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Multicenter Evaluation of a New Automated Fourth-Generation Human Immunodeficiency Virus Screening Assay with a Sensitive Antigen Detection Module and High Specificity

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Fourth-generation assays for the simultaneous detection of human immunodeficiency virus (HIV) antigen and antibody that were available on the international market until now have antigen detection modules with relatively poor sensitivity and produce a higher rate of false-positive results than third-generation enzyme immunoassays (EIAs). The new Cobas Core HIV Combi EIA with an improved sensitivity for HIV p24 antigen was compared to alternative fourth- and third-generation assays, the p24 antigen test, and HIV type 1 (HIV-1) RNA reverse transcriptase PCR (RT-PCR). A total of 94 seroconversion panels ($n = 709$ sera), samples from the acute phase of infection after seroconversion ($n = 32$), anti-HIV-1-positive specimens ($n = 730$) from patients in different stages of the disease, 462 subtyped samples from different geographical locations, anti-HIV-2-positive sera ($n = 302$), dilutions of cell culture supernatants ($n = 62$) from cells infected with different HIV-1 subtypes, selected performance panels from Boston Biomedica Inc., 7,579 unselected samples from blood donors, 303 unselected daily routine samples, 997 specimens from hospitalized patients, and potentially interfering samples ($n = 1,222$) were tested with Cobas Core HIV Combi EIA. The new assay showed a sensitivity comparable to that of the Abbott HIV-1 AG Monoclonal A for early detection of HIV infection in seroconversion panels. The mean time delay of Cobas Core HIV Combi EIA (last negative sample plus 1 day) in comparison to that for HIV-1 RT-PCR for 87 panels tested with both methods was 2.75 days. The diagnostic window was reduced with Cobas Core HIV Combi EIA by between 3.6 and 5.7 days from that for third-generation assays. The specificities of Cobas Core HIV Combi EIA in blood donors were 99.84 and 99.85% (after repeated testing). Overall, 30 repeatedly reactive false-positive results out of 10,031 HIV-negative samples were obtained with Cobas Core HIV Combi EIA. Our results show that a fourth-generation assay with improved specificity such as Cobas Core HIV Combi EIA is suitable for blood donor screening because of its low number of false positives and because it detects HIV p24 antigen with a sensitivity comparable to that of single-antigen assays.

Since the first enzyme immunoassays (EIA) for blood donor screening and laboratory diagnosis of human immunodeficiency virus (HIV) infection were licensed over 15 years ago, the quality of these tests has been continuously improved by the use of recombinant antigens and synthetic peptides (second test generation) and the sandwich EIA technology (third test generation) (10, 36). There is however a residual risk for false-negative results. The potential causes include the diagnostic window in the preseroconversion phase, genetic variability, atypical seroconversions, a delayed or absent immune response in the very early or advanced stages of infection, respectively, and laboratory reporting errors (6).

The highest risk (>90%) of a false-negative result is observed in the preseroconversion phase during primary HIV infection (diagnostic window) (6). The residual risk of an HIV infection by a seronegative blood donor during acute HIV infection is estimated to be 1/493,000 to 1/1,866,000 per transfused unit in healthy, unpaid donors in the United States and Germany (2). In emergency department patients and in high-risk groups, it ranges between 0.14 and 0.17% (9, 18).

Early detection of HIV infection is important for reasons of infection security, prevention, and individual prognosis. An antiretroviral combination therapy during primary HIV infection reduces the likelihood of a rapid progression to the AIDS stage. Moreover, the frequency of opportunistic infections,

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skin and mucous membrane diseases, and respiratory infections is reduced (4).

Nucleic acid amplification technology (NAT) and HIV antigen (Ag) detection make it possible to reduce the residual risk of HIV transmission by blood and blood products and to improve the early detection of primary HIV infection in high-risk groups. With NAT testing, the diagnostic window (about 21 days) is reduced by 11 days and the residual risk is reduced by over 50% (2). In the primary HIV infection, a localized viral replication (eclipse) takes place first and lasts for approximately 10 days. In exceptional cases, it can last for many months. Experiments conducted with the animal model indicate that the HIV-infected subject is not infectious during this phase of the incubation period. In the subsequent viremic phase, HIV RNA is the first and only detectable virus-specific marker for 1 to 5 days. In theory, all potentially infectious viral carriers are excluded by using the NAT technique, because no infectivity is observed during primary infection in the animal model before appearance of HIV type 1 (HIV-1) RNA (22). The advantages of NAT are offset by its technical limitations. The automated processing of large numbers of samples, especially nucleic acid extraction, is not possible at this time or is possible only with limitations. HIV-1 RNA amplification is currently performed on pooled samples from donors in European blood banks as a cost-effective alternative to single-sample testing (25). Depending on the pool size and detection threshold of NAT, the gain in sensitivity may be relatively low in comparison to HIV p24 Ag testing (17). However, NAT testing of pools is more cost-effective than Ag determination in individual donations (24).

Fourth-generation HIV screening assays that make combined HIV Ag and antibody detection possible in one test batch have been licensed in Europe since 1997 (12, 19, 20, 23, 26, 30–35). These combined assays offer the advantage of early detection of HIV infection via p24 Ag detection at a technical burden and financial cost more or less equal to those for HIV antibody testing using third-generation EIAs. The diagnostic window is reduced by 4 to 5 days compared with that for antibody detection alone (third-generation assays).

Approximately one-third of the solid phase is coated with monoclonal antibodies for HIV p24 Ag detection. The remaining binding capacity is available for antibody detection (sandwich or indirect EIA). Generally, fourth-generation assays exhibit a relatively high limit for detection of the HIV Ag module (>30 pg of p24 Ag/ml). For this reason, they cannot replace single-Ag determination with sensitive commercially available EIAs (detection limit: 3 to 5 pg/ml) (34). The risk of interference is potentially higher with the HIV combined assays than with single-Ag or -antibody EIAs, since both serological markers are determined in one test batch. The specificity for blood donor screening (99.6 to 99.8%) is lower than that of third-generation HIV EIAs (12, 19). The higher rate of false-positive results (0.2%) of HIV combined assays has a particularly negative effect in cases of high sample throughput and low prevalence as in blood donor screening mainly because a separate Ag detection must be carried out in addition to the antibody confirmatory assay (Western blotting) in order to rule out an HIV infection (12).

MATERIALS AND METHODS

Recently, new fourth-generation assays with an improved sensitivity of the Ag detection module and optimized specificity have been established. In a multicenter study (Table 1 shows a list of the participants), the new automated Cobas Core HIV Combi EIA was compared to different fourth- and third-generation HIV screening assays, p24 Ag tests, and HIV-1 reverse transcriptase PCR (RT-PCR).

Cobas Core HIV Combi EIA. Cobas Core HIV Combi EIA is a double-Ag immunoassay for the detection of total antibodies to HIV-1 (including group O) and HIV-2, combined with a sandwich assay for the detection of HIV-1 p24 Ag. HIV-specific antibodies and the HIV-1 p24 Ag are detected within one determination.

Samples, digoxigenin-conjugated anti-p24 antibodies, and digoxigenin-conjugated RT were incubated with polystyrene beads coated with recombinant Ags derived from the Pol and Env regions of HIV-1 and HIV-2 as well as monoclonal antibodies against HIV-1 p24 Ag.

For Ag detection, HIV-1 p24 Ag reacted simultaneously with the anti-p24 antibodies on the bead and with the digoxigenin-labeled anti-p24 antibodies to form a sandwich complex.

