Quantification of HBsAg in serum: characteristics of the assays

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Abstract

Introduction

The presence of hepatitis B virus (HBV) surface antigen (HBsAg) in serum is the hallmark of HBV infection, and qualitative tests for its detection have been used since 1969. Recently, HBsAg quantification has been applied for monitoring chronic HBV infection during its natural history as well as for the prediction of the response to treatment. As HBsAg titres are becoming an integral part in the monitoring of chronic hepatitis B, knowledge of the characteristics of HBsAg quantitative assays is of great importance. Two automated assays are currently commercially available, namely the Architect QT (Abbott Laboratories) and the Elecsys HBsAg II Quant (Roche Diagnostics). Although the correlation between the currently approved methods for HBsAg quantification is quite good, the use of the same assay in the monitoring of individual patients with chronic HBV infection appears to be mandatory. The aim of this review is to discuss the characteristics and comparisons of available HBsAg quantitative assays.

Conclusion

Correlation of HBsAg quantification is good, but using the same assay in the monitoring of patients with chronic HBV infection is mandatory. Dilution steps for commercial assays minimize human error: We call for further studies to be conducted so that we are able to improve the techniques we have and further our understanding.

Introduction

The hepatitis B surface antigen (HBsAg), first referred to as Australia antigen, was discovered by Blumberg in 1965, and since 1969, it has been widely used in qualitative serological assays for the diagnosis of hepatitis B virus (HBV) infection. Another HBV antigen, the hepatitis B e antigen (HBeAg) discovered in 1972, was also used initially as a serological marker of HBV replication, while subsequently its presence, together with HBV DNA and ALT aminotransferase levels, has permitted the recognition of the initial phases in the natural history of chronic HBV infection, namely the ‘immune-tolerant’ and the ‘immune-active’ phases.

HBsAg is the surface envelope glycoprotein of HBV, being translated from two HBV subgenomic mRNA transcripts (pre-S1 and pre-S2/S) after transcription of cccDNA in the nucleous of infected hepatocytes that produce three different-sized [small (S), medium (M) and large (L)] proteins, with the S protein being expressed in the highest quantity during HBV infection. HBV DNA replication is accomplished through a separate RNA transcript (also after transcription from cccDNA), the pregenomic RNA (pgRNA), which also codes for the precore/core proteins. Thus, the production of HBV proteins and HBV DNA replication run through different molecular pathways, either parallel or dissociated, as it can happen during anti-HBV treatment with oral nucleos(t)ide analogues. Moreover, HBsAg is produced not only by cccDNA but it can also be derived from HBV DNA sequences of the surface antigen open-reading frame (ORF) integrated into the genome of hepatocytes. Integrated HBV DNA appears to be the major template for HBsAg production in HBe-negative chronic hepatitis B (CHB) as well as in cases of HBV-related hepatocellular carcinoma (HCC).

The surface protein of HBV is an important protein involved not only in HBV virion secretion but also in viral infectivity and is the target of neutralizing antibodies (anti-HBs) that develop either naturally during the course of the infection or after vaccination. HBsAg is produced in excess amounts and circulates in the serum of HBV-infected individuals in the form of large numbers of non-infectious HBsAg spherical particles and filamentous structures (known as empty subviral particles) as well as the envelope protein covering the mature viral particles (Dane particle) (Figure 1). The circulating HBsAg contains all forms of L, M and S proteins that cannot be distinguished by HBsAg quantitative and qualitative tests, which use antibodies that target epitopes in the S protein contained in all three forms (Figure 2). With the use of specific monoclonal antibodies in enzyme immunoassays, pre-S1 and pre-S2 Ag titres have been shown to positively correlate with total HBsAg levels.

The first HBsAg tests were performed with the two-dimensional micro-Ouchterlony immunodiffusion technique, but subsequently methods with higher sensitivity [such as radioimmunoassay (RIA) and enzyme immunoassay (EIA)] were...
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The aim of this review is to assess the HBsAg quantitative assays and compare them to analyse their effectiveness.

