

Performance Evaluation of New Automated Hepatitis B Viral Markers in the Clinical Laboratory

Two Quantitative Hepatitis B Surface Antigen Assays and an HBV Core-Related Antigen Assay

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Key Words: Hepatitis B surface antigen; HBsAg; Hepatitis B core-related antigen; HBcAg; Quantitative HBsAg assay; Elecsys HBsAg II quant; Architect HBsAg QT; Lumipulse HBcAg

DOI: 10.1309/AJCP8QDN7NAUXJFJ

Upon completion of this activity you will be able to:

- describe the relationship of hepatitis B surface antigen (HBsAg) and hepatitis B virus core-related antigen (HBcAg) to viral production in the liver.
- discuss the potential utility of quantitative serum assays for HBsAg and HBcAg for monitoring chronic hepatitis B patients.

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The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose. Questions appear on p 837. Exam is located at www.ascp.org/ajcpeme.

Abstract

We evaluated quantitative hepatitis B surface antigen (qHBsAg) assays and a hepatitis B virus (HBV) core-related antigen (HBcAg) assay. A total of 529 serum samples from patients with hepatitis B were tested. HBsAg levels were determined by using the Elecsys (Roche Diagnostics, Indianapolis, IN) and Architect (Abbott Laboratories, Abbott Park, IL) qHBsAg assays. HBcAg was measured by using Lumipulse HBcAg assay (Fujirebio, Tokyo, Japan). Serum aminotransferases and HBV DNA were respectively quantified by using the Hitachi 7600 analyzer (Hitachi High-Technologies, Tokyo, Japan) and the Cobas AmpliPrep/Cobas TaqMan test (Roche). Precision of the qHBsAg and HBcAg assays was assessed, and linearity of the qHBsAg assays was verified. All assays showed good precision performance with coefficients of variation between 4.5% and 5.3% except for some levels. Both qHBsAg assays showed linearity from 0.1 to 12,000.0 IU/mL and correlated well ($r = 0.9934$). HBsAg levels correlated with HBV DNA ($r = 0.3373$) and with HBcAg ($r = 0.5164$), and HBcAg also correlated with HBV DNA ($r = 0.5198$; $P < .0001$). This observation could provide impetus for further research to elucidate the clinical usefulness of the qHBsAg and HBcAg assays.

Hepatitis B virus (HBV) is a common pathogen, and it causes acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Hepatitis B surface antigen (HBsAg) was first identified more than 45 years ago¹ and has been used as a serologic marker for the diagnosis of HBV infection. Numerous assays for detecting HBsAg are commercially available, although most of them are intended only for qualitative or semiquantitative use.

In the late 1990s, the first quantitative HBsAg (qHBsAg) assay was developed for use with the Architect automated analyzer (Abbott Laboratories, Abbott Park, IL).² The clinical usefulness of this assay (Architect HBsAg QT) had not been extensively investigated until quite recently. Recent research has focused on investigating the correlation between HBsAg and HBV DNA levels according to the phases of the disease.³⁻⁵

HBsAg is known to be produced by the translation of transcriptionally active intrahepatic covalently closed circular DNA (cccDNA), which has a role as a template in viral replication and translation of virus genes transcribed from the integrated HBV DNA in the host genome.^{3,6,7} The serum HBsAg level was correlated with transcriptionally active cccDNA in previous studies and is regarded as a surrogate marker of infected cells.^{5,8} HBsAg, as the name suggests, is a component of the envelope of infectious virions. In addition, HBsAg is also found in the noninfectious form of spheres or filaments, which is produced at least 100-fold more than infectious particles.⁹

Although it is considered the most accurate marker for the number of infected hepatocytes, measuring cccDNA can be done only in hepatic tissues with complex techniques. To complement this limitation of cccDNA, serum HBV DNA quantification using polymerase chain reaction is widely used in current clinical practice for the diagnosis and monitoring of HBV infection. A decline of the serum HBV DNA level can reflect a reduction in viral replication, while a decrease in the serum HBsAg level usually represents a reduction in the translation of messenger RNA molecules transcribed from cccDNA.⁵ Thus, a quantitative determination of the HBsAg level may provide helpful information on infection status that is complementary to information provided by the HBV DNA level.⁵

