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RECENT ADVANCEMENTS IN THE DIAGNOSIS OF HEPATITIS B INFECTION

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ABSTRACT

HBV infection is a highly still a major hazard to health issues all around the world and It can cause liver illnesses. The Diagnosis of HBV patients, both acute and chronic, It is a critical step in minimizing the infection's impact. One important way to reduce the impact of the disease is to serological diagnosing using biomarkers is to detect antigens and antibodies against these virus such as HBs Ag (Hepatitis B surface antigen), HBs Ab (Antibody to HBV surface antigen), HBc Ag (Antibody to HBV core antigen), HBe Ag (Hepatitis B e antigen), Anti-HBe (Antibody to HBV e antigen), Anti-HBc (Antibody to HBV core antigen), HBcr Ag (Hepatitis B core-related antigen) and advance serological techniques are such as Enzyme-linked immunosorbent assay (ELISA), Western Blot, Chemiluminescent Enzyme Immunoassay (CLEIA), Rapid test, Micro-particle Enzyme Immunoassay (MEIA). Quantitative and qualitative molecular tests are utilized to confirm a primary diagnosis, measure the viral load, detect genotypes, and resistance mutations, and molecular techniques are including such as Polymerase chain reaction (PCR),

Biosensor and paper based analytical device are needed for effective diagnosis of HBV infection. The most recent available serological and molecular techniques for diagnosing HBV infection are summarized in this review article.

Keywords: Hepatitis B virus (HBV), HBs Ag, HBe Ag, HBc Ag, HBcr Ag, HBV DNA, Serological diagnosis, Molecular diagnosis

INTRODUCTION

Hepatitis B virus can cause liver a potentially fatal liver infection and as well as damage and the virus spreads during sexual contact or exposure to tainted blood or bodily fluids and HBV transmits from mother to kid and can cause Acute and Chronic disease of the liver **[1]**. HBV is *Hepadnaviridae* virus, which means it is a dsDNA virus **[1]**. The genome is made up of four different types of DNA to open reading frames that overlap encodes an exterior lipid envelope and an inner nucleocapsid core **[2]**. In 2020, According to the WHO estimated the chronic hepatitis B affects around 296 million individuals globally, with around 900,000 people dying each year from HBV-related complications **[3]**. Based on the WHO report overview in this below the table:

Table 1: WHO Report for the occurrence of HBV infection region

Region	HBV infection
Western Pacific	116 million
African	82 million
South-East Asia	60 million
Eastern Mediterranean	18 million
European	14 million
Americans	6 million

[Source: Global progress Report 2021, viral hepatitis and sexually transmitted infection] [4]

From 2021, the WHO published a worldwide health report on HBV infection, to eliminate new chronic hepatitis B virus infections by 90 percent **[1]**. It has been reported that India has 40 million persons infected with HBV and India is in the pathophysiology of hepatitis B is intermediate phase **[5]**. The finest diagnosis on HBV infections is required, the death rate is reduced and acute and chronic HBV infection is one important strategy for reducing the disease's burden. HBs Ag detects antibodies to anti-HBc IgM, while IgM antibodies are utilized to diagnose acute infection **[6]**. Chronic infection is determined by the resolution of HBs Ag in serum and HBV markers include HBs Ag and HBV DNA **[6]**. For serological diagnosis, ELISA, MELA, and fast assay have been employed and HBV detection DNA is crucial tool is

used for diagnosis by serum samples are frequently used in molecular techniques **[7]**. The goal of this review is to reduce the burden of HBV infection by examining the most recent available serological and molecular methods for diagnosing HBV infection.

HBV infection detection by serology:

Table 2: Serology tests for HBV infection

Disease	HBs Ag	Anti-HBs	HBe Ag	Anti-HBe	Anti-HBc	HBcr Ag	HBV DNA
Acute	+	-	-	-	+	-	+
Chronic	+	-	+/-	+/-	+	+	+/-

[Source: Luke Dang *et al*, Hepatitis B Serologic Testing methods, Infectious Disease. 2021] [8, 9]

Serological markers for HBV infection:

