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TOPIC HIGHLIGHT

2015 Advances in Hepatitis B virus

Rapid and quantitative detection of hepatitis B virus

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Abstract

Despite availability of a universal vaccine, hepatitis B virus (HBV) infection has a huge impact on public health worldwide. Accurate and timely diagnosis of HBV infection is needed. Rapid developments have been made in the diagnostic and monitoring methods for HBV infection, including serological and molecular assays. In clinical practice, qualitative hepatitis B surface antigen (HBsAg) testing has long served as a diagnostic marker for individuals infected with HBV. More recently, HBsAg level has been used to predict treatment outcome when determined early during treatment or at baseline. However, identification of HBV DNA positive cases that do not have detectable HBsAg has encouraged the application of molecular tests. Hence, combination of quantitative detection of HBV DNA and HBsAg can be used to discriminate patients during the course of HBV infection and to monitor therapy. This article reviews the most commonly used quantitative methods for HBsAg and HBV DNA.

Key words: Hepatitis B virus; Biosensor; Polymerase chain reaction; Isothermal amplification methods; Quantitative assay

Core tip: The combination of quantitative detection of hepatitis B surface antigen (HBsAg) and hepatitis B virus (HBV) DNA can be used to classify individuals during the course of HBV infection and to monitor therapy. The most popular platforms for HBsAg detection are based on chemiluminescent microparticle immunoassay, while polymerase chain reaction based methods are widely used for HBV DNA assay. Recently, isothermal amplification and biosensors offered a lower cost and more rapid alternative for HBV quantification. This article reviews the most commonly used quantitative methods for HBV.

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INTRODUCTION

Hepatitis B virus (HBV) is an enveloped virus with a small (3.2 kb) partially double-stranded DNA genome that causes acute and chronic infections[1]. The impact of HBV infection on public health is enormous, with an estimated prevalence of 2 billion infected and 360 million chronically infected[2]. The diagnosis of HBV infection relies heavily on serological and molecular tests. Serological tests use serum-based blood tests that can be analyzed by enzyme immunoassays either qualitatively or quantitatively[3]. The tests identify virus-encoded antigens and their corresponding antibodies: hepatitis B surface antigen (HBsAg), anti-HBs, hepatitis B e antigen (HBeAg), anti-HBe, and antibodies to hepatitis core antigen (anti-HBc). In contrast, the molecular tests focus on quantitative viral load, genotyping, drug resistance mutations, and core promotion/pre-core mutation assays.

In clinical practice, qualitative assays for HBsAg have served as diagnostic markers for patients with HBV infection. More recently, quantitative methods for HBsAg have been used to predict treatment outcome when determined early during treatment or at baseline[4]. However, identification of HBV-DNA-positive cases, which do not have detectable HBsAg, is greatly encouraging for the application of molecular testing. This article reviews the most commonly used quantitative detection methods for HBsAg and HBV DNA (Figure 1).

QUANTITATIVE METHODS FOR HBsAg

Since the discovery of HBsAg in 1965[5], it has served as a biomarker for the diagnosis of HBV infection. Methods for HBsAg detection were first described in the 1970s using electron microscopy, radioimmunoassay, and enzyme immunoassays[6-8], which were cumbersome, labor intensive, and restricted to a research setting. Since then, various diagnostic methods have been developed for quantitative HBV detection.

In the early 1990s, HBsAg quantification was considered to be a simple, promising, and inexpensive method to monitor viral replication in chronic hepatitis B (CHB) patients who were receiving interferon (IFN) therapy[9]. HBsAg quantification is associated with the concentration of covalently closed circular DNA (cccDNA), the persistent intrahepatic form of HBV DNA[10,11]. The amount of circulating HBsAg is hypothesized to be predictive for response to antiviral therapy. Currently, standard quantitative HBsAg assays have been developed, which are fully automated and have high throughput. Two commercially available assays are briefly introduced here.

The Architect HBsAg QT (Abbott Laboratories, Abbott Park, IL, United States) is an automated chemiluminescent microparticle immunoassay method, which is the most widely used assay in clinical practice[12]. The Architect HBsAg QT assay is a two-step immunoassay with flexible assay protocols, referred to as Chemiflex, for quantitatively measuring HBsAg concentrations in serum and plasma[13]. The Architect HBsAg system can detect as low as 0.2 ng/mL HBsAg with a dynamic range of 0.05-250.0 IU/mL (1 IU/mL is equivalent to 1-10 ng/mL HBsAg)[14]. The assay is capable of processing up to 800 tests per hour.

