Dry Blood Spots As Alternative To Conventional Serum Samples In Diagnosting Some Viral Infections

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Abstract

The purpose of this work was to validate the utility of dried blood spot (DBS) samples for diagnosting HBV infection in comparison with the conventional serum samples.

Pair-matched blood samples obtained from six HBsAg positive and four HBsAg negative persons by two ways; venipuncture and fingerstick by lancet and collect blood drops on filter papers (Zelpa type). Blood spots left to dry for two hours on flat bench then placed in nylon bag with dessicant to reduce humidity and stored in the refrigerator for one week. Before examination, blood spots were removed from filter papers and placed in tubes containing phosphate-buffered saline plus tween 80 and left overnight. ELISA for HBsAg screening was applied on sera and blood spot samples. Statistical analysis was done using pearson correlation test to test the optical densities of samples.statistical analysis showed no significant differences between serum and DBS samples and there is a strong linear correlation between the two types of samples.

Dried blood spots can be used as successful alternative for serum samples in diagnosing certain viral diseases.

Keywords: Dried blood spot (DBS), Enzyme linked immunosorbent assay (ELISA), Hepatitis B surface antigen (HBsAg).

بقع الدم الجاف كبديل لعينات المصل التقليدية في تشخيص بعض الاصابات الفايروسية

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الملخص

الهدف: تم اجراء هذا البحث لغر ض اختبار صلاحية عينات بقع الدم الجافة في تشخيص الاصابة بفايرس التهاب الكبد نمط بي بالمقارنة مع عينات المصل التقليدية.
المواد وطرق العمل: تم اخذ عينات دم مزدوجة لستة مرضى موجبين لفحص HBsAg وأربعة سالبين للفحص للمقارنة وبطريقتين (طريقة السحب من الوريد وطريقة وخز الابهام وجمع قطرات الدم على اوراق ترشيح من نوع Zelpa). تركت الاوراق لتجف على سطح مستوي لمدة ساعتين. بعد ذلك وضعت اوراق الترشيح في كيس نايلون مع مادة مانعة للرطوبة desiccant وخزنت في الثلاجة لمدة اسبوع. قبل الفحص تم رفع المناطق الحاوية على قطرات الدم الجاف ووضعها في انابيب تحوي دارئ الفوسفات الملحي مع منظف tween 80 لغرض الفحص في اليوم التالي.
أجري فحص الاليزا على عينات المصل والدم الجاف للكشف عن المستضد السطحي للفايرس HBsAg.
النتائج: اظهرت النتائج عدم وجود فروق معنوية بين عينات الدم الجاف وعينات المصل وكذلك وجود علاقة خطية قوية بين قيم عينات المصل وعينات الدم الجاف.
الاستنتاج: يمكن استخدام عينات الدم الجاف كبدائل ناجحة لعينات المصل ان لم تكن ذات اولوية في تشخيص بعض الاصابات الفايروسية.

الكلمات المفتاحية :- يقع الدم الجافة (DBS) , فحص الاليزا (ELISA) , المستضد السطحي للفايروس (HBsAg)

Introduction

It is clear that the availability of clinical specimens that can be easily collected, stored and transported is advantageous in areas that lack appropriate infrastructure for blood processing. Furthermore, obtaining the required amount of blood from neonates and certain ages is an operation not empty from difficulties. Moreover, venipuncture is a relatively invasive procedure that must be performed by a trained phlebotomist (usually in a clinical setting), and it requires readily accessible facilities where blood samples can be promptly processed and stored under controlled conditions. Consequently, assays using whole blood dried on filter paper may provide a viable alternative.

The concept that capillary whole blood obtained by heel or fingerprick and blotted on to a filter paper (Guthrie card), could be used to screen for metabolic disease in large populations of neonates was introduced in Scotland by Guthrie and Susie in 1963(1). Neonatal screening for phenylketonuria became nationwide in 1969/70. Since then, Guthrie card samples from babies have been collected routinely in over 20 countries to screen for phenylketonuria and more recently for congenital hypothyroidism and sickle cell disorders(1,2).The detection of markers of disease, such as medium chain acyl CoA dehydrogenase (MCAD deficiency)(3), human chorionic gonadotrophin (hCG) in Down syndrome(4), and glycated haemoglobin in insulin dependent diabetes(5), and the estimation of drug levels have been also been investigated(6-8). The use of dried blood spots in Virology was restricted for many years because of limitations of sensitivity and specificity when screening such small volumes of blood (equivalent to 5-10 ul)(7).

