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Advances in Rapid Nucleic Acid Diagnostics for Hepatitis B and C Viruses: A Comprehensive Review

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ABSTRACT

Chronic viral hepatitis, predominantly caused by HBV and HCV infections, remains a major global health burden and a leading cause of cirrhosis, hepatocellular carcinoma (HCC), and liver-related mortality. Achieving the World Health Organisation (WHO) target of diagnosing 90% of HBV and HCV infections by 2030 necessitates a fundamental transition from centralised laboratory-based testing towards rapid, accessible, and decentralised diagnostic solutions. This review provides a comprehensive and critical assessment of the current diagnostic landscape for viral hepatitis, spanning established commercial assays and emerging nucleic acid testing (NAT) technologies. We systematically examine recent advances in biosensor-enabled diagnostics, with particular emphasis on the integration of isothermal amplification and CRISPR-based molecular recognition into electrochemical and field-effect transistor (FET) platforms. These electronic sensing technologies enable label-free signal transduction, scalable miniaturisation, and seamless integration with point-of-care (POC) workflows, thereby addressing key limitations of conventional diagnostic pipelines. In addition, we discuss persistent challenges in sample preparation, signal amplification, and system integration, and highlight strategies that couple biochemical amplification with solid-state electronics to streamline diagnostic workflows. By synthesising the state of the art and identifying critical technological bottlenecks, this review describes the technological progression of next-generation diagnostic platforms required to accelerate the global elimination of viral hepatitis and to improve patient outcomes through earlier and more equitable access to diagnosis.

Abbreviations: +ssRNA, positive-sense single-stranded RNA; ACEL, Alternating-current electroluminescence; anti-HBs, anti-HBsAg antibodies; AuNP, gold nanoparticle; cccDNA, covalently closed circular DNA; CIA, Chromatographic immunoassay; CLIA, chemiluminescent immunoassay; CRISPR, clustered regularly interspaced short palindromic repeat; crRNA, CRISPR RNA; E-CLIA, Electrochemiluminescence Immunoassay; ECL, electrochemiluminescence; EGFET, extended-gate field-effect transistor; ELISA, enzyme-linked immunosorbent assay; FET, field-effect transistors; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCR, hybridisation chain reaction; IgG, Immunoglobulin G; IgM, Immunoglobulin M; ISFET, ion-sensitive field-effect transistor; ITO, indium tin oxide; LAMP, loop-mediated isothermal amplification; LFA, lateral flow assay; LFIA, lateral flow immunoassays; LMICs, low- and middle-income countries; LOD, limit-of-detection; MOSFET, metal-oxide-semiconductor field-effect transistor; MSM, men who have sex with men; NAT, nucleic acid testing; PAM, protospacer adjacent motif; PFS, protospacer-flanking site; pgRNA, pregenomic RNA; POC, point-of-care; POCT, point-of-care testing; PWID, people who inject drugs; RAA, recombinase-aided amplification; rcDNA, relaxed circular DNA; RDTs, Rapid Diagnostic Tests; RPA, recombinase polymerase amplification; TMA, transcription-mediated amplification; WHO, World Health Organisation.

1 | Introduction

Chronic viral hepatitis, primarily driven by HBV and HCV, remains a leading cause of global liver-related morbidity and mortality, accounting for a substantial proportion of liver cirrhosis and hepatocellular carcinoma (HCC) cases [1, 2]. According to the World Health Organisation (WHO), approximately 254 million individuals were living with chronic HBV infection and 50 million with chronic HCV infection in 2022, despite the availability of effective vaccines for HBV and curative antiviral therapies for HCV [3]. Although updated epidemiological data suggest a modest decline in incidence compared with previous years, the absolute global burden of viral hepatitis remains unacceptably high, particularly in low- and middle-income countries (LMICs).

The geographic distribution of HBV and HCV infections is heterogeneous, reflecting differences in transmission routes, public health infrastructure, and access to preventive and diagnostic services. HBV infection remains highly prevalent in sub-Saharan Africa and East Asia, with nearly half of all chronic cases concentrated in China, India, Nigeria, and Indonesia [4]. In contrast, HCV infection exhibits a broader global distribution, with the highest prevalence reported in the Eastern Mediterranean and European regions [5]. In the US, genotype 1 accounts for about 60% of cases of HCV [6]. Taiwan has a unique hepatitis epidemiological profile, characterized by a major HBV epidemic dominated by genotypes B and C in the 1980s and a high prevalence of HCV genotype 1b, which accounts for approximately 50%–70% of infections [7–9].

HBV and HCV are primarily transmitted through exposure to infected blood and body fluids; however, the relative contribution of specific transmission routes varies substantially across geographic regions and socio-economic settings [3]. Historically, blood transfusion and transplantation of organs or tissues were major routes of transmission for both viruses. However, through the widespread implementation of nucleic acid testing (NAT) for donor screening, together with routine serological and molecular testing in high-income countries, the risk associated with transfusion and transplantation and iatrogenic exposure has been substantially reduced. Nevertheless, transmission dynamics have evolved; men who have sex with men (MSM), people who inject drugs (PWID), and individuals receiving unregulated tattooing have emerged as increasingly prominent high-risk populations [10, 11]. These groups now account for a growing proportion of new HBV and HCV infections in many high-income settings. In contrast, in many LMICs, incomplete screening coverage, limited access to advanced diagnostics, and fragmented healthcare infrastructure continue to permit transfusion and transplantation-associated transmission. Apart from healthcare-associated transmission, HBV is efficiently transmitted through perinatal exposure and horizontal transmission during early childhood, particularly in endemic regions such as sub-Saharan Africa and East Asia [11, 12]. As for HCV, unsafe blood transfusion and non-sterile injection practices remain major drivers of transmission in low-income settings, underscoring persistent global inequalities in infection control and healthcare access.

1.1 | Critical Gaps and Biological Barriers to Elimination

Globally, the current status of HBV and HCV infections continues to present considerable challenges for public health management. By the end of 2022, only 13% of individuals with chronic HBV infections had been diagnosed and only 3% had received therapy, whereas only 36% of those with HCV were diagnosed and 20% received curative treatment [3]. This disparity highlights the critical gaps in diagnosis and treatment across regions, particularly in LMICs, where approximately 67% of the 71 million people with HCV suffer from poor access to testing facilities and treatment services in the area they reside [3, 10].

Although HBV vaccination, which was introduced in 1982, has led to a dramatic decline in global HBV infections [13], its high cost limits its implementation in several LMICs where the virus remains prevalent [14, 15]. In contrast, the development of an effective HCV vaccine has been hindered by the virus's rapid mutation rate and extensive genetic heterogeneity. The six major HCV genotypes and more than 100 subtypes exhibit sequence divergence of up to 50%, which facilitates immune evasion and further complicates vaccine development [16]. Despite the availability of highly effective direct-acting antiviral agents achieving cure rates of up to 97% in treated individuals [17], the HCV continues to spread, exacerbated by the rising injection-based drug use in Western countries [18]; moreover, the persistent infection in a significant percentage of immunocompetent hosts underscores the complex nature of HCV pathogenesis [19].

Collectively, these factors underscore the persistence of viral hepatitis as a substantial public health challenge despite major therapeutic advances for both HBV and HCV [20]. Figure 1 illustrates the relationship between hepatitis infection–associated biomarkers and the evolution of diagnostic technologies. While serological markers are predominantly detected using established commercial assays, molecular markers, specifically HBV DNA and HCV RNA, are increasingly targeted by emerging point-of-care–oriented nucleic acid–based platforms. This review summarizes commercially available diagnostic assay kits targeting key HBV and HCV biomarkers and further highlights emerging detection methodologies with the potential to enhance disease management and improve public health outcomes.

