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Fourth-Generation Enzyme-Linked Immunosorbent Assay for the Simultaneous Detection of Human Immunodeficiency Virus Antigen and Antibody

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The VIDAS HIV DUO Ultra, a fourth-generation immunoassay under development for the simultaneous detection of human immunodeficiency virus type 1 (HIV-1) p24 antigen and antibodies to HIV-1 and HIV-2, was evaluated. The enzyme-linked fluorescence immunoassay, performed on the automated VIDAS instrument, is claimed to detect early and established HIV infection. The assay was challenged with a total of 2,847 samples that included 74 members of 10 seroconversion panels, 9 p24 antigen-only-reactive members of a panel of group M clades, 503 consecutively collected samples from individuals seeking care in the University of Maryland Medical System, 1,010 samples from U.S. blood donors, 1,141 samples from patients in a high-incidence population in Trinidad, 83 samples from a clinic for sexually transmitted diseases in the Bahamas, 10 confirmed HIV-1 group O samples, and 16 confirmed HIV-2 samples from the Cote d'Ivoire. Reference tests were U.S. Food and Drug Administration-licensed HIV antibody screening, p24 antigen tests, HIV confirmatory assays, and the Roche Diagnostics Amplicor HIV-1 Monitor. The VIDAS HIV DUO Ultra demonstrated 100% sensitivity and 99.5% specificity overall, with a 99.7% specificity in low-risk individuals. The analytical sensitivity, as assessed by seroconversion panels and p24 antigen in samples, was equivalent to the sensitivity of the reference assays used to characterize these panels. The VIDAS HIV DUO Ultra is accurate, offers potential advantages over conventional HIV testing for time and cost savings, has walk-away capability, and correctly identifies both early and established HIV infections.

Since 1986, a number and variety of commercial assays have been available to screen blood, diagnose infection, and monitor disease progression in individuals infected by human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2). These assays are categorized in four main classes, including tests that detect HIV antibody, detect p24 antigen, detect or quantify viral nucleic acids, and estimate T-lymphocyte numbers (cell phenotyping) (5). The enzyme-linked immunosorbent assay (ELISA) is the most common immunoassay utilized for the detection of HIV antibody and antigen. This technique has evolved from the first-generation viral lysate-based immunoglobulin G (IgG) tests, to the second-generation tests incorporating recombinant and/or synthetic peptide antigens, to the third-generation tests which detect IgG and IgM (antigen sandwich techniques), and finally to the third-generation-plus assays which also detect HIV-1 group O (5).

Specific antibody to HIV is synthesized soon after infection, although the precise time may depend on several factors, including both host and viral characteristics. Significantly, antibody may be present at low levels during early infection; however, these levels may be below the minimum concentration detectable by some assays (5). Antibody is detected in a majority of individuals within 6 to 12 weeks after infection with the earlier generations of assays, but antibody levels can be detected within 3 to 4 weeks after infection when the newer third-generation antigen sandwich assays are used (3). This window period can be shortened to about 2 weeks using p24 antigen assays or to 1 week with the implementation of nucleic acid detection assays (10). Consequently, the window period between infection and detection of infection may be less than 2 weeks if a comprehensive testing approach is utilized (6).

In addition to increased sensitivity and specificity with the incorporation of recombinant proteins and synthetic peptide antigens, the ELISA offers several advantages over other types of assays in that it is inexpensive, relatively simple, suitable for testing sizeable numbers of samples, and easily adapted to automated platforms. Although nucleic acid testing and viral culture are highly sensitive and specific methods to identify infection, respectively, these procedures are time-consuming, laborious, and expensive (5).

The detection of p24 antigen by ELISA is a simple and cost-effective technique to demonstrate viral components in blood, thereby verifying infection and/or identifying early infection, and offers the same performance advantages as the ELISAs for antibody detection (6). The antigen assay measures viral capsid (core) p24 protein in blood usually earlier than antibody during acute infection due to the initial burst of virus replication after infection (8). In the United States, antigen testing was implemented in 1995 to supplement antibody screening of donated blood components and has identified

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antibody-negative, HIV-contaminated units (11). Consequently, screening blood for both antibody and antigen results in almost 30 million tests for the 15 million blood units donated per year in the United States. Not only does this double the cost of screening and increase the turnaround time of results, but it also requires additional personnel and instrumentation.

