Rapid Enzymatic Analysis for Human Immunodeficiency Virus Type 1 DNA in Clinical Specimens

J. Sanders Sevall,1 Harry Prince,2 George Garratty,2 William A. O'Brien,3 and Jerry A. Zack4

A clinical procedure for rapid detection of human immunodeficiency virus type 1 (HIV-1) by DNA amplification is demonstrated. The rapid procedure reduces handling requirements and amplification time and eliminates use of radioactivity for the detection of the amplification product. Total leukocyte lysates are the amplification substrates. Two conserved regions in the HIV-1 genome are amplified by 45 cycles of a two-temperature thermal cycle and the amplification products are detected by ultraviolet light after electrophoresis on agarose gels. Twenty-four specimens clinically diagnosed by detection of antibody (IgG) to HIV-1 were confirmed by the rapid DNA amplification procedure. In a blind study, 56 samples positive for HIV-1 DNA were detected in 503 individuals by the current classical polymerase chain reaction method; the same 56 positive samples were also detected by the rapid amplification protocol. No false-positive or false-negative results were obtained. The turnaround time for analysis has been reduced to <24 h without compromising test results.

Indexing Terms: polymerase chain reaction • leukocytes • electrophoresis, agarose • uridine • uracil DNA glycosylase • β-globin

Rapid, low-cost, and reliable detection of human immunodeficiency virus type 1 (HIV-1) is of primary concern for laboratories involved in HIV-1 diagnosis. General serologic screening with enzyme immunoassay and confirmation by Western blot is the standard detection system capable of identifying individuals who seroconvert after HIV-1 exposure (1, 2). However, individuals who have not seroconverted may still carry HIV-1 DNA and transmit the virus. High-risk individuals who experience multiple exposures before seroconversion may be the most common latently infected individuals (3–8). False-positive serology has been observed with infants of mothers who carry HIV-1-specific antibodies yet lack viral DNA (9, 10). Further, 10–20% of sera that are reactive with an enzyme immunoassay can have indeterminate HIV-1 Western blots that remain indeterminate on retesting (11). In all these cases, methods other than antibody testing are required to determine whether virus is present.

The polymerase chain reaction (PCR) is the most sensitive and direct test for HIV-1 DNA detection in peripheral blood mononuclear cells (PBMCs) (12, 13). A serious limitation of DNA amplification is its extreme sensitivity to cross-contamination by minute quantities of amplified product from prior amplifications (14). A procedure was developed for the clinical laboratory that includes maintaining strict laboratory technique, amplification controls, multiple-site amplification, and confirmation by serology before positive results are reported (15). However, the technical difficulties and manipulations involved in sample preparation increase the probability of possible target contamination. Detection of the PCR product by hybridization with a radioactive probe complementary to the internal sequence of the amplification product requires time (3 days) for detection, increases the unit cost per assay, and creates radioactive waste disposal problems, all of which reduce the utility of enzymatic DNA amplification in the clinical laboratory.

This study demonstrates the feasibility of a rapid PCR protocol for HIV-1 DNA detection in clinical material in the diagnostic laboratory. By use of a simple and rapid method of sample preparation, an anti-contamination technique to minimize false-positive results (16), and a rapid, nonradioactive detection method, HIV-1 DNA can be detected within 24 h after receipt of the clinical specimen. We conclude that the rapid DNA detection method favorably compares with the current classical laboratory PCR protocol.

