Evaluation of the new assay LIAISON XL Murex HBsAg Quant for the quantification of Hepatitis B surface Antigen in patients with chronic hepatitis B

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Introduction and Purpose. There is renewed interest in measuring serum levels of the hepatitis B virus (HBV) surface antigen (HBsAg) as a surrogate marker to monitor the natural history of chronic HBV infection (CHB) and predict the efficacy of therapy, indeed the clinical relevance of HBsAg level stems from the apparent correlation with intrahepatic cccDNA levels and influences treatment response. Recent technologic innovations allow for the quantitative assessment of hepatitis B surface antigen (HBsAg) levels in serum. LIAISON-XL Murex HBsAg Quant assay (DiaSorin, Saluggia, I) is the newest direct two-step chemiluminescence-based immunoassay (CLIA) CE approved to quantify HBsAg. The aim of the study was to compare LIAISON-XL performances with ARCHITECT-QT HBsAg (Abbott Diagnostics, IL, US), as reference test. We determined the correlation between the two assays for the accurate measure of the concentration of the HBsAg in sequential serum specimens from patients with CHB undergoing antiviral therapy and followed at the Department of Gastroenterology and Hepatology, University of Turin, North-West Italy.

Methods. Sequential serum samples (n=152) from 14 HBe-negative patients with CHB, the majority of them infected by HBV genotype D and undergoing antiviral treatment, were retrospectively tested with both assays. Twelve patients underwent antiviral treatment (3 with Lamivudine, 5 with Entecavir and 4 with Peg-Interferon). Serum samples were extracted from the laboratory serum bank, thawed, and subsequently analyzed for HBsAg levels using both LIAISON-XL and ARCHITECT-QT. The 2nd HBsAg WHO Standard subtype adw2, genotype A 00/588 was used to assess assay performances. Serial dilutions of this standard within a range of 0.041-10 IU/mL were analyzed in triplicates.

Results. Correlation between LIAISON-XL and ARCHITECT-QT was excellent (r=0.9508, 95% CI: 0.932-0.964, p<0.0001) (Fig 1a). Bland-Altman analysis revealed a close agreement between the two assays and mean quantitation difference was 0.21±0.15 log_{10} IU/mL (95% CI: −0.07-0.5 log_{10} IU/mL) (Fig.1b). In 59% of paired samples the difference between the two assays was ≤0.25 log_{10} IU/mL, while in 74% ≤0.3 log_{10} IU/mL, in 86.8%% ≤0.4 log_{10} IU/mL and in 98% ≤0.5 log_{10} IU/mL. Four samples showed a quantitation difference above ±2SD (N=4) and were re-tested on both platforms; observed results in retested samples were highly correlated. Three samples whose HBsAg diverted ≥0.5 log_{10} IU/mL underwent re-testing, but differences between first and second measurements were within ±0.02 log_{10} IU/mL with both assays. High divergence was not related to HBV genotype.
Overall mean level of HBsAg as detected by LIAISON-XL and ARCHITECT-QT in the study-series was similar: 3498 and 5512 IU/mL, respectively (range 0.04-54,916 IU/mL). Distribution of HBsAg concentrations with ARCHITEC-QT and LIAISON-XL is shown in Fig. 2: 12.5% of samples measured ≤1 log_{10} IU/mL with both the two immunoassays, 4% and 6.5% tested ≤ 2 logs, 13.8% and 18.5% ≤ 3 logs, 49.3% and 59.3% ≤4 logs and 20.4% and 3.2% ≤5 logs with the two assays, respectively, constantly giving ARCHITEC-QT higher values than LIAISON-XL across the entire series of samples.
Dilution experiments with nine different concentrations of the 2nd WHO standard from 10 to 0.041 IU/mL showed excellent concordance to the expected values with both the systems (LIAISON-XL: r=0.998, p<0.0001, 95% CI: 0.993-0.999; ARCHITEC-QT: *r*=0.996, p<0.0001, 95% CI: 0.981-0.999). LIAISON-XL results were nearer to the expected WHO standards when compared to the results of the ARCHITECT-QT for all the standard concentrations (Fig.3). With LIAISON-XL, in 83.5% of samples the final result was given with a single analysis because they had a final level which was measurable within the initial onboard dilution (1:400). In 16.5%% of samples, the first measurement was below the detection range, and re-analysis of undiluted serum was required.
In patients undergoing antiviral treatment median baseline HBsAg levels before antiviral treatment was similar with the two methods: 4550 and 8003 IU/mL with LIAISON-XL and ARCHITECT-QT, respectively. At one year of antiviral treatment, HBsAg decrease from baseline was -0.3 log_{10} IU/mL with ARCHITECT-QT and -0.2 with LIAISON-XL. Fig. 4 (a,b,c) shows some representative courses of quantitative HBsAg of different patients to demonstrate the variety of the courses with the two assays. Both assays detected a fluctuating course of HBsAg with pronounced decreases over a short time (<1 year) only in patients undergoing PEG-IFN treatment.

