

# COMPARISON OF CLINICAL APPLICATION OF THE ABBOTT HBV PCR KIT AND THE VERSANT HBV DNA 3.0 TEST TO MEASURE SERUM HEPATITIS B VIRUS DNA IN TAIWANESE PATIENTS

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With an estimated 350–400 million people worldwide chronically infected with hepatitis B virus (HBV), and the subsequent serious complications caused by liver damage including cirrhosis, liver failure, and hepatocellular carcinoma, HBV infection remains a global health issue, particularly in Taiwan, an HBV-hyperendemic area. Sensitive and accurate quantification of HBV DNA is necessary to monitor patients with chronic hepatitis B who are receiving antiviral therapy to determine treatment response and adapt therapy. We evaluated and compared the clinical performance of two HBV DNA assays based on different technologies: the RealArt™ HBV™ PCR Kit (Abbott HBV DNA PCR kit, real-time polymerase chain reaction assay, detection limit: 27 IU/mL) and the VERSANT bDNA 3.0 assay (Bayer, branched DNA signal amplification assay, detection limit: 357 IU/mL). Serum levels of HBV DNA in 173 chronic HBV carriers were determined using both the RealArt™ HBV™ PCR Kit and the VERSANT bDNA 3.0 test. Of the 173 samples analyzed for baseline viral load detection, HBV DNA was quantifiable in 147 patients (82.1%) by the RealArt™ HBV™ PCR Kit, which was significantly higher than the 92 (53.2%) samples quantified by the VERSANT bDNA 3.0 assay. A total of 86 (49.7%) samples were quantifiable by both assays, whereas 25 (14.5%) were below the detection limit of both assays. The HBV DNA quantification values measured by the RealArt™ HBV™ PCR Kit and the VERSANT bDNA 3.0 assay were positively correlated (Spearman's rank correlation coefficient  $r=0.932$ ,  $p<0.001$ ). On average, the results derived from the RealArt™ HBV™ PCR Kit were 0.67 log lower than those of the VERSANT bDNA 3.0 assay. HBV DNA concentrations were significantly higher in 63 HBeAg-seropositive patients than in 110 HBeAg-seronegative patients ( $5.42 \pm 2.34$  logs vs.  $3.21 \pm 2.27$  logs,  $p<0.001$ ). The RealArt™ HBV™ PCR Kit is more sensitive and has a wider dynamic range than the VERSANT bDNA 3.0 assay in the clinical setting of chronic hepatitis B patients. The sensitivity and wide dynamic range of the PCR assay allow optimal monitoring and timely adaptation of



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antiviral therapy. Nevertheless, the HBV DNA values measured by the RealArt™ HBV™ PCR Kit and the VERSANT bDNA 3.0 assay were significantly correlated.

**Key Words:** bDNA 3.0 assay, HBeAg, HBV DNA PCR Kit, HBV DNA quantification, HBV infection

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An estimated 350–400 million people worldwide are infected with hepatitis B virus (HBV), which is one of the leading causes of death worldwide [1]. In Taiwan, where HBV infection is hyperendemic [2,3] and is mainly vertically transmitted, HBV is the most important agent responsible for chronic liver disease. Most of the infections are asymptomatic and cause subsequent serious complications, including cirrhosis, liver failure and hepatocellular carcinoma [4,5]. Recent studies have demonstrated a direct link between HBV viral load and the risk of developing disease complications such as cirrhosis and hepatocellular carcinoma [6]. In infected individuals, antiviral drugs such as lamivudine [7], adefovir [8,9] and entecavir [10] are effective in reducing the viral load, normalizing liver function and consequently reduce the incidence of long-term complications [11]. However, these treatments do not generally allow complete eradication of HBV from the patients, and continuous long-term therapy is required to maintain effective viral suppression and symptom control [12]. In addition, the long-term effectiveness of these drugs is compromised by the emergence of resistant strains, which allows viral breakthrough and resumption of disease activity [13]. Furthermore, the current standard of care is to adapt antiviral therapy in patients with drug resistance as early as possible, namely at the time of viral breakthrough, as defined by an increase in viremia levels by 1 log unit of copies/mL compared with the nadir value [14].

Although numerous tests have been developed to diagnose and monitor the course and outcome of chronic liver disease caused by HBV infection, direct detection of HBV DNA in serum is considered to be the most reliable method to diagnose infection, particularly for some HBV genetic variations [15] or occult HBV infection [16,17].