1.2 | The Diagnostic Landscape: From Serological Markers to Molecular Assays

The diagnosis of HBV and HCV infection relies on a complementary combination of serological and molecular biomarkers, which reflect different stages of viral infection and host immune response. Serological markers are widely used for population screening and clinical staging, whereas molecular markers are indispensable for early diagnosis, viral load quantification, and donor screening [21].

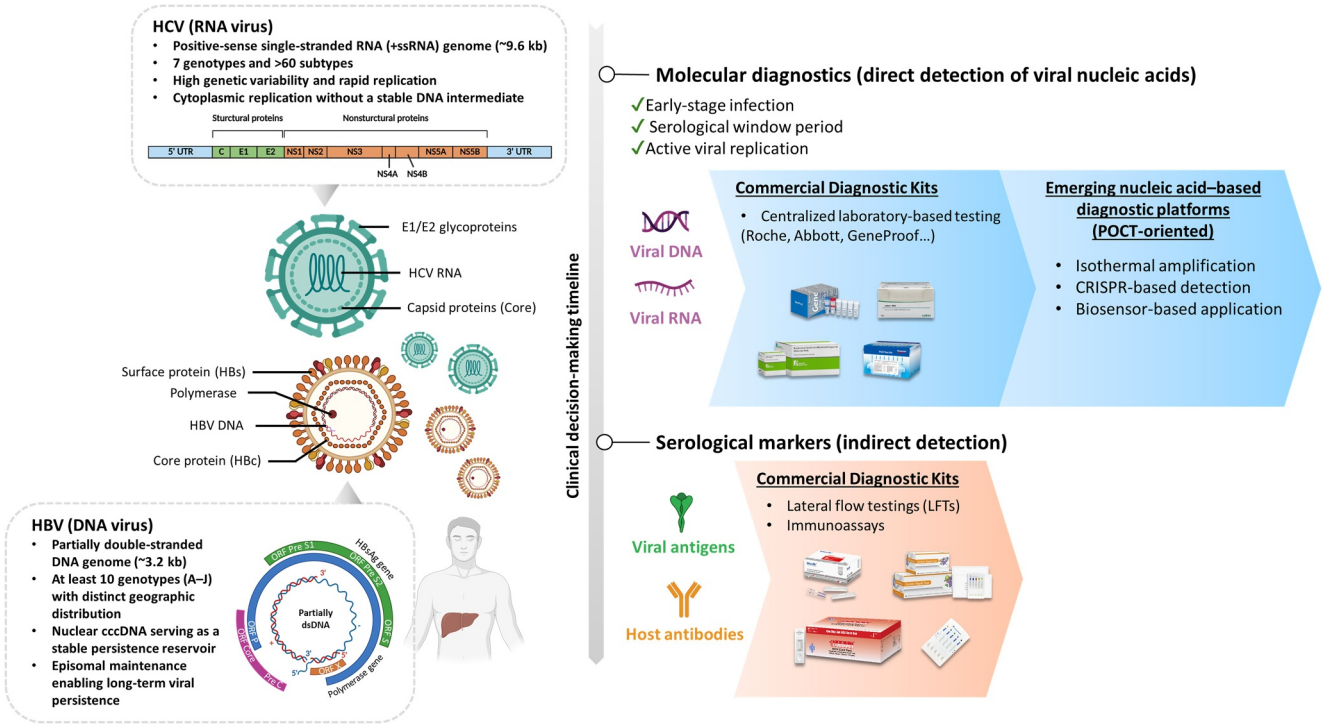

FIGURE 1 | Biological characteristics and diagnostic landscape of HBV and HCV.

1.2.1 | Hepatitis B Virus Serological Markers

For HBV infection, acute HBV infection is often asymptomatic; however, about one-third of patients develop flu-like symptoms during the early stage of infection. The incubation period spans approximately 30–180 days, during which serological markers appear in a specific chronological sequence [21, 22]. Hepatitis B surface antigen (HBsAg) remains the primary marker for identifying active infection, and its persistence for more than 6 months is the defining criterion for chronic HBV infection [23, 24]. During the acute phase, the host immune response generates various antibodies, including Immunoglobulin M (IgM) and Immunoglobulin G (IgG) against the core protein [25]. Hepatitis B e antigen (HBeAg) serves as a key indicator of active viral replication and high infectivity, whereas its clearance and the subsequent emergence of anti-HBe antibodies often signify a transition towards lower replicative phases [26]. Although hepatitis B core antigen (HBcAg) remains undetectable in serum, anti-HBc antibodies are essential indicator of past or current infection and are extensively used in blood donor screening [27, 28]. Lastly, anti-HBsAg antibodies (anti-HBs) indicate protective immunity, whether acquired through natural infection or successful vaccination [26]. While these markers are helpful for staging, their clinical sensitivity is notably limited during the early diagnostic window period (Figure 2a).

1.2.2 | Hepatitis C Virus Serological Markers

HCV is characterised by an incubation period of approximately 7–8 weeks, during which most individuals remain asymptomatic, with up to 75% unaware of their infection [29, 30]. Only 20%–30% of patients present with mild symptoms, which further complicates early detection [31]. Current HCV diagnosis relies heavily on serological markers such as anti-HCV IgM and IgG antibodies (Figure 2b). However, these antibody-based tests cannot distinguish between active infections and resolved ones. Furthermore, false negatives may frequently occur in immunocompromised populations, such as haemodialysis patients, due to a delayed or diminished immune response [32, 33].

1.2.3 | The Role of Molecular Markers and NAT

During the diagnostic window period, when serological markers may be absent or inconclusive, diagnosis relies primarily on the detection of virological markers, specifically serum HBV DNA and HCV RNA, which directly indicate active viral replication (Figure 1, Table 1).

From a virological perspective, HBV is a small, enveloped virus containing a partially double-stranded, relaxed circular DNA (rcDNA) genome of approximately 3.2 kb. Upon infection, this rcDNA is transported into the host cell nucleus and converted into covalently closed circular DNA (cccDNA). This stable episomal reservoir serves as the essential transcriptional template for all viral mRNA and pregenomic RNA (pgRNA), the latter of which is reverse-transcribed into new DNA progeny. The persistence of cccDNA within the hepatocyte nucleus is the primary reason for chronic infection and the source of detectable DNA in the bloodstream [24, 34].

