

Quantitation of hepatitis B surface antigen by an automated chemiluminescent microparticle immunoassay

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Abstract

A fully automated chemiluminescent microparticle immunoassay (Architect HBsAg QT) was used for the detection and quantitation of hepatitis B surface antigen (HBsAg). The assay is capable of processing up to 800 HBsAg tests per hour. The concentration of HBsAg is determined by utilizing a previously generated Architect HBsAg calibration curve. Architect HBsAg QT sensitivity was found to be around 0.2 ng/ml which is equivalent or superior to other known and commercially available enzyme immunoassays and/or chemiluminescent immunoassays. We performed a quantitative study of HBsAg, HBeAg, HBV-DNA and HBV-DNA polymerase in over 733 sera obtained from 43 chronic hepatitis B carriers. Serum HBsAg levels detected by Architect HBsAg QT were found to be higher in HBeAg-positive than in anti-HBe-positive HBV chronic carriers and correlated with the level of serum HBV-DNA and HBV-DNA polymerase.

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1. Introduction

Several solid-phase enzyme immunoassays have been used for the detection of HBsAg. The availability of Architect HBsAg QT (Deguchi *et al.*, 1999, 2000), a fully automated chemiluminescent microparticle immunoassay made it possible for us to quantitate large number of samples of HBsAg-positive sera obtained from hepatitis B virus (HBV) chronic carriers in Japan. Quantitative HBsAg in paired sera taken 1 month apart has been found to be reliable in differentiating acute from chronic HBV cases (Frosner *et al.*, 1982) and in managing chronic HBV patients (Zoulim *et al.*, 1992).

In this study, we quantitated HBsAg by the Architect HBsAg QT and compared this hepatitis serologic marker to

other HBV serum markers. Architect HBsAg QT was found to be a reliable, reproducible, sensitive and specific assay for HBsAg detection and quantitation.

2. Materials and methods

All of the 733 sera included in this study were serial bleeds obtained from 43 randomly selected HBV chronic carriers hospitalized in our institution (the tenets of the Declaration of Helsinki were observed and the study had the approval of our Internal Review Board). Sera were stored at -20°C until tested for a variety of HBV serum markers. All samples were tested and quantitated for HBsAg by Architect HBsAg QT (ABBOTT Japan Corp.) according to the manufacturer's Package Insert. Briefly, in the first step, sample and anti-HBs-coated paramagnetic microparticles are combined. HBsAg present in the sample binds to the anti-HBs-coated microparticles. After washing, acridinium-labeled anti-HBs conjugate is added in the

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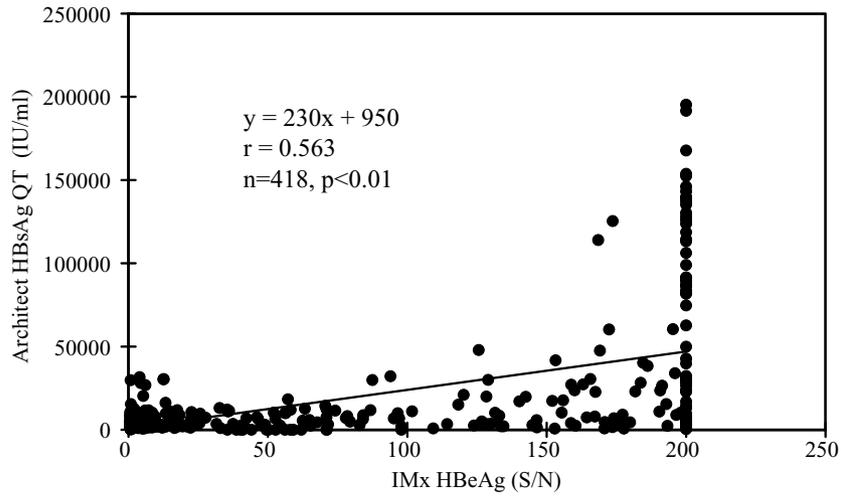


Fig. 1. Correlation of HBsAg and HBeAg.