Elecsys HBsAg Ⅱ (Roche Diagnostics, Indianapolis, IN, United States) is another popular assay for HBsAg quantification[15]. Elecsys HBsAg Ⅱ is a "sandwich" assay with a total testing time of 18 min. The results are reported as a cutoff index (signal sample/cutoff). The sample is considered nonreactive when the index is < 0.9, while samples with index value > 1.0 are interpreted as reactive[15].

These two methods can be confidently used for HBsAg quantification for the most prevalent HBV genotypes. These tests are easy to use, inexpensive, and have a rapid turnaround time; the analytical performance of the assays is generally satisfactory.

QUANTITATIVE METHODS FOR HBV DNA

With the increased prevalence of serologically negative HBV infections (HBeAg negative CHB and occult HBV infection) and the rapid advent of diagnostic escape mutants, the detection of HBV DNA has gained more attention in clinical medicine[16]. The detection of HBV DNA in peripheral blood is a reliable marker of HBV activity, while high levels of HBV DNA are associated with a higher incidence of hepatocellular carcinoma (HCC) and more rapid progression to cirrhosis[17]. In addition, HBV DNA detection is beneficial in routine clinical practice to identify individuals who need anti-viral treatment and provide them the most suitable therapy[18]. Nowadays, a variety of molecular technologies have been used in HBV DNA quantification, such as ultraviolet (UV) spectrophotometry, real-time polymerase chain reaction (PCR), digital PCR, isothermal amplification methods, and biosensors[19-22]. Here, we review the rapid and quantitative methods that have been used for HBV DNA detection.

UV spectrophotometry

The aromatic rings of the bases absorb UV light with a maximum peak at 260 nm. When a beam of UV light shines through a sample containing DNA or RNA, the amount of UV absorption by the sample depends on the DNA or RNA concentration. The amount of UV light absorbed by a series of standard DNA amounts is measured to calibrate the technique, and the ratio of UV light absorbed by the unknown sample is measured and plotted on the calibration curve to deduce the concentration of DNA or RNA.

NanoDrop instruments (Thermo Scientific, Wilmington, DE, United States) are based on UV

