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# Modern Obstacles and Prospects for Hepatitis B Virus Diagnosis: A Comprehensive Review study

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**Abstract:**

**Background-** About 5% of adults with Hepatitis B Virus (HBV) develop chronic hepatitis B (CHB). Most chronic liver diseases, cirrhosis, and liver cancer cases stem from HBV acquired in childhood. HBV spreads through blood and body fluids, with a 2-5 week incubation period, leading to serious liver conditions. In low–medium resource countries, mother-to-child transmission is common, along with factors like IV drug use and unsafe sexual practices. Despite vaccines, HBV remains a major health issue, infecting 260 million people and causing 890,000 deaths annually. One-third of the global population contracts HBV, though most clear the virus without chronic disease.

**Aims objectives-** To achieve the goal of eradicating HBV, it's essential to identify every stage of the infection. This can be accomplished through a comprehensive strategy that integrates new marker assays with cutting-edge pretesting and testing techniques.

**Conclusion-** Mass testing for hepatitis B is crucial in regions with high prevalence and limited resources. Developing sensitive and standardized tests is urgent to detect viral markers accurately. Standardizing reporting for occult infections is vital. Enhancing disease awareness and refining surveillance are key to WHO's goal of eradicating viral hepatitis by 2030.

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**Key words-** Diagnosis, Genome, Genotypes,Hepatitis B, Procedure, Prevention, WHO,

**Introduction:**

Around 5% of cases where Hepatitis B Virus (HBV) is acquired in adulthood persist and progress into chronic hepatitis B infection (CHB). In contrast, the majority of chronic liver disease cases, including cirrhosis and hepatocellular carcinomas (HCC), result from HBV acquired in infancy and childhood, accounting for approximately 95% and 20–30% respectively [1, 2]. HBV, primarily transmitted through blood and body fluids, has an incubation period of approximately 2–5 weeks and infects hepatocytes (liver cells), leading to severe conditions like cirrhosis and hepatocellular carcinoma [3]. In low–medium resource countries (LMIC), the predominant mode of HBV transmission is from mother to child. Additional factors contributing to HBV transmission include intravenous (IV) drug abuse, occupational exposure to infected blood products, engaging in sexual activity with multiple partners, and insufficient awareness [4, 5, 6].

Hepatitis B virus (HBV) infection continues to be a significant health issue despite widespread vaccination efforts. Worldwide, 260 million people live with chronic HBV infection, and 890,000 deaths occur annually due to complications arising from the progression of the disease [7, 8]. HBV's involvement in the development of chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC) is notable. Approximately one-third of the world's population contracts HBV at some stage in their lives, with acute hepatitis B infection typically resolving upon viral clearance [9, 10].

HBV, a hepatotropic enveloped DNA virus belonging to the family Hepadnaviridae, serves as the etiological agent for both acute and chronic hepatitis B infection in humans. Despite a decrease in HBV infections attributed to vaccination and the use of antiviral therapy (which reduces the viral load in chronically infected patients), approximately 3.5% of the global population remains chronically infected with HBV [9, 11]. In 2016, the WHO Global Hepatitis Health Sector Strategy outlined the goal of eliminating viral hepatitis by 2030. Pursuant to this strategy, achieving the elimination target hinges on bolstering diagnosis to facilitate timely testing, care, and treatment [10]. This article delves into the present challenges associated with diagnosing HBV and highlights recent advancements in the field [12, 13].

