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Enzyme-Linked Oligosorbent Assay for Detection of Polymerase Chain Reaction-Amplified Human Immunodeficiency Virus Type 1

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An enzyme-linked oligosorbent assay (ELOSA) was developed for the detection on microtiter plates of polymerase chain reaction (PCR)-amplified human immunodeficiency virus type 1 (HIV-1) DNA. The denatured PCR product was hybridized with a passively adsorbed oligonucleotide capture probe and a horseradish peroxidase-labeled oligonucleotide detection probe. The sensitivity and specificity of the PCR-ELOSA technique depended to some extent on the nucleotide sequences of the oligonucleotide primer and probe quartet used in the amplification and detection. We evaluated five oligonucleotide quartets located in the *gag*, *pol*, *vpr*, *env*, and *nef* regions of HIV-1. DNAs from 39 HIV-1-seropositive individuals and 27 healthy HIV-1-seronegative controls were amplified by the PCR procedure, and the products were detected by ELOSA. Ten copies of HIV-1 DNA against a background of 1 μg of human DNA were specifically detected by PCR-ELOSA. Specificities and sensitivities were, respectively, 100 and 95% for the *gag* system, 100 and 97% for the *pol* system, 100 and 85% for the *vpr* system, 96 and 95% for the *env* system, and 100 and 95% for the *nef* system. The simplicity of ELOSA makes it suitable for automation and applicable to genetic testing and detection of viral and bacterial DNAs or RNAs in most routine laboratories.

The routine diagnostic procedures used to identify individuals infected with human immunodeficiency virus type 1 (HIV-1) rely on the testing of blood products for antibodies against viral HIV-1 antigens. However, direct identification of the virus is required in several circumstances, such as in children born to HIV-1-seropositive mothers (23), primary infections before the appearance of antibodies (25), and monitoring of treatment for HIV-1 infection (2). Alternative approaches based on the identification of circulating antigen lack sensitivity, and isolation of the virus by cocultivation is time-consuming and requires laboratory facilities.

It has been shown that the sensitive detection of HIV-1 nucleic acids by polymerase chain reaction (PCR) amplification (34) of conserved sequences of HIV-1 proviral DNA is of interest for the diagnosis of HIV-1 infection (11, 23–25, 40) and could be a potential parameter for clinical status (9) or treatment evaluation (2). The variability of the viral genome, which can lead to problems of specificity and sensitivity, has encouraged many groups to develop primer pairs in various conserved regions of the viral genome, generally *gag* and/or *pol* (7, 23, 25, 30, 31), less frequently *env* and/or the long terminal repeat (7, 30), or *nef* or *vif* (7).

These PCR assays normally require analysis by Southern blotting (30), dot blotting (37), restriction mapping (22), or a second round of amplification with nested primers (1, 35) in order to discriminate the correct PCR product from nonspecifically amplified sequences. These techniques are generally used in specialized laboratories and are not adapted to large-scale screening. Like routine enzyme-linked immunosorbent assays applied to the detection of proteins, there is a need for a simple, rapid, nonisotopic, and sensitive technique for the detection of amplified HIV-1 genomic material. Because the microtiter plate or tube format is well adapted to clinical applications, these formats have been already used for the detection of DNA (39). With such an assay format, PCR products must be captured on a solid phase, directly or indirectly, and further detected by direct or indirect labeling. Generally, proteins like streptavidin, which are directed against biotinylated PCR products (14, 35), or antibodies directed against biotin (38), DNA-binding protein (20, 26, 32, 36), or antibodies against DNA-DNA or DNA-RNA hybrids (3–5, 38) either are coated on the plastic to capture the DNA or RNA target or are used as a detection probe. Nagata et al. (29) first described the direct binding of the DNA target onto a microtiter plate. In the nucleic acid-based sandwich system (8, 33), two nucleic acid probes are used as a capture or a detection probe. They can be either an insert cloned in a vector like m13, phage λ, or pBR322 (15, 17, 33), a PCR product, or a restriction fragment (16). DNA fragments or PCR products larger than 300 bp can be coated onto a solid phase either covalently (15) or by passive adsorption (13, 17). Oligo(dT)$_{14}$ covalently linked to the solid phase was used to capture a poly(A) tail (12, 27).

