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ORIGINAL ARTICLE

Hepatitis B virus genotyping by enzyme-linked immunosorbent assay in Taiwan

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Abstract
Purpose Restriction fragment length polymorphism (RFLP) and enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies (mAbs) were used in this study to detect genotypes of HBV, and the efficiency and precision of ELISA using the mAbs for HBV genotype detection were also estimated.
Methods The ELISA with mAbs method was used for the detection of HBV genotype in a Taiwanese population. The HBV genotypes of 100 chronic hepatitis B patients were determined by ELISA and were then compared with those obtained using RFLP.
Results Genotype B was found to be the most prevalent in this study (63% by RFLP; 62% by ELISA) followed by genotype C (31% by RFLP; 35% by ELISA). There was no significant difference between the results obtained by RFLP and ELISA ($P = 0.75$). The ELISA overall genotypeable rate, the correct genotyping rate from genotypeable specimens, and the concordance of the HBV genotyping assay was 96.00, 94.79, and 91.00%; for the ELISA HBV genotyping assay for genotype B specimens was 96.77, 100.00, and 96.77%; and for genotype C specimens was 97.14, 91.18, and 88.57%, respectively. The mean HBV DNA level was higher in the specimens that could be genotyped by both RFLP and ELISA samples (6.24 ± 1.77 vs. 2.34 ± 0.90, log IU/ml), and a significant difference in terms of HBV DNA level of more than 2×10^3 IU/ml was identified between the genotyped RFLP samples ($P < 0.001$).
Conclusions ELISA is a practical and a useful method for HBV genotyping in a clinical setting in Taiwan, in particular for patients with lower levels of HBV DNA.

Keywords Hepatitis B virus · Genotype · ELISA · RFLP

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Background and purpose of the report

Hepatitis B virus (HBV) infection is a serious health problem worldwide: about 350 million people are suffering from chronic HBV infection [1], and approximately 15–40% of HBV patients will develop cirrhosis, chronic liver disease, hepatic failure, or hepatocellular carcinoma (HCC) [2–5].

HBV is a 3.2 kb, partially double-stranded deoxyribonucleic acid (DNA) virus of the class Hepadnaviridae. HBV has four open reading frames: core (C), surface (S), X, and polymerase (P). Couroucé-Pauty et al. [6] identified a variety of w-subtypic determinants, which they designated w1, w2, w3, and w4. These various serotypes of HBV according to different subtypes of HBsAg, have been found to be related to HBV genotype [7, 8]; e.g., serotypes adr and ayr are related to genotype C, ayw2 and ayw3 to genotype D, ayw4 to genotype E, and adw4 to genotype F.

HBV has more recently been classified into eight genotypes, A–H, based on a divergence over the entire genomic sequence of >8% [7, 9]. HBV genotypes I [10] and J [11] have been discussed in brief in previous studies, but these two genotypes need to be further investigated. Different serological genotypes of HBsAg have distinct geographic distributions: genotypes A and B are frequent in Europe and north, and central Africa; genotype C is dominant in Asia; genotype D is found in India; genotype E is found in west Africa; genotype F is present in Polynesia; and genotypes G and H are dominant in France, Germany, and Mexico [12, 13]. In Taiwan, genotypes B and C are the most and second most prevalent, respectively (B 68%, C 32%) [14]. In addition, different genotypes of HBV exhibit specific clinical progression, presence, outcome, and response characteristics anti-virus therapy. Genotypes A and B have a better therapeutic response to interferon therapy than genotypes C and D, but there is no difference in response to adefovir dipivoxil among the different HBV genotypes [15–19]. In previous studies, genotype C has been identified as being usually associated with more severe liver disease [20], and another study showed that, different genotypes of HBV give rise to different rates of development of HCC [14]. Therefore, detection of HBV genotype is very important in clinical treatment and management.

Nowadays, many genotyping methods developed for HBV depend on nucleotide sequencing, such as the direct sequence method, restriction fragment length polymorphism (RFLP) [21], the type-specific primers method [22, 23], etc. Although the direct sequence method is the most direct and precise technique for confirming HBV genotype, it is costly in terms of time and money. The type-specific primers method takes less time, but is not as precise, and the cost of RFLP is also high in terms of time and money. These methods are also not suitable for large-scale surveys. To solve these problems, the ELISA with monoclonal antibodies (mAbs) method for the detection of HBV genotype was developed by Usuda et al. [24], a technique that uses mAbs b, m, k, s, and u against specific epitopes of pre-S2 region products in order to identify HBV genotypes A, B, C, D or E, and F [24, 25]. The advantages of this method are the short time required, the low cost, and its suitability for large-scale surveys.

