

# HBV DNA ASSAYS: (METHODS AND PRACTICAL USE) AND VIRAL KINETICS

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## ABSTRACT

The presence of HBV DNA in peripheral blood is a reliable marker of active HBV replication. HBV DNA detection and quantification can be achieved by means of signal amplification or target amplification methods. HBV DNA quantitative units used in the various assays are not yet standardized, but an HBV DNA international unit (IU) has been defined that should now be implemented in all commercial HBV DNA quantitative assays. HBV DNA detection and quantification are useful in the diagnosis of infection, therapeutic decision-making, and assessment of the response to therapy. However, clinically relevant decision thresholds have not been precisely defined, preventing from drawing simple management guidelines based on HBV DNA level assessment. Sensitive and accurate HBV DNA quantification methods can be used to study HBV kinetics in various clinical settings. Progress in standardization and better understanding of the mechanisms of HBV infection and response to therapy should help improve the practical use of HBV DNA assays in the future.

Virological diagnosis and monitoring of hepatitis B virus (HBV) infection are based on serologic assays detecting specific anti-HBV antibodies, and assays that can detect, quantify or characterize the components of HBV viral particles, such as HBV DNA and various viral antigens. HBV DNA detection and quantification now play a key role in the diagnosis of infection, therapeutic decision-making, and assessment of the response to therapy. However, clinically relevant HBV DNA thresholds remain to be established in various settings. HBV DNA quantification can also be used to monitor viral replication kinetics to better understand the mechanisms of infection and the virologic response to antiviral therapy.

## HBV DNA, A MARKER OF VIRAL REPLICATION

The presence of HBV DNA in peripheral blood is a reliable marker of active HBV replication. HBV DNA is detectable within a few days after infection. It generally increases to reach a peak at the time of acute hepatitis, before progressively decreasing and disappearing when the infection resolves spontaneously [1]. In the patients progressing towards chronic HBsAg carriage, chronic infection evolves through successive phases. HBV DNA levels are not stable over time and depend on the infection phase : the immunotolerance after acute infection is characterized by high levels of viral replication ; the immuno-elimination phase is characterized by generally lower, often fluctuating HBV DNA ; the “clinical latency” phase is characterized by very low or undetectable levels of viral replication, depending on the sensitivity of the assay used ; during reactivation phases, that are facilitated by immunosuppressive treatments, viral replication is generally reaches high levels. During all phases in chronic HBsAg carriers, HBV DNA and supercoiled DNA (cccDNA, the persistent form of HBV in hepatocytes) are detectable in the liver with sensitive techniques. The HBV DNA load does not appear to be affected by the severity of liver lesions, but a non causal relationship may exist, because liver lesions develop principally during the immuno-elimination phase.

## AVAILABLE HBV DNA TESTS

The principles of the molecular biology-based techniques that can be used to detect and quantify HBV DNA in peripheral blood have been reviewed recently [2]. These techniques are based on signal amplification following molecular hybridization (including the “hybrid capture” and the “branched DNA” methods) or target amplification (including polymerase chain reaction, PCR, and transcription-mediated amplification, TMA) [2]. **Table 1** shows the commercial assays that can currently be used to detect and quantify HBV DNA. HBV

DNA quantitative units used in the various assays do not represent the same actual amount of HBV DNA in a given clinical sample. The World Health Organization has established an international standard for universal standardization of HBV DNA quantification units, and an HBV DNA international unit (IU) has been defined [3]. International unit conversion factors for the non standardized units used in commercial HBV DNA quantitative assays are currently being calculated. This IU must be preferred to any other quantitative unit and should now be implemented in all commercial HBV DNA quantitative assays for establishing clinically relevant thresholds and recommendations for clinical decisions based on HBV DNA load.

The dynamic ranges of quantification of the available assays are shown in table 1 (in non standardized units). Samples with a viral content above the upper limit of a given assay must be retested after 1/10 or 1/100 dilution for accurate quantification. These assays have been shown to be specific and accurate within their respective dynamic ranges of quantification [4-13]. The possible influence of the HBV genotype on quantification has not been studied. Whatever the assay, differences or variations of less than 0.5 log (i.e. less than three-fold) should not be taken into account, as they may be due to intrinsic or between-patient variability.