The benefits of testing for both antibody and antigen are justifiable due to the need to identify individuals with both established and early HIV infections not only within the blood donor population but also in clinical application. Early detection of infection via antigen testing promotes the prompt referral of infected individuals for the initiation of treatment, counseling, and prevention interventions to reduce the risk of transmission (6). Further, the existence of an assay that may provide for simultaneous antigen and antibody detection would be of great benefit for the diagnosis of HIV infection by clinical laboratories in hospitals or private organizations. Recently, reports of a new generation of combination ELISAs that simultaneously detect both antigen and antibody demonstrate promise in reducing the window period to diagnosis of infection as well as decreasing the time, personnel, and costs necessary to perform both assays (14, 24, 25, 26).

The VIDAS HIV DUO Ultra (bioMérieux, Marcy l'Etoile, France) is claimed to be a more sensitive screening test for early and established HIV infection because it is able to detect both antigen and antibody. This report describes an evaluation of a fourth-generation test that screens for HIV-1 p24 antigen, anti-HIV-1 (including HIV-1 group O), and anti-HIV-2 simultaneously.

MATERIALS AND METHODS

VIDAS instrument. The VIDAS immunoanalysis system includes a test processing unit, a computer, and a printer. The test-processing unit consists of five independently operating sections. Each unit is a tray with channels for six reagent strips (tests). The instrument holds pipette tip-like solid-phase receptacles (SPR) coated internally with p24 monoclonal antibody and HIV antigens. The SPR has a straight tip with a small hole for the aspiration or dispensing of liquid by the instrument. Under the control of the computer, the SPR moves vertically in and out of the wells in the reagent strip and the reagent strip tray moves horizontally so that the SPR accesses the required well at each step. In the procedure, all liquids are located in the reagent strip and are aspirated into the SPR for any antibody-antigen reaction to occur and for pneumatic transfer to and from the reagent strip. A fluorometric scanner mechanically moves horizontally to sequentially read the optical density of each of the reaction cuvettes in the reagent strip (1). The instrument performs a background reading and two fluorescence experimental readings, with the computer automatically analyzing the results. The background reading is taken after the enzyme-substrate reaction occurs in the lower surface of the SPR. The first test value is obtained as a relative fluorescence value (RFV) for antibody detection and is calculated by subtracting the background reading. The second experimental fluorescence reading is taken after the conjugate-substrate reaction of the entire surface of the SPR. An RFV for the antigen detection result is derived from a calculation model, where two test values are calculated by dividing the sample RFV for each section of the SPR by a corresponding standard RFV. The computer interprets test values of ≥ 0.25 as a positive result. Reactive samples are generated when one or both of the antibody and antigen test values are positive. A nonapplicable (N/A) result may appear for either the antibody or antigen result in some cases if the individual determination of one cannot be calculated, but the final interpretation is based on the positive test value of the other.

VIDAS HIV DUO Ultra assay. The VIDAS HIV DUO Ultra (DUO Ultra), a product under development, is an ELISA that combines two immunoassay reactions with two final fluorescence detections as described by the manufacturer (VIDAS HIV DUO Ultra kit insert; bioMérieux). Coated with a cocktail of three different monoclonal p24 antibodies, the surface of the upper section of the SPR enables the detection of p24 antigen. The lower surface of the SPR allows for the detection of anti-HIV-1 and anti-HIV-2 immunoglobulin because it is coated with an entire gp 160 protein and two peptides representing the immunodominant regions of gp41 for HIV-1 group O and gp36 of HIV-2. Each strip has 10 wells; plasma or serum is dispensed in the first well. The next eight wells contain factory-dispensed diluent, wash solution, and conjugate. The last well is an optical cuvette that contains the fluorescent substrate and receives the final reactants for detection and reading.

