Rapid Culture and Quantitation of Human Immunodeficiency Virus Type 1 from Patient Cells without the Use of Mitogen-Stimulated Donor Cells

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We report the development of a rapid, sensitive virus culture method for direct quantitation of human immunodeficiency virus (HIV) in peripheral blood mononuclear cells (PBMCs). This new method involves culturing 10^7 PBMCs from HIV-seropositive persons in 10 ml of medium containing phorbol 12-myristate 13-acetate and interleukin-2. Both agents stimulate cell activation and hence viral replication. Cell-associated virus and free virus are quantitated by a commercially available HIV p24 antigen capture enzyme immunoassay. Detection of cell-associated p24 antigen by flow cytometry was less sensitive than by the enzyme immunoassay. In this preliminary study, HIV was detected in 20 of 23 HIV-seropositive patients and in none of the 11 HIV seronegative low-risk individuals. One HIV-seronegative person with Guillain-Barré syndrome following high-risk activity was found to be rapid-HIV-culture positive. The overall sensitivity and specificity were 87% and 100%, respectively. By comparing the quantity of virus produced in infected cells with the amount of virus produced in chronically infected U1 monocyes and ACH-2 lymphocytes stimulated with phorbol 12-myristate 13-acetate and interleukin-2, the approximate number of infected cells per sample is calculated.

In the same patient specimens, quantitation of the number of HIV infected cells by the HIV rapid-culture method correlated with the results of the 21-day cell dilution coculture assay (correlation coefficient r = 0.5; 95% confidence interval, 0.07 to 0.77). Advantages of the rapid HIV culture include no requirement for donor PBMCs or change of media, shortened culture time, and the ability to detect p24 viral antigen from cell-associated virus for quantitation of viral load.

Surrogate markers are currently used for studies of human immunodeficiency virus (HIV) pathogenesis and transmission. The absolute CD4^+ helper T-cell count is presently the best surrogate marker for staging and progression of HIV infection and AIDS (4). Other markers used include the CD4/CD8 ratio, β2-microglobulin, neopterin, and interferon levels (7, 11). However, these assays do not directly monitor the virus (7, 11, 31, 34). Estimation of the patient's viral load has been suggested as a more direct approach and may be a better predictor of disease (11, 51). Studies correlating viral load with HIV transmission or response to antiretroviral therapy in large numbers of patients have not been published.

Present methods of assessing viral load measure the amount of HIV-1 p24 antigen in serum, plasma, or cells; the number of peripheral blood mononuclear cells (PBMCs) expressing HIV in culture; the number of virions cultured per volume of plasma; the number of copies of viral RNA in plasma; or the amount of viral RNA or DNA in peripheral blood monocytes or lymphocytes (5, 7, 12, 15, 21, 28, 29, 32, 33, 38, 43). HIV gene amplification by PCR is sensitive but highly susceptible to cross-contamination of amplified products, which makes PCR less clinically applicable (7, 26, 52). The viral load determined from quantitative culture of PBMCs or plasma parallels the stage of HIV disease and is used for studies of antiretroviral therapy (12, 21). Quantitative viral coculture requires HIV-seronegative donor PBMCs and takes 21 or more days (but may be completed in as little as 14 days). The frequent exchange of culture medium, every 3 to 4 days, is labor intensive and increases the possibility of microbial contamination, which makes the standard coculture less clinically applicable. Because of the need for a rapid, sensitive method to detect HIV and assess viral load, an alternative approach was taken. We report the development of a simple viral culture assay for detection and quantitation of HIV that requires no donor PBMCs and can be completed in 7 days or less.

