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**PERFORMANCE OF THE COBAS AMPLIPREP/COBAS TAQMAN  
(CAP/CTM) REAL-TIME POLYMERASE CHAIN REACTION ASSAY  
FOR HEPATITIS B VIRUS DNA QUANTIFICATION**

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**Running Title: CAP/CTM FOR HBV DNA QUANTIFICATION**

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## ABSTRACT

Hepatitis B virus (HBV) DNA quantification is used to establish the prognosis of chronic HBV-related liver disease, to identify those patients who need to be treated, and to monitor the virologic response and resistance to antiviral therapies. Real-time polymerase chain reaction (PCR)-based assays are gradually replacing other technologies for routine quantification of HBV DNA in clinical practice. The goal of this study was to evaluate the intrinsic characteristics and clinical performance of the real-time PCR Cobas Ampliprep/Cobas Taqman (CAP/CTM) platform for HBV DNA quantification. Specificity was satisfactory (95% confidence interval: 98.1-100%). Intra-assay coefficients of variation ranged from 0.22% to 2.68% and inter-assay coefficients of variation from 1.31% to 4.13%. Quantification was linear over the full dynamic range of quantification of the assay (1.7 to 8.0 Log<sub>10</sub> international units (IU)/ml) and was not affected by dilution. The assay was accurate, regardless of the HBV genotype. Samples containing HBV DNA levels above 4.5 Log<sub>10</sub> IU/ml were slightly underestimated relative to another accurate assay based on branched DNA technology, but this is unlikely to have noteworthy clinical implications. Thus, the CAP/CTM HBV DNA assay is sensitive, specific and reproducible, and accurately quantifies HBV DNA in patients chronically infected by HBV genotypes A to F. Samples with HBV DNA concentrations above the upper limit of quantification need to be diluted then retested. Broad use of fully automated real-time PCR assays should improve the management of patients with chronic HBV infection.

## INTRODUCTION

The presence of hepatitis B virus (HBV) DNA in peripheral blood is a reliable marker of active HBV replication. HBV DNA quantification is the best method for monitoring the level of HBV replication in chronically infected patients. Detection and quantification of HBV DNA are useful: (i) for establishing the prognosis of liver disease, notably the risk of progression towards cirrhosis and hepatocellular carcinoma, (ii) for identifying patients who need antiviral therapy, and (iii) for monitoring the virologic response and resistance to antiviral therapies (reviewed in (10)). Commercial assays have been available for several years for detecting and quantifying HBV DNA in clinical practice. Most of them, however, have not been calibrated to the World Health Organization (WHO) standard (15) and still use the nonstandardized “copies/ml”, whereas international units (IU/ml) should be preferred (10). These assays are based either on signal amplification following molecular hybridization or on target amplification. Signal amplification assays include the Digene Hybrid-Capture assay and assays based on branched DNA (bDNA) technology (Versant™ HBV DNA 3.0 Assay, Siemens Medical Solutions Diagnostics, Tarrytown, New York). Target amplification techniques are based on the polymerase chain reaction (PCR) and include the Amplicor HBV Monitor® assay (Roche Molecular Systems, Pleasanton, California) and its semi-automated version Cobas® Amplicor HBV Monitor® (reviewed in (11)).

HBV DNA quantification assays should ideally be sensitive, specific, precise, reproducible, well calibrated (accurately providing HBV DNA levels in IU/ml), automated, and rapid, with minimal hands-on time (20). They should also have clinically relevant dynamic ranges of quantification and should be able both to detect

and to quantify HBV DNA, regardless of the HBV genotype or sequence polymorphisms. None of the commercially available HBV DNA assays based on signal amplification or classical PCR meet all these criteria (10, 11). The recent development of “real-time” PCR techniques represents a major advance in the field of viral genome quantification (1, 6, 7, 22). Indeed, these new methods are, at least theoretically, more sensitive than classical PCR techniques, they are not prone to carryover contamination, and they generally have a wide dynamic range of quantification, meaning they can be used to quantify the full range of viral genome levels observed in treated and untreated patients. Real-time PCR is gradually replacing other technologies for routine quantification of HBV DNA in clinical practice.

A frequently used commercial real-time PCR assay for HBV DNA quantification is the Cobas Ampliprep/Cobas Taqman (CAP/CTM) platform (Roche Molecular System) (8). CAP/CTM combines the Cobas Ampliprep<sup>®</sup> instrument, that performs fully automated extraction of HBV DNA from 850  $\mu$ l of patient plasma, and the Cobas Taqman 48<sup>®</sup> Analyzer that performs fully automated real-time PCR amplification and detection, followed by interpretation of HBV DNA levels by means of Amplilink<sup>®</sup> software. The manufacturer’s lower limit of detection (LOD) for the HBV DNA assay is 12 IU/ml (1.1 Log<sub>10</sub> IU/ml) and the dynamic range of quantification is from 54 to 1.1 x 10<sup>8</sup> IU/ml (1.7 to 8.0 Log<sub>10</sub> IU/ml).

The goal of this study was to evaluate the intrinsic characteristics and clinical performance of the CAP/CTM assay for HBV DNA quantification.

## **MATERIALS AND METHODS**

### **Materials**

**Standards.** A standard panel of plasma samples (OptiQuant™ HBV DNA, AcroMetrix, Benicia, California) containing different concentrations of HBV DNA from a single source (a patient chronically infected by HBV genotype A) was used to study the analytical performance of the assay. The seven panel samples are designated NAP-000, NAP-HBV2E2, NAP-HBV2E3, NAP-HBV2E4, NAP-HBV2E4, NAP-HBV2E5, NAP-HBV2E6 and NAP-HBV2E7, and contain 0,  $2 \times 10^2$  IU/ml ( $2.3 \text{ Log}_{10}$  IU/ml),  $2 \times 10^3$  IU/ml ( $3.3 \text{ Log}_{10}$  IU/ml),  $2 \times 10^4$  IU/ml ( $4.3 \text{ Log}_{10}$  IU/ml),  $2 \times 10^5$  IU/ml ( $5.3 \text{ Log}_{10}$  IU/ml),  $2 \times 10^6$  IU/ml ( $6.3 \text{ Log}_{10}$  IU/ml) and  $2 \times 10^7$  IU/ml HBV DNA ( $7.3 \text{ Log}_{10}$  IU/ml), respectively.

