Review

Advancing HBV Diagnostics: The Role of Ultrasensitive HBsAg Testing

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Advancing HBV Diagnostics: The Role of Ultrasensitive HBsAg Testing

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Abstract

Hepatitis B virus (HBV) represents a significant global health challenge, affecting over 254 million individuals and contributing to 1.1 million deaths from liver-related complications in 2022. The World Health Organization has set ambitious targets to reduce HBV infections and mortality by 2030. However, only a small proportion (13%) of infected individuals receive timely diagnosis and treatment. HBV elimination efforts necessitate substantial improvements in HBV diagnosis, particularly in identifying occult HBV infections (OBI), early-stage infections, and breakthrough cases. The hepatitis B surface antigen (HBsAg) is a key biomarker in HBV diagnosis, serving as a reliable indicator of infection status and treatment response. Conventional HBsAg assays, with a lower limit of detection (LoD) between 0.03-250 IU/mL, often fail to detect OBI and HBV reactivation. In contrast, ultrasensitive HBsAg assays, with an LoD as low as 0.005 IU/mL, can improve the identification of low concentration levels of HBsAg, facilitating earlier diagnosis, monitoring of therapeutic response, and assessing for functional cure. Research confirms the superiority of ultrasensitive assays in detecting HBV in cases missed by conventional assays, detection of NAT-yield samples, and enabling earlier detection of HBV reactivation. This review examines the challenges in HBV diagnostics and the clinical utility of ultrasensitive HBsAg assays in improving progress toward global HBV elimination.

Keywords: hepatitis B virus; chronic hepatitis infection; HBV diagnostics; diagnostic sensitivity; lower limit of detection; functional cure; early detection; therapeutic monitoring; HBV elimination

1. Introduction

Hepatitis B virus (HBV) remains a critical global health challenge, with over 254 million individuals living with chronic infection in 2022 and 1.2 million new infections reported annually [1]. The virus contributes to substantial morbidity and mortality from liver-related complications, such as cirrhosis and hepatocellular carcinoma (HCC), causing 1.1 million deaths in 2022 [1]. Alarmingly, only 13% of individuals with hepatitis B are aware of their infection, and just 3% of those with chronic hepatitis B (CHB) receive treatment [1]. Recognizing this urgency, the World Health Organization (WHO) set targets to reduce new infections by 90% and deaths by 65% between 2016 and 2030 [2]. However, progress has been hindered by lack of accurate and sensitive diagnostic assays, limited uptake of testing, and the COVID-19 pandemic, which put a strain on national healthcare systems [3,4]. If the current trajectory continues, annual global deaths from HBV could increase by 39% by 2030 [5], underscoring the need for a renewed focus on early detection and management.

Diagnostics are integral to HBV elimination efforts: early and accurate detection helps to decrease spread and initiate suppressive treatment [6]. The HBV surface antigen (HBsAg) has long been a reliable serological marker for predicting clinical and therapeutic outcomes, with increased assay sensitivity enabling earlier detection and reducing the diagnostic window [7]. Yet, there remain challenges in detecting mutants, occult infections, reactivations, and breakthrough infections. Current commercial serological assays with a sensitivity of 0.05 IU/mL may still miss infections in the

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early window period or cases of apparent HBsAg loss [7]. High-sensitivity HBsAg detection assays are urgently needed to address these gaps and improve HBV screening [7].

Emerging ultrasensitive HBsAg assays can detect low antigen levels, identifying occult HBV infections (OBIs) and residual viremia in patients previously considered cured [3,7,8]. Their significance lies in identifying true infection status, which can facilitate assessment of HBV reactivation (HBV-R) risk and allow physicians to confidently determine achievement of functional cure [7,8]. Hence, in addition to screening, such advancements can guide decisions regarding therapy cessation and help address critical gaps in patient management. Despite their clinical importance, comprehensive reviews on these assays are scarce.

Therefore, this review examines the current challenges in HBV diagnosis and the need for enhanced assay sensitivity. It focuses on ultrasensitive HBsAg assays and their role in addressing gaps in detection, monitoring, and therapeutic decision-making.

