Yassin et al., Surgery 2013, S12
DOI: 10.4172/2161-1076.S12-002

Surgery: Current Research

Research Article Open Access

Evaluation of Dry Blood Spot (DBS) Stored at Different Temperature for Detection of HIV Antibodies by Using Different Rapid Tests

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Abstract

Objective: To evaluate the uses of dry blood spot stored at 37°C, 45°C, and 50°C for detection for HIV antibodies by using Rapid test.

Methods: 95 blood specimens were used in this study, 50 specimens were collected from HIV positive and 45 collected from HIV non reactive patient, Dry Blood Spots (DBS) were prepared by applying 50 µl of whole blood on Whatman 3 filter paper (Whatman International® Ltd.), specimens of all DBS were stored at 37°C, 45°C and 50°C.

All DBS were tested with two rapid tests (*ACON* ® One Step Test Device) and (*ACCURATE*® test device) and to evaluate the effect of storage temperature and time on sensitivity and specificity, DBS were tested after 2 weeks, 4 weeks and 8 weeks.

Results: After 2 weeks 48 out of 50 HIV-positive DBS were positive, and all 45 HIV-negative DBS were negative, at the different temperatures with both ICT test devices, with sensitivity of (96%) and specificity of (100%). After 4 weeks at 37°C, no change was reported in sensitivity and specificity with the both rapid tests. There was decrease in sensitivity after 8 week storage, especially at higher temperatures 45°C and 50°C.

Conclusion: When tested with rapid test, DBS stored at 37°C and 45°C showed good stability until 8 weeks, but when stored at 50°C it showed good stability until week 4 and it had less stability when stored for 8 weeks. The present study concluded that in area with high temperature such as Sudan DBS can be stored at room temperature and tested with rapid test within 4 weeks.

Keywords: HIV; Dry Blood Spot (DBS); Intraoperative Computerized Tomography (ICT); Sudan

Introduction

At the end of 2010, an estimated 34 million people (31.6 million–35.2 million) were living with HIV worldwide, up 17% from 2001. Sub-Saharan Africa remains the region most heavily affected by HIV. In 2010, about 68% of all people living with HIV resided in sub-Saharan Africa, a region with only 12% of the global population. Sub-Saharan Africa also accounted for 70% of new HIV infections in 2010 [1].

Dried blood specimens are clinical specimens collected by careful applying of a few drops of blood, freshly drawn by finger stick with a lancet from adults, or by heel stick from infants, onto specially manufactured absorbent specimen collection (filter) paper [2], for example Schleicher and Schuell (S and S) 903, S and S 2992, and Whatman grade 1 or 3 [2,3]. The blood is allowed to thoroughly saturate the filter paper and is air dried for a minimum of three hours [2]. It is a well- accepted means of collection, transport and storage of blood samples for various epidemiologic, serologic and molecular assays for Human Immunodeficiency Virus (HIV) studies [4]. Particularly important for mother to infant transmission studies of affected individual living in remote areas, and for large-scale, population based screening programs [4].

Testing of blood dried on filter paper introduced by Robert Guthrie (hence it also called Guthrie spot) in 1960s to collect blood by heel stick, for measurement of phenylalanine in newborns to detect Phenylketonuria (PKU) [5].

Testing neonatal DBS for maternal HIV antibodies, using Enzyme Immunoassay (EIA) and immunoblot (IB) was first described in 1987 [6]. In 1994 testing of DBS for the presence of HIV type 1 proviral DNA by PCR was first described [7].

Materials and Methods

Confirmed HIV positive specimens (ELISA and Immunoplot positive) and HIV test negative specimens (ELISA and Immunoplot negative) were enrolled into this study**.** Three ml of venous blood was collected into EDTA container, and DBS was prepared on Whatman 3 (Whatman International® Ltd.). Filter paper was cut into strips, each strip used for preparation of 4 DBS. Three DBS strips were prepared from each sample.

One end of each strip was used for sample identification, including labelling number, date of collection, and temperature of storage. DBS was prepared by applying 50 µl of EDTA anticoagulated whole blood on filter paper, then it was positioned horizontally at rack, allowed to dry at room temperature for about 3 hours, and then each strip was enclosed separately in a plastic bag. From each sample three DBS strips were made and one was stored at 37°C, the second one was stored at 45°C and the 3rd one was stored at 50°C. Storage temperatures were selected to mimic ambient temperatures in Sudan that could reach more than 45°C in the summer season.

