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USE OF A HIGHLY SENSITIVE ELECTROPHORESIS—PRECIPITATION METHOD TO DETECT VIRUS HEPATITIS B ANTIGEN IN HUMAN BLOOD SERUM

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A modification of the highly sensitive method of electrophoresis—precipitation in polyacrylamide gel for the detection of virus hepatitis B antigen (HBsAg) and antibodies against it is described. The method completely preserves the specificity of immunodiffusion in gel, but is 1000-2000 times more sensitive for the detection of HBsAg. The method has given good results with the sera of patients with various diseases. It detects HBsAg more efficiently than other methods used previously.

KEY WORDS: *1 antigen of hepatitis B (HBsAg); electrophoresis—precipitation method.*

The antigen of virus hepatitis B (Australian antigen HBsAg) is nowadays universally tested for in blood service establishments in order to detect carriers among donors, and in clinical and diagnostic laboratories, hemodialysis units, and so on.

The sensitivity of the methods used to detect HBsAg (double immunodiffusion in gel and counterimmunoelectroosmophoresis) is too low to guarantee completely the absence of HBsAg in the sera tested. The development of highly sensitive methods for the determination of HBsAg and of antibodies against it is thus an urgent matter.

In this paper a modification of the method of electrophoresis precipitation in polyacrylamide gel (EPPG) for the determination of HBsAg is described.

The EPPG method suggested by Abelev [1] combines the resolving power and specificity of the immunodiffusion method with the sensitivity of the radioimmunological test. The method is based on concentration of the test antigen in an interrupted buffer system followed by its detection by a precipitating test system, whereby the samples detected can be identified. The sensitivity of the method for α-fetoprotein lies between $3 \cdot 10^{-4}$ and $6 \cdot 10^{-4}$ μg/ml.

In the present writers' variants the method cannot be used to detect HBsAg because of the large size of its molecules: High-molecular-weight HBsAg will not penetrate into finely porous polyacrylamide gel; the method had therefore to be modified by using agarose gel instead of polyacrylamide gel.

The method consisted of two stages: concentration of HBsAg, development of the antigen with an immunological diagnostic serum for HBsAg, detection of invisible precipitates by ^{125}I-labeled antigammaglobulin, followed by autoradiography of the precipitate. Changes were introduced into the first and second stages: concentrations and immunodiffusion detection of the antigen, in which the polyacrylamide gel was replaced by 0.8 and 1% agarose.

The reaction is carried out in the flat chamber (30 × 90 × 1 mm) of an apparatus for vertical electrophoresis in gel, full details of which were described earlier [1]. For

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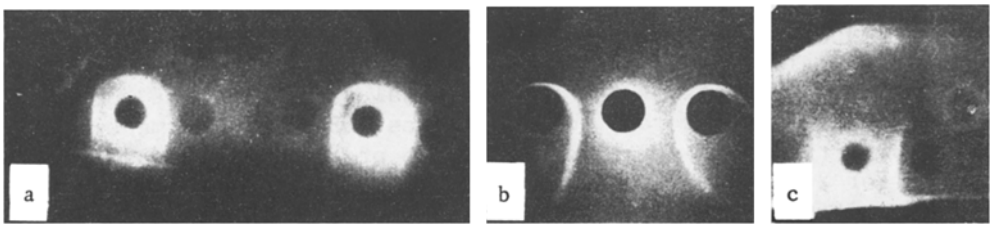

Fig. 1. Detection of HBsAg and antibodies against it by electrophoresis—precipitation reactions in 1% agarose (autoradiography): a) donors' serum containing HBsAg; b) donors' serum containing anti-HBsAg; c) donors' serum negative for both HBsAg and anti-HBsAg.

TABLE 1. Detection of HBsAg in Sera of Blood Donors and Patients with Acute Virus and Chronic Hepatitis

Material tested	No. of sera	ID	CIEOP	EPPG	
				visible zone	invisible zone
Donors' sera	150	—	—	2,1%	4,4%
Sera of patients with acute virus hepatitis	60	—	—	12%	18%
Sera of patients with chronic hepatitis	140	—	—	3,5%	9%

