

Comparison of the Cobas Ampliprep™/Cobas TaqMan™ HBV Test versus the Cobas Amplicor HBV monitor for HBV-DNA detection and quantification during antiviral therapy

Tiziano Alice¹, Francesco Cerutti¹, Fabrizia Pittaluga¹, Silvia Varetto¹, Silvia Gabella¹, Alfredo Marzano², Alessandro Franchello², Valeria Ghisetti¹

¹Microbiology Laboratory;

²Gastroenterology Department and Liver Transplantation Centre, Molinette Hospital, Turin, Italy

SUMMARY

Performances of the new automatic system COBAS AmpliPrep™/COBAS TaqMan™ 48 (CAP/CTM) (Roche, Branchburg, NJ) for HBV DNA extraction and real-time PCR quantification were assessed and compared with the end-point PCR COBAS AMPLICOR HBV Monitor (CAHBM, Roche). Analytical evaluation with proficiency panels from UK National External Quality Assessment Scheme (UK NEQAS) over a 1-year period of distribution showed that CAP/CTM correctly measured HBV DNA levels with a close correlation between expected and observed values ($r=0.995$). Clinical evaluation as tested with samples from 11 HBsAg-positive patients undergoing antiviral therapy (71 serial specimens of plasma), demonstrated excellent correlation with CAHBM ($r=0.958$, mean difference in quantitation: 0.14 \log_{10} IU/ml), but CAP/CTM detected longer period of residual viremia. HBV DNA reduction was much higher in the combination schedule (Lamivudine+Adefovir), than in Adefovir monotherapy (5.1 vs. 3.5 logs). In conclusion, CAP/CTM allows for an accurate and standardized quantification of HBV DNA in high through-put laboratories. Due to its high sensitivity, it may further improve the detection of emerging drug resistance strains and the assessment of antiviral therapy.

KEY WORDS: Quantification, HBV DNA, TaqMan, Automation, Antiviral therapy

Received July 17, 2007

Accepted August 08, 2007

INTRODUCTION

Hepatitis B virus (HBV) infection is a major cause of chronic liver disease. It is estimated that nearly 2 billion people worldwide are infected by HBV and more than 350 million have persistent and chronic infection (Yim and Lok, 2006). HBV-carriers have a high risk of developing long-term se-

quelae of hepatitis B, including cirrhosis and hepatocellular carcinoma. In countries at moderate prevalence of HBV infection, like Italy, HBV-related cirrhosis accounts for nearly 25% of the indications to for liver transplantation in reference centres.

Post-transplantation outcome largely depends on the prevention of allograft HBV reinfection, such as it is achieved with specific immunoprophylaxis and anti-HBV therapy (Salizzoni *et al.*, 2005).

Antiviral therapy against HBV is currently based on nucleos(t)ide analogues that have dramatically improved chronic hepatitis B management including the prevention of allograft reinfection in

Corresponding author

Valeria Ghisetti

Laboratory of Microbiology

Molinette Hospital

Corso Bramante, 88/90 - 10126 Torino, Italy

E-mail: vghisetti@molinette.piemonte.it

liver transplantation for HBV-related disease. The success of antiviral therapy requires highly sensitive tests for monitoring HBV DNA levels in plasma, whose main purposes is to define the activity of HBV infection, the selection of patients for treatment and the efficacy of antiviral therapy identifying the development of HBV drug-resistant strains (Werle *et al.* 2004; Yim and Lok, 2006).

Polymerase Chain Reaction (PCR) allows a very sensitive quantification of HBV DNA that can be achieved with either the end-point or real-time technology, both commercially available for HBV DNA.

Quantification with end-point PCR is assessed at the end of the reaction; requires a post-PCR step to detect the amplified target, it is labour intensive, prone to contamination and suffers from a narrow dynamic range, allowing quantification within 3-4 \log_{10} . In fact, results obtained with the end-point PCR are subjected to errors due to the "plateau" effect, when the efficiency of PCR is at its minimum due to the exhaustion of reagents, thus affecting the quantification of moderate-high levels of viremia.

Real-time PCR is designed to quantify nucleic acid in the exponential phase of the reaction through the evaluation of the threshold cycle (Ct) when amplified products begin to be detected. For this reason, real-time PCR allows a more reliable and precise quantification over a much wider dynamic range without a post-PCR step. If many are the advantages of nucleic acid amplification by real-time PCR are many, reliable results also depend on sample preparation and nucleic acid extraction from biologic specimens is the most technically demanding step of PCR-based assays.