MATERIALS AND METHODS

Materials, reagents, and cell lines. The following materials and reagents were used: RPMI 1640, penicillin/streptomycin, L-glutamine, Dulbecco's phosphate-buffered saline (PBS), and fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.); N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES) buffer solution (Whittaker Bioproducts, Inc., Walkersville, Md.); 50-ml conical centrifuge tubes and interleukin-2 (IL-2) (Becton Dickinson, Bedford, Mass.); phytohemagglutinin (PHA), Ficol, Polybrene, and phorbol 12-myristate 13-acetate (PMA) (Sigma Chemical Co., St. Louis, Mo.); 76% Hypaque (Winthrop Pharmaceutical, New York, N.Y.); 25-cm^2 tissue culture flasks and 24-well tissue culture plates (Corning Glass Works, Corning, N.Y.); 0.9% NaCl solution and sterile water (Abbott Laboratories, North Chicago, Ill.); HIV-1 p24 antigen detection kit and neutralization assay (Coulter Corp., Hialeah, Fla.); and EL340 automated microplate reader (Bio-Tek Instruments, Inc., Winooski, Vt.).

The chronically HIV-infected cell lines used in this study were obtained from Thomas Folks, Centers for Disease Control and Prevention, Atlanta, Ga.; they consist of a monocyte cell line (U1) and a T-cell clone (ACH-2) (16–18, 42). The parent cell lines, U937 and A3.01 (not infected with HIV),
from which U1 and ACH-2, respectively, were derived (16–18) were obtained from American Type Culture Collection, Rockville, Md., and Thomas Frohlich. They were cultured in complete medium at 37°C under 5% CO₂. HIV p24 antigen production in U1 and ACH-2 cells is enhanced by PMA (18, 27, 42).

Isolation of PBMCs from HIV-seropositive and -seronegative persons. A 15-ml sample of heparinized (50 U/ml) peripheral blood was collected by venipuncture from each of 24 patients (23 of whom were HIV seropositive) from the wards and Center for Special Studies of The New York Hospital. Of the 23 HIV-seropositive patients, 22 were adults (54% were men) and 1 was a boy (patient 23) infected by vertical transmission; 18 of the 23 patients were symptom free, and 5 had AIDS-defining illness (patients 1, 2, 3, 23, and 24). One HIV-seronegative patient (patient 9) presented with Guillain-Barré syndrome following high-risk homosexual activity and had mild pleocytosis on cerebrospinal fluid examination. To determine the specificity of the rapid HIV culture assay, blood samples from HIV-seronegative healthy volunteers or donors from the New York Blood Center were collected as heparinized peripheral blood or as citrate-leukocyte-rich buffy coats, respectively. Each sample of PBMCs was isolated within 24 h by 9% Ficoll–33.9% Hypaque gradient centrifugation (23), washed twice with PBS, and resuspended in complete medium containing RPMI 1640, 10% fetal bovine serum, 2 mM l-glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 25 mM HEPES. HIV serology and flow cytometry for T-cell subset quantitations and participates in national and Centers for Disease Control and Prevention-sponsored proficiency testing.

Detection of HIV p24 antigen in rapid HIV culture and standard 21-day quantitative coculture by EIA and neutralization assay. HIV p24 viral antigen in the rapid HIV culture and viral cocultures was measured by a commercial solid-phase enzyme immunoassay (EIA) (Coulter). A 200-μl sample was considered reactive for p24 antigen when the A₄₅₀ detected by an EL340 microplate reader was greater than the cutoff value (0.55 + absorbance of the mean negative control). By using a p24 antigen neutralization kit (Coulter), these samples were confirmed positive in a subsequent EIA if antibodies in a neutralization reagent prevented binding of sample p24 antigen to mononuclear antibodies in the sample well. A standard curve was prepared with the use of the HIV p24 antigen kit. Standards ranging from 7.8 to 250 pg of p24 antigen per ml were used in the EIA to estimate the concentration of p24 antigen in experimental samples.

Rapid HIV culture and isolation of p24 antigen. In a 50-ml centrifuge tube, PBMCs from HIV-seropositive patients were suspended in 10 ml of complete medium containing 10% IL-2 (20 U/ml), 50 ng of PMA per ml, and 2 μg of Polybrene per ml. Loosely capped tubes were incubated at a 45° angle at 37°C under 5% CO₂. The rapid HIV assay for seven patients was compared after 1 and 7 days. Of the seven rapid cultures positive at 7 days, two were positive after 1 day of culture. In addition, preliminary experiments in which whole blood was stimulated with PHA for 3 to 7 days resulted in significant cell death. After performing preliminary trials with various time points (1, 7 days), 7 days was chosen as a time point which most probably would (i) result in an acceptable sensitivity, (ii) reduce the number of days needed to perform the culture relative to the standard coculture method, and (iii) minimize cell death because of the lack of medium replacement. After 7 days, cells were centrifuged at 175 × g for 5 min; the supernatant containing released HIV p24 antigen was removed and saved.

