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# Comparison between Elecsys HBsAg II and Architect HBsAg QT Assays for Quantification of Hepatitis B Surface Antigen among Patients Coinfected with HIV and Hepatitis B Virus

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**Hepatitis B surface antigen (HBsAg) quantification has been steadily gaining interest as a clinical marker of therapeutic efficacy, for which two commercial assays are currently available: Architect HBsAg QT (Architect) and Elecsys HBsAg II (Elecsys). HBsAg quantification was evaluated using both assays in 126 human immunodeficiency virus (HIV) and hepatitis B virus (HBV)-coinfected patients initiating treatment with tenofovir dipivoxil fumarate. Linear regression and correlation were used to establish the relationship between the two methods. Bland-Altman analysis was performed to determine mean between-assay difference and limits of agreement (LOA) (±2 standard deviations [SD]) both overall and stratified on HBV (hepatitis B envelope antigen [HBeAg] status, replication, genotype, HBV mutants) or HIV ($CD4^+$ cell count) cofactors. There was a significant correlation between Elecsys and Architect assays (correlation coefficient, $r = 0.959$; $P < 0.001$). HBsAg quantification using the Elecsys assay was on average 0.200 $\log_{10}$ IU/ml (LOA, −0.500, 0.800) higher than that using Architect, which was consistent across levels of $CD4^+$ cell count, presence of precore and YMDD mutations, and HBeAg status. A slightly larger mean between-assay difference was observed with genotypes A and G (0.196 and 0.201, respectively) versus HBV genotypes D and E (0.036 and 0.030, respectively). Mutations on the S region at position s120/s145 were the only determinant in which the mean between-assay difference in HBsAg quantification was lower than the null value (−0.078). In conclusion, the Elecsys assay, with automatic on-board dilution, is capable of quantifying serum HBsAg levels in HIV-HBV-coinfected patients, with very high correlation with the Architect assay.**

Hepatitis B (HBV) DNA quantification is the most often used marker for therapeutic efficacy during follow-up in patients chronically infected with HBV and treated with either pegylated interferon or HBV reverse transcriptase (RT) nucleos(t)ide analogs (7). The ultimate goal of anti-HBV treatment is HBs antigen (HBsAg) clearance, as it leads to the improvement of long-term clinical outcomes, including longer survival (28). However, it cannot be adequately predicted by obtaining an undetectable HBV DNA viral load (14). Recent studies among HBV-monoinfected patients have shown the clinical utility in HBsAg quantification, whose levels before and during treatment with pegylated alpha interferon were able to accurately predict patients with 3-year HBsAg loss (4, 12, 13, 17, 27). A nascent body of literature has also been published on HBsAg quantification during treatment with various nucleos(t)ide analogs but are for the most part preliminary (2, 8, 10, 16, 26, 30, 33).

To date, the Architect HBsAg QT (Architect) assay is the most widely used system for quantifying HBsAg levels (6). HBsAg quantification may also be performed using the Elecsys HBsAg II (Elecsys) assay, which has been recently validated for use within a study population of treated chronic hepatitis B (CHB)-infected patients (18, 29, 32). However, there is a lack of data concerning its performance in more difficult-to-treat populations, particularly HIV-HBV-coinfected patients, in whom HBsAg quantification with the Architect assay has been recently assessed during treatment with tenofovir dipivoxil fumarate (TDF) (3, 19). The aim of the present study was then to evaluate the performance of the Elecsys HBsAg II assay by comparing it with the Architect assay, specifically in coinfected HIV-HBV patients initiating treatment with TDF. Differences in HBsAg quantification between the two systems were also investigated with respect to a variety of HBV and HIV cofactors, particularly genetic determinants able to impact quantification by both assays.

## MATERIALS AND METHODS

**Study population and samples.** A total of 126 treatment-experienced, HIV-HBV-coinfected patients initiating treatment with 300 mg TDF per day as part of their combined antiretroviral therapy were included in this study with the following inclusion criteria: HIV positivity confirmed by a complete Western blot analysis, HBsAg seropositivity for at least 6 months, TDF initiation as part of their antiretroviral regimen, and an available serum sample at TDF initiation. Patients with previous or concomitant therapy with lamivudine, adefovir dipivoxil, emtricitabine, and/or interferon-based treatment were included. All patients took part in the multicenter, prospective French HIV-HBV Cohort Study and were enrolled from May 2002 to May 2003 (9). All patients gave their written informed consent to participate in the cohort, and the study received ethical approval in accordance with the Helsinki declaration.