2. Challenges in HBV Diagnosis

2.1. Global HBV Burden

The global burden of HBV exhibits significant geographic variation, with transmission routes influenced by regional prevalence [6,9]. High-endemic regions, such as parts of Asia, Sub-Saharan Africa, and the South Pacific, experience mostly perinatal or early childhood transmission. Intermediate-endemic areas, including the Mediterranean, Alaska, and India, see similar early-life transmission, with additional spread through sexual contact, intravenous drug use, and unsafe injections in adulthood. In low-endemic regions, primarily in the developed world, transmission occurs mainly through sexual contact and intravenous drug use and among homeless and incarcerated individuals [6,9]. HBV is further categorized into distinct genotypes (A–H), each with specific geographic distributions. Multiethnic populations may harbor multiple genotypes [9].

Approximately 90% of infants born to HBsAg-positive or HBV e antigen (HBeAg)-positive mothers are at risk of developing CHB, presenting a significant global public health concern [6]. Moreover, HBV accounts for 50–80% of HCC cases, making it a major contributor to global morbidity and mortality. HCC is the sixth most common cancer and fourth-leading cause of cancer-related deaths worldwide [6]. To address this, the WHO set ambitious targets to eliminate HBV as a public health threat by 2030 [2]. However, the COVID-19 pandemic severely disrupted the cascade of viral hepatitis care, reducing consultation and referral of new patients by 31%. Additionally, HBsAg carriers received less stringent follow-up, with HBsAg and HBV DNA testing decreasing by 31% and 39%, respectively [4]. The pandemic's economic effects also put additional pressure on public health initiatives [4]. A 1-year delay in viral hepatitis elimination programs is anticipated to increase HCC cases by 44,800 and liver-related deaths by 79,400 between 2020 and 2030 (relative to no delay) [10]. Other challenges include complexities in patient selection, such as identifying individuals who require antiviral therapy, and the need for optimal drug regimens.

Disparities in access to vaccination, diagnostics, and treatments and limited awareness, particularly in resource-limited settings, further exacerbate the situation [11]. Expanding access to high-sensitivity HBV diagnostics, particularly in resource-limited settings, is essential to improve early detection.

2.2. Limitations of Current Diagnostic Tools

Conventional biomarkers include HBV DNA, HBsAg, HBeAg, and antibody to the HBV core antigen (anti-HBc) [12]. Of them, HBsAg and HBV DNA are the most common markers used for diagnosis and monitoring, as well as measuring viral load [3]. They are critical for therapeutic decision-making, including the initiation and monitoring of antiviral therapy [12]. However, conventional diagnostic tools are not without limitations.

Traditional assays often lack the sensitivity to identify certain phases of HBV infection, limiting their efficacy in guiding timely therapeutic interventions [13]. Early and accurate detection is

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particularly challenging in high-risk populations, such as immunocompromised individuals or those with OBI or HBV-R [13–16]. OBI refers to the persistence of viral genomes in hepatic nuclei, with an absence of detectable HBsAg and presence of very low levels of serum HBV DNA [3,17]. In such cases, common serological tests may be unreliable owing to low or intermittent HBV replication, and liver histology may be impractical due to its invasive nature [18]. Further, the negative HBsAg findings may be attributable to low circulating concentrations of HBsAg and/or low sensitivity of assays [3,17].

HBV-R is characterized by the reappearance of HBV DNA and/or HBsAg seroreversion in individuals with resolved HBV or rise in viral load in patients with CHB [19]. HBV-R can occur spontaneously or be induced by immunosuppressive medication, chemotherapy, or biological agents that target immunological pathways. The current reported rate of HBV-R varies across studies, ranging from 10% to 16.9% or more [20,21]. A retrospective evaluation of patients with hematopoietic cell transplantation revealed a cumulative incidence of 9%, 21.7%, and 42.9% at 1, 2, and 4 years, respectively [22]. HBV-R can result in asymptomatic viral replication or even acute liver failure and death [23].

At present, early detection is the most effective measure to mitigate complications of HBV-R [20]. As intrahepatic HBV DNA can produce small amounts of HBsAg, monitoring HBsAg could help detect CHB early or reclassify a "cured" status to a "low-HBsAg-level chronic carrier" status [3,8]. Therefore, highly-sensitive and highly-specific diagnostic tools with a lower limit of detection (LoD) are essential not only to identify active infections but also to monitor treatment responses and determine therapy endpoints [3,7].