HBsAg was first proposed as a marker for monitoring responses to the interferon therapies in patients with chronic hepatitis B (CHB) in 1994,¹⁰ but at that time, the absence of commercially available qHBsAg assays restricted its application as a monitoring tool. As technology has developed, qHBsAg assays have been improved, and their clinical usefulness is under investigation. Inactive HBV carriers could be discriminated from patients with reactivated disease by qHBsAg and HBV DNA levels,¹¹ and qHBsAg seems to be helpful for predicting responses to treatment with pegylated interferon and/or oral nucleoside/nucleotide analogs.¹²⁻¹⁴

The measurement of serum HBV core antigen (HBcAg) levels has been problematic because it is localized to the infected hepatic tissues and not released into blood. A new assay for measuring HBV core-related antigen (HBcrAg), which consists of a core, e antigen (HBeAg), and the precore proteins of HBV (22-kD precore fragment, amino acids 28-150), was developed; HBcrAg levels correlated well with HBV DNA levels.¹⁵ A recent study showed that the serum HBcrAg concentration was related to the level of intrahepatic cccDNA.¹⁶

Until recently, only 1 commercial qHBsAg assay had been available for more than a decade. The analytic performance of the novel HBcrAg assay and of the 2 commercially available qHBsAg assays has not been sufficiently evaluated in clinical laboratories. Verifying the laboratory performance of these assays should precede their general application in clinical practice. The aim of this study was to evaluate the analytic performance of 2 currently available qHBsAg assays and to compare the results between the assays and between those of other parameters, including serum HBV DNA levels. We also evaluated the precision performance of the newly developed HBcrAg assay and compared the concentrations of serum HBcrAg with those of HBsAg and HBV DNA.

Materials and Methods

Specimens for Comparison Between Assays

This study was approved by the institutional review board of Severance Hospital, Seoul, Korea. During March

to April 2010, a total of 529 serum samples from different patients with CHB, regardless of treatment, were consecutively collected at the Severance Hospital. These specimens were requested for HBV DNA quantification by the Division of Hepatology, Department of Internal Medicine, to screen HBV infection or to monitor CHB. All specimens were divided into aliquots immediately after arrival, and each was assayed for HBV DNA, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels. Remaining aliquots were stored at -70°C until tested, and with these aliquots, HBsAg and HBcrAg levels of all 529 samples were determined by the respective assays following the manufacturers' instructions.

qHBsAg assays

Two methods were used for quantifying HBsAg levels. One used the Roche Cobas e 411 analyzer with Elecsys HBsAg II Quant reagent kits (Roche Diagnostics, Indianapolis, IN). This test uses the electrochemiluminescence immunoassay with a sandwich complex formed from the 2 biotinylated monoclonal anti-HBsAg antibodies and a mixture of monoclonal and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex as a chemiluminescence molecule. The resulting chemiluminescence reactions were measured and converted to HBsAg concentrations in the specimens using the calibration curve generated by 2-point calibrators. The total assay duration was 18 minutes. The analyzer provided an onboard dilution function, which prediluted the samples automatically after they were loaded; the analytic measurement range (AMR) suggested by the manufacturer is between 5 and 13,000 IU/mL when the samples are 100-fold diluted using the automated dilution function. Diluted samples with HBsAg levels less than 5 IU/mL were retested without predilution, and the specimens were manually diluted by 20-fold before being loaded on the analyzer (total dilution, 2,000-fold) when the HBsAg concentration was greater than 13,000 IU/mL.

The other method used the Abbott Architect i4000SR analyzer and the Architect HBsAg QT reagents (Abbott Laboratories). This assay uses a chemiluminescent microparticle immunoassay principle with 2-point calibration. The total assay time was about 35 minutes, and the AMR of this assay proposed by the manufacturer is from 0 to 250 IU/mL. All samples were tested after a 500-fold manual dilution with the diluent provided, which is recommended by the manufacturer. The serum samples with HBsAg levels greater than 125,000 IU/mL were retested in a 1,000-fold manual dilution, and the samples with an HBsAg level less than 250 IU/mL were assayed again without dilution.

HBcrAg assay

HBcrAg in the serum samples was quantified by using the Lumipulse G1200 automated analyzer with the Lumipulse HBcrAg reagent kits (Fujirebio, Tokyo, Japan). This assay

uses the chemiluminescent enzyme immunoassay with 5 levels of calibrators. The AMR of this test was between 1.0 and 10,000.0 kU/mL, and the total duration of assay was about an hour, including 30 minutes of manual pretreatment with a detergent, which exposes HBcrAg.

