Comparison of Three Luminescent Immunoassays for Hepatitis B Virus Surface Antigen Quantification during the Natural History of Chronic Hepatitis B Virus Infection

Xiao-Dong Cheng, Liu-Wei Song, Lin-Lin Fang, Lin Yang, Yong Wu, Sheng-Xiang Ge, Quan Yuan, Jun Zhang, Ning-Shao Xia, Xiao-Ke Hao

Hepatitis B surface antigen (HBsAg) quantification has garnered attention because of its high predictive value in determining treatment responses. The HBsAg quantification assays, such as Architect and Elecsys, are commercially available, and more assays are in development. We aimed to compare the results of the Architect and Elecsys assays with those of a new assay, WTultra. The WTultra HBsAg assay is a sandwich chemiluminescent microplate enzyme immunoassay and provides an alternative choice which is more cost-effective and potentially applicable in developing or resource-constrained countries and areas. A total of 411 serum samples were collected from patients during various phases of chronic hepatitis B (CHB) infection. The samples were assessed using the three assays, and the results were compared and analyzed. The results for the Architect, Elecsys, and WTultra assays were well correlated according to the overall results for the samples (correlation coefficients, r_Architect versus Elecsys = 0.952, and r_WTultra versus Elecsys = 0.981) and the various infection phases (r_Architect versus WTultra ranging from 0.67 to 0.975, r_Architect versus Elecsys ranging from 0.695 to 0.982, and r_WTultra versus Elecsys ranging from 0.877 to 0.99). Additionally, consistent results were observed according to genotype (genotype B: r_Architect versus WTultra = 0.976, r_Architect versus Elecsys = 0.978, and r_WTultra versus Elecsys = 0.979; genotype C: r_Architect versus WTultra = 0.950, r_Architect versus Elecsys = 0.963, and r_WTultra versus Elecsys = 0.981) and hepatitis B virus (HBV) DNA levels (r_Architect = 0.540, r_WTultra = 0.553, and r_Elecsys = 0.580). In conclusion, the Elecsys and WTultra assays were well correlated with the Architect assay, irrespective of the CHB infection phase or genotype. All of these assays are reliable for HBsAg quantification.

Hepatitis B virus (HBV) infection is a global health problem that results in more than one million deaths per year (1). Since the discovery of the hepatitis B surface antigen (HBsAg) by Blumberg in 1965, HBsAg detection has been the leading hallmark for diagnosing an HBV infection (2). HBsAg persistence longer than 6 months is the key diagnostic criterion for chronic hepatitis B (CHB) infection (3). HBsAg seroclearance reflects the immunological control of the infection and confers an excellent prognosis in the absence of preexisting cirrhosis or concurrent infections with other viruses (3–5).

HBsAg quantification assays have been available for several years; however, the clinical application of HBsAg quantification has recently drawn attention. Several studies initially demonstrated an association between the levels of HBsAg and the levels of covalently closed circular (ccc) DNA, the template for viral replication inside the nuclei of hepatocytes (6, 7). Subsequently, serum HBsAg levels were hypothesized to be a marker of an immunological response to therapy, independent of the virological response detected from HBV DNA levels. Recently, evidence has suggested that the HBsAg levels change during the natural course of chronic HBV infection (8, 9), and a rapid decline in HBsAg levels indicates a strong response to therapy, regardless of the treatment approach. Monitoring HBsAg levels can help identify nonresponders to therapy (peginterferon [PEG-IFN] or nucleoside analogues) and determine the best management strategy for many patients (10–15).

Currently, the most widely used assay for HBsAg quantification is the Architect assay (16), followed by the Elecsys platform. These assays are fully automatic, based on microparticle and automated large-scale instruments. The WTultra HBsAg assay is a novel chemiluminescent microplate enzyme immunoassay (CLEIA) that requires manual dilution and operation, and the assay cost is much lower, which is a potential requirement in some developing countries and areas. The HBsAg level varies greatly during the natural phases of HBV infection, and the Architect assay has been proven to be reliable in various scenarios; however, other assays have not been fully evaluated in each natural phase, highly limiting their clinical usage. The aim of this study was to compare these three assays for HBsAg quantification during the various phases of natural infection.