## **PRACTICAL USE OF HBV DNA QUANTIFICATION**

### Diagnosis of HBV infection

Acute hepatitis B. HBV DNA detection or quantification is not necessary for the diagnosis of acute hepatitis B, which is based on serologic testing.

Chronic HBsAg carriage. In chronic HBsAg carriers, HBV DNA detection-quantification is necessary to determine whether or not HBV is replicating [14]. The quantitative result does not really matter in the presence of HBeAg, because the diagnosis of replicating chronic hepatitis B can be made whatever the viral load. In contrast, the interpretation of HBV DNA quantification is difficult in HBeAg-negative/anti-HBeAg-positive patients. Indeed, the “inactive carriers” appear to have lower mean HBV DNA levels than the patients with clinically active chronic hepatitis B [15, 16]. However, the discriminating HBV DNA threshold remains to be established in appropriate clinical studies using highly sensitive and accurate HBV DNA assays, and standardized international units [17].

### Assessment of disease severity and prognosis

HBV DNA detection provides valuable prognostic information. Indeed, the presence of HBV DNA is associated with a significant risk of progression to chronic hepatitis B complications including cirrhosis and hepatocellular carcinoma [14]. This risk is low in the absence of detectable HBV DNA, except in patients with cirrhosis who may subsequently develop hepatocellular carcinoma despite the absence of HBV replication. The possible prognostic significance of HBV DNA load, i.e. what level of HBV DNA is associated with progressive liver disease, remains to be determined in appropriate clinical studies.

### **Treatment of HBV infection.**

**Decision to treat.** The decision to treat chronic hepatitis B must be made in patients with elevated serum alanine aminotransferase (ALT) activity, a liver biopsy showing chronic hepatitis with or without cirrhosis, and the presence of significant levels of HBV DNA. The decision to treat is practically easy if HBeAg is present. It is more difficult in HBeAg-negative patients with detectable HBV DNA and mild to moderate lesions on liver biopsy, because no precise clinically relevant HBV DNA thresholds are known. Prospective trials are needed to determine HBV DNA loads (in IU/ml) above which patients with chronic hepatitis B should be treated (and below which they should not).

**Selection of optimal therapy.** The current treatment of chronic hepatitis B is based on standard interferon (IFN)- $\alpha$  or lamivudine [18-23]. The choice will change in the near future for the following reasons : promising preliminary results have been reported with pegylated IFN- $\alpha$  24, 25]; new potent antiviral molecules, such as

adefovir dipivoxil and others, will soon be available [26-28] ; combination therapy has not been appropriately evaluated to date and might yield interesting results. HBV DNA quantification could help selecting optimal therapy. The patients with a low HBV DNA level could be more likely than those with a high HBV DNA level to have a sustained response to IFN- $\alpha$ -based therapy. Conversely, the patients with a high HBV DNA level might be the best candidates to antiviral therapy with nucleoside/nucleotide analogs. Again, the precise HBV DNA cutoff that discriminates between “low” and “high” pretreatment replication needs to be determined in prospective clinical trials, using standardized quantification units.

***Treatment monitoring.*** HBV DNA quantification, together with repeated ALT determinations and HBeAg/anti-HBe antibody assessments in HBeAg-positive patients, is critical in treatment monitoring [18-23]. Non responders to IFN- $\alpha$ -based treatment have little or no change in HBV DNA load during therapy, whereas responders show a significant decrease. Successful IFN- $\alpha$  treatment is characterized by HBe seroconversion in HBeAg-positive patients, and a reduction in HBV DNA load below the detection cutoff of signal amplification assays. Small amounts of HBV DNA often remain detectable in HBe seroconverters with more sensitive target amplification assays. In patients receiving nucleoside/nucleotide analogs, the viral load significantly and rapidly decreases, but low-level replication remains detectable with sensitive assays in most cases. To what level HBV DNA should be reduced to ensure sustained virologic and clinical remission remains to be prospectively determined.

