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Temporal trend and characteristics of recent HIV-1 infections: application of an algorithm for the identification of recently acquired HIV-1 infections among newly diagnosed individuals over a 10-year period

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SUMMARY

Identification of recent infections (RI) may contribute to improve the quality of human immunodeficiency virus (HIV) surveillance, monitoring ongoing transmission and planning and evaluating prevention programs. Our study applied an algorithm combining clinical and serological information to identify RI in individuals newly diagnosed with HIV in Rome, during the years 1999-2008, in order to describe the trend and characteristics of recently infected individuals. RI were documented seroconverters, or people with an HIV avidity index (AI)<0.80. Individuals with advanced infection (CD4 count <200 cells/μL or AIDS-defining illness) or with AI ≥0.80 were considered long-standing infections. Overall, we observed 2,563 new HIV diagnoses. The algorithm was applied in 2124/2563 (82.9%). Of these, 355 were RI (16.7%). RI was found independently associated with calendar year (adjusted odds ratio [aOR]= 1.06, 95% confidence intervals [CI]=[CI 1.02-1.11], for every year of increase), HIV-risk category (men having sex with men: aOR=1.44, [CI 1.04-1.98]; injecting drug users: aOR=1.58, [CI 1.03-2.42] vs. heterosexuals), country of origin (foreign-born: vs Italians: aOR=0.46, [CI 0.33-0.62]), and recruitment site (inpatient vs outpatient clinic: aOR=0.49, [CI 0.37-0.66]). By the application of our algorithm we could characterize the pattern of ongoing HIV transmission, identifying groups needing more urgent prevention programs.

KEY WORDS: Newly diagnosed HIV infection, Serological testing algorithm for recent HIV seroconversion, Antibody avidity, Recent infection, Long-standing infection.

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INTRODUCTION

Identifying recent infections (RI) of HIV allows the ongoing transmission of HIV infection to be monitored to disclose emerging trends in the epidemic and to target and evaluate prevention policies. For this purpose, some high-income countries have implemented RI surveillance at regional or national level, applying new testing algorithms in which a serological test for recent infections (TRI) is performed for individuals newly diagnosed with HIV (Hall *et al.,* 2008; Semaille *et al.,* 2008; Bätzing-Feigenbaum *et al.,* 2009; Romero *et al.*, 2009; Guy *et al.*, 2010).

TRIs are based on the evolution of specific properties of HIV-1 antibodies during the early phase of infection, and offer the possibility to discriminate recently acquired infections (on an average within the past six months) from long-standing infections, using a single serum sample (Janssen *et al.*, 1998; Suligoi *et al.*, 2002; Murphy, 2008, Re

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et al., 2012). To increase TRI accuracy and reduce misclassifications, a number of strategies have been proposed such as the use of two sequential TRI to improve the limitations associated with measuring response to a single assay (Braunstein, 2011), the associated use of clinical information available at diagnosis, such as the CD4 cell count (Busch *et al.*, 2010), or the combined use of 2 serological assays with CD4 cell count and HIV load in a multiassay algorithm (Laeyendecker *et al.*, 2013).

In recent years, cross-sectional surveys in selected groups or in large populations have been conducted using different algorithms to define RI (Schupbach *et al.*, 2007; Schwarcz *et al*, 2007; Cortes Martins, 2008; Hall *et al.*, 2008; Semaille *et al.*, 2008; Bätzing-Feigenbaum *et al.*, 2009; Romero *et al.*, 2009; Guy *et al.*, 2010) However, the majority of the studies had data from a short period of observation, and thus only limited information is available on the trend over time of the proportion and characteristics of individuals identified with RI among those diagnosed with HIV.

In order to monitor and describe the characteristics of RI among persons newly diagnosed with HIV in Rome, Italy, over a 10-year period (1999-2008), we applied an algorithm including a TRI based on the avidity of HIV antibodies, in addition to conventional laboratory methods, and clinical information at diagnosis.

MATERIALS AND METHODS

Study population

We reviewed data on adults (18 years or older) with newly diagnosed HIV infection observed from January 1999 to December 2008 at the "Lazzaro Spallanzani" National Institute for Infectious Diseases, Rome. The Institute is the regional reference centre for diagnosis and care of HIV/AIDS disease and includes a Voluntary Counseling and Testing Site (VCTS), an outpatient department and hospital wards. It identifies approximately 50% of AIDS cases and more than 30% of new HIV infections yearly occurring in Lazio (Pezzotti *et al.*, 2011), a region located in central Italy, including the metropolitan area of Rome (almost six million inhabitants). During the years considered in the study, the Region constantly accounted for 13-14% of the Italian cases reported to the National AIDS Surveillance System (Suligoi *et al.*, 2009).

