Journal of Virological Methods 194 (2013) 190–193

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Evaluation of commercialized rapid diagnostic testing for some Hepatitis B biomarkers in an area of intermediate endemicity

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Article history:
Received 24 May 2013
Received in revised form 13 August 2013
Accepted 21 August 2013
Available online 1 September 2013

Keywords:
Hepatitis B markers
Rapid diagnostic testing
Reliability
Anti-HBc
Anti-HBs
Anti-HBe

A B S T R A C T

Hepatitis B virus (HBV) is a major public health threat. Enzyme immune assay (EIA) of HBsAg is the screening method used in most settings, including in blood banks. Other markers are used to evaluate the HBV replication, immunity and the infectious level of the patient. Testing negative for HBsAg, however, does not always mean the absence of infection, and testing other markers using EIA is costly. This study evaluated the diagnostic reliability of commercially available non-HBsAg HBV biomarkers to detect their usefulness to screen for HBV infection. INTEC rapid tests for HBV markers were evaluated in 508 HBsAg negative blood donors and were compared to EIA as a reference method. Only anti-HBs, anti-HBc and anti-HBe could be evaluated. Sensitivities of all tests (64.2, 85.48, and 82.78 respectively) were much lower than those claimed by the manufacturer. The specificities and negative predictive values for all tests exceeded 95% and 93% respectively and were lowest for anti-HBs. Anti-HBe had the highest accuracy (99.02%), while anti-HBs had the lowest (90.16%). It was concluded that the rapid test performance was lower than the manufacturer's reports. They are reliable tools to prove negativity, but less efficient to confirm reactivity. The best performance was for anti-HBe. The positive results of anti-HBs was significantly associated with higher ELISA titer levels, which is therefore recommended to be used for screening of immunity.

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1. Introduction

Hepatitis B virus (HBV) infection is a worldwide major public health problem and it contributes substantially to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The global prevalence of HBV varies. In most developing countries, 5–15% of the population are chronic HBV carriers owing to failure to adopt appropriate measures to confine the spread of infection (Abbas and Siddiqui, 2011; Qirbi and Hall, 2001). HBV prevalence is estimated to be 6.7% among healthy Egyptian populations (Lehman and Wilson, 2009).

There are several HBV-specific antigens and antibodies, and detection of these serologic markers individually or in combination is useful in diagnosing HBV infections (Laperche et al., 2001).

Among these markers, the surface antigen (HBsAg) is essential for diagnosing ongoing HBV infection. It is widely accepted that the diagnosis of HBV infection rests on the presence of HBsAg in the blood stream (Hino et al., 2002), which can generally be detected even during the incubation period (Badur and Akgun, 2001; Giarcia-Montalvo et al., 2005).

The absence of HBsAg can be interpreted as 1) no current or past HBV infection if no other HBV markers are detected; 2) recovery from past infection if detectable anti-HBs and anti-HBc are still present; 3) immunity due to vaccination if detectable isolated anti-HBs occurs; or 4) occult infection if HBV-DNA is present in serum or liver, with or without other markers (Giarcia-Montalvo et al., 2005). This later category has many explanations including presence of mutant HBsAg or circulating level of HBsAg below the detection limit of a screening assay (Carman, 1997).

Antibodies directed against the core antigen (anti-HBc) appear early during the acute phase of the infection, and remain detectable for life (Behzad-Behbahani et al., 2006). Therefore, a number of countries, but not including Egypt screen blood donation for anti-HBc (Antar et al., 2010). This marker is also associated with non-response to hepatitis C antiviral therapy, and testing for it may be recommended prior to starting therapy (Emara et al., 2010).

The presence of anti-HBc with or without antibodies against HBsAg (anti-HBs) indicates previous infection; false positivity is not ruled out in cases of anti-HBc alone. The presence of

Abbreviations: HBV, Hepatitis B virus; ELISA, enzyme linked immunosorbent assay; HBsAg, Hepatitis B surface antigen; anti-HBs, Hepatitis B surface antibody; anti-HBc, antibodies against the core antigen; anti-HBe, antibodies against the e antigen; RDT, rapid diagnostic testing; PPV, positive predictive value; NPV, negative predictive value; DNA, deoxyribonucleic acid; ALEXREP, Alexandria Research Enhancement project.

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http://dx.doi.org/10.1016/j.jviromet.2013.08.026

anti-HBs indicates immunity from either past infection or importantly from vaccination (Laperche et al., 2001). Consequently, screening for anti-HBs would determine the need for vaccination, evaluate the vaccine response, and identify the need for a booster.

A negative "e" antigen (HBeAg) result indicates very minimal or lack of HBV replication. Positive antibodies against HBeAg (anti-HBe) results usually indicate inactivity of the virus and low infectivity. Positive anti-HBe results in the presence of detectable HBV DNA in serum indicate active viral replication in these patients. Screening of these markers in chronic HBV patients is essential in treatment decisions (Lok and McMahon, 2009).

Despite the importance of testing non-HBsAg HBV markers for diagnosis and management, they are neither ordered commonly in routine practice nor used for screening.

