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Immune Responses in Ugandan Women Infected with Subtype A and D HIV Using the BED Capture Immunoassay and an Antibody Avidity Assay

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Abstract

Introduction: Analysis of samples from Uganda using serologic HIV incidence assays reveal that individuals with subtype D infection often have weak humoral immune responses to HIV infection. It is unclear whether this reflects a poor initial response to infection or a waning antibody response later in infection.

Materials and Methods: Samples (N=2614) were obtained from 114 women aged 18-45 in the Ugandan GS Study cohort (2001-2009; 82 subtype A, 32 subtype D; median 23 samples/women, range 3-41 samples, median follow-up of 6.6 years). Samples were analyzed using the BED capture immunoassay (cutoff 0.8 OD-n) and the avidity assay (cutoff 90% AI). Antibody maturation was assessed by having the BED-CEIA or avidity value exceed the assay cutoff 1 or 2 years after infection. A waning antibody response was measured by having the BED-CEIA or avidity value fall >20% below the maximum value.

Results: For the BED-CEIA, eight women with subtype A infection and three women with subtype D infection never progressed past the cutoff value (median 5.9 years follow-up after infection). Six women with subtype D infection never achieved an AI >90%. Subtype did not impact the proportion of women whose assay values regressed by >20% of the maximal value (for BED-CEIA: 33% for A, 41% for D, p=0.51; for avidity: 1% for A, 6% for D, p=0.19).

Discussion: The higher frequency of misclassification of individuals with long-term subtype D infection as recently infected using serologic incidence assays reflects a weak initial antibody response to HIV infection that is sustained over time.

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Keywords

subtype; incidence; BED-CEIA; avidity; immune response; Uganda

Introduction

Analysis of HIV incidence is the most direct approach for measuring the efficacy of interventions for HIV prevention.¹ HIV incidence estimates can be obtained through repeated testing of individuals in longitudinal cohorts.² However, longitudinal cohorts may be difficult to establish and costly to maintain.³ They may also suffer from bias related to loss of follow-up.⁴ An alternative approach for HIV incidence estimation relies on tests that distinguish recent from non-recent infection in a cross-sectional survey.⁵ HIV incidence can be estimated from cross-sectional surveys by measuring biomarkers that evolve during the course of HIV infection.⁶ Many cross-sectional incidence assays measure antibody maturation as a marker of duration of HIV infection (reviewed by Murphy⁷ and Guy⁸).

One limitation of using serologic assays for cross-sectional HIV incidence estimation is that some individuals have immature-appearing antibody a year or more after infection. Many factors are associated with “false-recent” misclassification, including low HIV viral load, low CD4 cell count, and long-term use of antiretroviral therapy (ART).⁹⁻¹³ We previously reported that the frequency of false-recent misclassification varies in different regions of Africa.¹⁴ Particularly high rates of false-recent misclassification were observed using the BED capture enzyme immunoassay (BED-CEIA)¹⁵ or an antibody avidity assay¹⁶ in Eastern Africa, where subtypes A and D predominate.¹⁷ The frequency of false-recent misclassification is higher in those infected with subtype D HIV, compared to those with subtype A infection.¹⁸ Other studies have also noted subtype-based differences in cross-sectional incidence assay performance.¹⁹⁻²¹ In Uganda, women with subtype D infection were more likely to have low BED-CEIA results and lower antibody avidity than women with subtype A infection.^{18,22} Subtype D HIV has been shown to be more pathogenic than subtype A HIV.²³ It was not clear whether the high frequency of false-recent misclassification in subtype D-infected individuals was due to faster disease progression (e.g., faster progression to AIDS, with a waning antibody response²⁴) or some other mechanism associated with a weak initial humoral response to HIV infection that was sustained over time. In this study, we used the BED-CEIA and avidity assay to analyze the humoral response to HIV infection in adult women with subtype A and D HIV infection.