At diagnosis of HIV infection, from each newly diagnosed individual, epidemiological (age, sex, country of origin, risk exposure group) and clinical data (CD4 cell count, HIV viral load, AIDS diagnosis) are routinely collected and anonymously reported to a database.

This study was exempt from ethical approval since it was based on a review of routinely collected data and analyses from anonymous subjects in the database.

Recent Infection Testing Algorithm

For the identification of recent HIV-1 infection we used an algorithm including the following sequential criteria. At first, according to the conventional laboratory definition of HIV-1 seroconversion, we identified RI from diagnoses of HIV infection - made using a commercial third generation HIV-1 immunoassay (Genscreen HIV1/2, Biorad) and an HIV-1 Western blot (New Lav Blot-1, Biorad) - by the presence of p24 antigen, or HIV-1 RNA in the plasma, with simultaneous negative or indeterminate HIV-1 antibody test (HIV-1/2 ELISA and Western blot). All specimens yielding negative or indeterminate Western blots (<2 envelope bands, core bands or both) were tested again after approximately 1 month to document seroconversion.

Secondly, we considered RI all seroconverter individuals documented to have been seronegative during the 6 months before diagnosis. Individuals with an AIDS-defining illness or with a CD4 cell count lower than 200 cells/μL within 30 days of diagnosis were considered long-standing infections.

For the remaining individuals, we calculated the avidity index (AI) of antibodies by an automated third generation anti-HIV enzyme immunoassay (EIA), the AxSYM HIV 1/2gO (Abbot Diagnostics Division, Delkenheim, Germany) according to a procedure already described in detail elsewhere (Selleri *et al.*, 2007). To define an RI we used an AI lower than 0.80, because this has been identified as the threshold with the highest accuracy (area under the receiver operating curve: 0.958) corresponding to a sensitivity of 93.0% and a specificity of 98.5% (Galli *et al.*, 2008); this threshold was found to be associated with a mean win-

dow period of 202 days (standard error: 18.4 days) (Sweeting *et al.*, 2010).
Avidity test was performed on residual samples of serum obtained for diagnostic purposes, taken within 2 months after the initial diagnosis, and stored at -80°C.
Since early treatment was found to affect the evolution of HIV antibody avidity (Selleri *et al.*, 2007), we considered only AI from patients not on combination anti-retroviral therapy. Moreover, long-standing infections were not tested by the TRI, as the avidity of HIV antibodies was found to be influenced by the late stage of HIV infection (Braunstein, 2011). Consequently, individuals having a CD4 cell count higher than 200 cells/μL or missing, for whom there was no serum sample available within two months after HIV diagnosis and before start of treatment were not tested by TRI and defined as non-classified diagnoses.

Statistical analysis
Demographic and clinical characteristics were compared between persons with RI and those with a long-standing infection, and between RI identified using AI and those identified by conventional laboratory methods. The Chi-square test, Fisher's Exact test and Wilcoxon test for independent samples were performed.
Univariable and multivariable logistic regression models were used to assess the association between the likelihood of being a RI and a priori-selected covariates (the year of diagnosis as continuous variable, gender, country of origin, age at diagnosis, risk exposure and site of recruitment). Results were shown in terms of odds ratios (OR) with a relative 95% confidence interval (CI). Pair-wise interactions between the year of diagnosis and the other covariates were tested using a likelihood ratio test. All tests were two sided and p-values<0.05 were considered significant.
To evaluate the potential bias in the estimates due to missing information on the response variable, we used the multiple imputation method. Thirty imputed datasets were obtained by using the multiple imputation by chained equations approach as implemented in user contributed ice command in Stata (StataCorp. STATA Statistical Software: Release 10. College Station, TX: StataCorp LP; 2007), then mim command was used to analyze the datasets and combine the estimates (White *et al.*, 2011).