Yu et al. [26] used an immunoblot ELISA method to determine the reactivities of antibodies to serotype-specific epitopes of HCV, and reported that, it was a useful method for HCV typing in a clinical setting in Taiwan. Tanaka et al. [27] also reported that HBV genotyping by ELISA is reliable, sensitive and simple, and that this method would be useful for clinical use. In this study, we used RFLP and ELISA with mAbs to detect genotypes of HBV, and also to estimate the efficiency and precision of the ELISA with mAbs method for HBV genotype detection in Taiwan. We also explored the relationship between average HBV DNA level, genotyping results, and sensitivity, specificity, concordance, obtained by RFLP and ELISA.

Materials and methods

Patients and sera collection

We collected sera from 100 symptom-free chronic hepatitis B patients in Chung-Ho Memorial Hospital, Kaohsiung Medical University, whose HBsAg had been persistently positive for at least 6 months, and whose HBV DNA was also positive. All of the sera were preserved in a freezer at −20°C.

Hepatitis virus markers

The HBsAg and HbeAg levels of all 100 sera samples were ascertained using Ausria-II and IMx HBe 2.0 (Abbott Laboratories, North Chicago, IL, USA), respectively. Anti-HCV and anti-hepatitis D virus testing were performed using commercially available assays (HCV EIA II and Anti-Delta; Abbott Laboratories).

Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)

One hundred samples of HBV DNA were extracted from HBV present in patients' sera using a Qiagen Kit (Qiagen, Hilden, Germany), then processed by the semi-Nested PCR reaction [23]. We used 25 pmol/μl of primers RF3 (5′-YCCTGCTGGTGGCTCCAGTTC-3′) and RFIA (5′-AAG CCANACARTGGGGGAAAGC-3′) for the first PCR reaction for 25 cycles (96°C, 2 min; 94°C, 15 s; 60°C, 45 s; and 72°C, 45 s), followed by 7 min at 72°C, which was carried out on an Applied Biosystems 9700 machine (Applied Biosystems, Foster City, CA, USA). We subsequently used 25 pmol/μl of primer RFIA and primer RFIS (5′-GTCTAGACTCGTGGTGGACTTCTCTC-3′) for the second PCR reaction, following the same procedure. The three primers used are fragments of the S gene of HBV DNA. Finally, a 485 bp PCR product was obtained on 3% agarose gel. The specific PCR products were digested by the restriction enzymes *Alw*I, *Ear*I, *Nci*I, *Hph*I, and *Nla*IV (New England BioLabs, Ipswich, MA, USA). In this study, we used the digestive enzyme *Alw*I in the first step, and if the PCR product could be cut, we identified it as genotype C; if the PCR product could not be cut by *Alw*I, we used *Ear*I in the second step to identify whether this product was of genotype B; similarly, we used *Hph*I and *Nci*I in the third step to identify genotype E (379 bp) or F (438 or

485 bp), and *Nla*IV in the fourth step to identify genotype A (219 or 220 bp) or D (186 bp) [23]. The length of the band expected for RFLP at 37°C after 3 h was found in order to classify the different genotypes of HBV.

Enzyme-linked immunosorbent assay (ELISA)

Sera samples obtained from all 100 patients were diluted 5-fold in sodium phosphate buffer (20 mM, pH 7.2) containing 130 mM NaCl and 0.01% NaN_3,[24] and 50 µl diluted sera were placed in 4 of 96 wells immunoplated with a coating with anti-HBs (Institute Immunology Co. Ltd, Tokyo, Japan), then covered with a membrane at room temperature. The plate was shaken for 2 h at 450 times per min, and the solution in the wells was then discarded.

The wells were washed five times with 150 mM NaCl containing 0.05% Tween 20, and then four wells were each filled with 2.5 µg/ml of the different mAbs, m, u, s, and k, which were labeled with horseradish peroxidase (Institute of Immunology Co. Ltd, Tokyo, Japan). The plate was then covered with a membrane at room temperature, shaken for 2 h at 450 times per min, then washed five times with 150 mM NaCl containing 0.05% Tween 20. At room temperature, and avoiding direct illumination of samples, the solution in each well was reacted with H_2O_2 and tetramethylbenthizine for 30 min, after which, a reaction stopper was added to each well. After shaking the immunoplate until the blue solution turned yellow, the plate was subjected to ELISA (Stat Fax 2100, Awareness) at 450 nm, and the different genotypes classified by EIA using a HBV Genotype EIA kit (code 1A64, Institute of Immunology Co. Ltd, Tokyo, Japan).

