Comparison of the Reversed Passive Hemagglutination with Radioimmunoassay Methods for Hepatitis B Antigen

W.-D. LEERS* AND G. M. KOUROUPIS

Department of Microbiology of The Wellesley Hospital and Department of Medical Microbiology, University of Toronto, Toronto, Ontario, Canada*

Received for publication 1 April 1975

Radioimmunoassay for the detection of hepatitis B antigen has been accepted in many diagnostic laboratories now. The question of nonspecific positives has always been a matter of controversy. Two improved radioimmunoassay tests, namely Ausria II-125 by Abbott Laboratories and a radioimmunoassay method by Connaught Laboratories Limited (Hebria), were compared with the original Ausria ¹²⁵I. Included in the comparison was the reversed passive hemagglutination test (Auscell, Abbott). Five hundred sera of clinical patients were tested. Fifty-five or 11% were found to have hepatitis B antigen. Three tests, Ausria ¹²⁵I, Ausria II-125, and Hebria showed the same number of positive sera, whereas Auscell missed one. However, Ausria ¹²⁵I indicated two additional false positives. Dilution experiments, however, indicated that Ausria II-125 and Hebria were the most sensitive tests, with the reversed passive hemagglutination showing the least sensitivity. Therefore, the new Ausria II-125 and the Hebria radioimmunoassay tests are preferable in view of their sensitivity and specificity.

A variety of tests are generally available for the laboratory diagnosis of hepatitis B antigen (HB-Ag), such as the agar gel diffusion, counter-immunoelectrophoresis (CIEP), complement fixation test (CFT), immunoelectromicroscopy, reversed passive hemagglutination (RPH), and direct radioimmunoassays (RIA) (3-7).

RPH and RIA tests were introduced to the market by Abbott Laboratories (North Chicago, Ill.) under the trade names of "Auscell" and "Ausria ¹²⁵I", respectively. Recently, Abbott developed a variation of Ausria ¹²⁵I with the trade name "Ausria II-125." Lately, Connaught Laboratories (Willodale, Ontario, Canada) introduced "Hebria," a direct RIA method based on the same principles as those of Ausria ¹²⁵I (10).

The purpose of this study was to determine the degree of sensitivity between CIEP, RPH, Ausria-I, Ausria-II, and Hebria. We have excluded the agar gel diffusion because of its lack of sensitivity (4). The sensitivity of the CFT has been established previously (4, 7). It has not gained widespread use because of its technical complications, although it is more sensitive than the CIEP. The CFT was necessary in this study because it provided a titer as reference point.

(This paper was presented in part at the Joint Meeting on Infectious Diseases of the Canadian

Public Health Association, 27-30 November 1974, Ottawa, Ontario, Canada.)

MATERIALS AND METHODS

Antisera. The antisera for this study were provided in a kit by Abbott and Connaught Laboratories. These antisera were prepared either in guinea pigs (Ausria) or in chimpanzees (Hebria) by injection of purified and concentrated HB-Ag by a process similar to that employed by Gerin et al. (2).

CIEP and CFT. CIEP and CFT were performed by the method described previously (7).

RPH test. We employed the RPH technique developed by Abbott (Auscell). This test is based on the agglutination of Duracyte (Abbott Laboratories) cells by HB-Ag. Duracyte cells are specially prepared, stabilized lyophilized human erythrocytes sensitized with hepatitis B antibody (HB-Ab). In the presence of sufficient HB-Ag, the antibody-sensitized cells form a complex leading to visible agglutination. Agglutinated antibody-sensitized Duracyte cells form a disperse pattern on the bottom of the well in the disposable "V" plate, whereas lack of agglutination is indicated by the antibody-sensitized Duracyte cells settling into a sharp compact button (1, 5).

RIA tests. (i) **Ausria-I.** The Ausria-I is a solid phase RIA technique which uses the sandwich principle to detect HB-Ag (10).

(ii) **Ausria-II.** The Ausria-II also uses the sandwich principle with the solid phase RIA technique. Plastic beads instead of tubes as in Ausria-I, coated

with guinea pig HB-Ab are supplied with the Ausria-II kit. These beads are placed in small tubes and 0.2 ml of patient's serum is added. During incubation, any HB-Ag from the patient's serum would attach to the antibody, then antibody tagged with iodine-125 is added, which binds to any HB-Ag on the bead creating an antibody-antigen-antibody sandwich complex. Ausria-II employs a heterologous system utilizing antibodies from two different species. An antibody of guinea pig origin is used in the solid phase of the bead and an antibody of human origin is used for the radiolabeled antibody. This combination should minimize potential cross-reactions with immune globulins of the sera tested.