Description of HBsAg quantitative methods

Two commercial assays, applied on automated analysers, are widely available for HBsAg quantification: the Architect QT (Abbott Laboratories) and the Elecsys HBsAg II Quant (Roche Diagnostics) (Table1).

Quantification of HBsAg has been tried early on, mainly with RIA but also with EIA methods. In recent years, automated tests for the quantification of serum HBsAg levels have been made commercially available to practicing clinicians. In contrast to HBsAg, HBeAg serum quantification remains more of a research tool, although its levels have been associated with the prediction of early seroconversion in HBeAg-positive CHB. The use of HBsAg quantification in clinical practice has increased recently, particularly during the last 5 years, after the publication of numerous articles on the value of serum HBsAg quantification for the monitoring of HBV infection during its natural history as well as for the prediction of the response to treatment of CHB.

As HBsAg titres are becoming part of the monitoring of CHB, knowledge of the characteristics of the available quantitative assays is of great importance.

Figure 1: HBsAg production in HBV infection.

Figure 2: The three forms of HBsAg.
mesures up to 250 IU/mL of HBsAg in undiluted sera. Manual dilution is needed for measurement of higher HBsAg levels (maximum authorized dilution, 1:999). Recently, a new assay file was developed for the Abbott Architect HBsAg assay to allow the user to select an on-board dilution of 1:500, decreasing the possibility of human error and offering a wider range of quantification. Auto dilutions demonstrate better precision values within and between run, and the total coefficient of variation (CV%) is approximately three times lower than that in manual dilutions. In fact, it was demonstrated that CVs 9.05–10.73% by manual dilutions were minimized to 2.84–3.76% when the auto dilution option was used.

The Elecsys HBsAg assay, which has been available since 2011, is an automated electrochemiluminescence immunoassay that utilizes two biotinylated monoclonal and a mixture of monoclonal and polyclonal anti-HBs antibodies labelled with ruthenium to form a sandwich complex with the target HBsAg. The complex then binds to streptavidin-coated microparticles, via biotin–streptavidin interaction, and in the measuring cell, the microparticles are magnetically captured onto the surface of the electrode where application of voltage induces chemiluminescent emission, which is measured by a photomultiplier. Results are determined using a two-point calibration curve and a master curve provided by the reagent barcode. The calibration method is standardized against the WHO International Standard (NIBSC code: 00/588; WHO second International Standard for HBsAg, subtype adw2, genotype A; IU/mL). The total duration of the assay is 18 minutes. The Roche assay has an automatic on board 1:100 or 1:400 dilution step (depending on the analyser—1:100 for the Elecsys 2010 and cobas e411, 1:400 for the Modular Analytics E170, cobas e601 or e602) and subsequently a range of HBsAg measurement from 5–20 to 13,000–52,000 IU/mL. Lower (down to 0.05 IU/mL) and higher levels are determined with testing undiluted serum or further manual dilution, respectively. In a study comprised of 611 CHB patients with HBV genotypes A–G, approximately 72% of samples could have HBsAg level measured on the first analysis by the Elecsys II assay without the need of further manual dilution. The assay’s performance in precision, linearity, carryover rate and specificity has been shown to be good. The intra- and inter-assay CVs are <5–10%, there is no cross-reactivity with potentially interfering substances and the carryover rate is 0.0002%.

Modifications of qualitative assays for measurement of HBsAg concentration have been tried, and these early efforts could serve as a base for the development of accredited tests by more manufacturers. In such an effort, the ETI-MAK-4 assay (Diasorin, Turin, Italy) and Monolisa HBsAg Ultra (Biorad, Redmond, WA) classic ELISA assays and the Roche HBsAg II CMIA test on the Cobas analyser were slightly modified for HBsAg quantification and compared with the Abbott Architect QT. The Diasorin, Bio-Rad and Roche assays generated slightly different median logarithmically-transformed values, and differences above 0.5 log10 IU/mL were seen in 9%, 13% and 10% of the results, respectively.