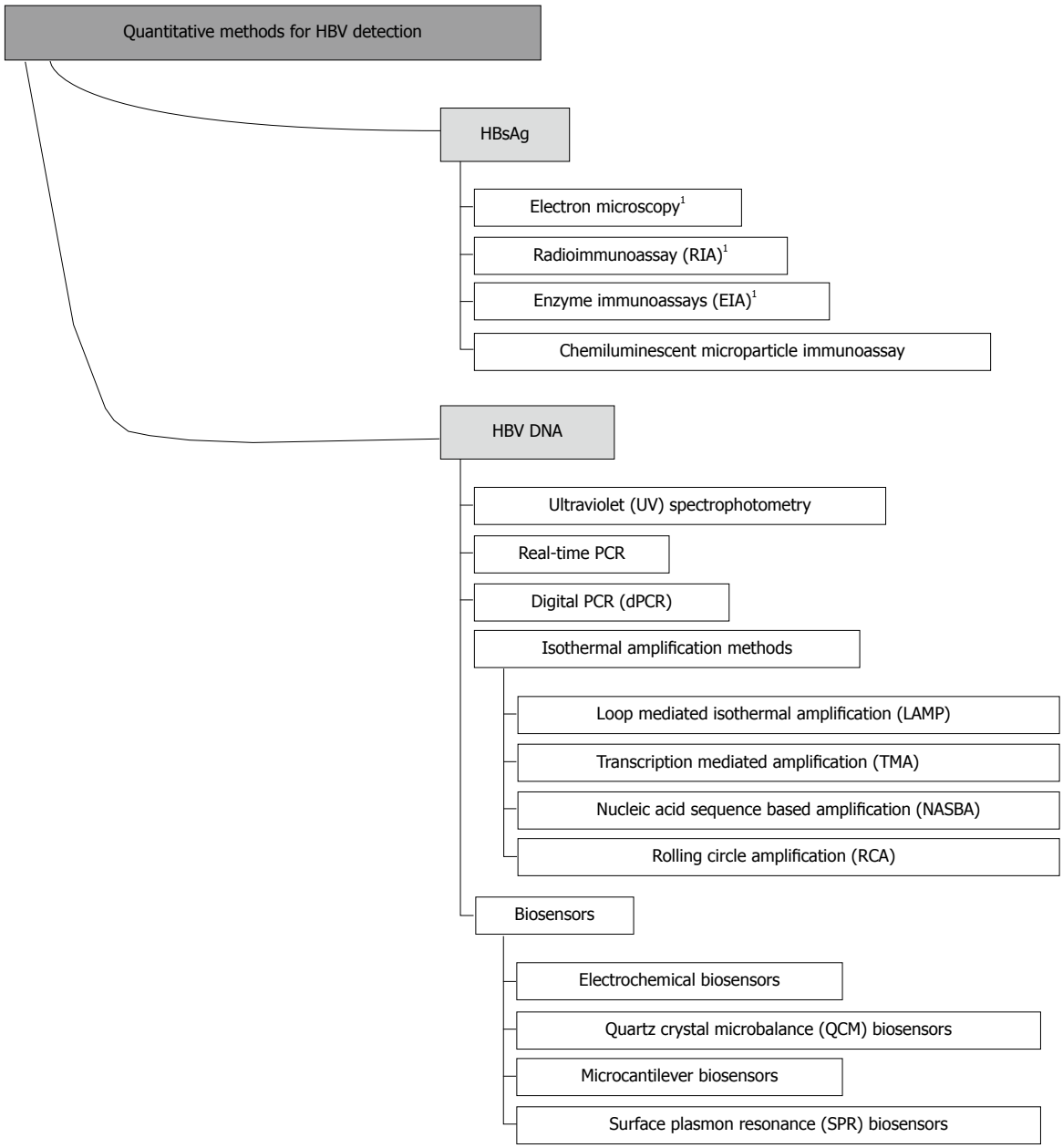

Figure 1 Quantitative methods for hepatitis B virus detection. [1]These methods are qualitative or semi-quantitative for HBsAg. HBsAg: Hepatitis B surface antigen.

spectrophotometry and utilize a patented sample-retention system that allows the quantification of DNA or RNA from 1-2 μL samples[23]. The usable concentration ranges are 0.4-15000 ng/μL. No specific sample preparations are needed for NanoDrop instruments[23].

Real-time PCR

Since developed in 1983 by Kary Mullis[24], PCR has become an essential tool for molecular biologists, and its application in nucleic acids detection system has revolutionized the quantitative analysis of DNA and RNA. PCR techniques have rapidly evolved over the last few years, and their quantitative applications have favored the development of real-time PCR. Real-time PCR follows the general principle of conventional PCR, and its key feature is that the amplified DNA is detected as the reaction progresses in real time. This new approach has a broader dynamic range compared to conventional PCR. The most commonly used reagents for real-time PCR are TaqMan probes[25]. TaqMan probes are hydrolysis probes that were designed in 1991 to increase the specificity of quantitative PCR[26]. Figure 2 outlines the reaction mechanism of real-time PCR based on TaqMan probes.

In the study by Abe *et al*[27], a sensitive, accurate, and reproducible assay for HBV DNA quantification based on real-time PCR using TaqMan probes was reported. Their results demonstrated that the limit of detection was as few as 10 copies/reaction, with a

