Multicenter Evaluation of New Double-Antigen Sandwich Enzyme Immunoassay for Measurement of Anti-Human Immunodeficiency Virus Type 1 and Type 2 Antibodies

PHILIPPE BÜRGISSER,^{1*} FRANÇOIS SIMON,² MARTIN WERNLI,³ THOMAS WÜST,⁴
MARIE-FRANÇOISE BEYA,⁵ AND PHILIPPE C. FREI¹

*Division d'Immunologie et Allergie, Centre Hospitalier Universitaire Vaudois, Lausanne,¹
Regionales Blutspendezentrum, Kantonsspital Aarau, Aarau,³ and Roche Diagnostic
Systems, Basel,⁵ Switzerland; Laboratoire de Virologie, Groupe Hospitalier
Bichat-Claude Bernard, Paris, France²; and Städtisches
Klinikum, Pforzheim, Germany⁴*

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A new enzyme immunoassay (EIA), the Cobas Core Anti-HIV-1/HIV-2 EIA DAGS (also referred to as Roche DAGS), for the detection of antibodies to human immunodeficiency virus type 1 (HIV-1) and HIV-2 was evaluated in four centers. The assay is based on the double-antigen sandwich (DAGS) format, which enables the detection of all classes of antibodies. The antigens consist of recombinant proteins in their native conformation and of synthetic peptides. Of a total of 5,836 negative serum samples, including 95 samples likely to produce false reactivities, 6 were false positive, resulting in a specificity of 99.9%. None of 35 sera that were from noninfected individuals but contained p24-cross-reacting antibodies as revealed by Western blot (immunoblot) analysis were reactive by the Roche DAGS assay. In samples from individuals infected with HIV-1 group M ($n = 499$) and HIV-2 ($n = 200$), the sensitivity of the assay was 100%. Although containing antigens with sequences from subtype B only, the assay was also able to correctly identify with high optical density/cutoff ratios samples from subjects infected with HIV-1 subtype O ($n = 10$). In 17 of 19 seroconversion panels tested, the assay detected the presence of HIV-1 antibodies as early as another sandwich EIA. Eight of these panels were also analyzed by an indirect second-generation assay, which detected antibodies 2 to 10 days later than did the DAGS assay under evaluation. The excellent specificity and sensitivity of the new Cobas Core Anti-HIV-1/HIV-2 EIA DAGS are the result of the DAGS format as well as of the native, naturally folded form of the recombinant protein used as the gag antigen.

The diagnosis of human immunodeficiency virus (HIV) infection still relies on the detection of specific antibodies to HIV type 1 (HIV-1) and HIV-2 with some exceptions, such as in children born to seropositive mothers. A primary objective of serodiagnosis is the earliest detection of seroconversion. In the course of seroconversion, low-affinity immunoglobulin M antibodies appear in the blood before immunoglobulin G antibodies (6, 7, 20). However, since immunoglobulin M antibodies are generally not detected by enzyme immunoassays (EIAs) based on the indirect (anti-gamma globulin) format, the double-antigen sandwich (DAGS) format has recently been introduced. This format enables the detection of all classes and subclasses of antibodies to HIV-1 and HIV-2, thus allowing an earlier detection of seroconversion (7, 22). However, a drawback of this type of assay is its frequent inability to detect antibodies to the highly divergent HIV-1 subtype O strains (14). On the other hand, while the use of recombinant proteins and synthetic peptides has considerably decreased the rate of false-positive reactions (5), there is still a need for increased specificity. The Cobas Core Anti-HIV-1/HIV-2 EIA DAGS is a new assay which combines the DAGS format with antigens consisting of recombinant proteins in their native conforma-

tion and synthetic peptides. Here, we present the results of the evaluation of this test performed in four different centers.