ELISAs are the most widely used laboratory tests for detection of HBV markers. Although ELISAs are sensitive and specific, they are time consuming, involve complicated procedures, and are relatively expensive and logistically difficult in field settings and during the household surveys (Soeung et al., 2009).

Rapid diagnostic testing (RDT) has been widely used in the public health context particularly in developing countries. It is simple, easy, cheap and obviously rapid (RDT-Info). Commercial RDT kits for detecting HBV markers are available. Their application in clinical and field practice remains questionable as to date only a few studies have evaluated their performance, most of which were particularly concerned with HBsAg.

This study was carried out to evaluate the diagnostic reliability of commercially available RDT for non-HBsAg HBV biomarkers in view of the possibility of using them in primary care facilities and for survey purposes.

2. Material and methods

2.1. Sampling

Through a cross-sectional study described elsewhere (El-Ghitany et al., 2012), 508 HBsAg negative blood donors were included voluntarily in this study. A blood sample was obtained from each participant and the serum was separated and preserved in different aliquots at −20 °C.

2.2. Reference method

The sera were screened for HBV markers by ELISA as the gold standard reference method. Serology comprised total anti-HBc, anti-HBs, anti-HBe, anti-HBc IgM and HBeAg. Every test was performed twice using ELISA (Dialab, Wiener Neudorf, Austria). The tests were done manually in accordance with the manufacturer's instructions. The reported sensitivities and specificities of all the tests were 99–100%. All samples were tested twice and positivity was only considered if the reaction was evident in both tests. The fiftieth percentile of the cutoff points was calculated for all positive tests and the positive values were plotted against it. Values above or below this percentile were respectively considered relatively high or low.

2.3. Rapid testing

RDT was done for all samples using the colloidal-gold-enhanced immunochromatography strip tests "ADVANCED QUALITY^{TM} ONE STEP TEST (Intec Products, Fujian, China)". The tests were performed according to the manufacturer's instructions and were interpreted visually after 15–20 min. According to the tested biomarker, the presence or absence of a red bar on the nitrocellulose strip indicated the reactivity. The presence of the red bar on the control window was essential otherwise an invalid result was considered. The documented sensitivity and specificity of the tests ranged from 95% to 100% according to the manufacturer. Laboratory staff were blinded to the ELISAs results, which were reviewed only after testing was completed.

2.4. Ethics statement

Research was done in accordance with the declaration of Helsinki. Ethical approval was obtained from the High Institute of Public Health (HIPH) Ethics Committee. Informed consent was obtained from all participants.

2.5. Data analysis

Data were analyzed using Epiinfo (version 6). Epi Info^{TM} software (Centers for Disease Control and Prevention, Atlanta, GA). Categorical data were compared using χ^2 test and the *P* value was considered significant if <0.05.

Sensitivity, specificity, the predictive value of a positive (PPV) or negative (NPV) test result, the likelihood ratios and 95% confidence intervals (CI) were used as the estimates of the effectiveness of the rapid tests. They were assessed by comparing the results obtained by ELISA as a gold standard.

Sensitivity was calculated as the proportion of positive test results obtained among the positive samples revealed by ELISA, specificity as the proportion of negative test results obtained among samples whose ELISA results were negative. PPVs and NPVs were calculated as the proportion of true-positive results among all positively reacting samples and as the proportion of true negative results among all negatively reacting samples, respectively. The likelihood ratio of a positive test result (LR+) is sensitivity divided by 1 − specificity. The likelihood ratio of a negative test result (LR−) is 1 − sensitivity divided by specificity. Probabilities, odds and accuracy were calculated manually. Pre-test odds = prevalence/(1 − prevalence). Post-test odds = pre-test odds × LR. Post-test Probability = post-test odds/(post-test odds + 1). Accuracy was calculated as a proportion of true results (positive and negative) relative to the total results obtained.

3. Results

Anti-HB IgM and HBeAg could not be evaluated because they were reactive in only 3 and 2 samples respectively and even though RDT failed to detect them. The results could only be shown for anti-HBs, anti-HBc and anti-HBe. All the parameters used to evaluate their reliability are shown in Tables 1 and 2. The accuracy of any of the 3 tests was at least 90% (Table 1). The specificity and NPV exceeded 95% and 93% respectively for all tests (Table 1). The lowest sensitivity and PPV was for anti-HBs. Anti-HBe had the best values of reliability measures except for sensitivity, for which it was exceeded by that of anti-HBc. Anti-HBs testing had the lowest sensitivity and PPV (Table 1). For all markers, the +LR was high and the −LR was low (Table 2). Similarly, the post-test probability in case of positive results was high, particularly for anti-HBc and anti-HBe, and that in case of negative results was low (Table 2).

Regarding the relation between the results of the rapid tests and ELISA titer levels (Table 3), RDT of anti-HBs was the only marker whose positive result was significantly associated with higher ELISA titer.

4. Discussion

Unfortunately, many developing countries lack the required number of clinical and field laboratory facilities for the detection of infection markers. Particularly, there is a paucity of rapid

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