Molecular Testing in the Diagnosis and Management of Chronic Hepatitis B

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INTRODUCTION

Hepatitis B virus (HBV) causes a highly complex chronic infection that impacts a significant proportion of the world's population. Diagnostics for chronic hepatitis B have evolved from the simple detection of HBsAg through the complex antibody response against individual viral proteins and to the detection and quantification of viral DNA. Implementation of increasingly sensitive methods of HBV DNA quantification has greatly aided the diagnosis and management of disease. Assays are also available to determine HBV genotypes and to detect the presence of viral mutants, including those that confer drug resistance and others that downregulate HBV e antigen. In this review, an overview of the virus and chronic hepatitis B infection is provided. The current utility of the different types of molecular diagnostic tests is discussed, and the performance characteristics of the available assays are described.

HBV PROTEINS AND REPLICATION

HBV is an enveloped virus containing a 3.2-kb, partially double-stranded, relaxed circular genome. The genomic coding scheme is extraordinarily efficient; every nucleotide is a part of at least one open reading frame. The major viral proteins (polymerase, core, envelope, X, and e antigen) and their activities are shown in Table 1. HBsAg and HBeAg are particularly important in the management of chronic hepatitis B. Detectable HBsAg in serum is a marker of chronic infection. HBeAg in serum is a marker of high viral replication levels in the liver. Loss of HBeAg in serum and emergence of anti-HBeAg antibody (termed HBeAg seroconversion) is associated with clinical improvement of hepatitis (reduced HBV DNA, normalized serum aminotransferase levels, and quiescence of inflammation in the liver) (9).

HBV replicates primarily in human hepatocytes, although viral DNA can be found in peripheral blood mononuclear cells. Entry is mediated by envelope binding to an unknown receptor. After entry and virion uncoating, nucleocapsids are translocated into the nucleus, where cellular DNA repair enzymes complete virion DNA synthesis. The resultant covalently closed circular DNA (cccDNA) is the template for viral mRNA transcription, which is mediated by host polymerase. Replication-competent nucleocapsids comprised of core protein, encapsidated full-length pregenomic RNA, and viral polymerase are assembled in the cytoplasm. Genomic DNA is synthesized by reverse transcription of pregenomic RNA by viral polymerase. Encapsidated, relaxed, open circular DNA can be transported to the nucleus to become cccDNA and additional mRNA template, or it can be released from the host cell via a process that requires cytosolic packaging (along with polymerase) by envelope glycoproteins, budding into endoplasmic reticulum, and release after Golgi transit.

HBV infection is noncytolytic. Clearance of infected cells is believed to be mediated in part by the noncytolytic intracellular activity of cytokines secreted by T cells (23). Cytotoxic T lymphocytes also lyse infected hepatocytes and induce liver injury (23).

EPIDEMIOLOGY

The worldwide burden of HBV is enormous. The World Health Organization (WHO) currently estimates that 2 billion people have been infected with HBV and that 360 million are chronically infected (153). HBV is a significant contributor to morbidity worldwide. Current estimates suggest that it causes

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TABLE 1. HBV proteins

Protein	Function
Envelope proteins: small (HBsAg), medium, large	Glycoproteins located on virion surface; bind unknown cellular receptor to initiate virion entry into host cell
Core protein (HBcAg)	Encapsidates pregenomic RNA and partially double-stranded DNA genome in cytoplasm
e antigen (HBeAg)	Synthesized as precursor protein; proteolytically cleaved in endoplasmic reticulum; ultimately secreted extracellularly and found in peripheral blood; has diverse activities, including immunomodulation (tolerogenic) (21) and replication inhibition (45, 76, 127)
Polymerase	Reverse transcriptase, RNase H (degrades pregenomic RNA template during reverse transcription), DNA polymerase
X protein	Transcriptional transactivator; cofactor for hepatocellular carcinoma (14)

30% of cirrhosis and approximately 50% of hepatocellular carcinoma (HCC) globally (121).

HBV can be transmitted perinatally, percutaneously, and sexually. Routes of transmission are dependent on the regional prevalence of infection (131). In areas of high endemicity such as Asia and the South Pacific, where seroprevalence is ≥8%, most infections are acquired perinatally or horizontally during childhood. In regions of intermediate seroprevalence (2 to 7%), such as sub-Saharan Africa, Alaska, the Mediterranean, and India, infection is transmitted during childhood (perinatally or horizontally) and later in life (sexually, by intravenous drug use, or by unsafe health care-related injection practices). In low-prevalence regions (most of the developed world and parts of Central and South America; seroprevalence, <2%), HBV is usually transmitted sexually and by intravenous drug use.

Historically, the scheme for distinguishing HBV subtypes was based on surface antigen serotyping. The current scheme is genetically based, with a genotype defined as greater than 8% divergence of the complete genome sequence at the nucleotide level (110). Eight genotypes (A to H) have been defined (6, 105, 109, 110, 136). HBV genotypes have distinct geographic distributions (128) (Fig. 1). Multiethnic populations tend to have multiple genotypes (25).

HBV INFECTION

HBV can cause acute and chronic infection. The incubation period between exposure and clinical presentation of acute infection is 1 to 4 months. The likelihood of development of symptomatic acute infection is age related, with a small proportion of cases (approximately 10%) occurring in children younger than 4 years old and a greater proportion (approximately 30%) in adults older than 30 years (99). Symptoms of acute HBV infection are similar to other causes of acute hepatitis, including influenza-like syndrome and right upper-quadrant pain. The clinical syndrome of acute hepatitis B has been well described and is diagnosed through the detection of viral proteins and host anti-HBV antibodies (53, 70). There is currently no role for molecular testing in the diagnosis of acute hepatitis B other than in the detection of asymptomatic patients during pretransfusion screening of blood products (67, 71). In contrast, chronic hepatitis B has been evolving as a clinical concept, and various paradigms have been utilized and

FIG. 1. Geographic distribution of HBV genotypes. Letter sizes indicate relative prevalences in regions with multiple genotypes. Lowercase letters indicate HBV subtypes.