For antibody detection, antibodies directed to RT formed a complex with immobilized Ag on the bead and digoxigenin-labeled Ag. Antibodies directed to HIV-1 Env Ag (including group O) and HIV-2 bound to the immobilized Ags. After beads were washed, the Env-specific antibodies were detected by incubation with recombinant Ags from the Env regions of HIV-1 and HIV-2 conjugated to horseradish peroxidase and a peroxidase-labeled antidigoxigenin monoclonal antibody.

After a second washing step, the bead was incubated with Cobas Core substrate. The intensity of the resulting color was roughly proportional to the amount of anti-HIV antibodies and HIV-1 p24 Ag detected in the specimen.

The cutoff was calculated from the following formula: $0.12 \times$ positive control + negative control. A result was considered negative if the index value (signal sample/cutoff value) was <0.9. A sample is positive if its index value is ≥ 1.0. The grey zone ranges between index values ≥ 0.9 and <1.0.

Alternative HIV screening assays. Cobas Core HIV Combi EIA was compared to different third- and fourth-generation assays established as routine methods in the different centers participating at the study. Included were HIV-1/HIV-2 3rd-gen. Plus EIA, IMx HIV-1/HIV-2 III Plus, AxSYM HIV-1/2 gO, Prism HIV-O Plus (Abbott, North Chicago, Ill.), VIDAS HIV DUO (Biomérieux, Marcy-l'Etoile, France), Genscreen HIV-1/2 (Bio-Rad, Marnes la Coquette, France), Enzygnost Anti-HIV1/2 Plus, Enzygnost HIV Integral, Enzygnost Anti-HIV (Dade Behring, Marburg, Germany), Enzymun-Test HIV Combi, and Cobas Core Anti-HIV 1+2+O EIA (Roche Diagnostics, Penzberg, Germany). All the tests were performed and interpreted in accordance with the manufacturers' recommendations.

HIV Ag detection assays. For p24 Ag detection, different commercially available assays, i.e., HIV-1 AG Monoclonal A (Abbott), VIDAS HIV p24 II assay (Biomérieux), Coulter HIV p24 Ag assay, Elecsys HIV Ag (Roche Diagnostics), were used. All the HIV Ag tests were performed and interpreted in accordance with the manufacturers' recommendations.

HIV antibody confirmatory assays. Repeatedly reactive samples were subjected to Western blot or immunoblot testing. The assays used included commercially available reagents, i.e., NEW LAV Blot I, NEW LAV Blot II (Bio-Rad), Diagnostic Biotechnology Western blot HIV 1, version 2.2 (Genelabs, Geneva, Switzerland), HIV Blot 2.2 (Abbott Diagnostics, Delkenheim, Germany), INNO-LIA HIV Confirmation (Innogenetics, Ghent, Belgium), RIBA HIV-1/-2 SIA (Chiron, Emeryville, Calif.), and in house Western blots: SBL-6669 (HIV-2; Swedish Institute for Infectious Disease, Solna, Sweden) and MVP-899/87 (HIV-1), MVP-5106/91 (HIV-O), and MVP 11971/87 (HIV-2) (Friedrich-Löffler-Institut für Med. Mikrobiologie, Greifswald, Germany).

Positive results were defined in accordance with World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) criteria (NEW LAV Blot I and II), Food and Drug Administration and WHO criteria (Diagnostic Biotechnology Western blot), and the Deutsches Institut für Normierung (DIN 58969-41 [1992]) definition (Friedrich-Loefler-Institute für Med. Microbiology).

Nucleic acid amplification. HIV-1 RNA was amplified by using different assays including Amplicor Monitor HIV 1, version 1.5, Ampliscreen HIV 1, version 1.5 (Roche Diagnostics, Branchburg, N.J.), and in-house HIV-1, HIV-2, and HIV-O PCR (Friedrich-Loefler-Institute für Med. Microbiology).

Specimens. The following specimens were tested to evaluate the sensitivity of Cobas Core HIV Combi EIA in comparison to different third- and fourth-generation assays and p24 Ag detection (Table 1). (i) Ninety-four HIV-1

TABLE 1. Participants involved in the study, methods used, and samples tested

Center	Comparison method(s)	Confirmation HIV antibody or antigen assay	Sample type or source (no. of panels or serum samples tested)
Friedrich-Loeffler-Institut für Med. Microbiology	Enzygnost Anti-HIV1/2 Plus, Enzygnost anti-HIV 1, Enzygnost HIV Integral, Enzymun-Test HIV Combi, AxSYM HIV-1/2 gO	In-house Western blotting for HIV-1, -2, and -O	Early infection (2), HIV-1 positive (112), HIV-1 subtyped (89), HIV-2 or SIV positive (10)
Swedish Institute for Infectious Disease Control	Enzygnost Anti-HIV1/2 Plus, IMx HIV-1/HIV-2 III Plus, Cobas Core Anti-HIV 1+2+O EIA	In-house Western blotting for HIV-2, Diagnostic Biotechnology HIV 1 Western blotting, HIV AG-1 Monoclonal A	Seroconversion (7), early infection (7), HIV-1 positive (100), HIV-2 positive (100)
Division d'Immunologie et d'Allergie, Département de Médecine Interne, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland	Genscreen HIV1/2, AxSYM HIV-1/2 gO, VIDAS HIV DUO	BioRad NEW LAV Blot 1, BioRad NEW LAV Blot 2, Elecsys HIV Ag	Early infection (13), HIV-1 positive (71), HIV-1 subtyped (15), HIV-2 positive (16), HIV Ag positive (30), potentially interfering (140)
Landeskrankenanstalten Salzburg Blutzentrale, Salzburg, Austria	Enzygnost Anti-HIV1/2 Plus	NEW LAV Blot 1, Elecsys HIV Ag, Coulter HIV p24 antigen assay	HIV Ag positive (20), Blood donors (3,550)
Centro de Transfusión de Galicia, Santiago de Compostela, Spain	Prism HIV-O Plus	RIBA HIV-1/-2 SIA,[a] AmpliScreen HIV-1, version 1.5, Ortho HIV-1/2 antibody capture ELISA	Blood donors (1,522)
Red Cross Transfusion Service Upper Austria, Allgemeines Krankenhaus, Zentrallabor, Linz, Austria	Prism HIV-O Plus	NEW LAV Blot 1, Coulter HIV p24 antigen assay	Blood donors (1,025)
National Blood Centre, Thai Red Cross Society, Bangkok, Thailand	Prism HIV-O Plus	Abbott HIV Blot 2.2, Coulter HIV p24 antigen assay	Blood donors (1,482)
Hospital de Curry Cabral, Laboratorio Microbiologia, Lisbon, Portugal	Enzymun-Test HIV Combi	VIDAS HIV p24 II, NEW LAV Blot II, Diagnostics Biotechnology HIV-1 Western blotting	HIV-1 infected (87), HIV-1 subtyped (50), HIV-2 positive (20), HIV Ag positive (14), unselected hospitalized patients (420)
Institut für Med. Virologie, Universitätskliniken Frankfurt, Frankfurt, Germany	HIV-1/HIV-2 3rd gen. Plus EIA, AxSYM HIV-1/2 gO	NEW LAV Blot I, NEW LAV Blot II, HIV AG-1 Monoclonal A	HIV-1 infected (69), HIV Ag positive (27)
Laboratories Réunis, Kutter-Lieners-Hastert, Junglinster, Luxembourg	VIDAS HIV DUO, HIV-1/HIV-2 3rd gen. Plus EIA, Enzymun-Test HIV Combi, Enzygnost HIV Integral	NEW LAV Blot I, NEW LAV Blot II, HIV AG-1 Monoclonal A, VIDAS HIV p24 II, INNO-LIA HIV Confirmation	HIV-1 subtyped (17), Virus lysates (5), Routine samples (303), potentially interfering (37)
Roche Diagnostics, R & D Infectious Diseases	Enzygnost HIV Integral, Cobas Core Anti-HIV 1+2+Q EIA, Genscreen HIV 1/2, version 2, Enzymun-Test HIV Combi, AxSYM HIV-1/2 gO	Elecsys HIV Ag, Coulter HIV p24 antigen assay, NEW LAV Blot I, NEW LAV Blot II	Seroconversion (n = 87), early infection (10), HIV-1 positive (181), HIV-1 subtyped (197), HIV-2 positive (159), HIV Ag positive (29), virus lysates (57), dilutions of HIV positive sera (10), performance panels (8), selected hospitalized patients (577), potentially interfering (1,048)

