Journal of Infection and Public Health 12 (2019) 224–228

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Performance of ELISA and Western blot to detect antibodies against HSV-2 using dried blood spots

Santa García-Cisneros[a], Miguel Á. Sánchez-Alemán[a], Carlos J. Conde-Glez[b], Sergio J. Lara-Zaragoza[c], Antonia Herrera-Ortiz[a], Tanya Plett-Torres[d], Maria Olamendi-Portugal[a,*]

[a] Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México
[b] Centro de Investigación en Salud Poblacional, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México
[c] Facultad de Biología, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México
[d] Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, México

ARTICLE INFO

Article history:
Received 24 April 2018
Received in revised form
20 September 2018
Accepted 15 October 2018

Keywords:
Herpes simplex virus type 2
Dried blood spots
Western blot
ELISA

ABSTRACT

Background and aims: Herpes simplex virus type 2 (HSV-2) is a sexually transmitted agent and is detected worldwide. HSV-2 is the main cause of genital ulcers and is diagnosed mainly with serological tests. The objective of current study was to evaluate the use of DBS samples to detect HSV-2 antibodies using commercial ELISA and Western blot tests.
Materials and methods: IgG-G2 ELISA (Human® Diagnostics, Germany) and Western blot IgG/IgM (EUROLINE-WB, Euroimmun® Germany) tests were modified to use DBS samples. Samples were processed by both methods to determine ELISA cutoff points using ROC curves. ELISA was performed with 100 μl and the Western blot with 200 μl of eluted DBS. A 1:5 dilution was used and the incubation times were increased for the Western blot.
Results: 908 DBS samples were processed and the following cutoff points were determined: negative (0–3.79), undetermined (3.8–4.6) and positive (≥4.61), with sensitivity and specificity close to 95%.
Conclusion: Modifications of the cutoff points of the ELISA test were obtained with technical adjustments done to detect HSV-2 antibodies by ELISA and Western blot using DBS samples.

Introduction

Herpes simplex virus type 2 (HSV-2) is a sexually transmitted infection, a risk factor for HIV acquisition and transmission, and the main cause of genital ulcers [1–3]. Antibodies against HSV-2 have been widely used as a biomarker of sexual behaviors among different populations around the world [4]. In Mexico, 5.9% of college students had HSV-2 antibodies, pregnant women 14.5%, adult women 29.8%, persons living with HIV 48.5% and female sex workers 85.7% [5–9]. HSV-2 detection can be carried out by cell culture, molecular tests (PCR and real time PCR) or serological methods (ELISA and Western blot), each with different sensitivity and specificity values [3,10]. The commercial serological tests use specific viral proteins, purified or recombinant; the viral proteins used in these tests must be antigenically different, Gg-1 for HSV-1 and gG-2 for HSV-2 [11].

Generally, the tests use serum to detect antibodies, however, there are several advantages using dried blood spots (DBS). First, the collection of samples is simple, non-invasive and requires small blood volumes (less than 0.5 ml). The samples do not need equipment or centrifugation, and can be mailed without refrigeration. Likewise, materials used to obtain DBS like sterile lancets, cotton paper and cotton swabs are cheap [12–15]. The samples can be stored at room temperature for 2 weeks or frozen at −20 °C for longer periods of time.

These advantages are evident when used in studies of large populations or population of remote areas.

Different studies have used DBS samples for antibody detection, particularly against HIV, HBV, HCV, HSV-2 and DENV. Hemagglutination, agglutination, immunochromatographic strips, EIA, ELISA,

* Corresponding author at: Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, México. Av. Universidad No. 655 Colonia Santa María Ahuacatitlán, Cerrada Los Pinos y Caminera C.P. 62100, Cuernavaca, Morelos, México.
E-mail address: molamendi@insp.mx (M. Olamendi-Portugal).

https://doi.org/10.1016/j.jiph.2018.10.007
1876-0341/

and PCR assays, have been used in these studies [16]. In each case, the procedure needs to be standardized for DBS samples, depending on the biomarker to be detected, as most of these techniques have been designed and previously standardized only for serum samples. The aim of this study was to evaluate a commercial ELISA test (IgG-G2 HUMAN®, Human Diagnostics, Germany) and a Western blot (Anti-HSV-1/HSV-2-gG-2 EUROLINE-WB (IgG/IgM), EUROIMMUN®, Germany) for the detection of HSV-2 specific antibodies using DBS, and to calculate the sensitivity, specificity and predictive values of the ELISA test.

