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Detection of markers of hepatitis B infection in serum dried on to filter-paper: an application to field studies*

HUI ZHUANG,[1] ANTHONY G. COULEPIS,[2] STEPHEN A. LOCARNINI,[2] & IAN D. GUST[2]

In an attempt to find a cheap, reliable, and convenient method for the transportation and storage of serum specimens during seroepidemiological surveys, a technique in which serum was dried on to pieces of filter paper was developed and evaluated. For the evaluation, a total of 382 sera were selected from the extensive serum collection held by the WHO Collaborating Centre for Virus Reference and Research at Fairfield Hospital, Australia. These sera were dried on to pieces of filter-paper, stored at different temperatures and then tested for the presence of the various markers of infection with hepatitis B virus by solid-phase radioimmunoassay. The results were in complete agreement with those obtained on whole serum specimens. In addition, storage at 4 °C, room temperature (22 °C), or 37 °C for up to 30 days did not alter the sensitivity of the test. This technique may be useful in field surveys, not only for the detection of hepatitis B infection, but also in the study of other diseases and metabolic disorders.

Seroepidemiological surveys are of importance in developing countries to determine the prevalence of infection with different agents, to study the natural history of diseases within given communities, and to provide the data needed to design and evaluate alternative methods of disease control. Despite their importance, large-scale seroepidemiological surveys are often difficult because of technical and logistic problems encountered in the field. While it is relatively easy to collect blood by either venepuncture or ear- or heel-prick, problems are encountered in separating the serum and refrigerating it before and during transportation to the laboratory where the tests are to be performed, a process that may take several days. These difficulties are magnified when the population being studied is remote or spread over a large area, and all the supplies needed must be carried by hand. A simple, cheap, and reliable procedure for processing and transporting the serum specimens, which alleviates the need for refrigeration and does not require expensive equipment, is urgently needed.

Blood samples dried on to pieces of filter-paper have been used to screen for metabolic disorders (*1-4*), and to detect markers of HBV infection (*5-6*). However, these techniques have not been evaluated in large-scale epidemiological surveys, and in each case the small quantity of blood used (*25-50* µl) made it difficult to detect low titres of antigens or antibodies (*6*). In an attempt to provide a more sensitive system that would have greater application in field studies, we modified the procedure, using relatively large volumes of serum dried on to filter-paper, and evaluated its sensitivity for detecting evidence of HBV infection under conditions likely to be encountered in the field.

MATERIALS AND METHODS

Tests performed

The markers of HBV infection studied were hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), antibody to hepatitis B surface antigen (anti-HBs), antibody to hepatitis B e antigen (anti-HBe), and antibody to hepatitis B core antigen (anti-HBc).

Source of sera tested

A total of 382 sera were tested. These sera were selected from the extensive serum collection held by the virus laboratory at Fairfield Hospital, Victoria,

* From the WHO Collaborating Centre for Virus Reference and Research, Fairfield Hospital, Yarra Bend Road, Fairfield, Victoria, 3078, Australia. Requests for reprints should be addressed to Dr Ian D. Gust, Director of the WHO Collaborating Centre.

[1] Visiting Scientist from: Department of Epidemiology, School of Public Health, Beijing Medical College, Beijing, People's Republic of China.

[2] Virologists.

and had been tested for the presence of markers of HBV infection by solid-phase radioimmunoassay (SPRIA).

HBsAg. A panel of 282 sera was tested. These sera comprised a group of 25 freeze-dried sera making up the Australian hepatitis B reference panel, 73 sera from patients with acute hepatitis B (including serial bleeds from five patients), 4 sera from chronic carriers of HBsAg, and 180 sera from two populations in which HBV infection is known to be endemic.

HBeAg. Sera from 25 HBsAg positive patients with acute or chronic HBV infections and HBeAg titres ranging from 1/5 to 1/1000 by RIA, were tested.

Anti-HBs. Sera from the 25 subjects with anti-HBs detectable by RIA and with titres ranging from 1/5 to 1/10 000 were selected. These were obtained from healthy blood donors and patients admitted to Fairfield Hospital with hepatitis or other diseases.

Anti-HBe. A group of 25 HBsAg positive sera from subjects with acute or chronic infection with HBV and with anti-HBe titres ranging from 1/5 to 1/100 by RIA, were tested.

Anti-HBc. A group of 25 sera from patients with active or past infection with HBV, and with anti-HBc titres ranging from 1/5 to 1/10 000 by RIA, were selected.