3. Paradigm Shift in HBV Treatment Goals

3.1. Treatment Endpoints

Conventional treatment endpoints are based on assessment of viral suppression, biochemical response, serologic response, and histologic improvement [24]. The management of CHB has traditionally focused on eliminating or suppressing HBV replication to prevent disease progression to cirrhosis and HCC [25]. While HBV DNA levels have previously served as the gold standard for treatment monitoring, undetectable levels do not reliably predict sustained remission. The optimal level of HBV DNA suppression that should be attained to achieve these benefits is not well defined, and more than 50% of patients relapse upon discontinuation of nucleos(t)ide analog (NA) therapy [24,26]. Biochemical response, indicated by aminotransferase normalization, is another classic endpoint in HBV treatment. Although associated with a reduced risk of complications, its utility as a therapy endpoint is limited by its non-specificity for CHB, inability to reflect disease severity, and variability in the upper limit of normal between different laboratory methods and trials [24]. In HBeAg-positive patients, spontaneous reduction or loss of HBeAg is a positive indication of immune suppression of the virus but liver disease progression can still occur [24]. Histologic improvements, including fibrosis regression, can be achieved with prolonged antiviral therapy. However, biopsies have been replaced with noninvasive tests in clinical practice (serum fibrosis biomarkers, transient elastography), which primarily evaluate fibrosis severity rather than necro-inflammatory activity and require further research [24].

Given these limitations, a more precise and clinically relevant treatment endpoint is needed to guide therapeutic strategies and improve long-term outcomes.

3.2. Functional Cure and Emerging Focus on HBsAg

A 2016 workshop by the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL) on HBV treatment endpoints classified potential HBV cure as sterilizing, functional, and partial [27,28]. Sterilizing cure, or complete cure, refers to undetectable serum HBsAg and elimination of HBV DNA, including intrahepatic covalently closed circular DNA (cccDNA) and integrated genomic HBV DNA [27,28]. Functional cure is defined

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as sustained HBsAg loss and undetectable HBV DNA in serum (6 months post-treatment), with or without seroconversion to hepatitis B surface antibody (anti-HBs), resolution of the residual liver injury, and persistence of low levels of intrahepatic cccDNA and HBV DNA integration [27–29]. Partial cure, a less stringent endpoint, refers to detectable HBsAg but persistently undetectable serum HBV DNA after completing a finite course of treatment [27,28]. Although the ultimate therapeutic goal is a sterilizing cure, complete eradication of HBV cccDNA and integrated HBV DNA from infected cells is currently impossible [30]. Sterilizing cure has not yet been observed naturally in individuals with CHB or those who have recovered from acute infection. Further, no available treatment can reliably eliminate cccDNA [27]. While research into cccDNA-silencing compounds is ongoing, targeting a functional cure remains the most realistic and clinically relevant option [27,30].

The current most reliable indicator of functional cure is sustained HBsAg loss, confirmed at least twice over 6 months, with undetectable HBV DNA [24]. It captures both virological (undetectable serum HBV DNA) and clinical aspects (improved clinical outcomes) of functional cure [29]. Assessing HBsAg loss allows clinicians to safely discontinue antiviral therapy [26]. However, it is rarely achieved with current treatments. In patients receiving NA therapy, functional cure is observed in fewer than 10% of cases after a decade of continuous treatment. Similarly, pegylated interferon (peg-IFN) therapy has demonstrated HBsAg loss rates of 2–3% at the end of treatment (EoT), increasing to 8–14% over 3–5 years of post-treatment follow-up [31–33]. The risk of HCC persists even after HBsAg loss in patients older than 50 years or those with cirrhosis or coinfection with hepatitis C or D virus [34].

Despite these limitations, functional cure remains a feasible endpoint, particularly when achieved at a younger age and in the absence of significant fibrosis, and lowers risk of HCC [26]. Functional cure is being increasingly recognized as a key endpoint in determining long-term prognosis, guiding therapy cessation, and monitoring disease progression. Importantly, HBsAg seroclearance can remove the stigma linked to HBV infection, easing social and professional restrictions and representing a major milestone from a patient's perspective [35].