Samples. The performance of the DUO Ultra assay was challenged with sera from eight populations of patients totaling 2,838 samples. The populations included 1,141 samples from patients in a high-incidence population from Trinidad who were at high likelihood of being p24 antigenemic (16); 83 samples from a high-risk sexually transmitted disease clinic cohort in the Bahamas; 503 samples sequentially collected from patients seeking HIV testing at the University of Maryland Medical System (prevalence of HIV infection, 2%); 1,010 samples from blood donors in the United States (The Blood Center, Houston, Tex.); 16 HIV-2-positive samples and 1 nonreactive sample from Cote d'Ivoire, 10 confirmed HIV-1 group O samples from Cameroon and the United States; 10 HIV seroconversion panels representing a total of 74 members (Boston BioMedical Inc. [BBI], Bridgewater, Mass.); and one panel of HIV-1 group M antigen-reactive clades (BBI) consisting of nine members (eight samples of clades A to H and a panel diluent). The seroconversion panels (A, C, E, I, J, K, L, P, Q, and R) were selected based on the presence of antigenemia by the reference tests in at least one time point during the seroconversion process. All samples were either serum or plasma. Sera from Cote d'Ivoire were characterized by HIV-1 and -2 ELISA (Genetic Systems, Redmond, Wash.) and by the HIV-1 and -2 Western blot 2.2 (Genelabs Diagnostics, Singapore). To be classified as HIV-2 reactive, samples had demonstrated reactivity with the gp36 HIV-2 specific peptide but did not meet the criteria for HIV-1 positivity by the Genelabs Western blot. Among the 10 confirmed HIV-1 group O samples, eight were from Cameroon and were characterized by an HIV-1 and -2 ELISA, an HIV-1 competitive ELISA, and a V3 loop peptide EIA (Ant-70 and 5180) and by V3 loop sequencing as described elsewhere (4). The two group O samples from the United States were purchased from Serologicals, Inc. (Clarkston, Ga.), and represented the two confirmed cases of HIV-1 group O infection from California and Maryland, as reported by the Centers for Disease Control and Prevention (Atlanta, Ga.).

Analysis. Testing was performed at the University of Maryland in a blinded fashion utilizing two VIDAS instruments and the DUO Ultra assay. Reference testing was performed using a routine HIV-1 and -2 antibody ELISA (HIV-1 and HIV-2 peptide enzyme immunoassay; Genetic Systems, or the HIV-1 and -2 recombinant DNA enzyme immunoassay and HIV AG-1 monoclonal p24 ELISA; Abbott, Abbott Park, Ill.) according to the manufacturers' instructions. The reference test results for the blood donor samples were obtained by testing performed with routine Food and Drug Administration-approved assays at The Blood Center, and those for the seroconversion samples were obtained from results published by Boston Biomed, Inc.; however, the University of Maryland performed the remainder of the reference testing. Samples that produced results that were discordant between any of the three assays (reference antibody tests, reference antigen test, and the DUO Ultra) were repeated in duplicate following a test algorithm (Fig. 1). Additional reference tests, such as a Western blot assay (Novapath HIV-1 Immunoblot; Bio-Rad, Hercules, Calif.), incorporating the Association of State and Territorial Public Health Laboratory Directors/Centers for Disease Control and Prevention criteria for positivity, and an ultrasensitive, quantitative HIV reverse transcriptase PCR (RT-PCR) assay (Amplicor HIV-1 Monitor test; Roche Diagnostics Corporation, Indianapolis, Ind.), were used to further analyze repeatedly discordant samples. The level of sensitivity of the PCR assay is 50 RNA copies/ml. Final results were classified based on a concordant positive or negative result by both the reference tests and the DUO Ultra, while discordant samples were classified based on the Western blotting and PCR results. Samples that produced indeterminate Western blotting results and had negative PCR results were considered noninfected, although early infection could not be totally ruled out. Any samples that could not be tested by all reference tests to fully resolve their HIV status were not included in the calculation of test indices.

RESULTS

Of the 2,773 samples (excluding the seroconversion panels), there were 126 that produced reactive results by at least two of the three screening assays (reference antibody or reference antigen test and the DUO Ultra assay) and 2,631 that were

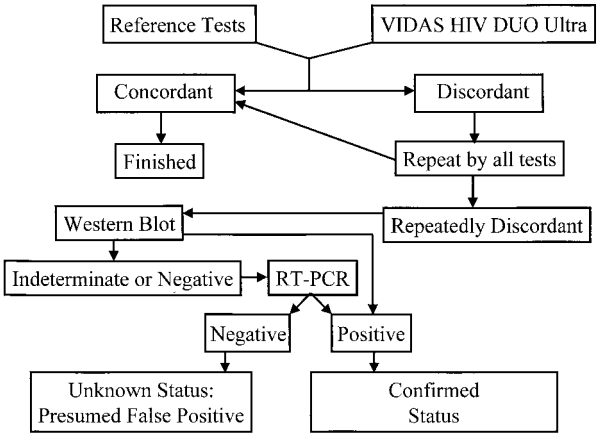

FIG. 1. Evaluation algorithm. If discordant results were found for any assays, Western blotting and then RT-PCR (if necessary) were used to determine whether the sample's status was presumed false positive or confirmed.

nonreactive by all assays. The remaining 16 samples produced results discordant between the assays.

