

Supplementary Material

A contaminant-free assessment of Endogenous Retroviral RNA in human plasma

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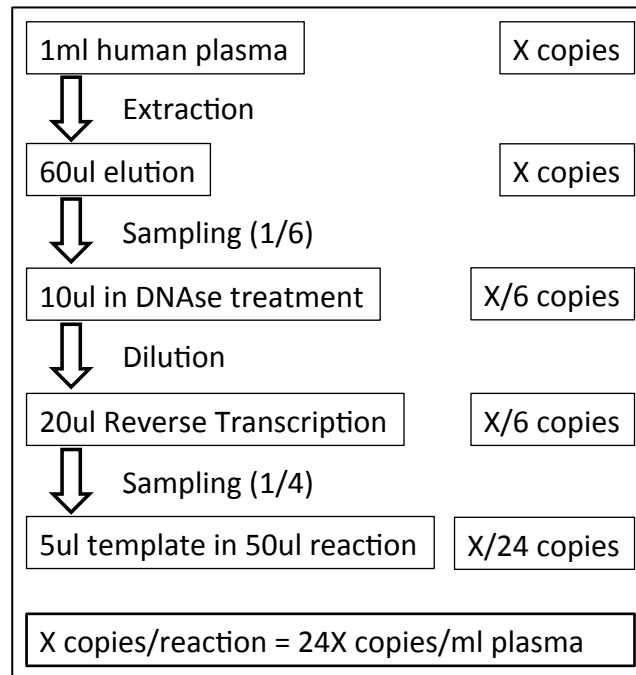
Suppl. Table 1: Sensitivity of the assay in biological material. Absolute quantification of HK2 pol in undiluted and in log10-serial-diluted NCCIT cell-supernatant in HIV plasma (spiking).

Type	Cq	Amplification	Estimated copies/reaction	Average copies/re action	Estimated*	Expected**
					copies/ml	copies/ml
NCCIT SUPERNATANT	30.86	TRUE	4397.14	4236.33	101672	
NCCIT SUPERNATANT	31.92	TRUE	2073.52			
NCCIT SUPERNATANT	30.37	TRUE	6238.33			
SUPERNAT plasmaSpike 1/10	35.10	TRUE	216.39	346.11	8307	10167
SUPERNAT plasmaSpike 1/10	34.10	TRUE	440.31			
SUPERNAT plasmaSpike 1/10	34.30	TRUE	381.62			
SUPERNAT plasmaSpike 1/100	37.19	TRUE	48.97	27.63	663	1017
SUPERNAT plasmaSpike 1/100	39.11	TRUE	12.51			
SUPERNAT plasmaSpike 1/100	38.35	TRUE	21.41			
SUPERNAT plasmaSpike 1/1000	39.64	TRUE	8.57	7.67	184	102
SUPERNAT plasmaSpike 1/1000	39.97	TRUE	6.77			
SUPERNAT plasmaSpike 1/1000		FALSE				
Standard, 10000 copies/reaction	30.23	TRUE	6863.48	10590.70		
Standard, 10000 copies/reaction	29.20	TRUE	14317.92			
Standard, 1000 copies/reaction	32.63	TRUE	1251.27	1039.42		
Standard, 1000 copies/reaction	33.21	TRUE	827.56			
Standard, 100 copies/reaction	36.61	TRUE	73.71	103.52		
Standard, 100 copies/reaction	35.78	TRUE	133.33			
Negative		FALSE				

