# Performance of version 2.0 of the Cobas AmpliPrep/Cobas TaqMan real-time PCR assay for hepatitis B virus DNA quantification.

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# *Performance of version 2.0 of the Cobas AmpliPrep/Cobas TaqMan real-time PCR assay for hepatitis B virus DNA quantification*

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## Abstract

**Hepatitis B virus (HBV) DNA detection and quantification are essential to diagnose and treat chronic HBV infection. The use of real-time PCR assays for HBV DNA quantification is strongly recommended. The goal of this study was to evaluate the intrinsic characteristics and clinical performances of version 2.0 (v2.0) of the COBAS® AmpliPrep/COBAS® TaqMan® (CAP/CTM) assay, a fully automated platform for HBV DNA quantification in serum or in plasma with a claimed lower limit of detection of 20 IU/mL and a claimed upper limit of quantification of $1.7 \times 10^8$ IU/mL. The specificity of the assay was 99% (95% confidence interval: 94.7–100%). Intra-assay and inter-assay coefficients of variation ranged from 1.10% to 3.07%, and 0.82% to 2.95%, respectively. Calibration of the assay was found to be satisfactory. Study of blood specimens from patients infected with HBV genotypes A to F showed a good correspondence between HBV DNA levels measured with CAP/CTM v2.0, version 1.0 of the same assay and the third generation " branched DNA" assay. CAP/CTM v2.0 equally quantified HBV DNA levels in serum or plasma from the same patients. In conclusion, the new version of the CAP/CTM assay is sensitive, specific and reproducible. It accurately quantifies HBV DNA levels in patients chronically infected with HBV genotypes A to F. Improvements made to ensure equal quantification of HBV DNA in serum and plasma have been successful. Overall, the CAP/CTM assay version 2.0 is well suited to monitoring clinical HBV DNA levels according to current Clinical Practice Guidelines.**

## INTRODUCTION

Chronic hepatitis B virus (HBV) infection is associated with a large spectrum of liver diseases, ranging from a low viremia inactive carrier state to chronic active hepatitis, which may subsequently evolve towards cirrhosis and hepatocellular carcinoma (HCC). Morbidity and mortality are linked to the persistence of viral replication and hepatic complications develop in 15% to 40% of patients chronically infected with HBV. Overall, HBV-related end-stage liver disease and HCC are responsible for over 750,000 deaths worldwide per year (5 ).

HBV DNA detection and quantification is essential to diagnose ongoing HBV infection and to establish the prognosis of related liver disease, influences the decision to treat, and is indispensable to monitor the virological response to antiviral therapy and the emergence of resistance in order to tailor therapy (4 ). A number of HBV DNA detection and quantification assays are available. For many years, such methods were based on either hybrid-capture, signal amplification by means of branched DNA technology, or classical polymerase chain reaction (PCR), all of which suffered from poor analytical sensitivity and a narrow range of HBV DNA quantification (3 ). More recently, assays based on real-time PCR quantification have been developed. Their use for routine detection and quantification of HBV DNA is recommended, because of their excellent analytical sensitivity (lower limit of detection: 10–20 international units per milliliter (IU/mL)), their specificity, their accuracy and their broad dynamic range of linear quantification that fully covers the clinical needs (9 ). Among these assays, we recently evaluated the first-generation COBAS® AmpliPrep/COBAS® TaqMan® (CAP/CTM, Roche Molecular Systems, Pleasanton, California). This assay was found to be sensitive, specific, and reproducible, and to accurately quantify HBV DNA levels in patients chronically infected by HBV genotypes A to F (2 ). However, this assay could only be used for HBV DNA quantification in plasma.

A second version of the CAP/CTM assay (v2.0) has been recently released. Several changes have been made; in particular, the assay can be used on both serum and plasma and it requires 650 µL of sample instead of 850 µL. Its claimed dynamic range of quantification is 20 to $1.7 \times 10^8$ IU/mL (1.3 to 8.2 $\text{Log}_{10}$ IU/mL). The goal of this study was to evaluate the intrinsic characteristics and clinical performances of the CAP/CTM v2.0 assay.

## MATERIALS AND METHODS

## Materials

### *Standards*

A standard panel of HBV genotype A plasma samples (OptiQuant™ HBV DNA, AcroMetrix, Benicia, California) was used to study the analytical performance of the assay. The panel was made of 7 members (NAP-000 to NAP-HBV2E7) that contained no HBV DNA, 2 × $10^2$ IU/mL (2.3 $Log_{10}$ IU/mL), 2 × $10^3$ IU/mL (3.3 $Log_{10}$ IU/mL), 2 × $10^4$ IU/mL (4.3 $log_{10}$ IU/mL), 2 × $10^5$ IU/mL (5.3 $Log_{10}$ IU/mL), 2 × $10^6$ IU/mL (6.3 $Log_{10}$ IU/mL), and 2 × $10^7$ IU/mL (7.3 $Log_{10}$ IU/mL) respectively.