We describe a rapid and easy method for the nonradioactive detection on microtiter plates of PCR-amplified nucleic acid sequences from HIV-1. The detection method that we developed is based on sandwich hybridization, in solution and at 37°C, between a single-stranded DNA template and short synthetic oligonucleotides (18- to 27-mer) as capture

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TABLE 1. Primers and capture and detection probes used in the present study[a]

Target gene	Oligonucleotide function	DNA sequence (5′ to 3′)	Name	Location
gag	PCR	ATAATCCACCTATCCCAGTAGGAGAAAT	SK38	1089–1116
	PCR	TTTGGTCCTTGTCTTATGTCCAGAATGC	SK39	1176–1203
	Capture	ATTAAATAAAATAGTAAG	CG1105	1141–1158
	Detection	TATAAAAGATGGATAATCCTGG	DG375	1118–1139
pol	PCR	CATGGGTACCAGCACACAAAGGAATT	AP714	3695–3720
	PCR	TCACTAGCCATTGCTCTCCAATTACT	AP715	3823–3848
	Capture	TGAACAAGTAGATAAATTAG	CP759	3729–3748
	Detection	GGAATCAGIAAAGTACTATT	DP760	3757–3776
env	PCR	CAGGAAGCACTATGGGCGC	AE552	7346–7364
	PCR	GCTGCTTGATGCCCCAGAC	AE553	7477–7495
	Capture	CCAGACAITTAITGTCTGGTATAGTGC	CE1093	7391–7417
	Detection	AACAATTTGCTGAGGGCTAT	DE330	7426–7445
vpr	PCR	TGGAACAAGCCCCAGIAGACC	AV659	5105–5125
	PCR	TGCTATGTIGACACCCAATTCTG	AV660	5320–5342
	Capture	TATGAAACTTATGGGGATAC	CV1115	5242–5261
	Detection	GAAGCTGTTAGACATTTTCCTAG	DV736	5188–5210
nef	PCR	AAGATGGGTGGCAAITGGTC	AN635	8339–8358
	PCR	CATTGGTCTTAAAGGTACCTG	AN1116	8558–8578
	Capture	GGATGGCCTICTITAAGGGAAAGAATG	CN763	8375–8401
	Detection	GAGGAGGTIGGTTTTCCAGTCA	DN753	8531–8552
ras	PCR	TGGTTATAGATGGTGAAACCTG	AR732	20–41
	PCR	CTGTAGAGGTTAATATCCGCAAA	AR170	157–179
	Capture	CAGTGCCATGAGAGACCAAT	CR1092	81–100
	Detection	TTCCTCTGTGTATTTGCCAT	DR284	121–140

[a] HIV-1 nomenclature is according to HIVHXB2R (28); the *Ras* exon 2 position is with reference to the HUMRASN2 sequence (Gen Bank).

and detection probes. The fixation of the capture oligonucleotides was done by passive adsorption on the surface of the microtiter wells by using phosphate-buffered saline (PBS) containing high salt concentrations. The detection probe was a horseradish peroxidase-labeled oligonucleotide. We evaluated five different oligonucleotide primer pairs and five corresponding probe pairs for the amplification and detection of HIV-1. Primers in the *gag* (30), *pol* (31), *env*, *vpr*, and *nef* regions were chosen; the internal capture and detection probes are defined below. Inosine was substituted for highly significant mutations, either in amplification primers or capture and detection probes. The five primer pairs and the five probe pairs were used for HIV-1 DNA amplification and detection in samples from 25 HIV-1-seronegative patients and 39 HIV-1-seropositive patients. The PCR amplification and enzyme-linked oligosorbent assay (ELOSA) procedures were found to be simple, rapid, specific, and sensitive.

MATERIALS AND METHODS

Patient samples. Blood samples from 25 HIV-1-seronegative individuals and 35 HIV-1-seropositive subjects (11 Centers for Disease Control [CDC] stage II, 13 CDC stage III, 1 CDC stage IVB, 4 CDC stage IVC1, 6 stage CDC IVC2) were collected at Centre Hospitalier Régional Universitaire Bretonneau, Tours, France, and blood samples from 2 subjects with AIDS-related complex and 2 patients with AIDS were kindly provided by D. Rigal, Blood Transfusion Center, Lyon, France.

