Hepatitis B virus markers on dried blood spots. A new tool for epidemiological research

ERICA VILLA, ROBERTO CARTOLARI, STEFANO BELLENTANI, PAOLO RIVASI,* GIANCARLO CASOLO, FEDERICO MANENTI *Institute of Semeiotica Medica and *Blood Transfusion Centre, University of Modena, Italy*

HBsAg testing is widely used as a marker of hepatitis B virus (HBV) infection in epidemiological surveys, but the detection of anti-HBs and anti-HBc antibodies and possibly those of the HBeAg—anti-HBe system, has now become increasingly important.[1 2] Large scale surveys, however, often require facilities not readily available in most of the countries where HBV infection is endemic. Furthermore, all problems are increased when the population under investigation is spread over a large area far from the study centre, especially when repeated controls are required. This needs a simple and reliable method for acquiring and transferring blood samples.

In 1978, Farzadegan *et al.*[3] proposed a method for detection of HBsAg on the eluates from dried blood spots. This technique has not been applied to other HBV-related antigen and antibodies. We describe here a modified technique for testing eluates from dried blood spots for HBsAg, HBeAg, anti-HBe, anti-HBs and anti-HBc.

Material and methods

Two groups of patients were studied:
Group 1: 12 HBsAg-positive, anti-HBc-positive patients (six chronic asymptomatic carriers, six with chronic active hepatitis). The titre of anti-HBc in all patients was >1/1000. One of these patients was HBeAg-positive (titre 1/100 000) and four were anti-HBe-positive (titre 1/1000).
Group 2: 12 HBsAg-negative, anti-HBs- and anti-HBc-positive patients (two with chronic persistent hepatitis, five with chronic active hepatitis, five with liver cirrhosis). For both antibodies, titres ranged between 1/1 and 1/1000.

COLLECTION OF SAMPLES

Capillary blood was collected on filter paper (spots of about 8 mm diam), air-dried, and stored at room temperature. Similar samples, prepared from eight patients of the second group, were also stored at +4°C and at −20°C. Venous blood was also obtained from the same patients and aliquots of serum kept frozen.

ELUTION AND TESTING

Blood spots were tested after 1, 7, 15, 30, 60, and 180 days from sampling. Spots were extracted with either 250 or 600 μl 0·9% saline for HBsAg, anti-HBs and HBeAg assays and with either 150 or 600 μl for anti-HBc and anti-HBe assays; in the first six patients of each group, spots were eluted for 1, 6, and 12 hours at room temperature; in the remaining patients, spots were eluted overnight.

Eluates and sera were tested by radioimmunoassay (Ausria II, Ausab, Corab, e—anti e RIA kit, Abbott Laboratories). Serial tenfold dilutions of sera were made to estimate the titre of antibodies. Results obtained at different times with kits differing in specific activity were compared for each patient by calculating the ratio between "samples cpm" and "cutoff value" for each experiment.

Results

For each of the tests, the best separation between positive and negative results was found when spots were extracted for 12 hours. In subsequent experiments, therefore, dried specimens were eluted overnight.

In the HBsAg-positive patients, tests for HBsAg on dried spots gave positive results after each time interval, without differences due to the elution volumes of 250 and 600 μl. Anti-HBc testing in the same group of patients consistently yielded positive results, both with 150 and 600 μl of elution volume. Dried specimens showed a slight loss of activity after 30, 60, and 180 days storage in comparison with serum, although all results were still strongly positive (Fig. 1). In the same group, one patient was HBeAg-positive and four anti-HBe positive, both on serum and eluate, after each time interval. After 15, 30, 60, and 180 days HBeAg and anti-HBe results on eluates decreased on average by 20% in comparison with the corresponding sera.

In the HBsAg-negative, anti-HBs, and anti-HBc positive group anti-HBs and anti-HBc tests gave positive results, with elution volumes of 250 and 150 μl respectively, when the antibody titre in serum was ⩾ 1/100. When the titre was 1/10, the test on eluates gave inconsistent results while eluates were always negative when antibody determination was positive on undiluted serum only. When spots were eluted with 600 μl, tests were positive only when the antibody titre was at least 1/1000.

