Human Immunodeficiency Virus (HIV) Antibody Avidity Testing To Identify Recent Infection in Newly Diagnosed HIV Type 1 (HIV-1)-Seropositive Persons Infected with Diverse HIV-1 Subtypes[▽]

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A guanidine-based antibody avidity assay for the identification of recently acquired human immunodeficiency virus type 1 (HIV-1) infection was evaluated. The kinetics of maturation of antibody avidity were determined prospectively in 23 persons undergoing acute seroconversion followed for up to 1,075 days. Avidity indices (AI) of ≤ 0.75 and ≤ 0.80 reproducibly identified seroconversion within the previous 125 (95% confidence interval [CI], 85 to 164) and 142 (95% CI, 101 to 183) days, respectively. To validate the assay, 432 serum samples from newly diagnosed patients were tested by both the avidity assay and the detuned assay. Results were highly concordant (kappa value for agreement, 0.85). The avidity assay was subsequently used to screen 134 consecutive newly diagnosed patients, including 55/134 (41%) infected with non-B subtypes (A, C, D, G, CRF01, CRF02, CRF06, CRF13, and CRF16). In this cohort, 25/79 (32%) persons with the B subtype and 7/55 (13%) with non-B subtypes showed an AI of ≤ 0.75 , and there were 16/25 (64%) and 3/7 (43%) persons, respectively, with a documented history of acute seroconversion illness within the predicted seroconversion interval. An AI of ≤ 0.75 was also observed for four patients (three with the B subtype and one with a non-B subtype) who presented with AIDS-defining conditions. In multivariate analysis, an AI of ≤ 0.75 was associated with younger age, higher HIV-1 plasma RNA load, and being born in the United Kingdom or Ireland rather than in Africa but not with gender, ethnicity, risk group, HIV-1 subtype, or CD4 counts. In conclusion, HIV antibody avidity testing provides a reliable method for identifying recently acquired HIV-1 infection. Results are affected by advanced disease and should therefore be interpreted in the context of other clinical parameters.

The identification of newly acquired human immunodeficiency virus type 1 (HIV-1) infection provides important information on the dynamics of the epidemic, transmission networks, and patterns of transmitted drug resistance, guides public health intervention programs, and identifies candidates for clinical trials and vaccine strategies targeting early infection. The serologic testing algorithm for recent HIV seroconversion (STARHS) assay, or detuned assay, differentiates between recent and established HIV infection by using a high-sensitivity/low-sensitivity dual enzyme immunoassay (EIA) (11). The method was first introduced in 1998 and has been applied successfully to epidemiological studies of newly diagnosed HIV-1 infections worldwide (13, 16, 22). However, the assay suffers from a number of recognized limitations. Firstly, its availability is limited to a few centers worldwide. In addition, the assay employs an indirect first-generation EIA that requires exacting laboratory conditions, rigorous standardization, and quality assurance procedures for its reliable and reproducible use (9, 16). An additional consideration is that discontinuation or modification of the basic platform assay may adversely affect the STARHS application.

Avidity is a measure of the strength of the binding between immunoglobulin G antibodies and the corresponding antigen, a property that increases over a period of months in newly acquired infections (20). Antibody avidity assays classically employ urea or guanidine to elute low-avidity and low-affinity antibodies after antigen-antibody bonds have formed. The assays are well established in clinical care. Examples of current applications include the management of rubella and cytomegalovirus infection during pregnancy, in which immunoglobulin G avidity is used to differentiate between primary and established infections, thereby allowing an estimation of the risk of mother-to-child transmission (1, 8). A guanidine-based antibody avidity assay has also been proposed for the evaluation of newly diagnosed HIV infection, although performance with persons with diverse HIV-1 subtypes has not been characterized (12, 18, 19). The aim of this study was to evaluate an automated guanidine-based antibody avidity assay for the identification of recently acquired HIV-1 infections in a diverse HIV-1-seropositive cohort and to correlate the results to clinical, immunological, and virological parameters, including HIV-1 subtype.

