

Evaluation of a Red Cell Agglutination Test for Detection of Australia Antigen (HB Ag)

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An investigational red cell agglutination (RCA) test was evaluated for sensitivity in detecting and titering hepatitis B antigen (HB Ag) in comparison with two counterelectrophoresis (CEP) systems and a solid-phase radioimmunoassay (RIA). The RCA procedure was found to be significantly more sensitive than the CEP methods and compares favorably in sensitivity with the solid-phase RIA, detecting even lower concentrations of the HB Ag. Since the RCA test can be completed in 2 to 3 h and requires relatively inexpensive equipment, it offers a highly sensitive and rapid procedure suitable for use in blood banks to screen donors or detect low levels of antigen in serum of patients.

The identification of Australia antigen or hepatitis B antigen (HB Ag) by Blumberg et al. (1) and its subsequent correlation with hepatitis (2) has allowed, for the first time, the screening of blood donors for potentially infectious blood. The methodology has ranged from Ouchterlony double diffusion (7) to counterelectrophoresis (CEP) (3) and radioimmunoassay (RIA) (4-6, 9) with RIA being described as the most sensitive method (4, 6). However, RIA is not a practical procedure for many laboratories and requires at least an overnight incubation. A passive hemagglutination test described by Vyas et al. (8) and Hollinger et al. (4) has been shown to exhibit a high order of sensitivity. An experimental hemagglutination test termed "red cell agglutination" (RCA) was made available for evaluation. This test system was supplied by Abbott Laboratories and compared with counterelectrophoresis and solid-phase radioimmunoassay for relative sensitivity in titering and detecting the hepatitis B antigen.

MATERIALS AND METHODS

Test systems: RCA. The test was supplied as lyophilized sensitized "duracytes." These duracytes are human erythrocytes stabilized by double-aldehyde-fixation and sensitized with hepatitis-associated antibody (HB Ab) from guinea pigs as described by Sultan et al. (Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 214, 1972). When reconstituted to 0.25% with supplied buffer, the sensitized cells may be used to screen serums or titer samples for presence of antigen. In preparing sera, all dilutions were

made with the diluting buffer supplied with the RCA system. In screening serums, 0.025 ml of sensitized cells are added to 0.025 ml of a test serum (1:16); in titration of serums, serial dilutions of test material (0.025 ml) are prepared in a microtiter system using disposable "V" plates, and then the same volume of cells (0.025 ml) is added to each dilution. The plates are agitated on a Vortex mixer to afford uniform distribution of reagents within each well. The test is incubated for 2 h at room temperature and read for agglutination patterns. Serums showing positive agglutination at the 1:16 dilution in the screening procedure are confirmed by subsequent titration of the specimen. A duracyte control (0.25% unsensitized duracyte cells) is run with each serum along with a positive and negative control.

Radioimmunoassay. The Ausria-125 solid-phase radioimmunoassay of Abbott Laboratories was employed for testing of selected serum panels. The sera (0.1 ml) were added to polypropylene tubes coated with guinea pig HB Ab and then incubated for 16 h. Unbound antigen is removed by washing each tube five times with 2 ml of either tris(hydroxymethyl)aminomethane (Tris) buffer or sterile distilled water. This is followed by a second 90-min incubation after adding 0.1 ml of ¹²⁵I-labeled HB Ab, thus creating an antibody-antigen-¹²⁵I antibody "sandwich." The tubes again were rinsed as above to remove unbound label, and the radioactivity was determined in a gamma counter. With each unit of tests, seven negative and three positive controls were included to establish the mean and standard deviations. In these tests, the positive limit was defined as 2.1 times the mean count of the seven negative controls. Serums consistently giving a count in a range from 1.5 to 2.1 times the above mean value for negative controls are considered questionable, and for screening of donors the blood would not be used.

Counterelectrophoresis. Two commercial CEP systems (Ortho Hapindex and Abbott AUS-tect) were employed for comparison with the RCA test. The total Hapindex system was used, and the AUS-tect plates, buffer, and wicks were used with a Gelman electrophoresis chamber and Heathkit power supply. Sera were tested along with a standard positive serum according to manufacturer's directions.

Serum panels: library sera panel. A library of sera from human hepatitis-related cases, collected in the Midwest since 1955 and maintained in our laboratory, was available for testing. This library contains sera from patients and blood donors implicated in hepatitis cases. One panel of 173 known HB Ag-positive sera (95 hepatitis patients and 78 blood donors) was selected to titer quantity of antigen. A second panel of 96 sera composed of 23 implicated donors and 73 hepatitis patients was used for screening evaluations.

Hospital patient panel. Sera from 60 hospitalized patients comprised this panel. Tests for HB Ag were requested by the admitting physician on these patients, and the group also includes sera from 20 renal dialysis patients.

Random populations panel. Sera from 200 blood donors and 100 routine hospital admissions at Baptist Memorial Hospital, Memphis, Tenn., were used in this evaluation. In addition, sera from 100 hospital laboratory personnel were included as a part of the random panel.

American Association of Blood Banks panel. A panel of 20 sera supplied to participating hospitals by the American Association of Blood Banks (AABB) for a survey on HB Ag testing was included in the investigation.

RESULTS

Sensitivity in titering HB Ag. Each of the 173 library sera known to be HB Ag positive by a prior CEP assay was titered by the two CEP systems and the RCA test. Sera were checked initially undiluted, and then subsequent twofold dilutions were prepared in normal saline starting with a 1:10 dilution for the CEP assays. Table 1 gives the distribution according to titer of the 173 serums by the three systems. The figures represent the number of sera showing a particular titer by each of the systems. Hapindex detected lower levels of HB Ag than AUS-tect, showing 21 positive sera at 1:160, whereas AUS-tect detected only 1. All 173 sera were positive by RCA with 171 having titers equal to or greater than 1:1280.

