Comparison of the AdvanSure HBV Real-time PCR Test with Three Other HBV DNA Quantification Assays

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Abstract. Background: We compared the AdvanSure hepatitis B virus real-time polymerase chain reaction (AdvanSure HBV) kit with three other HBV DNA quantification assays and evaluated its performance. Methods: The AdvanSure HBV real-time PCR assay was compared with the Abbott RealTime HBV Quantification Kit, the COBAS TaqMan HBV Test, and the VERSANT HBV branched DNA 3.0 assay. The precision, linearity, accuracy, limit of detection (LOD), cross reactivity, and genotype inclusivity of the assays were compared, and any influence of the sampling tube type was evaluated. Results: The AdvanSure HBV PCR showed good correlations with the three other HBV DNA assays. The R² coefficients were 0.944, 0.939, and 0.921 with the Abbott RealTime HBV Quantification Kit, the COBAS TaqMan HBV Test, and the VERSANT bDNA 3.0 assay, respectively. Linearity was good in the tested range of 1.15-8.45 log₁₀ IU/ml. The lower LOD result was consistent with the 18 IU/ml claimed by the manufacturer. HBV genotypes A-F were all correctly amplified, and no cross reactivity was found in samples with high HCV RNA levels or high protein concentrations. The results were not influenced by the sample preparation tube (i.e. plain tube, SST, and EDTA containing tubes). Conclusion: The AdvanSure HBV real-time PCR assay is a reliable method for quantifying HBV DNA levels in routine laboratory testing.

Key words: hepatitis B virus; DNA quantification; real-time PCR; AdvanSure

Introduction

Current consensus guidelines recommend determining the level of hepatitis B virus (HBV) DNA to characterize the course of chronic HBV infection, to determine treatment, and to demonstrate the effects of antiviral drugs on HBV replication [1, 2]. Serum HBV-DNA quantification plays an important role in detecting an emergence that might be resistant to antiviral treatment, and in evaluating the risk factors for cirrhosis and the progression of hepatocellular carcinoma [3].

Various commercial polymerase chain reaction (PCR) assays for quantifying HBV DNA are available. These include the VERSANT HBV bDNA 3.0 Assay (Siemens Healthcare, New York, USA) using branched DNA (bDNA) technology, and the Digene HBV Hybrid-Capture II (Murex Diagnostics, Dartford, Kent, UK). Hybridization methods and endpoint PCR-based assays have proved useful in measuring the upper range of HBV viremia, but they lack the sensitivity to detect low levels of HBV DNA and have relatively narrow detection ranges [4, 5]. The real-time PCR assays have improved the sensitivity and detection range of viral measurements significantly. The COBAS TaqMan HBV Test (Roche Molecular Systems, Branchburg, NJ, USA), Abbott RealTime HBV Quantification Kit (Abbott Molecular Inc., USA), and AccuPower HBV Quantitative PCR Kit (Bioneer Corp., Daejeon, S. Korea) are real-time PCR assays commonly used in HBV DNA measurement.

Some HBV DNA assays use coupled automation instruments for DNA extraction, but automatic DNA extraction using magnetic beads might be inefficient for removing particular inhibitors (e.g., clot activators). In our experience, the incomplete removal of clot activators sometimes inhibits PCR reactions; in such cases, only plain red top tubes could be safely used.
The AdvanSure HBV real-time PCR assay (LG Life Sciences, Ltd., Seoul, S. Korea) is a new method using TaqMan chemistry [6]. The aim of this study was to compare its performance with that of commercially available HBV DNA quantification assays and to validate it for detecting and quantifying HBV DNA.

Materials and Methods

Clinical serum samples were prepared from patients with acute or chronic hepatitis B who visited Uijeongbu St. Mary's Hospital, the Catholic University of Korea. Between June 2009 and May 2010, 150 patients were enrolled. Serum samples were collected from the patients using serum separator tubes (SSTs). EDTA treated plasma samples and plain tube samples were also collected from 48 of the patients. The samples were stored at -70°C. Informed consent was obtained from all patients and the study was approved by the Institutional Review Board of the Catholic Medical Center (IRB number: XC10SSM10011U). The dilution solution used pooled serum from clinical samples, which were hepatitis B surface (HBs) antigen negative, HBe antigen and antibody negative, and negative in the AdvanSure HBV real-time PCR assay.