MATERIALS AND METHODS

Study populations. Serum samples collected prospectively from 23 acute HIV-1 seroconverters (persons undergoing seroconversion) were used to establish the kinetics of HIV antibody avidity after infection using a guanidine-based assay and a urea-based assay. Seroconverters were defined as those who presented either with HIV EIA antibody results evolving from negative ($n = 4$) or

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FIG. 1. Evolution of HIV-specific antibody avidity (expressed as avidity index) after HIV-1 seroconversion as determined for a patient infected with subtype B HIV-1 by either a guanidine-based assay or a urea-based assay and for a patient infected with F/complex non-B subtype mosaic HIV-1 by a guanidine-based assay.

equivocal ($n = 12$) to positive in closely consecutive samples or with positive HIV EIA antibody results and evolving Western blot patterns ($n = 7$). Between 1 and 7 (median, 4) prospective samples were available from each seroconverter, for a total of 86 HIV antibody-positive samples from a period spanning up to 1,075 days from the date of the first HIV antibody-positive result (day 0). Serum samples obtained from 432 newly diagnosed HIV-1-infected patients were used to compare the performance of the guanidine-based avidity assay to that of the detuned assay. In addition, 134 serum samples obtained from all patients diagnosed as being HIV-1 seropositive between April 2004 and May 2005 were tested by the guanidine-based avidity assay, and results were correlated to clinical, immunological, and virological parameters, including HIV-1 subtype. The study received approval from the local Ethics Committee to be conducted using anonymous specimens after the collection of relevant demographic and clinical data.

HIV antibody avidity assays. Paired sera in duplicate or, in cases in which enough sample was available, triplicate wells were tested for HIV antibodies by the anti-HIV-1 and -2 VITROS ECI assay (Ortho-Clinical Diagnostics, United Kingdom) following incubation for 10 min at a 1:10 dilution in either phosphate-buffered saline (reference dilution) or 1 M guanidine (test dilution). The Vitros ECI assay (with enhanced chemiluminescence) is an EIA-like fully automated assay that employs a light-emitting substrate to detect the presence of bound antibody by a luminometer. Results are reported as the sample-to-cutoff (S/CO) ratio, with positive values being ≥ 1 . The avidity index (AI) was calculated with the equation of the S/CO ratio of the test dilution (mean of replicate wells) over the S/CO ratio with the reference dilution (mean of replicate wells). A total of 63 samples with a mean AI of 0.6 ± 0.2 (range, 0.2 to 1.0) underwent repeat testing to assess assay reproducibility, particularly at an AI of ≤ 0.8 (60/63 samples). Samples in duplicate or triplicate wells were also tested by a urea-based avidity assay using the anti-HIV-1/HIV-2 antibody-capture enzyme-linked immunosorbent assay (Ortho-Clinical Diagnostics) either according to the standard EIA protocol (reference condition) or with an additional wash with 8 M urea following the first incubation step (test condition). The AI was calculated by dividing the averaged test condition optical density (OD) value by the averaged reference condition OD value. Samples were tested by the detuned assay according to the established STARHS algorithm, using a modified anti-HIV assay (bioMérieux, Hampshire, United Kingdom) and following the CDC interpretive criteria as previously described (11). Results were reported as standardized OD (SOD) values, and values of ≤ 1.0 indicate seroconversion within the previous 170 days (95% confidence interval [CI], 145 to 200) (11). A subset of samples was also tested by the AxSYM HIV 1/2 gO MEIA assay (Abbott Diagnostics, Berkshire, United Kingdom) modified to incorporate an incubation step with guanidine, as previously described (18), and by the commercially available BED Calypte HIV-1 incidence EIA (Calypte Biomedical Corporation, OR), as previously described (6).

HIV-1 subtyping. HIV-1 subtypes were determined from HIV-1 *pol* gene sequences (reverse transcriptase codons 1 to 335 and protease codons 1 to 99) obtained with the ViroSeq HIV-1 genotyping system (Celera Diagnostics) and the ABI 3100-Avant genetics analyzer. Briefly, RNA was manually extracted from plasma and a 1.8-kb cDNA fragment generated by reverse transcription-

FIG. 2. Avidity index values observed with 72 serum samples collected from 23 HIV-1-infected persons at multiple points between day 0 and day 262 after HIV antibody seroconversion and tested by a guanidine-based HIV antibody avidity assay. The data points spanning the range of day 32 to day 153 (17 points) were analyzed by linear regression. The regression line (solid line) and 95% confidence prediction intervals (dotted lines) are shown.

PCR. Cycle sequencing was carried out using seven different primers to cover a 1.3-kb region of cDNA. Subtype assignment was done by phylogenetic analysis using reference sequences derived from the Los Alamos database (<http://hiv-web.lanl.gov>) and including previously characterized HIV-1 subtypes and circulating recombinant forms (CRFs), using ClustalX and PAUP version 4.0.

