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SHORT PAPER

Detection of antibodies against hepatitis A in blood spots dried on filter paper. Is this a reliable method for epidemiological studies?

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SUMMARY

Diluted dried blood drops on filter paper were compared with serum samples as a specimen source for qualitative anti-HAV antibody determination by ELISA. A total of 298 serum samples and dried blood drops were collected from a population of healthy adolescents ($15{\cdot}3 \pm 1{\cdot}2$ years old). The prevalence of anti-HAV antibody obtained by testing serum samples was 7·7% (95% CI: 4·8–10·1). Compared with serum sampling the sensitivity and specificity of diluted dried blood drops were 91·3 and 99·3%. The positive and negative predictive values were 91·3 and 99·3%, respectively, and the likelihood ratios of positive and negative results were 91 and 0·09. It is proposed that this test represents a reliable procedure for anti-HAV antibody testing.

Large-scale seroepidemiological surveys are important in assessing the prevalence of different infections, as well as providing data for the design and evaluation of methods of disease control [1]. However, these studies often require facilities not available in the countries where they should be carried out [2]. Frequently, it is relatively easy to collect blood samples by venepuncture, but problems are encountered due to inadequate storage facilities or means of transport [1]. These problems are increased when the population under investigation is located in a remote area [1, 2]. Blood samples dried on a piece of filter-paper have been used in various screening tests, including that for metabolic disorders [3, 4] and detection of markers of hepatitis B infection [2, 5, 6]. The present study was designed to assess the accuracy of anti-HAV antibody ELISA determination in diluted dried blood drops on filter paper.

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Blood samples were obtained for a cross-sectional seroepidemiological study carried out in students of a public school of Madrid, Spain. The study protocol was approved by the Research Ethics Committee of Hospital San Carlos, School of Medicine, Madrid. Written informed consent was obtained from parents and participants.

On recruitment day all students between 14 and 17 years of age were invited to participate in the study. Four drops of whole blood [spots of at least 15 mm diam.] were obtained by finger-stick, collected on filter paper (Schleicher and Schuell 2992, Acefe S.A., Barcelona, Spain), air-dried and stored at +4 °C until antibody determination 90 days later. A 10 ml venous blood sample was simultaneously obtained by venepuncture. This sample was centrifuged; serum was separated and frozen at −20 °C until antibody determination 90 days later.

Total anti-HAV antibody in serum samples was determined by ELISA method (IMx, Abbott Laboratories, Chicago, Ill, US). Spots were eluted with 1 ml

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Table 1. *Results obtained by ELISA anti-HAV antibody testing of serum samples and diluted dried blood spots*

	Serum samples		
Test results	Positives	Negatives	Total
Blood spots			
Positives	21	2	23
Negatives	2	273	275
Total	23	275	298

of 0·115% saline solution plus 1·5% bovine albumin for 12 h at room temperature [6]. Eluates were tested by ELISA method (IMx, Abbott Laboratories, Chicago, Ill, US). All antibody determinations were performed in the laboratory of the Department of Public Health, School of Medicine, Complutense University, Madrid, Spain.

Prevalence (and the corresponding 95% confidence intervals) of anti-HAV antibody obtained by both methods was calculated and compared by Chi-square test. Sensitivity, specificity, negative and positive predictive values anti-HAV antibody ELISA determination in diluted dried blood drops on filter paper were calculated (with the 95% CI), using as reference the anti-HAV antibody determination in serum samples. All statistical analyses were carried out using EPIINFO 6.0 software (CDC, Atlanta, GA, US). The likelihood ratio for positive and negative results of the test was also calculated as previously described [7].

A total of 298 serum samples and blood samples on filter paper were collected in a population of healthy adolescents (15·3 ± 1·2 years old; 48% males). The prevalence of anti-HAV antibody obtained on serum samples was 7·7% (95% CI: 4·8–10·1). Table 1 shows the results obtained by anti-HAV determination on serum samples and diluted dried blood drops. The calculated sensitivity and specificity for anti-HAV antibody ELISA testing of diluted dried blood drops were 91·3% (95% CI: 70·5–98·5) and 99·3% (95% CI: 97·1–99·9), respectively. The positive and negative predictive values for this test were 91·3% (95% CI: 70·5–98·5) and 99·3% (95% CI: 97·1–99·9), and the likelihood ratios of positive and negative results were 91 and 0·09, respectively.

Detection of HBsAg on the eluates from dried blood spots was described by Farzadegan and colleagues in 1978 [5]. Later this technique was applied to other hepatitis B-related markers [2, 6] and other viral diseases including hepatitis A [8–11]. The accuracy of a screening test should be determined by verifying the results of the test against a reference standard that defines true infection status [12]. The ELISA test has been shown to be highly sensitive and specific for screening of the presence of anti-HAV antibody in serum samples [13, 14], and therefore it can be considered as a reference standard for the assessment of the efficiency of the test performed on eluted dried blood spots.

The results obtained in this study indicate that anti-HAV is stable when dried on filter paper and that ELISA determination on diluted dried blood is a reliable method for anti-HAV determination. In addition, this test provides a high level of true positive results with a minimum of false positives; and the same can be applied to negative results [7]. However, it is foreseeable that the sensitivity of this test might decrease after the introduction of mass vaccination against hepatitis A, when levels of antibody in vaccinated individuals may be lower than those reached by natural infection [15]. This problem may be solved by increasing the size of the blood spot to obtain a higher concentration of the solution after this is eluted [6].

This technique would be suitable for epidemiological studies or large-scale population screening projects. Samples on filter paper are easy to obtain (specially in groups of population, as newborns or children, from whom it is difficult to obtain venous blood or who may refuse this procedure), to handle (resistance to unintentional breakage or spillage), to store and to be readily transported or posted from remote areas [2, 5]. In addition, this test has in common with other techniques, such as salivary or urinary antibody testing, that many infectious diseases markers can be determined, including HAV and hepatitis B virus [1, 2, 5, 6, 11, 16, 17]. In contrast with these techniques, blood spots dried on filter paper have the additional advantage of being suitable for detecting haemoglobinopathies and metabolic disorders [3, 4].

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