Methods

Study Population

The Genital Shedding and Disease Progression (GS) Study evaluated the use of hormonal contraceptives, genital shedding of HIV, and HIV disease progression among 303 Ugandan and Zimbabwean women with known dates of seroconversion.²⁵ We analyzed 2,614 samples from a subgroup of Ugandan women, aged 18-45 who were infected with HIV subtype A (N=84) or subtype D (N=34) who had samples available from at least three study visits after HIV seroconversion, including at least one sample collected a year or more after seroconversion (2001-2009). The median number of samples per woman was 23 (range 3-41 years) and the median follow-up was 6.56 years (range 0.13-9.19 years). During the course of follow-up, 38 women initiated antiretroviral therapy (ART). CD4 cell count, viral load, and HIV subtype data were determined previously.^{25,26} Date of HIV seroconversion was defined as either the midpoint between the last negative HIV antibody test and the first positive HIV antibody test, or fifteen days after acute infection was documented (defined as HIV RNA positive / HIV antibody negative).²⁵

Laboratory Methods

The BED-CEIA was performed according to the manufacturer's directions (Calypte Biomedical Corporation, Lake Oswego, OR, USA), with one exception: samples were run in duplicate and the average result was reported; results were reported as normalized optical density units (OD-n).^{14,18} The BED-CEIA measures the proportion of total IgG that binds to a branched synthetic tri-peptide that contains three 18-amino acid components derived from an immunodominant region of gp41 (region corresponding to positions 590 to 607 of HXB2 gp160 in HIV subtypes B, E and D).²⁷ The avidity assay was performed using the Genetic Systems HIV-1/HIV-2 PLUS O EIA (Bio-Rad Laboratories, Redmond, WA, USA) with the following modifications: plate covers were used rather than sealers; an incubation time of 30 minutes rather than of 60 minutes was used after adding cold diluent to each sample; and deionized water was used rather than wash buffer solution when preparing the diethyl amine (DEA) solution.¹⁶ The Genetic Systems HIV-1/ HIV-2 PLUS O EIA is an enzyme immunoassay that uses a direct antibody sandwich technique to detect anti-HIV antibodies. The percent avidity (Avidity Index, AI) is calculated for each sample by dividing the optical density of the DEA-treated well by the optical density of the non-treated well for the same sample and multiplying by 100.¹⁶ Maturation of the immune response to HIV infection assessed within the first year and within the first two years after seroconversion; for each time interval, we assessed whether the BED-CEIA result rose above the cutoff of 0.8 OD-n, and whether the AI rose above the cutoffs of 40%^{16,28-30} or 90%. Antibody maturation was classified as 'normal' if the BED-CEIA value was >0.8 OD-n and the AI was >40% by two years after seroconversion. Antibody regression was defined as decline in the BED-CEIA or the AI result of at least 20% compared to the maximum value measured for the same women at an earlier time point.

Statistical Analysis

For samples collected more than 2 years after seroconversion, Fisher's exact test or the Chi Square test were used to identify factors associated with false-recent misclassification using the BED-CEIA and the avidity assay. These factors included: age, HIV viral load, CD4 cell count, duration of ART at the time of sample collection, and year of sample collection). Logistic regression using general estimating equations (GEE) was performed to determine the odds of false-recent misclassification for all factors analyzed.³¹ Factors that were associated with false-recent misclassification in the univariate analysis with $P < 0.15$ were included in a multivariate logistic regression model. All analyses were performed using STATA v11 (StataCorp, College Station, TX).

Results

Subtype distribution in the study cohort

The women included in this study had either subtype A or subtype D infection (overall, 72% and 28%, respectively). The relative proportions of these subtypes were not significantly different for most of the subgroups included in the analysis (Table 1). The following exceptions were noted: (1) for women on ART <1 year: 56.5% (87/154) samples were subtype A and 43.5% (67/154) samples were subtype D ($p < 0.05$), (2) for women on ART >1 year: 27.0% (55/204) samples were subtype A and 73.0% (149/204) samples were subtype D ($p < 0.01$). Most of the samples were collected >2 years after seroconversion: 68.5% (1256/1833) for subtype A and 71.5% (558/781) for subtype D. There were 385 samples collected between 1 and 2 years after seroconversion, none of which were from individuals on ART.

