International Journal of Technology and Applied Science (IJTAS)
E-ISSN: 2230-9004 ● Website: www.ijtas.com ● Email: editor@ijtas.com

A comparison of rapid screening test and ELISA for the diagnosis of Hepatitis B in a tertiary care hospital in Central India

Dr. Nishu Sharma[1], Dr. Neha Sharma[2], Dr. Priyanka Kalbhor[3], Dr. Vaishali Rahangdale[4], Dr Sunanda Shrikhande[5]

[1]Junior Resident, [2]Senior Resident, [3]Assistant Professor, [4]Associate Professor, [5]Professor and Head
[1,2,3,4,5]Department of Microbiology, Government Medical College, Nagpur

INTRODUCTION

Hepatitis B virus (HBV) infection is a significant public health concern and is often characterized by silent transmission[1]. Every year, over a million people succumb to liver cirrhosis or hepatocellular carcinoma due to HBV infection, which affects nearly 350 million individuals globally. Approximately 45% of the world's population resides in areas with high chronic HBV prevalence rates[2]. HBV infections pose a serious risk to the community, as false negative results create the potential for silent transmission, enabling the spread of the virus[3]. Among the various viral antigens associated with Hepatitis B, HBsAg is a key marker used for detecting HBV infection. Formerly known as the Australia antigen, HBsAg is the first serological marker to appear in the blood of infected individuals, often 2-4 weeks before clinical symptoms manifest. HBsAg levels are typically highest during the symptomatic phase and subsequently decline[4]. HBV is transmitted through blood and blood products, needle-stick injuries, sexual contact, and vertical transmission from mother to foetus[5]. As Hepatitis B is highly infectious and can lead to severe complications, early identification of true positives is crucial for effective treatment. Timely and precise detection of HBV using sensitive and specific methods helps assess the infection status and implement strategies to prevent transmission. Therefore, it is essential to validate detection methods before their use in laboratories to minimize the risk of false positives and false negatives[6]. Currently, several techniques are available for detecting HBsAg in patient samples, including rapid ICT, ELISA, Enzyme Immunoassay (EIA), Nucleic Acid Amplification Tests (NAT), and Polymerase Chain Reaction (PCR). Of these, ELISA, EIA, NAT, and PCR methods are more expensive and generally used in advanced laboratories, while rapid kits offer a cost-effective solution and require minimal technical expertise[7].

MATERIALS AND METHODS

A cross-sectional study was carried out from January 2024 to March 2024 in a tertiary care centre in central India.

A total of 5439 blood samples were received for HBsAg detection in the Microbiology Department, Government Medical College, Nagpur, India.

Inclusion criteria: Samples received for HBsAg testing from various departments of the hospital.

Exclusion criteria: Stored (>3 days)/contaminated samples, heat inactivated samples, samples that used sodium azide as preservative.
Sample processing: All 5439 blood samples were centrifuged and serum was separated. Serum of all the 5439 received blood samples were tested for HBsAg using both rapid ICT methods (Mediline-SA HBsAg Combo Rapid Test, S.A. - Medline Pvt. Ltd.) and ELISA Trustwell (HBsAg ELISA Kit, Athenese-Dx Pvt. Ltd.) method.

PROCEDURE

Rapid Card Test process:
The Mediline-SA (HBsAg Combo Rapid Test, S.A.- Medline Pvt. Ltd.) is a lateral flow chromatographic immunoassay designed for HBsAg detection. The test cassette includes: 1) a conjugate pad, which is burgundy in colour, and contains mouse anti-HBsAg antibodies conjugated with colloidal gold (HBsAg Ab conjugates), and 2) a nitrocellulose membrane strip featuring a test band (T band) and a control band (C band). The T band is pre-coated with non-conjugated HBsAg antibodies, while the C band is coated with goat anti-mouse IgG antibodies. When a sufficient amount of the test sample is applied to the sample well, the specimen migrates across the cassette via capillary action. If HBsAg is present in the specimen, it binds to the HBsAg Ab conjugates. The resulting immunocomplex is captured by the pre-coated non-conjugated HBsAg antibody on the membrane, forming a burgundy-colored T band, which indicates a positive HBsAg result. The absence of the T band suggests a negative result. The test also includes an internal control (C band), which should show a burgundy-colored band of the immunocomplex of goat anti-mouse IgG and HBsAg Ab-gold conjugate, regardless of whether the T band is present. If the C band is absent, the test result is considered invalid, and the specimen must be retested using a new device.

ELISA Method:
The Trustwell (HBsAg ELISA Kit, Athenese-Dx Pvt. Ltd.) is a solid-phase enzyme-linked immunosorbent assay designed for the qualitative detection of hepatitis B virus surface antigen (HBsAg) in human serum or plasma. The kit consists of two main components:

1. Solid microwells pre-coated with monoclonal anti-HBsAg antibodies, and
2. Liquid conjugates containing polyclonal anti-HBsAg antibodies conjugated with horseradish peroxidase (HRP-HBsAg conjugates).

During the assay, the test specimen and HRP-HBsAg conjugates are simultaneously incubated with the coated microwells. If HBsAg is present in the specimen, it binds to both the anti-HBsAg antibody on the microwell surface and the HRP-HBsAg conjugates, forming a sandwich complex. Unbound conjugates are then removed through washing. The presence of the complexed conjugates is indicated by a blue color after further incubation with the TMB substrate. The reaction is halted by adding Stop Solution, and the absorbance is measured using a spectrophotometer at 450/620-690 nm.