Materials and methods

Biological samples and survey

The National Health and Nutrition Survey 2012 (ENSANUT 2012) was a national probabilistic survey of Mexico, with a multistage and stratified design, ENSANUT 2012 was approved by the Ethics, Research and Biosafety Commissions of the Instituto Nacional de Salud Pública (INSP). A total of 48,067 people were selected with an age range of 15–49 years; of these, 40,578 subjects answered a questionnaire and 32,934 individuals signed informed consent and provided capillary blood that was collected on filter paper (Whatman No. 903); the serum samples were obtained from a subsample of these individuals. The DBS samples and serum samples were sent to INSP and were frozen at −20 °C for their subsequent testing [17,18]. The current study used a convenience sub-sample of 908 individuals with an over-representation of samples with index (S/CO) from 2.0 to 6.0, from sexually active subjects.

ELISA (IgG-G2 HUMAN®, Human Diagnostics, Germany)

A volume of 100 μl of 1:101 serum sample dilution was added to the ELISA plate and incubated for 30 min at room temperature (17–25 °C). After four washing rounds, the conjugate was added (rabbit anti-human IgG antibodies conjugated with peroxidase) and incubated for 30 min at room temperature (17–25 °C). After four washing rounds, the plate was incubated for 15 min at room temperature (17–25 °C) with the substrate (TMB). A stop solution was added and the absorbance was measured at 450 nm. The washing rounds and the absorbance measurement were performed with Elisys Uno (Human Diagnostics, Germany).

DBS samples were cut into 6 mm circles. Every spot was eluted with 400 μl of sample diluent buffer (included in the kit) for 16 h at 4 °C. The filter paper was removed and 100 μl of the eluate was used for the ELISA test. In both cases, serum and DBS samples, were classified according to the antibody index (absorbance of the sample/cut-off value; S/C) recommended by the manufacturer as: Negative (≤0.84), undetermined (0.85–1.14) and positive (≥1.15). All ELISA plates complied the quality control, according to the manufacturer's conditions. The antibody index of the paired samples was compared (serum *vs* DBS) using the statistical software GraphPad Prism 6.

To evaluate differences among samples considering the ELISA results, the quantification of total protein was performed with NanoDrop 1000 V3.7 (ThermoScientific ®) on 7 serum samples (diluted 1:101), and among three groups of DBS samples (with 10 samples in each group) according to ELISA results (negative, positive and undetermined). The information was processed with GraphPad Prism 6 software.

Western blot (Anti-HSV-1/HSV-2-gG-2 EUROLINE-WB, EUROIMMUN®, Germany)

Serum samples were processed according to manufacturer's instructions (diluted 1:51 with universal buffer included in the kit). Nitrocellulose strips contained HSV-1 extract, electrophoretically separated and a membrane chip contained HSV-2 G2 protein. Nitrocellulose strips were incubated with the sample dilution for 30 min at room temperature, washed three times with universal buffer and incubated with the conjugate (goat anti-human IgG conjugated with alkaline phosphatase) at room temperature for 30 min. After three washing rounds, strips were incubated for 10 min at room temperature with the substrate (NBT/BCIP). Finally the liquid was removed and the strips were washed three times with distilled water to stop reaction. The results were compared with the band pattern supplied by the manufacturer. For DBS samples, one spot was eluted with 200 μl PBS for 16 h at 4 °C. The first dilution tested was 1:51, then, more concentrated DBS dilutions were tested (1:26, 1:17 and 1:5). Finally, the incubation time for the sample was increased together with the conjugate and the substrate dilution. Western blot results were used as gold standard, the sensitivity, specificity, positive predictive value and negative predictive value were calculated with confidence intervals of 95% ($CI_{95\%}$). New cut-off points with ROC analysis were proposed to obtain 95% sensitivity and 95% specificity, SPSS 15.0 software was used for the statistical analysis.