Storage at room temperature did not affect results during the whole period of observation, when the antibody titre was ⩾1/1000 but eluates became

Accepted for publication 5 November 1980

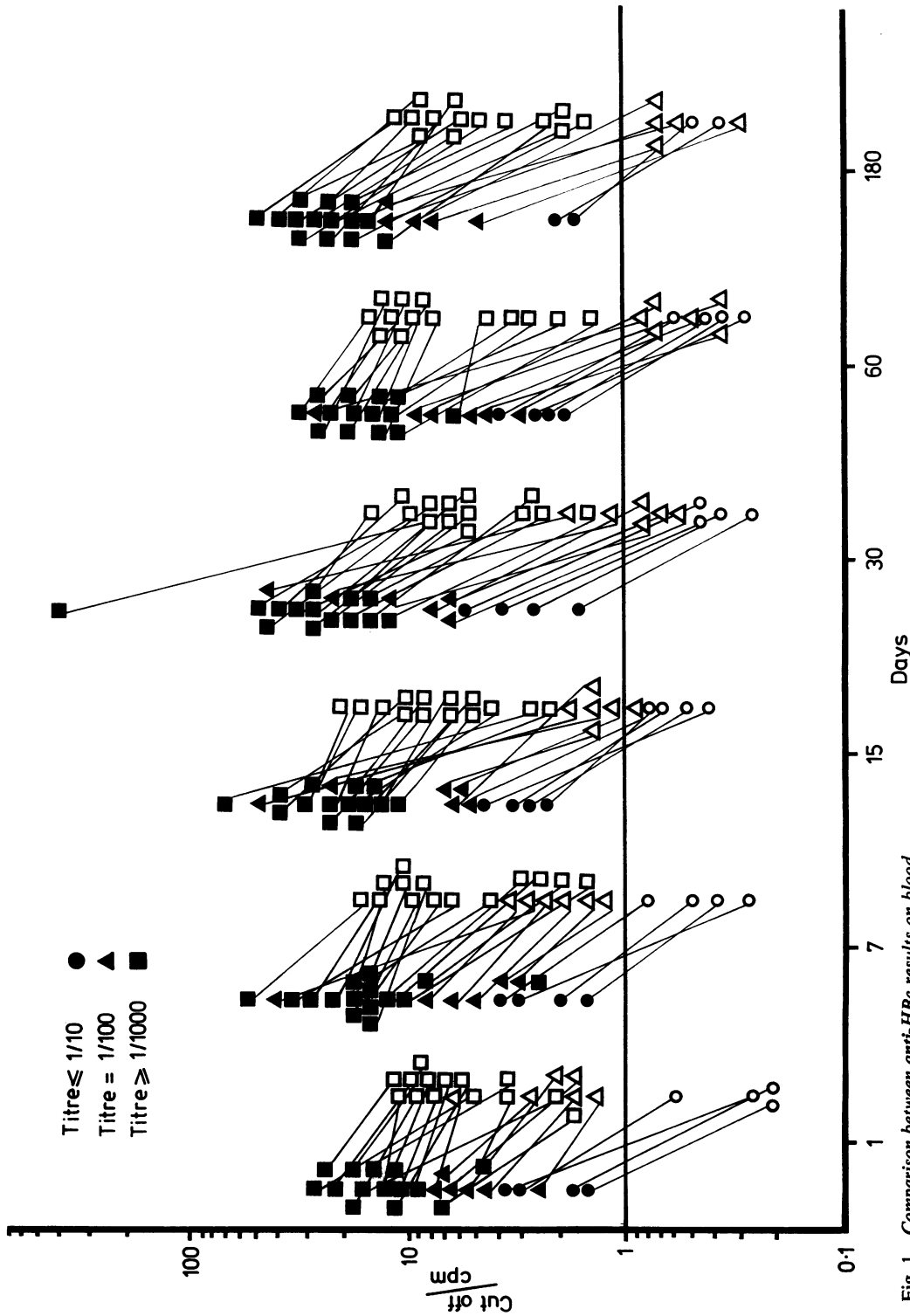

Fig. 1 *Comparison between anti-HBc results on blood (closed symbols) and on eluates (open symbols) at intervals after sampling.*