Regarding the 126 reactive samples, the DUO Ultra correctly classified all 10 HIV-1 group O positive samples, all 16 HIV-2 samples, all six antigen-reactive HIV-1 group M clades that were detected by the reference antigen test, and a total of 94 HIV-1 and -2-reactive samples from the other populations. The 94 HIV-1 and -2-reactive samples included 61 that were antibody-only reactive by both the reference test and the DUO Ultra, 28 that were both antibody and antigen reactive by the reference assays and antibody reactive with the DUO Ultra (N/A antigen test value by the DUO Ultra), and 5 samples that were antigen-only reactive by the reference antigen test and the DUO Ultra assay.

Of the samples from the United States, the DUO Ultra correctly classified 100% (503 of 503) of the hospital samples (10 reactives and 493 nonreactive) and 99.7% (1,007 of 1,010) of the blood donor samples (Table 1). The DUO Ultra correctly classified 1,131 of 1,141 samples from Trinidad and 80 of 83 samples from the Bahamas. In these populations, there were 76 reactive and 1,055 nonreactive samples from Trinidad and 8 reactive and 72 nonreactive samples from the Bahamas. There was also one sample from Cote d'Ivoire that was correctly classified as nonreactive; this sample functioned as an HIV nonreactive control during blinded testing of the HIV-2-reactive samples.

The 16 discordant samples included 10 samples from Trinidad, 3 blood donor samples from the United States, and three samples from the Bahamas (Table 2). Of the 10 discordant samples from Trinidad, 8 were reactive by the DUO Ultra (1 was antigen only and 7 were antibody only), but all were nonreactive by the reference tests and indeterminate by Western blotting. The remaining two samples were antibody reactive by the DUO Ultra, nonreactive by the reference tests, and negative by Western blotting. Of the three U.S. blood donor samples which were reactive by the DUO Ultra, one was antigen-only reactive and two were antibody-only reactive. All three were negative by Western blot. Using the Roche Amplicor HIV-1 Monitor, HIV RNA was not detected in any of 13 samples. The three remaining samples from the Bahamas were classified as antibody-only reactive by the DUO Ultra, nonreactive by reference tests, and indeterminate by Western blotting; PCR could not be used to analyze these three samples because of an insufficient volume of specimens.

Results of the DUO Ultra using the commercially available HIV-1 seroconversion panels were compared to results from a number of antibody and antigen tests as published by BBI. Comparison of the results was made based on the first bleed detected by the DUO Ultra versus the first bleed by the most sensitive assay(s) performed by BBI. In the 10 panels, the final interpretation by the DUO Ultra showed detection of infection one bleed ($n = 2$), two bleeds ($n = 3$), three bleeds ($n = 4$), and five bleeds ($n = 1$) earlier than the most sensitive antibody assay. In seven panels, the DUO Ultra detected infection at the same time as the most sensitive antigen assay. In two panels, the DUO Ultra detected infection four bleeds and one bleed earlier than the most sensitive antigen assay(s); in one panel, the DUO Ultra detected infection one bleed (2 days) later than the most sensitive antigen assay. In addition, the DUO Ultra detected antigen in six of the nine members of the panel of HIV-1 group M clades; according to BBI, the Abbott HIVAG-1 also detected p24 antigen in the same six samples. In this panel, one sample acted as a nonreactive diluent con-

TABLE 1. Populations tested and summary of results[a]

Sample sources[b]	Total no. tested	Total no. R (reference and HIV DUO Ultra)	Total no. discordant (reference NR and HIV DUO Ultra R)	Total no. NR (reference and HIV DUO Ultra)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
University of Maryland	503	10	0	493	100	100	100	100
U.S. blood donors	1,010	0	3[c]	1,007	N/A	99.7	N/A	100
Trinidad	1,141	76	10	1,055	100	99.1	88	100
Bahamas	83	8	0[e]	75	100	100[e]	100[e]	100
Cote d'Ivoire[d]	1	0	0	1	N/A	100	N/A	N/A
HIV-2	16	16	0	0	100	N/A	100	N/A
HIV-1 group O	10	10	0	0	100	N/A	100	N/A
HIV-1 group M clades	9	6	0	3	100	N/A	100	100
All sources	2,773	126	13	2,634	100	99.5	90.8	100

[a] R, reactive; NR, nonreactive; PPV, positive predictive value; NPV, negative predictive value.
[b] Excludes seroconversion panels.
[c] HIV RNA not detected by RT-PCR.
[d] Control (see text).
[e] The calculation of specificity is based on eight samples; three samples were excluded because RT-PCR could not be performed.

trol, and the other two samples (clades A and C) did not have a detectable level of antigen by either the Abbott assay or the DUO Ultra.