Results

ELISA

The index value of 13 serum samples (1:101 dilution) was between 0.289 and 7.248, six samples between 0.0 and 2.9 S/CO and seven samples between 3.0 and 8.0 S/CO. The S/CO of 13 DBS samples (elution in 400 μl) was from 0.487 to 7.866, five samples between 0 and 2.9 S/CO and eight samples from 3.0 to 8.0 S/CO. Fig. 1 shows results between serum samples and DBS, the correlation coefficient (r^2) was 0.887, the concordance values with Kappa test was 0.843, and the agreement was 92.3%.

Western blot

From 14 serum samples, antibodies against HSV-2 were detected in eight samples while six samples were negative. All DBS samples (1:51 diluted) were negative for the antigen-antibody reaction. For the other three dilutions of the DBS eluate, only the 1:5 dilution showed concordant results with the serum samples. The incubation time with the sample and with the conjugate increased from 30 to 45 min, while with the substrate increased from 10 to 20 min, enhancing the intensity of the antigen-antibody reaction (Fig. 2). The final conditions to perform the Western blot with DBS samples were: a spot eluted with 200 μl of PBS at 4 °C for 16 h, adding 800 μl of universal buffer (dilution 1: 5), the first two incubation rounds (sample and conjugate) for 45 min and the last incubation (substrate) for 20 min.

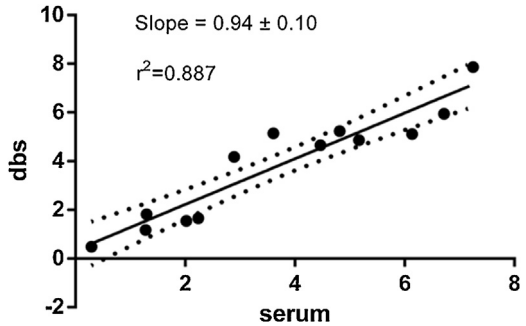

Fig. 1. Agreement of antibody index of anti-HSV-2 by ELISA among paired serum-DBS samples. Concordance 92.3%, Kappa test 0.843.

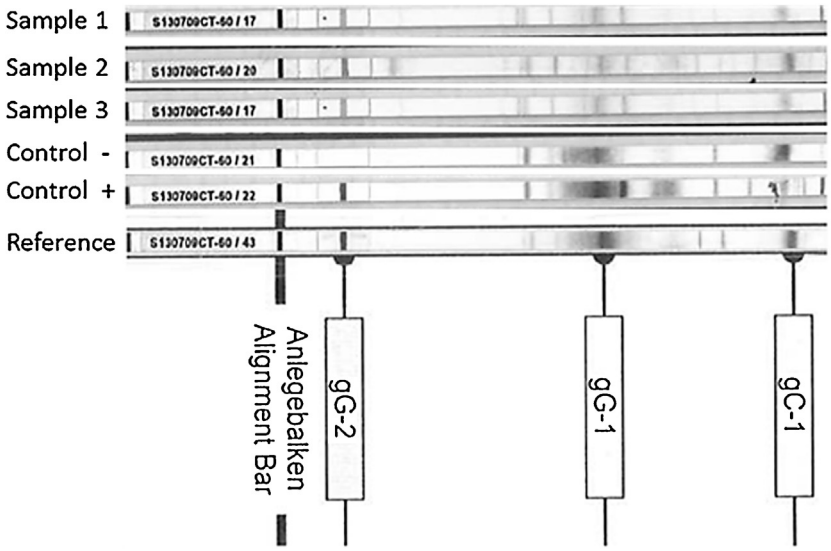

Fig. 2. Western blot results of DBS samples. Samples 1–3 show the presence of antibodies against gG-2 protein (samples positive for HSV-2), the negative control did not show the band corresponding to the antibody recognition of gG-2 protein and the positive control showed the recognition of gG-2 protein.