Quantitation of HBV DNA

We used the COBAS AmpliPrep/COBAS TaqMan HBV Test (Roche Diagnostics, Laval, QC, Canada), a nucleic acid amplification test, with automated real-time PCR assay for the quantitation of HBV DNA for all 100 samples. The primer used for the quantitation of HBV DNA was a fragment of the S gene of HBV DNA. The lower detection limit of quantification was 54 IU/ml and of qualification was 12 IU/ml.

Sequencing of HBV DNA

We used 310 Genetic Analyzer Polymers (Applied biosystems) to sequence HBV DNA, with PCR elongation based on the Sanger dideoxy nucleotide triphosphate (ddNTP) terminator method [28], from eight samples for which the results of genotyping by RFLP and ELISA were not in agreement.

Statistical analysis

The HVB DNA levels were analyzed by descriptive statistics, such as the mean, standard deviation, maximum and minimum, and the means were compared using the Mann–Whitney *U* test. Differences between proportions were analyzed using the Chi-square test, and the results were considered significant if the *P* value was ≤ 0.05. The genotypeable rate for ELISA was defined as the number of genotypeable specimens/total number of specimens; the correct genotyping rate from genotypeable specimens for ELISA was defined as the number of correct genotypes/total number of genotypeable specimens; and concordance was defined as the number of correct genotypes/total number of specimens. Analysis was performed using the SPSS statistical package (SPSS Inc., Chicago, IL, USA).

Results

The demographic characteristics of the 100 hepatitis B virus patients included in the study are listed in Table 1. The average age of the patients was 39.17 ± 12.92 years (range 16–74 years), and males were dominant (81 males; 19 females). The average levels of AST, ALT, and HBV DNA (using logs) were 149.23 ± 268.54 IU/ml (range 15–1487 IU/ml), 258.13 ± 429.59 IU/ml (range 15–2193 IU/ml), and 6.08 ± 1.90 IU/ml (range 1.38–8.74 IU/ml), respectively. The HbeAg and anti-HBe positive rates were 45% and 52%, respectively. Hepatitis C and hepatitis D were not detected in these patients (not shown in table).

A total of 100 hepatitis B specimens were genotyped by RFLP and ELISA. Genotype B was found to be the most prevalent in our study (63 specimens, 63% by RFLP; 62 specimens, 62% by ELISA), followed by genotype C (31 specimens, 31% by RFLP; 35 specimens, 35% by ELISA). Four (4%) HBV specimens could not be genotyped by RFLP and two (2%) by ELISA (Table 2). There were no significant differences in the results obtained by RFLP and

Table 1 Basic demographic characteristics of the 100 chronic hepatitis B patients included in the study

Age[a]	39.17 ± 12.92	16–74[b]
Gender (male/female, *n* (%))	81/19 (81.0/19.0)	
AST (IU/l)[a]	149.23 ± 268.54	15–1487[b]
ALT (IU/l)[a]	258.13 ± 429.59	15–2193[b]
HBV DNA[a] (log, IU/ml)	6.08 ± 1.90	1.38–8.74
HBeAg (positive/negative, *n* (%))	45/55 (45/55)	
Anti-HBe (positive/negative, *n* (%))	58/42 (58/42)	

[a] Mean ± standard deviation

[b] Range, no anti-HCV or -HDV markers were detected in any of the 100 patients

ELISA ($P = 0.75$) (Table 2). The overall genotypeable rate for ELISA, the correct genotyping rate from genotypeable specimens for ELISA, and the concordance of the ELISA HBV genotyping assay was 96.00, 94.79, and 91.00%; for the ELISA HBV genotyping assay for genotype B specimens was 96.77, 100.00, and 96.77%; and for genotype C specimens was 97.14, 91.18, and 88.57%, respectively (Table 2).