HBV DNA Quantification and Other Assays

The HBV DNA viral load (IU/mL) was determined by using Cobas AmpliPrep/Cobas TaqMan HBV Test, version 2.0 (Roche Molecular Systems, Pleasanton, CA) according to the manufacturer's instructions. Serum AST and ALT levels were measured by using the Hitachi 7600 DDP modular chemistry analyzer (Hitachi High-Technologies, Tokyo, Japan).

Precision Performance Evaluation

Precision performance of the qHBsAg and HBcrAg assays was assessed based on the guidelines from the Clinical and Laboratory Standards Institute document EP4-A2. The testing was performed by using a single lot of reagents, calibrators, and control samples for each analyzer. For the evaluation of the 2 qHBsAg assays, positive quality control (QC) material provided by Roche, positive and negative QC materials provided by Abbott, and 3 levels of pooled serum panels were divided into aliquots and frozen at -20°C . The QC materials provided by the manufacturers were assessed to evaluate the precision performance of the respective assays. Each aliquot was assayed in replicates of 2 at 2 separate times per day for 20 days (80 tests per single level) except for the negative and positive QC materials for the Architect qHBsAg assay, which were tested during 14 and 10 days owing to the small sample quantity. In addition, 2 pooled serum samples were also tested for HBcrAg in the same manner with the

evaluation of the qHBsAg assays. All analyzers used a single calibration curve throughout the study.

Validation of the Linearity Ranges of the qHBsAg Assays

The tests to validate the linearity of the qHBsAg assays were performed based on the Clinical and Laboratory Standards Institute document EP6-A. Samples with 5 HBsAg levels were prepared by mixing high and low levels of pooled patient serum samples and were tested in quadruplicate. The linearity of the results was assessed by linear regression analysis.

Statistical Analysis

All statistical analyses were performed by using Analyse-it Method Evaluation Edition, version 2.22 software (Analyse-it Software, Leeds, England). A nonparametric Spearman rank test was used to determine the correlation coefficients between the levels of HBsAg, HBcrAg, and other indices. All values were used without logarithmic transformations. The slope and intercept for the linear regressed line between the HBsAg levels by the 2 qHBsAg assays were calculated by using Passing-Bablok regression analysis. *P* values of less than .05 were considered statistically significant in all analyses.

Results

Precision Performance of the qHBsAg and HBcrAg Assays

The precision performance of all evaluated assays is summarized in **Table 1**. The Elecsys qHBsAg assay showed total imprecision between 4.5% and 5.3% coefficient of variation

Table 1
Precision Performance of the qHBsAg and HBcrAg Assays During 80 Runs for Each Level for a 20-Day Evaluation Period Except for QCs of the Architect qHBsAg Assay

Analyte/Assay	Material	Mean	Imprecision, SD (Coefficient of Variation, %)				
			Repeatability	Between Run	Between Day	Total	
HBsAg (IU/mL)	Elecsys	QC	0.077	0.003 (3.6)	0.011 (14.3)	0.007 (9.6)	0.014 (17.6)
		PS low	40.603	1.038 (2.6)	0.811 (2.0)	1.285 (3.2)	1.840 (4.5)
		PS medium	4,929.9	93.8 (1.9)	75.8 (1.5)	229.1 (4.9)	258.9 (5.3)
	Architect	PS high	11,900.2	223.4 (1.9)	199.4 (1.7)	550.2 (4.6)	626.4 (5.3)
		QC negative*	0.006	0.009 (152.5)	0.000 (0.0)	0.000 (0.0)	0.009 (152.5)
		QC positive†	0.250	0.009 (3.4)	0.003 (1.3)	0.007 (2.7)	0.011 (4.5)
		PS low	38.166	1.419 (3.7)	0.996 (2.6)	0.233 (0.6)	1.749 (4.6)
		PS medium	4,472.9	569.3 (12.7)	0.0 (0.0)	184.5 (4.1)	598.5 (13.4)
		PS high	12,262.5	705.1 (5.8)	293.9 (2.4)	796.7 (6.5)	1,103.8 (9.0)
		HBcrAg (kU/mL)	Lumipulse	PS low	0.13	0.05 (39.8)	0.04 (34.7)
PS high	312.74			9.76 (3.1)	9.06 (2.9)	4.43 (1.4)	14.04 (4.5)

HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; PS, pooled serum samples; QC, quality control; qHBsAg, quantitative HBsAg.