**Genome Hepatitis B Virus (HBV):**

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Blumberg et al. [14] made a groundbreaking discovery in 1965 when they identified an antigen initially dubbed the "Australian antigen" in an Australian aborigine, later recognized as the hepatitis B surface antigen [16]. Dane et al. [15] then utilized electron microscopy in 1970 to visualize HBV particles for the first time. Their observations revealed three distinct types of HBV particles in the serum of infected patients. Among them were two spherical structures measuring 42 and 22 nm in diameter, alongside a filament-like structure also measuring 22 nm in diameter, with varying lengths [15, 16]. The infectious virion, known as the Dane particle, boasts a larger spherical structure measuring 42 nm in diameter. Encased within a lipid membrane are three viral surface antigens (HBs), encircling a nucleocapsid comprising hepatitis B core protein (HBc), viral polymerase (Pol), and the viral genome DNA. This genome, spanning 3.2 kb, consists of circular partially double-stranded DNA, referred to as relaxed-circular DNA (rcDNA), comprising a closed (−) strand and an open (+) strand. Within the HBV genome lie 4 overlapping open reading frames (ORFs): C, P, S, and X.

The functional proteins of Hepatitis B Virus (HBV) are determined by overlapping Open Reading Frames (ORFs). ORF C encodes for the 22-kDa precore protein (p22cr), as well as HBc and HBeAg. ORF P is responsible for encoding the viral polymerase (Pol). ORF S dictates the viral surface antigen, including L (Large)-HBs, M (Middle)-HBs, and S (Small)-HBs. Lastly, ORF X is accountable for encoding the HBV X protein (HBx). Hepatitis B virus e antigen (HBeAg), emerging from the reading frame of HBc, serves as a gauge for cccDNA replication. Upon cell infiltration, HBV's rcDNA undergoes metamorphosis into covalently closed circular DNA (cccDNA), prompting the genesis of viral RNAs of varying lengths via transcription initiation from diverse promoters. These RNA strands measure approximately 3.5 Kb, 2.4 Kb, 2.1 Kb, and 0.7 Kb respectively. Back in 1983, Rall et al. unveiled that HBV genome transcription is orchestrated by RNA polymerase II from the infected host, under the governance of four distinct promoters and two enhancers (Enhancer I and Enhancer II), catering to preS1, preS2, core, and X sequences [17].

**Important clinical factors and Genotypes of HBV:**

Throughout the world, one encounters four prevalent HBV genotypes (A, B, C, and D). However, the occurrence of genotypes B and C predominates in Eastern and Western Asia,

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while genotypes A and D prevail in North America, Africa, and Europe, with genotype E prevalent in West Africa [18, 19]. The HBV genotype potentially correlates with disease advancement, the course of infection chronicity, and treatment response [20]. Distinctive disease progression, outcomes, and responses to antiviral therapy may be observed among patients infected with different HBV genotypes. Nonetheless, approved HBV vaccines demonstrate efficacy against all HBV genotypes [20, 21].

The HBV genotypes, distinguished by an 8% or greater deviation in the HBV genome sequences, have undergone classification. Approximately 10 genotypes (A through J), alongside several subtypes, have thus far been recognized, with their prevalence differing across geographical regions. The presence of specific HBV genotypes within a populace can bear epidemiological significance, unveiling origins and aiding in transmission pattern surveillance. Immigrants' HBV genotypes are typically linked back to their homelands [19, 22].

**The HBV Infection history:**

The pathogenic nature of HBV infection hinges on a complex interplay of the host's immune response, viral replication and evolution, and various environmental influences. When it comes to chronic HBV infection, the age at which the individual contracts the virus is crucial. The likelihood of acute HBV evolving into a chronic condition is approximately 95% if the infection occurs during the perinatal period, drops to 20-30% for children aged 1 to 5 years, and falls below 5% for adults [23].

**a.** HBeAg-Positive Chronic Hepatitis or Immune-Active Phase: Is triggered by the host's immune response to HBV, leading to liver cell damage. This is evident through elevated ALT levels and signs of moderate to severe liver injury. During this period, HBV replication decreases, causing a decline in HBV DNA, HBeAg, and HBsAg levels. The phase concludes with a reduction in HBV DNA and the seroconversion of HBeAg to anti-HBe positivity[9].

**b.** HBeAg-Positive Chronic Infection or Immune-Tolerant Phase: In this phase the virus replicates vigorously while causing minimal inflammation. It is marked by elevated viral loads, typically with HBV DNA levels exceeding 10^7 IU/mL. During this

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stage, both HBeAg and HBsAg are detectable in the blood, yet alanine aminotransferase (ALT) levels and liver histology remain normal [24, 25].

