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Evaluation of an HIV recent infection testing algorithm with serological assays among men who have sex with men in Mexico

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ABSTRACT

Background: Human immunodeficiency virus (HIV) incidence should be calculated in cross-sectional studies using recent infection testing algorithms (RITA) that consider clinical variables and serological test results such as enzyme-linked immunosorbent assay (ELISA) and dried blood spot (DBS) analysis.
Methods: The correlation between serum samples and DBS was evaluated using two commercial ELISA kits: SediaTM BED HIV-1 Incidence EIA (BED-Sedia) and Maxim HIV-1 Limiting Antigen Avidity (LAg-Avidity). Eight different RITAs were developed; all of them included serological assays. A combination of the variables viral load, antiretroviral therapy (ART) and CD4 count was used to build the RITAs. The sensitivity, specificity, Youden index, predictive positive value, predictive negative value, false recent rate (FRR) and false long-term rate were evaluated.
Results: The correlations between serum samples and DBS were 0.990 and 0.867 for BED-Sedia and LAg-avidity, respectively. Using only serological assays, the Youden index was higher for LAg-avidity than BED-Sedia (82.1–83.0% *versus* 69.2–69.6%). The best RITA was ART-serology, which showed a Youden index of 91.2–93.9% and FRR of 1.8–2.2%.
Conclusions: Using DBS samples to determine HIV incidence is a good tool for epidemiological surveillance. The RITA that included ART and serological tests (BED-Sedia or LAg-avidity) showed the highest sensitivity and specificity and a low FRR.

Background

Knowing HIV incidence allows us determine the risk factors to be infected, evaluate the effectiveness to treatment and monitor changes in transmission patterns [1]. There are three approaches to determine the HIV incidence in a population: cohort studies, mathematical models and laboratory tests [2]. Cohort studies are the gold standard to calculate HIV incidence; however, their design is expensive, very susceptible to recruitment bias and, if the incidence is low, then a large sample size is required [1–3]. An estimation based on mathematical modelling requires the knowledge of HIV prevalence, information about mortality, migration and sexual behaviour among people living with HIV (PLWH). The third option detects recent infections and estimates the incidence of HIV through the detection of antigens, antibodies and/or nucleic acids [4,5].

Serological and molecular methods have been developed to detect recent infections with cross-sectional samples, serum or dried blood spots (DBS). DBS have a lower cost, do not require specialised equipment, no cold chain is needed for their storage, and these tests are better accepted by the population [6,7]. ELISA is based on the maturity, affinity and titres of antibodies generated during an HIV infection. In this sense, different tests have been developed, the proportion of HIV antibodies and the avidity of HIV antibodies. The limitations of ELISA are that people with long-term infections are often severely immunocompromised or are using antiretroviral therapy (ART), and thus could be misclassified as a recent infection [8–11]. To improve diagnostic accuracy, the recent infection testing algorithm (RITA) has been implemented. RITAs combine laboratory methods and clinical indicators such as a diagnosis of AIDS, the use of ART, HIV viral load and CD4 count [9].

