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Evaluation of the Diagnostic Accuracy of the HBsAg Rapid Test for Hepatitis B Virus Infection

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Abstract: Hepatitis B virus (HBV) infection presents a notable worldwide health burden, affecting millions of people globally. It can lead to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Accurate diagnosis of HBV infection is vital for effectively managing and preventing disease progression. In this investigation, we aimed to assess the diagnostic efficacy of the HBsAg Rapid Test. To assess its sensitivity, specificity, and accuracy, we conducted a comprehensive analysis of 602 specimens. This test exhibited an accuracy of 99.7%, with a sensitivity exceeding 99.9% and a specificity of 99.4%. Additional evaluations on cross-reactivity affirmed the test's specificity, as it consistently produced negative outcomes for different viral strains. Manufactured by Hangzhou AllTest Biotech Co., Ltd, the HBsAg Rapid Test provides rapid and reliable results, demonstrating a high level of accuracy. Consequently, it serves as a valuable diagnostic instrument in clinical settings, especially in resource-constrained environments. The timely detection enables prompt intervention and effective Hepatitis B management, resulting in improved patient outcomes and reduced transmission of the virus.

1. Introduction

Hepatitis B is a viral infection that affects the liver, leading to both acute and chronic illness. According to the World Health Organization (WHO), around 254 million individuals were estimated to have chronic hepatitis B infection in 2022, with approximately 1.2 million new infections occurring each year. Cirrhosis and hepatocellular carcinoma, a type of primary liver cancer, were the leading causes of the estimated 1.1 million deaths associated with hepatitis B in 2022.[1] The transmission of Hepatitis B Virus (HBV) commonly occurs through various routes, including vertical transmission from an infected mother to her child during childbirth, horizontal transmission through engaging in unprotected sexual intercourse, sharing of needles and other paraphernalia used for drug administration, and exposure to infected blood or blood products.[2] HBV infection typically presents as either asymptomatic or mild, underscoring the significance of proactive screening for individuals at potential risk. The timely identification of HBV allows healthcare providers to promptly initiate

tailored management approaches, encompassing antiviral therapy, lifestyle modifications, and regular surveillance. Early intervention plays a pivotal role in preventing or minimizing liver damage, decreasing the likelihood of chronic infections, and alleviating long-term complications like cirrhosis and hepatocellular carcinoma. By ensuring timely diagnosis and treatment, a substantial majority of fatalities associated with the Hepatitis B virus can be averted.[3]

The standard diagnosis for hepatitis B virus (HBV) involves the utilization of serum or plasma samples and encompasses enzyme immunoassay (EIAs) and electrochemical luminescence (ECLIA) techniques.[4] Nevertheless, the routine application of these tests in low- and middle-income countries may encounter obstacles due to several limitations. These include the need for trained personnel, the availability of essential infrastructure, and relatively lengthy turnaround times.[5] In addition to standard procedures, rapid tests (RTs) offer several advantages as an alternative option. These tests are characterized by their ease of performance and ability to provide conclusive results within minutes. Furthermore, they can be conducted on a case-by-case basis without the need for laboratory infrastructure. Moreover, the execution of RTs requires minimal training.[6]

At the core of diagnosing and managing hepatitis B is the crucial task of detecting the hepatitis B surface antigen (HBsAg). This protein is found on the surface of HBV particles and plays a pivotal role in the diagnostic algorithm for hepatitis B, enabling the identification of both acute and chronic infections. Rapid HBsAg testing has emerged as a prominent approach due to its convenience and its potential to significantly impact timely patient management. HBsAg, the outer coat of HBV, is abundantly produced during infection and can be readily detected in blood samples. It serves as a valuable marker for both acute and chronic hepatitis B infections, often being detectable before the onset of symptoms. Alongside HBV DNA, HBsAg acts as an early indicator of acute infection and remains present in individuals with chronic infection, enabling screening and detection of hepatitis B at various stages of the disease.[7]

2. Materials and Methods

2.1 Study Design

The objective of this investigation was to evaluate the diagnostic accuracy and efficacy of the HBsAg Rapid Test, in detecting Hepatitis B Virus (HBV) infection. In order to enhance the credibility of the findings, a comprehensive set of 602 specimens, encompassing both positive and negative samples collected at various time intervals, were included in the study. The determination of the specimen size was predicated on rigorous statistical considerations, ensuring the desired level of precision necessary to achieve sufficient statistical power for the evaluation.

Reference testing using serum or plasma specimens was performed by diagnostic reference laboratories. The HBsAg Rapid Test offers the flexibility to employ either serum or plasma specimens for detection. To ensure accurate results, it is crucial to promptly separate the serum or plasma from the blood to prevent hemolysis, which refers to the rupture of red blood cells. It is recommended to conduct testing promptly, without leaving the specimens at room temperature for an extended duration. To ensure proper storage, short-term preservation of serum and plasma specimens should involve storing them at temperatures ranging from 2-8 ℃ for a maximum duration of 3 days. For long-term storage, it is essential to store the specimens below -20 ℃. Before commencing the test, it is important to allow the specimens to reach room temperature.