Manual specimen preparation is labour-intensive, a great source of run-to-run variability and specimen-to-specimen contamination. As a result, there is a growing clinical need for automated sample processing systems to provide accurate and reliable quantification of nucleic acid for clinical purposes.

The recently introduced real-time PCR technique for HBV DNA quantification represents the method of choice compared to previous, conventional end-point PCR due to a very sensitive quantification of viral load over a wide dynamic range as it is required for HBV infected patients (Loeb

et al., 2000; Chen *et al.*, 2001; Ide *et al.*, 2003; Mukaide *et al.*, 2003; Leb *et al.*, 2004; Stelzl *et al.*, 2004; Sum *et al.*, 2004; Weiss *et al.*, 2004; Gordillo *et al.*, 2005; Zhao *et al.*, 2005; Lole *et al.*, 2006). Since sample preparation is a major weakness in molecular tests, improvements are constantly introduced, with the purpose of decreasing the variability of the techniques and the risk of contamination, like ready-to-use reagents and automation of the extraction procedure. The COBAS AmpliPrep™/COBAS TaqMan™ HBV (CAP/CTM HBV) (Roche Molecular Systems, Inc., Branchburg, NJ) is a fully automated real-time PCR system, consisting of two integrated platforms: the COBAS AmpliPrep™ for the automated nucleic acid extraction from plasma specimens and the COBAS TaqMan™ 48, a real-time PCR assay based on the TaqMan™ technology. The system has recently been developed and introduced on the market (Weiss *et al.*, 2004; Hochberger *et al.*, 2006, Alice *et al.*, 2007). CAP/CTM is suitable for large routine series and has been demonstrated to equally quantitate HBV genotype A through G (Weiss *et al.*, 2004; Hochberger *et al.*, 2006, Alice *et al.*, 2007).

In the present study, performances of CAP/CTM HBV were evaluated and compared with the end-point PCR assay COBAS AMPLICOR HBV Monitor™ (CAHBM) (Roche Molecular Systems, Inc, Branchburg, NJ). Analytical sensitivity and precision were assessed with HBV proficiency panels from the UK National External Quality Assessment Scheme (UK NEQAS) over a 1-year period of distribution and on HBV DNA reference standards from Acrometrix (Acrometrix Corp., Benicia, CA).

Correlation and differences in DNA quantification were evaluated in specimens from patients with chronic HBV hepatitis on antiviral therapy. Discordant results were evaluated with a more sensitive home-made reference PCR for HBV DNA.

MATERIALS AND METHODS

Analytical evaluation

The evaluation of the assay was carried out analysing the UK National External Quality Assessment Scheme (UK NEQAS) proficiency panels for HBV DNA quantification sent to par-

participants from September 2005 to July 2006 (3 panels, 12 specimens). Panels were distributed by NEQAS as freeze-dried plasma and participants were requested to reconstitute the samples by adding 500 µl Dnase-free water. For CAP/CTM testing, 550 µl HBV DNA-negative plasma were added to obtain the final working volume of 1050 µl as requested by the system. CAP/CTM results were reported to the UK NEQAS for each distribution. Detection rate and correlation with expected values as stated by the UK NEQAS reports, were analysed.

To test CAP/CTM reproducibility, a commercially available panel of HBV DNA standards at concentrations of 20,000, 2,000 and 200 IU/ml (OptiQuant HBV-DNA; Acrometrix Corp., Benicia, CA) was used in four different runs in subsequent days and interassay coefficient of variation (CV%) was evaluated.

Clinical evaluation

Serial plasma specimens (N=71) from 11 HBsAg positive patients with HBV chronic disease (10 anti-HBe positive, 1 HBeAg positive) and undergoing antiviral therapy with Lamivudine (LAM) in combination with Adefovir (ADF) (5 patients) or ADF alone (6 patients) were tested with CAP/CTM. These specimens were sent to the Microbiology Laboratory, Molinette Hospital of Turin for routine quantitative HBV DNA testing by the CAHBM. Patients were followed for a mean of 542 days (range 377-974 days) after the introduction of ADF due to the onset of lamivudine-resistant and HBV DNA was evaluated in plasma every three months. Aliquots of plasma were stored at -20°C before testing with CAP/CTM.