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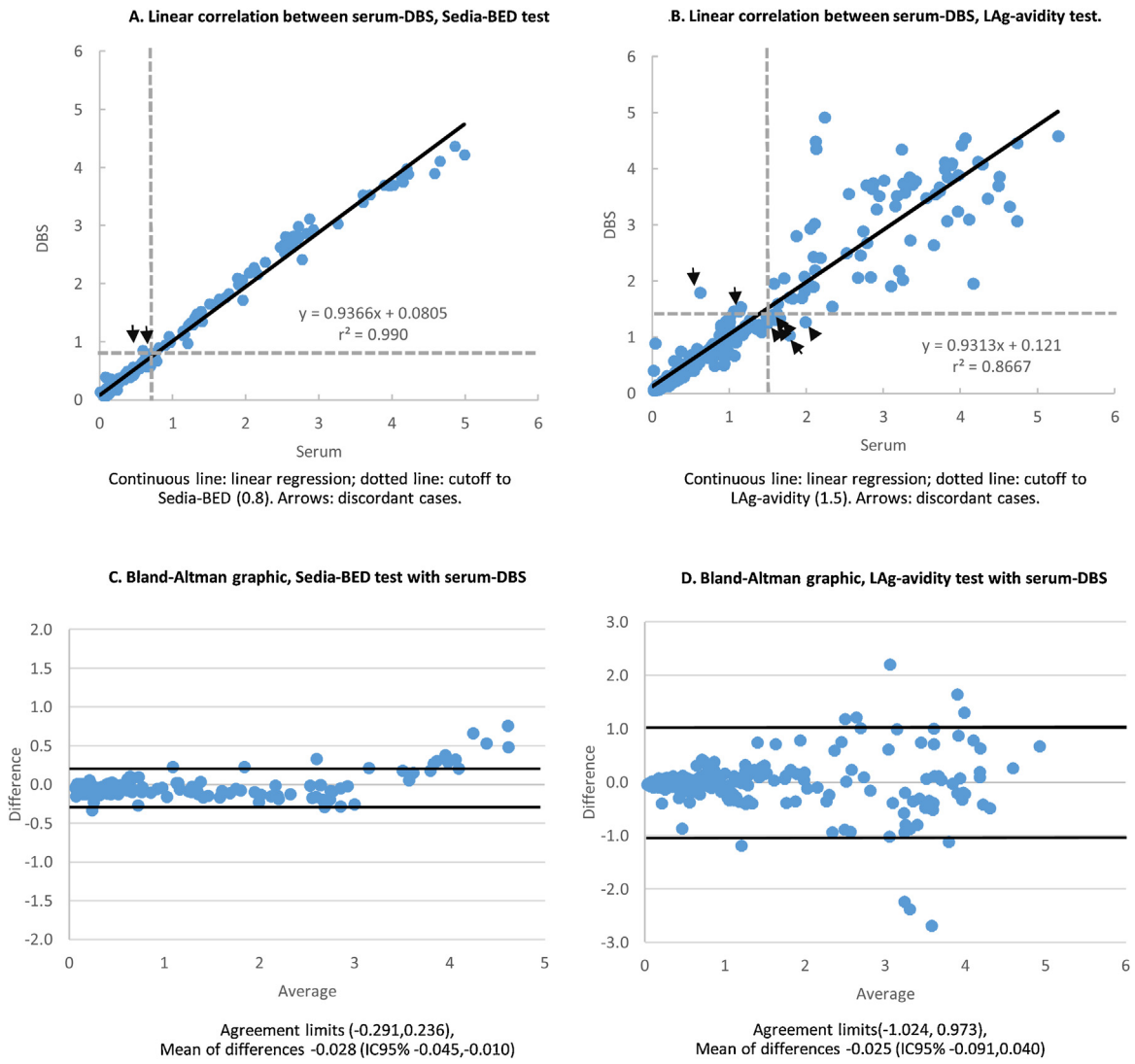

Fig. 1. Correlation between serum and DBS samples.
a and b. Lineal correlation between serum and DBS, Sedia-BED and Lag-avidity respectively. c and d, Bland–Altman graphic correlation between serum and DBS, Sedia-BED and Lag-avidity respectively.

The World Health Organisation (WHO) suggests that each country must validate the serological tests of recent infection in each population and validate the use of DBS to determine a correction factor to estimate the HIV incidence from cross-sectional studies [3]. Thus, the aim of this study was to develop a highly accurate RITA to detect recent HIV infections in Mexican men who have sex with men (MSM).

Methods

Description of samples

After patient authorisation, Clinica Especializada Condesa (CEC) carried out HIV detection in serum samples. Biological samples were stored at −20 °C until use. This study was approved by the Ethics, Biosafety and Research committees of the Instituto Nacional de Salud Pública in Mexico. In total, 224 blood samples were collected and divided into two groups. Group 1 contained 139 samples classified as recent infection (subjects who had a positive result, but with a negative result six months before), while group 2 included 85 samples classified as long-term infections (subjects who had two consecutive positive results within 12 months). HIV viral load, CD4 count and ART data were used.

