Multicenter Evaluation of a Semiautomated, Standardized Assay for Detection of Hepatitis B Virus DNA in Blood Donations

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We evaluated the COBAS Ampliscreen hepatitis B virus (HBV) test using standards, seroconversion panels, consecutive donations, and samples from patients with abnormal alanine aminotransferase and chronic hepatitis C. Specificity was 100% and sensitivity was 20 IU/ml. In seroconversion panels, HBV DNA was detected up to 4 to 18 days before HBsAg, suggesting that this assay is useful in shortening the infectious window phase.

Over the past decade, the risk of acquiring blood-borne viruses through transfusion has dramatically declined in industrialized countries. A residual risk due to donations of blood during the window period (time from infection to reactivity by serological assays) still exists and can be shortened by using nucleic acid testing (NAT) assays (1–3, 5, 7, 10, 12, 14, 17). Such assays are already implemented in the United States and in most European countries for the detection of hepatitis C virus (HCV) RNA and human immunodeficiency virus (HIV) RNA. For hepatitis B virus (HBV), the estimated probability of having a potentially infectious donation released in the blood supply is even higher than those expected for HCV and HIV. Hence, the introduction of HBV DNA assays may be a useful tool in the quest of approaching zero risk (5, 6, 8, 15).

In this study we evaluated a semiautomatized PCR test, COBAS Ampliscreen HBV (CAS HBV), that allows for the detection of HBV DNA in minipools and makes use of the same extract that can be processed with the respective HCV and HIV assays (16).

The analytical sensitivity of the CAS HBV was assessed using the HBV DNA nucleic acid panel (NAP; Acrometrix, Benicia, CA) consisting of seven plasma, of which one was negative and six were positive, with concentrations ranging from 2 × 10^4 to 2 × 10^7 IU/ml. Serial dilutions of the 2 × 10^6 IU/ml sample in HBV-negative human plasma were also tested in duplicate to define the assay’s detection limit.

Five seroconversion panels, PHM 929, PHM 932, PHM 925, PHM 928 (Boston Biomedical Inc., West Bridgewater, MA), and SB-0405 (NABI Biochemicals, Bocaraton, FL), comprising serial plasma collected at close intervals during the seronegative window phase, were used to evaluate the test’s ability to detect HBV DNA. Each of these panels had been previously tested by the manufacturers using different HBsAg assays.

To assess specificity, plasma samples were tested from individuals (n = 43) with isolated alanine aminotransferase (ALT) elevation (>60 IU/liter), from patients (n = 23) with chronic hepatitis C, and from repeat donors (n = 81) previously detected to be negative for both HBsAg and HBV DNA by an in-house PCR. In addition, 9,547 plasma samples were collected from repeat consecutive donors and analyzed in five different blood banks.

All specimens were processed according to the CAS HBV Multiprep Sample Processing procedure, which includes assembling minipools of 24 samples by mixing 100 μl of the sample under testing and 2.3 ml of HBV-negative human plasma. An aliquot of 1 ml was pelleted by ultracentrifugation, and HBV DNA was manually extracted by chaotropic lysis. In some experiments, to increase the test’s sensitivity the total volume (2.4 ml) of the minipool was tested.

Extracted samples and controls were then processed for amplification and detection using the automated system COBAS Amplior, according to the manufacturer’s instructions (4, 9).

The standard sample processing procedure, used for testing of individual samples (volume, 200 μl) according to the manufacturer’s instructions, was further added when testing the seroconversion panels.

All seven NAP samples (Acrometrix) were correctly identified. Analysis of serial dilutions of the 2 × 10^6 panel members tested in duplicate revealed a detection limit of 20 IU/ml with the minipool procedure.

When assessing the results obtained on the five seroconversion panels (Table 1), HBV DNA was positive 4 to 18 days (mean, 10 days) prior to the appearance of HBsAg with the single-sample procedure and 0 to 11 days (mean, 3.7 days) with the minipool procedure (total volume, 1 ml). A 2-to 3-day shortening of the window phase was observed when samples were analyzed in a 2.4 ml final minipool volume instead of a 1-ml volume. Comparison was made with data reported by the seroconversion panel manufacturers regarding the first HBsAg detection with the most sensitive assay.

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The CAS HBV test showed 100% specificity, as no reactive samples were detected in samples from 81 healthy donors and 66 patients with abnormal ALT or chronic hepatitis unrelated to HBV. Finally, of the 9,547 consecutive donations, one resulted positive for both HBV DNA and HBsAg.

In recent years a semiautomated PCR system, the COBAS Ampliscreen, was developed for the detection of HIV and HCV in minipools.

In this study we evaluated the performance of the CAS HBV test, the last to be developed on this platform for the detection of HBV DNA. Despite the increased sensitivity of serological HBsAg tests, a residual risk of HBV transmission still exists, and the introduction of HBV NAT in blood screening may be warranted. Using a real-time PCR-based assay, developed with the same primer set included in the CAS HBV panel, the CAS HBV allowed detection of an active infection on average of 10 and 3.7 days earlier than the HBsAg test when used in the single-sample format and in the minipool format, respectively. A certain additional shortening of the window phase was seen with the latter procedure when samples were analyzed in a 2.4-ml final volume.

Of the 9,547 donations tested, only one was HBV DNA positive in the presence of HBsAg. Absence of reactivities in the window phase (i.e., HBV DNA positive and HBsAg negative) might be due to the limited sample size collected in an area where the HBV residual risk is very small, approximately 13.9 per 106 donations (18).

The potential throughput and flexible configuration of this assay, which allows for different pool size and sample volume, were appreciated by the participating centers, where its introduction did not significantly increase the screening workload nor alter the laboratory workflow. CAS HBV has, in fact, the advantage of using an aliquot of the same extract already obtained for the CAS HCV and HIV tests, while the remaining steps of the procedure are performed in automation by the COBAS Ampliprimer.

In conclusion, our data showed that the CAS HBV is a reliable assay that can help to improve the safety of blood supplies by shortening the preseroconversion infectious window phase.

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