Journal of Clinical Virology 45 (2009) 232–236

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Hepatitis B virus (HBV) genotype determination by the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 in serum and plasma matrices☆

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ARTICLE INFO

Article history:
Received 7 May 2009
Accepted 11 May 2009

Keywords:
HBV genotypes
Quantification
COBAS
AmpliPrep
TaqMan
Fully automated

ABSTRACT

Background: Viral load quantification is established in the clinical management of chronic Hepatitis B virus (HBV) infection for assessing efficacies and resistance developments in anti-viral drug treatment.
Objectives: The fully automated COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 was evaluated for the linear measuring range and the inclusivity of HBV genotype determination in EDTA plasma and serum samples.
Study design: Two kit lots of the test were used to determine the linear measuring range as well as linearity and limit of detection applying different concentration levels of specimens representing HBV genotypes A to H along with a pre-core mutant and the WHO Standard.
Results: The COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 displayed a linear measuring range of seven $\log_{10}$ steps from 20 IU/mL (lower limit of quantification) to 2.3E+08 IU/mL (upper limit of quantification) yielding similar results for EDTA plasma and serum. Inclusivity was shown by reliable quantification of HBV genotypes A to H at different concentration levels. The ≥95% hit rate LOD was 15 IU/mL for genotypes C, D, F, G, the pre-core mutant and the WHO Standard and 20 IU/mL for genotypes A, B, E and H matching the test's lower limit of quantification. 95% PROBIT analysis yielded concentrations of 8.9 IU/mL for the WHO Standard and of 6.0–16.4 IU/mL for the genotypes.
Conclusions: The COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 provides genotype inclusivity for accurate viral load monitoring in serum and EDTA plasma samples and supports clinical routine in the management of HBV infection.

1. Background

Hepatitis B virus (HBV) infection represents a major health concern with over 350 million of chronically infected carriers, facing high risks for chronic hepatitis, cirrhosis and hepatocellular carcinoma.[1–4] Genetic variability and adaptive capacity of HBV are very high and led to the emergence of eight genotypes A to H as well as of naturally occurring mutants e.g. pre-core mutants all showing distinct geographic distribution patterns.[5,6] For instance, genotype A predominates in Northern Europe and Sub-Saharan Africa and genotype E in Central Africa, whereas genotypes B and C are prevalent in Asia and Australia. In contrast, genotypes F, G and H are characteristic for the American continent and genotype D is more evenly distributed. During the past years it became more evident, that HBV genotype variability has an impact on the severity and the progression of the disease as well as on the efficacy of anti-viral therapy, in particular of the standard interferon treatment.[7–9]

In the clinical management of HBV infection it is generally accepted that a reduction in plasma virus levels is associated with a decreased risk of clinical progression.[10,11] Recent findings confirmed that early and profound suppression of viral replication improves the probability of sustained virologic response to treatment[12] and reduces the likelihood of drug induced resistance.[3,13,14] Thus, changes of viral load need to be accurately monitored independent of HBV genotype. HBV DNA testing elucidates an individual's true hepatitis B status[15,16] and has a high prognostic value for the outcome of acute and chronic HBV infections and their treatment.[17–20] Today, viral load quantification is the major tool to evaluate the efficacy of treatment regimens and to identify treatment resistances in the management of chronic HBV infection.[13–15,21]

The COBAS® AmpliPrep/COBAS® TaqMan® system (Roche Molecular Systems Inc., Branchburg, NJ, USA) offers fully automated extraction of HBV DNA combined with automated amplification and real-time determination. The COBAS® AmpliPrep/COBAS®

Abbreviations: HBV, Hepatitis B virus; IU, International Units; LOD, limit of detection.

☆ This test is currently not available in the US.

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doi:10.1016/j.jcv.2009.05.021

Table 1
Designation of genotype plasmid DNA.

Plasmid designation	Genotype	Parent specimen origin
p3952-c1	A	Cameroon
p1767-c1	B	China
p1786-c1	C	China
p3953-c2	D	North Africa
p3963-c2	E	Nigeria
p1009-c1	F	Spain
p00042975-4	G	United States
pEFHBV20	H	Nicaragua

TaqMan® HBV Test v1.0 was developed to meet the high demands for clinical monitoring of HBV infection in plasma samples.[22,23] The improved version COBAS® AmpliPrep/COBAS® TaqMan® HBV Test v2.0 is now applicable to samples in EDTA plasma and serum matrices.

2. Objectives

The present study evaluates the performance of the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 with regard to the linear measuring range and examines HBV genotype inclusivity according to linearity and limit of detection (LOD) in serum and EDTA plasma matrices. Two production kit lots of the test were used to measure clinical specimens representing HBV genotypes A to H at different concentration levels along with the most common HBV pre-core mutant and the WHO Standard.