### Discussion

The author has referenced some of his/her own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

The precision of both available automated assays has been thoroughly examined in a recent study that confirmed previous results but also demonstrated some greater variability (CV >10%) by Elecsys for QC material with a mean HBsAg level of 0.077 IU/mL and by Architect for the pooled serum with a mean HBsAg of 4,472.9 IU/mL.

Both assays have a sensitivity of 0.05 IU/mL, which is equivalent to 0.2 ng/mL of HBsAg or about 4 × 107 HBsAg particles/mL of serum. The linearity of the assays has been found to be excellent across HBsAg levels between 0.1 and 12,000 IU/mL with an r2 > 0.99. Although these tests have achieved an excellent level of sensitivity, negative sera should be also tested with qualitative tests with even higher sensitivity to determine HBsAg loss. Moreover, high titres of HBsAg that are usually seen in the early phase of HBsAg-positive chronic HBV infection need manual dilutions of the samples, increasing the probability of human error.
The correlation between the HBsAg logarithmically transformed measurements by these two assays has been studied and proven to be excellent \((r = 0.96)\)\(^2\). This high correlation was true for all genotypes \((r = 0.89–0.97)\), even in drug-resistant HBV YMDD strains \((r = 0.94)\), and values with differences above 0.5 \(\log_{10}\) IU/mL were seen in 6% of the tested samples\(^{21,22}\). Generally, the Elecsys assay has been shown to produce higher values than the Architect test, on average only 0.01 \(\log_{10}\) IU/mL higher than the Architect assay but with a range ±2 standard deviations – 0.55–0.52 \(\log_{10}\) IU/mL. Comparison studies between the two assays have also been made in HBV–HIV co-infected individuals with similar results\(^{21}\). In this population, the correlation between Elecsys and Architect was again significant \((r = 0.959)\), and the Elecsys assay produced on average 0.200 \(\log_{10}\) IU/mL higher results. This difference was consistent across sera of patients with different levels of CD4 cells and independent of the presence of precore and YMDD mutations or HBeAg status. A slightly larger mean difference was observed with genotypes A and G \((0.196 and 0.201 \log_{10} \text{IU/mL})\) versus HBV genotypes D and E \((0.036 and 0.030 \log_{10} \text{IU/mL})\). Mutations at position s120/s145 were the only ones where the Architect assay produced higher results than Elecsys \((\text{mean difference } 0.078 \log_{10} \text{IU/mL})\). Interestingly, in a recent study, HBsAg levels determined by the Elecsys assay were 0.8-fold lower than those measured by the Architect assay\(^{20}\). In this study, HBV genotypes were not known, but genotype C is known to be predominant in that area. These discrepant results when compared with other studies need to be further investigated with more extensive comparisons of the assays. In our laboratory, we have also observed lower values by Elecsys compared with Architect in a population of patients with HBeAg-negative CHB infection consisting predominantly of HBV genotype D (unpublished data).

Most of the published clinical studies have used the Architect analyser where HBsAg quantification was first available, and manual dilutions were performed in all sera with HBsAg values >250 IU/mL. Differences between the assays, when considered in the frame of monitoring and decision making depending on suggested HBsAg cut-off points, may be critical.

