Evaluation of Four Simple/Rapid Assays and Two Fourth-Generation ELISAs for the Identification of HIV Infection on a Serum Panel Representing the HIV-1 Group M Genetic Diversity in Cameroon

Avelin Fobang Aghokeng, MSc,† Léonard Ewane, BSc,* Bih Awazi, BSc,* Aubin Nanfack, BSc,* Eric Delaporte, MD, PhD,† Martine Peeters, PhD,† and Léopold Zekeng, MD**

Summary: The performance of 4 rapid and simple assays: Camstix-HIV 1+2 (Camdiagnostix, Yaoundé, Cameroon); Determine HIV 1+2+0 (Abbott Laboratories, Tokyo, Japan); Genie II HIV-1/HIV-2 (Bio-Rad, Marnes la Coquette, France); ImmunoComb II HIV 1 & 2 BiSpot (Orgenics, Yavne, Israel); and 2 fourth-generation ELISAs: Enzygnost HIV Integral (Dade Behring, Marburg, Germany) and Genscreen plus HIV Ag-Ab (Bio-Rad, Marnes la Coquette, France) currently used in Cameroon to detect HIV infections were evaluated on a local serum panel. A total of 503 samples were collected, using the Camstix-HIV 1+2 assay. Overall, 280 samples were confirmed HIV positive, 181 were negative, and 42 were indeterminate. All positive samples belonged to group M: CRF02_AG (73.5%), A1 (7.1%), A2 (1.2%), G (4.7%), F2 (5.1%), D (1.6%), CRF11 (1.6%), CRF06 (1.2%), and CRF01_AE (1.6%). Sensitivity, specificity, test efficiency, and positive and negative predictive values were calculated both including and excluding indeterminate samples. Except for Genie II and ImmunoComb II (98.9 and 99.3%, respectively), sensitivities were 100% for the remaining 4 tests. Specificities, efficiencies, and positive predictive values of all assays were negatively affected by the addition of HIV-indeterminate samples in the calculations. These data show the importance of prior test evaluations on local serum panels and in field conditions before a national policy for HIV screening is decided on and stress also the need to use tests and algorithms that can reduce the high number of HIV-indeterminate results in Africa.

Key Words: HIV, anti-HIV antibody detection, simple/rapid assays, ELISA, mutations

(*J Acquir Immune Defic Syndr* 2004;37:1632–1640)

The HIV type 1 genome exhibits an extraordinary degree of variability mainly due to the error-prone and recombinogenic nature of the viral reverse transcriptase enzyme and the fast turnover of virions.[1,2] The genetic characterization of many HIV isolates obtained from diverse geographic localities led to the classification of HIV-1 into 3 groups: M as major, O as outlier, and N as non-M/non-O.[3,4] Within group M, HIV-1 is also subdivided into 9 subtypes (A–D, F–H, J, and K) and 15 circulating recombinant forms (CRFs).[5] Group O is characterized by several heterogeneous viruses,[6] but group N is identified in only a limited number of individuals.[7] This genetic classification has led to increased understanding of the molecular evolution of HIV-1 and the geographic distribution and patterns of spread of HIV strains.

Cameroon and neighboring countries in Central Africa are characterized by the co-circulation of a large number of HIV variants among its population; all 3 HIV-1 groups, almost all subtypes, and at least 5 CRFs have been documented.[8–12] Cameroon is the only African country where group M, N, and O HIV-1 strains co-circulate, and group O and N were first discovered in this country, respectively, in 1994[3,13] and 1998.[4] The overall HIV prevalence in the general adult population in urban areas has increased significantly from 0.5% in 1987 to 11% in 2000.[14] Similarly as to urban areas, several recent studies have reported a high prevalence and genetic diversity of HIV-1 in rural Cameroon.[11,15]

The identification of group O, group N, and intergroup M/O recombinants in Cameroon[16–18] illustrates the necessity of a continuous monitoring of circulating HIV strains in this region.

As part of the efforts to reduce the transmission of HIV, there is a need for reliable diagnostic assays, capable of ensuring the correct identification of infected individuals and the safety of blood transfusion.[19] The identification of HIV-1 group O demonstrated the weakness of certain assays to diagnose these strains and led to the development of more sensitive and specific assays, with either the inclusion of HIV group O–specific antigens or by using broadly cross-reacting HIV antigens.[20] In addition, it has been shown that most of the commercially available third-generation HIV antibody assays are less efficient at detecting early seroconversions in non-B-infected individuals as compared with B. The introduction of p24 antigen in HIV fourth-generation detection assays has

Received for publication December 12, 2003; accepted April 5, 2004.
From the *Laboratoire de Santé Hygiène Mobile, Yaoundé, Cameroon; and †UMR 145, IRD, Université de Montpellier I, Montpellier, France.
Supported by the Agence Nationale de Recherche sur le SIDA (ANRS), France.
Reprints: Martine Peeters, Laboratoire Retrovirus, IRD, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France (e-mail: martine.peeters@mpl.ird.fr).

considerably reduced the diagnostic window.[21,22] The availability of simple/rapid and low-cost assays is now facilitating access to diagnosis in resource-poor settings.[23–25] Despite all these efforts, an ongoing evaluation of performance of HIV assays remains important, because genetic subtype distribution is a dynamic process. It is also important to evaluate the tests on a panel of African sera and in field conditions.

Since the majority of HIV serologic assays rely on antibody responses to the structural proteins of the virus, especially the envelope glycoprotein gp41, mutations observed in this domain, especially in the immunodominant region (IDR) composed of the CTL epitope (LAVERYLKDQQLL) and the cysteine loop (CSGKLIC), could affect the sensitivity of serologic assays.[26,27] Many studies have reported several amino acid mutations in the gp41 domain among all subtypes of HIV-1 group M viruses, as well as group O and group N.[28,29] The aim of our study was to evaluate selected HIV diagnostic assays currently used in Cameroon on a panel of HIV-positive and -negative sera, representing the predominant viral strains and monitor the impact of mutations within the IDR of the gp41 domain on antibodies detection.