MATERIALS AND METHODS

Cobas Core Anti-HIV-1/HIV-2 EIA DAGS. The Cobas Core Anti-HIV-1/HIV-2 EIA DAGS test was performed according to the manufacturer's instructions. The Cobas Core Anti-HIV-1/HIV-2 EIA DAGS is a solid-phase direct EIA for the detection of all classes and subclasses of human antibodies against HIV-1 and HIV-2 in serum and plasma. In a first incubation step, samples and controls (100 μ l) were incubated with recombinant viral core (capsid) protein (p24) as well as recombinant transmembrane gp41 (HIV-1) and gp36 (HIV-2) nonglycosylated proteins, which were immobilized on the solid phase (beads). On completion of the binding of antibodies reactive to HIV-1 or HIV-2, unbound material was removed by washing the beads. Bound human antibodies were then labeled with antigen-peroxidase conjugates in a second incubation step. Synthetic peptide antigens were used for the HIV-1 (gp41) and HIV-2 (gp36) envelope-peroxidase conjugates, while folded native recombinant protein was used for the p24-peroxidase conjugate. After a second washing step, the fraction of bound enzyme was determined by a chromogenic reaction by using tetramethyl benzidine and hydrogen peroxide as the substrates. The intensity of the color reaction is proportional to the amount of antibodies to HIV-1 and HIV-2 (anti-HIV-1/HIV-2 antibodies) present in the sample. The absorbance was measured and compared with a cutoff (CO) value calculated from the positive controls (PC) and negative controls (NC) assayed in parallel: $CO = NC + (0.16 \times PC)$. The same reagent lot was used throughout the entire evaluation.

Reference tests. The reference tests used were as follows: Abbott Recombinant HIV-1/HIV-2 3rd generation EIA, also referred to here as Abbott 3rd (Abbott Laboratories, North Chicago, Ill.); Detect-HIV (BioChem ImmunoSystems Inc., Montreal, Quebec, Canada); and HIV-2 Ab EIA (Clonatec, Paris, France). All assays were performed according to the instructions provided by the manufacturers. No additional analysis of samples from seroconversion panels was performed. All other positive samples as well as discrepancies were investi-

* Corresponding author. Mailing address: Division d'Immunologie et Allergie, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland. Phone: 41 21 314 08 00. Fax: 41 21 314 07 91.

TABLE 1. Clinical category of the subjects seropositive for HIV-1 and HIV-2

Clinical category	No. of subjects		
	HIV-1 group M positive	HIV-1 group O positive	HIV-2 positive
CDC A	51	4	168
CDC B	103	2	12
CDC C	150	3	18
Not known		1	2
Total	304	10	200

gated further for anti-HIV antibodies by Western blotting (immunoblotting) (WB) with NEW LAV-BLOT I and NEW LAV-BLOT II (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France). In addition, samples reactive by the Roche DAGS or the reference EIA, but negative or indeterminate by WB analysis, were tested for HIV-1 p24 antigen (p24 Ag) with Abbott HIVAG-1 Monoclonal (Abbott Laboratories). The identification of HIV-1 subtype O-positive specimens has been described previously (19). Western blots were interpreted according to the criteria of the Association of State and Territorial Public Health Laboratory Directors-Centers for Disease Control (2).

Other methods. Rheumatoid factor titers were determined with the Behring nephelometer analyzer. PCR detection of HIV-1 DNA was performed essentially as previously described (11) with *gag* (SK38/SK39 and SK145/SK431) and *pol* (17) primers. Amplicons were detected by liquid-phase hybridization with the corresponding *gag* (SK19 and SK102) and *pol* (reference 17, no. 1) probes that were labeled with ^{32}P at the 5' end (11).

Equipment. The Cobas EIA semiautomated system as well as the fully automated Cobas Core Immunoanalyzer were used in this study. In a previous evaluation, the two systems had been shown to produce identical results for standard serum samples (data not shown).

Serum samples. In total, 6,663 samples were analyzed. They were divided into eight categories as follows: samples from blood donors ($n = 5,207$); samples from pregnant women ($n = 200$); samples from noninfected individuals with repeatable anti-p24 reactivity in HIV-1 WB ($n = 35$); negative samples likely to produce false-positive results ($n = 95$); unselected samples from routine diagnosis ($n = 300$); and samples from subjects infected by HIV-1 group M ($n = 499$), HIV-1 group (subtype) O ($n = 10$), or HIV-2 ($n = 200$). In addition, a series of 19 commercially available seroconversion panels (BBi A, C, D, H, I, J, K, N, R, Q, S, U, X, and Y and NABI SV-91, 1436, 1571, 5237, and 5338), representing a total of 117 samples, were analyzed. Details of clinical category and geographical origin of HIV-infected subjects are given in Tables 1 and 2, respectively.

Intra-assay variation. Each of the two kit controls (negative and positive) and each of three different serum samples (including one negative) were measured 21 times on two occasions, i.e., once on the first day of the evaluation and once on the last day.