### *Clinical specimens*

Plasma and serum samples were obtained from patients attending the Department of Hepatology and Gastroenterology of Henri Mondor Hospital and from blood donors diagnosed with an HBV infection at the Institut National de la Transfusion Sanguine. Group A comprised 103 HBV-seronegative individuals (no marker of past or ongoing HBV infection); group B comprised 97 patients with a serological profile of resolved HBV infection (presence of both anti-HBc and anti-HBs antibodies); Group C comprised 51 patients with chronic HBV infection, all of whom had detectable HBsAg, anti-HBc antibodies, and HBV DNA. Based on sequencing of a portion of the S gene followed by phylogenetic analysis, this group comprised 12 patients with HBV genotype A, 9 with genotype B, 8 with genotype C, 10 with genotype D, 10 with genotype E, and 3 with genotype F, as previously described (2 ). Group D included 16 patients receiving nucleoside/nucleotide analogue therapy who were serially sampled on treatment. They included 4 patients infected with genotype A, 5 with genotype C, 1 with genotype D and 6 with genotype E. In total, 51 plasma and 51 serum specimens sampled at the same time were available from group D patients (on average 3 time points per patient, range 1–7).

## Assessment of CAP/CTM v2.0 performance

### *Analytical sensitivity*

To determine the analytical sensitivity of the assay, the NAP-HBV2E4 standard was serially diluted from 50 IU/mL (1.7 $Log_{10}$ IU/mL) to 6.25 IU/mL (0.8 $Log_{10}$ IU/mL). Twenty-one replicates of each HBV DNA concentration were tested in different experiments.

### *Specificity*

The specificity of CAP/CTM v2.0 was assessed by testing the 103 and 97 clinical specimens from groups A and B.

### *Precision and reproducibility*

To assess precision (intra-assay reproducibility), each sample in the OptiQuant™ HBV DNA standard panel was tested in triplicate. To assess inter-assay reproducibility, the low-positive control (LPC) and the high-positive control (HPC) provided with the kits were tested 18 times in the corresponding runs on different days.

### *Linearity, accuracy and influence of the HBV genotypes*

The linearity of quantification in CAP/CTM v2.0 was assessed by testing the 7 members of the OptiQuant™ HBV DNA standard panel. Each panel member was tested three times in the same experiment with CAP/CTM v2.0. The average measured values were then compared with the expected values. The 51 plasma specimens from group C were tested in parallel with CAP/CTM v2.0, CAP/CTM v1.0 and the bDNA assay. If needed, dilutions were made with the Nucleic Acid Test (NAT) dilution matrix (AcroMetrix), a defibrinated, delipidized normal human plasma.

### *Assessment of equal quantification in serum and plasma*

The 51 plasma and 51 serum specimens sampled at the same time point in patients from group D were tested with version 2.0 of the CAP/CTM assay and the results were compared. These samples were tested in parallel with CAP/CTM v1.0 in plasma and with bDNA in both serum and plasma.

## HBV DNA quantification

### *CAP/CTM v1.0 and v2.0*

HBV DNA was extracted from 850 µl of plasma for version 1.0 and from 650 µl of plasma or serum for version 2.0 by means of the automated extractor COBAS® AmpliPrep, according to the manufacturer's instructions. The COBAS® TaqMan 96 Analyzer was used for automated real-time PCR amplification and detection of PCR products according to the manufacturer's instructions. The data were analyzed with the Amplilink® software. HBV DNA levels were expressed in IU/mL. The dynamic ranges of quantification of CAP/CTM v1.0 and CAP/CTM v2.0 are 54 to 110,000,000 IU/mL (1.7 to 8.0 $Log_{10}$ IU/mL) and 20 to 170,000,000 IU/mL (1.3 to 8.2 $Log_{10}$ IU/mL), respectively.

***bDNA***

In the Versant HBV DNA 3.0 Assay (Siemens Medical Solutions Diagnostics, Tarrytown, New Jersey), HBV DNA was recovered from 50 µl of plasma or serum and quantified by the semi-automated System 340® bDNA analyzer (Siemens Medical Solutions Diagnostics), according to the manufacturer's instructions. HBV DNA levels were expressed in IU/mL. The dynamic range of quantification of this assay is 357 to 17,857,000 IU/mL (2.5 to 7.3 $Log_{10}$ IU/mL).

