The Analytic Sensitivity and Mutant Detection Capability of Six Hepatitis B Surface Antigen Assays

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Abstract

Hepatitis B virus surface antigen (HBsAg) mutants occur in clinical specimens. We studied the analytic sensitivity and ability to detect recombinant and native mutants of 6 HBsAg assays. The ARCHITECT, AUSZYME MONOCLONAL, and AxSYM assays (Abbott Diagnostics, Abbott Park, IL); the ADVIA Centaur assay (Bayer Diagnostics, Tarrytown, NY); and the Test System 3 and VITROS ECi assays (Ortho Clinical Diagnostics, Raritan, NJ) showed comparable sensitivity with wild-type HBsAg. The ARCHITECT, AUSZYME, and AxSYM assays detected all mutants that were tested. The Test System 3 and VITROS ECi assays failed to detect mutants with amino acid substitutions at positions 143, 144, and 145, which are located in the immunodominant “a” determinant. The ADVIA Centaur failed to detect substitutions at position 145 and showed negative or very low positive results for substitutions at position 143. The inability to detect HBsAg mutants may lead to misdiagnosis of hepatitis B virus infection. Further studies on the prevalence of HBsAg mutants and the ability of commercial assays to detect them are needed.

Around 2 billion people have been infected with the hepatitis B virus (HBV), and approximately 350 million of them are chronic carriers worldwide.1 Approximately 20% to 25% of carriers eventually have chronic liver disease, including cirrhosis and hepatocellular carcinoma. The World Health Organization (WHO) estimates that HBV infection is responsible for 1 to 2 million deaths each year.2 In the United States in 2003, there were an estimated 73,000 new infections with HBV.3 Approximately 1.2 million persons in the United States have chronic HBV infection and are sources of HBV transmission to others.4

Although HBV is a DNA virus, it contains a polymerase that lacks proofreading activity, so error frequencies are comparable to those seen for retroviruses and other RNA viruses. The mutation rate is 1.4 to 3.2 × 10⁻⁵ substitutions per site per year in chronic infections and nearly 100-fold higher in the liver transplantation setting.5,6 Owing to the low fidelity of the polymerase, the high replication rate, and overlapping reading frames, mutations occur throughout the HBV genome, including the sequence coding for the immunodominant “a” determinant of the viral envelope that produces hepatitis B surface antigen (HBsAg) mutants. These mutants have been described in patients who have been vaccinated, patients who have received hepatitis B immune globulin therapy, and in chronic infection, including in patients with hepatitis B surface antibody (anti-HBs) directed against epitopes not shared by the circulating antigen. The inability to detect HBsAg mutants using commercial assays has been identified as a potential problem.7-11 We studied the analytic sensitivity and the ability of 6 commercial HBsAg assays available or in development for the United States to detect native and recombinant mutants.
Materials and Methods

Six commercial HBsAg assays were used according to the manufacturer’s instructions. The ARCHITECT, AUSZYME MONOCLONAL (Procedure B), and the AxSYM HBsAg assays were from Abbott Diagnostics (Abbott Park, IL). The AUSZYME assay used monoclonal capture and detection antibodies. The ARCHITECT and AxSYM assays used monoclonal capture and polyclonal detection antibodies and are in development for the US market, but not yet available in this country. The ADVIA Centaur HBsAg assay was from Bayer Diagnostics (Tarrytown, NY) and used monoclonal capture and detection antibodies. The Test System 3 and the VITROS ECi HBsAg assays were from Ortho Clinical Diagnostics (Raritan, NJ). The Test System 3 and VITROS ECi assays both used monoclonal capture and detection antibodies. A signal/cutoff ratio (S/CO) of 1.0 or more was used to identify reactive samples by 5 methods. An assay index value of 1.0 or more identified reactive samples on the ADVIA Centaur. Confirmatory testing was not performed in the present study. The AUSZYME and Test System 3 assays are manual methods, whereas the ADVIA Centaur, ARCHITECT, AxSYM, and VITROS ECi assays were performed on random access automated analyzers.

