Comparison of Enzyme Immunoassays for Detection of Antibodies to Hepatitis D Virus in Serum

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Serology remains critical for diagnosing hepatitis D virus (HDV) infection, which affects 15 to 20 million people worldwide, but the literature on characterizing commercial enzyme immunoassays (EIAs) dates back to 15 years ago. We evaluated 2 commercial EIAs currently available for detecting anti-HDV antibodies. The DiaSorin assay demonstrated 100% sensitivity and specificity. Using a modified cutoff value, the Cusabio assay demonstrated a sensitivity of 81.3% and specificity of 90.9%. Our data show that recently developed EIAs are reliable for anti-HDV antibody detection.

Hepatitis D virus (HDV) is a small, defective RNA virus that requires hepatitis B virus (HBV) surface antigen (HBsAg) for replication and propagation. Among the estimated 240 million people worldwide with chronic HBV infection, 15 to 20 million are estimated to be coinfected with HDV (1). Individuals coinfected with both viruses have more severe liver disease, faster progression, and poorer prognosis than those with HBV infection alone (2, 3). While serology remains critical for diagnosing HDV infection, most studies in this area were performed in the late 1980s and early 1990s (4–7). The assays described in those studies have been discontinued or are no longer available in the United States. More recently developed HDV immunoassays have been mainly limited to research purposes or clinical use in local institutions (8–10). In this study, we evaluated two commercially available enzyme immunoassays (EIAs) for detecting anti-HDV antibodies and compared the data with those obtained in two reference laboratories.

A total of 87 serum specimens initially submitted to ARUP Laboratories (ARUP) or Focus Diagnostics Reference Laboratory (Focus) between March 2014 and June 2014 for evaluation of HDV antibodies were randomly selected and analyzed with both reference enzyme-linked immunosorbent assays (ELISAs). All 87 deidentified specimens were kept at −80°C before being sent to University of Washington and tested for HDV antibodies using commercially available kits from DiaSorin (Saluggia, Piedmont, Italy) and Cusabio (Wuhan, Hubei, China). Each of the kits used different HDV antigen preparations, as well as a variety of conjugate detection methods. The DiaSorin kit measures total Ig to HDV as a qualitative competitive ELISA, while the Cusabio ELISA kit quantitatively measures IgG. According to the manufacturer, the cutoff value for the DiaSorin kit is defined as (0.5 × mean negative control) + (0.5 × mean positive control), whereas the cutoff for the Cusabio kit is set as 0.2 + mean negative control. Microtiter plates were read on the Epoch Microplate Spectrophotometer (BioTek, Winooski, VT) using the Gen5 data analysis software.

HDV total antibodies were measured at Focus using a proprietary laboratory-developed assay. Briefly, serum samples diluted 1:101 in phosphate-buffered saline with 0.1% Tween 20 (PBST) containing 0.1% bovine serum albumin were added to microtiter wells coated with a proprietary recombinant HDV protein (GenScript, Piscataway, NJ). After incubation at room temperature (RT) for 1 h and 3 washes with PBST, wells received horseradish peroxidase (HRP)-conjugated F(ab′)2 fragment goat anti-human IgG+IgM+IgA (Jackson ImmunoResearch, West Grove, PA). After incubation at RT for 30 min, wells were washed and then received tetramethylbenzidine (Moss Inc., Pasadena, MD). The optical density at 450 nm (OD450) was measured using an ELISA control (0.14 + mean negative control) was used.

### TABLE 1 Performance of Cusabio and DiaSorin ELIA kits compared to that of reference laboratory methods

<table>
<thead>
<tr>
<th>ELISA Kit</th>
<th>No. of samples giving the following results compared to reference laboratory methodsa</th>
<th>Sensitivity (% [95% CI])b</th>
<th>Specificity (% [95% CI])d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
<td>+/−</td>
<td>−/−</td>
</tr>
<tr>
<td>Cusabio</td>
<td>26</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>DiaSorin</td>
<td>32</td>
<td>0</td>
<td>55</td>
</tr>
</tbody>
</table>

a +/+ , true positive; +/− , false positive; −/− , true negative; −/+ , false negative.
b CI, confidence interval.
c A proposed cutoff value (0.14 + mean negative control) was used.