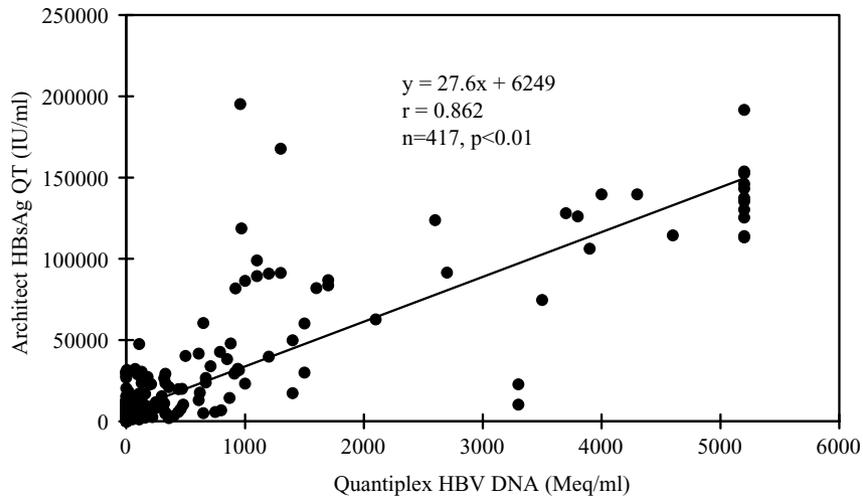


Fig. 2. Correlation of HBsAg and HBV-DNA.

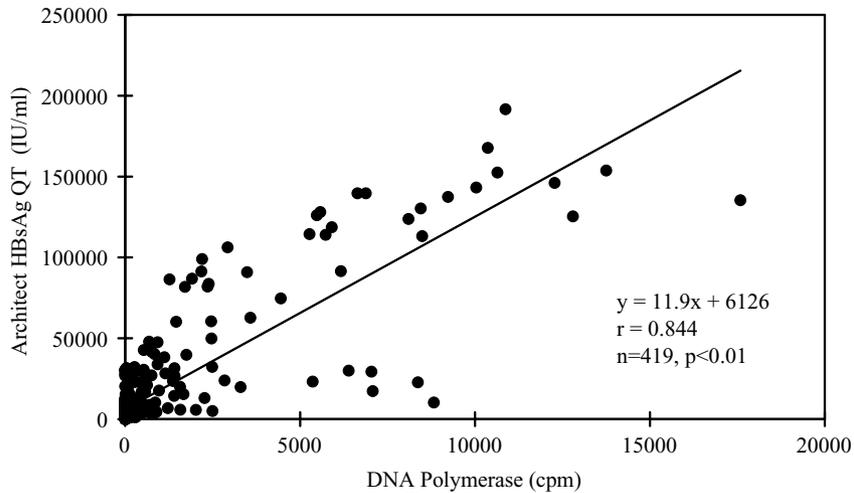


Fig. 3. Correlation of HBsAg and HBV-DNA polymerase.

second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured in relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by Architect HBsAg QT. The Architect AUSAB (ABBOTT Japan Corp.) was used for the quantitation of anti-HBs. IMx HBeAg DainaPack AX and IMx HBeAb DainaPack AX (ABBOTT Japan Corp.) were used for the quantitation of HBeAg and anti-HBe, respectively. Quantiplex HBV-DNA (Chiron Corp.) was used for HBV-DNA detection and quantitation. HBV-DNA polymerase assay was used as described (Lin et al., 1983). The quantitation methods were utilized as described previously (Zoulim et al., 1992). Statistical analysis was done by parametric methods and minimum χ^2 correlation. A P value less than 0.05 (two-tailed) was considered to be significant.