Interassay variation. The two kit controls and the three serum samples used to assess the intra-assay variation were measured in triplicate in 13 separate experiments prior to assaying the clinical samples.

RESULTS

Blood donors. Samples from 5,207 blood donors were assayed in two centers. Five samples were repeatedly positive by the Roche DAGS test, and four others were repeatedly positive by the reference assays (three of 2,000 by Detect-HIV and one of 3,207 by Abbott 3rd). No sample tested positive by both the Roche DAGS test and the reference assays.

Pregnant women. Serum samples from 200 pregnant women (week 5 to week 38 of gestation) were tested. One hundred ninety-nine serum samples were negative by both the Roche DAGS and the Abbott 3rd assays, while one sample was highly reactive by both assays but yielded an indeterminate WB pattern for HIV-1, with the reactivity limited to *gag* proteins (p24 and/or p55). WB analysis for anti-HIV-2 antibodies and the p24 Ag test was negative.

Negative samples likely to produce false-positive results. Samples ($n = 95$) were from patients with liver cirrhosis ($n = 15$), chronic hepatitis ($n = 10$), monoclonal gammopathy ($n = 20$), rheumatoid factor concentrations of ≥ 200 IU/ml ($n = 20$),

and systemic lupus erythematosus ($n = 10$) and from subjects undergoing long-term dialysis ($n = 20$). Such specimens are likely to elicit false-positive reactions (1, 16). All were negative by both the Roche DAGS and Abbott 3rd tests.

Unselected routine diagnosis. Of a total of 300 samples, 298 tested negative by both the Roche DAGS and Abbott 3rd assays. One sample was positive by both EIAs and was confirmed to be positive by WB analysis. Another sample was negative by the Roche DAGS assay (optical density [OD]/CO, 0.03 and 0.04) but reproducibly weakly positive by the Abbott 3rd test (OD/CO, 1.38 and 1.56). WB analysis for HIV-1 was indeterminate (weak p24 *gag* and p32 *pol* bands), while that for HIV-2 and the p24 Ag test were negative. No follow-up sample was available.

Noninfected individuals with persistent *gag* reactivity by WB analysis for HIV-1 antibodies. We investigated 35 individual samples that were indeterminate by HIV-1 WB analysis with p24 *gag* reactivity (alone or in combination with other *gag* or *pol* proteins) and that were either negative or indeterminate by HIV-2 WB analysis. All samples were reproducibly reactive by a second-generation (indirect) EIA. However, early seroconversion was considered unlikely in view of the negative p24 Ag test and was subsequently ruled out by the absence of serological changes in later samples taken after 2 to 11 weeks ($n = 9$) (according to the criteria of the Société Française de Transfusion Sanguine [8]) or after 3 months or longer ($n = 23$). In another three subjects, seroconversion was excluded by the negative PCR result for HIV-1 DNA in peripheral blood mononuclear cells by using three pairs of primers (two in *gag* and one in *pol*). When tested with the Roche DAGS assay, all these samples were clearly negative (OD/CO: mean, 0.08; range, 0.05 to 0.27).

HIV-1 group M-positive samples. All of the 499 WB-confirmed HIV-1 group M-positive samples tested positive by the Roche DAGS assay. For 497 samples, OD/CO ratios of >9 were observed, independent of the clinical category or geographical origin of the subjects. The two remaining samples (taken from the same patient) yielded OD/CO ratios of 4.85 and 7.66. The samples were taken 7 and 13 days, respectively, after an initial sample that showed indeterminate WB HIV-1 results (with p17, p24, and p55 bands) and positive p24 Ag results, indicating ongoing seroconversion. Because of insufficient volume, this initial sample could not be tested with the Roche DAGS assay.

HIV-2-positive samples. All 200 HIV-2-positive samples, independent of the clinical category or geographical origin of the subjects, tested highly positive (OD/CO, >9) in this evaluation.

HIV-1 subtype O-positive samples. The 10 HIV-1 subtype

TABLE 2. Geographical origin of HIV-1- and HIV-2-positive subjects

Geographical origin	No. of subjects		
	HIV-1 group M positive	HIV-1 group O positive	HIV-2 positive
Europe	264	1	12
Northern Africa	12		1
Sub-Saharan Africa	22	9	185
United States	1		
Caribbean	1		2
South America	1		
Asia	3		
Total	304	10	200

FIG. 1. Example of the performance of the Roche DAGS test, one of the reference assays (Abbott 3rd), and the Roche 2nd generation indirect EIA, in six seroconversion panels.