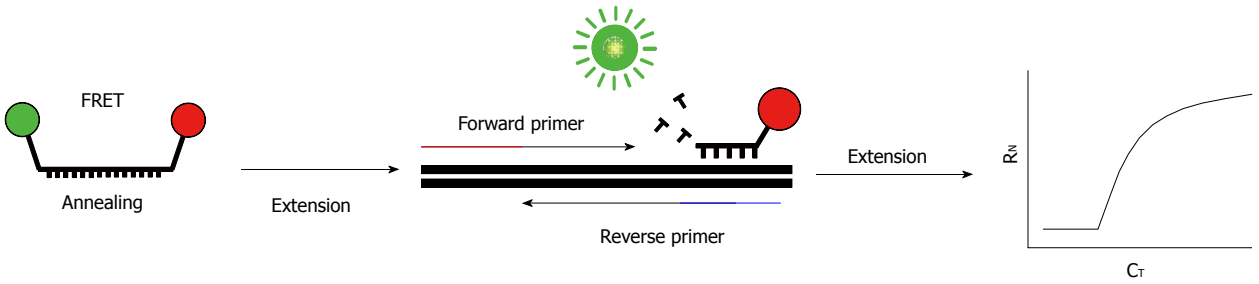

Figure 2 Reaction mechanism of real-time polymerase chain reaction based on TaqMan probe technology. TaqMan probe is an oligo-nucleotide probe that has a fluorescent reporter at the 5' end and a quencher attached to the 3' end. Once hybridized to the target sequence during annealing, TaqMan probe is cleaved by DNA polymerase, which separates the fluorescent reporter from the quencher. Once they are separated, the signal is emitted and detected in the real-time machine. The intensity of fluorescence is proportional to the amount of PCR product produced. FRET: Fluorescent resonance energy transfer.

linear standard curve between 10 and 10^8 DNA copies/reaction. The coefficient of variation for both inter- and intra-experimental variability indicated remarkable reproducibility.

Sitnik *et al*[28] developed a real-time PCR assay for HBV DNA quantification with TaqMan and minor groove binder (MGB) probes. In this assay, primers and probes were designed using an alignment of sequences from all HBV genotypes in order to amplify all the genotypes equally. The assay had a dynamic range from 50 to 10^8 IU/mL.

Although many laboratories have developed in-house real-time PCR-based assays, all of them had a lack of quality control and standardization[3], which restricted their application in clinical diagnosis. The major diagnostics companies also provide commercial quantitative assays. These assays vary in their detection limit and dynamic range: the first version suffered from a narrow dynamic range from 10^2 to 10^5 copies/mL, while the detection limit of the newer assay is lower than 50 IU/mL with a dynamic range of approximately 10^8 IU/mL[29-32]. Recently, the World Health Organization has established a universal standard for HBV DNA quantification measured in IU/mL, with the purpose of correlating different results using a single reference unit[33]. However, significant variability in quantification among different assays can occur randomly in spite of the standardization of reporting units and the generally good correlation between different assays[34,35]. Hence, patients are suggested to be monitored by a single assay[1].

Digital PCR

Digital PCR, developed by Vogelstein *et al*[36], is a refinement of conventional PCR methods that can be used to quantify and clonally amplify nucleic acids directly, including DNA, cDNA, and RNA. The key difference between digital and conventional PCR lies in the measuring method; digital PCR is a more precise method than conventional PCR[37]. A sample is partitioned in digital PCR so that individual nucleic acid molecules within the sample are localized and concentrated within many separate regions. The partitioning of the sample allows for estimation of the number of molecules, by assuming that the molecular population follows a Poisson distribution. Hindson *et al*[38] developed a fundamentally distinct method of partitioning, droplet digital PCR, which partitions a sample into 20000 droplets and provides digital counting of nucleic acids.

High-sensitivity techniques distinguish differences in the number of HBV copies among samples, especially in cases of low copy number (*e.g.*, nucleic acids extracted from tissues). Huang *et al*[39] applied droplet digital PCR to measure the number of HBV copies in formalin-fixed paraffin-embedded (FFPE) HCC tissue. A total of 131 HCC FFPE samples with different tumor stages and clinical features were classified by their serological tests. The number of HBV copies were successfully determined by droplet digital PCR for all FFPE tissues, with copy numbers ranging from 1.1 to 175.5 copies/μL. These results showed that droplet digital PCR improved the analytical sensitivity and specificity of nucleic acids measurement to a single-molecule level and was suitable for HBV DNA quantification.

Isothermal amplification methods

PCR-based assays are the most widely used methods for HBV DNA quantification; however, they need a thermo-cycling machine to separate DNA strands and amplify the fragments[21]. Isothermal amplification methods are carried out at a constant temperature, and do not require a thermal cycler. The isothermal amplification methods have been developed according to new findings in the molecular biology of DNA/RNA synthesis and *in vitro* nucleic acid amplification function of some accessory proteins. Here, we describe several isothermal amplification methods that have been used to quantify HBV DNA.

Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) is a single-tube technique for DNA amplification[40]. LAMP may be combined with a reverse transcription step to allow the detection of RNA[41]. In LAMP, the target sequence is amplified at a constant temperature of 60 ℃-65 ℃ using either two or three sets of primers and a polymerase with high activity of strand

displacement and replication activity. An additional pair of loop primers can accelerate the amplification[42]. Due to the specific nature of the primers, the amount of DNA products in LAMP is considerably higher than in PCR-based amplification[42]. Compared with PCR, LAMP was less sensitive to inhibitors in complex samples, such as blood, due to the use of a different DNA polymerase. However, complex primer design has been considered as a weakness of LAMP, and it may limit its application in some aspects of molecular biology[21,41].