DNA extraction and PCR. DNA preparations from 5-ml blood samples were obtained by using an Applied Biosystems 340A nucleic acid extractor. HIV-1 DNA was amplified by PCR by using 1 μg of extracted genomic DNA in a 100-μl volume containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 mM each dATP, dCTP, dGTP, and dTTP, 50 pmol of each primer, and 1.5 U of recombinant *Taq* polymerase (Perkin-Elmer Cetus). Cycling conditions were denaturation for 1 min at 95°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C for 35 cycles and then a final extension for 7 min at 72°C. The *Ras* cellular gene was amplified as a control for DNA quality and PCR performance by using the same PCR conditions described above, except that cycling was limited to 25 cycles.

Primers and probes. All the oligonucleotides (Table 1) were prepared by the phosphoramidite method on an Applied Biosystems 394 synthesizer and were purified by reverse-phase high-pressure liquid chromatography (HPLC). The oligonucleotides used for detection and capture were synthesized with an amine arm at the 5′ end. The addition was performed on a synthesizer with the Aminolink II reagent (Applied Biosystems). Horseradish peroxidase labeling of the oligonucleotides used for detection was performed as described by Keller and Manak (18). Preparation of the oligonucleotide-enzyme conjugate was as follows. A total of 200 μg of oligonucleotide synthesized with an Aminolink II arm was dried and mixed with 500 μl of 1,4-phenylene diisothiocyanate (Sigma Chemical Co.; 20 mg/ml in dimethylformamide) and 25 μl of sodium borate buffer (0.1 M; pH 9.3). The tube was incubated in the dark for 2 h before three extractions with *n*-butanol. The aqueous phase containing the activated oligonucleotide was dried and mixed with 200 μl of 0.1 M sodium borate (pH 9.3) containing 2 mg of horseradish peroxidase. After one night of incubation at room temperature, the conjugate was purified by ion-exchange HPLC. The labeled detection probe can be stored at −20°C in glycerol for several months.

ELOSA. The passive adsorption of the capture probes was performed as follows. A total of 100 μl of 3× PBS buffer (10× PBS buffer is 1.37 M NaCl, 27 mM KCl, 43 mM Na_2HPO_4, 14 mM KH_2PO_4) containing the capture probe (150 nM) was dropped into each well of a microtiter plate (Nunc). After incubation for 2 h at 37°C or overnight at room temperature, the plate was washed three times with 1× PBS buffer–0.05% (wt/vol) Tween 20. The oligonucleotide-coated plates were stable for several months at 4°C. Of the 100 μl of the amplified double-stranded HIV-1 DNA, 25 μl was diluted in 65 μl of hybridization buffer (0.1 M sodium phosphate [pH 7.0], 0.5 M NaCl, 0.65% [wt/vol] Tween 20, 0.14 mg of salmon sperm DNA [Boerhinger] per ml, 2% polyethylene glycol 4000) and denatured by the addition of NaOH (final concentration, 0.2 M), incubated at room temperature for 5 min, and neutralized by the addition of acetic acid (final concentration, 0.2 M). After the denaturation step, the DNA reaction mixture was adjusted to 125 μl with the hybridization buffer. Some 50 μl of this solution was dropped into a well of a microtiter plate that was previously coated with a specific capture probe. Immediately thereafter, 50 μl of a detection probe (15 nM in hybridization buffer) was added to each well. After 1 h of hybridization at 37°C, the microtiter plate was washed three times with 1× PBS buffer–0.05% Tween. For detection, 100 μl of *o*-phenylenediamine (Sigma; 4 mg/ml in 0.05 M citric acid, 0.1 M Na_2HPO_4 [pH 4.9]) containing H_2O_2 (0.03 volume) was added to each well. After 30 min, the reaction was stopped by the addition of 100 μl of 1 N H_2SO_4. The signal was read at 492 nm in an automatic microtiter plate reader and optical densities (ODs) were expressed as $A_{492} \times 1{,}000$.

Reproducibility of the ELOSA method. A total of 1 μg of HIV-1 DNA extracted from patient samples was amplified by PCR with the *gag*, *pol*, *env*, *vpr*, and *nef* primer pairs, and PCR products were analyzed by the ELOSA as described above. Ten independent ELOSA determinations were performed, with each one done in two separate wells.