Statistical analysis. The coefficient of variation between replicate samples was calculated from the following formula: (standard deviation/average) $\times 100$. Kappa statistics were used to assess agreement between results of the avidity assay and the detuned assay using a cutoff of 0.8 for the avidity assay and either 1.0 or 0.9 for the detuned assay. Generalized linear models with log link and Poisson error (using generalized estimating equations with an unstructured covariance) were used to assess multivariable (adjusted) relative risks for the association between various covariates and risk of the avidity index being ≤ 0.75 . Analyses were done using SAS 8.2.

RESULTS

Kinetics of HIV antibody avidity in acute HIV-1 seroconverters. Serum samples ($n = 86$) collected prospectively from 23 HIV-1 seroconverters and spanning a period of up to 1,075 days after seroconversion were used to establish the kinetics of HIV antibody avidity in newly acquired infections. The panel included 4 persons with HIV antibody results evolving from negative to positive in successive samples collected over 7 to 23 days, 12 persons with HIV antibody results evolving from equivocal to positive over 7 days, and 7 persons with positive HIV antibody results and evolving Western blot patterns. Of the 23 seroconverters, 22 were infected with subtype B HIV-1 and 1 was infected with F/complex non-B subtype mosaic virus. All were antiretroviral drug naïve at the time of sampling. Samples were tested by both a guanidine-based avidity assay and a urea-based avidity assay. As shown in the example given in Fig. 1, AI values obtained in the guanidine-based assay increased gradually over time. In contrast, the urea-based assay showed a more rapid evolution and across the entire sample panel gave AI values of >0.8 within the first four weeks (data not shown). The kinetics of antibody avidity maturation in the patient infected with the mosaic non-B subtype were similar to those observed for patients with subtype B infection (Fig. 1).

Figure 2 shows the AI values obtained with the guanidine-

FIG. 3. Correlation between the HIV antibody avidity assay and the detuned assay. Samples obtained from 432 newly diagnosed HIV-1-infected patients were tested in by the guanidine-based HIV avidity assay and results reported as avidity indices, with values of ≤ 0.8 indicating seroconversion within the previous 142 days. Samples were also tested by the detuned assay and results reported as SOD, with values of ≤ 0.9 indicating seroconversion within the previous 150 days.

based assay in 72/86 samples collected from the 23 seroconverters during the first 262 days after seroconversion. On day 0 (first antibody-positive sample), the median AI was 0.3 (range, 0.1 to 0.4), and median AI were lowest in the four persons whose consecutive samples progressed from HIV antibody negative to positive and were highest in the seven persons who presented with positive HIV antibody results and evolving Western blot patterns. The remaining 14/86 samples, collected from 11/23 seroconverters between day 337 and day 1,075, all showed AI values of 1.0 (data not shown). In cases in which sufficient amounts of specimen were available, samples underwent repeat testing. With 39 samples showing an average AI of 0.5 (± 0.2) in the first test and tests replicated an average of three times (range, 2 to 4), the mean interassay coefficient of variation was 3.2% ($\pm 4.8\%$).

The data points spanning the range of day 32 to day 153 (17 points) were analyzed by linear regression (Fig. 2). The correlation coefficient was 0.91, the residual standard deviation was 18 days, and the mean and variance of AI were 0.65 and 0.011, respectively. The results showed that the time in days (d) since seroconversion was predicted from the AI to be, for AI in the range of 0.45 to 0.80, as follows: $d = 350\text{AI} - 138$. The predictions (95% CI) for AI cutoff values of 0.50, 0.75, and 0.80

were days 37 (-3 to 78), 125 (85 to 164), and 142 (101 to 183), respectively.

Comparison of the guanidine-based avidity assay with the detuned assay. Stored samples from 432 newly diagnosed patients underwent retrospective testing by both the guanidine-based avidity assay and the detuned assay. The overall correlation between the two assays is shown in Fig. 3. With a few exceptions, patients were clearly categorized into two groups: those presenting soon after infection and those with established infections.