The first assays developed to measure HBV DNA were based on membrane hybridization or solution hybridization of a radiolabeled probe to the denatured strand of HBV DNA in the serum [18]. Then,

nonradioactive hybridization assays based on chemiluminescent probes were developed to overcome the disadvantages of radioisotopes. The branched DNA (bDNA) assay, which can detect the HBV DNA level at the lower limit of detection (LLOD) of  $0.7 \times 10^6$  equivalents/mL, was reported to be better than other assays for measurement of samples with low HBV DNA levels [19,20]; this assay was used as the standard to measure HBV DNA in an antiviral trial [21]. However, in this assay, the LLOD is still too high to allow detection of lower viral load in some patients, particularly those who respond to antiviral treatment. It was found that pronounced decreases in HBV DNA after treatment were related to better clinical outcomes, whereas a persistent rebound in viral replication may indicate the selection of drug-resistant viruses in patients receiving antiviral therapy [21,22]. Thus, a more sensitive assay of HBV DNA is needed for the evaluation of patients.

Methods to assess HBV DNA by quantitative polymerase chain reaction (PCR) have been developed [23,24]. The RealArt™ HBV™ PCR Kit (RealArt Test; Artus Biotech, Hamburg, Germany) is an automatic, PCR-based assay of HBV DNA in serum. In our study, we measured the concentrations of HBV DNA using the RealArt™ HBV™ PCR Kit in Taiwanese patients with chronic HBV infection. The performance characteristics and clinical usefulness of the RealArt™ HBV™ PCR Kit were evaluated, and the correlation between this assay and the VERSANT bDNA 3.0 assay to measure HBV DNA levels was examined.

## METHODS

### *Patients and serum samples*

A total of 173 Taiwanese treatment-naïve patients with chronic hepatitis B were enrolled in this study. All patients were seropositive for hepatitis B surface antigen for more than 6 months, and all of these patients

were tested for hepatitis B e antigen (HBeAg) by radioimmunoassay (General Biological Cooperation, Hsinchu, Taiwan). All serum samples were separated from whole blood, collected in serum separation tubes, and stored immediately at  $-70^{\circ}\text{C}$  in several aliquots. Alanine aminotransferase (ALT) concentrations (normal upper limit of serum ALT, 40 IU/L) and aspartate aminotransferase (AST) concentrations (normal upper limit of serum AST, 42 IU/L) were measured on a multichannel autoanalyzer. All samples were negative for antibodies to hepatitis C virus (HCV) according to the third-generation HCV antibody (anti-HCV) enzyme immunoassay test (Abbott Laboratories, North Chicago, IL, USA).

### **Measurement of serum HBV DNA**

Serum HBV DNA levels were measured by two assays, the VERSANT bDNA 3.0 assay (Bayer Diagnostics, Tarrytown, NY, USA) and the RealArt™ HBV™ PCR assay (Applied Biosystems, Branchburg, NJ, USA) according to the manufacturer's instructions.

#### *VERSANT bDNA 3.0 assay*

Briefly, HBV genomic DNA is captured onto a microwell by a set of specific, synthetic oligonucleotide capture probes during an overnight incubation. A set of target probes is also added. The capture probes and the target probes bind to the HBV genome. A preamplifier probe that hybridizes to the target probes is then added. The amplifier probe subsequently hybridizes to the preamplifier, forming a bDNA complex. Multiple copies of an alkaline phosphatase-labeled probe are then hybridized to this immobilized complex. Detection is achieved by incubating the alkaline phosphatase-labeled complex with a chemiluminescent substrate. Light emission is directly related to the amount of HBV DNA present in each sample, and results are recorded as relative light units by the Bayer System 340bDNA Analyzer. The dynamic range of HBV DNA measurements was 357–17,857,100 IU/mL.