**c.** HBeAg-Negative Chronic Infection or Immune Control Phase: During this phase, the hepatitis B e antigen (HBeAg) transitions to its antibody form (anti-HBe), resulting in low or undetectable levels of HBV DNA and normal ALT levels. Nonetheless, approximately 10–30% of patients who undergo HBeAg seroconversion continue to exhibit elevated ALT and HBV DNA levels. These individuals are categorized as HBeAg-negative chronic hepatitis B (CHB) patients, often due to mutations in the core promoter or pre-core region, with antibodies targeting only the core protein [9, 24].

**d.** HBeAg-Negative Chronic Hepatitis or HBeAg-Negative Immune Reactivation Phase: Approximately 10-20% of inactive hepatitis B virus (HBV) carriers experience a resurgence of HBV DNA replication after years of dormancy. This recurrence is often associated with mutations in the core promoter or pre-core regions, accompanied by liver histology revealing necroinflammation and fibrosis [9].

**e.** Acute HBV Infection: It manifests as elevated levels of HBsAg and alanine aminotransferase (ALT), typically resolving within six months. These elevated levels normalize within this period. Most acute HBV cases are asymptomatic, with only about 30% of individuals exhibiting clinical symptoms such as jaundice and hepatitis. This infection has an "eclipse" phase lasting around eight days, during which no obvious signs are apparent. While acute HBV infections are generally self-limiting, they can linger as a residual infection and become active in immunocompromised individuals. Initially, it was believed that HBV DNA was completely eradicated, but sensitive PCR assays can detect trace amounts of DNA in the serum and liver. If the infection persists beyond six months, it can develop into chronic HBV infection, characterized by high levels of HBsAg and ALT due to a weak cytotoxic T-cell response [26, 27, 28].

**f.** Occult HBV Infection (OBI):Characterized by the presence of replication-competent HBV DNA (specifically, episomal covalently closed circular DNA) within the liver and/or detectable HBV DNA in the bloodstream, all while standard tests fail to detect HBsAg . This condition arises due to the suppression of viral replication and protein production, a result of the host's immunological and epigenetic defenses, leading to undetectable HBsAg levels [29, 30]. OBI is further classified into two types:

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- Sero-positive OBI**:** This form tests positive for anti-HBc and/or anti-HBs antibodies.
- Sero-negative OBI**:** Representing 1-20% of all OBI cases, this form tests negative for both anti-HBc and anti-HBs antibodies.

**HBV Diagnosis:**

The enduring scrutiny and monitoring of persistent infection hinge upon the scrutiny of laboratory viral indicators. Within the realm of HBV biomarker assays, two pivotal domains emerge: the first, serology, encapsulates the identification and measurement of viral-specific antibodies and/or antigens; the second, nucleic acid testing (NAT), delves into the detection and measurement of the HBV genome and its RNA transcripts [27].

By embarking on WHO's visionary voyage to vanquish viral hepatitis by the year 2030, we set sail towards a horizon where diagnosis, care, and treatment burgeon [11]. The quest for elimination entails a noble endeavor, aiming to slash the incidence rate by a resounding 90% and curtail fatalities by a remarkable 65% from the anchor point of 2015 [12,31]. Casting our nets wider, screening and diagnosis must unfurl across the populace, embracing those oblivious to their HBV status or yet to tether themselves to the realm of care and treatment [32]. For it is amidst the ranks of the unwittingly infected that the viral whispers persist, weaving their clandestine saga of transmission [33, 34]. Serologic assays pinpointing HB surface antigen (HBsAg) serum concentrations, HB surface antibodies (anti-HBs), and HB core antibodies (anti-HBcs) unveil those exposed to HBV. Meanwhile, NAT tests divulge insights into viral replication intensity, emergence of distinct strains, and reservoir occurrences. Innovations in testing extend to gauging intrahepatic HBV replication rates. These biomarkers serve to identify HBV infection, track disease evolution, and gauge treatment response and efficacy of novel therapeutics in clinical assessments. Diagnosis of HBV hinges on a gamut of markers including hepatitis B surface antigens (HBsAgs), hepatitis B surface antibodies (anti-HBs), hepatitis B e-antigen (HBeAgs), hepatitis B e-antibodies (anti-HBes), and hepatitis core antigens (anti-HBcs).

