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 readout in:

HIV-1/Cell 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.

Figure 1

Figure 2

Figure 3

Figure 1) QuantStudio 5 for PCR measurement of HIV DNA and cell number. Figure 2) Beckman Coulter Aquios for CD3+ and CD4+ absolute cell numbers. Figure 3) Sysmex XN for CBC / Diff.

METHODS

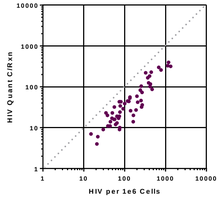
Whole blood (EDTA) from 58 HIV-1+ patients were extracted with the Promega RSC Blood kit and then analyzed for HIV-1 DNA and total cells with a PCR assay supplied by Ultrabio Technologies (Bothel, WA).

The Sysmex XN 9000 was used to determine CBC and Diff results for all 58, while a subset of 25 samples were also evaluated for Lymphocyte subsets using a Beckman Coulter Aquios flow cytometer. Precision estimates for these two instruments were taken from company product literature.

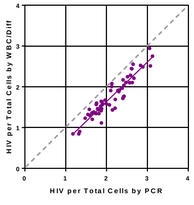
Comparison of Methods to Estimate Cell Quantity

- **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**

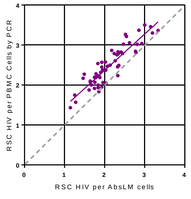
Quant vs Ratio Whole Blood



Ratios for Whole Blood and PBMCs

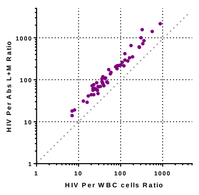


PCR vs WBC Whole Blood
Slope = 0.97
R^2 = 0.88
Bias = 0.35

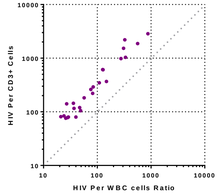


AbsL+M vs PCR PMNs
Slope = 0.91
R^2 = 0.83
Bias = 0.34

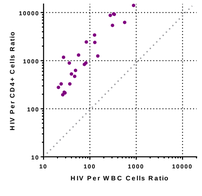
Ratios Utilizing WBC and T Cell Numbers



WBC vs AbsL+M
Slope = 1.02
R^2 = 0.97
Bias = 0.33 Log



WBC vs CD3+
Slope = 1.07
R^2 = 0.95
Bias = 0.53 Log



WBC vs CD4+
Slope = 1.15
R^2 = 0.87
Bias = 1.19 Log

RELATIVE ASSAY PRECISION

Assay Method	N=	Mean	S.D.	C.V.
PCR – High Ctl HIV c / Rxn	16	177,262	6,670	38.6%
PCR – Low Ctl HIV c / Rxn	10	467	95	20.3%
PCR – High Ctl α-actin c / Rxn	16	334,493	115,054	34.4%
PCR – Low Ctl α-actin c / Rxn	10	416,091	97,279	23.4%
Sysmex WBC # / uL	>10			<3.0%
Sysmex Abs Lymph # / uL	>10			<8.0%
Sysmex Abs Monocyte #/uL	>10			<20.0%
Aquios – High Ctl CD3 # / uL	311	831		3.3%
Aquios – Low Ctl CD3 #/uL	307	388		3.7%
Aquios – High Ctl CD4 # / uL	311	555		3.6%
Aquios – Low Ctl CD4 # / uL	307	123		5.0%

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.