MATERIALS AND METHODS

Assays and methods. HBsAg was quantified using the Architect (Abbott Laboratories, Abbott Park, IL, USA), Elecsys HBsAg (Roche Diagnostics, Indianapolis, IN, USA), and WTultra HBsAg (Wantai Biotechnology, Beijing, China) platforms. The HBsAg testing was performed according to.

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Address correspondence to Quan Yuan, yuanquan@xmu.edu.cn, or Xiao-Ke Hao, haoxkg@fmmu.edu.cn.
X.-D.C. and L.-W.S. contributed equally to this article.
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the manufacturer’s instructions on the package inserts using test kits from a single lot.

The Architect assay is a two-step chemiluminescent microparticle immunoassay and was performed following the manufacturer’s recommendations. The detection range of the assay is 0.05 to 250 IU/ml. After a 1:100 dilution was obtained using the serum diluent provided by the manufacturer, the samples with HBsAg levels of >250 IU/ml were retested at dilutions of 1:500 and 1:1,000. The samples with HBsAg levels of <0.05 IU/ml at a 1:100 dilution were retested undiluted. The conversion factor from Abbott Architect IU/ml to the World Health Organization (WHO) IU/ml was 1.31.

The Elecsys HBsAg assay is a two-step sandwich chemiluminescent microparticle immunoassay. All of the samples were tested at a 1:100 dilution in serum that was negative for both HBsAg and anti-HBs. When the results revealed a cutoff index (COI) of >1, the final result was calculated as COI × 100. When the COI was <1, the sample was retested undiluted to yield a final result. When the COI was ≥1,000, the sample was retested at a 1:5,000 dilution, and the final result was calculated as COI × 5,000. The signal/cutoff ratio obtained was converted to IU/ml using the WHO standard conversion factor of 0.055 IU/ml.

The WTultra HBsAg assay is a one-half-step sandwich chemiluminescent microparticle enzyme immunoassay, which does not need to be washed before addition of the enzyme conjugates. Briefly, for the assay procedure, sample diluent and samples or standards were pipetted onto the microplate and mixed gently. Then the microplate was incubated at 37°C for 1 h, and the enzyme-conjugated polyclonal antibody for HBsAg were added. The microplate was incubated for another 30 min at 37°C. After a washing step, the substrate was added, and the results were produced by a microplate luminometer (Orion II; Berthold Detection Systems GmbH, Pforzheim, Germany). The detection range of the assay is 0.02 to 50 IU/ml. All of the samples were diluted 1:500 with diluent and tested. The samples with HBsAg levels of >50 IU/ml were retested at a dilution of 1:10,000. The samples with HBsAg levels of <0.02 IU/ml at a 1:500 dilution were retested undiluted. The conversion factor from WTultra HBsAg IU/ml to WHO IU/ml was 0.575.

### Patients and samples
A total of 411 serum samples were collected from patients at Xijing Hospital, Xi’an, China. The patients were diagnosed with CHB infection and were not receiving treatment. None of the patients included in the analysis presented hepatitis delta virus, hepatitis C virus, or human immunodeficiency virus coinfection. The patients were classified into the following 6 natural phases according to published criteria (3): 43 patients with immune tolerance (IT), 51 patients with immune clearance (IC), 76 patients with low-replicative (LR), 25 patients with hepatitis B e antigen (HBeAg)-negative hepatitis (ENH), 93 patients with liver cirrhosis (LC), and 94 patients with hepatocellular carcinoma (HCC). A total of 283 samples were successfully genotyped using whole-genome sequencing. The serum samples were stored at -20°C before testing, and all of the samples were assessed using the three assays. This study received ethical approval from the institutional review board of Xijing Hospital of PLA according to the Declaration of Helsinki.