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Patients were predominantly male ($n = 112$, 88.9%) with a median age of 41.4 years (25th to 75th percentile, 36.1 to 46.2). A total of 27 patients (21.4%) came from a country of HBsAg seroprevalence of >8% and had an estimated median (25th to 75th percentile) duration of HBsAg- and HIV-positive serostatus of 8.2 (4.1 to 12.4) and 11.9 (6.5 to 15.5) years, respectively. Transaminase levels were above normal for most patients, as median (25th to 75th percentile) alanine and aspartate aminotransferase levels were 44 (31 to 73) and 40 (29 to 60) IU/ml, respectively.

**HBV genotype and mutations.** HBV genetic information was obtained on a subset of patients with >190 IU/ml HBV DNA. Precore nucleotide 1896, basal core promoter (BCP) dinucleotide 1762/1764, and clade genotyping were determined by DNA sequencing or DNA chip technology (bioMérieux, Marcy l'Etoile, France). The probe was designed to determine the nucleotide at position 1896 (G versus A) in the precore region and positions 1762 (A versus T) and 1764 (G versus A/T) in the BCP region. Mutations in the YMDD motif (RT domain of the *pol* gene) were determined using PCR and direct sequencing. The detection of mutations in the S open reading frame, including immune vaccine escape sP120T and sG145R, was based on duplex amplification of the whole HBV genome followed by a DNA chip hybridization using the Affymetrix HBV DNA-Chip assay (bioMérieux, France) as recommended by the manufacturer's instructions (24, 25).

**HBsAg quantification assays.** In this study, we compared the results of HBsAg titer as measured by the Modular E170 assay (Roche Diagnostics, Meylan, France) and the Architect i2000 assay (Abbott Laboratories, Rungis, France).

The Elecsys (Roche Diagnostics, Meylan, France) is a two-step sandwich chemiluminescent microparticle immunoassay. Briefly, samples were mixed with antibody conjugates labeled with a biotin and ruthenium complex. The resulting antibody/antigen complexes were captured after washing with streptavidin-coated magnetic microparticles. When voltage was applied, a chemiluminescent signal was produced and measured using a photomultiplier. Results were compared to a cutoff value obtained through HBsAg calibration using the second World Health Organization (WHO) international standard for HBsAg (subtype adw2, genotype A; IU/ml; code number 00/588). The Elecsys II assay had an automatic onboard dilution at 1:400 with a range of HBsAg measurements from 20 to 52,000 IU/ml. In our assay, all samples were tested automatically at 1:400. Samples with HBsAg titers of >52,000 IU/ml were manually diluted to 1:10 or 1:100 to bring the reading within calibration range. Samples quantified at <20 IU/ml using the 1:400 predilution were retested as undiluted, with a resulting threshold of <0.05 IU/ml.

The Architect (Abbott Laboratories, Rungis, France) is a two-step immunoassay based on the use of chemiluminescent microparticles. Briefly, samples were mixed with paramagnetic beads presenting anti-HBs antibodies (anti-HBsAb). After a washing step, a conjugate and reactant were added and a light signal was emitted, which was proportional to HBsAg concentration within a linear-wide range (0.05 to 250 IU/ml). Standardized calibration of the Architect assay was performed using the first WHO international standard for HBsAg (subtype adw2, genotype A; IU/ml; code number 80/549). HBsAg titer in serum was quantified according to the manufacturer's instructions. An initial manual dilution of 1:100 was performed on all samples. Samples with HBsAg titers of >250 IU/ml were manually diluted at 1:500 to 1:2,000 to bring the reading within calibration range. Samples with HBsAg levels of <0.05 IU/ml at 1:100 dilution were retested undiluted.