Currently, dried blood spot samples have proved useful in detection, quantification and identification of avariety of infectious pathogens including viruses (10-19), bacteria (20-25), and different parasitic (26-31) and helimenthic infections (32,33).

Materials & methods

Six HBsAg positive and four HBsAg negative control subjected for this study. Blood samples obtained from subjects by two ways ; 2ml of blood drown by venipuncture, and blood obtained by fingerbrick as follows:

1- The finger was cleaned with alcohol – rinsed cotton then punched by asterile lancet.

2- A filter paper (zelpa type, Belgium,11.0 cm, thickness: 0.33mm) was lightly touched to alarge blood drop. Three drops of blood were collected on separated locations of the filter paper for each person tested.

3- The blood spots were allowed to dry for tow hours on aflat surface.

4- Blood spots were kept for seven days at refrigerator (2 – 8 C̊).

Blood spot preparation

1- Dried blood spots were cut from filter papers by scissors avoiding attachment of the spot borders.
2- Each blood spot was transferred to kahn tube containing 1ml of phosphate buffered saline containing phenol and 0.05% tween 80 and left for 24hrs at refrigerator.
3- Before testing, the tubes were shaked several times to homogenate the reactants and centrifuged at low speed for 5 minutes.The supernatant was used for further analysis.
4- The supernatant obtained from the previous step and serum samples were tested for HBsAg by ELISA screening test (HBsAg ELISA kit, Plasmatec, UK).

Statistical analysis was performed using spss 17 version software. pearson correlation test was uesd to compare the significance of the differences between the two types of sampling methods.

Results & discussion

The results of optical densities of DBS, serum, and HBsAg negative samples as detected by ELISA are shown in the table below.

Sample Number	O.D readings			Cut off Value
	HBsAg (+ve)		HBsAg (-Ve)	
	serum	DBS		
1	2.8	2.0		0.14
2	2.1	1.7		
3	2.4	2.0		
4	2.7	2.2		
5	2.4	1.8		
6	1.6	1.2		
7			0.11	
8			0.13	
9			0.09	
10			0.14	

Correlation between blood spot and serum values found to be linear and high (Pearson correlation *R* 5 0.941).

Several community-based applications have shown dried blood spot sampling method to be a convenient and reliable means to facilitate sample collection, storage, and transportation, and laboratory methods have been validated for a growing number of analytes. This study aproved that DBS samples are an effective alternative to conventional serum samples in HBV diagnosis . moreover DBS sampling method has many advantages in which:

1- Sample do not requires highly trained technician. It can be collected on filter paper easily by nonmedical personnel: The patient's finger or heel in neonate is pricked with a sterile, disposable lancet, and a drop of blood (50 µL) is spotted onto standardized filter paper.

2- Dried blood spot samples are a simple and inexpensive sampling method, especially useful for blood collection in resource-poor settings with limited access to diagnostic facilities.

1. Traditionally, the monitoring of HBV infection is done by serological assays involving serum and plasma samples that require frozen storage conditions. Blood samples must be processed within 6 hours of collection. If the assay is not available immediately, the samples must be frozen at -20 for serum, or -70 degree C for plasma. In developing countries where cold storage and transportation present special problems, the use of DBS should be considered since DBS samples are stable for long periods of time, and can be transported to a reference laboratory at minimal cost (34-36).

3- Non-invasive, less hazardous does not involve the risks associated with the use and disposal of needles and syringes.

4- Many viruses, especially enveloped such as HIV-1 and -2, human T cell leukaemia/lymphoma virus (HTLV) –I and -II, and hepatitis C virus (HCV) that are known to be present in serum or plasma lose infectivity owing to disruption of their envelope on drying

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