

## Original Article

# Reactivity of Genotypically Distinct Hepatitis B Virus Surface Antigens in 10 Commercial Diagnostic Kits Available in Japan

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**SUMMARY:** Hepatitis B virus (HBV) surface antigen (HBsAg) is one of the most important serological markers of current HBV infection. However, there are significant antigenic variations of HBsAg caused by genotypic diversity as well as mutation of the HBV genome. It is predictable that amino acid substitutions occurring in the HBsAg "a" determinant of a particular HBV genotype will affect the sensitivity of some diagnostic kits, since all the diagnostic kits currently available utilize monoclonal and/or polyclonal antibodies against the "a" determinant. One possible concern is that there may be a significant variation in the sensitivity of HBsAg diagnostic kits to HBsAg encoded by HBV of different genotypes, which might result in a failure to detect HBsAg of a particular HBV genotype. In this study, we assessed the reactivity of HBsAg specimens derived from three different HBV genotypes (A, B, and C) that are prevalent in Japan by 10 commercially available EIA (enzyme immunoassay), CLIA (chemiluminescent immunoassay), and CLEIA (chemiluminescent enzyme immunoassay) diagnostic kits. Specimens included both clinical samples and recombinant HBsAg. Our results showed that all the diagnostic kits evaluated were able to detect HBsAg irrespective of HBV genotypes. At the same time, it is apparent that some, but not all of the kits showed clear differences in sensitivity to the three HBV genotypes.

## INTRODUCTION

Antigenic variation of the hepatitis B virus (HBV) surface antigen (HBsAg) "a" determinant should be taken into consideration when a diagnostic kit with high specificity/sensitivity is designed. Since all the diagnostic kits for detection of HBsAg utilize an antibody against the major epitope, i.e., the "a" determinant, amino acid substitution in this region would be accountable for diagnostic failures. There are three major causes of variations in HBsAg: differences in subtype, differences in genotype, and mutations. HBsAg is classified into four major serological subtypes, i.e., adr, adw, ayr, and ayw (1,2). The diagnostic kits currently available are able to detect all of them with only a slight sensitivity variation. There is, however, a significant variation in the sensitivity of diagnostic kits for detection of naturally occurring or vaccine-inducing mutants mainly in the "a" determinant region (3). Therefore, when designing diagnostic kits, if we employ a monoclonal antibody (mAb) that recognizes amino acid residues but is subject to mutation, the result will be a failure to detect the mutant HBsAg. For performing sensitive and accurate blood donor screenings, we need a robust assay system that does not overlook any positive specimens. Since the incidence of HBV mutation in specimens from the general population is rather low, this may not be a major problem. In contrast, genotypic variation in HBV could become a serious problem if some diagnostic kits fail to detect HBsAg of particular genotypes.

Based on an intergroup divergence of 8% or more in the complete nucleotide sequence, HBV has been classified into eight genotypes, designated as A to H (4-7). The prevalence of specific genotypes varies geographically (8, 9). Genotypes B and C are prevalent in Japan, and only a small population contracts HBV of genotype A (9,10). Recently, however, there has been an increase in the number of acute hepatitis B patients infected with HBV of genotype A, especially in metropolitan areas in Japan (11). And there is an accumulating body of evidence that certain HBV genotypes correlate with the severity of liver disease (12-14) and with the susceptibility to anti-viral drugs (15-18). It is thus important to detect HBsAg derived from various HBV genotypes without a sensitivity divergence. To date, there has been no published study examining whether or not commercially available HBsAg diagnostic kits are able to detect genotypically distinct HBsAg - e.g., HBsAg derived from HBV genotypes A, B, and C - with equal efficacy. The lack of such information threatens to undermine our confidence in the reliability of these diagnostic kits. Accordingly, the objective of the present study was to compare the sensitivity of 10 diagnostic kits available in Japan to serum/plasma samples containing HBsAg as well as recombinant HBsAg derived from HBV of genotypes A, B, and C. None of the diagnostic kits examined here failed to detect HBsAg of genotypes A, B, and C at a concentration of 0.2 IU (international units)/ml. There were, however, obvious differences in the sensitivity to various HBV genotypes in some kits. Possible explanations for the reactivity differences found in genotypically distinct HBsAg in some diagnostic kits will be discussed.

