Application of Dried Blood Spots on Filter Paper for Detection of HIV Antibodies: Effect of Temperature and Duration of Storage

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ABSTRACT

Background: Acquired immunodeficiecy syndrome (AIDS) is now adays a worldwide health problem affecting both the developed and developing countries. It has greater burden on the developing countries because of the increased cost of testing including specimen collection, transport, storage and laboratory examination. Moreover the present technique of collecting blood for diagnosis is associated with increased risk of infection, loss and contamination of specimen. This study aimed at evaluating the use of blood spot dried on filter paper (under different storage conditions) for detection of HIV antibodies in patient's serum as an alternative cheap and relatively safe method.

Materials and methods: venous blood was collected from 100 known HIV infected patients and 50 healthy volunteers. Filter papesr (Whattman number 1) were soaked with the specimens, air-dried and then divided into 3 groups. Each group is containing a sample from each subject under study. The three groups were subjected to different storage conditions (room temperature, incubator at 37 ^{o} C and refrigerator at 4^{o}C). PBS elutes from each group were tested for HIV antibodies using ELISA at regular intervals (48 hrs., 7,15, 30, 45, 60, 75, 90 and 105 days).

Results: All the dried blood on filter paper remained positive for HIV antibodies for one month. Thereafter some of the specimens started to progressively show negative results. Specimens stored in the refrigerator were more stable for HIV antibodies than those kept at room temperature and the incubator.

Discussion: In this study, the results of testing elutes of dried blood spots on filter paper for HIV antibodies were found to be 100% positive in concordance with those of testing serum samples. HIV antibodies were stable in all specimens for one month under the different storage conditions.

Conclusion: dried blood spot on filter paper can remain positive for HIV antibodies for at least one month under different storage conditions. It can therefore be recommended as a cheap, simple and reliable technique for collection of blood for HIV testing under field surviellance as it does not require complex setting and instruments and moreover it eases transportation of specimen.

Key words: immunodeficiency syndrome (AIDS), storage, heparinized, ELISA.

Acquired immunodeficiency syndrome (AIDS) first appeared as a health problem in the summer of 1981. Isolation of the etiological agent was first reported in 1983 by Luc Montagnier and his colleagues from Pasteur institute in Paris[1].

In 1985, the first serological test named enzyme linked immunosorbent assay (ELISA) became available for detection of Human Immunodeficiency Virus (HIV) antibodies. This made possible a more realistic estimation of the extent of HIV infection[2]. The laboratory diagnosis is usually achieved by the detection of antibodies to HIV in patient serum or plasma using ELISA or any screening test followed by a confirmatory test. HIV/AIDS is nowadays an important health problem in Sub-Saharan Africa, despite that

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the epidemiology of HIV in many countries in this region (including Sudan) is not yet clear. The reason for that is the sample collection which is expensive and is associated with hazard of encountering the infection to the collector. Collection of blood needs many tools such as: syringes, vacutainers, centrifuges and cryogenic tubes to separate the serum. Special measures for transportation and storage are also needed. These collectively increase the cost of HIV screening. For these reasons the need for simpler and more affordable laboratory methods for sample collection becomes of major importance.

This study was done to evaluate the stability of HIV antibodies in a dried blood spot on filter paper as a simple less expensive and safe method of collection of blood for diagnosis of HIV infection.

Materials and methods

Study area:

The study was conducted in Khartoum Teaching Hospital AIDS Unit, where confirmed cases of HIV infection allover the state are cared for.

Study population:

Confirmed HIV infected individuals admitted to the hospital during the period of the study from November 2003 to March 2004 were recruited. A total of 100 patients were included in the study and 50 persons known to be negative for HIV by conventional testing were included as control group.

Data collection:

The purpose and research procedure were first explained to each subject. After a verbal consent to participate in the study, data were collected from each individual under study by interviewed questionnaire.

Sample collection:

From each patient and control subject under study 5 mls of venous blood were collected. Three mls were added to sterile plain container and 2mls into heparinized container for preparation of dried blood spot on filter paper strips.