3. Results

3.1. Comparison of HBsAg and HBeAg concentration

A total of 418 serum samples from HBV chronic carriers were tested and quantitated for HBsAg and HBeAg. Fig. 1 shows the correlation between HBsAg concentration expressed as IU/ml and HBeAg concentration expressed as S/N. The concentration is expressed as $y = 230x + 950$ (where x is HBeAg S/N value and y is HBsAg concentration). The correlation coefficient, r is 0.563 ($n = 418$, $P < 0.01$).

3.2. Comparison of HBsAg and HBV-DNA concentration

A total 417 serum samples from HBV chronic carriers were tested for both HBsAg and HBV-DNA concentration. Fig. 2 shows the correlation between HBsAg level expressed as IU/ml and HBV-DNA concentration expressed as meq./ml. The correlation is expressed as $y = 27.6x + 6249$ (where x is HBV-DNA concentration and y is HBsAg concentration). The correlation coefficient, r is 0.862 ($n = 417$, $P < 0.01$).

3.3. Comparison of HBsAg and HBV-DNA polymerase concentration

A total of 419 samples obtained from chronic HBV carriers were tested for both HBsAg and HBV-DNA polymerase levels. Fig. 3 shows the correlation between HBsAg concentration expressed as IU/ml and the HBV-DNA polymerase expressed as counts per minute (cpm). The correlation is expressed as $y = 11.9x + 6126$ (where x is HBV-DNA polymerase level and y is HBsAg concentration). The correlation coefficient, r is 0.844 ($n = 419$, $P < 0.01$).

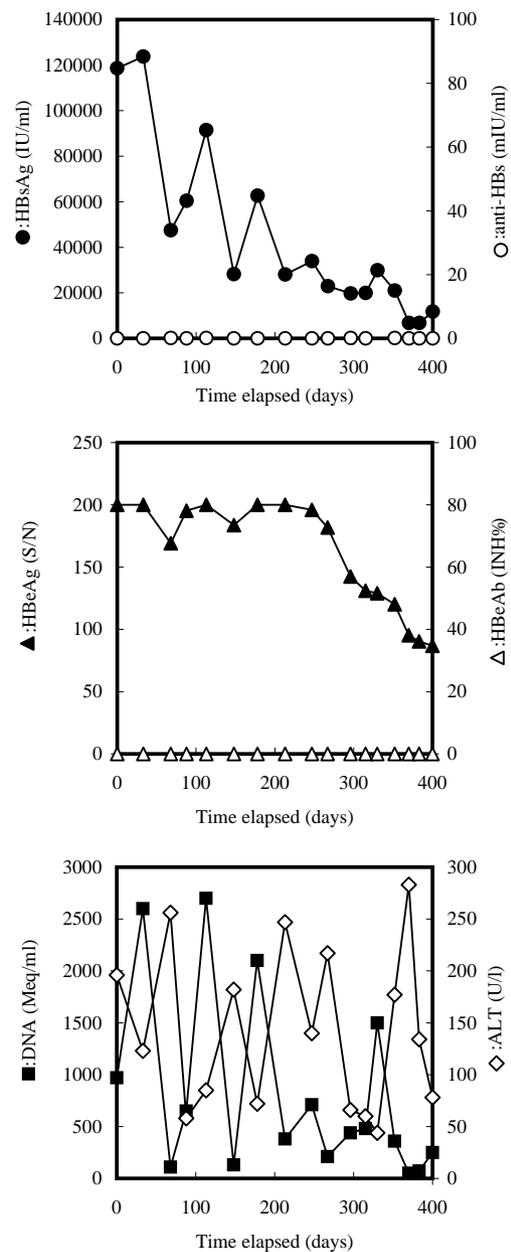


Fig. 4. Patterns of HBV serum markers during chronic HBV infection (profile 1).

