

DETECTION OF HBV VIRUS NUCLEIC ACID BY SEROLOGICAL METHODS

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ABSTRACT

Since Cumitech 18 was published in 1984, significant advances have occurred in the understanding of the viruses that cause hepatitis. These advances include laboratory diagnosis, prevention, and the treatment of viral hepatitis. Hepatitis (liver inflammation) has many etiologies, one of which is viral infection. Although the differences in the clinical course of infection with each of the hepatitis viruses give some indication as to the viral etiology, diagnosis is usually laboratory based. Laboratory diagnosis of these infections is based on serological or nucleic acid detection techniques, because members of this group of viruses grow either poorly or not at all in cell culture. Therefore, protection against the hepatitis viruses will require developing an effective vaccine for each individual agent. In this article from valid sources of Google Scholar, search for, in this paper, which is convenient and fast method for detection of viruses to you introduce the readers.

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INTRODUCTION

HBV is a 42-nm spherical particle consisting of a 7-nm outer shell and a 27-nm inner core that encloses the viral DNA and DNA polymerase. surface component of the complete virion, sometimes referred to as the Dane particle, contains hepatitis B surface antigen (HBsAg), and the inner core component contains hepatitis B core antigen (HBcAg) [1]. In addition to complete virus particles, numerous HBsAg-containing, 22-nm spherical particles and tubular structures of the same diameter and variable length circulate in the blood of HBV-infected individuals. HBV strains have been classified into nine different subtypes, ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq+, and adrq-, according to the antigenic determinants and subdeterminants of their HBsAg HBV has also been classified into six genotypes, A to F, based on nucleotide sequence differences [2]. HBcAg, unlike HBsAg, is not directly detectable in the serum, but removal of the outer HBsAg component of the complete HBV particle with nonionic detergents results in the release of serologically reactive HBcAg Solubilization with sodium dodecyl sulfate and 2-mercaptoethanol of either the inner HBcAg component of HBV particles or core components from infected liver results in the appearance of hepatitis B e antigen (HBeAg) and the disappearance of HBcAg reactivity [3], suggesting that HBeAg is a structural component of the inner core of the HBV particle HBeAg is also present as a readily detectable soluble protein in the serum of HBV-infected individuals who have high levels of viremia. During HBV infection, HBsAg, HBcAg, and HBeAg can induce an antibody response in the host with the development of anti-HBs, anti-HBc, and anti-HBe, respectively. The inner core component of the HBV virion contains an endogenous DNA polymerase with reverse transcriptase activity and a unique viral genome that consists of partially single-stranded, noncovalently closed circular DNA and is only 3,200 nucleotides (nt) in length [4]. The complete nucleotide sequence of the HBV genome and the coding sequences for all of the HBV proteins have been identified. The unique characteristics of HBV classify it as the prototype member of the hepadnavirus family of hepatitis B-like viruses. which includes hepatitis viruses infecting woodchucks Beechey ground squirrels and Pekin ducks, These viruses have several characteristics in common,

including ultrastructure, antigenic composition (except for the duck virus), DNA size and structure, DNA polymerase, a tropism for liver, an association with persistent infection, and, for the primary hepatocellular woodchuck carcinoma [5]. virus and HBV, Transmission of HBV in humans is primarily through intimate physical contact or, less frequently, through exchange of blood products, such as transfusion or hemodialysis [6].

Clinical findings

The incubation period for HBV is usually about 3 months, but varies from 4.5 to 180 days. Approximately 30 to 40% of adults infected with HBV develop symptomatic acute hepatitis, and fulminant hepatitis resulting in death occurs in 1 to 3% of these individuals [7]. At least 50% of persons infected with HBV exhibit a transient subclinical infection [8]. In symptomatic HBV infection, the onset of acute hepatitis is preceded by a short prodromal phase characterized by fever, fatigue, malaise, anorexia, myalgia, nausea, and vomiting. The icteric phase usually begins within 10 days of the initial prodromal symptoms and is characterized by the appearance of dark, goldenbrown urine followed several days later by pale stools and jaundice [9]. A chronic HBsAg carrier state develops in 5 to 10% of adults after either symptomatic or subclinical acute hepatitis B (54). Up to 90% of infants who acquire HBV infection from HBsAg-positive mothers become chronically infected. Chronic HBV infection may progress to chronic persistent hepatitis, chronic active hepatitis with or without cirrhosis, or even primary hepatocellular carcinoma [10].