Sensitivity of the PCR-ELOSA on an in vitro model. A total of 1 μg of human DNA containing 10 copies of plasmid pBH10 and 1 μg of human DNA used as a negative control were amplified by PCR with the *gag*, *pol*, *env*, *vpr*, and *nef* primer pairs. Five independent PCRs were performed and the PCR products were analyzed by ELOSA as described above.

Statistical analysis. Differences in the ability of the PCR-ELOSA to detect the control cellular gene and the amplified HIV-1 sequences between HIV-1-seronegative controls and HIV-1-seropositive individuals were assessed by the Mann-Whitney U test. Differences between CDC stages were assessed by the Kruskal-Wallis test. Detection data are displayed graphically in "box-whisker" plots (StatView II; Abacus Concepts, Inc., Berkeley, Calif.): the tops and bottoms of the boxes represent the 75th and 25th percentiles, respectively; the middle 50% of the ODs are contained within the span defined by the box boundaries, and the line inside the box represents the 50th percentile or median; the lines extending above and below the box are referred to as "whiskers"; the top whisker is drawn from the 75th percentile to the 90th percentile; the bottom whisker is drawn from the 25th percentile to the 10th percentile; the values (outliers) under the lower whisker and above the upper whisker represent observed values below and above the 10th and 90th percentiles, respectively.

TABLE 2. Reproducibility of the ELOSA method[a]

Patient	Probe	Mean OD	CV (%)
L	*gag*	2,041	6.3
L	*pol*	1,433	5.5
L	*env*	586	5.2
L	*nef*	642	7.3
H	*nef*	2,154	5.1
L	*vpr*	311	6.1
H	*vpr*	1,482	7.2

[a] DNAs from HIV-1-seropositive patients were amplified by PCR with the *gag*, *pol*, *env*, *vpr*, and *nef* primer pairs. ODs are expressed as the mean of 10 independent ELOSA detections as described in Materials and Methods. Coefficients of variation (CV) were expressed at various ODs. The oligonucleotide probe pairs were *gag*, *pol*, *env*, *nef*, and *vpr*. H, symptomatic patient; L, asymptomatic patient.

RESULTS

Reproducibility of the ELOSA method. Preliminary experiments performed on various biological and synthetic models have shown that no less than between 1/100th (1 μl) and 1/25th (4 μl) of the PCR mixture is required to detect between 1 and 10 copies of a target gene, depending on the oligonucleotide quartet used, i.e., the primer pairs and subsequent probe pairs (data not shown). Because the aim of the present study was not to quantify the HIV-1 proviral DNA but to detect at least 10 copies in 1 μg of total DNA, we chose to use 1/10th of the PCR mixture.

Coefficients of variation of the *gag*, *pol*, *env*, *nef*, and *vpr* probe systems ranged from 5.1 to 7.3% for ODs ranging from 311 to 2,041 (Table 2).

Sensitivity of the PCR-ELOSA on an in vitro model. By using 10-fold serial dilutions of a PCR which had reached its plateau, preliminary experiments showed that the sensitivity of the ELOSA method was similar to that of overnight autoradiography of Southern blots revealed by a radiolabeled oligonucleotide probe (data not shown). Before testing of the primer and probe quartets on DNAs isolated from patients, we first checked their sensitivities. The results of five independent PCR reactions and ELOSA analyses are plotted in Fig. 1. Each oligonucleotide quartet was able to detect 10 copies of HIV-1 DNA in a background of 1 μg of DNA, which is equivalent to 150,000 peripheral blood mononuclear cells.

Sample analysis by the PCR-ELOSA method. DNA samples were amplified by PCR with the five HIV-1 primer pairs and with one primer pair corresponding to a control cellular gene for 35 and 25 cycles, respectively. Each PCR product was tested by ELOSA in two different wells. The panel consisted of blood samples from 27 HIV-1-seronegative controls and 39 HIV-1-seropositive patients, including 11 CDC stage II, 13 CDC stage III, and 15 CDC stage IV individuals. No significant difference in the level of detection of the control cellular gene (*Ras*) was observed between the 39 HIV-1-seropositive patients and the 27 HIV-1-seronegative controls ($P = 0.700$, Mann-Whitney U test). Highly significant differences in the level of detection of the amplified HIV-1 genome (*gag*, *pol*, *env*, *nef*, and *vpr*) were observed between the HIV-1-seropositive population and the HIV-1-seronegative population ($P = 0.0001$, Mann-Whitney U test). No significant difference in the level of detection of the control cellular gene (*Ras*, $P = 0.622$, Kruskal-Wallis test) and the amplified HIV-1 genome (*gag*, $P = 0.380$; *pol*, $P = 0.963$; *env*, $P = 0.882$; *nef*, $P = 0.964$; *vpr*, $P = 0.818$; Kruskal-Wallis test) was observed for