HCV, conversely, exhibits a markedly different genomic and replicative architecture. It possesses a positive-sense single-

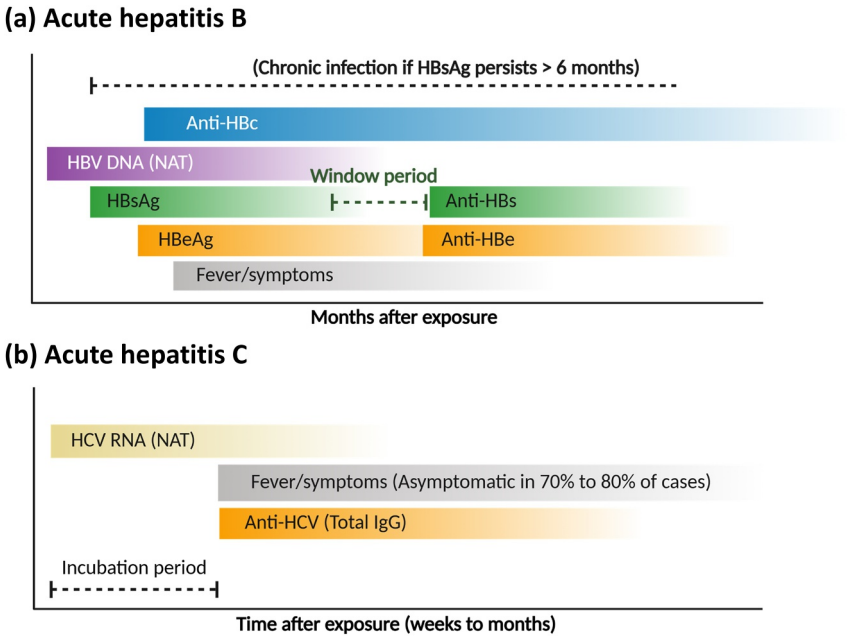

FIGURE 2 | Temporal profile of molecular and serological biomarkers in (a) acute hepatitis B and (b) acute hepatitis C. The schematic illustrates the kinetics of key viral markers, including NAT and antigens/antibodies, relative to the clinical incubation period and symptom onset. Image adapted from Kramvis et al., *Nat Rev Gastroenterol Hepatol*, 2022 [24] and Mondelli et al., *J Hepatol*, 2005 [36].

stranded RNA (+ssRNA) genome of approximately 9.6 kb. This genome consists of a single open reading frame encoding a large polyprotein that is subsequently cleaved into structural and non-structural proteins. Unlike HBV, HCV replicates exclusively in the cytoplasm and lacks a stable DNA intermediate. However, due to the absence of proofreading activity in its RNA-dependent RNA polymerase, HCV exhibits exceptionally rapid replication kinetics and high genetic diversity. Reported serum RNA titres can reach 10^5–10^7 IU/mL during the acute phase, which underscores the critical diagnostic value of nucleic acid-based assays for detecting the viral genome during this early stage [11, 32].

The presence of viral nucleic acids in the bloodstream serves as a reliable indicator of ongoing infection and a prognostic marker closely associated with liver disease progression, reflecting both viral replication dynamics and the severity of hepatic involvement [35]. Accordingly, NAT substantially shortens the diagnostic window period and enables the detection of low-level viraemia and occult infection. These advantages have established NAT as the gold standard for blood and organ donor screening. In addition, quantitative viral load measurements provide essential information for assessing transmission risk, monitoring treatment response, and guiding clinical disease management.

2 | Commercial Diagnostic Kits for HBV and HCV

A wide array of commercial diagnostic kits has been developed to address the diverse needs of HBV and HCV screening, ranging from rapid on-site testing to high-throughput laboratory quantification. Table 2 summarises several commercial kits currently utilised for identifying hepatitis-related antigens, antibodies, and viral genomes.

2.1 | Serological and Rapid Diagnostic Tests (RDTs)

Conventional serological screening predominantly utilises enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassays (CLIA), or lateral flow immunoassays (LFIA). Among these, LFIA are particularly valued for their rapid turnaround time, typically providing results within 15–20 min. This efficiency is crucial in clinical triage and epidemiological surveillance. For instance, the HBV-5 Rapid Test has been successfully deployed to assess HBV biomarkers in population-based studies, revealing a seroprevalence of 10.78% in specific cohorts [37, 38]. Similarly, the HCV Ab Plus Combo Rapid Test has demonstrated its utility in high-risk environments, such as correctional facilities in Nigeria, where it effectively identified high-seroprevalence rates of HIV, HBV, and HCV, highlighting the necessity for targeted interventions [39]. However, the trade-off for this speed can be sensitivity; comparative analyses of kits like the CTK Biotech HCV Ab Rapid Test have shown sensitivity and specificity rates of 84.72% and 100%, respectively, suggesting a potential risk of false negatives during early or low-titre infections [40].

2.2 | Molecular Diagnostic Platforms: The Gold Standard

In contrast to serological screening, molecular diagnostic kits are indispensable for confirming active infection, quantifying

TABLE 1 | Clinical significance of HBV and HCV serology and nucleic acid biomarkers.

Virus	Marker	Clinical significance	Primary clinical application
HBV	HBsAg	– HBsAg is the initial serological marker can be detected after HBV infection.	Diagnosis of active and chronic HBV infection; population screening; blood donor screening
		– Persistent positivity for over 6 months may indicate a potential transition to chronic carrier status.	
		– HBsAg is essential for diagnosing chronic HBV infection.	
	HBeAg	– HBeAg denotes active HBV infection with high infectivity and represents stages of active viral replication.	Assessment of viral replication status; treatment decision-making
		– HBeAg is associated with an increased risk of HCC.	
	Anti-HBe	– Anti-HBe appears between 8 and 16 weeks post-infection.	Monitoring disease phase and treatment response
		– Seroconversion from HBeAg to Anti-HBe denotes a lower level of infectivity and replication.	
		– Anti-HBe is associated with the asymptomatic carrier state.	
		– Anti-HBe indicates a reduction in the patient's infectivity and viral activity.	
	HBcAg	– HBcAg only be detected exclusively in liver tissue; absent in the bloodstream.	Not used as a routine serum biomarker
	Anti-HBc IgM	– Elevated levels are typically observed in acute hepatitis B infection.	Differentiation of acute infection from chronic flare or superinfection
		– Lower levels of Anti-HBc IgM are characteristic of chronic hepatitis B infection.	
		– Anti-HBc IgM indicate either current or previous HBV infection.	
		– It is useful for differentiating acute hepatitis B from superinfection by other hepatitis viruses.	
	Anti-HBc IgG	– Anti-HBc IgG indicates a past infection with HBV.	Epidemiological studies; infection history assessment
	Total Anti-HBc	– It is typically negative in healthy individuals.	Blood donors
		– Anti-HBc assesses past exposure to hepatitis B virus.	
		– It is crucial for detecting HBV infection during the 'window period' when HBsAg has cleared and HBsAb has not yet been produced.	
	Anti-HBs	– Anti-HBs appears following recovery from hepatitis B infection or post-vaccination, indicating immunity to HBV.	Confirmation of immunity; vaccination efficacy
		– The presence of anti-HBs confirms successful hepatitis B vaccination.	
		– Anti-HBs signifies recovery from hepatitis B and provides protective immunity against HBV reinfection.	