**Precision of HBsAg quantification assays.** Three serum samples for each assay were arbitrarily chosen to represent various levels of HBsAg (~2, ~3, and ~4 $\log_{10}$ IU/ml) in the study population. Precision of both assays was then tested on each sample using ASTM International protocol E177-10 (http://www.astm.org). Under strict repeatability conditions, each sample was retested 21 times by the same technician, in the same laboratory, using the same apparatus, and during the same day. Intermediate precision and reproducibility conditions were not tested.

**TABLE 1** Precision of Architect and the Elecsys assays under repeatability conditions

| Sample[a] | IU/ml HBsAg | | % CV | Intra-assay correlation[b] |
|---|---|---|---|---|
| | Mean | SD | | |
| Architect | | | | |
| 1 | 319.9 | 14.4 | 4.5 | 0.999 |
| 2 | 3334.1 | 208.0 | 6.2 | 0.999 |
| 3 | 45 556.2 | 1232.7 | 2.7 | 0.999 |
| Elecsys | | | | |
| 4 | 633.6 | 26.4 | 4.2 | 0.999 |
| 5 | 6390.1 | 174.0 | 2.7 | 0.999 |
| 6 | 35 270.6 | 1190.7 | 3.4 | 0.999 |

[a] All samples were retested 21 times.
[b] Determined using a one-way analysis of variance model, pooled for all HBsAg levels.

**Statistical analysis.** Levels of quantified HBsAg were log transformed. In order to guarantee that our results would not be biased by outliers from undetectable levels, all values of <0.05 IU/ml were imputed as zero ($n = 2$).

When analyzing assay precision, intraclass correlation for both methods was evaluated using a one-way analysis of variance model. In addition, the mean, standard deviation (SD), and coefficient of variation (CV) were given at each HBsAg level.

In an initial analysis, values from both methods were plotted and compared using Pearson's correlation. A linear regression model was then fit using the Elecsys assay as the dependent variable and the Architect assay as the independent variable. Second, for each pair of measurements, the differences between Elecsys and Architect methods were plotted against their means in a Bland-Altman analysis. Assuming normal distributions, the mean between-assay difference and its limits of agreement (LOA; ±2 SD) were calculated on the overall population and then stratified on HBeAg serostatus; level of serum HBV DNA (>2,000 IU/ml, ≤2,000 IU/ml); HBV genotype (A, D, E, G); presence of precore, YMDD, or s120/s145 mutations; presence of HCV- or hepatitis D virus (HDV)-positive serology; and level of $CD4^+$ T cells (>500 cells/mm$^3$, 350 to 500 cells/mm$^3$, <350 cells/mm$^3$). Mean differences were compared between strata using a Student *t* test. Since HBsAg level could potentially influence between-assay differences, we also performed these analyses in patients with HBsAg quantification above or below median HBsAg levels (as determined by the Elecsys assay).

All statistics were performed using STATA statistical software (version 11.0; College Station, TX). Significance was determined using a *P* value of <0.05; however, *P* values of <0.1 were also reported.

## RESULTS

**Comparison between Architect and Elecsys HBsAg quantification.** A total of 126 samples were tested using both methods, all of which were obtained at TDF initiation. When using the Elecsys assay, HBsAg levels were measured in 86 (70%) samples without the need for further manual dilution. When using the Architect assay, only 11 (10%) samples were tested without dilution, giving results of <0.05 to 250 IU/ml. Subsequently, the majority of samples ($n = 70$, 55%) required an additional manual dilution at 1:100. Precision of both assays at various HBsAg levels is reported in Table 1.

Levels of HBsAg given by each quantification method are shown in Fig. 1. Overall, correlation between Architect and Elecsys methods of HBsAg quantification was extremely high ($r = 0.959$). Using a linear regression model, the relationship between methods was as follows: $\log_{10}\text{HBsAg}_{\text{Elecsys}} = 0.975 \cdot (\log_{10}\text{HBsAg}_{\text{Architect}}) + 0.257$.