The analytic sensitivity of each method was determined by using 3 HBsAg panels diluted with normal human plasma. Two native antigen panels were prepared using subtypes ad and ay wild-type native antigen samples. Each of these panels contained 9 samples and ranged in concentration from 0 to 3.7 ng/mL and 0 to 3.8 ng/mL for the panels contained 9 samples and ranged in concentration of about 3 ng/mL. The recombinant mutant panel members prepared in normal human plasma at an HBsAg concentration were characterized previously by DNA sequence analysis. Mutant samples were analyzed in duplicate, and the mean result was reported.

Results

The analytic sensitivity for an S/CO or index of 1.0 for wild-type HBsAg panels ranged from 0.10 to 0.34 ng/mL. There was approximately a 2-fold range in HBsAg concentrations producing an S/CO or index of 1.0 for both ad and ay subtypes across methods, and the ay subtype was detected slightly better by all methods than was the ad subtype. The analytic sensitivity for an S/CO or index of 1.0 for the WHO International Standard ranged from 0.03 to 0.08 IU/mL. There was nearly a 3-fold difference in sensitivity for the WHO Standard across the 6 methods. The AUSZYME and VITROS ECi assays were the least sensitive methods for all 3 panels used to estimate analytic sensitivity.

Although the sensitivity of the 6 assays to detect wild-type HBsAg was roughly comparable, their ability to detect HBsAg mutants differed considerably. The AUSZYME and AxSYM methods detected all 9 recombinant mutants that were tested, whereas the Test System 3 and VITROS ECi methods failed to detect 6 of the 9 recombinant mutant samples. Owing to a lack of sufficient sample volume, only 3 recombinant mutants were tested by the ADVIA Centaur and ARCHITECT methods. The ADVIA Centaur failed to detect a recombinant mutant with a glycine to arginine substitution at position 145 but detected mutants with substitutions at positions 129 and 133. The ARCHITECT assay detected all 3 recombinant mutants that were tested.

Three native mutant samples were tested by all 6 methods. The ADVIA Centaur assay detected 1 native mutant as a very low positive result and failed to detect the other 2. The ARCHITECT, AUSZYME, and AxSYM assays detected all 3 native mutants that were tested with high S/CO values for all samples. The Test System 3 and VITROS ECi methods failed to detect all 3 native mutants that were tested.

Table 1

Summary of HBsAg Concentrations at the Assay Cutoff*

<table>
<thead>
<tr>
<th>HBsAg Standard</th>
<th>ADVIA Centaur</th>
<th>ARCHITECT</th>
<th>AUSZYME MONOCLONAL</th>
<th>AxSYM</th>
<th>Test System 3</th>
<th>VITROS ECi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtype ad (ng/mL)</td>
<td>0.13</td>
<td>0.14</td>
<td>0.31</td>
<td>0.15</td>
<td>0.17</td>
<td>0.34</td>
</tr>
<tr>
<td>Subtype ay (ng/mL)</td>
<td>0.12</td>
<td>0.12</td>
<td>0.21</td>
<td>0.13</td>
<td>0.10</td>
<td>0.22</td>
</tr>
<tr>
<td>WHO International Standard (IU/mL)</td>
<td>0.05</td>
<td>0.03</td>
<td>0.07</td>
<td>0.03</td>
<td>0.05</td>
<td>0.08</td>
</tr>
</tbody>
</table>

HBsAg, hepatitis B surface antigen; WHO, World Health Organization.
*Signal/cutoff ratio or index = 1.0. ARCHITECT, AUSZYME MONOCLONAL (Procedure B), and AxSYM assays, Abbott Diagnostics, Abbott Park, IL; ADVIA Centaur assay, Bayer Diagnostics, Tarrytown, NY; Test System 3 and VITROS ECi assays, Ortho Clinical Diagnostics, Raritan, NJ.
Discussion

The ability to accurately detect the presence of HBsAg has a critical role in the diagnosis of HBV infection, especially in asymptomatic people, including blood donors and pregnant women. The 6 commercial assays we studied all have the ability to detect wild-type HBsAg at concentrations below 0.4 ng/mL or 0.1 IU/mL. The appearance of escape mutants in people with medically or naturally induced immune pressure raises the question of whether these mutants can be detected by commercial HBsAg assays.