(Continues)

TABLE 1 | (Continued)

Virus	Marker	Clinical significance	Primary clinical application
	HBV DNA	– DNA positivity indicates an active replication phase of hepatitis B. – HBV DNA represents the viral load of hepatitis B in the body. – It is utilised as a monitoring marker during antiviral therapy.	Gold standard for active infection, window-period detection, treatment monitoring, and donor screening
HCV	Anti-HCV IgM	– Anti-HCV IgM marks the initial immune response to hepatitis C virus infection.	
	Anti-HCV IgG	– Anti-HCV IgG signifies either past or ongoing exposure to the hepatitis C virus, but does not confer immunity or indicate current infectious status. – Further assessment with HCV-RNA testing is advised to confirm infection status.	Initial screening test
	HCV core antigen (HCV Ag)	– Core antigen contains encapsulated HCV RNA, serving as a valuable alternative for quantifying viral nucleic acids. – Low sensitivity in samples with lower HCV NA levels	Alternative to NAT in limited settings
	HCV RNA	– RNA is utilised for assessing HCV viral load at various stages to determine the efficacy of antiviral treatment. – RNA positivity indicates ongoing hepatitis C virus activity and is recommended for patients who test positive for anti-HCV antibodies.	Gold standard for diagnosis of active infection, window-period detection, donor screening, and treatment monitoring

viral load, and determining genotypes. The commercial landscape is dominated by real-time PCR and transcription-mediated amplification (TMA) assays, which are widely integrated into centralised clinical and donor screening laboratories. Beyond standalone kits like the GeneProof HBV and HCV series, major diagnostic manufacturers provide fully automated, validated platforms. These include Roche (COBAS AmpliPrep/COBAS TaqMan), Abbott (RealTime HBV/HCV), Bio-Rad, and Grifols (Procleix Ultrio Elite). These high-throughput systems offer exceptional analytical sensitivity, typically ranging from 10 to 20 IU/mL for HBV DNA and 20–1000 IU/mL for HCV RNA [41–43]. Such precision is vital for monitoring treatment efficacy and closing the diagnostic 'window period' that limits serological assays.

2.3 | Challenges in Decentralised Testing

While laboratory-based molecular assays provide the highest diagnostic accuracy, their reliance on sophisticated instrumentation and stable infrastructure limits their use in decentralised or resource-limited settings. Conversely, while LFIA offer portability and speed, they often lack the sensitivity required for early detection. This technological gap underscores an urgent need for next-generation nucleic acid-based platforms that can deliver molecular-level sensitivity in a portable, point-of-care (POC) format. The following sections explore emerging molecular strategies designed to address these limitations.

3 | Emerging Methodologies for Hepatitis Virus Nucleic Acid Detection

NAT is pivotal for the accurate confirmation of hepatitis infections by directly identifying viral genetic material. This section focuses on the latest breakthroughs in HBV DNA and HCV RNA detection, highlighting innovative research transitioning towards clinical application and point-of-care testing (POCT) miniaturisation (Figure 3, Table 3).

3.1 | Advancements in Isothermal Amplification and CRISPR-Based Diagnostics for POC Diagnostics

Isothermal amplification has emerged as a robust alternative to conventional PCR-based techniques, particularly well-suited for POCT environments due to its constant temperature requirement [44]. These methods, such as Recombinase Polymerase Amplification (RPA) and Loop-mediated Isothermal Amplification (LAMP), leverage enzyme-driven mechanisms to eliminate the need for sophisticated thermal cyclers, thereby streamlining diagnostic workflows. Parallel to these

TABLE 2 | Current commercially available test kits for HBV- and HCV-related biomarkers.

Product	Brand	Methods	Turnaround time	Analyte	Sensitivity (LOD)
AMD Human Hepatitis B Virus (HBV) kit	Advanced molecular diagnostics	Real time PCR	—	HBV genotype A, B, C, D, E, F and H	20 IU/mL
AccuPower HBV Quantitative PCR Kit	Bioneer corporation	Real-time PCR	—	HBV genotype A, B, C, D, E, F and H	—
Cobas HBV	Roche diagnostics	Real-time PCR	3 h	HBV genotype A, B, C, D, E, F and H	10–20 IU/mL
Diagnostic Kit for Quantification of Hepatitis B Virus DNA	Da An Gene Co. Ltd.	Real-time PCR	—	HBV DNA	10 IU/mL
AltoStar HBV PCR Kit 1.5	Altona diagnostics GmbH	Real-time PCR	—	HBV genotypes A to H	10.2 IU/mL
GeneProof Hepatitis B Virus (HBV) PCR Kit	GeneProof a.s.	Real-time PCR	—	HBV genotypes A–H (P gene)	13.9 IU/mL
RevoDx HBV qPCR Kit	Idil Biotech	Real-time PCR	—	All HBV genotypes	10 IU/mL
HBV Quantitative PCR Test Kit	ACON Laboratories, Inc.	Real-time PCR	—	Hepatitis B virus	20 IU/mL
Hepatitis B virus (HBV) TaqMan PCR Detection Kits	Norgen Biotek Corp.	Real-time PCR	33 min	HBV DNA	—
Architect HBsAg Qualitative II Assays	Abbott Diagnostics, Abbott Laboratories	Electro-CLIA (E-CLIA)	30–40 min	HBsAg	0.05 IU/mL
HBV-5 Rapid Test	CTK Biotech Inc.	LFIA	15 min	HBsAg, HBsAb, HBeAg, HBeAb, HBcAb	—
Anti-HBc IgM ELISA Test Kit	InTec PRODUCTS INC.	ELISA	—	Anti-HBc IgM	—
HBsAg Rapid Quantitative Test	Biotime Biotechnology Co. Ltd.	LFIA	10 min	HBsAg	—
Wondfo One Step HBsAb Serum/Plasma Test	Wondfo Biotech Co. Ltd.	LFIA	15 min	HBsAb	—
HBV 5 in 1 Combo Test Panel	Henso Medical (Hangzhou) Co. Ltd.	LFIA	10–15 min	HBsAg, HBsAb, HBeAg, HBeAb, HBcAb	—
Healgen HBsAg Rapid Test Strip	Healgen Scientific Limited Liability Company, Houston	Chromatographic immunoassay (CIA)	—	HBsAg	
Hepatitis C (HCV RNA) PCR kit	Sacace Biotechnologies	Real-time PCR	—	HCV genotypes (tested genotypes: 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 4a, 4c, 4d, 5a, 6a) (UTR)	20 IU/mL
COBAS AmpliPrep/ COBAS TaqMan HCV Test	F. Hoffmann-La Roche Ltd	Real-time PCR	3 h	HCV genotypes 1–6	15–25 IU/mL
GeneProof Hepatitis C Virus (HCV) Diagnostic PCR kit	GeneProof a.s.	Real-time PCR	—	HCV genotypes 1–8 (5‘ UTR)	> 7.95 IU/mL
HCV Quantitative PCR Test Kit	ACON Laboratories Inc.	Real-time PCR	—	HCV RNA	30 IU/mL

(Continues)

TABLE 2 | (Continued)