HBV DNA quantification

COBAS AmpliPrep™/COBAS TaqMan™ HBV (CAP/CTM HBV)

COBAS AmpliPrep/COBAS Taqman™ HBV (CAP/CTM HBV) is an automated real-time PCR test based on a dual labeled hybridization probe targeting the pre-core/core region associated with an HBV DNA automated extraction based on the affinity of DNA for silica gel-covered magnetic beads.

The procedure starts from 1050 µl of plasma and consists of subsequent steps of lysis with caothropic agents and proteinase K, glass-particle DNA capture and purification. An internal

quantitation standard is added in the sample during the processing step. After DNA elution at high temperature (80°C), a robotic arm loads the nucleic acids in microvials containing the PCR master mix prepared by the same robotic arm. The real-time PCR test is performed by the Cobas TaqMan™ 48 using a TaqMan assay, simultaneously amplifying two targets: HBV DNA and the HBV quantitation standard (QS), that is a non-infectious construct with primer binding regions identical to those of the HBV target sequence but with a different detection. Results are expressed as HBV-DNA IU/ml and a 5.82 copies/IU conversion factor was previously reported. Sensitivity was set at 12 IU/ml with a dynamic range from about 54 to 1.1×10^8 IU/ml (Mukaide *et al.*, 2003; Sum *et al.*, 2004). Prevention of carry over contamination and sample integrity are ensured by the use of Amperase® (Longo *et al.*, 1990). The system is designed for the extraction of 24 plasma specimens in about 2 hours. The entire procedure of HBV DNA extraction and amplification from 24 samples takes about 4 hours.

COBAS AMPLICOR HBV Monitor (CAHBM)

The COBAS AmpliCor™ HBV Monitor System is an end-point PCR test for HBV DNA quantification associated with a manual DNA extraction from 100 µl of plasma sample. The sensitivity of CAHBM is 200 copies/ml (~74 IU/ml, conversion factor: 2.7 copies per IU) with a linear range up to 200,000 copies/ml.

Nested-PCR for HBV DNA detection

A reference home-made nested-PCR for the core gene of HBV (Ghisetti *et al.*, 2004) was assessed on CAP/CTM and CAHBM discordant samples. Two sets of primers mapping the core gene from position 2230-2660 and leading to a final 221 bp amplified product were used. Five µl of DNA extracted from 200 µl plasma with a manual procedure based on nucleic acid silica-gel affinity (QIAgen, MI, I) were subjected to PCR. The sensitivity of the assay was previously established at 10 copies/5 µl of DNA, testing known amount of a plasmid containing the entire HBV genome.

HBV polymerase gene mutants

HBV lamivudine resistant strains were detected with a reverse hybridization line probe assay (INNO-LIPA, Innogenetics, Ghent, Belgium) after

amplifying domains B and C of the viral polymerase gene with specific primers.

Serologic markers of HBV infection

HBV serology was performed with the ChLIA method (Abbott Laboratories, Abbott Park, IL USA).

Statistical analysis

Mean and standard deviation were calculated using conventional statistical tests. Correlation between CAP/CTM and CAHBM was determined by linear regression analysis and mean differences in quantification by the Bland-Altman plot. HBV DNA results were expressed as \log_{10} IU/ml. Differences were considered statistically significant for p value <0.05 (Student T test).

RESULTS

CAP/CTM analytical performances for HBV DNA quantification were assessed on the UK NEQAS distributions from September 2005 to July 2006 (3 distribution, 12 specimens) and on HBV DNA reference standards. Clinical evaluation was assessed on 71 specimens from 11 HBsAg-positive patients with chronic hepatitis B undergoing anti-HBV therapy.

Analytical evaluation

Twelve freeze-dried plasma specimens derived from 3 HBV DNA UK NEQAS panels, reconsti-

tuted with 500 μ l Dnase-free water plus 550 μ l HBV DNA-negative plasma to obtain the final working volume of 1050 μ l, were tested with CAP/CTM. Expected values ranged from 0.3 to 4.63 \log_{10} IU/ml. HBV genotype distribution included HBV genotype D in 10 and genotype A in 2 specimens, respectively.

CAP/CTM observed results were very close to the expected ones for each NEQAS specimens with excellent correlation ($r=0.995$) and linearity ($R^2=0.989$). For expected values of 1, 0.49, 3.64, 2.72, 1.32, 0.79, 4.63, 3.24, 4.15, 3.67, 1.73 and 0.3 \log_{10} IU/ml reported values of 1.17, 0.65, 3.98, 2.79, 1.59, 1.12, 4.94, 3.33, 4.91, 3.97, 1.82 and 0.52 \log_{10} IU/ml were observed, respectively (Table 1). Differences between expected and observed values ranged from -0.07 to -0.76 logs. Bland-Altman plot demonstrated that the mean difference between expected vs observed values for averaged quantitation logs was -0.26 ± 0.19 (Fig. 1). Only one samples (#8026, genotype D) with an HBV DNA level >4 logs gave a significantly different results (4.91 log vs. 4.15 expected value, Table 1, Figure 1).