Comparison of the AdvanSure HBV real-time PCR with three other commercial HBV PCR tests. The results of the AdvanSure HBV real-time PCR (Advansure QPCR) were compared with results from three other HBV DNA assays: the Abbott RealTime HBV Quantification Kit (Abbott QPCR; n=101), the Roche COBAS TaqMan HBV assay (COBAS TaqMan; n=88), and the VERSANT bDNA assay (VERSANT bDNA; n=138). Not all samples were tested with all four assays because of small sample amounts. The VERSANT bDNA assay uses a signal amplification method,

<table>
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<th>Principle</th>
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<th>VERSANT</th>
<th>Abbott</th>
<th>COBAS</th>
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Abbreviations: AdvanSure, LG AdvanSure HBV real-time PCR assay; VERSANT, VERSANT HBV bDNA 3.0 Assay; Abbott, Abbott RealTime HBV Quantification assay; COBAS, Roche COBAS TaqMan HBV assay; PC, positive control; NC, negative control; LP, low positive control; HP, high positive control.
with a solid-phase, sandwich hybridization assay, incorporating multiple sets of synthetic oligonucleotide probes. The Abbott QPCR and COBAS TaqMan assays are both real-time PCR methods. Analyses were performed according to the manufacturers’ specifications. The characteristics, including the principles used and the linear detection limit of each assay, are summarized in Table 1.

**Performance evaluation of the AdvanSure QPCR.**

**Precision.** Precision was evaluated using pooled sera with low (4.38 × 10^2 IU/ml) and high (3.43 × 10^6 IU/ml) concentrations measured by the AdvanSure QPCR. Interassay variability was determined by testing the HBV concentrations at two levels for 20 consecutive days, and the intra-assay variability was determined from duplicate tests.

**Linearity.** Linearity was evaluated using an HBV-positive clinical specimen (2.7 × 10^8 IU/ml, by AdvanSure QPCR) diluted directly (i.e., not serially) into nine concentrations with HBV-negative serum. The investigated samples were quantified twice.

**Accuracy.** Accuracy was evaluated using the HBV DNA PHD801 Panel (BBI Diagnostics, West Bridgewater, MA, USA). This panel consists of one negative and nine positive samples, and the tests were quantified twice.

**Limit of detection (LOD).** The limit of detection (LOD) was determined using the WHO International Standard for HBV, which was directly diluted with HBV-negative serum, resulting in five concentration levels (i.e. 10, 20, 40, 60, and 100 IU/ml). For each concentration level, the percent positivity was calculated for five repeated tests.

**Cross Reactivity.** Cross reactivity was evaluated using the COBAS TaqMan HCV test on pooled serum samples with high HCV RNA levels (46,350,000 IU/ml) and on pooled samples with high protein levels (total protein 9.7 mg/dl) of HBV-negative samples. Ten HBV-negative samples (as assessed using the AdvanSure QPCR) were mixed 1:1 with high HCV RNA and high total protein sera, respectively. After mixing, each of the 10 samples was tested using the AdvanSure QPCR.

**Genotype inclusivity.** The HBV DNA Genotype Performance Panel PHD201 (BBI Diagnostics, MA 02379, USA) was used for testing genotype inclusivity. These panels consist of eight positive members – genotypes A (three kinds), B, C, D, E, and F – and one negative member.

**Matrix effect from the sample collection tubes.** Samples collected with different sampling tubes were tested to analyze any interference (e.g., from clot activators) in this study. The results of HBV DNA from EDTA treated plasma, SST collected, and plain tube collected serum samples from the same patient were compared. Forty-eight samples were tested to evaluate the potential matrix effect of interference produced by the sample collection type.

**Statistical Analysis**

Any correlation between paired commercial methods was tested by determining Pearson correlation...
coefficients and by linear regression analysis: $P < 0.05$ was considered significant. The mean differences between other tests were determined using Bland-Altman plots.

**Results**

**Comparison of AdvanSure QPCR with three commercial HBV PCR tests** (Figure 1, Table 2).

The qualitative agreement rate of AdvanSure QPCR and Abbott QPCR ($n=101$) was 95.0% (six samples were below the lower LOD, and 90 samples showed positive results in both assays). The positive samples were within the range of 1.43 - 8.42 $\log_{10}$ IU/ml in the AdvanSure QPCR. The $R^2$ coefficient was 0.944 ($P < 0.001$) and the mean difference (AdvanSure QPCR - Abbott QPCR ± SD) was 0.65 ± 0.46 $\log_{10}$ IU/ml. Five samples were under the lower LOD (<10 IU/ml) with the Abbott QPCR but showed HBV concentrations of 39 - 189 IU/ml with the AdvanSure QPCR.

The qualitative agreement rate of AdvanSure QPCR and COBAS TaqMan ($n=88$) was 94.3% (four samples were under the lower LOD, and 79 samples showed positive results in both assays). The positive samples were within the range of 0.93-8.35 $\log_{10}$ IU/ml in the AdvanSure QPCR. The $R^2$ coefficient was 0.939 ($P < 0.001$) and the mean difference (AdvanSure QPCR - COBAS TaqMan ± SD) was -0.27 ± 0.44 $\log_{10}$ IU/ml.