Overall, 425/432 (98%) samples showed concordant results when breakpoints of 0.8 for the avidity assay (average seroconversion interval, 142 days; 95% CI, 101 to 183 days) and 1.0 for the detuned assay (average seroconversion interval, 170 days; 95% CI, 145 to 200 days) (11) were used, with a kappa value for agreement of 0.90. The remaining 7/432 (2%) samples showed discordant detuned/avidity assay results. With six samples, the detuned assay indicated an established infection (SOD values, 1.1 to 7.3), whereas the avidity assay indicated a recent infection, with AI values of ≤ 0.8 in repeat experiments (Table 1). One sample with an SOD of 3.5 showed discordant AI values in the replicate experiments and was therefore classified as indeterminate. The seven detuned/avidity-discrepant samples were also tested by the BED HIV-1 incidence EIA and the AxSYM guanidine-based avidity assay. The results of the additional tests were in agreement with each other and with those of the detuned assay for four samples but discordant with each other for three other samples (Table 1). Among the seven discrepant samples, four were from patients with evidence of advanced HIV-1 infection. Results of the avidity assay and the detuned assay were also compared by using a cutoff for the detuned assay of ≤ 0.9 , which reduces the average seroconversion interval to 155 days (11). The kappa value for agreement was 0.85. A further four samples showed discrepant avidity/detuned results for a total of 11/432 (2.5%) discordant samples. The SOD values were 1.0 in these four samples, whereas the AI values were 0.3, 0.5, 0.6, and 0.7, respectively. There was insufficient specimen available for further testing.

Characterization of patients diagnosed as being HIV-1 seropositive between April 2004 and May 2005. In the period between April 2004 and May 2005, 134 patients were diagnosed as being HIV-1 positive at the local HIV testing clinic (Tables 2 and 3). The entire cohort underwent both HIV antibody avidity testing and HIV-1 subtyping. Overall, 79/134 (59%) patients were infected with subtype B and the remaining

TABLE 1. Analysis of seven samples showing discrepant results by the HIV antibody avidity assay and the detuned assay^a

Sample	Detuned assay SOD	Vitros avidity index				Avidity index (interpretation)		CD4 count (cells/mm ³)	HIV-1 subtype	Ethnicity (country of birth)
		First test	Second test	Third test	Interpretation	BED assay	AxSYM assay			
1	1.1	0.55	0.7	0.6	RI	2.4 (LT)	0.7 (RI)	273	C	BA (Somalia)
2	2.4	0.7	0.8	0.7	RI	0.4 (RI)	0.9 (LT)	254	A	BA (Kenya)
3	1.4	0.7	0.8	0.7	RI	1.9 (LT)	0.9 (LT)	205	B	W (United Kingdom)
4	3.5	0.6	0.7	0.9	Indeterminate	2.8 (LT)	0.8 (RI)	28	C	BA (Zambia)
5	7.3	0.7	0.75	0.7	RI	2.5 (LT)	0.9 (LT)	42	CRF02	BA (Ghana)
6	5.1	0.7	0.7	0.7	RI	3.0 (LT)	1.0 (LT)	15	A	BA (Congo)
7	2.0	0.7	0.6	0.7	RI	2.1 (LT)	0.9 (LT)	19	D	BA (Kenya)

^a RI, recent infection; LT, long-term infection; BA, black African; W, white.

TABLE 2. Characteristics of the cohort newly diagnosed with HIV-1 infection between April 2004 and May 2004 and of persons with recently acquired infection (avidity index, ≤ 0.75)

Characteristic	No. of patients among whole population (%)	No. of patients with avidity index of ≤ 0.75 (%)		
		Subtype B	Non-B subtype	Total
Male gender	94 (70)	24 (96)	3 (43)	27 (84)
Risk group ^a				
MSM	76 (57)	24 (96)	1 (14)	25
Heterosexual	56 (42)	1 (4)	6 (86)	7
IDU/other	2 (1)	0	0	0
Ethnic group				
White	73 (54)	23 (92)	2 (29)	25 (78)
Black African	44 (33)	0	4 (57)	4 (13)
Black Caribbean	10 (8)	2 (8)	0	2 (6)
Other	7 (5)	0	1 (14)	1 (3)
Total	134 (100)	25 (19)	7 (5)	32 (24)

^a MSM, men who have sex with men; IDU, intravenous drug users.