Reproducibility of CAP/CTM was evaluated on HBV DNA standards from AcroMetrix (expected value: 20,000, 2,000 and 200 IU/ml). CAP/CTM observed values were very close to the expected ones: 27,550, 2560 and 186 IU/ml for the 20,000, 2,000 and 200 IU/ml standards. Interassay CV for standards 200, 2×10^3 and 2×10^4 IU/ml were 37%, 18% and 15%, respectively.

TABLE 1 - Evaluation of 12 HBV DNA standards from 3 NEQAS proficiency panels with CAP/CTM system.

Expedition month/day/year	ID	Genotype	Expected value (\log_{10} IU/ml)	Observed value (\log_{10} IU/ml)	Difference between expected and observed values (\log_{10} IU/ml)
09/26/2005	7643	A	1	1.17	-0.17
	7644	A	0.49	0.65	-0.16
	7645	D	3.64	3.98	-0.34
	7646	D	2.72	2.79	-0.07
03/06/2006	7844	D	1.32	1.59	-0.27
	7845	D	0.79	1.12	-0.33
	7846	D	4.63	4.94	-0.31
	7847	D	3.24	3.33	-0.09
07/03/2006	8026	D	4.15	4.91	-0.76
	8027	D	3.67	3.97	-0.30
	8028	D	1.73	1.82	-0.09
	8029	D	0.3	0.52	-0.22

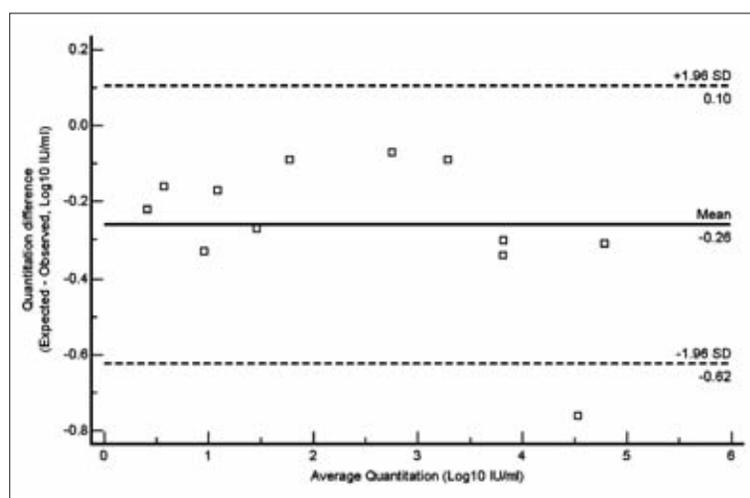


FIGURE 1 - Bland-Altman analysis of the mean difference between expected and observed values for 12 HBV DNA standards from 3 NEQAS proficiency panel distributions as evaluated with CAP/CTM.

Clinical evaluation and systems comparison

Plasma samples from 11 patients with chronic HBV LAM-resistant hepatitis submitted for routine HBV DNA testing were studied. HBV viral load was measured with CAHBM and CAP/CTM from the time ADF was started (baseline), in combination with LAM (5 patients) or alone (6 patients), then at months 6, 9 and 12. At the time samples were tested with CAP/CTM, 35 were HBV DNA positive and 36 HBV DNA negative by CAHBM (Table 2).

Concordant results were determined in 90% of samples (32 positive and 32 negative concordant samples). CAP/CTM detected HBV DNA in 4 samples (HBV DNA levels from 54 to 196 IU/ml) that tested negative by CAHBM. A more sensitive nested PCR for the core region of HBV confirmed these as true positive samples, whereas CAHBM positive/CAP/CTM negative samples (N=3) were not confirmed with the reference PCR test (false positive results with CAHBM). Comparison between CAP/CTM and CAHBM showed an excellent correlation ($r=0.958$, 95% CI 0.932-0.973) (Figure 2a).

Mean differences (CAHBM-CAP/CTM) for averaged logs were within 1 log: $0.14 \pm 0.71 \log_{10}$ IU/ml (mean \pm SD) as shown in Figure 2b.