3.4. HBV serum markers during chronic HBV infection

Analysis of the 43 chronic HBV carriers for markers of HBV infection revealed five serological profiles as shown in Figs. 4–8. All of the patients showed persistent HBs antigenemia. Some patients showed also either HBeAg (Fig. 4) or anti-HBe persistence (Fig. 5). Others showed persistent HBe antigenemia followed by seroconversion to anti-HBe (Figs. 6–8). Among these patients, some of them like this one (Fig. 8) showed a reverse seroconversion for anti-HBe positivity to HBeAg positivity followed by a seroconversion to anti-HBe. This is perhaps due to the reactivation of the disease. HBsAg and HBV-DNA flared up before the peak

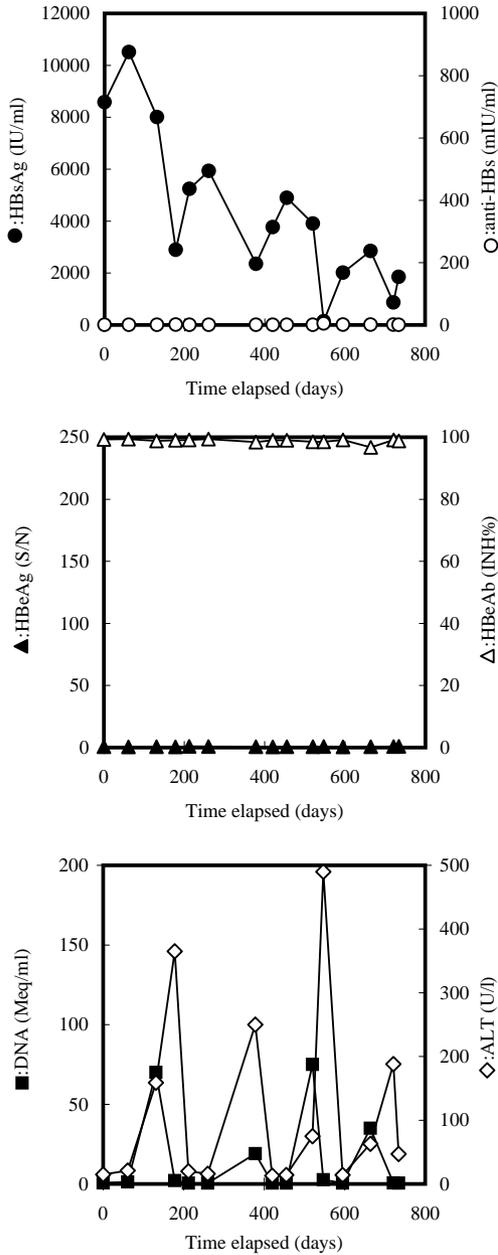


Fig. 5. Patterns of HBV serum markers during chronic HBV infection (profile 2).

of ALT elevation. Clinical changes of HBsAg concentration seemed to be same as the changes of HBeAg, HBV-DNA and HBV-DNA polymerase.

4. Discussion

Since HBsAg is produced in 10^4 excess over intact virions during replication, a detectability goal of 10^4 HBsAg particles/ml would be needed to approach a zero false negative assay. Over the past years, assays for the detection of HBsAg have been improved consistently to detect 0.1 ng HBsAg/ml of serum. This calculates to about 2×10^7 HBsAg particles/ml of serum. This is roughly equivalent

