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Emerging Biomarkers and Diagnostic Innovations in Hepatitis B Virus Infection

Hepatit B Virüsü Enfeksiyonunda Yeni Biyobelirteçler ve Tanısal Yenilikler

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ABSTRACT

Hepatitis B infection remains a global public health challenge despite the availability of vaccines and antiviral therapies. Conventional diagnostic approaches rely on serological and virological markers, including hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), hepatitis B e antigen, and hepatitis B virus (HBV)-DNA quantification. Although these biomarkers remain fundamental to screening, diagnosis, and disease monitoring, they are limited in their ability to reflect intrahepatic viral transcriptional activity, covalently closed circular DNA (cccDNA) persistence, and treatment outcomes. Advances in molecular diagnostics have introduced novel biomarkers that provide insights into viral replication, cccDNA activity, and host-virus interactions. These include serum HBV-RNA, hepatitis B core-related antigen, quantitative HBsAg, quantitative anti-HBc, and ultrasensitive HBsAg assays. Investigational biomarkers such as HBV nucleic acid-related antigen, together with advanced molecular approaches, including rapid amplification of cDNA ends, have expanded the understanding of HBV transcriptional dynamics and viral persistence. In parallel, innovative diagnostic strategies, including reflex HBV-DNA testing and point-of-care molecular assays, are improving diagnostic accessibility, efficiency, and patient management. Moreover, non-invasive tools for assessing liver fibrosis, such as transient elastography, including vibration-controlled transient elastography, have transformed the evaluation of HBV-related liver disease. Contemporary hepatitis B diagnostics are undergoing substantial methodological evolution, integrating advanced molecular techniques, quantitative viral and host biomarkers, and highly sensitive analytical platforms into routine clinical assessment. Continuous updates to diagnostic methodologies are enhancing the accuracy of disease staging, therapeutic monitoring, and

ÖZ

Hepatit B enfeksiyonu, etkili aşıların ve antiviral tedavilerin bulunmasına rağmen küresel bir halk sağlığı sorunu olmaya devam etmektedir. Geleneksel tanısal yaklaşımlar; hepatit B yüzey antijeni (HBsAg), hepatit B çekirdek antijenine karşı antikorlar (anti-HBc), hepatit B e antijeni ve hepatit B virüs (HBV)-DNA kantifikasyonu gibi serolojik ve virolojik belirteçlere dayanmaktadır. Bu biyobelirteçler tarama, tanı ve hastalık izlemi açısından temel önemini korusa da, intrahepatik viral transkripsiyonel aktiviteyi, kovalent kapalı dairesel DNA (cccDNA) persistansını ve tedavi sonuçlarını yansıtmada kısıtlılıklara sahiptir. Moleküler tanıdaki gelişmeler, viral replikasyon, cccDNA aktivitesi ve konak-virüs etkileşimleri hakkında bilgi sağlayan yeni biyobelirteçlerin ortaya çıkmasını sağlamıştır. Bunlar arasında serum HBV-RNA, hepatit B core-related antigen, kantitatif HBsAg, kantitatif anti-HBc ve ultra-duyarlı HBsAg testleri bulunmaktadır. HBV nucleic acid-related antigen gibi araştırma aşamasındaki biyobelirteçler ile cDNA uçlarının hızlı amplifikasyonu gibi ileri moleküler yaklaşımlar, HBV transkripsiyonel dinamikleri ve viral persistansın anlaşılmasını genişletmiştir. Bununla birlikte, refleks HBV-DNA testi ve hasta başı moleküler testler gibi yenilikçi tanısal stratejiler; tanısal erişilebilirliği, klinik verimliliği ve hasta yönetimini geliştirmektedir. Ayrıca transient elastografi ve *vibration-controlled transient elastography* gibi karaciğer fibrozis değerlendirmesinde kullanılan non-invaziv yöntemler, HBV ile ilişkili karaciğer hastalığının değerlendirilmesini dönüştürmüştür. Sonuç olarak, günümüz hepatit B tanısı, gelişmiş moleküler teknikleri, kantitatif viral ve konak biyobelirteçlerini ve son derece hassas analitik platformları rutin klinik değerlendirmeye entegre ederek önemli bir metodolojik dönüşüm geçirmektedir. Tanı yöntemlerindeki sürekli güncellemeler, hastalığın evrelemesi, tedavi izlemi ve risk sınıflandırmasının doğruluğunu

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risk stratification, while paving the way for more precise, adaptive, and personalized approaches to chronic hepatitis B management.
Keywords: Hepatitis B virus, emerging biomarkers, precision medicine, HBcrAg, HBV-RNA

artırırken, kronik hepatit B tedavisine yönelik daha hassas, uyarlanabilir ve kişiselleştirilmiş yaklaşımların önünü açmaktadır.
Anahtar Kelimeler: Hepatit B virüsü, yeni ortaya çıkan biyobelirteçler, hassas tıp, HBcrAg, HBV-RNA

Introduction

Despite the availability of effective vaccines and potent antiviral therapies, chronic hepatitis B virus (HBV) infection remains a major global health burden. According to the 2026 update to the World Health Organization's (WHO's) Global Hepatitis Report, an estimated 240 million people worldwide were living with chronic HBV infection; approximately 0.9 million new infections and nearly 1.1 million hepatitis-related deaths occurred annually, primarily due to cirrhosis and hepatocellular carcinoma (HCC). HBV prevalence remains disproportionately high in sub-Saharan Africa and the Western Pacific region, where vertical and early childhood transmission continue to play a dominant role in disease persistence (1).

The diagnosis and clinical monitoring of HBV infection are primarily based on the combined interpretation of conventional serological and virological markers, including hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), antibody to hepatitis B surface antigen (anti-HBs), hepatitis B e antigen (HBeAg)/antibody to hepatitis B e antigen (anti-HBe), and quantitative HBV-DNA assays. HBsAg positivity remains the cornerstone of HBV diagnosis, whereas persistence of HBsAg for more than six months defines chronic HBV infection. The presence of anti-HBc indicates prior or ongoing exposure to HBV and, when interpreted together with anti-HBs, helps differentiate immune status, resolved infection, and isolated anti-HBc serological patterns. HBeAg and anti-HBe assays provide important information regarding viral replication, infectivity, and disease phase, whereas quantitative HBV-DNA measurement is considered the principal marker for treatment indication, monitoring of virological response, and assessment of antiviral suppression (2). In recent years, there has been an emphasis on expanding access to HBV-DNA testing to improve access to diagnosis across the HBV care pathway, as well as on the importance of reflex HBV-DNA testing strategies and the use of point-of-care molecular tests where appropriate (1).

Although conventional serological and virological assays remain indispensable in HBV management, they have important limitations in accurately reflecting intrahepatic covalently closed circular DNA (cccDNA) quantity and transcriptional activity (3). Serum HBV-DNA reflects circulating viral replication; however, low-level ongoing HBV infection and persistent intrahepatic cccDNA activity may continue despite nucleos(t)ide analogue (NA) therapy (4). Similarly, quantitative HBsAg (qHBsAg) may not accurately reflect intrahepatic cccDNA transcriptional activity, particularly in HBeAg-negative patients, where HBsAg production may substantially derive from integrated HBV-DNA sequences (5). In addition, HBeAg negativity does not necessarily indicate inactive disease, since precore and basal core promoter mutations may lead to active hepatitis despite negative HBeAg results (2). Consequently, currently available biomarkers may be insufficient for accurately predicting treatment withdrawal outcomes, relapse risk, or the probability of achieving functional cure in chronic hepatitis B (6).

The current therapeutic goal in chronic hepatitis B extends beyond simple HBV-DNA suppression and increasingly focuses on achieving functional cure, defined by durable HBsAg loss with sustained viral control. Achieving this objective requires novel biomarkers capable of more accurately characterizing disease phase, reflecting intrahepatic viral activity, and predicting treatment response at earlier stages (2). Serum HBV-RNA has emerged as a promising non-invasive biomarker because of its close association with pregenomic RNA (pgRNA) transcription and cccDNA activity (3). Hepatitis B core-related antigen (HBcrAg), which reflects a composite of hepatitis B core antigen (HBcAg), HBeAg, and related precore/core proteins, has been associated with serum HBV-DNA levels, intrahepatic cccDNA activity, and post-treatment relapse risk (7). The combined use of qHBsAg, quantitative anti-HBc (qAnti-HBc), ultrasensitive HBsAg assays, HBV-RNA, and HBcrAg may facilitate more individualized treatment strategies, optimized monitoring, and safer antiviral discontinuation approaches in chronic hepatitis B management (4). The HBV life cycle, together with the formation and clinical relevance of major conventional and emerging HBV biomarkers, is summarized in Figure 1.

Conventional Diagnostic Markers in HBV Infection

Serological Diagnostic Panel

Serological assays remain the foundation of HBV screening, diagnosis, and infection status classification and primarily rely on the combined interpretation of HBsAg, anti-HBs, total anti-HBc, immunoglobulin M (IgM) anti-HBc, HBeAg, and anti-HBe (2). Current recommendations increasingly support the use of a "triple panel" screening strategy consisting of HBsAg, anti-HBs, and total anti-HBc because this approach enables identification of current infection, previous exposure, vaccine-induced immunity, and susceptible individuals requiring vaccination (Figure 2A) (8).

HBsAg is considered the principal serological marker of current HBV infection because large amounts of surface antigen are secreted into the bloodstream during viral replication, facilitating detection even in early phases of infection (9).

