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**Title:**

Do tests devised to detect recent HIV-1 infection provide reliable estimates of incidence in Africa ?

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**Running head :** Recent HIV infection and incidence in Africa

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**Abstract:**

The objective of this study is to assess the performance of four biological tests designed to detect recent HIV-1 infections in estimating incidence in West Africa (BED, Vironostika, Avidity, and IDE-V3).

These tests were assessed on a panel of 135 samples from 79 HIV-1-positive regular blood donors from Abidjan, Côte d'Ivoire, whose date of seroconversion was known (ANRS 1220 cohort). The 135 samples included 26 from recently infected patients ( $\leq 180$  days), 94 from AIDS-free subjects with long standing infection ( $> 180$  days), and 15 from patients with clinical AIDS. The performance of each assay in estimating HIV incidence was assessed through simulations.

The modified commercial assays gave the best results for sensitivity (100% for both), and the IDE-V3 technique for specificity (96.3%). In a context like Abidjan with a 10% HIV-1 prevalence associated with a 1% annual incidence, the estimated test-specific annual incidence rates would be 1.2% (IDE-V3), 5.5% (Vironostika), 6.2% (BED) and 11.2% (Avidity). Most of the specimens falsely classified as incident cases were from patients infected for  $> 180$  days but  $< 1$  year.

Authors conclude that None of the four methods could currently be used to estimate HIV-1 incidence routinely in Côte d'Ivoire, but further adaptations might enhance their accuracy.

**Keywords:** HIV incidence, STARHS assay, surveillance, Africa, recent infection, immunoassay.

## **Introduction**

In sub-Saharan Africa, the human immunodeficiency virus type-1 (HIV-1) epidemic has been fast growing for more than two decades [1]. To monitor its trend, two indicators are required: the prevalence, which estimates the number of people living with HIV infection at a given time, and the incidence, which estimates the number of new infections during a given time interval. In Africa, surveillance systems have focused on estimating the HIV-1 prevalence through serological testing of samples collected from sentinel populations, while the HIV incidence rate has rarely been estimated. However, estimates of incidence rates are essential for understanding the natural dynamics of the epidemic as well as for measuring the impact of prevention strategies.

Direct measurement of HIV incidence through large prospective cohort studies with repeated HIV antibody testing of the participants has been the gold standard to estimate the rate of acquisition of new infections. However, such studies are very expensive, time consuming, may be subject to participation bias, and are logistically difficult to implement, especially in the African context. Therefore, only a handful of such community-based prospective surveys have been undertaken so far [2-5].

Mathematical models have been developed to estimate incidence using single or serial age-specific prevalence data and survival distribution after HIV-1 infection [6-11]. Although these methods are of great interest and give age and time trends of HIV-1 incidence, they require assumptions which lead to unavoidable uncertainty in point estimates [12]. Furthermore, they were designed in settings where patients had limited or no access to care and treatment, a situation which will hopefully become increasingly rare with the scaling-up of treatment programs in Africa [13].

Over the past decade, for the purpose of estimating HIV incidence, a number of enzyme-immuno assays (EIA) have been developed to differentiate between recent and long-standing HIV-infections [14-20]. These methods rely on features of the evolving HIV-1 antibody response during the months following primary HIV-1 infection. Some of these methods have found wide acceptance. For instance the IgG capture BED EIA assay [15] is being used by the US Centers for Disease Control and Prevention (CDC) to estimate national HIV-1 incidence in the USA, superseding the so-called ‘detuned’ assay [14, 17] that has been used by many groups to estimate HIV incidence in specific population groups [21-23]. In France, the technique developed by Barin et al [19] is now routinely used during the ‘mandatory notification’ of HIV infection [24]. An antibody avidity assay has also been developed and is being routinely used in Italy [25]. Each of these approaches takes advantage of different features of the early immune response to HIV infection, though all were developed with antigens derived from HIV-1 subtype B only, or B, E and D, and are thus optimised for populations among which these subtypes predominate, such as those of America, Asia and Europe. By contrast, these techniques have had only limited evaluation on specimens from Africa where HIV-1 incidence is generally higher and where non-B subtypes predominate.