The original article on LAMP, which was published in 2000 by Notomi *et al*[40], showed that 600 and 6000 copies of HBV DNA were detected at 13 and 11 min, respectively, which indicated a high specificity and efficiency for HBV detection. Cai *et al*[43] developed an accurate and rapid real-time fluorogenic LAMP protocol to quantify HBV. Their study demonstrated a dynamic range of eight orders of magnitude, a lower detection limit of 210 copies/mL, low inter-assay and intra-assay variability (4.24%-12.11%), and excellent correlation with real-time PCR (R^2 = 0.96). Similar LAMP assays have been reported by others, indicating that LAMP may be useful in the future as a low-cost alternative for HBV DNA quantification[44,45].

Transcription-mediated amplification

Transcription-mediated amplification (TMA) is an isothermal amplification technique used in molecular biology research and in clinical laboratories for the rapid diagnosis of infections. In contrast to PCR, this method involves RNA transcription (*via* RNA polymerase) and DNA synthesis (*via* reverse transcriptase). There are several more differences between TMA and PCR: (1) TMA is isothermal; (2) TMA produces RNA rather than DNA amplicons. Since RNA is more labile in a laboratory environment, this reduces the possibility of carry-over contamination; and (3) TMA produces 100-1000 copies per cycle (PCR only produces two copies per cycle), which results in a 10 billion-fold increase in nucleic acid products within 15-30 min[46].

Kamisango *et al*[47] developed a sensitive and quantitative assay using TMA and hybridization protection assay for the detection of HBV DNA in serum. The assay achieved a detection range of 5 × 10^3 to 5 × 10^8 genome equivalents/mL. It takes about 5 h to complete a moderately sized manual assay. Ide *et al*[48] noted that TMA was more useful in understanding the changes of HBV DNA level than branched DNA signal amplification assay in lamivudine-treated CHB patients. Kubo *et al*[49] examined the usefulness of TMA for evaluation of the active degree of hepatitis and estimation of recurrence after resection of HBV-related HCC.

Nucleic acid sequence-based amplification

Nucleic acid sequence-based amplification (NASBA) is similar to TMA, which was developed by Compton[50] in 1991 to amplify RNA sequences. This technique can also be used for amplification of DNA with modifications in the basic method, such as primer design, sample extraction, and template denaturation[16]. Compared with PCR, major advantages of NASBA are: (1) it works under isothermal conditions - usually at a constant temperature of 41 ℃; and (2) it is more rapid and sensitive than PCR in medical diagnostics[51].

Yates *et al*[52] developed an HBV DNA quantification system based on amplification with NASBA and real-time detection with molecular beacon technology. The detection range of the assay is 10^3-10^9 copies/mL in plasma or serum, with good reproducibility and precision. Deiman *et al*[53] used NASBA, including a restriction enzyme digestion for HBV DNA amplification, and found that the sensitivity of normal NASBA was improved 100-1000 times when restriction enzyme digestion was performed prior to amplification. The limit of detection was 10 IU/mL with a dynamic detection range of 10^2-10^9 IU/mL.

Rolling circle amplification

Rolling circle amplification (RCA) is a simple, reliable, and isothermal amplification method, which is driven by DNA polymerase to generate a long tandem repeat product based on a circular DNA template[54]. This technique does not require advanced laboratory equipment or experimental expertise. Compared with PCR, the main advantages of RCA include: (1) it is resistant to inhibitors present in clinical samples and requires almost no assay optimization[16]; and (2) it can amplify targets on solid support or in solution, offering opportunity for microarray and biosensor application[55] (Figure 3).

The property of a circular template for RCA makes it ideal for detection of HBV DNA, especially cccDNA. Margeridon *et al*[56] reported that cccDNA from liver biopsies could be amplified from as few as 13 copies using RCA. Martel *et al*[57] developed a method using *in vitro* completion/ligation of plus-strand HBV relax circular DNA and amplification using RCA. The method can amplify complete HBV genomes from serum with viral loads ranging from 10^3 to 10^8 IU/mL.