#### *RealArt™ HBV™ PCR assay*

The HBV™ Master contains reagents and enzymes for the specific amplification of a 134-bp region of the HBV virus genome. The QIAamp DSP Virus Kit (Qiagen Inc., Valencia, CA, USA) is used for DNA isolation according to the manufacturer's instructions. The RealArt™ HBV™ PCR constitutes a ready-to-use system for the detection of HBV DNA using PCR in

the ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Briefly, 30  $\mu\text{L}$  of the Power SYBR Green Master Mix is added to each required reaction tube or well of the 96-well reaction plate. Subsequently, 20  $\mu\text{L}$  of the eluate from the DNA isolation is added to each well. The solution is mixed well by repeated up-and-down pipetting. The reaction tubes are closed with the corresponding caps and centrifuged (in a storage rack for PCR tubes) for 30 seconds at 1,780g (4,000 revolutions/minute) to collect the prepared reaction volume in the bottom of the tube. If this type of centrifuge is not available, both the master mix and the sample volume should be pipetted to the bottom of the tubes. The prepared reactions are stored at  $+4^{\circ}\text{C}$  until the ABI PRISM® SDS Instrument is programmed; then the reactions are transferred into the instrument. The amplicon is detected by measuring the FAM fluorescence. In addition, the RealArt™ HBV™ PCR assay contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an internal control by measuring the JOE (6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein) fluorescence. The internal control can optionally be used exclusively to check for possible PCR inhibition. The detection limit and the linearity range of the RealArt™ HBV™ PCR assay are 27 IU/mL and  $9-4 \times 10^9$  IU/mL, respectively.

### **Statistical analysis**

All HBV DNA results are expressed as mean  $\pm$  standard deviation (SD) after logarithmic transformation of the original values. The  $\chi^2$  test with Yates' correction, Fisher's exact test, Student's *t* test, Pearson correlation coefficients, simple linear regression, stepwise multiple linear regression and multiple logistic regression were used as appropriate. Results were deemed significant if *p* values were less than 0.05.

## **RESULTS**

### **Background of the 173 patients**

The clinical characteristics of the 173 patients, aged  $35.5 \pm 13.5$  years (range, 17–76 years), are shown in Table 1. The mean  $\pm$  SD ALT and AST concentrations were  $145.5 \pm 269.0$  and  $231.9 \pm 416.4$  IU/L, respectively (Table 1). Sixty-three (36.4%) patients were positive for HBeAg. Serum HBV DNA levels were significantly higher in patients with abnormal AST and ALT levels

**Table 1.** Backgrounds of hepatitis B virus (HBV)-infected patients and their serum HBV DNA levels measured by VERSANT bDNA 3.0 assay and RealArt™ HBV™ PCR Kit\*

Characteristics	Mean ± SD or n (%)	VERSANT bDNA 3.0 assay <sup>†</sup>	<i>p</i>	RealArt™ HBV™ PCR Kit <sup>†</sup>	<i>p</i>
All patients	173	4.69 ± 2.01		3.96 ± 2.60	
Sex			0.132		0.183
Male	140 (80.9)	4.58 ± 2.01		3.84 ± 2.61	
Female	33 (19.1)	5.16 ± 1.94		4.50 ± 2.40	
AST	145.47 ± 268.94		<0.001		<0.001
Normal (≤42 IU/L)	77 (44.5)	3.76 ± 1.84		2.86 ± 2.46	
Abnormal (>42 IU/L)	96 (55.5)	5.47 ± 1.82		4.85 ± 2.32	
ALT	231.90 ± 416.41		<0.001		<0.001
Normal (≤40 IU/L)	54 (31.2)	3.59 ± 1.76		2.86 ± 2.46	
Abnormal (>40 IU/L)	119 (68.8)	5.19 ± 1.92		4.61 ± 2.43	
Hepatitis B e antigen			<0.001		<0.001
Negative	110 (63.6)	4.06 ± 1.80		3.11 ± 2.32	
Positive	63 (36.4)	5.78 ± 1.89		5.46 ± 2.33	

\*Data presented as mean ± standard deviation (SD) or n (%); <sup>†</sup>expressed as mean ± SD of logarithmic transformation of log IU/mL. AST = aspartate aminotransferase; ALT = alanine aminotransferase.

and positive HBeAg by both VERSANT bDNA 3.0 assay and RealArt™ HBV™ PCR Kit (all  $p < 0.001$ ; Table 1).