Product	Brand	Methods	Turnaround time	Analyte	Sensitivity (LOD)
Hepatitis C Virus Genotype Diagnostic Kit	Sansure Biotech Inc.	Real-time PCR	—	HCV genotypes 1b, 1, 2, 3, 4, 5 and 6	1000 IU/mL
Real-TIME PCR Detection Kit (HCV)	DNA-technology Research & Production	Real-time PCR	45 min	HCV genotypes 1a, 1b, 2, 3, 4, 5, 6	15 MU/mL
AltoStar HCV RT-PCR Kit 1.5 RUO	Altona Diagnostics GmbH	Real-time PCR	78 min	HCV genotypes 1 to 6	—
ELISA Diagnostic Kit for Antibody to Hepatitis C Virus	InTec PRODUCTS INC.	ELISA	—	HCV antibodies	—
HCV Rapid Test Kit	NewScen Coast Bio-Pharmaceutical Co. Ltd. (NewScen)	CIA	20 min	HCV antibodies	—
HCV Ab Plus Combo Rapid Test	CTK Biotech Inc.	LFIA	—	Anti-hepatitis C virus antibodies (IgG, IgM, IgA)	—
First Response HCV Card Test	Premier Medical Corporation Private Limited	CIA	15–20 min	HCV Ab	
HCV IgG ELISA	CTK Biotech Inc.	Solid phase ELISA	—	HCV IgG	—
Nucleic Acid Test Kit for HBV, HCV, HIV (Type1 + 2)	Sansure	Real-time PCR	4.5–5 h	HBV DNA, HCV RNA, HIV-1 RNA and HIV-2 RNA	HBV 2.41 IU/mL HCV 12.38 IU/mL HIV-1 31.68 IU/mL HIV-2 48.34 IU/mL
AmpliSens HCV/HBV/HIV-FRT	MoBiTec GmbH	Multiplex PCR	90 min	HCV/HBV/HIV-1/HIV-2	HCV 10–100 IU/mL HBV 5–50 IU/mL HIV-1 20-200 copies/mL HIV-2 60-600 copies/mL
GenesigPLEX qPCR Kit HBV/HCV/HIV1/HIV2	Primerdesign Ltd	Multiplex qPCR	72 min	Human hepatitis viruses B and C and human immunodeficiency viruses 1 & 2	100 copies
HIV-1 HIV-2 HCV and HBV Four-in-One Nat Test Kit	Suzhou Bacme Biotech Co. Ltd.	Real-time PCR	—	HIV-2, HIV-1, HBV, HCV	HBV DNA 4.2 IU/mL HCV RNA 12.5 IU/mL HIV RNA 42.3 IU/mL

(Continues)

TABLE 2 | (Continued)

Product	Brand	Methods	Turnaround time	Analyte	Sensitivity (LOD)
Nucleic Acid Test Kit for HBV, HCV HIV (Real-time PCR)	Da an Gene Co. Ltd.	Real-time PCR	60 min	HBV/HCV/HIV-1	100–500 IU/mL
Procleix Ultrio Elite Assay	Grifols Diagnostic Dolutions Inc.	TMA	3–4 h	HCV genotypes 1–6 (subtypes 1a, 1b, 2a/c, 2b, 3a, 3b, 3e, 4a, 4b/c, 5a, 6a), HBV genotypes A-H	—
HIV, HBsAg, HCV & Syphilis Combo Rapid Test	Biopanda Reagents Ltd	LFIA	10 min	HIV type 1 and type 2 antibodies, HBsAg, HCV antibodies, *Treponema Pallidum* (TP) syphilis antibodies (IgG and IgM)	—
HBsAg/HCV Ab Rapid Test	CTK Biotech	LFIA	15 min	HBsAg and anti-hepatitis C virus antibodies (IgG, IgM, IgA)	—
Accu-Tell HBsAg/HCV Combo Rapid Test Cassette (serum/plasma)	AccuBioTech Co. Ltd.	CIA	20 min	HBsAg and anti-hepatitis C virus antibodies	—
HBsAg HIV HCV Panel Test Card	Xiamen Boson Biotech Co. Ltd	LFIA	20 min	HBsAg, HIV I&II antibodies, HCV antibodies	—

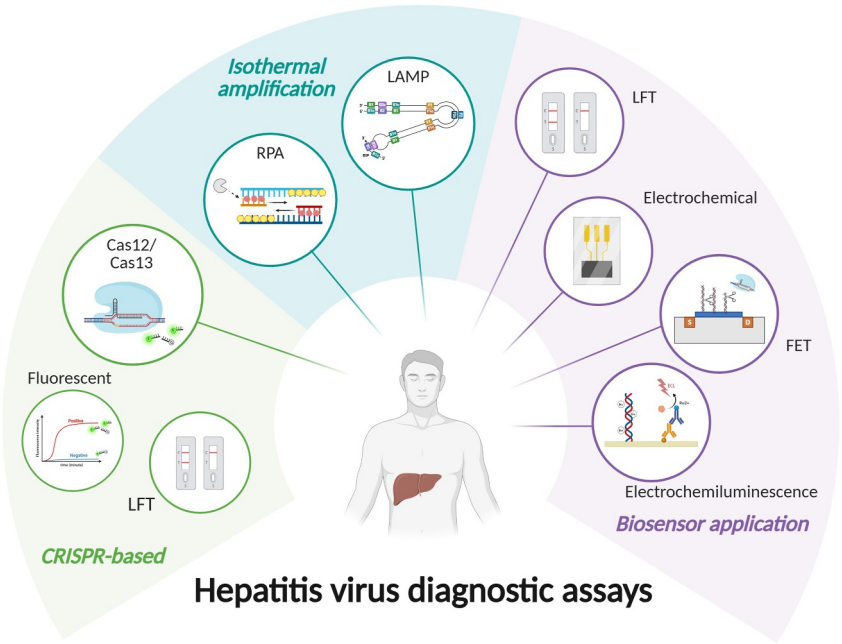

FIGURE 3 | Biosensing technologies for hepatitis virus detection. FET, field-effect transistor; LAMP, loop-mediated isothermal amplification; LFT, lateral flow testing; RPA, recombinase polymerase amplification.

advancements, CRISPR-Cas systems have revolutionised POC diagnostics by providing a highly specific molecular recognition and signal-reporting mechanism. While isothermal techniques facilitate rapid target enrichment, their integration with CRISPR-based detection ensures high precision in identifying viral sequences. The following sections detail the progress in these synergistic technologies, focussing on RPA, LAMP, and their integration with CRISPR platforms.

TABLE 3 | Summary of methods, detection time, and LOD of existing diagnostic methods.

Target	Method	Sample	Amplification	Preparation time	Detection time	LOD	Ref.
HBV	Paper-based electrochemical sensor (oSlip-DNA)	HBV DNA	—	195 min	< 5 min	85 pM	[82]
HBV	Electrochemical LFA, acpcPNA	HBV DNA	—	30 min	2 min	7.23 pM	[102]
HBV	Lateral flow strip combined with RPA (LF-RPA)	HBV DNA	RPA	25 min	30 min	10 copies/reaction	[74]
HBV	Automated centrifugal microfluidic chip (RPA-T7-cas13a)	HBV DNA	RPA	2 h	1 h	—	[103]
HBV	RAA-CRISPR-Cas13a (fluorescence assay/strip assay)	HBV DNA	RAA	30 min	30 min/ 3 min	10 copies/μL	[61]
HBV	LAMP-Cas12a (fluorescent readout and by lateral flow test strips)	HBV DNA	LAMP	> 30 min	13 min/ 20 min	1 copy/μL	[60]
HBV	Fluorescence signal/ immuno-chromatography strip	HBV DNA	LAMP		60 min/ 10 min	10 copies	[49]
HBV	Fluorescence signal	HBV DNA (genotypes/sub-genotypes A1/2/ 3/B/C/D/E/f)	LAMP		60 min	40 IU/mL	[52]
HBV	Electrochemical or fluorescent method	HBV DNA (genotype A-F)	LAMP		60 min	6.18 fg/μL	[54]
HCV	Electrochemical biosensor (differential pulse voltammetry)	Surface antigen (E2)	—	> 20 min	—	2.1×10^{-5} mg/mL	[104]
HCV	Electrochemical aptasensor (electrochemical impedance spectroscopy)	HCV core antigen	—	> 1 h	—	3.3 pg/mL	[105]
HCV	Colourimetric assay	Anti-HCV antibody	RCA	—	30 min	0.998 pM	[106]
HCV	Differential pulse voltammetry	HCV RNA	—	> 6 h	—	1×10^{-15} mol/L	[83]
HCV	Cyclic voltammetry	HCV RNA	—	3 h	20 min	3.1×10^{-22} M	[88]
HCV	Alternating-current electroluminescence (ACEL)	HCV cDNA	—		30 min	1.96 pM	[102]
HCV	Nucleic acid fluorescence-based	HCV RNA genotypes 1–6	RT-RPA		15 min	10 copies/reaction	[46]
HCV	Lateral flow dipstick	HCV C/E1 genes	RT-RAA	5–15 min	5–15 min	10 copies/μL	[75]
HCV	RT-LAMP-coupled CRISPR–Cas12 assay (lateral flow strip/ fluorescence detector)	HCV RNA (5' NCR)	RT-LAMP	60 min	30 min	10 ng/μL	[62]

