JOURNAL OF CLINICAL MICROBIOLOGY, May 1997, p. 1284–1286 Vol. 35, No. 5
0095-1137/97/$04.00+0

Detection of Genetically Diverse Human Immunodeficiency Virus Type 1 Group M and O Isolates by PCR

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Received 18 October 1996/Returned for modification 17 December 1996/Accepted 4 February 1997

A panel of 136 genetically diverse group M and 5 group O adult isolates from outside the United States and Europe were evaluated by PCR with the Roche AMPLICOR HIV-1 test, a modified version of the AMPLICOR HIV-1 test, and a new primer pair/probe system. Detection of some of these isolates was less efficient with the AMPLICOR HIV-1 test; however, the assay was significantly improved by reducing the sample input and lowering the annealing temperature. The new primer pair/probe set detected 140 of 141 isolates, including the 5 group O isolates that were not detected with either of the AMPLICOR HIV-1 test formats.

Human immunodeficiency virus type 1 (HIV-1) has been recognized as one of the causative agents of AIDS (2, 19). Genetic variability of HIV-1 has been shown among isolates from different geographic locations and different individuals and within individuals during disease progression (7, 21). HIV-1 has recently been categorized into groups M and O (4, 5, 9, 14, 16). Although the genomic organization of the HIV-1 group O is similar to group M, the sequences of isolates vary significantly (4, 5, 16). Group M consists of subtypes A to J, of which A to E are presently found in the centers of the pandemic (8, 10). Group O, which includes MVP5180, ANT70, and VAU strains, was originally found in Cameroon and Gabon (4, 5). Believed to be endemic in those countries, group O has been identified recently in Europe and the United States (11, 20).

The AMPLICOR HIV-1 test (Roche Diagnostic Systems, Somerville, N.J.) for proviral DNA has been tested extensively on adult isolates from the United States and Europe with a demonstrated sensitivity of greater than 99% (3). However, the genetic diversity of HIV-1 has raised concern about the ability of current serological and nucleic acid procedures to detect all variants (1), including those not commonly found in the United States and Europe (such as subtypes A and E) and, particularly, the recently described group O isolates (11, 12, 20, 22). To address this, we compared, in a blinded study, a new consensus primer pair/probe set with the AMPLICOR HIV-1 test on provirus from 136 genetically diverse group M and 5 group O isolates obtained from adults in different geographic locations.

Three panels of samples were evaluated. Sample panel one consisted of Vacutainer CPT (Becton-Dickinson, Franklin Lakes, N.J.)-isolated peripheral blood mononuclear cells (PBMCs) from 31 Thai patients which included 26 subtype E and 5 subtype B HIV-1 isolates. The subtypes were determined by seroreactivity with subtype-specific peptides of the V3 loop of the *env* gene (18). The cells were extracted with Roche Specimen Extraction Reagent (Roche Molecular Systems, Somerville, N.J.) without metal ions as described in the AMPLICOR HIV-1 qualitative test product insert. Briefly, 200 μl of extraction reagent was added to 5×10^5 cells which were then incubated first at 60°C for 30 min and then at 100°C for 30 min. Sample panel two consisted of 105 PBMC samples representing HIV-1 subtypes A to G based on sequencing of the *env* region of the genome (15). The PBMCs were isolated from whole blood by using LeucoPrep tubes (Becton-Dickinson) as described in the product insert, and proviral DNA was extracted as described previously (17). Sample panel three consisted of 5 cell lines representing the major arms of the group O phylogenetic tree (6, 11, 13) extracted as described for sample panel one. The group O viruses were established in HUT 78 cells, and growth was monitored by p24 and reverse transcriptase activity as previously described (5). Negative and positive controls were included in all the studies. The positive control was an HIV-1 plasmid DNA (Perkin-Elmer, Norwalk, Conn.) that was diluted in negative cell lysates and analyzed at 10 copies per reaction. The negative control was prepared from PBMCs from a seronegative individual. All samples and controls were coded and tested with each assay system simultaneously.

Amplifications were performed with the AMPLICOR HIV-1 assay, a modified AMPLICOR HIV-1 assay, and a new prototype primer pair system, RAR1032-1033. The AMPLICOR HIV-1 assay was performed according to the manufacturer's instruction. The modified AMPLICOR HIV-1 assay incorporated two changes: a reduction in the sample input volume and a lowering of the annealing temperature. The standard AMPLICOR HIV-1 assay uses 50 μl of the extracted sample, whereas the modified protocol uses 25 μl of the extracted sample and 25 μl of sterile distilled water to make up the volume difference. In addition, a 50°C annealing temperature

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TABLE 1. Evaluation of AMPLICOR and RAR1032-1033 PCR assays of 31 Thai patient samples[a]

HIV-1 group M subtype	No. of patients	% Sensitivity of assay		
		AMPLICOR		RAR1032-1033[d]
		Standard[b]	Modified[c]	
B	5	100	100	100
E	26	57.7	96.2	100

[a] Sample panel one.
[b] Assay conditions: 50-μl volume sample extract; 55°C annealing temperature; 3.75 mM $MgCl_2$ final concentration.
[c] Assay conditions: 25-μl volume sample extract; 50°C annealing temperature; 1.88 mM $MgCl_2$ final concentration.
[d] Assay conditions: 50-μl sample extract; 55°C annealing temperature; 2.5 mM $Mn(OAC)_2$ final concentration.

was used for all 35 cycles of amplification. Amplified products were detected by the microwell plate assay as described in the AMPLICOR HIV-1 assay product insert.