* Evaluated during 14 days; analytic sensitivity of this assay, suggested by the manufacturer, is 0.05 IU/mL.

† Assessed during 10 days.

(CV) for the specimens with mean HBsAg levels of 40.603, 4,929.9, and 11,900.2 IU/mL, except the QC material with a mean HBsAg level of 0.077 IU/mL, for which the imprecision was 17.6% (CV) and 0.014 IU/mL (SD).

The Architect qHBsAg assay also showed imprecision less than 5% CV for the positive QC (mean HBsAg, 0.250 IU/mL) and the low-level serum (mean HBsAg, 38.166 IU/mL). Imprecision of this assay was 9.0% (CV) for the serum with a mean HBsAg level of 12,262.5 IU/mL but was greater than 10% (CV) for the pooled serum with a mean HBsAg of 4,472.9 IU/mL.

The Lumipulse HBcrAg assay also showed total imprecision less than 5% for the pooled serum with the mean HBcrAg concentration of 312.7 kU/mL. The total imprecision of this assay was 52.9% (CV) and 0.07 kU/mL (SD) for serum with a mean HBcrAg of 0.13 kU/mL.

Linearity of the qHBsAg Assays

The linearity of the 2 qHBsAg assays was evaluated by testing the serum panel with 5 concentrations in quadruplicate. The measured HBsAg levels by both assays showed linear responses to the expected concentrations with an R^2 greater than 0.99. The Elecsys qHBsAg assay was verified to be linear in the range between the HBsAg levels of 0.081 and 12,708.0 IU/mL (linear fit, $y = 0.9840x + 1.7730$; $F = 8,651.20$; $P < .0001$; $R^2 = 0.9979$), and the linearity of the

Architect qHBsAg assay was validated from 0.003 to 12,237.9 IU/mL (linear fit, $y = 0.9833x + 11.443$; $F = 2,204.95$; $P < .0001$; $R^2 = 0.9919$), where x means the expected HBsAg concentration and y represents the measured HBsAg levels.

Correlation Between the Two qHBsAg Assays

The results obtained with the 2 qHBsAg assays correlated well with a correlation coefficient (r) of 0.9934 (95% confidence interval [CI], 0.9921-0.9944; $n = 529$; $P < .0001$) (Figure 1A). However, there were proportional and constant biases between the results by the 2 assays, and the following equation was established by using Passing-Bablok regression:

$$y = 0.8042x - 110.7340$$

where x indicates the HBsAg levels by the Architect assay and y represents the levels by the Elecsys assay ($n = 529$) (Figure 1B). There was a nonlinear relationship between x and y ($P < .01$). The 95% CI for the proportional bias (slope) was between 0.7897 and 0.8195, and that for the constant bias (intercept) was from -143.9137 to -88.3642.

Correlation of HBsAg and HBcrAg Levels With Other Indices

The correlations for the serum levels of HBsAg, HBcrAg, HBV DNA, and aminotransferases are shown in Table 2. In the 529 specimens included in this study,