### Statistical analysis
The results of the three assays were transformed to 
\[ \log_{10}(IU/ml) \] and then compared pairwise using Pearson’s correlation coefficient and Bland-Altman analyses. Unpaired *t* tests were used to compare the serum HBsAg levels between two groups; the one-way analysis of variance (ANOVA) was used to compare variables among the various groups and the three tests. The least significant difference (LSD) method was used to compare pairwise differences between means. A *P* value of <0.05 was considered statistically significant. The Statistical Package for the Social Sciences (SPSS) v17.0 was used to carry out all of the statistical analyses.

### RESULTS

#### Clinical characteristics of the patients during the various phases of HBV infection

The cohort was divided into 6 groups according to the various phases of HBV infection. The clinical characteristics of the patients in these phases are listed in Table 1. The HBsAg data were obtained using the Architect assay. The HBsAg levels varied among the different groups (*P* < 0.0001) (Fig. 1A). The HBsAg levels were significantly higher during the IT phase than during the LR phase (*P* < 0.001) and ENH (*P* < 0.001) phases. The HBsAg levels were lowest during the LC (*P* < 0.001 versus LR) and HCC (*P* < 0.001 versus LR) phases (Fig. 1A). The HBsAg/HBV DNA ratio was highest in the LR group (*P* < 0.001 versus any other group). This ratio was significantly higher in the HCC group than in the IT group (*P* = 0.029) (Fig. 1B). No significant differences were observed in the HBsAg levels of patients with genotypes B and C during the IT, IC, and LR phases (Fig. 1C).

#### Correlations among the Architect, WTultra, and Elecsys assays

(i) Correlations for the overall results. No significant differences were observed among the three assays (*P* = 0.91). Correlations between the Architect and WTultra assays (*r* = 0.936, *P* < 0.001), the Architect and Elecsys assays (*r* = 0.952, *P* < 0.001), and the WTultra and Elecsys assays (*r* = 0.981, *P* < 0.001) are shown in Fig. 2. Using regression analysis, we determined that

\[ \text{HBsAg}_{\text{Architect}} = 1.048 \times \text{HBsAg}_{\text{WTultra}} - 0.193, \text{HBsAg}_{\text{Architect}} = 1.042 \times \text{HBsAg}_{\text{Elecsys}} - 0.162, \text{and HBsAg}_{\text{Elecsys}} = 0.958 \times \text{HBsAg}_{\text{WTultra}} + 0.143. \]

Furthermore, a Bland-Altman analysis was performed, and the results are shown in Fig. 2 and Table 2. The Architect assay detected HBsAg levels that were 0.033 log_{10} IU/ml higher than those detected by the WTultra assay and 0.023 log_{10} IU/ml higher than those detected by the Elecsys assay. The WTultra assay detected HBsAg levels that were 0.010 log_{10} IU/ml lower than those detected by the Elecsys assay.

### Table 1 Clinical characteristics of patients according to different natural phases of HBV infection

| Characteristic       | IT (n = 43) | IC (n = 51) | LR (n = 76) | ENH (n = 54) | LC (n = 93) | HCC (n = 94) |
|----------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Age (mean [range]) (yr) | 22 (10–32) | 30 (16–56) | 32 (10–61) | 42 (15–76) | 51 (24–80) | 52 (22–79) |
| Gender (no. male/no. female) | 25/18 | 46/5 | 45/31 | 44/10 | 73/20 | 75/19 |
| Genotype (B/C)       | 11/29      | 5/28       | 7/23       | 5/45       | 5/52       | 4/69        |
| HBeAg-positive (no. [%]) | 43 (100) | 51 (100) | 0 (0) | 0 (0) | 35 (38) | 31 (33) |
| ALT (mean [range]) (U/liter) | 27 (16–40) | 204 (80–1,146) | 23 (5–39) | 334 (80–1,040) | 52 (9–339) | 74 (14–644) |
| HBV DNA log_{10} (mean [range]) (copies/ml) | 8.38 (7.8–9.1) | 6.7 (2.8–10.2) | 2.5 (0.7–4.0) | 6.17 (3.5–9.3) | 4.8 (1.0–7.7) | 4.5 (1.0–8.3) |
| HBsAg log_{10} (mean [range]) (IU/ml) | 4.7 (3.9–5.6) | 3.9 (1.0–5.4) | 3.2 (0.9–4.9) | 3.4 (1.7–5.2) | 2.7 (0–1.1) | 2.8 (0–4.1) |