**Sequence analysis of the preC-C region**

Sequence analysis of the preC-C gene, the target of PCR amplification and probe hybridization in CAP/CTM v2.0, was performed on one HBV DNA-positive sample from each patient from group D. A hemi-nested PCR reaction was performed to amplify a 772-base pair fragment by means of sense primers HBPr86 and HBPr87 and antisense primer HBPr303, as previously described (10 ). Briefly, HBV DNA was extracted from 200 µl of serum or plasma using the QIAamp MinElute Virus Vacuum Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Amplification included an initial denaturation step at 95°C for 10 min, followed by cycles of denaturation at 95°c for 30 s, annealing at 50°C for 30 s and elongation at 72°C for 1 min, and a final elongation step at 72°C for 7 min. The amplification products (5 µl) were run on 1.5% agarose gels. The gels were stained with SYBR® safe (Invitrogen, Carlsbad, California). They were then purified with Montage PCR Centrifugal Filter Devices (Millipore Corporation, Bedford, Massachusetts), according to the manufacturer's protocol. PCR products were directly sequenced by means of the Big-Dye Terminator Cycle v3.1 sequencing kit on the ABI 3100 sequencer (Applied Biosystems, Foster City, California), according to the manufacturer's protocol.

**Statistical analysis**

Descriptive statistics are shown as the mean ± standard deviation (SD) or the median and interquartile range as appropriate. Comparisons between groups were made using the Kruskall-Wallis test or Mann-Whitney test. The relationship between quantitative variables was studied by means of regression analysis. P values <0.05 were considered significant.

## RESULTS

**Intrinsic performance of the CAP/CTM v2.0 assay**

***Analytical sensitivity***

The lower limit of detection of the assay is claimed to be 20 IU/mL by the manufacturer. Twenty-one tested replicates containing 50 IU/mL (1.7 $Log_{10}$ IU/mL), 25 IU/mL (1.4 $Log_{10}$ IU/mL) and 12.5 IU/mL (1.1 $Log_{10}$ IU/mL) tested HBV DNA-positive in CAP-CTM v2.0. However, in 12 out of the 21 HBV DNA-positive replicates containing 25 IU/mL and in the 21 HBV DNA-positive replicates containing 12.5 IU/mL, the result was expressed as “HBV DNA-positive, below 20 IU/mL“. Nineteen of the 21 replicates containing 6.25 IU/mL (0.8 $Log_{10}$ IU/mL) were HBV DNA-positive in the CAP/CTM v2.0 assay. The result was expressed as “below 20 IU/mL“ in all of them.

***Specificity***

All but one of the 103 HBV-seronegative specimens from group A were HBV DNA-negative in CAP-CTM v2.0, whereas one tested HBV DNA-positive (specificity: 99.0%; 95% confidence interval (95%CI): 94.7%–100%). Ninety-six out of the 97 specimens with a profile of resolved infection from group B (positive anti-HBs and anti-HBc antibodies) were found to be HBV DNA-negative, whereas one sample tested HBV DNA-positive (specificity: 99.0%; 95%CI: 94.4%–100%). In both samples with detectable HBV DNA, the result was expressed as “target detected“, below the lower limit of detection of 20 IU/mL. Both samples were retested with another real-time PCR assay (m $2000_{RT}$, Abbott Molecular, Des Plaines, Illinois) and found to be HBV DNA-negative in this assay.

***Precision and reproducibility***

As shown in Table 1 , the intra-assay coefficients of variation (precision) and inter-assay coefficients of variation (reproducibility) ranged from 1.10% to 3.07% and 0.82% to 2.95%, respectively.

**Accuracy, linear quantification and influence of the HBV genotype**

***Linear quantification of standard panel dilutions***

Quantification of the OptiQuant™ HBV DNA panel in triplicate showed a significant relationship between the average measured HBV DNA levels in the CAP/CTM v2.0 assay and the expected HBV DNA levels ($r = 0.9987$, $p < 0.0001$). The differences between the average measured and expected HBV DNA levels ranged from 0.01 to 0.36 $Log_{10}$ IU/mL, with a modest underestimation with CAP/CTM v2.0 over 5 $Log_{10}$ IU/mL (Figure 1 ).

***Quantification of HBV DNA in clinical samples containing HBV genotypes A to F***

HBV DNA levels were measured in the 51 samples from patients from group C, infected with HBV genotypes A to F. Thirty-eight of them (75%) fell within the dynamic range of quantification of the CAP/CTM v2.0 assay, whereas the remaining 13 samples (25%) had to be retested after dilution. As shown in Figure 2 , there was a significant relationship between the HBV DNA levels obtained with CAP/CTM v2.0 and the bDNA assay (Figure 2A ), and with CAP/CTM v1.0 and v2.0 (Figure 2B ), regardless of the HBV genotype.

Figure 3A shows a Bland-Altman plot of HBV DNA levels measured with the second-generation CAP/CTM and bDNA methods. The figure plots the difference between the two measured values (CAP/CTM v2.0 minus bDNA) as a function of the mean of the two measurements. A moderate underestimation of HBV DNA levels by CAP/CTM v2.0 as compared to the bDNA method was observed in 34 (66.7%) of the 51 samples containing HBV genotypes A to F (median CAP/CTM minus bDNA difference: −0.16 $Log_{10}$ IU/mL). HBV DNA levels were underestimated by CAP/CTM v2.0 as compared to bDNA in almost all samples over 5 $Log_{10}$ IU/mL (mean difference: −0.29±0.18 $Log_{10}$ IU/mL). Below 5 $Log_{10}$ IU/ml, HBV DNA levels were often moderately overestimated by CAP/CTM v2.0 as compared to the bDNA method (mean difference: +0.11±0.21 $Log_{10}$ IU/mL) (Figure 3A ). Six samples had a difference over 1.96 times the mean difference, including three (two with genotype A and one with genotype B) that were underquantified and three (one with genotype D and two with genotype E) that were overquantified with CAP/CTM v2.0 relative to bDNA. However, the individual differences between CAP/CTM v2.0 and bDNA values were always below 1.0 $Log_{10}$ IU/mL in these samples.

