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Detection of antibodies against hepatitis A virus in eluates of blood spotted on filter-paper: a pilot study in Rio de Janeiro, Brazil

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Abstract

The validity of blood spotted on to filter-paper (BSOFP) eluates for the detection of antibodies against hepatitis A virus (HAV) was investigated in 718 individuals (children and adults) during a field study in a small area in Rio de Janeiro State, Brazil. Serum samples were considered the 'gold standard'. BSOFP eluates were analysed by 2 different techniques: microplate competitive enzyme-linked immunosorbent assay (ELISA) of the whole study group and microparticle enzyme immune assay (MEIA) of a subsample of 59 individuals. For BSOFP eluates by ELISA, sensitivity and specificity were 89·6% (95% CI: 84·7–93·1) and 97·5% (95% CI: 95·6–98·7), respectively. For a seroprevalence of anti-HAV antibodies of 32%, the positive predictive value was 94·5% (95% CI: 90·3–97·0) and the negative predictive value was 95·2% (95% CI: 92·8–96·8). The test efficiency was 95·0% (95% CI: 93·1–96·4). Similar results were found for BSOFP eluates by MEIA. Agreement between the 2 techniques used for BSOFP (ELISA and MEIA) was also high ($\kappa = 0.93$). These results encourage the more widespread application of BSOFP as a means of surveillance for large-scale epidemiological studies for hepatitis A.

Keywords: hepatitis A, epidemiological survey, filter paper blood collection, validation, Brazil

Introduction

Epidemiological surveys of antibody prevalence provide useful information for assessing the immune status in populations. Serological surveillance can be used to identify groups at risk, by age or geographical location for example, making it possible to change or improve public health policies. However, the potential of serosurveillance has not been fully realized, in part because of the invasive nature of blood collection and associated ethical and technical issues for large-scale surveys. In our experience, refusal to enter surveys based on venepuncture can reach 13% (AZEVEDO NETO *et al.*, 1995).

The use of blood spotted on filter-paper (BSOFP) is a convenient and cheap method for the collection and storage of samples, as shown by VAI *et al.* (1987). This method has many advantages for large-scale serosurveys and has been used successfully for detection of antibodies against rubella, varicella and measles (MASSAD *et al.*, 1995; AZEVEDO-NETO *et al.*, 1996).

The disadvantages of this method were pointed out in the detection of antibodies against hepatitis A virus (HAV) and hepatitis B virus, for its reduced sensitivity compared to analysis of serum (ZOULEK *et al.*, 1985; GIL *et al.*, 1997).

In order to evaluate the performance of eluates of BSOFP in large-scale seroepidemiological surveys and surveillance of hepatitis A, having blood samples obtained by venepuncture as the 'gold standard', a pilot study was carried out. This study was part of a survey designed to evaluate the seroprevalence for this disease in 2 areas of Great Rio de Janeiro City, Brazil, prior to the construction of a new system of water and sewage draining.

Subjects and Methods

Setting and research design

A cross-sectional pilot study was conducted from August to November 1996 to estimate the prevalence of antibodies against HAV by age, and to validate the use of eluate of BSOFP for epidemiological studies on hepatitis A (ALMEIDA *et al.*, 1997). The study was carried out in 3 small areas in Rio de Janeiro State, Brazil: 2 of them (Sector 111 and 112) in Campos Elyseos (Duque de Caxias City) and the 3rd (Sector 9) in Ilha do Governador. In Sector 112 the studied population was composed of all residents aged 1–9 years and a sample of 60 individuals equally divided in 3 age strata: 10–14, 15–19 and ≥20 years. In Sectors 9 and 111 blood samples were collected from all residents aged 1–15 years and a small sample aged >15 years.

Survey team and protocol

Visits to all dwellings in the 3 districts resulted in a map of the area with addresses and family age-composition of each home. Home visiting was carried out by 20 interviewers and 12 individuals experienced in nursing practice, supervised by at least 2 researchers during the field work. Visitors were instructed to explain the objectives of the study. Written informed consent was obtained from each family as well as information regarding house water-supply and sewage-system through a questionnaire.