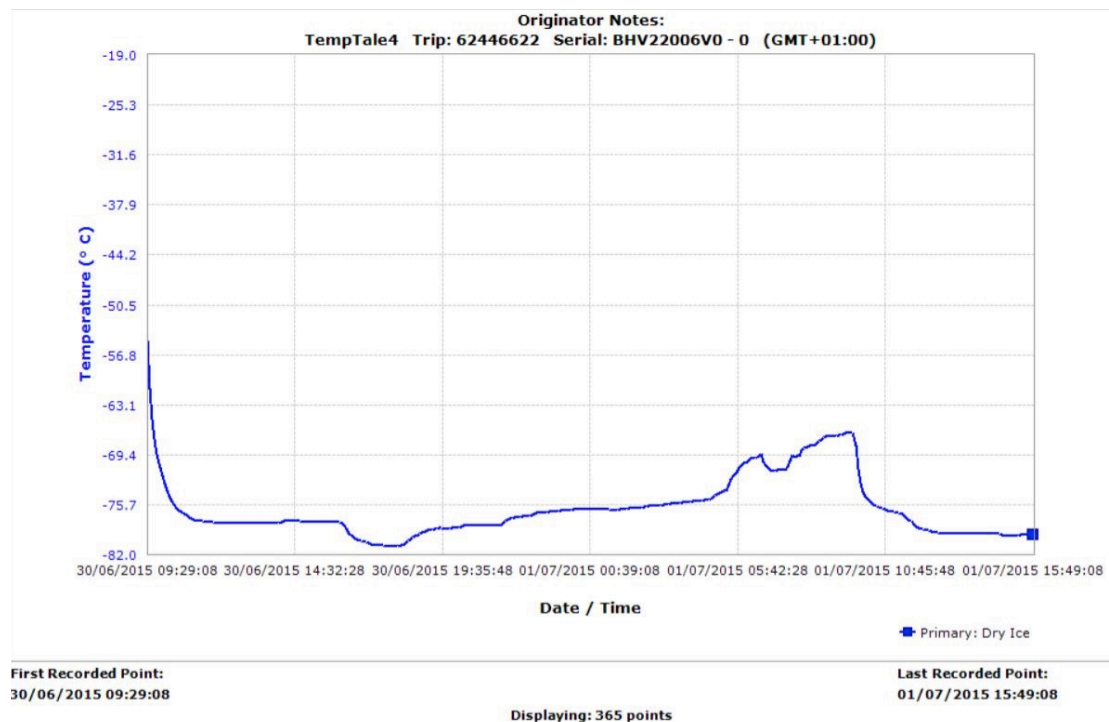
* Rounded, based on the formula presented in Suppl. Figure 1.

**Expected copies after plasma spiking

Suppl. Figure 1: Calculation of equivalence between copies/reaction and copies/ml plasma

