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Semiautomated Quantification of Hepatitis B Virus DNA in a Routine Diagnostic Laboratory

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The Cobas Amplicor HBV Monitor test for quantitative determination of hepatitis B virus (HBV) DNA in serum has recently been introduced. To evaluate the performance of this assay in a routine diagnostic laboratory, reproducibility of results was determined with the First European Union Concerted Action HBV Proficiency Panel and the Accurun 325 HBV DNA Positive Control, Series 300. Results for 270 routine serum samples were additionally evaluated. To avoid the retesting of a large number of samples due to titers exceeding the upper limit for the linear range of the assay, sera of patients with chronic hepatitis B (CHB) were diluted prior to the assay to 10^{-4} in normal human plasma, which is included in the assay. The mean coefficient of variation was 22.9% for all input HBV DNAs. Of 270 routine serum samples, 182 (150 sera from transplant donors and 32 sera from patients who had recovered from CHB) tested negative. Eighty-six sera were found to be HBV DNA positive; in six sera, HBV DNA levels were found to exceed the upper limit for the linear range of the assay and had to be retested. In the remaining two sera, inhibition occurred. The semiautomated Cobas Amplicor HBV Monitor test showed sufficient reproducibility and helped in avoiding human error. The relatively narrow linear range of detection is a limitation of the new assay.

In the routine diagnostic laboratory, PCR-based molecular assays are gaining importance in the diagnosis and monitoring of infectious diseases. For detection of hepatitis B virus (HBV) DNA in serum, home-brew PCR-based assays lack standardization and reproducibility of results, as has been shown by the results of the EUROHEP proficiency study, in which more than 50% of participating laboratories failed to meet either the sensitivity or the specificity criteria (11, 15). The standardized quantitative Amplicor HBV Monitor test (Roche Diagnostic Systems, Pleasanton, Calif.), which is based on coamplification of the HBV template and an internal quantitation standard followed by hybridization and detection of captured amplification products using the enzyme immunoassay technique, has been introduced recently. This assay was found to be a valuable tool for the detection of HBV DNA in serum and revealed a sensitivity superior to that of other commercially available molecular assays (2, 7, 12). However, it lacks automation of the hybridization and detection steps, limiting its utility in the routine diagnostic laboratory.

The Cobas Amplicor instrument allows the automation of the amplification and detection steps of a PCR test and was found to be an easy, quick, and reliable way to perform high-volume PCR for detection of several infectious agents (1, 3, 5, 8, 14). The Amplicor HBV Monitor test has recently been adapted for automated processing by the Cobas Amplicor instrument. The new assay (Cobas Amplicor HBV Monitor test) has proved to be highly sensitive, but the upper limit for the linear range of the assay has been reduced from 10^7 to 10^5 HBV DNA copies/ml compared with the manual Amplicor HBV Monitor test (10).

The aim of this study was to evaluate performance of the Cobas Amplicor HBV Monitor test in a routine diagnostic laboratory. In a first step, the reproducibility of results was determined, and in a second step, routine serum samples were tested.

The Cobas Amplicor HBV Monitor test was performed according to the manufacturer's package insert instructions. Briefly, HBV DNA was manually isolated from 100 μl of serum by polyethylene glycol precipitation, followed by virion lysis and neutralization. A known quantity of an internal quantitation standard was introduced into each specimen and carried through the whole molecular assay. The Cobas Amplicor instrument automatically performed PCR amplification, hybridization, and detection. According to the manufacturer's package insert, the Cobas Amplicor HBV Monitor test shows linearity from 2.0×10^2 (lower detection limit) to 2.0×10^5 HBV DNA copies/ml.

For determination of reproducibility of results, the First European Union Concerted Action HBV Proficiency Panel and the Accurun 325 HBV DNA Positive Control, Series 300 (Boston Biomedica, West Bridgewater, Mass.), were used. The First European Union Concerted Action HBV Proficiency Panel contained HBV strains ad (1.0×10^3, 2.0×10^5, 2.0×10^6, and 1.0×10^7 HBV DNA copies/ml) and ay (2.0×10^6 and 1.0×10^7 copies/ml). All samples containing more than 2.0×10^5 HBV DNA copies/ml were diluted prior to the assay in HBV-negative serum to fall within the linear range of the Cobas Amplicor HBV Monitor test. The Accurun 325 HBV DNA Positive Control, Series 300, contained 10^3 HBV DNA copies/ml of the HBV strain ad. All standards were tested five times on different days.