The 2019 EASL-AASLD HBV Treatment Endpoints Conference suggested using functional cure as a primary endpoint of phase 3 trials, with HBsAg loss in ≥30% patients after 1 year of therapy voted as the desired response rate by over two-thirds of the participants [29]. However, the lower LoD of several available immunoassays is 0.05 IU/mL. With the paradigm shift in HBV treatment goals, there is a growing emphasis on lowering the HBsAg threshold to detect even minute quantities of the viral antigen, enabling better understanding of the HBsAg status prior to treatment cessation [36].

4. HBsAg: A Key Biomarker in HBV Diagnosis

HBsAg, a component of the viral envelope, is widely regarded as the "hallmark of HBV infection." It is the first serological marker to appear in the blood after infection, and its levels reflect the transcriptional activity of cccDNA in the liver [13,37]. HBsAg serves as a simple, reliable, and direct viral marker for identifying both acute and chronic infections [38,39].

4.1. Current Landscape of HBsAg Assessment

HBsAg assessment helps differentiate between disease phases and monitor treatment response, including sustained virological response [12]. For instance, patients with acute infection typically exhibit HBsAg levels >4 log10 IU/mL, while lower levels (<4 log10 IU/mL) are linked to CHB [12]. Similarly, persistent HBsAg detection beyond 6 months indicates CHB, whereas its absence suggests recovery from acute infection [39]. Evaluation of HBsAg kinetics also holds prognostic value and can help predict likelihood of HBsAg loss and risk of liver fibrosis and HCC [12,37]. Low baseline HBsAg (<50 IU/mL) and a significant decline during therapy (<70 IU/mL by week 8 of treatment) predict a good treatment response with HBsAg clearance [12]. Clearance of HBsAg, whether spontaneous or treatment-induced, represents a "functional cure" of HBV infection; however, the risk of HCC persists [12].

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Coexistence of serum HBsAg and anti-HBs, observed in 5–10% of chronic HBV carriers, presents an additional challenge [8]. Immune complexes of HBsAg and anti-HBs can mask HBsAg, affecting its detectability [40]. Therefore, ultrasensitive HBsAg assays capable of dissociating HBsAg from immune complexes that bind HBsAg to anti-HBs are needed to enhance detection [14].

4.2. Ultrasensitive HBsAg Assays

A recent advance in HBV diagnosis, ultrasensitive HBsAg assays, have a lower LoD (0.005 IU/mL), allowing early detection of acute infection, HBsAg clearance, HBV-R or subclinical levels of HBsAg following seroclearance, and OBI [13,39].

5. Clinical Role of Ultrasensitive HBsAg Assays

5.1. Improved Specificity and Sensitivity

False positive results are a common concern with HBsAg assays. A recent study from Japan reported high false-positive rates of 33.1% in the HBsAg range of 0.005–0.049 IU/mL and 1.2% when HBsAg >0.05 IU/mL [41]. Consequently, HBsAg weak-reactives/false positives require confirmatory testing, including neutralization tests, which incur extra time and resources [39,41]. Ultrasensitive HBsAg assays that have high specificity offer a potential solution by enhancing diagnostic accuracy and reducing the need for additional testing.

Prakash et al. investigated the ability of an ultrasensitive HBsAg assay to overcome the challenge of weak-reactives [39]. Of 248 samples testing positive with a standard assay, 180 (72.58%) were repeat reactive. Notably, 159 (64.11%) samples tested negative with the ultrasensitive assay ($p<0.0001$). The standard and ultrasensitive assays had a concordance rate of 57.67%. With a much lower LoD compared to the standard assay (0.0046 IU/mL versus 0.021 IU/mL), the authors concluded that the ultrasensitive assay was better positioned to resolve weak reactive samples [39]. Further, between 1% and 48% of samples testing negative with standard HBsAg assays (lower LoD, 0.05 IU/mL) yield a positive result with ultrasensitive HBsAg assays (lower LoD, 0.005 IU/mL) [14]. Lou et al. compared the sensitivity of an ultrasensitive assay with that of other commercially available assays and found 3.2- to 7.1-fold greater sensitivity. Of 27 seroconversion panels included in the study, the investigational assay detected more panel members (191 of 364) than the comparator assays (144–160) [42].