*IT,* immune tolerance; *IC,* immune clearance; *LR,* low replicative; *ENH,* HBeAg-negative hepatitis; *LC,* liver cirrhosis; *HCC,* hepatocellular carcinoma.

* ALT, alanine aminotransferase.

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Comparison of Three HBsAg Quantification Assays

During the IC, LR, ENH, LC, and HCC phases, the correlations were weaker during the IT phase ($r = 0.670, 0.695,\ and\ 0.877\ for\ Architect\ versus\ WTultra,\ Architect\ versus\ Elecsys,\ and\ WTultra\ versus\ Elecsys,\ respectively$). A Bland-Altman analysis of the three assays revealed biases of $-0.098$ to $0.175$ between the Architect and WTultra assays, $-0.238$ to $0.155$ between the Architect and Elecsys assays, and $-0.140$ to $0.082$ between the WTultra and Elecsys assays.

(iii) Comparison of the samples with relatively lower and higher HBsAg levels. According to the HBsAg quantification results of the Architect assay, we divided the serum samples into two groups: 154 samples with HBsAg levels $<1,000$ IU/ml and 257 samples with HBsAg levels $\geq 1,000$ IU/ml. Table 3 shows the results of the Bland-Altman analysis and the correlations among the three assays using samples with lower and higher HBsAg levels. The correlations between the assays using samples with lower HBsAg levels ($r = 0.800, 0.826,\ and\ 0.962$ for Architect versus WTultra, Architect versus Elecsys, and WTultra versus Elecsys, respectively) were weaker than those from samples with higher HBsAg levels ($r = 0.917, 0.945,\ and\ 0.963$ for Architect versus WTultra, Architect versus Elecsys, and WTultra versus Elecsys, respectively). Furthermore, the results for the samples with higher HBsAg levels indicated biases of $-0.002, -0.0003,$ and $0.002,$ respectively, among the assays, whereas the results for the samples with lower HBsAg levels indicated biases of $0.090, 0.062,$ and $-0.029,$ respectively.

(iv) Comparison of the assays according to HBV genotype. We obtained the genotypic information from 283 samples. Overall, 37 samples of HBV genotype B and 246 samples of HBV genotype C were found. The three assays correlated well, irrespective of genotype (Table 4).

Correlation between HBsAg and HBV DNA levels. The correlations between HBsAg and HBV DNA levels were concordant according to each of the three assays. The correlation coefficients were $0.540$ for the Architect assay, $0.553$ for the WTultra assay, and $0.580$ for the Elecsys assay.

**FIG 1** (A) Distribution of HBsAg levels during the various phases of CHB infection. (B) Distribution of the HBsAg/HBV DNA ratio during the various phases of infection. (C) Comparison of the HBsAg levels in patients with HBsAg levels $<1,000$ IU/ml and $\geq 1,000$ IU/ml. Table 3 shows the results of the Bland-Altman analysis and the correlations among the three assays using samples with lower and higher HBsAg levels. The correlations between the assays using samples with lower HBsAg levels ($r = 0.800, 0.826,\ and\ 0.962$ for Architect versus WTultra, Architect versus Elecsys, and WTultra versus Elecsys, respectively) were weaker than those from samples with higher HBsAg levels ($r = 0.917, 0.945,\ and\ 0.963$ for Architect versus WTultra, Architect versus Elecsys, and WTultra versus Elecsys, respectively). Furthermore, the results for the samples with higher HBsAg levels indicated biases of $-0.002, -0.0003,$ and $0.002,$ respectively, among the assays, whereas the results for the samples with lower HBsAg levels indicated biases of $0.090, 0.062,$ and $-0.029,$ respectively.

