Discrepancies Between Two Automated Immunoassay Systems in Determining Hepatitis B Virus Markers in Serum Samples with Concomitant Presence of Antigens and Antibodies

Liming Cheng, Qing Guan, Junxia Zhang, and Ziyong Sun
Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Abstract. This study compared the assay results of HBV markers obtained with two automated immunoassay systems in serum samples with concomitant HBV antigens and antibodies. Eighty-nine samples of concomitant HBsAg and anti-HBs and 74 samples of concomitant HBeAg and anti-HBe were analyzed by the Abbott Architect i2000 and the Roche Elecsys 2010 immunoassay systems. The overall concordance rates of the Architect and Elecsys results were 78.6% for samples with concomitant HBsAg and anti-HBs and 77% for samples with concomitant HBeAg and anti-HBe. The data show that substantial differences exist between the results obtained by these two systems, especially in determining anti-HBs and HBeAg at low concentrations.

Keywords: hepatitis B viral markers, HBsAg, HBeAg, anti-HBs, anti-HBe

Introduction

A series of antigens and antibodies including HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc are produced in patients infected with hepatitis B virus (HBV). The appearance and disappearance of these serum markers in persons infected with HBV follow certain rules and generally adhere to characteristic patterns. However, unusual profiles of HBV markers have been reported recently, which may be caused by virus mutation or reflect the state of the human immune system.

Serum HBV markers are usually detected by enzyme immunoassay (EIA), radioimmunoassay (RIA), micro-particle enzyme immunoassay (MEIA), or chemiluminescence. The development of automated immunoassay systems has greatly improved the sensitivity, specificity, and accuracy of serum HBV marker detection. However, standardization of immunoassay methods is difficult, which leads to inconsistency among the results obtained by different analytical systems. Moreover, discrepancies of assay results may be especially frequent in samples with unusual serological profiles. Therefore, we decided to compare the analytical results obtained by two automated immunoassay systems in a set of 89 serum samples with concomitant HBsAg/anti-HBs and in a set of 74 samples with concomitant HBeAg/anti-HBe.

Methods and Materials

Serum samples. From 4,281 serum samples that were HBsAg positive, we collected a set of 89 samples of concomitant HBsAg/anti-HBs positive sera and a set of 74 samples of concomitant HBeAg/anti-HBe positive sera, which were detected using an Architect i2000 analyzer (Abbott Diagnostics, Abbott Park, IL) during the period from September, 2007 to July 2008. The two sets of serum samples were retested using an Elecsys 2010 analyzer (Roche Diagnostics, Mannheim, Germany). Roche first generation reagents were used when testing HBsAg. All 89 samples of concomitant HBsAg/anti-HBs positive sera were collected from different patients; among the 74 samples of concomitant HBeAg/anti-HBe positive sera, two samples were from one patient with a 3-mo blood sampling interval.
Automated immunoanalysis systems. The Architect i2000 analyzer uses a chemiluminescent microparticle immunoassay (CMIA); serum HBsAg and anti-HBs are quantitatively determined, while serum HBeAg, anti-HBe, and anti-HBc are qualitatively determined. Positive cut-off values for HBsAg and anti-HBs are ≥0.05 IU/ml and ≥10 mIU/ml, respectively. HBeAg, anti-HBe, and anti-HBc are interpreted using the ratio of the sample’s relative light unit (RLU) rate to the cut-off RLU (S/CO), where S/CO ≥1.00 indicates HBeAg or anti-HBc positive, while S/CO ≤1.00 indicates anti-HBe positive.

The Elecsys 2010 analyzer uses an electrochemiluminescence immunoassay (ECLIA); serum anti-HBs is quantitatively determined, while serum HBsAg, HBeAg, anti-HBe, and anti-HBc are qualitatively determined. HBsAg, HBeAg, anti-HBe, and anti-HBc are interpreted using the ratio of the sample signal to the cut-off signal (S/CO), where COI ≥1.00 indicates HBsAg or HBeAg positive and COI ≤1.00 indicates anti-HBe or anti-HBc positive. Results of anti-HBs with concentration values ≥10 IU/L are positive.

For samples with concomitant presence of HBsAg/anti-HBs, when the results of HBsAg were inconsistent between the two systems, that is, positive on the Architect i2000 analyzer and negative on the Elecsys 2010 analyzer, we examined HBsAg with HBsAg confirmation reagents on the Architect i2000 analyzer and with second generation HBsAg reagents on the Elecsys 2010 analyzer, respectively. For samples of HBeAg that were positive on the Architect i2000 analyzer but negative on the Elecsys 2010 analyzer, we rechecked HBeAg using an Axsym immunoanalysis system (Abbott Diagnostics).

Statistics. For comparison of anti-HBs titers obtained by the Elecsys 2010 and Architect i2000 assays, the correlation coefficient was calculated by SPSS 12.0.

Results

Samples with concomitant HBsAg and anti-HBs detected by Architect i2000 and Elecsys 2010. As shown in Table 1, 74 serum samples with concomitant HBsAg/anti-HBe positivity were detected by the Architect i2000 immunoanalysis system. When these samples were analyzed by the Elecsys 2010 immunoanalysis system, all 74 were anti-HBe positive (concordance rate 100%), but only 57 samples were HBeAg positive (concordance rate 77%). For all 74 samples, using the Architect i2000 analyzer, the S/CO value of HBeAg ranged from 1.131 to 46.075, and the median was 2.69. The HBeAg S/CO values of the 17 samples with inconsistent results ranged from 1.195 to 2.284, except for one sample with S/CO value of 10.711. Among these 17 samples, 14 samples were HBeAg negative when tested with the Axsym immunoanalysis system. (The remaining 3 samples could not be analyzed due to insufficient sample volume.) Four of these 17 samples were HBsAg negative and anti-HBsAb positive with the Architect i2000 system, and 2 of the 4 samples were from one patient with a 3-mo blood sampling interval.