DISCUSSION

In this study, the performance of the DUO Ultra immunoassay was evaluated with samples having diverse origins. Excluding the 74 members of seroconversion panels, 2,773 samples representing 126 reactive samples (prevalence = 6.8%) and 2,647 nonreactive samples were tested. The DUO Ultra correctly classified 2,757 (99.4%) of the samples. Although all 126 reactive samples were correctly classified as reactive by the DUO Ultra (100% sensitivity), 16 samples were identified as reactive only by the DUO Ultra. Thirteen of these samples were analyzed by RT-PCR, but levels of RNA above 50 copies/ml could not be demonstrated. The remaining three samples were interpreted as inconclusive based on the results of Western blot analysis (indeterminate), since the volumes of these specimens were insufficient for RNA testing. Since the true status of these three samples cannot be determined, they were excluded from the calculations of specificity. Therefore, the epidemiological sensitivity of the DUO Ultra was 100% (126 of 126), including a sensitivity of 100% for HIV-2, HIV-1 group M clades, and HIV-1 group O samples. The specificity of the DUO Ultra was at least 99.5% (2,647 of 2,660) overall and 99.7% (1,010 of 1,013) in low-risk individuals when compared to Food and Drug Administration-licensed tests.

In regard to the 16 discordant samples, the results of Western blot analysis provided little additional information toward resolution. As noted, 11 of the 16 discordant samples exhibited reactions almost exclusively to core components (p18, p24, and p55). Reactions to these components do not differentiate early infection from nonspecific reactions in noninfected individuals (2). This is also true for the five samples which produced negative results, since it is well documented by seroconversion panels that Western blot results can be negative during early infection when screening tests are reactive (2). It is presumed that the DUO Ultra produced false-reactive results at least in the 13 that were tested for the presence of RNA. However, early infection cannot be ruled out since the integrity of specimen processing was not under our control and EDTA may not have been used to collect the blood specimen as recommended for PCR analysis. Assuming that the samples were processed appropriately, it is likely that these results represent false-positive results by the DUO Ultra, since RNA was not detected in the samples that could be tested and detectable levels of RNA would have been expected during early infection. Furthermore, if these samples had been from persons with established infection undergoing antiviral therapy where RNA levels may have been undetectable, the Western blot profiles should have been more advanced. The reason for these presumably false-positive results is uncertain since the DUO Ultra incorporates gp36, gp41, and gp160. There was not any reactivity to the envelope antigens on the Western blot. It should also be noted that the false-positivity rate of the DUO Ultra (0.5%) must be compared to the collective false-positivity rate of both the reference antibody and antigen tests. Therefore, the specificity of the DUO Ultra can be considered to be

TABLE 2. Samples which produced discordant results for the HIV DUO Ultra and other assays[a]

Sample identification		Results for:									
		DUO ULTRA run 1									
Prefix	No.	Final	Antibody	Antigen	DUO ULTRA runs 2 and 3	HIV antibody	HIV antibody repeat	p24 antigen	p24 antigen repeat	Western blot (Bio-Rad)	HIV RNA (Roche PCR)	Final interpretation	Source
M	85-03-763	R	R	N/A	R	NR	NR/NR	NR	NR	I (24, 55)	QNS	I[b]	Bahamas
F	97-16-39	R	R	NR	R	NR	NR/NR	NR	NR	I (24)	QNS	I[b]	Bahamas
M	98-20-195	R	R	NR	R	NR	NR/NR	NR	NR	I (18, 24,+/−55)	QNS	I[b]	Bahamas
CRC	00450	R	R	N/A	R	NR	NR/NR	NR	NR	I (24, +/−55)	Not detected	FP	Trinidad
CRC	00544	R	R	NR	R	NR	NR/NR	NR	NR	I (18, 24, +/−32, +/−24, 55)	Not detected	FP	Trinidad
CRC	00687	R	R	NR	R	NR	NR/NR	NR	NR	I (24, +/−55)	Not detected	FP	Trinidad
CRC	00712	R	NR	R	R	NR	NR/NR	NR	NR	I (+/−24)	Not detected	FP	Trinidad
CRC	00716	R	R	NR	R	NR	NR/NR	NR	NR	I (+/−24)	Not detected	FP	Trinidad
CRC	00984	R	R	NR	R	NR	NR/NR	NR	NR	I (24, +/−55)	Not detected	FP	Trinidad
CRC	01088	R	R	NR	R	NR	NR/NR	NR	NR	I (+/−18, +/−24)	Not detected	FP	Trinidad
CRC	01335	R	R	N/A	R	NR	NR/NR	NR	NR	I (+/−18)	Not detected	FP	Trinidad
CRC	00422	R	R	NR	R	NR	NR/NR	NR	NR	Negative	Not detected	FP	Trinidad
CRC	01266	R	R	NR	R	NR	NR/NR	NR	NR	Negative	Not detected	FP	Trinidad
BD	579	R	NR	R	R	NR	NR/NR	NR	ND	Negative	Not detected	FP	Blood donor
BD	698	R	R	NR	R	NR	NR/NR	NR	ND	Negative	Not detected	FP	Blood donor
BD	795	R	R	NR	R	NR	NR/NR	NR	ND	Negative	Not detected	FP	Blood donor