Filter-paper

Glass microfibre paper, GF/A[a], was cut into squares 3 cm × 3 cm or 1.5 cm × 1.5 cm and placed into sterile Petri dishes. The larger squares were used for 200-μl aliquots of serum, and the smaller ones for the addition of 100-μl aliquots.

Application of serum

Filter-paper squares were folded twice and placed into 5-ml sterile plastic screw-cap tubes with the aid of forceps. For the detection of HBsAg, anti-HBs, and HBeAg, 200 μl of serum was slowly applied to the filter-paper squares, while for the detection of anti-HBe and anti-HBc, 100-μl aliquots were used. The serum samples were completely absorbed by the respective filter-paper squares and left at room temperature (22 °C) overnight to dry.

Replicate samples were stored at 4 °C, 22 °C, and 37 °C and tested 1 and 30 days later.

Test for HBV markers

Test methods. HBsAg was detected by SPRIA and reverse passive haemagglutination assay (RPHA) using commercial reagents.[b] HBeAg, anti-HBe and anti-Hbc were detected by SPRIA, using commercial reagents.[c]

Filter-paper testing procedure. Briefly, for detection of HBsAg, HBeAg, and anti-HBs, 400 μl of saline (8.5 g/litre) were dispensed into each tube containing filter-paper impregnated with serum. The moist filter-paper was gently unfolded with a plastic disposable microtitre tip[d] and the appropriate bead added. The filter-paper was then wrapped around the bead using the same dispensing tip, and left for 18–22 hours at room temperature, after which the beads were transferred to reaction trays provided with the kit, and the test completed as described by the manufacturer (see Fig. 1).

[a] Whatman, Maidstone, England.

[b] Ausria II-125, Abbott Laboratories, North Chicago, Illinois, USA; and Hepatest, Wellcome, Beckenham, England.

[c] Abbot-e, Ausab, Corab, Abbott Laboratories.

[d] Oxford Laboratories Inc., Foster City, California, USA.

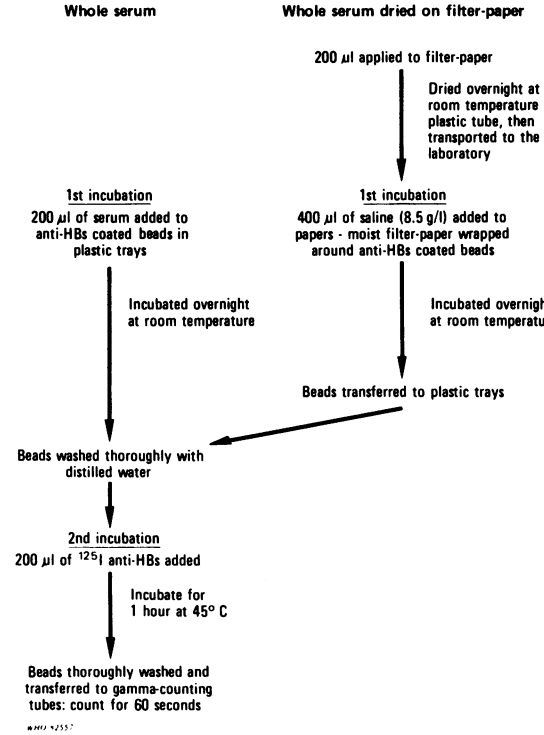

Fig. 1. Detection of HBsAg, HBeAg, and anti-HBS by the solid-phase radioimmunoassay. The procedure shown is that for HBsAg. For HBeAg, the test is the same except that anti-HBe coated beads are used and ^{125}I anti-HBe is reacted with the beads for 3 hours at 45 °C for the second incubation period. For anti-HBs, HBsAg coated beads are used and ^{125}I HBsAg is reacted with the beads for 4 hours at room temperature during the second incubation period.

For the detection of anti-HBe (a neutralization test) and anti-HBc (a competitive RIA test), 100 µl of saline (8.5 g/litre) were added to each tube containing filter-paper impregnated with serum, followed by 100 µl of neutralizing reagent (for anti-HBe) or iodinated anti-HBc (for anti-HBc). The beads were then incubated, and the procedure completed as shown in Fig. 2.