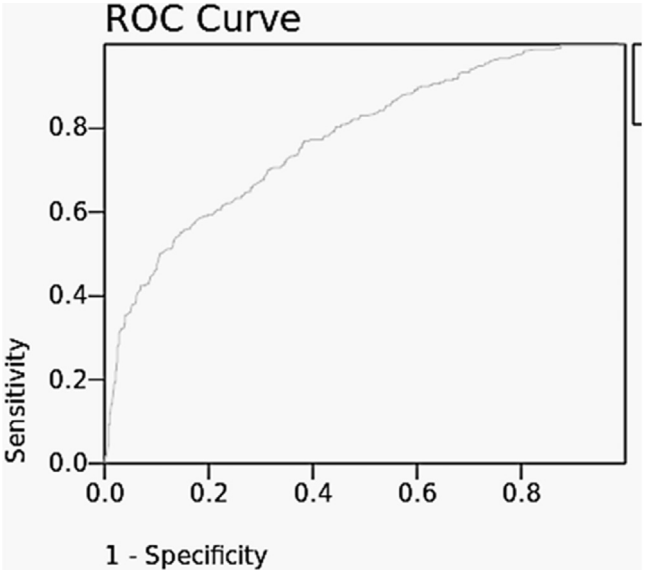

Fig. 3. ROC analysis for ELISA antibody index compared to Western blot result.

The same strip also include the gG1 HSV-1 antigen, the HSV-1 seroprevalence from all the samples was 96%. This finding will not be discussed anymore as it was not part of the study's objectives.

ELISA vs Western blot

From the 908 DBS samples tested for anti-HSV-2 antibodies, 36.3% and 97.0% were positive by Western blot and ELISA (cut-off point 1.15), respectively. The comparison between ELISA and Western blot as gold standard showed a sensitivity of 99.7% (95% CI 98.3–100%) and specificity of 4.5% (95% CI 3.0–6.5%). The comparison also showed a positive predictive value of 37.3% (95% CI 34.1–40.6%) and a negative predictive value of 96.3% (95% CI 81.0–99.9%). The ROC curve was constructed (Fig. 3) and the proposed cut-off points for a better classification of the samples were as follows: negative (0–3.79), undetermined (3.8–4.6) and positive (≥4.61). With the new cut off points for ELISA, the sensitivity and specificity values were near of 95%, as shown in Fig. 4. A total of 144 samples were positive, 162 were negative and 602 were undetermined. Without the undetermined samples, the sensitivity was 90.4% (95% CI 84.1–94.4), the specificity 87.1% (95% CI 81.2–91.4), positive predictive value 84.7% (95% CI 77.9–89.8) and negative predictive value 92.0% (95% CI 86.7–95.4%).

Regarding protein concentration, the serum samples had an average concentration of 61.9 mg/ml and the diluted serum (1:101) showed a mean of 1.2 mg/ml. DBS samples that were positive, undetermined, and negative for anti-HSV-2 by ELISA, had a mean protein concentration of 5.2 mg/ml, 4.8 mg/ml, and 4.8 mg/ml, respectively. The difference between these concentrations was not significant. DBS samples showed 4 times more protein concentration than diluted serum samples (Fig. 5).

Discussion

Serological assays generally have been developed using serum obtained from whole blood draw by venipuncture. The difficulties for the specimen collection, transport, handling and storage serum and plasma samples, are well recognized, particularly in resource-limited setting. Because of above, the use of dried blood spots facilitates the serological diagnosis of STIs [19]. ELISA (IgG-G2 HUMAN®) protocol uses a 1:101 dilution of serum, the current study found similar results using one spot (DBS) eluted with 400 μl of the kit's sample diluent buffer. In studies where one spot was eluted in 150 μl of PBS, a 1:4 dilution from the eluate was comparable to a 1:101 serum dilution [20]. Our study also evaluated the mean concentration of protein from the eluted DBS, finding that it was four times higher than the one found in the serum samples dilutions used in ELISA, and that it was enough for anti-HSV-2 antibodies detection.