Table 3: Serological HBV markers

	HBV Biomarker	Characteristics
(1)	HBs Ag (Hepatitis B surface antigen)	It is a biomarker made from combined HBV DNA that's used to diagnose acute and chronic infection.
(2)	HBs Ab (Antibody to HBV surface antigen)	Indicates immunity to HBV and protection from disease.
(3)	HBc Ab (Antibody to HBV core antigen)	Total-indicates part or active infection. IgM-early indicator of acute infection. Antibody against the core protein(anti-HBc)
(4)	HBe Ag (Hepatitis B e antigen)	It is important for persistence and is utilised to distinguish the chronic hepatitis B phase and if HBV DNA is undetectable, it is linked to lower morbidity and mortality.
(5)	Anti-HBe (Antibody to HBV e antigen)	Prognostic for infection resolution
(6)	HBcr Ag (Hepatitis core-related antigen)	The structural nucleocapsid protein cannot be detected directly, the HBcr Ag assay was proposed as a straightforward technique for monitoring chronic hepatitis B patients.
(7)	Anti-HBc (Antibody to HBV core antigen)	The most reliable indicator of previous HBV infection. In the diagnosis of acute.

[Source: Kathy Jackson *et al*, Available diagnosing biomarkers for HBV infection, 2018.] [10]

Recent available Serological methods for HBV diagnosis:

Present-day to following serological procedures are used:

- ELISA (Enzyme-linked immuno-sorbent Assay):

  The enzyme-linked methodology is still the most used way to detect HBV infection, requires an antibody-dependent enzyme tag **[11]**. Type of solid-phase immunoassay which enzyme can catalyze substrate into colored products **[12]**. HBV consists of a nucleocapsid DNA associated with core protein and a capsid. The primary component is a protein called a surface antigen (HBs Ag), which is the initial sign of HBV infection in HBs Ag detection which is most important for the diagnosis of

acute and chronic hepatitis. Pregnant women and control of blood preparation and derivatives intended for transfusion. The performance of the ELISA test is determined by the coordination of the coated antibody, conjugated, and sera, as well as the availability of a kit. In most cases, kits are evaluated against pretested catalog **[12]**.

These can be detected by the ELISA method. In **Figure 1**: (a)Microwells are covered with seizing antibodies that recognize Hepatitis B Surface antigen (b)Blood plasma is added to permit antigen to be bound (c)A noticing antibody linked to an enzyme is added which binds seize antigen(d)An enzyme substance is added and a color reaction is catalyzed. The emitted light is computed as an indicator of Hepatitis B surface antigen concentration in blood **[13]**.

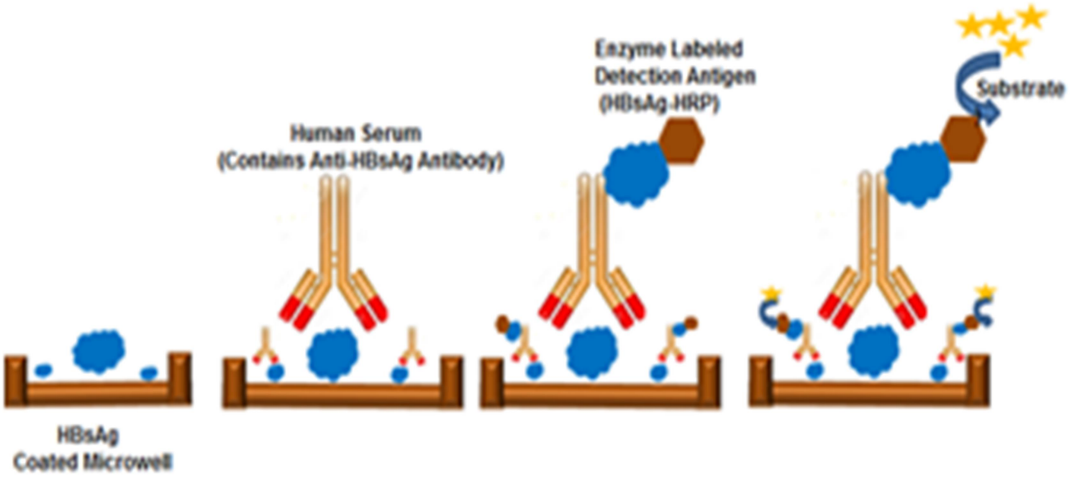

Figure 1: Hepatitis B surface antigen ELISA detection

[Source: Yucel F *et al*, Development of sandwich ELISA systems for the diagnosis of HBs Ag. 2018] [14]

- CLEIA (Chemiluminescent enzyme immunoassay):

  This fast immunoassay approach is more sensitive than ELISA because it uses antigens or antibodies that are tagged with imminent molecules **[12]**. In comparison to PCR, CLEIA has a sensitivity of 96 percent and researcher's various modifications have fascinated me even more **[12]**. A mixture of monoclonal antibodies specific for HBs Ag epitopes is used in the CLEIA approach for quantitative detection of HBs Ag. This assay had a correlation coefficient of 0.921 when compared

to a commercially available light-activated chemiluminescence test **[12]**.