There was an overall mean difference of +0.200 $\log_{10}$ HBsAg

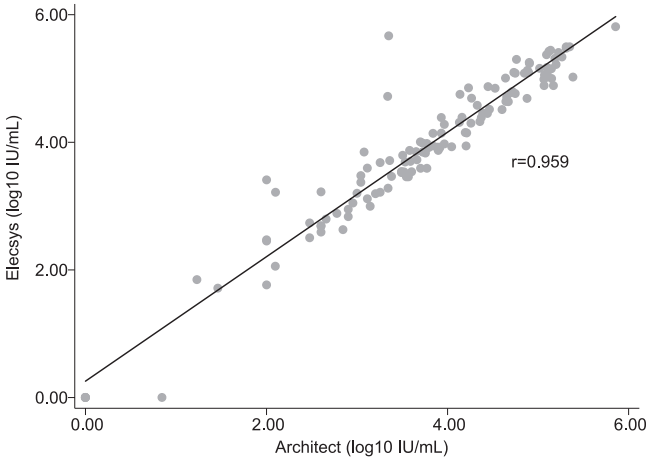


**FIG 1** Correlation between Elecsys and Architect HBsAg quantification systems. Individual levels of HBsAg are plotted (gray dots), with Architect as the independent and Elecsys as the dependent variable. Univariate linear regression (black line) has also been fitted.

between Elecsys and Architect methods, corresponding to an LOA (±2 SD) of −0.5 to 0.9. A plot of the paired difference between the two methods versus their mean is shown in a classic Bland-Altman plot (Fig. 2). The mean difference of HBsAg quantification between Architect and Elecsys methods was maintained irrespective of HBsAg levels (Fig. 2).

**Effect of various clinical parameters and between-assay comparability.** Comparison of the mean differences between HBsAg quantification methods according to various virological and immunological parameters is shown in Table 2 and stratified on below- or above-median HBsAg levels in Table 3.

**(i) CHB phase.** A total of 69 (54.8%) patients were HBeAg positive. There was no difference in quantification between these assays according HBeAg status (mean difference of 0.182 and 0.143, respectively, for HBeAg-positive and HBeAg-negative patients; $P = 0.5$), regardless of HBsAg level (Table 3). Likewise, no between-assay differences in HBsAg quantification were observed between high and low levels of HBV DNA (defined at 2,000 IU/ml; $P = 0.5$) (Table 2), although slightly higher discrepancies were observed when both HBV DNA and HBsAg levels were high ($P = 0.06$, comparing mean differences between HBV DNA strata at high HBsAg levels) (Table 3).

**(ii) HBV genetic variability.** A subset of patients with detectable HBV DNA also had data on HBV genotype ($n = 88$), precore mutation ($n = 86$), YMDD mutations ($n = 80$), and s120/s145 mutations ($n = 87$). HBV genotypes were distributed as follows: A, $n = 59$; D, $n = 7$; E, $n = 7$; G, $n = 14$; and mixed A/G, $n = 1$. During analysis, G and A/G mixed populations were merged into one group. A small difference between methods was observed particularly for HBV genotypes D and E (mean difference = 0.036

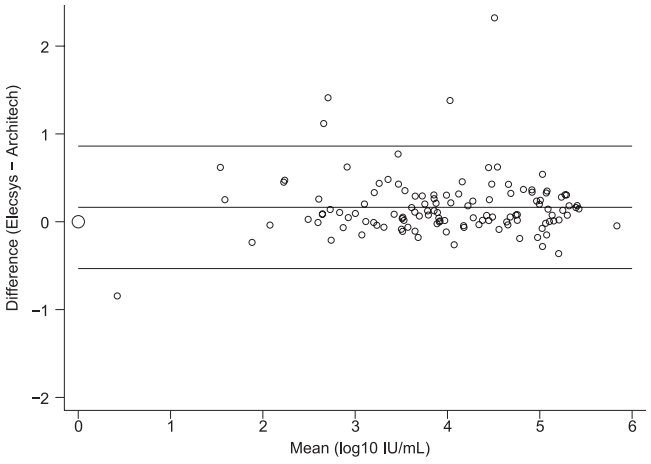


**FIG 2** Overall Bland-Altman plot. HBsAg techniques are compared by plotting the differences of Elecsys to Architect ($y$ axis) with their means ($x$ axis). The average between-method difference (0.2 $\log_{10}$ IU/ml) is represented by the middle solid line, while the limit of agreement (−0.5, 0.9 $\log_{10}$ IU/ml) is represented by the two outer solid lines. Five values lie outside this range.