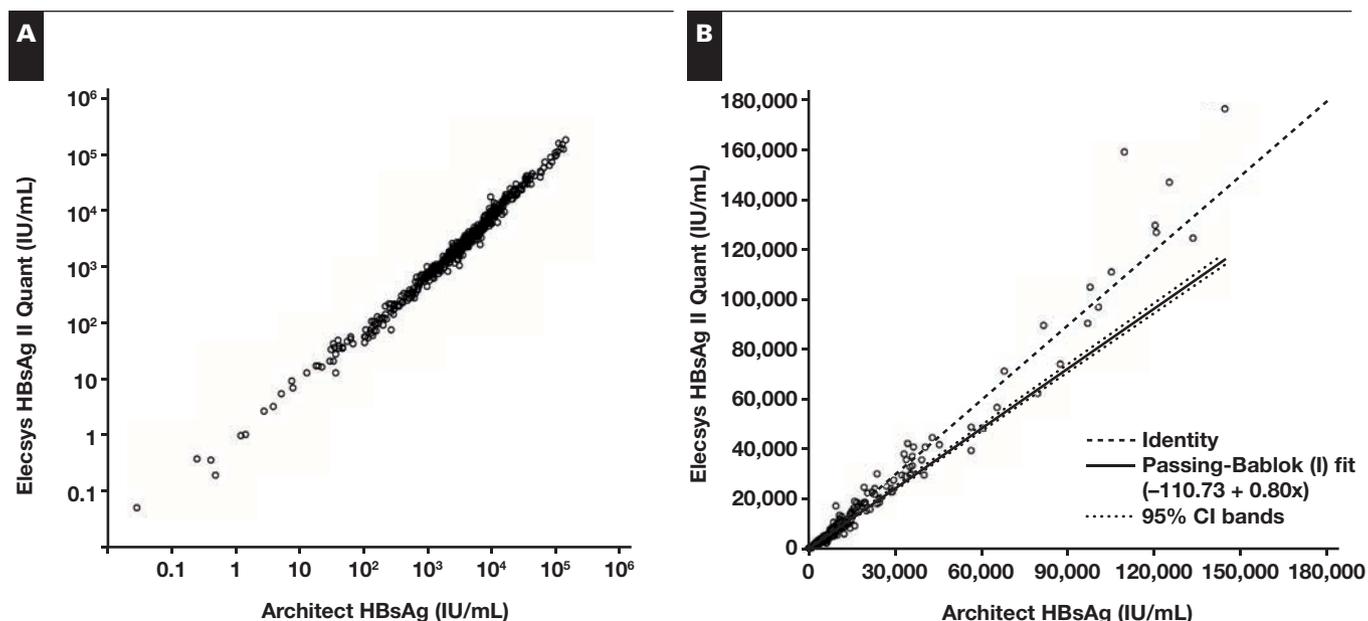


Figure 1 Correlation between the Elecsys and Architect quantitative hepatitis B surface antigen (HBsAg) assays. **A**, HBsAg levels by the Elecsys HBsAg II Quant assay correlated well with those by the Architect HBsAg QT with a correlation coefficient (r) of 0.9934 (95% confidence interval, 0.9921-0.9944; $n = 529$; $P < .0001$). Both axes are log scaled. **B**, There were significant proportional and constant biases between the results by the 2 assays, and the following equation was established: $y = 0.804x - 110.734$, where x indicates the HBsAg levels by the Architect assay and y represents those by the Elecsys assay.

the serum HBV DNA levels correlated only weakly with HBsAg levels, $r = 0.3373$ (Elecys, $P < .0001$) **Figure 2A** and $r = 0.3336$ (Architect, $P < .0001$) **Figure 2B**. No correlation was found between the serum HBsAg concentrations determined by the Elecys qHBsAg assay and the levels of aminotransferases (AST, $r = -0.0433$; $P = .3204$; ALT, $r = 0.0890$; $P = .0408$).

The serum HBcrAg levels were moderately correlated with the HBV DNA levels ($r = 0.5198$; $P < .0001$) **Figure 3A** and with HBsAg levels determined by the Elecys assay ($r = 0.5164$; $P < .0001$) **Figure 3B**. Also, there was a weak correlation between the levels of HBcrAg and aminotransferases (AST, $r = 0.2371$; $P < .0001$; ALT, $r = 0.2698$; $P < .0001$).

Discussion

We evaluated 2 commercially available qHBsAg assays, one of which was recently developed specifically for quantification, and a novel newly developed HBcrAg assay. The Elecys qHBsAg assay showed better precision performance of around 5% CV for the pooled serum samples at 3 levels compared with the Architect qHBsAg assay. Although all assays evaluated in our study, including the HBcrAg assay, showed a high degree of imprecision of between 17.6% and 152.5% (CV) for the respective samples with low concentrations, their imprecision would be negligible because the SD ranged from only 0.009 to 0.07. In addition, the 2 qHBsAg assays showed excellent linearity across HBsAg levels of

Table 2
Correlation in Serum Levels of HBsAg, HBcrAg, HBV DNA, and Aminotransferases in 529 Specimens Studied

