

## Rapid and Sensitive Viral Culture Method for Human Immunodeficiency Virus Type 1

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**Peripheral blood mononuclear cells from 142 consecutive patients with antibodies to human immunodeficiency virus type 1 (HIV-1) were cultured for HIV-1. All 72 patients with symptoms of HIV-1 infection were culture positive, as were 69 of 70 asymptomatic patients. Of the 142 patients, 132 (93%) were culture positive within 10 days after initiation of the culture.**

Screening and confirmatory tests for evidence of human immunodeficiency virus type 1 (HIV-1) infection are currently based on detection of antibodies to HIV-1 in serum or plasma. The presence of antibodies to HIV-1 presumably indicates current HIV-1 infection. However, rates of HIV-1 isolation from peripheral blood mononuclear cells (PBMC) of antibody-positive persons by cell culture techniques in conjunction with reverse transcriptase or HIV-1 antigen assays have been reported to range in some studies from only 30 to 89% (1, 3, 6, 10, 12). Our first reported HIV-1 isolation rate of 57% in patients with acquired immunodeficiency syndrome (AIDS) at the University of Minnesota was similar (5). However, after modifying our former culture method as described below, we were able to isolate HIV-1 from 99% of HIV-1 antibody-positive individuals. Moreover, HIV-1 was detected significantly sooner in symptomatic HIV-1 antibody-positive patients than in asymptomatic patients.

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PBMC of 142 consecutive HIV-1 antibody-positive patients were cultured (57 HIV-1 antibody-positive AIDS patients, 15 HIV-1 antibody-positive patients with AIDS-related complex [ARC], and 70 HIV-1 antibody-positive asymptomatic patients). With the exception of two women, all subjects were adult homosexual males who were referred to the AIDS Clinical Trials Unit at the University of Minnesota. Of the 142 patients, 85 were subsequently enrolled in one of five AIDS Clinical Trials Unit treatment protocols. All patients had Western blot (immunoblot)-positive HIV-1 serum antibody tests. At the time of venipuncture, none of the patients was taking drugs that had anti-HIV-1 activity. Blood samples from 30 HIV-1 antibody-negative blood donors with no recognized risk factors for AIDS were also cultured.

PBMC were obtained from the buffy coats of whole-blood donors negative for HIV-1 antibodies. Each buffy coat was diluted 1:3 with sterile phosphate-buffered saline (pH 7.3 at 24°C) within 8 h of donation. Thirty milliliters of diluted buffy coat was then layered over 15 ml of sterile Ficoll-Paque (Pharmacia, Inc., Piscataway, N.J.) and centrifuged at 350 ×

g for 30 to 45 min at room temperature. The layer containing the PBMC was removed and washed twice in sterile phosphate-buffered saline. Pelleted cells were suspended and pooled in stimulation medium (fresh RPMI 1640 medium [GIBCO Laboratories, Grand Island, N.Y.] containing 20% heat-inactivated fetal bovine serum [GIBCO], 2 mM glutamine, 5 µg of Polybrene [Sigma Chemical Co., St. Louis, Mo.] per ml, 200 U of penicillin per ml, 200 µg of streptomycin per ml, and 4 µg of phytohemagglutinin-P [Sigma] per ml) and placed in upright 275-ml tissue culture flasks at a concentration of 10<sup>6</sup> cells per ml. After 2 to 4 days in culture at 37°C in a 5% CO<sub>2</sub> atmosphere, the supernatant above the settled cells was removed to bring the volume to one-fourth that of the original. Unwashed samples of 5 × 10<sup>6</sup> or 3 × 10<sup>6</sup> of these donor PBMC were then used to feed cultures of PBMC from patients. Donor PBMC older than 4 days were discarded.

For separation of PBMC obtained from patients, 20 to 30 ml of heparinized blood was diluted 1:3 with sterile phosphate-buffered saline within 24 h of collection. Thirty-milliliter portions of diluted blood were then layered over 15 ml of sterile Ficoll-Paque and centrifuged at 350 × g for 30 to 45 min at room temperature. The layer containing the PBMC was removed and washed twice in sterile phosphate-buffered saline. Pelleted cells were suspended and pooled in 10 ml of T-cell growth factor medium (fresh RPMI 1640 medium containing 20% heat-inactivated fetal bovine serum, 5% interleukin-2 [Cellular Products, Buffalo, N.Y.], 2 mM glutamine, 5 µg of Polybrene per ml, 200 U of penicillin per ml, and 200 µg of streptomycin per ml). A cell suspension volume equal to 10<sup>7</sup> PBMC from patients was placed in an upright 50-ml flask along with 5 × 10<sup>6</sup> phytohemagglutinin-P-stimulated donor PBMC. The final cell suspension volume was diluted to 15 ml with T-cell growth factor medium. These cocultures were incubated in at 37°C in a 5% CO<sub>2</sub> atmosphere for as long as 28 days. Approximately 7 ml of culture medium above the settled cells was removed every 3 to 4 days for HIV-1 antigen detection and replaced with an equal volume of fresh T-cell growth factor medium. An additional 3 × 10<sup>6</sup> phytohemagglutinin-P-stimulated donor PBMC were added every 7 days.