Antibody maturation in women with subtype A and D infection

The proportion of women whose BED-CEIA results remained <0.8 OD-n was similar for subtype A (Figure 1A) and subtype D (Figure 1B) during the first year after seroconversion (48% [39/82] vs. 50% [16/32], $p=0.83$) and during the first two years after seroconversion (21% [17/82] vs. 16% [5/32], $p=0.60$, Table 2). Over the entire follow-up period (median follow-up: 5.9 years), 10% (8/82) of women with subtype A and 9% (3/32) of women with subtype D infection never attained a BED-CEIA result above 0.8 OD-n.

The proportion of women whose avidity assay result remained $<40\%$ was 0% (0/82) for subtype A (Figure 2A) and 6% (2/32) for subtype D infection (Figure 2B) during the first year after seroconversion ($p=0.084$); the avidity assay result rose above 40% for all women by 2 years after seroconversion (Table 2). The proportion of women whose avidity result did not rise above 90% after seroconversion was lower for women with subtype A than for women with subtype D (within 1 year: 17% [14/82] vs. 56% [18/32], $p<0.01$; within 2 years: 0% [0/82] vs. 34% [11/32], $p<0.01$). Over the entire follow-up period (median follow-up: 5.9 years), none of the women with subtype A and 19% (6/32) of women with subtype D infection never attained an avidity result $>90\%$.

Antibody regression in women with subtype A and D infection

Regression in antibody response was defined by a decrease of $>20\%$ in the BED-CEIA or avidity results compared to the maximum value obtained for the same women at an earlier study visit. Antibody regression was observed for 40 women using the BED-CEIA and for three women using the avidity assay. For both assays regression was more common for the subtype D infected women for BED-CEIA: 33% [27/82] for subtype A vs. 41% [13/32] for subtype D, $p=0.51$; for the avidity assay: 11% [1/82] for subtype A vs. 6% [2/32] for subtype D, $p=0.19$, Table 2). Antibody regression was associated with ART initiation. In this cohort of women, 24 women with subtype A and 14 women with subtype D infection initiated ART during follow-up. Among the subtype A women who initiated ART, 63% [15/24] saw regression in the BED-CEIA values, while none of these women saw a decrease in avidity values. For the 14 subtype D women initiating ART, 79% [11/14] had regression in their BED-CEIA and 21% [3/14] had regression in their avidity values. The timing of ART initiation was also associated with antibody regression. The proportion of women who had antibody regression measured with the BED-CEIA was higher for those who started ART more than 3 years after seroconversion (78% [22/26]) than for those who started ART earlier (15% [4/26], $p<0.01$). In contrast, there was no significant difference in antibody regression measured with the avidity assay among women who started ART less than vs. more than 3 years after seroconversion (50% [1/2] for both groups). Some women with subtype A infection (8% [2/24]) had increasing BED-CEIA values after starting ART; in contrast, none of the women had increasing avidity values after starting ART (0% [0/24]).

Because ART treatment can confound the impact of subtype on the regression of response as measured by the BED-CEIA and avidity assay, we performed an analysis that was limited to those who were never treated. Individuals who were never treated displayed similar progression trends as those presented in Table 2. One individual with subtype A infection and one individual with subtype D infection demonstrated antibody regression by the avidity assay (with AI $<40\%$ and AI $<90\%$, respectively). The proportion of individuals who had a regression of BED-CEIA values was higher for individuals with subtype A compared to subtype D infection; however, this difference was not statistically significant (12/58=21% vs. 2/18=11%, $p=0.36$). Among individuals who had a regression in BED-CEIA values (both on ART and not), a higher proportion of non-treated individuals with subtype A had regression compared to those with subtype D infection; this difference was of borderline significance (12/27=44% vs. 2/13=15%, respectively, $p=0.07$). When this analysis was

restricted to individuals on ART, there was no significant difference in regression of BED-CEIA values obtained for those with subtype A vs. D infection (15/24=63% vs. 11/14=79%, respectively, $p=0.30$). In contrast, a difference of borderline statistical significance was observed for the avidity assay for these two groups (0.24=0% vs. 2/14=14%, respectively, $p=0.057$).