O-positive samples were all highly reactive by the Roche DAGS test (OD/CO, >11). Most of these samples had been investigated in the past with various EIAs (14, 19). In those studies, the Roche indirect assay had correctly identified the seven specimens tested (OD/CO: range, 1.0 to 5.2), while three of eight specimens had been missed by one or more sandwich EIAs (14, 19).

Seroconversions. The Roche DAGS and Abbott 3rd assays detected the appearance of antibodies at the same time in 17 of 19 panels. In the remaining 2 panels (BBI C and R), the reference test, Abbott 3rd, detected antibodies in an earlier blood sample (taken 2 or 5 days before) than did the Roche DAGS assay. Eight seroconversion panels were also investigated by a second-generation assay, the Roche indirect. This test detected the presence of antibodies in serum 2 to 10 days (mean, 5 days) later than did the Roche DAGS assay. Furthermore, the Roche DAGS test showed a constant increase in the OD/CO ratio during seroconversion, which was in contrast to the Abbott 3rd assay, for which in certain samples (e.g., BBI Q) the OD/CO ratio declined after an initial positive peak. Examples of seroconversions are depicted in Fig. 1.

Specificity and sensitivity. A total of 5,836 true-negative samples (samples from blood donors, pregnant women, and unselected routine diagnosis; samples considered at risk to induce false-positive reactions; and samples containing antibodies cross-reactive with p24 *gag*) were analyzed. Six false-positive results (five from the group of blood donors and one from the group of pregnant women) were recorded, yielding an

overall specificity of 99.89% (95% confidence limits, 99.79 to 99.96%). The specificity of the Roche DAGS assay was unchanged (99.90%; 95% confidence limits, 99.77 to 99.96%) when blood donors were considered separately (5 false-positive reactions in 5,207 samples).

The sensitivity was 100% for the HIV-1 group M- and group O-positive samples as well as for the HIV-2-positive samples. Figure 2 displays the distribution of OD/CO ratios obtained with the Roche DAGS assay in this study.

Intra-assay variation. Coefficients of variation for the intra-assay variation ranged from 3.5 to 16.6% for the negative samples and from 2.6 to 5.8% for the positive samples.

Interassay variation. Coefficients of variation ranging from 8.5 to 22.2% for the negative samples and from 2.2 to 4.7% for the positive samples were obtained for the interassay variation.

DISCUSSION

We have evaluated the performance of a new anti-HIV-1/HIV-2 assay based on the DAGS format as well as on the use of recombinant proteins in their native conformation and synthetic peptides.

The specificity of 99.9% was due to 6 of 5,836 samples, which were found to be repeatedly reactive by the Cobas Core Anti-HIV-1/HIV-2 EIA DAGS. Three of these samples were clearly negative by another sandwich assay (Abbott 3rd), by HIV-1 and HIV-2 WB analysis, and by the p24 Ag test and were therefore considered to be false positives by the Roche DAGS assay. Two others tested unambiguously negative by the reference test (Detect-HIV), by the p24 Ag test, and by HIV-2 WB but were indeterminate by HIV-1 WB, with the reactivity limited to the *gag* proteins p24 and p55. In those two cases, a seroconversion to HIV-1 was excluded by the absence of serological evolution in specimens collected 7 months later. The sixth false-positive sample, taken from a pregnant woman, was known to contain cross-reactive antibodies to p24 from an earlier test carried out 4 years earlier during a previous pregnancy. The sample had revealed the presence of p24-reactive antibodies on HIV-1 WB, as well as a positive reaction by the Roche DAGS and Abbott 3rd assays (data not shown). However, the negative p24 Ag finding and the absence of new WB bands, especially *env*, after a 4-year period, coupled with the

FIG. 2. Distribution of OD/CO ratios for all sera except seroconversion panels ($n = 6,546$, i.e., 5,830 true negatives, 6 false positives, and 710 true positives). a, false-positive samples; b, one case of seroconversion present among the HIV-1 group M-positive samples. The scale of the ordinate is logarithmic.

subject's low-risk lifestyle, strongly argue against an HIV infection.

