Comparison of the Second-Generation Digene Hybrid Capture Assay with the Branched-DNA Assay for Measurement of Hepatitis B Virus DNA in Serum

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The optimal hepatitis B virus (HBV) DNA quantitative assay for clinical use remains to be determined. We examined the sensitivity, linearity, and variability of a novel second-generation antibody capture solution hybridization assay, the Digene Hybrid Capture II assay (HCII), and compared it with another widely used solution hybridization assay, the branched-DNA (bDNA) assay (Quantiplex; Chiron Corp.). Our results showed similar and satisfactory assay linearity values, as well as interassay and intra-assay variability values, for both HCII and bDNA assays across different ranges of HBV DNA. Ninety-one percent of 102 serum samples from hepatitis B surface antigen-positive patients showed concordant results with the two assays. The HCII assay was more sensitive than the bDNA assay by 1 dilution, with the lowest reading being 0.9 pg/ml (3.8 pg/ml by bDNA assay). The HBV DNA seropositivity rates for the 102 samples were 58, 67, and 97% by bDNA, HCII, and nested PCR, respectively. While the relationship between results obtained with the bDNA assay and those with the HCII assay was nonlinear, with the bDNA assay yielding values 2.83 ± 0.92-fold higher than those of the HCII assay, especially at high HBV DNA levels, a linear relationship was observed between the two sets of data after logarithmic conversion. The formula for interassay conversion of results was derived as follows: HBV DNA by HCII (picograms per milliliter) = 3.19 + [HBV DNA by bDNA (megaequivalents per milliliter)]^{0.866}. The HCII assay was technically less complex and required a shorter assay time (4 h) than the bDNA assay (24 h). We conclude that the HCII assay compares favorably with the bDNA assay and offers the additional advantages of increased sensitivity and shorter assay time. The increased sensitivity should be particularly useful in monitoring the efficacy of antiviral therapies and detecting the emergence of drug-resistant HBV mutants.

Hepatitis B virus (HBV) infection is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma and accounts for 1 million deaths annually (5, 13). Information on the virus load and the replicative activity of HBV is of paramount importance in the management of patients with chronic HBV infection, especially after the recent advent of medications which can effectively suppress HBV replication, such as the nucleoside analogues. Serological parameters which have been used in this regard include the hepatitis B e antigen (HBeAg) status, DNA polymerase, HBV DNA status by qualitative methods such as PCR or semiquantitative dot blot hybridization, and more recently HBV DNA quantitation by solution hybridization (8, 11, 13). The direct and quantitative nature of the latter makes it a useful clinical test to monitor serially the efficacy of antiviral therapy (7, 12, 14).

Solution hybridization techniques for HBV DNA quantitation can utilize radioactive (e.g., Genostics; Abbott Laboratories), antibody capture (e.g., Hybrid-Capture; Murex Diagnostics Ltd.), or branched-DNA (bDNA) signal detection systems (e.g., Quantiplex; Chiron Corp.). Comparisons among these assays are complicated by variations in methodology, standards, and units. The bDNA assay is the most sensitive and precise among the three (1, 2, 16). Since a universal standard is lacking and the bDNA assay both is technically intricate and requires a long assay time, the optimal choice of quantitative HBV DNA assay for clinical use remains to be defined.

Recently, a novel (second-generation) antibody capture solution hybridization HBV DNA quantitative assay (Digene Hybrid Capture II assay [HCII]; Digene Corp., Beltsville, Md.) has offered sensitivity improved over that of the bDNA assay. In this study, we evaluated the sensitivity, specificity, linearity, and technical complexity of this novel HCII assay and compared it with the bDNA assay. Correlations between results obtained with the HCII and those with the bDNA assay were examined, and an equation was derived for the conversion of results between the two quantitative assays.

MATERIALS AND METHODS

Serum samples from 102 hepatitis B surface antigen (HBsAg)-positive patients and 22 HBsAg-negative controls were assayed for HBV DNA by (i) HCII assay, (ii) bDNA assay, and (iii) an in-house nested PCR (nPCR) assay. The patients were randomly included from known chronic HBV carriers attending regular follow-up for serial monitoring of their liver status. Blood samples were centrifuged within 4 h to obtain the serum fractions, which were then aliquoted and kept at −80°C before assay.

HCII assay. The Digene HCII (standard) assay (Digene Corp.) quantitates HBV DNA by solution hybridization, followed by immunocapture and chemiluminescent signal detection. The assay protocol was according to the manufacturer’s instructions. Briefly, 30 μl of denaturing reagent was added to each microplate well containing 30 μl of test samples or HBV DNA standards (0 to 6,000 pg/ml). The plate was incubated at 65°C for 30 min for the lysis of HBV DNA. The hybrid-containing wells were then transferred into RNA-DNA capture wells and washed (Thermolyne Maxi-Mix III) at 1,100 rpm at room temperature for 60 min. The solution in the wells was then removed by aspiration. Seventy-five microliters of alkaline phosphatase-conjugated antibodies to RNA-DNA hybrids was added to each well and incubated at room temperature for 30 min. After six washings, 75 μl of chemiluminescent substrate was added, and light emission after 15 min was measured by a chemiluminometer (DML 2000 luminometer; Digene Corp.). Results were...
expressed in picograms per milliliter according to the plot of standards. The sensitivity according to the manufacturer was 0.5 pg/ml or $3 \times 10^6$ copies/ml.