Culture supernatant fluids were tested for the presence of HIV antigen by using an enzyme-linked immunosorbent assay produced by Abbott Laboratories, North Chicago, Ill.

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TABLE 1. Proportion of positive HIV-1 cultures from antibody-positive patients by day in coculture

Group (no. of patients)	No. (%) positive on culture day:					
	3	7	10	14	21	28
AIDS (57)	34 (60)	55 (96)	56 (98)	56 (98)	57 (100)	
ARC (15)	9 (60)	13 (87)	14 (93)	15 (100)		
Asymptomatic (70)	13 (19)	48 (68)	62 (89)	64 (91)	66 (94)	69 (99)

This assay primarily detects the p24 core antigen of HIV-1 and has a reported lower limit of detection of 50 to 100 pg/ml (4). Culture supernatant fluids were removed every 3 to 4 days and frozen in 1-ml aliquots at  $-20^{\circ}\text{C}$ . Specimens were thawed within 1 month of collection and tested in accordance with manufacturer directions. A culture was considered to be positive for HIV-1 antigen if two serial supernatant samplings were positive, with the later sampling showing greater reactivity. Abbott Laboratories claims that its assay is specific for HIV-1 antigens and that it does not cross-react with human T-cell lymphotropic virus type 1, cytomegalovirus, Epstein-Barr virus, herpes simplex virus, hepatitis B virus, or PBMC from HIV-1 antibody-negative individuals (2, 8). Culture supernatant fluids from known HIV-1-positive and -negative cell cultures were included in every assay as positive and negative controls.

The Wilcoxon rank sum test was used to determine the significance of median times to positivity of cultures of PBMC from symptomatic and asymptomatic groups of patients. Significant results are reported as two tailed.

HIV-1 was isolated from the PBMC of 141 (99.3%) of 142 HIV-1 antibody-positive patients (all 57 AIDS patients, all 15 ARC patients, and 69 [98.6%] of 70 asymptomatic HIV-1 antibody-positive patients). The cultures of 60% of the AIDS and ARC patients were positive by day 3 of culture compared with 19% of those of asymptomatic patients (Table 1). The cultures of all AIDS and ARC patients were positive by day 21, compared with 94% of those of the asymptomatic group. The median time to positive culture of symptomatic patients was significantly less than that of asymptomatic patients (less than 3.0 days versus 4 to 7 days, respectively;  $P < 0.001$ ; Wilcoxon rank sum test). None of the 30 HIV-1 antibody-negative blood donors had positive cultures.

Possible factors for the improved sensitivity of our culture assay may include one or all of the following modifications.

(i) A reverse transcriptase assay was replaced with the Abbott Laboratories HIV-1 antigen detection assay. The Abbott system has been reported to be at least 100-fold more sensitive than reverse transcriptase assays by serial dilution studies of culture supernatant (2, 7). (ii) Amphotericin B was eliminated from the cell culture medium. Amphotericin B has been reported to inhibit expression of HIV-1 virus protein antigens (11). In our laboratory, we found amphotericin B to delay the time to positivity by 4 to 10 days at a concentration of 2.5  $\mu\text{g/ml}$ . (iii) Donor PBMC that were phytohemagglutinin-P stimulated for 2 to 4 days rather than 3 to 14 days before use were added weekly.

Because we made all three modifications simultaneously, we cannot be certain what combination of changes was responsible for the markedly improved results. Nevertheless, our culture results verified that the presence of HIV-1 antibodies indicated persistent HIV-1 infection in both symptomatic and asymptomatic individuals, not just past exposure to HIV-1. Moreover, the shorter time to positivity of cultures of PBMC from HIV-1-infected symptomatic versus asymptomatic patients suggested that ill patients

either have a greater viral load or more efficiently replicating strains of HIV-1.

In terms of practical application, this sensitive culture assay may be useful for confirming HIV-1 infection in individuals with indeterminate Western blots or in neonates with passively transferred maternal HIV-1 antibodies. Cultures of HIV-1 antibody-negative individuals with known sexual exposure to HIV-1-infected partners may be warranted in view of a recent study, which reported that the period of latent HIV-1 infection preceding overt seroconversion was 6 to 18 months in certain patients (9). By allowing monitoring of time to positivity, this culture technique could be helpful in evaluating the antiviral effects of drugs.

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