The presence of anti-HSV-2 antibodies is detected by diagnostic tests that usually throw undetermined results. These results undetermined should be confirmed by another test, like Western blot [21]. As the EUROLINE-WB recommends its use only for serum or plasma [22], the Western blot conditions were adjusted for DBS: one spot eluted with 200 μl PBS, 1:5 dilution of the eluate, 45 min for sample and conjugate incubation, and 20 min for substrate incubation.

Eing et al. reported the Western blot (Euroline WB, Euroimmun) sensitivity and specificity of 98.9% and 100%, respectively; samples were analyzed with three commercial ELISA kits, being Western blot decisive in special situations [23]. Other study reported

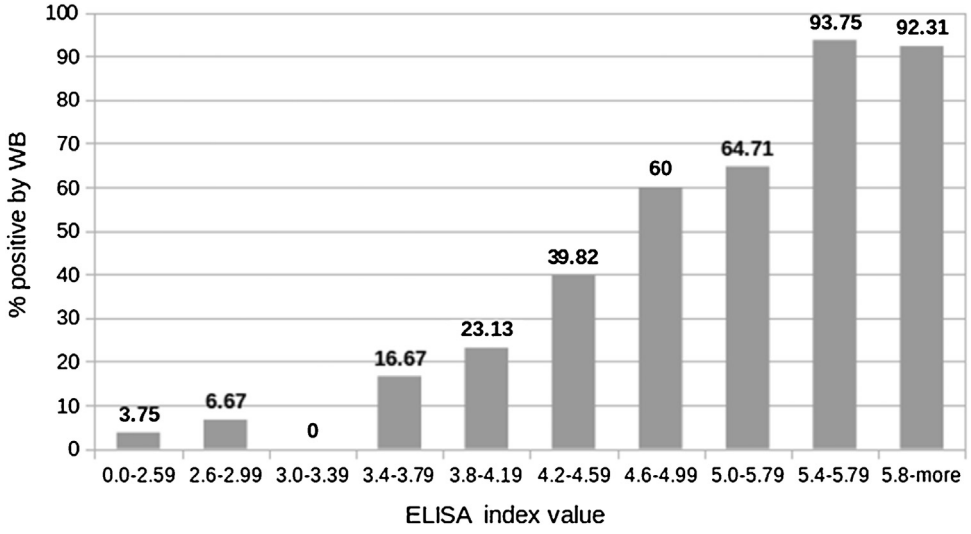

Fig. 4. Percentage of positive samples by Western blot stratified by ELISA antibody index.

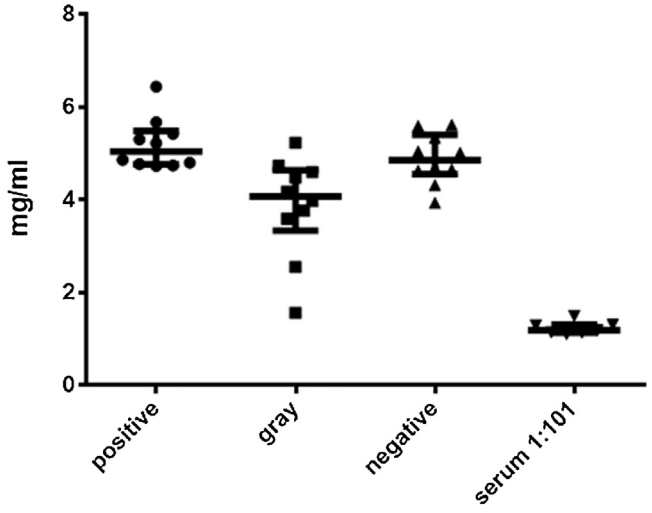

Fig. 5. Total protein quantification among DBS groups and serum samples.

that Western blot (Euroline WB, Euroimmun) had a sensitivity of 96.9–98.7% and specificity of 65.4–89.7% among samples from different populations around the word, being the specificity the parameter with the higher variability among different locations [24].