TABLE 2. Useful markers for distinguishing chronic hepatitis B phases

Phase	ALT	HBsAg	HBeAg	HBeAg antibody	HBV DNA (IU/ml)[a]	Liver histology
Immune tolerance	Usually normal	Present	Present	Absent	≥20,000	Usually normal; can have mild inflammation
Immune clearance	Elevated; can be episodic	Present	Present	Absent	≥20,000	Active inflammation
Inactive HBsAg carrier	Usually normal; can have flares	Present	Absent	Present	<20,000*	Degree of abnormality dependent on disease severity during clearance phase (mild inflammation to inactive cirrhosis)
HBeAg⁻ CHB	Periodic flares	Present	Absent	Present	>20,000 or <20,000	Active inflammation
Occult hepatitis B	Can be elevated	Absent	Absent	Present in recovered HBV infection	<20,000†	Ranges from normal to cirrhosis and HCC

[a] Symbols: *, can be undetectable by PCR; †, usually (often detectable only with highly sensitive molecular tests).

discarded. Importantly, molecular assays have begun to play increasingly significant roles in chronic hepatitis B. The content of this review is therefore focused on chronic hepatitis B and the commercial molecular assays that are available for its diagnosis and management.

The probability of progression from acute to chronic hepatitis B infection is in part dependent on when acute infection occurs (158). The highest rates of chronicity are observed as a consequence of perinatal acquisition (90%) and in children less than 5 years of age who are infected horizontally through household contacts (25 to 30%). The incidence of chronic infection is lowest after acute infection during adulthood (<10%).

A paradigm of four phases of chronic infection, consisting of immune tolerance, immune clearance, inactive carrier, and HBeAg-negative chronic hepatitis B (HBeAg⁻ CHB), is now widely accepted; however, not all patients go through each of the four phases (158). Markers that are helpful in distinguishing these phases are depicted in Table 2. The first phase, immune-tolerant chronic HBV, is observed primarily in perinatally acquired infection; it is characterized by high levels of viral replication (HBeAg seropositivity, high levels of HBV DNA in peripheral blood) and normal to minimally elevated alanine aminotransferase (ALT) levels indicating mild hepatitis, if any. This phase can last for decades. It occurs variably when infection is acquired later in childhood or as an adult. The immune tolerance phase is fairly benign, with a low incidence of cirrhosis and HCC. In a longitudinal study of HBsAg-positive Taiwanese adults who likely acquired infection perinatally, the annual incidence of cirrhosis was 0.5%, the cumulative probability of cirrhosis after 17 years was approximately 13%, and no HCC was observed during a mean follow-up period of 10.5 years (27). The majority of patients (85%) underwent HBeAg seroconversion between the ages of 20 and 39.

The second phase, immune clearance, is associated with HBeAg seropositivity, high or variable HBV DNA concentrations in peripheral blood, periodic abnormalities in liver enzymes, and histologic evidence of active inflammation (158). The overall risk of progression to cirrhosis and HCC is directly related to disease activity during this phase, including overall duration and the number and severity of flares (26). This inflammatory phase also leads commonly to HBeAg seroconver-

sion and entry into inactive HBsAg carrier status (the third phase of chronic infection). Spontaneous HBeAg seroconversion has been observed in 66% to 98% of prospectively studied cohorts (26) and occurs at an estimated rate of 10% per year (10, 100).

Patients in the inactive HBsAg carrier state have normal liver enzymes and are HBeAg negative and anti-HBe antibody positive. Most individuals have low HBV DNA levels in peripheral blood (<5.0 $\log_{10}$ copies/ml); a minority have undetectable viral loads by PCR (165). Biopsy findings can range from mild inflammation and minimal fibrosis to inactive cirrhosis if disease was severe during immune clearance (158).

The HBsAg carrier state can have a number of potential outcomes, including indefinite persistence, resolution of chronic infection (manifested by HBsAg clearance and appearance of anti-HBsAg antibody), or disease reactivation due either to recrudescence of the original infection or the emergence of mutant viruses that fail to express HBeAg (HBeAg⁻ CHB). Approximately 70% of patients remain as inactive carriers indefinitely; however, their course and outcome are not necessarily benign. ALT flares have been observed in 33% of inactive carriers (55). In addition, ongoing inflammation can cause progressive liver disease. Cirrhosis and HCC were observed in 8% and 2% of patients after HBeAg seroconversion (55). Clearance of HBsAg and the emergence of anti-HBsAg antibody has been reported to occur at a rate of 0.5% to 0.8% per year (82, 100). Half of these patients continue to have detectable HBV DNA (82, 100). A small proportion of inactive carriers serorevert to HBeAg-positive status (HBeAg seroreversion). In two Asian cohorts, 3 to 4% of HBeAg-negative patients experienced HBeAg seroreversion during long-term follow-up (55, 165). Higher seroreversion rates (14%) were observed in an Alaskan cohort (100).

The fourth phase of chronic infection, HBeAg⁻ CHB, is characterized by lack of detectable HBeAg, the presence of anti-HBeAg antibody, detectable HBV DNA, fluctuating liver enzymes, and active inflammation upon biopsy (Table 2). Progression to this phase occurs spontaneously or in the setting of immune suppression in inactive carriers. Some patients can progress directly from HBeAg⁺ CHB to HBeAg⁻ CHB (55). Replicating viruses have mutations that prevent HBeAg expression, including two sequence changes in the basal core promoter that drives HBeAg transcription (A1762T and

G1764A) (110) or a stop codon in the second-to-last codon of the precore region (codon 28, nucleotide 1896) (12). These mutations can occur singly or in combination. The stop codon mutation is found only after infection with certain genotypes, due to constraints imposed by secondary structure (46). Nucleotide 1896 is base-paired to nucleotide 1858 within a stem loop structure required for HBV replication. Nucleotide 1858 varies according to genotype (T or U in B, D, E, G and some C strains; C in A, F, and some C strains). The G-to-A stop codon mutation at nucleotide 1896 stabilizes the stem loop in B, D E, G, and some C strains. The opposite effect occurs in A, F, and some C strains, likely leading to a relative lack of replicative fitness. The stop codon mutation is therefore found in infections with genotypes B, D, E, and G (and some C strains).

HBeAg$^-$ CHB was once thought to be uncommon outside the Mediterranean region, but it is now recognized to be variably prevalent, ranging from 15% of total chronic hepatitis B in the United States, northern Europe, and Asia-Pacific to 33% in the Mediterranean region (38). The prevalent mutation (basal core promoter versus stop codon) varies by geographic locale, with the basal core promoter mutation found in Asia (median, 77%) and the stop codon mutation found in the Mediterranean region (median, 92%), likely due to the genotypes present in each region (38).