RESULTS

Study population
During the study period, 2,563 newly diagnosed HIV infections in adults were observed. No evidence for a trend in the number of reported cases was observed (average, 256 per year, range 222-304).
Men accounted for 75.6%, with a significant increase in proportion over time, from 70.8% in 1999-2000 to 80.7 % in 2007-2008 (p=0.002). Median age at diagnosis was 36.6 years (range 18-85) for men and 33.9 years (range 18-73) for women.
The majority of newly diagnosed individuals (86.6% for females and 28.4 % for males) reported heterosexual contacts, whereas 38.0% (29.2% in 1999-2000 up to 45.7% in 2007-2008) declared they were men having sex with men (MSM). Less than 10% were injecting drug users (IDU). Concerning the country of origin, out of 722 foreign-born individuals, 39.6% were from sub-Saharan African countries, 31.6% from Latin America, and 12.6% from Eastern Europe.
More than half of the new HIV diagnoses were from VCTS of the Institute (1597, 62.3%); 189 (7.4%) were made at out-patient clinics, and 777 (30.3%) new diagnoses were made during a hospital stay.
Overall, the median CD4 cell count at diagnosis was 299 cells/μL (from 242 cells/μL in 1999-2000 to 333 cells/μL in 2007-2008, p<0.001). More than one third of the individuals (931, 36.3%) had <200 CD4 cells/μL, and 25.5% (654) had >500 CD4 cells/μL. The median viral load at diagnosis did not change significantly during the study period.

Recent infections
The classification of HIV diagnoses according to the algorithm applied is shown in Figure 1. Overall, of the 2,124 cases for whom it was possible to apply the algorithm, 355 (16.7%) were RI: 173 were identified by the conventional definition and 182 by the TRI. Of the 1,769 long-standing infections, 829 were classified on the basis of the TRI results and 940 on the basis of clinical and/or immunological criteria (353 had an AIDS-defining illness and/or 587 a CD4 cell count lower than 200 cells/μL).
Four hundred and thirty-nine newly diagnosed individuals (17.1%) were non-classified as recent

or long-standing because they did not meet any of the algorithm criteria. A decreasing trend of non-classified diagnoses (from 20.7% to 11.6%, p<0.001) was found during the study period. Compared with classified subjects, non-classified individuals were younger and more frequently diagnosed at the VCTS.

Non-significant differences between non-classified and classified individuals were found regarding gender, risk exposure and country of origin (data not shown).

Table 1 shows the characteristics of RI, and the comparison between RI identified by conventional serologic methods and RI identified by AI. Overall, we found a higher proportion of RI among younger individuals, and in MSM and IDU. Moreover, the proportion of RI was higher in persons born in Italy than in foreigners. Among 61 foreign-born individuals identified as RI, 19 were from Sub-Sahara Africa and 22 from Latin America.

Regarding the recruitment site, the proportion of RI was higher in individuals receiving HIV diagnosis at the VCTS and at out-patient clinics than in hospitalized patients.

Finally, we found that RI identified by conventional serologic methods, when compared with RI identified by AI, were more frequently MSM or IDU, diagnosed during hospitalizations, and with higher HIV-1 plasma RNA loads.