To the cell pellet was added 900 μl of fresh medium, and the suspended cells were transferred to a 1.5-ml Eppendorf tube. To lyse and release intracellular p24 antigen from the cells, 10 μl of 20% Triton X-100 was added and the cell suspension was incubated at room temperature for 15 min. The samples were frozen in a −70°C dry ice-ethanol bath and thawed in a 37°C water bath three times at 3 min per cycle. Samples were centrifuged in a microcentrifuge at 7,000 × g for 5 min, and the supernatants containing intracellular p24 antigen were transferred to fresh Eppendorf tubes. Samples containing released or intracellular p24 antigens were stored at −70°C for subsequent p24 assays.

Released (supernatant) and intracellular (cell pellet) p24 antigens were assayed separately by commercial EIA. A rapid viral culture is positive if either the supernatant or cell pellet was reactive in the first EIA and is neutralized by ≥50% in the second EIA. HIV p24 antigen in the supernatant and cell pellet, expressed as picograms per milliliter, is obtained from the standard curve for each assay. The rapid-culture results are expressed as picograms per 10⁶ cells to allow quantitation of the number of HIV-infected patient cells when compared with chronically infected cell lines. To derive the amount of p24 antigen produced by 10⁶ PBMCs, the following formula was used: p24 antigen per 10⁶ PBMC = [(p24 antigen in cell pellet) + (p24 antigen in supernatant) × 10³]/10, where p24 antigen in cell pellet is the total p24 in 10⁷ PBMC and p24 antigen in supernatant is the amount of p24 antigen detected in 1 ml of supernatant (and since 10⁷ cells were cultured in 10 ml of medium, this value was multiplied by 10). The numerator is divided by 10 to adjust for p24 antigen per 10⁶ PBMC.

Standard 21-day quantitative viral coculture assay. PBMCs from HIV-seronegative donors were suspended in complete media at 3 × 10⁶ to 5 × 10⁶ cells per ml and stimulated with PHA (2 μg/ml) for 24 h at 37°C under 5% CO₂. For quantitation of cell-associated HIV, 2 × 10⁶ PHA-stimulated donor PBMCs were cocultured with 10-fold dilutions of patient PBMCs (2 × 10⁶ to 2 × 10⁴) in 1.5 ml of medium containing 10% IL-2 at 37°C under 5% CO₂ in 24-well tissue culture plates (21). After 24 h and then every 3 days (for 21 days), half of the supernatant was removed, saved, and replaced with fresh medium containing 10% IL-2. The presence of HIV antigen in culture supernatants was determined by commercial HIV antigen capture EIA (Coulter Corp.). A positive coculture was each dilution was maintained. The EIA was used to quantify the p24 antigen values of either (i) ≥30 pg/ml and a later harvest value of at least four times the first value or (ii) two consecutive harvest p24 antigen values of ≥250 pg/ml.

Quantitation of HIV cell infection by using HIV chronically infected cell lines. U1 and ACH-2 cells were used to create a standard curve for the quantitation of HIV infection by rapid HIV culture. Cells chronically infected with HIV were maintained in continuous culture for no more than 3 months. U1 and ACH-2 cells were washed twice in PBS, twofold dilutions (1,600, 800, 400, 200, 100, 50, 25, and 0 cells) were made, and each dilution was cultured with 10⁶ of the respective parent cells in 10 ml of medium containing 20 U of IL-2 (10%) per ml and 50 ng of PMA per ml at 37°C under 5% CO₂ as described for the rapid-culture assay. Cells pelleted by centrifugation at 175 × g for 5 min were separated from the supernatant. Pelleted cells resuspended in 900 μl of fresh medium were lysed as previously described. Cell-free and cell-associated p24 antigen was assayed as described above.