[a] SIA, strip immunoblot assay.

seroconversion panels were provided by Boston Biomedica Inc. (BBI; West Bridgewater, Mass.), BioClinical Partners (Franklin, Mass.), North American Biologicals Inc. (NABI; Boca Raton, Fla.), and the Swedish Institute for Infectious Disease Control. For most of the seroconversion panels, HIV-1 RNA detection was performed by using quantitative PCR (Amplicor HIV-1 Monitor; Roche Diagnostics; see Table 2). The detection limits of the PCR protocol employed for panels BCP 9010 to 9034 and ANT 6240 to 6248 were 50 and 400 copies of HIV-1 RNA/ml of plasma, respectively. All seroconversion samples were tested by Western blotting. HIV Ag detection (HIV-1 AG Monoclonal A) was performed for all the seroconversion panels with the exception of SIIDC35. (ii) Single serum samples ($n = 32$) from the acute phase of infection after seroconversion were obtained. (iii) Anti-HIV-1-positive specimens ($n = 610$) from patients in different stages of the disease were obtained. (iv) For the assessment of the effect of genetic variability on HIV-1 detection, 373 subtyped samples from different geographical locations (Cameroon, Germany, Luxembourg, Belgium, Portugal, Switzerland, South Africa, Thailand, and Zimbabwe), including group M (subtypes A to J and putative subtype S) and group O sera were selected. The total number of group M non-B sera was 324. Samples from patients infected with recombinant group M virus and simian immunodeficiency virus (SIV)-positive monkey sera were analyzed. Subtype determination was by competitive EIA using HIV-1 subtype A- to E-specific gp120 V3 peptides in accordance with the protocol of Kasper et al. (P. Kasper, A. N. Smith, G. Duraisamy, B. Ofenloch, and E. Faatz, Abstr. Deutscher AIDS-Kongreß, abstr. V089, p. 32, 1996) or by using a V3 loop-based research enzyme-linked immunosorbent assay from Dade-Behring (14, 27) and PCR-amplified sequencing of the C2V3 region after isolation of HIV from peripheral blood mononuclear cells from citrated blood. Alignment of the amino acids to the known consensus sequences was done by using the Los Alamos database (15). Also included were the Worldwide HIV-1 performance panel WWRB301 (BBI) consisting of 47 HIV-1 subtyped and HIV-2-positive samples from different geographic locations (Argentina, Cameroon, Canada, United States, China, Egypt, Ghana, India, South Africa, Thailand, Uganda, and Zimbabwe) and panel AfrRB1 (BBI) including 42 HIV-1 group M (A, B, and C) and 5 HIV-2-positive samples from Africa. (v) Anti-HIV-2-positive samples ($n = 302$) were from different geographic regions in West Africa and Portugal and included three SIV type 2 (SIV-2)-positive samples from monkeys. (vi) HIV-1 Ag- and HIV-1 antibody-positive samples were from patients at different stages of disease ($n = 120$). (vii) Dilutions of cell culture supernatants ($n = 57$) from cells infected with different HIV-1 subtypes, including group M subtypes A to H, group O, HIV-2, and unknown subtypes were tested in order to investigate the influence of the genetic variability of HIV on Ag detection. Virus isolates had been genotyped by sequencing PCR-amplified fragments of the C2V3 genome region (16). All the supernatants were diluted in anti-HIV-negative serum. (viii) Selected performance panels from BBI included low-titer panels PRB104, -105, -106, and -107 and anti-HIV-1 mixed-titer panels PRB202 and PRB203. Criteria for inclusion in mixed-titer performance panels were a positive Western blot using CDC criteria or an indeterminate blot when HIV Ag is present. Inclusion criteria for low-titer panels were sample absorbance-to-cutoff ratios of less than 4.0 on the basis of at least two Food and Drug Administration-licensed tests and a positive Western blot by Association of State and Territorial Public Health Laboratory Directors and CDC criteria or an indeterminate Western blot when HIV p24 Ag is present.

For the evaluation of specificity, the following unselected and selected speci-

mens were comparatively tested with Cobas Core HIV Combi EIA and alternative assays (see Table 3): (i) 7,579 unselected samples from blood donors from different blood transfusion centers, (ii) 303 unselected daily routine samples, (iii) 420 unselected specimens from hospitalized patients, (iv) 577 selected samples from hospitalized patients tested negative for acute or chronic hepatitis B, (v) a high number of potentially interfering samples (n = 1,222) including rheumatoid factor-, anti-hepatitis C virus-, and human T-cell leukemia virus-positive serum samples, sera from patients suffering from acute viral, bacterial, and fungal infections, liver cirrhosis, or autoimmune diseases, dialysis patients, and pregnant women.

Data evaluation and statistical analysis. Determinations were carried out in single measurements. Initially reactive (IR) specimens and discrepant specimens were repeated in single measurements or double determinations if enough sample material was available. Repeatedly reactive (RR) and discrepant samples were subjected to antibody and/or Ag confirmation with Western blotting and/or a single-Ag assay.

The performance of Cobas Core HIV Combi EIA was compared with that of alternative screening assays and HIV Ag and HIV-1 RNA detection for the seroconversion panels. The mean number of days by which the diagnostic window period was reduced with Cobas Core HIV Combi EIA in comparison to results for alternative third- and fourth-generation assays was calculated. The statistical significance of the reduction for each test was determined by using the Wilcoxon test for matched pairs (5).

The time delay between blood sampling points in commercially available seroconversion panels used for the present study is on average relatively short (2 to 7 days) but may last up to 37 days, for example, for panel BBI W. The calculation model for time delays between assays established by the Paul Ehrlich Institute (11) was used. This method considers that seroconversion is theoretically possible the following day after the last negative follow-up sample. The total number and the average number of days of time delay for the 94 panels were compared with those for the most sensitive assay.

For the calculation of sensitivity and specificity, samples were considered HIV-1 positive if any of the following tests were positive: Western blotting (interpreted according to CDC criteria [7]), HIV-1 p24 Ag assay, and HIV-1 RNA assay. Patients were considered HIV negative if all the screening assays were negative or, for the EIA reactive samples, if the Western blotting result was negative or indeterminate and the HIV-1 p24 Ag assay was negative.