Table 1. Positive samples and concordance rates (%) between the two immunoanalysis systems.

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>HBsAg/Anti-HBs positive (n)</th>
<th>HBeAg/Anti-HBe positive (n)</th>
<th>HBsAg positive (n)</th>
<th>Anti-HBs positive (n)</th>
<th>HBeAg positive (n)</th>
<th>Anti-HBe positive (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architect i2000</td>
<td>89</td>
<td>74</td>
<td>89</td>
<td>89</td>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>Elecsys 2010</td>
<td>70</td>
<td>57</td>
<td>84</td>
<td>75</td>
<td>57</td>
<td>74</td>
</tr>
<tr>
<td>Concordance rates (%)</td>
<td>78.6%</td>
<td>77%</td>
<td>94.4%</td>
<td>84.2%</td>
<td>77%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Other observations. During the period from September 2007 to July 2008, we identified two serum samples that were HBsAg negative, anti-HBs positive, HBeAg positive, anti-HBe negative, anti-HBc positive by the Architect i2000 analyzer. These assay results were completely consistent with those obtained by the Elecsys 2010 analyzer (data not shown).

Discussion

The typical serological feature of chronic HBV infection is the presence of circulating HBsAg in patients but the absence of anti-HBs. However, coexistence of HBsAg and anti-HBs in patients with chronic HBV infection has been frequently reported. Screening of 411 patients with chronic HBV infection from different regions of China revealed that a relatively high percentage (4.9%) of these persons had detectable serum anti-HBs levels [1]. Another report showed that the incidence of concomitant HBsAg/anti-HBs was 4.69% in HBV asymptomatic carriers, 11.11% and 5.45% respectively in patients with chronic hepatitis B and hepatitis B cirrhosis, and 0.0% in acute hepatitis B patients [2]. The following factors may partly explain this phenomenon: (i) infection of different HBV subtypes [3]; (ii) gene mutation of HBV [4,5]; (iii) presence of HBsAg “a” determinant mutant [6]; and (iv) HBsAg and anti-HBs forming an immune complex [7]. In the present study, the incidence of concomitant HBsAg/anti-HBs was 89/4281 samples (2.08%) when the Architect i2000 immunoanalytical system was used, and it was evidently affected by the analytical method.

There may have been HBsAg variant strains in 2 serum samples, which led to false negative results with the Elecsys 2010 system using the first generation reagent. The second generation reagents of the Elecsys 2010 system has improved ability to detect HBsAg variant strains, compared to the first generation reagents. The concordance rate of anti-HBs was low in this research, and the inconsistent results occurred mainly in samples with low titers of anti-HBs (anti-HBs <50 mIU/ml). Kim et al [8] reported that the concordance rate of anti-HBs between the Elecsys 2010 and Architect i2000 systems was 97.3%, with high correlation (r = 0.918), and that inconsistent results occurred mainly in samples with anti-HBs levels <100 mIU/ml. Differences between the two studies may reflect the samples we tested, since our anti-HBs titers were <50 mIU/ml (range, 10.37-512.46; median, 22.57 mIU/ml) in 70 of the 89 samples. The results of our study show that discrepancies exist in assay results obtained by the Architect i2000 and Elecsys 2010 systems for detecting anti-HBs of low concentration. However, considering the factors that cause coexistence of HBsAg and anti-HBs, such discrepancies probably have little clinical significance.

On the other hand, HBeAg positivity indicates active viral replication and high contagiousness. Anti-HBe is produced after serum HBeAg converts from positive to negative, which indicates clearance of some viruses and relief of chronic hepatitis B infection. Seroconversion to anti-HBe serves as a treatment goal for HBeAg-positive patients [9]. Treatment of HBeAg-positive patients can increase seroconversion to anti-HBe-positive status by 23%, which reduces cirrhosis and hepatocellular carcinoma by 47% and 78% respectively [10]. Thus, HBeAg and anti-HBe testing play important roles in routine monitoring. Kim et al. [8] and Chen et al. [11] reported concordance rates for HBeAg of 98.7% and 96.97% respectively between the Elecsys 2010 and Architect i2000 systems, which were significantly different from our results. Low concentrations of HBeAg and concurrent presence of anti-HBe in the samples we studied may have contributed to the difference. Moreover, we only compared serum samples that were HBeAg positive rather than those HBeAg negative. Fourteen of the 17 samples with inconsistent results were HBeAg negative with the Axsym immunoanalytical system, which may indicate a high false positive rate for the Architect i2000 analyzer.

In summary, this study shows substantial differences between the results for HBV markers obtained by two automated immunoassay systems, especially for serums containing low concentrations of anti-HBs and HBeAg. Given the importance of HBV markers in the diagnosis and management of hepatitis B, these discrepancies should be taken into account in the interpretation of serum HBV
markers; otherwise they can cause serious confusion to the clinicians.

References