Calibration of the volume of blood absorbed by filter paper:

The first step was to determine the size of Filter paper (Whatman No 1, Whatman Company) soaked by known volumes of blood. Filter papers were cut into pieces of different lengths but with an equal diameter of one Cm. Using an automatic pipette different volumes (10 µl, 20 µl, 30 µl, 40 µl and 50 µl) of heparinized venous blood were added into different areas of a clean glass slides. Pieces of filter paper were used to absorb the different volumes of blood on the slides. By touching the surface of the blood by the tips of the pieces of filter paper and allowing blood to soak the filter paper by capillary pressure until the whole volume of blood was completely absorbed and then the length of the filter paper soaked by each blood volume was measured by a ruler. This was repeated 10 times for each of the volumes mentioned above. The average length of filter paper soaked by each volume of blood was calculated.

The final results obtained were approximated and found to be as follows:-

10 µl, 20 µl, 30 µl, 40 µl, and 50 µl of the blood spread on the paper to distances of 5 mm, 10 mm, 15 mm, 20 mm and 25 mm respectively.

Sample processing:

The unheparinized blood specimens were allowed to clot and after clot retraction centrifuged and serum separated into cryogenic tubes and kept in refrigerator at 4-8°C until tested. From the heparinized blood as much as possible strips of filter paper (Whattman no 1) with diameter of 1cm were prepared as previously described. The total length of strips aimed at was 45cm for each blood sample. For each patient the strip of filter paper were divided into 3 groups, each group including strips soaked with blood with a total length of 15cm. One group was stored

at room temperature (20°C -25°C). The 2nd group stored in
refrigerator at 4°C to 8°C. The 3rd group stored at 37 °C (in incubator). Strips for the control subject were treated in the same way as the test subjects. All strips were stored in plastic bags, tightly closed to avoid humidity.

Laboratory examination:

a) Venous blood

Venous bloods were tested for HIV antibodies by ELISA (Human, Human company) as described by manufacturer. The negative controls were also tested for HIV antibodies.

b) Dried blood spot on filter paper:

Elution of blood:

The day before the assay was to be performed; strips of dried blood spot were removed from the bags. Strips of 2cm length (approximately soaked with 40 µl of blood) were cutout for each subject and placed in disposable glass test tubes.200 µl of Elution buffer were added to each tube. The tubes were covered tightly. The total volume aimed at was 200 µl with concentration of 40 µl kept in refrigerator at 4-8°C for 24 hr then samples were removed from refrigerator and rotated at 350 rpm for 15 min and left for 2h.at room temperature then tested for HIV antibodies by ElISA (Human, Human company)

Test procedure

The microtitre strips were washed before use by adding working wash solution, incubated for 30 sec. Then 100 µls of the diluent were added to all micro wells for the control wells (three negative controls and two positive controls). 20 µls from each control were added to the corresponding wells. 120 µl of the elutes were added to the corresponding wells. The strips were incubated at 37°C for 30 minutes. After incubation the strip was washed 5 times using working wash solution .Then 100 µls of the working conjugate were added to all wells and incubated for 15 minutes at 37°C. The strips were then washed 5 times and 100 µls of the substrate added to all wells and incubated for 15 minutes at room temperature. The reaction was stopped by addition of 100 µls of sulfuric acid. The optical density for the samples and controls was measured by spectrophotometer using filter 450 nm.

Validation of the test

The test was validated and the cut off point calculated according to the manufacturer's instructions. Samples with optical density equal or more than the cut off were considered as positive. The optical density less than cut off were considered negative

Results

All blood samples (100) from the confirmed HIV positive cases proved to be positive by ELISA test and all the 50 negative control samples were negative for HIV antibodies. The sensitivity and specificity of testing elutes of dried blood spot on filter paper for HIV antibodies was estimated to be 100%. Results of dried blood spot on filter paper were found to be 100% in concordance with the result of serum.

Dried blood spot on filter paper:-

Control samples: - Dried blood spot on filter paper were found to be negative for HIV antibodies on days 2, 7, 15, 30,45,60,75 and 90 without any false positive results

Samples kept at room temperature:

All dried blood samples continued to be positive for HIV antibodies for one month, 95% remained positive after 45days, 75% for 60 days, 40% for 75days and all were negative after 90days (Fig. 1).