**Clinical specimens.** Plasma samples were obtained from patients managed in the Department of Hepatology and Gastroenterology of Henri Mondor Hospital and from blood donors diagnosed with HBV infection at the Institut National de la Transfusion Sanguine. Group A comprised 205 HBV-seronegative individuals, i.e. subjects with neither HBs antigen (HBsAg) nor anti-HBc antibodies in a third-generation enzyme immunoassay (Vitros ECi, Ortho-Clinical Diagnostics, Raritan, New Jersey). Group B comprised 52 patients with chronic HBV infection, who all had detectable HBsAg, anti-HBc antibodies and HBV DNA. Initial genotype determination was done with the INNO-LiPA HBV genotyping assay (Innogenetics, Gent, Belgium), a reverse hybridization assay that targets a portion of the sequence encoding both HBsAg and subdomains B and C of the reverse transcriptase domain of the HBV DNA polymerase (overlapping open reading frame). The HBV genotype was confirmed in all cases by sequencing the gene encoding both HBsAg and the HBV polymerase, followed by phylogenetic analysis. Based on these analyses, group B

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. Plasma was separated from whole blood by centrifugation, placed in sterile tubes and stored at -70°C until use in this study.

### **Assessment of CAP/CTM performance.**

*Specificity.* The specificity of the CAP/CTM assay was assessed by testing the 205 HBV-seronegative clinical specimens from group A.

*Linearity, accuracy and influence of the HBV genotype.* The linearity of quantification by CAP/CTM was assessed by testing the seven members of the OptiQuant™ HBV DNA standard panel, which contain up to  $2 \times 10^7$  IU/ml (7.3 Log<sub>10</sub> IU/ml). Each panel member dilution was tested three times in the same experiment with both CAP/CTM and the third-generation bDNA-based Versant® HBV DNA 3.0 assay. This assay has a dynamic range of quantification between 357 and 17,857,000 IU/ml (2.5 to 7.3 Log<sub>10</sub> IU/ml). The average measured values were then compared with the expected values. In addition, serial one-fifth dilutions down to signal extinction were tested in 12 genotype A, 9 genotype B, 8 genotype C, 9 genotype D, 9 genotype E and 3 genotype F samples from group B. The dilutions were made with the Nucleic Acid Test (NAT) dilution matrix (AcroMetrix), a defibrinated, delipidized normal human plasma. We compared the results of CAP/CTM with those obtained for the same samples with the third-generation bDNA-based assay in the 52 group B samples. This latter assay has been shown to be precise and accurate and to equally quantify HBV genotypes A to F, through the use of a set of 16 capture and 65 extender oligonucleotide probes spanning the full-length HBV genome.

*Precision and reproducibility.* In order to assess precision (intra-assay reproducibility), each member of the OptiQuant™ HBV DNA panel was tested in triplicate. In order to assess inter-assay reproducibility, the low positive control (LPC) and the high positive control (HPC) provided with the kits were tested 35 times in the corresponding runs on different days.

### **HBV DNA quantification.**

*CAP/CTM.* HBV DNA was extracted from 850 µl of plasma by the automated extractor Cobas AmpliPrep®, according to the manufacturer's instructions. The Cobas TaqMan 48® Analyzer was used for automated real-time PCR amplification and detection of PCR products according to the manufacturer's instructions. The data thus generated were analyzed with Amplilink® software. HBV DNA levels were expressed in IU/ml.

*bDNA.* In the Versant HBV DNA 3.0 Assay, HBV DNA was recovered from 50 µl of plasma 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.

**HBV genotype determination.** The HBV genotype was determined by directly sequencing a portion of the overlapping genes encoding HBsAg and the B and C subdomains of the reverse transcriptase domain of HBV polymerase, followed by phylogenetic analysis.

*DNA extraction.* DNA was extracted from 200 µl of plasma by using the QIAamp® MinElute™ Virus Vacuum Purification kit (Qiagen, Hilden, Germany),



according to the manufacturer's instructions. The DNA pellet was eluted with 150  $\mu$ l of RNase-free water containing 0.04% NaN<sub>3</sub> (w/v) and stored at -20°C until analysis.

*PCR amplification.* A hemi-nested PCR procedure was used to amplify a 492-base-pair (bp) fragment. The first round used external sense and antisense primers POL-1 and POL-2 (13). Five microliters of DNA was added to the PCR mixture containing GeneAmp<sup>®</sup> 10X PCR Gold buffer and 10 pmol of the primers plus AmpliTaq Gold<sup>®</sup> DNA polymerase (Applied Biosystems, Foster City, California). Amplification included an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 30 s, and elongation at 72°C for 1 min, followed by a final elongation step at 72°C for 10 min. The second-round PCR used the same sense primer POL-1 and the internal antisense primer HBPr-94 (18) and included an initial denaturation step at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 1 min, followed by a final elongation step at 72°C for 10 min. Amplification products (5  $\mu$ l) were run on 1.5% agarose gels. The gels were stained with ethidium bromide and DNA was viewed under UV light.

*Purification of PCR products.* The PCR products were purified using Microcon 100 centrifugal filters (Millipore, Bedford, Massachusetts) according to the manufacturer's instructions. Amplicons were eluted in 20  $\mu$ l of sterile water and stored at -20°C until analysis.

*Sequencing of PCR products.* PCR products were directly sequenced in both directions by using the Big-Dye Terminator Cycle v3.1 sequencing kit on the ABI 3100 sequencer (Applied Biosystems) according to the manufacturer's protocol; the primers used for sequencing were HBPr94 and POL1.

*Phylogenetic analysis.* The sequences were aligned by the Clustal W program (19), together with prototype sequences of HBV genotypes A to H obtained from GenBank (16, 17). Phylogenetic relationships were inferred by the DNADIST and NEIGHBOR modules of PHYLIP (Phylogeny Inference Package) version 3.65. For neighbor-joining analysis, a distance matrix was calculated as described by Kimura, using a transition to transversion ratio of 2.0. Trees were drawn with TREEVIEW or NJPlot (9).

**Statistical analysis.** Descriptive statistics are shown as the mean  $\pm$  standard deviation (SD) or the median and interquartile range as appropriate. Comparisons between groups were made using the Kruskal-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 assay**

*Specificity.* The specificity of CAP/CTM was assessed by testing 205 samples from anti-HBV-seronegative patients (group A). None of these sample tested positive (above the LOD of 12 IU/ml), the results being expressed as “target not detected“ in every case (specificity: 100%, 95% confidence interval: 98.1-100%).

*Precision and reproducibility.* Precision (intra-assay reproducibility) was assessed by testing the seven members of the OptiQuant™ HBV DNA standard

range, that contain 0, 2.3, 3.3, 4.3, 5.3, 6.3 and 7.3 Log<sub>10</sub> IU/ml, respectively, three times in the same experiment. As shown in Table 1, the coefficients of variation ranged from 0.22% to 2.68%. Inter-assay variability was assessed by testing both the high positive control (HPC) and the low positive control (LPC) 35 times in different experiments. The coefficients of variation were 1.31% and 4.13%, respectively (Table 1).