Box plots of individual differences between the two methods are shown for each genotype in Figure 3B . They confirm the global, moderate underestimation of HBV DNA levels by CAP/CTM v2.0 compared to the bDNA method, independently of the HBV genotype. The median differences were −0.17 $Log_{10}$ IU/mL for genotype A, − 0.37 $Log_{10}$ IU/mL for genotype B, −0.32 $Log_{10}$ IU/mL for genotype C, +0.05 $Log_{10}$ IU/mL for genotype D, and −0.03 $Log_{10}$ IU/mL for genotype E (not significant). HBV genotype F is not shown in Figure 3B because only three samples were tested.

***HBV DNA monitoring in patients receiving antiviral therapy***

Sixteen patients chronically infected with HBV (4 with genotype A, 5 with genotype C, 1 with genotype D and 6 with genotype E) included in group D were serially sampled on treatment with nucleoside/nucleotide analogues. For these patients, 51 plasma and 51 serum specimens sampled at the same time were available. The 51 plasma samples were tested in parallel with CAP/CTM v1.0, CAP/CTM v2.0 and bDNA, while the 51 serum samples were tested with CAP/CTM v2.0 and bDNA. As shown in Table 2 , CAP/CTM v2.0 equally quantified HBV DNA in plasma or serum (mean difference range: 0.04 to 0.11 $Log_{10}$ IU/mL, according to the HBV genotype). As shown in Table 3 , both versions of the CAP/CTM assay equally quantified HBV DNA levels in plasma (mean difference range: 0.04 to 0.20 $Log_{10}$ IU/mL, according to the HBV genotype).

Figure 4 shows individual examples of HBV DNA level kinetics measured with the two versions of the CAP/CTM assay and bDNA in patients infected with HBV genotypes A, C, D and E. The differences between CAP/CTM v2.0 and bDNA were always less than 0.5 $Log_{10}$ IU/mL, except in two patients. Indeed, in one patient infected with genotype C (Pt non 7) and in one patient infected with genotype E (Pt non 11), HBV DNA levels were underestimated by CAP/CTM v2.0 by −1.38 and − 1.41 $Log_{10}$ IU/mL on average, respectively, relative to the third-generation bDNA assay (Figure 4 ).

***Target sequence analysis in clinical specimens from patients included in the longitudinal analysis***

The nearly full-length preC-C region of the HBV genome, which is the target of CAP/CTM primers and probes, was sequenced in the 16 patients from group D in order to determine the possible role of nucleotide sequence polymorphisms in the underestimation of HBV DNA levels in patients 7 and 11. In spite of the presence of polymorphisms in the pre-C region, no nucleotide signature was found to explain underestimation of HBV DNA levels in the two patients (data not shown).

## DISCUSSION

Real-time PCR assays have become the reference for quantification of viral genomes in clinical practice. The most recent international guidelines on management of HBV therapy recommend the use of real-time PCR assays to detect and quantify HBV DNA in order to diagnose HBV infection, orientate the indication of therapy and monitor antiviral treatment responses and resistance (4 , 8 ). The first-generation CAP/CTM assay showed excellent analytical performance (2 ), but could be used only on plasma specimens. A second generation was recently developed. In this second generation, HBV DNA levels can be measured in both plasma and serum from only 650 µL of material, and the assay has a claimed lower limit of detection of 20 IU/mL.

In the present study, we showed that CAP/CTM v2.0 has an excellent analytical sensitivity, with a lower limit of detection of the order of the value claimed by the manufacturer, i.e. 20 IU/mL. This assay was also precise and reproducible, as previously reported (6 ). In addition, we observed a strong, significant relationship between HBV DNA levels measured by CAP/CTM v2.0 and the third-generation bDNA-based assay. The use of the third-generation bDNA assay as a comparator was justified by the fact that this assay is accurate,

precise, reproducible, well-calibrated to the World Health Organization HBV DNA standard and quantifies HBV DNA levels independently of the HBV genotype, due to the presence of a large number of capture and extender probes located at various positions along the HBV genome (7 , 11 ). There was also an excellent correspondence between versions 1.0 and 2.0 of the CAP/CTM assay in plasma in our experiments, regardless of the HBV genotype.