The isothermal amplification methods mentioned above offer several advantages over PCR, most importantly, they do not require an expensive and cost-intensive thermal cycler. Comparisons between isothermal amplification methods and conventional PCR are shown in Table 1.

Biosensors

Biosensors are analytical devices used for detection, which combine a biological component with a physicochemical detector[22]. Recently, an increasing number of biosensors have been used in clinical research; a common example is the blood glucose biosensor, which uses glucose oxidase to break blood glucose down[58,59]. Most of the clinical research on

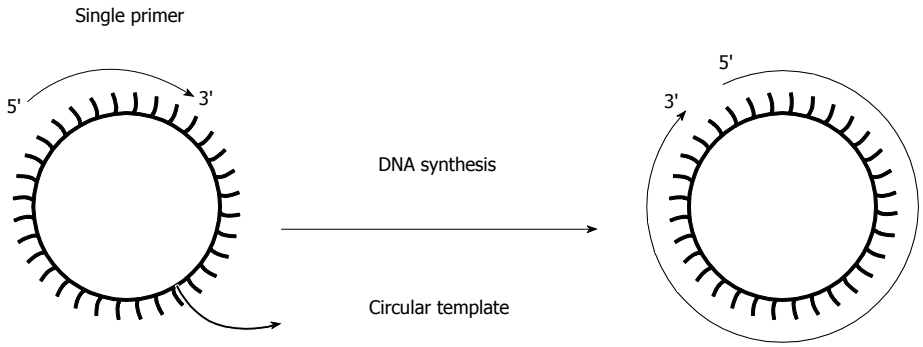

Figure 3 Scheme of the rolling circle amplification reaction. RCA rapidly synthesizes multiple copies of a single circular template with use of a single primer. RCA: Rolling circle amplification.

Table 1 Characteristics of isothermal amplification methods and polymerase chain reaction reviewed in this article

Method	Temperature requirement (℃)	No. of enzymes	Primer design	Product detection method	Rapid detection possibility	Tolerance to biological components
PCR	55-95	1	Simple	GE, ELISA, Real-time	Yes	No
IAM						
LAMP	60-65	1	Complex	GE, turbidity, Real-time	Yes	Yes
TMA	50-60	2	Simple	GE, ELISA, Real-time, ECL	Yes	No
NASBA	37-41	2 or 3	Simple	GE, ELISA, Real-time, ECL	Yes	No
RCA	37	1	Simple	GE, Real-time	Yes	No

ECL: Electrochemiluminescence; GE: Gel electrophoresis; PCR: Polymerase chain reaction; IAM: Isothermal amplification methods; LAMP: Loop-mediated isothermal amplification; TMA: Transcription-mediated amplification; NASBA: Nucleic acid sequence-based amplification; RCA: Rolling circle amplification.

biosensors was based on immunological reactions or DNA hybridization, and the biosensors always yielded rapid results with high sensitivity[60-62].

Electrochemical biosensors: Electrochemical biosensors work by detecting current or potential changes caused by binding reactions that occur on or near the electrode surface[63]. Recently, electrochemical biosensors have offered sensitivity, selectivity, and low-cost detection for DNA sequences and have attracted considerable attention. Ding *et al*[64] described a label-free electrochemical biosensor for the detection of oligonucleotides related to HBV sequences *via* the interactions of DNA with the redox-active complex 2,9-dimethyl-1,10-phenantroline cobalt[Co(dmp)(H_2O)(NO_3)$_2$]. The experiment was performed by hybridizing 21-mer DNA probes modified on glassy carbon electrode with target DNA and cobalt[Co(dmp)(H_2O)(NO_3)$_2$], whose sizes were comparable to the small groove of native double-helix DNA that was used as an electrochemical indicator. Under optimal conditions, the electrical signal had a linear relationship, with the concentration of target DNA ranging from 3.96×10^{-7} to 1.32×10^{-6} mol/L, and the detection limit was 1.94×10^{-8} mol/L.

Zhang *et al*[65] developed an electrochemical biosensor using diaquabis[N-(2-pyridinylmethyl)-benzamide-kappa N-2,O]-cadmium(Ⅱ) dinitrate as a new electroactive indicator for the detection of human HBV DNA. The hybridization between the probe and its complementary single-stranded DNA was determined by differential pulse voltammetry. Experiments with non-complementary oligonucleotides were carried out to assess the selectivity of the developed electrochemical DNA biosensor. HBV DNA could be quantified in a range from 1.01×10^{-8} to 1.62×10^{-6} mol/L with good linearity ($\gamma = 0.9962$). The detection limit was 7.19×10^{-9} mol/L.