The qualitative agreement rate of AdvanSure QPCR and the VERSANT bDNA ($n=138$) was 84.8% (10 samples were under the lower LOD, and 107 samples showed positive results in both assays). The positive results were within the range of 0.71 -8.45 $\log_{10}$ IU/ml in the AdvanSure QPCR. The $R^2$ was 0.921 ($P < 0.001$) and the mean difference (AdvanSure QPCR - VERSANT bDNA results ± SD) was -0.56 ± 0.65 $\log_{10}$ IU/ml. Twenty samples with results under the lower LOD (<357 IU/ml) of the VERSANT bDNA showed positive results with the AdvanSure QPCR (21-214 IU/ml). Fifteen of the 107 positive samples in both assays showed results over the upper LOD (>1 x $10^8$ IU/ml) with VERSANT bDNA but showed measurable results with the AdvanSure QPCR (6.81-8.45 $\log_{10}$ IU/ml).

One patient’s sample tested negative (HBV DNA levels below the lower LOD) in three assays, but gave a positive result in the VERSANT bDNA test. Though the HBV DNA levels of two patients exceeded the upper LOD of both the VERSANT bDNA and COBAS TaqMan assays, they appeared measurable in the AdvanSure QPCR and Abbott QPCR systems. Discrepancies between the various HBV DNA assays were found mainly at lower concentrations (Table 3).
Evaluation of the AdvanSure QPCR.

**Precision.** The SD and coefficient of variation (CV) of intra-assay (within run) variability were 0.05 log10 IU/ml and 2.4% at a low concentration (4.38 x10^2 IU/ml) and 0.06 log10 IU/ml and 0.75% at a high concentration (3.43 x10^6 IU/ml). The SD and CV of interassay variability (between day precision) were 0.14 log10 IU/ml and 5.7% at a low concentration and 0.16 log10 IU/ml and 2.4% at a high concentration.

**Linearity.** Nine HBV DNA levels (range 1.15-8.45 log10 IU/ml) of clinical samples were used for the linearity test, and the Advansure QPCR showed good linearity in the tested range. The linear regression equation was y = 0.9977x + 0.3358, and the correlation coefficient was R^2 = 0.997 (Figure 2).

**Accuracy.** By using the HBV DNA Panel PHD801 (BBI Diagnostics, USA), one negative sample showed a negative result with the AdvanSure QPCR. The mean difference between the expected values and observed values of the nine samples tested was 0.59 log10 IU/ml. The differences in each of the nine samples, expressed first as the expected value and second as the difference from the observed value measured by the AdvanSure QPCR, were (6.38, -0.16), (5.84, -0.26), (5.08, -0.61), (4.72, -0.71), (4.3, -0.78), (3.23, -0.9), and (2.15, -0.68).

**Limit of detection (LOD).** All five repeated tests showed positive LOD results at expected values for the 20, 40, 60, and 100 IU/ml concentrations, and two of five tests showed positive results at 10 IU/ml.

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Table 3. Patient samples that showed discrepancies in paired comparisons among four HBV DNA assays (Unit: IU/ml)

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* Only patients who had at least one positive serologic HBV marker result within one month before and after the HBV DNA sampling time are listed in this table.

Abbreviations: AdvanSure, LG AdvanSure HBV real-time PCR assay; Versant, VERSANT HBV bDNA 3.0 Assay; Abbott, Abbott RealTime HBV Quantification assay; COBAS, Roche COBAS TaqMan HBV assay; NT, not tested; p (or numeric results), positive results; n, negative results.
All 10 HBV-negative samples that were mixed (1:1) with high HCV (46,350,000 IU/ml) serum and high-protein pooled serum (total protein 9.7 g/ml) samples showed negative results with the AdvanSure QPCR.

Genotype inclusivity. The PHD201 (BBI Diagnostics, USA) panel consists of eight positive members and one negative member. One negative sample showed negative results and all other samples with HBV-DNA of A-F viral genotypes showed positive results with the AdvanSure QPCR (Table 4).

Matrix effect by the sample collection tubes. There were no significantly different results according to the sample preparation tubes: as measured by the AdvanSure QPCR system, 43 samples showed positive results and five samples were under the lower LOD in all four different sampling tubes. The HBV DNA level of the positive sera ranged from 1.21 to 8.45 log10 IU/ml. The correlation coefficient R² was 0.987 between EDTA plasma and SST serum, 0.977 between plain tube serum and EDTA plasma, and 0.994 between EDTA plasma and SST serum. The correlation graphs and Bland-Altman plots are presented in Figure 3.