HBV resistance, which has been shown to be frequent with lamivudine monotherapy, is characterized by a relapse of HBV replication while the drug is still administered [29-39]. Patients with lamivudine resistance are generally kept on lamivudine or may be switched to adefovir dipivoxil in case of rapidly progressing liver disease.

## **HBV VIRAL KINETICS**

The advent of sensitive, reproducible and accurate tools to measure HBV DNA levels has recently open the way to the monitoring of HBV replication kinetics in various clinical settings.

### HBV kinetics during acute infection.

The kinetics of acute HBV infection have been recently characterized in patients infected from a single source [1]. HBV DNA was shown to replicate rapidly. The HBV DNA peak occurred on average  $127 \pm 47$  days after infection and reached very high levels, in the order of  $10^{10}$  copies/ml. In these patients (who spontaneously recovered), HBV DNA clearance started at the time of symptomatic acute hepatitis. It followed a two or three phase decay pattern with an initial rapid decline of unclear mechanism, and a final phase that might be related to infected hepatocyte loss. The peak HBV production rate was estimated to be on average in the order of  $10^{13}$  virions per day, with a maximum production rate of each infected hepatocyte of 200 to 1000 virions per day only [1]. The authors estimated that at this peak rate of virion production, every possible single and most double mutations would be created each day [1]. The latter finding suggests that viral escape is not the primary mechanisms of HBV persistence, which occurs in approximately 5% of adult patients with acute hepatitis B.

### HBV kinetics during chronic infection.

At the chronic stage of infection, HBV DNA kinetics are at a steady state, which explains that HBV DNA levels remain relatively stable over time within each phase of infection. The daily production rate of HBV virions has been estimated to be on average  $10^{11}$  viral particles per day, with a mean half-life of free HCV virions in the order of 1 day [40, 41]. The minimum half-life of infected cells remains debated [40, 41].

### HBV kinetics during antiviral therapy.

HBV kinetics during antiviral therapy with various molecules have been recently described. Given the small number of reports and the small numbers of patients in each study, these results should be considered

preliminary, pending further studies on larger groups of patients. In a Greek study, pegylated IFN-a 2b, 100 µg qw and 200 µg qw, induced a 29.4% and 67.4% mean HBV replication blocking effectiveness, respectively. The addition of lamivudine to 100 µg qw of pegylated IFN-a 2b increased the mean blocking effectiveness to 90.7% [25]. The mean death rate of infected cells was increased with the higher dose of pegylated IFN-a and when lamivudine was added [25].

The administration of nucleosidic/nucleotidic antiviral molecules, such as lamivudine, adefovir dipivoxil or entecavir, was shown to be associated with a dose-dependent HBV replication blocking effectiveness, over 95% on average with the usual dosages. Various patterns of response, including both bi- and triphasic patterns, have been reported, the significance of which remains unclear [42-46]. The second slope of viral decrease during lamivudine therapy was shown to be associated with baseline ALT levels in one study, emphasizing the relationship between disease activity and the immune response responsible for infected cell death [42]. The recent report of a sensitive and accurate method to quantify cccDNA in the liver [47] might improve interpretation of viral kinetics analyses in patients with chronic hepatitis B receiving antiviral therapy.

## CONCLUSION

HBV DNA detection and quantification plays a key role in the management of patients with HBV infection. However, few practical guidelines can be drawn at the present time due to the lack of published data and standardization of HBV DNA quantification units. Various clinical studies need to be performed in order to establish clinically relevant thresholds. A better understanding of the effects of antiviral drugs and the HBV DNA levels that should be targeted is also mandated. The study of HBV kinetics might help better understand the mechanisms of action of antiviral drugs and improve the global management of these patients.

**Table 1-** Available HBV DNA detection and quantification assays. The dynamic ranges of quantification are given in non standardized units. Conversion factors to IUs are currently being calculated.

