Comparison of Accuracy of ELISA Technique and RAPID Screening Techniques for the Diagnosis of Hepatitis B Surface Antigen (HBsAg).

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Abstract

Hepatitis B Virus (HBV) infection is a worlwide public health problem and the primary marker for screening and diagnosis of HBV infection is hepatitis B surface antigen (HBsAg). RAPID screening kits are the most frequently used for screening of HBsAg, although the method is less reliable than Enzyme-Linked Immunosorbent Assay (ELISA). The present study was conducted to compare the diagnostic accuracy of ELISA technique and RAPID screening techniques for the diagnosis of Hepatitis B Surface Antigen (HBsAg).A total of 400 samples were tested for HBsAg using both RAPID and ELISA technique. Out of 400 samples, ELISA test showed positive results for 4 samples (1%) while negative results for 396 samples (90%). Out of 400 samples, RAPID test showed negative results for 400 samples (100%). Sensitivity of ELISA test and RAPID test was found to be 100 % and 0 %, respectively with 100 % specificity for both the methods. The obtained results highlight towards higher accuracy of ELISA technique than more popular RAPID screening kits used widely in developing nations. Therefore, there is a need to develop more sensitive, accurate and safe methods for screening of HBsAg by combining RAPID tests with ELISA method along with other Nucleic Acid Testing (NAT) strategies.

Keywords: ELISA, Hepatitis B, HBsAg, RAPID screening test.

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Introduction

Hepatitis B Virus (HBV) infection having severe impact on liver damage is one of the major of causes of death globally, especially in developing nations. The HBV infection comes under World Health Organization target towards its complete eradication by 2020 [1,2]. As per reports, approximately 2 billion people suffer from HBV infection and around 280 million are carriers of HBsAg with virus harboured in their liver [3]. The infectious HBV has dsDNA which encodes for P, X, core, and surface proteins. Hepatitis B surface antigen (HBsAg) is a complex surface antigen of HBV and important marker of past or present infection of HBV [4]. HBsAg can be detected 2 to 5 weeks before the actual onset of symptoms of jaundice in both acute and chronic HBV infection [5]. For HBV infection diagnosis, patients are screened both on serological and molecular level. Owing to its simplicity, sensitivity, and fast response, nucleic acid test (NAT) should be preferred for performing screening tests of the presence of HBsAg [6]. Serum samples from 37 HBV infected patients covering genotypes A-G have been assessed for HBsAg titer using high quality NAT-based Roche Elecsys HBsAg II point-of-care (POC) assay [7]. Although sensitive, the limitations such as false positive especially, in case of occult HBV, requirement of well-equipped labs, and high cost with decreased window period have restricted the usage of NAT based assays on large scale [8].

Since 1990, RAPID screening kits are the most commonly used methods of diagnosing HBV infection attributed to their low cost, ease of handling, robust working, and requirement of neither complex instrumentation nor trained personnel [9]. The kits are extensively used for emergency, field survey diagnosis, laboratory, and home testing [10]. RAPID test employs monoclonal or polyclonal antibody immobilized lateral flow immunoassay (LFIA) for determination of HBsAg in blood, serum, and plasma samples. The preliminary studies have also been reported for evaluation of RAPID kits for the screening of HBsAg in blood samples [11]. For e.g., RAPID POC strips developed by Quest Diagnostics have been explored for screening serum samples from 297 high HBV infection risk individuals in local clinical settings [12]. POC testing was found to be 73.7% sensitive and 97.8% specific for HBsAg have evaluated another RAPID kit "Nano Sign(®)HBs" as POC chromatographic immunoassay for detection of HBsAg of different genotypes [13]. Although the POC testing strips demonstrated sufficiently high sensitivity for varied genotypes, frequent false negatives were observed. Despite of several economic advantages, RAPID kits exhibit less sensitivity and specificity as compared to NAT resulting into false positive and false negative results. Another frequently used technique for the diagnosis of HBV infection is ELISA, which is sandwich type enzymatic immunoassay employing monoclonal antibody. ELISA is highly sensitive and specific due to enzymatic amplification of the signal and monoclonal antibodies recognized by WHO for most parts of varied HBV strains, respectively [14].

The present work compares the RAPID screening kit and ELISA technique for detection of HBV infection in blood samples of students studying in tertiary healthcare institutions in terms of analytical sensitivity and specificity.