Assay	Comparative Index	Correlation Coefficient* (r) (95% CI)	P
Elecys qHBsAg	HBV DNA	0.3373 (0.2595 to 0.4108)	<.0001
	AST	-0.0433 (-0.1281 to 0.0421)	.3204
	ALT	0.0890 (0.0037 to 0.1729)	.0408
Architect qHBsAg	HBV DNA	0.3336 (0.2556 to 0.4073)	<.0001
	Elecys qHBsAg	0.9934 (0.9921 to 0.9944)	<.0001
Lumipulse HBcrAg	HBV DNA	0.5198 (0.4547 to 0.5793)	<.0001
	Elecys qHBsAg	0.5164 (0.4510 to 0.5763)	<.0001
	AST	0.2371 (0.1550 to 0.3159)	<.0001
	ALT	0.2698 (0.1889 to 0.3471)	<.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; HBcrAg, hepatitis B core-related antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; qHBsAg, quantitative HBsAg.

* Correlation coefficients were calculated by using the Spearman rank test.

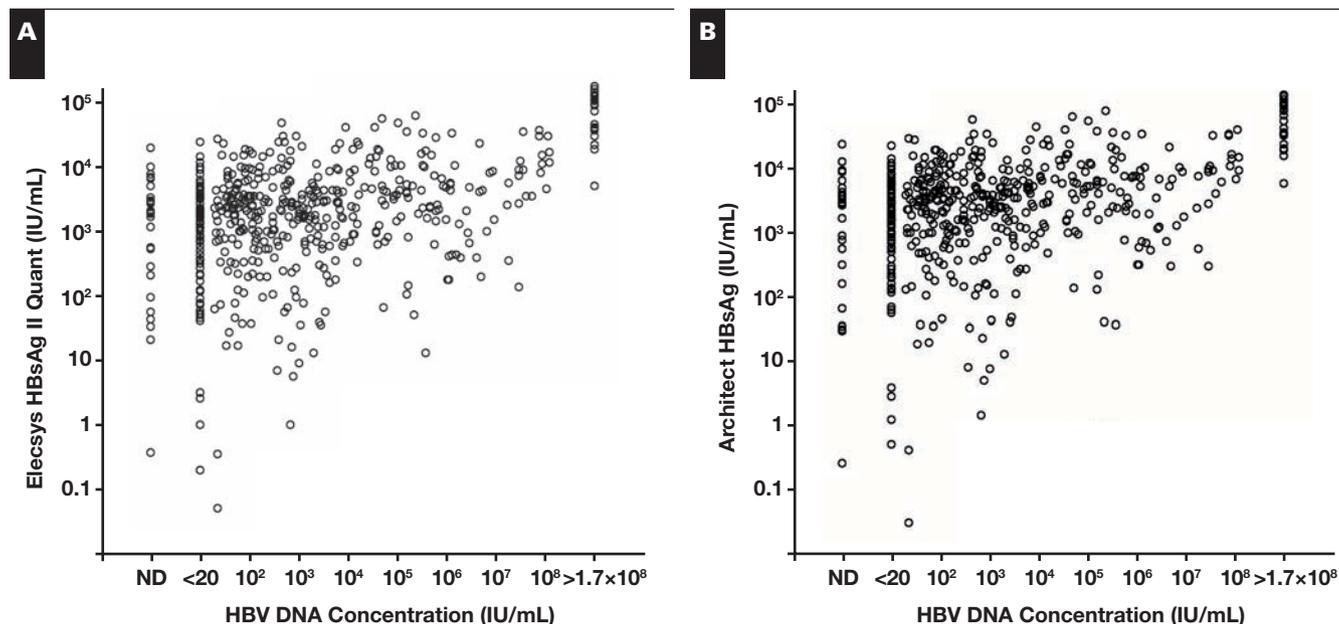


Figure 2 Correlation between serum levels of hepatitis B surface antigen (HBsAg) and hepatitis B virus (HBV) DNA. **A**, HBsAg levels determined by the Elecys HBsAg II Quant assay slightly correlated with HBV DNA concentrations ($r = 0.3373$; 95% confidence interval [CI], 0.2695-0.4108; $n = 529$; $P < .0001$). **B**, The levels measured by the Architect HBsAg QT assay also showed a similar degree of correlation with HBV DNA levels ($r = 0.3336$; 95% CI, 0.2556-0.4073; $n = 529$; $P < .0001$).

between 0.1 and 12,000 IU/mL with an R^2 greater than 0.99. While the Architect assay should be performed after manual dilution of the samples, the Elecsys assay can be carried out using the automated predilution after a sample is loaded on the analyzer. The manufacturer suggests that the 400-fold automated dilution function is installed in the autoanalyzers, including Cobas e 601/e 602 and Modular Analytics E170, which are different from those used in this study. Therefore, the AMR of the Elecsys assays can be between 20 and 52,000 IU/mL when those analyzers are used, which represents an advantage of the Elecsys assay over the Architect assay.