Occult hepatitis B infection, defined as detectable HBV DNA in peripheral blood or liver by sensitive nucleic acid detection methods in patients without detectable HBsAg, is now widely recognized to occur, although it has not been discussed as a phase of chronic hepatitis B in any recent guidelines. Occult hepatitis B has been observed in patients with unexplained liver disease (hepatocellular carcinoma and cryptogenic cirrhosis), in HBV-seropositive individuals with resolving or recovered chronic infection (spontaneously or after interferon [IFN] therapy), and in HBV-seronegative patients without evidence of liver disease, such as hemodialysis patients and blood donors (11, 20, 44, 101, 102, 139). Although data from properly designed cross-sectional studies are lacking, the available data suggest that the prevalence of occult HBV is correlated to the rate of endemic infection (29). Within a given population, occult HBV appears to be found more frequently in patients with evidence of liver disease than in other subjects. For example, occult hepatitis B was found in 16% of North American patients with HCC (62) versus 2% of hepatitis B core antibody-positive blood donors (50) and 4% of hemodialysis patients (101). Occult HBV is found more frequently in patients with serologic evidence of HBV infection (anti-hepatitis B core antibody positive) than in core antibody-negative individuals (11, 102). Finally, occult HBV is found in a significant proportion of patients with chronic hepatitis due to hepatitis C virus, with HBV DNA detectable in up to 30% of serum samples and 50% of liver biopsies (11).

Although viruses with replication deficits could theoretically explain occult HBV, the finding of cccDNA, RNA transcripts, and pregenomic replicative RNA intermediates in a large proportion of patients suggests that most occult infections are due to low-level replication of wild-type virus (97, 98). In addition, the transmissibility of acute HBV via liver transplant or blood product transfusion from donors with occult infection (5, 85, 88) provides evidence against the presence of defective viruses.

Occult HBV may also result from mutations in HBsAg coding or transcription control regions that alter antigenicity or expression levels (19, 54, 61). Such mutant viruses have been reported as the sole circulating strain in up to 40% of patients with occult HBV (2, 15, 54, 101). A higher prevalence of HBsAg mutants (~60%) in individuals with occult HBV from an isolated Inuit community has recently been reported and awaits further study (102).

Serum virus loads in occult HBV cases are usually below the limits of detection of hybridization assays, and detection usually requires more-sensitive methods, such as nested or real-time PCR. In anti-core antibody-positive individuals with occult HBV, virus loads are usually <10,000 copies/ml, or <2,000 IU/ml with the approximate conversion factor of 5 copies per international unit for PCR methods (50, 68, 102, 108). A single study reported viral loads of >4 million copies/ml in anti-core antibody-positive individuals (145); however, further description of patients with such high-titer viremia was not provided.

The consequences of occult HBV infection are not clear. Given the prevalence of HBV DNA in the livers of affected patients (11, 20), it may play a role in the development of cirrhosis and HCC. However, an overall risk of disease progression has not been defined. In hepatitis C virus-infected patients, the data on the effects of occult HBV on fibrosis are conflicting, with some studies documenting more-severe fibrosis (139) and others demonstrating no effect (57). However, a number of studies suggest that occult HBV is associated with an increased risk of HCC in chronic hepatitis C (104, 134, 139).

There are currently no consensus guidelines for diagnosis of occult HBV. Testing has been advocated for a variety of settings, including cryptogenic liver disease, impending immunosuppression in patients with HBV risk factors (who may experience flares), and isolated positivity for core antibody, since the presence of HBV DNA may affect solid-organ-donation and HBV vaccination decisions (29, 139). From the perspective of diagnostic yield, liver biopsy samples may be better specimens than serum, as HBV DNA positivity rates have been found to be higher in liver than in serum in studies of paired samples (2, 11, 40, 162). There are currently no available reports on treatment for occult HBV.

ANTIVIRAL AGENTS

Six drugs have been approved by the U.S. Food and Drug Administration for therapy of chronic hepatitis B, including IFN-α, pegylated IFN-α, lamivudine, adefovir dipivoxil, entecavir, and telbivudine (Table 3). Tenofovir disoproxil fumarate is currently not approved for use in chronic hepatitis B but has been shown to be effective in human immunodeficiency virus type 1 (HIV-1)/HBV-coinfected patients (Table 3). IFN-α (and pegylated formulations) is the only drug that eliminates cccDNA from hepatocytes and is therefore potentially curative. In comparison, prolonged treatment with other agents is required due to greater relapse rates. The therapeutic gain of this approach is balanced by the potential emergence of antiviral resistance. The incidence of resistance varies among the different drugs (Table 3). The highest rates are observed for lamivudine; reported resistance to other nucleoside and nucleotide reverse transcriptase inhibitors is much lower.

TABLE 3. Antiviral therapeutics for chronic hepatitis B

Drug[a]	Mechanism of action[b]	Efficacy[c]	Resistance
IFN-α	Immune-mediated clearance	HBeAg$^+$ CHB: 20% greater VR, 6% greater CR than untreated controls (150) HBeAg$^-$ CHB: avg 24% SR-12 vs 0% for untreated controls (91)	None
Pegylated IFN-α2a and IFN-α2b*	Immune-mediated clearance	HBeAg$^+$ CHB: 10% greater BR and VR at 6 mo posttreatment than with lamivudine (13, 79); pegylated IFN-α2a and IFN-α2b similarly efficacious (60, 79) HBeAg$^-$ CHB: 15% greater BR and VR 6 at mo posttreatment than with lamivudine (95) No added benefit of combined pegylated IFN plus lamivudine for HBeAg$^+$ or HBeAg$^-$ CHB (60, 79, 95)	None
Lamivudine	NRTI; cytidine analog	12-mo regimen: BR and VR at 12 mo posttreatment 10–15% greater than untreated controls for HBeAg$^+$ and HBeAg$^-$ CHB (46, 91) Prolonged treatment: HBeAg seroconversion rates progressively increase to 50% after 5 yr in HBeAg$^+$ CHB; BR and VR peak after 24–36 mo (~50%) and then decline in HBeAg$^-$ CHB (91)	HBeAg$^+$ and HBeAg$^-$ CHB: 1 yr, 20%; 2 yr, 40%; 3 yr, 60%; 4–5 yr, 70% (18, 80, 115)
Adefovir dipivoxil	NRTI; dATP analog (chain terminator)	HBeAg$^+$ CHB (end of treatment): VR, ~20%; BR, ~50% (compared to placebo) (94) HBeAg$^-$ CHB: VR, 70%; BR, 40% (47)	HBeAg$^+$ and HBeAg$^-$ CHB: 1 yr, 0% (47, 149) HBeAg$^-$ CHB: 2 yr, 3%; 3 yr, 6% (47); 5 yr, 29% (48)
Entecavir	2′-Deoxyguanosine analog; inhibits polymerase priming activity and chain elongation	HBeAg$^+$ CHB: higher rates of undetectable DNA by PCR at end of treatment than with lamivudine; HBeAg loss, seroconversion, and BR equivalent to that with lamivudine (17) HBeAg$^-$ CHB: higher rates of undetectable DNA by PCR at end of treatment than with lamivudine; BR equivalent to that with lamivudine (75)	HBeAg$^+$ and HBeAg$^-$ CHB: 1 yr, 0% (17, 75); lamivudine-resistant strains have reduced susceptibility in vitro (155) and in vivo (28)
Telbivudine	NRTI; dTTP analog	Not yet published in peer-reviewed literature	High-level cross-resistance to lamivudine in vitro (155)
Tenofovir disoproxil fumarate	NRTI; dATP analog (chain terminator)	HIV-1/HBV coinfection: undetectable DNA by PCR in 30–75% of patients (8, 73) HBV monoinfection: undetectable DNA by PCR in 80–100% of patients (72, 141)	1 yr, 0% (30)
Emtricitabine	NRTI; nucleoside analog of cytidine	Improved histology, higher rates undetectable DNA by PCR, normalized ALT compared to controls (83); approved for use with HIV-1; may be useful in HIV-1/HBV coinfection (133)	1 yr, 13% (83); 2 yr, 18% (42); high-level cross-resistance to lamivudine in vitro (155)

