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Performance of the cobas Hepatitis B virus (HBV) test using the cobas 4800 system and comparison of HBV DNA quantification ability between the COBAS AmpliPrep/COBAS TaqMan HBV test version 2.0 and cobas HBV test

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ABSTRACT

Background: Hepatitis B virus (HBV) DNA levels are used to predict the response to therapy, determine therapy initiation, monitor resistance to therapy, and establish treatment success.
Objective: To verify the performance of the cobas HBV test using the cobas 4800 system for HBV DNA quantification and to compare the HBV DNA quantification ability between the cobas HBV test and COBAS AmpliPrep/COBAS TaqMan HBV version 2.0 (CAP/CTM v2.0).
Study design: The precision, linearity, and limit of detection of the cobas HBV test were evaluated using the 4th World Health Organization International Standard material and plasma samples. Clinical samples that yielded quantitative results using the CAP/CTM v2.0 and cobas HBV tests were subjected to correlational analysis.
Results: Three hundred forty-nine samples were subjected to correlational analysis, among which 114 samples showed results above the lower limit of quantification. Comparable results were obtained ([cobas HBV test] = 1.038 × [CAP/CTM v2.0]–0.173, r = 0.914) in 114 samples, which yielded values above the lower limit of quantification. The results for 86.8% of the samples obtained using the cobas HBV test were within 0.5 $\log_{10}$ IU/mL of the CAP/CTM v2.0 results. The total precision values against the low and high positive controls were 1.4% (mean level: 2.25 $\log_{10}$ IU/mL) and 3.2% (mean level: 6.23 $\log_{10}$ IU/mL), respectively. The cobas HBV test demonstrated linearity (1.15–6.75 $\log_{10}$ IU/mL, y = 0.95 × 6 + 0.17, r^2 = 0.994).
Conclusion: The cobas HBV test showed good correlation with CAP/CTM v2.0, and had good precision and an acceptable limit of detection. The cobas HBV test using the cobas 4800 is a reliable method for quantifying HBV DNA levels in the clinical setting.

1. Background

Hepatitis B virus (HBV) infection is a major public health problem worldwide. Individuals with chronic HBV infection are at risk for cirrhosis, liver failure, and hepatocellular carcinoma. Treatment against HBV aims to maintain undetectable HBV DNA levels and to improve the prognosis of HBV-related liver disease. Despite advances in vaccination and treatment, the global burden of HBV remains high. Approximately 30% of the world's population shows serological evidence of a current or past HBV infection, and an estimated 257 million people were living with chronic HBV infection globally in 2015 [1,2]. The presence of HBV DNA in the blood reflects virus replication and the course of chronic infection [3]. Thus, detection of the HBV DNA level is important for the diagnosis of HBV infection, therapy initiation, monitoring resistance to therapy, and establishing treatment success [1,4].

Most HBV DNA assays that are currently in use are real-time PCR assays [1]. Real-time PCR assays have better analytical sensitivity, specificity, accuracy, and a dynamic range of linear quantification than other technologies [5]. Several commercially available assays based on real-time PCR are fully or partly automated. The COBAS AmpliPrep/COBAS TaqMan HBV, version 2.0 (CAP/CTM v2.0; Roche Molecular Systems, Pleasanton, CA, USA) and Abbott RealTime HBV assay (Abbott Molecular, De Plaines, IL, USA) are the most widely used tools for HBV DNA quantification. The cobas HBV test (Roche Molecular Systems) is a newly developed assay for HBV DNA quantification and accompanies the cobas® 4800/6800/8800 system (Roche Molecular Systems). The

Abbreviations: Anti-HBc Ab, hepatitis B core antibodies; CAP/CTM v2.0, COBAS AmpliPrep/COBAS TaqMan HBV version 2.0; CI, confidence interval; HBsAg, Hepatitis B surface antigen; HBV, hepatitis B virus; LOD, limit of detection

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Cobas HBV test and CAP/CTM v2.0 are both based on fully automated nucleic acid extraction and real-time PCR, but the Cobas HBV test has a broader range of quantification and a lower limit of detection with a low turnaround time than the CAP/CTM v2.0 according to the manufacturer.

To our knowledge, only a few studies have evaluated the performance of the cobas HBV test, and none has evaluated the performance of the cobas HBV test using the cobas 4800 system.

2. Objectives

The aims of our study were to verify the performance of the cobas HBV test using the cobas 4800 system to quantify HBV DNA and to compare the HBV DNA level estimated using the cobas HBV test to that obtained with CAP/CTM v2.0 using the COBAS AmpliPrep/TaqMan system in plasma samples.

3. Methods

3.1. Correlation samples

The remaining plasma from clinical specimens submitted to a medical laboratory from January 2016 to July 2017 were used for the analysis. To assess the accuracy of the cobas HBV test for identifying people not infected with HBV, samples from 121 individuals who participated in an annual community health screening survey and had negative results for hepatitis B surface antigen (HBsAg) and hepatitis B core antibodies (anti-HBc Ab) were obtained. To assess the accuracy of the cobas HBV test for patients with chronic hepatitis B, samples from 260 individuals who were indicated for HBV viral load testing were obtained.