Table 3 shows the relationship between the HBV DNA levels of each RFLP/ELISA HBV genotyped group. The mean HBV DNA level of the specimens genotypeable by RFLP was higher than that of the specimens non-genotypeable by RFLP (6.24 ± 1.77 vs. 2.34 ± 0.90, log IU/ml). In addition, the lowest HBV DNA level was 1.78 log HBV DNA (IU/ml) in the group genotypeable by RFLP, and the highest was 3.17 log in the specimens non-genotypeable by RFLP.

Table 4 shows the relationship between the HBV DNA levels of the samples genotyped by RFLP and ELISA, from which it can be seen that there was a significant difference between the HBV DNA levels with cut-off value of 2×10^3 IU/ml and whether or not the sample could be genotyped by RFLP ($P < 0.001$), and all samples with an HBV DNA level greater than 2×10^3 IU/ml could be genotyped by RFLP in this study, but there was no significant difference between specimens that could and could not be genotyped by ELISA in terms of HBV DNA level ($P = 0.19$).

Table 2 ELISA genotyping results for the 100 genotyped specimens (*n* (%))

Genotype	No. of specimens in each RFLP HBV serotype						Genotypeable rate for ELISA (%)	Correct genotyping rate from genotypeable specimens for ELISA (%)	Concordance (%)
ELISA	A	B	C	F	N[a]	Total	(95% CI)[b]	(95% CI)[c]	(95% CI)[d]
A	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	–	–	–
B	0 (0.0)	60 (60.0)	0 (0.0)	0 (0.0)	2 (2.0)	62 (62.0)	96.77 (87.83–99.44)	100 (92.50–100.00)	96.77 (87.83–99.44)
C	1 (1.0)	1 (1.0)	31 (31.0)	1 (1.0)	1 (1.0)	35 (35.0)	97.14 (83.38–99.85)	91.18 (75.19–97.69)	88.57 (72.32–96.27)
D or E	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	100.00 (5.46–100.00)	–	–
N[a]	0 (0.0)	1 (1.0)	0 (0.0)	0 (0.0)	1 (1.0)	2 (2.0)	50.00 (2.68–97.33)	–	–
Total	1 (1.0)	63 (63.0)	31 (31.0)	1 (1.0)	4 (4.0)	100 (100.0)	96.00 (89.49–98.71)	94.79 (87.70–98.07)	91.00 (83.17–95.54)

Chi-square test for RFLP and ELISA $P = 0.75$

RFLP restriction fragment length polymorphism, *ELISA*, enzyme-Linked ImmunoSorbent Assay, *CI* confidence interval

[a] *N* could not be genotyped

[b] Genotypeable rate for ELISA (number of genotypeable specimens/total number of specimen)

[c] Correct genotyping rate from genotypeable specimens for ELISA (number of correct genotypes/total number of genotypeable specimens)

[d] Concordance (number of correct genotypes/total number of specimens)

Table 3 HBV DNA level of each RFLP/ELISA HBV genotyped group (log IU/ml)

	RFLP/ELISA	Number	Mean	Standard deviation	Minimum	Maximum
Samples genotypeable by RFLP	A/C	1	6.29			
	B/B	60	6.12	1.90	1.78	8.74
	B/C	1	7.25			
	B/D or E	1	5.10			
	B/N	1	6.98			
	C/C	31	6.45	1.63	2.14	8.38
	F/C	1	5.77			
Sum		96	6.24	1.77	1.78	8.74
Samples non-genotypeable by RFLP	N/B	2	3.10	0.09	3.06	3.17
	N/C	1	1.38			
	N/N	1	1.77			
Sum		4	2.34	0.90	1.38	3.17
Total		100	6.08	1.90	1.38	8.74

RFLP restriction fragment length polymorphism, *ELISA* enzyme-linked immunosorbent assay

Table 4 Relationship between HBV DNA level and the samples genotyped by RFLP and ELISA (n (%))

	RFLP			ELISA		
	Could not be genotyped	Could be genotyped	*P*	Could not be genotyped	Could be genotyped	*P*
HBV DNA $\leq 2 \times 10^3$ IU/ml	4 (100.0)	12 (12.5)	<0.001	1 (50.0)	15 (15.3)	0.19
HBV DNA $>2 \times 10^3$ IU/ml	0 (0.0)	84 (87.5)		1 (50.0)	83 (84.7)	

RFLP restriction fragment length polymorphism, *ELISA* enzyme-linked immunosorbent assay

Table 5 Characteristics of discrepant genotyping results obtained by RFLP, ELISA, and DNA sequencing

