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Comparison of In-House and Commercial Sample Preparation and PCR Amplification Systems for Detection of Human Immunodeficiency Virus Type 1 DNA in Blood Samples from Tanzanian Adults

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This study compared the performance of several in-house nested PCR systems and the Amplicor human immunodeficiency virus type 1 (HIV-1) PCR kit in the detection of HIV-1 DNA in Tanzanian samples prepared by two different methods. All six of the in-house primer sets evaluated had a higher sensitivity for HIV DNA detection in samples prepared by the Amplicor PCR sample preparation method than in those prepared by the Ficoll-Isopaque (FIP) density gradient centrifugation method. A sensitivity of 100% was achieved by combining two in-house primer sets. The sensitivity of the standard Amplicor HIV-1 PCR kit was only 59%, whereas a modified Amplicor HIV-1 PCR test had a sensitivity of 98%. Our data show that Tanzanian samples prepared by the Amplicor preparation method are more suitable for HIV-1 PCR testing than samples prepared by the FIP method. The modified, but not the standard, Amplicor HIV-1 PCR kit provides an alternative to the nested in-house PCR technique for the diagnosis of HIV infection.

Detection of viral nucleic acid in peripheral blood mononuclear cells (PBMCs) by PCR is one of the reliable diagnostic methods for the laboratory detection of human immunodeficiency virus (HIV) infection (2, 6, 17, 19, 20). PCR for the diagnosis of HIV type 1 (HIV-1) infection is particularly useful in situations where serological tests give inconclusive results or fail to detect any antibody response to HIV, i.e., for confirming HIV diagnosis in newborns and infants whose mothers are HIV infected (20, 21) and during the serological window in early infection (5, 13). Several factors determine the success of this highly sensitive method, including proper specimen preparation, use of optimal primers representing conserved regions of the viral genome, appropriate composition of the reaction mixture, and the thermal cycling profile. Ficoll-Isopaque (FIP) density gradient centrifugation has conventionally been used for the separation of PBMCs. Some alternative PCR sample preparation methods are commercially available, and these need to be evaluated to ascertain their suitability for use in PCR sample preparation. It is also important to evaluate newly designed primers to establish their performance in the detection of HIV, especially in samples from regions like Africa, where extensive HIV sequence diversity has been reported (14, 15). In this report, we present results of an evaluation of the sensitivities of various primer sets, including the Amplicor HIV-1 test for detection of HIV-1 DNA in PBMCs by PCR by using blood samples from HIV-1-infected Tanzanian adults, prepared by the FIP and the Amplicor whole blood preparation methods.

Blood samples were collected from 73 pregnant mothers attending an antenatal clinic and from 14 adult females recruited into ongoing studies on the incidence and natural history of HIV-1 infection in Dar es Salaam, Tanzania. The study subjects were asymptomatic for HIV infection. Levels of $CD4^+$ and $CD8^+$ T-lymphocyte subsets in the whole blood samples were measured by FACScount (Becton Dickinson Immunocytometry Systems). HIV serologic analysis was done by using two rapid tests: the HIV spot (Diagnostic Biotechnology, Science Park, Singapore) and Capillus (Cambridge Biotech, Worcester, Mass.) tests. Reactivity was confirmed by the Behring Enzygnost anti-HIV-1–HIV-2 enzyme-linked immunosorbent assay (Behring, Marburg, Federal Republic of Germany). Of the 87 subjects included in the study, 65 were HIV seropositive and 22 were seronegative.

Preparation of samples for PCR was done in Dar es Salaam. PBMCs were separated by FIP (Pharmacia Biochemicals, Uppsala, Sweden) density gradient centrifugation in accordance with the manufacturer's recommendations. Remaining erythrocytes were lysed by using fluorescence-activated cell sorter lysing solution (Becton Dickinson), followed by thorough washing with phosphate-buffered saline, pH 7.2. Crude cell lysates were prepared as previously described (1). Sample preparation by the Amplicor whole blood PCR method (Roche Diagnostic Systems, Inc., Nutley, N.J.) was performed in accordance with the manufacturer's instructions. The samples prepared by both methods were stored at −70°C and transferred to the Swedish Institute for Infectious Disease Control in Stockholm, where they were tested once by each PCR system.