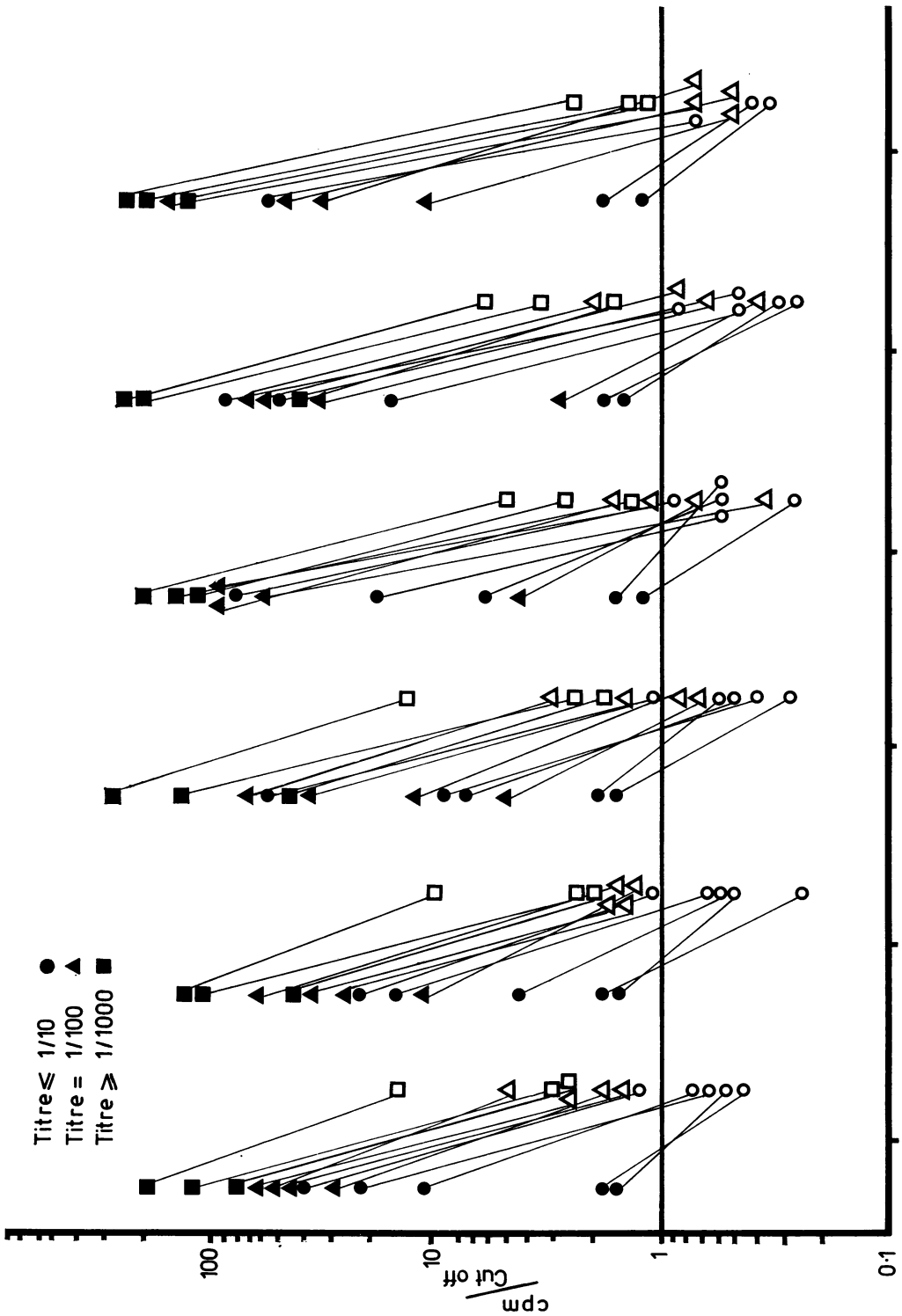

Fig. 2 *Comparison between anti-HBs results on blood (closed symbols) and on eluates (open symbols) at intervals after sampling.*

borderline-positive or even negative, after 15 days of storage or more, when the titre in serum was 1/100 (Figs. 1 and 2). Storage at +4°C or at −20°C did not significantly improve results when compared with those stored at room temperature. In patients with the highest antibody titres, the slight loss in activity after 30, 60, and 180 days was, for all three periods of storage, in the range of one dilution in respect to serum levels. In patients with low titres, storage at +4°C or at −20°C did not prevent the test becoming negative.