55/134 (41%) showed subtypes other than B (5, A; 28, C; 6, D; 3, G; 3, CRF01; 5, CRF02; 3, CRF06; 1, CRF13; 1, CRF16). Among patients infected with subtype B, 25/79 (32%) showed AI values of ≤ 0.75 (Tables 2 and 3). Within the predicted seroconversion period of up to 164 days, 5/25 (AI range, 0.1 to 0.6; CD4 range, 104 to 642 cells/mm³) patients had HIV antibody seroconversion documented in consecutive samples, 11/25 (AI range, 0.1 to 0.6; CD4 range, 260 to 701 cells/mm³) patients gave a reliable clinical history consistent with seroconversion illness, and 6/25 (AI range, 0.2 to 0.7; CD4 range, 302 to 845 cells/mm³) patients gave a history of unprotected homosexual intercourse with casual partners. The remaining 3/25 patients (AI range, 0.4 to 0.6; CD4 range, 6 to 57 cells/mm³) presented with an AIDS-defining condition. In addition, three white homosexual males infected with subtype B (CD4 counts of 443, 626, and 658 cells/mm³) showed an AI of 0.8, including one with a well-documented clinical history consistent with seroconversion illness and two with a record of activities in which they were prone to exposure within the previous 183 days.

Among persons with non-B subtypes, 7/55 (13%) showed AI values of ≤ 0.75 (Tables 2 and 3). These included 3/7 persons with a well-documented clinical history consistent with seroconversion illness within the predicted seroconver-

sion interval, comprising one 21-year-old black African heterosexual woman from Ghana (AI, 0.3; subtype CRF02; CD4 count, 667 cells/mm³), one 31-year-old black African heterosexual woman from Kenya (AI, 0.4; subtype CRF16; CD4 count, 539 cells/mm³), and one 46-year-old white homosexual male from Singapore (AI, 0.4; subtype CRF06; CD4 count, 678 cells/mm³). In addition, one 39-year-old white heterosexual male from the United Kingdom (AI, 0.7; subtype C; CD4 count, 1,100 cells/mm³) reported sexual intercourse with multiple casual partners within the predicted seroconversion interval, whereas one 26-year-old black African heterosexual male from Somalia (AI, 0.7; subtype C; CD4 count, 273 cells/mm³) gave a history of multiple sexual partners within the predicted seroconversion interval and presented with a diagnosis of pulmonary tuberculosis. Finally, 2/7 persons (AI, 0.65 and 0.7; subtypes D and CRF01; CD4 counts, 2 and 46 cells/mm³) presented with AIDS-defining conditions. An additional four persons showed an AI of 0.8. These included a 40-year-old heterosexual woman from Brazil (subtype C; CD4 count, 374 cells/mm³) who gave a history of assault and blood exposure within the previous 183 days and three black African persons (two with subtype C and one with subtype D; CD4 counts were 19, 76, and 53 cells/mm³) who presented with AIDS-defining conditions.

To evaluate reproducibility of the avidity assay at AI values close to the assay cutoff of 0.8, 24 samples showing an average AI of 0.7 (± 0.1) in the first test underwent an average of three (range, two to four) repeat avidity tests. The mean interassay coefficient of variation was 3.7% ($\pm 5.8\%$).

Heterosexual risk group, infection with a non-B subtype, African country of origin, and black ethnicity were all highly correlated in this population (pairwise kappa values around 0.8). Table 4 shows the univariable (unadjusted) and multivariable (adjusted) relative risks of an AI value of ≤ 0.75 for several demographic variables as well as HIV-1 plasma RNA load, HIV-1 subtype, and CD4 count. In the multivariate analysis, age, country of birth, and HIV-1 plasma RNA load were independently associated with AI values of ≤ 0.75 (Table 4). Younger patients, those born in the United Kingdom or Ireland and those with a higher HIV-1 plasma RNA load, were more likely to show an AI of ≤ 0.75 than other patients. Gender, risk group, ethnic group, HIV-1 subtype, and CD4 counts were not independently associated with AI values of ≤ 0.75 .

TABLE 3. Data for the cohort newly diagnosed with HIV-1 infection between April 2004 and May 2004 and for persons with recently acquired infection (avidity index, ≤ 0.75)

Group	Median value (range)			
	Age (yr)	CD4 count (cells/mm ³)	HIV-1 RNA load (log ₁₀ copies/ml)	Avidity index
Persons with an AI of ≤ 0.75				
Subtype B infected	33 (19–55)	359 (6–845)	5.4 (4.2–6.2)	0.3 (0.1–0.75)
Non-B subtype infected	30 (21–46)	539 (2–1,100)	4.9 (3.7–5.8)	0.6 (0.3–0.7)
Total	32 (19–55)	408 (2–1,100)	5.3 (3.7–6.2)	0.4 (0.1–0.75)
Whole population	35 (19–68)	348 (1–1,170)	4.9 (2.1–6.9)	0.9 (0.1–1.0)