Anti-HBs indicates protective immunity and may develop either after successful hepatitis B vaccination or after recovery from natural HBV infection, following HBsAg clearance. Total anti-HBc develops after exposure to HBV and generally persists lifelong in individuals with previous or ongoing infection, whereas isolated vaccine-induced immunity does not result in anti-HBc positivity (8). Isolated anti-HBc positivity may be observed in different clinical settings, including resolved infection with waning anti-HBs levels, occult HBV

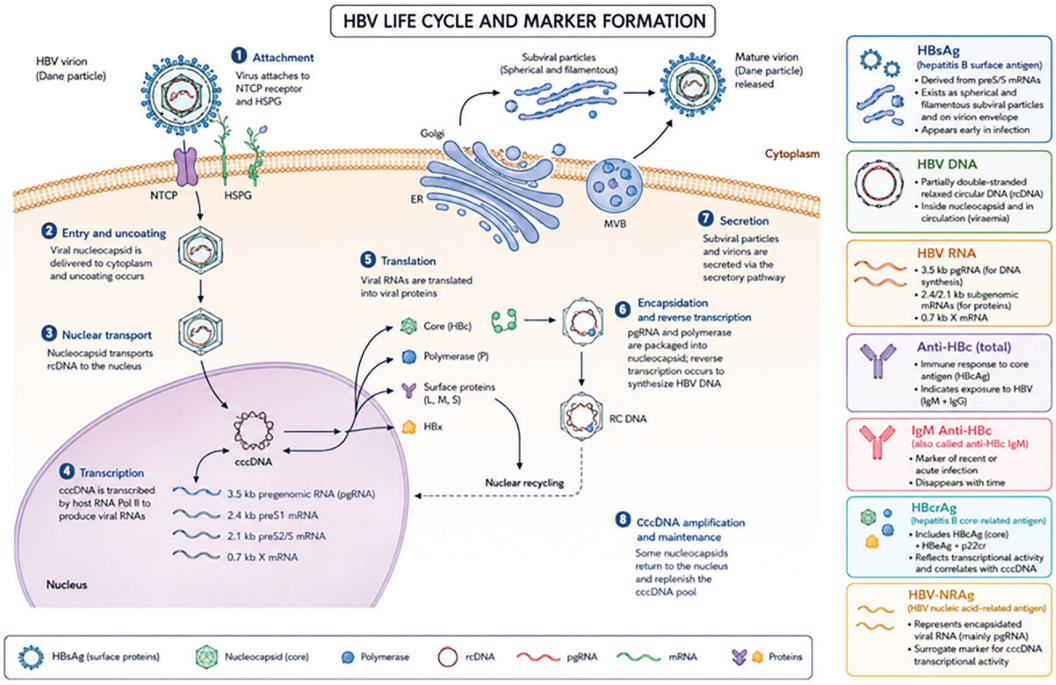

Figure 1. HBV life cycle and formation of circulating viral markers

Anti-HBc: Antibody to HBcAg, cccDNA: Covalently closed circular DNA, DNA: Deoxyribonucleic acid, ER: Endoplasmic reticulum, HBc: Hepatitis B core protein, HBcAg: Hepatitis B core antigen, HBeAg: Hepatitis B e antigen, HBcrAg: Hepatitis B core-related antigen, HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virus, HBV-NRAg: Hepatitis B virus nucleic acid-related antigen, HBx: Hepatitis B virus X protein, HSPG: Heparan sulfate proteoglycan, IgM: Immunoglobulin M, kb: Kilobase, L, M, and S: Large, middle, and small HBV surface proteins, respectively, mRNA: Messenger RNA, MVB: Multivesicular body, NTCP: Sodium taurocholate cotransporting polypeptide, p22cr: 22-kDa precore protein, pgRNA: Pregenomic RNA, preS1 and preS2: Pre-surface 1 and pre-surface 2 regions, rcDNA: Relaxed circular DNA, RNA: Ribonucleic acid

infection, false-positive serology, or the window phase of acute HBV infection (6,9).

Anti-HBc IgM is primarily associated with acute HBV infection and represents an important marker for differentiating acute hepatitis B from chronic infection. It is typically present at high concentrations during acute HBV infection and declines to undetectable levels within approximately 6 months. However, anti-HBc IgM may also become detectable during severe hepatic flares or reactivation episodes in patients with chronic HBV infection, limiting its specificity for newly acquired infection in selected clinical contexts (2,8). Acute hepatitis B and acute exacerbation of chronic hepatitis B may therefore present with similar biochemical and serological patterns, including marked alanine aminotransferase (ALT) elevation and anti-HBc IgM positivity. Consequently, qualitative anti-HBc IgM positivity alone has limited specificity for distinguishing these two clinical conditions and should be interpreted together with HBsAg, total anti-HBc, HBeAg/anti-HBe status, HBV-DNA level, ALT kinetics, coagulation parameters, previous HBV history, and epidemiological context (10).

HBeAg is a secreted viral protein translated from the precore/core genomic region of HBV and is distinct from the nucleocapsid core antigen despite sharing part of the same open reading frame (11). In clinical practice, HBeAg is used as a conventional serological marker that contributes to the assessment of viral replication, infectivity, and disease phase in HBV infection. European Association for the Study of the Liver (EASL) 2025 recommends testing for HBeAg in patients with HBV infection for disease-phase assessment. HBeAg-positive chronic HBV infection is generally characterized by high levels of HBV replication; consequently, HBeAg status is used to classify chronic HBV infection into HBeAg-positive and HBeAg-negative phases. In HBeAg-positive patients, the level of HBV-DNA and the degree of liver inflammation vary, and disease-phase assignment requires integration of HBeAg status with HBV-DNA, ALT, fibrosis assessment, and clinical context (Figure 2B) (2).

Loss of HBeAg and subsequent HBeAg seroconversion are frequently used as clinically relevant endpoints in HBeAg-positive chronic hepatitis B, particularly in studies evaluating antiviral or pegylated interferon (peg-IFN)-based treatment (6). However, HBeAg negativity alone should not be interpreted as inactive disease, because HBeAg-negative chronic hepatitis B can occur in the presence of ongoing viral replication and liver injury (2).

anti-HBe is conventionally interpreted in relation to HBeAg status and is most clinically meaningful when evaluated together with HBV-DNA and ALT levels. EASL 2025 states that anti-HBe may be tested for disease-phase assessment, particularly when HBeAg is negative (2). The transition from HBeAg positivity, referred to as HBeAg seroconversion, is commonly considered an important virological and serological milestone in HBeAg-positive chronic hepatitis B (2,12). HBeAg seroconversion is often accompanied by a decline in HBV replication, but anti-HBe may be present despite persistent HBV-DNA replication and active hepatic inflammation. Thus, anti-HBe should not be interpreted as a simple marker of inactive infection; rather, it should be evaluated as part of a broader serological and



Figure 2. Conventional diagnostic markers in HBV infection. A. Interpretation of conventional serological markers. B. Relationship between HBeAg/Anti-HBe status and viral replication. C. Clinical role of HBV DNA quantification. D. Limitations of conventional biomarkers and transition toward emerging biomarkers

ALT: Alanine aminotransferase, Anti-HBc: Antibody to hepatitis B core antigen, anti-HBe: Antibody to hepatitis B e antigen, anti-HBs: Antibody to hepatitis B surface antigen, cccDNA: Covalently closed circular DNA, DNA: Deoxyribonucleic acid, HBeAg: Hepatitis B e antigen, HBcrAg: Hepatitis B core-related antigen, HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virus, IgM: Immunoglobulin M, IU: International unit, PCR: Polymerase chain reaction, pgRNA: Pregenomic RNA, qAnti-HBc: Quantitative antibody to hepatitis B core antigen, qHBsAg: Quantitative hepatitis B surface antigen, RNA: Ribonucleic acid.

virological profile that includes HBeAg, HBV-DNA, ALT, and fibrosis status (2).

HBV-DNA Quantification

Quantitative HBV-DNA measurement is considered the principal virological marker for evaluating viral replication, determining treatment eligibility, monitoring antiviral response, and assessing virological suppression in chronic HBV infection (2). HBV-DNA assays are based on nucleic acid amplification techniques (NATs), primarily real-time polymerase chain reaction (PCR), and highly sensitive assays with lower limits of detection below 10-20 IU/mL are recommended for clinical use (13). HBV-DNA level is one of the major determinants used to define the different phases of chronic HBV infection, and it is used as a key criterion for treatment indication. Quantitative HBV-DNA testing is therefore recommended during treatment follow-up to evaluate virological response, adherence, and potential antiviral resistance (Figure 2C) (2).

Despite its central role in HBV management, serum HBV-DNA has important limitations: circulating viral load does not always accurately reflect intrahepatic cccDNA quantity or transcriptional activity. Persistent intrahepatic cccDNA activity and integrated HBV-DNA transcription may continue despite long-term suppression of serum

HBV-DNA with antiviral treatment (14). In areas where resources are limited, HBV-DNA testing poses significant challenges. Conventional real-time PCR methods require specialised infrastructure, trained personnel, and substantially prolonged turnaround times (Figure 2D) (2). The course of serological parameters and quantitative HBV-DNA levels during acute and chronic HBV infection is summarized in Figure 3. Thus, HBV-DNA remains the central virological marker for treatment decisions and monitoring, but it does not fully capture intrahepatic viral activity.

