Hepatitis B virus (HBV) currently infects approximately 300 million people throughout the world (5). It has been estimated that this infection causes 1.5 million deaths from cirrhosis and hepatocellular carcinoma each year (8).

HBV infection may present with a broad clinical spectrum ranging from mild hepatitis to aggressive disease that ultimately leads to posthepatitis cirrhosis and hepatocellular carcinoma (4). In addition, HBV infection can manifest itself as viral antigenemia only without clinical disease or as a marker of clinical entities not usually recognized as HBV disease like polyarteritis nodosa and glomerulonephritis (8). Consequently, accurate and rapid diagnosis is of utmost importance.

Specific serologic assays that detect the presence of HBV were developed some 30 years ago. Immunoassays have since progressed from manual, labor-intensive radio immunoassay and enzyme immunoassay procedures to procedures that use automated batch-processing analyzers and, most recently, to procedures that use sophisticated random-access systems capable of processing a variety of tests simultaneously.

In this study, we compared the results obtained by two microparticle enzyme immunoassays (IMx and AxSYM) with those of a chemiluminescent immunoassay (IMMULITE) for the detection of HBV surface antigen (HBsAg).

**MATERIALS AND METHODS**

The study included blood specimens referred prospectively to the clinical laboratory of the Providence Portland Medical Center for routine testing for HBsAg. Blood samples were clotted and centrifuged prior to testing. All sera were then tested for the presence of HBsAg by the IMx (Abbott Laboratories, Abbott Park, Ill.) and the IMMULITE (DPC, Los Angeles, Calif.) assays. All reactive serum samples were retested, and all repeat positive results were confirmed by the confirmatory assays provided by the respective manufacturer.

An aliquot of 2 ml of each serum sample was frozen at −20°C and was shipped to the Laboratory for Medical Microbiology at the Diakonessen Hospital Utrecht, Utrecht, The Netherlands. Samples were tested at the Diakonessen laboratory for HBsAg by the AxSYM (Abbott Laboratories) assay. Frozen sera were centrifuged at 10,000 × g for 10 min prior to testing. If an HBsAg-reactive serum sample was identified, the test was repeated, and the results for all samples with positive results by the repeat test were confirmed by the AxSYM confirmatory assay.

The IMx and AxSYM immunoassay systems (Abbott Laboratories) are based on the microparticle enzyme immunoassay technology (2). The IMx assay system uses a carousel for batch-wise processing, while the AxSYM assay system is a fully automated system with a random-access menu that uses primary tube sampling with bar code reading.

The DPC IMMULITE immunoassay system uses enzyme-amplified chemiluminescence chemistry for the detection of HBsAg. The DPC IMMULITE system is a bench-top immunoassay analyzer with continuous random-access capabilities and patient identification with bar codes (1). The three immunoassay systems were carried out according to the manufacturers’ protocols, and the results for all samples reactive for HBsAg were confirmed by the confirmatory assays provided by the respective manufacturer.

Statistical analysis of frequency counts was performed by Fisher’s exact test for small samples by using the Instat statistical package.

**RESULTS**

Serum samples from 200 patients, 176 women (mean age, 28.9 ± 7.0 years) and 24 men (mean age, 39.3 ± 9.6 years), were analyzed by the IMx and IMMULITE assays. Insufficient serum was available from four patients for testing with the AxSYM immunoassay system. The four patients with insufficient serum volumes were previously identified as nonreactive by both the IMx and the IMMULITE assays. In the population studied, 12 patients were identified and confirmed to be positive for HBsAg by all three systems.

The results of the initial testing for HBsAg are shown in Table 1. The results obtained after confirmation of the original results are presented in Table 2. One sample confirmed to be positive with the AxSYM system only was determined to be nonreactive by both the IMx and IMMULITE assays. Upon further testing, this sample with discrepant results was found to be negative by the AxSYM total anti-HBV core assay (Abbott Laboratories), and therefore, the outcome of detection of HBsAg was considered to be a false-positive result by the AxSYM assay.

The sensitivity after confirmation of the results was calculated to be 100% for the three systems, with specificities of 100, 100, and 99.4% for the IMMULITE, IMx, and AxSYM assay systems, respectively.

Prior to confirmation of the results, 2 additional samples were initially identified as positive with the IMx assay system and 16 other samples were positive with the AxSYM assay system. The specificities prior to confirmation of the results
TABLE 1. Comparison of test results initially obtained by the three immunoassays evaluated

<table>
<thead>
<tr>
<th>Result</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AxSYM assay</td>
</tr>
<tr>
<td>HBsAg positive</td>
<td>29</td>
</tr>
<tr>
<td>HBsAg negative</td>
<td>167</td>
</tr>
<tr>
<td>Total</td>
<td>196</td>
</tr>
</tbody>
</table>

were calculated to be 100% for the IMMULITE system and 98.9 and 91.3% for the IMx and AxSYM systems, respectively. The AxSYM system yielded significantly more false-positive results prior to confirmation of the results than the IMMULITE system (P < 0.05).

DISCUSSION

Classification of an HBV infection requires the identification of several serologic markers. The first marker to appear in patient serum is HBsAg (3). The presence of this antigen indicates an ongoing infection with HBV and is detectable in both acutely ill patients and chronic carriers of HBV, thus the importance of accurate testing for this marker. Detection of HBsAg has evolved from a cumbersome and time-consuming procedure by manual radioimmunoassay or enzyme immunoassay to procedures with systems that partially or fully automate the process with random-access capabilities. The results obtained with these various systems, like the AxSYM system, can be downloaded in real time to the laboratory and hospital information system. Although the test kits for both the AxSYM and the IMx systems for detection of HBsAg for various reasons are not marketed in the United States, they are widely used in Europe and other parts of the world.

In the present study, the AxSYM system yielded significantly more positive results that could not be confirmed by the neutralization assay or detection of the presence of anti-HBV core antibodies. It can be argued that this may be attributed to the shipping, i.e., the freezing-thawing, procedure. However, the samples were centrifuged prior to analysis, as recommended by the manufacturer. In a separate study described previously, a similar phenomenon was reported for serum samples that had not been frozen (R. Benne, presentation to the Dutch Society for Clinical Virology, Amsterdam, The Netherlands, July 1999).

The IMMULITE system uses a chemiluminescent substrate, adamantyl dioxetane, which after hydrolysis yields a sustained emission of light. Theoretically, the low background signal of the system allows a high degree of discrimination between negative and (true) positive serum samples (6, 7). The performance of the IMMULITE assay for detection of HBsAg has previously been shown to be equivalent to that of the Abbott Auszyme assay (C. Cervantes, T. E. Schutzbank, D. Hovanec-Burns, M. Ghadhessi, and A. S. Shami. 1998. Evaluation of the IMMULITE HBsAg assay using commercially available seroconversion and performance panels. Clin. Chem. vol. 44, abstract A3). In our study, the results obtained by the IMx, AxSYM, and IMMULITE assays were comparable. Prior to neutralization the IMMULITE system yielded significantly fewer false-positive results than the AxSYM system. It is concluded that the DPC IMMULITE test is a sensitive assay with high specificity for the detection and confirmation of HBsAg in patient sera.

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REFERENCES