9	14,000	++	++
P10	16,347	+++	+++
P11	20,500	+++	+++
P12	22,462	+++	+++
P13	24,363	+++	+++
P14	24,500	+++	+++
P15	30,170	+++	+++
P16	37,091	+++	-
P17	41,445	+++	+++
P18*	42,871	+++	+++
P19	49,179	+++	+++
P20*	54,731	+++	++
P21	68,574	+++	+++
P22	114,000	+++	+++
P23	136,000	N/A	+++
P24*	159,362	+++	++
P25	580,000	+++	+++

* Plasma dried immediately after collection. All other samples were dried after initially being frozen, shipped, and then thawed. N/A = matched frozen sample not available for this subject.

Fig 2: High concordance of phenotypic drug susceptibility between frozen and dried matched plasma samples.

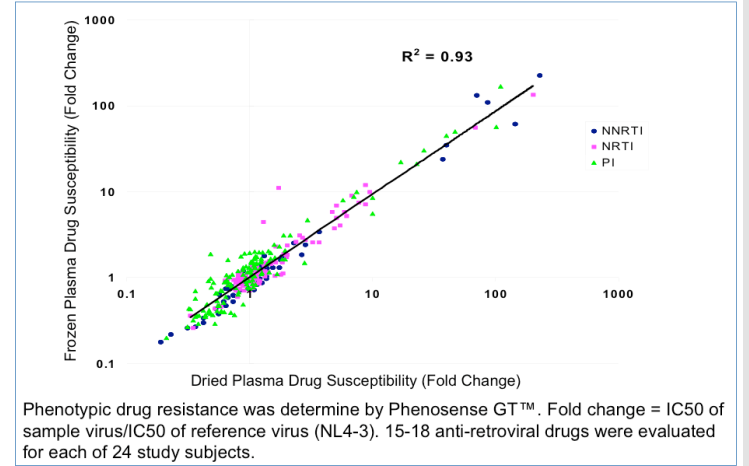


Table 2: Genotypic pol data from frozen and dried matched plasma had a high degree of concordance. The average sequence identity was 98.3% with only minor differences detected in relative proportions of nucleotide mixtures.

Patient ID	Viral Load	Plasma Source	% Seq Identity	RTI Resistance-Associated Mutations		PI Resistance-Associated Mutations	
				Frozen	Dried	Frozen	Dried
P3	3,304	Frozen	97.0	none	none	L10LW, L63AT	L10LW, L63AT
P4	3,492	Frozen	96.0	M184MV	none	H3V, G16G/E, K20L, M36L, L63P, H69K, L89M	H3V, G16G/E, K20L, M36L, L63P, H69K, L89M
P5	6,079	Frozen	99.4	M41L, M184V, T215Y	none	H3V, K20L, M36L, L63P, H69K, L89M	H3V, K20L, M36L, L63P, H69K, L89M
P6	6,960	Frozen	99.7	M41L, M184V, T215Y	none	L10L, K43T, M46L, I54V, L63P, A71T, V82T	L10L, K43T, M46L, I54V, L63P, A71T, V82T
P7	8,600	Frozen	97.5	K65R, M184V, L100I, K103N	none	L10L, K43T, M46L, I54V, L63P, A71T, V82T	L10L, K43T, M46L, I54V, L63P, A71T, V82T
P8	10,353	Frozen	98.2	none	none	M36I	M36I
P9	14,000	Frozen	97.5	none	none	H3V, L33V, L63LP	H3V, L33V, L63LP
P10	16,347	Frozen	97.6	M184V	none	L10V, M36M, Q58Q/E, V77V	L10V, M36M, Q58Q/E, V77V
P11	20,500	Frozen	98.4	M184V	none	L10V, Q58Q/E, V77V	L10V, Q58Q/E, V77V
P12	22,462	Frozen	98.9	D67N, K70R, M184V, T215F, K219Q, K103N, V106V, E138A, K238T	none	L10L, E35D, M36M, I54L, L63P, A71AT	L10L, E35D, M36M, I54L, L63P, A71AT
P13	24,363	Frozen	98.8	M41L, E44D, D67N, L74I, V75M, V118I, L210W, T215F, K219Q, K103N, V106V, E138A, K238T	none	L63Q, V77I	L63Q, V77I
P14	24,500	Frozen	98.8	M41L, E44D, D67N, L74I, V75M, V118I, L210W, T215F, K219Q, K103N, V106V, E138A, K238T	none	L10L, H3V, L63LARS, A71T, V77V	L10L, H3V, L63LARS, A71T, V77V
P15	30,170	Frozen	96.9	none	none	L10L, H3V, L63LARS, A71T, V77V	L10L, H3V, L63LARS, A71T, V77V
P17	41,445	Frozen	99.3	none	none	M36I	M36I
P18	42,871	Frozen	97.9	none	none	M36M	M36M
P19	49,179	Frozen	98.9	D67N, K70R, V118V, M184V, T215F, K219Q	none	L10V, H3V, K20T, L33F, M46L, G48V, I54L, Q58E, L63LAPV, V82A, B4V	L10V, H3V, K20T, L33F, M46L, G48V, I54L, Q58E, L63LAPV, V82A, B4V
P21	68,574	Frozen	97.3	D67N, K70R, M184V, T215F, K219Q, K103N, V106V, E138A, K238T	none	H3V, L63P	H3V, L63P
P22	114,000	Frozen	97.8	D67N, K70R, M184V, T215F, K219Q, K103N, V106V, E138A, K238T	none	L10L, L63P, V77I	L10L, L63P, V77I
P25	580,000	Frozen	99.0	M41L, D67N, K70R, L74I, V75M, V118I, M184V, L210W, T215Y, K103NS, Y181I	none	L10L, H3V, L63P, V77I	L10L, H3V, L63P, V77I
Average			98.3				

Fig 3: Phenotypic susceptibility of frozen and dried samples were within 2 fold for 99.4% of all drug comparisons.