Flow cytometry to assess the level of intracellular p24 antigen. Isolated PBMCs or cells chronically infected with HIV
were fixed in 20 μg lysolceithin per ml-1% parafomaldehyde for 2 min, cold 100% methanol for 15 min on ice, and 0.1% Nonidet P-40 for 5 min on ice. No wash es were performed between fixation steps. Fixed cells were stained with anti-p24 antigen phycocerythrin-labeled monoclonal antibodies (KC57-RD1; Coulter) for 15 min and washed twice with PBS. The samples were analyzed for the amount of red fluorescence per cell on an EPICS Profile II (Coulter) flow cytometer.

**Statistical analysis.** Regression analysis was performed with the EPI-INFO version 5.1 statistical analysis package.

## RESULTS

**Sensitivity and specificity of the HIV rapid-culture assay.**

The HIV rapid culture was performed on blood samples from 23 HIV seropositive individuals, on 11 HIV seronegative individuals at low-risk for HIV, and on 1 HIV-seronegative patient who was participating in high-risk homosexual activities and was found to have developed Guillain-Barré syndrome with low-level pleocytosis on examination of the cerebrospinal fluid. Of the 23 HIV-seropositive patients, 22 were adults (54% were men) and one was a boy infected by vertical transmission of HIV. Seven had CD4+ T-cell counts below 200/μl, five had clinical AIDS, and the remaining patients were symptom free with HIV infection.

Of the 23 HIV-seropositive persons, 22 had p24 antigen detected by rapid culture in either or both the supernatant and cell-associated fractions (Table 1); 20 (87%) of the 23 were confirmed by neutralization assay and were considered positive. Patient 10 was negative in the rapid culture because the p24 antigen EIA was nonreactive. For patients 15 and 19, the first p24 antigen EIA of the rapid culture was reactive but the second EIA showed <50% neutralization; the culture was therefore considered negative (Table 1). To test the specificity of the rapid HIV assay, 11 HIV-seronegative low-risk persons were used as negative controls. Three of these individuals had optical density units that were slightly above background in the first EIA (negative control of the kit), and all 11 were negative by the second EIA. The rapid culture was positive in the HIV-seronegative patient who, after high-risk sexual activity, presented with Guillain-Barré syndrome and mild pleocytosis in the spinal fluid. In this patient the detection of p24 antigen was neutralized in the second EIA by the competing anti-p24 monoclonal antibody. Overall, the sensitivity of the assay in HIV seropositive persons is 87% (20 of 23) and the specificity in HIV-seronegative low-risk persons is 100% (11 of 11).

Excluding the HIV-seronegative patient with Guillain-Barré syndrome from the analysis, the positive predictive value is 100% (20 of 20) and the negative predictive value is 78% (11 of 14).

**HIV p24 antigen production by known numbers of cells chronically infected with HIV.** U1 monocytic cells and ACH-2 T cells were used to reflect levels of HIV infection in monocytes and T cells. The amounts of p24 antigen produced by known numbers of these chronically HIV-infected cells were used to generate a standard curve from which the number of HIV-infected patient PBMCs was determined. The production of p24 antigen by U1 monocytic and ACH-2 T cells cultured with 10^6 parent cells not infected with HIV is illustrated in Fig. 1. HIV p24 antigen is not detected in U1 or ACH-2 cells cultured for up to 72 h in medium alone at cell concentrations below 10^4 per well in 24-well plates (data not shown). In response to PMA and IL-2, a linear relationship between the number of infected U1 or ACH-2 cells and p24 antigen production was observed (Fig. 1). ACH-2 cells produced slightly larger amounts of p24 antigen than U1 cells did when larger numbers of cells were infected with HIV but produced comparable amounts at cell counts of 400 or fewer infected cells per 10^6 cells. Since peripheral blood contains similar numbers of CD4+ T cells and monocytes, and since both cell types harbor HIV, a standard curve of the mean of the p24 antigen produced by both cells can be constructed.