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Serological Markers Andimmunodiagnosis

Because HBV is not readily propagated in cell cultures or in animals other than high-order primates, the diagnosis of HBV infection is accomplished with sensitive immunoassays for the identification of HBV-associated antigens and antibodies in the blood [11]. Third-generation immunoassays including RIAs, EIAs, microparticle enzyme immunoassays (MEIAs), and a reversed passive hemagglutination (RPHA) test are commercially available for the detection of HBsAg, anti-HBs, anti-HBc, IgM anti-HBc, HBeAg, and anti-HBe. Additional serum markers for HBV infection include the following: DNA polymerase, HBcAg, and HBV DNA (see “Nucleic Acid Diagnosis”). Tests for the presence of these markers do not add substantially to the information provided by the commercially available assays for HBeAg and are thus not included in most routine diagnostic evaluations. Measuring HBV DNA levels may be useful in chronic hepatitis B patients who are receiving antiviral therapy. This complicated system of HBV-associated serological markers may define the phase of infection, degree of infectivity, prognosis, and immune status of the patient. HBsAg is the first serological marker to appear after HBV infection. HBsAg becomes detectable during the late incubation period, 2 to 5 weeks before the onset of symptoms, and persists for 1 to 5 months in symptomatic acute hepatitis B. HBsAg is the first serological marker to appear after HBV infection. HBsAg becomes detectable during the late incubation period, 2 to 5 weeks before the onset of symptoms, and persists for 1 to 5 months in symptomatic acute hepatitis B. Although HBsAg is detectable in the sera of most patients with symptomatic acute hepatitis B, it may be undetectable by current third-generation assays in more than 10% of these cases. HBsAg is frequently absent, or present for only days, in transient subclinical HBV infection. In chronic HBV infection, HBsAg usually persists at high titer for a minimum of 6 months to as long as decades [12]. The presence of HBsAg should always be considered indicative of ongoing HBV infection. Some individuals with chronic HBV infection have undetectable levels of circulating HBsAg. Such persons are referred to as “low-level carriers”. HBeAg appears at the same time as or shortly after HBsAg, and its presence correlates with early and still active disease as well as high infectivity in acute HBV infection [13]. HBeAg persists for 1 to 9 weeks after the onset of symptoms in symptomatic acute hepatitis, and although it may occasionally remain detectable for several weeks after the clearance of HBsAg, it usually disappears before HBsAg. Persistence of HBeAg for more than 10 weeks after the onset of illness predicts the development of chronic HBV infection. HBeAg remains detectable for several months to years in HBsAg-positive chronic HBV infection and will eventually disappear in many patients with persistent HBsAg. HBsAg-positive chronic HBsAg carriers tend to be young, to have developed the carrier state within the previous 10 years, to have some evidence of chronic liver disease, and to have high titers of hepatitis B virions [14].

Mother-to-infant transmission of HBV infection is highly associated with the presence of HBeAg. Anti-HBc appears shortly after the appearance of HBsAg and HBeAg and shortly before or at the onset of clinical

