Improvements to the HIV-1 DNA Assay to Make a “Clinical Laboratory Friendly” and Precise Method

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BACKGROUND

Utilizing an assay to assess the level of DNA in HIV-1 infected patients is becoming increasingly important as the number of patients on HAART increases and a variety of new therapies to "cure" HIV integration are being developed.

Traditional ways of measuring HIV-1 DNA have involved isolation of peripheral blood mononuclear cells (PBMCs), manual extractions, 2 quantitative PCRs, and a final result read-out in:

HIV-1/Cel Ratio = HIV-1 Copies per 1x106 PBMCs

This ratio method requires 2 PCR reactions, one to measure the amount of HIV-1 present and one to measure the number of cells present.

We previously validated a new method to utilize whole blood samples (See our separate CVS poster), and in this study evaluated the best way to determine the quantity of cells present in the whole blood. The PCR method was compared to Hematology instrumentation (for WBC and Absolute Lymphocyte+Monocytes) and flow cytometry (CD3+, CD4+). Each method was evaluated to determine the effect on the calculated HIV-1 Copies/Cell ratio.

METHODS

Whole blood (EDTA) from 58 HIV+ patients were extracted with the Promega RSC kit and then analyzed for HIV-1 DNA and total cells with a PCR assay supplied by Ultrabio Genetics (Bothell, WA). The Sysmex XN 9000 was used to determine CBC and Diff results for all 58, while a subset of 25 samples was also evaluated for Lymphocyte subsets using a Beckman Coulter Aquios flow cytometer. Precision estimates for these two instruments were taken from company product literature.

Comparison of Methods to Estimate Cell Quantity

1) HIV-1 DNA Copies / mL

2) Ratio’s calculated from HIV-1 Copies per one of 6 different measurements of cell quantity:
   1. Per 1x106 Cells by PCR
   2. Per 1x106 PBMCs by PCR
   3. Per WBC
   4. Per Absolute Lymphocytes + Monocytes
   5. Per Absolute CD3+
   6. Per Absolute CD4+ cells

CONCLUSIONS:

1. Multiple methods to quantify the cells present worked equally well.
2. The most precise measurement based on industry data would be the WBC value. However, the CD3 and CD4 values are almost as precise and offer the possibility of higher clinical relevance.
3. Because of method bias, it would be best to stick with a single method to estimated cell quantity for serial studies.