TABLE 4. Factors associated with an avidity index of ≤ 0.75 (recently acquired HIV-1 infection) in univariable and multivariable analysis^a

Characteristic	Relative risk (95% confidence interval) (<i>P</i> value)	
	Univariable	Adjusted
Female gender	0.44 (0.18–1.05) (0.06)	0.96 (0.21–2.24) (0.92)
Risk group		
MSM ^b	1.00	1.00
Heterosexual	0.39 (0.18–0.84) (0.02)	0.87 (0.38–2.00) (0.74)
Age (per 10 yr increment)	0.62 (0.39–0.98) (0.04)	0.58 (0.38–0.89) (0.01)
Ethnic group		
White	1.00	1.00
Black	0.29 (0.11–0.78) (0.01)	0.86 (0.19–4.03) (0.85)
Country of origin		
United Kingdom/Ireland	1.00	1.00
Africa	0.24 (0.10–0.59) (0.002)	0.24 (0.10–0.55) (0.0009)
Other	0.51 (0.22–1.19) (0.12)	0.51 (0.23–1.13) (0.1)
Non-B subtype	0.40 (0.19–0.86) (0.02)	1.29 (0.49–3.39) (0.61)
CD4 count (per 100 cell/mm ³ increment)	1.09 (0.98–1.21) (0.11)	1.05 (0.93–1.18) (0.43)
HIV-1 plasma RNA load (per 1 log ₁₀ copy/ml increment)	1.53 (1.04–2.26) (0.03)	1.47 (1.03–2.10) (0.03)

^a Values in bold represent significant associations (AI ≤ 0.75).

^b MSM, men who have sex with men.

DISCUSSION

In this study, we evaluated a guanidine-based HIV antibody avidity assay employing a third-generation HIV antibody testing method and an automated platform that is widely available in routine diagnostic settings. The assay differentiated between early and established HIV-1 infection with high reproducibility and was simple to perform and inexpensive. Following the necessary validation, the method can be adapted to various assay formats, including both manual and automated platforms used in laboratories worldwide, thus allowing data obtained in different geographic areas and populations to be compared.

Following HIV antibody seroconversion, avidity increased slowly in the guanidine-based assay but more rapidly when using a urea-based assay, making the latter a less useful option. The guanidine-based avidity assay is quantitative and allows avidity to be determined as a continuous variable ranging from 0 to 1.0. Although there exists individual variability in the rapidity of avidity maturation, relevant breakpoints can be identified. Using a large panel of samples obtained from seroconverters over the course of up to 1,075 days of follow-up, an AI of ≤ 0.75 reproducibly identified seroconversion within the previous 125 days (95% CI, 85 to 164), whereas a value of ≤ 0.8 predicted seroconversion within 142 days (95% CI, 101 to 183). A cutoff of 0.8 was previously proposed for the AxSYM HIV antibody avidity assay (18). As also seen for the detuned assay (11), the choice of the breakpoint ultimately depends on the intended application of the assay. Adopting a lower AI cutoff value increases specificity but may result in some loss of sensitivity. Thus, AI values in general and those just above or below the chosen cutoff in particular should be confirmed by repeat testing and interpreted in the context of other clinical

parameters. Of note, the maturation of avidity in a seroconverter infected with a complex non-B mosaic virus followed similar kinetics to those seen in patients with subtype B.

The guanidine-based avidity assay showed excellent agreement with the detuned assay, with a small number of discrepant results and excellent kappa values for the agreement. When two additional methods including the BED assay were used for the discrepant samples, results did not consistently agree, indicating that a subset of samples could not be classified using the currently available methods. The BED assay, which is commercially available, is an alternative to the detuned assay, although some concerns about its use in populations with diverse subtypes have been raised. No large-scale comparison of the BED assay with other assays of recent infection has been described so far in United Kingdom populations.

