Sensitive Matrix Gel Diffusion Test for the Detection of Australia Antigen

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A matrix gel diffusion (MGD) procedure with a sensitivity comparable to the complement fixation test (CF) has been developed for detecting Australia antigen in serum. The test utilizes a thin layer of agar (0.1 mm) with an applied plastic matrix. Reactants are introduced directly onto the surface of the agar through wells in the plastic matrix. End points obtained by CF with a panel of 11 sera varied from 1:8 to 1:512. When these sera were tested by MGD, end points for detection of antigen were within one dilution of that obtained by CF.

The association of Australia antigen with long-incubation hepatitis in humans has been shown by gel diffusion and complement fixation (CF) tests (2, 5). Recent studies have indicated that the CF procedure may be more sensitive than gel diffusion for detection of Australia antigen in serum (3, 5).

A sensitive matrix gel diffusion (MGD) procedure was developed in this laboratory for use in detecting antibodies to infectious agents (1). With slight modification, this procedure was found to detect Australia antigen in sera with a sensitivity comparable to the CF test. The following report describes this procedure and compares its sensitivity to that of the CF test.

MATERIALS AND METHODS

Serum specimens containing Australia antigen (Au) or antibody (anti-Au) were obtained from several sources (Table 1). The MGD method utilized a thin agar layer with a matrix overlay. Depth of the agar diffusion medium was approximately 0.1 mm. Microscope slides were dipped in 0.5% clarified Ionagar no. 2 and permitted to dry. Clarification was obtained by centrifugation of molten, aqueous 1% agar solution at $14 \times 10^4 \times g$ for 20 min. Blank glass matrices with vinyl tape 0.1 mm thick attached to each end were placed in the center of the coated microscope slides so that the tape touched the slides. The bottom of each slide was heated briefly in an open flame until the agar coat had melted, and a 0.5% aqueous solution of melted clarified agar (Ionagar no. 20 Consolidated Laboratories, Inc., Chicago Heights, Ill., or "Seakem" Agarose, lot 251207, Bausch & Lomb, Inc., Rochester, N.Y.) containing 0.1% sodium azide was applied with a Pasteur pipet to the space between the blank matrix and the microscope slide. When the slide and agar had cooled, usually after 5 min at room temperature, the blank matrix with attached spacer strips was slid from the agar and a plastic matrix of 3 mm thickness with a selected well pattern was immediately slid into place on the agar. Twenty microliters or less of antigen or antibody preparation was placed in opposing wells. Slides were placed in racks within a covered chamber containing a thin layer of water at the bottom and were incubated 24 to 48 hr at 25°C. Patterns of each matrix were arranged in four rows of four wells or in a hexagonal configuration with a center well. Diameter of wells was fixed at 5 mm, and distance between centers of opposing wells was 7.5 mm with a minimum of 2.5 mm separating opposing edges.

After incubation, excess reagents were flushed from the slide with distilled water, and the matrix was slid off the agar. Slides were returned to the rack and excess protein was eluted in the chamber with three 1-hr washings in 0.85% saline and a single 1-hr wash in distilled water.

Slides were stained by immersion in a solution of 1% tannic acid for 20 min or 0.1% thiazine red in 5% acetic acid for 5 min. Excess stain was removed from slides treated with tannic acid with two 20-min washes in distilled water, and slides were photographed wet. Slides stained with thiazine red were washed three times for 5-min intervals with 5% acetic acid and once for 5 min with distilled water, air dried, and photographed. For detecting reactions and for photography, better contrast was obtained with tannic acid.

The CF test used for sensitivity comparison was performed by using the Microtiter method modified by Sever (4). All sera were heated undiluted at 56°C for 30 min to destroy endogenous complement. Sera were tested at dilutions of 1:2 through 1:512 by using four units of antibody. Test sera, antibody, and 1.7 exact units of complement were mixed and incubated overnight at 4°C. The next morning sensitized sheep red blood cells were added. After incubation at 37°C for 30 min, Microtiter plates containing the tests were centrifuged 2 to 5 min at $2 \times 10^4$ rev/min and read. The last dilution showing at least 3+ fixation was the
TABLE 1. Reagents and sources