Sickinger et al. compared the specificity and sensitivity of an ultrasensitive immunoassay with those of an on-market HBsAg qualitative/confirmatory assay [38]. They studied 450 HBsAg PCR-positive specimens from patients with acute and chronic infections. Both investigational and comparator assays showed a diagnostic specificity of 100.00%. However, their analytical sensitivity ranged between 4.5–6.14 mIU/mL and 17–22 mIU/mL, respectively, and clinical sensitivity was 100.00% and 99.78%, respectively. The four-fold increased sensitivity enhances clinical diagnosis and screening, mutant detection, and seroconversion sensitivity [38]. Consistent with these findings, another study showed that the HBsAg detection rate increased by 6–7% when compared with conventional assays [43].

Notably, greater sensitivity of an HBsAg assay is often achieved at the cost of specificity [38]. However, Sickinger et al. [38] and Lou et al. [42] observed that the enhanced sensitivity of the investigated ultrasensitive HBsAg assay did not adversely impact its specificity. This combination of enhanced sensitivity and specificity offered by ultrasensitive HBsAg assays could aid clinicians in making early and accurate decisions, reducing the likelihood of unnecessary treatments.

5.2. Detecting Early Infection, OBI, and HBV-R

At present, clinical gaps persist in the detection of early and late acute HBV infection, as well as OBI [13]. With an exceptionally low LoD, ultrasensitive HBsAg assays hold vast potential for diagnosing asymptomatic HBV-infected patients in the low replication phase [3]. Coupled with

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sensitive HBV DNA assays, they could identify carriers of HBV, those at risk of liver-related complications and HBV-R, as well as minimize risk of HBV transmission [43].

The period before HBsAg becomes detectable is the early window period, which can be shortened through enhanced HBsAg assay sensitivity [13]. Ultrasensitive HBsAg assays have been found to reduce the early window of detection by an average of 6.3 days compared to standard HBsAg assays [42]. Notably, Wong et al. observed that an ultrasensitive assay can detect HBsAg an average of 14 days earlier than standard assays [43].

Gupta et al. evaluated the performance characteristics of an ultrasensitive assay in 439 clinical samples from patients and healthy donors [3]. Notably, the assay demonstrated a lower LoD of 0.0033 IU/mL, with incremental detection of HBsAg in 11 additional samples (including 5 samples with OBI) compared to a standard HBsAg assay [3]. In another study by Steve et al., an ultrasensitive assay demonstrated an LoD of 0.0043 IU/mL. Of 116 patients with HBsAg loss after treatment with entecavir, adefovir, lamivudine, and/or interferons, 18 (15.5%) tested positive with the ultrasensitive assay. Similarly, of 54 patients with spontaneous clearance, 15 (27.7%) tested positive with the ultrasensitive assay [7]. This assay demonstrated superior signal intensity compared to a standard assay across different phases of HBV infection, including samples with prozone effect. Further, it allowed for cost-effective in-house neutralization to confirm low HBsAg levels [7].

Kuhns et al. assessed incremental detection by an ultrasensitive assay in 347 samples of acute infection and OBI of patients from the USA, South Africa, Spain, Cameroon, and Vietnam [44]. The assay showed improved detection of both early acute infection (nucleic acid testing [NAT] yield) and OBI samples, with incremental detection of 33.6% and 22.3%, respectively. Moreover, the assay improved the detection of HBsAg in the presence of anti-HBs. Eighteen of 86 (20.9%) NAT yield and OBI samples incrementally detected by the assay were anti-HBs-positive. Anti-HBs ≥10 mIU/mL was present in 28.6% of samples testing positive with the assay and in 45.2% OBI samples testing negative with the assay. The authors speculated that anti-HBs >300 mIU/mL coupled with an extremely low viral load may result in limited HBsAg detection [44]. However, other research indicates that ultrasensitive assays can identify low HBsAg levels even when complexed with anti-HBs and in the presence of 2500 mIU/mL anti-HBs in CHB and OBI cases [40,45].