The samples were stored at −70 °C for a period of up to 6 months before the analysis. Subsequently, the samples were thawed and subjected to the CAP/CTM v2.0 assay and cobas HBV test. Both tests for a given specimen were completed within 24 h. If a test failed due to insufficient sample volume, the sample was excluded from the analysis.

3.2. Determination of analytical performance

Two levels of controls were used to determine precision. Triplicate controls were prepared and measured over a period of 5 days to evaluate repeatability: intra-assay precision, between-day precision, and within-laboratory precision. Serial dilutions of a high-titer sample were used to determine linearity. The expected values were based on the average of prior measurements of the sample using the CAP/CTM v2.0 assay and corrected for the dilution factor. A series of dilutions were prepared with nominal concentrations from 1.15 $\log_{10}$ to 6.75 $\log_{10}$ IU/mL to evaluate linearity, with triplicate measurements being performed. To verify the limit of detection (LOD), 14 replicates at concentrations of 0, 0.5, 2.5, 5, 10, 15, and 25 IU/mL were tested with each assay according to the guidelines of the 4th World Health Organization International Standard for Hepatitis B Virus DNA Nucleic Acid Amplification Techniques (National Institute for Biological Standards and Control [NIBSC] code 10/266; National Institute for Biological Standards and Control, United Kingdom).

3.3. Efficiency of the cobas HBV test using the cobas 4800 system and CAP/CTM v2.0 using the COBAS AmpliPrep/COBAS TaqMan system

The cobas HBV test was performed using the cobas 4800 system, which consists of separate devices for the sample preparation/amplification mix setup (cobas x 480 instrument) and amplification/detection (cobas z 480 analyzer). CAP/CTM v2.0 incorporates an automated sample extraction and PCR assembly with thermocycling and detection. The transfer of assembled reaction mixtures to the TaqMan can be automated using an optional docking station [18]. The assay targets the precore and core regions of the HBV genome. HBV DNA levels are expressed in international units per milliliter (IU/mL). For the cobas HBV test and CAP/CTM v2.0, the lower LODs are 4.4 and 9 IU/mL and the ranges of quantification are 10–1.0×10^9 and 20–1.7×10^8 IU/mL, respectively, according to the manufacturer. The cobas HBV test and CAP/CTM v2.0 test utilizes three external controls (a high titer positive, low titer positive, and negative control) and an internal control, which is a non-competitive armored quantitation standard for viral load quantification.

3.4. Statistical analysis

The data were analyzed using IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA), and a MedCalc Version 17.7.2-based probit analysis (95% detection rate) was performed for the LOD. The Kappa and Pearson chi-squared tests were used for qualitative results, and Passing-Bablok regression analysis was performed for quantitative results. The differences between the two methods were illustrated using Bland-Altman plots. For all analyses, p-values < 0.05 indicated statistical significance. This study protocol was reviewed and approved by the institutional review board of Pusan National University Hospital (H-1611-001-001).

4. Results

4.1. Agreement and correlation between the cobas HBV test and CAP/CTM v2.0

A total of 381 samples were analyzed, of which 32 had an insufficient volume for testing. Thus, the remaining 349 samples that yielded quantitative results using the CAP/CTM v2.0 assay and cobas HBV test were used to evaluate the correlation between the two assays. The kappa coefficient between the qualitative results of the two assays was 0.781 (Table 1).

Among 104 samples that yielded negative results for HBsAg and anti-HBc Ab, 101 samples demonstrated undetectable HBV loads in both assays, and 3 samples yielded detectable loads (< 10 IU/mL) in only the cobas HBV test. Of the 245 samples that previously yielded positive results for HBV viral load, 71 did not yield detectable levels in both assays, 24 yielded detectable levels in only the cobas HBV test, 11 samples yielded detectable levels in only the CAP/CTM v2.0 assay, and 139 samples yielded detectable levels in both assays.

A total of 235 samples yielded results below the lower limit of quantification (< 10 IU/mL with the cobas HBV test or < 20 IU/mL with CAP/CTM v2.0) from more than one assay. The correlation in terms of HBV DNA quantification observed between the cobas HBV test and CAP/CTM v2.0 was very good in the remaining 114 samples ([cobas HBV test] = 1.038 × [CAP/CTM v2.0] − 0.173, r = 0.914; 95% confidence interval [CI] of intercept: −0.316 to −0.045, slope: 1.000–1.076, r: 0.878–0.940; $p < 0.001$; Fig. 1). The samples with HBV DNA levels below 2000 IU/mL using the cobas HBV test (n = 72) showed high correlations ([cobas HBV test] = 0.928 × [CAP/CTM v2.0] + 0.0992, r = 0.708; 95% CI of intercept: −0.192 to 0.410, slope: 0.808–1.039, r: 0.570–0.870; $p < 0.001$). The samples with HBV

Table 1
Comparison of HBV DNA levels using the cobas HBV test and CAP/CTM v2.0.

		CAP/CTM v2.0 (IU/mL)		
		Not detected	< 20	> 20
cobas HBV test (lU/mL)	Not detected	172	11	0
	< 10	25	19	1
	> 10	2	5	114

HBV, hepatitis B virus; CAP/CTM v2.0, COBAS AmpliPrep/COBAS TaqMan HBV version 2.0.