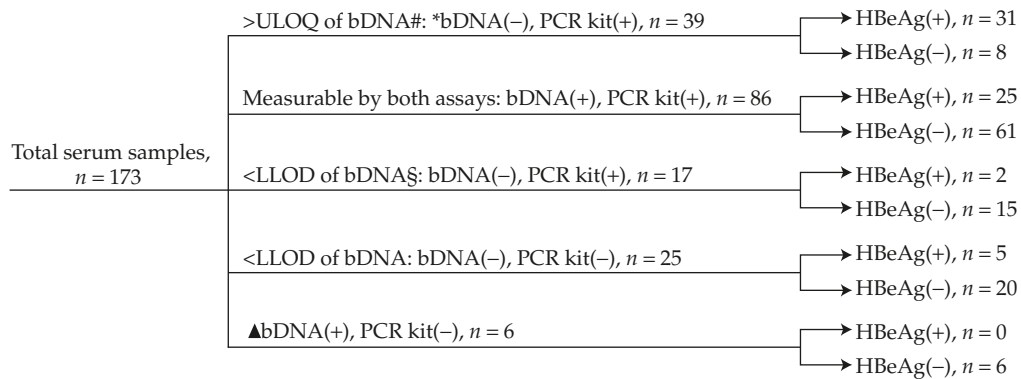
### Quantitative results of HBV DNA

Eighty-six (49.7%) of 173 samples were quantifiable by both assays, whereas 25 (14.5%) were below the detection limit of both assays. Seventeen of 42 (40.5%) samples with HBV DNA values below the LLOD for the VERSANT bDNA 3.0 assay could be measured by the RealArt™ HBV™ PCR Kit. Six HBeAg-negative samples that could not be measured by the RealArt™ HBV™ PCR Kit were measurable by the VERSANT bDNA 3.0 assay. All 39 samples (31 HBeAg-positive and 8 HBeAg-negative) with HBV DNA values higher than the upper limit of quantification (ULOQ) for VERSANT bDNA 3.0 assay (17,857,100 IU/mL) were successfully measured by the RealArt™ HBV™ PCR Kit. The successful rate of quantification was significantly higher with the RealArt™ HBV™ PCR Kit than with the VERSANT bDNA 3.0 assay for 63 HBeAg-positive samples (92.1% [58 of 63] vs. 39.7% [25 of 63],  $p < 0.001$ ) and 110 HBeAg-negative samples (76.4% [84 of 110] vs. 60.9% [67 of 110],  $p = 0.014$ ). The concentration of HBV DNA was higher than the ULOQ of the VERSANT bDNA 3.0 assay in 31 of 63 HBeAg-positive patients. In addition, HBV DNA could not be measured in 42 (38.2%) of 110 HBeAg-negative

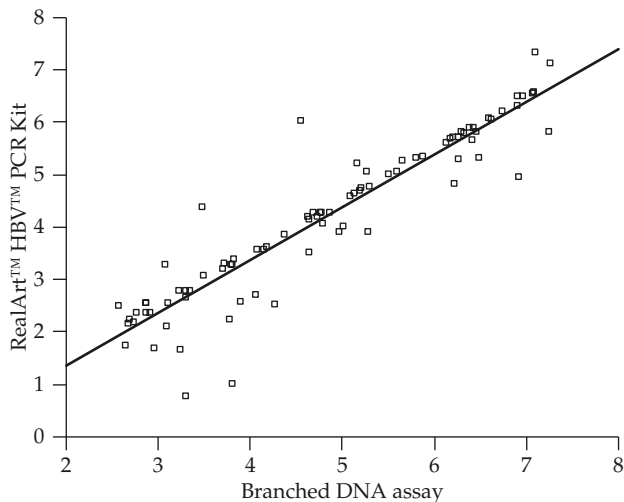
samples by the VERSANT bDNA 3.0 assay, because the DNA level was below the LLOD (<357 IU/mL). However, the RealArt™ HBV™ PCR Kit successfully measured 58 (92.1%) of 63 HBeAg-positive samples. Sixty-one (91.0%) of the 67 HBeAg-negative samples measurable by the VERSANT bDNA 3.0 assay could also be measured by the RealArt™ HBV™ PCR Kit, and 17 (40.5%) of the 42 samples with HBV DNA values below the LLOD for the VERSANT bDNA 3.0 assay could be measured by the RealArt™ HBV™ PCR Kit. The overall frequency of HBV DNA measurement for the RealArt™ HBV™ PCR Kit was 82.1%, which was significantly higher than that for the VERSANT bDNA 3.0 assay (53.2%,  $p = 0.001$ ; Figure 1). The control samples could not be measured by either assay.

