Passive Hemagglutination Test for Antibody to Hepatitis B Core Antigen

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Available tests for antibody to hepatitis B core antigen (anti-HBc) are complicated. A passive hemagglutination assay for anti-HBc was developed by sensitizing sheep erythrocytes with hepatitis B core antigen synthesized by a recombinant DNA technique. It was compared with a commercial passive hemagglutination assay and an enzyme-linked immunosorbent assay for anti-HBc and showed good agreement with both. It is rapid, simple, and sensitive.

Antibody (anti-HBc) to hepatitis B core antigen (HBcAg) is a useful marker for hepatitis B virus infections (4,5). The development of tests for anti-HBc has been hampered by the limited supply of HBcAg. Recently, an HBcAg synthesized by a recombinant DNA technique has been successfully used in a radioimmunoassay (3,7,9) and an enzyme-linked immunosorbent assay (8) for anti-HBc, but both are technically complicated. To simplify testing for anti-HBc, we devised a passive hemagglutination assay (PHA) for anti-HBc that uses synthetic HBcAg.

Sheep erythrocytes (SRBC) were collected in Alsever solution, washed three times in 0.15 M phosphate-buffered saline (PBS) (pH 7.2) and packed by centrifugation at 2,000 rpm for 5 min in a Sorvall GLC 2 centrifuge with a swing-out rotor. Packed SRBC (0.1 ml) were mixed with 1.2 ml of PBS (pH 7.2)–0.25 ml of 2.5% glutaraldehyde in water, rotated at room temperature for 2 h, and washed three times in PBS (pH 7.2). The glutaraldehyde-preserved SRBC were tanned by mixing 2.5% SRBC in PBS (pH 7.2) with an equal volume of tannic acid diluted 1:20,000 in PBS (pH 7.2), incubating the mixture at 37°C for 30 min, washing the mixture three times in PBS (pH 7.2), and sensitizing the mixture with an HBcAg synthesized in Escherichia coli by a recombinant DNA technique (9) (this HBcAg was donated by Biogen S.A., Geneva, Switzerland). Sensitization with the HBcAg was done by mixing 1 volume of 2.5% tanned SRBC with 1 volume of the HBcAg, both diluted in 0.15 M PBS (pH 6.4), and incubating the mixture at 37°C for 30 min. The HBcAg-sensitized SRBC were washed three times in PBS (pH 7.2), made into a 0.5% suspension in PBS (pH 7.2) containing 0.5% bovine serum albumin and 0.1% NaCl, and lyophilized. The optimal dilution of HBcAg used for the sensitization of SRBC was determined by box titration at a twofold dilution against positive and negative sera.

The PHA was done in U-bottom microtiter plates with 0.025 ml of serum diluted to 1:16, 1:32, and 1:64 in PBS (pH 7.2) containing 3% normal rabbit serum, 1% bovine serum albumin, and 0.1% NaCl. The 1:32 and 1:64 serum dilutions, 0.025 ml of HBcAg-sensitized SRBC was added. To the 1:16 serum dilution, 0.025 ml of control SRBC (0.5% glutaraldehyde-preserved SRBC in PBS (pH 7.2)) was added. A 2+ hemagglutination at a minimum serum dilution of 1:32 was considered positive for anti-HBc. Nonspecific hemagglutination occurred in less than 1% of sera; these sera were absorbed for 1 h with an equal volume of 20% glutaraldehyde-preserved SRBC in normal saline and retested.

Two serum specimens with PHA titers of 1:4,096 and 1:1,048,356 were tested for within-run repeatability by endpoint titration on the same day, 20 times each. To test for between-run repeatability, we divided four anti-HBc-positive serum specimens into 10 aliquots each, froze them at −40°C, and titrated them daily for 10 days, using new aliquots each day. The PHA showed excellent within-run and between-run repeatability. Within-run and between-run assays with 1:64 serum dilutions were made 20 times each with 20 sera, 10 of which were PHA positive, and the results in all runs were identical.