The objective of the present study was to assess the performance of four tests devised to detect recent infections (TRI) in estimating HIV-1 incidence in West Africa. The assessment utilised a panel of specimens from HIV-1 seroconverters enrolled in the ANRS 1220 PRIMO-CI cohort with a known date of infection [26], and mainly infected with HIV-1 CRF02\_AG strains in Abidjan, Côte d’Ivoire, West Africa [27].

## **Material and methods**

### **Laboratory methods**

In our study, four assays that identify recent HIV-1 infections were assessed: one assay commercialised by Calypte Biomedical Corporation (Rockville, Maryland, USA), the IgG-capture BED-EIA (BED) [15]; two modified commercial assays: the Vironostika HIV-1 EIA from BioMerieux, Raleigh, NC, USA (Vironostika) [16, 17]; and the Abbott HIV1 1/2gO assay modified by Suligoi et al to assess the avidity of anti-HIV (Avidity) [18]; and an in-house assay developed by Barin et al [19], the IDE-V3 EIA. With the exception of the avidity assay the results were quantitative ratios calculated as optical densities obtained with serum specimens divided by the assay cut-off which was derived from the reactivity of controls and/or calibrators incorporated in each run. The avidity assay provides a quantitative result based on the relative signal given by the specimen with and without treatment with a chaotropic agent (1M guanidine). Each approach employs a defined cut-off and window period which allows the distinction of recent from non-recent infections. The exact methods for the performance, standardization and interpretation of each assay have been described previously [15, 17, 19, 25]. Table 1(A) summarizes the cut-offs employed for each of the TRI, the associated window periods during which recent infection can be inferred, and the sensitivity and specificity estimates that have been described in the literature for each of the four tests. These values were established and validated on Caucasian populations harbouring a predominance of HIV-1 subtype B viruses. For the BED method, we applied the window period of 160 days and the associated cut-off of 1.0 to be closer to the other TRI window periods and for the Avidity test, we used the 0.85 cut-off as it is the one that has been selected [28] and used as previously described [29].

## Study population and samples

We used data from the ANRS 1220 PRIMO-CI cohort, constituted of HIV-1 and HIV-1+2 positive regular blood donors with a known or estimated date of sexual transmission [26]. Enrolment into this cohort began in June 1997 and involved 199 individuals as of December 2003. In the present study, the four above mentioned TRIs were assessed on 135 serum samples from 79 cohort subjects. All selected specimens were sent to the virology laboratory in Tours, France, where the IDE-V3 immunoassay was carried out. The Vironostika, BED and Avidity assays were performed in the Virus Reference Department of the Health Protection Agency Centre for Infections, London, UK.

The window period associated with TRI  $x$  ( $x$  could be either BED, Vironostika, IDE-V3, or Avidity) is denoted by the symbol  $\omega_x$ . Samples were defined as ‘recently infected’ if the time elapsed between their last negative HIV serological result and their first positive result, obtained on specimens taken at inclusion in the cohort, was  $\leq \omega_x$ . Individuals for whom this same interval exceed  $\omega_x$  were assumed not to have been infected recently (established infection). The selection criteria for serum specimens were as follows: (i) all available samples taken at recruitment in the ANRS 1220 PRIMO-CI cohort from subjects defined as recently infected, i.e. with the time period between last HIV-negative test and sample  $< \omega_x$ . However, the number of recently infected samples available differed according to the window period,  $\omega_x$ , defined for each TRI employed. Indeed, for the TRI with the longest window,  $\omega_x=180$  days, there were 26 samples which met the definition for recent infection. Two of these were not suitable for the definition of recent infection with the window period  $\omega_x=170$  days ( $n=24$ ), and only 14 of them were suitable with the window period  $\omega_x=160$ ; (ii) 94 specimens from asymptomatic subjects with established infection; and (iii) 15 specimens from subjects who were diagnosed with clinical AIDS. Among the 109 specimens from patients



infected for >180 days, 27 were infected for 181-365 days, and 70 for more than one year. The 12 remaining specimens could not be classified within one of the two previous groups as the time period between their last negative HIV test and the date of sampling was too large (>one year), while the period between their first positive HIV test and the date of sampling was too short (<one year).

### **Statistical methods**

The sensitivity (in our case, the ability to correctly classify as recent infection a patient infected with HIV in the past  $\omega_x$  days) and the specificity (the ability to classify a patient infected for more than  $\omega_x$  days as having a non-recent infection) were calculated for each test from the samples described above.