MATERIALS AND METHODS

Sample Collection

The strategy defined by the Cameroonian National AIDS Committee for most of the public hospitals is to screen all blood donations for the presence of HIV antibodies by a simple and rapid test, Camstix-HIV 1+2 (Camdiagnostix, Yaoundé, Cameroon), a test partially manufactured in Cameroon. To further evaluate the efficiency of other commonly used HIV screening assays, a serum panel of HIV-positive and -negative samples was constituted in 2001, among blood donors attending blood banks in 7 distinct localities: Yaoundé (Center Province) (n = 211), Douala (Littoral Province) (n = 164), Bamenda (North-West Province) (n = 36), Baffousam (West Province) (n = 29), Bertoua (East Province) (n = 25), Limbé (South West Province) (n = 22), and Ebolowa (South Province) (n = 16). A total of 503 (306 reactive and 197 nonreactive) samples were colleted, using the Camstix-HIV 1+2 assay without any discrimination in age, sex, marital status, or occupation. Samples were shipped in coolers to the Laboratoire de Santé Hygiéne Mobile at Yaoundé and stored at +4 ± 2°C for a maximum of 5 days.

For all samples, plasma was separated, aliquoted, and stored at −20°C. Peripheral blood mononuclear cells (PBMCs) were also isolated from the remaining blood on Ficoll gradients (Amersham Pharmacia Biotech, Uppsala, Sweden). Each PBMC sample was aliquoted and stored in liquid nitrogen.

Serologic Testing and Evaluation

Plasma samples were tested with a total of 4 rapid and simple tests and 2 fourth-generation enzyme-linked immunosorbent assays (ELISAs; tests based on the simultaneous detection of antigens and antibodies). The following rapid tests were used: Camstix-HIV 1+2 (Camdiagnostix, Yaoundé, Cameroon), Determine HIV 1+2+0 (Abbott Laboratories, Tokyo, Japan), Genie II HIV-1/HIV-2 (Bio-Rad, Marnes la Coquette, France), and ImmunoComb II HIV 1 & 2 BiSpot (Orgenics, Yavne, Israel). Enzygnost HIV Integral (Dade Behring, Marburg, Germany) and Genscreen plus HIV Ag-Ab (Bio-Rad, Marnes la Coquette, France) were the 2 ELISAs evaluated. All assays were performed according to the manufacturers' protocols, and their characteristics are summarized in Table 1.

Samples nonreactive in all assays were considered as HIV negative. Samples with discordant results among the different HIV assays were further tested with a confirmation assay; Inno-Lia HIV Confirmation (Innogenetics, Gent, Belgium) or HIV Blot 2.2 (Genelabs Diagnostics, Singapore Science Park, Singapore). All samples classified as HIV positive and HIV indeterminate were further genetically characterized in gp41 and other regions of the genome, to identify subtypes and confirm presence of HIV, respectively. Samples reactive in all assays were also tested with a competitive enzyme immunoassay (EIA), Wellcozyme HIV recombinant (Abbott Laboratories, Abbott Park, IL) to discriminate between HIV-1 group M and O based on optical density (OD) ratios as previously described.[30,31]

DNA Extraction, PCR, and Sequencing Reactions

Polymerase chain reactions (PCR) were performed on all samples that reacted with ≥1 of the HIV screening assays. Proviral DNA was extracted from uncultured PBMCs with the QIAamp Viral DNA Mini Kit Handball (QIAGEN, Courtaboeuf, France). The gp41 region of the *env* gene was amplified by nested PCR with the following primers: gp40F1 (forward) 5′-TCTTAGGAGCAGCAGGAAGCACTATGGG-3′ (nucleotides [nt] 7789-7816) and gp41R1 (reverse) 5′-AACGACAAAGGTGAGTATCCCTGCCTAA-3′ (nt 8347-8374) used as outer primers and gp46F2 (forward) 5′-ACAATTATTGTCTGGTATAGTGCAACAGCA-3′ (nt 7850-7879) plus gp48R2 (reverse) 5′-TCCTACTATCATTATGAATATTTTTATATA-3′ (nt 8265-8294) as inner primers. These primers were previously described by Yang et al[32,33] as highly sensitive and specific for the detection of HIV type 1 groups M, N, and O and simian immunodeficiency viruses (SIV) from chimpanzees. Using these primers, a final fragment of about 460 bp spanning 40% of the gp41 region, including the IDR, was expected from as few as 1–5 copies of viral DNA.[29] First-round PCR was carried out in a final volume of 50 μL containing 1X GeneAMP PCR buffer II, 1.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate, 20 pmol of each primers, 2.5 U of Ampli*Taq* DNA polymerase (Perkin-Elmer Cetus, Foster City, CA), 5 μL of the DNA extract, and finally distilled water. After initial denaturation at 94°C for 2 minutes, 35 cycles were performed at 94°C for 15 seconds, 50°C for 30 seconds, and 72°C for 1 minute, with a final extension step at 72°C for 5 minutes. The second round was done in a final volume of 100 μL with the same concentration of reagents excepted for Ampli*Taq* DNA polymerase 2.6 U and the first-round PCR product: 3 μL. Only 30 cycles were performed during this second round. The nested PCR products were electrophoresed in 1% agarose gels with an appropriate bp marker and visualized under ultraviolet light by ethidium bromide staining.

TABLE 1. Characteristics of the HIV Diagnostic Assays Evaluated as Described by Manufacturers

Number	Test Name	Manufacturer	Test Type	Antigen/Antibody Type	Solid Phase	Local Price (US$)
Rapid assays						Per test
1	Camstix-HIV 1+2	I.M.P.M/Camdiagnostix (Yaoundé, Cameroon)	Dot immunoassay	Synthetic peptides	Polystyrene comb	1.5
2	Determine HIV 1/2	Abbott Laboratories (Tokyo, Japan)	Immunochromatographic assay	Recombinant antigens and synthetic peptides	Membrane	2.5
3	Genie II HIV-1/HIV-2	Bio-rad (Marnes la Coquette, France)	Immunochromatography assay and immunoconcentration	Recombinant antigens and synthetic peptides	Membrane	4
4	ImmunoComb II HIV 1&2	Orgenics (Yavne, Israel)	Dot immunoassay	Synthetic peptides	Polystyrene comb	3.7
ELISAs						
5	Enzygnost HIV Integral	Dade Behring (Marburg, Germany)	Sandwich ELISA	Synthetic peptides, recombinant protein, and polyclonal antibodies	Microplate	4
6	Genscreen plus HIV Ag-Ab	Bio-rad (Marnes la Coquette, France)	Sandwich ELISA	Synthetic peptides, recombinant protein, and monoclonal antibodies	Microplate	3.5
Discriminatory assay						
7	Wellcozyme HIV Recombinant	Abbott murex (Abbott Park, IL)	Competitive ELISA	Recombinant protein	Microplate	3.5
Confirmation assays						
8	Inno-Lia HIV Confirmation	Innogenetics (Gent, Belgium)	Blot	Synthetic peptides and recombinant protein	Nylon	13.5
9	HIV Blot 2.2	Genelabs Diagnostics (Singapore Science Park, Singapore)	Blot	Viral proteins and synthetic peptides	Nitrocellulose strip	26.6

On HIV-positive or indeterminate samples for which gp41 could not be amplified, the glucose-6-phosphate dehydrogenase (G6PDH) gene was amplified to verify the DNA quality. When G6PDH PCRs were positive, we attempted to amplify a more conserved region of the HIV genome using the previously described diagnostic HPOL primers.[34] For samples that remained negative in this assay, more universal PCR primers known to amplify a large variety of HIVs and SIVs in the *pol* gene were used.[35,36]

The amplified gp41 fragments were purified with the QIAquick gel extraction kit (QIAGEN) and directly sequenced with the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Orsay, France), using an automated sequencer (373A stretch model, Applied Biosystems, Foster City, CA).