In the in-house PCR assays, a sample volume of 10 μl, corresponding to 100,000 PBMCs, was amplified in a total volume of 50 μl (3, 8). After nesting and a second amplification, the amplimer was visualized with ethidium bromide in an agarose gel. Primer sets OG228-OG572 (*gag*) (9), OG154-OG197 (*pol*) (8), OG462-OG502 (*vif*) (8), and OG7253-

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TABLE 1. Sensitivity of different primer sets for detection of HIV-1 DNA in FIP and Amplicor whole blood sample preparations

PCR assay	Primer pair	No. of samples reactive/no. tested (% reactive)	
		FIP	Amplicor
In-house nested	OG154-OG197	46/53 (87)	60/64 (94)
In-house nested	OG288-OG572	37/53 (70)	44/54 (81)
In-house nested	OG462-OG502	46/53 (87)	62/64 (97)
In-house nested	OG7253-OG7541	47/53 (89)	60/64 (94)
In-house nested	JA79-JA82	49/53 (92)	62/64 (97)
In-house nested	JA171-JA174	48/53 (91)	55/64 (86)
Amplicor standard	SK431-SK462	NT[a]	38/64 (59)
Amplicor modified	SK431-SK462	NT	63/64 (98)

[a] NT, not tested.

OG7541 (*env*) (9) have been presented earlier. Sequences of primer set JA79-JA82 (*pol*) were as follows: outer sense, 5′-ACAGGAGCAGATGATACAGTATTAG-3′; inner sense, 5′-GAAGATGGAAACCAAAAATGATAGG-3′; inner antisense, 5′-CAATTATGTTGACAGGTGTAGGTCC-3′; and outer antisense, 5′-CCTGGCTTTAATTTTACTGGTACAG-3′. Primer set JA171-JA174 (*pol*) was as follows: outer sense, 5′-CCCCAAAGTC AAGGAGTAGTAGAA-3′; inner sense, 5′-CTTAGGACAGCA GTACAAATGGCAG-3′; inner antisense, 5′-TGCTGTCCCTG TAATAAACCCGAAA-3′; outer antisense, 5′-TACTACTGC CCC TTCACCTTTCCA-3′.

The standard Amplicor HIV-1 PCR test (Roche Diagnostic Systems, Somerville, N.J.) was done in accordance with the manufacturer's instructions. Briefly, 50 μl of sample in a total volume of 100 μl was amplified with biotinylated primers SK431 and SK462. The amplified products were hybridized to a specific nucleic acid probe, immobilized on microwell plates, and detected by color formation. In the modified Amplicor PCR, only 25 μl of a test or control sample was amplified in a final volume of 100 μl (18). The annealing temperature was changed to 50°C in all 35 cycles. All other parameters were identical to those of the standard Amplicor PCR.

Recommended laboratory routines were strictly followed to avoid contamination in the PCR, and relevant controls were included in each analysis (12). The detection limits for the Amplicor and the JA in-house PCR systems have been shown to be ≤10 copies of HIV-1 (4, 10).

Fifty-nine FIP preparations (from 53 HIV-1 seropositive and 6 seronegative subjects) and 86 Amplicor preparations (from 64 HIV-1 seropositive and 22 seronegative subjects) were subjected to PCR amplification with the various OG and JA primers. Fifty-two and six of the samples from seropositive and seronegative individuals, respectively, were prepared by both PCR sample preparation methods. None of the samples from HIV-1-seronegative individuals were found to be positive by any of the in-house PCR assays used with both preparation methods.

The sensitivities of the in-house nested PCR systems with the samples prepared by the two methods are shown in Table 1. For each of the primer sets evaluated, the sensitivity was higher with the Amplicor preparations than with samples prepared by the FIP method. The highest sensitivity for the detection of HIV-1 DNA (97%, 62 of 64) was shown when the OG462-OG502 or the JA79-JA82 primers were used with the Amplicor preparations. Of the 53 FIP samples from HIV-1-seropositive individuals tested, 31 were PCR positive with each of the OG and JA primer sets, 21 had discordant primer pair results, and 1 was PCR negative with all of the primer sequences. This single HIV PCR-negative sample and all of the other samples were reactive when amplified with primers specific for the human β-globin gene. Of the 64 samples from HIV-1-seropositive subjects that were extracted with the Amplicor sample preparation procedure, 45 were PCR positive with each of the primer pairs tested (OG, JA, and Amplicor in the modified version) and 19 had discordant PCR results. With the Amplicor PCR sample preparation method, HIV-1 DNA was detected by all primers in the HIV-1-seropositive individual whose FIP PBMC sample was found to be PCR negative.

The combined sensitivities of the OG462-OG502 and OG7253-OG7541 primer sets were 96% (51 of 53) and 100% (64 of 64) for HIV-1 DNA detection with the FIP and Amplicor sample preparation methods, respectively. The sensitivities obtained by combining the JA79-JA82 and JA171-JA174 primer sets were 92% (49 of 53) and 98% (63 of 64) for HIV-1 DNA detection with the FIP and Amplicor PBMC sample preparation methods, respectively.