Reflex Testing Strategy

A potential strategy to improve access to nucleic acid testing following a positive HBsAg result and to facilitate earlier linkage to care and treatment initiation is implementing reflex HBV-DNA testing. The reflex testing strategy is defined as automatic HBV-DNA testing in all individuals with a positive HBsAg result. WHO describes two main reflex HBV-DNA testing approaches: laboratory-based and clinic-based reflex testing. In laboratory-based reflex testing, the same or a duplicate sample obtained for initial HBsAg testing is automatically used for HBV-DNA testing if the HBsAg result is positive, without requiring an additional patient visit or blood draw. In clinic-based reflex testing, a second blood sample is collected immediately after a positive HBsAg rapid diagnostic test result during the same clinical encounter, and HBV-DNA testing is subsequently performed either in a laboratory or using an on-site point-of-care assay (15).

According to the systematic review and meta-analysis summarized in the WHO 2024 HBV guidelines, reflex HBV-DNA testing strategies were associated with improved HBV-DNA testing uptake, faster linkage to care, and shorter turnaround times compared with non-reflex approaches. The review included eight studies evaluating both laboratory-based and clinic-based reflex testing strategies. Sites

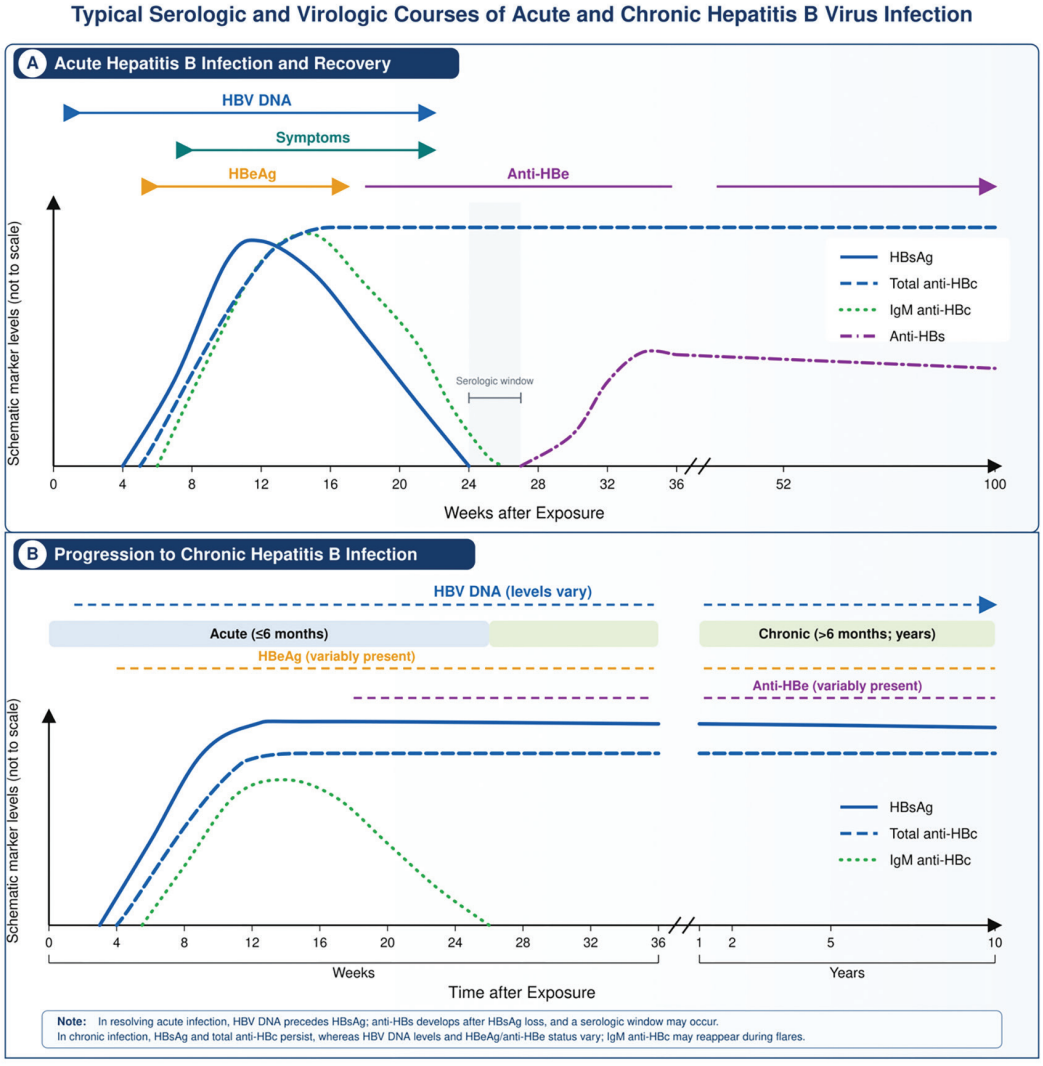

Figure 3. Typical temporal profiles of serologic and virologic markers in acute and chronic HBV infection. A. Acute HBV infection followed by recovery, characterized by clearance of HBsAg and HBV DNA, decline of IgM anti-HBc, and subsequent development of anti-HBs. B. Progression to chronic HBV infection, characterized by persistence of HBsAg, HBV DNA, and total anti-HBc beyond six months. Curves represent schematic relative concentrations and are not quantitative

Anti-HBc: Antibody to hepatitis B core antigen, anti-HBe: Antibody to hepatitis B e antigen, anti-HBs: Antibody to hepatitis B surface antigen, DNA: Deoxyribonucleic acid, HBeAg: Hepatitis B e antigen, HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virus, IgM: Immunoglobulin M

implementing reflex HBV-DNA testing achieved higher rates of timely testing following positive HBsAg screening; laboratory-based reflex testing enabled HBV-DNA testing in all HBsAg-positive individuals in several studies. Reflex testing also substantially shortened the interval between screening and treatment initiation, with some clinic-based approaches initiating treatment within a few hours of HBsAg positivity. However, the overall certainty of the evidence was low because most included studies were observational and had a moderate to high-risk of bias (15).

EASL strongly supports implementing HBV-DNA testing and particularly emphasizes reflex HBV-DNA testing strategies in resource-limited settings. However, it is also stated that simplified approaches may be practical in settings with unrestricted access to treatment, whereas important challenges may arise in regions where access to NA is limited and treatment prioritization is required (2). Recommendations from the WHO and EASL guidelines on reflex HBV-DNA testing strategies are summarized in Figure 4.

Reflex HBV-DNA testing strategies have the potential to improve linkage to care, accelerate treatment evaluation, and simplify the HBV diagnostic pathway. The optimal reflex testing approach should be selected according to national health-care infrastructure, laboratory capacity, sample transport systems, and accessibility of testing and treatment services. While laboratory-based reflex testing may be more appropriate in settings with centralized high-capacity laboratories, clinic-based reflex testing combined with point-of-care molecular assays may improve access to care in vulnerable populations with limited laboratory access. In many regions, a combined use of laboratory-based and clinic-based reflex testing strategies may be required to address the needs of different patient populations and health-care systems (15).

Emerging Molecular Biomarkers in HBV Infection

Serum HBV-RNA

HBV is a partially double-stranded DNA virus that replicates its genome through reverse transcription of pgRNA. During the HBV replication cycle, cccDNA within the nuclei of infected hepatocytes serves as the major transcriptional template for the production of several viral transcripts, including pgRNA. The generated pgRNA is subsequently encapsidated into nucleocapsid particles and reverse transcribed by HBV polymerase into relaxed circular DNA (rcDNA), after which rcDNA-containing virions acquire an envelope and are released from infected hepatocytes (16). Serum HBV-RNA has emerged as a novel viral biomarker that predominantly represents circulating encapsidated pgRNA and may directly reflect the transcriptional activity of intrahepatic cccDNA. Circulating HBV-RNA may exist as full-length, spliced, or 3'-terminally truncated forms, and the predominant species detected by reverse transcription PCR (RT-PCR) is usually 3.5-kb HBV-RNA (Figure 5) (16,17,18).