In those patients undergoing the combination schedule (LAM+ADF) of therapy, the mean reduction of HBV DNA level from baseline was 3.5 \log_{10} IU/ml at month 6, 3.7 logs at month 9 and 5.1 logs at month 12 (Table 3).

In the ADF monotherapy group, the mean reduction of HBV DNA from baseline was 3.0 logs

at month 6, 3.6 at month 9 and 3.1 logs at month 12 (Table 3). At month 12, 8 out of 11 patients (10/11 with CAHBM) were below the lower detection limit of the CAP/CTM assay while 3 patients, two on LAM+ADF and one on ADF monotherapy were still HBV DNA positive (HBV DNA values: 2.5, 2.3 and 3.9 \log IU/ml, respectively). CAHBM detected HBV DNA in only one of the 3 patients. Therefore, CAP/CTM detected residual HBV DNA for longer periods than CAHBM.

The reduction of HBV DNA levels after 12 months of ADF administration was much more significant on the combination schedule (5.1 log decline) than on ADF monotherapy (3.5 log decline)

TABLE 2 - Clinical performance of CAP/CTM HBV assay compared to COBAS AMPLICOR HBV Monitor (CAHBM) in a group of 71 samples from HBsAg-positive patients.

CAP/CTM (N. of samples)	Cobas Amplicor Monitor (N. of samples)	
	Positive	Negative
Positive	32	4*
Negative	3 [§]	32
Total	35	36

*True positive samples by CAP/CTM as confirmed by a more sensitive reference nested-PCR assay. See text for details. [§]False positive samples by Cobas Amplicor Monitor as confirmed by a more sensitive reference nested-PCR assay. See text for details.

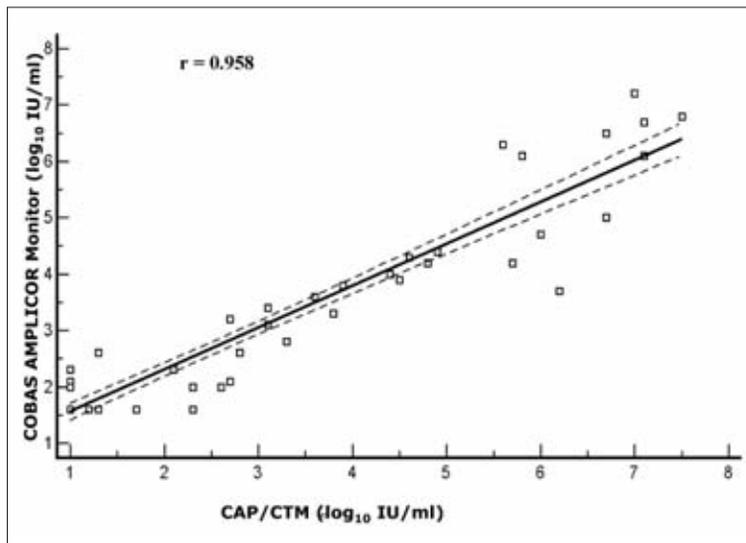


FIGURE 2a - Correlation between CAHBM and CAP/CTM as assessed on 71 plasma specimens from HBsAg-positive patients undergoing antiviral therapy.

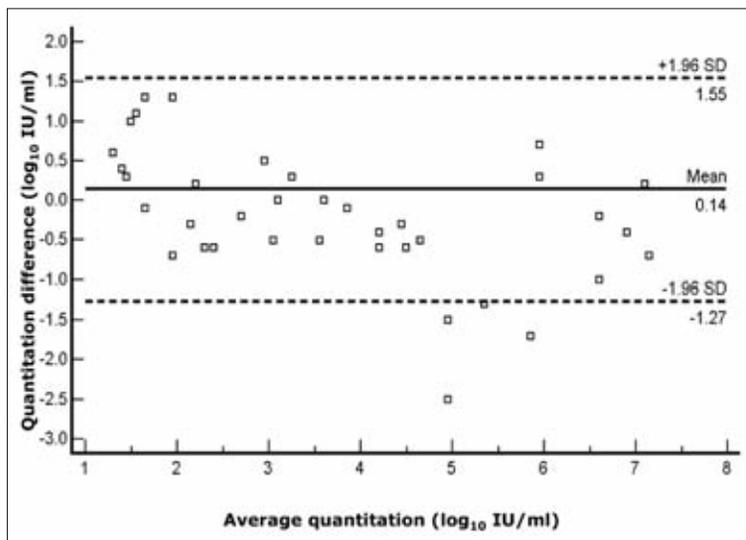


FIGURE 2b - Bland-Altman analysis of the differences in HBV DNA quantification between CAHBM and CAP/CTM tests as assessed on 71 clinical samples (CAHBM-CAP/CTM).

suggesting a stronger effect of the association of two or more drugs against HBV.