Materials and Methods

Specimen collection and amplification controls. Two groups of specimens were collected. Group one included whole-blood samples (heparinized or treated with acid citrate dextrose) from patients with clinically diagnosed acquired immunodeficiency syndrome (AIDS) at the West Los Angeles Veterans Medical Center (Los Angeles, CA). These specimens were the positive controls for assay characterization. Group two included remnant whole-blood samples submitted to Specialty Laboratories, Inc. (Santa Monica, CA), for assay of HIV-1 DNA by the current PCR method. These specimens were received blind from asymptomatic individuals who were thought to be exposed to HIV-1. The samples were
transported at ambient temperature and processed for amplification within 24 h of receipt. The detection limit was determined with leukocytes isolated from a seronegative low-risk donor to which serial dilutions of the ACH-2 T-cell line were added (17). The T-cell line has a single integrated copy of the HIV-1 genome (cat no. 349; AIDS Research and Reference Program, Public Health Service, National Institutes of Health, Bethesda, MD). Serial dilution gave from 6000 (240 000 copies/mL) to 0.6 copies (24 copies/mL) per assay. Control amplification of genomic DNA with γ-globin-specific oligonucleotides confirmed the presence of amplifiable DNA in the cell lysates.

**PCR conditions.** Preparation of specimens and reagents and analysis of PCR products were conducted in specified, physically separated laboratory spaces with equipment designated for use in each of the respective areas to minimize the possibility of contamination (14). All reagents were tested and shown to be free of amplified product contamination. The two amplification protocols are compared in Table 1.

**Current clinical method.** Three steps are required for clinical amplification: sample preparation, DNA amplification, and detection of the amplification product. For the current HIV-1 DNA PCR amplification protocol used in clinical laboratories, PBMCs are prepared by centrifugation with Ficoll-Hypaque (Pharmacia, Piscataway, NJ) and washed twice in phosphate-buffered saline (GIBCO-BRL, Gaithersburg, MD), and a cell lysate at 6 × 10⁹ cells/L is prepared. The cell lysate (per liter: 100 mmol of KCl, 10 mmol of Tris-HCl, pH 8.3, 2.5 mmol of MgCl₂, 10 mL of Tween 20, and 10 g of NP40) is prepared by incubating the cell solution with 120 mg/L proteinase K for 1 h at 60 °C and inactivating the proteinase K at 95 °C for 10 min.

All PBMC lysates were determined amplifiable by detection of an amplification product of an HLA-DQα or γ-globin human DNA amplification target (specific sites are listed under primers). For amplification, an equivalent of 150 000 PBMCs (50 μL of cell lysate) was amplified with 30 thermal cycles. A single thermal cycle comprised denaturation at 95 °C for 30 s, renaturation at 55 °C for 30 s, and extension at 73 °C for 1 min (18). For each sample, two targets were amplified in duplicate along with a positive and negative clinical control, a sensitivity control, and a blank (19). For PCR product detection, 25 μL of the amplification mixture was hybridized with a 32P-end-labeled internal oligonucleotide in 0.15 mol/L NaCl, 0.01 mol/L EDTA, pH 8.0, at 55 °C for 15 min. The product was separated from the hybridized probe by electrophoresis on 12.5% polyacrylamide gels in 90 mmol/L Tris base, 90 mmol/L boric acid, and 10 mmol/L EDTA (1 × TBE) and exposed to autoradiography for ≥12 h.

**Rapid PCR method.** The sample was prepared by determining the total leukocytes from the collected specimens with a Sysmex™ K-1000 Cell Analyzer (TOA Medical Electronics, Inc., Los Alamitos, CA). Leukocytes from 2–3 mL of whole blood were isolated by erythrocyte lysis with hypotonic sterile ammonium chloride lysis buffer (per liter: 0.15 mol of NH₄Cl, 0.01 mol of KHCO₃, and 0.1 mmol of EDTA, pH 7.4) followed by two 15-min washes at room temperature. Cells were collected by a 5-min centrifugation at 700–735 × g in a Beckman (Fullerton, CA) TJ6 table-top centrifuge. The leukocyte pellet was suspended in 1 mL of phosphate-buffered saline and the cells in a 10-fold dilution of the cell suspension were counted. The cells were pelleted at 700–735 × g (in an Eppendorf Microfuge, Madison, WI) for 10 min. A cell lysate was made by suspending the cell pellet in a cell-lysis buffer modified from that of Higuchi (20) (per liter: 50 mmol of NaCl, 10 mmol of Tris-HCl, pH 8.3, 2.5 mmol of MgCl₂, 4.5 g of MP40, and 4.5 mL of Tween 20) at 12 × 10⁹ cells/L. The suspension was incubated at 60 °C for 1 h in the presence of 1 g/L proteinase K, which was then inactivated at 95 °C for 10 min. The cell lysate can be stored at 4 °C.