3. Study design

3.1. Linear measuring range

The linearity of HBV DNA quantification by the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 in EDTA plasma and serum matrices was analysed by using 12 and 13 concentration levels, that were prepared by serial dilutions of highly concentrated HBV positive certified clinical specimens of genotype A.

To verify correct concentration assignment, one of the concentration levels was determined according to the Calibrator Bracketing Method with the HBV Secondary Standard as calibrator traceable to the WHO International Standard for Hepatitis B virus DNA (NIBSC Code 97/746 and NIBSC 97/750), genotype A.[24] All concentration levels were measured with 10 replicates per each of two kit lots. The linear range was defined as the range of the nominal input concentrations, with mean $\log_{10}$ deviations between observed and nominal concentrations falling within the accuracy range of $\pm 0.3 \log_{10}$.

3.2. Linearity of HBV genotype determination

Plasmids representing genotypes A to H were used (Table 1) and diluted with EDTA plasma or serum to concentration levels of 1.0E+07 IU/mL, 1.0E+05 IU/mL and 1.0E+03 IU/mL, respectively. Analysis was performed with 10 replicates per concentration level, matrix and kit lot.

3.3. HBV genotypes for LOD determination

Genotypes A to F and H were represented by one HBV positive certified clinical specimen each, and were provided by Teragenix (Ft. Lauderdale, FL, USA). Genotype G was included as purified plasmid DNA, as well as the most common pre-core mutant represented by the plasmid piTmut1896, both provided by Roche Molecular Systems Inc. (Pleasanton, CA, USA). Furthermore, the WHO Standard was used. Nominal concentrations for each genotype were assigned using the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0. Traceability of this concentration assignment to the WHO Standard was shown for an Intermediate Stock Solution (~250 IU/mL), applying the Calibrator Bracketing Method with the HBV Secondary Standard as a calibrator, directly traceable to the WHO Standard.[24]

A verification of the concentration assignment was performed using the High Pure Viral Nucleic Acid Kit and the COBAS® TaqMan® 48 HBV Test.[25] Comparing the $\log_{10}$ concentration results of the two methods, the mean deviations were within $\pm 0.2 \log_{10}$ confirming the accuracy of genotype concentration assignment.

For LOD determination, the assigned Intermediate Stock Solutions (250 IU/mL) of each genotype and the WHO Standard were used to prepare the concentration levels of 28 IU/mL, 24 IU/mL, 20 IU/mL, 15 IU/mL and 5 IU/mL in HBV-negative EDTA plasma or serum. Additionally, HBV-negative EDTA plasma and serum samples were tested as blanks. 24 replicates per concentration level, matrix and kit lot were analysed.

3.4. COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0

The COBAS® AmpliPrep/COBAS® TaqMan® HBV Test v2.0 (Roche Molecular Systems Inc., Branchburg, NJ, USA) is a nucleic acid amplification test, combining automated specimen processing on the COBAS® AmpliPrep Instrument with real-time amplification, quantification and data documentation on the COBAS® TaqMan® Analyzer. The Test v1.0 requires 1000 μL of input volume of EDTA plasma only,[22] whereas the Test v2.0 uses 650 μL of EDTA plasma as well as of serum specimens. Results are reported in IU/mL and traceable to the WHO International Standard (NIBSC 97/746 and NIBSC 97/750). Test v1.0 claims a linear measuring range from 54 IU/mL to 1.1E+08 IU/mL and a LOD of 12 IU/mL. HBV DNA determination by the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 was performed on the COBAS® TaqMan® or COBAS® TaqMan® 48 Analyzer following the manufacturers instructions.

3.5. Data analysis

The hit rate (%) of each individual genotype was calculated as: number of valid positive results/number of total valid results × 100. The LOD for each genotype was determined as the lowest concentration level with a hit rate ≥95% for which all higher concentration levels also had a hit rate ≥95%. Additionally, the values for each genotype, matrix and kit lot were subjected to PROBIT analysis yielding the 95% PROBIT hit rate concentration and the 95% PROBIT confidence interval. The overall LOD was obtained by combining the data of both kit lots and matrices for each genotype. Linear regression analysis of $\log_{10}$-transformed results was performed using the JMP statistical analysis software. The measuring range was analysed with the polynomial fit evaluation according CLSI EP6-A.

4. Results

4.1. Linear measuring range of the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0

The test was evaluated with HBV positive clinical specimens diluted in serum or EDTA plasma. Linear regression analyses for both matrices are presented in Figs. 1 and 2. The hit rate for all concentration levels tested was 100%. The nominal concentration levels from 17 IU/mL to 4.3E+08 IU/mL ($1.24 \log_{10}$–$8.64 \log_{10}$) for plasma and from 20 IU/mL to 2.3E+08 IU/mL ($1.31 \log_{10}$–$8.35 \log_{10}$) for serum fell within the accuracy range of $\pm 0.3 \log_{10}$ for EDTA plasma and serum. Accordingly, independent of the matrix used the test displayed a linear measuring range of 20 IU/mL (lower limit