Sample collection and preparation

Two techniques were used to collect blood samples: venepuncture and finger prick. The sample from each individual was stored in 2 ways: serum and eluate. Blood collected by venepuncture was obtained by a vacuum-containing system (Vacutainer™, Becton Dickinson, USA) or by syringes and needles. The serum obtained after centrifugation of clotted samples was stored at −20°C. The second technique involved the use of a commercial device primarily designed to get samples from diabetic patients for blood glucose dry-chemistry testing (Glucolet® 2 and Ames Minilet™, Bayer AG, Germany). In brief, after the lancing of a fingertip with this automatic device, blood drops were spread on 2 circles (25-mm diameter each) printed in a filter-paper strip (Whatman® No. 1), until both circles were completely covered. BSOFP was allowed to air-dry, covered with a cellophane sheet and then stored in freezer bags at 4–8°C for up to 2 months. BSOFP samples (both circles) were folded to fit 1·5-mL plastic tubes and soaked overnight (12 h at least) with 500 µL of phosphate-buffered saline containing 0·2% Tween 20® and 5% bovine serum albumin (PBST–BSA) at 4–8°C. Eluates

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obtained this way were separated from the filter-paper with a disposable stick and frozen at −20°C until analysed.

Laboratory tests

Total antibodies to HAV (anti-HAV) were determined by 3 different techniques.

(1) Competitive enzyme-linked immunosorbent assay, ELISA (HAVAB EIA, Abbott Laboratories, Chicago, IL, USA) was used to detect anti-HAV in 718 serum samples. This technique was regarded as the 'gold standard'.
(2) Competitive ELISA (ETI-AB-HAVK-3, Sorin Biomedica Diagnostics S.p.A., Saluggia (Vercelli), Italy) was used to detect anti-HAV in 718 BSOFP eluates without modification of the manufacturer's instructions. Besides kit control samples, we have used 2 BSOFP eluate controls, collected from 2 known seropositive individuals, in every microplate to check between-run variation and calculate the cut-off value for eluate samples.
(3) Microparticle enzyme immune assay, MEIA (Ax-SYM HAVAB, Abbott Laboratories, Chicago, IL, USA) was used to analyse 60 randomly duplicated BSOFP samples.

Statistical analysis

Validity. Validity of a serological test measures its ability to identify correctly seropositive and seronegative people. It is evaluated by estimating measures as sensitivity, specificity, predictive values and test efficiency (ROTHMAN & GREENLAND, 1998). In this case, sensitivity is defined as the probability that a test classifies as positive people who have antibodies against HAV. On the other hand, specificity is the probability that a test classifies as negative people without antibodies against HAV. The predictive value positive is the proportion of people with a positive test who have antibodies against HAV. The predictive value negative is the proportion of people with a negative test who have no antibodies against HAV. Test efficiency is the total proportion of people correctly classified by the test.

Global and age-specific sensitivity, specificity, test efficiency and predictive values (STRONGIN, 1992) of BSOFP were estimated by comparing the results of 718 BSOFP eluates analysed by competitive ELISA with results of 718 serum samples from the same individuals analysed by competitive ELISA. The same strategy was used to investigate the validity of BSOFP analysed by MEIA in a subsample of 59 individuals.

Reliability. Different from validity, the reliability of a test is its capacity to give the same result—positive or negative, whether correctly or incorrectly—using the same technique in duplicated samples of BSOFP (intra-test reliability) or using different techniques (inter-test reliability).

To evaluate the possible consequences of using kits from 2 different manufacturers, 60 randomly duplicated BSOFP eluates were analysed by competitive ELISA (Sorin Biomedica) and MEIA (Abbott). Results were compared and reliability between the 2 techniques was estimated by the κ statistic. It measures the agreement between the observed results using the 2 methods corrected by the agreement expected by chance (FLEISS, 1981). Ninety-five percent confidence interval (95% CI) was estimated using a procedure based on a χ^2 goodness-of-fit test (DONNER & ELIASZIW, 1992). This approach provides coverage levels that are accurate in small samples.

Results

Age-stratified seroprevalence

Overall, HAV antibodies were detected by ELISA technique applied to serum samples in 230 (32·03%) out of 718 individuals; 218 individuals (30·36%) were positive for HAV antibodies from the 718 paired BSOFP eluates analysed by ELISA. The age-stratified data and 95% CI comparing serum and BSOFP samples are shown in Table 1.