(Continues)

TABLE 3 | (Continued)

Target	Method	Sample	Amplification	Preparation time	Detection time	LOD	Ref.
HCV	ITO-based EGFET biosensor	HCV RNA	RT-LAMP	1 day	20 min	1 genomic copy/reaction	[101]
HBV/HCV	Lateral-flow strip	HBV DNA, HCV RNA	m-LAMP	—	25 min	HBV: 10 genomic copies/reaction, HCV: 10^3 genomic copies/reaction	[78]

3.1.1 | Recombinase Polymerase Amplification (RPA)

RPA employs recombinase and single-stranded binding proteins to facilitate primer insertion, coupled with strand-displacement DNA polymerase for rapid amplification [45]. For instance, studies have demonstrated that RPA can achieve detection limits as low as 25 copies per reaction for HCV, showcasing its potential for rapid and accurate diagnosis in clinical settings [46]. Additionally, Yi et al. suggested that the betaine-assisted RPA assay could reduce non-specific amplification, thereby increasing the overall specificity of the assay [47]. This optimised assay successfully detected HBV in clinical samples, demonstrating a high correlation with results obtained from conventional gold-standard PCR.

3.1.2 | Loop-Mediated Isothermal Amplification (LAMP)

The LAMP assay utilises Bst DNA polymerase, which possesses high auto-cycling strand displacement activity, in combination with a set of six specialised primers [48]. This enzyme enables the reaction to proceed at a constant temperature between 60°C and 65°C, typically using a simple water bath or heating block, and is capable of producing up to 10^9 target copies within a single hour. The resulting amplicons can be detected using handheld fluorescence scanners, a feature that significantly enhances its suitability for POCT in resource-limited settings [49].

The high sensitivity and specificity of LAMP for HBV detection have been extensively documented. For instance, studies have shown that LAMP can detect HBV DNA in serum samples with a detection limit as low as 6 copies, making it highly effective for identifying occult HBV infections that might be missed by conventional serological methods [50]. Furthermore, the assay has been validated for its ability to differentiate between HBV genotypes B and C or detect pan-genotype detection through the design of primers targeting highly conserved viral regions [51, 52]. Such rapid turnaround is particularly beneficial for maternal viral load screening in antenatal care within LMICs, where timely results are critical for determining treatment protocols to prevent vertical transmission [53]. Moreover, the integration of LAMP with portable platform enhances its applicability in field settings [54–56].

3.1.3 | CRISPR-Based Approaches for Hepatitis Testing

Originally recognized for its revolutionary role in precision genome editing, Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) technology has recently expanded into the diagnostic realm, emerging as a transformative tool through the exploitation of the collateral cleavage activity inherent in specific Cas enzymes (e.g., Cas12a or Cas13). Once triggered by the specific recognition between the CRISPR RNA (crRNA) and the target sequence, these enzymes non-specifically degrade adjacent quenched reporters to yield a detectable signal [57–59]. Integrating CRISPR with isothermal pre-amplification (e.g., LAMP and RPA) significantly boosts sensitivity and specificity, with results readable via visual lateral flow strips or fluorescence detectors [60–62]. However, while this pre-amplification process is essential for achieving low detection limits, it also increases the risk of cross-contamination. To minimise these risks, one-pot assays that combine nucleic acid amplification and CRISPR detection in a single step have been developed [63–66], although these systems can suffer from poor sensitivity due to reagent competition, which can be effectively mitigated by the addition of glycerol [67]. Beyond these procedural challenges, a primary limitation of CRISPR-based diagnostics is the requirement for short sequence motifs, such as the protospacer adjacent motif (PAM) or protospacer-flanking site (PFS), which can restrict the selection of target regions and complicate assay design [68, 69]. Recent advancements suggest that optimising and adjusting these PAM sequences may help further improve the sensitivity and specificity of hepatitis detection assays [70].

3.1.4 | Lateral Flow-Based Assays for Hepatitis Virus Detection

In the field of viral hepatitis diagnostics, lateral flow assays (LFAs) are primarily established for the qualitative screening of serological markers, including viral antigens and host antibodies. By simplifying operational procedures and minimising equipment requirements, these protein-based LFAs offer a convenient and cost-effective solution for initial screening in diverse clinical environments. The functional performance of an LFA depends on its architecture, which generally consists of several zones comprising different membrane materials [71, 72]. To further enhance sensitivity, implementing specialised architectures, such as irregular sample pad geometries or

integrated sponge shunts, can significantly modulate fluid flow rates. These modifications facilitate more thorough interactions between target analytes and detection reagents, effectively enhancing analytical sensitivity by tenfold and reducing the HBV DNA detection limit from 10^4 to 10^3 copies/mL [73].

For hepatitis NAT, the most straightforward application of LFA is its use as a visual readout integrated with isothermal amplification techniques, such as RPA [74], RAA [75] or LAMP [55, 76, 77]. This integration combines the high sensitivity of molecular amplification with the simplicity of paper-based detection. A recent study by Guo et al. developed a multiplex LAMP-LFA test strip capable of simultaneously detecting HBV and HCV [78]. This device achieved limit-of-detections (LODs) of 10^1 and 10^3 genomic copies per reaction for HBV and HCV, respectively. The authors further validated the feasibility of this device using clinical samples spanning various genotypes, confirming its reliability for decentralised testing. By bridging isothermal amplification with LFA, these integrated platforms enable highly sensitive and stable detection of hepatitis viral nucleic acids, facilitating effective POC management even in remote or resource-limited settings.

3.2 | Advanced Electrochemical and Electronic Biosensing Platforms

3.2.1 | Electrochemical and Electrochemiluminescence-Based Biosensors

The development of biosensors for medical diagnostics has rapidly expanded, with electrochemical sensing emerging as a promising approach due to its high sensitivity, rapid response, and compatibility with miniaturised POC platforms. Electrochemical biosensors transduce specific biochemical recognition events into measurable electrical signals, enabling stable and quantifiable nucleic acid detection [79–81].

For the detection of hepatitis viruses, redox-based or electroactive electrochemical sensing strategies have been widely reported. For instance, Li et al. developed a paper-based electrochemical sensor for HBV DNA detection using a metalloimmunoassay format (Figure 4a) [82], while Roohizadeh et al. demonstrated a label-free electrochemical nanobiosensor for HCV RNA detection based on differential pulse voltammetry using a fluorine-doped tin oxide electrode modified with copper oxide and gold nanoparticles on nitrogen-doped graphene, achieving high recovery rates in clinical samples (Figure 4a) [83].