Analysis of factors associated with false-recent misclassification

Overall, 17.6% (319/1,814) of the samples from women more than 2 years after seroconversion had BED-CEIA values <0.8 OD-n; in contrast, only 3.0% (55/1,814) had avidity assays $<40\%$ and only 3.9% (70/1,814) had avidity values $<90\%$ in this time frame.

The following factors were associated with false-recent misclassification using the BED-CEIA in univariate analysis (Table 3a): CD4 cell count <200 cells/ μ l ($p<0.05$); missing CD4 data ($p<0.05$); viral load of <400 copies/ml ($p<0.01$); and on ART <1 year ($p<0.01$). In a multivariate model, false-recent misclassification was associated with missing CD4 cell count data ($p<0.05$); and on ART <1 year ($p<0.01$) and on ART >1 year ($p<0.01$). In a multivariate model, false-recent misclassification using the avidity assay was associated with duration of infection (48-72 months, $p<0.01$); and HIV subtype ($p<0.05$). We compared results obtained using the BED-CEIA and avidity assays. False-recent misclassification with the BED-CEIA was seen in 17.3% (305/1759) of samples that had avidity assay results $>40\%$ and in 25.5% (14/55) of samples with avidity assay results $<40\%$; this difference was not statistically significant.

For samples collected more than 2 years after seroconversion and stratified by subtype, 0.0% (0/1256) of samples from individuals with subtype A infection were misclassified by the avidity assay, compared to 9.9% (55/558) of samples from individuals with subtype D infection ($p<0.001$). Similar differences were observed for the BED-CEIA (15.5% (195/1256) of samples from individuals with subtype A infection were misclassified, compared to 22.2% (124/558) of samples from individuals with subtype D infection ($p<0.001$).

There were 157 samples (95 subtype A and 65 subtype D) that did not have CD4 cell count data associated with them. Ninety two (62 subtype A and 30 subtype D) of those samples were collected at a time point early in infection (median 110 days after seroconversion) from women who were not on ART. 86% (79 [56 subtype A and 23 subtype D]/92) misclassified by the BED-CEIA, 46% (42[31 subtype A and 11 subtype D]/92) misclassified by the avidity assay and 95% (40/42) of samples that misclassified by the avidity assay misclassified by the BED-CEIA. 2 samples both subtype D infected of the remaining 65 samples were collected early in infection (<2 years) and on ART. Both samples misclassified by the BED-CEIA and neither misclassified by an AI cutoff of 40%. The remaining 63 (33 subtype A and 30 subtype D) samples were collected later in infection (>2 years) and 76% (48/63) were recently placed on ART (median 28 days). 8% (5/63) misclassified by the BED-CEIA all subtype D and 10% (6/63) misclassified by an avidity index of cutoff $<40\%$ all of which were subtype D. No sample that misclassified by the avidity assay, misclassified by the BED-CEIA.

Discussion

Previous studies in other cohorts have demonstrated that individuals with long-standing subtype D HIV infection often have low levels of anti-HIV antibodies detected with serologic HIV incidence assays.^{14,18} This type of false-recent misclassification lowers the precision of these assays for HIV incidence estimation in populations where subtype D is prevalent. This report investigated the biologic basis for false-recent misclassification in

adults with subtype D infection by evaluating antibody maturation and antibody regression in a cohort of women with subtype A and D infection. We found that differences in the performance of the BED-CEIA and avidity assays in these two subtypes reflect a weaker initial antibody response to HIV infection in those with subtype D infection. We did not see a significant difference in antibody regression (decline in BED-CEIA and avidity assay results) in these two subtypes. For both subtypes, antibody regression was associated with initiation of ART and duration of treatment.

