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Blood collection on filter paper for HIV antibodies detection: experience of SampaCentro Project

Colheita de sangue em papel filtro para detecção de anticorpos anti-HIV: experiência do Projeto SampaCentro

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ABSTRACT

Introduction: Blood samples collected on filter paper (dried blood spot [DBS]) is an immunoassay that has been used for antibodies screening. **Objective:** To evaluate the strategy of DBS blood collection for detection of HIV antibodies, evaluation of Q-Preven HIV 1 + 2 – DBS kit lot, and to analyze the stability of DBS samples. **Method:** Blood collection on DBS was performed according to World Health Organization (WHO) recommendations. The evaluation of the kit lot for HIV antibodies detection was performed using delta (d) values from the results of 774 DBS samples from volunteers men who have sex with men (MSM) recruited in the central region of São Paulo city, Brazil. **Result:** DBS blood collection was performed without complications. The positive (5.26) and negative (5.23) delta values allowed to clearly differentiate HIV antibodies reactive and non-reactive samples. We observed good performance of the kit lot and samples were stable on DBS form. **Conclusion:** Blood collection on DBS is feasible for the study of MSM population and is suitable for laboratory routine. The overall performance of Q Preven HIV-1 + 2 – DBS kit was satisfactory, having reached the quality levels required for the development of this study.

Key words: filter paper; HIV diagnosis; ELISA; antibodies.

INTRODUCTION

The acquired immunodeficiency syndrome (Aids) epidemic in Brazil began in the early 1980s. Over these thirty years, it was characterized as a "concentrated" epidemic, reaching differently certain population groups, specifically men who have sex with men (MSM), injected drug users, and prostitutes. These groups who have shown high prevalence of infection with human immunodeficiency virus (HIV), greater than 5%, while in the general population the prevalence remains low, less 1%[1, 2].

Estimates of HIV prevalence in certain population are important for understanding the epidemiological characteristics, evaluating prevention programs, and developing new strategies. In Brazil, the prevalence rate of HIV infection among MSM was estimated between 11.1% to 14.2%, based on data from 2009. This prevalence was about two and three times higher than estimated for prostitutes and drugs users, respectively, in the same period[3].

Several studies have shown the importance of behavioral surveillance, to monitore the risk practices associated with HIV infection in population groups at highest risk[4]. In Brazil, these studies have not been conducted in a systematic way[1].

Together with the behavioral surveillance, access to diagnosis of HIV infection has been essential for preventive and therapeutic measures to be imposed and, therefore, contribute to the interruption of virus transmission[5, 6].

In order to ensure early diagnosis of infection, studies and public health programs search the most vulnerable population in other areas than health services, such as prisons, sociability spaces, among others. In these scenarios, to enable access to diagnosis, it is essential to adopt strategies to simplify the blood collection and facilitate the transport of samples from field to the laboratory[7, 8].

Since its introduction in the phenylketonuria diagnosis (Guthrie and Susi 1963)[9], the blood samples collected on filter paper (dried blood spot [DBS]) have been widely used in many

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countries in neonatal screening for a wide range of metabolic and genetic disorders.

DBS samples have been used in clinical diagnosis and seroepidemiological studies of infectious diseases[10, 11]. This technique enables the performance of large-scale epidemiological studies, which can be conducted faster, allowing making strategic decisions to populations at risk[12]. In HIV scenario, blood samples on filter paper were initially used for antibody screening tests to estimate the seroprevalence of HIV-1 infection in pregnant women[13].

The capillary blood collection on filter paper has significant advantages over venipuncture, it is simple to perform, requires minimal training, and does not involve the risks associated with the use and disposal of needles and syringes[14, 15]. Additionally, the use of DBS sample minimizes the risks associated with biological transport and, thus, reduces the risk of work accidents with potentially infected materials[16].

DBS reduces the risk of contamination of professional during handling virus samples, such as, for example, HIV 1 and 2, human T-lymphotropic virus (HTLV) I and II, and hepatitis C virus (HCV), which lose their infectivity due to envelope rupture, during blood drying[12].