DNA amplification of 300 000 total leukocytes (2 μg of DNA) was done in 50 μL total reaction volume containing, per liter, 25 mmol of Tris-HCl, pH 8.0, 4 mmol of MgCl₂, 50 mmol of NaCl, 2 mmol of dNTP (50-fold dilution from a 25 mmol/L stock of each of the four deoxynucleotide triphosphates—uridine, cytosine, adenosine, and guanine; US Biochemicals, Cleveland, OH), 50 pmol of each primer, and 5 U of Taq polymerase (Promega, Madison, WI). Ionic, nucleotide, primer, and magnesium concentrations were determined with different cell lines that contained, individually, HIV-1, HIV-2, HTLV-I, and HTLV-II by promoting amplification with minimal nonspecific background. Forty-five two-temperature thermal cycles each consisted of denaturation at 95 °C for 30 s and annealing and extension at a predetermined optimal temperature (60 °C for all primers used in the rapid PCR method) for 30 s (21). For amplification product detection, 20–25 μL of the PCR reaction mixture was analyzed on 3% Nusieve, 1% agarose in 0.5 × TBE gel containing 0.25 mg/mL ethidium bromide (EtBr). The amplification product was made visible with ultraviolet light and photographed with P/N Type 55 Polaroid film.

**Use of uracil DNA glycosylase (UDG).** Incorporation of uridine in place of thymine in the PCR product makes

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**Table 1. Amplification Protocols**

<table>
<thead>
<tr>
<th>Clinical protocol (2–3 days)</th>
<th>Rapid protocol (1 day)</th>
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<tbody>
<tr>
<td>Lysate preparation (5 h)*</td>
<td>Lysate preparation (2 h)*</td>
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<tr>
<td>Ficoll-Hypaque whole-blood</td>
<td>hypotonic lysis of</td>
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<tr>
<td>lysate; leukocytes at 6 ×</td>
<td>erythrocytes lysis;</td>
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<tr>
<td>10⁹/L</td>
<td>leukocytes at 12 × 10⁹/L</td>
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<tr>
<td>Amplification (4 h): 30 three-step</td>
<td>Amplification (2 h): 45 two-step</td>
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<tr>
<td>thermal cycles</td>
<td>thermal cycles</td>
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<td>30 s at 95 °C</td>
<td>30 s at 95 °C</td>
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<td>30 s at 55 °C</td>
<td>30 s at 60 °C</td>
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<tr>
<td>60 s at 73 °C</td>
<td>Product detection (24–36 h):</td>
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<td></td>
<td>hybridization with end-labeled,</td>
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<td>internal oligonucleotide,</td>
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<td>followed by polyacrylamide</td>
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<td>autoradiography</td>
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<td>Product detection (2 h):</td>
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<tr>
<td></td>
<td>agarose gel electrophoresis;</td>
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<td></td>
<td>ultraviolet visualization of</td>
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<td>ethidium bromide-stained gel</td>
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* Best approximation of the time required to complete step.
it susceptible to the bacterial enzyme UDG, which 
deglycosylates uridine in the PCR product but essen-
tially leaves the native DNA target intact (16). Degly-
cosylation makes the PCR products liable to high-
temperature hydrolysis. UDG is inactive at high 
temperatures; thus, amplification products produced 
in the presence of UDG are stable provided they remain 
above 72 °C. Analysis of the PCR products in the pre-
ence of UDG (Perkin-Elmer Cetus, Norwalk, CT) re-
quired programming the thermal cycler for extended 
incubation at 73 °C after completion of PCR, before gel 
analysis of the PCR products. Standards indicated that 
sensitivity was not compromised with UDG. In the 
current experiments, UDG was not used in the clinical 
amplifications; however, all thymidine triphosphates 
were replaced with deoxyuridine triphosphates, allow-
ing the effect of deoxyuridine on amplification to be 
determined.