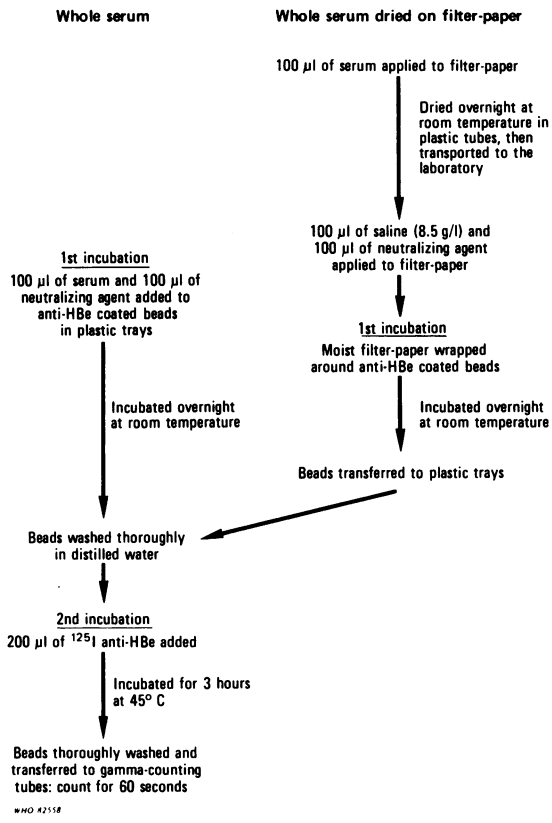

Fig. 2. Detection of anti-HBe and anti-HBc. The procedure shown is that for anti-HBe (neutralization test). For anti-HBc the procedure is the same except that no second incubation period is required as this test is a competitive SPRIA. HBcAg-coated beads and 100-µl aliquots of ^{125}I anti-HBc are used during the first incubation, after which the beads are washed thoroughly and counted for 60 seconds.

For RPHA, eluates from the filter-paper were used. Aliquots of 800 µl of buffer, supplied by the manufacturer, were added to the filter-paper containing dried serum and left to soak overnight. The next day, 25 µl of eluate (a one in five dilution of serum)

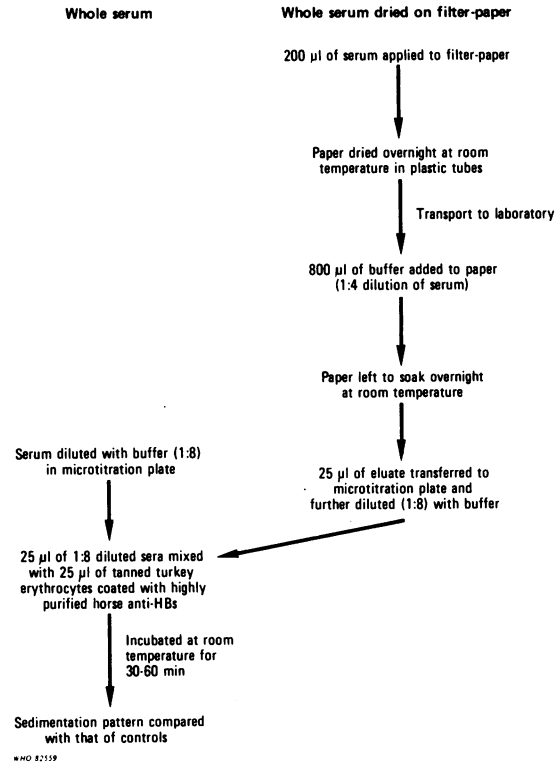

Fig. 3. Testing for HBsAg by reverse passive haemagglutination assay.

was removed from each tube and tested for HBsAg by the standard technique (see Fig. 3).

RESULTS

The results obtained by RIA and RPHA for detection of HBsAg in 282 serum specimens dried on filter-paper squares are shown in Table 1. The results are in complete agreement with those obtained on whole serum. In particular, the technique detected all 20 positive sera in the Australian reference panel, and end-point titrations performed on 8 sera containing high and low titres of HBsAg gave identical results to those obtained with whole serum.

Identical results were also obtained when the two techniques were compared for the detection of anti-HBs, anti-HBe, and anti-HBc. False-positive results were not encountered in any of the assay procedures. In addition, storage at 4 °C, room temperature, and 37 °C for up to 30 days did not alter the sensitivity of the test.

Table 1. Comparison of results obtained by SPRIA and RPHA for detection of HBsAg in serum and in serum dried on filter-paper

Sera	No. tested	No. positive by SPRIA (Ausria 11-125)		No. positive by RPHA (Hepatest)	
		Serum	Serum dried on filter-paper	Serum	Serum dried on filter-paper
Australian hepatitis B reference panel	25	20	20	17	17
Sera from patients with acute hepatitis B	73	61	61	61	61
Sera from chronic HBsAg carriers	4	4	4	4	4
Sera from two populations in which hepatitis B is endemic	180	5	5	5	5

DISCUSSION

The results obtained in this study indicate that aliquots of sera can be dried on to filter-paper pieces and held for up to 30 days at 37 °C without decreasing the detection rate of HBsAg, HBeAg, anti-HBs, anti-HBc, and anti-HBe.