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## MATERIALS AND METHODS

**Specimens:** Serum samples from HBsAg-positive blood

donors were kindly provided by Hokkaido Red Cross Blood Center. They were genotyped by direct sequencing and found to be of either genotype B or C. Genotype A HBsAg-positive plasma samples were obtained from International Reagents Corporation (Kobe, Japan). Recombinant HBsAg of genotypes A, B, and C were obtained from culture supernatants of genotype-defined HBV gene-transfected HuH-7 cells. In brief, extracted HBV-DNA from HBsAg-positive plasma was amplified by using a TaKaRa LA PCR™ Kit (ver.2.1; TaKaRa, Tokyo, Japan) with sense primer **GGCTCTTCTTTTCA CCTCTGCCTAATCA** (1821-1841) and antisense primer **GGCTCTTCAAAAAGTTGCATGGTGCTGG** (1825-1806), both of which were modified by Günther et al. (19). SapI sites sequences (bold letters) were added to remove HBV DNA from vector. The amplified HBV full genomes were cloned using a TOPO XL-PCR cloning kit (Invitrogen, Carlsbad, Calif., USA) and their genotypes were determined by sequencing. The plasmids containing HBV full genomes were transfected into HuH-7 by lipofectin reagent (Invitrogen). Culture supernatants were harvested after 2 days and stored at -20°C until use.

**Diagnostic kits for HBsAg:** The 10 diagnostic kits utilized in this study are listed in Table 1. Tests were performed according to the manufacturer's instruction.

**Statistical analysis:** Statistical analyses were carried out using Student's *t* test.

## RESULTS

The HBsAg concentration of each specimen was first determined by utilizing ARCHITECT HBsAg QT (Abbott Japan Co., Ltd., Chiba, Japan), which is the only quantitative assay kit approved in Japan, and expressed in IU/ml. The concentration of each specimen was adjusted to 10 IU/ml with a multi-marker negative matrix (Accurun 810; BBI Co., Ltd., Boston, Mass., USA). Specimens were then diluted to make test samples of three different concentrations, i.e., 0.04, 0.2, and 1.0 IU/ml. These test samples derived from various HBV genotypes were then analyzed with 10 diagnostic kits, including ARCHITECT HBsAg QT, as shown in Table 1. In the preliminary experiments, each specimen was analyzed by 10 different diagnostic kits and the reactivity, which was expressed as IU/ml, COI (cut off index), or other measures depending on the kit utilized, was plotted along with the arbitrary HBsAg concentration pre-determined by ARCHITECT HBsAg QT. The measures were intentionally omitted

from the y-axes of Fig. 1 so that the kits could not be identified. Each curve showed a good linearity, at least in the range from 0.04 IU/ml to 1.0 IU/ml of HBsAg (data not shown). Therefore, values corresponding to the concentration of 0.2 IU/ml HBsAg were chosen and plotted for each genotype as shown in Fig. 1. It was concluded that all the HBsAg samples, irrespective of their genotypes, tested positive in every assay kit, at least at the concentration of 0.2 IU/ml. There were, however, considerable differences in the sensitivity to HBsAg of various HBV genotypes in some assay kits. For example, in kit no. 7, sensitivity to genotype B was significantly lower than those to genotypes A and C. Figure 2 summarizes the difference in sensitivity to various genotypes in each assay kit. Assay kits no. 1 to 4 and 8 to 10 showed a marginal variability in sensitivity to the HBsAg of the three different genotypes as the ratio against genotype A was close to 1.0 in each of these kits. In contrast, assay kits no. 5 to 7 showed a considerable variability in sensitivity to each genotype.

## DISCUSSION

In the present study, 10 highly sensitive diagnostic kits (EIA [enzyme immunoassay], CLIA [chemiluminescent immunoassay], and CLEIA [chemiluminescent enzyme immunoassay] kits) currently available in Japan were examined for their sensitivity to HBsAg encoded by HBV of three distinct genotypes, A, B, and C. It was concluded that all the kits examined were able to detect HBsAg of all the genotypes at the concentration of 0.2 IU/ml. This is a sufficient level of sensitivity according to the "Guidance for Industry" issued by the FDA (20) or the "CTS" (Common Technical Specification) defined by the EU (21). Our results thus validated the sensitivity of all the diagnostic kits to HBsAg of genotypes A, B, and C, which are dominant in Japan. However, it was concurrently demonstrated that some diagnostic kits showed a substantial difference in sensitivity to the three genotypes (Figs. 1 and 2, kit no. 5, 6, and 7). This apparent sensitivity difference may stem from the antibodies employed in the "capture" or "detection" phase in the diagnostic kits. As shown in Table 1, kits no. 1 to 4 employed a mAb for the "capture" phase and a polyclonal antibody (pAb) for the "detection" phase. These four kits showed little or no sensitivity differences to the three genotypes (Figs. 1 and 2). A similar result was obtained for kit no. 8, which employed a pAb for "capture" and a mAb for "detection". In the case of kit no. 7, however, there was a noticeable difference in sensitivity to the three genotypes. As shown in Table 1, kit no. 7 employed a mAb for both "capture" and "detection". Both of these mAbs may have had a poor affinity to the amino acid residues unique to genotype B. On the other hand, kits no. 5, 6, and 7 had a higher sensitivity to genotype C than to the other genotypes (Figs. 1 and 2), probably because the antibodies employed in these kits have a high affinity to the amino acid residues unique to genotype C. Kits no. 9 and 10 showed only a slight difference in sensitivity to the three genotypes (Figs. 1 and 2), despite the fact that both kits employed a mAb for both the "capture" and "detection" phases. The manufacturer's unpublished information revealed that they employed two mAbs with different epitope specificities. One of the mAbs recognizes the "loop 1" region (a.a. 124-137) of the "a" determinant, whereas the other recognizes the "loop 2" region (a.a. 139-147). Since the "loop 1" region is assumed to be more conserved among various genotypes than the