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Received December 28, 2012; **Accepted** January 25, 2013; **Published** February 03, 2013

Citation: Yassin MEM, Eldaif WA, Elkhider IM (2013) Evaluation of Dry Blood Spot (DBS) Stored at Different Temperature for Detection of HIV Antibodies by Using Different Rapid Tests. Surgery S12: 002. doi:10.4172/2161-1076.S12-002

Elution of DBS for testing

DBS was punched with manual 1-hole punch (Staples® Brands Group) for 1/4 inch (6.35 mm) punch, in an area that is fully saturated with blood. Elution was prepared with 200 µl phosphate buffered saline with 0.05% Tween 20 (PBST).

Elution with 200 µl PBST was selected on basis of preliminary results that indicated superiority to other volume tested (100 µl and 300 µl).

Elutions were prepared after 2 weeks, 4 weeks and 8 weeks for testing with ICT.

Two rapid tests were used: *ACON* HIV 1/2 (Human Immunodeficiency Virus Ultra Rapid Test Device. *ACON* Laboratories and *ACCURATE* HIV 1/2 (Human Immunodeficiency Virus Ultra Rapid Test Device), these tests are used widely in Sudan as screening test at the low resources regions.

Test method

Test device, specimen and buffer were allowed to equilibrate to room temperature prior testing. The tests were carried out according to manufactures direction. Positive result was indicated by appearance of two red lines within 10 minutes.

Results

Replicates of 50 HIV positive DBS stored at 37°C, 45°C, and 50°C were tested with rapid test (*ACCURATE*® test device) after 2 weeks, 4 weeks and 8 weeks intervals (Table 1). 48 DBS samples were still positive at different temperature until 4 weeks, after 8 weeks storage at 37°C and 45°C two samples were changed from positive to negative, at 50°C six samples were changed to negative. On the other hand all HIV negative DBS were negative at the different temperature and storage time. As shown in (Table 2), 50 HIV positive DBS stored at 37°C, 45°C, and 50°C were tested with rapid test (*ACON*® One Step Test Device), after 2 weeks, 4 weeks and 8 weeks intervals. 48 DBS samples were positive after two weeks at the different temperature. After four weeks at 37°C 48 sample were still positive, where as 47 samples were found positive at a temperature of 45°C and 46 samples positive at 50°C. A Few discrepancies were observed after 8 weeks storing, 46 DBS samples were positive at temperature of 37°C, 45 samples were positive at 45°C, while 42 samples were found positive at 50°C.

As illustrated in (Figure 1) out of 50 HIV positive samples, after 4 weeks storage at 37°C the two rapid test gave same result (48 positive DBS), while at 45°C 48 DBS samples were positive with *ACCURATE* compared with 47 DBS positive samples with *ACON*, whereas 46 DBS samples were positive with *ACON* compared with 48 DBS positive with *ACCURATE* at a temperature of 50°C.

In figure 2 after 8 weeks storing at 37°C, 45°C & 50°C, 46 DBS stored at 37°C were positive with both rapid tests, at 45°C 45 DBS samples were still positive for HIV with *ACON* compared with 46 DBS samples with *ACCURATE*, whereas only 42 DBS stored at 50°C were positive with both tests.

As indicated in (Table 3) the sensitivity & specificity of both rapid simple tests were 84%- 96% and 100% respectively.

Discussion

In this study we used Dry Blood Spot (DBS) stored for various times at different temperatures for detection of HIV-1/2 antibodies with two rapid test (*ACON*® One Step Test Device) and (*ACCURATE*® test device) [8].

Storing duration	Temperature	*ACCURATE* ® test device	
		Positive n (%)	Negative n (%)
2 weeks	37°C	48 (96)	2 (4)
	45°C	48 (96)	2 (4)
	50°C	48 (96)	2 (4)
4 weeks	37°C	48(96)	2 (4)
	45°C	48 (96)	2 (4)
	50°C	48 (96)	2 (4)
8 weeks	37°C	46 (92)	4 (8)
	45°C	46 (92)	4 (8)
	50°C	42 (84)	8 (16)

Table 1: The effect of temperature and storage time on testing of 50 HIV positive DBS samples with *ACCURATE* ® rapid test.

Storing duration	Temperature	*ACON* ® One Step Test Device	
		Positive n (%)	Negative n (%)
2 weeks	37°C	48 (96)	2 (4)
	45°C	48 (96)	2 (4)
	50°C	48 (96)	2 (4)
4 weeks	37°C	48(96)	2 (4)
	45°C	47 (94)	3 (6)
	50°C	46 (92)	4 (8)
8 weeks	37°C	46 (92)	4 (8)
	45°C	45 (90)	5 (10)
	50°C	42 (84)	8 (16)

Table 2: The effect of temperature and storage time on testing of 50 HIV positive DBS samples with *ACON*® rapid test.