Generally, in the early phase of acute HBV infection, HBV DNA is the only detectable serum marker before HBsAg appears, whereas in the late phase, as HBsAg becomes undetectable and anti-HBc appears, HBV DNA levels decline before resolving completely with the emergence of protective anti-HBs. Improvements in HBsAg assay sensitivity have shortened these diagnostic windows [13]. Kuhns et al. investigated the ability of an ultrasensitive assay to detect early and late acute infection and OBI [13]. Compared to a standard assay, the investigational assay increased detection of NAT yield samples (28/77, 36.4%), late acute infection (up to ≥13 days longer detection), and OBI (11/101, 10.9%). Genotypes A1, A2, B2, B4, C1, C2, C5, D3, E, and H were all adequately detected [13]. Similarly, Bourdin et al. observed the ability of an ultrasensitive assay in detecting weak positive samples, with improved sensitivity and specificity across varied genotypes [40]. They included 253 samples split into four panels: (1) routine prospectively screened serum samples (n=196), (2) retrospective serum samples before HBV-R (n=18), (3) OBI (n=10), and (4) a selection of wild-type HBV genotype samples (n=29) [40]. Although Panel 1 showed robust agreement between the ultrasensitive and standard assays, seven false positive samples with the standard assay tested negative with the ultrasensitive assay. This result was further corroborated with confirmatory testing. The ultrasensitive assay also identified one case of OBI. Interestingly, in Panel 2, four of 18 samples (22%) tested positive for HBsAg with the ultrasensitive assay. These findings indicate potential time savings of 1 to 6 months in diagnosing HBV-R, which could have significant clinical implications. In Panel 3, the 10 samples were previously shown to be HBsAg-negative and HBV DNA-positive. All samples were anti-HBc-positive and anti-HBs negative. The findings highlighted the ability of the ultrasensitive assay in detecting OBI. In Panel 4, the ultrasensitive assay detected all different genotypes with a greater tendency for detection compared to a standard assay; however, this difference was not significant. The authors concluded that the ultrasensitive assay was effective in

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resolving a high proportion of weak-reactive samples in HBV-R and OBI [40]. Consistent with these findings, Wong et al. observed that seroconversion sensitivity increased by 22–25% on using an ultrasensitive assay [43].

These findings highlight the significant clinical utility of ultrasensitive HBsAg assays in enabling early detection, identifying OBI, and improving monitoring of HBV-R, making them a potentially valuable tool in clinical practice.

5.3. Detecting Vaccine Breakthrough Infections

Although HBV vaccines are highly effective, breakthrough infections can still occur. In these cases, index samples often test negative for anti-HBc but positive for HBV DNA, even when protective levels of anti-HBs are present. HBsAg detection may be delayed or absent, and some breakthrough infections have been linked to HBsAg mutations [46].

Kuhns et al. found that an ultrasensitive HBsAg assay was more sensitive in detecting breakthrough infections than standard HBsAg assays or preS2 antigen and HBcoreAg testing [46]. Their study included serial samples from two commercially available plasma donor panels. The ultrasensitive assay detected panel 6272 at day 51, 43-46 days earlier than the comparator HBsAg assays [46].

5.4. Assessing Treatment Response and Functional Cure

The importance of attaining functional cure in chronic hepatitis B has continued to increase in recent years, with research showing the prognostic utility of HBsAg clearance. Several studies indicate that the HBsAg level at the time of stopping NA treatment is a strong predictor of whether HBV infection will remain inactive or relapse [47–49]. For instance, the global RETRACT-B study analyzed off-therapy outcomes after NA cessation to identify factors that could assist selection of patients for NA withdrawal [47]. At 4 years post-EoT, the cumulative probability of HBsAg loss at EoT was higher among patients >50 years than those <50 years; among Whites than Asians; and among those with HBsAg levels <100 IU/mL than between 100–1000 or >1000 IU/mL at EoT. Interestingly, 83.4% of the cohort had virologic relapse, 54.6% had clinical relapse, and 54.7% had started retreatment [47]. Additionally, a post-hoc analysis of UMIN000001299 evaluated the efficacy of an ultrasensitive assay (lower LoD, 0.005 IU/mL) for samples of lymphoma patients whose HBV infection resolved with anti-CD20 antibody, rituximab-containing chemotherapy [50]. A positive result with the assay at baseline was an independent risk factor for HBV-R. The sensitivity of the standard (lower LoD, 0.05 IU/mL) and ultrasensitive assays at HBV-R were 18.2% and 77.3%, respectively, indicating the superior performance of the ultrasensitive assay [50].