**DISCUSSION**

The recent availability of commercial quantitative assays for serum HBsAg detection has garnered interest in HBsAg as a biomarker for prognosis and treatment responses in CHB patients (17). The current studies have demonstrated that in HBeAg-positive patients receiving PEG-IFN therapy, an HBsAg quantification level at 12 weeks of $<1,500$ IU/ml is a strong predictor for HBeAg seroconversion, whereas an HBsAg level of $>20,000$ IU/ml is a strong indicator for no response (18, 19). In HBeAg-negative patients receiving PEG-IFN, a combination of no decline in HBsAg and a $<2$ log$_{10}$ IU/ml decline in HBV DNA seems to be a predictor of nonresponse, indicating that the therapy can be stopped immediately (20, 21). In addition, an HBsAg level of $<100$ IU/ml is a strong predictor for spontaneous HBsAg loss (22, 23). The clinical usage of HBsAg quantification had been suggested by several international treatment guidelines (3, 24).

The Architect assay is the most frequently used platform for HBsAg quantification, followed by the Elecsys assay. Several studies have compared the accuracy of these two assays for HBsAg quantification and found that the Elecsys assay is as reliable as the Architect assay (25, 26). However, comparisons of these assays according to the various phases of HBV infection were limited by the small number of samples (26). In addition, other assays have
not been fully evaluated. Therefore, we enrolled a large cohort of patients in each of the six phases of CHB infection to evaluate the two assays. Furthermore, we compared these assays with a CLEIA HBsAg quantification assay, WTultra, which is a more cost-effective test.

According to the overall results, the assays correlated well, although with a slight bias, indicating that the three assays produced consistent quantification results. However, we found several small differences. The correlation between the WTultra and Elecsys assays was slightly better than the correlation between the Architect assay and the other two assays; however, the bias was slightly smaller. This slight difference may have been due to the avidity of the antibodies used in the WTultra and Elecsys assays compared with those in the Architect assay. The differences among the three assays were insignificant in terms of affecting the accuracy of HBsAg quantification. It is impossible to obtain precisely the same result using different assays. Nevertheless, the differences indicate that the same assay should be used during treatment monitoring for one patient. The three assays presented similar results regarding the correlation between serum HBsAg and HBV DNA levels, indicating that the assays exhibited significant agreement.

During the various phases of CHB infection, HBsAg levels vary significantly. HBsAg levels peak during the IT phase due to high levels of HBV replication, and these levels are the lowest during the inactive (LR or IC) phase (8, 9). In this study, the highest HBsAg levels were observed during the IT phase, followed by the IC, LR, and ENH phases, results which is in agreement with the findings of a previous study. In addition, we observed two other groups of patients with end-stage liver disease, including liver cirrhosis (LC) and hepatocellular carcinoma (HCC). In these patients, the HBsAg levels observed during the LC phase were the lowest among those for the six phases of natural infection. The HBsAg levels during the HCC phase were higher than those of the LC phase and lower than those of the LR phase (Fig. 1A). During the advanced phases of infection, especially the LC and HCC phases, an increase in x can produce defective and aborted viral HBsAg particles (27). This phenomenon may suppress HBsAg levels during the LC and HCC phases. We observed that the
TABLE 2 Results of correlations and Bland-Altman analyses for the three assays*  