[a] R, reactive; NR, nonreactive; ND, not done; I, indeterminate; QNS, quantity not sufficient; FP, presumably false positive by the HIV DUO ULTRA (see text).
[b] Samples not included in calculations of test indices.

TABLE 3. Comparison of fourth-generation HIV assay evaluations[a]

Test used and reference(s)	Sample size	Sensitivity (%)	Specificity (%)
HIV DUO/HIV DUO Ultra			
26	17 SC panels/255 CR	91.4 (SC)	88.2% (CR)
27	34 SC pts, 236 +	100	NDI
15	141 +, 300 −, 387 CR, 3 SC panels	100	98.2–100
18	29,657 pts	100	99.51
Saville et al., this study	10 SC panels, 2,773 (1,010 BD)	100	99.4–99.7
Vironostika HIV Uni-Form II Ag/AB			
24, 25	Unknown	100	NDI
Enzymun-Test HIV Combi			
14	7,659 (6,649 BD)	100	99.3–99.6
26	17 SC panels/255 CR	88.2 (SC)	97.2 (CR)

[a] BD, blood donor; NDI, not done or indicated; CR, cross-reactive; SC, seroconversion; pts, patients; +, HIV reactive; −, HIV nonreactive; ∗, current evaluation.

equivalent to or better than that of routine HIV assays that detect antibody and antigen.

When the assay was challenged for its analytical sensitivity, a large number of seroconversion panels and a panel of HIV-1 group M clades that had detectable antigen were utilized. These panels have been well characterized for the detection of HIV antibody and antigen using a variety of commercially available tests and are useful for evaluating the ability of assays to detect early infection (2). As noted, the DUO Ultra produced results equivalent to or better than those of the tests that were used to characterize the seroconversion panels. On average, the DUO Ultra detected infection 12.4 days earlier than the reference commercial, third-generation, antibody assays. Compared to the commercial antigen assays, the DUO Ultra detected infection on the same day as one or more of the reference antigen assays. In 2 of the 10 panels, the DUO Ultra detected p24 antigen 18 days (four bleeds) and 5 days (one bleed) earlier than one or more of the reference antigen assays. In just one bleed of 1 of the 10 seroconversion panels was the DUO Ultra less sensitive for antigen detection (2 days later) than only one of the five antigen assays used to characterize the panel. In addition, the DUO Ultra correlated exactly with the Abbott HIVAG-1 for the detection of antigen in the HIV-1 group M clade samples. This high analytical sensitivity of detection by the DUO Ultra is most likely attributed to the combination of a third-generation format (antigen sandwich) for antibody detection and the ability to simultaneously detect antigen.

To our knowledge, there are presently eight commercial fourth-generation assays for detection of both antibody and antigen. In addition to the DUO Ultra, there is the Enzymun-Test HIV Combi (Boehringer Mannheim), Vironostika HIV Uni-Form II Ag/AB (Organon Teknika), AxSYM HIV Ag/AB (Abbott), Enzygnost HIV Integral (Dade Behring Marburg), Genescreen Plus HIV Ag-AB (Bio-Rad), and COBAS Core HIV Combi (Roche Diagnostics GmbH). The eighth assay is an 18-min, double-antigen sandwich combination assay called the Elecsys HIV Combi (Boehringer Mannheim) that has been reported to have a specificity of 99.8% when challenged with a cohort of hospitalized patients (F. Donie, B. Upmeier, E. Hoess, and E. Faatz, Abstr. 12th World AIDS Conf., abstr. 163/41102, 1998). This rapid assay is based on electrochemiluminescence and is reported to reduce the window period by 5 days. A ninth, unidentified, noncommercial assay is a lineal immunoenzymatic assay evaluated to have a sensitivity of only 99.5% and a specificity of 94.8%, and an evaluation reported that the time needed to diagnose acute infection was shortened to approximately 2 weeks (19).

