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PCR as a Confirmatory Test for Human Immunodeficiency Virus Type 1 Infection in Individuals with Indeterminate Western Blot (Immunoblot) Profiles

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A combination of two PCR systems which amplify human immunodeficiency virus type 1 (HIV-1) *gag* and *pol* DNA was used to elucidate the HIV-1 infection status of a group of individuals screening positive for anti-HIV but having indeterminate Western blot (immunoblot) profiles. The results obtained suggest PCR could be used to resolve such cases.

The standard diagnostic procedure for human immunodeficiency virus type 1 (HIV-1) infection is to screen blood for specific HIV-1 antibodies by the enzyme immunoassay (EIA) and to confirm reactive specimens by the Western blot (WB) (immunoblot) test. Problems arise with a small number of individuals who present with reactive screening results but with indeterminate WB profiles. These individuals could either (i) be in the early seroconversion stage or (ii) be uninfected but give false EIA-reactive results and inconclusive WB tests because of some interfering substances in their serum.

Currently, patients with these unresolved results are monitored for weeks to months, resulting in anxiety and delay in the treatment of infected individuals.

The application of the PCR method to detect HIV-1 infection prior to seroconversion has been documented for high-risk homosexual men (13), intravenous drug users (5), and neonates born to HIV-positive mothers (12). The exquisite sensitivity of PCR in the detection of HIV-1 DNA has prompted us to study its usefulness in resolving the HIV-1 status of patients with indeterminate WB profiles.

Twenty-six samples of peripheral blood mononuclear cells (PBMC) from 23 individuals who were EIA reactive and who had indeterminate WB profiles were studied. PBMC samples from 50 individuals whose anti-HIV WB tests were confirmed to be positive and from 50 individuals who were negative by the EIA served as test controls. Each sample was tested by two PCR assays, the Amplicor HIV-1 test kit (Roche Molecular System), which amplified the HIV-1 *gag* gene, and an in-house nested PCR test targeted at the HIV-1 *pol* gene. The Abbott recombinant HIV-1/HIV-2 third-generation EIA, the Genelabs Diagnostic Western blot HIV 2.2 (Singapore), and the Virion Serofluor indirect immunofluorescent antibody assay for the detection of immunoglobulin G antibodies to HIV-1 (Cham, Switzerland) were used for HIV serology testing and confirmation. Manufacturers' recommendations were followed for all test procedures. For the in-house nested PCR, PBMC collected by gradient centrifugation were digested, using 2% sodium dodecyl sulfate and 1 mg of proteinase K per ml in phosphate-buffered saline, for 2 h at 65°C. DNA was purified with phenol-chloroform-isoamyl alcohol and precipitated with ethanol. Sample DNA (0.5 μg) was tested by nested PCR with primer pairs JA17 and JA20 and JA18 and JA19 (1). The inner primers were modified: JA18 was biotinylated, and a GCN4 tail was added to JA19 (8). The first round of PCR consisted of 30 cycles at 94°C for 30 s, 42°C for 30 s, and 75°C for 45 s, and the second round consisted of 25 amplification cycles of 94°C for 30 s, 50°C for 30 s, and 75°C for 30 s. Amplified products were analyzed by the Captagene-GCN4 enzyme-linked assay (AMRAD Corporation, Melbourne, Australia).

A complete correlation with the PCR results was obtained for HIV-1-positive and -negative samples, giving results that were 100% sensitive and specific. None of the 26 samples obtained from the 23 individuals with indeterminate WB tests was positive by either PCR method. However, HIV serology results varied among individuals (Table 1). After being monitored for various periods, one became seronegative (by EIA and WB), two remained anti-HIV positive after screening but became negative by WB testing, and five screened as nonreactive but remained indeterminate by WB. One individual was lost before follow-up. Anti-HIV screening tests and WBs remained reactive and indeterminate, respectively, for the remaining 14 individuals.

The eight individuals who were shown to have seroreverted by the screening and/or WB method were considered free from HIV infection.

Early HIV-1 seroconversion was excluded as a possibility for the 14 individuals with persistent EIA-reactive and indeterminate WB results on the basis of the following determinations. (i) Antibody profiles may vary among individuals early in seroconversion, but an individual should have seroprogressed or seroconverted after a follow-up period lasting weeks, months, or years. WB profiles remained exactly the same for 10 patients and one seroregressed, showing fewer WB bands. These individuals were therefore unlikely to be HIV-1 infected. (ii) Three individuals whose test results showed more WB bands during the follow-up period had very faint bands. These specimens gave clear-cut nonreactive results by the immunofluorescent antibody ssay and two other immunoglobulin G with immunoglobulin M HIV screening tests, the Serodia whole-virus lysate particle agglutination test (Fujirebio), and the Genelavia Mixt EIA (Sanofi Diagnostics Pasteur).