Table 1. HBsAg diagnostic kits used in this study

No.	Method	Antibody (capture/detection)
1	CLIA	monoclonal/polyclonal
2	EIA	monoclonal/polyclonal
3	CLIA	monoclonal/polyclonal
4	EIA	monoclonal/polyclonal
5	EIA	monoclonal/monoclonal( $\times 2$ ) <sup>1)</sup>
6	CLEIA	polyclonal/monoclonal( $\times 2$ ) <sup>1)</sup>
7	CLEIA	monoclonal/monoclonal( $\times 2$ ) <sup>1)</sup>
8	CLEIA	polyclonal/monoclonal
9	CLIA	monoclonal/monoclonal
10	CLIA	monoclonal/monoclonal

CLIA: chemiluminescent immunoassay.

EIA: enzyme immunoassay.

CLEIA: chemiluminescent enzyme immunoassay.

<sup>1)</sup>( $\times 2$ ): Two different monoclonal antibodies.

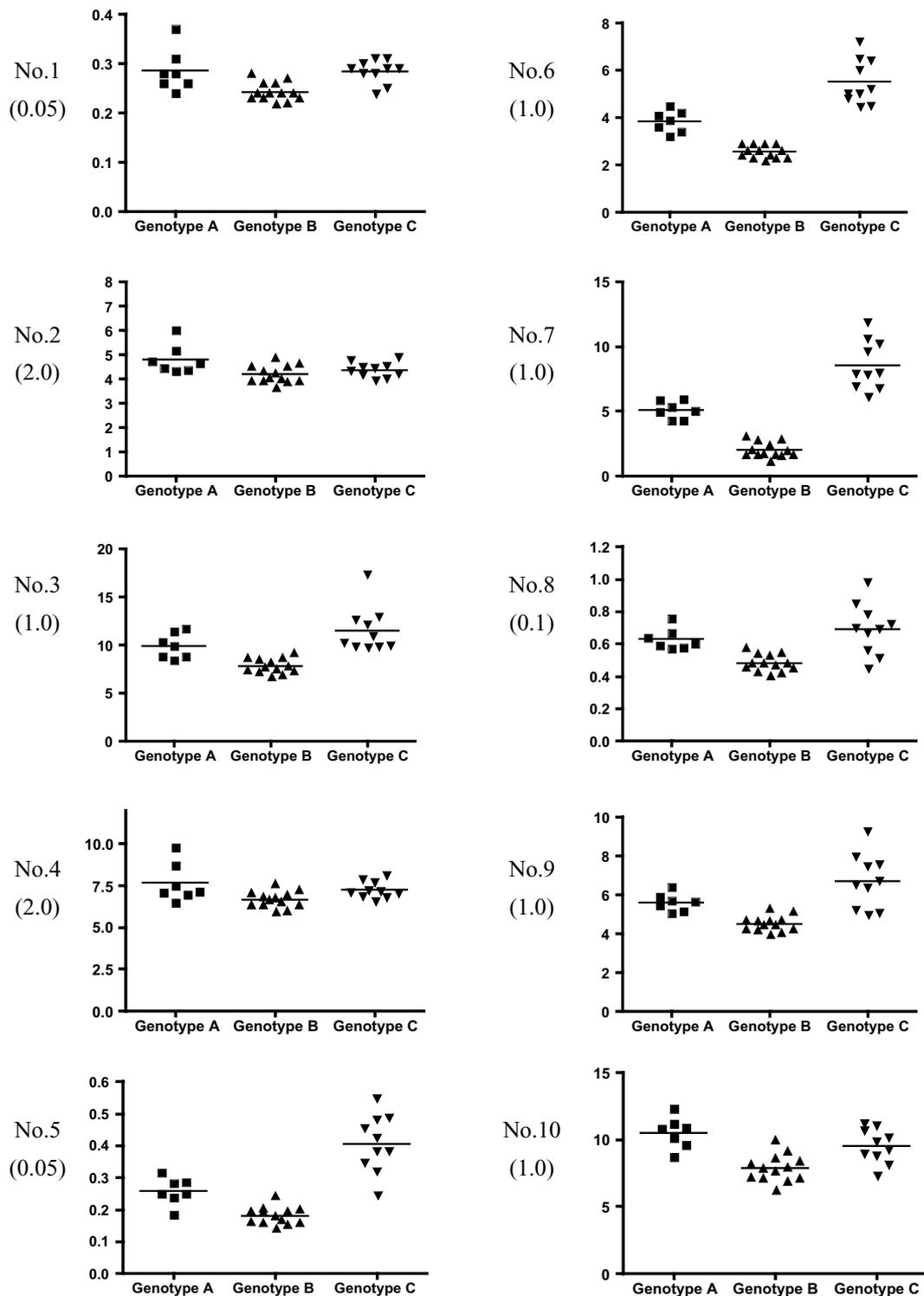


Fig. 1. HBV genotypic preferences of each test kit. Detection of HBsAg derived from HBV of genotypes A, B, and C. A total of 30 specimens containing HBsAg (0.2 IU/ml), including 7 samples of genotype A (■), 13 samples of genotype B (▲), and 10 samples of genotype C (▼), were assayed by utilizing 10 diagnostic kits listed in Table 1. Horizontal bars indicate the means of measured values. The number in the parenthesis is the cut-off value for each diagnostic kit to evaluate the reactivity of a specimen. Statistically significant ( $P < 0.1$ ) differences in the mean measures between two genotypes were noticed in the following kits. No. 5: B vs. C. No. 6: A vs. B and B vs. C. No. 7: A vs. B, B vs. C, and C vs. A.

“loop 2” region, kits no. 9 and 10 were able to detect all the genotypically distinct HBsAg with only minimal divergence.

These results are reminiscent of the previously published studies that pointed out the failure of some diagnostic kits in detecting mutant HBsAg (3,22-25). In those studies, monoclonal-based assays often failed to detect mutant