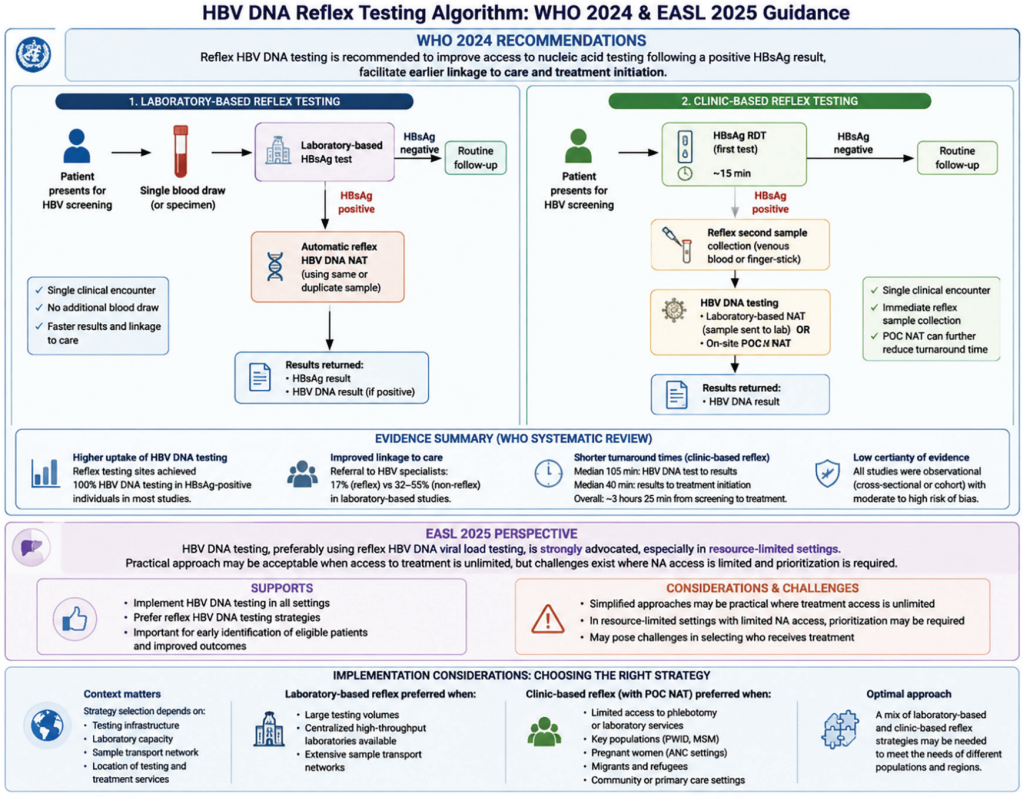

Figure 4. Reflex HBV-DNA testing strategies: synthesis of the WHO 2024 recommendations and EASL 2025 guidance

ANC: Antenatal care, DNA: Deoxyribonucleic acid, EASL: European Association for the Study of the Liver, HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virus, MSM: Men who have sex with men, NA: Nucleos(t)ide analogue, NAT: Nucleic acid testing, POC: Point-of-care, PWID: People who inject drugs, RDT: Rapid diagnostic test, WHO: World Health Organization

During NA therapy, serum HBV-DNA declined markedly, whereas HBV-RNA remained detectable in a substantial proportion of patients, supporting its potential role in monitoring ongoing viral transcription and treatment response. HBV-RNA may reflect persistent cccDNA transcriptional activity despite virological suppression (19). In the majority of studies, end-of-treatment HBV-RNA levels—either alone or particularly when interpreted in combination with qHBsAg and HBcrAg kinetics—have been shown to help predict the risk of relapse or the likelihood of sustained response in HBeAg-negative patients considered for treatment discontinuation, as well as in mixed cohorts including both HBeAg-positive and HBeAg-negative patients (20,21,22,23). However, the predictive value of HBV-RNA for relapse in HBeAg-negative patients appears to be limited by intergenotypic heterogeneity, low rates of biochemical relapse/flares, single time-point HBV-RNA measurement, different sampling time points between cohorts, and the inability of the applied HBV-RNA assay to precisely distinguish the quantified HBV-RNA species (24). Thus, HBV-RNA may provide complementary information for treatment response and relapse assessment, particularly when interpreted together with other biomarkers.

The prognostic value of HBV-RNA for HCC risk is also increasingly recognized, as limited early on-treatment HBV-RNA decline in NA-treated patients has been independently associated with HCC development and may improve risk stratification when added to PAGE-B, mPAGE-B or aMAP scores (25). In one study, higher serum HBV-RNA levels observed in HBV-related HCC patients compared with cirrhotic controls suggested that HBV-RNA may not only reflect treatment response, but also represent persistent transcriptionally active viral reservoirs and potentially serve as a biomarker of residual oncogenic viral activity (26). Moreover, in special patient populations with low-level viremia or suboptimal virological response, HBV-RNA is being investigated as a complementary biomarker to HBV-DNA for treatment optimization, guidance of antiviral strategy modifications, and identification of the need for closer monitoring (27). These findings support a potential complementary role for HBV-RNA in selected patients for whom residual viral activity or treatment response require further characterization.

Multiple molecular techniques are available to detect and quantify HBV-RNA, including reverse transcription quantitative PCR, droplet digital PCR (ddPCR), fluorescence-based isothermal nucleic acid amplification assays, and rapid amplification of cDNA ends. These methods differ in their analytical principles, sensitivity, quantification capacity, and ability to detect distinct HBV-RNA isoforms (3). Automated RT-PCR-based platforms are widely used for HBV-RNA quantification owing to their high analytical sensitivity, broad dynamic range and high-throughput capacity. In contrast, ddPCR technology has attracted attention particularly for the assessment of low-level viremia because of its ability to achieve lower quantification limits (13,17). Nevertheless, variability among assays persists because of differences in amplification targets, non-standardized protocols, sample processing procedures, and the heterogeneous nature of circulating HBV-RNA species. Therefore, the development of international reference standards and the standardization of HBV-RNA quantification remain important

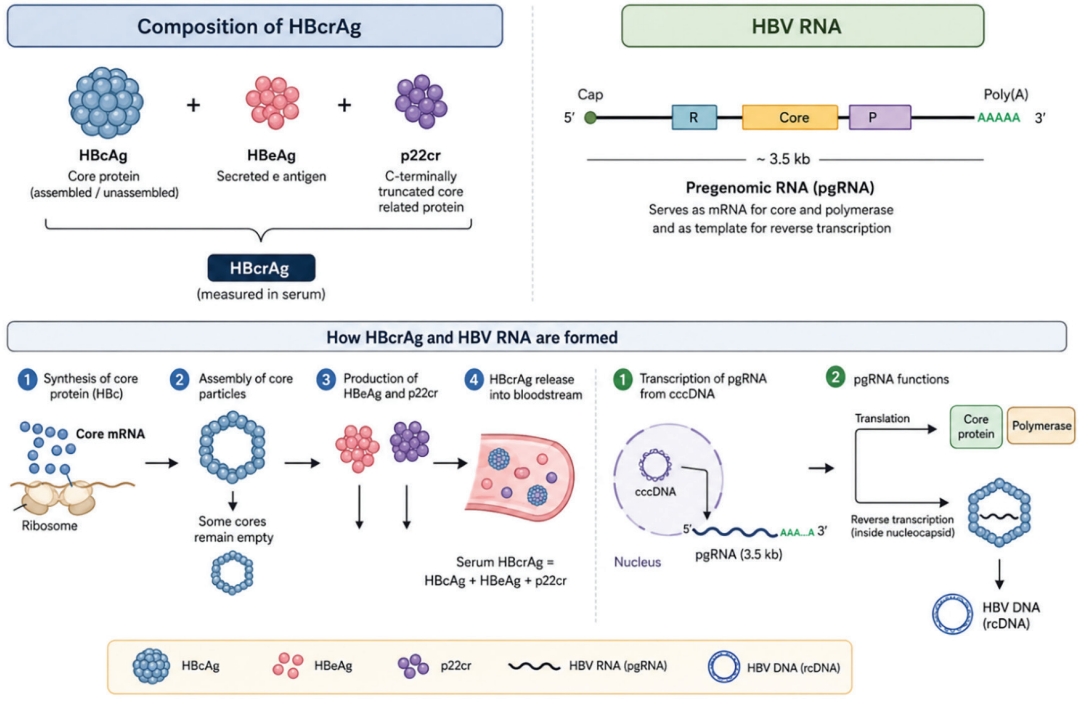

Figure 5. Composition, biogenesis, and functional roles of HBcrAg and HBV pgRNA

cccDNA: Covalently closed circular DNA, DNA: Deoxyribonucleic acid, HBc: Hepatitis B core protein, HBcAg: Hepatitis B core antigen, HBeAg: Hepatitis B e antigen, HBcrAg: Hepatitis B core-related antigen, HBV: Hepatitis B virus, kb: Kilobase, mRNA: Messenger RNA, P: Polymerase-coding region, p22cr: 22-kDa precore/core-related protein, pgRNA: Pregenomic RNA, poly(A): Polyadenylate tail, R: Terminal redundancy region, rcDNA: Relaxed circular DNA, RNA: Ribonucleic acid

requirements for the integration of HBV-RNA into clinical practice (28). Although serum HBV-RNA quantification represents a promising emerging biomarker, its measurement requires advanced molecular diagnostic platforms and technical expertise, and it has not yet been incorporated into routine first-line screening or diagnostic clinical practice (13,18).

HBcrAg

HBcrAg is a composite biomarker consisting of HBeAg, HBcAg, and p22cr proteins derived from the precore/core region of HBV, and serum HBcrAg levels strongly correlate with the transcriptional activity of intrahepatic cccDNA (29). HBcAg is produced through translation of the 3.5 kb pgRNA transcribed from cccDNA, whereas HBeAg is generated from the precore protein, which is translated from precore mRNA, following proteolytic removal of its N-terminal signal peptide. In contrast, p22cr is a 22-kDa truncated precursor core protein generated by both N-terminal and C-terminal processing of the precore protein. The simultaneous detection of these viral proteins enables HBcrAg to reflect not only serum viremia but also ongoing intrahepatic viral transcription and residual cccDNA activity (Figure 5) (3,7,28,30,31).

Testoni et al. (5) reported that HBcrAg-negative patients exhibited lower intrahepatic cccDNA levels and lower cccDNA transcriptional activity than HBcrAg-positive patients. The authors further demonstrated that serum HBcrAg levels significantly correlated with serum HBV-DNA levels, intrahepatic total HBV-DNA levels, cccDNA levels, and pgRNA levels, as well as with cccDNA transcriptional activity assessed by the pgRNA/cccDNA ratio. Notably, among HBeAg-negative patients with comparable amounts of intrahepatic cccDNA, HBcrAg-positive individuals showed significantly higher pgRNA levels and pgRNA/cccDNA ratios, suggesting that HBcrAg reflects not only the quantity of intrahepatic cccDNA but also its transcriptional activity (5).