**TABLE 2** Comparison of mean differences between HBsAg quantification methods by various clinical parameters[a]

| Clinical parameter | No. of samples[e] | Mean difference[b] | Limit of agreement (±2 SD) |
|---|---|---|---|
| HBeAg status | | | |
| HBeAg positive | 69 | 0.182 | −0.560, 0.923 |
| HBeAg negative | 57 | 0.143 | −0.500, 0.786 |
| HBV DNA viral load | | | |
| >2,000 IU/ml | 75 | 0.180 | −0.655, 1.014 |
| ≤2,000 IU/ml | 51 | 0.141 | −0.284, 0.566 |
| HBV genotype[c] | | | |
| A | 59 | 0.196 | −0.564, 0.956 |
| D | 7 | 0.036 | −0.165, 0.237 |
| E | 7 | 0.030 | −0.251, 0.311 |
| G+A/G | 15 | 0.201 | −0.275, 0.676 |
| Precore (W28) mutation | | | |
| Yes | 23 | 0.111 | −0.279, 0.500 |
| No | 63 | 0.195 | −0.543, 0.933 |
| YMDD mutation | | | |
| Yes | 60 | 0.156 | −0.543, 0.855 |
| No | 20 | 0.234 | −0.403, 0.871 |
| s120/s145 mutations[d] | | | |
| Yes | 6 | −0.078 | −0.403, 0.247 |
| No | 81 | 0.186 | −0.487, 0.859 |
| HCV-positive serology | | | |
| Yes | 15 | 0.160 | −0.924, 1.243 |
| No | 110 | 0.164 | −0.473, 0.802 |
| HDV-positive serology | | | |
| Yes | 9 | 0.251 | −0.485, 0.988 |
| No | 116 | 0.157 | −0.541, 0.856 |
| $CD4^+$ T cell count | | | |
| >500 cells/mm$^3$ | 42 | 0.195 | −0.341, 0.732 |
| 350–500 cells/mm$^3$ | 36 | 0.155 | −0.791, 1.102 |
| <350 cells/mm$^3$ | 48 | 0.143 | −0.464, 0.751 |

[a] Bland-Altman analysis stratified on various HBV and HIV cofactors.
[b] Difference of Elecsys to Architect.
[c] $P$ values of <0.1 when mean between-assay differences are compared betwen D versus G+A/G and E versus G+A/G.
[d] $P$ values of <0.1 when mean between-assay differences are compared between strata.
[e] Total number of samples with available data for each stratum.

**TABLE 3** Comparison of mean differences between various clinical parameters stratified on below/above-median HBsAg levels[a]