RESULTS

HIV Ag was detected with Cobas Core HIV Combi EIA one bleeding earlier in 4 of 93 seroconversion panels tested in parallel with HIV AG-1 Monoclonal A (Table 2). HIV Ag was not detected with HIV AG-1 Monoclonal A until the end of follow-up in four cases of primary infection (panels BBI AQ, BCP 9014, 9031, and 9032). Conversely, HIV-1 AG Monoclonal A detected acute HIV infection one or more bleedings earlier than Cobas Core HIV Combi EIA in seven seroconversion panels. The Coulter HIV p24 Ag assay detected HIV-1 Ag one or two bleedings earlier in 26 of 64 seroconversion panels tested in parallel with Cobas Core HIV Combi EIA (see Table 2). Cobas Core HIV Combi detected HIV-1 primary infection one or more bleedings earlier in four seroconversions.

Cobas Core HIV Combi EIA was more sensitive than the alternative fourth-generation assays for early detection of HIV infection. HIV Ag was detected earlier in 2 of 16, 6 of 15, and 21 of 29 seroconversion panels than with VIDAS HIV DUO, Enzymun-Test HIV Combi, and Enzygnost HIV Integral, respectively (Table 2). In panel BCP 9029, Enzygnost HIV Integral was transiently positive on day 16 and remained negative until the end of follow-up. Since this sample was HIV-1 RT-PCR, HIV Ag, and Western blotting negative, the result should be considered false positive. Primary HIV infection was detected one or more bleedings earlier than with AxSYM HIV-1/2 gO, Prism HIV-Oplus, Enzygnost Anti-HIV1/2 Plus, and Genscreen HIV1/2, version 2, in 49 of 62, 25 of 36, 34 of 40, and 20 of 31 seroconversion panels, respectively. Conversely, seroconversion was detected one bleeding earlier with one or more third-generation assays in panels BCP 9017 and 9032 and NABI SVO-0251-1. A second diagnostic window with Cobas Core HIV Combi EIA was observed in three seroconversion panels. A borderline result (index value, 0.92) was observed on day 45 in BCP 9010. Cobas Core HIV Combi EIA became negative in panel SIIDC13 on day 50 when antigenemia was no longer detectable; this sample reacted weakly in the IMx HIV-1/HIV-2 III Plus and was tested negative with Enzygnost Anti-HIV1/2 Plus. Cobas Core HIV Combi EIA was borderline reactive (index values on duplicate testing: 0.87 and 0.92) in a follow-up sample obtained 4 days later that was positive in the third-generation assays. A borderline result was observed in the second follow-up sample of SIIDC35 with Cobas Core HIV Combi. In panel BCP 9012, the HIV-1 RNA copy number was transiently under the detection limit of HIV-1 RT-PCR (day 2). The viral load was below the detection limit in panel BCP 9014 in the last four follow-up samples (days 12 to 31).

The mean time delay of Cobas Core HIV Combi EIA (last negative sample plus 1 day) in comparison to HIV-1 RT PCR for 87 panels tested with both methods was 2.75 days. The difference in sensitivity between Cobas Core HIV Combi EIA and HIV-1 RT-PCR was statistically significant ($P < 0.0001$). Table 3 gives an overview of the mean time delays obtained for Cobas Core HIV Combi EIA and alternative assays in comparison to PCR for seroconversion panels. Cobas Core HIV Combi EIA showed a significantly higher sensitivity for the detection of primary HIV infection than alternative fourth-generation (with the exception of VIDAS HIV DUO) and third-generation assays ($P < 0.05$) (Table 3).

All the 32 single-serum samples obtained during seroconversion were tested positive with Cobas Core HIV Combi EIA. A variable number of samples gave false-negative results with third-generation EIAs (Table 4).

All 368 HIV-1 group M subtypes and group O and five samples positive for SIV-1 or -3 were found positive with Cobas Core HIV Combi EIA and the alternative assays. Only one sample (putative HIV-1 subtype S) was negative with Enzygnost Anti-HIV1/2 Plus and positive with Cobas Core HIV Combi EIA.

All the 302 sera from HIV-2-infected individuals, 3 SIV-2-positive sera from monkeys, and subtyped samples from BBI panel WWRB301 (M) (n = 47) and AfrRB1 (n = 42) were tested positive with Cobas Core HIV Combi EIA and the alternative assays (Table 4). One HIV-2-positive serum sample from Guinea-Bissau gave an index value (1.16) close to the cutoff with Cobas Core HIV Combi EIA and Enzygnost Anti-HIV1/2 Plus.

All the cell culture supernatants (n = 62) infected with different HIV-1 subtypes, including group M subtypes A to H, group O, HIV-2, and unknown subtypes were detected with a twofold- to more-than-fivefold-higher sensitivity with Cobas Core HIV Combi EIA than with Enzymun-Test HIV Combi (data not shown). Of 57 dilutions of virus lysates, including 1 HIV-1 subtype B, 2 group O, and 8 untyped HIV-1 lysates, tested in parallel with the Coulter HIV p24 Ag assay, HIV Ag was detected in higher dilutions in 11 lysates with Coulter HIV

TABLE 2. Time delay in days for the detection of HIV primary infection in comparison with the most sensitive assay for each panel

Sero-conversion panel	Bleeding day with first positive result (last negative bleeding day + 1 day) in:													
	Cobas Core HIV Combi EIA	VIDAS HIV DUO	Enzygnost HIV Integral	Enzymun-Test HIV Combi	Genscreen HIV1/2, version 2	IMx HIV-1/HIV-2 III Plus	AxSYM HIV-1/2 gO	Prism Anti-HIV-1/2	Enzygnost Anti-HIV1/2 Plus	HIV-1/HIV-2 3rd gen. Plus EIA	Cobas Core Anti-HIV 1+2+O EIA	HIV AG-1 Monoclonal A	Coulter HIV p24 Ag	HIV-1 RT-PCR
BBI Q	1				61				61			1		0
BBI R	0				3				3			0		
BBI S	0								1			0	0	
BBI T	0								4			0		
BBI U	0								4			0		
BBI V	0								8			0		
BBI W	1	3	3	3		3			13			1		0
BBI X	0					1			1			0		0
BBI Y	0	0				0			1			0		0
BBI Z	1	1	8	8		2			10			1		0
BBI AB	0						1		0			0	0	0
BBI AC	0	0					0		0			0	0	0
BBI AD	1	1	1	1			18		18			1	0	0
BBI AE	0	1	1	1			4		4			1	0	0
BBI AF	1	1		1			1		1			1	0	0
BBI AG	0	0		0			0		0			0	0	0
BBI AH	0			0			0		0			0	0	0
BBI AI	0	0	0	0			1		1			0	0	0
BBI AJ	1			1			5		5			1	1	0
BBI AK	3	3	3	3			10		15			3	3	0
BBI AL	3						10		10			3	3	0
BBI AM	0						4		4			0	0	0
BBI AN	1				8		10		10			2	1	0
BBI AP	1						8		8			1	1	0
BBI AQ	5						5		5			26	1	0
BBI AR	1						15		15			1	1	0
BBI AS	1		3	3	10		10		10			3	1	0
BBI AT	3		10	8			10		10			1	3	0
BBI AU	8				8		14		14			8	8	0
BBI AV	1						12		12			1	1	0
BBI AW	1				1		1		1			1	1	0
BBI AX	1				10		10					1	1	0
BBI AY	4				4		13		13			4	4	0
BBI AZ	1		4	4	4		4		4			1	0	0
BBI BA	1		4	1	8		8		8			0	0	0
BBI BB	0				1		1		5			0	0	0
BBI BC	4				4		8		8			4	4	0
BBI BD	4	4	8									4	8	0
BBI BE	4	4	8				8					4	1	0
BBI BF	3	3	8				11					3	3	0
BBI BG	3		3				10					3	1	0
BBI BH	8		10				10					8	3	0
BBI BI	1		1				8					0	0	0
ANT 6240	1				3		8	8	8			1		0
ANT 6241	0				3	3		9	3		3	0		0
ANT 6243	8						10	10	10	10	10	8	3	0
ANT 6244	2						9	4	4		4	4	1	0
ANT 6245	1						14	10	10	10	10	1	1	0
ANT 6246	1					8	8	8	8	8	8	1	0	0
ANT 6247	1						8		3	8	8	1	0	0
ANT 6248	1				5		5			5	5	5	1	0
BCP 9010	1						10	10		10	10	1	1	0
BCP 9011	29						29	29	29		29	29	24	0
BCP 9012	17						17	17	17		17	15	10	0
BCP 9013	8						8	8	8		8	6	1	0
BCP 9014	5						5	5	5		5	0	0	0
BCP 9015	1						10	10	10		10	1	1	0
BCP 9016	3						8	8	8		8	3	1	0
BCP 9017	29						25	25	25		25	29	25	0
BCP 9018	5						8	8	8		8	5	5	0
BCP 9019	0						0	0	0			0	0	0
BCP 9020	8	8	12				15	12				5	1	0
BCP 9021	5		12		8		12	12				1	1	0
BCP 9022	7		9		9		9	9				7	7	0
BCP 9023	8				13		15	15				8	1	4
BCP 9024	1		5		5		5	5				1	1	0
BCP 9025	0		1		1		7	1				0	0	0
BCP 9026	0		1		0		0	1				0	0	0
BCP 9028	1		3		3		3	3				0	0	0
BCP 9029	1		1		1	1	9	9				1	0	0
BCP 9030	3		10		10		10	10				3	3	0
BCP 9031	8		8		8		8	8				37	16	0
BCP 9032	8		8		6		6	8				40	40	0
BCP 9033	0	0	3				3	3				0	0	0