In the setting of NA therapy, the clinical relevance of HBcrAg becomes more apparent because NAs efficiently suppress reverse transcription and serum HBV-DNA, but they do not directly eliminate intrahepatic cccDNA or fully silence cccDNA-derived transcriptional activity (32). Accordingly, HBcrAg may remain detectable despite complete virological suppression by HBV-DNA assays, indicating residual intrahepatic viral transcription that is not captured by serum HBV-DNA alone (33). In a study including 149 NA-treated patients with chronic HBV infection, Lin et al. (34) reported that serum HBcrAg and HBV pgRNA levels differed according to treatment duration, supporting the concept that variable degrees of cccDNA transcription may persist during antiviral therapy. Similarly, Sandmann et al. (35) showed that HBcrAg has measurable kinetics during antiviral treatment and may provide additional information on residual viral activity beyond conventional HBV-DNA monitoring. Therefore, in HBV-DNA-suppressed patients receiving NA therapy, HBcrAg may serve as a useful non-invasive marker of residual cccDNA transcriptional activity and may help refine on-treatment monitoring (35,36).

Beyond its role in reflecting residual cccDNA activity during NA therapy, HBcrAg has also been investigated as a predictive marker for serological response and functional cure. Both baseline and on-treatment HBcrAg levels have been shown to predict subsequent HBeAg seroconversion in antiviral-treated HBeAg-positive patients (37). In another study, Mak et al. (38) showed that early reductions in HBcrAg and HBV pgRNA at week 4 predicted favourable long-term HBsAg response, defined as HBsAg seroclearance or decline to ≤100 IU/mL during long-term follow-up. This observation suggests that early HBcrAg decline may identify patients in whom NA therapy is accompanied by a deeper reduction in cccDNA-derived antigen production and a greater probability of approaching functional cure (38). Taken together, these findings indicate that low baseline HBcrAg levels, marked on-treatment HBcrAg decline, and low end-of-treatment HBcrAg may help predict HBeAg seroconversion, HBsAg loss, and durable functional cure, particularly when interpreted together with HBsAg, anti-HBs, HBV-RNA, and treatment modality (3).

Studies performed in both HBeAg-positive and HBeAg-negative patients discontinuing NA therapy have shown that HBcrAg, particularly when interpreted together with HBV-RNA and HBsAg, improves the prediction of clinical relapse, ALT flares, and HBsAg loss, whereas low end-of-treatment or follow-up HBcrAg levels are associated with a lower risk of relapse (39,40). Moreover, higher end-of-treatment HBcrAg levels have been associated with an increased risk of off-treatment ALT flares and HBV relapse, while combining HBcrAg with qHBsAg may further improve relapse risk stratification (40). These findings suggest that low HBcrAg levels may help identify more suitable candidates for NA discontinuation, whereas persistently detectable or high HBcrAg levels should be interpreted as markers of residual viral transcription requiring closer biochemical and virological monitoring after treatment cessation (41).

HBcrAg has emerged as a potential biomarker for HCC risk stratification in patients with chronic HBV infection. Recent studies have shown that persistently elevated HBcrAg levels are associated with an increased risk of HCC development, even in patients with low or undetectable serum HBV-DNA during NA therapy, suggesting that residual cccDNA transcriptional activity may contribute to ongoing hepatocarcinogenesis despite virological suppression (3,4,31). HBcrAg has also been investigated as a potential biomarker for hepatic fibrosis progression and cirrhosis in chronic HBV infection. Higher HBcrAg levels have been shown to be associated with advanced liver fibrosis, cirrhosis, and ongoing hepatic inflammation, suggesting that persistent cccDNA transcriptional activity may contribute not only to viral persistence but also to progressive liver injury and fibrogenesis (3,42,43).

HBcrAg measurement is currently performed primarily using chemiluminescent enzyme immunoassay (CLEIA)-based methods; the most widely used platform is the fully automated Lumipulse system developed by Fujirebio. CLEIA simultaneously detects HBcAg, HBeAg, and precore-derived proteins and has become widely used in clinical studies because of its high analytical sensitivity, broad dynamic range, and automated workflow (33,44). While the lower limit of detection of the conventional Lumipulse HBcrAg assay is approximately 1000 IU/mL, the recently developed high-sensitivity iTACT-HBcrAg assay can detect HBcrAg levels down to

approximately 126 IU/mL, thereby enabling the identification of low-level HBcrAg positivity in patients receiving antiviral therapy. The increased sensitivity of the iTACT-HBcrAg assay has been achieved through optimization of sample pretreatment procedures, modification of the measurement system, and refinement of reagent composition, whereas the underlying antibody principle remains largely comparable to that of the conventional Lumipulse assay (44). Recent studies using ultrasensitive HBcrAg assays have suggested that these assays may improve the detection of residual viral activity despite HBV-DNA suppression during NA therapy and may also contribute to HCC risk stratification (45).

HBcrAg has several limitations that currently restrict its widespread clinical application, including incomplete assay standardization, quantitative variability among different platforms, and inconsistent sensitivity (31). In addition, HBcrAg levels may be influenced by HBV genotype, HBeAg status, and treatment phase, making the establishment of universally applicable cut-off values challenging. HBcrAg testing is also not yet widely accessible in many clinical settings, and issues related to laboratory availability continue to limit its routine implementation. Furthermore, additional prospective studies are still needed to better define the interpretation of low HBcrAg levels in deeply suppressed patients and to establish optimal thresholds for predicting functional cure (2).

qHBsAg

qHBsAg represents the numerical measurement of serum HBsAg, expressed in IU/mL, and, unlike qualitative HBsAg assays, provides information not only on the presence of infection but also on viral antigen burden. Although qHBsAg partially reflects intrahepatic cccDNA transcriptional activity, particularly in HBeAg-positive phases, HBsAg production from integrated HBV-DNA—especially in HBeAg-negative infection—limits its interpretation as a direct surrogate marker of active viral replication (2,46).

QHBsAg is measured using standardized automated chemiluminescent or electrochemiluminescent immunoassays, and the results are reported in IU/mL. These methods are relatively low-cost, reproducible, high-throughput, and suitable for automated quantification in routine clinical laboratory practice. Currently, the most widely used commercial qHBsAg assays include Architect QT (Abbott Laboratories), Elecsys HBsAg II Quant (Roche Diagnostics), and DiaSorin Liaison XL, and strong analytical correlation has been demonstrated between these assays in comparative studies (47). These assays have been based on in-house quantitative enzyme-linked immunosorbent assays (ELISA) or Western blot analysis using monoclonal antibodies directed against the S domain, the preS1, and the N-glycosylated preS2 domain. Currently available commercial assays cannot differentiate among the three HBs protein isoforms (47,48).

In general, qHBsAg levels are highest in HBeAg-positive high-replicative phases and lower in HBeAg-negative chronic infection; therefore, they may contribute to phase classification when interpreted together with HBV-DNA, ALT, and HBeAg status (2,49). In a meta-analysis of 17 studies comprising 4,134 patients, qHBsAg levels showed an overall moderate correlation with quantitative HBV-DNA in patients with chronic hepatitis B. The correlation was more pronounced in HBeAg-positive patients, whereas only a weak association was observed in HBeAg-negative individuals. These findings suggest that qHBsAg cannot be considered a standalone surrogate marker replacing HBV-DNA; however, when interpreted together with HBV-DNA, ALT levels, HBeAg/anti-HBe status, and fibrosis assessment, it may contribute to a more comprehensive evaluation of disease activity and infection phase (48).

In HBeAg-negative patients with low-level viremia, qHBsAg <1000 IU/mL, particularly when combined with HBV-DNA <2000 IU/mL and persistently normal ALT, supports the diagnosis of inactive chronic HBV infection (50,51,52).

However, low HBV-DNA levels do not always indicate a completely inactive disease state. In HBeAg-negative individuals with HBV-DNA <2,000 IU/mL, qHBsAg levels >1,000 IU/mL have been associated with a higher risk of transition from HBeAg-negative chronic infection to HBeAg-negative chronic hepatitis, suggesting that this subgroup may have residual immunological or transcriptional activity despite low-level viremia (47,53). Moreover, data from Asian cohort studies showed that among HBeAg-negative patients with HBV-DNA <2,000 IU/mL, those with HBsAg levels >1,000 IU/mL had a significantly higher risk of HCC than those with HBsAg levels <1,000 IU/mL, indicating that qHBsAg may provide additional prognostic information beyond HBV-DNA in low-viremic patients (53).

Although HBV-DNA can be effectively suppressed in most patients receiving NA therapy, HBsAg loss remains uncommon. Therefore, a longitudinal decline in qHBsAg may serve as a complementary biomarker reflecting not only virological suppression but also a progressive reduction in antigenic burden and proximity to HBsAg clearance. Zhou et al. (54) reported that the annual rate of qHBsAg decline progressively accelerated as patients approached HBsAg loss, with a marked reduction in qHBsAg levels particularly during the final year preceding HBsAg clearance. In patients with chronic hepatitis B receiving peg-IFN therapy, low baseline qHBsAg levels, rapid early on-treatment qHBsAg decline, and low qHBsAg levels at weeks 12 and 24 may help identify patients with a higher probability of HBsAg loss and seroconversion, thereby facilitating the selection of optimal candidates for peg-IFN (55).