[a] *, IFN-α2b is not currently FDA approved for treatment of chronic hepatitis B.
[b] NRTI, nucleoside reverse transcriptase inhibitor (causes chain termination).
[c] VR, virologic response (decrease in serum HBV DNA level to <1 × 10^5 copies/ml and loss of HBeAg in patients with HBeAg$^+$ CHB); CR, complete response (combined virologic response, biochemical response, and loss of HBsAg); SR-12, sustained response 12 months after therapy cessation; BR, biochemical response (normalization of ALT).

MOLECULAR ASSAYS IN THE DIAGNOSIS AND MANAGEMENT OF HBV INFECTION

Four types of molecular assays are available for the diagnosis and management of HBV infection: quantitative viral load tests, genotyping assays, drug resistance mutation tests, and core promoter/precore mutation assays. Viral load tests that quantify HBV in peripheral blood (serum or plasma fractions) are currently the most useful and most widely used. The applications of other assays are currently more specialized, and their use is more limited. The utility of these assays and their performance characteristics are reviewed below.

Quantitative HBV DNA Assays: Utility

Practice guidelines for the management of chronic hepatitis B have been published by a number of professional societies (32, 65, 81, 90). These consensus documents recommend the quantifica-

tion of HBV DNA in the initial evaluation of chronic hepatitis B and during management, particularly in the decision to initiate treatment and in therapeutic monitoring. High-sensitivity molecular assays are clearly important for the diagnosis of HBeAg$^-$ CHB and occult HBV, where viral loads can be quite low.

Detectable HBV DNA in plasma or serum is one of the criteria for chronic hepatitis B in all guidelines. Assessment of HBV DNA in plasma or serum should therefore be performed along with other tests to establish this diagnosis. Several guidelines (65, 90, 91) specify a threshold of ≥20,000 IU/ml (100,000 copies/ml) for active viral replication during HBeAg$^+$ CHB. This value corresponds to the limit of detection of hybridization-based quantitative HBV DNA assays and was originally proposed in an early consensus statement on HBV management (89) without much supporting evidence. Subsequent studies have demonstrated that ~90% of patients with HBeAg$^+$ CHB had viral loads of >20,000 IU/ml (24) and 98%

of inactive HBsAg carriers consistently had levels of <20,000 IU/ml (96), suggesting that this threshold is reasonable.

Measurement of HBV DNA is critical for the diagnosis and management of HBeAg⁻ CHB (core promoter, precore stop mutation), as it is the only marker of viral replication that can be monitored. Distinguishing HBeAg⁻ CHB from the inactive carrier state can be difficult due to fluctuating ALT and HBV DNA levels. A cutoff of 2,000 IU/ml was found to optimally differentiate HBeAg⁻ CHB from the inactive carrier state after short-term and long-term follow-up, particularly in patients with normal ALT levels (93, 166). Application of a higher threshold (20,000 IU/ml) was not found to be useful in this setting given the inability to identify 30% of patients with HBeAg⁻ CHB even after multiple sequential tests (24). Despite the observed predictive value of the 2,000-IU/ml threshold, serial testing should be performed in patients with low HBV DNA and normal ALT levels to optimally distinguish the inactive carrier state from HBeAg⁻ CHB (32). Viral loads can fall below the detection limit of low-sensitivity hybridization-based assays in 40 to 60% of patients with HBeAg⁻ CHB (93); therefore, more-sensitive assay formats should be used in this setting.

The detection of HBV DNA in peripheral blood (plasma or serum) is also important to establish the diagnosis of occult HBV (by definition, detectable HBV DNA in peripheral blood or liver in the absence of HBsAg). Testing for occult hepatitis B has been recommended in the following settings: (i) in cryptogenic liver disease, especially if serology is positive for previous exposure to HBV (anti-core antibody-positive individuals); (ii) prior to immunosuppression, due to the potential for hepatitis flares; and (iii) in solid organ transplant donors whose only serological marker of HBV infection is anti-core antibody, due to the potential for transmission (29, 139).

There is general agreement between practice guidelines that the decision to initiate therapy should be based on the demonstration of active viral replication (defined as an HBV DNA level of ≥20,000 IU/ml in HBeAg⁺ CHB) and moderate to severe disease, as evidenced by persistent ALT elevation (3 to 6 months) and/or histologic demonstration of moderate to severe hepatitis (32, 65, 81, 90). HBV DNA levels are not primary determinants of therapy, because viral loads are not necessarily reflective of disease severity. Median viral loads in inactive chronic hepatitis B are lower than in HBeAg-positive patients; however, there is some overlap in viral load between individuals in these phases (66, 96, 129). Cirrhosis has also been observed in patients with low viral loads (<2,000 IU/ml) (160).

One recent set of treatment guidelines (65) has applied viral load thresholds more extensively than others. For example, these guidelines suggest that viral loads can be helpful in the decision to obtain a biopsy from HBeAg⁺ CHB or HBeAg⁻ CHB patients with normal ALT levels. Individuals with viral loads greater than threshold (20,000 IU/ml for HBeAg⁺ CHB; 2,000 IU/ml for HBeAg⁻ CHB) may benefit from biopsy to identify histologic evidence of disease requiring initiation of treatment. Additionally, these guidelines recommend treatment for patients with compensated cirrhosis and viral loads greater than 2,000 IU/ml, while those with lower viral loads could either be treated or observed. Of note, the threshold for HBeAg⁺ CHB without cirrhosis (20,000 IU/ml) was greater than that for HBeAg⁻ CHB and for compensated cirrhosis

(2,000 IU/ml), largely reflecting the lower viral loads associated with the latter two states.