**Comparison between rapid HIV culture and 21-day coculture assays.** Of the 23 HIV-seropositive persons evaluated by the HIV rapid-culture assay, 19 had sufficient cells left for comparison with the results of the 21-day coculture assay. All HIV-seropositive persons assayed by the 21-day coculture method gave positive results. In the HIV-seronegative patient with Guillain-Barré syndrome, the rapid HIV culture was positive but the 21-day coculture was negative.

Many of the assayed patients were receiving antiretroviral therapy and chemoprophylaxis for opportunistic infections, and 5 of the 23 HIV-seropositive patients had clinical AIDS-defining illness. Viral load, as defined by the tissue culture infective dose (TCID) per 10^7 PBMCs, correlated poorly with the absolute CD4+ T-cell numbers. The Pearson correlation

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<th>Patient*</th>
<th>Level of HIV p24 Ag&lt;sup&gt;a&lt;/sup&gt; (pg/10^6 cells) in RHC&lt;sup&gt;b&lt;/sup&gt;</th>
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* Patients 1, 2, 3, 23, and 24 had AIDS-defining illness; patient 23 was the only child tested. Patient 9 had Guillain-Barré syndrome and was HIV seronegative. Patients 10, 15, and 19 were rapid HIV culture negative. Patient 10 was nonreactive to p24 antigen EIA; patients 15 and 19 had positive EIA for p24 antigen but did not show neutralization by ≥50% on a second EIA.

* Abbreviations: Ag, antigen; RHC, rapid HIV culture; ND, not done because of insufficient cells; NEG, negative; NA, not available.

* HIV-p24 antigen in the rapid culture is expressed as picograms per 10^6 cells and is the sum of released and intracellular fractions. Of the 20 positive rapid HIV cultures, 3 (15%) were positive only in the cell-associated fraction and the remainder were positive in both cell-associated and supernatant fractions.

* For the rapid HIV culture, TCID<sub>50</sub> was established by using either standard curve A, B, or C illustrated in Fig. 1. For the 21-day coculture, 10-fold dilutions of patient PBMCs were cocultured with PHA-stimulated HIV-seronegative donor cells, and the lowest dilution of patient's cells positive for p24 antigen was expressed in TCID<sub>50</sub>. Four patients had insufficient cells for the 21-day coculture.
FIG. 1. HIV quantitation with HIV chronically infected cells. The production of HIV p24 antigen by ACH-2 and U1 cells is illustrated for cell dilutions up to 1,600 HIV chronically infected cells per 10^6 cells (A) and for cell dilutions up to 400 HIV chronically infected cells per 10^6 cells (B). ACH-2 cells were cultured and were each cultured with 10^6 parent cells in 10 ml of medium containing 20 U of IL-2 per ml and 50 ng of PMA per ml. Cell-free and cell-associated HIV p24 antigens were assayed. Standard curves A, B, and C were constructed by using HIV chronically infected cell lines (U1 monocytic cells and ACH-2 T cells) or the mean of the two cell lines, respectively. The U1 cell standard curve (curve A) is the mean (± standard error of the mean [SEM]) for U1 cells cultured with 10^6 U937 parent cells not infected with HIV (n = 6). The ACH-2 cell standard curve (curve B) is the mean (±SEM) for ACH-2 cells cultured with 10^6 A3.01 parent cells not infected with HIV (n = 6). Standard curve C is the mean (±SEM) for U1 and ACH-2 cells.

FIG. 2. Correlation between HIV p24 antigen detected by the rapid culture assay and TCID obtained by the 21-day coculture assay. Mononuclear cells from HIV-seropositive patients were cultured by the rapid HIV assay and by the 21-day coculture method. The level of HIV p24 antigen in the rapid culture is expressed as picograms per 10^6 cells and is the sum of released and intracellular fractions. Of the 20 positive rapid HIV cultures, 3 (15%) were positive only in the cell-associated fraction and the remainder were positive in both cell-associated and supernatant fractions. The amounts of HIV p24 antigen produced per 10^6 cells detected by the rapid HIV culture is compared with the TCID defined by the 21-day coculture. The Pearson correlation coefficient was 0.55 (95% confidence interval, 0.16 to 0.80; P < 0.02 [two tailed]).

