Comparative Characterization of Two Tests for Measurement of Hepatitis B Virus DNA in the Blood Serum and Plasma, Based on the Use of Two Different Detection Methods

A. Juman Awadh, K. K. Kyuregyan*, O. V. Isaeva*, and M. I. Mikhailov*

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Two tests for measurements of hepatitis B virus DNA in human serum and plasma, VERSANT HBV 3.0 (based on the use of branched DNA chains) and Biotitre-B (a real-time PCR variant), were compared. Serum specimens from patients (n=56) with documented viral hepatitis were tested. For specimens with DNA values in the linear range in both tests the correlations were evaluated by Pearson's method. The sensitivity of the two tests, and reproducibility of the results of HBV DNA measurements were evaluated using a panel of recombinant virus DNA dilutions with a step of 1 lg HBV DNA copies per ml (from 7 to 3 lg). Three measurements for each concentration of the reference sample were carried out in each test. Clinical specificity of the two tests was evaluated by the analysis of HBV-negative serum samples, collected from donors not reactive by HBsAg and anti-HBc (n=60). Of 56 samples with documented infection, the results of testing by VERSANT HBV 3.0 and Biotitre-B did not agree in 4 (7.1%) samples. Pearson's correlation coefficient of for results obtained in VERSANT HBV 3.0 and Biotitre-B in linear range for both tests was 0.712. Evaluation of reproducibility of the tests using a panel of recombinant HBV DNA showed higher reproducibility of VERSANT HBV 3.0 test with coefficient of variations from 0.79 to 2.79% *vs.* 2.39-10.69% for Biotitre-B reference test. All 60 serum samples from donors areactive by HBsAg and anti-HBc were negative by HBV DNA when tested by VERSANT HBV 3.0 and Biotitre-B. Hence, clinical specificity of both tests was 100%. The results indicate high specificity of both tests and good agreement of their results, the reproducibility of VERSANT HBV 3.0 test being higher.

Key Words: *VERSANT HBV 3.0; Biotitre-B; clinical specificity; sensitivity; reproducibility of tests*

Evaluation of the virus load became a routine procedure in patients infected by hepatites B and C viruses (HBV and HCV). The significance of precise evaluation of the virus load for optimal treatment of patients implies that quantitative tests should provide reliable results, which should be comparable in case of using different diagnostic agents. Methods for measurements of nucleic acids, including those of infectious agents, can be divided conditionally into 2 groups: based on amplification of

Peoples' Friendship University of Russia; *M. P. Chumakov Institute of Poliomyelitis and Viruses Encephalitis. Russian Academy of Medical Sciences, Moscow, Russia

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DNA target site and on luminescent signal. One of the recent methods for quantitative evaluation of virus load in the former group is real-time PCR [9]. The other group is presented by the analysis of branched DNA chains (bDNA analysis). Due to technological and working characteristics of bDNA analysis it became a routine method in many clinical and research laboratories, because it does not require a high degree of purification of the analyzed nucleic acids and is free from DNA amplification (a potential source of contamination by amplicons) [1].

We compared two tests intended for quantitative evaluation of HBV DNA in human serum and plasma: VERSANT HBV 3.0 (method of branched DNA chains; Bayer) and Biotitre-B (real time PCR method; LITECH).

MATERIALS AND METHODS

The results of VERSANT HBV 3.0 and Biotitre-B tests were compared for serum specimens from patients with clinically verified HBV infection (n=56). Of these, 33 (58.9%) presented with chronic hepatitis B (CHB), 15 (26.8%) were HBsAg carriers, 2 (3.6%) had HBV-associated cirrhosis of the liver, 3 (5.4%) HBV and HCV coinfection, 2 (3.6%) HBV and hepatitis delta virus (HDV) coinfection, and 1 (1.8%) HBV, HCV, and HDV coinfection. Serum specimens from these patients were tested in parallel in VERSANT HBV 3.0 and Biotitre-B tests. Results of two tests differing by at least 1 lg HBV DNA copies/ml were considered as discordant. For specimens with values in the linear range in both tests (VERSANT HBV 3.0 and Biotitre-B), the correlation of the values was evaluated by Pearson's method.