- Western Blot:

  Western blot is a confirmatory test for hepatitis B infection. HBV is double-stranded DNA molecule. The capacity to detect HBx Ag could be influenced by the cell line utilized to express it. In the livers of carriers, HBx Ag is a frequent marker to expression confirmed using Western blots between cells in which the protein was introduced by immunoblotting **[15]**.

- Rapid Immunochromatographic test:

  Rapid immunochromatographic test uses two sets of antibodies that are HBs Ag and HBe Ag **[16]**. The detection of specific antigens catches in a typical test tube and the initial set of antibodies is colloidal gold-labeled and imbedded in a gel. For testing the second pair is immobilized on two opposing lines to be a bi-folded card with a test pad **[17]**. The WHO has pre-qualified HBV immunochromatographic RDTs.

- MEIA (micro-particle enzyme immunoassay)

  MEIA is a method for determining the quality of hepatitis B surface antigen by using very small microparticles in a liquid suspension and signal amplification to provide quantitative results and it is found that the received sample is positive. It is done in a shorter time than other immunological procedures due to the active surface area of microparticles, which disrupts the kinetics study and minimizes the incubation period **[18]**.

  The accuracy of HBV serological diagnostic testing has improved because they can be stored at room temperature, there may be a requirement for the trained and rapid tests insensitivity. Although trained technicians can employ ELISA for automated results, MEIA can be used because of its great sensitivity.

Resent available Molecular methods for HBV diagnosis

The molecular diagnostic techniques are used for HBV DNA quantification, genotyping and recently HBV PCR assay, approved nucleic acids amplification tests (NATs) used for diagnosis of HBV infection.

- HBV DNA

HBV DNA can be used as the sole approach to track viral replication in chronic infection and diagnosis and monitor the risk of cirrhosis advancement for the diagnosis of HBs Ag chronic hepatitis B, a highly sensitive molecular assay is certainly necessary, and viral levels might to quite low **[19, 20]**. Amplification techniques such as branched DNA technology, hybrid capture are the most used ways for identifying and measuring HBV DNA. HBV DNA can be detected in HBe Ag-negative samples using the Bayer diagnostic approach, which can be utilized for monitoring the chronic HBV infection **[18]**.

Table 4: Characteristic of hepatitis B virus molecular techniques

HBV molecular diagnosis	Application	Method
HBV DNA Qualitative methods	**Diagnosis infection HBV occult cases identification**	**PCR**
HBV DNA Quantitative methods	**Define the beginning of antiviral treatment**	**bDNA**

[Source: Livia melo et al, Updates on hepatitis B and C virus diagnosis. 2015] [18]

- HBV DNA quantification

  Individual infectivity and infectivity of HBsAg-positive pregnant women are determined using HBV DNA by NAT to prevent and cure illness **[21]**. UV spectrophotometers, real-time PCR, digital PCR, and surface plasma resonance biosensors are commonly used to measure results **[22]**.

- HBV genotyping

  HBV has a high genetic heterogeneity because it reproduces via a reverse transcription that has insufficient proofreading capability and sequence divergence **[23]**. HBV can be divided into ten genotypes, HBs Ag seroclearance due to the presence of a mutation **[24]**. HBV genotyping is not required for initial diagnosis and there are many genotyping systems **[24]**, thus it can be confirmed using diverse methods such as reverse hybridization, genotype-specific PCR assay, real-time PCR, restriction fragment-polymorphism, fluorescence -length polarization assay etc. use in detection of HBV infection **[7]**. HBV genotype identification by molecular means could be utilized to diagnose HBV infection.

- PCR (Polymerase chain reaction)

  In individuals with chronic hepatitis B, PCR is a highly versatile and sensitive method for detecting HBV DNA that is efficient in diagnosing

infection, as well as following up on HBV infection in liver transplantation patients to the specific surface gene was amplified through PCR **[25]**. For the detection of HBV, the isolated DNA was submitted to PCR **[25]**. For HBV diagnosis, PCR is a basic tool for automated amplification and detection of DNA, covalently circular DNA of HBV level observed in the hepatocytes is the most precise way of estimating the number of infected hepatocytes **[15]**. The two most important advantages of PCR are its high sensitivity and the ability to create non-radioactive probes for quick tests **[15]**.