The results of our study with recombinant HBsAg mutants are consistent with those of previous studies of the same recombinant mutant panel that we used.7,12 Mutations at amino acid positions 144 and 145 in the “a” determinant of the S gene product are detected by some commercial assays but not others. The substitution of arginine for glycine at position 145 is the best documented mutation in HBsAg.10 It is noteworthy that the concentration of recombinant mutant HBsAg in our study was higher than was used by Coleman et al7 (3 vs 1 ng/mL), yet the mutants at positions 145 that were detected by the ARCHITECT, AUSZYME, and AxSYM assays were not detected by the ADVIA Centaur, Test System 3, and VITROS ECi assays. Unfortunately, some of the recombinant mutants were not available for testing on the ADVIA Centaur and ARCHITECT assays owing to a lack of sufficient volume.

The 3 native mutants that were tested gave an S/CO greater than 25 by the ARCHITECT and AxSYM assays but were either negative or only weakly positive by the ADVIA Centaur assay and negative by the Test System 3 and VITROS ECi assays. The inability to detect HBsAg mutants in this study is likely because the monoclonal capture and detection antibodies used by the ADVIA Centaur, Test System 3, and VITROS ECi assays are directed toward regions in the “a” determinant of the S gene product where the mutations are occurring. One strategy to prevent this from occurring is to use antibodies that are directed at regions of the S gene that are not frequent mutational sites. This approach likely has been used by the ARCHITECT, AUSZYME, and AxSYM assays. We concur with a previous recommendation that manufacturers use standardized samples containing recombinant mutants encountered in clinical practice to test assay susceptibility to mutants.7 Antibodies should be selected that bind to commonly encountered mutants of HBsAg.

Although the ARCHITECT, AUSZYME, and AxSYM assays detected the T118V/M,K133I/Y,F134N/P142S/S,T143L/G145K native mutant and the ADVIA Centaur, Test System 3, and VITROS ECi did not, the ARCHITECT and AxSYM assays produced substantially higher S/CO values than the AUSZYME assay, whereas for the other mutants, the S/CO values were fairly comparable. The difference in the AUSZYME and the ARCHITECT and AxSYM S/CO values for this sample might be because of different assay platforms or because the AUSZYME assay uses monoclonal antibodies for detection and capture and the ARCHITECT and AxSYM assays use monoclonal capture and polyclonal detection antibodies. It is noteworthy that the ARCHITECT and AxSYM assays gave comparable results for most mutants that were tested. However, results were
approximately 10-fold different for the S,T143L/Y206G/S207R and S,T143L/V190A/Y200C/Y206R,T native mutants. These interassay differences may result from differences in the dose-response curves of the assays.

All of the recombinant mutants studied originally were identified in patient samples from around the world. Although it is possible that other mutants that were not tested in this study might be detected by the ADVIA Centaur, Test System 3, or VITROS ECI assays and not by the ARCHITECT, AUSZYME, or AxSYM assays, it is important that commercial assays be capable of detecting the most common mutants. Two recent articles identified HBsAg mutants in North America. In the report by Minuk et al., a high prevalence of the substitution of arginine for glycine at position 145 in the “a” determinant was reported. Further studies on the prevalence of mutants in the general population and in selected groups are needed to identify the most prevalent and clinically relevant mutants. If mutants are identified, it would be desirable to prepare the corresponding recombinant antigen, as discussed, to know which commercial assays would detect them and which ones would not.

In patients who have unusual HBV serologic findings such as isolated hepatitis B core antibody, hepatitis B e antigen positive but HBsAg negative, positive for anti-HBs and HBsAg, or HBV DNA positive but HBsAg negative, one should consider the possibility of an HBsAg mutant. Testing by an alternative HBsAg method, preferably one that detects the most common mutants, and/or quantitative HBV DNA testing also should be considered. Further studies of the ability of other HBsAg assays that are available commercially in the United States to detect HBsAg mutants also are needed.

References


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