In order to investigate the adequacy of the window period of each TRI to our sample, we categorised non-recent infections according to two time durations since infection: i) those infected >180 days but  $\leq 1$  year, ii) those infected for >1 year, and calculated the specificity for each group.

The adequacy of the cut-offs were assessed using receiver operating characteristic (ROC) curves. The ROC curve represent the sensitivity by (1-specificity) plotted considering each quantitative value of the TRI as a possible cut-off to set apart recent from non-recent infections.

Although the performance of the tests in estimating incidence depends on their sensitivity and specificity, the link is not straightforward as, when estimating a population incidence, a false negative can be compensated for by a false positive. This can lead to an accurate number of recent infections overall, though the individual diagnosis may not be correct. The ultimate goal of a TRI is usually to estimate incidence from a cross-sectional survey. We can not

directly assess the ability of the TRIs to estimate incidence as we do not have a sample with known incidence rate to which we could apply the TRIs and compare the true incidence with the estimated. Therefore, we simulated such populations with values for prevalence  $P$  and incidence  $I$ , and calculated the incidence that would be estimated with each TRI, according to its sensitivity and specificity characteristics that had been derived from their evaluation against the specimen panel described above.

Let denote  $N_{inc}$ =number of recent infections,  $N_{neg}$ =number of HIV seronegative individuals,  $N_{pos}$ =number of HIV-1 antibody positive subjects in the survey obtained using a standard HIV test,  $N=N_{pos}+N_{neg}$ , and  $k=365/\omega$  to allow results to be annualised .

Surveys simulated of size  $N=135$  were constructed as follows: from the true prevalence, we deduced the number of HIV negative subjects in the survey:  $N_{neg} = \frac{1-P}{P} \cdot N_{pos}$  . From the true incidence, we deduced the number of new infections in the survey:  $N_{inc} = N_{neg} \frac{I}{k(1-I/2)}$  .

For each TRI, the incidence estimated in the simulated survey was calculated as follows: the number  $d_{inc}$  of new infections that would be diagnosed in the simulated survey was calculated using the following relation:  $d_{inc} = Se \cdot N_{inc} + (1-Sp) \cdot (N_{pos} - N_{inc})$ , where  $Se$  and  $Sp$  are the sensitivity and specificity of the TRI. We deduced the estimated incidence for each TRI using the consensus formula agreed upon at the US CDC for calculating incidence from a cross-sectional survey [30]:  $I_{est} = \frac{k \cdot d_{inc}}{N_{neg} + k \cdot d_{inc} / 2}$  .

$$I_{est} = \frac{k \cdot d_{inc}}{N_{neg} + k \cdot d_{inc} / 2} .$$

This can be written as a function of the true prevalence  $P$  and incidence  $I$  in the simulated sample, and the sensitivity and specificity of the TRI, by replacing  $d_{inc}$  using the relations given above, and finally:

$$I_{est} = \frac{I \cdot (1-P)(Se + Sp - 1) + (1-Sp)(P \cdot k \cdot (1-I/2))}{(1-P) \cdot (1-I/2) + [I \cdot (1-P)(Se + Sp - 1) + (1-Sp)(P \cdot k \cdot (1-I/2))] / 2} \quad (a)$$

It is clear from the formula (a) that the incidence estimated depends on the sensitivity and specificity of the test, as expected, but also on the real prevalence and incidence of HIV in the population sample. Therefore, we simulated different scenarios of surveys with given  $P$  and  $I$  in a population, and calculated the estimated incidence using each test with the formula (a), and compared it to the simulated incidence by calculating the relative bias  $\frac{I-I_{est}}{I}$ .

The estimated variance of the incidence is  $I_{est}^2/d_{inc}$  [30]; this variance is used to calculate the 95% confidence intervals using the Normal approximation.

## **Results**

### **Performance in individual detection of recent infection**

Table 1(B) summarizes the analytical performance of the four tests in detecting recent infections derived from applying them to our Abidjan specimen panel. In terms of sensitivity, the Vironostika and the Avidity tests gave the best results (both 100%), whereas the IDE-V3 technique performed poorly (42.3%). However, in terms of specificity, the IDE-V3 was the most powerful (96.3%) whereas the Avidity showed the worst result (49.5%). The 15 patients with AIDS were all classified as non recent infections by three tests (Vironostika, BED and IDE-V3), while five were misclassified by the Avidity test (figure 1) which had the poorest specificity.