A comparison was made with a commercial PHA for anti-HBc (Corecell; Green Cross Corp., Inc., Osaka, Japan) with 1:64 dilutions of sera from 150 hospital personnel. Both tests showed good agreement (Table 1), except for an insignificant difference between 1+ hemagglutination and 2+ hemagglutination in nine specimens; these results indicate a similarity in sensitivity and specificity. Corecell was positive while our test was negative for three serum specimens which were also negative for both hepatitis B surface antigen (HBsAg) and antibody to HBsAg. PHA titers in these two PHAs for 10 anti-HBc-positive samples were identical, except for one serum sample that showed a difference of fourfold; these results indicate equal sensitivity.

A comparison was made with an enzyme-linked immunosorbent assay for anti-HBc (Corzyme; Abbott Laboratories, North Chicago, Ill.) with a different lot of 53 coded serum specimens. The PHA was done with 1:32 and 1:64 serum dilutions. Both tests showed an agreement of 98% (Table 2).

### Table 1. Comparison of two PHAs for anti-HBc with 1:64 dilutions of 150 serum specimens

<table>
<thead>
<tr>
<th>Test result</th>
<th>Corecell</th>
<th>No. (%) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>70 (46.66)</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>68 (45.33)</td>
</tr>
<tr>
<td>+*</td>
<td>−*</td>
<td>9 (6)</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>3 (2)</td>
</tr>
</tbody>
</table>

* Our PHA showed 2+ hemagglutination, while Corecell showed 1+ hemagglutination, which was considered negative.

* Corresponding author.
TABLE 2. Comparison between our PHA with 1:32 serum dilutions and an enzyme-linked immunosorbent assay for anti-HBc with 53 serum specimens

<table>
<thead>
<tr>
<th>Test result</th>
<th>Enzyme-linked immunosorbent assay</th>
<th>No. (%) positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>28 (52.83)</td>
</tr>
<tr>
<td>-</td>
<td></td>
<td>24 (45.28)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>1 (1.88)</td>
</tr>
</tbody>
</table>

For 1,000 specimens submitted for anti-HBc and HBsAg testing, our PHA was positive for 43.6%, a result which was compatible with the incidence of anti-HBc in Thailand obtained with Corezyme (C. Wasi, unpublished data). For 123 HBsAg-positive serum specimens in this group, 122 were anti-HBc positive in the PHA. The prozone phenomenon was found in 2.4% of HBsAg-positive sera.

Comparable results obtained with the two PHAs was due to the use of identical synthetic HBcAg in both tests; however, small differences in results reflected differences in standardization. The control for nonspecific hemagglutination included only in our PHA optimized specificity. We found occasional false-positives with Corecell, owing to heterogeneous antibody, despite the presence of SRBC stroma in its diluent. An agreement of 98% between Corezyme and our PHA indicated that both tests were approximately equal in sensitivity and specificity, but the PHA is simpler, rapid, and inexpensive. When HBcAg is purchased, our PHA is 10 and 20 times less expensive than Corecell and Corezyme, respectively. In addition to describing a useful test, this study supported the findings of other studies (3, 7–9) that synthetic HBcAg is the antigen of choice for developing tests for anti-HBc.

Anti-HBc, used in conjunction with HBsAg and anti-HBsAg, is very useful in the screening of candidates for receipt of hepatitis B virus vaccine (6; C. J. Oon, L. Chan, and R. Guan, Letter, Lancet i:1272, 1983). The benefit of screening blood donors for anti-HBc remains controversial (1, 2, 10), but the PHA is highly applicable for this purpose. Our PHA can be used for immunoglobulin M anti-HBc by adding HBcAg-sensitized SRBC to immunoglobulin M anti-HBc captured to U-bottom microtiter plates, thereby forming a sheet of SRBC on the bottom of the well; an evaluation of this procedure is in progress.

A simple, rapid, inexpensive, sensitive, and specific PHA for anti-HBc is highly applicable in developing countries where hepatitis B virus infections are hyperendemic.

P. Wingfield of Biogen S.A. and K. Murray of the University of Edinburgh donated HBcAg for this study. F. Deinhardt made the necessary correspondence leading to these donations.

LITERATURE CITED