Sensitivity in screening for HB Ag. To further evaluate the sensitivity of the RCA test for detecting HB Ag, the random population panels consisting of blood donors, hospital admissions, and laboratory personnel were tested by the RCA screening procedures and the AUS-tect CEP system. In addition, the

TABLE 1. *Distribution of 173 HB Ag-positive sera as to titer by three detection systems*

HB Ag titer	Detection system		
	CEP		RCA
	Aus-Tec	Hapindex	
Undilute	59	36	0
1:10	52	24	0
1:20	39	23	0
1:40	15	41	0
1:80	7	28	0
1:160	1	21	0
1:320	0	0	0
1:640	0	0	2
≥1:1280	0	0	171

AABB reference panel was tested by both CEP systems for comparison with the RCA technique. The results of this screening study are presented in Table 2. No additional positive sera were detected by RCA in the laboratory personnel, admissions, or donors. However, the RCA test revealed 11 positives out of 20 serums in the AABB panel, whereas the AUS-tect detected only 3 and the Hapindex 9.

Comparison with radioimmunoassay. The RCA procedure also was compared with the RIA test for detecting HB Ag in the screening of different serum panels. For this study, the second panel of 96 library sera and the hospitalized patient panel were tested by both screening techniques along with the AUS-tect CEP system. These results are summarized in Table 3 and show that HB Ag was detected in 34 of 156 sera by either the RCA or the RIA technique, whereas only 8 sera were positive by the CEP method. Of the 34 HB Ag-positive sera, 30 were detected by both RCA and RIA; two sera were positive by RCA and negative by RIA, whereas two were negative by RCA and positive by RIA. Therefore, both the RCA and RIA showed the same relative sensitivity for detecting HB Ag in screening tests with the serum panels from clinically ill and hospitalized patients.

Since the RCA and RIA procedures showed comparable results in detecting HB Ag in the panel sera by screening techniques, 25 HB Ag-positive serums were selected at random and tested to compare the absolute sensitivities between the two methods. A comparison of the end-point HB Ag titers of these sera determined by the RCA and RIA methods is summarized in Table 4. The results show that the RCA titer is higher than the RIA titer for each of the 25 serums and suggests that the RCA test

TABLE 2. Detection of HB Ag by red cell agglutination and counterelectrophoresis

Serum panel	Total no. of serums	No. of serums HB Ag-positive by:	
		RCA	CEP
Blood donors	200	1	1
Hospital admissions	100	4	4
Laboratory personnel	100	5	5
AABB reference panel ^a	20	11	3

^a CEP by Hapindex system detected nine positive samples.

TABLE 3. Comparative evaluation of serum panels for detection of HB Ag by red cell agglutination (RCA), radioimmunoassay (RIA), and counterelectrophoresis (CEP)

Serum panel	Total no. of serums	No. of serums HB Ag-positive tested by:		
		RCA	RIA	CEP
Blood donors (selected by recipient case history)	23	3	3	0
Hepatitis patients ^a (selected by case history and clinical diagnosis)	73	22	21	2
Hospital patients ^b	60	9	10	6

^a Two positive by RCA and negative by RIA; one negative by RCA and positive by RIA.

^b One negative by RCA and positive by RIA.

TABLE 4. Comparison of titers in twenty-five HB Ag-positive sera by red cell agglutination (RCA) and radioimmunoassay (RIA)

RCA HB Ag titer	No. of sera	RIA HB Ag titer						
		1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
1:640	4	2	2					
1:1,280	2		1		1			
1:2,560	6			3	3			
1:5,120	8				3	5		
1:10,240	2					1	1	
1:20,480	2					1		1
1:81,921	1							1

is capable of detecting HB Ag at even lower concentrations than the RIA test. In fact, the RCA test detected antigen at a four- to eight-fold greater dilution in 22 of the 25 serums. Two serums were positive at a 16-fold greater dilution and one serum was positive at only a

twofold greater dilution by the RCA method. Hence, it appears that the RCA test is capable of detecting even lower concentrations of HB Ag in serum than the RIA technique.

DISCUSSION

The objective of this study was to evaluate the relative sensitivity of the RCA test for the detection and titration of HB Ag in serum. When the RCA test was compared with two CEP methods as to titers of antigen in known HB Ag-positive sera, the serums showed significantly higher antigen titers by RCA than with either of the two CEP procedures. In studies designed to test the ability of RCA to detect HB Ag-positive sera in comparison with CEP and solid-phase RIA, and RCA did not detect any additional positive sera over the CEP system in groups of 200 blood donors, 100 laboratory personnel, and 100 random hospital admissions. This is due, possibly, to the expected low incidence in "normal" populations, and much larger samples would be needed to detect a difference. In testing the AABB reference panel and selected hepatitis-related sera, the RCA system detected more positive sera than either the AUS-tect or Hapindex CEP systems. On the other hand, the RCA and RIA tests appeared to be of similar sensitivity for detection of HB Ag in screening of sera. However, the RCA test may be capable of detecting even lower concentrations of HB Ag than theoretically could be present in apparently healthy blood donors whose sera still might be capable of transmitting hepatitis. By virtue of the fact more rapid results can be achieved using relatively inexpensive equipment, the RCA method offers a practical and highly sensitive means to screen blood donors for HB Ag and quantitate antigen in suspected patients.

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