| Biomarkers | Specification | Significance |
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| | | |
|---|---|---|
| HBV DNA | Signs of an active HBV presence, scrutinize viral proliferation | • Detectable within the period of peering through the window.<br>• Discerns the hidden presence of HBV occult infection (OBI) amidst patients grappling with liver ailments or co-infections.<br>• Detection of HBV concentration serves as a compass guiding therapeutic interventions and ongoing assessment of treatment effectiveness |
| HBsAg | Hallmark of infection | • The inaugural emblem on the serological canvas<br>• Endures beyond half a year amidst chronic HBV's grasp<br>• A crucial compass in deciphering infection's tale, mirroring its transcriptional dance |
| Anti-HBs | Neutralizing antibody | • Regeneration or resilience against HBV<br>• Solely marked, the sentinel of immunity post HBV vaccination |
| HBeAg | Sign of bustling HBV replication | • Reflections of sero-status unveil the intricate tapestry of disease's natural chronicles<br>• Beacons to discern HBeAg-positive chronic infections and hepatitis from their HBeAg-negative counterparts<br>• Harbingers signaling the reawakening of subdued chronic infections with low replication rates |
| Anti-HBc | Manifestation of HBV Exposure | • Signs of engagement with HBV emerge<br>• HBV reawakening post-potent immune quelling<br>• Amid the HBsAg-negative realm of ailment |
| Anti-HBe | Detected swiftly in the onset of acute hepatitis B, it swiftly vanishes post ALT zenith | • Reduced HBV bustling<br>• Diminished infectivity, ailment's retreat<br>• Mutations in the precore and core promoters |
| IgM anti-HBc | Recent infection with hepatitis B virus | • During the fiery onset of HBV infection, its notable presence shines bright, yet fades within a fleeting six-month span<br>• In the realm of chronic hepatitis B, a curious 10-20% exhibit flares accompanied by the faint glimmer of IgM anti-HBc, its index value meek but discernible |
| IgG anti-HBc | Indicates a chronic infection | • A lack of neutralizing antibodies alongside HBV presence signifies prior exposure<br>• Detection of solitary IgG anti-HBc may suggest occult HBV infection |

Table 1. : HBV biomarkers and their significance in diagnosis.

**a.** Commonly Used HBV biomarkers:

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**Hepatitis B surface antigen (HBsAg)**: stands as the pivotal serological indicator for both acute and chronic hepatitis B infections. In epidemiological research, HBsAg is employed to determine the prevalence of chronic HBV infection. Typically, the presence of HBsAg in tests conducted six months apart signals chronic HBV infection. Conversely, the absence of HBsAg in the serum signifies recovery from an acute HBV infection [35, 36].

The gene ORF S encodes for the viral surface antigen, producing small (SHBs), medium (MHBs), and large (LHBs) proteins [37]. Transmission occurs when SHBs and/or LHBs, containing the pre-S1 region, bind to sodium taurocholate co-transporting polypeptide and heparan sulphate proteoglycan. HBsAg, a component of the viral envelope, also exists as non-infectious subviral particles that may inhibit the host immune system. Measuring HBsAg in body fluids is crucial for diagnosis in clinical practice and trials [38, 39].

Persistently elevated HBsAg levels for more than six months signify chronic HBV infection, while these levels become undetectable following recovery from acute HBV infections. Monitoring HBsAg in body fluids is vital for determining the infection stage, as it indicates the transcriptional activity of cccDNA, which is higher in HBeAg-positive patients compared to HBeAg-negative ones [40, 41].