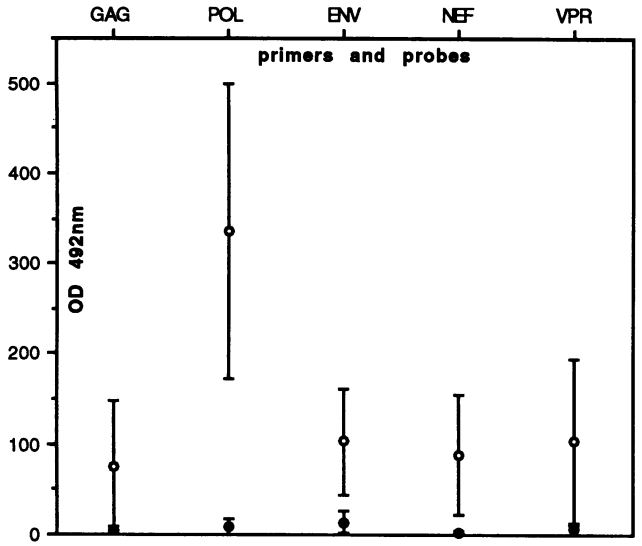

FIG. 1. Sensitivity of the PCR-ELOSA. Four independent PCR and ELOSA detections were performed with 1 μg of human DNA (●) or 10 copies of HIV-1 pBH10 plasmid diluted in 1 μg of human DNA (○). Means ± standard deviations are plotted. ODs at 492 nm are expressed as OD × 1,000. Oligonucleotide quartets, primer pairs, and probe pairs are referred to as *gag* (GAG), *pol* (POL), *env* (ENV), *nef* (NEF), and *vpr* (VPR).