The sensitivity and reproducibility of the results of measurements of HBV DNA in VERSANT HBV 3.0 in comparison with Biotitre-B test were evaluated using a panel of diluted HBV DNA reference sample. The panel presented dilutions of the reference sample containing recombinant HBV DNA (Avicenna) in HBV-negative human plasma and consisted of 5 dilutions at a step of 1 lg HBV DNA copies/ml with concentrations from 7 to 3 lg HBV DNA copies/ml. Three measurements for each concentration of the reference sample were carried out in each test.

Clinical specificity of the two tests was evaluated by the analysis of HBV-negative serum samples from blood donors, areactive by HBsAg and anti-HBc in IFA (n=60).

RESULTS

Evaluation of the tests with a panel of recombinant HBV DNA dilutions showed higher reproducibility of VERSANT HBV 3.0 test, its coefficient of variations being 0.79-2.79%, while for Biotitre-B reference test the coefficient varied from 2.39 to 10.69% (Table 1). The coefficient of variation was lower in VERSANT HBV 3.0 test for each dilution in the panel (10^3, 10^4, 10^5, 10^6, and 10^7 copies/ml).

Testing of 60 blood samples from HBV-areactive blood donors showed that all samples were negative by HBV DNA in VERSANT HBV 3.0 and Biotitre-B test, and hence, clinical specificity of both tests was 100%.

Of 56 samples from patients with verified HBV infection, the results in VERSANT HBV 3.0 and Biotitre-B were discordant in 4 (7.1%) specimens. All these four samples were collected from patients with CHB with high viral load (more than 10^6 copies/ml in both tests). Distribution of concentrations in linear ranges of both tests (2×10^3-10^8 copies HBV DNA/ml in VERSANT HBV 3.0 and 10^3-10^7 copies HBV DNA/ml in Biotitre-B) were within the range obtained for 30 (53.6%) samples (Fig. 1). Pearson's coefficient of correlation for the results obtained by VERSANT HBV 3.0 and Biotitre-B in the linear range for both tests was 0.712.

The distribution of HBV DNA concentrations in HBV-infected patients according to VERSANT HBV 3.0 was as follows: the levels of HBV load were low (<2000 copies/ml) or medium (<10^6 copies/ml) in HBsAg carriers, patients with HCV and

TABLE 1. Evaluation of HBV DNA with a Panel of HBV Reference Sample (Recombinant HBV DNA)

HBV DNA concentrations (HBV DNA copies)	10^7	10^6	10^5	10^4	10^3
Mean titer in VERSANT HBV 3.0, lg copies/ml	1.7×10^6	5.7×10^5	5.3×10^4	3.0×10^3	0
	6.23	5.76	4.72	3.48	
Coefficient of variation, %	2.54	1.34	0.79	2.79	—
Mean titer in Biotitre-B, lg copies/ml	6.2×10^6	6.6×10^5	9.4×10^4	5.5×10^4	0
	6.79	5.82	4.97	3.74	
Coefficient of variation, %	4.36	10.69	2.39	5.67	—

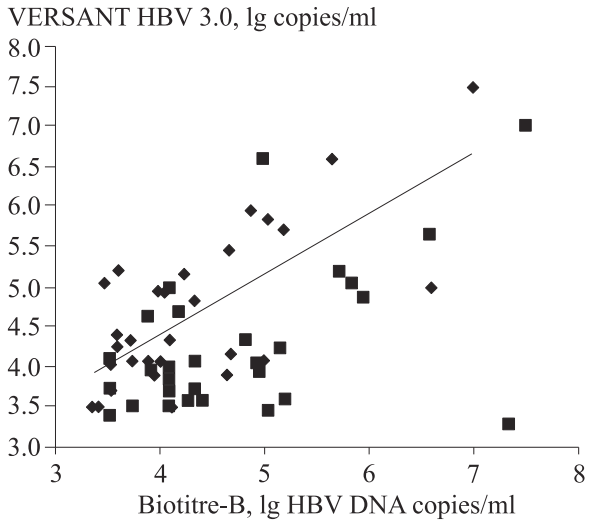

Fig. 1. Correlations of results of VERSANT HBV 3.0 and Biotitre-B tests with serum samples from patients with HBV infection.