### **Suitability of the window periods**

For each of the four TRI, the specificity was much higher when calculated only on those specimens collected from individuals infected for more than one year (table 2). This was especially true for the Vironostika and the BED tests whose specificity increased from 44% to 93% and from 48% to 89% respectively.

### **Suitability of the cut-offs**

The ROC curves associated with each TRI are shown in figure 2, with arrows indicating the cut-offs used based on manufacturers' recommendations or previous published reports. It is apparent from these graphs that the recommended cut-offs might not be the most appropriate ones on the samples from Abidjan. For example, with the Vironostika and Avidity assays, a lower cut-off would improve greatly the specificity without a corresponding magnitude of loss of sensitivity. The Vironostika gave the highest area under ROC curve (0.96), indicating its potential to provide the best balance of sensitivity and specificity.

### **Performance in estimating incidence:**

The incidence figures estimated with each TRI according to several simulations of incidence and prevalence in a population, are presented in table 3. The situations where the simulated incidence falls within the confidence intervals of the estimated incidence are in bold. This happened only with the method with the best specificity (IDE-V3), and only in those scenarios for which the simulated incidence was high relative to the prevalence. When the simulated incidence is low compared to the prevalence, the incidence can be greatly overestimated by the TRI. For example, in a population with a 30% prevalence and 1% incidence, the over-estimation of incidence was 3 times with the IDE-V3 test, 17 times with the Vironostika, 20 times with the BED, and 36 times with the Avidity assay (table 3). For a given prevalence, and for all the TRIs, the bias in the estimated incidence increases when the simulated incidence decreases. This means that the tests can not be used reliably to monitor the time trends of incidence as, with a constant prevalence over time, it would underestimate a decrease in incidence, or overestimate an increase. For example, in a setting like Abidjan with a prevalence about 10% [31] and incidence around 1% [11], if HIV incidence is halved (that is a 50% relative decrease with the incidence going down to 0.5%) and if the prevalence

remains stable, all four methods would fail to identify the decrease in incidence: the BED would estimate the incidence to decrease from 6.2 to 5.9% (that is a 5% relative decrease), the Vironostika from 5.5 to 5.1% (that is a 7% decrease), the IDE-V3 from 1.2 to 1.0% (a 17% relative decrease), and the Avidity from 11.2 to 11% (a 2% relative decrease).

## **Discussion**

This study is the first to assess these four reference assays for the detection of recent HIV-1 infections among a West African population. We have shown that none of the four methods can be used in their present form on Abidjan's population to provide accurate point estimates of incidence or follow trends in incidence.

On the basis of our findings on the poor specificity among patients infected for more than 180 days but less than one year (table 2), it is likely that our results are pessimistic in terms of specificity compared to a general HIV-infected population. Indeed, among the 109 selected specimens from patients infected for more than 180 days, 27 (25%) were from patients infected for less than one year, which is the period with the poorest specificity. Although it is difficult to know what would be the distribution of time since infection among a randomly selected sample of the general population, it is likely that this borderline population was over-represented in our sample of seroconverting blood donors. For example, if we suppose that the number of new infections is constant over a 12-month period, when the prevalence is 10% and the incidence 1% as it has been previously estimated in Abidjan [11], there should be about 4.5% of the non-recently infected individuals infected for less than one year. Nevertheless, even if we take an optimal sample, that is when all the non-recent infections are older than 1 year, the specificity of each of the TRIs is still lower than the specificity of the IDE-V3 test on the overall sample (table 2), and this will lead to biased estimates of

incidence. This emphasises that although our validation sample probably over-represents the frequency of infections of 6-12 months duration, this does not provide an explanation for the overall bias obtained when estimating incidence.