Similarly to the first-generation CAP/CTM assay, we observed a modest underestimation of HBV DNA levels in the 6 members of the standard panel, mainly above an HBV DNA level of 5.0 $Log_{10}$ IU/mL. This finding was confirmed in clinical samples when CAP/CTM v2.0 values were compared to values obtained with the bDNA assay. This modest underestimation was independent of the HBV genotype and has no implications in clinical practice. Indeed, in contrast with HCV therapy, where accurate quantification is needed to make treatment decisions (3 ), this technical issue has little clinical implication in HBV therapy with nucleoside/nucleotide analogues, as the goal of this treatment is to maintain HBV DNA levels in the long term below the lower limit of quantification of the assay.

In two cases, however, we observed a substantial underestimation of HBV DNA levels in CAP/CTM relative to bDNA. These samples contained HBV genotypes C and E, respectively. The underestimations were consistent in several serial samples from the two patients, suggesting that they were due to the nature of the infecting HBV strain in these patients. This may be seen when mismatches occur between the primers and/or the Taqman probe and the target viral sequence as a result of natural nucleotide polymorphisms (1 ). We thus sequenced the target region (preC-C) in order to identify whether nucleotide polymorphisms could differentiate the patients who were equally quantified in CAP/CTM and bDNA from these two individuals. No sequence signature has been identified that could explain underestimation of HBV DNA levels in these two patients.

The main improvement in the second generation of the CAP/CTM assay is the possibility to quantify HBV DNA in both serum and plasma, whereas the first-generation assay was for plasma only. We thus assessed whether values obtained in plasma with both versions of the assay and in serum and plasma with CAP/CTM v2.0 were concordant. As shown here, there was an excellent correspondence between the values obtained in different matrices. In addition, serial follow-up in patients from group D showed almost perfect superimposition in all cases, confirming that CAP/CTM v2.0 can be used equally on serum or plasma samples in clinical practice.

In conclusion, this study shows that the new version of the CAP/CTM assay is sensitive, specific and reproducible, and that it accurately quantifies HBV DNA in both plasma and serum samples from patients with chronic HBV infection. Quantification is linear over the full dynamic range of quantification, which covers values observed in both treated and untreated patients with chronic hepatitis B. However, the upper limit of quantification (8.2 $Log_{10}$ IU/mL) is still too low to cover the full range of possible values. Any sample falling above the upper limit of quantification of the assay needs to be retested after dilution, a step that does not affect quantification. In our hands, the new version of the CAP/CTM assay appeared to be suitable for large-scale routine analysis of samples containing HBV genotypes A to F. Broad use of fully automated real-time PCR assays, as recommended in recent international guidelines for the management of HBV therapy (4 , 8 ), will improve the management of patients with chronic HBV infection, as well as the monitoring of antiviral responses and drug resistance.

## Ackowledgements:

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**Figure 1**

CAP/CTM v2.0 quantification of a commercial standard panel containing $2 \times 10^2$ (2.3 $Log_{10}$) to $2 \times 10^7$ (7.3 $Log_{10}$) HBV DNA IU/mL (OptiQuant™ HBV DNA, AcroMetrix, Benicia, California). The average measured values are shown as a function of the expected values (actual HBV DNA content of the panel member). The dashed line is the equality line.

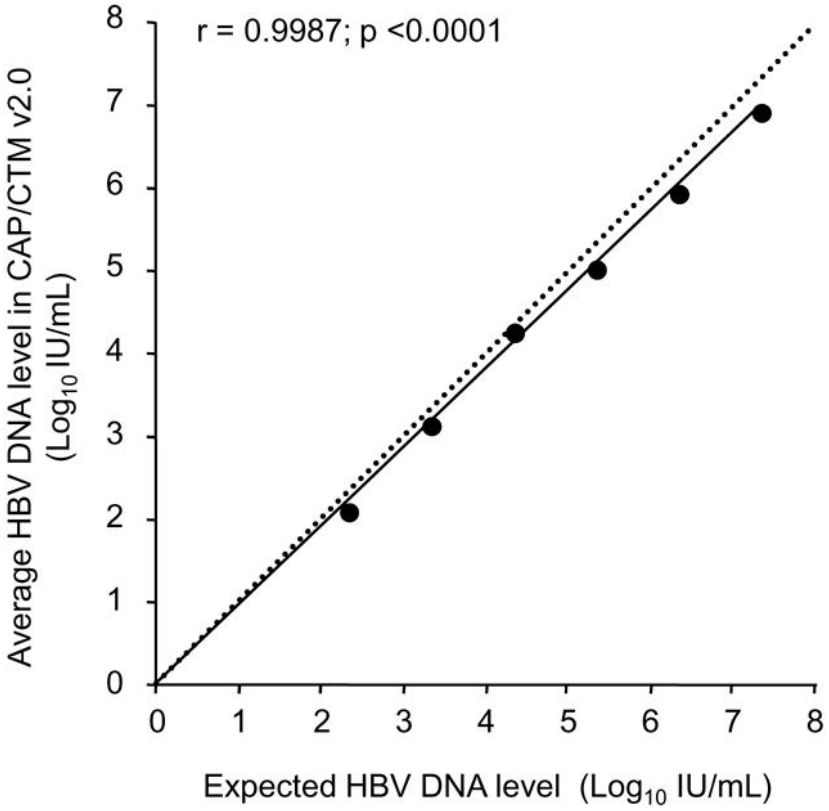

**Figure 2**

Correlation between HBV DNA levels measured by CAP/CTM v2.0 and bDNA (A), and CAP/CTM v2.0 and v1.0 (B) in 51 clinical samples (group C) containing HBV genotypes A (n=12), B (n=9), C (n=8), D (n=9), E (n=10) and F (n=3).