2.2 Screen Test

(1)RADT

The HBsAg Rapid Test Cassette (Serum/Plasma) is a qualitative assay based on solid-phase

technology, employing a dual-site sandwich method for the detection of Hepatitis B surface antigen within serum or plasma specimens. The cassette's test line region is pre-coated with antibodies against HBsAg, while the particles used in the test are coated with anti-HBsAg antibodies. During the test, the serum or plasma specimen interacts with the antibody-coated particles. Through capillary action, the mixture moves upward on the chromatographic membrane, reacting with anti-HBsAg antibodies on the membrane and producing a colored line. The presence of a colored line in the test area signifies a positive outcome for HBsAg, whereas no line in this region suggests a negative result. To ensure the test has been performed correctly and that the membrane has functioned as intended, a colored verification test line always appears in the control area, affirming that the specimen volume was added correctly and the membrane's wicking action was effective. The researchers performed the test in accordance with the manufacturer's instructions and adhered to the provided guidelines. Visual readout serves as the outcome for all these tests.

(2)ELISA

The Enzyme-Linked Immunosorbent Assay (ELISA) is a pivotal laboratory technique utilized in clinical medicine for the detection of specific proteins or peptides within a sample. One of its primary applications is in the detection of the hepatitis B surface antigen (HBsAg), an essential marker that becomes detectable in the bloodstream during a hepatitis B virus (HBV) infection. The underlying principle of ELISA for HBsAg detection involves the utilization of specific antibodies that can bind to the target antigen. A capture antibody is immobilized on a microtiter plate, and the patient's sample is added. If the sample contains HBsAg, it will bind to the immobilized capture antibody. Subsequently, a detection antibody, enzymatically labeled, binds to the HBsAg already bound to the capture antibody. After washing to remove any unbound components, a substrate for the enzyme is introduced, which generates a detectable product that can be measured utilizing colorimetric or spectrophotometric methods. The intensity of the signal generated is directly proportional to the quantity of HBsAg present within the sample.

The procedure for conducting an ELISA for HBsAg involves a series of steps that include coating the microtiter plate with the capture antibody, adding the sample, washing to remove unbound components, adding the enzymatically labeled detection antibody, washing again, and finally adding a substrate for the enzyme. The resulting color change or signal is then measured and interpreted to determine the presence or absence of HBsAg, indicating either a current or past HBV infection.

3. Results and Discussion

3.1 Results

3.1.1 Sensitivity and Specificity

The HBsAg Rapid Test Cassette (Serum/Plasma) underwent sensitivity testing using a panel that ranged from 0 to 300 ng/mL. Positive results were obtained for all 10 HBsAg subtypes on the HBsAg rapid test cassette (Serum/Plasma). This test can detect HBsAg in serum/plasma at concentrations as low as 1 PEI ng/mL.

The antibodies used in the HBsAg rapid test cassette (Serum/Plasma) were specifically developed against the entire Hepatitis B antigen obtained from the Hepatitis B virus. Furthermore, the specificity of the HBsAg rapid test cassette (Serum/Plasma) was evaluated using laboratory strains of Hepatitis A and Hepatitis C, all of which yielded negative results.

Table 1 revealed excellent sensitivity and specificity for the HBsAg Rapid Test Cassette (Serum/Plasma). The test exhibited a sensitivity of over 99.9%, indicating its ability to accurately detect true positive cases. On the other hand, the specificity was determined to be 99.4%, highlighting its capability to correctly identify true negative cases. Overall, the test demonstrated an accuracy of

99.7%, emphasizing its overall reliability in providing accurate results.

Table 1: Performance Characteristics of HBsAg Rapid Test.

Method		EIA		Total Results
HBsAg Rapid Test Cassette (Serum/Plasma)	Results	Positive	Negative	
	Positive	241	2	243
	Negative	0	359	359
Total Results		241	361	602

Relative Sensitivity: >99.9% (95%CI*: 98.8%-100%)
Relative Specificity: 99.4% (95%CI*: 98%-100%)
Accuracy: 99.7% (95%CI*: 98.8%-100%)
*: Confidence Intervals

3.1.2 Precision

To assess intra-assay precision, 15 replicates were analyzed for each of the three specimens with HBsAg concentrations of 0ng/mL, 1ng/mL, and 5ng/mL. The results demonstrated accurate identification of both negative and positive controls, with a precision exceeding 99% in the majority of cases. For inter-assay precision, the performance was evaluated over a period of 10 days by conducting 15 separate assays using the same three specimens with HBsAg concentrations of 0ng/mL, 1ng/mL, and 5ng/mL. To verify consistency across different production lots, three lots of the HBsAg Rapid Test Cassette (Serum/Plasma) were tested. The assay exhibited a remarkable level of precision and specificity, with accurate identification of the specimens occurring in over 99% of instances.