**bDNA assay.** The bDNA assay (Quantiplex; Chiron Corp.) is a sandwich nucleic acid hybridization assay. A set of oligonucleotide probes bind single-stranded HBV DNA to a solid phase, which is detected by a second set of oligonucleotide probes. bDNA then serves to amplify the signal, which is generated by adding alkaline phosphatase-conjugated probes for bDNA and dioxetane as substrate (6). The assay protocol was according to the manufacturer's instructions. Briefly, 10 ml of test samples or HBV DNA-positive standards (0.5 to 4,400 MEq/ml) was added to 10 ml of lysis reagent on a microwell plate coated with oligonucleotide probes and incubated for 30 min at 63°C to release HBV DNA from the viral particles. Ten microliters of the second set of probes in denaturing buffer was then added to the wells and incubated for 30 min at 63°C. Ten microliters of neutralizing reagent was then added, and the hybridization process continued for 16 h. After washing, 40 ml of bDNA amplifiers was added and incubated for 30 min at 53°C. Forty microliters of alkaline phosphatase-conjugated probes was then added to each well and incubated for 15 min at 53°C. After washing, 30 ml of dioxetane substrate was added. The plate was incubated for 25 min at 37°C in the Chiron luminometer, and light emission was measured for each well. The result of HBV DNA level, expressed as megaequivalents per milliliter, was generated by software supplied by the manufacturer. The sensitivity according to the manufacturer was 0.7 MEq/ml ($3 \times 10^6$ copies/ml) or 2.5 pg/ml (1 MEq/ml $5 \times 3.53$ pg/ml [9]).

**nPCR assay.** The nPCR was performed according to a previously published protocol (3). The primer sets for the HBV core region were GGAGTGTGGA TTCGCACTCCTCC (map positions 2269 to 2288) and ATACTAACATTGAG ATTCCC (2457 to 2438) for the first-round PCR and AGACCACAAATGC CUCTAT (2299 to 2318) and GATCTTCTGCGACGCGGGA (2429 to 2410) for the second round. Briefly, 10 ml of serum was mixed with 8 ml of 0.2 M NaOH solution and incubated at 37°C for 1 h and then at 98°C for 5 min. Eighty microliters of the PCR solution, with 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl$_2$, 50 mM KCl, 200 μM (each) deoxyribonucleoside triphosphate, 1 μM (each) first-round primers, and 1.25 μl of Taq polymerase, was then added. The mixture was heated to 95°C for 5 min, followed by 30 PCR cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min in a thermal cycler (PTC-100; MJ Research, Watertown, Mass.). For the second-round PCR, 5 μl of the first-round PCR product was added to 40 μl of a polyacrylamide gel and visualized under UV light after staining with ethidium bromide. Detection of a DNA band of 130 bp denoted positivity. All the samples were tested in duplicate, and positive and negative controls were included in each run. Our in-house nPCR assay had a sensitivity of 1 fg/ml (300 copies/ml).

**Statistics.** Correlation between HBV DNA results obtained with the HCII and those with bDNA assays was examined by Pearson's method.

### RESULTS

A serum sample with a high HBV DNA concentration (7,081 pg/ml by bDNA assay and 2,164 pg/ml by HCII assay) was tested upon serial dilution with normal human serum at 1/10, 1/100, 1/1,000, and 1/2,000 dilutions in order to determine sensitivity.

![Graph showing the linearity of the HCII and bDNA assay results as determined by testing a standard serum sample with a high HBV DNA concentration at serial dilutions.](http://jcm.asm.org/)

**TABLE 1. Comparison of corresponding values and intra- and interassay variations when identical serum samples with L, M, and H levels of HBV DNA were tested with the HCII and the bDNA assays**

<table>
<thead>
<tr>
<th>Variation and specimen identification</th>
<th>HCII</th>
<th>bDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>CV (%)</td>
</tr>
<tr>
<td>Intra-assay (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>22.4 ± 1.3</td>
<td>5.8</td>
</tr>
<tr>
<td>M</td>
<td>355.9 ± 24.3</td>
<td>6.8</td>
</tr>
<tr>
<td>H</td>
<td>2,884.3 ± 96.6</td>
<td>6.8</td>
</tr>
<tr>
<td>Interassay (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>20.8 ± 2.3</td>
<td>11.1</td>
</tr>
<tr>
<td>M</td>
<td>337.4 ± 26.0</td>
<td>7.7</td>
</tr>
<tr>
<td>H</td>
<td>2,871.0 ± 177.7</td>
<td>6.1</td>
</tr>
</tbody>
</table>

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and compare the sensitivity and performance linearity values of the bDNA and HCII assays. Repeated testing of this dilution series three times yielded consistent results. The sensitivity limit for the bDNA assay was at a 1/1,000 dilution, giving an assay result of 3.8 pg/ml, while that for the HCII assay was at a dilution of 1/2,000, yielding an assay result of 0.9 pg/ml. Linearity values of the two assays were similar (Fig. 1).

