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 efficiencies 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 126 whole blood, 26 PBMC, 14 blood RSC, 27 whole blood EZ1, 20 PBMC RSC, and 48 blood EZ1 samples.
- Automated extraction was performed with the Promega RSC instrument and the Promega Blood Kit and the Qiagen EZ1 Advanced instrument on the Qiagen EZ1 Whole Blood kit.

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.

Stability Studies
- 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.

Conclusion
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