The observations noted above support the validity of the negative PCR results and, hence, reflect the true anti-HIV-negative status of these 14 individuals. In addition, we have determined that the sensitivity of the PCR assay is consistently reliable for six copies and, on occasion, that the assay can

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TABLE 1. Summary of HIV-1 serology results for 23 individuals with indeterminate WB results[a]

Sample	Anti-HIV status of patients			Length of follow-up period
	Initially by WB banding[b]	After follow-up period by:		
		EIA[c]	WB[d] (banding)	
1001[e]	gp160	R	I (gp160)	6 to 7 months
1002	p24	R	Neg	8 to 9 months
1003	p55	R	I (p55)	1 month
1004	p24	R	I (p24)	10 days
1005	p55	R	Neg	4 to 5 months
1006	p24	R	I (p24)	2 weeks
1007	p55	NR	Neg	4 months
1008	p17, p24	ND	ND	NS[f]
1009	p24	NR	I (p24, p51, p55)	1 year
1010	p17	R	I (p17)	1 month
1011	p31	NR	I (p24, p31)	2 weeks
1012	p17, p31, p51, p55	R	I (p17, p51, p55)	9 days
1013	p24, p55	R	I (p17, p24, p55, gp160)	2 to 3 months
1014	p17, p51, p55, gp160	R	I (p17, p51, p55, gp160)	1 week
1015	p55	R	I (p51, p55)	1 month
1016	p24, p51, p55	NR	I (p24)	10 days
1017	p51, p55	NR	I (p51, p55)	2 weeks
1018	p31, p51, p55, p66, gp160	NR	I (p51, p55)	2 weeks
1019	p51, p55	R	I (p51, p55)	3 months
1020	p24, p51, p55, gp160	R	I (p24, p51, p55, gp160)	14 months
1021	p51, p55, gp160	R	I (p51, p55, gp160)	2 months
1022	p17, p51, p55	R	I (p17, p51, p55)	4 weeks
1023	p51, p55, gp160	R	I (p17, p51, p55, gp160)	3 months

[a] All patients had tested negative by nested and Amplicor PCR, and all patients were initially reactive by EIA.
[b] All patients initially had indeterminate results by WB testing. For banding, p denotes protein and gp denotes glycoprotein.
[c] R, reactive; NR, nonreactive; ND, not determined.
[d] I, indeterminate; Neg, negative; ND, not determined.
[e] This individual had three PCR follow-up specimens. All three specimens were negative by PCR.
[f] NS, no follow-up specimen.

detect as little as a single copy of HIV-1 DNA (unpublished observations). This level of sensitivity minimizes the probability of false-negative results, since HIV can be recovered from PBMC early on and throughout the course of HIV-1 infection (3). Unlike PCR, sensitivity in detecting HIV infection prior to seroconversion (or in cases of EIA-negative screening) has not been proven for the indirect immunofluorescent antibody assay, a U.S.-licensed supplementary confirmation test for HIV in cases of indeterminate WBs.

Abbott EIA-reactive and indeterminate WB results could be attributed to false biological reactions such as those produced after vaccination with hepatitis B virus (9) and influenza virus (7). p55, the *gag* precursor, is also characterized as a nonviral antigen, corresponding to the HLA cellular antigen (4). In addition, antibodies to p24, gp120, and gp160 HIV antigens have been shown to cross-react with human platelets (2, 6).

This study shows the potential usefulness of PCR in elucidating the status of HIV-1 infection of individuals with indeterminate WB profiles, thus enabling an early resolution of HIV-1 status, which otherwise could take months or years to determine by serological testing. However, primer sets specific for HIV-1 may not detect infections caused by HIV-2 or an HIV-1 variant, such as the recently described subtype O (11). However, in this study, HIV-2 infections were unlikely since the WB HIV-2-specific band (gp36) was not detected in any of the samples. In addition, HIV-1 subtype O has not been reported in Asia. The use of PCR with specific primer pairs for HIV-1 subtype O and HIV-2 or with common PCR primers for both HIV-1 and HIV-2 (10) should not only resolve the indeterminate status of individuals with predominant HIV-1 infection but also detect HIV-2 and HIV-1 subtype O infections.

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