In the context of NA discontinuation, qHBsAg is considered a supportive biomarker for predicting clinical relapse and estimating the likelihood of HBsAg loss, particularly in selected non-cirrhotic HBeAg-negative patients with long-term virological suppression. Lower end-of-treatment qHBsAg levels have been associated with a reduced risk of relapse and a higher probability of HBsAg loss; however, qHBsAg alone is insufficient for decision-making, and close clinical and biochemical monitoring remains essential after treatment withdrawal (2,56).

Major advantages of qHBsAg include its availability on standardized automated immunoassay platforms, its broad applicability in routine clinical practice, its potentially greater accessibility and lower cost compared with PCR-based HBV-DNA testing, its ability to reflect

circulating antigen burden, and its usefulness for longitudinal monitoring in treatment response evaluation and functional cure studies (2,47). However, in vitro HBsAg production has been shown to be associated with host-related factors such as age and sex, as well as HBV genotype (2). *In vivo* serum HBsAg levels were found to be higher in individuals infected with genotype A than in those infected with genotypes B, C, and D (57,58,59). In addition, preS/S mutations may influence HBsAg production, as these variants have been reported to show a negative correlation with serum HBsAg levels (60).

Major limitations of qHBsAg include its inability to directly and independently reflect cccDNA transcriptional activity, its interpretative challenges caused by HBsAg production from integrated HBV-DNA, particularly in HBeAg-negative and long-standing infection, its phase-dependent correlation with HBV-DNA, and its absence of universally applicable cut-off values for clinical decision-making. Therefore, qHBsAg should not be considered a standalone surrogate marker replacing HBV-DNA, but rather a complementary biomarker that should be interpreted together with HBV-DNA, ALT, HBeAg/anti-HBe status, fibrosis assessment, HBcrAg, HBV-RNA, and relevant clinical risk factors (2,46,48).

Ultrasensitive HBsAg Assays

Ultrasensitive HBsAg assays are next-generation high-sensitivity diagnostic methods developed to detect extremely low levels of HBsAg that remain below the detection limits of conventional qHBsAg assays. While the analytical sensitivity of standard HBsAg assays is generally around 0.05 IU/mL, ultrasensitive assays can reduce the limit of detection to approximately 0.005 IU/mL or even lower (61,62). This improvement has important implications for the assessment of occult HBV infection, residual viral activity during antiviral therapy, low-level viremia, and minimal antigen persistence after functional cure (63,64,65). Currently available ultrasensitive HBsAg assays include the HBsAg NEXT Qualitative assay (Abbott Diagnostics, Illinois, USA), the HBsAg NEXT Confirmatory assay (Abbott Diagnostics, Illinois, USA), and the iTACT-HBsAg assay (Fujirebio Inc., Tokyo, Japan) (63,64,65,66).

Studies have demonstrated that minimal HBsAg production associated with persistent cccDNA transcriptional activity may continue despite complete serum HBV-DNA suppression during NA therapy; ultrasensitive assays may reveal this residual antigenemia. These findings suggest that viral activity may persist even in patients who appear HBsAg-negative by standard assays and have therefore contributed to a re-evaluation of the concept of "functional cure" (2,67,68). Recent studies have further shown that ultrasensitive HBsAg positivity may predict relapse risk and HBsAg seroreversion after NA discontinuation, supporting a potential role for these assays as biomarkers in finite-treatment strategies and treatment withdrawal decisions (68). Ultrasensitive HBsAg assays may also contribute to earlier detection of HBV reactivation in high-risk patients receiving potent immunosuppressive therapies. In addition, these assays may provide more sensitive monitoring of treatment responses during evaluation of emerging antiviral and cure-directed therapeutic strategies (69). The EASL 2025 HBV guidelines likewise emphasize the growing importance of qHBsAg and novel viral biomarkers in biomarker-driven and personalized HBV management (2).

qAnti-HBc

qAnti-HBc refers to measurement of serum anti-HBc levels and has recently emerged as a novel biomarker for assessing immunological activity in chronic hepatitis B infection. Because anti-HBc antibodies largely reflect HBcAg-specific B-cell activation and the host immune response, qAnti-HBc levels are considered associated not only with viral replication but also with hepatic inflammation and immune activity. In addition, qAnti-HBc may provide supplementary information regarding the magnitude of the humoral immune response against HBV and the intrahepatic HBcAg load (70,71). An international reference standard for the quantification of HBV core antibodies has been established and is expressed in WHO IU/mL (72). qAnti-HBc levels are mainly measured using chemiluminescent microparticle immunoassays, ELISA, and double-sandwich immunoassay-based techniques (71).

One of the most important potential clinical applications of qAnti-HBc is the non-invasive assessment of hepatic inflammation. Several studies have demonstrated that elevated qAnti-HBc levels are associated with significant histological inflammation even in patients with normal or mildly elevated ALT levels (71,73,74). Some reports have suggested that qAnti-HBc may predict significant liver inflammation more accurately than ALT alone (75). Therefore, qAnti-HBc may contribute to treatment decision-making, particularly for patients who cannot undergo liver biopsy or are considered to be in the "gray zone" of chronic hepatitis B management.

qAnti-HBc has potential value in predicting antiviral treatment response. Higher baseline qAnti-HBc levels have particularly been associated with HBeAg seroconversion and favorable on-treatment outcomes in patients receiving peg-IFN therapy or NAs (76). Particularly when interpreted together with low qHBsAg levels, qAnti-HBc may contribute to predicting the likelihood of functional cure (13,77). Furthermore, qAnti-HBc is being investigated as a supportive biomarker for estimating relapse risk after NA discontinuation in selected patients (13). Nevertheless, because anti-HBc levels reflect not only viral activity but also the host immune response, their interpretation may vary across different clinical phases of HBV infection. HBeAg status, HBV genotype, antiviral treatment exposure, and immunological status may all influence qAnti-HBc levels (71). Although qAnti-HBc has been reported to be relatively genotype-independent in some treatment-response analyses, natural-history data show phase-dependent differences between genotypes B and C, and many proposed treatment-response cut-offs derive from Asian cohorts dominated by these genotypes. Therefore, qAnti-HBc should be interpreted in conjunction with HBV genotype and clinical phase (71,76,78).

HBV Nucleic Acid-Related Antigen (HBV-NRAg)

HBV-NRAg is one of the newly identified investigational biomarkers in chronic HBV infection and is considered a candidate

marker reflecting viral replication activity (30). Current assays are mainly based on ELISA-based combined detection of HBV preS1 antigen and HBcAg (30,78). Early studies have suggested that HBV-NRAg positivity may correlate with serum HBV-DNA levels and active viral replication (79). Therefore, HBV-NRAg has been proposed as a potential alternative or complementary biomarker for assessing viral replication activity (30). However, HBV-NRAg measurement remains investigational and has not entered routine clinical practice. Because currently available evidence remains limited, the role of HBV-NRAg in predicting treatment response, functional cure, or outcomes after NA discontinuation has not yet been clearly established. HBV-NRAg is an emerging investigational biomarker that may reflect HBV replication activity, although further large-scale prospective studies and assay standardization are needed before its clinical utility can be clearly defined (30,80).

Non-Invasive Assessment of Liver Disease in HBV

Accurate assessment of liver fibrosis and cirrhosis in chronic HBV infection is essential for treatment decisions, prognostic evaluation, and HCC risk stratification. Although liver biopsy has long been considered the reference standard, its invasive nature, sampling variability, and limited patient acceptability have led to increasing interest in non-invasive assessment methods. In this context, serum-based non-invasive tests (NITs) and liver stiffness measurement (LSM) have become widely used tools for evaluating liver stiffness and fibrosis stage, providing important clinical value, particularly for monitoring antiviral treatment and for identifying patients at risk for advanced fibrosis or cirrhosis.

NITs

NITs are serum-based methods developed to evaluate liver fibrosis and disease severity without the need for liver biopsy in patients with chronic hepatitis B. In recent years, NITs have become increasingly important in HBV management because liver biopsy, although historically considered the reference standard for fibrosis staging, is invasive, costly, subject to sampling error, and associated with potential complications (81,82).

Among serum-based NITs, aspartate aminotransferase to platelet ratio index (APRI) and fibrosis-4 index (FIB-4) are the most widely used because they are inexpensive, easily accessible, and recommended by WHO guidelines, particularly in low-resource settings. These scores are primarily used to identify patients with significant fibrosis or cirrhosis and to guide treatment eligibility decisions in chronic HBV infection (15). The principal non-invasive serum-based biomarkers and composite indices currently used for the assessment of liver fibrosis in chronic hepatitis B are summarized in Table 1 (2,15,81,82,83,84).

Clinically, NITs are used in several settings in HBV infection. First, they are widely used for baseline fibrosis staging and treatment decision-making (82,85). Patients with advanced fibrosis or cirrhosis identified by NITs are generally considered candidates for antiviral therapy even when ALT levels are not markedly elevated, particularly when HBV-DNA remains detectable (2). Second, NITs are used longitudinally to monitor fibrosis progression or regression during antiviral therapy (86). Third, NITs, particularly LSM-based methods, contribute to HCC risk stratification because advanced fibrosis and cirrhosis remain major predictors of HCC development in chronic HBV infection (87).