Primers. For the current HIV-1 DNA amplification, the 
primers were directed to the group-specific antigen gene 
(gag) of HIV-1 [5'-ATAATCCACCTATCCGAGGAGAAG-3' (sense) and 5'-TTTGGTCCTGCT-TATCCGAGAATGCT-3' (antisense)] with the internal 
probe SK 19 5'-ATCTGGGATATAAAAAATGTAA-
GAATGTAGCCTACT-3']. The SK38/39 amplification 
product was 115 bp (19). The second primer set for the 
current HIV-1 DNA amplification method was also de-
erived from the conserved region within gag of HIV-1 
(SK101 5'-GCTATGCTCGTCCCTTGTTGCT-3' and 
SK145 5'-AGTGCGGACATCAAGCCAGCCATGCAA-
AT-3' with the internal probe SK102 5'-GAGACCAT-
CAATAGGAGAAGCTGCGAGATGGAT-3'). The ampli-
ified product of SK101/145 was 140 bp (22). For the rapid 
PCR method, SK101/145 amplified one target site, and 
a second primer pair was designed to detect the conserved 
R/U5 sequence of the long terminal repeat [LTR; M667 
5'-GGCTAACTAGGAGGCCGCCAGT-3' (sense) and AA55 
5'-CTGCTAGAGATTTCTCA-CTGC-3' (antisense)] (23). The AA55/M667 primers produced a 140-bp amplifica-
tion product. The internal probe for AA55/M667 detection 
was 5'-GTTGTTGCCCCGTCTTGGTGTAG-GTC-
TCTG-3' [nucleotides 555-584 of the HIV-1 
JR-25F isolate (24)]. Primers to confirm the presence of 
ampifiable DNA in the cell lysates were as previously 
described for the HLA-DQA locus (25) or complementary 
to the first exon of the human β-globin gene positions 
14-35 [5'-ACACAAGTGTGATCTAC-3' (sense)] and 
123-104 [5'-CAAATCTAGCCACCCT-3' (anti-
sense)] relative to the translation initiation site (26). The 
specificity of the primers was confirmed by computer 
search of the Genbank library and the failure of the 
HIV-1-specific primers to yield a specific product from the 
HTLV-I and -II genomes and uninfected human leukocyte 
DNA. The LTR-specific primers did not amplify HIV-2 
sequences but SK101/145 could amplify a similar region 
in the HIV-2 genome.

Results

The rapid DNA amplification procedure amplified 
lysates of total leukocytes isolated by ammonium chlo-
ride hypotonic lysis of whole-blood erythrocytes. Whole 
blood from 500 individuals at risk for HIV-1 infection 
had an average leukocyte count of 6.58 × 10⁹/L ± 3.05 
× 10⁹/L with a lymphocyte/monocyte count of 3.01 × 10⁹/L ± 1.83 × 10⁹/L. The average yield of leukocytes 
from selective erythrocyte lysis was 50% of the 
leukocytes in whole blood. The high variation in cell 
counts between individuals and the need to amplify 
DNA from a minimum number of CD4+ lymphocytes 
for high HIV-1 DNA sensitivity dictated cell-counting 
in both whole blood and the phosphate-buffered saline-
ashed leukocytes before cell lysis. The total leukocyte 
concentration in the lysate mixture is 12 × 10⁹/L, which 
provides ~150 000 lymphocytes–monocytes/25 μL for 
the amplification reaction.

Primer pairs SK101/145 and AA55/M667 amplified 
conserved regions of the HIV-1 genome and had high 
specificity when amplified under optimized conditions 
(21, 27). Both primer pairs are specific for HIV-1, with 
SK101/145 also capable of amplifying HIV-2. Sensitivity 
under optimized amplification conditions was deter-
mined by adding serial dilutions of the T-cell line 
ACH-2, which carries a single integrated HIV-1 ge-
nome, to a constant amount of whole-blood leukocytes 
(12 × 10⁶ cells/L).