Fig. 1a: Overall correlation between the LIAISON-XL and ARCHITECT-QT assays (n: 152). Sample concentrations were between approximately 0.04 and 54,916 IU/mL.

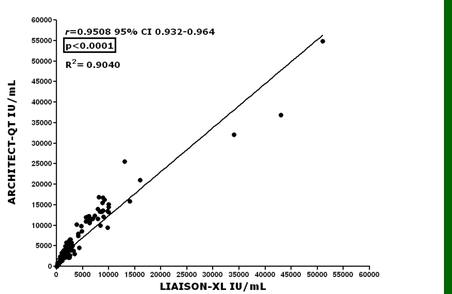


Fig. 1b: Bland–Altman plot of HBsAg measurements using the LIAISON-XL and the ARCHITECT-QT assays. Shaded area represents 95% confidence limits.

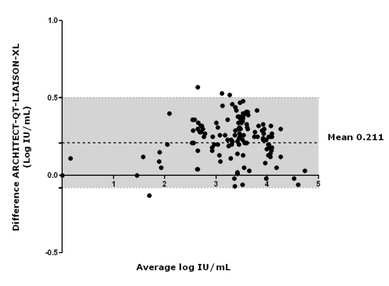


Fig. 2: Distribution of overall HBsAg concentration frequency with the LIAISON-XL and the ARCHITECT-QT assays.

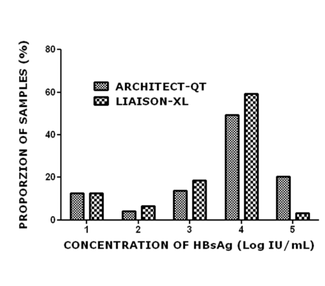


Fig. 3: Performance of the LIAISON-XL and the ARCHITECT-QT assays against the 2nd WHO standard. Values for LIAISON-XL:r=0.998, p<0.0001, 95% CI: 0.993-0.999. Values for ARCHITECT-QT: r=0.996, p<0.0001, 95% CI: 0.981-0.99.

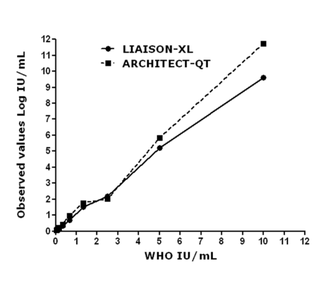


Fig. 4a: HBsAg kinetics with the two immunoassays LIAISON-XL and ARCHITECT-QT in a HBe-negative patient with CHB due to HBV genotype D, who underwent antiviral therapy with Lamivudine from August, 2001 to October, 2004, then followed by Adefovir add-on. After more than 12 years of antiviral therapy, in September 2013, HBsAg levels tested 230 and 523 IU/mL with LIAISON-XL and the ARCHITECT-QT, respectively.