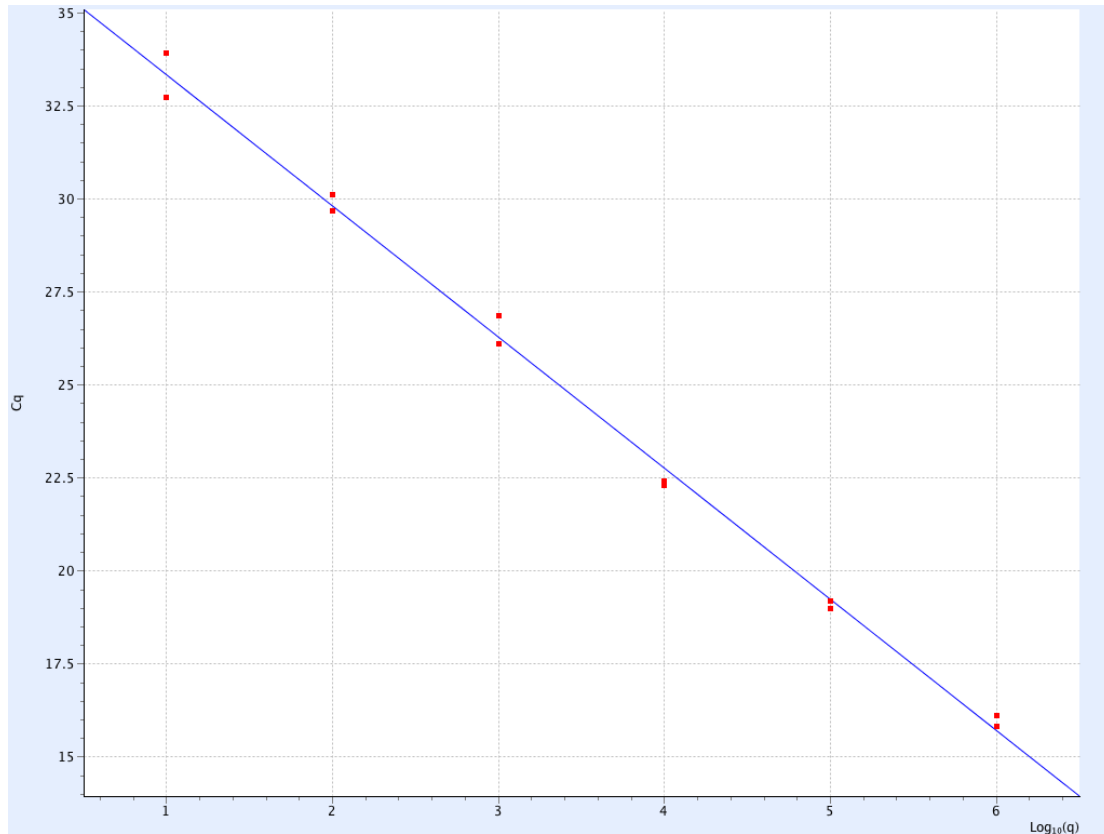


Suppl. Figure 2: Temperature log during the transport of the plasma samples from Greece to the United Kingdom. Dry ice was replenished during the shipment.



Suppl. Figure 3: SYBR-Green quantification of 14 HIV samples and comparison with pre-shipment measurements. Standards were built after absolute quantification of PCR amplicon with PicoGreen.

A) Standard Curve (E=1.922, r²=0.9961)