The major variables in the amplification are the ionic 
conditions, primer concentrations, and temperature of 
primer annealing. Ionic conditions are determined by 
the concentrations of potassium or sodium chloride and 
magnesium chloride in the reaction mixture and have 
been optimized by use of HIV-1 DNA standards (data 
not shown). These conditions are efficient for many 
amplification systems used in this clinical laboratory. 
The primers are maintained at 50 pmol, which is high 
for most reactions, but nonspecific amplification bands 
are minimized by the higher reannealing temperatures 
used in the two-temperature thermal cycle. Studies 
now indicate that 25 pmol of primers does not affect sen-
sitivity (data not shown). The amount of Taq poly-
merase (5 U/assay) used in these studies saturated and 
 promoted low copy amplification. Enzyme concentra-
tions can be reduced further to 2.5 U without loss of sensitivity (data not shown).

Figure 1 (A, B) shows the sensitivity of the rapid PCR 
amplification to one-copy HIV per assay with each 
individual HIV-1 primer set. Amplification with a two-
step thermal cycle with 60 °C for the reannealing step 
for 45 cycles provides sufficient specificity for the 
amplification product to be distinguished after electrophoresis 
on low-molecular-mass sieving agarose gels. Sensitivity of 
HIV-1 copy-level range is 1000 to 1 (Figure 1, A and 
B, lanes A–D). At low copy numbers (1–0 copies/assay; 
lanes D–G), some nonspecific bands are observed but are 
distinguished from the 140-bp amplification product by 
electrophoretic mobility. PCR products have been con-
firmed by the presence of a single internal restriction 
site (both SK101/145 and the AA55/M667 products can 
be cut by the restriction endonuclease Alu I, generating 
86- and 56-bp fragments or 38- and 102-bp fragments, 
respectively) or by probing the product with end-la-
amplification of whole-leukocyte DNA from 24 clinically diagnosed AIDS patients. The results were obtained within 24 h of specimen receipt, as opposed to 72 h with the radioactive detection assay. All 24 specimens were found positive by both the current clinical method and the rapid PCR amplification procedure. No false-negative results were observed under these isolation and amplification conditions.

Specimens submitted for HIV-1 DNA detection by DNA amplification to Specialty Laboratories, Inc. were assayed in parallel with the rapid PCR amplification method. The specimens received were generally from either individuals who are serologically positive for HIV-1 and require DNA confirmation or from asymptomatic individuals who may have been exposed to infection and want to know their DNA status before seroconversion. Case histories were generally not known. All specimens were prepared for amplification upon receipt. The rapid DNA analysis was finished within 24 h, whereas the results of the clinical amplification procedure required 3–5 days. The rapid amplification results were compared after clinical results were released (generally 1 week after receipt of the samples). All specimens found to be DNA positive were serologically positive; the only serologically positive samples found to have no DNA were from infants. All other negative DNA samples were also serologically negative. All HIV-1 DNA-positive samples (66 of the 503 specimens) were detected by both amplification methods, and both amplification procedures did not detect HIV-1 DNA in the other 447 samples. Therefore, the rapid PCR procedure could reduce the total turnaround time for HIV-1 DNA amplification without effect on the results obtained by the current clinical procedure.

Table 2 indicates an additional advantage of the rapid PCR procedure. Eleven percent of the current PCR amplifications had to be repeated, in contrast to 7% of the rapid PCR amplifications. Repeats are dictated by a discordant result from one of the amplifications of two target sites done in duplicate. Repeat amplifications were done on the cell lysates with the original two primer pairs and a third primer pair (SK 36/39). The greatest decrease in repeat amplifications was observed with the noninfected samples, where 11% had to be repeated with the current clinical amplification procedure, but only 6% had to be repeated with the rapid amplification procedure. This may reflect the decrease in the manipulations required by the rapid DNA amplification procedure compared with the current clinical amplification procedure.