The limitations of sensitivity and specificity, as regards screening small blood volumes (equivalent to 5-10 ul), restricted the use of DBS samples for many years. However, recent advances, such as the production of monoclonal antibodies, synthetic protein production, and the introduction of the polymerase chain reaction (PCR) overcome many of these problems by allowing the potential for a extensive DBS database stored to be used by biochemists, geneticists and microbiologists[12].

DBS technology has been applied to the screening of antibodies using different diagnostic techniques, for example, particle agglutination tests and enzyme immunoassay (EIA). The feasibility of this technology in HIV epidemiological surveillance studies have been evaluated and increasingly recognized by the need for an low cost and efficient means for monitoring the extent of HIV infection in Europe and in the United States[12, 14].

Since the discovery of HIV, many tests for HIV antibodies detection have been developed and introduced into the clinical laboratory[17]. These tests are an important tool in the evaluation of blood quality in blood banks and to achieve the clinical diagnosis[18]. Therefore, the results of serological tests must be accurate and properly interpreted, due to medical and social importance of testing positive for HIV[19].

In these nearly thirty years since the first tests for HIV antibodies detection have been developed, although tests have been increasingly improved, reagents that combine maximum sensitivity at maximum specificity have not been described yet[18-21]. For this reason, it is recommended the use of flowcharts tests for diagnosis of HIV infection, defined according to the purpose of testing – diagnosis, monitoring and screening blood donors.

The enzyme immunoassay (ELISA/EIA) method has been universally used as early serologic screening for HIV antibodies detection, and samples with positive reactivity in these tests are confirmed by the Western blot (WB) and indirect immunofluorescence (IIF) methods[22, 23].

In Brazil, procedures for the laboratory diagnosis of HIV infection are defined in a statutory instrument of the Ministry of Health, which regulates the type of biological material to be analyzed, the type of tests used, the interpretation, and the issuance reports. According to regulations in effect at the time of the survey[24], the blood samples collected on filter paper could be used only for screening.

The accomplishment of a study to estimate the prevalence of HIV infection among MSM attending sociability spaces in the central region of São Paulo city, Brazil, the SampaCentro Project, was configured as an appropriate scenario to test this technology in field, in a big city like São Paulo.

OBJECTIVE

To evaluate the strategy of blood collection on filter paper for detecting HIV antibodies in samples from SampaCentro Project volunteers using the commercial kit reagents Q-Preven HIV 1+2 for HIV antibodies (EIA), as well as to evaluate the lot of diagnostic kit used, and to analyze the stability of DBS samples.

MATERIAL AND METHODS

Blood samples on filter paper were analyzed for HIV antibodies detection from the SampaCentro study – sexual behavior and sexual practices, access to prevention, prevalence of HIV and other sexually transmitted infections among gay men, transgender and MSM – in central region of the city of São Paulo, Brazil.

The participants were approached at social spaces (pubs, nightclubs, restaurants, cinemas, concentrations in the street)

in the districts of República and Consolação, between November 2011 and January 2012, they were invited to participate in the study with written consent in the Informed Consent (IC), previously approved by the Research Ethics Committee of the Reference and Training Center in STD/AIDS (Comitê de Ética em Pesquisa do Centro de Referência e Treinamento em DST/Aids [CRT DST/AIDS]), of the Santa Casa de Misericórdia, the Municipal Health Department, and the Instituto Adolfo Lutz (IAL), all located in São Paulo, Brazil. Those who areed answering a structured questionnaire applied by trained interviewers, were then invited to collect blood for serologic HIV antibody test.

Sample collection

The volunteers collected blood in a vehicle van category, which was prepared for the procedure, parked near the sociability spaces; the collection was carried out by trained professionals from CRT DST/AIDS.

To obtain the DBS samples, blood was collected by a fingertip puncture, using a standardized collection kit (Bio-Oxford Importação Ltda, São Paulo, Brazil). The drops of blood (minimum three and maximum five) were placed on the filter paper Schleicher and Schuel - S&S 903 (Whatman/GE Healthcare Life Sciences do Brazil, São Paulo, Brazil) in the area previously defined. DBS sample of each individual was properly identified with unique code.