The linearity of HBcrAg was not verified in this study because there were insufficient numbers of samples with low and high HBcrAg levels. This assay uses calibrators with 5 levels from 0.0 to 10,000 kU/mL, whereas the 2 qHBsAg assays use calibration curves derived from 2 levels of calibrators. The analytic performance of the qHBsAg assays evaluated in this study suggests that they are appropriate for use in clinical laboratories, and those of the HBcrAg would need to be assessed by further studies. To the best of our knowledge, there are no published data regarding the precision performance of the HBcrAg assay.

In addition, the new Elecsys qHBsAg assay agreed well with the previously developed Architect assay with a high correlation of $r = 0.9934$ for the 529 specimens. Overall, the HBsAg levels determined by the Elecsys assay were 0.8-fold lower than those measured by the Architect assay; this

difference might be due to the differences in the antibodies used to capture the antigen. Two recently published studies also reported correlation coefficients of 0.97 and 0.96 between the Elecsys and Architect qHBsAg assays.^{17,18}

The HBsAg and HBcrAg levels in our analysis data were slightly or moderately correlated with the serum HBV DNA levels (Table 2). Both antigens were reported to be related to the intrahepatic cccDNA, and cccDNAs serve as the template of HBV DNA in the virions. Thus, it is plausible that the serum levels of HBV DNA are associated with those of the 2 antigens. The levels of HBsAg and HBV DNA have also been reported to correlate with each other with various correlation coefficients ranging from 0.121 to 0.862 in several previous studies.^{2,13,19-23} It is interesting that HBsAg and HBV DNA showed lower correlation ($r = 0.490$) in 20 patients with CHB during lamivudine treatment²⁴ and correlated better ($r = 0.709$) in 67 asymptomatic HBV carriers.²³ The degree of correlation was also different according to the disease stage and HBeAg status.^{19,20} There was also a trend toward decreased correlation according to the duration of treatment with interferon or nucleoside/nucleotide analogs observed in previous studies.^{13,22} Thus, variation in reported correlation coefficients could result from differences in disease stage, HBeAg status, HBV genotypes involved, and types/phases of treatment. These factors might also affect the correlation between the HBcrAg and HBV DNA levels. The correlation coefficients between HBcrAg