The usefulness of a serological assay to monitor HIV incidence in the population of the United Kingdom has been previously described (9). When the avidity assay was applied to a well-characterized population of recently diagnosed HIV-1-infected persons, 24% of the infections were defined as being recently acquired, using a conservative cutoff of ≤ 0.75 . One limitation of this study was that, given its anonymous design, no follow-up to determine maturation of the response was possible. Nonetheless, among 32 persons with an AI of ≤ 0.75 , 59% had a well-documented clinical history consistent with a recent acute seroconversion illness, while an additional 22% reported high-risk contacts within the predicted seroconversion interval, thus supporting the results of the avidity assay. Overall, the prevalence of recently acquired infection among patients infected with subtype B was higher than the prevalence among those infected with non-B subtypes, and this is consistent with epidemiological data indicating that most non-B subtype infections in the United Kingdom are imported through immigration, particularly from sub-Saharan Africa (7, 14). This is also consistent with the finding that clinical cohorts of persons with documented acute seroconversion in the United Kingdom predominantly include homosexual males of white ethnicity infected with subtype B (21). In agreement with these observations, the multivariate analysis determined that persons born in Africa were significantly less likely to show AI values of ≤ 0.75 than persons born in the United Kingdom or Ireland, whereas the ethnic group and HIV-1 subtype were not predictive of AI values. In addition, there was an association between age and AI values, indicating that new HIV-1 infections are predominantly occurring among younger adults. An independent positive association was also found between AI values of ≤ 0.75 and higher HIV-1 plasma RNA loads, consistent with the high rates of virus replication observed in the early phases of HIV-1 infection, before achievement of the viral load set point (4, 15). In contrast, there was no association with CD4 counts, reflecting the recognized variability in CD4 count decline and rate of recovery observed in newly infected patients (3).

Whereas AI values remained 1.0 during three years of follow-up after seroconversion, they appeared to decline in patients presenting with AIDS-defining conditions. Similar observations have been made when using the detuned assay (10). These findings are not surprising, as changes in qualitative and quantitative aspects of HIV-specific antibody responses can be

expected in advanced disease, as an expression and result of profound immune dysfunction (2, 5, 17). We are currently investigating the kinetics of antibody avidity in the course of disease progression.

In summary, HIV antibody avidity testing provides a reliable method for identifying recently acquired HIV-1 infection, and the guanidine-based Vitros assay shows a performance highly similar to that of the detuned assay. The assay performance does not appear to be affected by the infecting HIV-1 subtype, but the effects of advanced disease indicate that result interpretation should occur in the context of other clinical parameters.