Quantifying HBsAg carries significant prognostic value and has been integrated into risk scores to predict the likelihood of HCC and potential viral rebound risk after cessation of NUCs. Presently, standardized commercial assays for HBsAg quantification include the Architect HBsAg assay by Abbott Diagnostics, USA, the ElecsysHBsAg II quant assay by Roche Diagnostics, USA, and the DiaSorin Liaison XL by DiaSorin, Italy. While these assays are proficient in detecting and quantifying HBsAg, they lack the capability to distinguish among the three HBs proteins. Differentiation of these three HBs proteins requires in-house ELISA or Western blot analysis [42, 43].

**Anti-HBs (Antibody to HBsAg):** This key marker indicates the presence of antibodies capable of neutralizing HBV, typically signifying recovery from an acute infection. Post-recovery, both anti-HBs and anti-HBc may be present. A positive anti-HBs result with a negative HBsAg can occur due to HBV vaccination, recovery from acute hepatitis, or seroconversion in chronic HBV infection. An anti-HBs titer below

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10 IU/l is negative, between 10–100 IU/l is moderate, and above 100 IU/l is considered protective, even against high HBV viral loads [26, 27].

**Hepatitis B Core Antigen (HBcAg) and Anti-HBc:** The HBcAg, shielded by HBsAg, is not easily detected in the serum. While assays for anti-HBc are available, those for direct detection of HBcAg are rare [26].

In the early stages of acute hepatitis B infection, the immune system first produces IgM anti-HBc antibodies, which can be detected roughly one month after the appearance of HBsAg. Elevated levels of IgM anti-HBc indicate a recent infection and typically persist for about 4–6 months. As the infection resolves, IgM anti-HBc levels decline, while IgG anti-HBc levels rise. In cases of chronic HBV infection, low levels of IgM anti-HBc may remain and can increase with the severity of the disease. Testing for anti-HBc in blood is valuable for identifying occult HBV infection (OBI) in blood or organ donors, assessing individuals before they undergo immunosuppressive therapy, and conducting epidemiological studies [26].

**Hepatitis B e antigen (HBeAg) and its counterpart, anti-HBe**: Hold pivotal roles in the intricate dance of HBV infection. Encoded by the precore (Pre-C) segment within the core gene (C), HBeAg serves as a distinguishing emblem, separating the HBeAg-positive from the negative in chronic hepatitis B (CHB) infections [44]. The shift from the immune clearance to the immune control phase is heralded by the enigmatic phenomenon of HBeAg seroconversion. In the complex chronicles of HBV infection, HBeAg emerges as a sentinel, marking chronic active HBV infection during its immune-tolerant phase and signaling the resurgence of low-replicative chronic infection. Its presence in the serum of HBsAg-positive carriers signifies a bustling hive of viral replication and heightened infectivity [44, 45]. Detection assays for both HBeAg and anti-HBe are often bundled together, nestled within the confines of enzyme immunoassay kits, as they represent diametrically opposing forces in the battle against HBV. Moreover, HBeAg emerges as a cost-effective surrogate for HBV DNA, offering a lifeline for therapeutic management and efficacy assessment, particularly in resource-constrained settings [46, 47].

**Hepatitis B virus DNA (HBV DNA):**Unraveling the genetic code within serum unveils insights into HBV viral activity, a pivotal gauge in chronic HBV patients, warranting periodic assessment, typically every six months [24, 44]. The HBV DNA viral load not only guides therapeutic strategies but also reflects treatment

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effectiveness. While conventional methods seldom utilize viral cccDNA for HBV detection, its quantification sheds light on viral replication dynamics [48, 49]. The presence of cccDNA in hepatic cells poses a formidable obstacle to HBV eradication, serving as a blueprint for virion reproduction. The absence of HBsAg and cccDNA signifies a genuine HBV cure, whereas an uptick in HBV DNA levels signals resistance to ongoing therapies [9, 23].