Of the 64 samples from HIV-seropositive individuals extracted by the Amplicor sample preparation method, only 38 (59%) were positive by the standard Amplicor HIV-1 test. The sensitivity of the standard Amplicor PCR test was not significantly different when samples from individuals with low or high $CD4^+$ T-lymphocyte counts were tested (Table 2). Modification of the Amplicor test increased the sensitivity to 98% (63 of 64). One Amplicor PCR sample from a seropositive individual with a $CD4^+$ T-lymphocyte count of 245 cells/μl gave a false-negative PCR result in the modified Amplicor test but was PCR positive when amplified by the OG154-OG197, OG228-OG572, and JA79-JA82 primer sets.

Of 22 samples from HIV-seronegative individuals extracted by the Amplicor sample preparation method, 1 was reactive by the standard Amplicor test, while all 22 samples were negative by the modified Amplicor test and by all six in-house PCR systems.

To ensure that PCR results are reliable throughout the world, PCR primer sequences need to be chosen in regions of the genome which are conserved among all subtypes. This study shows the need to evaluate the sensitivities of different PCR systems by using samples from geographic regions like sub-Saharan Africa, where HIV-1 strain diversity is marked.

Results obtained in the present study show that the OG154-OG197, OG462-OG502, OG7253-OG7541, and JA79-JA82 primer sets evaluated have high sensitivities for HIV-1 DNA detection in PCR samples from Tanzania, especially when they are prepared by the Amplicor nucleic acid extraction method. Combining two primer pairs further increased the sensitivity of detection. Thus, we agree with other workers who have also recommended that more than one region should be used as targets for amplification (7, 16, 23).

An important finding in the present study is the observation that using PBMCs prepared by the Amplicor method gave better PCR results for all primers than using PBMCs separated by the conventional FIP method. The reason for this difference

TABLE 2. Sensitivity of the standard Amplicor PCR assay for detection of HIV-1 DNA in samples from HIV-seropositive individuals with different levels of $CD4^+$ T lymphocytes

$CD4^+$ T-lymphocyte count (cells/μl)	No. of samples reactive/no. tested	% Reactive
0–199	4/7	57.1
200–499	9/19	47.4
≥500	16/27	59.3

is not clear. In our experience, repeated lysis and washing of African blood samples is necessary when using the fluorescence-activated cell sorter lysing solution and this might affect the PCR samples prepared by the FIP method. The Amplicor whole blood preparation method for PCR samples is simple and fast, and we did not encounter any problems with lysis of the erythrocytes in the samples analyzed.

In the present study, a low sensitivity (38 of 64, 59%) was obtained when the Amplicor HIV-1 PCR test was done in accordance with the manufacturer's instructions. Other investigators have reported sensitivities of the standard Amplicor PCR test ranging from 93 to 100% in different study populations (7, 11, 24). In one of these studies (7), it was observed that African samples were more frequently false negative than European samples. Whereas a low virus titer may give false-negative PCR results (25), it is unlikely that a low proviral copy number is the only explanation for the false-negative results in the present study, since many patients with low CD4 counts, who are likely to have a high viral load, were also falsely negative. One of the Amplicor primers, SK462, has several mismatches with some African HIV-1 sequences, most notably those classified as subtype A. Therefore, the low sensitivity of the standard Amplicor test could be due to a primer mismatch. Information about HIV-1 subtypes in Tanzania is scanty, but subtypes A and D seem to predominate (3a, 22). In a previous evaluation (7), the Amplicor PCR test was shown to be capable of amplifying HIV-1 subtypes A, B, D, G, and H, but the sensitivity in relation to subtypes was not reported. We are currently investigating if the failure of the standard Amplicor test can be related to the HIV-1 subtype.

The higher sensitivity of the modified Amplicor assay was achieved by lowering the annealing temperature and decreasing the sample input. Theoretically, more unspecific binding of primers might occur when amplifying with lower stringency (i.e., at a reduced annealing temperature). However, in this study no lowering of specificity was seen when the modified protocol was used.

The present study shows that the nested in-house PCR is as sensitive as the modified Amplicor test. The nested PCR has a lower reagent cost but is somewhat more labour intensive than the Amplicor test.

In conclusion, PCR samples prepared by the Amplicor whole blood preparation method are more suitable for use in HIV-1 PCR testing than PBMCs separated by the conventional FIP gradient centrifugation method. The simple procedure involved in the Amplicor method also makes it a better procedure for the preparation of PCR samples. The modified Amplicor HIV-1 PCR test is sensitive, is easy to perform, gives rapid results, enables processing of multiple samples, and therefore provides an alternative to the conventional in-house PCR techniques for the diagnosis of HIV-1 infection.

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