### Correlation of RealArt™ HBV™ PCR Kit and VERSANT bDNA 3.0 assay

In the 86 samples successfully measured by both assays (excluding 48 low-titer and 39 high-titer samples for the VERSANT bDNA 3.0 assay and the RealArt™ HBV™ PCR Kit, respectively), there was a positive correlation between the two assays ( $r = 0.932$ ;  $p = 0.0001$ ; Figure 2). Accordingly, the formula derived for the conversion of results is as follows:  $\log(\text{HBV DNA value by RealArt™ HBV™ PCR Kit}) = 1.0086 \times \log(\text{HBV DNA value by bDNA 3.0 assay}) - 0.6624$ . On average, the results derived from



**Figure 1.** Flow chart showing the relationship between measurement of hepatitis B virus (HBV) DNA by RealArt™ HBV™ PCR Kit/ VERSANT bDNA 3.0 assay and hepatitis B e antigen (HBeAg) status. bDNA = VERSANT bDNA 3.0 assay; PCR kit = RealArt™ HBV™ PCR Kit; ULOQ of bDNA# = upper limit of quantification of bDNA; LLOD of bDNA§ = lower limit of detection of bDNA; \*bDNA(-), PCR kit(+) = HBV DNA values were not quantifiable by VERSANT bDNA 3.0 assay but were quantifiable by PCR kit; ▲bDNA(+), PCR kit(-) = VERSANT bDNA 3.0 assay could measure the HBV DNA values of these samples (520, 567, 1,135, 2,614, 4,537 and 7,452 IU/mL), but the PCR Kit could not detect HBV DNA of these serum samples.



**Figure 2.** Comparison between hepatitis B virus (HBV) DNA values in samples from 86 patients with chronic HBV infection that were quantifiable by both the RealArt™ HBV™ PCR Kit and the VERSANT bDNA 3.0 assay. There was a good correlation between all samples yielding values within the quantitative range of each of the assays. Pearson's correlation coefficient  $r = 0.932$ ,  $p < 0.001$ . HBV values are expressed as log IU/mL.

the RealArt™ HBV™ PCR test were 0.67 log lower than the bDNA values (Table 2). The mean differences between the two assays did not differ between samples that were positive or negative for HBeAg.

### Association between viral load and clinical characteristics

Of the 173 patients, the mean ± SD HBV DNA and ALT and AST values were  $3.96 \pm 2.60$  log IU/mL,

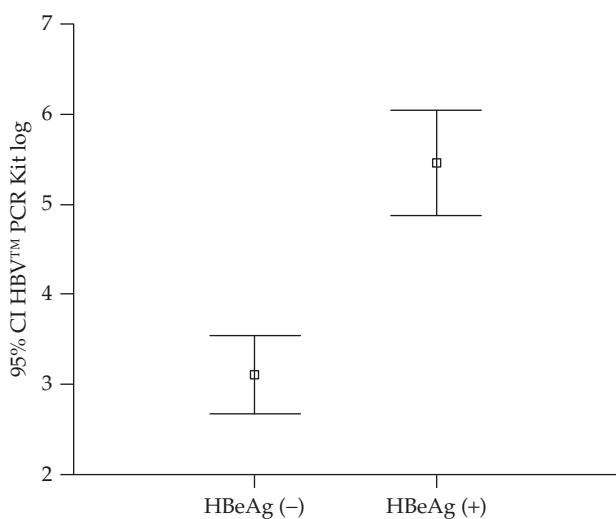
and  $240.4 \pm 421.4$  and  $150.2 \pm 272.5$  IU/L, respectively. The ALT concentrations of 96 patients (55.5%) were abnormal. The association between HBV viral load and clinical characteristics of HBV infection was evaluated. The median HBV DNA concentrations of the HBeAg-positive and HBeAg-negative samples, as assessed by the RealArt™ HBV™ PCR Kit, were  $3.11 \pm 2.32$  log IU/mL and  $5.46 \pm 2.33$  log IU/mL, respectively (Figure 3). According to univariate analyses, the HBV DNA values were significantly higher in HBeAg-positive than in HBeAg-negative patients ( $p < 0.001$ ; Table 1), higher in patients with an abnormal ALT concentration than in those with a normal ALT concentration ( $4.61 \pm 2.43$  log IU/mL vs.  $2.54 \pm 2.34$  log IU/mL;  $p < 0.001$ ), and higher in patients with an abnormal AST concentration than in those with a normal AST concentration ( $4.85 \pm 2.32$  log IU/mL vs.  $2.86 \pm 2.46$  log IU/mL;  $p < 0.001$ ). No significant association between HBV DNA value and sex or age was observed. Multivariate analysis revealed that HBeAg status and ALT value were associated with HBV DNA concentrations (both  $p < 0.0001$ ). The serum HBV DNA concentration was the only factor associated with ALT value (odds ratio, 1.70; 95% confidence interval, 1.30–2.1).