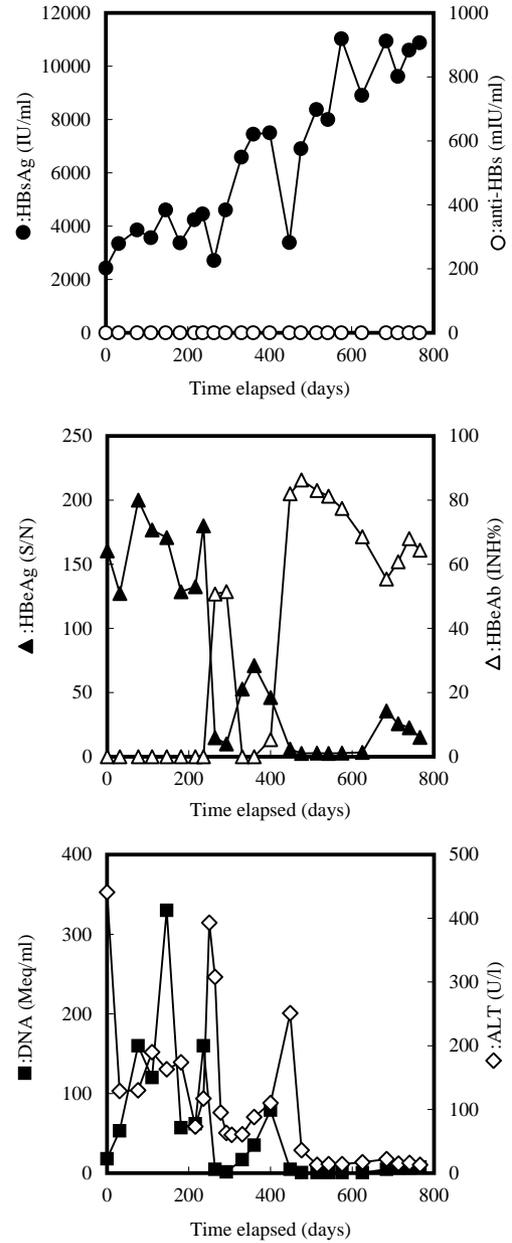


Fig. 6. Patterns of HBV serum markers during chronic HBV infection (profile 3).

to 2000 HBV/ml. Our quantitation of HBsAg utilizing Architect HBsAg QT is capable of detecting 0.05 IU/ml, which is equivalent to around 0.2 ng/ml of HBsAg. The quantitation of HBsAg by this instrument was based on a calibration curve that was standardized to the World Health Organization standard for HBsAg. It was found that the Architect HBsAg QT is capable of measuring a wide range of HBsAg concentration ranging from 0.05 to 250.0 IU/ml. Samples of HBsAg level of higher than 250 IU/ml require a 1:500 or greater dilution to bring them into the range of the calibration curve. The high sensitivity and specificity of the Architect HBsAg QT which includes a random access immunoassay analyzer together with its capability of

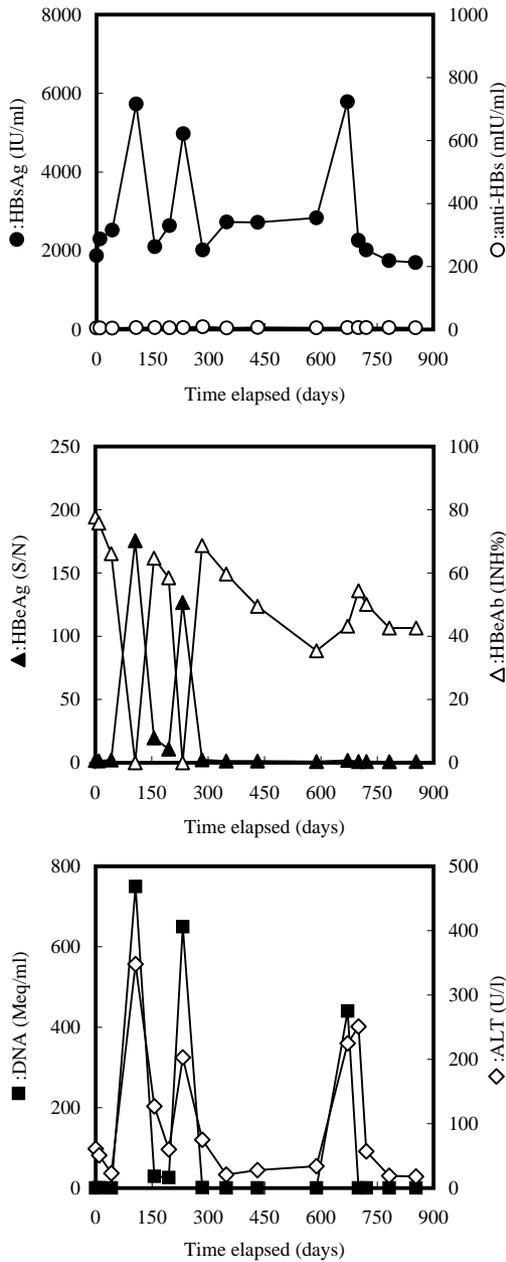


Fig. 7. Patterns of HBV serum markers during chronic HBV infection (profile 4).