A comparison of four of the commercial fourth-generation assays versus four third-generation antibody ELISAs using seroconversion panels reported that all four of the fourth-generation assays detected infection in fewer days than did all the third-generation ELISAs, thus indicating that these assays show promise in reducing the window period and detecting early infection (7). The VIDAS HIV DUO (bioMérieux), an earlier version, demonstrated the highest sensitivity by detecting infection in the lowest number of days. Additional promising performances by fourth-generation ELISAs have been reported, including both peer-reviewed studies (Table 3) and evaluations reported via meeting abstracts (7, 14, 15, 18, 20–27; M. Biron, J. Basse, J. Jego, and S. Gadelle, Abstr. XIII Int. AIDS Conf., abstr. TuPeA3000, 2000; S. Brust and S. Knapp, Abstr. XIII Int. AIDS Conf., abstr. MoPeA2111; E. Faatz, F. Donie, W. Melhior, B. Upmeier, and C. Seidel, Abstr. 12th World AIDS Conf., abstr. 41113, 1998; J. Schalken, J. van Binsbergen, A. Jacobs, R. Reddy, C. Deltmann, A. Siebelink, et al., Abstr. XIII Int. AIDS Conf., abstr. TuPeA2996; U. Schmitt, H. Andres, and E. Faatz, Abstr. XIII Int. AIDS Conf., abstr. MoOrA112, 2000; and D. West, G. Hall-Steele, D. Collins, D. Daghfal, and M. Mullner, Abstr. XIII Int. AIDS Conf., abstr. TuPeA2991, 2000).

These assays have demonstrated sensitivities ranging from 99.5 to 100%. Several studies with large sample sizes have reported sensitivities of 98 to 99.87% by the Vironostika HIV Uni-Form II Ag/AB, the Enzymun-Test HIV Combi, AxSYM HIV Ag/AB, and the Enzygnost HIV Integral (14, 23; Brust and Knapp, Abstr. XIII Int. AIDS Conf.; Faatz et al., Abstr. 12th World AIDS Conf.; and West et al., Abstr. XIII Int. AIDS Conf.) Using seroconversion panels, several evaluations have calculated a reduction in the diagnostic window by a minimum of 4 days and some have even reported a reduction of up to 9 days by using the fourth-generation assays (14, 24, 26). The specificities have ranged from 98 to 100%, excluding one study that reported a specificity of only 88.2% (26). This latter study tested the performance of the HIV DUO with a sample size of only 250 potentially cross-reactive samples, such as specimens

from pregnant women; patients with autoimmune disorders or rheumatoid factor; patients reactive for IgM antibodies to cytomegalovirus, herpes simplex virus, rubella virus, or toxoplasmosis; and patients infected with hepatitis C or Epstein-Barr virus.

In fact, all of the studies reviewed that have evaluated the bioMérieux system have been performed using the HIV DUO (15, 18, 26, 27). Three of the four studies have reported sensitivities of 100% when testing samples from individuals with early and established infections (15, 18, 27). The only study reporting a sensitivity of 91.4% used 17 seroconversion panels and thus assessed analytical sensitivity, not epidemiological sensitivity (ability to detect established infection) (26). In addition to the study noted above that reported a specificity of 88.2%, another study using the HIV DUO demonstrated a specificity ranging from 98.2 to 100% when small cohorts of potentially cross-reactive samples were tested (15, 26). The fourth study reported a specificity of 99.5% in a very large multicenter evaluation using samples from 29,657 patients (18). In addition to detecting all 453 HIV-infected individuals, the HIV DUO had the ability to detect early infection in 17 samples containing p24 antigen that were nonreactive by the reference third-generation assays and by Western blotting, further supporting the benefits of adding the antigen testing component to a screening test. Interestingly, only 11 of the 17 samples had detectable levels of RNA, and all 17 resolved as acute infections upon follow-up testing (18).