The technique has obvious implications for field studies as filter-paper can be readily transported, even from remote areas. Blood specimens collected in the field by venepuncture could be allowed to clot and aliquots of serum transferred to several pieces of filter-paper. These pieces of paper could then be carried or posted to the base laboratory for testing or storage for future reference. Specimens prepared in this way are dry, light, easy to handle and cannot be broken, spilled or leak. As each piece of filter-paper weighs approximately 70 mg, 1000 sera could be easily and cheaply posted in an envelope weighing less than 100 g. The pieces should first be thoroughly dried, separated by clean pieces of tissue-paper and sealed in a plastic bag. In addition, sera dried on filter-paper are easy to store, and can be kept for at least 30 days prior to testing, even at 37 °C, without any loss of reactivity.

The technique is potentially suitable for detection of both biological and non-biological markers and can be modified for use with a variety of detecting systems, including the enzyme-linked immunosorbent assay (ELISA). As such, it should be a valuable tool for use in many types of field study.

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RÉSUMÉ

DÉTECTION DES MARQUEURS DE L'INFECTION PAR L'HÉPATITE B DANS DU SÉRUM DESSÉCHÉ SUR DU PAPIER FILTRE : APPLICATION À DES ÉTUDES SUR LE TERRAIN

Les enquêtes séro-épidémiologiques à grande échelle sont souvent difficiles à effectuer en raison des problèmes techniques et logistiques rencontrés sur le terrain. S'il est relativement facile de recueillir du sang soit par ponction veineuse soit par piqûre à l'oreille ou au talon, on rencontre des difficultés pour séparer le sérum et le réfrigérer avant et pendant le

transport au laboratoire, ce qui peut prendre plusieurs jours. En outre, ces difficultés sont souvent aggravées lorsque la population étudiée habite des lieux écartés ou bien est répartie sur une grande superficie, toutes les fournitures devant être apportées ou remportées à la main. Afin de procurer un système qui trouverait une large application dans des études sur le terrain, une méthode a été mise au point à l'aide de volumes relativement grands de sérum séché sur du papier filtre et elle a été évaluée en ce qui concerne sa sensibilité pour la détection des signes d'infection par le HBV, dans les conditions susceptibles d'être rencontrées sur le terrain. Les marqueurs étudiés de l'infection par le HBV étaient: l'antigène de surface de l'hépatite B (HBsAg) et l'anticorps correspondant (anti-HBs), l'antigène *e* de l'hépatite B (HBeAg) et l'anticorps correspondant (anti-HBe), ainsi que l'anticorps à l'égard de l'antigène central de l'hépatite B (anti-HBc). En vue de l'évaluation de cette technique, un total de 382 sérums a été choisi à partir de la vaste collection de sérums conservée au Centre collaborateur OMS de référence et de recherche pour les virus à l'hôpital de Fairfield, en Australie. Les sérums éprouvés ont été desséchés sur des morceaux de papier filtre, conservés pendant 1 ou 30 jours à 4 °C, 22 °C et 37 °C, puis soumis à la recherche des différents marqueurs de l'infection par le HBV par dosage radio-immunologique en phase solide (SPRIA). Il y avait une complète concordance entre les résultats obtenus avec le sérum desséché et ceux qui ont été obtenus avec du sérum entier (sérum témoin). De même, des résultats identiques ont été obtenus en ce qui concerne la détection des anti-HBs, anti-HBe et anti-HBc. Pour aucun des marqueurs, il n'a été trouvé de résultats faussement positifs dans l'un quelconque des titrages. En outre, le stockage à 4 °C, à la température du laboratoire (22 °C) ou à 37 °C, pendant une période allant jusqu'à 30 jours, ne modifiait pas la sensibilité de l'épreuve.

La technique sur papier filtre décrite dans cet article sera utile pour les enquêtes sur le terrain, car elle facilitera le transport et le stockage des échantillons de sérum depuis le point de prélèvement jusqu'au laboratoire et diminuera le besoin de réfrigération. De plus, les échantillons préparés de cette manière sont secs, légers, faciles à manipuler et à stocker et ne peuvent être brisés, renversés ou sujets à des fuites. Chaque morceau de papier filtre portant du sérum desséché pèse approximativement 70 mg, et 1000 sérums pourraient donc être expédiés par la poste, facilement et à bon marché, dans une enveloppe pesant moins de 100 grammes. Il est probable que cette technique sera précieuse non seulement pour la détection de l'infection par l'hépatite B, mais également pour l'étude d'autres maladies et troubles métaboliques.

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