Sensitivity, specificity and predictive values

Tables 2 and 3 present the results of detection of HAV antibodies in 718 BSOFP eluates by ELISA compared to detection in serum samples by ELISA. Global sensitivity and specificity of BSOFP eluates were high: 89·6% and 97·5%, respectively. For a seroprevalence of anti-HAV of 32%, positive predictive value was 94·5% and negative predictive value was 95·2%. Test efficiency was 95·0%. For all these values, 95% CI are shown in Table 3.

The results of detection of HAV antibodies in 59 BSOFP eluates by MEIA compared to detection in

Table 1. Age-stratified seroprevalence of hepatitis A virus antibodies in Rio de Janeiro State (Brazil), estimated by competitive enzyme immunoassay (ELISA) from serum and BSOFP eluates

		BSOFP samples		Serum samples	
Age (years)	Sample size	Number positive	Prevalence, % (95% CI)	Number positive	Prevalence, % (95% CI)
1	40	4	10·0 (3·2–24·6)	6	15·0 (6·2–30·5)
2	50	3	6·0 (1·6–17·5)	4	8·0 (2·6–20·1)
3	49	5	10·2 (3·8–23·0)	5	10·2 (3·8–23·0)
4	56	12	21·4 (12·0–34·8)	13	23·2 (13·4–36·7)
5	47	17	36·2 (23·1–51·5)	16	34·0 (21·3–49·4)
6	41	10	24·4 (12·9–40·6)	10	24·4 (12·9–40·6)
7	55	16	29·1 (18·0–43·1)	18	32·7 (21·0–46·8)
8	51	12	23·5 (13·2–37·8)	12	23·5 (13·2–37·8)
9	58	26	44.8 (31·9–58·4)	26	44·8 (31·9–58·4)
10	32	4	12·5 (4·1–29·9)	4	12·5 (4·1–29·9)
11	45	13	28·9 (16·8–44·5)	15	33·3 (20·4–49·0)
12	36	12	33·3 (19·1–51·0)	12	33·3 (19·1–51·0)
13	41	16	39·0 (24·6–55·5)	19	46·3 (31·0–62·4)
14	26	9	34·6 (17·9–55·6)	11	42·3 (24·0–62·8)
15	39	23	59·0 (42·2–74·0)	22	56·4 (39·8–71·8)
16–19	34	19	54·3 (38·1–72·4)	19	54·3 (38·1–72·4)
>20	18	17	94·4 (70·6–99·7)	18	100·0 (78·1–100·0)
Total	718	218		230	

BSOFP, blood spotted on filter-paper.

Table 2. Comparative results from competitive enzyme immunoassay (ELISA) for hepatitis A virus antibodies in both serum samples and eluates from blood spotted on filter-paper (BSOFP)

	Serum samples		
BSOFP	Positive	Negative	Total
Positive	206	12	218
Negative	24	476	500
Total	230	488	718

Table 3. Performance of eluates from blood spotted on filter-paper (BSOFP) analysed by competitive enzyme immunoassay (ELISA) in 718 individuals in Rio de Janeiro State, Brazil (serum samples as 'gold standard')

	BSOFP analysed by ELISA % (95% CI)
Sensitivity	89·6 (84·7–93·1)
Specificity	97·5 (95·6–98·7)
Positive predictive value	94·5 (90·3–97·0)
Negative predictive value	95·2 (92·8–96·8)
Test efficiency	95·0 (93·1–96·4)

serum samples are shown in Tables 4 and 5. Global sensitivity and specificity of BSOFP eluates were high: 100·0% and 97·5%, respectively. For a seroprevalence of anti-HAV of 32%, positive predictive value was 95·0% and negative predictive value was 100·0%. Test efficiency was 98·3%. For all these values, 95% CI are shown in Table 5.

Reliability of duplicated BSOFP

Sixty BSOFP samples were randomly duplicated in order to evaluate the concordance between the 2 techniques: competitive ELISA and MEIA. Detection of total antibodies to HAV by ELISA and by MEIA in eluates from BSOFP was compared (Table 6). Reliability was high expressed by a κ of 0·93 (95% CI 0·74–0·98).

Table 4. Comparative results for hepatitis A virus antibodies in serum (competitive enzyme immunoassay, ELISA) and in eluates from blood spotted on filter-paper (microparticle enzyme immune assay, MEIA)

	Eluates from BSOFP (MEIA)		
Serum (ELISA)	Positive	Negative	Total
Positive	19	0	19
Negative	1	39	40
Total	20	39	59

BSOFP, blood spotted on filter-paper.