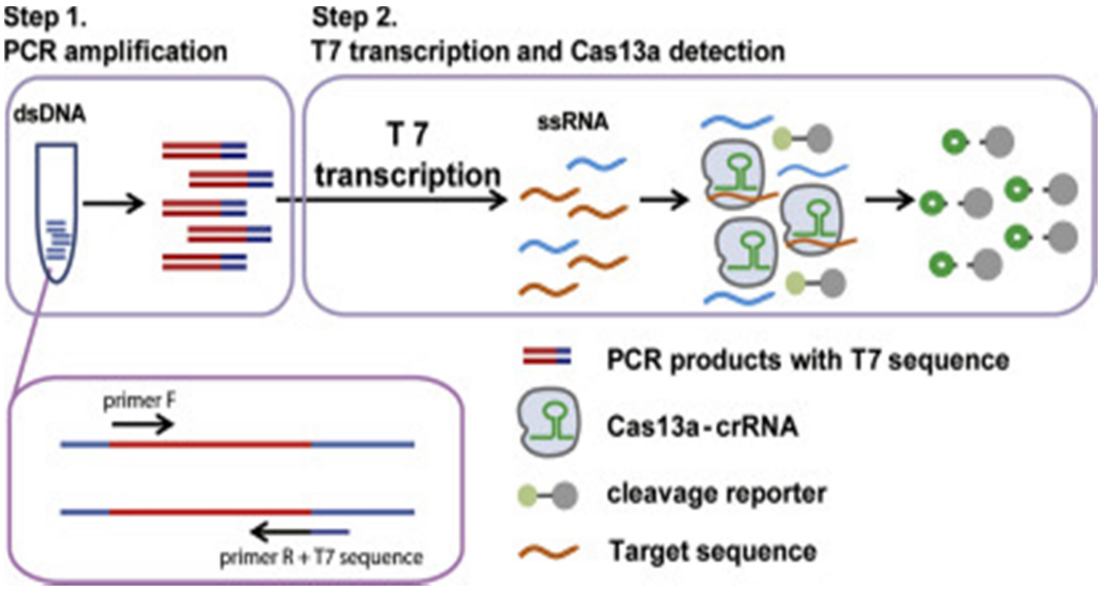

Figure 2: Detection of hepatitis B virus DNA by PCR

[Source: S. Wang *et al*, Highly sensitive and specific detection of hepatitis B virus. 2021] [26]

PCR amplification of the target gene sequence requires a template DNA molecule and transcription and detection Cas13a for each specific primer. Complete the sequencing process, followed by a final DNA extraction phase in which DNA polymerase stretches the primer and creates a new complementary DNA strand. To detect low viral loads and this time several real-time PCR assays are available to diagnosis test **[18]**.

- Immunofluorescence technique

  Immunofluorescence is an antigen detection technique that is performed on frozen tissue slices and cell smears to detect antigen by the binding of specifically modified, although it binds to an agent to the sample matrix

with agent-specific antibodies, it emits light at a longer wavelength. Specialized microscope equipped with filters for the fluorochrome emission wavelength detects optically emitted light, which can be directly attached to the agent of interest antibody (direct immune-fluorescence) or it can be attached to an anti-immunoglobulin molecule that recognizes the agent specific antibody (indirect immune-fluorescence) **[15]**.

- Biosensors

  The biosensor is currently in this state of the biosensor is that it is capable of detecting HBV in an efficient and in a timely and specific manner **[27]**. HBs Ag should be detected by a biosensor based on localized SPR was developed **[22]**. The results show that a gold nanorod-based LSPR biosensor capable of monitoring HBs Ag concentration may be developed **[27]**. The sensing element is made up of antigen or antibody bands that are immobilized, and the transducer converts the binding event into a detectable signal and the purpose of an immunosensor is to produce a signal proportional to the concentration of the analyte **[15]**.