Comparison of the VERSANT bDNA 3.0 assay and the RealArt™ HBV™ PCR Kit resulted in patient population patterns. The number and proportion of samples with levels of HBV DNA that were below the LLOD or within various ranges were compared (Table 3). Lower levels of HBV DNA that could not be

**Table 2.** Correlation between the results of the RealArt™ HBV™ PCR Kit and the VERSANT bDNA 3.0 assay in 86 samples quantifiable by both assays

	n	HBV DNA value*		Difference <sup>†</sup>	Spearman's rank	
		VERSANT bDNA 3.0 assay	RealArt™ HBV™ PCR Kit		Correlation coefficient	p
All	86	4.81 ± 1.43	4.19 ± 1.55	0.62	0.947	<0.001
HBeAg						
Positive	25	4.95 ± 1.43	4.36 ± 1.43	0.59	0.930	<0.001
Negative	61	4.76 ± 1.43	4.12 ± 1.60	0.64	0.902	<0.001

\*Expressed as mean ± standard deviation of logarithmic transformation (log IU/mL); <sup>†</sup>Difference = log(bDNA 3.0 assay) – log(RealArt™ HBV™ PCR Kit). HBV = hepatitis B virus; HBeAg = hepatitis B e antigen.



**Figure 3.** Comparison between hepatitis B virus (HBV) DNA values in hepatitis B e antigen-negative and -positive samples from patients with chronic HBV infection measured by the RealArt™ HBV™ PCR Kit. CI=confidence interval; HBeAg=hepatitis B e antigen.

measured by the VERSANT bDNA 3.0 assay (log HBV DNA 0.5–1, 1–2 IU/mL) could be quantified by the RealArt™ HBV™ PCR Kit. These results indicate that the RealArt™ HBV™ PCR Kit has better sensitivity for lower levels of HBV DNA and a wider dynamic range.

## DISCUSSION

Because HBV infection is endemic in Taiwan, being able to adequately diagnose and manage patients with the aid of diagnostic tools, including virological

tests, is important. The viral load varies greatly during the different phases of HBV infection, ranging from undetectable levels to >10<sup>9</sup> copies/mL. Dramatic changes may occur in response to antiviral treatment; therefore, the range of quantitative measurement for HBV DNA detection assays needs to be wide. Assays that use signal amplification, including the bDNA assay (Bayer Diagnostics, Inc.), generally have a wide dynamic range, but their sensitivity is lower compared with assays based on target amplification (i.e. PCR-based assays); however, the latter often have a restricted dynamic range [25]. Quantitative HBV DNA assays that use real-time PCR have recently been developed; these assays are very sensitive and have a wider linear range compared with PCR- or bDNA-based assays.

Our study showed a correlation between RealArt™ HBV™ PCR Kit and VERSANT bDNA 3.0 assay for the detection and quantification of HBV DNA over a wide range of HBV DNA levels. Both assays shared significant concordance of values included in the overlapping dynamic range ( $r=0.932, p<0.001$ ). This implies that both assays can provide consistent indications for decision making on the use and adaptation of antiviral therapy. The RealArt™ HBV™ PCR Kit improved the sensitivity by extending the detection range; therefore, patients in whom the viral load was unmeasurable by the VERSANT bDNA 3.0 could be evaluated. A study by Chan et al [26] revealed that approximately half of those patients with undetectable HBV DNA values by bDNA assays were positive for HBV DNA in an in-house PCR assay. All HBeAg-positive samples that could be detected by the VERSANT bDNA 3.0 assay could also be measured by the RealArt™ HBV™ PCR Kit. We found

**Table 3.** Comparison of results using RealArt™ HBV™ PCR Kit and VERSANT bDNA 3.0 assay at different levels of hepatitis B virus DNA