Dried blood spots

DBS were prepared as follows: a blood sample was collected from an individual without an HIV infection. Erythrocytes and serum were separated by centrifugation. Erythrocytes were washed three times with phosphate buffered solution (PBS) (1:1 ratio). Then, the blood was reconstituted using 25 μl of erythrocytes and 25 μl of serum. Next, 10 μl of reconstituted blood was placed on Whatman 903 filter paper and allowed to dry for 1 h at room temperature. 5 mm circles were cut out and stored at −20 °C until use. DBS from infected patients were prepared in the same manner. Five DBS were prepared from every sample.

ELISA

Two ELISA tests were performed using commercial kits: 1) Sedia™ BED HIV-1 Incidence EIA (BED-Sedia) (Sedia Biosciences, Portland OR) and 2) Maxim HIV-1 Limiting Antigen Avidity (LAg-Avidity) (Maxim Biomedical, Inc., Rockville, MD). Both ELISA methods distinguish recent infection from long-term infection. Bed-Sedia measures the ratio of HIV-1 specific immunoglobulin class G (IgG) from total IgG ($Ig_{HIV\text{-}1}/Ig_{total}$). In a recent infection,

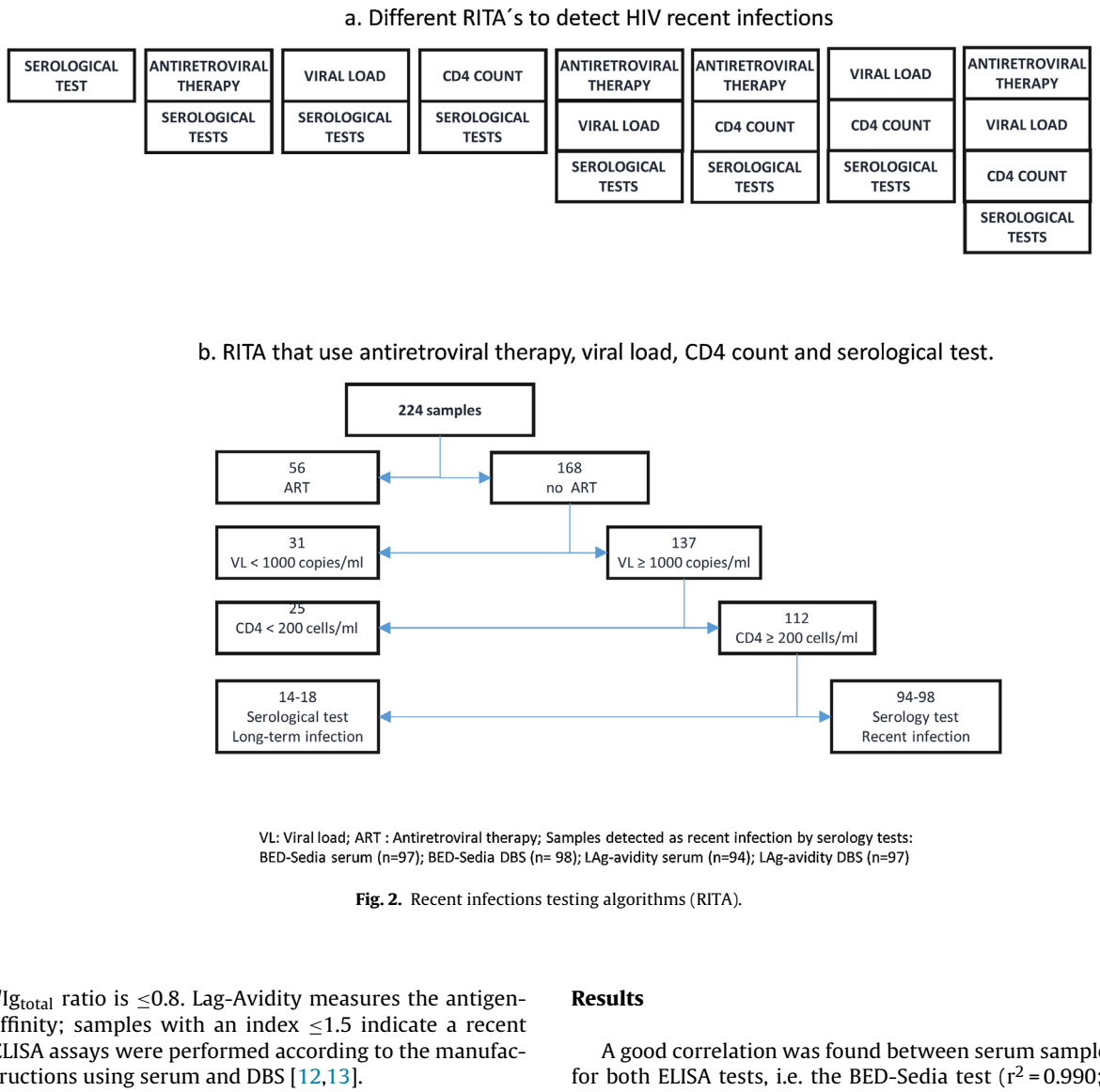

Fig. 2. Recent infections testing algorithms (RITA).

the $Ig_{HIV\text{-}1}/Ig_{total}$ ratio is ≤0.8. Lag-Avidity measures the antigen-antibody affinity; samples with an index ≤1.5 indicate a recent infection. ELISA assays were performed according to the manufacturer's instructions using serum and DBS [12,13].

Statistical analysis

For the quantitative results, a Spearman correlation analysis between DBS and serum was performed, while for qualitative results, the kappa of concordance was calculated. For both statistical tests, the p value was calculated. A Bland-Altman graph was prepared [14], and the concordance limits and mean of the difference were calculated by comparing serum and DBS with both ELISA tests.

Recent infection testing algorithm