There are few reports in the world regarding the use of DBS to detect HSV-2. Luseno et al. evaluated two types of samples, serum and DBS for HSV-2 and HIV detection in Kenya (Kalon Biological Ltd.) and Zimbabwe (HerpesSelect 2; Focus Diagnostics). They found that DBS samples were better than serum for fieldwork [25]. Nsobya et al. evaluated also plasma and DBS for HSV-2 with commercial kits (Kalon Biological Ltd). The sensitivity and specificity using DBS were lower than using plasma, and recommended a dilution 1:2 for DBS [26]. Finally, Hogrefe et al. found high agreement between DBS and plasma with other commercial kit (HerpeSelect 2) [20].

Among young Mexican students, DBS samples were used to detect HSV-2 antibodies with Focus HSV-2 ELISA test, (HerpesSelect 2). The study found a global seroprevalence of HSV-2 of 21%, suggesting that good results may be obtained when DBS samples are used to detect HSV-2 antibodies with this method [27]. Schneider et al. reported a sensitivity and specificity of 100% between DBS and serum by ELISA (HerpeSelect 2). Later, from 12,617 DBS samples, 4.7 were positive for anti-HSV-2 [28]. This result is similar to that reported by Sgaier et al. in India which after DBS validation, the seroprevalence of HSV-2 was 10.1% [29].

A very low specificity (4.5%) of the ELISA test (IgG-G2 HUMAN®) with DBS was detected in the current study, possibly because the kit was standardized for serum samples without hemolysis. The manufacturer reported 92% sensitivity and 98% specificity with serum samples [30]. This kit includes HSV-2 antigen purified from cell culture, other kits include purified recombinant antigen (Euroimmun, Focus). To improve the sensitivity with DBS samples, we suggest changing the cut-off point for the ELISA test (HUMAN®): negative from 0 to 3.79, and undetermined of 3.8–4.6. Also, to increase the specificity of the test, Western blot must be used to further analyze samples within of the undetermined.

The modification of the cut-off point for different ELISA tests has been reported to optimize the performance of the assay [31]. Ng'ayo et al. reported a cut-off point of 3.5 (before 1.1) for HerpeSelect Focus HSV-2 ELISA, to increase specificity from 52.3% to 84.9% [32]. Mujugira et al. used the same test and concluded that the optimal cut-off value was 2.1 [33]. In the current study with a new cut-off point, the sensitivity was 96.9% and the specificity increased from 4.5% to 82.5%. We consider this method as an alternative to perform anti-HSV-2 detection using DBS.

One limitation of the current report is that we quantified the total protein in the samples instead of quantifying the specific immunoglobulins. Despite this, the amount of total protein in the DBS samples was enough to perform the Western blot (Euroimmun®), with the corresponding modifications of the technique. The concentration of total protein was also enough to use the samples for ELISA (HUMAN®) as presumptive HSV-2 diagnosis with the recommendation to change the cut-off point of the test and confirm all the results within gray zone. Another limitation was the small number of the paired serum-DBS samples used to modify the conditions of both methods.

Funding

No funding sources.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgement

The authors acknowledge the National Council for Science and Technology for financial support (CONACYT No. 162418).