Phylogenetic and Sequence Analyses

Nucleotide sequences were aligned using CLUSTAL W with minor manual adjustments, bearing in mind the protein sequences.[37] Sequences that could not be aligned unambiguously, due to length or sequence variability, were omitted from the analysis. Phylogenetic trees using the neighbor-joining method and reliability of the branching orders using the bootstrap approach were implemented with CLUSTAL W. Genetic distances were calculated with the Kimura 2-parameter method (ratio T/T = 2.0). The newly determined HIV-1 *env* sequences were aligned with the known HIV-1 sequences representing the different genetic subtypes, and reference strains from the circulating recombinant forms (CRFs) documented in West and West Central Africa (CRF01_AE, CRF02_AG, CRF06_CPX, CRF11_CPX, and CRF13_CPX). To clearly identify whether a sequence belonged to a subgroup corresponding to a CRF within a certain subtype, phylogenetic analysis was done for each sequence individually. Then, different trees were constructed for each group of new sequences that were thought to cluster together, and finally a general tree was obtained to visualize all the results. The clustering of each new sequence was compared and should be concordant between all trees. The reference strains used were indicated in the legend of phylogenetic trees.

The amino acid (AA) sequences deduced from the fragments corresponding to the IDR were compared with the subtype B prototype sequence [= LAI prototype strain: LAVERYLKDQQLLGIWGCSGKLIC (AA 581–604)] to analyze the mutations that occurred in non-B HIV-1 strains from our serum panel and check whether these mutations could be associated with reduced sensitivity of anti-HIV antibody detection assays.

Analysis of the Performance of the Serologic Assays

The sensitivity, the specificity, the efficiency, and the predictive values were calculated for each test. Sensitivity was calculated as the number of HIV-positive samples detected by the assay under evaluation, divided by the total number of confirmed HIV-positive samples, multiplied by 100. Specificity

was calculated as the number of HIV-negative samples detected by the assay under evaluation, divided by the total number of confirmed HIV-negative samples, multiplied by 100. The efficiency was calculated as the number of HIV-negative and positive samples correctly detected by the test, divided by the total number of HIV-positive and negative samples detected plus the false results, multiplied by 100. The 95% CLs of the estimated sensitivities, specificities, and efficiencies were calculated using the formula: $P \pm 1.96 \sqrt{P(1-P)/n}$, where "P" is the sensitivity, the specificity, or the efficiency and "n" is the number of specimens tested. For sensitivities, specificities and efficiencies of 100%, a value corresponding to 99.9% was assigned to "P" in this equation.

The positive and negative predictive values (PPV and NPV) were determined for each assay based on the highest and the lowest estimated Cameroonian HIV prevalences at year 2000.[38,39]

Sensitivity, specificity, test efficiency, and PPV and NPV values were calculated both including and excluding indeterminate samples. When included, the indeterminate samples were assumed to represent negative samples, and hence reactivity to represent false positives.

RESULTS

Serum Panel

A total of 503 samples, obtained from 7 different geographic localities in Cameroon, were tested with the 4 rapid and simple assays and the 2 ELISAs described above. Among the 503 samples, 181 were negative in all assays and were therefore considered as HIV-negative samples; 280 samples were considered as HIV-positive because they were reactive in all screening assays (n = 277), or gave discordant results in the screening assays and were further confirmed positive by an HIV confirmatory assay (n = 3). All positive samples were also tested with the HIV-1 competitive ELISA (Wellcozyme) and were identified as HIV group M according to the OD ratios.

Importantly, the remaining 42 samples representing 8.4% of the serum panel were considered as indeterminate because they were not reactive in all screening assays and could not be confirmed as HIV positive. All of them had antibodies to ≥1 HIV antigens but did not fulfill criteria of HIV positivity in Inno-Lia or Western blot. In none of these HIV-indeterminate samples could proviral HIV DNA be amplified with diagnostic HPOL PCR, known to have a high sensitivity (>93%) on a large panel of HIV variants including divergent HIV-1 O, N, and SIVcpz strains.[34] In addition, we tested these samples also with universal HIV/SIV *pol* primers known to amplify a large variety of HIV/SIV strains. These 42 samples were then further tested by the G6PDH PCR to check the DNA quality, and only for 6 samples, DNA was degraded. In addition, all the indeterminate samples were negative in the competitive HIV screening assay previously used to discriminate between HIV-1 group O and M.

Performance of HIV Screening Assays

Samples with an indeterminate HIV serology are often reported in Africa and especially in Central Africa. However, indeterminate sera are usually excluded from calculations of sensitivity, specificity, etc. Since our data and previous reports showed that such sera represent an important proportion of samples in Africa,[23,25] we studied the performance of the HIV screening assays according to 2 different scenarios: one without the indeterminate sera and another where we included the HIV-indeterminate sera, which we added to the number of HIV-negative samples due to the absence of proviral HIV DNA. Tables 2A and B show the specificities, the sensitivities, the efficiencies, and the predictive values of the different assays, respectively, with and without inclusion of the HIV indeterminate samples.

Sensitivity

The sensitivity of all assays was by definition identical in both scenarios. Camstix, Determine, and the 2 ELISAs detected all HIV-positive sera with a 100% sensitivity. Genie II and ImmunoComb II did not detect 3 and 2 HIV-positive sera, respectively, resulting in sensitivities of 98.9 and 99.3%, respectively.