A longer time on ART was associated with lower OD-n by the BED-CEIA, but not a lower avidity index by the avidity assay due to the nature of the assay. The BED-CEIA measures the proportion of IgG antibody that is directed to an immunodominant region of gp41. When the level of circulating HIV antigens decreases (with viral suppression) antibody production may be down-regulated, and the titers of anti-HIV antibodies may fall. This decrease in antibody titers may result in lower BED-CEIA values.^{13,14,32} In contrast, the avidity assay measures the strength of antibody binding to target antigens in the presence of a chaotropic agent. A decrease in the amount of circulating antigen would not necessarily impact antibody avidity, and is, therefore, less likely to impact the results obtained with an avidity assay. A previous study demonstrated a lack of impact of decreasing antigen (as measured by decreasing HIV viral load) on the avidity assay used in this report.³²

In a previous study of individuals from the Rakai Community Cohort Study (RCCS), we observed more frequent false-recent misclassification among women with subtype D compared to subtype A; this association was not observed for men with these subtypes.¹⁸ In this report, we extended that study by showing that 9% of women with subtype D infection did not attain a mature antibody response even after many years of infection; this failure of antibody maturation was uncommon in women with subtype A infection. This study also extends our previous study of the RCCS, since the GS Cohort analyzed in this report included women who initiated ART during study follow-up. In the GS Cohort, women with subtype D were less likely to attain BED-CEIA values above the assay cutoff; this indicates that they maintained low levels of HIV-specific IgG in their serum, even years after seroconversion.²² BED-CEIA values also declined over time in more women with subtype D than subtype A; this effect, which we refer to as antibody regression, was associated with ART use.

The findings in this report support the use of multi-assay algorithms (MAAs) for cross-sectional HIV incidence estimation.²² The performance of MAAs is likely to be improved when they include two serologic assays that measure different features of the immune response to HIV infection; in this study, false-recent misclassification by the BED-CEIA and avidity assay were not associated.²² In this report, low CD4 cell count and duration of time on ART were associated with misclassification by the BED-CEIA. Use of ART for more than one year more than doubled the frequency of false-recent misclassification by the BED-CEIA; the duration of ART was also associated with false-recent misclassification by the BED-CEIA in previous studies.^{14,33} Inclusion of non-serologic biomarkers in MAAs, such as CD4 cell count and HIV viral load, may further reduce the frequency of false-recent misclassification.¹⁴ For both subtypes A and D, the performance of the avidity assay was superior to that of the BED-CEIA.

The high prevalence of subtype D HIV infections in Uganda likely explains the high frequency of false-recent misclassification observed with serologic incidence assays in populations from Eastern Africa.¹⁴ Our results suggest that even higher frequencies of false-recent misclassification will be observed as ART use increases in those populations. Increasing ART use may limit the utility of serologic HIV incidence assays in populations where subtype D is prevalent, even where those assays are used in parallel or in serial testing

algorithms.¹⁸ Though these findings reveal differences in the immune responses to HIV infection in individuals with subtype A and D infection, further experiments are needed to determine if these differences are specific to the two incidence assays used in this study, or are more general in nature. In order to evaluate whether the antibody responses are generally weaker in individuals with subtype D infection, it would be necessary to evaluate the antibody responses to a broader spectrum of HIV antigens and to evaluate other features of the humoral immune response to HIV infection (e.g. antibody neutralization, antibody isotype).

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A.

B.

Figure 1. BED-CEIA values by time from seroconversion

Antibody maturation measured by the BED-CEIA is shown as a function of time since seroconversion (x-axis). The assay cutoff (>0.8 OD-n) is shown as a horizontal line. Panel A: women with subtype A infection (82 women, 1833 samples). Panel B: women with subtype D infection (32 women, 781 samples).

A.

B.