The collection procedures, transport and storage of DBS samples were carried out according to the instructions in the manufacturer's manual of the diagnostic reagent kit Q-Preven HIV 1+2 – DBS (Symbiosis Diagnostica Ltda, São Paulo, Brazil), based on the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS)[25] and the World Health Organization (WHO)[26].

Procedures for drying and storage DBS samples

The critical factor for DBS samples stability has been keeping them in dry condition, i.e. without moisture. After blood collection, the samples were sent to the CRT DST/AIDS offices, there, they were kept at room temperature for up to 24 hours in appropriate shelves to avoid cross contamination[26]. When completely dry, they were placed in individual envelopes containing desiccant packets (silica-gel) and sent to the HIV/Aids Laboratory – Immunology Center (Centro de Imunologia [CIM]) of the IAL, São Paulo, Brazil, and they were stored and maintained in freezer at -70ºC to completion reactions by ELISA/EIA – Q-Preven HIV 1+2 – DBS for detecting HIV antibodies.

Evaluation of ELISA/EIA – Q-PREVEN HIV 1+2 – DBS lot

The simplified evaluation of the diagnostic reagent kit Q-Preven HIV 1 + 2 – DBS lot (1022000140) was held at the HIV/Aids Laboratory, CIM-IAL, São Paulo, Brazil, using a panel of 26 blood samples previously characterized for HIV antibodies presence, with known clinical, epidemiological and laboratory data, 11 samples with nonreactive results and 15 with reactive result for HIV and detectable viral load. The panel samples were obtained in DBS form and subjected to the same conditions of the volunteer's samples.

HIV antibodies detection: Q-PREVEN HIV 1+2 – DBS

It is ELISA/EIA standard for detecting HIV antibodies (HIV 1; HIV 2 and HIV 1 subtype O), specifically for blood samples collected on filter paper.

The cards containing the DBS samples were removed from the freezer -70ºC and the punches infused with dried blood (diameter 4.7 mm) and, in parallel, the control punches (positive and negative) of the reagent diagnostic kit. Samples and controls were eluted in microplates, as recommended on the package. After elution, samples and controls were transferred on microplates sensitized with recombinant antigens (p24, gp41 do HIV 1 e gp35 do HIV 2) and synthetic peptide (gp41 do HIV 1 subtype O) for ELISA/EIA performance. In the first step of the assay, incubation of eluate sample was carried out compared to the antigens present in the microplate cavity. After washing, conjugate incubation was performed, composed of recombinant and synthetic antigens of HIV 1 and 2 linked to peroxidase. After further washing, the assay development was carried out by adding chromogen solution (tetramethylbenzidine). Then the enzymatic reaction was interrupted by adding stop solution (H2SO4, 1N) and the absorbances were measured with microplate reader (Tecan – Sunrise A – 5082, Salzburg, Austria), with 450/620 nm filters, with results expressed in optical density units. The procedures were performed according to the manufacturer's instructions, and the interpretation of results according to the current legislation in Brazil.

For individuals with HIV antibody reactive result in Q-Preven HIV 1+2 – DBS that attended the CRT DST/AIDS to be aware of their serological status, was offered the rapid diagnosis methodology –rapid test for HIV-1/2-Bio-Manguinhos (Fundação Oswaldo Cruz [Fiocruz], Rio de Janeiro, Brazil) and Rapid Check HIV 1 and 2 (Center of Infectious Diseases [Núcleo de Doenças Infecciosas], Universidade Federal do Espírito Santo, Brazil) or Determine HIV-1/2 (Abbott Laboratories, Abbott Park, IL, USA).

Data analysis

The results of the samples were analyzed by the values of optical density (OD) and the corresponding cut-off (CO) ratio obtained in each reaction (mean of negative controls + 0.180).