Once the decision to treat has been made, viral load testing is useful at baseline to predict the response to antivirals and the emergence of antiviral resistance, particularly to lamivudine (16, 74). Low viral loads are associated with response to IFN-α (120) and pegylated IFN-α (60) in patients with HBeAg⁺ CHB. The influence of pretreatment viral load on IFN responsiveness in HBeAg⁻ CHB has not been reported. The value of baseline viral load as a predictor of response to lamivudine appears to depend on the patient cohort. In HBeAg⁺ CHB, it has not been found to be a significant variable for initial response, defined as HBeAg loss (52, 119), but was found to be associated with relapse (143). In HBeAg⁻ CHB, high viral loads were observed to be associated with lack of response to lamivudine (116). The influence of pretreatment viral load on response and the emergence of resistance to newer nucleoside/nucleotide antivirals has not yet been reported.

Viral load measurement plays a significant role during therapy, as most guidelines propose that suppression of HBV replication is a major therapeutic goal. Measurement of viral load at 3- to 6-month intervals during treatment has been recommended (32, 81). Monitoring intervals can also be guided by specific treatment, with shorter intervals (every 3 months) for lamivudine and longer intervals (at least every 6 months) for other nucleoside/nucleotide reverse transcriptase inhibitors due to the more rapid emergence of resistance during lamivudine therapy (65).

The treatment duration for IFN is relatively fixed (4 to 6 months for HBeAg⁺ CHB; 12 to 24 months for HBeAg⁻ CHB). However, viral load measurement is helpful in determining when to end treatment with nucleoside/nucleotide reverse transcriptase inhibitors. One study has suggested that viral load can be used as a primary end point to assess the response to nucleoside antiviral therapy, since a correlation between a decline in viral load, an improved histologic necroinflammatory score, and HBeAg seroconversion was observed (103). However, most guidelines also incorporate serologic testing (HBeAg and anti-HBeAg antibody to assess HBeAg seroconversion) in patients with HBeAg⁺ CHB. In this setting, therapy can be stopped 4 to 12 months after HBeAg seroconversion occurs and HBV DNA levels decrease to less than 20,000 IU/ml (32, 90) or become undetectable by PCR (<40 IU/ml) (65, 81). Data documenting the relative utility of high (20,000 IU/ml) versus low (40 IU/ml) thresholds are not available. In HBeAg⁻ CHB, HBV DNA is the only virologic marker that can guide the decision to end treatment; however, no specific stopping point for nucleoside/nucleotide reverse transcriptase inhibitors has been recommended in practice guidelines largely due to the higher relapse rates in these patients (37, 116).

The identification of nonresponders through determination of viral load kinetics at early time points posttreatment compared to baseline has become the standard of care in pegylated IFN/ribavirin treatment for chronic hepatitis C. A similar paradigm for chronic hepatitis B therapy has not yet been adopted, largely due to the lack of trials designed to address this question; however, preliminary data are emerging. In one recent study of IFN-α treatment in HBeAg⁺ CHB, nonresponders could be predicted accurately at week 8 and week 12

TABLE 4. Commercially available assays and reagents for HBV DNA quantification

Test or reagent (manufacturer)	Method	Analytical sensitivity[a]		Linear range[a]		Reference(s)	Regulatory status[b]
		IU/ml	Copies/ml	IU/ml	Copies/ml		
RealTime HBV PCR (Abbott Molecular)	Real-time PCR	4*		$9–4 \times 10^{9}$*			Europe, CE; United States, ASR
Hybrid Capture II (Digene)	Hybrid capture		1.9×10^{5}		$1.9 \times 10^{5}–1.7 \times 10^{9}$ $3.0 \times 10^{6}–1.0 \times 10^{9}$	107 118	United States, RUO
Ultrasensitive Hybrid Capture II (Digene)	Hybrid capture		8×10^{3}		$8.0 \times 10^{3}–1.7 \times 10^{9}$	107	United States, RUO
Artus HBV PCR (QIAGEN Diagnostics)	Real-time PCR			$54–3.6 \times 10^{9}$ $2.5 \times 10^{2}–1.0 \times 10^{9}$		56 135	Europe, CE; United States, not available
COBAS Amplicor HBV Monitor (Roche Diagnostics)	Semiautomated PCR		2×10^{2}		$2.0 \times 10^{2}–2.0 \times 10^{5}$ $1 \times 10^{3}–3 \times 10^{5}$	69, 90 118	Europe, CE; United States, RUO
COBAS TaqMan HBV (Roche Diagnostics)	Real-time PCR		35†		$1.7 \times 10^{2}–8.5 \times 10^{8}$†	146	Europe, CE; United States, ASR, RUO (with HighPure extraction)
		12‡ ~35§ ~2.5¶		$54–1.1 \times 10^{8}$‡ $30–1.1 \times 10^{8}$§ $6–2.1 \times 10^{8}$¶		51 124 39	
VERSANT HBV DNA 3.0 (Siemens Medical Solutions Diagnostics)	bDNA		3.3×10^{3}		$3.3 \times 10^{3}–1.0 \times 10^{8}$	156	Europe, CE; United States, RUO

[a] Symbols: *, data from company website (http://www.international.abbott.molecular.com/HBVPCRkit_1137.asp); †, nucleic acid extraction with HighPure (Roche Diagnostics); ‡, extraction with COBAS AmpliPrep and HBV-specific chemistry (Roche Diagnostics); §, extraction with COBAS AmpliPrep and Total Nucleic Acid chemistry (Roche Diagnostics); ¶, extraction with MagNAPure (Roche Diagnostics).

[b] CE, Conformité Européen (approval according to European In Vitro Diagnostic Directive 98/79/EC); ASR, analyte-specific reagent; RUO, research use only.

of treatment, although sustained responders were identified more accurately by measuring the response at week 12 (142).

HBV viral load measurement has been widely recommended as part of a panel of follow-up tests (including transaminases and HBeAg/HBeAg antibody, if relevant) to assess the durability of the antiviral response after treatment cessation. Recommended posttreatment monitoring intervals vary from every 1 to 3 months for 12 months and then every 6 to 12 months (32) to monthly for 3 months then once again at 6 months, with continued monitoring only for nonresponders to identify delayed therapeutic responses or the need to reinitiate treatment (81).