Materials and methods

This prospective study included assessment of comparison of ELISA technique and RAPID screening techniques for the diagnosis of Hepatitis B Surface Antigen (HBsAg). Institutional ethical approval was obtained and written consent was obtained from all subjects after explaining in detail the entire research protocol. A market survey was done to determine the most commonly used RAPID screening kit brand in different labs and hospitals of Mysore. It was found that Hepa Card brand employed on large scale and thus, was selected for the study. A total of 400 samples were included in the present study. During the entire period, the blood samples were collected and testing was done for the presence of HBsAg using mentioned RAPID screening kits. Both HBsAg positive and negative samples were properly labeled and marked. Re-testing of the samples was done using gold standard ELISA technique based SURASE B-96 (TMB) ELISA kit. For this, 100 µL of sample to be tested was incubated with peroxidase enzyme conjugated antibody followed by washing and addition of tertramethylbenzidine substrate solution. The binding of conjugate and subsequent peroxidase activity is proportional to the presence of HBsAg. The colorimetric reaction was stopped and absorbance of the formed product was measured by dichromatic reading at 450/620 nm resulting into qualitative and quantitative determination of HBsAg. Further, the sensitivity and specificity values were calculated for both the employed methods.

Sensitivity determines the ability of diagnostic test to give a true positive in the presence of disease in the tested patient and is expressed as:

%Sensitivity=(No.of cases detected with disease using the test X 100)/(Total no.of cases diagnosed for disease)

On the other hand, specificity is the ability of screening test to give true negative when the patients tested under the study are free from disease.

%Specificity=(No.of negative cases in non-infected patients X 100)/(Total no.of non-infected persons diagnosed with the test)

All the data were collected and analyzed using computer statistical package of social sciences (SPSS) Version 21.0.The statistical analysis included calculation of percentages, mean, and bi-variate t-test and chi-square analysis. The differences were considered significant only when $p \leq 0.05$.

Results

In the present study, a total of 400 samples were analyzed. Comparison of results of ELISA test and RAPID test is shown in Table 1. Out of 400 samples, ELISA test showed positive result for 4 samples (1%) while negative for 396 samples (99%). On the other hand, RAPID tests showed negative results for 400 samples (100%). Sensitivity of ELISA test and RAPID test is shown in Table 2. Sensitivity of ELISA test and RAPID test was found to be 100% and 0% respectively. The 95% Confidence Intervals (CI) of sensitivity for ELISA and RAPID test were found to be 39.76% to 100 % and 0% to 60.24%, respectively. Table 3 shows specificity of ELISA and RAPID test which was found to be 100% for both the methods. Further, the 95% CI of specificity for ELISA and RAPID test were found to be 99.07% to 100%, each.

Table 1. *Comparison of results of ELISA and RAPID test.*

Test	Total samples	Samples positive		Samples negative	
		Number	Percentage	Number	Percentage
ELISA	400	4	1	396	99
RAPID	400	0	0	400	100

Table 2. *Sensitivity of ELISA and RAPID test.*

Test	Sensitivity (%)	95% CI
ELISA	100	39.76% to 100%
RAPID	0	0% to 60.24%

Table 3. *Specificity of ELISA and RAPID test.*

Test	Specificity (%)	95% CI
ELISA	100	99.07% to 100%
RAPID	100	99.07% to 100%

Discussion

Hepatitis B virus (HBV) remains a common cause of viral hepatitis, with possible long-term complications of cirrhosis and hepatocellular carcinoma in patients with developed chronic infection. Immunoassays are the most convenient and reliable detection platforms and their improvement for detection of viral antigens is an active research area. In the present study, the comparative analysis of RAPID and ELISA techniques was done to evaluate sensitivity and specificity for detection of HBsAg in HBV infected patients. It was observed that 10% of total samples which tested negative with RAPID kits were actually found to be positive for HBsAg when tested with ELISA technique. However, equal specificity was observed with both the methods i.e., 100% each. This highlighted towards improved sensitivity.

Conclusion

Therefore, superior applicability of ELISA tests for detection of HBsAg. The finding is consistent with earlier reports of comparison of RAPID kits and ELISA test performed in different regions of world [1,2,5,7]. The low performance of RAPID kits may be attributed to inadequate coating of antigen essentially required in the process, suboptimal testing, and genotypic heterogeneity of tested virus [15]. The discordant testing results between the two diagnostic assays may pose serious consequences for diagnostic industry and patient health. Therefore, RAPID screening kits need to be combined with ELISA and NAT methods to ensure timely and accurate detection of HBsAg for prevention of serious health hazards in HBV infected patients.

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