<i>Manufacturer</i>	<i>Assay</i>	<i>Method</i>	<i>Dynamic range of quantification</i>
Digene Corp., Gaithersburg, Maryland, USA	HBV Digene Hybrid-Capture™ I	Hybrid capture signal amplification in tubes	700,000-560,000,000 copies/ml
	HBV Digene Hybrid-Capture™ II	Hybrid capture signal amplification in microplates	142,000- 1,700,000,000 copies/ml
	Ultra-sensitive HBV Digene Hybrid-Capture™ II	Hybrid capture signal amplification in microplates after centrifugation	4,700-57,000,000 copies/ml
Roche Molecular Systems, Pleasanton, California, USA	Amplicor HBV Monitor™	Manual quantitative RT-PCR	1,000-4,000,000 copies/ml
	Cobas Amplicor HBV Monitor™	Semi-automated quantitative RT-PCR	200-200,000 copies/ml
Bayer Corporation, Tarrytown, New York, USA	Versant™ HBV DNA 1.0 Assay	Manual branched DNA signal amplification	700,000-5,000,000,000 genome equivalents/ml
	Versant™ HBV DNA 3.0 Assay	Semi-automated branched DNA signal amplification	In development : assessment of the dynamic range of quantification under way

\*The dynamic ranges of quantification are given in non standardized units. Conversion factors to IUs are currently being .

## REFERENCES

1. Whalley SA, Murray JM, Brown D, Webster GJM, Emery VC, Dusheiko GM, et al. Kinetics of acute hepatitis B virus infection in humans. *J Exp Med* 2001;193:847-53
2. Pawlotsky JM. Molecular diagnosis of viral hepatitis. *Gastroenterology* 2002;122:1554-68
3. Saldanha J, Gerlich W, Lelie N, Dawson P, Heermann K, Heath A. An international collaborative study to establish a World Health Organization international standard for hepatitis B virus DNA nucleic acid amplification techniques. *Vox Sang* 2001;80:63-71
4. Aspinall S, Steele AD, Peenze I, Mphahlele MJ. Detection and quantitation of hepatitis B virus DNA: comparison of two commercial hybridization assays with polymerase chain reaction. *J Viral Hepat* 1995;2:107-11
5. Barlet V, Cohard M, Thelu MA, Chaix MJ, Baccard C, Zarski JP, et al. Quantitative detection of hepatitis B virus DNA in serum using chemiluminescence: comparison with radioactive solution hybridization assay. *J Virol Methods* 1994;49:141-51
6. Krajden M, Minor J, Cork L, Comanor L. Multi-measurement method comparison of three commercial hepatitis B virus DNA quantification assays. *J Viral Hepat* 1998;5:415-22
7. Chan HL, Leung NW, Lau TC, Wong ML, Sung JJ. Comparison of three different sensitive assays for hepatitis B virus DNA in monitoring of responses to antiviral therapy. *J Clin Microbiol* 2000;38:3205-8
8. Hendricks DA, Stowe BJ, Hoo BS, Kolberg J, Irvine BD, Neuwald PD, et al. Quantitation of HBV DNA in human serum using a branched DNA (bDNA) signal amplification assay. *Am J Clin Pathol* 1995;104:537-46
9. Ho SK, Chan TM, Cheng IK, Lai KN. Comparison of the second-generation Digene hybrid capture assay with the branched-DNA assay for measurement of hepatitis B virus DNA in serum. *J Clin Microbiol* 1999;37:2461-5
10. Niesters HG, Krajden M, Cork L, de Medina M, Hill M, Fries E, et al. A multicenter study evaluation of the Digene hybrid capture II signal amplification technique for detection of hepatitis B virus DNA in serum samples and testing of EUROHEP standards. *J Clin Microbiol* 2000;38:2150-5
11. Pawlotsky JM, Bastie A, Lonjon I, Remire J, Darthuy F, Soussy CJ, et al. What technique should be used for routine detection and quantification of HBV DNA in clinical samples? *J Virol Methods* 1997;65:245-53
12. Pawlotsky JM, Bastie A, Hezode C, Lonjon I, Darthuy F, Remire J, et al. Routine detection and quantification of hepatitis B virus DNA in clinical laboratories: performance of three commercial assays. *J Virol Methods* 2000;85:11-21
13. Poljak M, Marin IJ, Seme K, Brinovec V, Maticic M, Meglic-Volkar J, et al. Second-generation Hybrid capture test and Amplicor Monitor test generate highly correlated hepatitis B virus DNA levels. *J Virol Methods* 2001;97:165-9
14. Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B 2000: summary of a workshop. *Gastroenterology* 2001;120:1828-53
15. Martinot-Peignoux M, Boyer N, Colombat M, Akremi R, Pham BN, Ollivier S, et al. Serum hepatitis B virus DNA levels and liver histology in inactive HBsAg carriers. *J Hepatol* 2002;36:543-6
16. Manesis EK, Papatheodoridis GV, Hadziyannis SJ. Serum HBV-DNA levels in inactive hepatitis B virus carriers. *Gastroenterology* 2002;122:2092-3
17. Chu CJ, Lok ASF. Clinical utility in quantifying serum HBV DNA levels using PCR assays. *J Hepatol* 2002;36:549-51
18. Perrillo RP, Schiff ER, Davis GL, Bodenheimer HC, Jr., Lindsay K, Payne J, et al. A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. *N Engl J Med* 1990;323:295-301

