Evaluation of Turkey Erythrocyte Hemagglutination Assay for the Detection of Hepatitis B Antigen

RAMON I. RAMIREZ, MOTI L. TIKU, AND PEARAY L. OGRA*

Departments of Pediatrics* and Microbiology, School of Medicine, State University of New York at Buffalo, and Division of Virology, Children's Hospital, Buffalo, New York 14222

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A recently described hemagglutination test for hepatitis B antigen (HB₄Ag) using turkey erythrocytes coated with horse antibody to HB₄Ag purified by affinity column chromatography was evaluated on a comparative basis with 100 HB₄Ag-positive and -negative serum samples. The turkey erythrocyte hemagglutination test (TEHA) was found to be less sensitive than radioimmunoassay (RIA) but gave far better results than counterimmunoelectrophoresis. Quantitative titration of HB₄Ag in serial dilutions of the samples appeared to be more reliably performed by TEHA than by RIA. TEHA is a simple and sensitive technique for the detection of HB₄Ag and may offer several practical advantages over RIA.

Considerable evidence has accumulated to suggest a strong association between hepatitis B antigen (HB₄Ag) and serum (type B, MS2, transfusion) hepatitis in man (8). The antigen has been demonstrated in peripheral blood and various other human body fluids (7). Although a number of testing procedures are currently available for the detection of HB₄Ag, there is a great need for a simple, sensitive, rapid and relatively inexpensive testing procedure in blood bank services, clinical laboratories, and epidemiological surveys. Of the test systems available at present, radioimmunoassay (RIA) is considered one of the most sensitive. However, this test is expensive and needs sophisticated equipment and highly trained personnel. Counterimmunoelectrophoresis (CEP) is commonly employed in blood bank service units as a screening test to detect HB₄Ag. CEP, although a quick test, is far less sensitive than RIA. Various other tests have been described, such as Ouchterlony double diffusion, red cell agglutination, reverse passive hemagglutination, latex agglutination, complement fixation, and immunoelectron microscopy (2, 4, 5, 10, 11), to name a few. Recently, a rapid hemagglutination test has been described (Wellcome Laboratories, England) which uses turkey erythrocytes coated with horse antibody to hepatitis B antigen purified by affinity column chromatography.

The present study was undertaken to evaluate, on a comparative basis, the sensitivity and specificity of turkey erythrocyte hemagglutination (TEHA) with RIA, CEP, and latex agglutination tests (10) for the detection of HB₄Ag.

MATERIALS AND METHODS

The test sera utilized in this study were obtained from the inmates of West Seneca State School, presently being followed as a prospective group for the study of the epidemiology of hepatitis B infection in a closed institutionalized population. One hundred specimens of HB₄Ag-positive serum were obtained from 100 antigen-positive subjects. The presence of HB₄Ag was initially detected and subsequently confirmed by RIA. In addition, 100 specimens of serum found to be HB₄Ag negative by RIA were included as controls.

TEHA was performed as described previously (1). The technique is summarized briefly below. U-bottom, rigid disposable plates (Cookes Engineering Co.) were used. Serial dilutions of the test serum starting at 1:8, were made in phosphate-buffered saline (pH 7.2) to which 4% normal human serum and 2% normal horse serum were added. One drop of antibody-coated cells was added to each well. The test was read over an illuminated translucent background after 30 min at room temperature. When hemagglutination was present, the cells did not settle into a tight ring or button at the bottom of the wells.

Formalized turkey erythrocytes were tanned and coated with affinity column-purified hepatitis B antibody by the method described by Sequeira and Eldridge (9) for coating erythrocytes with sonically treated Treponema pallidum. The antibody-coated erythrocytes were kindly provided by Wellcome Laboratories (Kent, England).