<table>
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<tr>
<th>HIV-1 status</th>
<th>Current PCR procedure</th>
<th>Rapid PCR procedure</th>
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<tbody>
<tr>
<td>56 positive</td>
<td>7 (12.5%)</td>
<td>8 (14.3%)</td>
</tr>
<tr>
<td>447 not detected</td>
<td>50 (11.2%)</td>
<td>28 (6.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>57 (11.3%)</td>
<td>36 (7.2%)</td>
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A multiplex amplification (β-globin primers and a single HIV-1 primer set) also exhibits HIV sensitivity to one copy per assay of HIV-1 (Figure 1C). However, nonspecific amplification targets appear more frequently, possibly reflecting the higher concentration of primers. To minimize the nonspecific amplification products, multiplex amplification was not generally done. Therefore, 45 cycles of the two-temperature thermal cycle (95 and 60 °C) followed by gel electrophoresis in the presence of EtBr can detect single-copy amounts of HIV-1 DNA in the amplification sample. Thirty cycles of amplification have similar sensitivity only after hybridization of the amplified product with radioactive, end-labeled oligonucleotide followed by electrophoresis and autoradiography (data not shown). The two-temperature, 45-cycle amplification can reduce the time required for detection of the target DNA in clinical samples. The clinical utility of the rapid PCR detection for HIV-1 DNA in whole blood was initially determined by

beled, internal complementary oligonucleotides (data not shown).

Fig. 1. Detection of PCR amplification products by visualization under ultraviolet light after EtBr staining.

A serial dilution of ACH-2 T cells in lysate of 12 × 10⁶/L leukocytes amplified with a range of 1000 to 0.1 copies of HIV-1 per amplification. Lanes A–E, cell dilutions from 1000–0.1 cells; lane F, leukocyte lysate standard control; lane G, water control; lanes H and I, 25 μL of negative and positive clinical lysate controls, respectively, and lane M, DNA molecular mass marker. Panel A, amplification product from the A55/M687 primer pair (140 bp); panel B, amplification product from the SK101/145 primer pair (141 bp); panel C, products from the multiplex amplification of β-globin primers (110 bp) and the A55/M687 primer pairs (140 bp).
samples amplified by the rapid procedure with the LTR (AA55/M667) and SK101/145 primer pair is shown in Figure 2 (A and B, respectively). The standard controls (Figure 2, lanes 1–7) range in orders of magnitude from 10,000 to 0 HIV-1 copies/assay. Figure 2C represents the sample amplified with the β-globin primer set, indicating that DNA capable of amplification is present in the sample. The 140-bp amplification product from both the SK101/145 and the AA55/M667 primer sets can be detected by comparison with the molecular mass standards (lane M). Two major nonspecific bands can be observed at >220 bp and another at ~170 bp. An appropriate confirmation for the rapid amplification method is the use of an internal restriction site to identify the appropriate amplification product (data not shown).

Discussion

The most sensitive test for detecting the presence of HIV-1 uses PCR to amplify minute amounts of viral DNA (28). Two major concerns with DNA amplification as a general diagnostic test for HIV-1 infection are the time required for results and the low technical reliability in diagnosing seronegative individuals because of false-positive amplification as a consequence of carryover contamination (14). In light of the clinical need for improved diagnostic methods, the PCR amplification methodology has been modified to provide more reliable amplification results.

The current clinical amplification method for HIV-1 proviral DNA (29) consists of three steps (Table 1). All three steps have been modified to provide a more efficient and rapid amplification procedure. With the current modifications, turnaround time for analysis has been reduced to 24 h without compromising test results and reducing the number of repeat amplifications of inconsistent results (Table 2). The use of total leukocytes as a source of amplification substrate has a distinct advantage over the current procedure of Ficoll-Hypaque isolation of mononuclear cells. A single tube required for leukocyte isolation minimizes physical manipulations of the specimen, reducing the possibility of carryover contamination.