HBsAg, such as the G145R mutation in the “a” determinant region. It was also suggested that mutations affecting immunoassay performance occurred mainly in the “loop 2” region (3).

In conclusion, all the diagnostic kits examined in this study were able to detect HBsAg regardless of their HBV

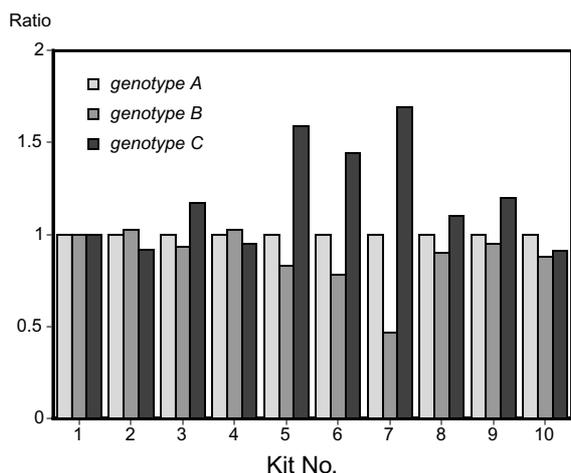


Fig. 2. Comparison among various HBsAg diagnostic kits. Variation in sensitivity of 10 diagnostic kits to HBsAg of genotypes A, B, and C. The mean value obtained by each diagnostic kit was divided by the corresponding value obtained by ARCHITECT HBsAg QT to normalize it. Then the calculated value for genotype B specimen was divided by the calculated value for genotype A to obtain the ratio of "genotype B vs. genotype A". In the same way, the ratio of "genotype C vs. genotype A" was calculated. For each diagnostic kit, therefore, the ratio for genotype A is always "1" and the ratios for genotypes B and C are expressed as the bar's height. Since we utilized ARCHITECT HBsAg QT as a tentative standard, the ratios of this kit are theoretically "1". If the ratio is close to "1", the variation in sensitivity to genotype B (or genotype C) would be minimum, whereas, if not, the variation would be substantial.

genotypes. In some kits, however, sensitivity was significantly diversified among the three HBV genotypes. When mAbs are utilized for both the "capture" and "detection" phases, it is recommended that at least one antibody recognizes an epitope that is conserved among HBV genotypes.

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