Different RITAs were performed to detect recent infections. The simplest algorithm considered only the ELISA results, while the most complex also considered ART, viral load (1000 copies/ml) and CD4 count (200 cells/ml). Sensitivity (Sens), specificity (Spe), positive predictive value (PPV), negative predictive value (NPV), false recent rate (FRR), false long-term rate (FLR) and the Youden index (J) [15] were calculated with 95% confidence intervals (95% CI), considering cohort results as the gold standard. The Youden index = sensitivity + specificity − 100. The statistical analysis was performed with SPSS 15.0 software.

Results

A good correlation was found between serum samples and DBS for both ELISA tests, i.e. the BED-Sedia test ($r^2 = 0.990$; $p < 0.001$) and the LAg-avidity test ($r^2 = 0.867$; $p < 0.001$). However, greater variability was detected in the LAg-avidity test, as shown in Fig. 1a and b. Fig. 1c shows the Bland–Altman graph for the BED-Sedia test. The mean difference between serum and DBS was −0.028 with a random distribution. The measurements with an index >4 presented the biggest differences, with a higher index in the DBS samples than in the serum samples. Fig. 1d shows greater variability in the Bland–Altman graph for the LAg-avidity test, with concordance limits of −1,024 to 0.973. For the qualitative results, the kappa agreement test was 0.978 ($p < 0.001$) between serum and DBS for the BED-Sedia test. Two discordant results were found in serum samples; they were detected as recent infections, but by DBS they were detected as long-term infections. For the LAg-avidity test, the kappa value was 0.920 ($p < 0.001$). Eight samples were discordant; in serum, two were detected as recent infections, while in the DBS these were detected as long-term infections, while a further six DBS were detected as recent infections while in serum they were detected as long-term infections. Discordant samples in both methods are indicated with arrows in Fig. 1a and b.