Quartz crystal microbalance biosensors: Piezoelectric materials, typically crystals, generate an electrical potential in response to a mechanical force[19]. Piezoelectric biosensors are mass-sensitive, and the additional mass to the sensor causes a detectable change in the resonance frequency of the crystal. The most common type of piezoelectric biosensor is quartz crystal microbalance (QCM). QCM biosensors have gained increasing attention in recent years because of their high sensitivity, good specificity, low-cost, label-free detection, and rapid response[66].

Zhou *et al*[67] developed a highly sensitive piezoelectric HBV DNA biosensor based on the sensitive mass-transducing function of the QCM and the specificity of nucleic acid hybridization reaction. HBV nucleic acid probes were immobilized onto the gold electrodes of a 9 MHz AT-cut piezoelectric quartz crystal *via* the polyethyleneimine adhesion, glutaraldehyde cross-linking method or the physical adsorption method. The frequency shifts of hybridization have a good linear relationship with the amount of HBV DNA, when the amount was 0.02-0.14 mg/mL.

Peptide nucleic acid (PNA)-based piezoelectric

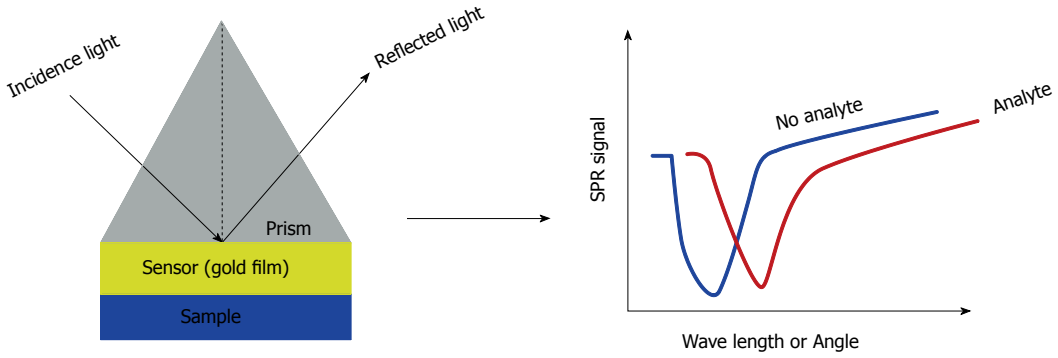

Figure 4 Scheme of the surface plasmon resonance biosensor. The incidence light causes the changes in wavelength or angle. Changes were detected in real time monitor. SPR: Surface plasmon resonance.

Table 2 Quantitative detection methods for hepatitis B virus discussed in this review

Method	Target	Detection range	Ref.
Architect	HBsAg	0.05-250.0 IU/mL	[12]
Elecsys	HBsAg	NA	[15]
UV spectrophotometry	HBV DNA	0.4-15000 ng/μL	[23]
In-house assays	HBV DNA	10^1-10^8 copies/reaction	[27]
based on real-time PCR	HBV DNA	50-10^8 IU/mL	[19]
Digital PCR	HBV DNA	Single copy	[39]
IAM			
LAMP	HBV DNA	48-10^8 IU/mL	[43]
TMA	HBV DNA	5×10^3-5×10^8 GE/mL	[47]
NASBA	HBV DNA	10^3-10^9 copies/mL	[52]
	HBV DNA	10^2-10^9 IU/mL	[53]
RCA	HBV DNA	10^3-10^8 IU/mL	[57]
Biosensors			
Electrochemical biosensors	HBV DNA	3.96×10^{-7}-1.32×10^{-6} mol/L	[65]
	HBV DNA	1.01×10^{-8}-1.62×10^{-6} mol/L	[66]
QCM biosensors	HBV DNA	0.02-0.14 mg/mL	[67]
	HBV DNA	8.6 pg/L[1]	[68]
Microcantilever biosensors	HBV DNA	23.1 fmol/L-2.31 nmol/L	[69]
SPR biosensors	HBV DNA	2 fg/mL[1]	[45]