| Clinical parameter | HBsAg levels below median | | | HBsAg levels above median | | |
|---|---|---|---|---|---|---|
| | No. of samples[d] | Mean difference[b] | Limit of agreement (±2 SD) | No. of samples[d] | Mean difference[b] | Limit of agreement (±2 SD) |
| Overall | 62 | 0.100 | −0.500, 0.800 | 64 | 0.200 | −0.500, 0.900 |
| HBeAg status | | | | | | |
| HBeAg positive | 13 | 0.116 | −0.384, 0.615 | 56 | 0.197 | −0.591, 0.985 |
| HBeAg negative | 49 | 0.132 | −0.553, 0.817 | 8 | 0.208 | −0.044, 0.460 |
| HBV-DNA[c] | | | | | | |
| >2,000 IU/ml | 54 | 0.125 | −0.550, 0.800 | 21 | 0.321 | −0.799, 1.440 |
| ≤2,000 IU/ml | 8 | 0.153 | −0.292, 0.597 | 43 | 0.139 | −0.288, 0.565 |
| HBV genotype | | | | | | |
| A | 15 | 0.118 | −0.205, 0.442 | 44 | 0.222 | −0.634, 1.079 |
| D | 3 | 0.003 | −0.268, 0.273 | 4 | 0.061 | −0.095, 0.216 |
| E | 4 | 0.077 | −0.215, 0.369 | 3 | -0.033 | −0.293, 0.228 |
| G+A/G | 9 | 0.168 | −0.310, 0.647 | 6 | 0.249 | −0.248, 0.746 |
| Precore (W28) mutation | | | | | | |
| Yes | 12 | 0.087 | −0.241, 0.415 | 11 | 0.136 | −0.322, 0.595 |
| No | 16 | 0.117 | −0.209, 0.443 | 47 | 0.222 | −0.608, 1.051 |
| YMDD mutation | | | | | | |
| Yes | 20 | 0.081 | −0.188, 0.349 | 40 | 0.194 | −0.634, 1.022 |
| No | 5 | 0.152 | −0.266, 0.570 | 15 | 0.262 | −0.437, 0.960 |
| s120/s145 mutations[c] | | | | | | |
| Yes | 2 | 0.043 | 0.034, 0.052 | 4 | −0.139 | −0.482, 0.205 |
| No | 29 | 0.108 | −0.238, 0.454 | 52 | 0.230 | −0.559, 1.019 |
| HCV-positive serology | | | | | | |
| Yes | 13 | 0.158 | −1.010, 1.326 | 2 | 0.170 | −0.092, 0.431 |
| No | 49 | 0.121 | −0.315, 0.557 | 61 | 0.200 | −0.559, 0.958 |
| HDV-positive serology | | | | | | |
| Yes | 6 | 0.268 | −0.660, 1.196 | 3 | 0.219 | 0.139, 0.298 |
| No | 56 | 0.114 | −0.500, 0.727 | 60 | 0.198 | −0.568, 0.963 |
| $CD4^+$ T cell count | | | | | | |
| >500 cells/mm³ | 16 | 0.116 | −0.607, 0.839 | 26 | 0.244 | −0.119, 0.607 |
| 350-500 cells/mm³ | 16 | 0.141 | −0.610, 0.892 | 20 | 0.167 | −0.930, 1.264 |
| <350 cells/mm³ | 30 | 0.129 | −0.435, 0.693 | 18 | 0.167 | −0.521, 0.856 |

[a] Bland-Altman analysis stratified on various HBV and HIV cofactors, presented among patients with high or low values of HBsAg quantity. HBsAg levels are given by the Elecsys technique (median = 3.98 $\log_{10}$ Iu/ml).
[b] Difference of Elecsys to Architect.
[c] *P* values of <0.1 when mean between-assay differences are compared between strata at HBsAg levels above the median.
[d] Total number of samples with available data for each stratum.

and 0.030, respectively). A slightly larger difference was observed with genotypes A and G+A/G (mean difference, 0.196 and 0.201, respectively; D versus G, $P = 0.09$; E versus G, $P = 0.09$) (Table 2). A similar magnitude of effect was observed at both high and low levels of HBsAg; however, no significant differences were found between genotypes (Table 3).

A total of 23 (26.7%) patients also harbored precore mutations, with an amino acid change at position sW28*, and considering the majority of patients (79.7%) had been treated with lamivudine prior to inclusion, 60 (75.0%) had an HBV mutation on the YMDD motif. With respect to these HBV mutations, there were no discernible differences between the two methods (Table 2), regardless of HBsAg level (Table 3).

A total of 6 (6.9%) patients had mutations on the S domain of the *env* gene, with amino acid changes at the following sites: sP120T ($n = 2$), sG145K/A ($n = 1$), sG145K ($n = 2$), and sG145R ($n = 1$). On average, between-assay difference tended to be lower with the presence of s120/s145 mutations ($P = 0.06$), as HBsAg quantities from the Elecsys assay were barely lower than those from the Architect assay in patients harboring s120/s145 mutations (Table 2). This effect was more apparent at higher HBsAg levels ($P = 0.07$, comparing mean differences between s120/s145 strata at high HBsAg levels) (Table 3).