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

*Linear quantification of standard panel dilutions.* The OptiQuant™ HBV DNA genotype A standard panel, composed of samples containing  $2 \times 10^2$  IU/ml (2.3 Log<sub>10</sub> IU/ml) to  $2 \times 10^7$  IU/ml (7.3 Log<sub>10</sub> IU/ml), was used to assess the linearity of HBV DNA quantification in the CAP/CTM assay. The panel was tested three times in the same experiment with both the CAP/CTM and bDNA assays. As shown in Figure 1A, a significant relationship was found between the average measured HBV DNA levels and the expected levels in the CAP/CTM assay ( $r = 0.9988$ ,  $p < 0.0001$ ). The difference between the average measured and expected HBV DNA levels ranged from 0.13 to 0.52 Log<sub>10</sub> IU/ml, and was larger for the highest HBV DNA levels. A significant relationship was also found between the average measured and expected HBV DNA levels in the bDNA assay ( $r = 0.9995$ ,  $p < 0.0001$ ; Figure 1B). The difference between the average measured and expected HBV DNA levels ranged from 0.00 to 0.13 Log<sub>10</sub> IU/ml. The HBV DNA level of two standards, NAP-000 and NAP-HBV2E2, was below the LOD of the bDNA assay (357 IU/ml, i.e. 2.55 Log<sub>10</sub> IU/ml).

*Quantification of HBV DNA in clinical samples containing HBV genotypes A to F.* Fifty-two samples from patients with chronic hepatitis B due to genotypes A to F (group B, see Methods) were tested with both the CAP/CTM and the third-generation bDNA assay. All these samples were within the dynamic range of quantification of both assays. As shown in Figure 2, there was a significant relationship between the HBV DNA levels obtained in each sample with CAP/CTM and bDNA methods, whatever the HBV genotype. The regression lines were always slightly below the expected equality line, as a result of lower values obtained with CAP/CTM than with the bDNA method for most samples, which were usually in the higher range of HBV DNA levels (Figure 2).

Figure 3A shows a Bland-Altman plot of HBV DNA levels measured in the 52 group B samples with the CAP/CTM and bDNA methods. The figure plots the difference between the two measured values (CAP/CTM minus bDNA) as a function of the mean of the two measurements. A moderate underestimation of HBV DNA levels by CAP/CTM as compared to the bDNA method was observed with 39 (75.0%) of the 52 samples, containing all HBV genotypes (median difference, CAP/CTM minus bDNA:  $-0.36 \text{ Log}_{10} \text{ IU/ml}$ ). HBV DNA levels were underestimated by CAP/CTM as compared to bDNA in almost all samples over  $4.5 \text{ Log}_{10} \text{ IU/ml}$  (on average  $-0.42 \pm 0.19 \text{ Log}_{10} \text{ IU/ml}$  for samples over  $4.5 \text{ Log}_{10} \text{ IU/ml}$ ). Below  $4.5 \text{ Log}_{10} \text{ IU/ml}$ , HBV DNA levels were often moderately overestimated by CAP/CTM as compared to the bDNA method (average difference:  $+0.11 \pm 0.35 \text{ Log}_{10} \text{ IU/ml}$ , significantly different from the average difference above  $4.5 \text{ Log}_{10} \text{ IU/ml}$ ,  $p < 0.0001$ ) (Figure 3A). None of the samples had a CAP/CTM minus bDNA difference less than  $-1.96$  times the mean difference, whereas four samples (two genotype A, one genotype D and one genotype E) had a CAP/CTM minus bDNA difference more than  $+1.96$  times the

mean difference. However, the individual differences between CAP/CTM and bDNA values were always below 1.0 Log<sub>10</sub> 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 compared to the bDNA method, independently of the HBV genotype. The median differences were -0.50 Log<sub>10</sub> IU/ml for genotype A, -0.40 Log<sub>10</sub> IU/ml for genotype B, -0.38 Log<sub>10</sub> IU/ml for genotype C, -0.03 Log<sub>10</sub> IU/ml for genotype D, and -0.25 Log<sub>10</sub> IU/ml for genotype E (differences not significant). HBV genotype F is not shown in Figure 3B because only three samples were tested.

Figure 4 shows the distribution of individual CAP/CTM minus bDNA differences for each HBV genotype (represented by different colors). This figure confirms the global, modest underestimation by the CAP/CTM assay relative to the bDNA method, independently of the HBV genotype. In the majority of cases, underestimation was less than -0.5 Log<sub>10</sub> IU/ml, and it was never more than -1.0 Log<sub>10</sub> IU/ml (11 samples had a difference between -0.54 and -0.83 Log<sub>10</sub> IU/ml). Overestimation relative to bDNA values was rare and exceeded +0.5 Log<sub>10</sub> IU/ml in only two cases (Figure 4).

*Linear quantification of serial dilutions of HBV-infected plasma.* Serial one-fifth dilutions down to signal extinction were tested for 12 genotype A, 9 genotype B, 8 genotype C, 9 genotype D, 9 genotype E and 3 genotype F samples from group B. The curves were always linear, whatever the HBV genotype, with significant Pearson's coefficients ranging from 0.9916 to 0.9994 for HBV genotype A, 0.9978 to 0.9988 for HBV genotype B, 0.9982 to 0.9999 for HBV genotype C, 0.9919 to 0.9999 for HBV genotype D, 0.9927 to 0.9992 for HBV genotype E and 0.9946 to 0.9997 for

HBV genotype F. Figure 5 shows individual examples of HBV DNA levels measured by CAP/CTM in serial 1/5 dilutions of samples containing HBV genotypes A, B, C, D, E and F. The expected difference between two successive 1/5 dilutions was 0.70  $\text{Log}_{10}$  IU/ml. The mean $\pm$ SD differences between the undiluted sample and the first 1/5 dilution, and between each dilution and the subsequent dilution were 0.76 $\pm$ 0.14, 0.68 $\pm$ 0.09, 0.64 $\pm$ 0.11, 0.69 $\pm$ 0.09, 0.77 $\pm$ 0.10, 0.77 $\pm$ 0.13 and 0.78 $\pm$ 0.16, respectively, for genotypes A, B, C, D, E and F (no significant difference).