Molecular Tests for HBV Quantification: Available Assays and Performance Characteristics

First-generation assays for HBV DNA quantification in peripheral blood (usually serum or plasma) were based on solution hybridization technology and measured HBV DNA in picograms per milliliter. Solution hybridization was successful due to high viral loads in many patients with chronic hepatitis B. This assay was relatively insensitive (approximately 5.0 $\log_{10}$ copies/ml), and its linearity ranged from 5.0 to 10.0 $\log_{10}$ copies/ml (90). Adaptation of advanced molecular technologies, such as signal and target amplification, led to the development of second-generation assays with enhanced sensitivity (as low as 200 copies/ml) (117).

The latest generation HBV quantification assays utilize real-time PCR and have improved analytical performance characteristics, including low limits of detection, broad linear ranges, and excellent precision (Table 4). These advances have been demonstrated to translate into better clinical performance as manifested by better detection of low virus concentrations and elimination of specimen dilution for a large proportion of specimens with high viral loads (43, 56).

A WHO international standard was created in response to the need for standardization of quantification (126). This virus preparation (code 97/746) was based on a high-titer genotype A EUROHEP reference preparation (49). Results are reported in international units per milliliter for most currently available assays. In the current WHO HBV standard preparation, 1 IU is equivalent to 5.4 genome equivalents (copies). However, accurate conversion factors are likely to be dependent on the chemistry used for HBV DNA quantification. In support of this, PCR-based quantification assays were demonstrated to have similar conversion factors (Amplicor, 7.3 copies per IU; SuperQuant, 6.2 copies per IU) that were higher than those for bDNA (VERSANT, v2.0, 4.5 copies per IU) and Hybrid Capture II (2.3 copies per IU) (132).

Significant variability in quantification among different assays can occur randomly (39, 69) despite the standardization of reporting units and the finding of generally good correlation between different assays (56, 77, 122). Patients should therefore be monitored with a single assay.

The HBV genotype is a variable that may influence quantification by different methods. Genotype F-dependent underquantification by conventional PCR (COBAS Amplicor Monitor) has been observed (77, 146). For real-time PCR, no bias in amplification has been observed (51, 146). Quantification by the Hybrid Capture II and Ultrasensitive Hybrid Capture II

tests also appears to be free of genotype-dependent bias (107). Data on genotype influence in quantification by VERSANT HBV DNA 3.0 have not been published.

Results of specificity studies have been reported for most of the assays described in Table 4. Excellent results (>97%) have been obtained in studies using samples from HBV-seronegative subjects (51, 107, 118, 146, 156). Problems with reproducibility at concentrations near the limits of quantification of Hybrid Capture II and Ultrasensitive Hybrid Capture II have been described (25 to 40% of samples originally determined to have 5,000 to 10,000 copies/ml were undetectable after repeat testing), suggesting that implementation of an equivocal zone should be considered for these assays (69). False-positive results (ranging from 1 to 3%) at the lower limit of quantification of VERSANT HBV DNA 3.0 have also been observed (39, 124, 156).

Real-time PCR assays have utilized a number of different methods for extraction of HBV DNA. Automated extraction methods with COBAS AmpliPrep (Roche Diagnostics) and MagNAPure (Roche Diagnostics) have been reported for RealArt HBV LC PCR reagents (Artus HBV PCR; QIAGEN Diagnostics) (56, 135) and COBAS TaqMan reagents (39, 51, 124). Manual column methods have also been reported for use with COBAS TaqMan reagents (84, 146).

HBV Genotyping: Utility

The HBV genotype is a variable that could potentially influence the outcome of chronic hepatitis B and the success of antiviral therapy. The influence of the HBV genotype on chronic infection has been most intensively studied in high-prevalence populations in Asia, where genotypes B and C predominate. Outcome data for these genotypes are probably the most valid, since infection duration (due predominantly to perinatal acquisition) is not a confounding variable (36). In general, patients with genotype B infections have more-favorable outcomes than those with genotype C, including younger age at HBeAg seroconversion, lower rates of active liver disease, and slower progression to cirrhosis (87). The influence of genotype on HCC is complex. In most cases, genotype C correlates with higher risk (112, 138, 159); however, the association may also be dependent on other factors, such as host age and geographically dependent virus strain (87). For example, a high rate of HCC has been observed in young patients (63, 106) and in Taiwanese patients with genotype B infection (63).

An HBV genotype-dependent response to antiviral therapy has been observed for some drugs but not for others. For IFN-α treatment of HBeAg$^+$ CHB, greater rates of HBeAg seroconversion have been observed for genotype A than for genotype D (49% versus 26%) (31) and for B than for C (39% versus 17%) (144). Similar results have been found with pegylated IFN-α2b (33, 60). A trend of higher HBeAg seroconversion rates after pegylated IFN-α2a treatment of HBeAg$^+$ CHB has been observed for genotype A compared to genotypes B, C, and D (79).

For nucleoside/nucleotide analogs, potential genotype-dependent predictive parameters include therapeutic response and emergence of resistance. On the whole, there seems to be little evidence for genotype-dependent responses to these agents. For lamivudine treatment of HBeAg$^+$ CHB, information is available primarily for genotypes B and C. There are

strong data demonstrating no effect of genotype (B versus C) on lamivudine response, including equivalent rates of HBeAg seroconversion, log HBV DNA reduction, and median aminotransferase normalization 12 months after end of therapy (163). Another study, measuring similar parameters at the same times (22), showed a greater sustained response in genotype B than in genotype C patients; however, the ratio of genotype B to C patients in this study was 3:1, introducing a potential for bias in the results. No genotype-dependent differences in HBV DNA responses have been observed for adefovir (148) or tenofovir (8).

Development of resistance appears to be equivalent among the different genotypes. Most data pertain to lamivudine, since it has the highest resistance rates of the nucleoside/nucleotide analogs. No difference in rates of resistance has been observed between genotypes B and C (3, 64). More rapid emergence of resistance has been reported for genotype A than for genotype D; however, the difference between the two genotypes was actually quite small (167). Recent data on adefovir resistance identified a potential association with genotype D that bears further investigation (35).

The above observations and data suggest that genotype determination in chronic hepatitis B has a more limited role in clinical management than it does in chronic hepatitis C virus infection. Genotyping is probably most important if IFN therapy is a consideration, and genotype determination has been suggested prior to initiation of IFN therapy in one set of practice guidelines (65). None of the current guidelines have advocated a role for genotyping in counseling patients on the outcome of chronic hepatitis B.