DISCUSSION

Quantification of HBV DNA as a marker of viral replication, is the most direct and reliable method for the accurate management of chronic hepatitis B.

Highly sensitive and reproducible molecular tests are required to assess the efficacy of the treatment and the development of HBV drug-resistant strains. End-point PCR techniques for HBV

DNA have shown a limited dynamic range, usually within 4 logs, affecting the quantification of moderate-high levels of viremia that are usually present in overt HBV disease (Mukaide *et al.*, 2003; Kohmoto *et al.*, 2003). On the contrary, real-time PCR is designed to quantify nucleic acids in the exponential phase of the reaction allowing for a more reliable and precise quantification over a much wider dynamic range.

Reliable PCR results depend on sample preparation that is technically demanding and a potential source of run-to-run variability and sample contamination (Weiss *et al.*, 2004; Gordillo *et al.*, 2005; Lindh and Hannoun, 2005). Real-time PCR

TABLE 3 - Evaluation of HBV DNA decrease (\log_{10} IU/ml) in LAM-resistant patients undergoing LAM+ADF combination therapy (5 patients) or ADF monotherapy (6 patients) from the time ADF has been administered (baseline value).

Months of therapy from baseline*	HBV DNA decrease from baseline* (\log_{10} IU/ml)	
	Combination schedule (LAM+ADF, 5 patients)	Monotherapy (ADF, 6 patients)
6	3.5	3.0
9	3.7	3.6
12	5.1	3.1

*Baseline corresponded to ADF administration either as combination schedule with LAM or monotherapy.

assays for HBV DNA based on either the TaqMan technology or other real-time platforms have been designed on manual, semi-automatic or automatic nucleic acid extraction procedures suitable for small series. So far, these formats have shown an excellent correlation with conventional end-point PCR systems, including CAHBM, that is the most commonly used assay in comparative evaluations (Ide *et al.*, 2003; Mukaide *et al.*, 2003; Leb *et al.*, 2004; Sum *et al.*, 2004; Stelzl *et al.*, 2004; Chen *et al.*, 2006; Lole *et al.*, 2006, Alice *et al.*, 2007).

The real-time PCR system COBAS TaqMan™ 48 for HBV DNA has been evaluated with both automatic and manual extraction platforms, i.e. the MagNA™ pure technology for small routine series and the generic extraction kit High Pure System Viral Nucleic Acid, respectively, being very reproducible and sensitive (Leb *et al.*, 2004; Weiss *et al.*, 2004; Gordillo *et al.*, 2005). The integration of the real-time PCR analyzer with a fully automated DNA extraction platform on the COBAS Ampliprep™ Instrument (CAP/CTM) for large routine series has been recently introduced (Weiss *et al.*, 2004; Hochberger *et al.*, 2006; Ronsin *et al.*, 2006; Alice *et al.*, 2007). In this report we provide an analytical and clinical evaluation of the integrated platform CAP/CTM based on proficiency panels from the UK NEQAS and other HBV DNA standards as well as on samples from patients with chronic hepatitis B.

The analytical evaluation of CAP/CTM with UK NEQAS proficiency panels over 1 year of activity, showed that CAP/CTM accurately quantitated HBV DNA levels with a close correlation between

expected and observed values. The mean difference for averaged logs was within $\pm 1.0 \log_{10}$. Reproducibility of CAP/CTM quantification as assessed on HBV DNA reference standards was excellent.

Comparison with the end-point PCR assay CAHBM was undertaken on clinical specimens, focusing on differences in quantification and viral kinetics in patients on antiviral treatment.