**(iii) Additional HCV and/or HDV infection.** Some patients were also infected with hepatitis C (HCV) or/and hepatitis D virus (HDV), resulting in the following coinfection combinations: HIV-HBV ($n = 106$), HIV-HBV-HCV ($n = 10$), HIV-HBV-HDV ($n = 4$), and HIV-HBV-HCV-HDV ($n = 5$). Due to the small

number of patients, we compared mean differences between the presence versus absence of additional HCV-positive (12.0%) or additional HDV-positive (7.2%) serology (Table 2). No substantial difference between methods was observed in HIV-HBV-coinfected patients with HCV-positive and HCV-negative serology (mean difference, 0.160 and 0.164, respectively; $P = 0.9$), independent of HBsAg level (Table 3). In contrast, the mean difference between methods was somewhat higher in HIV-HBV-coinfected patients with HDV-positive serology (0.251) than with HDV-negative serology (0.157). Nonetheless, this difference was not significant ($P = 0.4$), and the LOAs remained similar between HDV infection groups (Table 2).

**(iv) Level of CD4$^+$ T cells.** A third of patients ($n = 42$) were mildly immunocompromised (CD4$^+$ T cell count of $>500/mm^3$), while 28.6% ($n = 36$) and 38.1% ($n = 48$) had moderate (CD4$^+$ T cell count of 350 to $500/mm^3$) and more severe immunosuppression (CD4$^+$ T cell count of $<350/mm^3$), respectively. No significant differences were observed in the mean difference between methods across levels of CD4$^+$ cell count ($P$ values of $>0.3$ for each group-to-group comparison) (Table 2). A slightly larger LOA in difference was observed among those with CD4$^+$ cell counts of 350 to 500 ($-0.791$, 1.102). This effect did not differ between levels of HBsAg quantification (Table 3).

**Patients with highly divergent results.** A total of 5 patients (4.0%) had confirmed discordant results with a between-method difference greater than the overall LOA ($-0.5$, 0.9 $\log_{10}$ IU/ml) (Fig. 2). One of these patients had undetectable levels using the Elecsys method, whereas HBsAg was detectable with the Architect assay. Divergent results between Elecsys and Architect methods, respectively, were observed across a wide range of HBsAg levels, with the lowest being $<0.05$ versus 0.85 $\log_{10}$ IU/ml and the highest being 5.67 versus 3.35 $\log_{10}$ IU/ml.

With respect to clinical characteristics, only two patients had detectable HBV DNA (with HBeAg-positive serology), while both were harboring genotype A and one with a YMDD mutation. No mutations at position s120/s145 were observed in both patients. In the other three patients with undetectable HBV DNA, all were HBeAg negative, two were coinfected with HIV-HBV-HCV, and one was coinfected with HIV-HBV-HCV-HDV. Most patients ($n = 4$) were moderately immunocompromised (range of CD4$^+$ cell count, 256 to $473/mm^3$).

## DISCUSSION

Within a large population of HIV-HBV-coinfected patients at TDF initiation, we compared two major commercial platforms for HBsAg quantification, the Architect HBsAg QT and the Elecsys HBsAg II assays. A very high between-assay agreement was observed overall, indicating the similarities in epitopes targeted by the anti-HBsAb antibody used in each assay. The Elecsys assay tended to report higher levels of HBsAg than the Architect assay on an average of $+0.2$ $\log_{10}$ IU/ml, which is close to previously reported values (29). No discernible differences were noted across levels of HBsAg quantification, HBV genotypes, HBV mutations, HBeAg status, and levels of immunosuppression, thereby making this a clinically validated means of quantifying HBsAg in HIV-HBV-coinfected patients.

A compendium of recent studies has shown gaining interest in HBsAg quantification, making validation of other methods all the more important. With respect to the natural history of CHB, higher HBsAg levels have been observed in immune tolerant and clearance phases than in low-replicative or HBeAg-negative patients (15). Of note, HBsAg titers were also highly correlated with intracellular replication in the hepatocytes, explicitly for covalently closed circular DNA, in HBeAg-positive but not HBeAg-negative patients (11, 20).