Specificity

We first evaluated specificity excluding the 42 indeterminate sera (a best-case analysis). Genie II showed the highest specificity of 100.0%. ImmunoComb II misidentified 1 HIV-negative sample as positive, thus reducing its specificity to 99.5%. The specificities of Camstix, Determine, and Enzygnost HIV Integral were not statistically different from that of ImmunoComb II. In contrast, the specificity of Genscreen plus HIV Ag-Ab was only 95.0%, illustrating the high number of false positives yielded by this test. Overall, Determine, Camstix, Enzygnost HIV Integral, and Genscreen plus HIV Ag-Ab identified all HIV positives, but all yielded different levels of false-positive results. Genie II did not detect 3 HIV-positive samples but did not give any false-positive results. False-negative as well as false-positive results were observed only with the ImmunoComb II assay.

The inclusion of indeterminate samples in our calculations considerably reduced the specificity of all assays (Table 2B). Indeed, the 100.0% specificity of Genie II dropped to 98.2%. The specificities of Camstix, Determine, and Enzygnost HIV Integral also dropped significantly to 88.3, 90.6, and 92.3%, respectively. The Genscreen plus HIV Ag-Ab assay was most affected by the addition of indeterminate samples, because its specificity decreased to 82.5%. This important decrease in specificity of Camstix, Determine, Enzygnost HIV Integral, and Genscreen plus HIV Ag-Ab was due to the fact that being more sensitive, they identified the majority of the indeterminate samples as HIV reactive.

Efficiency

In the first scenario, the overall assay efficiency was >98.1%. The most efficient assay was Enzygnost HIV Integral, with an efficiency of 99.8%. However, as for the specificity, the efficiency of all assays decreased with the addition of indeterminate samples, especially for Camstix, Determine, Enzygnost HIV Integral, and Genscreen plus HIV Ag-Ab, whose specificities decreased significantly (Tables 2A and B).

TABLE 2A. Performance of Tests Evaluated, Without Indeterminate Samples

Test Number*	TSIC	Positive With Test	Negative With Test	True Positive	True Negative	False Positive	False Negative
Rapid assays							
1	461	283	178	280	181	3	0
2	461	283	178	280	181	3	0
3	461	277	184	280	181	0	3
4	461	278	182	280	181	1	2
ELISAs							
5	461	281	180	280	181	1	0
6	461	289	172	280	181	9	0

Test Number*	Sensitivity %	Specificity %	Efficiency %	Positive Predictive Values (%)		Negative Predictive Values (%)	
Rapid assays				11†	7†	11†	7
1	100.0 (99.6–100.0)	98.3 (97.1–99.5)	99.4 (98.7–100.0)	94.4	91.5	100	100
2	100.0 (99.6–100.0)	98.3 (97.1–99.5)	99.4 (98.7–100.0)	94.4	91.5	100	100
3	98.9 (98.0–99.8)	100.0 (99.6–100.0)	99.4 (98.7–100.0)	100	100	99.3	99.3
4	99.3 (98.5–100.0)	99.5 (98.9–100.0)	99.4 (98.7–100.0)	98.1	97.5	99.6	99.6
ELISAs							
5	100.0 (99.6–100.0)	99.4 (98.7–100.0)	99.8 (99.4–100.0)	98.2	97.2	100	100
6	100.0 (99.6–100.0)	95.0 (93.0–97.0)	98.1 (96.9–99.3)	85.3	78.7	100	100

*Test number as described in Table 1.
†11 and 7% are, respectively, the highest and the lowest HIV prevalences reported in Cameroon at year 2000.
TSIC, total samples included in the calculations.

Positive and Negative Predictive Values

Positive and negative predictive values were calculated taking into account the lowest and the highest HIV prevalence estimations in year 2000 in Cameroon.[38,39] With the exclusion of indeterminate samples, the PPVs varied between 85.3 and 100% in a population with 11% HIV prevalence. The lowest value was obtained with Genscreen plus HIV Ag-Ab and the best PPV was seen for the Genie II assay. As expected, the higher the HIV infection prevalence in the population, the greater the probability that a person with a reactive test result is

TABLE 2B. Performance of Tests Evaluated, Including Indeterminate Samples

Test Number*	TSIC	Positive With Test	Negative With Test	True Positive	True Negative	False Positive	False Negative
Rapid assays							
1	503	306	197	280	181	26	0
2	503	301	202	280	181	21	0
3	503	281	222	280	181	4	3
4	503	284	219	280	181	6	2
ELISAs							
5	503	297	206	280	181	17	0
6	503	319	184	280	181	39	0

Test Number*	Sensitivity %	Specificity %	Efficiency %	Positive Predictive Values (%)		Negative Predictive Values (%)	
Rapid assays				11†	7†	11†	7
1	100.0 (99.6–100.0)	88.3 (85.5–91.1)	89.7 (87.0–92.4)	67.9	57.4	100	100
2	100.0 (99.6–100.0)	90.6 (88.0–93.1)	91.7 (89.2–94.1)	72.4	62.5	100	100
3	98.9 (98.0–99.8)	98.2 (97.0–99.4)	98.4 (97.3–99.5)	92.9	88.9	99.3	99.4
4	99.3 (98.6–100.0)	97.4 (96.0–98.8)	97.6 (96.3–98.9)	89.8	84.6	99.5	99.6
ELISAs							
5	100.0 (99.6–100.0)	92.3 (90.0–94.6)	93.2 (91.0–95.4)	76.4	67.3	100	100
6	100.0 (99.6–100.0)	82.5 (79.2–85.8)	84.5 (81.3–87.7)	59.1	47.9	100	100

*Test number as described in Table 1.
†11 and 7% are, respectively, the highest and the lowest HIV prevalences reported in Cameroon at year 2000.
TSIC, total samples included in the calculations.

truly infected; therefore PPVs decreased when considering the HIV prevalence of 7%.

The addition of indeterminate samples in the calculations reduced the PPVs significantly. In this scenario, PPVs varied between 59.1 and 92.9% at the HIV prevalence of 11% and between 47.9 and 88.9% at the HIV prevalence of 7%. Overall, NPVs were very high (99.3–100%) and were not affected either by the HIV prevalence or by the addition of indeterminate samples.

Genetic Characterization of the gp41 Transmembrane Region

The gp41 transmembrane region was amplified and sequenced for 253 of the 280 samples considered as HIV positive. For 7 of the remaining 27 samples, PCRs were negative because DNA was degraded, and 20 could not be amplified for unexplained reasons.