### **Adjustment to improve the estimations**

One approach to circumvent the problem of imperfect specificity and sensitivity is to use a coefficient to adjust for misclassification as proposed by Parekh et al [15]. The incidence would then be estimated as follows:

$$I = \frac{F \cdot k \cdot N_{inc}}{N_{neg} + F \cdot k \cdot N_{inc} / 2},$$

$$\text{where } F = \frac{P_{obs} + Sp - 1}{P_{obs}(Se + Sp - 1)} \text{ and } P_{obs} = \frac{N_{inc}}{N}$$

The variability of this estimate depends on the variability of the sensitivity and specificity used. In the case where the sensitivity and specificity are not properly estimated, this correction could result in more harm than good, and even lead to negative incidence estimates when  $P_{obs} + Sp < 1$ . Taking a population resembling Abidjan, with a 10% prevalence and 1% incidence, the IDE-V3 test estimates the incidence to be 1.2% (table 3). If the specificity of this test is overestimated to the upper limit of its confidence interval, that is 99.5% instead of 96.3% (table 1(B)), the incidence estimated with the correction coefficient F would be 2.6%. If, on the other hand, the specificity of this test was underestimated to the lower limit of its confidence interval, that is 93% instead of 96.3% (table 1(B)), the incidence estimated with the correction coefficient F would be -0.9%. Both these estimations are further away from the real incidence than the non-corrected one and, moreover, one of them is negative. We conclude therefore, that this adjustment should be used with caution, and only when the sensitivity and specificity are well established.

### **Adaptation of the tests**

We have shown that the TRI with the best specificity gave the best incidence estimations (table 3). Raising the specificity of the TRIs would ameliorate their ability to estimate incidence.

The specificity of each of the four TRI investigated was shown to be poor (i.e. designed specimens as incident) during the six months following the described window period (table 2). These results suggest that a longer window period might be more appropriate to the Abidjan specimens. Furthermore, we showed that the cut-offs associated with each test validated on Caucasian samples might not be appropriate to African ones and should be re-evaluated, especially for the Vironostika and the Avidity methods (figure 2), in favour of improved specificity.

Differences in the response to HIV infection may exist between the Caucasian population amongst which the methods were originally developed and validated and the population of Abidjan, and this deserves further investigation. Some differences between African and European immune response to HIV have been reported already, especially concerning CD4 counts [32, 33]. Sera from AIDS cases were correctly classified as non-recent infection in the Abidjan study sample, with the exception of the Avidity method (figure 1), while it frequently led to a false positive diagnosis in the Caucasian population. Although this result correlates with a sustained anti-p24 antibody response among African patients, but not among Caucasians, when they progress to AIDS [34], it is a new information that should be validated on larger population samples of various origins.

The circulating viruses are predominantly subtype B in Europe and USA, and non-B, particularly CRF02\_AG, in West Africa. The difference in subtypes may be responsible for some performance loss, indeed, different performance characteristics in detecting recent

infections among individuals infected with subtypes B, A/D, C or E using BED or Vironostika methods have already been reported [20, 35]. This may be due to subtle differences in the binding of antibodies generated against infection with non-B subtypes of HIV with antigens derived predominantly from subtype B viruses, as utilised in most diagnostic tests. Alternatively, one could relate the differences of performance in different populations to the genetic background, or to environmental factors that may influence the immune response. Indeed, total IgG levels are known to be significantly elevated among African, and malnutrition, common in Africa, has been linked to immunological dysfunction [36, 37]. Such factors might be responsible for some of the performance differences but these hypotheses need further investigations.

Using a TRI to estimate incidence of HIV-1 infection remains a very attractive solution to monitor the epidemic on the African continent. Many countries already have a sero-surveillance system, and adding a TRI to the surveillance protocol would be simple and low-cost [38]. However, further investigations are needed to adapt the existing TRIs. Our data would indicate that the use of a longer window period, and/or a different cut-off favouring a better specificity might improve their accuracy in estimating HIV incidence. Also the size of the sample used in this study provides insufficient power to allow the definition of better adapted cut-offs and/or window periods. To bring together several cohorts of patients with known date of infection from various part of Africa, if possible including patients whose infection subtypes are characterized, is a necessary step to adapt the existing methods to permit accurate detection of recent infections on its continent. Our conclusion and recommendation support the recently released UNAIDS/WHO warning on the routine surveillance use of BED test and the need for further research on the matter [39].



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**Table 1.** Cut-offs, window periods, sensitivity and specificity associated with each of the four tests used to detect recent HIV infections.