| Assays compared | Phase | r   | Biasb | 95% limits of agreement | 95% limits of agreement |
|-----------------|-------|-----|-------|-------------------------|-------------------------|
| Architect vs WTultra | Overall | 0.936 | 0.033 | −0.761 to 0.827 | −2 SD to +2 SD |
| Architect vs WTultra | IT | 0.670 | −0.098 | −0.623 to 0.427 | −2 SD to +2 SD |
| Architect vs WTultra | IC | 0.975 | −0.094 | −0.512 to 0.323 | −2 SD to +2 SD |
| Architect vs WTultra | LR | 0.952 | 0.175 | −0.48 to 0.829 | −2 SD to +2 SD |
| Architect vs WTultra | ENH | 0.935 | −0.004 | −0.568 to 0.559 | −2 SD to +2 SD |
| Architect vs WTultra | LC | 0.843 | 0.029 | −0.990 to 0.932 | −2 SD to +2 SD |
| Architect vs WTultra | HCC | 0.868 | 0.130 | −0.841 to 1.100 | −2 SD to +2 SD |
| Architect vs WTultra | Overall | 0.952 | 0.023 | −0.653 to 0.700 | −2 SD to +2 SD |

| Architect vs Elecsys | IT | 0.695 | −0.238 | −0.768 to 0.292 | −2 SD to +2 SD |
| Architect vs Elecsys | IC | 0.982 | −0.012 | −0.418 to 0.394 | −2 SD to +2 SD |
| Architect vs Elecsys | LR | 0.962 | 0.155 | −0.345 to 0.655 | −2 SD to +2 SD |
| Architect vs Elecsys | ENH | 0.946 | 0.030 | −0.461 to 0.521 | −2 SD to +2 SD |
| Architect vs Elecsys | LC | 0.893 | −0.004 | −0.786 to 0.779 | −2 SD to +2 SD |
| Architect vs Elecsys | HCC | 0.883 | 0.078 | −0.736 to 0.892 | −2 SD to +2 SD |
| Architect vs Elecsys | Overall | 0.981 | −0.010 | −0.445 to 0.426 | −2 SD to +2 SD |

| WTultra vs Elecsys | IT | 0.877 | −0.140 | −0.461 to 0.181 | −2 SD to +2 SD |
| WTultra vs Elecsys | IC | 0.99 | 0.082 | −0.240 to 0.404 | −2 SD to +2 SD |
| WTultra vs Elecsys | LR | 0.984 | −0.019 | −0.464 to 0.425 | −2 SD to +2 SD |
| WTultra vs Elecsys | ENH | 0.970 | 0.034 | −0.349 to 0.418 | −2 SD to +2 SD |
| WTultra vs Elecsys | LC | 0.964 | 0.025 | −0.444 to 0.495 | −2 SD to +2 SD |
| WTultra vs Elecsys | HCC | 0.973 | −0.051 | −0.506 to 0.404 | −2 SD to +2 SD |

* For example, the Bland-Altman analysis of Architect versus WTultra is performed according to the formula: (Architect − WTultra) versus average.  
* Bias was calculated as the mean difference.  
* The 95% limits of agreement were computed as mean − 2 SD and mean + 2 SD.

HBsAg/HBV DNA ratio was highest during the LR phase, which has been previously observed (8, 28). Furthermore, during the HCC phase, the HBsAg/HBV DNA ratio was higher than those in the IT and IC phases. The association between HBsAg production and HBV DNA replication is weaker and “disconnected” during the later phases of infection, which may explain these findings (11).