To evaluate the effectiveness of Q-Preven HIV 1+2 – DBS assay by separating the HIV antibody reactive and non-reactive population from the cut-off, as described by Crofts (1988)[27] and Maskill (1998)[17], initially the distribution of the OD/CO ratio values was normalized by transforming them into $\log_{10}$. The delta values (d) were calculated by dividing the mean $\log_{10}$ (DO/CO) observed in the populations' assays of HIV antibodies reactive and non-reactive samples by $\log_{10}$ (OD/CO) set standard deviation of each population. Therefore, (d) value was interpreted as the distance (in standard deviation units) of the mean of distribution of these values in relation to the normalized cut-off[27]. The higher positive delta (d+) and negative delta (d-) values, the higher the probability of the test correctly identifying HIV antibodies reactive and HIV antibodies non-reactive, respectively[28].

RESULTS

From November 2011 to January 2012, 774 DBS samples were sent to CIM-IAL. These samples were analyzed using ELISA/EIA Q-Preven HIV 1+2 – DBS, within 30 days after collection, and the results were interpreted according to the reactivity of HIV antibodies, described in **Table 1**.

All DBS samples collected were viable without signs of deterioration or other aspects (presence of contamination) that could suggest loss of stability for tests performance. This shows that WHO recommendations[26] for DBS drying and storage would produce results according to the literature.

The DBS blood samples with known results ($n = 26$), used to evaluate the Q-Preven HIV 1+2 – DBS lot (1022000140) agreed 100% (26/26), 11/11 non-reactive and 15/15 reactive for HIV.

TABLE 1 – Results of HIV antibodies reactivity on DBS samples in ELISA/EIA Q-Preven HIV 1+2

Collection period	HIV antibodies results				Total	
	Reactive		Non-reactive			
November/2011	35	4.50%	277	35.80%	312	40.30%
December/2011	53	6.90%	251	32.40%	304	39.30%
January/2012	30	3.90%	128	16.50%	158	20.40%
Total	118	15.30%	656	84.70%	774	100%

In this evaluation, no sample has showed inconclusive result.
HIV: human immunodeficiency virus; DBS: dried blood spot; ELISA/EIA: enzyme-linked immunosorbent assay/enzyme immunoassay.

From 118 individuals with reactive results for HIV (Table 1), 37 (31.4%) attended the CRT DST/AIDS to be aware of their serological status. Of these, 25 (67.6%) agreed to collect a new blood sample for the rapid test HIV. The results showed 100% (25/25) agreement between the two methods.

The capacity of the Q-Preven HIV 1+2 – DBS assay to differentiate the two samples populations (HIV antibody reactive and non-reactive) was defined by units known as (d), which shows the distance of the mean of $\log_{10}$ OD/CO ratio of the population sample in relation to the cut-off value, and is expressed in standard deviation units. The **Figure** presents the evaluation of test performance with 774 DBS samples in populations with HIV antibody reactive (118 cases) and non-reactive (656 cases) results distributed as $\log_{10}$ (OD/CO).

The (d+) (5.26) and (d-) (5.23) values in the Figure show distance of $\log_{10}$ (OD/CO) value of samples in relation to cut value and the distribution of them according to the results obtained in the scale. Different reactivity profiles were observed in reactive samples, which can be explained by different concentrations of antibody present in these samples.

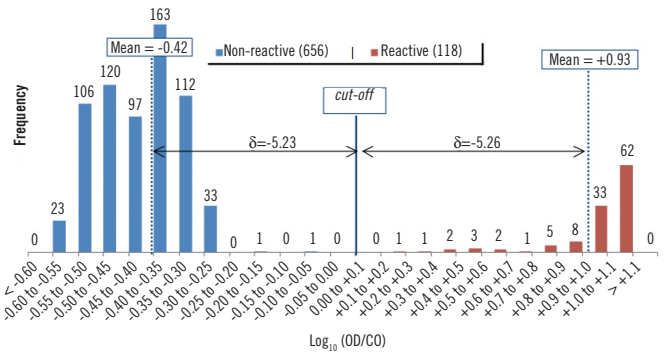

FIGURE – *Distribution of $\log_{10}$ (OD/CO) for DBS HIV reactive and non-reactive samples*
OD: optical density; CO: cut-off; δ: delta value; DBS: dried blood spot; HIV: human immunodeficiency virus.