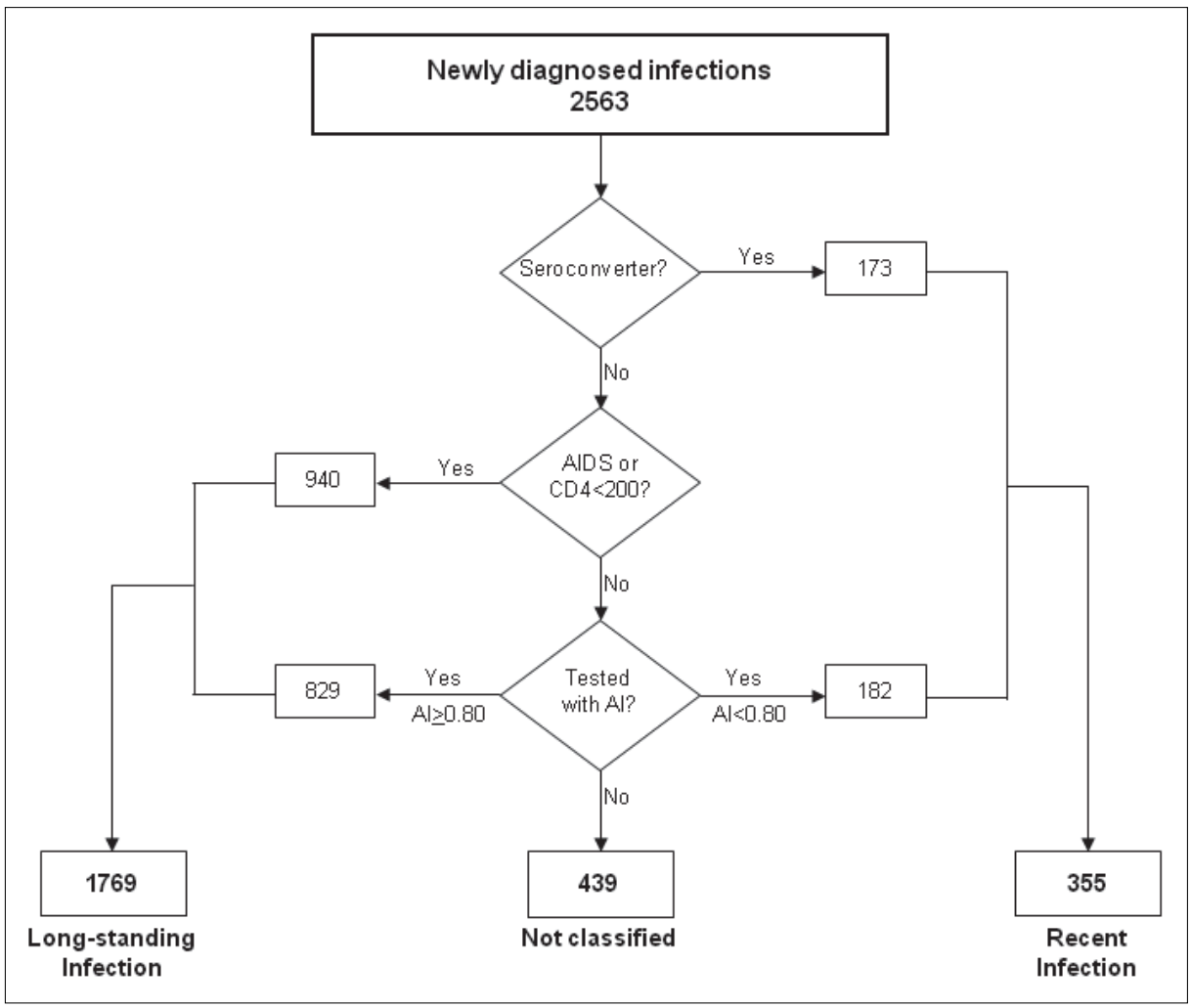

FIGURE 1 - *Algorithm for the identification of recent infection in newly diagnosed HIV infections, Rome, 1999-2008.*

The univariable analysis of classified diagnoses indicated a slight but significant increasing trend over time in the proportion of RI (OR: 1.08, 95% CI: 1.03-1.12, p<0.001) (Table 2).
In particular, among MSM the proportion of RI increased from less than 20% in the years 1999-2002 to more than 25% in years following 2002 (OR=1.09, 95% CI: 1.02-1.16, data not shown).
The association of being diagnosed as RI with the year of diagnosis was confirmed in multiple logistic regression (Adjusted OR 1.06, 95% CI 1.02-1.11; p=0.004) (Table 2). RI was also independently associated with being younger (p<0.001), being MSM or IDU (respectively, Adjusted OR 1.44, 95% CI 1.04-1.98; p=0.027 and 1.58, 95% CI 1.03-2.42; p=0.035), being of Italian origin (p<0.001), and having received the diagnosis at the VCTS or in an out-patient clinic (p<0.001).
Estimates obtained using multiply imputed data were similar to those obtained in the complete-case analysis (data not shown).

TABLE 1 - *Characteristics of classified HIV-1 diagnoses and Recent Infections, identified by serological conventional methods and Avidity Index (AI); Rome, 1999-2008.*