Continued on following page

TABLE 2—*Continued*

Seroconversion panel	Bleeding day with first positive result (last negative bleeding day + 1 day) in:													
	Cobas Core HIV Combi EIA	VIDAS HIV DUO	Enzygnost HIV Integral	Enzymun-Test HIV Combi	Genscreen HIV1/2, version 2	IMx HIV-1/HIV-2 III Plus	AxSYM HIV-1/2 gO	Prism Anti-HIV-1/2	Enzygnost Anti-HIV1/2 Plus	HIV-1/HIV-2 3rd gen. Plus EIA	Cobas Core Anti-HIV 1+2+O EIA	HIV AG-1 Monoclonal A	Coulter HIV p24 Ag	HIV-1 RT-PCR
BCP 9034	6	6	10				13	10		10		6	1	0
NABI SVO-0211-1	0				4		4					0		
NABI SVO-0241-1	0				4		4					0		
NABI SVO-0251-1	1				0		1					4		
NABI SVO-0261-1	0				0		1					0		
NABI SVO-0271-1	0	0		0	1		1					0		
NABI SVO-0281-1	1				1		1					0		
SIIDC1	0					0			0		0	0		
SIIDC5	0					0			0		0	0		
SIIDC13	0					0			34			0		
SIIDC18	0					0			0		0	38		
SIIDC22	0					0			0		0	0		
SIIDC24	0					1			1		1	0		
SIIDC35	0								4					

p24 Ag assay than with Cobas Core HIV Combi EIA. Conversely, Cobas Core HIV Combi EIA showed a higher sensitivity for one HIV-1 group O lysate, two HIV-2 lysates, and one untyped HIV lysate than the Coulter HIV p24 Ag assay.

Low index values were observed for two plasma samples (PRB107-06 [HIV p24 Ag positive, Western blot negative] and PRB 104-15 [HIV p24 Ag low positive, Western blot negative]) from BBI low-titer and mixed-titer performance panels. PRB107-06 was highly reactive in alternative third-generation assays. No results of third-generation assays were available for PRB104-15. One false-negative result was obtained with the Cobas Core Anti-HIV 1+2+O EIA in an HIV-1 p24-positive, Western blot-negative sample (PRB107-09).

From 7,579 unselected samples from blood donors, 16 (0.21%) were IR, and 14 (0.19%) were RR, in the Cobas Core HIV Combi EIA. Three samples were confirmed positive by Western blotting. The remaining 11 samples were negative in the Western blot and HIV-1 Ag test (Table 4). The Amplisscreen test was performed for 7 of the 11 RR samples from blood donors; all 7 were HIV-1 RNA negative. Cobas Core HIV Combi EIA allowed a high discrimination between negative and positive samples, since samples from only 3 of 7,579 blood donors gave an initially borderline reaction (index values: 0.9 to 1.0). The specificity of Cobas Core HIV Combi EIA for blood donors was high: 99.84% for IR samples and 99.85% for RR samples. The specificity of Enzygnost Anti-HIV-1/2 Plus was 99.83% (IR and RR samples). Prism Anti-HIV 1/2 showed 99.80% specificity for IR samples and 99.83% specificity for RR samples.

One hemolytic sample of 303 unselected routine sera was RR in the Cobas Core HIV Combi EIA but negative in the alternative assays, Western blotting and HIV p24 Ag EIA (Table 4). In a group of 420 unselected hospitalized patients with an extremely high prevalence of HIV, 50 HIV-positive confirmed samples were found with Cobas Core HIV Combi EIA and Enzymun-Test HIV Combi (Table 4). Three RR samples were Western blot indeterminate and HIV Ag negative. No follow-up sera were available, and samples were excluded from specificity analysis. One sample was initially negative in the Cobas Core HIV Combi EIA but positive in the Enzymun-Test HIV Combi. The sample was positive on repeated testing. Western blotting was indeterminate (gp160 reactive), and the sample was HIV Ag negative. This result was excluded from specificity analysis since no follow-up sample was available.

Fourteen false-positive results were obtained in the collec-

TABLE 3. Time delay in comparison to HIV-1 RT-PCR of Cobas Core HIV Combi EIA and alternative HIV screening assays in seroconversion panels

Cobas Core HIV Combi EIA time delay[a] (no. of days)	Alternative assay		No. of seroconversion panels tested with both assays	Mean time delay (days) between Cobas Core HIV Combi EIA and alternative assay	*P*
	Name	Time delay[a] (no. of days)			
237 (2.72)	HIV-1 AG Monoclonal A	308 (3.54)	70	−0.81	0.7922
216 (3.48)	Coulter HIV p24 antigen	184 (2.97)	63	0.51	0.0011
32 (2.00)	VIDAS DUO HIV	35 (2.19)	16	−0.19	0.5
83 (2.86)	Enzygnost HIV Integral	158 (5.45)	29	−2.59	<0.0001
14 (1.00)	Enzymun-Test HIV Combi	34 (2.43)	14	−1.43	0.0313
226 (3.65)	AxSYM HIV-1/2	519 (8.37)	62	−4.72	<0.0001
67 (5.57)	Prism Anti-HIV-1/2	275 (9.17)	30	−3.60	<0.0001
155 (3.30)	Enzygnost Anti-HIV1/2 Plus	423 (9.00)	47	−5.70	<0.0001
112 (7.00)	Cobas Core Anti-HIV 1+2+O EIA	174 (10.88)	16	−3.88	0.0059
67 (2.68)	Genscreen HIV1/2, version 2	194 (7.76)	25	−5.08	<0.0001

[a] Time delay is the last negative sample plus 1 day in comparison to result of HIV-1 RT-PCR. Values are totals. Values in parentheses are means. The calculation model for time delays between assays established by the Paul Ehrlich Institute (11) was used.