Identification of Genetic Subtypes in gp41

Phylogenetic tree analysis of all the isolates together showed that no laboratory contamination had occurred (data not shown). All isolates were of HIV-1 group M and represent the following subtypes and CRFs: 18 (7.1%) A1; 3 (1.18%) A2; 10 (3.95%) D; 13 (5.13%) F2; 12 (4.74%) G; 186 (73.5%) CRF02_AG; 4 (1.58%) CRF11_cpx; 3 (1.18%) CRF06_cpx and 4 (1.58%) CRF01_AE. Table 3 summarizes the subtype distribution in each province from which samples were collected. Although for some regions the number of samples was limited, CRF02-AG predominated in each region and represented 63–80% of the samples. Overall, 9 HIV-1 variants (subtypes or CRFs) co-circulate, and in each region, except in the south where only 15 samples were analyzed, at least 5 HIV-1 group M variants co-circulate. Compared with previously published studies on HIV-1 group M variants, our panel is representative for the strains that circulate in Cameroon.

Amino Acid Sequence Analysis

Comparison of the individual AA sequences from the IDR with the HIV-1 subtype B consensus sequence showed that 37 isolates (14.6%) were conserved in this domain while 216 sequences (85.4%) carried 1–5 AA substitutions. One AA change was found in 78 (30.8%) isolates, 2 AA changes were found in 101 (39.9%), 3 AA changes in 30 (11.9%), and 4 to 5 AA changes in 7 (2.8%) isolates. All these substitutions were minor AA substitutions with no impact on the protein active conformation: $V^{583} \rightarrow$ *L or I (85%);* $R^{585} \rightarrow$ *G, S, A, K or F (10%);* $K^{588} \rightarrow$ *R, G, Q, M, E or H (55%);* $Q^{591} \rightarrow$ *K or R (3%)* and $L^{592} \rightarrow$ *I or F (6%)* in the CTL epitope (AA 581–593), and $S^{599} \rightarrow$ *A or T (1%);* $K^{601} \rightarrow$ *R or I (3%);* $L^{602} \rightarrow$ *I, R or H (11%)* in the cysteine loop (AA 598–604). No major AA substitution leading to conformational changes of the gp41 protein was found. Figure 1 summarizes the different AA substitutions recorded in the CTL epitope and in the cysteine loop.

Effect of AA Substitutions on Serologic Detection

All the 216 specimens with single or multiple AA substitutions were anti-HIV antibody positive with all the assays previously evaluated, except for 3 samples that were not detected by Genie II or ImmunoComb II. However, the minor AA substitutions found in the gp41 IDR of these samples were also found in the gp41 IDR of several other samples that were correctly detected as HIV positive by Genie II and ImmunoComb II. Therefore, the minor AA substitutions observed within the CTL epitope and the cysteine loop had no impact on antibody detection of HIV-1 group M–positive sera.

DISCUSSION

A high HIV genetic diversity has been reported in urban and rural areas from Cameroon in several studies.[9,11,12,15,17,40,41] We here confirmed this high genetic variability of HIV-1 and the co-circulation in Cameroon of almost all HIV-1 group M subtypes, with the predominance of CRF02-AG-like viruses. HIV diversity in Cameroon is also characterized by the existence of 2 of the most divergent HIV-1 strains: HIV-1 group O and group N.[4,13] The low prevalence of these variants[12] could explain their absence in our serum panel. Alternatively, the preselection of samples by Camstix assay could have biased their representation in the panel, although in that case, possible false-negative group O or group N samples not detected with Camstix would have been reactive in at least one of the other tests used. However, the distribution of group M variants is similar to that previously reported in several studies, and therefore our panel can be considered as representative of the group M variants circulating in Cameroon.

To control the further spread of HIV infection, reduction of new HIV-infected cases is one of the main objectives of the Cameroonian National AIDS Committee. The achievement of

TABLE 3. Genetic Diversity of the gp41 Region of HIV-1 Group M in Distinct Cameroonian Provinces

	HIV-1 Group M Subtypes and CRFs									
Provinces	**CRF02_AG**	**A1**	**A2**	**G**	**F2**	**D**	**CRF11_cpx**	**CRF06_cpx**	**CRF01-AE**	**Total Province**
Littoral	77 (77.8%)	5 (5.1%)	0	6 (6.1%)	5 (5.1%)	3 (3.0%)	1 (1.0%)	0	0	99
Centre	27 (64.3%)	2 (4.8%)	2 (4.8%)	2 (4.8%)	3 (7.1%)	5 (11.9%)	1 (2.4%)	2 (2.0%)	0	42
West	14 (63.6%)	2 (9.1%)	0	1 (4.5%)	4 (18.2%)	0	0	0	1 (4.5%)	22
North-West	20 (69.0%)	4 (13.8%)	0	1 (3.4%)	1 (3.4%)	1 (3.4%)	0	0	2 (6.9%)	29
South-West	16 (76.2%)	1 (4.8%)	1 (4.8%)	2 (9.5%)	0	1 (4.8%)	0	0	0	21
South	12 (80.0%)	3 (20.0%)	0	0	0	0	0	0	0	15
East	20 (80.0%)	1 (4.0%)	0	0	0	0	2 (8.0%)	1 (4.0%)	1 (4.0%)	25
Total	186 (73.5%)	18 (7.1%)	3 (1.2%)	12 (4.7%)	13 (5.1%)	10 (4.0%)	4 (1.6%)	3 (1.2%)	4 (1.6%)	253

	CTL Epitope																	Cysteine loop						
aa position	581											593						598						604
Consensus B	L	A	V	E	R	Y	L	K	D	Q	Q	L	L	G	I	W	G	C	S	G	K	L	I	C
CRF02-AG n = 177	—	—	165 L 5 I 1 M	1 K	9 S 5 G 4 A 1 K	1 F	—	85 R 4 Q 4 G 2 M 1 H	—	1 K	—	5 I	—	—	6 L	1 R	—	—	1 A	—	4 R 1 I	8 I 1 H	1 R	—
A n = 11	—	—	8 L	—	1 G 1 A	—	6 R 1 Q	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1 I	—	—
A2 n = 1	—	—	1 I	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
D' n = 10	—	—	—	—	—	—	—	—	—	—	1 R	2 F	—	—	3 L	—	—	—	—	—	2 R	10 H	—	—
F2 n = 6	—	—	—	—	—	—	—	1 Q 1 R	—	—	2 R	1 F	—	—	3 L 1 M	—	—	—	—	—	—	—	—	—
G n = 6	—	—	2 L	—	—	—	—	2 Q 2 R	—	—	—	—	—	—	1 L	—	—	—	2 T	—	—	—	—	—
CRF01-AE n = 4	—	—	—	—	—	—	—	1 Q 1 E	—	—	4 K	4F	—	—	4 L	—	—	—	—	—	—	3 I 1 T	—	—
CRF06_cpx n = 2	—	—	2 L	—	—	—	—	2 R	—	—	—	—	—	—	—	—	—	—	—	—	—	1 I	—	—
CRF11_cpx n = 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1 R	—	—	—	—	—	—	—	—

