Detection of Hepatitis B Antigen by Counter-Immunoelectrophoresis: Enhancing Role of Homologous Serum Diluents

GORDON R. DREESMAN, F. BLAINE HOLLINGER, AND JOSEPH L. MELNICK

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77025

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Dilution of hyperimmune hepatitis type B antibody in undiluted homologous species normal serum has resulted in enhanced sensitivity for detecting hepatitis B antigen by a discontinuous counter-immunoelectrophoresis method compared to hepatitis type B antibody diluted in heterologous species normal serum or buffer solutions.

Counter-immunoelectrophoresis (CIE) (2, 4) is currently being used by blood banks and clinical laboratories to screen human blood for the presence of hepatitis type B antigen (HB Ag). A previous report (1) described the results of a two-dimensional titration between goat HB antibody (Ab) and HB Ag in which an extensive "prozone" of inhibitory activity occurred in the region of antigen excess. Dilution of the goat HB Ab in normal goat serum (NGS) minimized this phenomenon. The stabilizing effect of homologous normal serum has been further evaluated.

A discontinuous CIE (DCIE) technique (5) was used in which the ionic strength of the agarose buffer (0.015 μ) was fivefold less than that used to fill the chambers (0.075 μ) to enhance the movement of gamma globulin toward the anode by electroendosmosis. Plasma containing HB Ag was obtained from an anicteric volunteer blood donor. Hyperimmune HB Ab was prepared in goats and guinea pigs from purified antigen as previously described (1).

Goat HB Ab was diluted in various diluents and tested by two-dimensional DCIE titrations against the reference HB Ag-positive plasma diluted in normal human serum free of HB Ag or HB Ab. The use of NHS as diluent for the HB Ag was considered essential since sera are generally tested undiluted when screening donor blood by CIE. The results are summarized in Table 1. Maximum sensitivity for detecting HB Ag occurred when the goat HB Ab was diluted in fresh NGS or NGS heated at 56 C for 30 min, yielding HB Ag titers which were eightfold higher than those obtained when the antibody was diluted in low-ionic-strength (0.015 μ) Veronal buffer, pH 8.6. Increasing the ionic strength of the nonprotein buffered diluents also resulted in higher HB Ag titers (phosphate-buffered saline [PBS], pH 7.2, μ = 0.15 > Veronal buffer, μ = 0.075 > Veronal buffer, μ = 0.015). However, a prozone occurred more frequently and sensitivity was less than that observed when using undiluted NGS as diluent. Such results were not entirely unexpected since euglobulins are less soluble in low-ionic-strength buffer (3). Dilution of goat HB Ab in normal goat globulin obtained by salt precipitation yielded HB Ag titers which were slightly greater than those observed with PBS. An intermediate result was observed using the albumin-containing fraction as diluent. Dilution of goat HB Ab in heterologous species sera or serum protein fractions failed to enhance the sensitivity of the assay beyond that seen with PBS; in fact, decrease in titer as compared to NGS or PBS was seen when the goat antibody was diluted at titers of 1:160 (Table 1).

A similar four- to eightfold increase in sensitivity was obtained when guinea pig HB Ab was diluted in normal guinea pig serum compared to that obtained after dilution in low-ionic-strength buffers (Table 1).

Thus, two factors appear responsible for enhancing the sensitivity of HB Ag detection: (i) the ionic strength of the diluent and (ii) the globulin fraction of the homologous serum. It is postulated that homologous globulin stabilizes the antibody globulin during migration through the agarose gel. Enhancement by complement components was excluded since no change in

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the HB Ag titer was observed after dilution of HB Ab with either fresh or heat-inactivated NGS.

The importance of these observations was strengthened after the testing of a panel of 60 sera supplied by the National Institutes of Health, Division of Biological Standards (Table 2). The results indicate that dilution of HB Ag in homologous serum significantly increases the sensitivity of our DCIE test. In addition, by using higher dilutions, valuable HB Ab can be conserved, which represents an economic advantage to laboratories using this technique.

### Table 1. Effect on sensitivity of HB Ag detection by using different diluents to dilute hyperimmune HB Ab

| Dilution                     | HB Ag titer* observed when tested against goat HB Ab dilutions of: |
|------------------------------|---------------------------------------------------------------------|
|                              | 1:10 | 1:40 | 1:160 |
| Goat HB Ab diluted in:       |      |      |       |
| Fresh NGS                    | 160  | 160  | 160  |
| Heat-inactivated NGS         | 160  | 160  | 160  |
| Veronal buffer, μ = 0.015    | 20   | 20   | 20   |
| Veronal buffer, μ = 0.075    | 40   | 40   | 40   |
| PBS, μ = 0.15                | 80   | 80   | 80   |
| NGS globulin, 1%, in PBS*    | 80   | 160  | 80   |
| NGS albumin, 1%, in PBS†     | 80   | 80   | 80   |
| Fetal bovine serum           | 40   | 80   | 40   |
| Normal human serum           | 40   | 80   | <10  |
| Bovine γ-globulin (10%, PBS) | 40   | 40   | <10  |
| Bovine γ-globulin (1%, PBS)  | 40   | 80   | 20   |
| Bovine albumin (10%, PBS)    | 40   | 40   | <10  |
| Guinea pig HB Ab diluted in: |      |      |       |
| Normal guinea pig serum      | 80   | 160  | 80   |
| Veronal buffer, μ = 0.075    | 80   | 40   | 40   |
| Veronal buffer, μ = 0.015    | 20   | 20   | 20   |

* NHS used as diluent.
† (NH₄)₂SO₄ precipitant of NGS.
‡ (NH₄)₂SO₄ supernatant of NGS.

### Table 2. Comparative results of 60 coded human sera tested by DCIE by using different diluents for HB Ab*

| Goat HB Ab diluted 1:40 in: | Sensitivity |
|-----------------------------|-------------|
|                            | Positives identified | % Positive |
| Normal goat serum           | 31/60        | 52         |
| PBS, μ = 0.15               | 26/60        | 43         |
| Veronal buffer, μ = 0.075   | 25/60        | 42         |
| Veronal buffer, μ = 0.015   | 21/60        | 35         |

* National Institutes of Health, Division of Biological Standards Reference Hepatitis B Antigen Panel No. 2 contained 31 specimens positive for HB Ag by CIE. No false positives were observed. Subtypes ad and ay were evenly distributed among those specimens not identified in the lower-ionic-strength diluents.

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