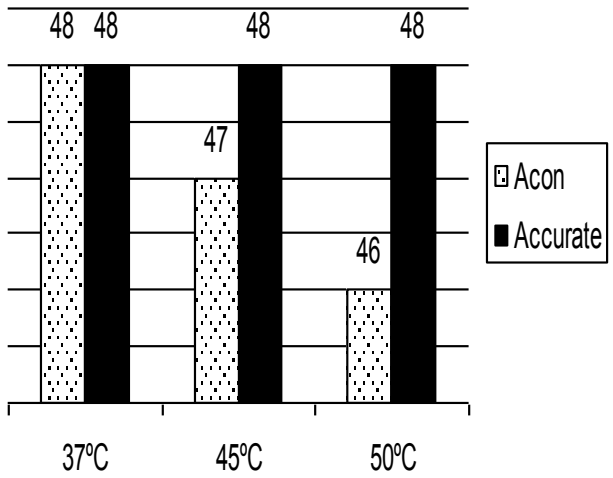

Figure 1: Result of DBS stored for 4 weeks at 37°C, 45°C, and 50°C tested with (*ACON*® One Step Test Device), and (*ACCURATE*® test).

Testing of DBS stored at 37°C, 45°C, and 50°C for two weeks, with rapid tests (*ACON*® One Step Test Device and *ACCURATE*® test device), showed sensitivity of 96% and specificity of 100% for both. This result is quite similar to study done by Thakar et al. [9] in who reported the sensitivity of (Immunocomb II HIV 1 and 2 bispot test kit) for detection of HIV-1/2 antibodies was 100%, and that of Ouwe-Missi-Oukem-Boyer et al. [10] in Niger [10] who used two rapid tests (Determine HIV 1/2 and Immunocomb II HIV 1 and 2 Bispot) to compare the dried blood spots and serum. The authors' reported similar results for the two tests used. This study also indicated some consent with the study done by Mei et al. in 2004 [11], who investigated the use of DBS with three rapid test (OraQuick, Determine and Uni-

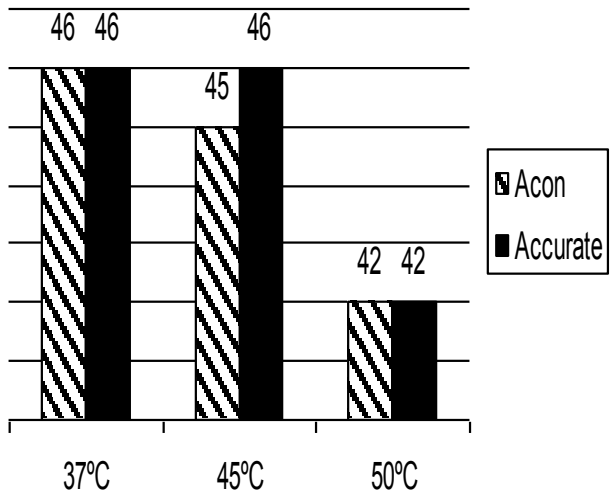

Figure 2: Result of DBS tested after 8 weeks storage at 37°C, 45°C, and 50°C, with (*ACON* ® One Step Test Device), and (*ACCURATE*® test device).

Storing duration	Temperature	Rapid Test			
		ACCURATE ® test device		*ACON* ® One Step Test Device	
		Sensitivity %	Specificity %	Sensitivity %	Specificity %
2 weeks	37°C	96	100	96	100
	45°C	96	100	96	100
	50°C	96	100	96	100
4 weeks	37°C	96	100	96	100
	45°C	96	100	94	100
	50°C	96	100	92	100
8 weeks	37°C	92	100	92	100
	45°C	92	100	90	100
	50°C	84	100	84	100

Table 3: Sensitivity and specificity of DBS stored at 37°C, 45°C, and 50°C tested with the two rapid tests after storage for two, four and eight week.

Gold) and reported that the specificity were 100%, 80%-89% and 73%-80% respectively.

All HIV negative DBS were negative at all temperature and time (specificity 100%).

In the current study, anti-HIV antibodies show good stability on DBS when stored at 37ºC and 45ºC for up to eight weeks. DBS stored at 50ºC were stable up to four weeks, but when stored for eight weeks, sensitivity was progressively declined. Specificity was not affected with temperature or time of storage.

Rapid test can be used successfully with DBS. In country with high temperature such as Sudan, DBS can be stored at ambient temperature without need for refrigerators up to two weeks.

Acknowledgements

Special thanks to all staff of Medical Microbiology department, Faculty of Medical Laboratory Sciences, Al-Neelain University, for their support and continues assistance through the whole process of this research. We would like to express our deep gratitude to Omar Nemairy center, Umdurman Teaching Hospital.

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