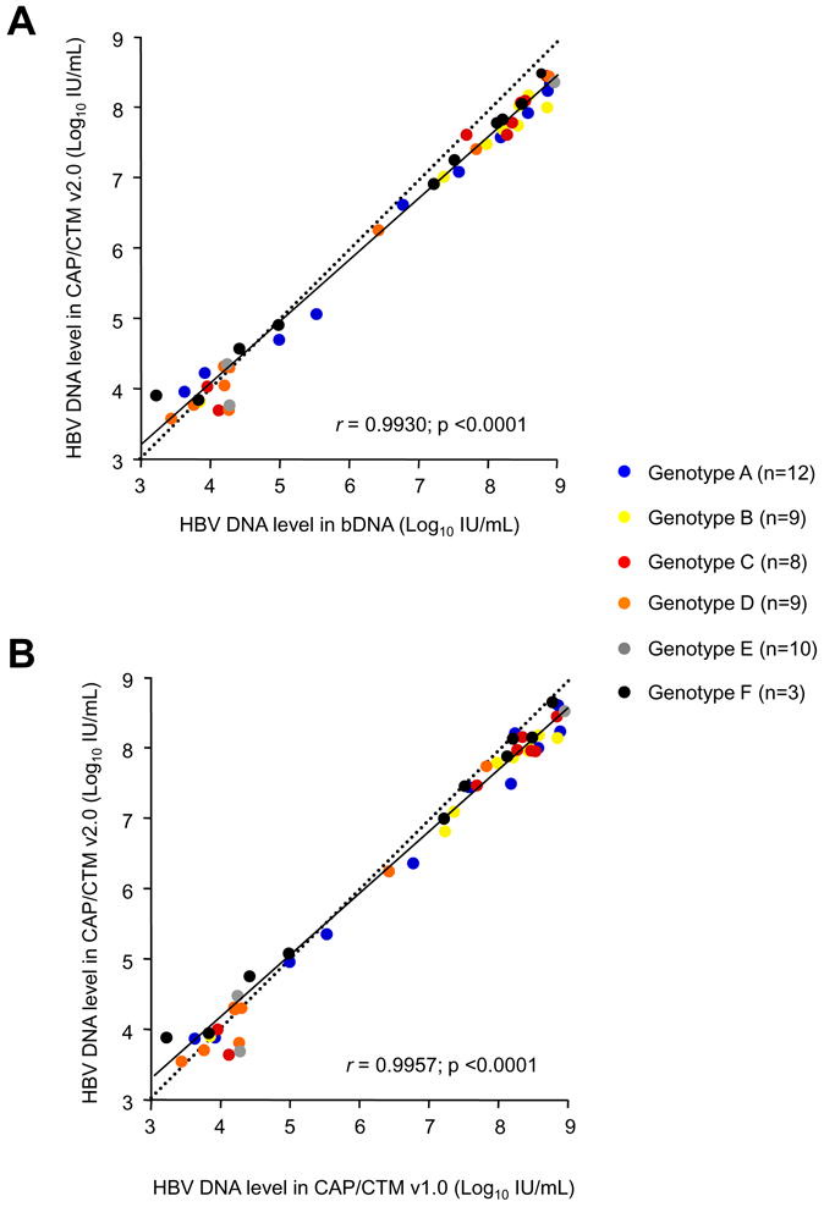

**Figure 3**

(A) Bland-Altman plot of HBV DNA levels measured by CAP/CTM v2.0 and bDNA in the 51 group B samples. The difference between HBV DNA levels measured by CAP/CTM v2.0 and bDNA is represented as a function of the mean of the two values. Different genotypes are represented by different colors. The gray area corresponds to the mean difference ±1.96 standard deviation. (B) Distribution of the differences between HBV DNA levels measured by CAP/CTM v2.0 and bDNA in the same samples, according to the HBV genotype (A to E). The difference was not significant.