CAP/CTM confirmed an excellent correlation with the end-point PCR system CAHBM. Differences for averaged \log_{10} between the two tests were within $\pm 1.0 \log_{10}$ IU/ml, in agreement with other studies (Weiss *et al.*, 2004; Gordillo *et al.*, 2005; Lindh and Hannoun, 2005; Hochberger *et al.*, 2006; Alice *et al.*, 2007). CAP/CTM was more sensitive than CAHBM, in detecting longer periods of viremia and more viremic patients. This is an important finding due to the increasingly recognized clinical significance of low viremia levels in predicting the response to antiviral therapy and the emergence of drug-resistant strains (Scotto *et al.*, 2005; Chen *et al.*, 2006; Fung *et al.*, 2006; Yim *et al.*, 2006; Yeon *et al.*, 2006; Liaw, 2007; Rapti *et al.*, 2007). Important decreases of HBV viral load were observed under ADF therapy, particularly in combination with LAM. After six months of treatment a mean of 3 logs reduction from baseline was observed and, at month 12, 72% of patients were below the lower detection limit of CAP/CTM (90% below the detection limit of CAHBM). The possibility of appreciating residual HBV DNA during treatment allows for improvements in the prediction of antiviral response, the development of HBV drug-resistant

strains, with a better definition of virologic end-points (Fung *et al.*, 2006; Yeon *et al.*, 2006). The mean reduction of HBV DNA level from baseline to month 12 was higher in patients on the combination (LAM+ADF) schedule than in those on ADF monotherapy (5.1 vs. 3.5 log₁₀ IU/ml). This data confirms a much higher genetic barrier offered by the combination schedule based on LAM+ADF than monotherapy with ADF alone (Rapti *et al.*, 2007). CAP/CTM was also more specific and less prone to contamination than the end-point PCR.

In conclusion, this study provides evidences that CAP/CTM allows for a rapid and accurate quantification of HBV DNA levels in patients on antiviral therapy. The system is a complete labour-saving platform allowing for a standardized quantification of HBV DNA in high through-put laboratories and can be considered an important step for the clinical application of PCR data in HBV disease. Due to its high sensitivity, it may further improve the detection of emerging drug resistance strains and the assessment of antiviral therapy.

REFERENCES

- ALLICE T., CERUTTI F., PITTALUGA F., VARETTO S., GABELLA S., MARZANO A., FRANCHELLO A., COLUCCI G., GHISSETTI V. (2007). COBAS AmpliPrep-COBAS TaqMan hepatitis B virus (HBV) test: a novel automated real-time PCR assay for quantification of HBV DNA in plasma. *J. Clin. Microbiol.* **45**, 828-834.
- CHEN C.J., YANG H.I., SU J., JEN C.L., YOU S.L., LU S.N., ET AL. (2006). REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* **295**, 65-73.
- CHEN R.W., PIIPARINEN H., SEPPANEN M., KOSKELA P., SARNA S., LAPPALAINEN M. (2001). Real-time PCR for detection and quantitation of hepatitis B virus DNA. *J. Med. Virol.* **65**, 250-256.
- FUNG S.K., CHAE H.B., FONTANA R.J., CONJEEVARAM H., MARRERO J., OBERHELMAN K., ET AL. (2006). Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J. Hepatol.* **44**, 283-290.
- GHISSETTI V., MARZANO A., ZAMBONI F., BARBUI A., FRANCHELLO A., GAIA S., MARCHIARO G., SALIZZONI M., RIZZETTO M. (2004). Occult hepatitis B virus infection in HBsAg negative patients undergoing liver transplantation: clinical significance. *Liver Transpl.* **10**, 356-362.
- GORDILLO R.M., GUTIERREZ J., CASAL M. (2005). Evaluation of the COBAS TaqMan 48 real-time PCR system for quantitation of hepatitis B virus DNA. *J. Clin. Microbiol.* **43**, 3504-3507.
- HOCHBERGER S., ALTHOF D., DE SCHROTT R.G., NACHBAUR N., ROCK H., LEYING H. (2006). Fully automated quantitation of Hepatitis B virus (HBV) DNA in human plasma by the COBAS AmpliPrep/COBAS TaqMan System. *J. Clin. Virol.* **35**, 373-380.
- IDE T., KUMASHIRO R., KOGA Y., TANAKA E., HINO T., HISAMUCHI A., ET AL. (2003). A real-time quantitative polymerase chain reaction method for hepatitis B virus in patients with chronic hepatitis B treated with lamivudine. *Am. J. Gastroenterol.* **98**, 2048-2051.
- KOHMOTO M., ENOMOTO M., YANO Y., OTANI S., MINAMITANI S., TAMORI A., ET AL. (2003). Detection of serum hepatitis B virus DNA by real-time quantitative polymerase chain reaction (TaqMan PCR) during lamivudine treatment: comparison with three other assays. *Hepatol. Res.* **26**, 125-133.
- LEB V., STOCHER M., VALENTINE-THON E., HOLZL G., KESSLER H., STEKEL H., ET AL. (2004). Fully automated, internally controlled quantification of hepatitis B Virus DNA by real-time PCR by use of the MagNA Pure LC and LightCycler instruments. *J. Clin. Microbiol.* **42**, 585-590.
- LIAW Y.F. (2007). Rescue therapy for lamivudine-resistant chronic hepatitis B: when and how? *Hepatology* **45**, 266-268.
- LINDH M., HANNOUN C. (2005). Dynamic range and reproducibility of hepatitis B virus (HBV) DNA detection and quantification by Cobas Taqman HBV, a real-time semiautomated assay. *J. Clin. Microbiol.* **43**, 4251-4254.
- LOEB K.R., JEROME K.R., GODDARD J., HUANG M., CENT A., COREY L. (2000). High-throughput quantitative analysis of hepatitis B virus DNA in serum using the TaqMan fluorogenic detection system. *Hepatology* **32**, 626-629.
- LOLE K.S., ARANKALLE V.A. (2006). Quantitation of hepatitis B virus DNA by real-time PCR using internal amplification control and dual TaqMan MGB probes. *J. Virol. Methods.* **135**, 83-90.
- LONGO M.C., BERNINGER M.S., HARTLEY J.L. (1990). Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene* **93**, 125-128.
- MUKAIDE M., TANAKA Y., KATAYOSE S., TANO H., MURATA M., HIKATA M., ET AL. (2003). Development of real-time detection direct test for hepatitis B virus and comparison with two commercial tests using the WHO international standard. *J. Gastroenterol. Hepatol.* **18**, 1264-1271.
- RAPTI I., DIMOU E., MITSOUA P., HADZIVANNIS S.J. (2007). Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. *Hepatology* **45**, 307-313.
- RONCIN C., PILLET A., BALI C., DENOYEL G.A. (2006). Evaluation of the COBAS AmpliPrep-total nucleic