Flow cytometry for detection and quantitation of intracellular HIV. For undiluted U1 cells, flow cytometry detected more than 50% of the cells expressing intracellular p24 antigen. For U1 cells diluted to 10 and 1%, flow cytometry detected intracellular p24 antigen in 10 and 1.6% of the cells, respectively. At the lower limit of detection, this value represented 10% infected cells or 10,000 TCID per 10^6. Because of the lower sensitivity, the flow cytometry method was abandoned as a potential comparison for the HIV rapid-culture assay.

DISCUSSION

We report a method for direct culture of patient mononuclear cells without donor cells that has a sensitivity of 87% and a specificity of 100%. The positive and negative predictive values were 100 and 79%, respectively. The sensitivity and
specificity are comparable to those of published HIV culture studies. These studies have reported sensitivities of 10 to 100%, depending in part on culture conditions and the stage of HIV disease, with AIDS patients most likely to yield a positive viral culture (1, 7, 8, 25, 26, 45, 48). The rapid direct culture assay is comparable in sensitivity to PCR with DNA from cells lysed directly (7, 26). The sensitivity of PCR can be less than 100% if the frequency of infected PBMCs is less than 1/250,000 cells or if the cells are contaminated with hemoglobin, a substance known to interfere with PCR (20, 37, 52). The rapid-culture assay is superior to detection of HIV p24 antigen in serum, which has a rate of positivity of 4 to 70% depending on the stage of HIV disease (4 to 17% in symptom-free patients, and 42 to 70% in AIDS patients) (12, 19, 24, 26). Flow cytometry for direct detection of intracellular p24 antigen using U1 and ACH-2 cell lines was found to be insensitive in our hands when compared with EIA.

There are several advantages to this new assay, including simplicity, rapidity, no need for donor cells, and the ability to quantitate viral load. The standard co-culture method requires medium replacement every 3 days and is generally maintained for 21 or more days. Reliable detection of HIV p24 antigen from coculture supernatant requires a minimum of 10 to 14 days (8, 25). This new method is completed in 7 days or less. The ability to cultivate HIV from patient cells without cocultivation with donor cells removes the potential of using seronegative but HIV-infected donor cells. In countries with a high prevalence of HIV, this potential is significant. Furthermore, in populations in whom HIV vaccines are tested and serologic assays may not discriminate between vaccine or infection-induced antibodies, this new assay will be able to determine the presence of true infection rapidly.

The HIV rapid-culture assay allows quantitation of the numbers of cells infected with HIV in 10<sup>7</sup> PBMCs by comparing the amounts of p24 antigen detected with those produced by known numbers of chronically infected T cells and monocytes used to construct the standard curves. Although U1 and ACH-2 cell lines were used to represent T-cell and monocyte levels of HIV infection, the U937 cell line from which U1 was derived acts more like T cells than monocytes with regard to HIV infection and load. For this reason, it is not surprising that the level of HIV detected was similar when either cell line was used to generate a standard curve. However, in the absence of a true "standard," one or more of these cell lines can be used for construction of a standard curve to determine the number of HIV-infected patient PBMCs (Fig. 1).

In standard curve C, an equal weight was given to T cells and monocytes because in patients both cells contain HIV proviral DNA and the numbers of monocytes approximate those of CD4<sup>+</sup> T cells in persons with absolute CD4<sup>+</sup> T-cell counts of 500 µl<sup>-1</sup>. By using the standard curve C that was generated with data from both T cells and monocytes, comparable numbers of HIV-infected cells were detected by the rapid and 21-day coculture assays. At a TCID of 5 or less detected by the 21-day coculture assay, the TCID determined by the rapid assay was approximately 50-fold higher. The presence of suppressor cells in the coculture assay may contribute to the discrepancy between the two assays. In preliminary experiments, the addition of anti-CD8 F(ab')<sub>2</sub> to the coculture resulted in a 10-fold or higher TCID. The use of PMA may induce maximal HIV replication, countering the effect of suppressor cells. In addition, the rapid culture assay uses twofold dilutions of chronically infected cells whereas the 21-day coculture assay uses 10-fold dilutions of patient cells, which may contribute to the lower correlation coefficient.