Comparatively, the present study is the first to evaluate the new version, the DUO Ultra, and to utilize the largest variety of samples and sample populations, including hospitalized patients, outpatients, blood donors, HIV-2 samples, HIV-1 group O samples, HIV-1 group M clades, patients from three geographical locations, and seroconversion panels. Challenged with 2,647 nonreactive samples from several settings and geographical locations, the specificity of at least 99.5% could have been higher, since 8 of the 13 presumably false-positive results were indeterminate by Western blotting, imparting some uncertainty with regard to their true status. In fact, the specificity was 99.7% when 1,010 blood donor specimens were analyzed.

HIV p24 antigenemia occurs early after infection; however, when antibodies become detectable, antigen is usually not demonstrable, most likely because of antigen-antibody complexes in the blood, thereby necessitating a test for HIV antibody as well (6). Although the presence of antigen is highly specific for infection, a significant limitation of assays may be insensitive levels of detection, because low concentrations of antigen are difficult to detect and antigenemia occurs only transiently during different stages of infection (13). The antigen test may be incapable of detecting 75% of low-risk individuals who are infected but are seronegative (12).

In order to further ensure the safety of the blood supply, the p24 antigen assay, in addition to HIV antibody tests, is used for screening blood, blood components, source leukocytes, and source plasma targeted for transfusion in the United States (11). Prior to 1995, one blood donation in every 210,000 to 1,140,000 in the United States was estimated to be from an HIV-infected individual during the window period, which is usually 22 to 25 days or longer (17). By implementing antigen screening of blood, an estimated four to six cases of transfusion-associated HIV infections may be prevented per year, lowering the estimated risk per unit transfused to a range of one in 562,000 to one in 825,000 (9, 17). Therefore, it appears that antigen testing has utility for helping to protect the blood supply, though at a cost exceeding $60 million annually. Due to their ability to detect p24 antigen, the DUO Ultra and other fourth-generation ELISAs will be of value in detecting early infection.

Based on its throughput and configuration of the DUO Ultra, the assay is highly applicable for the diagnosis of early and established HIV infection by hospital and private clinical laboratories and other laboratory settings. In these settings, individuals to be screened for infection come from higher-risk groups than does the blood donor population and thus require the use of testing methods with high levels of analytical sensitivity for primary infection. Of significance, the high level of analytical and epidemiological sensitivity demonstrated by the DUO Ultra with seroconversion and clade panels as well as with a variety of patient populations makes it ideal for use in a variety of testing situations for the diagnosis of early and established infection.

Eleven HIV-infected samples were identified by the DUO Ultra via antigen detection. In routine laboratory settings, these individuals would not have been identified by the usual screening antibody assays, since antigen testing of patients is not commonly performed as a screening tool outside of blood banks (6). The detection of early infection has been shown to be beneficial for the prompt initiation of appropriate antiretroviral therapy in a clinically relevant time frame. Additionally, early detection will help in the timely implementation of interventions, such as the counseling of patients, prevention of transmission, and management of infection.

Through the simultaneous testing of the presence of HIV antigen and antibody, the DUO Ultra will not only provide an increased initiation and efficiency of treatment and a reduction in the risk of transmission but offers several advantages over conventional antibody and antigen testing. It decreases the time necessary to perform both antibody and antigen testing by at least 50%, since the DUO Ultra will complete both tests simultaneously in a period comparable to that needed for accomplishing one of the assays independently. The DUO Ultra is an efficient, automated system; i.e., after introducing serum or plasma into the sample well of the reagent strip and entering identification into the computer program, the instrument will function independently to complete testing of up to 30 samples in 2.5 h in the absence of further hands-on time or supervision. Thus, the hands-on time for the technician is approximately 20 to 30 min per 30 tests to detect both antigen and antibody. Thirdly, it offers flexibility because any number of samples (1 to 28 with two controls) can be tested without the wastage of reagents or strip wells and because samples can be tested overnight without monitoring. Three sets of runs can easily be performed per day, each consisting of 30 samples for a total of 90 samples per day. Although the manufacturer has not quoted a price, it is reasonable to assume that the DUO Ultra's price will be competitive with the collective price of antibody and antigen tests.

HIV assays have evolved over the last decade to produce tests which possess novel characteristics and which can address those diagnostic issues which remain, e.g., detection of early infection, indeterminate results, infection in the newborn, the

need for rapid results, etc. The availability of a fourth-generation test capable of offering an increase in sensitivity over other antibody tests is yet another evolutionary step. The concept of simultaneous antigen and antibody detection is of great importance, as there is a definite need to continue testing for early infection and to apply cost-saving strategies. This study, along with the studies of others, has verified that this type of testing strategy is accurate and effective and offers a number of advantages over previous generations of antibody and antigen ELISAs.

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