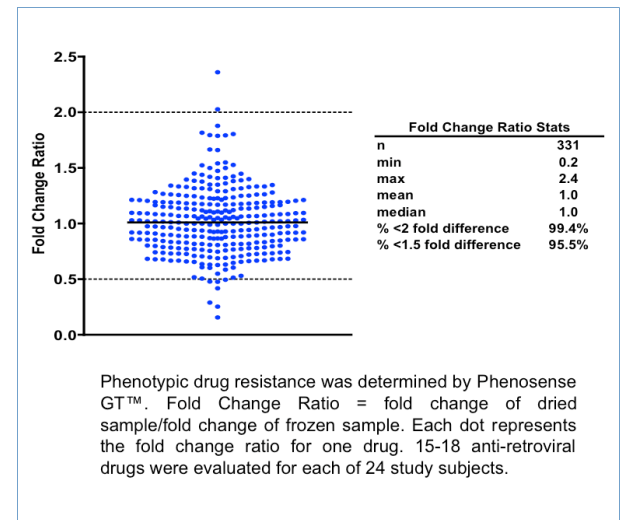


Table 3: Env RT-PCR amplification was successful in 20/23 frozen and 21/23 dried plasma samples.

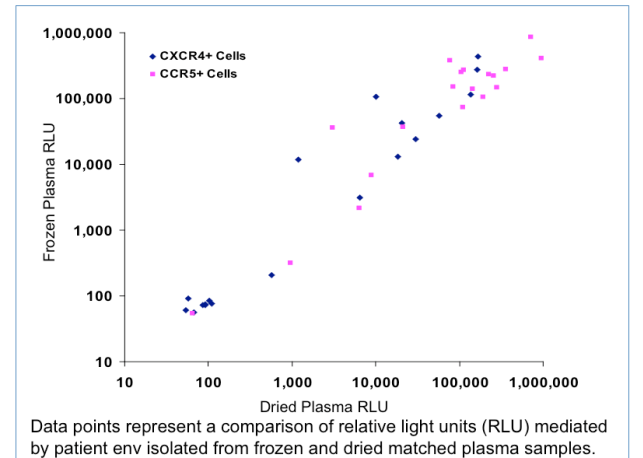
Patient ID Number	Viral Load (c/ml)	RT-PCR Band Intensity	
		Frozen Plasma	Dried Plasma
E1	< 50	+++	+++
E2*	134	-	-
E3	472	+	+
E4	833	+++	++
E5	1,269	-	-
E6	1,800	++	+
E7	3,507	+++	+++
E8	3,674	+++	+
E9	4,981	++	+++
E10	5,000	+++	++
E11	5,491	-	+
E12	7,212	++	+++
E13*	10,353	+++	+++
E14	40,000	+++	+++
E15*	42,871	+++	+++
E16	50,000	+++	+++
E17	70,000	+++	+++
E18	87,260	+++	+++
E19	130,000	+++	+++
E20	393,143	+++	+++
E21	508,011	+++	++
E22	> 700,000	+	+++
E23	750,000	+++	+++

* Plasma dried immediately after collection. All other samples were dried after initially being frozen, shipped, and then thawed.

Table 4: 100% concordance of Trofile™ coreceptor tropism calls between frozen and dried matched plasma samples.

Patient ID	Viral Load	Dried Plasma	Frozen Plasma
E3	472	R5	R5
E4	833	R5	R5
E6	1,800	DM	DM
E7	3,507	DM	DM
E8	3,674	R5	R5
E9	4,981	DM	DM
E10	5,000	DM	DM
E12	7,212	R5	R5
E13	10,353	DM	DM
E14	40,000	R5	R5
E15	42,871	DM	DM
E17	70,000	DM	DM
E18	87,260	DM	DM
E19	130,000	DM	DM
E20	393,143	DM	DM
E21	508,011	R5	R5
E22	750,000	R5	R5
E23	>700,000	X4	X4

Fig 4: Luciferase activity measurements from frozen and dried matched plasma samples



CONCLUSIONS

- Amplification sensitivity of HIV-1 *pol* and *env* genes is similar from dried and frozen plasma samples
- High concordance of phenotypic and genotypic NRTI, NNRTI, and PI drug resistance data from matched dried and frozen plasma
- 100% concordance of Trofile™ coreceptor tropism calls between dried and frozen matched plasma samples
- This study demonstrates the feasibility of using dried plasma for drug resistance and tropism testing

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