One serum sample from the routine diagnosis group tested unambiguously negative by the Roche DAGS assay but was reproducibly weakly positive by the Abbott 3rd test. On the basis of the WB and p24 Ag results, early seroconversion with a false-negative reaction by the Roche DAGS test was unlikely. Therefore, the sample was interpreted as a false-positive by the Abbott 3rd test.

Reactivity with p24 *gag* is seen fairly frequently in noninfected individuals (10). To circumvent this problem, some manufacturers have opted to omit *gag* sequences in the antigens used, at the risk of missing antibodies to non-B strains of HIV-1. This risk is of particular concern if the antigen consists of synthetic peptides representing short sequences encoded by the *env* gene (4, 12, 13).

The Roche DAGS test is based on an alternative approach using a p24 *gag* antigen in its native conformation. The results obtained in this study show that in most cases, the p24 *gag* cross-reactivity was abolished, thus improving the specificity of the assay. Moreover, the finding that cross-reacting antibodies recognize epitopes exposed preferentially on denatured p24 *gag* corroborates results previously obtained in immunoprecipitation studies (15, 18).

The sensitivity of the Roche DAGS assay was 100% in HIV-1 group M- and HIV-2-positive samples. In sera exhibiting seroconversion to HIV-1, the results obtained with the Roche DAGS assay were similar to those obtained with the Abbott 3rd test, although the decline in the OD/CO ratio seen for some samples with the latter assay did not occur with the former. This property of the Roche DAGS assay minimizes the risk of missing samples at the early stages of seroconversion.

All 10 samples from individuals infected with HIV-1 subtype O (3, 9, 21) yielded very high OD/CO values by the Roche DAGS test. Some of these samples had been missed in previous studies by one or more sandwich EIAs not containing sequences from HIV-1 subtype O at that time (14, 19). In addition, the Roche DAGS test produced higher OD/CO values than the Roche indirect assay (19). These findings indicate that DAGS EIAs using only subtype B antigens are able to reliably detect HIV-1 subtype O infections, as long as the proteins are presented in their native tridimensional structure.

In summary, our results confirm that the switch from the indirect assay to the DAGS format significantly improves the sensitivity of the assay by allowing earlier detection of seroconversions. They underscore the advantage of using antigens, particularly p24 *gag*, in their native conformation to achieve high sensitivity to HIV-1 subtype O and to dramatically reduce the number of false positives due to p24 cross-reactive antibodies. Thus, the remarkable specificity and sensitivity make the new Cobas Core Anti-HIV-1/HIV-2 EIA DAGS a very suitable test for the diagnosis of HIV-1, including subtype O, and HIV-2 infections.