FIGURE 1. Sequence variability within the gp41 immunodominant region (IDR) (aa 581 to 604) from HIV-1 group M specimens. n represents the number of isolates with mutation(s). Dashes represent conserved amino acids in all isolates, and number followed by letter represents the number of isolates with the same amino acid substitution.

this goal is directly dependent on the correct identification of infected persons (sexual partners, pregnant women) and donated blood by reliable, simple, and cost-effective diagnostic tests. Limitations in electric energy, high-standard laboratories, and trained personnel in developing countries led to the development of simple, rapid, and cost-effective diagnostic tools that combine ease of use and good performance. Camstix, Determine, Genie II, and ImmunoComb II are the 4 rapid tests mostly used in Cameroon. During this evaluation, we noted their ease of use and rapidity. Camstix and Determine showed a 100% sensitivity. In the absence of indeterminate results, specificities of the 4 assays varied between 98.3–100%, the most specific tests being Genie II and ImmunoComb II, although these latter tests showed some false-negative results, decreasing their sensitivity.

This is one of the rare independent evaluations of Camstix, because data available until now on its performances are those provided by Camdiagnostix, the structure in charge of its production in Cameroon. However, the sensitivity of the Camstix assay could be overevaluated in our study because of the preselection of the panel samples by this test. In contrast, Determine has been evaluated previously in several studies. The evaluation of Determine done by Urassa et al[25] in Tanzania reported a sensitivity and specificity (100 and 98.3%, respectively) similar to what we found. Two other studies carried out in Honduras/Dominican Republic and in Thailand reported a 100% sensitivity and specificity of Determine.[42,43] The discordance in specificities obtained in these studies could be due to the difference in sample origins. Compared with Determine, Genie II and ImmunoComb II have been less extensively evaluated. Three studies reported a 100% sensitivity of Genie II and ImmunoComb II for detecting HIV-1 group M.[24,44,45] Two of these studies reported specificities of 99.7 and 100%, respectively, for ImmunoComb II and Genie II.[24,44] However, another study conducted in Togo in 1999 reported sensitivity of only 90.7% for Genie II.[46] Our evaluation done in Cameroon showed sensitivities of 98.9 and 99.3% and specificities of 100 and 99.5% for Genie and ImmunoComb II, respectively. The number and the origin of samples could not explain the differences in test performances between these different studies, because all the evaluations included a high number of samples from all origins, especially from Africa. However, field conditions of evaluation could be responsible for these disparities. Indeed, the first 3 studies reporting very good results were carried out in high-standard laboratories (Centers for Disease Control, Abidjan, Ivory Coast; Institute of Human Virology, Baltimore, MD; Institute of Tropical Medicine, Antwerp, Belgium). This observation highlights the necessity of also evaluating HIV assays in field conditions. Despite all these disparities, Genie II was found to be a very specific assay in previous studies and in our evaluation, always showing a 100% specificity. It could be recommended as a second test in algorithms using 2 screening assays.

To shorten the diagnostic window between moment of infection and detection of antibodies, and to reduce the residual risk of HIV transmission by blood and blood products, fourth-generation assays combining the detection of antibodies and p24 antigens were developed.[47] Enzygnost HIV Integral and Genscreen plus HIV Ag-Ab are fourth-generation assays currently used in Cameroon. The sensitivities of both assays in this study were excellent (100%), similar to previous evaluations.[48] Other studies also reported their capacity to correctly identify HIV-1 group O and HIV-2 samples.[49,50] While Enzygnost HIV Integral showed a good specificity (99.4%), better than that obtained with rapid assays (Camstix and Determine), Genscreen plus HIV Ag-Ab showed a relatively low value (95.0%). Weber et al[48] also reported this better specificity of Enzygnost HIV Integral compared with Genscreen plus HIV Ag-Ab (93.4 and 98.4%, respectively).

Despite their high sensitivity, the use of fourth-generation assays for routine diagnosis in developing countries will probably be limited by the need for special algorithms for the confirmation of reactive results. Confirmation strategies proposed for these assays involve 2 parts (anti-HIV part and p24 Ag part),[48] requiring the use of very expensive assays such

as Western blot assays, and HIV p24 antigen tests, which are less efficient on African samples. For these reasons, their use in developing countries should be limited to the identification of potentially HIV-infected donated blood, and even in this situation, less specific assays such as Genscreen plus HIV Ag-Ab will result in the discard of many false-positive blood donations (>50%).

More than 8% of the samples tested in this study showed final indeterminate results that should be taken into consideration during such an evaluation. Many authors generally exclude indeterminate samples from statistical analysis, thus overestimating the performances of the test evaluated. We here considered both situations, and the discussion below on test performances focused on results obtained with indeterminate samples. Addition of these samples to our calculations as negative samples (all PCR results were negative) gave less satisfactory results. Indeed, the overall specificity was considerably reduced for all assays (82.5–98.2%), especially for the more sensitive tests (Camstix, Determine, Enzygnost HIV Integral, and Genscreen plus HIV Ag-Ab). Western blot assays, which are the worldwide gold standard for HIV infection confirmation, were unable to discriminate between positive and negative samples of this indeterminate panel, demonstrating their inefficiency in these situations. This observation also shows the advantages of testing strategies for African countries based on the use of simple/rapid assays or ELISA, which are reliable as well as cost effective, rather than screening strategies using Western blot assays. To reduce the number of indeterminate results, strategies using highly sensitive, rapid tests or ELISAs as first-line tests, and very specific rapid test or ELISA as second-line tests for positive samples, should be favored. Discordant samples could be screened with a third ELISA or rapid test of different principle or different antigen preparation, as recommended by the World Health Organization.[51] Fourth-generation assays with a very low specificity such as Genscreen plus HIV Ab-Ag should be avoided in routine diagnosis.

Successful identification of HIV-infected individuals is based on the correct detection of antibodies directed against the IDRs of the gp41. Many studies have reported AA substitutions in this region, capable of affecting the antibody reactivity.[26,27,52] The analysis of the gp41 IDRs in this study showed the existence of some AA mutations in the CTL epitope and the cysteine loop, but none of the AA substitutions found had an impact on serologic detection by the 6 HIV tests evaluated. This completely agreed with results obtained by Dorn et al[53] in their study using Food and Drug Administration–licensed EIA kits.