However, NITs have several important limitations in the management of chronic HBV infection. Simple serum-based indices such as APRI and FIB-4 frequently yield indeterminate results, and their diagnostic performance is modest, particularly for significant fibrosis and for distinguishing adjacent fibrosis stages (80). In chronic HBV infection cohorts, APRI has shown substantial heterogeneity across studies with variable thresholds, modest performance for significant fibrosis and cirrhosis, and 30-40% of patients may fall into indeterminate ranges (81,88,89).

FIB-4 has also been validated in chronic hepatitis B, but 30-40% of patients may have indeterminate scores, and optimal cut-offs remain insufficiently defined across different phases of HBV infection (81). In addition, variability in the natural history of chronic hepatitis B, immune activity, and inflammatory flares may affect the reliability of current NITs for fibrosis staging. Therefore, NITs are useful initial tools, especially when elastography is not available, but their results should be interpreted according to the clinical context and phase of HBV infection, and discordant or indeterminate results may require further assessment with elastography or, in selected cases, liver biopsy (2,15).

LSM

LSM refers to non-invasive elastography-based imaging techniques that estimate hepatic fibrosis by measuring liver stiffness. The main LSM modalities include vibration-controlled transient elastography (VCTE), acoustic radiation force impulse imaging (ARFI)-based techniques, and shear wave elastography (SWE) (2,81). Compared with liver biopsy, LSM-based elastography techniques are non-invasive, repeatable methods developed to reduce the need for histological assessment in routine fibrosis staging (81).

Among LSM techniques, VCTE, also known as transient elastography or FibroScan®, is one of the most widely validated methods in chronic hepatitis B and provides liver stiffness values expressed in kilopascals (kPa) (81,90). Liver stiffness values generally increase with fibrosis stage; however, hepatic inflammation and ALT elevation may affect stiffness measurements independently of fibrosis severity. In addition, inflammation, cholestasis, hepatic congestion, and food intake are important confounding factors that may influence LSM results. Acute inflammatory injury, such as that associated with HBV reactivation flares or acute HBV infection, may result in elevated LSM values independently of fibrosis stage. Because liver stiffness cut-off values may vary according to the etiology of chronic liver disease, LSM findings should be interpreted carefully by experienced, qualified clinicians, considering all variables that may affect measurement validity. Important limitations of VCTE include the requirement for operator training and experience to obtain reliable, valid measurements, and the inability to directly select a specific hepatic region of interest, because the technique does not

Table 1. Widely used non-invasive serum biomarkers for liver fibrosis assessment in chronic liver disease

Biomarker	Core components (serum variables)	Formula	Major clinical advantages	Key limitations	Suggested clinical thresholds
APRI	- AST - Platelet count	(AST/AST upper limit of normal)/platelet count (10^9/L) x 100	- Simple and inexpensive - Widely available - Good rule-out tool for advanced fibrosis	- Reduced accuracy in intermediate fibrosis - Influenced by necroinflammation and thrombocytopenia	- <0.5: Excludes significant fibrosis - 0.5-1.5: Gray zone - >1.5: Significant fibrosis likely - >2.0: Cirrhosis likely
FIB-4	- Age - AST - ALT - Platelet count	Age (years) x AST (U/L)/[platelet count (10^9/L) x √ALT (U/L)]	- Simple bedside calculation - Well validated in HBV and HCV patients - High negative predictive value for advanced fibrosis	- Age dependent variability - Lower specifity in older patients	- <1.45: Advanced fibrosis unlikely - 1.45-3.25: Gray zone - >3.25: Advanced fibrosis likely
GPR	- GGT - Platelet count	GGT (U/L)/platelet count (10^9/L)	- Better performance in some HBV-endemic populations - Simple and inexpensive	- Affected by cholestasis, alcohol intake, and enzyme induction - Less validated in Western populations	- <0.32: Significant fibrosis unlikely - 0.32-0.56: Significant fibrosis likely - >0.56: Cirrhosis likely
Forns index	- Age - GGT - Cholesterol - Platelet count	7.811-3.131 x ln(platelets) + 0.781 x ln(GGT) + 3.467 x ln(age)-0.014 x cholesterol	- Good rule-out method for significant fibrosis - Non-invasive alternative to biopsy	- Less commonly used in HBV - More complex calculation - Affected by cholesterol-lowering therapy	- <4.2: Excludes significant fibrosis - 4.2-6.9: Gray zone - >6.9: Significant fibrosis likely
Fibrotest (FibroSure)	- A-2 macroglobulin - Haptoglobulin - Apolipoprotein A1 - Total bilirubin - GGT	Proprietary algorithm combining 5 serum biomarkers	- High diagnostic accuracy - Extensively validated - Reproducible and robust	- Proprietary test - Affected by hemolysis, Gilbert syndrome, and acute inflammation	- <0.27: No or minimal fibrosis - >0.48: Significant fibrosis - >0.74: Cirrhosis
ELF score	- Hyaluronic acid - PIIINP - TIMP-1	Proprietary algorithm reflecting extracellular matrix turnover	- Reflects active fibrogenesis - Useful for longitudinal monitoring - Validated across etiologies	- Higher cost - Limited availability in resource-limited settings	- <7.7: No or mild fibrosis - 7.7-9.8: Moderate fibrosis - 9.8-11.3: Advanced fibrosis - >11.3: Severe fibrosis/ cirrhosis
M2BPGi	M2BPGi (glycoform level)	Serum M2BPGi level measured by immunoassay (reported as COI).	- Promising fibrosis and HCC risk marker - Dynamic monitoring possible	- Cut-offs vary by ethnicity and HBV phase - Limited availability outside Asia	- <1.0: Significant fibrosis unlikely - 1.0-3.0: Gray zone - >3.0: Advanced fibrosis/ cirrhosis

ALT: Alanine aminotransferase, APRI: AST to platelet ratio index, AST: Aspartate aminotransferase, COI: Cut-off index, ELF score: Enhanced liver fibrosis score, FIB-4: Fibrosis-4 index, GGT: Gamma-glutamyl transferase, GPR: GGT to platelet ratio, HBV: Hepatitis B virus, HCC: Hepatocellular carcinoma, HCV: Hepatitis C virus, ln: Natural logarithm, M2BPGi: Mac-2 binding protein glycan isomer, PIIINP: Procollagen III N-terminal propeptide, TIMP-1: Tissue inhibitor of metalloproteinases-1

provide simultaneous B-mode ultrasound imaging. In addition, the development of alternative probes such as the XL probe has reduced scan failure rates and poor reliability previously associated with the M probe in patients with obesity (90).

ARFI-based techniques and SWE are ultrasound-based elastographic methods that quantitatively assess tissue stiffness and can be performed during a conventional ultrasound examination. ARFI-based methods include point SWE, which measures stiffness within a small and fixed region of interest, and two-dimensional SWE (2D-SWE), which evaluates stiffness over a larger area while also providing a color-coded parametric stiffness map. Measurements obtained with ultrasound SWE techniques may be expressed as shear wave velocity in meters per second (m/s) or converted into Young's modulus values in kPa based on assumptions that may not always be entirely accurate (91,92). In a prospective chronic hepatitis B study, 2D-SWE LSMs showed a significant positive correlation with histological fibrosis stage, supporting its use as a non-invasive fibrosis assessment method (93). However, LSM results may be influenced by biological and technical factors, including inflammation, cholestasis, steatosis, obesity, and measurement-related variables; therefore, they should not be interpreted in isolation but rather in conjunction with other clinical, virological, and biochemical parameters.

Future Perspectives in HBV Diagnostics

The future of HBV diagnostics is increasingly focused on integrating novel viral, immunological, and host-related biomarkers

into individualized strategies for disease monitoring, treatment guidance, and prediction of clinical outcomes. This evolving approach has been emphasized in recent international guidelines and contemporary biomarker-focused reviews (2,13,30,94). A proposed diagnostic algorithm integrating point-of-care testing, molecular diagnostics, emerging biomarkers, and personalized monitoring strategies is summarized in Figure 6.

Rather than relying solely on conventional parameters such as HBV-DNA, ALT, and HBeAg status, emerging diagnostic approaches aim to characterize intrahepatic cccDNA transcriptional activity, immune control, and the likelihood of treatment response or relapse. In this context, biomarkers including qHBsAg, HBcrAg, serum HBV-RNA, qAnti-HBc, ultrasensitive HBsAg assays, and investigational markers such as HBV-NRAg are increasingly being evaluated as complementary tools in HBV management (2,30,95).

One of the major future applications of these biomarkers is the development of cccDNA-targeted therapeutic strategies. Since cccDNA persists within hepatocytes despite long-term NA therapy, it remains the principal barrier to HBV eradication and achievement of a true cure (93,95). Accordingly, novel antiviral approaches including small interfering RNA therapies, antisense oligonucleotides, capsid assembly modulators, entry inhibitors, therapeutic vaccines, immune modulators, and gene-editing technologies are being investigated to suppress or eliminate cccDNA transcriptional activity (Figure 7) (2,4,6,28,94,96). In this evolving therapeutic landscape, biomarkers capable of indirectly reflecting cccDNA activity—particularly HBcrAg and serum HBV-RNA assays—may become increasingly important for monitoring treatment efficacy and defining treatment endpoints (95).