To compare the precision of the bDNA and HCII assays, three serum samples with HBV DNA levels previously determined to be at the low (L) (2.5 to <40 pg/ml), medium (M) (40 to 3,900 pg/ml), and high (H) (>3,900 to 17,000 pg/ml) levels according to the standard ranges of bDNA assay (actual readings of 37.5, 909.2, and 10,525.3 pg/ml, respectively) were tested six times with both assays. The bDNA assay yielded HBV DNA levels 1.7 to 3.6 times higher than those obtained with the HCII assay (Table 1). The intra-assay and interassay coefficients of variation (CV) were similar for the two assays (P value not significant).

The prevalence of HBV DNA as determined by bDNA, HCII, and nPCR methods in the 102 HBsAg-positive samples and 22 HBsAg-negative samples is given in Table 2. The HBV DNA seropositivity rate was highest with the nPCR assay, followed by the HCII assay and then the bDNA assay, irrespective of the HBeAg-antibody status. The two quantitative (bDNA and HCII) assays gave 59 concordant positive and 34 concordant negative results, giving an overall concordance rate of 91% (93 of 102). All the bDNA-positive samples tested positive with the HCII assay. In contrast, nine samples were HCII positive but bDNA negative, giving a sensitivity gain of 13% (9 of 68) for the HCII assay. The HBV DNA concentration of the nine serum samples was 3.8 ± 4.9 pg/ml (range, 0.9 to 14.9 pg/ml). None of the three assays detected HBV DNA in the 22 HBsAg-negative serum samples.

HBV DNA levels measured with the HCII assay were compared to corresponding values determined with the bDNA assay in 55 of the 59 concordant positive samples (Fig. 2). Four samples yielded readings higher than the detection range of both assays and were not included in this comparison. The bDNA assay yielded increasingly higher values relative to the HCII readings as the HBV DNA concentration increased. The ratio of corresponding bDNA to HCII results (in picograms per milliliter) was 2.83 ± 0.92 (range, 0.65 to 4.31). A good linear relationship was observed when the logarithmic conversions of HBV DNA levels determined by the two assays were plotted against each other ($r^2 = 0.985$, slope = 1.155) (Fig. 3). The following formulae were derived accordingly for the inter-assay conversion of results:

$$\log [\text{HBV DNA by bDNA (picograms per milliliter)}] = 1.155 \times \log [\text{HBV DNA by HCII (picograms per milliliter)}] - 0.0337$$

or simplified as

$$\text{HBV DNA by bDNA (megaequivalents per milliliter)} = \frac{0.262 \times \text{HBV DNA by HCII (picograms per milliliter)}}{1.155}$$

$$\text{HBV DNA by HCII (picograms per milliliter)} = 3.19 \times \left(\frac{\text{HBV DNA by bDNA (megaequivalents per milliliter)}}{0.866}\right)^{2043}$$
serum samples as determined by the bDNA and HCII assays.

As shown in Table 3, the HCII assay required fewer reagents and less sample volume compared to the bDNA assay. The HCII assay also had a shorter detection time, which is an important factor in clinical practice.

**DISCUSSION**

Although the novel HCII assay claimed to offer improved sensitivity compared to other quantitative solution hybridization HBV DNA assays currently in use, these assays have not been directly compared against one another. Due to the lack of a universal HBV DNA standard, the identical blood sample can yield markedly different HBV DNA levels when tested by different assays. This represents a major difficulty in the interpretation of data in the literature. In this study, we not only examined the reliability of the HCII assay but also compared its results with those obtained by the bDNA assay and derived formulae for interconversion. In addition, the technical aspects and the assay duration of these two quantitative assays were compared, since these properties have a major bearing on their usefulness in the clinical management of patients.

TABLE 3. Comparison of the technical complexities of the HCII and bDNA HBV DNA assays

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HCII</th>
<th>bDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample vol (µl)</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>No. of reagents used (no. that need to be freshly prepared)</td>
<td>4 (1)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>No. of incubations</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>No. of washes</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Color indicator</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Assay time (h)</td>
<td>4</td>
<td>24</td>
</tr>
</tbody>
</table>

The University of Hong Kong.

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