Figure 2. Avidity assay values by time from seroconversion

Antibody maturation measured by the avidity assay is shown as a function of time since seroconversion (x-axis). The assay cutoff (>40%) is shown as a horizontal line. Panel A: women with subtype A infection (82 women, 1833 samples). Panel B: women with subtype D infection (32 women, 781 samples).

Table 1

Characteristics of the study population (Ugandan women, 2001-2009).

	Subtype A (82 subjects, 1833 samples)	Subtype D (32 subjects, 781 samples)
Age at infection, mean (SD)	26.5 (4.7)	27.3 (5.6)
Duration of Infection		
0-3 months	73.2% (41/56)	26.8% (15/56)
4-6 months	69.7% (101/145)	30.3% (44/145)
7-12 months	71.2% (153/215)	28.8% (62/215)
13-24 months	73.4% (282/384)	26.6% (102/384)
25-48 months	70.2% (552/786)	29.8% (234/786)
49+ months	68.5% (704/1028)	31.5% (324/1028)
CD4 (cells/μl)		
> 500	73.0% (832/1140)	27.0% (308/1140)
499-200	70.1% (808/1153)	29.9% (345/1153)
199-50	61.4% (94/153)	38.6% (59/153)
<50	36.4% (4/11)	63.6% (7/11)
No data	60.5% (95/157)	39.5% (62/157)
Viral load (copies/ml)		
> 50000	61.5% (235/382)	38.5% (147/382)
49,999-10,000	76.5% (248/324)	23.5% (76/324)
9,999-400	77.0% (245/318)	23.0% (73/318)
<400	47.7% (142/298)	52.3% (156/298)
No data	74.5% (963/1292)	25.5% (329/1292)
Time on ART		
Not on ART	74.9% (1691/2256)	25.1% (565/2256)
ART < 1 year	56.5% (87/154) [†]	43.5% (67/154) [†]
ART > 1 year	27.0% (55/204) [‡]	73.0% (149/204) [‡]

Abbreviations: SD: standard deviation; ART: antiretroviral therapy. Significant differences in groups are indicated using the following symbols:

[†] = p<0.05,[‡] = p<0.01.

Table 2

Assay maturation and regression in women with subtype A and D HIV.

	BED-CEIA OD-n >0.8		Avidity Index AI >40%		Avidity Index AI >90%	
	Subtype A	Subtype D	Subtype A	Subtype D	Subtype A	Subtype D
Total subjects tested	82	32	82	32	82	32
Progression						
Above assay cutoff within 1 year	52% (43/82)	50% (16/32)	100% (82/82)	94% (30/32)	83% (68/82)	44% (14/32)
Above assay cutoff within 2 years	79% (65/82)	84% (27/32)	100% (82/82)	100% (32/32)	100% (82/82)	66% (21/32)
Above assay cutoff during follow-up	90% (74/82)	91% (29/32)	100% (82/82)	100% (32/32)	100% (82/82)	81% (26/32)
Regression						
Antibody regression	33% (27/82)	41% (13/32)	1% (1/82)	6% (2/32)	1% (1/82)	9% (3/32)
Antibody regression on ART	56% (15/27)	85% (11/13)	0% (0/1)	100% (2/2)	0% (0/1)	66% (2/3)

Table 3A

Factors associated with false-recent misclassification by the BED-CEIA or avidity assay in women infected more than 2 years.