Samples kept in incubator at 37°C:

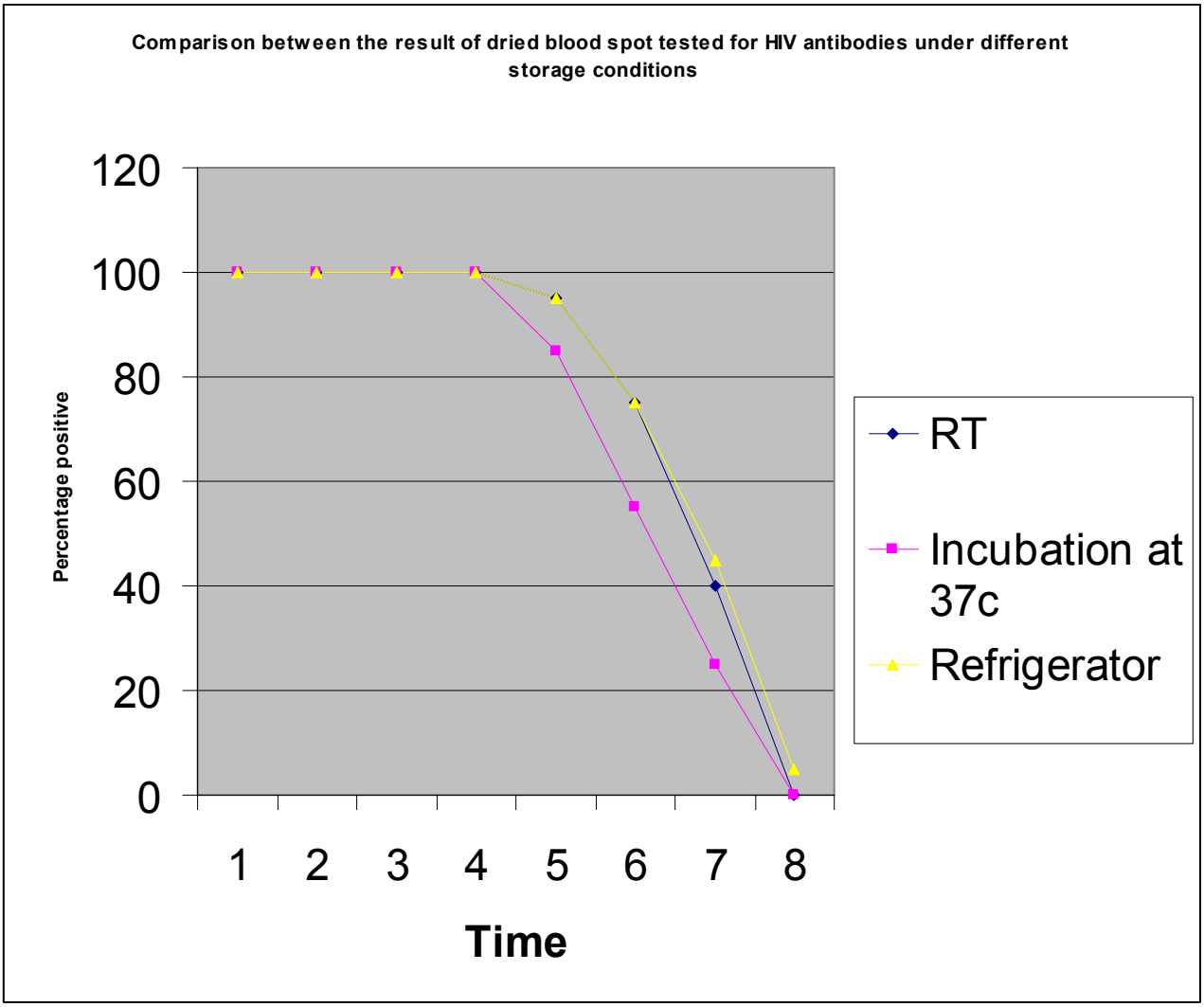
All dried blood samples continued to be positive for HIV antibodies for one month, 85% for 45days, 55% for 60 days, 25% for 75days and all were negative after 90 days (Fig. 1).