The profile of the frequency distribution of $\log_{10}$ (OD/CO) values showed that HIV antibody reactive and non-reactive samples showed 99.7% probability of classification as such, considering the distance of ± 3 standard deviations close to the mean of these values in their populations. No $\log_{10}$ (OD/CO) values were observed in the overlap region of the distribution curves of reactive and non-reactive populations samples, known as gray zone. Thus, we did not observe inconclusive results.

In **Table 2** the DBS HIV antibody reactive samples are grouped according to $\log_{10}$ (OD/CO) value obtained from Q-Preven HIV 1+2 – DBS assay and their respective (d) values.

DISCUSSION

The literature shows that studies that provide evidence of the advantages in collecting blood more easily and safely to the diagnosis of HIV infection are of public health significance[12, 14-16, 29]. In the present study we could observe that the blood collection on filter paper is a feasible alternative access to HIV antibody tests, under field conditions, in this case, sociability spaces which concentrate a large number of people, in many brazilian major cities for several of the most vulnerable groups to infection.

One measures to be taken when using DBS samples is the occurrence of significant delay between the time of collection and testing of samples in the laboratory, and exposure to heat and moisture conditions, which may compromise the results of HIV antibody tests[14]. According to WHO[26], another important factor is the storage stability of the DBS sample, which has been shown stable for at least two years if properly maintained. Considering that in this study these recommendations were strictly followed, the time and storage conditions for the performance of HIV antibody did not affect the quality of the samples. The procedures for the pre-analytical phase (collection, drying, transport and storage of DBS samples) were performed according to the protocol established[25, 26] in order to maintain the stability of the material and, therefore, the viability of HIV infection diagnosis. The same procedures were applied to the panel prepared in our laboratory composed of DBS samples known whether reactive and non-reactive for HIV, and the results obtained were satisfactory.

This study has some important limitations. Firstly it is essential understand the reason for choosing the strategy of blood collection on filter paper. Volunteers were recruited in public places, living spaces. Thus, we opted for the less invasive puncture method, with greater chance of acceptance by volunteers. Moreover, considering that the collection has been carried out in field situation, without adequate infrastructure for storage and transportation of blood tubes, DBS technique proved to be most convenient to obtain biological material for carrying out laboratory tests. Thus, the data presented here are based on a single type of sample (DBS), which made it impossible to conduct comparative tests with other standard techniques for serum or plasma samples, including conducting additional tests to serological screening tests in order to confirm the presence of specific antibodies against HIV, as recommended by the Brazilian Ministry of Health[24].

Another important limitation was related to the limited number of available tests, which crippled the analysis of a larger number of reference samples for HIV antibody profile. These two factors, therefore, made it impossible the performance evaluation of the reagents Q-Preven through conventional sensitivity and specificity measures.

Considering these limitations, we suggest EIA raw data analysis as an auxiliary measure to evaluate the performance of kit Q-Preven lot in the study samples. In this regard, the

TABLE 2 – Log_{10} (OD/CO) values of reactive DBS samples and respective delta values

	Log_{10} (OD/CO)							
	General	> 0.7	> 0.8	> 0.9	> 0.97	> 0.98	> 0.99	> 1
Delta	5.3	13.6	18.5	28.1	41.1	47.5	50.3	54.1
Mean	0.93	0.98	0.99	1.01	1.02	1.02	1.02	1.03
Standard deviation	0.1776	0.0725	0.0537	0.0358	0.0247	0.0215	0.0203	0.0189
Reactive sample (*n*)	118	108	103	95	83	74	70	62

OD/CO: optical density/cut-off; DBS: dried blood spot.

