EVALUATION OF A KIT-BASED HIV-1 DNA PCR PROTOCOL FOR CONFIRMING INFECTION

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Abstract

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

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 EIA (Bio-Rad 1-2+O) and Western blot.. The HIV-negative samples were from blood donors screened by an HIV-1 EIA and by 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 x 106 cells/pellet) and refrozen at -70oC 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 shows that the protocol consistently detected 10 infected cells in a frozen cell aliquot. An interim data analysis of 443 samples shows the protocol had a sensitivity of 99.28% (416/419) and a specificity of 98.94% (186/188). Eight (1.8%) samples with concordant EIA+WB/HIV-1 DNA results had total PBMC counts that were less than that desired for an aliquot (<1 x 106 cells) compared with 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 (40%) 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 and facilitators of kit use.

Introduction

- Evaluation of Roche HIV-1 DNA PCR kit for:
  - Specificity
  - Sensitivity
- Develop protocols for cell counting and external standards to monitor assay and user performance

Specimens

Clinical Samples:

HIV-1 positive whole blood specimens

- CDC's Validating Supplemental Testing to Confirm Preliminary Positive Rapid HIV Tests study (6 US cities)
- 18 to 55 years of age
- WB blot positive (Genetic Systems)
- ARV therapy-free for at least 3 months

HIV-1 negative whole blood specimens

- Blood donors (Memphis, TN)
- EIA negative (Abbott HIV AB HIV-1/HIV-2)
- Pooled HIV-1 RNA PCR negative (Roche Ampliscreen)

Materials & Methods

BD Biosciences CD45 monoclonal antibody and Trucount Tubes were used for cell counting by flow cytometry. The Roche Amplicor HIV-1 DNA Kit was used to detect HIV-1 DNA.

PBMCs from whole blood samples were obtained using a ficoll hypaque gradient and then cryopreserved at -125ºC. The cryopreserved PBMCs were thawed, washed and counted using a flow cytometric method (see cell counting). Counted PBMCs were aliquoted at $1x10^6$ cells/vial and stored at -70ºC until tested for HIV-1 DNA.

Cryopreserved PBMCs were thawed, resuspended in 20mL of cold wash solution (10% FBS in PBS), pelleted and resuspend in 5 mL of cold wash solution. Fifty microliters of the sample or CD Check Control (Streck Labs) was added to Trucount tubes with PerCP-CD45 monoclonal (20uL) and incubated in the dark (15 min). FACSLyse solution (300 ul, BD Biosciences) was added and PBMC counts were done using CD45+ gating to capture PBMC's with Cellquest software on FACSCalibur. Cell counts were calculated and PBMC's were aliquot at $1x10^6$ cells per aliquot.

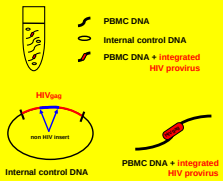
PCR controls:

HIV-1 negative: frozen aliquots of PBMC's ($1x10^6$)

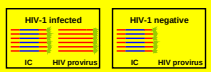
HIV-1 positive: frozen aliquots of PBMC's ($1x10^6$) spiked with 50 HIV-1 infected 8E5 cells

Assay Principles & Steps

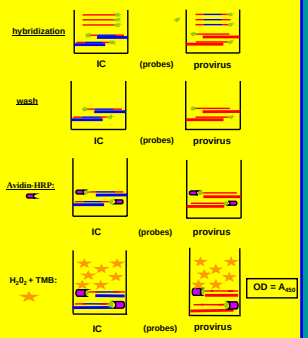
DNA extraction + internal control:



HIV-1 DNA PCR:



PCR detection (IC vs integrated provirus)



Sensitivity of the Roche HIV-1 DNA assay

Preparation of test samples:

1. Extract PBMC DNA (Roche Kit reagents)
2. Extract 8E5 DNA (1 integrated HIV-1 provirus/cell)
3. Serially dilute 8E5 DNA in PBMC DNA background
4. 6 independent PCR assays (triplicate PCR/assay/dilution)

Results:

Sample (total copies)	HIV-1 detection (%)
PBMC	0/18 (0)
HIV DNA:	
1000	18/18 (100)
100	18/18 (100)
10	18/18 (100)
1	12/18 (67)
0.1	1/18 (5)

Conclusion: The Roche Amplicor HIV-1 DNA assay is capable of consistently detecting 10 HIV-1 DNA copies/reaction.

Preparation of the External Control

1. Serially dilute 8E5 cells in background of PBMCs
2. Cryopreserve and store at -125ºC
3. Thaw and extract per kit instructions
4. Five independent PCR assays (triplicate PCR/assay/dilution)

Results:

Sample (total copies)	HIV-1 detection (%)
PBMC	0/15 (0)
HIV-1 DNA:	
1000	15/15 (100)
100	15/15 (100)
10	12/15 (80)
1	5/15 (67)
0.1	5/15 (67)

Conclusion: The positive external control was prepared to contain 50 HIV-1 infected cells per reaction mixture.

Total PBMC counts (10^7) among the tested whole blood samples

Percentile	All	Tested	HIV-1 DNA Pos.	HIV-1 DNA Neg.
95%	2.8	2.6	2.6	2.5
75%	1.7	1.7	1.7	1.7
median	1.3	1.3	1.2	1.3
25%	0.84	0.82	0.81	0.87
5%	0.26	0.27	0.27	0.25

Conclusion: There was no difference in the distribution of PBMC cell counts between HIV-1 DNA positive and negative samples.

Preliminary Results for Clinical Samples

Processed	904 (counted + aliquots)
Tested	703
PCR Positive	452
PCR negative	251
Inhibitory	0 (IC ODA450 < 0.2)

Sensitivity (PCR + / WB +)
99.28%
(416/419)

Specificity (PCR - / EIA plus pool RNA -)
98.94%
(186/188)

Summary and Conclusions

These preliminary results indicate that HIV-1 DNA testing may be applicable in a Western blot alternative confirmatory testing algorithm. We will examine samples with discordant results using other diagnostics tests in an attempt to identify their correct HIV-1 status.