Eight RITAs were tested to detect recent infections. Fig. 2a shows the sequence and combinations of the analysed RITAs. Fig. 2b shows the RITA sequence, which encompassed subjects who were using ART, with a viral load (VL) of less than 1000 copies/ml and a CD4 count of less than 200 cells/ml.

Table 1
Parameters evaluated for Sedia-BED test and LAg-avidity test with serum and DBS samples, compared with gold standard.

	RITA	Sens	Spe	PPV	NPV	FRR	FLR	J
BED-Sedia serum	Sero	98.6	70.6	84.6	96.8	11.2	**0.9**	69.2
	VL, Sero	88.5	94.1	96.1	83.3	2.2	7.1	82.6
	CD4, Sero	82.7	72.9	83.3	72.1	10.3	10.7	55.6
	ART, Sero	98.6	94.1	96.5	97.6	2.2	**0.9**	**92.7**
	ART, CD4, Sero	82.7	94.1	95.8	76.9	2.2	10.7	76.8
	ART, VL, Sero	88.5	97.6	98.4	83.8	**0.9**	7.1	86.1
	VL, CD4, Sero	72.7	95.3	96.2	68.1	1.8	17.0	68.0
	ART, VL, CD4, Sero	72.7	97.6	98.1	68.6	**0.9**	17.0	70.3
BED-Sedia DBS	Sero	97.8	71.8	85.0	95.3	10.7	**1.3**	69.6
	VL, Sero	87.8	94.1	96.1	82.5	2.2	7.6	81.9
	CD4, Sero	82.0	74.1	83.8	71.6	9.8	11.2	56.1
	ART, Sero	97.8	94.1	96.5	96.4	2.2	**1.3**	**91.2**
	ART, CD4, Sero	82.0	94.1	95.8	76.2	2.2	11.2	76.1
	ART, VL, Sero	87.8	97.6	98.4	83.0	**0.9**	7.6	85.4
	VL, CD4, Sero	71.9	95.3	96.2	67.5	1.8	17.4	67.2
	ART, VL, CD4, Sero	71.9	97.6	98.0	68.0	**0.9**	17.4	69.5
LAg-avidity serum	Sero	97.1	85.9	97.1	94.8	5.4	**1.8**	83.0
	VL, Sero	87.1	98.8	99.2	82.4	0.4	8.0	85.9
	CD4, Sero	80.6	87.1	91.1	73.3	4.9	12.1	67.7
	ART, Sero	97.1	95.3	97.1	95.3	1.8	**1.8**	**92.4**
	ART, CD4, Sero	80.6	95.3	96.6	75.0	1.8	12.1	75.9
	ART, VL, Sero	87.1	100	100	82.5	**0.0**	8.0	87.1
	VL, CD4, Sero	70.5	100	100	67.5	**0.0**	18.3	70.5
	ART, VL, CD4, Sero	70.5	100	100	67.5	**0.0**	18.3	70.5
LAg-avidity DBS	Sero	98.6	83.5	90.7	97.3	6.3	**0.9**	82.1
	VL, Sero	89.2	98.8	99.2	84.8	0.4	6.7	88.0
	CD4, Sero	82.0	85.9	90.5	74.5	5.4	11.2	67.9
	ART, Sero	98.6	95.3	97.2	97.6	1.8	**0.9**	**93.9**
	ART, CD4, Sero	82.0	95.3	96.6	76.4	1.8	11.2	77.3
	ART, VL, Sero	89.2	100	100	85.0	**0.0**	6.7	89.2
	VL, CD4, Sero	72.7	100	100	69.1	**0.0**	17.0	72.7
	ART, VL, CD4, Sero	72.7	100	100	69.1	**0.0**	17.0	72.7

Sero: serology test; VL: viral Load; CD4: Lymphocytes T type CD4; ART: antiretroviral therapy; Sens: sensitivity; Spe: specificity; PPV: positive predictive value. NPV: negative predictive value. FRR: false recent rate; FLR: false long-term rate; J: Youden index. Bold letter: greater Youden index values or lower FRR or FLR values. A total of 224 samples were used in every RITA's.