## DISCUSSION

This analysis of the intrinsic performance of the CAP/CTM HBV DNA quantification assay shows its excellent specificity. The CAP/CTM assay was also precise and reproducible, as previously reported (2, 5, 14). Although the LOD of the CAP/CTM assay was not specifically validated here, the fact that all the standards containing more than the stated LOD (i.e. 12 IU/ml) tested positive suggests that the analytical sensitivity of the assay is in keeping with that stated by the manufacturer. We observed a strong, significant relationship between HBV DNA levels obtained in a given sample by CAP/CTM and by the third-generation bDNA-based assay, regardless of the HBV genotype. The use of the third-generation bDNA assay as the comparator was justified by the fact this assay is accurate, precise and reproducible, is well calibrated to the WHO HBV DNA standard (as confirmed in this study, see Figure 1B), quantifies HBV DNA independently of the HBV genotype, and is relatively immune to sequence polymorphisms, owing to the use of a large number of capture and amplification probes located at various positions along the HBV genome (4, 12, 21).

Recently, we published an extensive analysis of the performance of the CAP/CTM assay for hepatitis C virus (HCV) RNA quantification (3) and identified two technical issues with possible clinical implications: (i) a substantial global overestimation of HCV RNA levels, of the order of +0.5 to +0.7 Log<sub>10</sub> IU/ml, likely due to the presence of substances interfering with the real-time PCR reaction in patients' blood, and (ii) marked underestimation of HCV RNA levels in approximately 15% of patients infected by HCV genotype 2 and 30% of those infected by HCV genotype 4, likely owing to mismatches in the primers and/or probe hybridization regions, related to natural polymorphisms (3). Neither problem was observed with the CAP/CTM HBV DNA assay. In particular, HBV DNA quantification was not affected by plasma dilution.

In contrast with the HCV assay, we observed modest underestimation of HBV DNA levels in the six members of the standard panel, mainly above an HBV DNA level of 4.5 Log<sub>10</sub> IU/ml. A similar modest underestimation of HBV DNA levels above 4.5 Log<sub>10</sub> IU/ml was observed in clinical samples relative to the bDNA assay, the calibration of which we confirmed as excellent (Figure 1B). This underestimation was independent of the HBV genotype. It is not surprising, as target amplification methods generally cannot reach the same level of accuracy as hybridization-based methods, this being the price to pay for better analytical sensitivity and broader dynamic ranges. Overall, the underestimation of HBV DNA levels was modest and should have no noteworthy clinical implications.

In conclusion, this study shows that the CAP/CTM HBV DNA assay is sensitive, specific and reproducible, and that it accurately quantifies HBV DNA in 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 ( $8.0 \text{ Log}_{10} \text{ IU/ml}$ ) is still too low to cover the full range of possible values, that may reach very high levels in some cases. Therefore, any sample above the upper limit of quantification will need to be retested after dilution, a step that does not affect quantification. In our hands, CAP/CTM appeared to be suitable for large-scale routine analysis of samples containing HBV genotypes A to F. The impact of occasional nucleotide polymorphisms remains to be tested. Broad use of fully automated real-time PCR assays should improve the management of patients with chronic HBV infection, as well as the monitoring of antiviral therapy and drug resistance.

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## FIGURE LEGENDS

**Figure 1.** CAP/CTM (A) and bDNA (B) quantification of a commercial standard panel containing  $2 \times 10^2$  ( $2.3 \text{ Log}_{10}$ ) to  $2 \times 10^7$  ( $7.3 \text{ Log}_{10}$ ) 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 and bDNA in 52 clinical samples (group B) containing HBV genotypes A (Figure 2A), B (2B), C (2C), D (2D), E (2E) and F (2F).

**Figure 3.** (A) Bland-Altman plot of HBV DNA levels measured by CAP/CTM and bDNA in the 52 group B samples. The difference between HBV DNA levels measured by CAP/CTM 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  $\pm 1.96$  standard deviation. (B) Distribution of the differences between HBV DNA levels measured by CAP/CTM and bDNA in the same samples, according to the HBV genotype (A to E). The difference was not significant.

**Figure 4.** Individual differences between HBV DNA levels measured by CAP/CTM and bDNA, ranked in increasing order of bDNA values, according to the HBV genotype. Different HBV genotypes are represented by different colors. Bars above the 0 line correspond to samples for which the CAP/CTM value was higher than the

bDNA value, whereas bars below the 0 line correspond to samples for which the CAP/CTM value was lower than the bDNA value.

**Figure 5.** HBV DNA levels measured by CAP/CTM in serial one-fifth dilutions of patients' clinical samples containing HBV genotypes A (Figure 5A), B (5B), C (5C), D (5D), E (5E) and F (5F). Two examples are shown for each genotype.

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**Table 1.** Intra-assay (precision) and inter-assay reproducibility of CAP/CTM HBV real-time PCR assay. For intra-assay reproducibility, the 7 members of the standard panel (NAP-000 to NAP-HBV2E7) containing 0 to  $2 \times 10^7$  IU/ml ( $7.3 \text{ Log}_{10}$  IU/ml) were tested in triplicate in the same experiment. For inter-assay reproducibility, the assay low positive control (LPC) and high positive control (HPC) were tested 35 times in different experiments.

	Standard	Control	Target HBV DNA ( $\text{Log}_{10}$ IU/ml)	No. of determinations	Mean (SD) measured HCV RNA ( $\text{Log}_{10}$ IU/ml)	Coefficient of variation (%)
<b>Intra-assay reproducibility</b>	NAP-000		0.00	3	Target not detected	-
	NAP-HBV2E2		2.30	3	2.03 (0.02)	0,75
	NAP-HBV2E3		3.30	3	3.17 (0.08)	2.68
	NAP-HBV2E4		4.30	3	4.09 (0.01)	0.22
	NAP-HBV2E5		5.30	3	4.96 (0.03)	0.56
	NAP-HBV2E6		6.30	3	5.79 (0.02)	0.39
	NAP-HBV2E7		7.30	3	6.81 (0.08)	1.24
<b>Inter-assay reproducibility</b>		LPC	LPC <sup>a</sup>	35	2.57 (0.11)	4.13
		HPC	HPC <sup>b</sup>	35	6.08 (0.08)	1.31

<sup>a</sup>Five kit lots were used, with LPC values between 2.08 and 3.08  $\text{Log}_{10}$  IU/ml, 2.04 and 3.04  $\text{Log}_{10}$  IU/ml, 1.97 and 2.97  $\text{Log}_{10}$  IU/ml, 2.03 and 2.99  $\text{Log}_{10}$  IU/ml, and 1.90 and 2.85  $\text{Log}_{10}$  IU/ml, respectively.

<sup>b</sup>Five kit lots were used, with HPC values between 5.59 and 6.59  $\text{Log}_{10}$  IU/ml, 5.52 and 6.52  $\text{Log}_{10}$  IU/ml, 5.69 and 6.69  $\text{Log}_{10}$  IU/ml, and 5.50 and 6.46  $\text{Log}_{10}$  IU/ml, respectively.