HBV Genotyping Methods

Given its limited utility, HBV genotype testing has not yet been widely adopted in clinical laboratories. A variety of methods have been used, including whole- or partial-genome sequencing, restriction fragment length polymorphism, genotype-specific PCR amplification, PCR plus hybridization, and serology (7). Whole-genome sequencing is the "gold standard," and it is particularly accurate for detecting recombinant viruses. However, it is cumbersome and time-consuming and has limited ability to detect mixed-genotype infections. Single gene sequencing, most commonly the S gene, is technically less demanding. The most common method of sequence-based HBV genotype determination has been searching of the GenBank database for homologous sequences using BlastN. This approach was noted to be complicated by the paucity of genotype-annotated HBV sequences within GenBank (7); however, the deposition of a growing number of annotated sequences into the database has made this approach more practical.

PCR plus hybridization has been adapted into a commercial product (INNO-LiPA; Innogenetics [research use only in Europe and the United States]). The amplification target lies in the major hydrophilic domain of HBsAg and is encoded within the pre-S1 gene, which has been found to be useful for genotype determination by direct sequencing. Amplicons are generated by nested PCR, although hybridization can be performed after the first PCR round if product is visualized by gel electrophoresis. Data on the analytical and clinical performance characteristics of this assay are limited to two reports

(58, 113). The reported accuracy of single-genotype identification was 97 to 99.9% compared to direct sequencing (58, 113). This method has several advantages over direct sequencing. It can effectively identify mixed infection, as 65% of mixed infections were verified by clonal analysis (113). In addition, it can be used to identify genotypes in samples that yield poor quality sequence data (113). One disadvantage is the potential for indeterminate results for viruses with single nucleotide polymorphisms or deletions in sequences that are complementary to probes used in the hybridization phase. An indeterminate rate of up to 5% has been observed (113). Reasonable analytical and clinical sensitivity has been achieved with a modified protocol incorporating a number of different improvements to the recommended PCR plus hybridization procedure, including automated extraction (MagNAPure; Roche Diagnostics), single-round PCR (versus the recommended nested PCR), and uracil N-glycosylase (123).

A commercial direct sequencing assay for genotype determination (TRUGENE HBV Genotyping Kit; Siemens Medical Solutions Diagnostics) is available as a research-use-only kit. Published assay performance data are limited. Correlation data with other direct sequencing methods or with INNO-LiPA have not been reported. The assay can be used in samples with fairly low HBV concentrations (200 to 900 IU/ml) (41).

Antiviral Resistance Testing: Utility and Assays

The emergence of drug-resistant HBV should be suspected when a 10-fold increase in viral load compared to nadir is confirmed in a patient with documented therapeutic response. Documenting the mutation that confers drug resistance has not been part of routine clinical practice. This may change with the growing armamentarium of antivirals and reported cross-resistance among drugs within the same structural class (for example, cross-resistance observed between lamivudine and other L-nucleosides such as entecavir, emtricitabine, and telbivudine [28, 155]).

Resistance can be documented by phenotypic or sequence analysis. Each strategy has advantages and disadvantages. Phenotypic analysis entails assessment of mutant replication in the presence of drug and requires some form of genetic engineering (either site-directed mutagenesis of wild-type sequence or construction of full-length mutant clones expressed in baculovirus models) followed by expression in cell culture systems (130). This approach is the most effective means of ascertaining whether a complex set of mutations confers antiviral resistance. However, it is far too cumbersome for standard clinical molecular laboratories and is usually limited to specialized laboratories with a specific interest in antiviral resistance.

Direct sequencing can identify known and potential new resistance mutations. However, this method is not sufficiently sensitive for the detection of emerging, resistant mutants that are present in low concentrations. These minor populations can be identified by large-scale cloning and sequencing protocols; however, this is cumbersome and beyond the capacity of clinical laboratories. In comparison, hybridization-based methods are more sensitive and less labor-intensive. This approach also has a number of disadvantages: (i) only known mutations can be identified, (ii) individual probes are required to detect each mutation, and (iii) single-nucleotide polymorphisms that

have no effect on phenotype can impair probe binding and produce false-negative results (92).

While sequence determination is relatively simple to perform compared to phenotypic analysis, interpretation of sequence data is not always straightforward. For example, some mutations confer resistance to multiple drugs (rtA181T is observed after long-term lamivudine therapy and in adefovir-resistant viruses) (34, 157). Other sequence changes may not confer resistance when present alone but apparently cause resistance in combination with other mutations. While lamivudine resistance has not been observed with the single mutation rtL180M, it does occur with the double mutant rtL180M/rtA181T, which apparently alters the position of rtM204, a critical residue in the nucleotide binding pocket (130). Finally, it has been noted that patients who have been treated with multiple drugs will likely have a combination of sequence changes that represent (i) drug resistance mutations, (ii) compensatory mutations that improve the fitness of drug-resistant viruses, and (iii) single-nucleotide polymorphisms that have no phenotype (130).

A small number of sequence determination assays are commercially available, including hybridization (biotinylated amplicons hybridized to membrane-bound oligonucleotides specific for each mutation) and direct-sequencing formats. The hybridization-based assay is in its second generation. The first-generation product (for the detection of lamivudine resistance mutations at amino acids 180, 204, and 207) was found to be more sensitive for the detection of emerging variants than sequencing and was able to detect mutations in specimens with low viral loads (approximately 200 IU/ml) (1, 92). The second-generation product (INNO-LiPA DR, version 2.0) has a refined, expanded lamivudine resistance panel (codons 80, 173, 180, and 204) and also detects adefovir resistance mutations (codons 181 and 236). This test demonstrated a high degree of concordance with direct sequencing (~95% of codons interrogated) (59, 114). INNO-LiPA DR, version 2.0, detected a greater number of mixed (resistance mutation plus wild-type virus) infections, and lamivudine resistance was detected earlier (mean, 6 to 7 months). Dual resistance (lamivudine and adefovir) was also demonstrated (59). Operational disadvantages have been noted, including a large number of reaction lines per strip (34 lines per strip), faint bands, absence of bands (false negatives), and indeterminate results due to lack of amplification by primers (114).

There is limited published experience with commercially available direct-sequencing resistance detection assays. The TRUGENE HBV genotyping kit (Siemens Medical Diagnostic Solutions), modified to incorporate automated extraction (versus the recommended manual method), was able to provide sequence data from specimens with low viral loads (<900 IU/ml) (41). In one comparison between TRUGENE and INNO-LiPA HBV DR, concordance was high (approximately 80%) with clinical samples from HIV-1/HBV-coinfected patients treated with lamivudine as part of antiretroviral therapy. However, the hybridization-based assay was better able to detect mixed populations (125), as has been reported in other comparisons between PCR plus hybridization and noncommercial direct sequencing protocols.