19. Dienstag JL, Perrillo RP, Schiff ER, Bartholomew M, Vicary C, Rubin M. A preliminary trial of lamivudine for chronic hepatitis B infection. *N Engl J Med* 1995;333:1657-61
20. Lok AS, Lai CL, Wu PC, Leung EK. Long-term follow-up in a randomised controlled trial of recombinant alpha 2-interferon in Chinese patients with chronic hepatitis B infection. *Lancet* 1988;2:298-302
21. Lai CL, Lok AS, Lin HJ, Wu PC, Yeoh EK, Yeung CY. Placebo-controlled trial of recombinant alpha 2-interferon in Chinese HBsAg-carrier children. *Lancet* 1987;2:877-80
22. Dienstag JL, Schiff ER, Wright TL, Perrillo RP, Hann HW, Goodman Z, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999;341:1256-63
23. Lai CL, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, et al. A one-year trial of lamivudine for chronic hepatitis B. *N Engl J Med* 1998;339:61-8
24. Cooksley WGE, Piratvisuth T, Wang YJ, Mahachai V, Chao YC, Tanwandee T, et al. Evidence for the efficacy of peginterferon alfa-2a (40kD) (Pegasys) in the treatment of HBeAg-positive chronic hepatitis B and impact of baseline factors. *J Hepatol* 2002;36 (Suppl. 1):8
25. Sypsa V, Tassopoulos NC, Chrysagis D, Mimidis K, Vassiliadis T, Raptopoulou M, et al. A viral kinetic study using pegylated interferon alpha-2b and lamivudine in naive patients with HBeAg(-)/HBV DNA(+) chronic hepatitis B: preliminary results. *J Hepatol* 2002;36 (Suppl. 1):94
26. Benhamou Y, Bochet M, Thibault V, Calvez V, Fievet MH, Vig P, et al. Safety and efficacy of adefovir dipivoxil in patients co-infected with HIV-1 and lamivudine-resistant hepatitis B virus: an open-label pilot study. *Lancet* 2001;358:718-23
27. Perrillo R, Schiff E, Yoshida E, Statler A, Hirsch K, Wright T, et al. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. *Hepatology* 2000;32:129-34
28. Xiong X, Flores C, Yang H, Toole JJ, Gibbs CS. Mutations in hepatitis B DNA polymerase associated with resistance to lamivudine do not confer resistance to adefovir in vitro. *Hepatology* 1998;28:1669-73
29. Zollner B, Petersen J, Schroter M, Laufs R, Schoder V, Feucht HH. 20-fold increase in risk of lamivudine resistance in hepatitis B virus subtype adw. *Lancet* 2001;357:934-5
30. Schalm SW. Clinical implications of lamivudine resistance by HBV. *Lancet* 1997;349:3-4
31. Yao FY, Terrault NA, Freise C, Maslow L, Bass NM. Lamivudine treatment is beneficial in patients with severely decompensated cirrhosis and actively replicating hepatitis B infection awaiting liver transplantation: a comparative study using a matched, untreated cohort. *Hepatology* 2001;34:411-6
32. Lau DT, Khokhar MF, Doo E, Ghany MG, Herion D, Park Y, et al. Long-term therapy of chronic hepatitis B with lamivudine. *Hepatology* 2000;32:828-34
33. Villeneuve JP, Condreay LD, Willems B, Pomier-Layrargues G, Fenyves D, Bilodeau M, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. *Hepatology* 2000;31:207-10
34. Benhamou Y, Bochet M, Thibault V, Di Martino V, Caumes E, Bricaire F, et al. Long-term incidence of hepatitis B virus resistance to lamivudine in human immunodeficiency virus-infected patients. *Hepatology* 1999;30:1302-6
35. Dienstag JL, Schiff ER, Mitchell M, Casey DE, Jr., Gitlin N, Lisoos T, et al. Extended lamivudine retreatment for chronic hepatitis B: maintenance of viral suppression after discontinuation of therapy. *Hepatology* 1999;30:1082-7
36. Tillmann HL, Trautwein C, Bock T, Boker KH, Jackel E, Glowienka M, et al. Mutational pattern of hepatitis B virus on sequential therapy with famciclovir and lamivudine in patients with hepatitis B virus reinfection occurring under HBIG immunoglobulin after liver transplantation. *Hepatology* 1999;30:244-56
37. Perrillo R, Rakela J, Dienstag J, Levy G, Martin P, Wright T, et al. Multicenter study of lamivudine therapy for hepatitis B after liver transplantation. *Hepatology* 1999;29:1581-6