**b.** Newer biomarker for HBV**:**

Constant pursuit for newer markers persists, aiming to enhance diagnostic precision, prognostic accuracy, and treatment efficacy. Among the recent additions to this quest are HBcrAg and HBV RNA. HBcrAg, or Hepatitis B core-related antigen, emerges as a pivotal player in the landscape of chronic HBV infection, where eradication remains elusive due to the resilient cccDNA nestled within the nuclei of afflicted hepatic cells. The conventional route of liver cell biopsy, requisite for cccDNA quantification, presents as a formidable and invasive procedure [50, 51]. Enter HBcrAg, a potential non-invasive surrogate marker for intrahepatic viral replicative activity. Studies unveil promising correlations between HBcrAg levels and cccDNA quantities across both HBeAg-negative and -positive patient cohorts. However, its association with HBsAg appears tenuous [52, 53].

Comprising HBeAg, p22cr, and HBcAg, HBcrAg emanates from the precore/core region, offering avenues for serological evaluation. These trio proteins share an identical 149-amino acid sequence, manifesting when HBV DNA and HBsAg elude detection. HBeAg, derived from a core gene, undergoes transformation into a circulating peptide, excreted by hepatocytes [53 54]. Meanwhile, HBcAg envelops viral DNA, with p22Cr residing within HBV DNA and HBcAg-negative Dane-like particles. HBcrAg emerges as a prospective surrogate for cccDNA, poised to revolutionize outcome prediction and management strategies in HBV-related afflictions [55, 56].

**Hepatitis B virus RNA (HBV RNA)**, serving as the genetic blueprint, orchestrates the synthesis of viral proteins. Remarkably, these transcripts, alongside pregenomic RNAs, emerge as distinctive markers for viral replication, coursing through the serum of afflicted individuals [57]. While an array of assays caters to the detection of HBV DNA, the elusive HBV RNA remains beyond the reach of commercial assays. In the void left by commercial tools, bespoke methodologies step in, capable of discerning

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HBV RNA, aligning with HBV DNA levels in untreated patients. Nevertheless, this pursuit is not devoid of challenges; variables like genotype variations and mutations can sway the accuracy of HBV RNA detection. Such insights, akin to those surrounding other diagnostic markers, could pave the way for enhanced reliability in future HBV RNA detection assays [58, 59].

Meanwhile, the saga of HBV biomarkers unfolds, with stalwarts like hepatitis B surface antigen (HBsAg), hepatitis core antigen (anti-HBc), hepatitis B e-antigen (HBeAg), hepatitis B surface antibody (anti-HB), and hepatitis B e antibody (anti-HBe) holding sway over the diagnosis of HBV's natural course and its myriad phases of infection. Yet, amidst this symphony of markers, the levels of HBV DNA emerge as the maestro, dictating the management of HBV infection with precision [59, 60].

**Standard Procedures for Hepatitis B Virus Detection**:

Detection and quantification of hepatitis B viral markers in body fluids typically involve a range of sophisticated techniques. These include enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), enzyme immunoassay (EIA), and polymerase chain reaction (PCR). More recently, innovative methods like microparticle enzyme immunoassay (MEIA), electrochemiluminescence immunoassay (ECLIA), and chemiluminescencemicroparticle immunoassay (CMIA) have emerged. However, these advanced HBV testing techniques are often costly, requiring substantial instrumentation and specialized training [61, 62].

| Procedures | Advantages | Disadvantages |
|---|---|---|
| PCR | • Low viral load detection ability<br>• Wide dynamic range<br>• Reduced likelihood of carryover contamination<br>• And full automation | • A steady supply of electricity, skilled technicians<br>• And sophisticated equipment |
| ECLIA/ CMIA | • An automated system possessing a significant level of sensitivity and specificity | • High price, advanced machinery, qualified specialists, and steady power supply |