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reader (BioTek). Results were expressed as an index value, calculated by dividing the patient sample OD by the OD of a calibrator serum included in every run. Index values of <0.90 were interpreted as negative, 0.90 to 1.10 as equivocal, and >1.10 as positive.

The reference assay at ARUP utilized a commercial kit that detects HDV total antibodies following the manufacturer’s instruction (International Immuno-Diagnostics, Foster City, CA). Briefly, proprietary HDV antigen was precoated to microtiter wells. A patient sample was added to the well in the presence of HRP-conjugated polyclonal antibodies for HDV. The plate was washed and then incubated with tetramethylbenzidine. The final OD was given by OD_{450} subtracted by OD_{620}. The result was interpreted as the ratio between the cutoff value and the specimen value, where the cutoff is defined as 0.2 × (mean negative control + mean positive control). Ratios of <0.90 were interpreted as negative, 0.90 to 1.10 as equivocal, and >1.10 as positive.

The HDV antigens used in the DiaSorin and Cusabio kits are proprietary. The DiaSorin kit has a competitive binding assay format, whereas the Cusabio kit is a direct binding assay. Each positive control was found to be specific to its own kit and showed no binding against the other (data not shown), suggesting that the HDV antigens in the kits are antigenically distinct. The lack of cross-reactivity between the two positive controls may be attributable to different types of HDV being used to raise antibodies. The genetic diversity of HDV, which confers different antigenic properties, has been shown to be related to the geographic origin of the isolates (11). The Cusabio and DiaSorin assays used in our study are manufactured in China and Italy, respectively.

The linearity of each assay was assessed by serially diluting the positive control materials provided in the kits. For the DiaSorin kit, the OD-concentration curve generated by 8 data points using 1:2 serial dilutions of the positive control yielded an $R^2$ value of 1 using the 4-parameter analysis of Gen5 data analysis software. For the Cusabio kit, 7 data points from 1:4 serial dilution of the positive control also yielded an $R^2$ value of 1 using the 4-parameter analysis. To examine the limit of detection, a positive sample was tested in dilutions with both kits. The sample was 1:5 serially diluted, and the lowest positive titer was 1:3,125 for the DiaSorin kit; the same sample was serially diluted 1:4, and the lowest positive titer for the Cusabio kit was 1:16.

Of 87 clinical samples tested at ARUP and Focus using their reference assays, 32 were positive and 55 were negative for anti-HDV antibodies. The samples were then tested with the DiaSorin and Cusabio kits. The DiaSorin kit demonstrated sensitivity and specificity of 100% relative to the reference laboratory results (Table 1). Using the cutoff suggested by the manufacturer (0.2 + mean negative control), the overall sensitivity of the Cusabio kit was 53.1%. Because of the low sensitivity, we proposed a new cutoff defined as 0.14 + mean negative control based on the distribution of the false-negative readouts (Fig. 1). By doing so, the overall sensitivity of the Cusabio kit increased to 81.3% (Table 1). Of the 11 discrepant results between the Cusabio assay and the reference methods, 6 were false negatives and 5 were false positives. The discrepancies were attributable to the lower sensitivity for the Cusabio assay and a lack of specificity when the lower cutoff OD was used for analysis. The limitation of this study includes the poorly understood structural and antigenic property of HDV antigens in the commercial kits.

In summary, results of this study show that the currently available commercial EIAs can effectively detect antibodies to HDV.
HDV. The DiaSorin kit appears to have better sensitivity and specificity and shows performance comparable to that of the reference methods.

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REFERENCES