TABLE 4. Specimens tested and results obtained with comparative assays for the evaluation of sensitivity and specificity of Cobas Core HIV Combi

Specimen collection	No. of positive samples/samples tested in:										
	Cobas Core HIV Combi	VIDAS HIV DUO	Enzygnost HIV Integral	Enzymun-Test HIV Combi	Genscreen HIV1/2, version 2	IMx HIV-1/HIV-2, III Plus	AxSYM HIV-1/2 gO	Prism Anti-HIV-1/2	Enzygnost, Anti-HIV1/2 Plus	Cobas Core Anti-HIV 1+2+O EIA	HIV AG-1 Monoclonal A	Coulter HIV p24 Ag assay
Seroconversion panels	306/709	54/144	61/276	53/97	80/282	48/106	140/597	54/364	159/410	56/186	238/700	185/525
Early infection	32/32	3/3		1/1	7/10	6/7			8/9	17/20		
HIV-1 positive specimens in different stages of the infection	620/620	68/68	1/1	113/113	62/62		92/92		202/202	181/181		
HIV-1-subtyped and SIV-positive samples	462/462	17/17	16/16	50/50	62/62	47/47	91/91		85/85	197/197		2/48
HIV-2- and SIV-2-positive specimens	305/305		6/6	70/70	15/15		13/13		100/100	104/104		
HIV p24 Ag- and antibody-positive samples	120/120	27/27		14/14			50/50			29/29	57/57	41/49
BBI Performance panels	102/102				28/28	23/23	37/37			13/14	36/88	
Blood donors[a]	14/7,579							7/4,029	2/3,550			
Unselected routine samples	1/303	0/303										
Hospitalized patients[b]	50/420			50/420								
Selected hospitalized patients[c]	10/577			10/577								
Potentially interfering serum samples	15/1,225	0/37	3/1,045		0/140							

[a] 3 of 14 RR Cobas Core HIV Combi samples were confirmed HIV-1 Western blot positive.
[b] 50 samples were confirmed positive with HIV-1 Western blotting.
[c] 10 samples were confirmed positive with HIV-1 Western blotting.

TABLE 5. Sensitivities and specificities of assays

Assay	Sensitivity[a] without seroconversions	Specificity[a] RR
Cobas Core HIV Combi	100 (99.82–100)	99.73 (99.61–99.82)
VIDAS HIV DUO	100 (99.43–100)	100 (99.12–100)
Enzygnost HIV Integral	100 (87.79–100)	99.71 (99.16–99.94)
Enzymun-Test HIV Combi	100 (98.8–100)	100 (99.68–100)
Genscreen HIV1/2, version 2	98.31 (95.13–99.65)	100 (97.88–100)
IMx HIV-1/HIV-2 III Plus	98.70 (92.98–99.97)	ND[b]
AxSYM HIV-1/2 gO	100 (98.50–100)	ND
Prism Anti-HIV1/2	ND	99.88 (99.64–99.93)
Enzygnost Anti-HIV1/2 plus	99.75 (98.60–100)	99.94 (99.80–99.99)
Cobas Core Anti-HIV 1+2+O EIA	99.27 (98.13–99.8)	ND
HIV AG-1 Monoclonal A	ND	ND
Coulder HIV p24 Ag assay	ND	ND

[a] Values are percentages. Values in parentheses are 95% confidence intervals.
[b] ND, not determined.

tion of 1,222 potentially interfering serum samples (Table 4). Most of the false positives ($n = 5$) were among the group of hepatitis A virus (HAV)-immunoglobulin M (IgM)-positive sera. Two sera each from pregnant women and human T-cell leukemia virus-infected patients and one sample each from dialysis patients, patients with autoimmune diseases or chronic hepatitis B virus infection, *Toxoplasma gondii* IgG-positive individuals, and one person vaccinated against influenza virus were also false positive.

Cobas Core HIV Combi EIA showed a sensitivity of 100% for HIV-1- and HIV-2-positive samples (Table 5). Overall, 30 RR false-positive results out of 10,031 HIV-negative samples, including a high number of potentially cross-reactive specimens, were obtained with Cobas Core HIV Combi EIA (Table 4). It is to be expected that about 1 of 334 specimens analyzed will be incorrectly identified as false positive. The specificity of Cobas Core HIV Combi EIA was 99.73% (Table 5). In most cases, the total number of samples measured with each one of the alternative assays was too low to get a statistically reliable result for specificity (Table 4).

DISCUSSION

The results of our study demonstrate that Cobas Core HIV Combi EIA permits an earlier diagnosis of HIV infection than third-generation EIAs. The time delay in comparison to HIV-1 RNA amplification is about 2.7 days (Table 3). By using fourth-generation assays with a more sensitive HIV Ag detection module (detection limit, $\leq$10 pg of p24 Ag/ml), such as Cobas Core HIV Combi EIA, the diagnostic window is reduced by about 1.5 to 2.5 days in comparison to that for less-sensitive combined p24 Ag and antibody assays (detection limit, $\geq$30 pg of p24 Ag/ml). As shown by the results obtained with Coulter HIV p24 Ag assay, the diagnostic window would be reduced by a further 0.5 days with a lower detection limit of the Ag module (Table 3). Of note, the new VIDAS Duo Ultra, with a detection limit of 3 pg of p24 Ag/ml (20), not investigated here, shows a sensitivity which is equal to that of one of the most sensitive HIV p24 Ag assays (detection limit, 2.2 pg of p24 Ag/ml) available on the market (32).

The diagnostic window would be reduced by 2.7 days in comparison to that for Cobas Core HIV Combi EIA by amplification of HIV-1 RNA by RT-PCR from plasma or serum

(single-unit testing). Cost-effectiveness analysis of expanded HIV testing protocols for donated blood has shown that RNA PCR testing would prevent annually eight more cases of transfusion-associated HIV infection than combined p24 and antibody detection at a net additional cost of $96 million per year in the United States (2). Current commercially available PCR protocols are not adapted to large-scale screening of individual blood donations. For these reasons, fourth-generation assays represent a valuable alternative in terms of cost-effectiveness calculations for the improvement of the sensitivity of blood donor screening.

The prevalence of HIV-1 group M non-B subtypes (subtypes A and C to J) and HIV-2 infections is increasing relatively quickly in Europe. HIV-1 group O infections remain a rarity. The epidemiological significance of HIV-1 group N, described only recently, is not yet known (28). The genetic variability represents a challenge in particular for the early detection of HIV infection. In the seroconversion phase, false-negative results and delayed detection of antibody response are observed in infections with HIV-1 non-B subtypes and HIV-2 (1, 8). In recent years, the sensitivity for detection of HIV-1 non-B subtypes has been optimized. However, obtaining seroconversion samples with HIV-1 non-B subtypes in order to permit an extensive evaluation of the sensitivities of newly developed tests is difficult. In the present evaluation, one seroconversion from a patient with HIV-1 subtype E infection (SIIDC24) was tested with Cobas Core HIV Combi EIA and HIV-1 AG Monoclonal A. Both assays were highly reactive in the first blood sample obtained 7 days after onset of symptoms. Tersmette et al. (29) reported the failure of a monoclonal antibody to detect p24 Ag from certain strains of HIV. HIV-1 subtype O, which is highly divergent from other HIV-1 subtypes known so far (13), may not be detected by assays using monoclonal antibodies for the capture of p24 Ag. Our results from dilution series of cell supernatants infected with different HIV-1 subtypes, including HIV-1 group O isolates and HIV-2, show that no commercially available assay (HIV p24 Ag EIA or fourth-generation EIA) is capable of detecting every HIV-1 virus lysate with an optimal sensitivity. Although a limited number of lysates were tested, Cobas Core HIV Combi EIA was likely to have a higher sensitivity for HIV-2 Ag detection than the Coulter HIV p24 Ag assay. Previous studies reported a variable sensitivity of fourth-generation assays for HIV-1 group M non-B subtypes and HIV-2 (12, 20, 33).