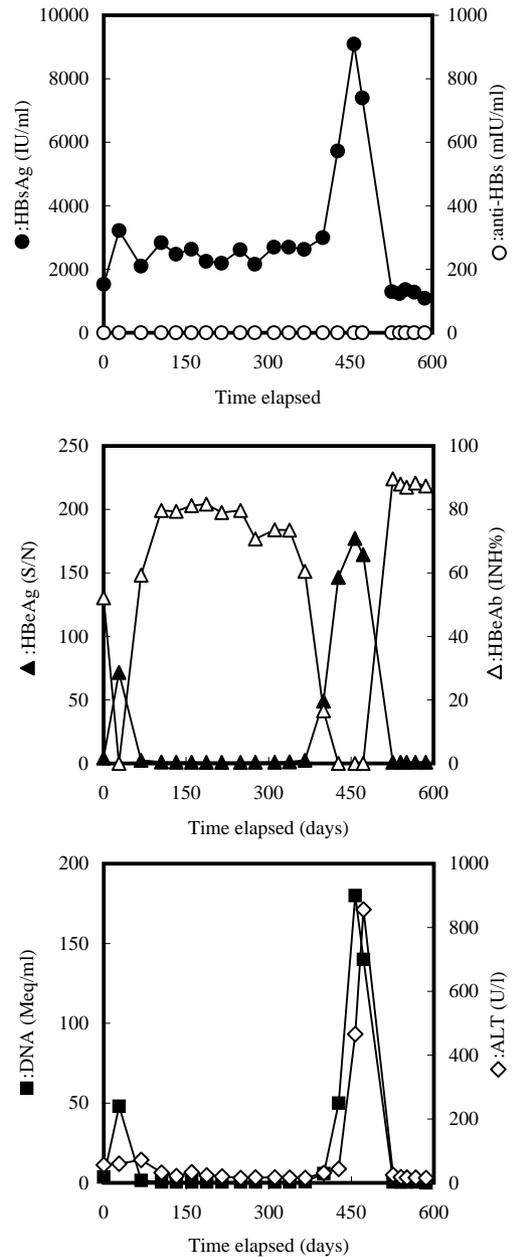


Fig. 8. Patterns of HBV serum markers during chronic HBV infection (profile 5).

detecting known HBsAg escape mutants (Coleman et al., 1999) makes it ideal for use in many diagnostic settings.

In this study, we compared also the results of the quantitation of HBsAg with HBeAg, HBV-DNA and HBV-DNA polymerase concentrations. As shown in Figs. 1–3, there was a positive correlation between these HBV serum markers and HBsAg concentration. No correlation was found between the length of persistence of HBeAg and the length of persistence of HBsAg and elevated ALT levels.

The majority of chronic HBV patients in our study can be classified into one of the three kinds of profiles shown in Figs. 4–8. About 98% (42 patients) of these chronic HBV

patients showed persistent HBs antigenemia with the serological profile illustrated in Figs. 4–7 and characterized by persistence of HBsAg and HBeAg. Our patients did not show circulating HBsAg and anti-HBs immune complexes. All of these patients were anti-HBs negative. Monitoring the changes of HBsAg concentration tells us the activities of HBV for the patients infected with HBV, as well as the HBeAg, HBV-DNA and HBV-DNA polymerase before ALT elevation. The unusual serological profile as shown in Fig. 8, is found frequently in HBV-infected homosexual men, illicit drug users and some hemophiliacs where frequent exposure and re-exposure to HBV is common (Mushahwar et al., 1981).

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