		BED-CEIA (OD-n < 0.8)	BED-CEIA OR (95% CI)	Avidity Index (AI < 40%)	Avidity Index OR (95% CI)
Percent (#/ total tested)		17.6% (319/ 1814)		3.0% (55/1814)	
Age at infection					
	18-24 years	18.1% (137/759)	1	2.9% (22/759)	1
	25-28 years	7.7% (48/623)	0.55 (0.17-1.81)	3.7% (23/623)	1.18 (0.09-15.06)
	> 28 years	31.0% (134/432)	1.45 (0.55-3.80)	2.3% (10/432)	0.77 (0.09-6.78)
Duration of infection					
	24-48 months	18.3% (144/786)	1	2.9% (23/786)	1
	48-72 months	16.5% (100/606)	0.87 (0.59-1.29)	2.5% (15/606)	0.65 (0.39-1.07)
	72-96 months	18.6% (69/371)	1.22 (0.72-2.07)	4.0% (15/371)	0.75 (0.27-2.07)
	> 96 months	11.8% (6/51)	1.53 (0.87-2.69)	3.9% (2/51)	1.58 (0.13-17.91)
CD4 (cells/μl)					
	500	25.6% (185/723)	1	1.4% (10/723)	1
	499-200	13.2% (116/877)	0.75 (0.49-1.13)	3.7% (32/877)	0.74 (0.40-1.38)
	<200	8.6% (13/151)	0.56 (0.32-0.99)[†]	4.6% (7/151)	0.80 (0.29-2.19)
	No data	7.9% (5/63)	0.40 (0.17-0.95)[†]	9.5% (6/63)	0.66 (0.06-7.51)
Viral load (copies/ml)					
	> 50,000	10.6% (20/188)	1	7.5% (14/188)	1
	49,999-400	17.4% (52/299)	1.31 (0.85-2.01)	1.0% (3/299)	0.92 (0.48-1.76)
	<400	42.2% (114/270)	2.80(1.40-5.60)[‡]	7.4% (20/270)	0.74 (0.11-5.08)
	No data	12.6% (133/1,057)	0.90 (0.65-1.24)	1.7% (18/1,057)	0.77 (0.36-1.62)
Time on ART					
	Not on ART	13.2% (193/1,463)	1	1.4% (21/1,463)	1
	< 1 year	18.9% (28/148)	1.91 (1.28-2.83)[†]	6.8% (10/148)	1.20 (0.62-2.34)
	> 1 year	48.3% (98/203)	4.30(1.92-9.62)[†]	11.8% (24/203)	0.73 (0.04-14.75)
Subtype					
	A	15.5% (195/1256)	1	0.0% (0/1256)	1
	D	22.2% (124/558)	1.52 (0.59-3.91)	9.9% (55/558)	2.95 (0.95-9.20)
Misclassified by other assay					
	BED-CEIA 0.8 OD-n	-	-	2.7% (41/1495)	1
	BED-CEIA < 0.8 OD-n	-	-	4.4% (14/319)	0.25(.0002-341.74)
	AI 40%	17.3% (305/1759)	1	-	-
	AI < 40%	25.5% (14/55)	1.82 (0.096-34.54)	-	-

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Table 3B

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	BED-CEIA aOR (95% CI)	Avidity Index aOR (95%CI)
Age at infection		
18-24 years	-	-
25-28 years	-	-
> 28 years	-	-
Duration of infection		
24-48 months	1	1
48-72 months	0.70 (0.49-1.02)	0.65 (0.50-0.85)[‡]
72-96 months	0.69 (0.47-1.02)	0.76 (0.46-1.24)
> 96 months	0.60 (0.34-1.08)	1.36 (0.41-4.58)
CD4 (cells/μl)		
500	1	-
499-200	0.80 (0.59-1.09)	-
<200	0.82 (0.60-1.14)	-
No data	0.52 (0.30-0.88) [†]	-
Viral load (copies/ml)		
> 50,000	1	-
49,999-400	1.17 (0.80-1.71)	-
<400	1.26 (0.75-2.12)	-
No data	0.94 (0.70-1.25)	-
Time on ART		
Not on ART	1	-
< 1 year	2.29 (1.25-4.20)[‡]	-
> 1 year	4.74 (2.33-9.66)[‡]	-
Subtype		
A	-	1
D	-	3.23 (0.99- 10.52)[†]

The association of factors with false-recent misclassification by the BED-CEIA and avidity assays was examined using univariate models table A (OR: odds ratio) and multivariate models table B (aOR: adjusted odds ratio). Significant OR and aOR values are indicated using the following symbols:

[†]=p<0.05,

[‡]=p<0.01.