symptoms. In acute hepatitis, anti-HBc is frequently the only detectable serological marker of HBV infection other than anti-HBe [15]. This serological pattern may occur during the acute phase in persons who do not develop detectable HBsAg and HBeAg, or during the convalescent phase after the disappearance of HBsAg but before the appearance of anti-HBs. Anti-HBc, alone or together with anti-HBe, is sometimes observed in individuals who have recovered from past HBV infection but did not develop or subsequently lost detectable anti-HBs. Anti-HBc initially consists of both IgG and IgM antibodies, but IgM anti-HBc decreases in titer and disappears within several months, to one or more years after acute infection, depending on the sensitivity of the assay [16]. Since IgG anti-HBc can persist for years or even a lifetime, the detection of IgM anti-HBc distinguishes HBsAg-negative, anti-HBc-positive patients with current or recent acute HBV infection from those with past infection. IgM anti-HBc has been demonstrated in more than 50% of acute hepatitis patients who are negative for both HBsAg and anti-HAV IgM but are positive for total anti-HBc, regardless of the presence or absence of anti-HBs. In chronic infections the initial anti-HBc response also consists of both IgG and IgM antibodies, but unlike the situation in acute infection, IgM anti-HBc titers decrease very slowly and low titers persist for years while total anti-HBc titers remain very high. Anti-HBc may be the only detectable serological marker in chronically infected HBsAg-negative individuals. The demonstration of IgM anti-HBc and high titers of total anti-HBc in these low-level carriers is indicative of chronic HBV infection. Because IgM anti-HBc can be detected in chronic HBV infection, its presence does not always distinguish between acute and chronic hepatitis B [17]. However, the sensitivity of commercial RIAs, EIAs, and MEIAs for IgM anti-HBc has been adjusted to a level where patients with current or recent acute HBV infection are IgM anti-HBc positive, but those with chronic infection are negative or only weakly positive [18]. Anti-HBe usually becomes detectable 2 to 6 weeks after the disappearance of HBeAg and 2 to 4 weeks before the clearance of HBsAg, and it may persist for months or years after acute infection. Anti-HBe positivity after acute infection has a duration generally less than that of anti-HBc and anti-HBs and may persist for only 6 months or less in about 5% of acute HBV infections. Early appearance of anti-HBe often predicts an uncomplicated course of acute hepatitis, whereas a delay of more than 6 weeks after the clearance of HBeAg may indicate a prolonged course or development of chronic hepatitis. Seroconversion from HBeAg to anti-HBe frequently occurs several years after the development of HBsAg-positive chronic hepatitis and is usually accompanied by the spontaneous resolution of chronic liver disease. Anti-HBe-positive chronic HBsAg carriers tend to be older than HBeAg-positive carriers, tend to have been chronically infected for many years, tend to have little or no evidence of chronic liver disease, and tend to be minimally infectious. Anti-HBs usually appears several months after the disappearance of HBsAg in acute hepatitis, although it may be present together with HBsAg as early as the onset of symptoms in 6% of cases. Seroconversion from HBsAg to anti-HBs usually reflects termination of HBV infection, clinical recovery, and immunity against reinfection by any HBsAg subtype by virtue of the common antigenic determinant. Anti-HBs persists for years after recovery from acute infection and is usually detected together with anti-HBc and anti-HBe, although it may be found alone in some individuals who have recovered from a past HBV infection with the subsequent loss of detectable anti-HBe and anti-HBc [19]. Anti-HBs is also the only detectable marker in individuals who have been immunized with the hepatitis B vaccine rather than infected with the virus. Anti-HBs is not usually detectable during chronic HBsAg-positive hepatitis, but it may occasionally be present due to previous or subsequent infection with a different HBsAg subtype or as a result of antigen-antibody complexes [20]. Tests for the detection of HBsAg, anti-HBc, and anti-HBs should be performed routinely for the laboratory diagnosis of current or past HBV infection. IgM anti-HBc, HBeAg, and anti-HBe determinations should only be performed when indicated by the results of HBsAg, anti-HBc, and anti-HBs assays. IgM anti-HBc testing can be used to distinguish between acute and chronic HBV infection in HBsAg-positive individuals [21]. HBeAg and anti-HBe detection should be attempted on HBsAg-positive sera when the relative infectivity of blood is of clinical significance or when early seroconversion from HBeAg to anti-HBe after acute HBV infection is used as a prognostic indicator of transient rather than chronic infection [22]. The detection of IgM anti-HBc or anti-HBe may be diagnostic of recent or current acute HBV infection when it is found together with total anti-HBc as the only detectable HBV-associated serological markers during the “window” phase of the infection between the disappearance of HBsAg and the appearance of anti-HBs. HBeAg and anti-HBe determinations may have therapeutic importance in chronic HBsAg-positive hepatitis because it has been suggested that antiviral therapy be reserved for HBeAg-positive patients with chronic liver disease [23].

Nucleic Acid diagnosis

Nucleic acid hybridization assays have been developed for the detection of HBV DNA in the serum of patients with HBV infection. Two (research use only) hybridization assays for the quantitation of HBV DNA in human serum are commercially available: a liquid-phase molecular hybridization assay (Genostics HBV DNA Assay; Abbott Laboratories) and a “sandwich” nucleic acid hybridization assay (Quantiplex HBV-DNA Assay; Chiron

Corporation, Emeryville, Calif.). Slot or dot blot hybridization assays have a detection limit of about 5 pg of HBV DNA per ml, corresponding to approximately 1.5×10^6 viral genomes per ml [24].