Table 1 shows four inputs: 1) BED-Sedia/serum, 2) BED-Sedia/DBS, 3) LAg-Avidity/serum and 4) LAg-Avidity/DBS. The RITAs that had the highest J values were ART-Serology (91.2–93.9%) and ART-VL-Serology (85.4–89.2%). The RITAs that had the lowest J values were CD4-Serology (55.6–67.9%) and CD4-VL-Serology (67.2–72.7%). Considering only the serological tests to detect recent infections, LAg-avidity had a higher specificity (83.5–85.9% *versus* 70.6–71.8%), higher J value (82.1–83.0% *versus* 69.2–69.6%) and a lower FRR (5.4–6.3% *versus* 10.7–11.2%) than the Sedia-BED test. However, the ART-Serology RITA had the highest J value, while the other parameters for both ELISA assays and samples were similar. ART-Serology showed J = 92.4–93.9% and 91.2–92.7%, FRR = 1.8% and 2.2%, FLR = 0.9–1.8% and 0.9–1.3% for the LAg-Avidity and BED-Sedia tests, respectively.

Of all the samples analysed, 25% (n = 56) were from patients using antiretroviral therapy and all (n = 56) were detected as long-term infections; 13.8% (n = 31) had 200 or fewer CD4 cells/ml, 74.2% (23/31) of which were recent infections. Finally, 32.1% (n = 72) had a viral load of 1000 or fewer copies/ml and of these 25% (18/72) were recent infections.

Discussion

Serological tests to detect HIV recent infections consist of quantifying antibodies, but quantification may be affected by sample quality, HIV subtype, low CD4 count, low viral load or ART use [3,8]. Two tests to identify recent infections were evaluated using serum and DBS; however, their previous validation is recommended for each region and population. In the current study, good correlations and agreement were found between serum and DBS in both ELISA tests to estimate recent HIV infections in Mexican MSM. Comparing only the serological tests with respect to the gold standard, the LAg-Avidity test was better than the BED-Sedia test. LAg-Avidity does not depend on the antibody titre, so the use of ART could influence it to a lesser extent and therefore lead to a lower frequency of false positives [16,17]. The suppression of viral replication probably diminishes the chronic stimulus of the immune system, resulting in a lower antibody titre, which could cause the misclassification of a long-term infection as a recent infection.

Due to the indirect effect of ART on serological tests to classify recent or long-term infections, the RITAs are necessary. In the current study, the RITAs that considered the use of ART showed better performance. Both serological tests showed similar performance if they considered the use of ART, without differences between serum or DBS. There are recommendations for the use of the CD4 counts in RITAs, because failure of the immune system is associated with the progression of an HIV infection; this could result in decreased antibody levels and impact the methods used to detect recent infections [16]. However, in the present study, the CD4 count had a negative influence on detecting recent infections, so the RITAs that considered the CD4 count were not used. A high percentage of recent infections was found among people with low CD4 counts, possibly due to an acute HIV infection since the CD4 count decreases during the first weeks of an HIV infection [18].

The beginning of ART was the best clinical parameter to distinguish between recent infections and long-term infections. The current study was carried out with samples collected between 2015 and 2016. In September 2018, a public health program called "Preparación para la Puesta en Marcha de la Prevención Combinada del VIH entre Poblaciones Clave" began in Mexico. The aim of the

program is to evaluate pre-exposure prophylaxis [19]; thus, it will be necessary to evaluate the impact of pre-exposure prophylaxis on serological measurements of recent infection. A low viral load was a good indicator of a long-term infection; however, there were some cases of recent infections with a low viral load, as some individuals were probably 'elite' cases or people with HIV infection not undergoing treatment but with undetectable viral load levels [20].

The measurement of HIV incidence is an essential parameter for epidemiological surveillance systems and to evaluate public health programs. The quantification of HIV incidence is facilitated with tools such as DBS (low cost samples, greater acceptance and no cold chain) and serological tests to detect recent infections (which require only one measurement over time) [5]. The current work validated the use of a RITA that considers ART use and serological tests that use serum samples or DBS among MSM in Mexico, obtaining the FRR parameter suggested by the WHO.