A total of 270 routine serum samples were studied. Because of the limited detection range of the Cobas Amplicor HBV Monitor test, an algorithmic approach based on recently published results was introduced: in the previous study, of 51 sera obtained from patients with chronic hepatitis B (CHB) (anti-HBc and HBsAg positive; HBeAg positive or negative; anti-HBs and anti-HBe negative), 50 had been found to contain more than 2.0×10^6 HBV DNA copies/ml (9). In the present

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TABLE 1. Reproducibility of results of the Cobas Amplicor HBV Monitor test

Dilution (DNA copies/ml)	Strain	Origin	Mean no. of DNA copies/ml detected	SD	Coefficient of variation (%)
1×10^3	ad	BB[a]	1.4×10^3	4.7×10^2	33
1×10^3	ad	EUCA[b]	2.6×10^3	4.9×10^2	19
2×10^5	ad	EUCA[b]	3.2×10^5	1.0×10^5	31
2×10^6	ad	EUCA[b]	4.0×10^6	1.5×10^6	39
1×10^7	ad	EUCA[b]	1.5×10^7	1.1×10^6	8
2×10^6	ay	EUCA[b]	2.7×10^6	6.7×10^5	25
1×10^7	ay	EUCA[b]	0.9×10^7	5.0×10^5	5

[a] BB, Boston Biomedica (Accurun 325 HBV DNA Positive Control, Series 300).
[b] EUCA, First European Union Concerted Action HBV Proficiency Panel.

study, we tried to keep the number of repetitions as low as possible. All sera from patients with CHB were diluted prior to the assay to 10^{-4} in HBV-negative normal human plasma, which is included in the Cobas Amplicor HBV Monitor test.

The Cobas Amplicor HBV Monitor test was found to be reliable, with coefficients of variation below 40%. Mean values, standard deviations, and coefficients of variation are shown in Table 1.

When 270 routine samples were tested with the Cobas Amplicor HBV Monitor test, 182 tested below the detection limit, 86 were found positive, and 2 showed an inhibition (negative quantitation standard). Of 182 samples that tested below the detection limit, 150 originated from transplant donors and 32 came from patients who had recovered from CHB (HBsAg and HBeAg negative; anti-HBc, anti-HBs, and anti-HBe positive) more than 12 months prior to blood collection. Of 86 positive samples, 30 were taken from patients with CHB, 23 came from patients after HBeAg seroconversion during anti-HBV therapy (HBsAg, anti-HBc, and anti-HBe positive; anti-HBs and HBeAg negative), and 33 came from inactive carriers with the same serological profile (HBsAg, anti-HBc, and anti-HBe positive; anti-HBs and HBeAg negative). In six sera, HBV DNA levels were found to be above the linear range. Five of these samples were taken from patients with CHB despite the applied algorithmic approach, and the remaining one came from an inactive carrier. Both of the inhibited samples originated from transplant donors. After 10-fold dilution, both of them gave valid results (negative).

With the algorithmic approach, the overall repetition rate because of titers above the upper detection limit was 2.2% (6 of 270) in this study. Of all positive samples, the repetition rate was 7.0% (6 of 86). None of the samples obtained from patients with CHB was found to contain levels of HBV DNA that were below the detection limit.

The molecular assay employed in this study proved to be suitable for a routine diagnostic laboratory that specialized in molecular diagnostics. The manual DNA isolation procedure can be carried out in 2.5 h; the automated amplification, hybridization, and detection on the Cobas Amplicor instrument take 6 h.

In the routine diagnostic laboratory, automation of molecular assays reduces hands-on work and thus helps to avoid human error. It has been demonstrated that the Cobas Amplicor HBV Monitor test has high sensitivity and reproducibility (10). It may be hypothesized that in comparison with the manual Amplicor HBV Monitor test, the Cobas Amplicor HBV Monitor test would provide better reproducibility of results because of more advanced automation. In the present study, the coefficient of variation ranged from 5 to 39%. In a recent evaluation study of the manual Amplicor HBV Monitor test, however, reproducibility testing, which had been done by the same technician, revealed a comparable coefficient of variation (7). Reproducibility of results of future molecular diagnostic assays may be improved by automation of sample preparation.

Because commercially available molecular assays are usually very expensive, the number of repetitions must be kept as low as possible. The Cobas Amplicor HBV Monitor test shows a very limited range of detection. In a routine diagnostic laboratory, use of this assay would not be cost effective because a very large number of samples would need to be retested. To reduce the number of repetitions, an algorithmic approach has to be introduced. When sera obtained from patients with CHB were diluted to 10^{-4} in HBV-negative plasma prior to the assay, only 5 of 32 sera had to be retested because they were still found to contain levels of HBV DNA that were above the linear range of detection. On the other hand, a sample with fewer than 10^6 copies of HBV DNA/ml might eventually be diluted below the detection limit (10^2 HBV DNA copies/ml). This problem, however, did not occur in the present study (using 30 sera from patients with CHB) and it would have occurred in only 1 serum sample of an earlier study (51 sera from patients with CHB), which would have had to be retested (9). The number of repetitions thus appears to be acceptable; use of this algorithm, however, requires knowledge about the serological profile of the patient.

Amplification may fail because of interference from PCR inhibitors, which include heparin, heme, and alcohol (4, 6, 13). In most cases, however, the nature of inhibitors remains a mystery. In this study, two specimens were found to be inhibitory and subject to repeat testing. After 10-fold dilution, both of them tested negative (by the positive quantitation standard).

In conclusion, the Cobas Amplicor HBV Monitor test proved to be useful for the routine diagnostic laboratory. It is reliable but has a limited range of detection that requires dilution of samples from patients with chronic hepatitis B prior to the assay. Future improvements should include automated HBV DNA isolation and extended linear range of detection.

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