The only minor AA substitution ($L^{602} \rightarrow H$) previously described in the cysteine loop by Horal et al[26] as capable of affecting the antibody reactivity of a single gp41 peptide-based assay was found in about 6% of the samples tested. However, all the isolates carrying this mutation were correctly identified as anti-HIV antibody positive by the 6 tests used. Whatever the case, most of the currently commercialized diagnostic assays include more than one antigenic component, including gp160, gp120, gp41, and p24 recombinant proteins or peptides only for HIV-1, and many other components for HIV-2. Consequently, minor variations of the gp41 IDRs should not significantly affect antibody detection, since a mutation in the gp41 IDRs capable of reducing antibody reactivity will be compensated by antibody reactivity with the other antigenic components of the assay.

In conclusion, diagnosis of HIV infection is a serious public health challenge in Cameroon, as in many other developing countries. Reliable and cost-effective assays are needed in this country to prevent the transmission of the virus and initiate treatments. Appropriate diagnostic algorithms involving the ELISAs and rapid tests evaluated in this study will probably yield good results. We clearly demonstrated their good performances as well as their limitations and the low risk of antibody detection mistakes due to the variability of the IDRs of the gp41 domain. However, there is a need to develop tests that can reduce the high number of indeterminate samples in Africa, without decreasing the capacity to identify HIV-positive samples. Our data also show the importance of prior test evaluations on local serum panel and in field conditions, before deciding on a national policy and guidelines for HIV screening.

REFERENCES

1. Hu D, Dondero TJ, Gayle TD. Global and molecular epidemiology of the HIV-1. In: Wormser IGP, ed. *AIDS and Other Manifestations of HIV Infection*. Philadelphia: Lippincott-Raven Publishers; 1998:27–40; discussion 230–221.
2. Preston BD. Reverse transcriptase fidelity and HIV-1 variation. *Science*. 1997;275:228–229.
3. Gurtler LG, Hauser PH, Eberle J, et al. A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. *J Virol*. 1994;68:1581–1585.
4. Simon F, Mauclere P, Roques P, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. *Nat Med*. 1998;4:1032–1037.
5. Robertson DL, Anderson JP, Bradac JA, et al. HIV-1 nomenclature proposal. *Science*. 2000;288:55–56.
6. Yamaguchi J, Vallari AS, Swanson P, et al. Evaluation of HIV type 1 group O isolates: identification of five phylogenetic clusters. *AIDS Res Hum Retroviruses*. 2002;18:269–282.
7. Ayouba A, Souquieres S, Njinku B, et al. HIV-1 group N among HIV-1-seropositive individuals in Cameroon. *AIDS*. 2000;14:2623–2625.
8. Fonjungo PN, Mpoudi EN, Torimiro JN, et al. Presence of diverse human immunodeficiency virus type 1 viral variants in Cameroon. *AIDS Res Hum Retroviruses*. 2000;16:1319–1324.
9. Mboudjeka I, Zekeng L, Takehisa J, et al. HIV type 1 genetic variability in the northern part of Cameroon. *AIDS Res Hum Retroviruses*. 1999;15: 951–956.
10. Nkengasong JN, Janssens W, Heyndrickx L, et al. Genotypic subtypes of HIV-1 in Cameroon. *AIDS*. 1994;8:1405–1412.
11. Zhong P, Burda S, Urbanski M, et al. HIV type 1 group M clades infecting subjects from rural villages in equatorial rain forests of Cameroon. *J Acquir Immune Defic Syndr*. 2002;31:495–505.
12. Vergne L, Bourgeois A, Mpoudi-Ngole E, et al. Biological and genetic characteristics of HIV infections in Cameroon reveals dual group M and O infections and a correlation between SI-inducing phenotype of the predominant CRF02_AG variant and disease stage. *Virology*. 2003;310: 254–266.
13. Vanden Haesevelde M, Decourt JL, De Leys RJ, et al. Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate. *J Virol*. 1994;68:1586–1596.
14. UNAIDS/WHO. Epidemiological fact sheets on HIV/AIDS and sexually transmitted infections. Available at: http://www.who.int/GlobalAtlas/PDFFactory/HIV/reportExporting.asp. Accessed December 5, 2003.
15. Nyambi P, Zekeng L, Kenfack H, et al. HIV infection in rural villages of Cameroon. *J Acquir Immune Defic Syndr*. 2002;31:506–513.
16. Takehisa J, Zekeng L, Miura T, et al. Triple HIV-1 infection with group O and Group M of different clades in a single Cameroonian AIDS patient. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997;14:81–82.