Thus, ultrasensitive assays have an important role in predicting the possibility of functional cure during follow-up. This assertion is further strengthened by the findings of the phase 2 REP 401 study (NCT02565719), where serum HBsAg levels declined to <0.05 IU/mL during therapy (in 24 of 40 participants) and were maintained at <0.005 IU/mL until EoT [51]. Further, HBsAg levels declined below 0.005 IU/mL during follow-up in 16 of 40 participants [51]. These findings underline the effect of HBsAg clearance during and after nucleic acid polymer-based combination therapy and the role of HBsAg assays to quantify the efficacy of combination therapies targeting complete suppression of HBsAg and thereby, functional cure [51].

Further, these findings indicate that ultrasensitive assays are a huge leap forward for novel drug trials. They can help generate reliable data to determine the optimum time for stopping treatment and beginning retreatment, increasing confidence among clinicians and patients.

6. Broader Implications of Ultrasensitive HBsAg Assays

6.1. Significance for the Immunosuppressed Population

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Immunosuppressed individuals, including those undergoing treatment for malignancies, autoimmune disorders, chronic rheumatic diseases, or post-transplantation care, face a heightened risk of HBV-R, particularly if they have undetected chronic, resolved, or occult infection [52]. Mortality from HBV-R in patients receiving chemotherapy for hematological disorders has been found to range between 5–40%, whereas the incidence of HBV-R in HBsAg-positive individuals with systemic inflammatory diseases can reach up to 67.5% [53]. Further, recent research revealed that in cancer patients with persistent HBV infection treated with PD-1/PD-L1 inhibitors, the overall prevalence of HBV-R was 5% [54].

Despite the availability of vaccines, screening tests, and antiviral therapies, breakthrough infections and HBV-R remain serious complications, often leading to severe hepatitis, liver failure, and even death [52,55]. These data highlight the increasing susceptibility of immunosuppressed persons, with the worldwide increase in HBV-R probably connected to the rising use of new immunosuppressive medications and inconsistent screening and management approaches [52]. In this setting, using highly sensitive diagnostic assays, such as ultrasensitive HBsAg assays, becomes crucial. Notably, an ultrasensitive HBsAg assay detected HBsAg 4 weeks before an HBV-R diagnosis in an individual receiving rituximab therapy [43]. Therefore, integrating an ultrasensitive HBsAg assay into routine screening programs may enhance prediction of HBV-R in immunocompromised individuals, including those requiring transplants or immunosuppressants, and decrease related complications and mortality.

6.2. Economic Impact

The use of ultrasensitive HBsAg assays across diverse clinical subgroups — stratified by age, race/ethnicity, baseline HBsAg level, genotype, and HBV S variants — has reduced misdiagnosis and the need for repeat testing [55,56]. These assays are positioned to effectively resolve weak reactive samples while demonstrating a good correlation with confirmatory/reflex tests and clinical disease [37]. This superior internal benchmarking can reduce the quantum of additional confirmatory testing, thereby also reducing the incurred costs [39]. By enhancing diagnostic efficiency and reducing unnecessary testing, ultrasensitive assays can also save valuable time, offering a cost-effective solution for laboratories and hospitals [40]. Such improvements could further increase the overall outcomes in addition to those already demonstrated with universal hepatitis B screening, which is estimated to save $262,857 (2020 USD) and help gain 135 quality-adjusted life years per 100,000 adults [57].