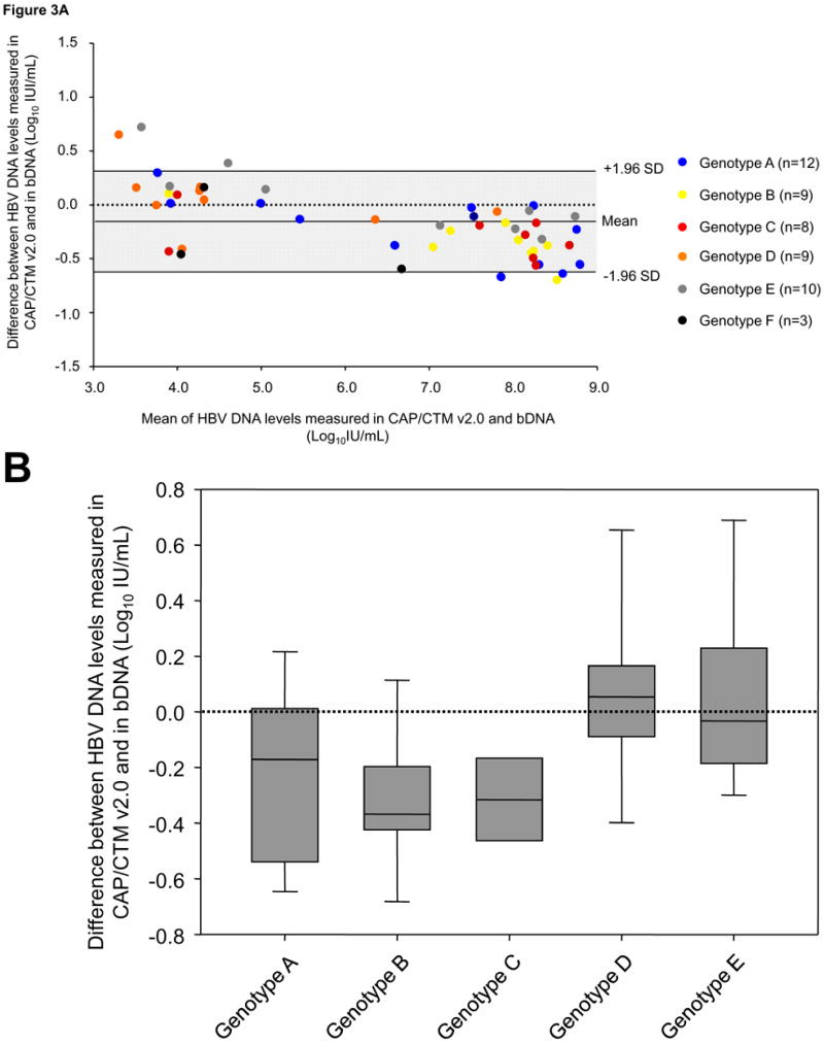

**Figure 4**

HBV DNA level kinetics measured in individual patients' plasma specimens with CAP/CTM v1.0 (black line, white circle), CAP/CTM v2.0 (black line, black circle) and bDNA (dashed line). The top and bottom gray areas correspond to the lower limit of detection of bDNA (2.55 $Log_{10}$ IU/mL) and CAP/CTM v2.0 (1.30 $Log_{10}$ IU/mL), respectively.