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| | | |
|---|---|---|
| EIA | • This automated system consistently delivers highly reproducible outcomes while keeping costs minimal | • This procedure demands significant time due to the necessity for advanced machinery and skilled personnel. Additionally<br>• it relies on a continuous power supply, making it impractical for on-site applications |
| RIA | • Sensitivity is very high | • This method comes with a steep price tag<br>• Operator faces potential hazards |
| MEIA | • An immunological technique boasting superior sensitivity and the quickest turnaround time compared to all other methods | • A steady supply of electricity, skilled technicians<br>• And sophisticated equipment |

Table 2. Advantages and disadvantages of the HBV diagnostic procedures

Dried Blood Spot (DBS): A practical sampling method facilitates large-scale population screening or testing in resource-limited settings with restricted access to testing facilities. A single drop of blood, obtained via a finger-prick, is collected on a specially treated paper card. The chemicals on the card preserve the HBV marker, allowing the sample to be transported at room temperature from the field to the laboratory. Once there, advanced molecular or immunoassays are used to test the samples [63, 64].

Point-of-Care Tests (POCT): Rapid diagnostic tests (RDTs), are revolutionizing the swift and accessible diagnosis of HBV. These user-friendly tests need just a drop or two of a sample and can be administered without specialized training, making them perfect for diverse community and outreach settings. However, it's important to note that while these kits offer convenience, their sensitivity is somewhat lower compared to other testing methods [65].

**Difficulties in Diagnosing HBV**:

In resource-limited settings or countries, gathering blood samples for HBV testing at remote locations often necessitates additional logistical support for transporting them to testing facilities. Typically, HBV diagnosis relies on plasma or serum, which are required for the

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most commonly available tests. However, sub-centers in low-resource settings frequently lack centrifuges to separate plasma and serum from blood samples [66]. Even if some sub-centers have centrifuges, securing supplies such as consumables and reliable power can be challenging. Transporting samples at room temperature generally does not affect serological test markers but can degrade molecular markers [67]. This issue can be addressed by employing the DBS method for sample collection and utilizing point-of-care tests. These approaches can mitigate the challenges of testing and reduce loss of follow-up in low-resource settings [68]. While nucleic acid testing for HBV screening is widely used in developed countries, its application in low-income countries is limited due to higher costs and the need for trained personnel.

**Perspectives for the Future:**

A precise grasp of diagnostic techniques is essential for effective hepatitis B treatment, as various therapies target distinct markers of the infection in different ways. Thus, diagnosis using a combination of hepatitis B markers is crucial for monitoring therapy effectiveness [23]. The seroclearance of HBsAg and the emergence of anti-HBs, which signal protective immunity, can be identified through the use of both HBs and anti-HBs markers. During the inactive and immune-tolerant phases, HBsAg serum levels and their sources vary, necessitating distinct diagnostic approaches. In HBeAg-negative patients, HBsAg levels below 100 IU/mL might suggest gradual clearance, detectable only when using both HBeAg and HBsAg markers [44]. Additionally, these markers, along with HBV genotypes, are useful for tracking antiviral therapy responses. HBV genotypes influence the effectiveness of antiviral treatments, so a comprehensive diagnosis involves assessing HBsAg, HBeAg, hepatitis B viral load, and HBV genotypes. This approach aligns with the WHO's objectives by determining the infection stage and guiding therapy decisions [69, 70].

**Conclusions:**

Despite ongoing research and unresolved issues surrounding the diagnosis of hepatitis B, mass testing remains essential, especially in regions where the virus is widespread and resources are scarce. There's an urgent call for developing sensitive, standardized, and

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validated testing procedures to detect HBsAg, HBV DNA (both in blood and liver), and other viral markers. These tests must be capable of identifying HBV S variants and HBsAg within immune complexes containing anti-HBs. establishing a standard report for occult blood infections is crucial to ensure consistent future reporting of such cases. In line with the WHO's mission to eradicate viral hepatitis by 2030, enhancing disease awareness, improving case identification, refining surveillance strategies, and optimizing treatment are critical steps towards this objective.

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