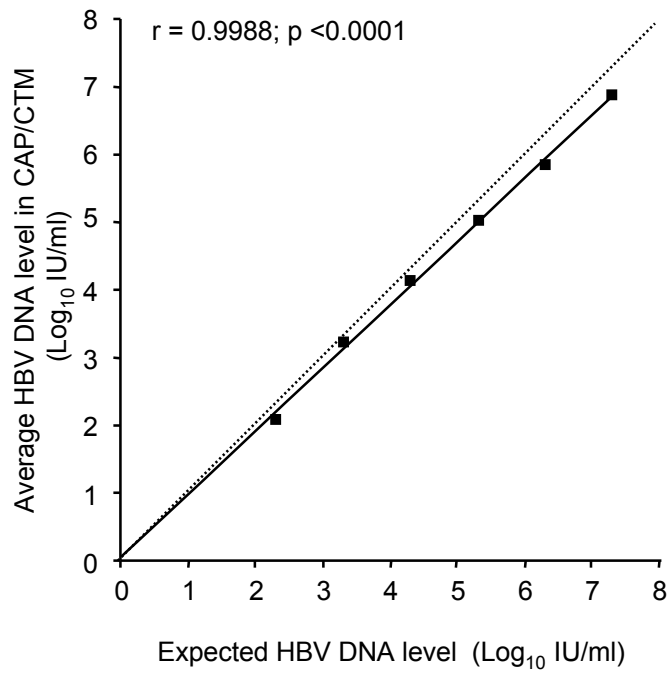
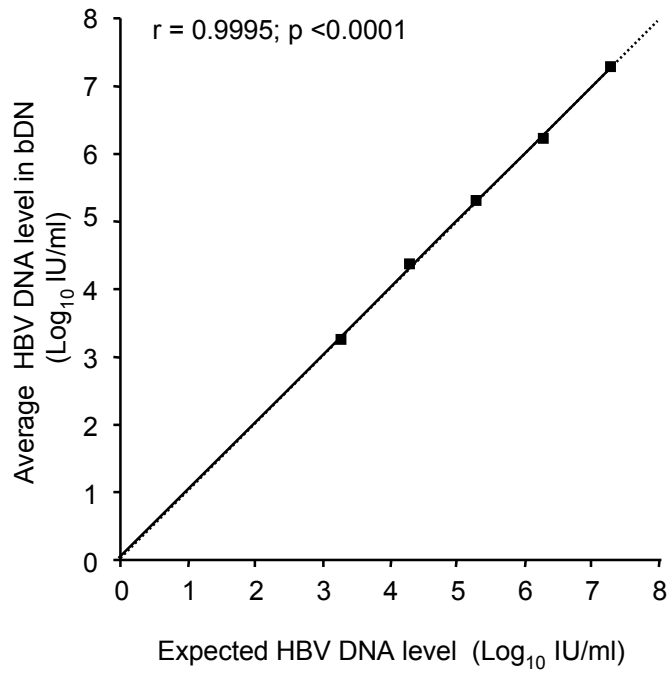
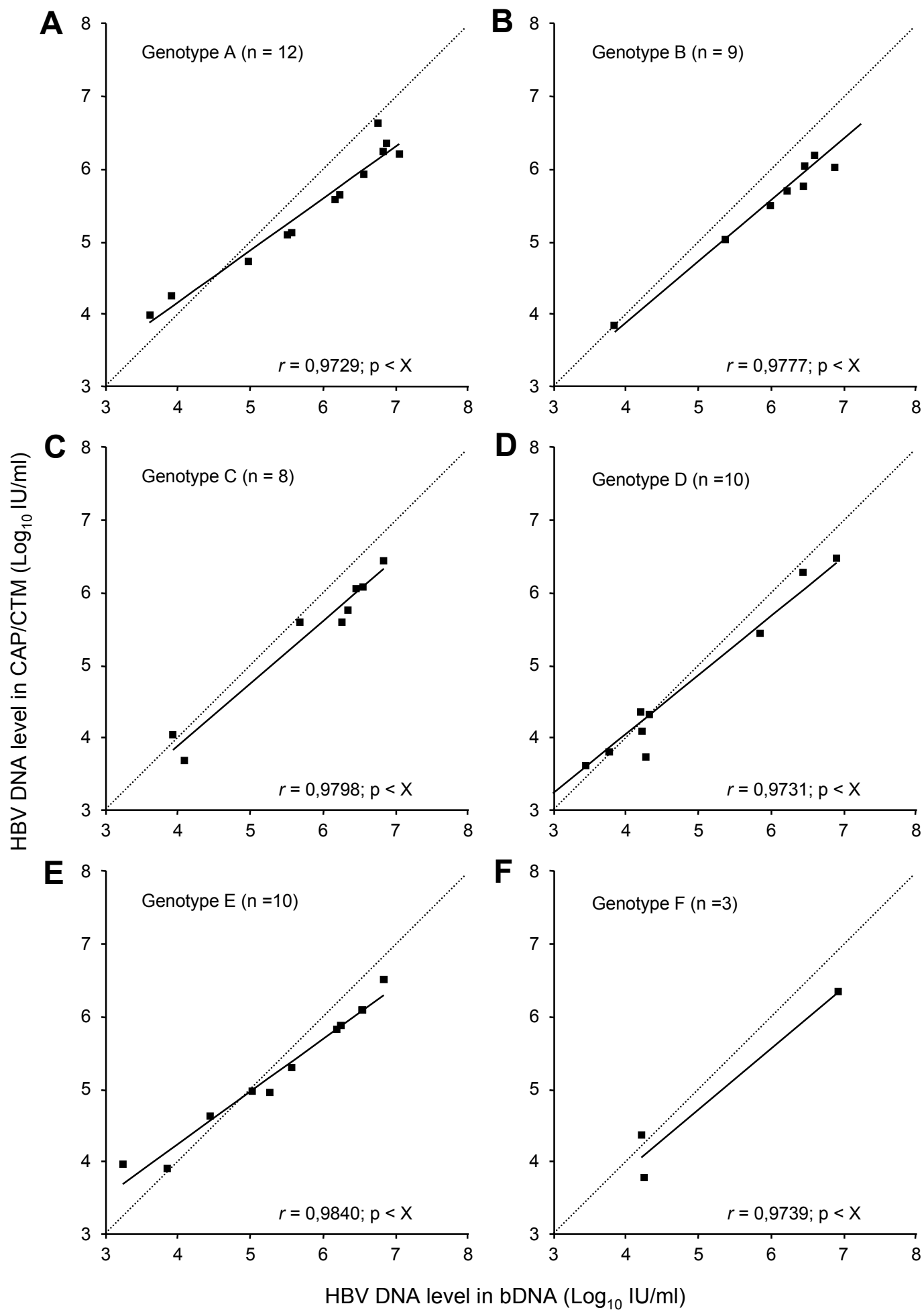
**A****B**