Given that viral load is closely associated with disease progression, electrochemiluminescence (ECL)-based platforms have been increasingly implemented for quantitative analysis. In these biosensors, recognition events are converted into optical signals through electrochemically driven reactions, offering enhanced sensitivity and reduced background noise. For HBV DNA detection, Nikolaou et al. utilised $[Ru(phen)_2dppz]^{2+}$ complex as a highly efficient intercalating dye, enabling detecting HBV genome by surface cooperative hybridisation and ECL transduction. Based on the specific hybridisation between the target viral genome nucleic acid and the surface-immobilised capture probe, the presence of the complete HBV viral genome modulates the local electrochemical environment, promoting efficient ECL emission of the luminescent material under an applied potential. Importantly, $[Ru(phen)_2dppz]^2$ provides strong signal amplification, enabling the detection of the HBV genome can reach a LOD of 0.1 a.m. [84] Furthermore, Gou et al. developed an 'off–on' ECL biosensor where DNA probes are immobilised on a glassy carbon electrode modified with ternary AgAuPt nanocubes [85]. While this method achieves an exceptional detection limit of 65 a.m., it necessitates repetitive washing steps to remove

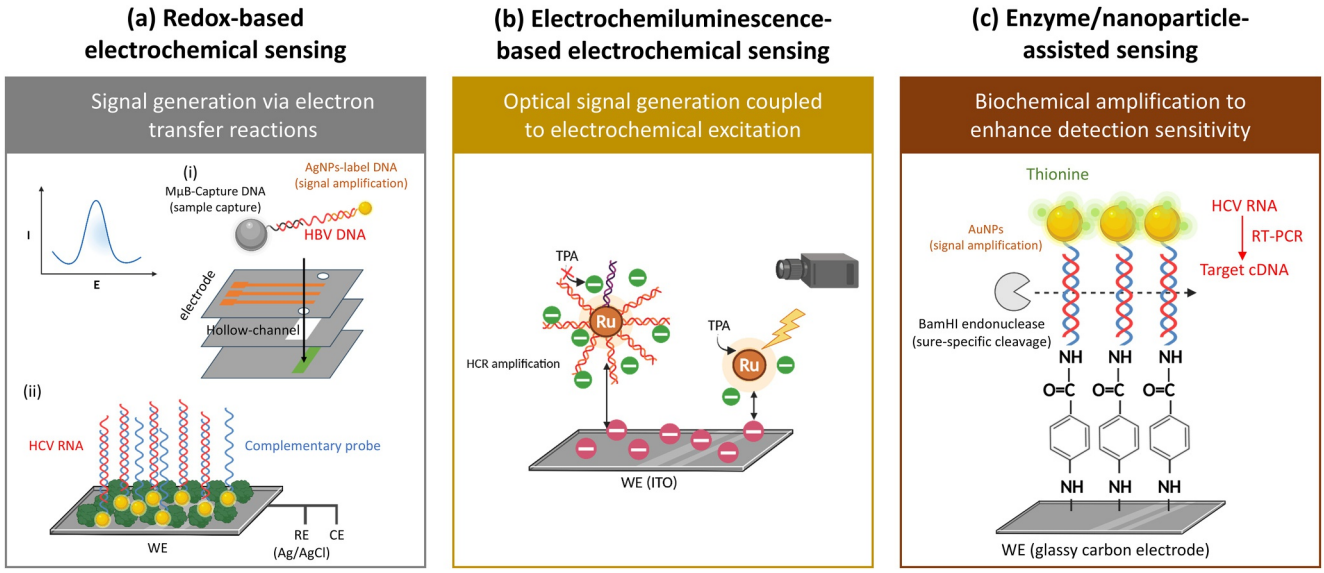

FIGURE 4 | Electrochemical biosensing strategies for viral nucleic acid detection. These platforms leverage surface functionalisation and electron transfer kinetics to generate quantifiable signal outputs. Shown are representative mechanisms of: (a) redox-mediated electrochemical sensing, (b) ECL-based signal generation, and (c) enzyme- or nanoparticle-assisted signal amplification. The schematic figures are redrawn from Li et al., *Anal Chem*, 2015 [82]; Roohizadeh et al., *Curr Res Biotechnol*, 2020 [83]; Luo et al., *Anal Chem*, 2023 [86]; Liu et al., *Anal chem*, 2011 [88].

unbound interferents from the electrode surface to ensure signal accuracy. To further simplify analytical procedures, Luo et al. reported a homogeneous (wash-free) biosensor that leverages the programmable specificity of CRISPR/Cas12a and the amplification efficiency of the hybridisation chain reaction (HCR) (Figure 4b) [86]. In this modality, $Ru(bpy)_3^{2+}$-doped silica nanoparticles ($Ru@SiO_2$ NPs) serve as charge-controlled luminescent bodies, where CRISPR-mediated collateral cleavage regulates the DNA length on the particles to modulate ECL efficiency, achieving a detection limit of 7.41 fM.

Furthermore, the integration of enzyme- and nanoparticle-assisted amplification strategies has been explored to further enhance analytical performance [87]. For example, Liu et al. developed an electrochemical platform combining gold nanoparticle (AuNP) amplification with sequence-specific endonuclease recognition for the identification of HCV genotype 1b (Figure 4c) [88]. In this approach, DNA probes immobilised on the electrode hybridise with target sequences to form structures containing genotype-specific cleavage sites. The *Bam*HI endonuclease selectively cleaves perfectly matched genotype 1b targets, thereby regulating the retention of thionine-labelled AuNPs. Because the electrochemical signal is directly correlated with the density of nanoparticles remaining on the electrode, this enzymatic recognition facilitates precise genotype-level discrimination.

Overall, electrochemical sensing strategies have demonstrated high sensitivity and selectivity for hepatitis virus nucleic acid detection by leveraging specific biorecognition events and nanomaterial-assisted signal amplification. However, optical signal readout introduces additional complexity in alignment, power consumption, and system integration, which can hinder seamless miniaturisation. These constraints highlight the growing need for simpler and more readily integrable electrical sensing platforms, thereby motivating the development of field-effect transistor (FET)–based diagnostic technologies.

3.2.2 | Field-Effect Transistor (FET) Technologies for Electrical Sensing

With advances in the semiconductor industry, solid-state diode and transistor technologies have become fundamental components of modern electronics, accelerating their adoption in chemical and biological sensing applications. The operating principles of metal-oxide-semiconductor field-effect transistors (MOSFETs), which have been predominantly used in integrated circuits during the past few decades, have been adapted to enable charge-based detection in chemical and biological sensing platforms [89]. A typical MOSFET consists of a silicon substrate where the source (S) and drain (D) terminals are connected by a semiconductor channel. The conductance of this channel is modulated by an electric field applied through a gate electrode (G) across a thin dielectric layer [90–92]. By extending this charge-modulation principle to biological interfaces, FET-based biosensors enable direct, label-free electrical transduction of nucleic acid recognition events. Recent advances in ion-sensitive field-effect transistor (ISFET) systems [93] and extended-gate field-effect transistors (EGFETs) further highlight the potential of these platforms for nucleic acid diagnostics. Based on their signal transduction mechanisms, these sensors can be categorised into probe-based FET, amplification-couple ISFET, and CRISPR-EGFET platform (Figure 5).

