Performance of an HIV-1 DNA assay with Whole Blood samples and a Comparison of Extraction Methods

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Introduction

Since the advent of modern highly active antiretroviral therapy (HAART) for HIV-1, most patients clear circulating virus as measured by plasma HIV-1 RNA PCR testing. Once the plasma is negative for virus, little additional information is gained by HIV-1 RNA testing unless the patient stops the medications or develops drug resistance. An alternative assay which measures the amount of HIV-1 DNA present in the peripheral blood mononuclear cells (PBMC) has also been developed.

PBMCs are not an ideal sample type in the clinical laboratory, we investigated whole blood as an alternative to PBMCs and evaluated two different extraction instruments/methods to compare the eluates for HIV-1 and genomic DNA concentration and purity. Utilizing whole blood may also have an additional advantage in that both complete blood counts with automated differentials as well as CD4/8 flow cytometry data could be used to generate very precise quantities of cells per volume.

Finally, we evaluated the entire extraction/PCR assay sensitivity, reproducibility, and sample stability over time.

Methods

- The final number of samples available for complete analysis were 57 whole blood RSC, 51 whole blood EZ1, 58 PBMC RSC, and 48 PBMC EZ1 samples.
- Automated extraction was performed with the Promega RSC instrument and the Promega Blood Kit and the Qiagen EZ1 Advanced instrument an the Qiagen EZ1 Whole Blood kit.

Promega RSC

Qiagen EZ1

Figure 1

	Promega RSC	Qiagen EZ1
Input	300 µL	350 µL
Output	50 µL	100 µL
Concentration	6X	3.5X

Table 1

- HIV primer set targeting 1) HIV IR gene region and 2) human α-actin gene; supplied by Ultrabio Technologies. Each have their own standard curve. Both used in calculation of HIV ratio.
- PCR was performed according to the manufacturer's instructions on a Thermofisher QuantStudio 5.

$$HIV\ Ratio = \frac{HIV\ quantity}{Cell\ quantity} * 1x10^6$$

RSC and EZ1

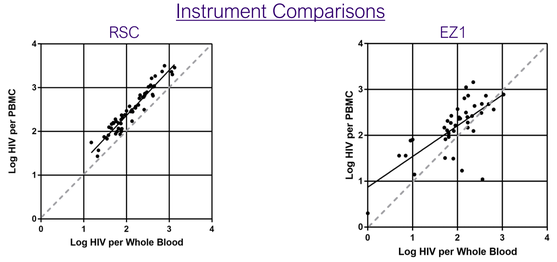
The RSC extractions consistently generated both HIV and cell DNA quantities 3-4 fold higher than the EZ1 extractions. This resulted in better sensitivity and reproducibility at lower HIV concentrations.

Table 2. Promega RSC consistently extracted 3x higher concentration of DNA

	Promega RSC Extractions				Qiagen EZ1 Extractions		
	HIV Qnt Copies/ Rxn	Cell Qnt Copies/ Rxn	HIV+ / 1e6 cells		HIV Qnt Copies/Rx	Cell Quant Copies/Rxn	HIV+ / 1e6 cells
1	26	185,787	140	1	8	39,214	212
2	46	182,357	252	2	17	48,764	349
3	20	121,948	164	3	7	52,449	126
4	27	141,681	190	4	4	48,996	90
5	117	273,966	427	5	14	100,628	143
6	73	273,349	267	6	13	83,467	156
7	4	203,806	21	7	0	75,450	0
8	397	334,064	1,188	8	90	140,250	642
9	14	86,363	162	9	5	26,987	170
10	36	134,044	269	10	15	40,596	362
11	9	123,685	75	11	1	49,590	22
SPC	17,222	193,757	88,885	SPC	1,751	87,868	19,928

Direct comparisons between the 2 instruments showed good correlation for both whole blood and PBMC for samples with HIV/cell ratios above 150. However, the EZ1 gave less accurate results for samples with ratios <150 and had several HIV detection failures at low HIV levels.