Figure 1





Figures 2



**B**

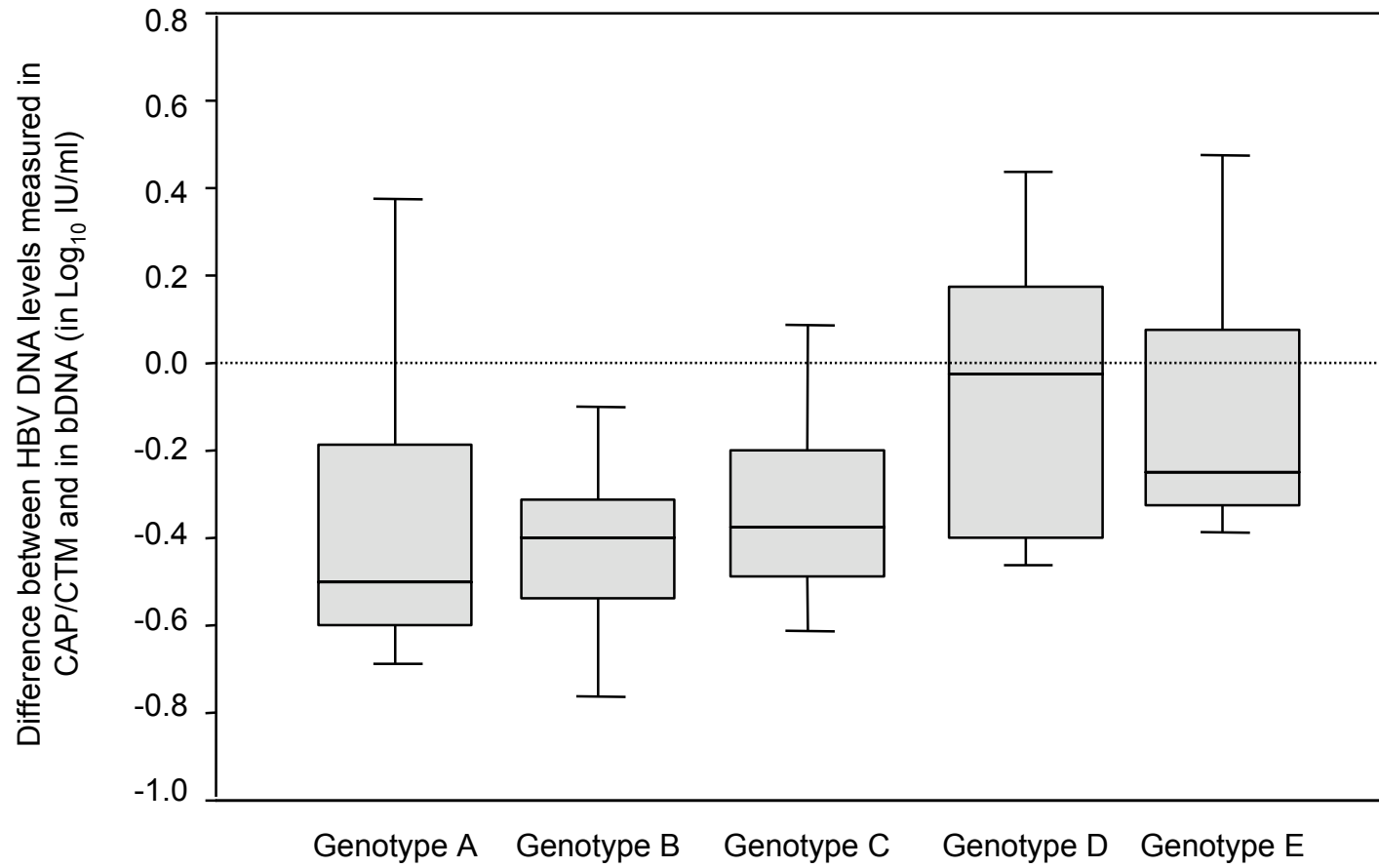


Figure 3B

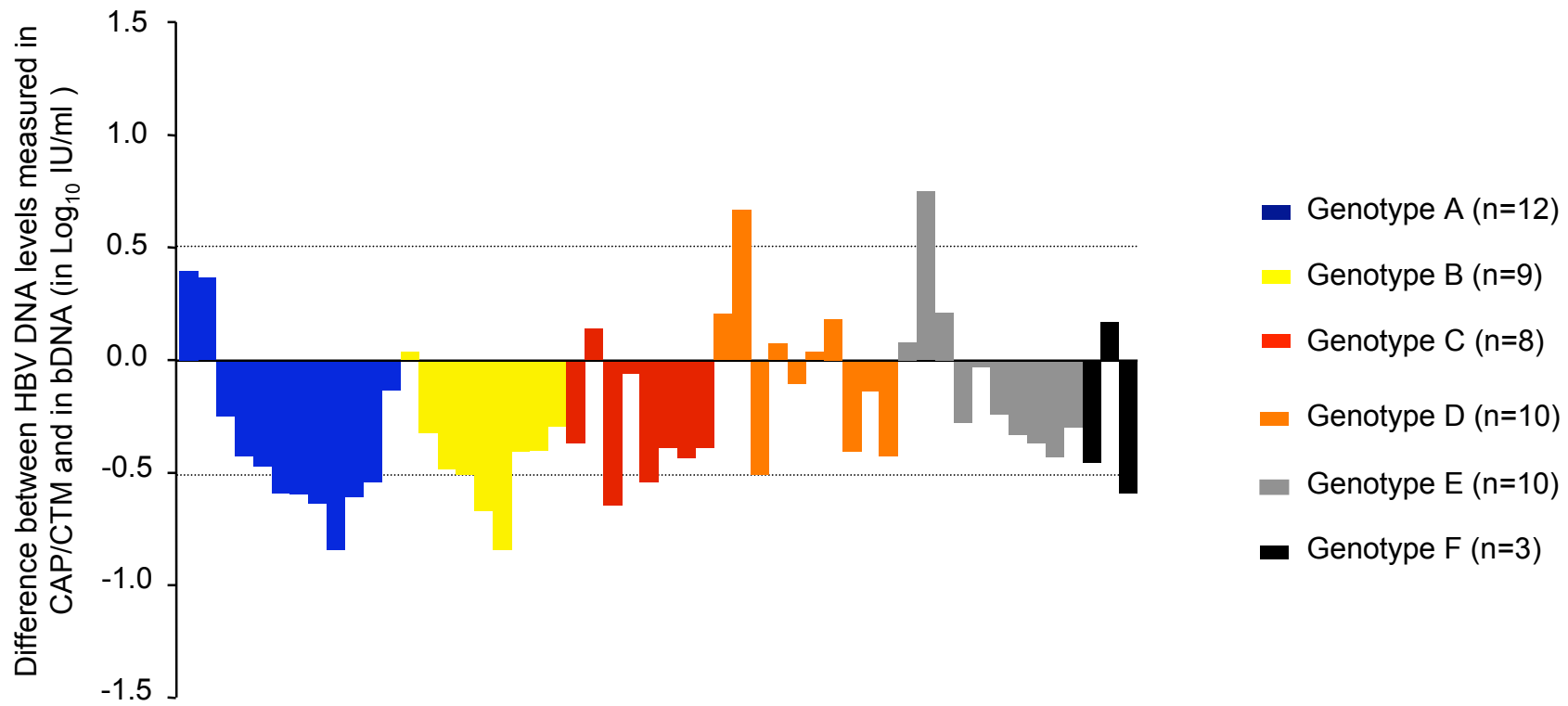
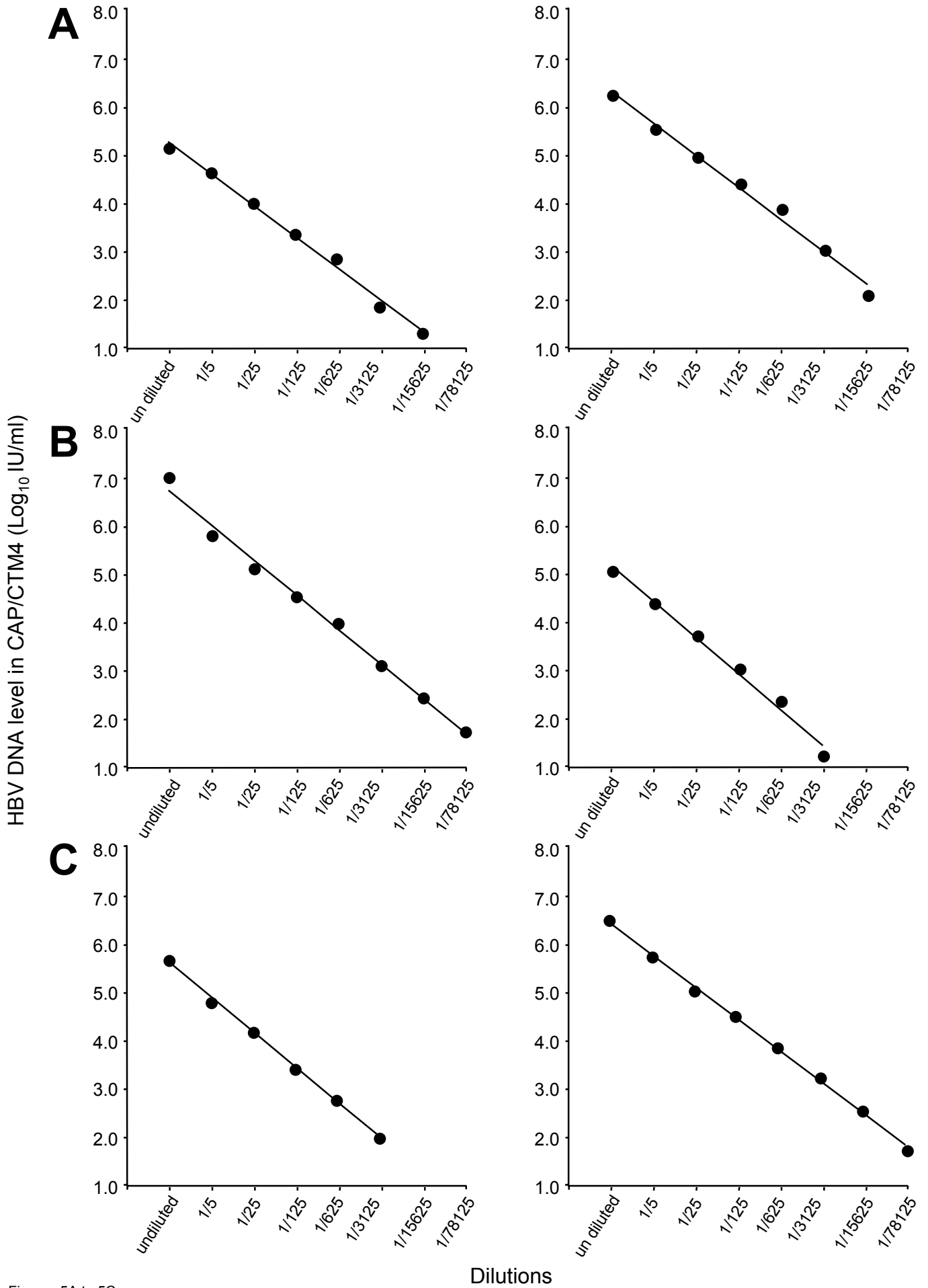