concentration an interrupted buffer system of Tris—HCl buffer, pH 6.7-8.9, is used. The chamber is half filled with a 7% solution of Cyanogum 41 (Serva) in Tris—HCl buffer, pH 8.9. At the end of polymerization of the 7% gel, 3 ml of 1% agarose made up in Tris—HCl buffer, pH 8.9, is layered above it. After the agarose has solidified (5 min) five glass strips measuring 15 × 30 mm are introduced from above into the chamber so that their lower ends rest snugly against the layer of agar. The space between the plates is filled with 4% solution of Cyanogum in Tris—HCl buffer, pH 6.7. At the end of polymerization of the 4% gel the glass plates are carefully removed, so that five reservoirs measuring 15 × 30 × 1 mm are formed. The test serum, in a volume of 0.5 ml, is mixed with an equal volume of 0.8% agarose in Tris—HCl buffer, pH 6.7, with the addition of a "witness" (bromphenol blue) and is introduced into the reservoirs. The flat chamber is placed in the vertical electrophoresis apparatus so that the top and bottom ends of the chamber lie in Tris—glycine buffer, pH 8.3. Electrophoresis is carried out with a current of 15 mA for 1-2 h. The end of electrophoresis is taken to be the time when the strips of "witness" have reached the middle of the layer of 1% agarose. At this stage concentration of the antigen and its incorporation into the reaction zone take place. In the second stage, immunodiffusion detection of the concentrated HBsAg is carried out with the aid of a commercial immunological diagnostic serum. For this purpose the chamber is taken from the apparatus, the top transparent plastic plate is removed, and three wells are punched out (diameter of the punch tubes 3 mm, distance between centers 5 mm) to a distance of 1 mm below the strip of dye, into which the components of the immunological diagnostic set are introduced.

The immunological diagnostic set for virus hepatitis B antigen and for antibodies against it marketed by the N. F. Gamaleya Institute of Epidemiology and Microbiology consists of two components: AG (antigen) and AT (antibodies); AG is donors' serum containing HBsAg, where AT is rabbit antiserum exhausted with dried donors' plasma. The order of introduction of the components of the immunological diagnostic set for detection of the antigen is as follows: Antibodies are added to the middle well and AG to the two outer wells (Fig. 1a). To detect antibodies against HBsAg the components are added in the opposite order (Fig. 1b). The precipitation reaction is carried out in a wet chamber for 18-24 h. To develop the concentrated antigen, the original dilutions of the immunological diagnostic set are used. If the concentration of antigen is low and no visible precipitates are formed, the third stage of the method is carried out, namely detection of invisible precipitates

with the aid of ^{125}I-labeled antibodies against rabbit γ-globulin, followed by autoradiography. In this case ^{125}I-labeled antibodies against rabbit γ-globulin obtained from Izotop (Moscow) are used. The components of the immunological diagnostic set for the detection of invisible precipitates are diluted four to eight times. Before treatment of the precipitate with labeled antibodies the strip of agarose with the precipitate is washed for 6 h in 10% NaCl solution, then for 24 h in 0.85% NaCl solution with constant mixing. To strengthen the brittle agarose plate it is coated with 1.5% Difco agar. The agarose plate, washed to remove protein, is immersed for 1 h in a solution of ^{125}I-labeled antibodies against rabbit γ-globulin. Full details of the method of treating the preparation with labeled antibodies were described previously [2]. For HBsAg, fixation with 70% ethanol is deleted from the scheme of treatment of the precipitate. Autoradiography is carried out on RF-3 film and the exposure is 6-24 h.

The method was worked out on blood sera from donors who were symptom-free carriers of HBsAg and patients with acute virus hepatitis with antigenemia. The EPPG method developed by the writers was compared with two other methods in use in the USSR: the method of double immunodiffusion in gel (ID) and the method of counterimmunoelectroosmophoresis (CIEOP).

The results obtained during investigation of 400 sera from donors who were HBsAg carriers showed that the sensitivity of the EPPG method in the visible zone is 16-32 times greater than the sensitivity of ID and eight times greater than the sensitivity of CIEOP, and in the invisible zone it was 1000-2000 and 500-1000 times more sensitive, respectively. Treatment of the precipitates with ^{125}I-labeled antibodies increases the sensitivity by 60 times.

Altogether 150 sera of healthy blood donors, 60 sera of patients with acute virus hepatitis, and 140 sera of patients with chronic hepatitis were investigated. No HBsAg was found in these sera by the ID and CIEOP methods (Table 1). Control tests on sera of patients with chronic hepatitis for the presence of HBsAg revealed antibodies against this antigen. Precipitation bands formed by the nitrogen of the immunological diagnostic set and antibodies of the test serum were located above the well containing antigen, and they formed a line of immunological identity with the precipitate of the test system (Fig. 1b).

The method of electrophoresis—precipitation can thus be used to detect HBsAg and antibodies against it. This method increases the sensitivity of the immunodiffusion reaction by 1000-2000 times while retaining all of its specificity. The method does not require preliminary purification of the serum for investigation or special purification of the test system. Introduction of the method into practice will increase the quality of testing of donors' blood.

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