Characteristics	*HIV diagnoses*[a]	*Recent Infections*						
		Total		*Conventional*[b]		*AI*[c]		*p-value*[d]
		N	*%*	*N*	*%*	*N*	*%*	
Total	2124	355	16.7	173	48.7	182	51.3	
Gender								0.304
Male	1611	279	17.3	140	50.2	139	49.8	
Female	513	76	14.8	33	43.4	43	56.6	
Country of origin								0.779
Italy	1537	294	19.1	142	48.3	152	51.7	
Other	587	61	10.4	31	50.8	30	49.2	
Age at diagnosis (years)								0.266
18-29	462	96	20.8	41	42.7	55	57.3	
30-39	890	169	19.0	91	53.8	78	46.2	
40-49	508	65	12.8	31	47.7	34	52.3	
50-85	264	25	9.5	10	40.0	15	60.0	
Risk exposure								0.031
Heterosexual contacts	925	122	13.2	48	39.3	74	60.7	
MSM	785	167	21.3	91	54.5	76	45.5	
IDU	206	41	19.9	24	58.5	17	41.5	
Other/Unknown	208	25	12.0	10	40.0	15	60.0	
Site of recruitment								0.015
VCTS & Outpatients	1393	286	20.5	130	45.5	156	54.5	
Inpatients	731	69	9.4	43	62.3	26	37.7	
Year of diagnosis[e]	2004 (2001-2006)	2005 (2002-2007)		2004 (2001-2007)		2005 (2002-2007)		0.683
CD4 cell count[e]	244 (68-80)	550 (388-788)		520 (338-786)		561 (422-795)		0.078
Log10 HIV-RNA[e]	4.8 (4.2-5.4)	4.70 (4.0-5.4)		4.81 (4.2-5.7)		4.52 (3.8-5.2)		<0.001

[a]Subjects for whom it was possible the application of the algorithm for the identification of Recent Infection. [b]Include RI identified by the presence of p24 antigen, or HIV-1 RNA in the plasma, with simultaneous negative or indeterminate HIV-1 antibody test, and seroconversions (a previous negative test within 6 months before diagnosis). [c]Avidity index (AI)<0.80. [d]Comparison between RI identified by conventional method and those identified by avidity index. [e]Median, and inter-quartile range in brackets. Abbreviations: MSM: Men having Sex with Men; IDU: Injecting Drug User; VCTS: Voluntary Counselling and Testing Site. CD4 available for 2019 individuals. HIV-RNA available for 1866 individuals.

TABLE 2 - *Factors independently associated with recent HIV-infections, Rome, 1999-2008. Results of logistic regression models.*

Characteristics	*Logistic Regression Models*			
	OR (95% CI)	*p-value*	*aOR (95% CI)*	*p-value*
Year of diagnosis	1.08* (1.03-1.12)	<0.001	1.06* (1.02-1.11)	0.004
Gender		0.186		0.472
Male	1.00		1.00	
Female	0.83 (0.63-1.09)		1.14 (0.80-1.61)	
Country of origin		<0.001		<0.001
Italy	1.00		1.00	
Other	0.49-(0.37-0.66)		0.46 (0.33-0.62)	
Age at diagnosis (years)		<0.001		<0.001
18-29	1.00		1.00	
30-39	0.89 (0.68-1.18)	0.432	0.85 (0.63-1.14)	0.278
40-49	0.56 (0.40-0.79)	0.001	0.51 (0.36-0.74)	<0.001
50-85	0.40 (0.25-0.64)	<0.001	0.40 (0.24-0.66)	<0.001
Risk exposure		<0.001		0.046
Heterosexual contacts	1.00		1.00	
MSM	1.78 (1.38-2.30)	<0.001	1.44 (1.04-1.98)	0.027
IDU	1.64 -(1.11-2.42)	0.014	1.58 (1.03-2.42)	0.035
Other/Unknown	0.90 (0.57-1.42)	0.650	0.97 (0.59-1.60)	0.920
Site of recruitment		<0.001		<0.001
VCTS & Outpatients	1.00		1.00	
Inpatients	0.40 (0.30-0.53)		0.49 (0.37-0.66)	

OR: Odds Ratio; aOR: Adjusted Odds Ratios; CI: Confidence Interval. Abbreviations: MSM: Men having Sex with Men; IDU: Injecting Drug User; VCTS: Voluntary Counselling and Testing Site. *For every year of increase.

DISCUSSION

Including the avidity assay as TRI in an algorithm for the identification of RI, we found that 16.7% of new HIV diagnoses had been acquired, on average, within the 6 months prior to diagnosis. Interestingly, adding the AI assay doubled the number of RI, compared to using only the conventional definition of seroconversion, which is, actually, a relatively rare event observed in a cross-section population (Welte *et al.*, 2010). Conventional methods were more likely to identify RI in MSM, IDUs, hospitalized individuals and individuals with higher HIV-1 viral load; including the AI increased RI diagnosis among heterosexuals, individuals attending VCTS and those with a lower viral load.