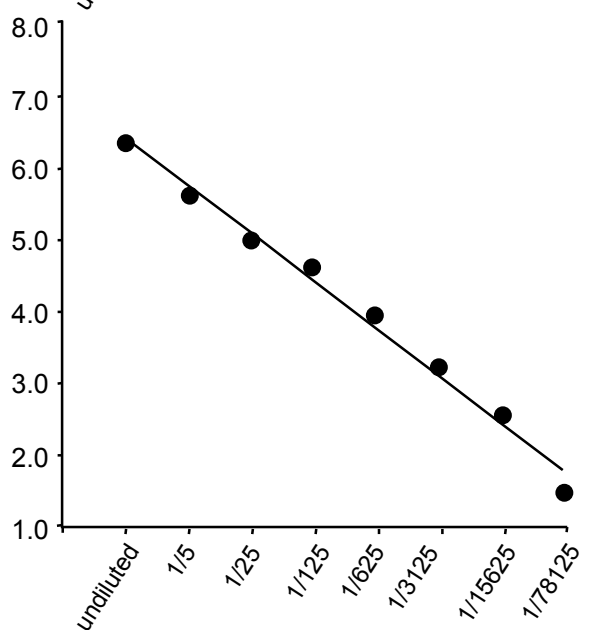
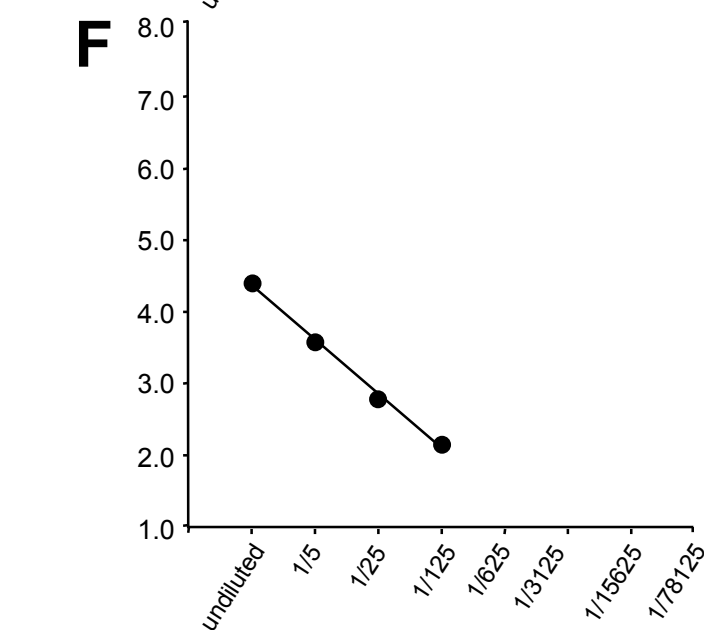
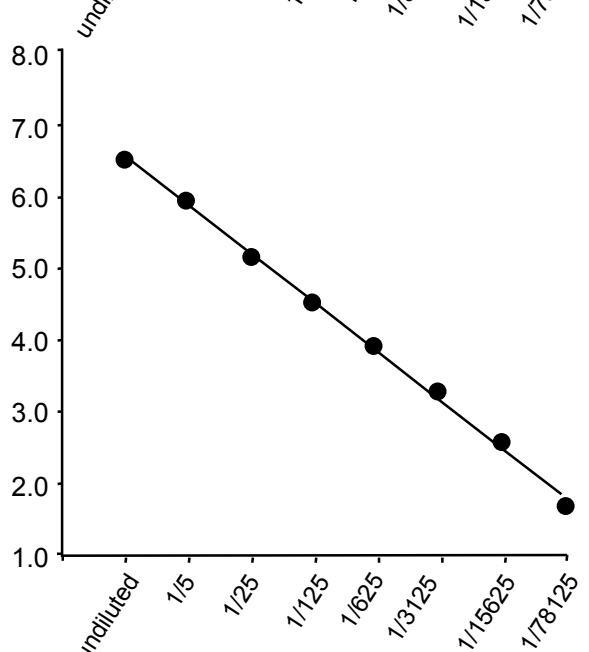
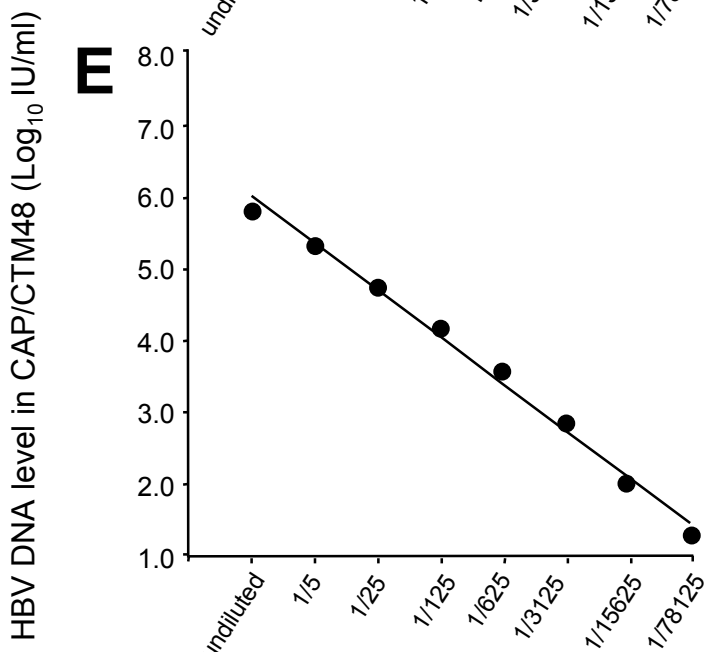