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REFERENCES

1. Aboudy, Y., B. Barnea, L. Yosef, T. Frank, and E. Mendelson. 2000. Clinical rubella reinfection during pregnancy in a previously vaccinated woman. *J. Infect.* **41**:187–189.
2. Carotenuto, P., D. Looij, L. Keldermans, F. de Wolf, and J. Goudsmit. 1998. Neutralizing antibodies are positively associated with CD4⁺ T-cell counts and T-cell function in long-term AIDS-free infection. *AIDS* **12**:1591–1600.
3. CASCADE Collaboration. 2003. Differences in CD4 cell counts at seroconversion and decline among 5739 HIV-1-infected individuals with well-estimated dates of seroconversion. *J. Acquir. Immune Defic. Syndr.* **34**:76–83.
4. Celum, C. L., S. P. Buchbinder, D. Donnell, J. M. Douglas, Jr., K. Mayer, B. Koblin, M. Marmor, S. Bozeman, R. M. Grant, J. Flores, and H. W. Sheppard. 2001. Early human immunodeficiency virus (HIV) infection in the HIV Network for Prevention Trials Vaccine Preparedness Cohort: risk behaviors, symptoms, and early plasma and genital tract virus load. *J. Infect. Dis.* **183**:23–35.
5. Chargelegue, D., C. M. Stanley, C. M. O'Toole, B. T. Colvin, and M. W. Steward. 1995. The affinity of IgG antibodies to gag p24 and p17 in HIV-1-infected patients correlates with disease progression. *Clin. Exp. Immunol.* **99**:175–181.
6. Dobbs, T., S. Kennedy, C. P. Pau, J. S. McDougal, and B. S. Parekh. 2004. Performance characteristics of the immunoglobulin G-capture BED-enzyme immunoassay, an assay to detect recent human immunodeficiency virus type 1 seroconversion. *J. Clin. Microbiol.* **42**:2623–2628.
7. Dougan, S., B. Patel, J. H. Tosswill, and K. Sinka. 2005. Diagnoses of HIV-1 and HIV-2 in England, Wales, and Northern Ireland associated with west Africa. *Sex. Transm. Infect.* **81**:338–341.
8. Eggers, M., U. Bader, and G. Enders. 2000. Combination of microneutralization and avidity assays: improved diagnosis of recent primary human cytomegalovirus infection in single serum sample of second trimester pregnancy. *J. Med. Virol.* **60**:324–330.
9. Gupta, S. B., O. N. Gill, C. Graham, A. D. Grant, P. A. Rogers, and G. Murphy. 2000. What a test for recent infection might reveal about HIV incidence in England and Wales. *AIDS* **14**:2597–2601.
10. Janssen, R. S., G. A. Satten, S. L. Stramer, B. D. Rawal, T. R. O'Brien, B. J. Weiblen, F. M. Hecht, N. Jack, F. R. Cleghorn, J. O. Kahn, M. A. Chesney, and M. P. Busch. 1998. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA* **280**:42–48.
11. Kothe, D., R. H. Byers, S. P. Caudill, G. A. Satten, R. S. Janssen, W. H. Hannon, and J. V. Mei. 2003. Performance characteristics of a new less sensitive HIV-1 enzyme immunoassay for use in estimating HIV seroincidence. *J. Acquir. Immune Defic. Syndr.* **33**:625–634.
12. Martró, E., B. Suligoi, V. Gonzalez, V. Bossi, A. Esteve, J. Mei, and V. Ausina. 2005. Comparison of the avidity index method and the serologic testing algorithm for recent human immunodeficiency virus (HIV) seroconversion, two methods using a single serum sample for identification of recent HIV infections. *J. Clin. Microbiol.* **43**:6197–6199.
13. Murphy, G., A. Charlett, L. F. Jordan, N. Osner, O. N. Gill, and J. V. Parry. 2004. HIV incidence appears constant in men who have sex with men despite widespread use of effective antiretroviral therapy. *AIDS* **18**:265–272.
14. Rice, B. D., L. J. Payne, K. Sinka, B. Patel, B. G. Evans, and V. Delpech. 2005. The changing epidemiology of prevalent diagnosed HIV infections in England, Wales, and Northern Ireland, 1997 to 2003. *Sex. Transm. Infect.* **81**:223–229.
15. Schacker, T. W., J. P. Hughes, T. Shea, R. W. Coombs, and L. Corey. 1998. Biological and virologic characteristics of primary HIV infection. *Ann. Intern. Med.* **128**:613–620.
16. Schwarcz, S., T. Kellogg, W. McFarland, B. Louie, R. Kohn, M. Busch, M. Katz, G. Bolan, J. Klausner, and H. Weinstock. 2001. Differences in the temporal trends of HIV seroincidence and seroprevalence among sexually transmitted disease clinic patients, 1989–1998: application of the serologic testing algorithm for recent HIV seroconversion. *Am. J. Epidemiol.* **153**:925–934.
17. Sciascia, C., E. Palomba, V. Gay, and P. A. Tovo. 1996. Anti-HIV-1 antibody avidity is correlated with clinical status in infected children. *Pediatr. AIDS HIV. Infect.* **7**:14–19.
18. Suligoi, B., C. Galli, M. Massi, F. Di Sora, M. Sciandra, P. Pezzotti, O. Recchia, F. Montella, A. Sinicco, and G. Rezza. 2002. Precision and accuracy of a procedure for detecting recent human immunodeficiency virus infections by calculating the antibody avidity index by an automated immunoassay-based method. *J. Clin. Microbiol.* **40**:4015–4020.
19. Suligoi, B., M. Massi, C. Galli, M. Sciandra, F. Di Sora, P. Pezzotti, O. Recchia, F. Montella, A. Sinicco, and G. Rezza. 2003. Identifying recent HIV infections using the avidity index and an automated enzyme immunoassay. *J. Acquir. Immune Defic. Syndr.* **32**:424–428.
20. Thomas, H. I., S. Wilson, C. M. O'Toole, C. M. Lister, A. M. Saeed, R. P. Watkins, and P. Morgan-Capner. 1996. Differential maturation of avidity of IgG antibodies to gp41, p24 and p17 following infection with HIV-1. *Clin. Exp. Immunol.* **103**:185–191.
21. UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance. 2001. Analysis of prevalence of HIV-1 drug resistance in primary infections in the United Kingdom. *BMJ* **322**:1087–1088.
22. Young, C. L., D. J. Hu, R. Byers, S. Vanichseni, N. L. Young, R. Nelson, P. A. Mock, K. Choopanya, R. Janssen, T. D. Mastro, and J. V. Mei. 2003. Evaluation of a sensitive/less sensitive testing algorithm using the bioMérieux Vironostika-LS assay for detecting recent HIV-1 subtype B⁺ or E infection in Thailand. *AIDS Res. Hum. Retrovir.* **19**:481–486.