Assay	Cut-off	Window period	Sensitivity (95% CI)	Specificity (95% CI)	N(RI-NRI)*
<b>(A) Published performances</b>					
<b>BED</b> [15]	1.0	160	82.7%	97.8%	
	0.8	153			
<b>Vironostika</b> [16, 17]	1.0	170	97%	>98%	
<b>IDE-V3</b> [19]	0.5	180	88.3%	97.6%	
<b>Avidity</b> [25]	0.6	180	26.4%	98.9%	
	0.7		61.8%	95.1%	
	0.8		79.4%	92.3%	
	0.9		88.2%	86.8%	
<b>(B) Calculated performances on the samples from Abidjan</b>					
<b>BED</b>	1	160	85.7% (79-92)	77.1% (70-84)	123(14-109)
<b>Vironostika</b>	1	170	100% (86-100)	79.8% (73-87)	133(24-109)
<b>IDE-V3</b>	0.5	180	42.3% (34-51)	96.3% (93-99)	135 (26-109)
<b>Avidity</b>	0.85	180	100% (87-100)	49.5% (41-58)	135 (26-109)

\* N= sample size, RI=number of recent infections, NRI=number of non recent infections  
 CI: confidence interval

**Table 2:** Distribution of false positive diagnosis of recent HIV infection according to time since infection groups, and associated specificity

<b>Time since infection</b>	<b>BED</b>		<b>Vironostika</b>		<b>IDE-V3</b>		<b>Avidity</b>	
	<b>False positive</b>	<b>Specificity</b>	<b>False positive</b>	<b>Specificity</b>	<b>False positive</b>	<b>specificity</b>	<b>False positive</b>	<b>specificity</b>
180 days to 1 year, n=27	14	48.2%	15	44.4%	4	85.2%	18	33.3%
More than 1 year, n=70	8	88.6%	5	92.9%	0	100%	29	58.6%
Total NRI, n=109	25	77.1%	22	79.8%	4	96.4%	55	49.5%

NRI: non-recent infections.

False positive : number of non-recent infections classified as recent.

Specificity: proportion of non-recent infections properly diagnosed.

**Table 3** : HIV incidence estimated with each test for recent infection (TRI), according to prevalence and incidence in the simulated populations.

Population		BED	Vironostika			IDE-V3		Avidity		
Prevalence	Incidence	Est. incidence (CI)	Bias*	Est. incidence (CI)	Bias*	Est. incidence (CI)	Bias*	Est. incidence (CI)	Bias*	
30%	10%	25.3% (17-30)	153%	23.8% (16-28)	138%	<b>7.0% (3-10)</b>	-30%	39.4% (31-44)	294%	
30%	5%	22.7% (15-27)	354%	20.4% (13-25)	307%	<b>5.1% (2-7)</b>	1%	37.7% (29-42)	654%	
30%	1%	20.6% (13-25)	1964%	17.7% (11-22)	1668%	3.5% (0.6-6)	254%	36.3% (28-41)	3532%	
10%	5%	8.6% (6-10)	73%	8.5% (6-10)	71%	2.8% (1-4)	-45%	13.0% (10-15)	160%	
10%	1%	6.2% (4-8)	524%	5.5% (4-7)	447%	<b>1.2% (0.3-2)</b>	21%	11.2% (9-13)	1021%	
10%	0,50%	5.9% (4-7)	1088%	5.1% (3-6)	917%	<b>1.0% (0.2-2)</b>	104%	11.0% (8-13)	10710%	
5%	1%	3.3% (2-4)	232%	3.0% (2-4)	204%	<b>0.8% (0.3-1.1)</b>	-22%	5.7% (4-7)	472%	
5%	0,50%	3.0% (2-4)	504%	2.6% (2-3)	429%	<b>0.6% (0.2-0.9)</b>	17%	5.5% (4-6)	997%	
5%	0,10%	2.8% (2-4)	2673%	2.3% (1-3)	2235%	0.4% (0.1-0.7)	333%	5.3% (4-6)	5195%	

\* Relative bias :  $(I_{est}-I)/I$

Est. incidence (CI): estimated incidence calculated as described in the method section, and confidence intervals.



**Figures titles:**

**Figure 1:** Distribution of the quantitative results obtained with each test according to the HIV infection category (RI: recent infection, NRI: non recent infection, AIDS: AIDS stage). The horizontal lines represent the recommended cut-off associated with the method. ANRS 1220 Cohort, Abidjan, Côte d'Ivoire.