(iii) **Hebria.** Connaught's RIA kit on the other hand uses incubation of the test samples in polystyrene tubes which have HB-Ab prepared in guinea pigs absorbed to their inside surfaces. The tubes are washed twice and then incubated with a solution containing HB-Ab prepared in chimpanzees and labeled with ¹²⁵I. After two further washes the radioactivity is determined.

Incubation time and incubation temperature. RIA-I, RIA-II, and Hebria were incubated at 47°C for 2 h as described previously (8).

RIA specificity determination by HB-Ab absorption. All RIA-positive sera were treated as follows: equal volumes of the serum sample and rabbit serum containing antibodies to HB-Ag were mixed and left at room temperature for at least 1 h. These sera were then again tested for the presence of HB-Ag by RIA. The absence of HB-Ag in the repeated RIA test would confirm specificity.

RESULTS

Five hundred sera from clinical patients were tested. The titer for HB-Ag was determined by CFT. We compared the HB-Ag content in those sera with CIEP, Auscell, Ausria-I, Ausria-II, and Hebria. Results are presented in Table 1. There was no difference in the results from sera with a CFT titer of 1:32 or higher. In sera with a CFT titer of 1:16 or lower, CIEP was less sensitive than the other four tests. There was no significant difference between the latter four tests, with RPH missing only one positive serum with a low HB-Ag titer.

To detect the true sensitivity of the five different test systems, a serum with a high HB-Ag content (CFT titer, 1:512) was diluted and compared by the five different methods (Table 2). The results indicate that Ausria-II and Hebria detected HB-Ag in the highest dilutions followed by Ausria-I, Auscell, and CIEP in order of decreasing sensitivity. Testing of additional sera with a high HB-Ag content produced a comparative pattern. All positive sera were retested after absorption with HB-Ab prepared in rabbits to confirm specificity. Results are shown in Table 3.

We detected no false positive sera in CIEP, Auscell, Ausria-II, or Hebria. In contrast, two false positive sera were found with Ausria-I.

TABLE 1. Comparison of 500 tested for HB-Ag by different methods

No. of specimens tested	CFT	No. of positives ^a				
		CIEP	Auscell	Ausria-I	Ausria-II	Hebria
438	<1:2	0	0	0	0	0
5	1:2	0	0	1	1	1
3	1:4	0	0	0	0	0
2	1:8	0	2	2	2	2
4	1:16	2	4	4	4	4
11	1:32	11	11	11	11	11
18	1:64	18	18	18	18	18
14	1:128	14	14	14	14	14
5	1:256	5	5	5	5	5

^a Auscell, Ausria-I, Ausria-II, Hebria, (Auscell-Abbott); (Ausria 125-I - Abbott); (Ausria II-125 - Abbott); (Hebria - Connaught).

TABLE 2. HB-Ag containing serum tested by different methods^a

Serial dilution of sample with CFT titer 1:512	Reciprocal of CFT titer	CIEP	Auscell	Ausria-I	Ausria-II	Hebria
1:2	256	+	+	+	+	+
1:4	128	+	+	+	+	+
1:8	64	+	+	+	+	+
1:16	32	+	+	+	+	+
1:32	16	-	+	+	+	+
1:64	8	-	+	+	+	+
1:128	4	-	+	+	+	+
1:256	2	-	±	+	+	+
1:512	<2	-	-	+	+	+
1:1024	<2	-	-	+	+	+
1:2048	<2	-	-	+	+	+
1:4096	<2	-	-	-	+	+
1:8192	<2	-	-	-	-	-

^a -, Absence of HB-Ag; +, presence of HB-Ag.

TABLE 3. Confirmation of specificity after absorption of HB-Ag positive sera by anti-HB rabbit serum

Test	Before confirmation	After confirmation	No. of false positives
CIEP	50	50	0
Auscell	54	54	0
Ausria-I	57	55	2
Ausria-II	55	55	0
Hebria	55	55	0

DISCUSSION

The continued search for greater sensitivity and specificity in the laboratory diagnosis of hepatitis B has led to the production of improved RIA tests. The most recent ones are the Ausria II-125 (Ausria-II), which is claimed to be

an improved test of Ausria ¹²⁵I (Ausria-I), both developed by Abbott Laboratories, and Hebria, a RIA test developed by the Connaught Medical Laboratories.

