

Dry Blood Spots As Alternative To Conventional Serum Samples In

Diagnosing 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 diagnosing 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 (Zelpha 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 لغرض اختبار صلاحية عينات بقع الدم الجافة في تشخيص الاصابة بفايروس 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 Zelpha). تركت الاوراق لتجف على سطح مستوي لمدة ساعتين. بعد ذلك وضعت اوراق الترشيح في كيس نايلون مع مادة مانعة للرطوبة desiccant وخزنت في الثلاجة

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لمدة اسبوع. قبل الفحص تم رفع المناطق الحاوية على قطرات الدم الجاف ووضعها في انابيب تحوي دارئ الفوسفات الملحي مع منظم 80 tween لغرض الفحص في اليوم التالي.

أجري فحص الاليزا على عينات المصل والدم الجاف للكشف عن المستضد السطحي للفايروس 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 كبديل 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 a variety of infectious pathogens including viruses (10-19), bacteria (20-25), and different parasitic (26-31) and helminthic 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 drawn by venipuncture, and blood obtained by fingerprick as follows:

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

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2- A filter paper (zelpa type, Belgium, 11.0 cm, thickness: 0.33mm) was lightly touched to a large 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 two hours on a flat surface.

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

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 a 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 shaken 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 used 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 $R = 0.941$).

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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 μ L) 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

References

1- Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* 1963, 32:338-343.

PubMed Abstract

2. Alvarez-Munoz MT, Zaragoza-Rodriguez S, Rojas-Montes O, Palacios-Saucedo G, Vazquez-Rosales G, Gomez-Delgado A, *et al.* High correlation of human immunodeficiency virus type-1 viral load measured in dried-blood spot samples and in plasma under different storage conditions. *Arch Med Res* 2005, 36:382-386. PubMed

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3.

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4. Dezateux C. Evaluating newborn screening programmes based on dried blood spots: future challenges. *Br Med Bull.* 1998;54(4):877-90.
5. Parker, SP and Cubitt, WD. The use of the dried blood spot sample in epidemiological studies. *J Clin Pathol.* 1999 september; 52(9):633-639.
6. Ramakrishnan Lakshmy, and Ruby Gupta. Measurement of Glycated Hemoglobin A1c from Dried Blood by Turbidimetric Immunoassay. *J Diabetes Sci Technol.* 2009 September; 3(5): 1203–1206.
7. Beaudette P, et al. Discovery Stage Pharmacokinetics Using Dried Blood Spots, *J Chromatograph B.* 2004,809:153-158.
8. Barfield M, et al. Application of Dried Blood Spots combined with HPLC-MS/MS for the quantification of acetaminophen in toxicokinetic studies, *J Chromatograph B.* 2008,870:32-37.
9. Spooner N. et al. Dried Blood Spots as a Sample Collection Technique for the Determination of Pharmacokinetics in Clinical Studies: Considerations for the Validation of a Quantitative Bioanalytical Method, *Anal Chem.* 2009,81:1557-1563.
10. Beck IA, Drennan KD, Melvin AJ, Mohan KM, Herz AM, Alarcon J, et al. Simple, sensitive, and specific detection of human immunodeficiency virus type 1 subtype B DNA in dried blood samples for diagnosis in infants in the field. *J Clin Microbiol* 2001, 39:29-33. PubMed Abstract | Publisher Full Text | PubMed Central Full Text
11. Jardi R, Rodriguez-Frias F, Buti M, Schaper M, Valdes A, Martinez M, et al. Usefulness of dried blood samples for quantification and molecular characterization of HBV-DNA. *Hepatology* 2004, 40:133-139. PubMed Abstract | Publisher Full Text
12. Barbi M, Binda S, Caroppo S. Diagnosis of congenital CMV infection via dried blood spots. *Rev Med Virol* 2006 , 16:385-392. PubMed Abstract | Publisher Full Text
13. Behets F, Kashamuka M, Pappaioanou M, Green TA, Ryder RW, Batter V, et al. Stability of human immunodeficiency virus type 1 antibodies in whole blood dried on filter paper and stored under various tropical conditions in Kinshasa, Zaire. *J Clin Microbiol* 1992, 30:1179-1182. PubMed Abstract
14. Youngpairoj AS, Masciotra S, Garrido C, Zahonero N, de MC, Garcia-Lerma JG. HIV-1 drug resistance genotyping from dried blood spots stored for 1 year at 4 degrees C. *J Antimicrob Chemother* 2008, 61:1217-1220. PubMed Abstract | Publisher Full Text | PubMed Central Full Text
15. Mcdade T W, Stallings J F, Angold A, Costello E J, Burleson M, Cacioppo J T, Glaser R., and Worthman C M. Epstein-Barr Virus Antibodies in Whole Blood Spots: A Minimally Invasive Method for Assessing an Aspect of Cell-Mediated Immunity. *Psychosomatic Medicine* 2000, 62:560–567.