The rapid HIV culture intentionally biases against amplification of the HIV by the short duration of culture. Therefore, the amount of p24 produced by cells cultured in the rapid assay is smaller than in the qualitative coculture assay. In contrast, the design of the qualitative coculture assay is intended to amplify HIV by multiple cycles of infection of mitogen-stimulated donor cells that result in high levels of p24 antigen. Although the amount of p24 detected by the rapid-culture assay is small in some cases, it is similar to that produced by chronically HIV-infected cell lines. Advantages of the rapid HIV culture over the standard coculture include its ability to overcome the variability of cell susceptibility from different donors to HIV-1 infection and its possibly higher sensitivity as a result of the culture of 10<sup>6</sup> cells compared with 2 x 10<sup>6</sup> cells used for the standard quantitative coculture.

Another advantage of the rapid-culture assay is the ability to monitor the viral load in relation to the stage of HIV disease and response to antiretroviral therapy. Although levels of HIV mRNA and DNA also provide information on the viral load, the viral culture method provides information on the replication competence of cells infected with HIV. Differences in viral quantitation by DNA PCR and viral culture have been observed and attributed to measurements of proviral copies of HIV and replication-competent virions (2, 3). Detection of large numbers of HIV mRNA copies by reverse transcription-PCR has been reported to correlate with disease progression (43). The use of newer antiretroviral agents, such as protease inhibitors, that act on posttranscriptional events may make the quantitation of cell-associated mRNA less likely to correlate with production of infectious virions. The ability of the rapid culture to detect intracellular and released viruses obviates this limitation. It is likely that the quantitation of the virus load in plasma by competitive PCR and plasma viral culture may be the only assays comparable to the rapid assay (12, 21, 38). These assays are limited by either their complexity or their potential for contamination.

The current clinically used surrogate markers of HIV disease and response to therapy are absolute CD4<sup>+</sup> T-cell num-
bers and HIV p24 antigen levels in serum. Quantitation of HIV p24 antigen levels in serum is insensitive, since up to 60% of patients may not have detectable levels even in the immune complex dissociation assay (38). Absolute CD4+ T cell numbers in persons given antiretroviral therapy may no longer be accurate, as observed in this study and by others (9, 11, 43). Furthermore, coinfection with human T-cell leukemia virus (HTLV) may also make the use of CD4+ T cells less reliable in areas with a high prevalence of HTLV (44, 51).

In the culture medium of the rapid assay, PMA provides the signal for HIV replication that replaces PHA-stimulated donor cells. IL-2 in the HIV rapid-culture assay probably serves to maintain cell growth, while PMA triggers viral replication (18, 27, 42). This is based on the inability of IL-2 to stimulate p24 production by HIV-seropositive patient cells without coculture with PHA-stimulated donor PBMC (22, 39). The signals transducing PMA include activation of protein kinase C (23, 27), induction of oxygen radical production (22, 47), production of tumor necrosis factor alpha and other cytokines (22, 39), and expression of IL-2 receptor, resulting in increased sensitivity to IL-2 (50). These factors in turn activate the transcriptional enhancer nucleus-binding protein, NF-κB (13, 14, 30, 39, 47, 49). NF-κB binds to specific DNA sequences in the long terminal repeat region of HIV and increases HIV transcription (13, 14, 39).

Testing in larger groups of HIV-seropositive symptom-free and AIDS patients and monitoring responses to antiretroviral therapy will be required to evaluate the application of the rapid-culture assay clinically. Most of the patients in this study were symptom free and had CD4+ T-cell counts of ≥200/μl. The sensitivity of the rapid assay may be improved by the use of additional cytokines such as TNF-α, IL-6, and IL-1 (6, 10, 14, 35, 36, 40, 41). Further refinements may additionally allow semiautomatization of the rapid-culture assay, making it even more practical.

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