Discussion

It seems apparent from our results that HBV markers are stable in a dried condition and that the technique for their determination on eluates from dried specimens is simple, reliable and reproducible.

As far as specificity is concerned, we have never encountered false-positive results although the eluates contained whole blood instead of serum. Sensitivity is not a problem for HBsAg determination. The failure of the method to give positive results when antibody titre in serum was less than 1/100 is probably related to the dilution of the small quantity of blood (about 50 μl) contained in a spot of 8 mm, once this is eluted. Elution with the minimal amounts necessary to perform the assay, 250 or 150 μl depending on the test, causes a three- to fivefold dilution of the antibody in comparison with serum, so that antibody activity becomes too weak to be detected. This could probably be obviated by increasing the size of the spot eluted, therefore obtaining a more concentrated solution.

This method seems suitable for epidemiological surveys or large-scale screenings, especially if mailing of specimens is required.

References

[1] Szmuness W, Harley EJ, Ikram H, Stevens CE. Socio-demographic aspects of the epidemiology of hepatitis B. In: Vyas GN, Cohen SN, Schmid R, eds. *Viral hepatitis*. Philadelphia: Franklin Institute Press, 1978:297-320.

[2] Perrillo RP, Gelb L, Campbell C, *et al*. Hepatitis BeAg, DNA polymerase activity and infection of household contacts with hepatitis B virus. *Gastroenterology* 1979;**76**: 1319-25.

[3] Farzadegan H, Noori KH, Ala F. Detection of hepatitis B surface antigen in blood and blood products dried on filter paper. *Lancet* 1978;i:362-3.

Requests for reprints to: Dr Erica Villa, Institute of Semeiotica Medica, Policlinico, Via del Pozzo 71, 41100 Modena, Italy.

Letters to the Editor

Plasma electrolytes in dangerous infectious diseases

Most of the common laboratory investigations necessary for the diagnosis and management of patients with hazardous infectious diseases can be done in a suitable safety cabinet.[1] However, plasma sodium and potassium present difficulties as their estimation requires the use of either ion-specific electrodes or a flame photometer.

Potentiometric assay appears to be simpler than flame photometry but we did not consider it appropriate in this case for two reasons: first, the necessary equipment is not generally available and second, flame photometers are rather simpler and more likely to function reliably at very short notice after long periods without attention or maintenance, a situation especially liable to obtain if an instrument is reserved for the relatively infrequent dangerous specimen. On the other hand, flame photometers should not be enclosed in a safety cabinet as they produce much heat and operate on gas and air under pressure.

A solution to this problem is to dilute the plasma or serum sample inside the safety cabinet using formalin as the diluent instead of water. After leaving the bottle of diluted sample in the dunk tank for at least 1½ h (ensuring that the outside is also sterilised) the contents can be analysed in an unenclosed bench flame photometer. We find that very little formaldehyde gets past the flame; even after 15 samples the smell of formaldehyde is minimal. Although the diluted sample comes into contact with only plastic and stainless steel, distilled water should be run through after use to clear the instrument of residual formaldehyde.

Plasma diluted in formalin in this way yields a faintly turbid suspension of precipitated protein. To check that micro-organisms trapped within such protein floccules are inactivated, we diluted in formalin (1/100), fresh human plasma to which *Bacillus globigii* spores had been added giving a final concentration of 10^8/ml of plasma. At 10, 20, 45, and 90 min after dilution the precipitates from 1 ml volumes of the diluted plasma were washed and one loopful was added to 5 ml of nutrient broth and another plated on nutrient agar. Growth was obtained only