There is a potential risk for false-negative results due to the absence of p24 Ag in the Ag mixture of Cobas Core HIV Combi. In general, anti-p24 antibodies are, together with antienvelope antibodies, the first to appear during seroconversion (21). HIV RT has been included in analogy to Enzymun-Test HIV Combi in the antigenic mixture of Cobas Core HIV Combi EIA in order to avoid false-negative results in the early seroconversion phase after disappearance of HIV antigenemia since p24 Ag would have interfered with anti-p24 for HIV Ag capture. As shown by the results of our study, in only 3 of 94 seroconversions were transient borderline-negative results measured with Cobas Core HIV Combi EIA in the early phase of seroconversion; this effect was also observed with Enzymun-Test HIV Combi in a former study (12) and could therefore possibly result from the absence of anti-p24 detection. The second reason for including RT as the Ag in the Cobas Core HIV Combi EIA is that, with gp41 alone, the sensitivity of the assay would have been impaired not only during seroconversion but also at all stages of the disease. The RT is highly conserved among HIV-1 group M and O isolates and HIV-2 and induces a cross-reactive, predominantly IgG response (W. Melchior, unpublished data).

A second diagnostic window may be observed not only in serological assays, since, in two cases of primary infection, the viral load decreased to under the detection limit of 50 copies of HIV-1 RNA/ml of plasma for HIV-1 RT-PCR after initially positive results. In panel BCP 9014 HIV-1 RNA was not detectable from day 12 (fourth follow-up sample) until the end of follow-up (day 31, seventh follow-up sample). With NAT, false-negative results are observed in infected subjects with a low viral load independent of HIV-1 subtype and stage of disease (3).

A second potential risk for impaired sensitivity is that a more limited area of the solid phase can be used for antibody detection since about one-third of the binding sites are occupied by anti-p24 antibody for HIV Ag detection. A time delay between third-generation assays and Cobas Core HIV Combi EIA was observed in three seroconversions with weak, delayed, or absent antigenemia. Gürtler et al. reported a delayed detection of primary infection with Enzymun-Test Combi in an HIV p24 Ag-negative seroconversion panel (12).

Anti-HAV-IgM antibody-positive samples possibly can lead to elevated signals with Cobas Core HIV Combi EIA, since 5 out of 125 anti-HAV-IgM-positive samples were tested as false positives. This interference cannot be explained by cross-reactive epitopes of HIV RT and HAV polymerase since these enzymes are not related, but rather most probably by immune complex formation.

The risk of interference is potentially higher with the HIV combined assays than with single-Ag or -antibody EIAs, since both serological markers are determined in one test batch. The specificity for blood donor screening with licensed fourth-generation assays (99.6 to 99.8%) was lower than that of third-generation HIV EIAs (12, 18). The higher rate of false-positive results (0.2%) of HIV combined assays has a particularly negative effect in blood donor screening, where a high sample throughput and low prevalence are to be expected, because a higher number of donations must be withdrawn and a separate Ag detection in addition to the antibody confirmatory assay (Western blotting) must be carried out in order to rule out an HIV infection. The specificities of the new Cobas Core HIV Combi EIA for 7,579 blood donors (99.84%) and for more than 10,000 samples (99.73%), including potentially interfering sera, were very high. Cobas Core HIV Combi EIA showed a better performance than Enzymun-Test HIV Combi, which achieved a specificity of 99.60% in 7,659 negative samples (12). The specificity of Cobas Core HIV Combi EIA was equivalent to that of third-generation assays, such as Enzygnost Anti-HIV1/2 Plus and Prism HIV-O Plus, which are routinely used for blood donor screening. Our results show that fourth-generation assays with improved specificity are suitable for blood donor screening in terms of specificity. Furthermore they detect HIV p24 Ag with a sensitivity comparable to that of single-Ag assays. Cobas Core HIV Combi EIA may represent an alternative to separate Ag determination in the diagnostic lab-

oratory and blood donor screening and possibly to NAT in (mini)pools.