Instrument Comparisons



Sample Comparisons

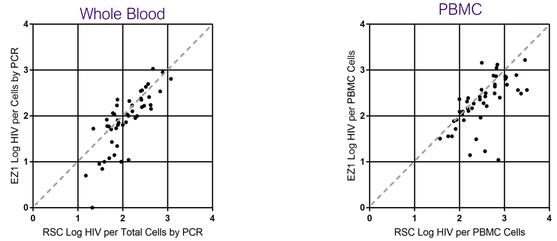
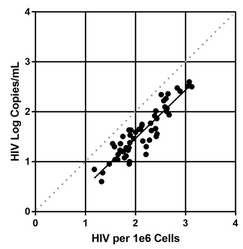


Figure 3. Log quant vs Ratio



Stability Studies

Figure 4. Four randomly selected HIV+ samples were run on day 1 for both short and long term. Aliquots were made and stored in either 4°C or frozen at -70°C.

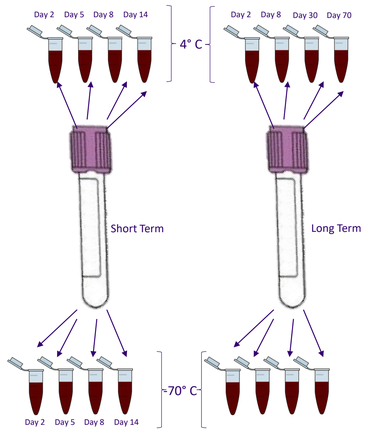
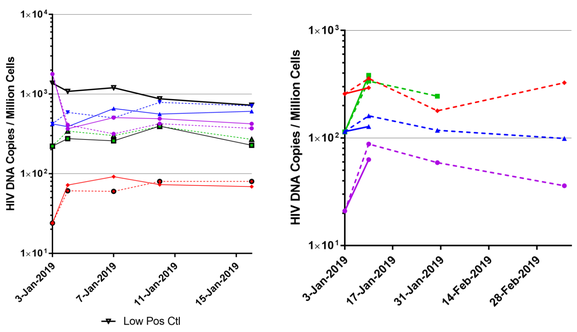


Figure 5. Ratio values pictured below. Frozen are depicted by dotted lines. Refrigerated depicted as solid lines.



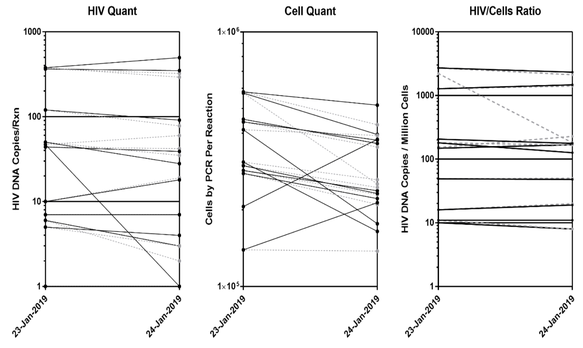


Figure 6. An additional 10 samples were tested only at day 1 and 2.

Assay Sensitivity and Reproducibility

- Serial 1:2 dilutions were made with a sample known to have a ratio of about 200.
- **Table 3** contains the results and average value obtained for each of the seven samples.

Table 3

	Theoretical	Actual HIV Quantity			Results	
Dilution	Average	Average	S.D.	C.V.	Number Tested	Number of +
Neat	200	197	31.45	15.9%	7	7
2	100	94	14.26	15.1%	7	7
4	50	38	3.70	9.7%	7	7
8	25	19	3.14	16.3%	7	7
16	12.5	7.7	1.30	17.0%	7	7
32	6.25	3.9	1.09	27.7%	7	7
64	3.13	1.60	1.29	80.7%	7	7
128	1.07	0.44	0.25	57.7%	7	5

Conclusion

- The RSC extractions consistently generated both HIV and cell DNA quantities 3-4 fold higher than the EZ1 extractions. This resulted in better sensitivity and reproducibility at lower HIV concentrations.
- This assay showed stability in 4 C for up to 14 days, and up to 70 days in -70 C.
- The sensitivity of the assay showed 3 copies/reaction or 25 copies/mL.