Result category (log HBV DNA IU/mL)	VERSANT bDNA 3.0 assay			RealArt™ HBV™ PCR Kit		
	No. of samples	%	Cumulative %	No. of samples	%	Cumulative %
<LLOD*†	44	24.6	24.6	32	17.9	17.9
0.5–1				1	0.6	18.5
1–2				15	8.4	26.9
2–3	13	7.3	31.8	25	14.0	40.9
3–4	23	12.8	44.6	15	8.4	49.3
4–5	17	9.6	54.2	18	10.1	59.4
5–6	14	7.8	62.0	21	11.6	71.0
6–7	23	12.8	74.8	15	8.4	79.4
7–8	45	25.2	100	37	20.6	100

\*HBV DNA was not detected or was detected below the limit of detection. For the bDNA 3.0 assay, the limit of detection is 2.255 log HBV DNA IU/mL (<357 IU/mL), and for the HBV PCR kit, the limit of detection is 0.301 log HBV DNA IU/mL (<4 IU/mL).

†Sensitivity in 147 patients (82.1%) measured by the HBV PCR kit was significantly higher than the 135 (75.4%) patients measured by the bDNA 3.0 assay ( $p=0.01$ ). HBV = hepatitis B virus; LLOD = lower limit of detection.

that 40.5% of samples with HBV DNA values below the LLOD of VERSANT bDNA 3.0 assay and 100% of samples with HBV DNA values higher than the ULOQ for the VERSANT bDNA 3.0 assay could be measured by the RealArt™ HBV™ PCR Kit. Approximately 69.1% of our samples for which the VERSANT bDNA 3.0 assay could not detect measurable HBV DNA (<LLOD or >ULOQ) were found to contain HBV DNA by the RealArt™ HBV™ PCR Kit. This PCR assay was shown to have a higher quantification rate of HBV DNA values than the VERSANT bDNA 3.0 assay in HBeAg-positive and HBeAg-negative samples. Our results are in strong agreement with a recent comparative analysis of the real-time PCR kit and the VERSANT bDNA 3.0 assay [27]; the sensitivity and dynamic range of the real-time PCR kit were superior to that of the VERSANT bDNA 3.0 assay. Our results were also consistent with studies that compared other real-time PCR assays and the VERSANT bDNA assay [28–30]. Therefore, the RealArt™ HBV™ PCR Kit might be more applicable than the bDNA 3.0 assay in clinical practice.

In our study, significant differences in HBV DNA values were found between HBeAg-positive and HBeAg-negative samples in both assays, and similar differences have been reported in studies analyzing real-time PCR and other assays [31–33]. The mean HBV DNA values were lower in HBeAg-negative than in HBeAg-positive patients, and the higher rate of HBV DNA values were below the LLOD of the bDNA 3.0 assay. The RealArt™ HBV™ PCR Kit, with

higher sensitivity, seems to be more suitable for accurate measurement of HBV DNA values in HBeAg-negative patients.

In our study, the HBV DNA values derived from the RealArt™ HBV™ PCR Kit were, on average, 0.67 log lower than the VERSANT bDNA 3.0 values. We revealed in our previous study that results derived by an automatic PCR-based assay (the COBAS AMPLICOR HBV MONITOR test; Roche Molecular Systems, Pleasanton, CA, USA) were, on average, 0.55 log lower than those of the bDNA assay [34].

HBV DNA values were also significantly associated with ALT values. A test allows detection of HBV DNA at a level well below the levels considered necessary for the control of disease or those associated with an elevated risk of hepatocellular carcinoma. The latest treatment concept advocates early introduction of rescue therapy in patients with viral breakthrough, which is defined as a 10-fold increase in HBV viral load from the nadir after treatment. This approach has been demonstrated to provide important benefits in terms of long-term clinical outcomes [14] and to adjust therapy during the 1<sup>st</sup> year when the viral load is not suppressed below 3 log<sub>10</sub> copies/mL [35]. This real-time PCR test with high sensitivity is well suited to the new treatment algorithm. Based on our analyses, the RealArt™ HBV™ PCR Kit is more useful when the HBV DNA values are below the LLOD of a bDNA assay.

Six HBeAg-negative samples could not be measured by the RealArt™ HBV™ PCR Kit; however,

the VERSANT bDNA 3.0 assay could measure the HBV DNA values of these samples (520, 567, 1,135, 2,614, 4,537 and 7,452 IU/mL). The reason why RealArt™ HBV™ PCR could not successfully determine the viral load in these samples deserves further investigation. A recent study [30] has shown some drawbacks of two widely used assays: (1) Cobas Monitor has a narrow dynamic range and underestimates genotype F sample viral loads; and (2) bDNA shows poor sensitivity and may fail to identify patients with low viral loads. Because of their higher performance in terms of analytical sensitivity combined with a larger dynamic range and an ability to quantify the main genotypes equally, real-time PCR methods appear to be more appropriate for accurate HBV DNA quantification. Furthermore, the impact of HBV genotyping on the performance of the testing methods needs to be clarified in further studies.