References

[1] Looker KJ, Margaret AS, Turner KM, Vickerman P, Gottlieb SL, Newman LM. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. PLoS One 2015;10:e114989, http://dx.doi.org/10.1371/journal.pone.0114989.
[2] Looker KJ, Elmes JAR, Gottlieb SL, Schiffer JT, Vickerman P, Turner KME, et al. Effect of HSV-2 infection on subsequent HIV acquisition: an updated systematic review and meta-analysis. Lancet Infect Dis 2017;17:1303–16, http://dx.doi.org/10.1016/S1473-3099(17)30405-X.
[3] Gupta R, Warren T, Wald A. Genital herpes. Lancet 2007;370:2127–37.
[4] Cowan FM, Johnson AM, Ashley R, Corey L, Mindel A. Antibody to herpes simplex virus type 2 as serological marker of sexual lifestyle in populations. BMJ 1994;309:1325–9, http://dx.doi.org/10.1136/bmj.309.6965.1325.
[5] Sánchez-Alemán MA, Conde-Glez CJ, Gayet C, García-Cisneros S, Uribe-Salas F. Sexual behavior and herpes simplex virus 2 infection in college students. Arch Med Res 2005;36:574–80, http://dx.doi.org/10.1016/j.arcmed.2005.03.028.
[6] Herrera-Ortiz A, Conde-Glez CJ, Vergara-Ortega DN, García-Cisneros S, Olamendi-Portugal ML, Sánchez-Alemán MA. Avidity of antibodies against HSV-2 and risk to neonatal transmission among Mexican pregnant women. Infect Dis Obstet Gynecol 2013;2013:14014, http://dx.doi.org/10.1155/2013/140142.
[7] Lazcano-Ponce E, Smith JS, Muñoz N, Conde-Glez CJ, Juárez-Figueroa L, Cruz A, et al. High prevalence of antibodies to herpes simplex virus type 2 among middle-aged women in Mexico City, Mexico: a population-based study. Sex Transm Dis 2001;28:270–6.
[8] Yáñez-Álvarez I, Martínez-Salazar MF, Conde-González CJ, García-Serrato AB, Sánchez-Alemán MA. Seroprevalencia y seroincidencia del Virus Herpes Simple tipo 2 en personas que viven con VIH. Enferm Infecc Microbiol Clín 2011;31:93–7.
[9] Uribe-Salas F, Conde-Glez CJ, Juárez-Figueroa L, Hernández-Castellanos A. Socio-demographic characteristics and sex practices related to herpes simplex virus type 2 infection in Mexican and Central American female sex workers. Epidemiol Infect 2003:131859–65.
[10] Whitley RJ, Roizman B. Herpes simplex virus infections. Lancet 2001;357:1513–8.
[11] Grabowska A, Jameson C, Laing P, Jeansson S, Sjögren-Jansson E, Taylor J, et al. Identification of type-specific domains within glycoprotein G of herpes simplex virus type 2 (HSV-2) recognized by the majority of patients infected with HSV-2, but not by those infected with HSV-1. J Gen Virol 1999;80(July (Pt 7)):1789–98.
[12] Mei JV, Alexander JR, Adam BW, Hannon WH. Use of filter paper for the collection and analysis of human whole blood specimens. J Nutr 2001;13(Suppl 5):1631S–6S.
[13] Li W, Tse FL. Dried blood spot sampling in combination with LC–MS/MS for quantitative analysis of small molecules. Biomed Chromatogr 2010;24:49–65.
[14] Déglon J, Thomas A, Mangin P, Staub Ch. Direct analysis of dried blood spots coupled with mass spectrometry: concepts and biomedical applications. Anal Bioanal Chem 2012;402:2485–98.
[15] Sullivan TJ, Antonio-Gaddy MS, Richardson-Moore A, Styer L, Bigelow-Saulsbery D, Parker MM. Expansion of VIH screening to non-clinical venues is aided by the use of dried blood spots for Western blot confirmation. J Clin Virol 2013;58(Suppl 1):123S–6S.
[16] Snijdewind IJ, van Kampen JJ, Fraaij PL, van der Ende ME, Osterhaus AD, Gruters RA. Current and future applications of dried blood spots in viral disease management. Antiviral Res 2012;93:309–21.
[17] Romero-Martínez M, Shamah-Levy T, Franco-Núñez A, Villalpando S, Cuevas-Nasu L, Gutiérrez JP, et al. Encuesta Nacional de Salud y Nutrición 2012: diseño y cobertura. Salud Pública Mex 2013;55(Suppl 2):332S–40S.