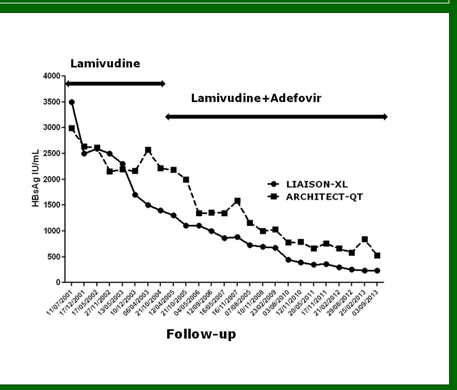


Fig. 4b: HBsAg kinetics with the two immunoassays LIAISON-XL and ARCHITECT-QT in a HBe-negative patient with CHB due to HBV genotype A, who underwent antiviral therapy with Pegylated-Interferon from November, 2011. After one year of antiviral therapy, in December 2012, HBsAg levels tested 84 and 503 IU/mL with LIAISON-XL and the ARCHITECT-QT, respectively.

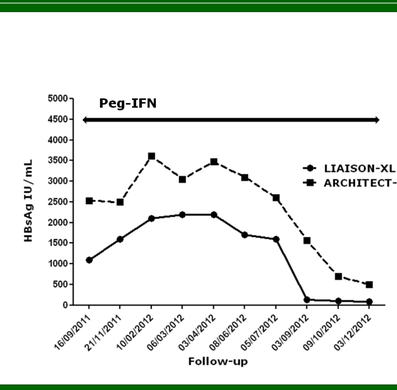
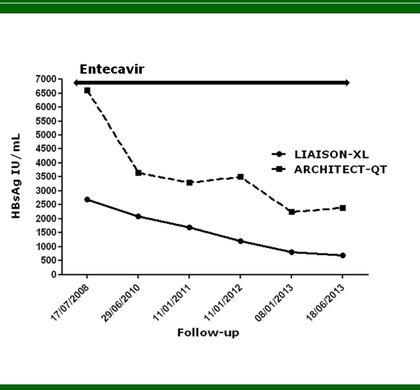


Fig. 4c: HBsAg kinetics with the two immunoassays LIAISON-XL and ARCHITECT-QT in a HBe-negative patient with CHB due to HBV genotype D, who underwent antiviral therapy with Entecavir from September 2008. After almost 5 years of antiviral therapy, HBsAg levels tested 700 and 2394 IU/mL with LIAISON-XL and the ARCHITECT-QT, respectively.



Conclusions. The quantitative measurement of HBsAg is becoming a tool in the clinical assessment and management of chronic hepatitis B. We compared against the gold standard the newly developed LIAISON-XL, a reliable test for HBsAg quantitation providing results that are standardized on the WHO 2nd International Standard.

In our study, the quantitation of HBsAg levels in routine clinical samples by LIASON-XL appeared overall to be accurate, with low variability and little discrepancy compared with ARCHITEC-QT. Therefore, threshold or prediction rules established for the ARCHITEC-QT platform may be transferred on the LIAISON-XL, without losing predictive accuracy. The kinetics of HBsAg with LIAISON-XL was also similar to ARCHITECT-QT in patients undergoing antiviral treatment, confirming that LIAISON-XL can be used for HBsAg quantification in clinical practice for the management of patients with CHB. However, the samples analyzed in our study were obtained from patients mainly infected by HBV genotype D, which is largely predominant in Southern Europe; further studies are required to establish if there is agreement also for other genotypes.

A major advantage of LIAISON-XL is its validation also for HBsAg screening in the setting of blood transfusion.

In conclusion, our study shows a high correlation and agreement between quantitative HBsAg measurement with ARCHITECT-QT and LIAISON-XL. On-board dilution has the advantage of lowering labor costs, turn-around-time and laboratory errors, improving accuracy and precision.

LIAISON-XL is therefore suitable for routine clinical use and can be applied for HBsAg quantification in the clinical practice and decision making for patients with chronic hepatitis B.