Another commercial platform (Affigene HBV DE/3TC assay; Sangtec Molecular Diagnostics AB) combines hybridization and direct sequencing in a microwell plate format to

detect lamivudine resistance mutations. PCR amplicons are immobilized by hybridization; codons 180 and 204 are subsequently interrogated by microsequencing. This assay demonstrated concordance with direct sequencing that was similar to INNO-LiPA DR, version 1.0 (111). Indeterminate rates were greater with Affigene than with INNO-LiPA (13 versus 3%).

Detection of Core Promoter/Precore Mutations in HBeAg⁻ CHB: Utility and Assays

The diagnosis of HBeAg⁻ CHB is made primarily through assessment of a combination of virological markers (HBsAg positive, HBeAg negative, detectable HBV DNA), serology (anti-HBeAg antibody positive), and evidence of hepatic injury (elevated aminotransferases and or histologic evidence of necroinflammation). Assays to detect mutations that abrogate HBeAg expression are commercially available. Test formats for the detection of antiviral resistance mutations have been applied to the identification of precore/core promoter mutations (PCR plus hybridization, INNO-LiPA HBV PreCore; hybridization/direct sequencing, Affigene HBV Mutant VL19 [Sangtec Molecular Diagnostics AB]). The PCR-plus-hybridization format detects three mutations (basal core promoter nucleotides 1762 and 1764 and precore codon 28), while the hybridization/direct-sequencing format detects two of these three mutations (basal core promoter nucleotide 1764 and precore codon 28). Both assays demonstrated high concordance with direct sequencing (approximately 90%); however, they more effectively detected mixed populations (mutant variant plus wild type) (111). Indeterminate results in low-viral-load specimens were observed only with the hybridization/direct sequencing assay, suggesting that PCR plus hybridization may be a more sensitive format (111). Procedural improvements to the PCR-plus-hybridization test (automated extraction, single round of PCR, incorporation of uracil N-glycosylase) have been published (123).

FUTURE TRENDS IN MOLECULAR DIAGNOSTIC TESTING FOR CHRONIC HEPATITIS B

The adoption of new technologies and the identification of new virological or host markers could potentially provide opportunities for growth and evolution of molecular testing in chronic hepatitis B. Emerging technologies that have not yet penetrated significantly into diagnostic laboratories may become useful in the future. For example, a high-density array that can compete with sequencing for the identification of mutations (polymerase-based resistance mutations and core promoter/precore mutations) and genotypes has been reported (140). This system has a number of advantages, including flexibility and throughput. Microarrays currently pose numerous technical, regulatory, and cost challenges that will have to be overcome in order for these types of platforms to become widely adopted in clinical laboratories.

Virtual phenotyping, the prediction of drug resistance from analysis of complex sequence changes, is used routinely in managing HIV-1 resistance to antivirals. This method has great applicability to the management of chronic hepatitis B. A database that can be used to process HBV DNA sequence information to identify genotype and detect mutations that

inhibit HBeAg expression and to predict resistance has been reported (SEQHEPB) (161). Access is provided on a subscription basis. Clinical information can be deposited so that mutations can be correlated with patient presentation, course, and treatment. One particularly interesting feature of this database is that the potential effects of mutations on drug binding can be visualized through a three-dimensional model of drug-bound reverse transcriptase. As the number of drugs to treat chronic hepatitis B expands, such databases may become increasingly useful.

New virological markers, such as cccDNA, the minichromosome that serves as the transcriptional template for viral RNAs, could become clinically useful. cccDNA quantification was once cumbersome but is now readily achievable by real-time PCR and other homogeneous amplification/detection chemistries such as cleavase (Invader HBV DNA; Third Wave Technologies). Studies utilizing these techniques are beginning to elucidate the dynamics of HBV replication during the course of infection and treatment. Median total intrahepatic HBV DNA and cccDNA levels have been found to be greater in HBeAg⁺ CHB than in HBeAg⁻ CHB (78, 147, 152, 154). Interestingly, higher levels of cccDNA transcriptional activity, defined as the ratio of pregenomic RNA to cccDNA, were observed in HBeAg⁺ CHB than in HBeAg⁻ CHB (78), providing a potential explanation for the higher levels of viral replication that occur in the former group. In HCC, tumor tissue has been found to contain higher median cccDNA levels and proportions of cccDNA to intrahepatic total HBV DNA than adjacent nontumor tissue (151), supporting the current paradigm that HBV replication is partially or completely downregulated in HCC. Total intrahepatic HBV DNA and cccDNA loads decline after antiviral therapy (137, 147, 154), reflecting response to therapy.

Preliminary data on the utility of intrahepatic cccDNA as a predictor of response to antiviral therapy are emerging. Whether cccDNA will be useful to predict a sustained response is not yet resolved. Data from studies with small cohorts are conflicting (137, 147). Initial evidence suggests that baseline intrahepatic cccDNA is useful for predicting HBeAg seroconversion after adefovir monotherapy or combination therapy with pegylated IFN-α (147, 154). Whether this applies to other therapies is unknown.

While novel intrahepatic markers of chronic HBV infection and response to therapy are promising and of interest, diagnostic targets that can be monitored less invasively in peripheral blood are likely to be more useful and more widely applicable. One potential candidate is cccDNA measurement in serum. This molecule has been detected in a greater proportion of patients with HBeAg⁺ CHB than with HBeAg⁻ CHB, and median levels have been found to be greater in HBeAg⁺ CHB (152). In addition, a statistically significant decrease in serum cccDNA was observed compared to placebo in patients with HBeAg⁺ CHB who responded virologically to a 52-week course of lamivudine (164). These studies indicate that measurement of cccDNA in peripheral blood is possible, but its potential clinical utility awaits further investigation.

Overall, the field of molecular testing for the diagnosis and management of HBV infection has shown steady improvement in technology and standardization. Emerging, more-sensitive technologies have facilitated diagnostics and therapeutics, particularly for HBeAg⁻ CHB and occult hepatitis B. They have

also helped to better define the natural history of chronic hepatitis B and how the host responds to therapy. Importantly, these new methods have also raised questions and spurred debate on the most useful virological markers for documenting the response to antivirals, which can only improve the application of molecular methods to this field (4, 86, 103). In the natural transfer of information that occurs between the basic and clinical sciences, new markers are being identified during investigation into chronic hepatitis B pathogenesis. As has happened in the past, the most promising molecules will be adapted for clinical use, propelling the field of clinical diagnostics forward yet again.

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