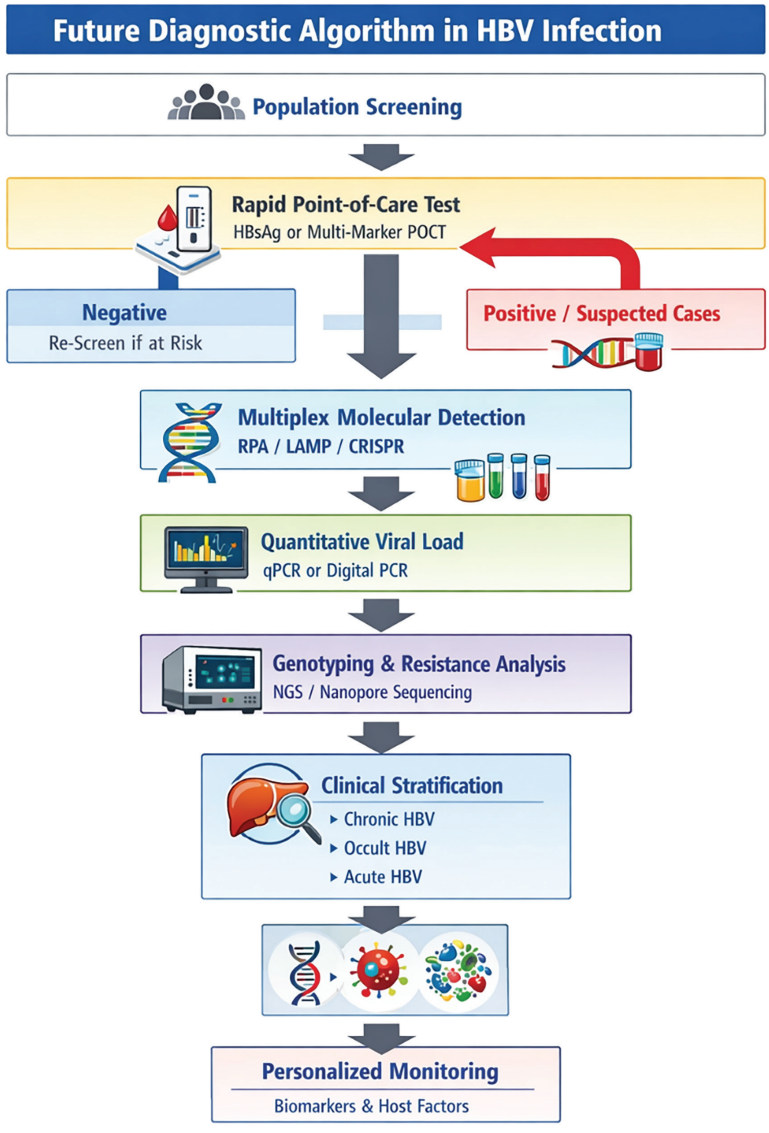

Figure 6. Proposed future diagnostic algorithm for HBV infection

CRISPR: Clustered regularly interspaced short palindromic repeats, HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virus, LAMP: Loop-mediated isothermal amplification, NGS: Next-generation sequencing, PCR: Polymerase chain reaction, POCT: Point-of-care testing, qPCR: Quantitative polymerase chain reaction, RPA: Recombinase polymerase amplification

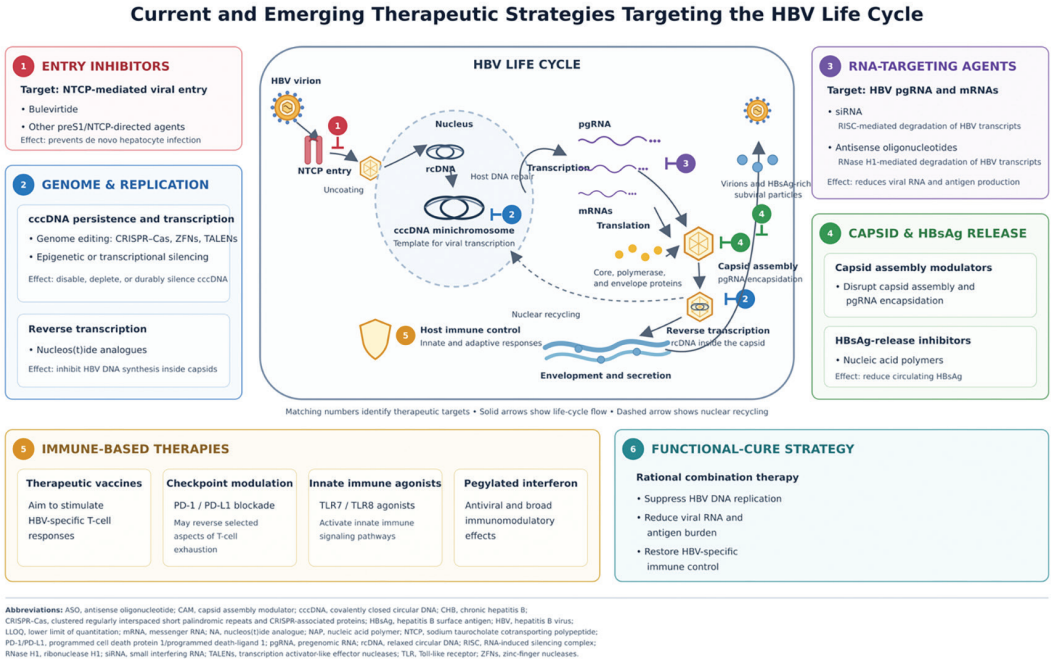

Figure 7. Current and emerging therapeutic strategies targeting the HBV life cycle

Anti-HBc: Antibody to hepatitis B core antigen, cccDNA: Covalently closed circular DNA, DNA: Deoxyribonucleic acid, ER: Endoplasmic reticulum, HBc: Hepatitis B virus core protein, HBcAg: Hepatitis B core antigen, HBcrAg: Hepatitis B core-related antigen, HBeAg: Hepatitis B e antigen, HBsAg: Hepatitis B surface antigen, HBV: Hepatitis B virus, HBV-NRAg: Hepatitis B virus nucleic acid-related antigen, HBx: Hepatitis B virus X protein, HSPG: Heparan sulfate proteoglycan, IgG: Immunoglobulin G, IgM: Immunoglobulin M, kb: Kilobase, L: Large surface protein, M: Middle surface protein, mRNA: Messenger RNA, MVB: Multivesicular body, NTCP: Sodium taurocholate cotransporting polypeptide, p22cr: 22-kDa precore-related protein, pgRNA: Pregenomic RNA, preS1: Pre-S1 region, preS2: Pre-S2 region, rcDNA: Relaxed circular DNA, RNA: Ribonucleic acid, S: Small surface protein

Another promising approach is the combined use of multiple viral and host-related biomarkers, rather than reliance on a single biomarker. Emerging evidence suggests that integrated biomarker strategies involving HBV-DNA, qHBsAg, HBcrAg, serum HBV-RNA, and fibrosis assessment tools may improve prediction of disease phase, fibrosis progression, HCC risk, antiviral treatment response, and relapse after treatment discontinuation. Integrated biomarker models combining qHBsAg, HBcrAg, HBV-RNA, HBV-DNA and ALT may therefore improve individualized patient management and support more accurate clinical decision-making (31,39,97,98). In addition, a novel spectrum of host-derived biomarkers, including host genetic biomarkers, microRNAs, serum immune markers, and cytokines, is expected to play an important role in predicting the risks of cirrhosis, fibrosis, and HCC, as well as in the interpretation of disease activity (98,99,100). HBV-specific CD8+ T-cell and natural killer cell phenotypes and functional profiles have also been investigated as potential predictors of HBsAg seroclearance (101). The combined use of host- and virus-related biomarkers may further improve the management of chronic hepatitis B, as host biomarkers may provide information on hepatic pathophysiology and immune status, whereas viral biomarkers reflect the level of HBV replication (30).

Because functional cure remains uncommon with currently available therapies, there is a growing need for sensitive biomarkers capable of accurately predicting HBsAg clearance, durable immune control, and relapse risk. Future diagnostic strategies will therefore likely focus not only on detecting viral replication, but also on defining the immunovirological state associated with long-term disease control and treatment-free remission (2,12).

Conclusion

HBV diagnostics is undergoing a major paradigm shift from conventional serological and virological testing toward integrated biomarker-driven approaches. Emerging biomarkers, such as serum HBV-RNA, HBcrAg, qHBsAg, qAnti-HBc, and ultrasensitive HBsAg assays, and investigational markers (including HBV-NRAg) provide deeper insights into intrahepatic viral activity, immune control, and treatment response. These novel biomarkers may improve disease staging, prediction of functional cure, relapse risk assessment, and individualized treatment strategies beyond the limitations of traditional HBV-DNA-based monitoring. In parallel, reflex HBV-DNA testing strategies, point-of-care molecular assays, and non-invasive fibrosis assessment tools are reshaping the diagnostic pathway and facilitating earlier linkage to care. The combined interpretation of viral, immunological, and host-related biomarkers will likely play a central role in future precision medicine approaches for chronic hepatitis B. As novel antiviral and cccDNA-targeted therapies continue to evolve, diagnostic algorithms will require ongoing updates to incorporate emerging biomarkers into routine clinical practice. Ultimately, integration of advanced molecular diagnostics with personalized treatment strategies may significantly improve long-term outcomes and accelerate progress toward a functional cure in chronic hepatitis B.

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Footnotes

Authorship Contributions

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