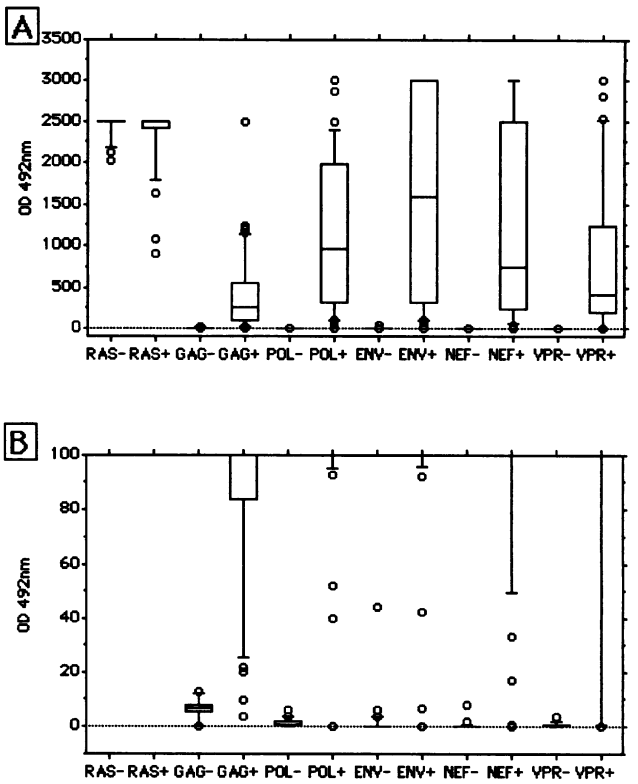

FIG. 2. Distribution of the ODs determined by PCR-ELOSA in relation to oligonucleotide quartets and clinical status. (A) Box plots indicate, for each group defined by an oligonucleotide quartet and a serological status, the distribution, and the median OD. The five horizontal lines on the boxes show the 10th, 25th, 50th, 75th, and 90th percentiles. Values above and below the 10th and 90th percentiles are represented as datum points (open circles). PCR amplification and ELOSA detection were performed on DNAs from 27 HIV-1-seronegative controls (RAS−, GAG−, POL−, ENV−, NEF−, VPR−) and DNAs from 39 HIV-1-seropositive patients (RAS+, GAG+, POL+, ENV+, NEF+, VPR+). Oligonucleotide quartets, primer pairs, and probe pairs are referred to as *ras* (RAS), *gag* (GAG), *pol* (POL), *env* (ENV), *nef* (NEF), and *vpr* (VPR). ODs at 492 nm are expressed as OD × 1,000. The mean ODs for PCR-negative controls ranged between 0 and 4. (B) Low ODs ranging from 0 to 100 were magnified in order to visualize data for samples from HIV-1-seronegative controls and samples from HIV-1-seropositive patients that were poorly reactive.

samples from patients at different CDC stages. This was expected, because the method was not meant to be quantitative.

The distributions of the five HIV-1 PCR-ELOSAs and the *Ras* control PCR-ELOSA (seronegative versus seropositive) determined by the box plot graphic method are presented in Fig. 2. Analysis of the ability of PCR-ELOSA to detect genes in samples from healthy seronegative controls (hereafter referred as gene negative) between each system showed that the *gag*-negative box and whiskers were "thicker" than those for the other gene systems (Fig. 2B), so we can conclude that the *gag* background is significantly higher, inducing a high OD cutoff (see below). All other systems had "thin" boxes and whiskers (Fig. 2B), a regular distribution similar to the *gag*-negative group, except for the *env*-negative group, which had a relatively high OD value for the sample from one patient (Fig. 2B, lane ENV−), inducing a dramatic increase in the OD cutoff (see below). Analysis of gene detection by PCR-ELOSA in samples from HIV-1-seropositive patients (hereafter referred as gene positive) between each system was done; because the boxes associated with *gag*-positive and *vpr*-positive PCR-ELOSA were thinner than the boxes associated with other gene systems (Fig. 2A), we conclude that the middle 50% of the OD distribution from the *gag*-positive and *vpr*-positive samples were more homogeneous than those for samples from other systems. Meanwhile, the fact that the whisker associated with the higher *gag*-positive, *vpr*-positive, and *nef*-positive ODs was longer than the whisker associated with the lower OD (Fig. 2A) suggests a positively skewed distribution. On the contrary, *pol*-positive and *env*-positive samples exhibited a symmetric distribution because of the positioning of the median near the middle of the box (Fig. 2A).

The OD cutoff, which was calculated as the mean negative value plus 3 standard deviations, was estimated for each gene system by using data for the 27 HIV-1-seronegative controls. The respective cutoffs were an OD of 19 for the *gag* system, an OD of 8 for the *pol* system, an OD of 30 for the *env* system, an OD of 8 for the *nef* system, and an OD of 4 for the *vpr* system. Specificities of 100% were obtained for the *gag*, *pol*, *nef*, and *vpr* systems, a specificity of 96% was obtained for the *env* system. The sensitivity was 97% for the *pol* system, 95% for the *gag*, *env*, and *nef* systems, and 85% for the *vpr* system. The specificity and sensitivity of gene detection by PCR-ELOSA were highlighted by comparison of the lower whiskers and outliers (Fig. 2B) for the HIV-1-seropositive patients with the higher whiskers and outliers for the HIV-1-seronegative controls; it shows the nonhomogeneous results obtained for eight individuals, including the previously described HIV-1-seronegative control (patient TA80) with a high *env* OD value of 44 (Fig. 2B, lane ENV−), and the significant overlap of *vpr*-positive and *vpr*-negative results, including those for six HIV-1-seropositive patients

who were not detected (Fig. 2B, lanes VPR+ and VPR−); one of them (patient TS220) was not detected by the *gag*, *pol*, *nef*, and *vpr* systems but had a low OD value, 42, by the *env* system. Added to the specificity and sensitivity criteria, the graphic method outlines the proximity of several *gag*-positive weak signals (<8% of the *gag*-positive signals were within 1× and 2× the cutoff value) with the *gag*-negative HIV-1-seronegative control. The *nef* system presented the same results with a lower amplitude (<2% of the *nef*-positive signals were within 1× and 2× the cutoff value). On the contrary, the lower positive *pol*-positive OD is largely distant from the higher *pol*-negative OD (>4× cutoff).