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Diagnosing Some Viral Infections

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16. Barbi, M., S. Binda, V. Primache, S. Caroppo, P. Dido, P. Guidotti, C. Corbetta, and Melotti D. Cytomegalovirus DNA detection in Guthrie cards: a powerful tool for diagnosing congenital infection. *J. Clin. Virol.* 2000, 17:159-165[Medline]
17. Barbi, M., S. Binda, Primache V, Luraschi C, and Corbetta C. Diagnosis of congenital cytomegalovirus infection by detection of viral DNA in dried blood spots. *Clin. Diagn. Virol.* 1996, 6:27-32.[Medline]
18. Barbi, M., S. Binda, V. Primache, and C. Novelli. Cytomegalovirus in peripheral blood leukocytes of infants with congenital or postnatal infection. *Pediatr. Infect. Dis. J.* 1996, 15:898-903.[Medline]
19. Binda, S., S. Caroppo, P. Dido, V. Primache, L. Veronesi, A. Calvario, A. Piana, and Barbi, M. Modification of CMV DNA detection from dried blood spots for diagnosing congenital CMV infection. *J. Clin. Virol.* 2004, 30:276-279.[Medline]
20. Johansson, P. J., M. Jonsson, K. Ahlfors, S. A. Ivarsson, L. Svanberg, and C. Guthenberg. Retrospective diagnostics of congenital cytomegalovirus infection performed by polymerase chain reaction in blood stored on filter paper. *Scand. J. Infect. Dis.* 1997, 29:465-468.[Medline]
21. Crawford D, C, Zimmer Sh, M, Morin C, A, Messonnier NE, Lynfield R, Qian Yi, Shephard C, et al. Integrating Host Genomics with Surveillance for Invasive Bacterial Diseases. *Emerg Infect Dis.* 2008 July; 14(7): 1138-40.
22. Patil SA, Ramu G, Sinha S, et al. Screening of anti-M leprae antibodies in the blood-samples eluted from filter-paper blood blots. *IJut* 7 Leprosy 1990, 58:123-6.
23. Chanteau S, Cartel JJ, Boutin JP, et al. Evaluation of gelatin particle agglutination assay for the detection of anti-PGLI antibodies-comparison with ELISA method and applicability on a large-scale study using blood collected on filterpaper. *Leprosy Rev* 1991, 62:255-61.
24. Huang Z, Dwyer B, Kaldor J. Helicobacter-pylori serology using specimens collected on filter-paper. *Clin Pathol* 1991, 44:167-9.
25. Takkouche B, Iglesias J, Alonso-Fernandez JR, et al. Detection of Brucella antibodies in eluted dried blood: a validation study. *Immunol Lett* 1995, 45:107-8.
26. Coates G, Guarenti L, Parker S, et al. Evaluation of the sensitivity and specificity of a Treponema pallidum dried blood spot technique for use in the detection of syphilis. *Trans R Soc Trop Med Hyg* 1998, 92:44.
27. Maeno Y, Nakazawa S, Dao ID, Yamamoto N, Giang ND, Van HT, et al. A dried blood sample on filter paper is suitable for detecting Plasmodium falciparum gametocytes by reverse transcription polymerase chain reaction. *Acta Trop* 2008, 107:121-127. PubMed Abstract | Publisher Full Text
28. Perez HA, Bolivar J. The feasibility of filter-paper collected blood for the serodiagnosis of malaria. *Meni Inst Oswaldo Cruz* 1989, 84:587-8.
29. Hsu HW, Grady GF, Maguire J, et al. Newborn screening for congenital Toxoplasma infection: five years experience in Massachusetts, USA. *Scand J Infect Dis* 1992, 84:59-64.

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30. Patel B, Holliman RE. Antibodies to *Toxoplasma-gondii* in eluates from filter-paper blood specimens. *Br. J. Biomed. Sci.* 1994;51:104-8.
31. Altukhi MH, Ackers JP, Alahdal MN, et al. Enzyme-linked immunosorbent-assay for the detection of anti-*Giardia* specific immunoglobulin-G in filter-paper blood-samples. *Trans R Soc Trop Med Hyg.* 1993;87:36-8.
32. Gupta S, Srivastava JK, Ray S, et al. Evaluation of enzyme-linked immunosorbent assay in the diagnosis of kala-azar in Malda district (West Bengal). *Ind Med Res Sect A Infect Dis.* 1993;97:242-6.
33. Evengard B, Hagi H, Linder E. A filter-paper technique for the detection of IgG and IgM class schistosome-specific antibodies in an endemic area. *Ann Trop Med Parasitol.* 1988;82:307-9.
33. Terhell AJ, Haarbrink M, Abadi K, et al. A filter paper technique for the detection of anti-filarial IgG4 in lymphatic filariasis. *Trans R Soc Trop Med Hyg* 1996, 90:196-8.
34. Karlsson H, Guthenberg C, von DU, Kristensson K. Extraction of RNA from dried blood on filter papers after long-term storage. *Clin Chem* 2003, 49:979-981.
- PubMed Abstract | Publisher Full Text
35. Zhang YH, McCabe ER. RNA analysis from newborn screening dried blood specimens. *Hum Genet* 1992, 89:311-314. PubMed Abstract | Publisher Full Text
36. Matsubara Y, Ikeda H, Endo H, Narisawa K. Dried blood spot on filter paper as a source of mRNA. *Nucleic Acids Res* 1992, 20:1998. PubMed Abstract.