Discussion

There are no data for the comparison of these four commercial HBV DNA quantification assays (AdvanSure QPCR, Abbott QPCR, COBAS TaqMan and VERSANT bDNA) in previous studies. In the present study, the AdvanSure QPCR showed good correlation with three other HBV DNA assays and showed a wider dynamic measurement than the VERSANT bDNA. Thirty-five of 138 samples (25.4%) for which results were either below the lower LOD (n = 20) or over the upper LOD (n = 15) with the VERSANT bDNA could be quantified by the AdvanSure QPCR. In our study, the HBV DNA values derived from the AdvanSure QPCR assay were on average 0.71 log10 lower than those obtained with the VERSANT bDNA, 0.27 log10 lower than those with the COBAS TaqMan, and 0.65 log10 higher than those with the Abbott QPCR. Small mean differences were found between different HBV DNA assays in our study. Other studies also reported that the VERSANT bDNA showed higher HBV DNA results (about 0.67 or 0.7 log10) than the COBAS TaqMan or other real-time PCR assays [6, 7]. Similarly, the Abbott QPCR results were reported to be 0.25 log10 IU/ml lower on average than the results of the COBAS TaqMan [8]. The different standardization process for PCR and the sample volume might cause these discrepancies between the HBV-DNA assays’ results.

Sample preparation tubes containing clot activators, thrombin, or other non-silica clot activators, could influence the assay performance. In fact, the Abbott QPCR might be inhibited by
clot activators, and the manufacturer’s instructions emphasize the possibility of aberrant results associated with different types of collection tube. The tube matrix effect on the AdvanSure QPCR was therefore investigated. There was no inhibition of PCR, and the correlation between samples collected in different tubes was good. These results might arise in part from the differences in automated DNA extraction methods. Some methods use silica-coated paramagnetic beads for which the basic principle of DNA extraction is similar to vacuum membrane column technology (e.g., the

Figure 3. Comparisons between HBV DNA levels measured from plain tube-collected, SST-collected serum samples and EDTA-treated plasma samples from the same patients. The correlation coefficient was 0.9844 and the mean difference was 0.20 Log_{10} HBV DNA IU/ml between the plain tube and SST serum samples (A and B). The correlation coefficient was 0.9766 and the mean difference was 0.22 Log_{10} HBV DNA IU/ml between the plain tube serum and EDTA plasma samples (C and D). The correlation coefficient was 0.9871 and the mean difference was 0.01 Log_{10} HBV DNA IU/ml between the EDTA plasma and SST serum samples (E and F).
LabTurbo 36 compact system for LG Advansure QPCR and QIAamp MinElute Virus Spin kits). The principal of DNA extraction is to isolate DNA based on its binding properties to silica at different salt concentrations. Insufficient mixing between samples and magnetic beads might occur with the paramagnetic bead method. However, the vacuum membrane column method represents conditions similar to those in manual centrifugation. Therefore, automated vacuum membrane column technology is more efficient in removing inhibitors than the automated paramagnetic bead method.

In the precision analysis of the AdvanSure QPCR, the between-day CV was 2.4 - 5.7% and the within-run CV was 0.8 - 2.4%. In previous HBV DNA studies, the between-run CV was 1.6 - 9.4% and the within-run CV was 6.5 - 20.7% with the VERSANT bDNA, while the between-run CV was 7.9 - 11.6% and the within-run CV was 13.0 - 18.6% with the COBAS TaqMan [9, 10]. Similarly, in a study using the Abbott QPCR, the between-run CV was 3.6 - 4.7% and the within-run CV was 3.0 - 5.0% [11]. Therefore, the precision of the AdvanSure QPCR was equivalent to or better than those of the previously used HBV PCR assays.

Linearity was found in the tested range of 1.15 - 8.45 log_{10} IU/ml, and this result confirmed the linear range of 20 - 108 IU/ml (1.3 - 8 log_{10} IU/ml) stated by the manufacturer. In our study, all of the five repeated tests at 20, 40, 60, and 100 IU/ml detected HBV DNA. These results coincided well with the LOD of 18 IU/ml claimed by the manufacturer.

In conclusion, the AdvanSure QPCR assays uses a relatively small sample volume (250 - 300 µl) compared to the other investigated assays (600 - 750 µl). It showed good sensitivity and a wider dynamic range than the VERSANT bDNA. Moreover, the results were not affected by the different collection tubes or by interfering materials. The results of the AdvanSure HBV QPCR correlated well with those of the VERSANT bDNA, Abbott QPCR, and COBAS TaqMan. Therefore, the AdvanSure QPCR can be used effectively to monitor patients with either low or high serum HBV DNA levels and to provide optimal monitoring and timely application of antiviral therapies.

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References