(d) values (Figure) provided a tool for ascertaining how the used assay may separate the different populations of HIV antibody reactive and non-reactive samples in relation to the cut-off. In this laboratory prior experience (unpublished data), with regard to immunoassays for HIV antibody detection, the greater the ability of the test in numerically distancing the means of OD of reactive samples from OD of non-reactive samples the more decisive is the referred assay. Thus, our data regarding the Q-Preven kit in DBS samples showed compliance to this requirement without loss of information about the different profiles of antibody concentration, which is an advantage for quantitative analysis based on immunoassay.

In this study, the profile of blood samples submitted to HIV antibody detection by Q-Preven HIV 1+2 – DBS test was heterogeneous, according to HIV antibody reactive values found and shown in Table 2. The (d+) and (d-) values obtained in the populations set of HIV antibody reactive and non-reactive samples, respectively, clearly indicated the separation of samples in relation to the cut-off, reflecting the ability of reagent diagnostic kit used in properly differentiate them.

When comparing the (d+) and (d-) values obtained from Q-Preven HIV 1+2 – DBS test with the (d) data available in the literature[17, 22], the evaluation of several reagent diagnostic kits, there was found that the results were similar.

As mentioned earlier, the collection plan of biological material for this study restricted the acquisition of serum or plasma samples that could be used for comparative testing and, in addition, imposed the most important limitation regarding confirmation of HIV antibody reactive results obtained from DBS. Considering the importance of confirmation reactive results in screening tests for HIV antibody, although it was not planned, we performed the Western blot test for HIV-1, commercially available (*Cambridge Biotech HIV-1 Maxim Biomedical*, Inc., Mariland, USA), using a protocol empirically modified (unpublished data) in order to verify the positivity of reactive samples in Q-Preven test. All samples analyzed showed results consistent with the presence of specific HIV antibodies.

The results of this study may be important when using blood samples collected on filter paper for programs of HIV/Aids prevention and control and for laboratory professional when selecting the appropriate reagent kit for HIV diagnosis.

CONCLUSION

Based on the results observed, and considering only the standardized technical conditions within the framework of research protocol, we can conclude that the volunteer blood samples collection on filter paper strategy enable to detect HIV antibody, using diagnostic reagents Q-Preven HIV 1+2 – DBS, obtaining satisfactory results and the quality levels required in the study plan.

The choice of DBS as a form of blood collection is feasible to conduct field studies and voluntary approach in public living spaces and restricted access to conventional infrastructure for collection of biological material for the purpose of laboratory analysis.

Despite the reported limitations, it was found in this study that the advantages of collecting blood samples on DBS complemented with alternative measures for performance analysis of laboratory tests carried out with these samples were superior to the weighted disadvantages.

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RESUMO

Introdução: *As amostras de sangue colhidas em papel filtro (DBS) têm sido utilizadas na triagem de anticorpos por meio de imunoensaios.* ***Objetivos:*** *Avaliar a estratégia de colheita de sangue em DBS para detecção de anticorpos contra o vírus da imunodeficiência humana (HIV), verificar o lote do* kit *Q-Preven HIV 1+2 – DBS e analisar a estabilidade das amostras DBS.* ***Método:*** *A colheita de sangue em DBS foi realizada conforme recomendações da Organização Mundial da Saúde (OMS). A avaliação do lote do* kit *para detecção de anticorpos anti-HIV foi feita por meio do valor de delta a partir dos resultados das 774 amostras DBS provenientes de voluntários homens que fazem sexo com homens (HSH) recrutados na região central da cidade de São Paulo, Brasil.* ***Resultado:*** *A colheita de sangue em DBS foi realizada sem intercorrências. O indicador delta positivo (5,26) e negativo (5,23) permitiu discriminar com clareza amostras anti-HIV reagentes e não reagentes. O lote do* kit *apresentou bom desempenho e as amostras permaneceram estáveis na forma de DBS.* ***Conclusão:*** *A colheita de sangue em DBS mostrou-se factível para o estudo realizado com a população HSH e foi adequada para a rotina laboratorial. O desempenho global do* kit *Q-Preven HIV 1+2 – DBS foi satisfatório, com a qualidade requerida para o desenvolvimento deste estudo.*

Unitermos: *papel filtro; diagnóstico anti-HIV; ELISA; anticorpos.*

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