Ausria-I is a useful sensitive test for the laboratory diagnosis of HB-Ag. However, it lacks a certain degree of specificity by showing false positive results. This fault could be corrected satisfactorily by absorbing the sera with anti-HB-Ab and retesting (7). Modification of the Ausria-I failed to solve the problem of specificity satisfactorily in our hands (9).

For the comparative study, 500 sera of clinical patients were tested by CIEP, RPH, and the three different RIA tests. The CFT was used only because it provides a titer as reference point.

The CIEP is a comparison to the RPH on one hand, whereas the Ausria-I, Ausria-II, and Hebria constitute a comparison of the three RIA methods.

Out of 500 sera, Ausria-II and Hebria detected 55 positive sera. Ausria-I detected two additional sera, that were found to be false positives on confirmation. All other 55 sera were found to contain HB-Ag on confirmation. This result indicates an improved specificity of both the Ausria-II and the Hebria, whereas 0.4% of the sera tested by Ausria-I were false positives ($P > 0.01$).

Dilution experiments for the detection of sensitivity showed that Ausria-I has a slightly reduced sensitivity as compared to Ausria-II and Hebria (Table 2). Therefore, both Ausria II and Hebria are superior to Ausria-I in specificity and sensitivity.

The RPH test (Auscell) was found to be considerably less sensitive than the RIA tests (Table 2). However, it was found to be more sensitive than CIEP and CFT which confirms a previous study (3). The fact that only one out of 55 positive sera was missed, was because these 55 sera contained a large proportion with a high HB-Ag content as shown by the CFT titer (Table 1). It is conceivable therefore that RPH could miss a certain number of sera with a low HB-Ag content; for example, in the range of a CFT titer of 1:4 or less. These sera would not be missed by RIA as has been shown previously (7).

In addition to its reduced sensitivity as compared to RIA, we encountered also some technical problems with the RPH. Sera with a low HB-Ag content, as indicated by CFT titer, had to be repeated to confirm clear results. These sera frequently produced a doubtful pattern of the settled erythrocytes, which prevented us from reading the results as clear positives or negatives. Only repetitive testing finally led to a reliable reading. This fact would make the RPH test a less reliable tool for the detection of HB-Ag in a routine diagnostic laboratory.

Our study, therefore, indicates that the new Ausria II-125 and Hebria are superior to both the Ausria ¹²⁵I and the RPH test (Auscell) in sensitivity and specificity.

ACKNOWLEDGMENTS

We wish to acknowledge the assistance of the technical staff of the Department of Microbiology and E. Mann for the preparation of the manuscript.

LITERATURE CITED

1. Cook, R. J. 1965. Reversed passive haemagglutination systems for the estimation of tetanus toxins and antitoxins. *Immunology* 8:74-80.
2. Gerin, J. L., R. H. Purcell, M. D. Hoggan, P. V. Holland, and R. M. Chanok. 1969. Biophysical properties of Australia antigen. *J. Virol.* 4:763-768.
3. Hirata, A. A., A. J. Emerick, and W. F. Boley. 1973. Hepatitis B virus antigen detection by reversed passive haemagglutination. *Proc. Soc. Exp. Biol. Med.* 143:761-763.
4. Holper, J. C., and A. Jambazian. 1971. Comparative sensitivity of complement fixation, counterimmunoelectrophoresis, radial and double diffusion of detection of Australia antigen. *Transfusion* 11:157-161.
5. Juji, T., and T. Yokochi. 1969. Haemagglutination technique with erythrocyte coated with specific antibody for detection of Australia antigen. *J. Exp. Med.* 39:615-620.
6. Kelen, A. E., A. E. Hathaway, and D. A. McLeod. 1971. Rapid detection of Australia/SH antigen and antibody by a simple and sensitive technique of immunoelectron-microscopy. *Can. J. Microbiol.* 17:993-1000.
7. Kouroupis, G. M., and W.-D. Leers. 1973. Radioimmunoassay, complement fixation and counter-immunoelectrophoresis in the laboratory diagnosis of hepatitis B. *Arch. Gesamte Virusforsch.* 43:112-118.
8. Kouroupis, G. M., and W.-D. Leers. 1974. A rapid radioimmunoassay method for the detection of hepatitis B antigen. *Arch. Gesamte Virusforsch.* 44:370-376.
9. Leers, W.-D., and G. M. Kouroupis. 1973. R.I.A. kit for assay of hepatitis-B antigen. *Lancet* 11:1334-1335.
10. Miller, U. P., and B. G. Mordecai. 1972. Evaluation of a solid-phase radioimmunoassay technique for the detection of hepatitis-associated antigen. *J. Nucl. Med.* 13:454.