Primers RAR1032 (5′GAGACACCAGGAATTAGATAT CAGTACAATGT3′) and RAR1033 (5′ CTAAATCAGATC CTACATATAAGTCATCCATGT 3′) and probe RAR1034 (5′CCACAAGGATGGAAAGGATCACCAGCTATATTCCA 3′) hybridize to a region of *pol* that is highly conserved in known HIV-1 group M and O isolates (16). The primers amplify a 170-bp region corresponding to nucleotide positions 2959 to 3128 of isolate HIV-1 HXB2 (GenBank accession no. K03455). The oligonucleotides were designed utilizing OLIGO 4.0 (National Biosciences, Plymouth, Minn.) primer analysis software. The RAR 1032-1033 system was originally developed for amplification with the r*Tth* DNA polymerase in a 50 mM bicine buffer–100 mM potassium acetate (OAc)–2.5 mM $Mn(OAc)_2$ system to allow RNA detection by reverse transcriptase-PCR. The system also amplifies proviral DNA in the presence of either 2.5 mM $Mn(OAc)_2$ or 2.5 mM $MgCl_2$ with equivalent efficiency. Sample panels one and three were tested with 50 μl of sample extract volume at 2.5 mM $Mn(OAc)_2$. Sample panel two was analyzed with a 50-μl sample extract volume at 2.5 mM $MgCl_2$. The thermocycling was performed as described in the product insert for the AMPLICOR HIV-1 assay, except the profile used for the first 5 cycles was extended to 10 cycles to give a total of 40 cycles of amplification. The amplification products were detected on microwell plates coated with bovine serum albumin-conjugated RAR 1034 probe in a format similar to that for the AMPLICOR HIV-1 assay.

For sample panel one (Table 1), standard conditions for the AMPLICOR HIV-1 assay yielded sensitivities of 100% (5 of 5) for subtype B but only 57.7% (15 of 26) for subtype E. The modified conditions of reducing the sample input volume to 25 μl and lowering the annealing temperature to 50°C significantly improved the detection rate to 96.2% (25 of 26) for subtype E without compromising detection of subtype B. The sensitivity of the RAR1032-1033 system was 100% for both subtypes B and E under standard conditions.

For sample panel two (Table 2), the AMPLICOR HIV-1 assay sensitivities for HIV-1 group M subtypes from outside the United States and Europe with standard conditions were 100% for subtypes C (1 of 1), D (25 of 25), and G (1 of 1), 87.2% for subtype A (41 of 47), 66.7% for subtype B (2 of 3), 66.7% for subtype E (10 of 15), and 84.6% for subtype F (11 of 13). Under the modified conditions, these sensitivities improved to 97.9% for subtype A (46 of 47), 100% for subtype B (3 of 3), 86.7% for subtype E (13 of 15), and 92.3% for subtype F (12 of 13) without compromising detection of isolates that were already efficiently amplified with the unmodified version. HIV-1 group O was not detected, and there were no false positives with the 34 negative controls (Table 2). Unfortunately, an insufficient amount of sample limited further analysis of the one South American subtype B isolate that was negative with the AMPLICOR HIV-1 assay.

The sensitivities of the RAR primer pair/probe set for group M variants were 100% for subtypes A (47 of 47), B (3 of 3), C (1 of 1), D (25 of 25), E (15 of 15), and G (1 of 1) and 92.3% for subtype F (12 of 13). The subtype F that was missed was different from the one missed by the AMPLICOR HIV-1 assay. Inadequate sample volume also prevented further testing of this sample. All five group O variants were detected, and there were no false positives with the 34 negative controls (Table 2).

While additional samples of all known subtypes need to be tested, these findings suggest that the modified conditions of reduced sample volume input and lowered annealing temperatures with the AMPLICOR HIV-1 assay may be better for detecting subtypes such as A, E, and F that originate primarily outside the United States and Europe. These findings also suggest that the new primer pair/probe system, RAR1032-

TABLE 2. Evaluation of AMPLICOR and RAR1032-1033 PCR assays of genetically diverse HIV-1 group M and O isolates

HIV-1 group	Origin (no. of patients)	% Sensitivity of assay		
		AMPLICOR[a]		RAR1032-1033[b]
		Standard	Modified	
M[c]				
Subtype A	Central Africa (47)	87.2	97.9	100
Subtype B	South America (3)	66.7	100	100
Subtype C	South America (1)	100	100	100
Subtype D	Central Africa (25)	100	100	100
Subtype E	Asia (15)	66.7	86.7	100
Subtype F	South America (9)	88.9	88.9	88.9
	Central Europe (4)	100	100	100
Subtype G	Central Africa (1)	100	100	100
O[d]	Cameroon (5)	0	0	100
Negative controls	34	0	0	0

[a] Assay conditions as described in footnotes to Table 1.
[b] Assay conditions as described in footnote to Table 1 except 2.5 mM $MgCl_2$ used instead of $Mn(OAc)_2$ for group M isolates.
[c] Sample panel two.
[d] Sample panel three.

1033/RAR1034, can detect a wide range of HIV-1 group M subtypes and the more divergent group O isolates.

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