Third Component, HB_eAg/3, of Hepatitis B e Antigen System, Identified by Three Different Double-Diffusion Techniques

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A third component, HB_eAg/3, of the hepatitis B e antigen system has been detected, and it was consistently detected in three variations of the double-diffusion technique.

With the discovery of the hepatitis B e antigen (HB_eAg) and its antibody (anti-HB_e) by Magnus and Espmark (2) and the identification of two components, HB_eAg/1 and HB_eAg/2, by Williams and Le Bouvier (4), it has become apparent that a third component, HB_eAg/3, of this system exists (1-3). Our laboratories have compared reagents and methods to demonstrate that this is a unique third component and that it is consistently detectable in three variations of the double-diffusion technique. All laboratories followed the same protocol using six different hexagonal patterns.

Variations of the double-diffusion technique were as follows. In method 1, glass slides 1 by 3 inches [ca. 2.54 by 7.62 cm] were prepared with 0.45% agarose dissolved in a tris(hydroxymethyl)aminomethane (0.05 M)-sodium chloride (0.15 M) buffer, pH 7.6, containing 0.1% sodium azide and 1% Dextran T500. Wells were cut in three staggered parallel rows, 5 mm in diameter and 8 mm center to center. All wells were filled three times within 4 h. Slides were incubated in a moist chamber at room temperature and read at 24-h intervals for 7 days.

In method 2, agarose was dissolved in distilled water to a concentration of 0.6% with 0.001% sodium azide and poured into tissue culture plates (35 by 10 mm). Wells were punched in a hexagonal configuration, 5 mm in diameter and 8 mm center to center. Wells were filled once; the plates were incubated at room temperature in a moist chamber and read at 24-h intervals for 7 days.

In method 3, rheophoresis plates (Abbott Laboratories, North Chicago, Ill.) containing 0.8% agarose in tris(hydroxymethyl)aminomethane buffer (0.01 M) with 0.1% sodium azide were

used. Each peripheral well was 5 mm in diameter, and the center well was 2 mm in diameter; the distance between wells was 8 mm center to center. Test wells were filled only once, but the center well (reagent antibody) was filled twice. Plates were incubated and read as described above.

All three methods gave identical results for each of eight reagent sera used by the four participating laboratories (Table 1). Clarity of the precipitin lines was comparable in each method and detected HB_eAg/1, HB_eAg/2, and the third component, HB_eAg/3. Figure 1 shows the positioning of the precipitins. Reagents containing the newly detected third components, HB_eAg/3 or anti-HB_e/3, were tested against se-

TABLE 1. Reagents used

Reagent	Source ^a	Components
Chimpanzee 16	BoB	HB _e Ag/1, HB _e Ag/2, HB _e Ag/3
ARC J (human)	BoB	anti-HB _e /1, anti-HB _e /2
T-69 (human)	BoB	anti-HB _e /1, anti-HB _e /2, anti-HB _e /3
Chimpanzee 926	CDC	HB _e Ag/1, HB _e Ag/2, HB _e Ag/3
Eaves (human)	CDC	anti-HB _e /2, anti-HB _e /3
Chimpanzee 109	CDC	anti-HB _e /1, anti-HB _e /2
CRC-124 (human)	Yale	HB _e Ag/1, HB _e Ag/2
CRC-28 (human)	Yale	anti-HB _e /1, anti-HB _e /2

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FIG. 1. Example of the detection of three HB_sAg components by agar gel diffusion.

rum samples from 50 healthy individuals, 82 individuals with acute hepatitis A virus infection, and 25 individuals with other liver disorders. No precipitins were detected in these individuals which is further evidence that the reaction is specific to hepatitis B.

ADDENDUM

Since the writing and submission of this note, A. M. Courouce-Pauty, et al., Centre National de Transfusion Sanguine, Paris, France, has published an article (1) recognizing three components of the HB_sAg system. We have exchanged reagents and are in agreement with regard to the immunological identity of the three components of the HB_sAg system. However, at the present time there continues to be a difference in terminology for these separate components. Our HB_sAg/1 is Courouce's HB_sAg/3, our HB_sAg/2 is her HB_sAg/1, and our HB_sAg/3 is her HB_sAg/2.

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