[1]Only the limits of detection were given in these two papers. HBsAg: Hepatitis B surface antigen; HBV: Hepatitis B virus; NA: Not available; LAMP: Loop-mediated isothermal amplification; TMA: Transcription-mediated amplification; NASBA: Nucleic acid sequence-based amplification; RCA: Rolling circle amplification; QCM: Quartz crystal microbalance; SPR: Surface plasmon resonance; IAM: Isothermal amplification methods.

biosensor for real time monitoring of hybridization of HBV genomic DNA was constructed by Yao *et al*[68]. PNA probes can combine target sequences more effectively and specifically than DNA probes. The PNA probes were designed and immobilized on the surface of the biosensor to be a substitute for the conventional DNA probes for direct detection of HBV genomic DNA without previous amplification by PCR. The hybridization assay was completed in 50 min. The detection limit was 8.6 pg/L, and the clinical specificity was 94.44% in comparison with real-time PCR.

Microcantilever biosensors: In the last two decades, microcantilevers have emerged as a sensitive tool for the detection of chemicals and bio-organisms. Because of their light weight, small size, and high surface-to-volume ratio, microcantilever sensors improve the detection and identification of biological agents by several orders of magnitude[69-71]. An HBV DNA detection method using a silica-nanoparticle-enhanced dynamic microcantilever biosensor was developed by Cha *et al*[72], with a 243-mer nucleotide of HBV DNA pre-core/core region used as the target DNA. In this study, the capture probe immobilized on the microcantilever surface and the detection probe conjugated with silica nanoparticles were designed specifically for the target DNA. HBV DNA was detected using a silica-nanoparticle-enhanced microcantilever biosensor with a concentration of 23.1 fmol/L to 2.3 nmol/L, which was obtained from the PCR procedure. The HBV target DNA of 243-mer was detected up to the picomolar level without nanoparticle enhancement and up to the femtomolar level using a nanoparticle-based signal amplification process.

Surface plasmon resonance biosensors: Surface plasmon resonance (SPR) biosensors are optical sensors that exploit special electromagnetic waves, surface plasmon polaritons, to probe interactions between analytes and biomolecular recognition elements immobilized on the SPR sensor surface[73] (Figure 4). SPR biosensors are a label-free, real-time analytical technology for the detection of biological analytes and the analysis of biomolecular interactions.

Chuang *et al*[45] constructed a simple, low-cost, SPR-sensing cartridge based on the LAMP method for the on-site detection of HBV. The HBV template mixed in 10 μL LAMP solution could be detected by the new system in 17 min, even at the detection-limited concentration of 2 fg/mL. They also analyzed the correlation coefficients between the initial concentrations of HBV DNA templates and the system response at varying amplification times to establish an

optimum amplification time endpoint of 25 min (R^2 = 0.98).

All rapid and quantitative methods for HBV detection mentioned above are summarized in Table 2.

FUTURE PERSPECTIVES

The level of HBsAg has been suggested as a marker for the amount of cccDNA or infected liver mass. Although commercial assays are now available for HBsAg quantification, their dynamic range is narrow, and manual dilution is required if HBsAg concentration exceeds the dynamic range. More sensitive assays with broader detection ranges are needed for rapid and quantitative detection of HBV in clinical practice.

Our review describes the most assays that have been used for quantification of HBV DNA, along with their merits and limitations. PCR is the most widely used method in HBV DNA quantification, but it is also known to have erroneous results caused by its hybridization mechanism and false-negative results because of low HBV copy numbers. Additionally, PCR requires efficient control of thermal cycles and thus is instrument intensive. The development of isothermal amplification methods makes the amplification of HBV DNA without a thermal cycler possible, which also offers an opportunity for biosensor miniaturization. In spite of isothermal amplification and high sensitivity, isothermal amplification techniques have remained less utilized for developing commercial detection assays for HBV quantification. Biosensors have major advantages, such as speed, sensitivity, and low cost. Detection and quantification of viral components and viruses using biosensors is a new concept and is still in its infancy[63]. Tests developed previously are now being integrated into other novel platforms, such as the combination of LAMP and biosensors[45].

Development of rapid quantification detection for HBV is progressing towards procedural simplicity, tolerance to crude samples, high sensitivity, specific amplification, and robust reliable performance. With the development of technologies, quantification of HBV may be moved away from centralized laboratory facilities to other locations, such as emergency rooms, physician's office, and even the family. Point-of-care and point-of-patient testing may be the mainstream in the future[19,20].

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