3.1.3 Cross-Reactivity

Extensive evaluations were conducted to assess the performance of the HBsAg Rapid Test Cassette (Serum/Plasma) in the presence of various cross-reacting antibodies and antigens. The test cassette was examined using experimental samples that tested positive for HAMA (Human Anti-Mouse Antibodies), Rheumatoid factor (RF), HAV (Hepatitis A Virus), Syphilis, HIV (Human Immunodeficiency Virus), H. Pylori (Helicobacter pylori), MONO (Mononucleosis), CMV (Cytomegalovirus), Rubella, and TOXO (Toxoplasma gondii). The results demonstrated no cross-reactivity, indicating the specificity of the test cassette for detecting HBsAg.

To further evaluate the cassette's resistance to interference from different types of matrices that could potentially impact test results, visibly hemolyzed and lipemic specimens were included in the assessment. No interference was observed, suggesting that the test cassette is not affected by these conditions. Additionally, the cassette exhibited no significant interference from specimens containing high levels of hemoglobin (up to 2,000 mg/dL), bilirubin (up to 1,000 mg/dL), and human serum albumin (up to 2,000 mg/dL). These findings indicate that the test cassette maintains its accuracy and reliability when used with a wide range of clinical specimen conditions.

3.2 Discussion

In clinical settings, the rapid and precise identification of Hepatitis B virus (HBV) infections is essential. This process is vital for the recognition of those who are infected, halting the advancement of the disease, and deploying effective management techniques. Therefore, the development of reliable diagnostic methods holds significant value.

The primary objective of our investigation was to assess the efficacy of the HBsAg rapid detection assay, a method designed to identify HBsAg. This assay utilizes antibodies that specifically bind to

the viral antigen, providing a qualitative assessment of HBsAg antibodies in serum or plasma. The results of our study highlight the utility of this assay as an effective diagnostic instrument for HBV infections. To evaluate the diagnostic performance of the HBsAg rapid test, we conducted a comprehensive analysis involving 602 specimens, both HBsAg-positive and negative. The results demonstrated exceptional sensitivity (>99.9%), specificity (99.4%), and accuracy (99.7%). Additionally, the test exhibited a detection capability of 1 PEI ng/mL of HBsAg in serum or plasma specimens. Furthermore, in cross-reactivity testing using samples from other infectious agents, no reactivity was observed, confirming the excellent specificity of this diagnostic method. Considering the cost-effectiveness and logistical simplicity of the rapid diagnostic test, it can serve as a reasonable alternative in resource-constrained settings. This makes it particularly suitable for epidemiologic surveys.[8]

It is important to acknowledge the limitations of the HBsAg rapid test kit. While this test provides a qualitative determination of the presence or absence of HBsAg, it does not yield quantitative values or concentration rates for HBsAg. Therefore, it should not be solely relied upon for diagnosing Hepatitis B virus infection. A comprehensive assessment requires considering all results in conjunction with other clinically available information. This approach ensures an accurate interpretation of the patient's condition. The natural history of diagnostic markers in chronic hepatitis B is more intricate compared to many viral infections. Uncomplicated chronic HBV cases often display transient low-level asynchronous quantitative fluctuations of HBsAg and DNA. While these cases are generally less severe and of lower priority than individuals with higher levels of viremia, they can significantly influence estimates of sensitivity and specificity.[9]

The HBsAg rapid test kit has a limitation in that it cannot detect HBsAg levels below 1 PEI ng/mL. Therefore, when the test yields a negative outcome but clinical symptoms persist, it is advisable to utilize additional clinical procedures for further examination. It is crucial to emphasize that a negative outcome at any given time cannot definitively exclude the presence of Hepatitis B infection. To address these limitations, the implementation of quality control measures during the manufacturing process of the test kit is essential. Stringent quality assurance protocols and regular calibration of the assay can help minimize potential defects and ensure consistent and reliable performance.

4. Conclusion

In conclusion, the HBsAg Rapid Test Cassette has been evaluated for its effectiveness compared to the ELISA method. The results have demonstrated high levels of accuracy, sensitivity, and specificity, with an accuracy rate of 99.7%, sensitivity exceeding 99.9%, and specificity of 99.4%. These findings establish the HBsAg Rapid Test Cassette as a reliable diagnostic tool in clinical scenarios, particularly in environments where access to existing laboratory infrastructure is limited or unavailable, such as remote areas or populations with limited healthcare access.[10] This diagnostic test facilitates early detection, facilitating prompt intervention and efficient management of Hepatitis B. As a result, it has the potential to improve patient outcomes and reduce the transmission of the virus.

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