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REFERENCES

1. **Apetrei, C., I. Loussert-Ajaka, D. Descamps, F. Damond, S. Saragosti, F. Brun-Vezinet, and F. Simon.** 1996. Lack of screening test sensitivity during HIV-1 non-subtype B seroconversions. AIDS **10:**57–60.
2. **Aubuchon, J. P., J. D. Birkmeyer, and M. P. Busch.** 1997. Cost-effectiveness of expanded human immunodeficiency virus-testing protocols for donated blood. Transfusion **37:**45–51.
3. **Barlow, K. L., J. H. C. Tosswill, J. V. Parry, and J. P. Clewley.** 1997. Performance of the Amplicor human immunodeficiency virus type 1 PCR and analysis of specimens with false-negative results. J. Clin. Microbiol. **35:**2846–2853.
4. **Berrey, M. M., T. Schlaker, A. C. Collier, T. Shea, S. J. Brodie, D. Mayers, R. Coombs, J. Krieger, T. W. Chun, A. Fauci, S. G. Self, and L. Corey.** 2001. Treatment of primary human immunodeficiency virus type 1 infection with potent antiretroviral therapy reduces frequency of rapid progression to AIDS. J. Infect. Dis. **183:**1466–1475.
5. **Bünung, H., and G. Trenkler.** 1978. Nicht-parametrierbare Methoden. Springer Verlag, Berlin, Germany.
6. **Busch, M. P., S. H. Kleinman, B. Jackson, S. L. Stramer, J. Hewlett, and S. Preston.** Committee report. Nucleic acid amplification testing of blood donors for transfusion-transmitted infectious diseases: report of the Interorganizational Task Force on Nucleic Acid Amplification Testing of Blood Donors. Transfusion **40:**143–159.
7. **Centers for Disease Control.** 1989. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. Morbid. Mortal. Wkly. Rep. **38**(Suppl. S-7)**:**1–7.
8. **Christiansen, C. B., T. E. Jessen, C. Nielsen, and P. Staun-Olsen.** 1996. False negative anti-HIV-1/HIV-2 ELISAs in acute HIV-2 infection. Vox Sanguis **70:**144–147.
9. **Clark, S. J., G. D. Kelen, D. R. Henrard, E. S. Daar, S. Craig, G. M. Shaw, and T. C. Quinn.** 1994. Unsuspected primary human immunodeficiency virus type 1 infection in seronegative emergency department patients. J. Infect. Dis. **170:**194–197.
10. **Constantine, N. T.** 1993. Serologic tests for the retroviruses: approaching a decade of evolution. AIDS **7:**1–13.
11. **Couroucé, A. M., and Groupe de Travail Rétrovirus de la S.F.T.S.** 1999. Tests de dépistage combiné des anticorps anti-VIH et de l'antigène p24. Gaz. Transfus. Sanguine **155:**4–18.
12. **Gürtler, L., A. Mühlbacher, U. Michl, H. Hofmann, G. G. Paggi, V. Bossi, R. Thorstensson, R. G. Villaescusa, A. Eiras, J. H. Hernandez, W. Melchior, F. Donié, and B. Weber.** 1998. Reduction of the diagnostic window with a new combined p24 antigen and human immunodeficiency virus antibody screening assay. J. Virol. Methods **75:**27–38.
13. **Gürtler, L., P. H. Hauser, J. Eberle, A. von Brunn, S. Knapp, L. Zekeng, J. Tsague, and L. Kaptue.** 1994. A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. J. Virol. **68:**1581–1585.
14. **Hoelscher, M., S. Hanker, F. Barin, R. Cheingsong-Popov, U. Dietrich, B. Jordan-Harder, D. Olaleye, E. Nägele, A. Markuzzi, D. Mwakagile, F. Minja, J. Weber, L. Gürtler, and F. Sonnenburg.** 1998. HIV type 1 V3 serotyping of Tanzanian samples: probable reasons for mismatching with genetic subtyping. AIDS Res. Hum. Retrovir. **14:**139–149.
15. **Korber, B. T., S. Osmanov, J. Esparza, and G. Myers.** 1994. The World Health Organization Global Programme on AIDS proposal for standardization of HIV sequence and nomenclature: W. H. O. network for HIV isolation and characterization. AIDS Res. Hum. Retrovir. **10:**1355–1358.
16. **Korber, B. T. M., E. E. Allen, A. D. Farmer, and G. L. Myers.** 1995. Heterogeneity of HIV-1 and HIV-2. AIDS **9:**S5–S18.
17. **Le Corfec, E., F. Le Pont, H. C. Tuckwell, C. Rouzioux, and D. Costagliola.** 1999. Direct HIV testing in blood donations: variation of the yield with detection threshold and pool size. Transfusion **39:**1141–1144.
18. **Ly, T. D., C. Edlinger, and A. Vabret.** 2000. Contribution of combined detection assays of p24 antigen and anti-human immunodeficiency virus (HIV) antibodies in diagnosis of primary HIV infection by routine testing. J. Clin. Microbiol. **38:**2459–2461.
19. **Ly, T. D., S. Laperche, and A. M. Couroucé.** 2001. Early detection of human immunodeficiency virus infection using third- and fourth-generation screening assays. Eur. J. Clin. Microbiol. Infect. Dis. **20:**104–110.
20. **Ly, T. D., L. Martin, M. Daghfal, A. Sandridge, D. West, R. Bristow, L. Chalouas, X. Qiu, S. C. Lou, J. C. Hunt, G. Schochetman, and S. G. Devare.** 2001. Seven human immunodeficiency virus (HIV) antigen-antibody combination assays: evaluation of HIV seroconversion sensitivity and subtype detection. J. Clin. Microbiol. **39:**3122–3128.
21. **McRae, B., J. A. M. Lange, M. S. Ascher, F. De Wolf, H. W. Sheppard, J. Goudsmit, and J. P. Allain.** 1991. Immune response to HIV p24 core protein during early phases of human immunodeficiency virus infection. AIDS Res. Hum. Retrovir. **7:**637–643.
22. **Murthy, K. K., D. R. Henrard, J. W. Eichberg, K. E. Cobb, M. P. Busch, J. P. Allain, and H. J. Alter.** 1999. Redefining the HIV-infectious window period in the chimpanzee model: evidence to suggest that viral nucleic acid testing can prevent blood-borne transmission. Transfusion **39:**688–693.
23. **Portincasa, P., R. Grillo, P. Pauri, M. G. Colao, P. P. Valcavi, D. Speziale, G. Mazzarelli, E. De Majo, P. E. Varaldo, G. Fadda, C. Chezzi, and G. Dettori.** 2000. Multicenter evaluation of the new HIV DUO assay for simultaneous detection of HIV antibodies and p24 antigen. New Microbiol. **23:** 357–365.
24. **Quinn, T. C., R. Brookmeyer, R. Kline, M. Shepherd, R. Paranjape, S. Mechendale, D. A. Gadkari, and R. Bollinger.** 2000. Feasibility of pooling sera for HIV-1 viral RNA to diagnose acute primary HIV-1 infection and estimate HIV incidence. AIDS **14:**2751–2757.
25. **Roth, W. K., H. Weber, and E. Seifried.** 1999. Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood bank setting. Lancet **353:**359–363.
26. **Saville, R. D., N. T. Constantine, F. R. Cleghorn, N. Jack, C. Bartholomew, J. Edwards, P. Gomez, and W. A. Blattner.** 2001. Fourth-generation enzyme linked immunosorbent assay for the simultaneous detection of human immunodeficiency virus antigen and antibody. J. Clin. Microbiol. **39:**2518–2524.
27. **Sherefa, K., A. Sönnerborg, J. Steinbergs, and M. Sällberg.** 1994. Rapid grouping of HIV-1 infection in subtypes A to E by V3 peptide serotyping and its relation to sequence analysis. Biochem. Biophys. Res. Commun. **205:** 1658–1664.
28. **Simon, F., P. Mauclere, P. Roques, I. Loussert-Ajaka, M. C. Muller-Trutwin, S. Saragosti, M. C. Georges-Courbot, F. Barré-Sinoussi, and F. Brun-Vézinet.** 1998. Identification of a new human immunodeficiency virus type 1 distinct from group M and Group O. Nat. Med. **4:**1032–1037.
29. **Tersmette, M., I. Winkel, M. Groenick, R. A. Gruters, R. P. Spence, E. Saman, G. van der Groen, F. Miedema, and J. G. Huisman.** 1989. Detection and subtyping of HIV-1 isolates with a panel of characterized monoclonal antibodies to HIV p24 gag. Virology **171:**149–155.
30. **van Binsbergen, J., A. Siebelink, A. Jacobs, W. Keur, F. Bruynis, M. van de Graaf, J. van der Heijden, D. Kambel, and J. Toonen.** 1999. Improved performance in seroconversion with a 4th generation HIV antigen/antibody assay. J. Virol. Methods **82:**77–84.
31. **van Binsbergen, J., W. Keur, A. Siebelink, F. M. van de Graaf, A. Jacobs, D. de Rijk, L. Nijholt, J. Toonen, and L. G. Gürtler.** 1998. Strongly enhanced sensitivity of a direct anti-HIV-1/-2 assay in seroconversion by incorporation of HIV p24 Ag detection: a new generation vironostika HIV Uni-Form II. J. Virol. Methods **76:**59–71.
32. **Weber, B., A. Berger, H. Rabenau, and H. W. Doerr.** 2002. Evaluation of a new combined antigen and antibody human immunodeficiency virus screening assay, VIDAS HIV DUO Ultra. J. Clin. Microbiol. **40:**1420–1426.
33. **Weber, B., E. M. B. Fall, A. Berger, and H. W. Doerr.** 1998. Reduction of diagnostic window by new fourth-generation human immunodeficiency virus screening assays. J. Clin. Microbiol. **36:**2235–2239.
34. **Weber, B., A. Mühlbacher, U. Michl, G. Paggi, V. Bossi, C. Sargento, R. Camacho, E. M. B. Fall, A. Berger, U. Schmitt, and W. Melchior.** 1999. Multicenter evaluation of a new rapid automated HIV antigen detection assay. J. Virol. Methods **78:**61–70.
35. **Yerly, S., F. Simon, and L. Perrin.** 1999. Early diagnosis of primary HIV infections: using a combined screening test (p24 antigen and anti-HIV antibodies). Schweiz. Med. Wochenschr. **27:**319–322.
36. **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.