No.	RFLP	ELISA	Sequence	HBV DNA (log IU/ml)
1	A	C	C	6.29
2	B	C	B	7.25
3	B	D or E	B	5.01
4	B	N	B	6.98
5	F	C	C	5.77
6	N	B	Unclassified	3.06
7	N	B	Unclassified	3.17
8	N	C	Unclassified	1.38

RFLP restriction fragment length polymorphism,*ELISA* enzyme-linked immunosorbent assay

Average log HBV DNA (IU/ml): Mismatch 4.87 ± 2.12 ($n = 8$); Match 6.19 ± 1.86 ($n = 92$), Mann–Whitney *U* test 0.06

Eight specimens were differently typed by RFLP and ELISA: one genotyped A by RFLP was genotyped C by ELISA; three genotyped B by RFLP were genotyped C, D or E by ELISA, and one specimen was not genotyped by ELISA; one specimen genotyped F by RFLP was genotyped C by ELISA; and three specimens that could not be genotyped by RFLP were genotyped B (2 samples) and C (1 sample) by ELISA (Table 5). The HBV DNA levels in the specimens for which mismatched results were obtained by RFLP and ELISA were lower than those of the specimens for which matching results were obtained (mismatch 4.87 ± 2.12 log IU/ml ($n = 8$); match 6.19 ± 1.86 log IU/ml ($n = 92$); Table 5). In addition, there was no significant difference in the HBV DNA level between the samples for which matching results were obtained by RFLP and ELISA and those for which differing results were obtained ($P = 0.06$). We performed DNA sequencing in order to genotype these eight mismatched samples, by which samples 1 and 5 were genotyped C, with the same results as those given by ELISA, and samples 2, 3, and 4 were genotyped B, with the same results as those given by RFLP. However, three samples (6, 7, and 8) could not be genotyped by analyzing the DNA sequence, and the DNA levels of these three samples were lower than those of samples 1–5 (Table 5).

Discussion

The ratio of HBV genotypes B and C genotyped by RFLP and ELISA in our study was similar to that of Kao's study in Taiwan, confirming again that genotypes B and C are the first and second most prevalent HBV genotypes in Taiwan [14]. Owing to this condition, we were only able in this study to perform more detailed analysis of the genotyping results for genotypes B and C of HBV, and not for the other genotypes. We, therefore, advocate that, with the exception of genotypes B and C, detailed analysis of genotyping by RFLP and ELISA for genotypes of HBV should be performed in the future using more samples and an advanced survey method.

Usuda et al. [24] mentioned that the pre-S2 region of HBV DNA is related to HBV genotype, and Tiollais and coworkers [29] showed that the P gene occupies more than 70% of the complete hepatitis B virus genome and also includes the entire pre-S and S genes and partial X and C genes. For this reason, the sequence of the S gene is more conserved than that of the pre-S region, because the S gene overlaps the reverse transcriptase active site in the P gene, which is encoded in a different frame [30]. Mizokami et al. [23] believed that the S gene is more suitable for genotyping than the pre-S region by RFLP.

The ELISA with mAbs method for the detection of genotypes of HBV has been developed over many years [24]; this method uses five mAbs to epitopes of the pre-S2 region products: b, m, k, s, and u. Monoclonal antibody b is a commonly expressed epitope on the pre-S2 region product, and serves as a control to avoid contamination by bacteria or proteinase; it is not related to the determination of genotype. Four other mAbs, m, k, s, and u, can be used to identify HBV genotypes A, B, C, D, and F according to the presence of the various mAbs [24, 25]. The advantages of this method are the time saving, low cost, and suitability for large-scale surveys. In the study by Tanaka et al. [27] the reproducibility, accuracy, and sensitivity of an ELISA-based HBV genotyping kit for the genotyping of A, B, C, or D samples by detecting genotype-specific epitopes in the pre-S2 region were evaluated. The genotyping results obtained by DNA sequencing for 91 samples were in complete accordance with 87 (95.6%) of those obtained by EIA genotyping. In our study, the genotypeable rate for

ELISA, the correct genotyping rate from genotypeable specimens for ELISA, and the concordance of ELISA genotyping of HBV genotypes B and C were around 90% those of the RFLP method, and we, therefore, believe that HBV genotyping by ELISA is suitable and appropriate for use in Taiwan.