6.3. Role in Public Health

With increasing clinical trial data being generated on the enhanced performance of ultrasensitive HBsAg assays, their direct role in refining patient-centric treatment outcomes for HBV management is evident. Moreover, these assays can influence public health at various levels, from improving antenatal screening in pregnant mothers to HCC surveillance monitoring during and after cessation of treatment [58]. They can also be used to screen other high-risk populations, including immunocompromised and incarcerated individuals [59]. Moreover, the risk of transfusion-transmitted HBV infections is high if these patients serve as blood donors [3]. High-sensitivity HBsAg assays address this concern and enable blood screening safety [3]. Recent guidance from the U.S. Food & Drug Administration (FDA) states that HBsAg testing of donated blood is not necessary when it is tested for HBV DNA by NAT and for anti-HBc using screening tests approved by the FDA. However, HBsAg testing of plasma donations should be continued as they are not tested for anti-HBc [60,61]. In many developing countries, NAT for HBV in donated blood is not mandatory, highlighting the need for ultrasensitive HBsAg assays to detect reduce risk of HBV transmission [40].

Holzmayer et al. showed that whole blood dried blood spot (DBS) is a feasible sample input for a specific HBsAg ultrasensitive assay, with no need for assay modification [62]. Therefore, detection of DBS samples with high-throughput serologic assay platforms may support global population surveillance programs [62].

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7. Future Scope in HBsAg Testing

The widespread use of HBsAg as a biomarker for screening and assessing active disease is hindered by the limited availability of rapid diagnostic tests, particularly in resource-limited settings [63]. Rapid tests generally have lower sensitivity compared to high-sensitivity HBsAg assays, making them less suitable for monitoring treatment response [63]. DBS-based screening could enable rapid, accurate detection in large surveys where conventional draws are impractical or in resource-limited regions [64].

Further technological advances in HBsAg assays should focus on universal screening and enhancing detection in asymptomatic cases of HBV. Strengthening public health initiatives could promote wider adoption of ultrasensitive assays and enhance case identification and linkage to care, accelerating progress toward HBV elimination, especially in endemic and resource-limited regions.

Although quantitative HBsAg testing allows assessment of cccDNA and/or integrated DNA transcriptional activity, there remain challenges in distinguishing between the two sources of surface antigen [65,66]. Further research on distinguishing HBsAg production originating from cccDNA versus integrated DNA could offer insight into the durability of a functional cure and help guide treatment adjustments for patients with CHB [65,66].

8. Conclusions

Ultrasensitive HBsAg assays demonstrate a substantial development in conquering screening, diagnostic, and monitoring constraints as well as enabling safe blood donation. Research indicates the enhanced performance of ultrasensitive assays in early HBV detection, including patients testing negative with conventional HBsAg assays. By identifying minimal antigen levels, these assays enhance diagnostic accuracy, particularly for patients with occult infection. As a reliable diagnostic tool, ultrasensitive assays enable clinicians to plan individualized treatment timelines by providing i) granular data on antigen decline over time, ii) reliable prognosis for estimating the probability of achieving sustained remission, and iii) evidence for extending or discontinuing therapy, especially in patients with borderline low HBsAg levels. These assays also play an important role in treatment monitoring, supporting clinical decisions regarding long-term therapy discontinuation.

In conclusion, with their increased sensitivity and predictive value, these assays greatly assist in improving HBV management, directing treatment cessation plans, and decreasing the risk of recurrence.

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Abbreviations

The following abbreviations are used in this manuscript:

AASLD American Association for the Study of Liver Diseases

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ALT	Alanine aminotransferase
Anti-HBc	HBV core antigen
Anti-HBs	Hepatitis B surface antibody
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
CI	Confidence interval
DNA	Deoxyribose nucleic acid
EASL	European Association for the Study of the Liver
EOT	End of treatment
FDA	U.S. Food & Drug Administration
HBeAg	HBV e antigen
HBV	Hepatitis B virus
HBsAg	HBV surface antigen
HBV-R	HBV reactivation
HCC	Hepatocellular carcinoma
LoD	Limit of detection
LoQ	Limit of quantification
NA	Nucleos(t)ide analog
NAT	Nucleic acid testing
NAP	Nucleic acid polymer
OBI	Occult HBV infection
PCR	Polymerase chain reaction
PD-1	Programmed death protein 1
Peg-IFN	Pegylated interferon
Peg-IFNα	Pegylated interferon alfa-2a
Q1W	Every week
Q4W	Every 4 weeks
Q8W	Every 8 weeks
TDF	Tenofovir disoproxil fumarate
ULN	Upper limit of normal
WHO	World Health Organization

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