

## Evaluation of a Kit-Based HIV-1 DNA PCR Protocol for Confirming Infection

<i>Abstract Category:</i>	Laboratory-based Confirmatory Algorithms Using Supplemental Western Blot, Indirect Immunofluorescence, or Nucleic Acid Amplifications Tests
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### OBJECTIVE

Evaluate 3,000 blood samples with a kit-based HIV-1 DNA PCR protocol for detecting HIV-infected cells as a supplemental test for confirmation of infection.

### METHODS

HIV-infected blood samples were obtained from CDC's Validating Supplemental Testing to Confirm Preliminary Positive Rapid HIV Tests study from persons who were 18-55 years of age, antiretroviral therapy-free for at least 3 months before blood collection, and confirmed positive for infection by both EIA (Bio-Rad 1-2+O) and Western blot. The HIV-negative samples were from blood donors screened by an HIV-1 EIA and using a pooled donor blood plasma HIV-1 RNA PCR testing algorithm. Peripheral blood mononuclear cells (PBMC) separated from whole blood samples were initially cryopreserved and then thawed, counted, aliquoted (goal of  $1 \times 10^6$  cells/pellet) and refrozen at  $-70^{\circ}\text{C}$  until analyzed using the Roche Amplicor HIV-1 DNA Test (version 1.5) per the kit protocol. The study outcomes were to determine protocol specificity and sensitivity for detecting HIV infection, and to identify factors that impact the performance of the protocol.

### RESULTS

A preliminary laboratory analysis using serial dilutions of an HIV-1-infected cell line found that the protocol consistently detected 10 infected cells in a frozen cell aliquot. An interim data analysis of 443 samples showed the protocol to have a sensitivity of 99.20% (370/373) and a specificity of 97.14% (68/70). Eight (1.8%) samples with concordant positive or negative EIA+WB and HIV-1 DNA results had total PBMC counts that were less than that desired for an aliquot ( $<1 \times 10^6$  cells) as did 1 of 3 samples that were EIA+WB positive/PCR negative and 1 of 2 samples that were EIA+WB negative/PCR positive. Significant hemolysis or red blood cell contamination was observed in 105 (24%) of 438 samples with concordant EIA+WB/HIV-1 DNA results versus 2 of 5 with discordant results; those 2 were EIA+WB negative/HIV-1 DNA positive. Additional EIA and HIV-1 RNA testing of samples with discordant results are ongoing.

### CONCLUSIONS

Preliminary findings using a kit-based HIV-1 DNA PCR protocol for confirmatory testing indicate high sensitivity but lower specificity. We are currently processing additional samples as well as collecting information on potential barriers to and facilitators of kit use.