Probe-based FET sensing is the most direct method for identifying targets. In this configuration, nucleic acid hybridisation probes are directly modified on the gate surface to modulate the electric field at the transistor channel. Shariati et al. developed an ultrasensitive, label-free FET biosensor based on ZnO-doped

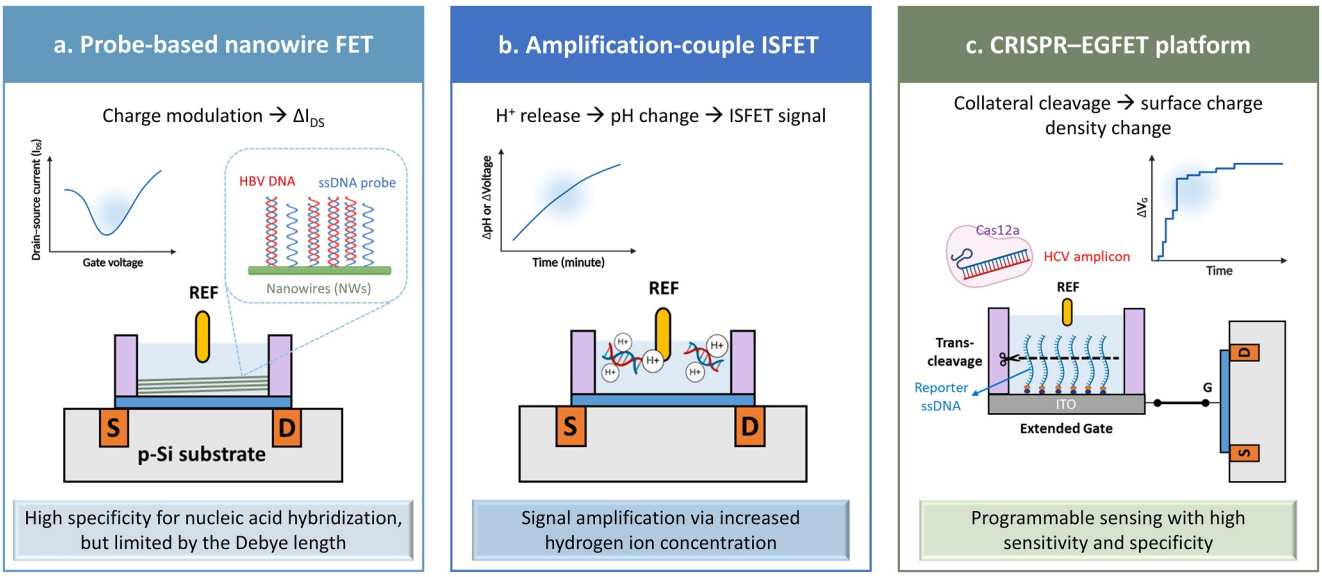

FIGURE 5 | Classification of FET-based viral nucleic acid biosensors according to their signal transduction mechanisms. (a) Probe-based nanowire FET, (b) Amplification-couple ISFET, (c) CRISPR-EGFET platform. The schematic figures are redrawn from Shariati et al., *Anal Chim Acta*, 2021 [94]; Jogezai et al., *Anal Methods*, 2018 [99]; Ho et al., *Sens Actuators B Chem*, 2023 [101].

MoS_2 nanowires for HBV DNA detection in blood serum (Figure 5a) [94]. In this platform, the nanowires function as the semiconducting channel, and hybridisation with complementary HBV DNA induces surface charge accumulation. This results in the electrostatic modulation of carrier density within the channel, leading to corresponding changes in drain–source current. The biosensor demonstrated a rapid response and a sensitivity of approximately ~1 fM.

However, FET biosensors also face challenges such as stringent requirements for physiological samples and Debye length limitations. Detection sensitivity depends on the increased negative charge density at the gate surface, but in high-salt environments, the Debye shielding effect screens this charge. This leads to a shorter Debye length and significantly reduced detection capability, as target charges outside this screening layer remain 'invisible' to the transistor channel [95–97]. To overcome these limitations, combining amplification reactions to increase the concentration of hydrogen ions generated in the solution is an effective solution to improve sensitivity. Toumazou et al. demonstrated an amplification-coupled detection method for directly measuring released protons during nucleotide incorporation using ISFET sensor [98]. Additionally, the hand-held ISFET–LAMP system (STALLION) developed by Jogezai et al. integrates loop-mediated isothermal amplification with ISFET-based pH sensing for the rapid detection of HCV RNA (Figure 5b) [99]. In this system, protons released during amplification are directly monitored by the ISFET, enabling real-time electrical readout and reducing reliance on bulky optical instrumentation.

Beyond ion-mediated sensing, integrating with programmable CRISPR nucleases extends the mechanistic synergy between CRISPR collateral cleavage activity and transistor-based electrical readout. Li et al. developed a CRISPR–FET biosensor that employs dual Cas13 RNP complexes to simultaneously target two distinct regions of the viral genome [100]. The use of a dual-RNP strategy markedly enhances sensing performance, achieving an approximately 20-fold improvement in sensitivity and an ultralow LOD of 1.56 a.m. without pre-amplification. Furthermore, many FET platforms currently employed for biomolecular sensing are based on conventional ISFET or channel-integrated FET architectures, in which the sensing interface is intrinsically coupled to the transistor channel. Such configurations complicate surface re-functionalisation for different biomarkers and raise practical concerns related to device reuse and hygiene when handling clinical samples. To overcome these limitations, Ho et al. introduced a miniaturised CRISPR-powered EGFET biosensor based on indium tin oxide (ITO) (Figure 5c) [101]. This EGFET architecture spatially decouples the disposable sensing surface from the reusable transistor unit, thereby enhancing modularity, operational flexibility, and cost efficiency. In this platform, a LAMP–CRISPR/Cas12a workflow is integrated to achieve rapid identification of multiple HCV genotypes within 20 min. Non-target ssDNA reporters immobilised on the ITO surface function as signal transducers; upon target recognition, activated Cas12a induces *trans*-cleavage of these probes, leading to changes in surface charge that are directly converted into electrical signals by the EGFET. This system achieved a detection sensitivity as low as one viral genomic copy per reaction, underscoring the potential of EGFET-based architectures for scalable and POC viral diagnostics.

4 | Conclusions

Despite therapeutic milestones including HBV vaccines and HCV direct-acting antivirals, identifying infected individuals remains the primary barrier to viral hepatitis elimination, particularly in high-prevalence, resource-limited regions. Achieving the WHO 2030 targets necessitates a decisive transition from centralised laboratory testing towards decentralised POC solutions. This review identifies a powerful synergy among isothermal amplification, CRISPR-based recognition, and electronic biosensing platforms, specifically electrochemical and FET-based technologies. These platforms enable label-free and sensitive detection, while EGFET architectures enhance clinical modularity by decoupling the sensing interface from the transducer. Overcoming current bottlenecks in sample preparation and charge-screening effects will require standardized, 'sample-to-answer' systems that bridge biochemical assays with solid-state electronics. Ultimately, the convergence of molecular biology and semiconductor engineering will provide the scalable and accessible diagnostic tools required to bridge the gap between treatment availability and disease detection, thereby accelerating global health initiatives towards the elimination of viral hepatitis.

Author Contributions

Hsin-Ying Ho: conceptualisation, investigation, data analysis, writing – original draft. **Chia-Yen Dai, I-Jung Feng, Yen-Hsu Chen, Ming-Lung Yu:** conceptualisation, writing – review, supervision, funding. **Che-Hsin Lin:** Research cooperator and experimental support for FET-based biosensing systems, funding. **Ling-Shan Yu:** conceptualisation, writing – review and editing, funding, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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