RIA for HB₄Ag was carried out with the Abbott Ausria I Kit, which employs a solid-phase sandwich method with antibody-coated polystyrene tubes and
was positive by rocytes. Furthermore, eliminated cytes samples positive All (Table serum samples. to HB.Ag-positive RIA-confirmed CEP gave serum samples. response glutinating activity by absorption positive in these samples, previously and CEP (Table 1). was applied the agarose. (3). HB.Ab plates previously (9). was applied the agarose. (3). HB.Ab plates previously (9). was applied the agarose. (3). HB.Ab plates previously (9).

RESULTS

One hundred HB.Ag-positive and -negative serum samples, previously tested and confirmed by RIA, were comparatively studied by TEHA and CEP (Table 1). TEHA testing gave HB.Ag-positive results in 94% of the RIA-positive test samples, whereas CEP detected only 80% of the RIA-positive samples.

Of the 100 RIA-negative samples, none gave a positive reaction when tested by CEP. However, TEHA gave a weakly positive hemagglutination response in two (2%) of the RIA HB.Ag-negative serum samples. The hemagglutinating activity in these two samples was effectively removed by repeated absorption with normal turkey erythrocytes. Furthermore, absorption with specific antibody to HB.Ag failed to inhibit the hemagglutinating activity of the two false-positive samples.

The results of CEP and latex agglutination testing for HB.Ag were compared in 27 HB.Ag-positive and 30 HB.Ag-negative samples of serum (Table 2). Both latex agglutination and CEP gave positive results in 19 out of 27 RIA-confirmed HB.Ag-positive serum samples. All samples positive by CEP were also positive by latex agglutination. Two serum samples gave false-positive results by latex agglutination, whereas none of the 30 RIA-negative samples was positive by CEP.

Twelve HB.Ag-positive serum specimens

were selected at random, and serial dilutions of the samples were tested by HB.Ag by TEHA, CEP, and RIA to compare the relative sensitivity of these techniques. The representative results of a single sample are presented in Table 3. The serum dilutions that were positive for HB.Ag by TEHA were also found to be positive by RIA, and the end points of antigen titer by TEHA and RIA were similar (Table 3). No HB.Ag could be detected by CEP in higher serum dilutions than those that were positive for HB.Ag by TEHA.

DISCUSSION

TEHA gave 94% positive results when tested against the RIA HB.Ag-positive sera. In an earlier study (1), only 1 of 111 RIA-positive sera gave a negative result. Of 100 RIA HB.Ag-negative serum samples tested by TEHA, false-positive reactions were observed only in two samples. Both of these sera were negative by CEP. The incidence of false-positive results in this study is slightly more than that reported in the earlier study (1). Although nonspecific agglutinins for turkey cells are infrequently seen in human sera, the false-positive reactions appeared to be largely due to such naturally
occurring agglutinins. Absorption of these serum samples with turkey erythrocytes eliminated nonspecific hemagglutination.

As a screening test, TEHA is less sensitive than RIA but gives far better results than CEP. The results can be read in less than 1 h, which may be of importance in certain clinical situations, such as hemodialysis units, and in transfusion services.

HB$_B$Ag may increase, decrease, or at times disappear in chronic carriers only to reappear later on. Therefore, a sequential quantitation of the antigen may perhaps be a more important parameter of the level of antigenemia than its simple presence in a single, undiluted sample. Quantitation done on serial serum dilutions by TEHA gives a reliable titer of HB$_B$Ag. Titration of HB$_B$Ag by RIA is an expensive procedure, and the end point of the titration is often difficult to interpret. The relative ease with which titration can be performed with TEHA may be one of its distinctive advantages over RIA.

TEHA is a reliable, sensitive, and inexpensive technique, and the results can be read in less time than RIA. Technically it is simple and does not need expensive equipment as does the RIA. TEHA gives more uniform titration results for HB$_B$Ag than does RIA, which may have importance in the follow-up evaluation of certain clinical situations with HB$_B$Ag. Latex agglutination, although rapid and simple and as sensitive as CEP, may occasionally give false-positive results.

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LITERATURE CITED