Correlations among the Architect, Elecsys, and WTultra assays were excellent during all phases of infection, including the LC and HCC phases. However, the correlations during the IT phase were weaker. The HBsAg levels in the samples from patients in the IT phase ranged from 3.67 to 5.19 log_{10} IU/ml for the Architect assay, from 3.67 to 5.19 log_{10} IU/ml for the WTultra assay, and from 3.72 to 5.41 log_{10} IU/ml for the Elecsys assay. However, the data range was small, which may have influenced the statistical analysis and led to a weak correlation during the IT phase. According to the correlation coefficient and Bland-Altman analyses during the various phases of infection, the three assays produced consistent results and performed well. Significant agreement was observed between the assays for genotypes B and C, which are the most common genotypes in Asia. The WTultra assay was associated with a larger bias for genotype B than those of the Architect and Elecsys assays; however, this bias may have occurred because too few patients with genotype B were included (n = 37). The numbers of patients in the IT, IC, and LR phases of HBV genotype B and C infections were comparable; we therefore compared the HBsAg levels during these phases according to genotype (Fig. 1C). The differences in the HBsAg levels during these phases of HBV infections with genotypes B and C were not significant, which is in contrast to a previous study that found lower HBsAg levels during the LR phase in patients with genotype B (8). However, the number of patients with HBV genotype B in this study may have been too small (n = 7), and the differences between the genotypes exhibited a trend toward significance (P = 0.08).

For the three assays, we found that the correlation was weaker and the bias was larger using samples with lower HBsAg levels (<1,000 IU/ml) compared with those for samples with higher levels (≥1,000 IU/ml). This phenomenon was observed in all of the comparisons from a previous study and is probably due to the complexity of the assays (26). For the three assays, the intra- and interassay coefficients of variance (CV) were larger using samples with lower HBsAg levels than using samples with higher levels. HBsAg was detected in most of the samples at the same dilution; therefore, this finding may indicate an important cause. Additionally, differences in the avidity of the antibodies used in the assays and the various detection systems may be a factor. The results therefore suggest that during clinical monitoring, low HBsAg levels may need to be validated using other assays.

TABLE 3 Results of correlations and Bland-Altman analyses in samples with different HBsAg levelsa  

| Group | Assay | r | Biasb | 95% limits of agreement |
|-------|-------|---|-------|-------------------------|
| Architect HBsAg of <1,000 IU/ml (n = 154) | Architect vs WTultra | 0.800 | 0.090 | −0.980 to 1.162 |
| Architect HBsAg of ≥1,000 IU/ml (n = 257) | Architect vs WTultra | 0.917 | −0.002 | −0.561 to 0.557 |

* For example, the Bland-Altman analysis of Architect versus WTultra is performed according to the formula: (Architect − WTultra) versus average.  
* Bias was calculated as the mean difference.  
* The 95% limits of agreement were computed as mean − 2 SD and mean + 2 SD.

TABLE 4 Results of correlations and Bland-Altman analyses according to genotype*  

| Genotype | Assays | r | Biasb | 95% limits of agreement |
|---------|-------|---|-------|-------------------------|
| B (n = 37) | Architect vs WTultra | 0.976 | 0.158 | −0.314 to 0.630 |
| B (n = 246) | Architect vs WTultra | 0.950 | −0.028 | −0.704 to 0.649 |

* For example, the Bland-Altman analysis of Architect versus WTultra was performed according to the formula: (Architect − WTultra) versus average.  
* Bias was calculated as the mean difference.  
* The 95% limits of agreement were computed as mean − 2 SD and mean + 2 SD.
Recently, researchers have shown great interest in HBsAg quantification. The Architect assay is the most widely used assay for HBsAg quantification; however, more assays are in development. It is important to validate these new assays. Previous studies have determined that the Elecsys assay is as dependable as the Architect assay, and our study validated this finding. Moreover, we validated the clinical application of another CLEIA assay, the WTultra, which offers an additional option for clinical HBsAg quantification. The Architect and Elecsys assays are fully automated and require large-scale equipment that may be prohibitively expensive in developing countries and areas. In comparison, although the WTultra assay requires manual operation, it can work with a small apparatus and is cheap enough for extensive application.

In conclusion, the Electys and WTultra assays performed well compared with the Architect assay, irrespective of the phase of CHB infection and the virus genotype. All of these assays are reliable for HBsAg quantification; however, the WTultra assay may be a better choice in developing or resource-constrained countries and areas.

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We declare no conflicts of interest.

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