More so, in low HBsAg levels (<1,000 IU/mL), the correlation of the two assays is positive but poorer \((r^2 = 0.64)\) than in higher antigen titres. In 25 tested samples with HBsAg <1,000 IU/mL by Architect, the Elecsys test produced again consistently higher values \((\text{range } 68–2,755 \text{ IU/mL} \text{ by Elecsys HBsAg II vs. } 53–996 \text{ IU/mL} \text{ by Architect})\)\(^{22}\). Also, although both tests have been optimized for detecting HBsAg mutants, discrepant results have been reported when HBsAg mutants generated \textit{in vitro} were tested\(^{21,24}\). On one hand, mutant T123A seemed to be underquantified by the Architect system and on the other, the mutants P142S, P142L and G145K produced lower results using the Elecsys. In clinical samples, these mutations occur in a low percentage of the total HBV population, and differences in HBsAg measurements cannot really be attributed to them. Also, these discrepancies cannot be solely attributed to specific HBsAg mutations but also to the low concentrations of HBsAg that were measured in this study. In any case, the observed differences imply that the cut-off points of <1,000 IU/mL proposed with the use of Architect should be retested and evaluated with Elecsys and with other quantitative HBsAg assays in development.

Recent studies with HBsAg measurements by the Elecsys assay in clinical settings have produced results similar to those in previous studies in which HBsAg was measured by Architect\(^{21,25–27}\). The stopping rule for PEG-IFNa treatment proposed for patients with HBeAg-positive CHB has been based on the presence or absence of a decline in serum HBsAg levels from baseline at week 12 of treatment. This was re-evaluated with both assays in 181 patients of the original study\(^{21}\). The negative predictive values (NPVs) obtained were very similar but not identical (NPVs: 96% for the Elecsys vs. 98% for the Architect). These findings taken together with the already mentioned differences between the two assays clearly suggest that the same assay must be used in HBsAg monitoring of individual patients. In this context, the previously proposed stopping rule of IFN treatment in HBeAg-negative patients on the basis of HBsAg quantification on Architect\(^{26,29}\) has to be also evaluated with comparison studies between Architect and Elecsys.

For HBsAg quantification with modified qualitative assays, although good agreement \((\text{correlation coefficient } r = 0.88–0.93)\) and no proportional bias for the three assays compared with the reference was reported, the differences observed, between assays in Bland–Altman plots, suggested that the same assay should be used for each patient throughout monitoring. Moreover, all results were reported in logarithmic scale, an approach that could minimize the differences with the approved reference assay. It should also be noted that modified qualitative tests might be used clinically for HBsAg quantification only following appropriate quantitative standardization and approval.

Although HBsAg titres are associated with the stage in the natural history of chronic HBV infection\(^{12,30,31}\) \((\text{Figure } 3)\), discrepancies in the mean HBsAg values, in different geographical areas (i.e. Europe vs. Asia) in HBeAg-positive patients, have been observed and are attributed to the differences in distribution of HBV genotypes. In experimental models, HBsAg expression has been reported to be highest...
in HBV genotype A2, followed by A1 and B2, less for B1 and C and the least for D3. Moreover, HBV basal core promoter mutations A1762T/G1764A have been found, in chronically infected HBeAg-positive Chinese patients, to be associated with genotype C and low serum HBsAg levels33. These findings are of importance since with increased immigration rates, there are no more homogeneous HBV genotype-infected populations, at least in most areas of Europe where in the past there was predominance of few or even of only one HBV genotype. Thus, proposed cut-off points in monitoring and treating chronic HBV infection should be tested not only with all approved methods, but it may also be necessary to be adjusted according to the infecting HBV genotype.

Conclusion

Although the correlation between the currently approved methods for HBsAg quantification is quite good, the use of the same assay in the monitoring of individual patients with chronic HBV infection appears to be mandatory. If different assays are used interchangeably, then misleading differences in HBsAg levels may be obtained. The on-board dilution steps, now available for both commercial automated assays, minimize handling of the specimens, consequently reducing human errors. In any case, whenever HBsAg titre is measured, the method applied should be reported.

Abbreviations list

CHB, chronic hepatitis B; CMIA, chemiluminescence microparticle immunoassay; CV, coefficient of variation; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NPV, negative predictive value; ORF, open-reading frame; pgRNA, pregenomic RNA

References