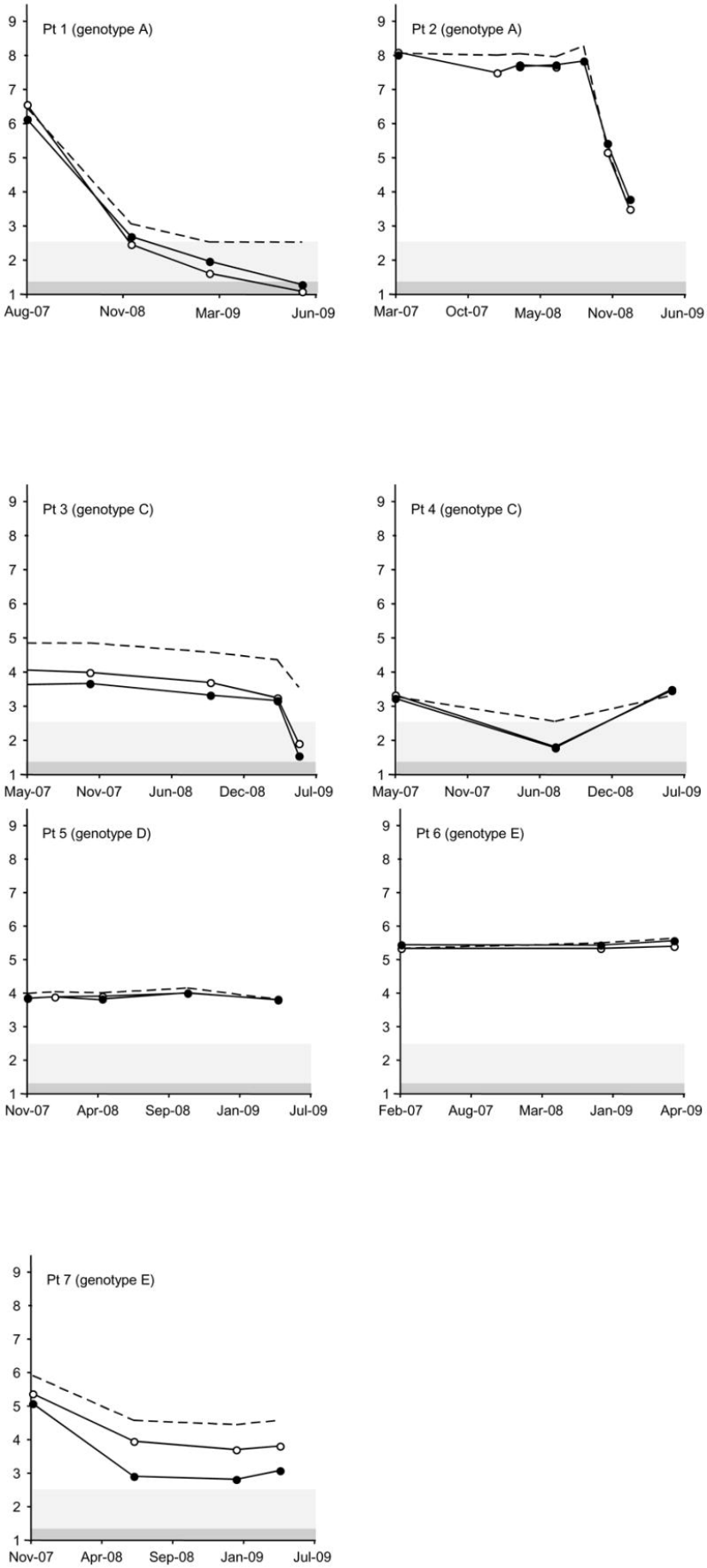

**Table 1**

Intra-assay (precision) and inter-assay reproducibility of CAP/CTM v2.0. For intra-assay reproducibility, the seven members of the standard panel (NAP-000 to NAP-HBV2E7) containing no HBV DNA up to $2 \times 10^7$ IU/mL, i.e. 7.3 $Log_{10}$ IU/mL, respectively, have been tested in triplicate in the same experiment. For inter-assay reproducibility, the assay low positive control (LPC) and high positive control (HPC) have been tested 18 times in different experiments.

| | Panel Standard Controls | Target HBV DNA ($Log_{10}$ IU/mL) | Number of determinations | Mean (SD) measured HBV DNA ($Log_{10}$ IU/mL) | Coefficient of variation (%) |
|---|---|---|---|---|---|
| **Intra-assay reproducibility** | NAP-000 | 0.00 | 3 | Target not detected | - |
| | NAP-HBV2E2 | 2.30 | 3 | 2.13 (0.06) | 2.58 |
| | NAP-HBV2E3 | 3.30 | 3 | 3.17 (0.06) | 1.98 |
| | NAP-HBV2E4 | 4.30 | 3 | 4.29 (0.01) | 3.07 |
| | NAP-HBV2E5 | 5.30 | 3 | 5.06 (0.03) | 1.10 |
| | NAP-HBV2E6 | 6.30 | 3 | 5.96 (0.02) | 1.58 |
| | NAP-HBV2E7 | 7.30 | 3 | 6.94 (0.08) | 1.16 |
| **Inter-assay reproducibility** | LPC[a] | variable[a] | 18 | 2.45 (0.07) | 2.95 |
| | HPC[a] | variable[a] | 18 | 6.01 (0.05) | 0.82 |

**a** Two kit lots have been used, with LPC values between 2.1 and 3.1 $Log_{10}$ IU/mL, and 2.0 and 3.0 $Log_{10}$ IU/mL, respectively, and HPC values between 5.7 and 6.7 $Log_{10}$ IU/mL, and 5.5 and 6.5 $Log_{10}$ IU/mL, respectively.

**Table 2**

Mean differences between HBV DNA levels measured with CAP/CTM v2.0 in serum and plasma specimens from the 51 patients with chronic HBV infection from group D (6 patients with genotype A, 5 with genotype B, one with genotype D and 6 with genotype E).

| HBV genotype | N | Mean difference ± SD ($Log_{10}$ IU/mL) |
|---|---|---|
| Genotype A | 16 | 0.11 ± 0.14 |
| Genotype C | 14 | 0.09 ± 0.17 |
| Genotype D | 5 | 0.09 ± 0.12 |
| Genotype E | 16 | 0.04 ± 0.16 |

**Table 3**

Mean differences between HBV DNA levels measured with CAP/CTM v1.0 and v2.0 in plasma specimens from the 51 patients with chronic HBV infection from group D (6 patients with genotype A, 5 with genotype B, one with genotype D and 6 with genotype E).

| HBV genotype | N | Mean Difference ± SD (in $Log_{10}$ IU/mL) |
|---|---|---|
| Genotype A | 16 | 0.04 ± 0,24 |
| Genotype C | 14 | 0.10 ± 0.22 |
| Genotype D | 5 | 0.04 ± 0.05 |
| Genotype E | 16 | 0.20 ± 0.34 |