These previous studies were conducted using mainly the Architect platform. Two recent studies have demonstrated the reliable and sensitive quantification of serum HBsAg given by the Elecsys assay, alongside its high correlation with the Architect assay (18, 29). The Architect assay can measure HBsAg levels within a relatively restricted range (0.05 to 250 IU/ml), which oftentimes requires manual dilution. The added advantage of the Elecsys assay is its automatic on-board dilution, allowing a range of HBsAg measurement of 20 to 52,000 IU/ml. The accessibility of this assay has already been noted in a multicenter study among 611 chronic hepatitis B patients, in which HBsAg levels were able to be quantified in 72% of the samples on the first attempt (1, 32). Similarly, 70% of our serum samples required no further dilution with the Elecsys assay, compared to 10% with the Architect assay.

Despite strong overall correlation between these assays, some discrepancies were noted within specific HBV or HIV cofactors. A slightly larger difference was observed with genotypes A and G, compared to very small differences with HBV genotypes D and E, albeit the limits of agreement never fell completely above or below a null difference for any particular genotype. The Elecsys assay has already been shown to quantify HBsAg at higher levels in HBV genotype A-infected patients (29). Considering that the majority of our population harbored either genotype A or G, confirmation of other genotypes within a larger number of samples is warranted.

There are several antigenic determinants of HBsAg important for anti-HBsAb recognition. The YMDD mutation in the RT domain of the *pol* gene, commonly associated with lamivudine resistance, also produces changes in the overlapping S domain of the *env* gene, which have been shown to alter HBsAg structure and promote defective antigenicity (22, 23). In our study, both assays adequately detected serum samples among patients harboring mutations on the YMDD motif. Other amino acid changes at positions s120 and s145 are also known to change antigenicity in the "a" determinant of the HBsAg (5, 21), with previous evidence suggesting higher levels of between-assay disagreement with mutations at position s120 (31). Interestingly, these mutations were the only factor overall for which the Elecsys assay quantified HBsAg at lower levels than the Architect.

Moreover, between-assay differences were also examined in a small subset of patients additionally infected with HCV or/and HDV. To a certain extent, we did observe higher HBsAg quantification with the Elecsys assay than with the Architect assay in HDV-seropositive patients; nevertheless, the LOA values in HDV-seropositive patients were consistent with those of the overall population. Although no difference in between-assay agreement was found when HCV-positive and -negative patients were compared, a very large LOA was observed among HCV-positive patients, and 60% of patients with highly divergent results had HCV-positive serology. Clinicians should heed these discrepancies when evaluating patients with multiple viral hepatitis infections.

From a diagnostic perspective, one must appreciate that HBsAg quantification detects all three forms of circulating HBsAg. An overall limitation of using quantitative enzyme immunoassays, like the Elecsys and Architect assays, is that antibodies target epitopes on the S protein and are therefore not capable of

distinguishing between different HBsAg proteins or between virion-associated HBsAg, subviral particles, and HBsAg produced from integrated sequences.

Several limits of our study are that, first, we were unable to determine if on-treatment changes in HBsAg were similar between the two methods. Since the kinetics of HBsAg during nucleos(t)ide analogue therapy are rather constrained, there were very few meaningful changes, that is, in the order of 1.0 to 2.0 $\log_{10}$ IU/ml, during the 3 years of available follow-up. Validation studies on long-term TDF use would therefore be warranted. Second, Bland-Altman analysis assumes that both the between-assay difference and its variance are constant over measurements and, for this reason, can be highly influenced by fixed and proportional biases. We attempted to address this issue by stratifying analysis on high and low HBsAg levels, assuming that the mean difference and its variance could be different above and below the median. Most analyses were rather consistent between HBsAg levels. Third, the Bland-Altman approach is based on confidence intervals, which assume that the distribution of mean differences is normal. In order to be consistent with these assumptions, we decided to report mean differences regardless of small sample size, wherein normality could have been questionable. Therefore, some caution in interpretation should be given for strata with very few patients.

In conclusion, the Elecsys assay is wholly capable of quantifying serum HBsAg levels in HIV-HBV-coinfected patients, with very high correlation and precision compared to those of the Architect assay. The Elecsys method can be applied with low interassay difference regardless of serum HBsAg levels, HBV genotypes, HBV mutations, CHB infection phase, and immunosuppression level. Further investigation examining the difference in HBsAg quantification methods is needed for patients harboring other genotypes, namely, E/F, that were hardly tested in recent studies (1, 18, 29).

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