**Figure 2 :** ROC curves associated with each test of recent HIV-1 infection. The arrows indicate the recommended cut-off values. ANRS 1220 Cohort, Abidjan, Côte d'Ivoire.

Figure 1 :

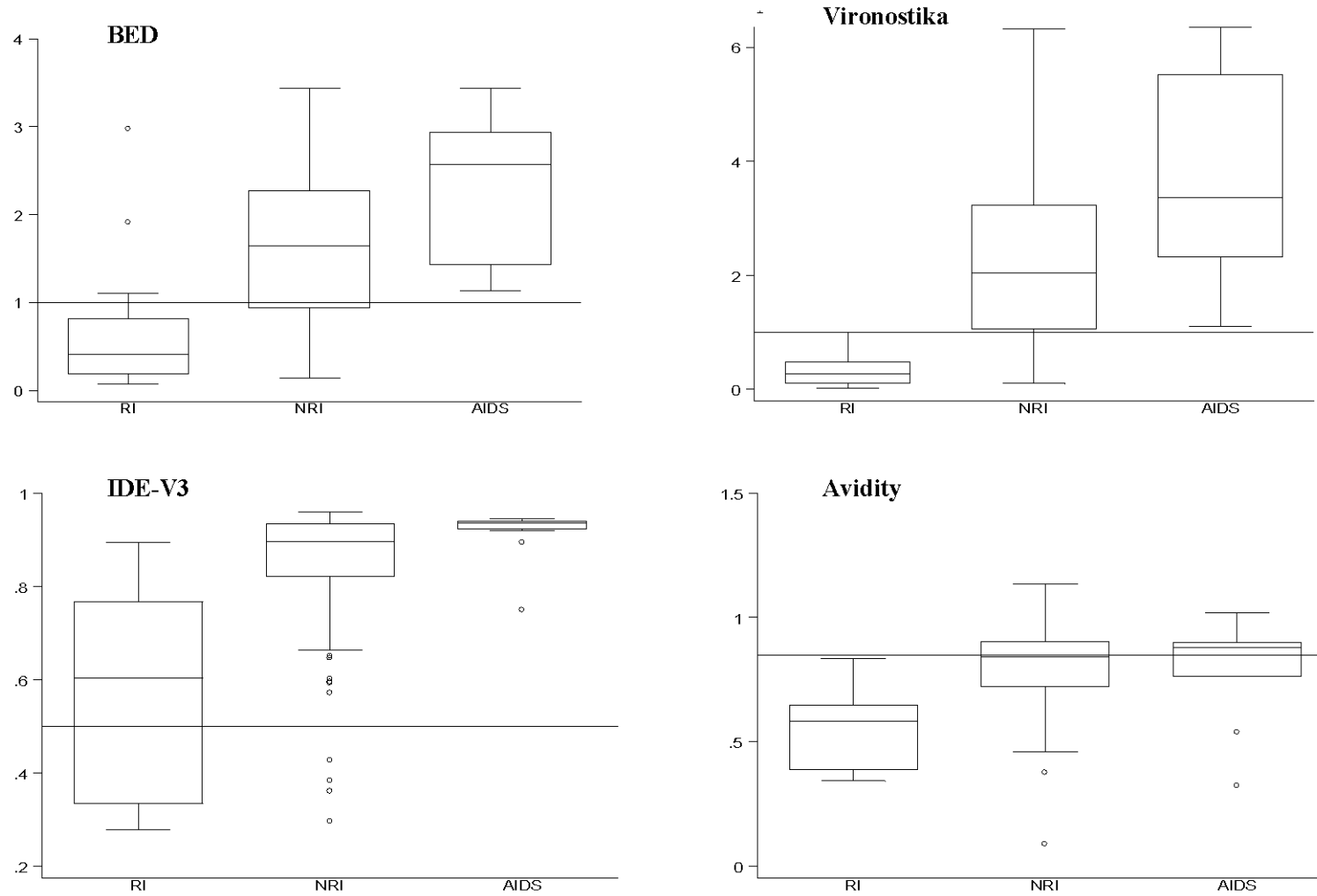


Figure 2 :