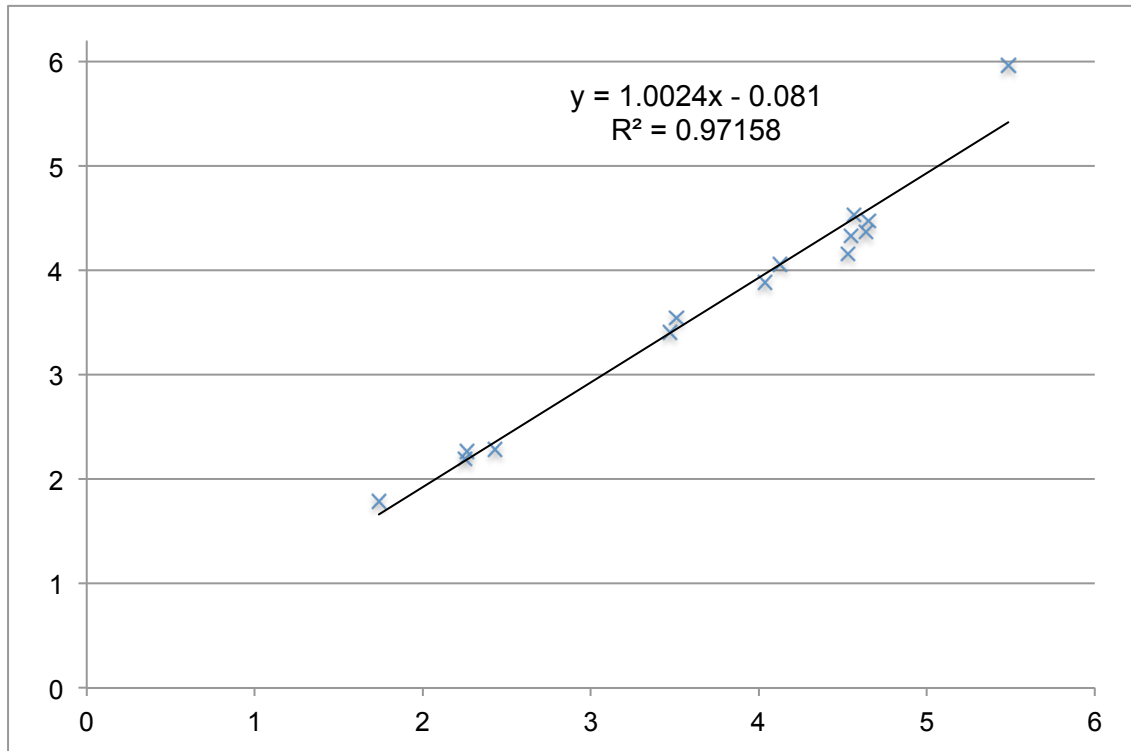


B) Pre- / post- shipment quantification

Sample	SYBR-green copies/reaction	Estimated* copies/ml	pre-shipment copies/ml
HIV29	2.55E+00	6.13E+01	5.48E+01
HIV30	1.47E+02	3.52E+03	3.23E+03
HIV31	6.45E+00	1.55E+02	1.78E+02
HIV32	8.04E+00	1.93E+02	2.70E+02
HIV33	9.73E+02	2.34E+04	4.33E+04
HIV34	3.81E+04	9.14E+05	3.06E+05
HIV35	1.42E+03	3.41E+04	3.70E+04
HIV36	3.19E+02	7.66E+03	1.08E+04
HIV37	8.91E+02	2.14E+04	3.55E+04
HIV38	1.06E+02	2.54E+03	2.96E+03
HIV39	4.71E+02	1.13E+04	1.34E+04
HIV40	1.23E+03	2.95E+04	4.49E+04
HIV41	7.65E+00	1.84E+02	1.84E+02
HIV42	6.05E+02	1.45E+04	3.38E+04

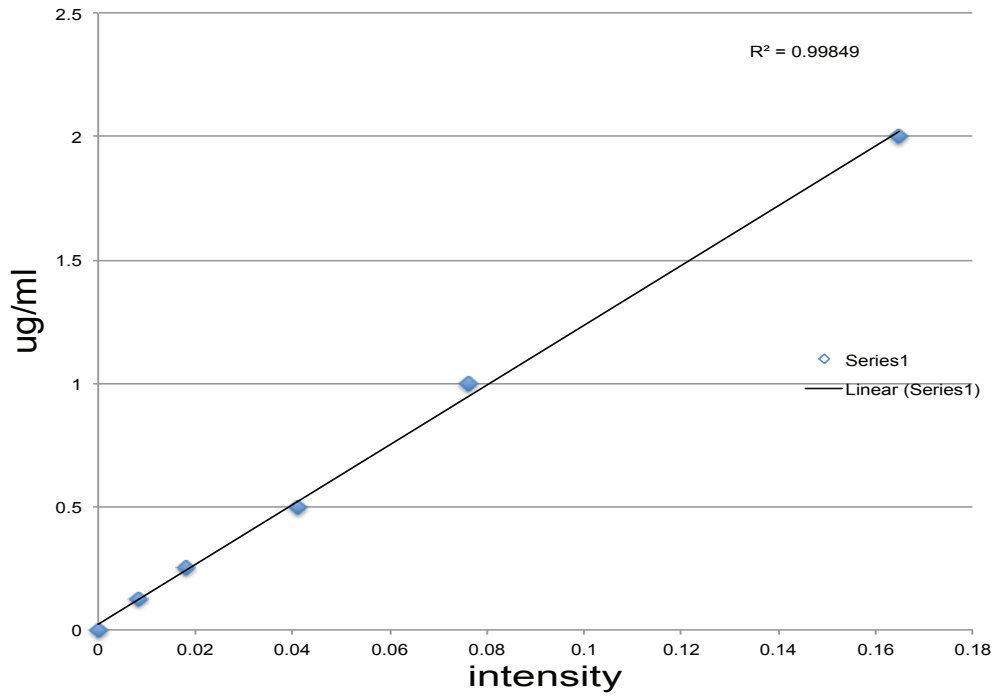
* Based on the formula presented in Suppl. Figure 1.

C) Regression line of pre- vs. post- shipment HIV viral loads (log10-transformed values). The 95% confidence intervals of the intercept and the slope were -0.5008 to 0.3388 and 0.8945 to 1.110 respectively.



Suppl. Figure 4: Absolute quantification total RNA with Ribogreen. RNA extracted from 14 HIV samples (1 ml plasma) was DNase treated and quantified using the kit-included rRNA standards.

Standard Curve



Total RNA in 14-HIV samples