Samples kept in the refrigerator:

All samples continued to be positive for HIV antibodies for one month, 95% for 45days, 75% for 60 days, 45% for 75days, 5% remained positive after 90 days and all were negative after 105 days (Fig. 1).

The cost of collection of specimens:

Each sample collected by conventional method was estimated to cost approximately 2.25 SDG, but by filter paper collection was estimated to cost 0.7 SDG.



RT = Room temperature

1 = 2 days, 2= 7 days, 3= 15 days, 4= 30 days, 5= 45 days, 6= 60 days, 7= 75 days, 8 = 90 days.

Fig 1: HIV antibodies at different conditions and at different times.

Table (1): Comparison between the cost of collection of one specimen by the conventional venous puncture and dried blood spot

Item	Cost by Sudanese Pounds (SDP)	
	Conventional method	Dried blood spot
Syringe	0.25	Not needed
Test tube	1.0	Not needed
Cryogenic tube	1.0	Not needed
Filter paper	Not needed	0.2
Lancet	Not needed	0.5
Total	2.25	0.7

Discussion

In this study all known HIV positive samples dried on filter paper gave positive result and all the negative samples gave negative results giving sensitivity and specificity of hundred percent. All specimens (100%) collected and dried on filter paper strips and stored under different condition remained positive for HIV antibodies for one month irrespective of the storage condition whether at room temperature, incubator at 37°C, or refrigerator at 4°C. However there was gradual reduction in the optical density (OD) of the specimens thereafter.

The specimens stored at room temperature: all (100%) remained positive for one month and all became negative after 90 days. This is consistent with a study done in Tokyo, in which recovery of HIV antibodies from finger-stick blood dried on filter paper after elution was comparable to those obtained by recovering antibodies from serum[3].

The results also agrees with another study done to detect human immunodeficiency virus type 1 (HIV-1) infection in infants in which maternal antibodies remained stable on dried blood spot for 3 months. Stability of HIV antibodies was similar to literature in which the dried blood spot samples remained stable at 22°C for three months[4].

The specimens stored at 37°C: all 100% were positive after month, and all become negative after 90 days. This goes with earlier reports which demonstrated that dried blood spots sample on filter paper stored at 37°C remained stable for HIV antibodies up to 7 weeks[5].

The specimens stored in refrigerator: they were all (100%) stable for HIV antibodies for up to one month, and 5% continued positive after 90 days. This contrasts the study done by Florence Fenollar and Didier Raoult, which demonstrated that the recovery of antibodies from finger-stick blood dried on filter paper after elution produces results comparable to those obtained by recovering antibodies from serum, and storing paper samples for 1 month at 4°C did not significantly affect the level of antibodies recovered[6]. Most studies were in agreement with this study except for small differences in the stability time, for example dried blood spot PSA specimens were stable for more than 14 days when stored at room temperature and for greater than 28 days when stored at -20 °C.

This study demonstrates that dried blood samples yielded results as accurate as collected venous samples. Test performance using the dried blood strip yielded 100% sensitivity and 100% specificity, or 100% concordance between conventionally collected venous blood and the dried blood spot samples. Collection of finger prick sample on filter paper was estimated in this study to cost 0.7 SDG per one sample compared to 2.25 SDG for the collection of one sample of venous blood.

Collection of 100 specimens using finger prick of blood dried on filter paper can save 218 SDG [104$] more than the conventional blood collection for screening testing.

Conclusion

Testing of dried blood spot on filter paper for HIV antibodies using ELISA was found to have sensitivity and specificity of 100%. Under different storage condition dried blood spot on filter paper remained stable for HIV antibodies for one month and deterioration into negative result start to occur in some specimen according to the storage condition.

Preservation of dried blood spot in refrigerator proved to relatively keep stability of HIV antibodies for longer period than the other methods. The use of dried blood spot for HIV antibodies was found to be a cheap, and a reliable method, for HIV antibody screening.

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