17. Takehisa J, Zekeng L, Ido E, et al. Various types of HIV mixed infections in Cameroon. *Virology.* 1998;245:1–10.
18. Takehisa J, Zekeng L, Ido E, et al. Human immunodeficiency virus type 1 intergroup (M/O) recombination in Cameroon. *J Virol*. 1999;73:6810–6820.
19. UNAIDS/WHO. *Technical Update: Blood Safety and HIV.* Geneva, Switzerland: Joint United Nations Programme on HIV/AIDS and the World Health Organisation; 1997.
20. Best SJ, Dax EM. Assays for HIV with improved sensitivity and specificity. *Exp Opin Invest Drugs*. 1997;8:965–983.
21. Weber B, Fall EH, Berger A, et al. Reduction of diagnostic window by new fourth-generation human immunodeficiency virus screening assays. *J Clin Microbiol*. 1998;36:2235–2239.
22. Gurtler L, Muhlbacher A, Michl U, et al. Reduction of the diagnostic window with a new combined p24 antigen and human immuno deficiency virus antibody screening assay. *J Virol Methods*. 1998;75:27–38.
23. Aidoo S, Ampofo WK, Brandful JA, et al. Suitability of a rapid immunochromatographic test for detection of antibodies to human immunodeficiency virus in Ghana, West Africa. *J Clin Microbiol*. 2001;39:2572–2575.
24. Koblavi-Deme S, Maurice C, Yavo D, et al. Sensitivity and specificity of human immunodeficiency virus rapid serologic assays and testing algorithms in an antenatal clinic in Abidjan, Ivory Coast. *J Clin Microbiol*. 2001;39:1808–1812.
25. Urassa W, Nozohoor S, Jaffer S, et al. Evaluation of an alternative confirmatory strategy for the diagnosis of HIV infection in Dar Es Salaam, Tanzania, based on simple rapid assays. *J Virol Methods*. 2002;100 (1–2):115–120.
26. Horal P, Svennerholm B, Jeansson S, et al. Continuous epitopes of the human immunodeficiency virus type 1 (HIV-1) transmembrane glycoprotein and reactivity of human sera to synthetic peptides representing various HIV-1 isolates. *J Virol*. 1991;65:2718–2723.
27. Oldstone MB, Tishon A, Lewicki H, et al. Mapping the anatomy of the immunodominant domain of the human immunodeficiency virus gp41 transmembrane protein: peptide conformation analysis using monoclonal antibodies and proton nuclear magnetic resonance spectroscopy. *J Virol*. 1991;65:1727–1734.
28. Leitner T. Genetic subtypes of HIV-1. In: Myers G, Korber B, Foley B, et al., eds. *Human Retrovirus and AIDS: A Compilation of Nucleic Acids and Amino Acid Sequences*. Los Alamos, NM: Los Alamos National Laboratory; 1996.
29. Pieniazek D, Yang C, Lal RB. Phylogenetic analysis of gp41 envelope of HIV-1 group M, N, and O. In: Korber B, Kuiken C, Foley B, et al., eds. *Human Retroviruses and AIDS*. Los Alamos, NM: Theoretical Biology and Biophysics Group; 1998:III-112–117.
30. Mauclere P, Loussert-Ajaka I, Damond F, et al. Serological and virological characterization of HIV-1 group O infection in Cameroon. *AIDS*. 1997;11:445–453.
31. Rehle TM, Mattke P, Liomba GN, et al. Evaluation of a quantitative double ELISA strategy for confirmation and differentiation of HIV infection. *J Virol Methods*. 1997;66:203–209.
32. Yang C, Dash BC, Simon F, et al. Detection of diverse variants of human immunodeficiency virus-1 groups M, N, and O and simian immunodeficiency viruses from chimpanzees by using generic pol and env primer pairs. *J Infect Dis*. 2000;181:1791–1795.
33. Yang C, Pieniazek D, Owen SM, et al. Detection of phylogenetically diverse human immunodeficiency virus type 1 groups M and O from plasma by using highly sensitive and specific generic primers. *J Clin Microbiol*. 1999;37:2581–2586.
34. Fransen K, Zhong P, De Beenhouwer H, et al. Design and evaluation of new, highly sensitive and specific primers for polymerase chain reaction detection of HIV-1 infected primary lymphocytes. *Mol Cell Probes*. 1994;8:317–322.
35. Clewley JP, Lewis JC, Brown DW, et al. A novel simian immunodeficiency virus (SIVdrl) pol sequence from the drill monkey, Mandrillus leucophaeus. *J Virol*. 1998;72:10305–10309.
36. Courgnaud V, Pourrut X, Bibollet-Ruche F, et al. Characterization of a novel simian immunodeficiency virus from guereza colobus monkeys (Colobus guereza) in Cameroon: a new lineage in the nonhuman primate lentivirus family. *J Virol*. 2001;75:857–866.
37. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*. 1994;22:4673–4680.
38. UNAIDS. *Epidemiology fact sheet on HIV/AIDS and sexually transmitted diseases*. 2001. Available at: www.unaids.org.
39. National AIDS Control Program. Ministry of Public Health, Yaounde, Cameroon, 2001 (annual report).
40. Carr JK, Torimiro JN, Wolfe ND, et al. The AG recombinant IbNG and novel strains of group M HIV-1 are common in Cameroon. *Virology.* 2001;286:168–181.
41. Tebit DM, Zekeng L, Kaptue L, et al. Genotypic and phenotypic analysis of HIV type 1 primary isolates from western Cameroon. *AIDS Res Hum Retroviruses*. 2002;18:39–48.
42. Palmer CJ, Dubon JM, Koenig E, et al. Field evaluation of the Determine rapid human immunodeficiency virus diagnostic test in Honduras and the Dominican Republic. *J Clin Microbiol*. 1999;37:3698–3700.
43. Arai H, Petchclai B, Khupulsup K, et al. Evaluation of a rapid immunochromatographic test for detection of antibodies to human immunodeficiency virus. *J Clin Microbiol*. 1999;37:367–370.
44. Beelaert G, Vercauteren G, Fransen K, et al. Comparative evaluation of eight commercial enzyme linked immunosorbent assays and 14 simple assays for detection of antibodies to HIV. *J Virol Methods*. 2002;105: 197–206.
45. Constantine NT, Zekeng L, Sangare AK, et al. Diagnostic challenges for rapid human immunodeficiency virus assays: performance using HIV-1 group O, HIV-1 group M, and HIV-2 samples. *J Hum Virol*. 1997;1:45–51.
46. Dagnra AY, Prince David M, Gaba J, et al. [Evaluation of eight diagnostic tests for HIV infection in Lome (Togo)]. *Med Trop*. 2002;62:507–510.
47. Ly TD, Martin L, Daghfal D, et al. Seven human immunodeficiency virus (HIV) antigen-antibody combination assays: evaluation of HIV seroconversion sensitivity and subtype detection. *J Clin Microbiol*. 2001;39: 3122–3128.
48. Weber B, Berger A, Rabenau H, et al. Evaluation of a new combined antigen and antibody human immunodeficiency virus screening assay, VIDAS HIV DUO Ultra. *J Clin Microbiol*. 2002;40:1420–1426.
49. Brust S, Duttmann H, Feldner J, et al. Shortening of the diagnostic window with a new combined HIV p24 antigen and anti-HIV-1/2/O screening test. *J Virol Methods*. 2000;90:153–165.
50. Shima T, Kondo M, Saito T, et al. [Evaluation of an HIV screening assay kit for the combined detection of anti-HIV-1/2 antibodies and HIV p24 antigen]. *Kansenshogaku Zasshi*. 2001;75:1014–1024.
51. World Health Organisation. Revised recommendations for the selection and use of HIV antibody tests. *Wkly Epidemiol Rec*. 1997;12:83–86.
52. Xu JY, Gorny MK, Palker T, et al. Epitope mapping of two immunodominant domains of gp41, the transmembrane protein of human immunodeficiency virus type 1, using ten human monoclonal antibodies. *J Virol*. 1991;65:4832–4838.
53. Dorn J, Masciotra S, Yang C, et al. Analysis of genetic variability within the immunodominant epitopes of envelope gp41 from human immunodeficiency virus type 1 (HIV-1) group M and its impact on HIV-1 antibody detection. *J Clin Microbiol*. 2000;38:773–780.

Aghokeng Fobang Avelin, Ewane L., Awazi B., Nanfack A., Delaporte Eric, Peeters Martine, Zekeng L. (2004)

Evaluation of four simple/rapid assays and two fourth-generation ELISAs for the identification of HIV infection on a serum panel representating the HIV-1 group M genetic diversity in Cameroon

Journal of Acquired Immune Deficiency Syndromes, 37 (5), 1632-1640

ISSN 1525-4135