DISCUSSION

PCR-ELOSA is a practical method for measuring the specific DNAs generated by PCR. The procedure combined a sandwich hybridization reaction with a conventional enzyme immunoassay. The denatured DNA template is captured by a specific oligonucleotide probe that is passively adsorbed onto the surface of a microtiter well. The hybridized template is then detected by using a horseradish peroxidase-labeled oligonucleotide probe. By using coated oligonucleotide plates, assays can be completed in less than 2 h. The reliability of the ELOSA method is suitable for use as a clinical assay, with coefficients of variation ranging for all systems from 5.1 to 7.3% for ODs ranging from 311 to 2,041. The PCR-ELOSA method detected at least 10 copies of HIV-1 plasmid DNA in a background of 1 μg of total DNA, corresponding to approximately 150,000 cells, with each of the five oligonucleotide primer pairs and probe pairs.

The specificities and sensitivities obtained for the *pol*, *nef*, *gag*, and *env* systems were very similar to those reported previously (5, 7, 24, 38, 39). The *gag*, *pol*, *nef*, and *vpr* oligonucleotide quartets described in this report were 100% specific for samples from the 63 patients tested. A disturbing result concerned the *env* system, whose specificity was 96%. The cutoff was determined to be an OD of 30; if we exclude the data for patient TA80, for whom the OD was 44, the cutoff would be shortened to 5. Because the *env* system is the only one that gave a significant OD, 42, for the sample from patient TS220, which was not detected by the four other systems, it would be of great interest to test the *env* system on a larger panel of samples to obtain a more significant cutoff. The sensitivity was 97% for the *pol* system, 95% for the *gag*, *env*, and *nef* systems, and 85% for the *vpr* system. A total of 82% of the HIV-1-seropositive patients were detected simultaneously by the five oligonucleotide quartets, and 92% were detected if we exclude the *vpr* system. Viral diversity could be an explanation for the different sensitivities of the oligonucleotide quartets, especially the low sensitivity of the *vpr* system; this hypothesis is reinforced by three observations. (i) The seven patients in whom HIV-1 was only partially detected did not belong to a specific CDC stage, excluding an association with clinical status; (ii) the patients' CD4 counts were not particularly low ($574 \pm 202/mm^3$), and (iii) patient TS220 was of African origin. Several investigators (7, 30) indicated that it is necessary to use multiple primer pairs for the detection of various viral genomes. Using the samples from the 39 HIV-1-seropositive patients, except for the sample from patient TS220, for whom the *env* system provided an ambiguous result, all results were positive with the *pol* system. Thus, it would be of interest to test the *pol*, *env*, and *nef* systems on a larger panel of samples, including samples from patients from different geographic areas, patients at risk of HIV-1 infection, and children born to HIV-1-seropositive mothers. Nevertheless, use of different primers and probes may overcome some striking differences in reactivity. These differences, potentially linked to viral variability, could be part of either the PCR step or the detection step, although the length of the oligonucleotides used in those detection systems allowed tolerance to point mutations. Increased sensitivity could be achieved by using two or more capture probes and two or more detection probes. This is more particularly feasible with a system with positive values far above the cutoff value, because of the risk of increasing the background value.

Optimization of the specificity and sensitivity of the PCR-ELOSA for HIV-1 detection could be achieved by using coamplification of several parts of the genome such as the *pol* and *env* genes, with the various products generated being detected simultaneously in a single well. Coamplification of one HIV-1 segment with a cellular gene as a reporter gene (19) could also be used as a quantitative approach to determining the viral load in patients, the result being expressed as the ratio of the OD of the HIV-1 gene and the OD of the cellular gene detected by ELOSA.

Furthermore, the ELOSA system has been used to detect, nonisotopically and at a single temperature, point mutations for HLA DR typing (6) by the microtiter plate format. It is also suitable for automation, and typing of human papillomavirus has been defined on the VIDAS (BioMérieux/Vitek) multiparametric immunoanalysis automated analyzer (1a). Furthermore, because ELOSA was able to detect single-stranded DNA and RNA, it either could be used for the direct detection of nucleic acids or could be coupled with amplification systems such as PCR or transcription-based amplification methods (10, 21). Because the ELOSA method proved to be efficient and practical and because it does not require highly specialized technicians or molecular biology equipment, it has the potential to become a widespread method for the detection of nucleic acids in clinical laboratories.

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