HDV coinfection, and HBV-associated cirrhosis of the liver. In the patient with HBV, HDV, and HCV coinfection, HBV load was <2000 copies/ml. Medium viral (HBV) load predominated among patients with CVH (n=20; 60.6%), but 7 (21.2%) presented with high viremia (>10^6 copies/ml) and 6 (18.2%) with low HBV viremia (<2000 copies/ml).

Evaluation of HBV load (concentration of viral DNA in blood serum or plasma) is of paramount importance for clinical practice, because the studies carried out in the patients showed the significance of high HBV load (more than 10^5 copies/ml) as a prognostic marker of an unfavorable outcome of the disease, eventuating in cirrhosis and hepatocellular carcinoma [3,4]. Along with biochemical values, measurement of HBV DNA is the main instrument of monitoring antiviral therapy and evaluation of its efficiency [5,6] and prediction of the treatment efficiency [7,11]. The value of HBV load is also an important factor determining the risk of perinatal transfer of HBV [10] and a criterion of permission for HBV infected medical workers to perform invasive procedures [2]. Evaluation of HBV load is extremely significant for clinical practice, and hence, the accuracy and reproducibility of the analysis are the main requirements to diagnostic agents for quantitative evaluation of HBV DNA. Despite stringent requirements to working characteristics of the tests registered for laboratory practice and standardization of the test by the International HBV standard, the majority of present recommendations allows the use of just one diagnostic agent during dynamic observation of a patient with HBV infection in order to rule out the discrepancies in evaluation of HBV load, which can appear if different tests are used.

Several tests for quantitative evaluation of HBV DNA are used in laboratory practice; these are mainly tests based on real-time PCR. In 2006, a new diagnostic agent for quantitative detection of HBV DNA based on the use of branched DNA chains (bDNA) appeared on the Russian market. A distinctive feature of this semiautomated test for the real-time PCR is no need in amplification of the DNA target, because detection in the bDNA method is based on a series of hybridizations with oligonucleotide probes labeled with fluorescent dyes, as a result of which the signal is amplified. No need in viral DNA amplification solved one of the main problems of the PCR method: risk of contamination with amplicons. One more advantage of bDNA test is no need in isolation and purification of nucleic acids from clinical samples, because native serum or plasma is pipetted into the reaction well. Both tests exhibited high clinical specificity by the results of testing specimens from blood donors negative for HBsAg and anti-HBc. Pearson's coefficient (0.712) obtained in linear regression analysis for 30 samples, in which DNA concentrations were within the linear range for both tests, indicates sufficiently high agreement between the values of HBV load determined by the two tests. However, this correlation between the results of two tests is not ideal. Comparison of two other tests for measurements of HBV DNA concentrations (COBAS AMPLICOR HBV MONITOR and AMPLICOR HBV MONITOR; Roche) exhibited a much higher correlation between the results of quantitative evaluation of HBV DNA (r=0.97; p<0.01) [8]. This high correla-

TABLE 2. Distribution of HBV DNA Concentrations among HBsAg-Positive Patients with Different Diagnosis

HBV DNA, copies/ml (VERSANT HBV 3.0)	CHB, n=33	HBsAg carriers, n=15	CHB+HDV, n=2	CHB+HCV, n=3	CHB+HCV+HDV, n=1	HBV-associated cirrhosis, n=2
<2000	6	6	1	1	1	1
2000-10^6	20	9	1	2	0	1
>10^6	7	0	0	0	0	0

tion could be expected, as both tests, based on quantitative PCR, are manufactured by the same company [8]. It seems that lower correlation between the results of two tests in our study can be explained by difference in the method of detection.

The results indicate high specificity of the studied tests and good agreement between their results, but VERSANT HBV 3.0 exhibited better reproducibility.

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