STATISTICAL ANALYSIS

A total of 5,439 serum samples were tested simultaneously using both the rapid card method and the ELISA method.

Data such as the optical density of the samples, positive control, negative control, cut-off value, and reactivity for both the rapid card method and ELISA were recorded and entered into an MS Excel spreadsheet for analysis.

RESULTS

Out of the 5,439 blood samples tested with the HBsAg rapid card, 112 samples were positive, and 5,327 were negative. When the same samples were subsequently tested using the ELISA method, 105 samples were positive, and 5,334 were negative. With ELISA serving as the gold standard confirmatory method, the observed data were recorded, and sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated using pre-established formulas.

Table.1 Comparison between Rapid card Test and ELISA results

TEST	ELISA POSITIVE	ELISA NEGATIVE	TOTAL
RAPID POSITIVE	104	08	112
RAPID NEGATIVE	01	5326	5327
TOTAL	105	5334	5439

Table.2 Performance Evaluation of Rapid Test compared to ELISA

SENSITIVITY	99.04 %
SPECIFICITY	99.85 %
PPV	92.85 %
NPV	99.98 %
DIAGNOSTIC ACCURACY	99.83 %

DISCUSSION

This study compared the effectiveness of rapid ICTs and ELISA for HBsAg screening. The primary aim was to assess how well rapid test kits perform against the more established ELISA method. The results clearly showed that ELISA is superior in both sensitivity and specificity. Specifically, the ICT method had a sensitivity of 99.04% and a specificity of 99.85% and there was one sample was rapid card test negative but positive by ELISA.

These findings align with previous research. Rahman M et al. reported a much lower sensitivity of 53% but a perfect specificity of 100% for ICT.[8] Similarly, Irwig L et al. found a sensitivity of 97% and specificity of 100%,[9] while Kaur H et al. recorded 93.4% sensitivity and 100% specificity. [10] A study from Iran by Ansari MHK et al. also showed similar performance between ICT and ELISA. [11]

CONCLUSION(S)

This study shows that ELISA is more sensitive than ICT in detecting HBsAg. Rapid test devices, like ICT, can be used for HBsAg screening in situations where resources are limited, such as in remote areas and peripheral health centers. Hepatitis B virus poses a significant risk of silent transmission and spread among populations, highlighting the need for more sensitive assays like ELISA. The main goal of this study is to recommend the use of ELISA kits for diagnosing HBV, regardless of the area's developmental or economic status, as they accurately detect positive cases.

REFERENCES:

1. Prabha P, Saikeerthana D, Vijayashree V, Gogan M. A comparison of rapid screening test and ELISA for the diagnosis of hepatitis B surface antigen in patients attending a tertiary care hospital, Tamil Nadu, India. Natl. J. Lab. Med. 2022;11:22-5.
2. Kumar M, Seema K, Kumar D, Kumar A, Sharma AK, Boipai M, Rani A. Seroepidemiology of hepatitis B virus (HBV) and relationship to serum transaminase levels in Indian population. Journal of Family Medicine and Primary Care. 2024 Jun 1;13(6):2410-5.
3. Tiwari YK, Pundir S, Saraf G, Pawan K, Dashora D, Pokra M, Patidar V, Rana J, Mishra RK. A comparison of rapid card test with enzyme-linked immunosorbent assay for the detection of hepatitis B surface antigen [HBsAg] in tertiary care hospital. Research & Reviews: A Journal of Microbiology and Virology. 2017;7(3):27-31.
4. Gitnick GL. Australia antigen and the revolution in hepatology (Medical Progress). Calif Med. 1972;116:28-34.
5. Mehta KD, Antala S, Mistry M, Goswami Y. Seropositivity of hepatitis B, hepatitis C, syphilis and HIV in antenatal women in India. J Infect Dev Ctries. 2013;7(11):832-37. Doi: 10.3855/jidc.
6. Sharma M, Golia S, Mehra SK, Jani MV. A comparative evaluation of rapid card test with enzyme-linked immunosorbent assay for the detection of HBsAg among pregnant women in a tertiary care hospital. Int Arch Bio Med Clin Res. 2019;5(1):31-33.
7. Hayder I, Ahmed W, Alam SE. Comparison of different ICT Kits for HBsAg and Anti HCV using Gold Standard ELISA. Pak J Med Res. 2012;51(3):72-76.
8. Rahman M, Khan SA, Lodhi Y. Unconfirmed reactive screening tests and their impact on donor management. Pak J Med Sci. 2008;24:517-19.
9. Irwig L, Bossuyt P, Glasziou P, Gatsonis C, Lijmer J. Designing studies to ensure that estimates of test accuracy are transferable. BMJ. 2002;324(7338):669-71.
10. Kaur H, Dhanao J, Oberoi A. Evaluation of rapid kits for detection of HIV, HBsAg and HCV infections. Ind J Med Sci. 2000;54:432-34.
11. Ansari MHK, Omrani MD, Movahedi V. Comparative evaluation of rapid immonuchromatographic rapid diagnostic tests (Strips and devices) and PCR method for detection of human hepatitis B surface antigen. Hepat Month. 2007;7:87-91.