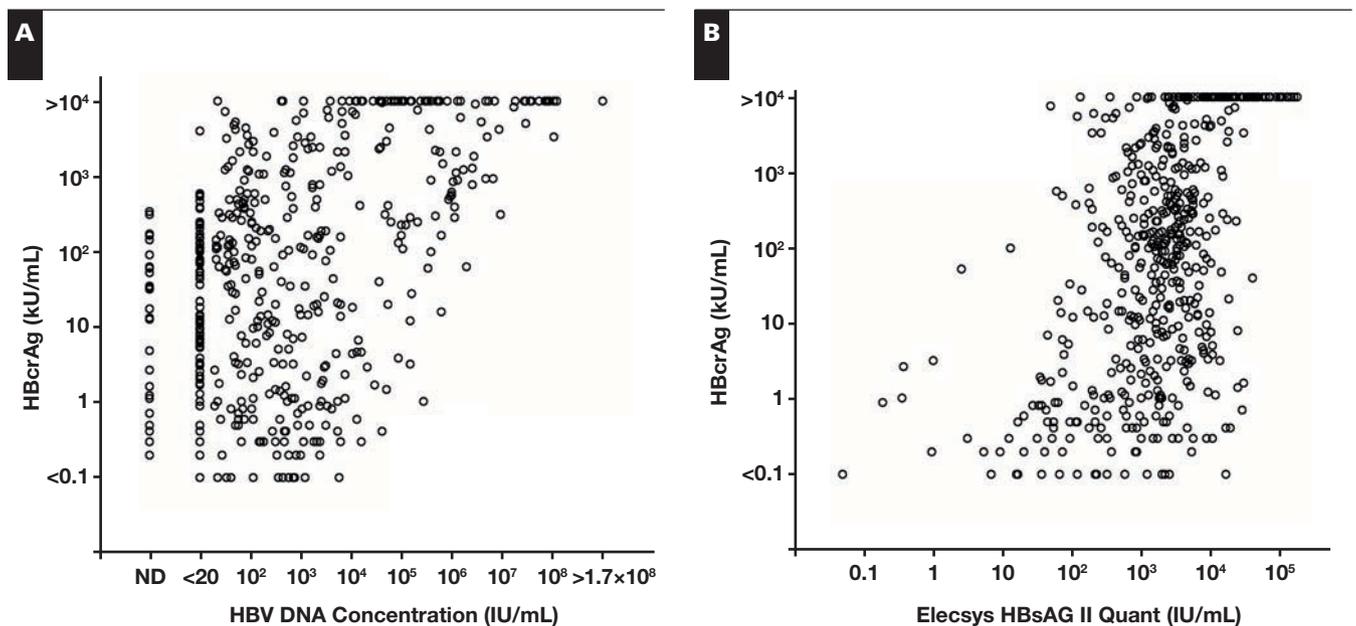


Figure 3 Correlation of serum hepatitis B core-related antigen (HBcrAg) concentrations with the levels of hepatitis B virus (HBV) DNA and hepatitis B surface antigen (HBsAg). **A**, The levels of HBcrAg and HBV DNA were moderately correlated with each other ($r = 0.5198$; 95% confidence interval [CI], 0.4574-0.5793; $n = 529$; $P < .0001$). **B**, There also was a moderate correlation between the serum concentrations of HBcrAg and HBsAg ($r = 0.5164$; 95% CI, 0.4510-0.5763; $n = 529$; $P < .0001$).

and HBV DNA in patients with CHB were 0.713 (n = 57) and 0.927 (n = 50), respectively, in 2 previous studies.^{15,16} The degree of correlation between HBcrAg and HBV DNA differed according to genotype ($r = 0.786$ for genotype B, n = 21; $r = 0.864$ for genotype C, n = 169) in another study.²⁵ In our study, HBcrAg correlated with HBV DNA levels in 529 specimens ($r = 0.5198$; $P < .0001$).

It is interesting that qHBsAg and HBcrAg also correlated with each other in our data ($r = 0.5164$; $P < .0001$). Moreover, the HBcrAg levels correlated slightly with the serum levels of aminotransferases, while the HBsAg levels did not. Since there are no published data regarding the correlation between qHBsAg and HBcrAg assays, our finding may provide an indication of a relationship in HBsAg, HBcrAg, and HBV DNA levels. Further research with well-defined patient groups (eg, treatment phases, HBV genotypes, and HBeAg status) would be needed to study the clinical usefulness of concurrent testing with qHBsAg and HBcrAg assays.

Unfortunately, we were unable to determine the potential influence of HBV genotype on correlations between the qHBsAg, HBcrAg, and HBV DNA levels in our study. However, the most prevalent HBV genotype in Korea is C2, which accounted for 98.1% (370/377) of the total HBV cases in a recent domestic study.²⁶ Thus, our conclusions may be regarded as being drawn from the C2 genotype. In a recent study, HBV genotypes did not significantly affect the correlation between the 2 qHBsAg assays,¹⁷ but further research would elucidate the effect of HBV genotype differences on the correlations in the levels of HBsAg, HBcrAg, and HBV DNA.

The qHBsAg and HBcrAg assays evaluated in our study showed acceptable analytic performance. In particular, the 2 qHBsAg assays were shown to have a wide AMR between 0.1 and 12,000 IU/mL of the HBsAg levels. In our study, the HBsAg, HBcrAg, and HBV DNA levels were only slightly to moderately correlated with each other, and this could provide an indication for the value of further research to elucidate the clinical usefulness of qHBsAg and HBcrAg assays.

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Acknowledgments: We thank Roche Diagnostics Korea and Abbott Korea for providing the reagents for the HBsAg quantification. We are also grateful to JW Pharmaceutical Korea for supplying the analyzer and reagents for the HBcrAg assay.

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