The proportion of RI we observed is lower than that reported by other studies: differences in study settings and populations, and in the algorithms used for the definition of RI may, at least in part, explain these differences.

In our population, individuals in an advanced stage of HIV disease could be over-represented due to the characteristics of our Institution, which is the largest regional center for HIV clinical care. Consistent with the data of a Spanish study that found differences in the proportion of RI according to the type of recruitment centre of HIV diagnosis (Romero *et al.*, 2009), we observed the highest proportion of RI (20.9%) among diagnoses from VCTS, and the lowest in individuals diagnosed with HIV (mainly with severe PHI) during hospitalization (Kelley *et al.*, 2007). Furthermore, different proportions of RI according to the different risk exposure groups were found in France (Semaille *et al.*, 2008). Finally, the very rigorous definition of RI used in the algorithm could have reduced the number of RI to be counted. Moreover, most of the previous stud-

ies applying TRI did not use avidity assay, but tests - like the BED assay - based on antibody concentration, susceptible to a higher rate of misclassification and of overestimating RI (Schupbach *et al.*, 2007; Schwarcz *et al.*, 2007).
We observed an increasing trend in RI over time, particularly evident among MSM. Although MSM have a higher risk perception and attitude to testing (Delpierre *et al.*, 2007; Schwarcz *et al.*, 2007), this could actually mirror an increase in HIV transmission, as neither new testing policies nor specific prevention and/or information campaigns to increase HIV awareness were conducted during the study period to justify an increased test attitude. Consistently, rising rates of newly acquired HIV and sexually transmitted infections, particularly in MSM, have also been observed in Italy (Giuliani *et al.*, 2005; Fisher *et al.*, 2007; Cicconi *et al.*, 2008) in recent years. Similarly, the higher proportion of RI observed among IDUs, compared with HET, could be explained by ongoing transmission of HIV as well as other bloodborne infections (Camoni *et al.*, 2010) in this population, as well as by a wider test availability at Italian drug treatment centers. We found that foreign-born individuals were less likely to be diagnosed as RI than Italians. Although late diagnosis of HIV is frequent among foreigners, mostly originating from Sub-Saharan Africa (Burns *et al.*, 2001), we found that two-thirds of the foreigners with RI were from resource-poor countries, suggesting that HIV transmission also occurs in these populations while living in Italy.
Our study has some limitations. Firstly, this investigation was conducted in a single institution and this may limit the generalizability of our findings. However, the new HIV diagnoses made at our institution account for one third of the total HIV diagnoses reported each year to the Regional Surveillance System (RSS), and this proportion is constant over time. Moreover, the distribution of HIV-infected individuals included in the present study is similar, by gender, age, risk exposure and country of origin, to that of diagnoses reported to the RSS during the same time period (LazioSanità-ASP, 2011).
Secondly, the limitations of the assays in detecting recent HIV infection are well-known (Schupbach *et al.*, 2007; Busch *et al.*, 2010). To avoid possible effects of the disease stage on TRI performance, people with AIDS or with a low CD4 cell count (with no laboratory evidence of an acute HIV infection, i.e. seroconverters) were considered long-standing infections and were not tested by avidity. In addition, many TRI were found to be affected by virus subtypes (Schupbach *et al.*, 2007; Busch *et al.*, 2010); nevertheless, avidity was found accurate in discriminating RI from long-standing infections also among individuals infected with non-B subtypes (Chawla *et al.*, 2007; Suligoi *et al.*, 2008).
Thirdly, although the potential bias due to non-classified samples was evaluated by using multiple imputations, it has to keep in mind that this method is based on the untestable assumption that missingness depends on observed data: biased estimates cannot be excluded.
In conclusion, the application of an avidity assay like TRI seems to be a relatively simple tool for identifying recently acquired HIV infections. Although the procedure for AI is generally inexpensive, for the identification of RI it is necessary to perform an additional HIV test. Including in the algorithm clinical and laboratory data routinely collected for surveillance purposes has the advantage of lowering the number of tests to perform, thus reducing costs.
By using this approach, we have characterized the pattern of ongoing HIV transmission over the last 10 years, and have identified the need for urgent prevention programs for groups at-risk for HIV infection, where HIV transmission may be on the rise. This method could therefore provide an affordable means to improve HIV surveillance systems and add remarkable value to single, cross-sectional surveys, also permitting HIV incidence estimations (Mastro *et al.*, 2010, Mastro, 2013).

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