From our results, we believe that HBV DNA level is not related to the products of the HBV gene, because we did not find a relationship between HBV DNA level and whether or not samples could be genotyped by the ELISA method using a cut-off value of 2×10^3 IU/ml, even though we did identify a significant difference in the HBV DNA levels between those samples that could be genotyped by RFLP with an HBV DNA cut-off value of 2×10^3 IU/ml, because all samples with an HBV DNA level greater than 2×10^3 IU/ml could be genotyped in this study. On the other hand, in the group of samples non-genotyped by RFLP, only three samples were genotypeable by ELISA, the average and maximum levels of HBV DNA were lower than those of the group of samples genotypeable by RFLP. We considered this reasonable, because we had hypothesized that the level of HBV DNA would be higher in those samples genotypeable by RFLP than in those genotypeable by ELISA. Since RFLP detects the amount of HBV DNA, the level of HBV DNA was not associated with the protein levels of sample genotypeable by ELISA.

In this study, we further analyzed by DNA sequencing the eight specimens (Table 5), for which different genotyping results were obtained by RFLP and ELISA, in order to definitively genotype these samples: two samples were genotyped as being of the genotype indicated by the ELISA results, and three samples as being of the genotype indicated by the RFLP results. In these eight specimens, no relationship was found between HBV DNA level and whether or not samples were genotyped, the same or differently by ELISA and RFLP ($P = 0.06$, Table 5); however, we believe that even though no significant difference was observed, this outcome could not be considered meaningful, because the sample size of the mismatching group was too small. In addition, the discrepancy between the RFLP and ELISA genotyping results could also reasonably explain the results obtained for samples 6–8 in Table 5, as discussed above, which had lower levels of HBV DNA and could be genotyped by ELISA, but not by RFLP. We believe that there was no difference in the accuracy of genotyping by RFLP and ELISA for the genotyping of HBV DNA, even though a discrepancy between these two methods was noted, and we, therefore, consider ELISA to be a reliable method for HBV genotyping.

A possible cause of this discrepancy in the results of HBV genotyping by the ELISA and RFLP methods is the difference in the mechanisms of these two tests: ELISA examines epitopes of the pre-S2 products of the HBV gene, while RFLP examines the different lengths of HBV DNA fragments digested by specific restrictive enzymes. In addition, the studies by Mizokami [23] and Usuda [24] included no HBV DNA samples from Taiwan, so it might be that the genotype classification methods reported in these two studies were not completely suitable for use in a Taiwanese population, which is another possible cause of the discrepancy. Amino acid mutations or deletions within targeted pre-S2 epitopes have also been mentioned as a possible reason for differing genotyping results obtained by ELISA and HBV DNA sequencing in a previous study [27], and may also explain the discrepancy in the genotyping results obtained by HBV DNA sequencing, and ELISA in this study for samples 2–4 (Table 5). We also thought that, mutation or other changes in other HBV DNA sites might be the cause of the different genotyping results obtained by RFLP and HBV DNA sequencing for samples 1–5 in Table 5. For samples 6–8 (Table 5), which could not be genotyped by RFLP or DNA sequencing but could be genotyped by ELISA, with lower DNA levels than samples 1–5, even the lowest HBV DNA level was 1.38 log IU/ml, which was between 100 and 200 IU/ml, but the levels of the other two samples were greater than 200 IU/ml (3.06 log and 3.17 log IU/ml). We cannot explain this result, although it could be that some pre-S2 products remained in the serum or plasma, but the level of HBV DNA was lower than can be genotyped by RFLP, and so these samples still could not be genotyped by RFLP and HBV DNA sequencing. We believe that the discrepancy between these methods used should be researched further in future studies. Other conditions, such as the primer targeting gene fragment used in RFLP, could also be a cause of this discrepancy; however, owing to the limited number of samples in this study, we were unable to perform advanced study of this discrepancy, and we propose to explore this in more details in the future.

In conclusion, even though a previous study reported that the S gene is more suitable for genotyping than the pre-S region by RFLP [23], our results showed that, HBV genotyping by ELISA is accurate and reliable, especially for HBV genotypes B and C, and we believe that the ELISA method is suitable for large-scale genotyping surveys of HBV in Taiwan. In addition, we found no relationship between HBV DNA level and whether or not the results of genotyping by RFLP and ELISA matched, and suggest that HBV DNA level cut-off value of 2×10^3 IU/ml be viewed as the threshold for the higher rate of genotyping by RFLP.

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Conflict of interest None.

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