In conclusion, the results of the RealArt™ HBV™ PCR Kit assay correlated well with those of the VERSANT bDNA 3.0 assay. The RealArt™ HBV™ PCR Kit had higher sensitivity and a wider dynamic range than the VERSANT bDNA 3.0 assay. This PCR assay can be used effectively to monitor patients with high serum HBV DNA levels and will remain effective if serum HBV DNA levels are decreased to levels that might be unmeasurable by the VERSANT bDNA 3.0 assay. The sensitivity and wide dynamic range of this PCR assay allow optimal monitoring and timely adaptation of antiviral therapy.

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# 針對台灣慢性 B 型肝炎患者以 Abbott HBV PCR Kit 和 VERSANT HBV DNA 3.0 Test 定量血清中 HBV DNA 值的臨床應用與比較

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全球估計約有三億五千萬至四億人感染慢性 B 型肝炎病毒，並導致慢性肝臟損傷，例如肝硬化、肝衰竭、肝細胞癌等嚴重後遺症。這使得慢性 B 型肝炎病毒感染已經成為全球健康的主要議題之一，尤其在 B 型肝炎病毒感染盛行的台灣更顯得重要。在接受 B 型肝炎抗病毒治療的過程中，靈敏與精確的 B 型肝炎病毒 (HBV DNA) 定量分析對治療反應評估與治療策略調整十分重要。因此，在本研究中，我們比較兩種新一代，但操作原理不同的 HBV DNA 定量分析方法：RealArt™ HBV™ PCR Kit (Abbott HBV PCR Kit，即時 PCR 分析法) 與 VERSANT bDNA 3.0 assay (Bayer branched DNA signal amplification assay)，分析這兩者在 HBV DNA 定量的靈敏度、偵測範圍 (sensitivity、dynamic range) 和臨床應用的優缺點。分別以 RealArt™ HBV™ PCR Kit 與 VERSANT bDNA 3.0 assay 定量分析 173 位慢性 B 型肝炎患者血清中的 HBV DNA 值，並分析 HBV DNA 值與 B 型肝炎 e 抗原 (HBeAg) 帶原情形、血清中 AST、ALT 值的關聯性。在所有 173 位患者的血清中，147 (82.1%) 個血清樣本可以 RealArt™ HBV™ PCR Kit 分析法測得 HBV DNA，明顯的優於 VERSANT bDNA 3.0 assay 分析法，其只測得了 92 (53.2%) 個血清樣本。這其中，86 (49.7%) 個血清樣本可同時由 RealArt™ HBV™ PCR Kit 與 VERSANT bDNA 3.0 assay 兩種方法測得 HBV DNA，而有 25 (14.5%) 個血清樣本因低於兩種方法的偵測值而皆無法測出 HBV DNA。在可同時被兩種方法定量分析出 HBV DNA 值者，分析的結果顯示兩種方法的定量值線性相關性佳 (Spearman's rank correlation coefficient  $r = 0.932$ 、 $p < 0.001$ )。平均而言，以 RealArt™ HBV™ PCR Kit 分析法定量的 HBV DNA 值較以 VERSANT bDNA 3.0 assay 分析法獲得之值低 0.67 log。同時，本研究亦顯示 B 型肝炎 e 抗原陽性患者 (HBeAg(+)) 較 B 型肝炎 e 抗原陰性患者 (HBeAg(-)) 有較高的 HBV DNA 值 ( $5.42 \pm 2.34$  logs vs.  $3.21 \pm 2.27$  logs、 $p < 0.001$ )。在偵測 HBV DNA 值上，RealArt™ HBV™ PCR Kit 分析法比 VERSANT bDNA 3.0 assay 分析法更具靈敏度和偵測範圍，因此在治療慢性 B 型肝炎患者時具更佳的應用性。但，兩個方法所定量分析出的 HBV DNA 值具有明顯的關聯性。

關鍵詞：bDNA 3.0 assay, HBeAg, HBV DNA PCR Kit, HBV DNA quantification, HBV infection  
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