Optimizing DNA amplification allows rapid detection of HIV-1 DNA, provided the primers are complementary for all known genotypes of HIV-1. The current amplification conditions have optimized the temperature of reannealing primers and take advantage of the thermal stability and the extension rate of Taq polymerase (60 nucleotides per second per enzyme molecule at 70 °C) (21, 27, 30, 31). The LTR primer set uses two target sites per integrated viral genome, enhancing sensitivity. The SK101/145 amplification site in gag of HIV-1 is highly conserved in HIV viral sequences, as determined by multiple computer alignments of known HIV-1 and -2 viral genomes. Other amplification sites can be identified by multiple alignments and optimization for routine amplification. In these studies, excess primer concentration and Taq DNA polymerase are used to ensure that low viral copy numbers can be detected in the clinical specimen and that sensitivity is not compromised by inhibitors that may be present in the clinical cell lysate (32). However, the high concentration of enzyme and primers with excessive thermal cycling can generate high-molecular-mass, nonspecific amplification products (33). In our experience, the nonspecific background has not interfered in the identification of amplification products on low-molecular-mass DNA-seieving agarose gels.

Alternative PCR product detection methods are being considered. First, unique internal restriction sites in the PCR product can be cut after amplification and identified by their mobility on polyacrylamide or agarose gels. Digesting the PCR product with an endonuclease restriction enzyme may depend on the specificity of the enzyme for the uridine-incorporated PCR product (34) and on the amount of nonspecific products. Should the nonspecific background be significant (which has not been our experience), an enzyme-linked oligonucleotide complementary to the internal sequence of the amplified PCR product can be hybridized to the PCR product and detected by colorimetric, chemiluminescence, or fluorescence methodology (35–37). Oligonucleotides with modified biotin in the 5' end as primers can produce 5' biotinylated PCR products that can be detected by hybridization to an enzyme-labeled probe in solution, and the hybrids collected on an avidin-coated matrix (38). This methodology will avoid the use of electrophoretic gels and may be adaptable for microtiter plate readers common in the clinical laboratory.

The main concern with using DNA amplification for diagnosing infectious disease is the risk of contaminat-
ing samples during preparation or by carryover contamination of the amplified target DNA from previous PCRs (14, 39, 40). The use of UDG to remove incorporated uracil from amplification products, thus rendering the products susceptible to thermal degradation, is an accepted procedure for reducing carryover contamination (16). However, better control of UDG inactivation must be developed for routine use in the clinical laboratory. The present data demonstrate that incorporation of deoxyuridine triphosphate in place of thymidine triphosphate still allows single-copy sensitivity. When the use of UDG to destroy carryover contamination is refined, the methodology can be incorporated into the current amplification procedure.

The risk of false-negative results because of HIV-1 sequence variation is reduced by using two primer pairs that are homologous to conserved regions within the HIV-1 DNA sequence of known HIV-1 genotypes. An advantage to using the LTR sequence primers is that full-length HIV DNA contains two copies of the LTR sequence, theoretically resulting in increased sensitivity with this target site. The use of enzyme immunoasay or Western-blot confirmation provides ancillary support to the PCR results and provides a monitoring system for those samples that are PCR positive yet seronegative (very rare). Retesting of samples that do not give consistent amplifications between the two amplification sites and duplicate amplifications can be rapidly done on the same sample or on freshly prepared material.

In summary, PCR offers a method of HIV detection that is highly sensitive and specific. No positive samples were missed by the rapid PCR amplification protocol compared with the current clinical amplification method and confirming serology. The rapid amplification protocol has a turnaround time of <24 h, reduces the amount of material required, does not require radioactive probes, and significantly reduces one source of contamination because of the speed and simplicity of sample preparation. By running the proper controls (amplification of two target sites in duplicate), the number of false-positive results is reduced dramatically and confidence in PCR diagnosis is increased. In vitro amplification for diagnosis can now be done with confidence.

Funded in part by the National Heart, Lung, and Blood Institute grant RO1 HL45352 to K.B. Mullis. We acknowledge the careful editorial assistance of Chang-Cheng Liu, Kary B. Mullis, Herr Reyes, and Miles Cabot. We thank Budi Tritorrahardjo for expert technical assistance.

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