38. Ono-Nita SK, Kato N, Shiratori Y, Masaki T, Lan KH, Carrilho FJ, et al. YMDD motif in hepatitis B virus DNA polymerase influences on replication and lamivudine resistance: a study by in vitro full-length viral DNA transfection. *Hepatology* 1999;29:939-45
39. Tipples GA, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DL. Mutation in HBV RNA -dependent DNA polymerase confers resistance to lamivudine in vivo. *Hepatology* 1996;24:714-7
40. Nowak MA, Bonhoeffer S, Hill AM, Boehme R, Thomas HC, McDade H. Viral dynamics in hepatitis B virus infection. *Proc Natl Acad sci USA* 1996;93
41. Zeuzem S, De Man RA, Honkoop P, Roth WK, Schalm SW, Schmidt JM. Dynamics of hepatitis B virus infection in vivo. *J Hepatol* 1997;27:431-6
42. Wolters LLM, Hansen BE, Niesters HGM, Levi-Drummer RS, Neumann AU, Schalm SW, et al. The influence of baseline characteristics on viral dynamics parameters in chronic hepatitis B patients treated with lamivudine. *J Hepatol* 2002;37:253-8
43. Lau GKK, Tsiang M, Hou J, Yuen ST, Carman WF, Zhang L, et al. Combination therapy with lamivudine and famciclovir for chronic hepatitis B-infected Chinese patients: a viral dynamics study. *Hepatology* 2000;32:394-9
44. Tsiang M, Rooney JF, Toole JJ, Gibbs CS. Biphasic clearance kinetics of hepatitis B virus from patients during adefovir dipivoxil therapy. *Hepatology* 1999;29:1863-9
45. Wolters LLM, Hansen BE, Niesters HG, De Hertogh D, De Man RA. Viral dynamics during and after entecavir therapy in patients with chronic hepatitis B. *J Hepatol* 2002;37:137-44
46. Neumann AU, Havlin Y, Tal R, Tsiang M, Wulfsohn M, Brosgart C, et al. Long-term HBV kinetics classification during treatment with adefovir dipivoxil. *J Hepatol* 2002;36 (Suppl. 1):121
47. Werle B, Wursthorn K, Petersen J, Bowden S, Locarnini S, James C, et al. Development of a quantitative assay for hepatic HBV cccDNA levels in patients with chronic hepatitis B. *J Hepatol* 2002;36 (Suppl. 1):4