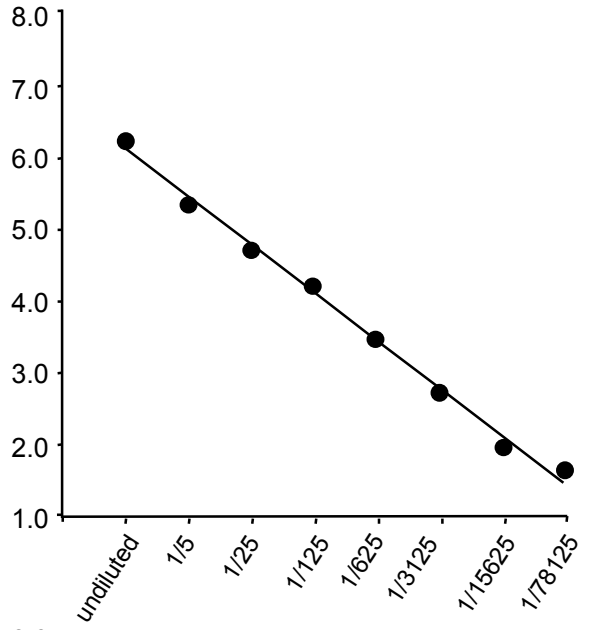
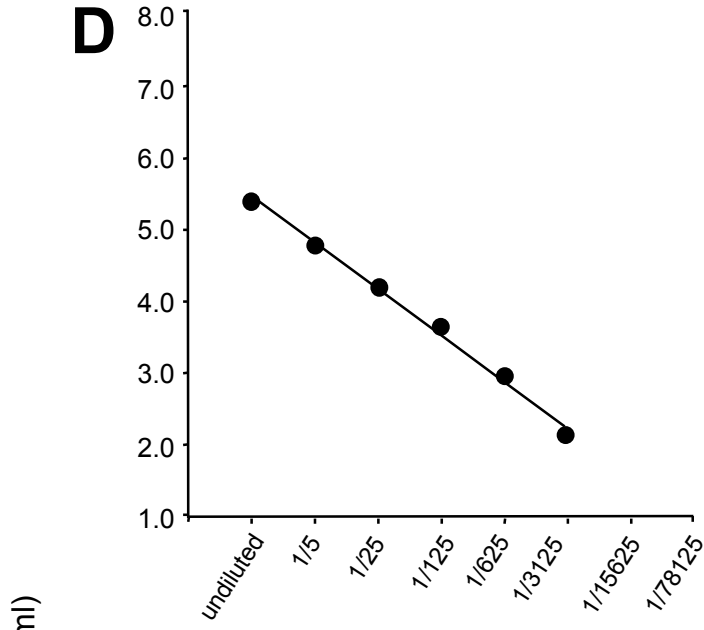


Figure 4



Figures 5A to 5C



Dilutions

Figures 5D to 5F