- acid isolation-COBAS TaqMan hepatitis B virus (HBV) quantitative test and comparison to the VERSANT HBV DNA 3.0 assay. *J. Clin. Microbiol.* **44**, 1390-1399.
- SALIZZONI M., CERUTTI E., ROMAGNOLI R., LUPO F., FRANCHELLO A., ZAMBONI F., ET AL. (2005). The first one thousand liver transplants in Turin: a single-center experience in Italy. *Transpl. Int.* **18**, 1328-1335.
- SCOTTO G., PALUMBO E., FAZIO V., TARTAGLIA A., SARACINO A., ANGARANO G. (2005). Clinical and virological response to adefovir dipivoxil for lamivudine-resistant HBeAg-negative hepatitis B. *New Microbiol.* **28**, 193-197.
- STELZL E., MULLER Z., MARTH E., KESSLER H.H. (2004). Rapid quantification of hepatitis B virus DNA by automated sample preparation and real-time PCR. *J. Clin. Microbiol.* **42**, 2445-2449.
- SUM S.S., WONG D.K., YUEN M.F., YUAN H.J., YU J., LAI C.L., ET AL. (2004). Real-time PCR assay using molecular beacon for quantitation of hepatitis B virus DNA. *J. Clin. Microbiol.* **42**, 3438-3440.
- WEISS J., WU H., FARRENKOPF B., SCHULTZ T., SONG G., SHAH S., ET AL. (2004). Real-time TaqMan PCR detection and quantitation of HBV genotypes A-G with the use of an internal quantitation standard. *J. Clin. Virol.* **30**, 86-93.
- WERLE B., CINQUIN K., MARCELLIN P., POL S., MAYNARD M., TREPO C., ET AL. (2004). Evolution of hepatitis B viral load and viral genome sequence during adefovir dipivoxil therapy. *J. Viral. Hepat.* **11**, 74-83.
- YEON J.E., YOO W., HONG S.P., CHANG Y.J., YU S.K., KIM J.H., ET AL. (2006). Resistance to adefovir dipivoxil (ADV) in lamivudine-resistant chronic hepatitis B patients treated with ADV. *Gut* **55**, 1488-1495.
- YIM H.J., LOK A.S. (2006). Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology* **43**, S173-S181.
- ZHAO J.R., BAI Y.J., ZHANG Q.H., WAN Y., LI D., YAN X.J. (2005). Detection of hepatitis B virus DNA by real-time PCR using TaqMan-MGB probe technology. *World J. Gastroenterol.* **11**, 508-510.