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Competing interests

None declared.

Ethical approval

Research Ethics Committee, National Institute of Public Health (Comite de Ética en Investigación, Instituto Nacional de Salud Pública).

References

[1] Kassanjee R, McWalter TA, Bärnighausen T, Welte A. A new general biomarker-based incidence estimator. Epidemiollogy 2012;23:721–8.
[2] Brookmeyer R, Laeyendecker O, Donnell D, Eshleman SH. Cross-sectional HIV incidence estimation in HIV prevention research. J Acquir Immune Defic Syndr 2013;63:S233–9.
[3] UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance. When and how to use assays for recent infection to estimate HIV incidence at a population level. Geneva: World Health Organization; 2011.
[4] Murphy G, Parry JV. Assays for the detection of recent infections with human immunodeficiency virus type 1. Euro Surveill 2008;13:314–20.
[5] Moyo S, Wilkinson E, Novitsky V, Vandormael A, Gaseitsiwe S, Essex M, et al. Identifying recent HIV infections: from serological assays to genomics. Viruses 2015;7:5508–24.
[6] Smit PW, Elliott I, Peeling RW, Mabey D, Newton PN. An overview of the clinical use of filter paper in the diagnosis of tropical diseases. Am J Trop Med Hyg 2014;90:195–210.
[7] Curtis KA, Ambrose KM, Kennedy MS, Owen SM. Evaluation of dried blood spots with a multiplex assay for measuring recent HIV-1 infection. PLoS One 2014;9(9):e107153.
[8] Mastro TD, Kim AA, Hallett T, Rehle T, Welte A, Laeyendecker O, et al. Estimating HIV incidence in populations using tests for recent infection: issues, challenges and the way forward. J HIV AIDS Surveill Epidemiol 2010;2(1):1.
[9] Hallett TB. Estimating the HIV incidence rate: recent and future developments. Curr Opin HIV AIDS 2011;6:102–7.
[10] Chaillon A, Le Vu S, Brunet S, Gras G, Bastides F, Bernard L, et al. Decreased specificity of an assay for recent infection in HIV-1-Infected patients on highly active antiretroviral treatment: implications for incidence estimates. Clin Vaccine Immunol 2012;19:1248–53.
[11] Kassanjee R, Pilcher CD, Keating SM, Facente SN, McKinney E, Price MA, et al. Independent assessment of candidate HIV incidence assays on specimens in the CEPHIA repository. AIDS 2014;28:2439–49.
[12] http://www.sediabio.com/products/bed-eia.
[13] http://www.sediabio.com/products/lag-avidity-eia.
[14] Altman DG, Bland JM. Measurement in medicine: the analysis of method comparison studies. Statistician 1983;32:307–17.
[15] Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32–5.
[16] Duong YT, Qiu M, De AK, Jackson K, Dobbs T, Kim AA, et al. Detection of recent HIV-1 infection using a new limiting-antigen avidity assay: potential for HIV-1 incidence estimates and avidity maturation studies. PLoS One 2012;7.
[17] Salustiano DM, de Lima KO, Cavalcanti AMS, Diaz RS, Lacerda HR. Comparison among the BED capture enzyme immunoassay test and AxSYM avidity index assay for determining recent HIV infection and incidence in two voluntary counselling and testing centres in Northeast Brazil. Braz J Infect Dis 2014;18:449–53.
[18] Vergis EN, Mellors JW. Natural history of HIV-1 infection. Infect Dis Clin North Am 2000;14:809–25.
[19] http://www.condesadf.mx/rokstories/comienza-el-protocolo-prep.htm. Consultada 7 de noviembre 2018.
[20] Sabina CA, Lundgrenb JD. The natural history of HIV infection. Curr Opin HIV AIDS 2013;8:311–7.