| Type                  | Source                                                                 |
|-----------------------|------------------------------------------------------------------------|
| Antigen-containing    |                                                                        |
| sera                  |                                                                        |
| Ag 1-5                | Institutional and drug-associated outbreaks and sporadic cases of hepatitis: verified and provided by M. Hatch, Laboratory Program, CDC, Atlanta, Ga. |
| Ag 6-7                | Chimpanzees 109 and 178: provided by J. Douglas, 6571st Aeromedical Research Laboratories, Holloman AFB, N. Mex. |
| Ag 8-10               | Sporadic hepatitis cases, Phoenix, Ariz. and E. Paso, Tex.: identified by CDC, Phoenix, Ariz. |
| Ag 11-16              | Sporadic hepatitis cases and institutional hepatitis outbreak, Anchorage, Alaska: identified by CDC, Phoenix, Ariz. |
| Antibody-containing   |                                                                        |
| sera                  |                                                                        |
| Ab-1                  | Aplastic anemia patient: verified and supplied by S. Rivers, Atlanta Regional Red Cross Blood Center, Atlanta, Ga. |
| Ab-2                  | Hemophiliac: verified and supplied by J. Sinclair, Banting Institute, University of Toronto, Canada. |
| Ab-3                  | Chimpanzee 208: provided by J. Douglas, 6571st Aeromedical Research Laboratories, Holloman AFB, N. Mex.; verified by A. M. Prince, New York Blood Center, New York, N. Y. |

end point. Ab-3 was the standard antiserum used at 1–8 dilutions in MGD and CF tests.

RESULTS

As illustrated in Fig. 1A, characteristic precipitin lines were formed in the MGD system when undiluted Au-containing sera were reacted with undiluted human antibody Ab-1. Identical results were obtained when these sera were reacted against human antibody Ab-2. Figure 1B shows the precipitin lines formed with the same antigen panel when chimpanzee serum antibody (Ab-3 diluted 1:8) was substituted in the center well. The identity patterns which appear as continuous lines without spurs in the precipitin reactions between chimpanzee and human sera containing Au or anti-Au are evident in both figures. Evidence supporting the identity pattern of the antigens was obtained when similar patterns were obtained by reacting each antigen against each anti-Au serum, as well as other human and chimpanzee sera known to contain anti-Au. No precipitin lines were formed when the above standard serums containing Au or anti-Au were reacted with sera from 10 healthy human controls and 20 chimpanzees with no history of hepatitis.

Results of MGD and CF tests performed on 11 Au-containing sera are presented in Table 2. The end points for detection of antigen were comparable for both procedures. Identical end points were observed in four sera, and a one-tube difference was seen between the two methods in the remaining seven.

DISCUSSION

Of the current tests available for detecting Au in serum, the radial immunodiffusion system offers a simple, inexpensive method adaptable for widespread use in a variety of laboratory circumstances. The MGD test used in this study and in subsequent replication trials was easy to perform with reproducible results. Furthermore, the results obtained indicated that this method will detect Au in sera with CF antigen titers as low as 1:8. The increased sensitivity of this method compared to the less satisfactory results reported by Purcell et al. (3) and Shulman and Barker (5), who used the Ouchterlony technique of gel diffusion, may relate to differences in concentration of reactants per unit of agar between the two procedures. With the MGD system, increased concentration of reactant per unit

TABLE 2. Comparison of matrix gel diffusion and complement fixation tests for detection of Australia antigen in serum

| Antigen-containing serum | Matrix gel diffusion | Complement fixation |
|--------------------------|---------------------|---------------------|
| Ag-6                     | 128                 | 256                 |
| Ag-7                     | 4                   | 8                   |
| Ag-8                     | 64                  | 128                 |
| Ag-9                     | 16                  | 32                  |
| Ag-10                    | 64                  | 128                 |
| Ag-11                    | 128                 | 128                 |
| Ag-12                    | 32                  | 64                  |
| Ag-13                    | 16                  | 32                  |
| Ag-14                    | 8                   | 8                   |
| Ag-15                    | 16                  | 16                  |
| Ag-16                    | 512                 | 512                 |

* Reciprocal of the highest serum dilution which gave precipitin reactions in gel diffusion or fixed complement.
of agar is obtained by using a thin agar layer. Diffusion of reagents through the surface at the bottom of the matrix well permits a longer distance for diffusion of the two reactants and a relatively larger area of clear agar for formation and detection of characteristic precipitin lines.

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