Table 5. Performance of eluates from blood spotted on filter-paper (BSOFP) analysed by microparticle enzyme immune assay (MEIA) in 59 individuals in Rio de Janeiro State, Brazil (serum samples as 'gold standard')

	BSOFP analysed by MEIA % (95% CI)
Sensitivity	100·0 (79·1–100·0)
Specificity	97·5 (85·3–99·9)
Positive predictive value	95·0 (73·1–99·7)
Negative predictive value	100·0 (88·8–100·0)
Test efficiency	98·3 (89·7–99·9)

Comparison of signal intensity

The ratio between optical density and cut-off value (OD/CO) was obtained for each sample analysed by competitive ELISA, for both BSOFP and serum. Table 7 shows the median and quartiles, range of variation, average and standard deviation of serum and BSOFP results. It can be noted that the reaction is slightly less intense on average for samples collected on to filter paper, with an almost identical median as compared to sera. However, there is a clear difference at the 25% quartile, indicating that sera have a stronger antibody activity, lowering OD/CO values, probably owing to a higher concentration of antibodies. These data are concordant with the sensitivity found for BSOFP samples.

Discussion

Accurate surveillance is a critical component of an effective infectious disease control programme. Large-scale seroepidemiological surveys can contribute objectively to the design and evaluation of methods applied to these programmes. However, the lack of resources, and the problems related to the population sample collection (by venepuncture) out of hospital or health centre facilities, limit the ability of health authorities to carry out such studies. Acceptability of the collection method by the population and the quality of samples are important considerations in the development of effective antibody detection techniques.

BSOFP is a good method for the collection, storage and transport of samples in field studies, especially in developing countries. Nevertheless, because the method is considered less sensitive than analysis of serum, it has been little used in epidemiological studies for hepatitis A.

In fact, our data from the comparison of serum and BSOFP OD/CO ratio have shown that the reaction for BSOFP is less intense, and consequently this method is

Table 6. Comparison of detection of total antibodies to hepatitis A virus by competitive enzyme immunoassay (ELISA) and microparticle enzyme immune assay (MEIA) in eluates from blood spotted on filter-paper (BSOFP)

	Eluates from BSOFP (ELISA)		
BSOFP (MEIA)	Positive	Negative	Total
Positive	19	1	20
Negative	1	39	40
Total	20	40	60

Table 7. Comparison of signal intensity (OD/CO) between BSOFP and serum samples analysed for antibodies against hepatitis A virus by competitive enzyme immunoassay (ELISA)

	OD/CO	
	BSOFP	Serum
25% Quartile	0·311	0·029
Median	1·469	1·461
75% Quartile	1·820	1·817
Range	0·135–5·044	0·000–3·273
Average	1·352	1·173
Standard deviation	0·919	0·913

OD/CO, ratio between optical density and cut-off value; BSOFP, blood spotted on filter-paper.

expected to be less sensitive. However, our study, carried out in the general population, showed high levels of global and age-specific sensitivity, specificity and predictive values. The exceptions were sensitivity and positive predictive value for children aged 1–2 years. Although we cannot reject that validity of the test can be low in this age-group, random error can also be responsible for this finding due to the small number of positive samples.

As 2 manufacturers' kits were used for each set of samples, the difference between the results obtained using BSOFP eluates and serum samples could be due to the choice of kit, instead of the type of specimen. But the reliability of BSOFP samples tested by both kits has been shown to be high, with a κ of 0·93, therefore introducing a small difference due to the kit selection.

The critical procedure to achieve better results with this alternative collection method seems to be the amount of blood to be eluated. In previous papers, authors described the collection procedure in number of drops (ZOULEK *et al.*, 1985). We propose a standard procedure where the volume of blood collected varies less between individuals because we determine the area to be spotted on a standard filter paper. Another improvement that could be made is to analyse the eluates by an antibody-capture assay, which has been shown to be very sensitive even for saliva and urine samples (CONNELL *et al.*, 1990; AZEVEDO-NETO *et al.*, 1995).

The present study indicates that use of eluates of BSOFP is reliable and valid for blood collection for the detection of antibodies against HAV in large-scale epidemiological surveys, producing similar seroprevalence results as serum samples.

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