[18] Gutiérrez JP, Sucilla-Pérez H, Conde-González CJ, Izazola JA, Romero-Martínez M, Hernández-Ávila M. Seroprevalencia de VIH en población mexicana de entre 15 y 49 años: resultados de la ENSANUT 2012. Salud Pública Mex 2014;56:323–32.
[19] Hobbs MM, Mwanyumba SW, Luseno WK, Hartman S, Halpern CT, Hallfors DD, et al. Evaluation of herpes simplex virus type 2 serological tests for use with dried blood spots in Kenya. Sex Transm Dis 2017;44:101–3.
[20] Hogrefe WR, Ernst C, Su X. Efficiency of reconstitution of immunoglobulin G from blood specimens dried on filter paper and utility in herpes simplex virus type-specific serology screening. Clin Diagn Lab Immunol 2002;9:1338–42.
[21] LeGoff J, Péré H, Bélec L. Diagnosis of genital herpes simplex virus infection in the clinical laboratory. Virol J 2014;11:83, http://dx.doi.org/10.1186/1743-422X-11-83.
[22] Euroimmun, Medizinische Labordiagnostika AG. Design verification: Anti-HSV-1/HSV-2 gG-2 EUROLINE-WB (IgG); 2011. Available from: http://www.euroimmun.ch/uploads/media/DY_2531_D_UK_A06.pdf. [Cited 22 May 2017].
[23] Eing BR, Lippelt L, Lorentzen EU, Hafezi W, Schlumberger W, Steinhagen K, et al. Evaluation of confirmatory strategies for detection of type-specific antibodies against herpes simplex virus type 2. J Clin Microbiol 2002;40(2):407–13.
[24] Neal JD, Tobian AA, Laeyendecker O, Ngo TD, Redd AD, Reynolds SJ, et al. Performance of the Euroline Western blot assay in the detection of herpes simplex virus type 2 antibody in Uganda, China and the USA. Int J STD AIDS 2011;22:342–4.
[25] Luseno WK, Hallfors DD, Cho H, Iritani BJ, Adze J, Rusakanico S, et al. Use de HIV and HSV-2 biomarkers in Sub-Saharan adolescent prevention research: a comparison of two approaches. J Prim Prev 2014;35:181–91.
[26] Nsobya SL, Hewett PC, Kalibala S, Mensch BS. Performance of Kalon herpes simplex virus 2 assay using dried blood spots among young women in Uganda. Afr J Lab Med 2016;5:429, http://dx.doi.org/10.4102/ajlm.v5i1.429. Epub 2016 Aug 16.
[27] Gutiérrez JP, Conde-González CJ, Walker DM, Bertozzi SM. Herpes simplex virus type 2 among Mexican high school adolescents: prevalence and association with community characteristics. Arch Med Res 2007;38:774–82.
[28] Schneider JA, Lakshmi V, Dandona R, Kumar GA, Sudha T, Dandona L. Population-based seroprevalence of HSV-2 and syphilis in Andhra Pradesh state of India. BMC Infect Dis 2010;10:59.
[29] Sgaier SK, Mony P, Jayakumar S, McLaughlin C, Arora P, Kumar R, et al. Prevalence and correlates of herpes simplex virus 2 and syphilis infections in the general population in India. Sex Transm Infect 2011;87:94–100.
[30] HUMAN, diagnostics worldwide design verification: ELISA HSV-2 IgG-G2; 2008. Available in: http://human.de/data/gb/vr/el-hsv2g.pdf. [Cited 27 March 2017].
[31] Laeyendecker O, Henson C, Gray RH, Nguyen RH, Horne BJ, Wawer MJ, et al. Performance of a commercial, type-specific enzyme-linked immunosorbent assay for detection of herpes simplex virus type 2-specific antibodies in Ugandans. J Clin Microbiol 2004;42:1794–6.
[32] Ng'ayo MO, Friedrich D, Holmes KK, Bukusi E, Morrow RA. Performance of HSV-2 type specific serological tests in men in Kenya. J Virol Methods 2010;163:276–81.
[33] Mujugira A, Morrow RA, Celum C, Lingappa J, Delany-Moretiwe S, Fife KH, et al. Performance of the Focus HerpeSelect-2 EIA for the detection of herpes simplex virus type 2 antibodies in seven African countries. Sex Transm Infect 2011;87:238–41.