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REFERENCES

1. Barthel, H. R., and D. J. Wallace. 1993. False-positive human immunodeficiency virus testing in patients with lupus erythematosus. *Semin. Arthritis Rheum.* **23**:1-7.
2. Centers for Disease Control. 1991. Interpretative criteria used to report Western blot results for HIV-1-antibody testing—United States. *Morbidity and Mortality Weekly Rep.* **40**:692-695.
3. Charneau, P., A. M. Borman, C. Quillent, D. Gutard, S. Chamaret, J. Cohen, G. Rmy, L. Montagnier, and F. Clavel. 1994. Isolation and envelope sequence of a highly divergent HIV-1 isolate: definition of a new HIV-1 group. *Virology* **205**:247-253.
4. Cohen, J. H. M., D. Gutard, F. Philbert, S. Chamaret, T. Tabary, R. Deleys, and L. Montagnier. 1995. Atypical HIV serological profile of novel HIV-1 variant distinct from subtype O. *Lancet* **345**:856-857.
5. Constantine, N. T. Serological tests for the retroviruses: approaching a decade of evolution. *AIDS* **7**:1-13.
6. Gaines, H., M. von Sydow, J. V. Parry, M. Forsgren, P. O. Pehrson, A. Snnerborg, P. P. Mortimer, and . Strannegrd. 1988. Detection of immunoglobulin M antibody in primary human immunodeficiency virus infection. *AIDS* **2**:11-15.
7. Gallarda, J. L., D. R. Henrard, D. Liu, S. Harrington, S. L. Stramer, J. E. Valinsky, and P. Wu. 1992. Early detection of antibody to human immunodeficiency virus type 1 by using an antigen conjugate immunoassay correlates with the presence of immunoglobulin M antibody. *J. Clin. Microbiol.* **30**:2379-2384.
8. Groupe de Travail Rtrovirus de la Socit Franaise de Transfusion Sanguine. 1994. Srologie HIV-algorithme aprs un dpistage positif. *Gaz. Transfusion* **105**:6-8.
9. Grtler, L. G., P. H. Hauser, J. Eberle, A. von Brunn, S. Knapp, L. Zekeng, J. M. Tsague, and L. Kaptue. 1994. A new subtype of human immunodeficiency virus type 1 (MPV-5180) from Cameroon. *J. Virol.* **68**:1581-1585.
10. Kmmerer, R., P. Brgisser, and P. C. Frei. 1995. Anti-human immunodeficiency virus type 1 antibodies of noninfected subjects are not related to autoantibodies occurring in systemic diseases. *Clin. Diagn. Lab. Immunol.* **2**:458-461.
11. Kellogg, D. E., and S. Kwok. 1990. Detection of human immunodeficiency virus, p. 337-347. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), *PCR protocols*. Academic Press, Inc., San Diego, Calif.
12. Lange, J. M. A., V. J. P. Teeuwssen, C. A. B. Boucher, A. Vahlne, F. Barin, S. Tjong-A-Hung, J. Dekker, U. Parkhede, F. de Wolf, and J. Goudsmit. 1993. Antigenic variation of the dominant gp41 epitope in Africa. *AIDS* **7**:461-466.
13. Leinikki, P., M. Lehtinen, H. Hyty, P. Parkkonen, M. L. Kantanen, and J. Hakulinen. 1993. Synthetic peptides as diagnostic tools in virology. *Adv. Virus Res.* **42**:149-186.
14. Loussert-Ajaka, I., T. D. Ly, M. L. Chaix, D. Ingrand, S. Saragosti, A. M. Courouc, F. Brun-Vzint, and F. Simon. 1994. HIV-1/HIV-2 seronegativity in HIV-1 subtype O infected patients. *Lancet* **343**:1393-1394.
15. Meilof, J. F., H. Arentsen, A. A. Kruize, R. J. Hen, J. W. J. Bijlsma, C. L. Van der Poel, R. J. T. Smeenk, and J. G. Huisman. 1992. Sjgren's syndrome and retroviral infection. *Arthritis Rheum.* **35**:1403-1404.
16. Novick, D. M., D. C. Des Jarlais, M. J. Kreek, T. J. Spira, S. R. Friedman, A. M. Gelb, R. J. Stenger, C. A. Schable, and V. S. Kalyanaraman. 1988. Specificity of antibody tests for human immunodeficiency virus in alcohol and parenteral drug abusers with chronic liver disease. *Alcohol. Clin. Exp. Res.* **12**:687-690.
17. Perrin, L. H., S. Yerly, N. Adami, P. Bachmann, E. Btler-Brunner, J. Burckhardt, and E. Kawashima. 1990. Human immunodeficiency virus DNA amplification and serology in blood donors. *Blood* **76**:641-645.
18. Ramirez, E., P. Uribe, D. Escanilla, G. Sanchez, and R. T. Espejo. 1992. Reactivity patterns and infection status of serum samples with indeterminate Western blot tests for antibody to human immunodeficiency virus type 1. *J. Clin. Microbiol.* **30**:801-805.
19. Simon, F., T. D. Ly, A. Baillou-Beaufils, V. Fauveau, J. De Saint-Martin, I. Loussert-Ajaka, M. L. Chaix, S. Saragosti, A. M. Courouc, D. Ingrand, C. Janot, and F. Brun-Vzint. 1994. Sensitivity of screening kits for anti-HIV-1 subtype O antibodies. *AIDS* **8**:1628-1629.
20. Stramer, S. L., J. S. Heller, R. W. Coombs, J. V. Parry, D. D. Ho, and J. P. Allain. 1989. Markers of HIV infection prior to IgG antibody seropositivity. *JAMA* **262**:64-69.
21. Vanden Haesevelde, M., J. L. Decourt, R. J. de Leys, B. Vanderborght, G. van der Groen, H. van Heuverswijn, and E. Saman. 1994. Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate. *J. Virol.* **68**:1586-1596.
22. Zaaijer, H. L., P. V. Exel-Oehlers, T. Kraaijeveld, E. Altena, and P. N. Lelie. 1992. Early detection of antibodies to HIV-1 by third-generation assays. *Lancet* **340**:770-772.