- Paper-based analytical devices

  Paper folding or three-D ePADs have recently been created to detect HBV DNA amplification. Hepatitis B was previously diagnosed **[28]**. The suggested device's detection principle utilized metalloimmunoassay, a type of hepatitis B tagged DNA with silver nanoparticles as the solid-phase support, and nitrocellulose membrane as the conjugate, pad with sample and absorbed sample **[28]**. Monoclonal anti-HBs Ag2 and polyclonal anti-HBs Ag were coated on the NC membrane as a test and control line **[28]**. The configuration of the gadget was similar to open and close the pop-up paper to assess the concentration of HBs Ag and the purpose of incubation step was performed to allow hybridized with HBV DNA in an open paper configuration allowing the reactant to react using HBV DNA that has been incubated and then detected to determine the amount of hepatitis B labeled DNA in the sample **[28]**.

  In patients with cryptogenic liver disease, Immunosuppressive therapy

and solid organ transplantation donors should be evaluated, due to the possibility of transmission **[23]**.

DISCUSSION

In this review, we strive to provide information on HBV infection diagnosis to distinguish between acute and chronic forms of infection. Each detection technique has their advantages and disadvantages.

Table 5: Advantages and Disadvantages of diagnostic techniques

TECHNIQUES	ADVANTAGES	DISADVANTAGES
ELISA	**Low cost, highly sensitive, easy to perform, the result can be quantitative and qualitative.**	**It takes time, requires the use of a plate reader, and the result is objective.**
CLEIA	**Instrumentation is rather straightforward, with a very low detection limit and a high sensitivity.**	**High cost, time- consuming, equipment and trained technicians.**
Western blotting	**High specificity**	**Time-consuming, costly, results are qualitative.**
Rapid Immunochromatographic test	**Fast performing, user- friendly, results within 20 minutes.**	**Shorter perceptive, with a higher risk of false positives and lower quality data.**
MEIA	**High sensitivity, faster than other immunological methods**	**Trained technicians, continuous supply of electricity**
PCR	**Extreme sensitive, highly specific.**	**Costly, need expertise, the problem of mutation will be there, specific primer needed**
Immunofluorescence	**Highly specific, subjective type, qualitative results.**	**Time-consuming, expertise and lab set up in needed, costly and time taking.**
Paper-based analytical device	**Simple method, rapid process.**	**Expensive wax, extra heating step after deposition.**

[Source: Pallavi kachhawah, Livia Melo *et al*, Current updates on Diagnostics for HBV infection. 2020] [15, 18]

- In the past, infection with hepatitis B virus was detected using a complement fixation test to demonstrate an antibody titer **[16]**.
- In diagnosis most acute detection usually anti-HBc IgM can be detected and HBV DNA present and the diagnosis of chronic infection based on the HBs Ag for 6 months, to detect HBV infection, DNA testing is widely employed **[15]**.
- A Second way was the gel diffusion technique, which was less sensitive for blood screening procedures and had the drawback of requiring more blood from both serological and molecular approaches have been used to diagnose the donor and HBV **[1, 18]**.

Recent available methods:

In this work, we intended to discuss the most recent serological and

molecular approaches. In the last few years, molecular biology techniques such as PCR has seen tremendous improvement in molecular diagnosis for HBV PCR-based amplification procedures such as ligase chain reaction are presently used to diagnose HBV infection. Currently, the detection of HBV DNA, antigen, or anti-HBV antibodies, a range of biosensors based on diverse goals have been developed by human antibodies and the most recent use of paper-based analytical devices for HBV detection as early as possible **[6, 15]**.

- The precision and reliability of enzyme immunoassay, as well as the high speed and technical simplicity of complete automation for make them ideal for high-volume clinical laboratories. Rapid diagnostic tests are quick and easy to use, and they can be used with serum or plasma. They can also be performed by trained by providers or healthcare workers in resource limited settings without the need for specialized apparatus.

Future scope:

As per recent research immunotherapy and biomarkers are a diagnosis of HBV infection in progress.

CONCLUSION

This article purpose is to provide current information on serological and molecular diagnosis approaches. According to need the following tests: ELISA, CLEIA, Western Blot, Rapid immunochromatographic test, MEIA, PCR, Immunofluorescence technique, HBV diagnosis includes new methods such as Biosensor and paper-based analytical device are discussed giving new advance information about this theme. In terms of diagnostics, each method of detection has advantages and downsides. In the present time, useful methods for serological diagnosis of HBV infection and in respect to molecular assay, digital PCR paper based analytical device is a most useful method for diagnosis of HBV infection. We look forward to more research outcomes from a global scientific fraternity to come out with new reliable markers and methods soon for the diagnosis of HBV infection.

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