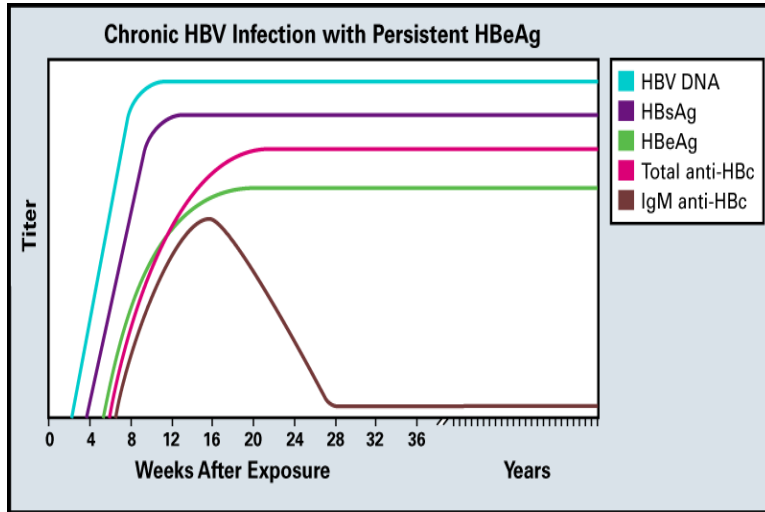


Fig. 1. Virologic and Serologic Markers in Patients who Progress to Chronic Hepatitis B Virus Infection with Persistent HBeAg.

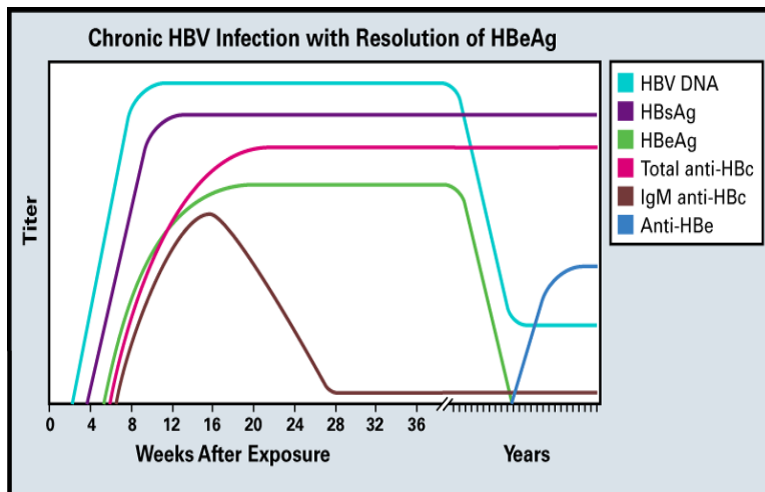


Fig. 2. Virologic and Serologic Markers in Patients who Progress to Chronic Hepatitis B Virus Infection with Resolution of HBeAg

The branchedchain DNA (bDNA) hybridization assay from Chiron Corporation can detect as few as 700,000 HBV DNA equivalents per ml (approximately 2.5 pg/ml) [25]. And the liquid-phase hybridization assay from Abbott has a detection limit of 400,000 HBV DNA equivalents per ml (approximately 1.5 pg/ml). The loss of detectable HBV DNA by a solution hybridization assay is an earlier indicator of response to treatment than loss [26]. PCR has been shown to detect as little as 10^3 pg of HBV DNA per ml, or approximately 100 to 1000 HBV DNA equivalents per ml, and is currently the most sensitive method available for the detection of HBV DNA in the serum. Most HBsAg-positive individuals have detectable HBV DNA in the serum, especially those who are also HBeAg positive. HBeAg-positive individuals usually have high levels of HBV DNA in the serum that are easily detectable by hybridization assays and PCR. HBsAg-positive individuals who are negative for HBeAg usually have lower levels of HBV DNA in the serum that may be undetectable by hybridization assays, but most of these individuals have detectable HBV DNA by PCR. HBeAg-negative patients with detectable serum HBV DNA have higher serum alanine aminotransferase [27].

levels than those who are PCR negative. HBV DNA may persist for several months after the clearance of HBsAg from patients with acute HBV infection. HBV DNA is also detectable in some patients with chronic HBV infection who are anti-HBc positive but HBsAg negative. Finally, in patients with chronic hepatitis B who become HBsAg negative following IFN- α treatment, HBV DNA may remain detectable by PCR for many months after the clearance of HBsAg. Thus, the PCR assay for HBV DNA appears to be more sensitive than HBsAg assays for detecting low-level viremia. The clinical significance of low levels of HBV DNA in HBsAg-negative patients is uncertain because HBV DNA eventually becomes undetectable in most of these patients [28].

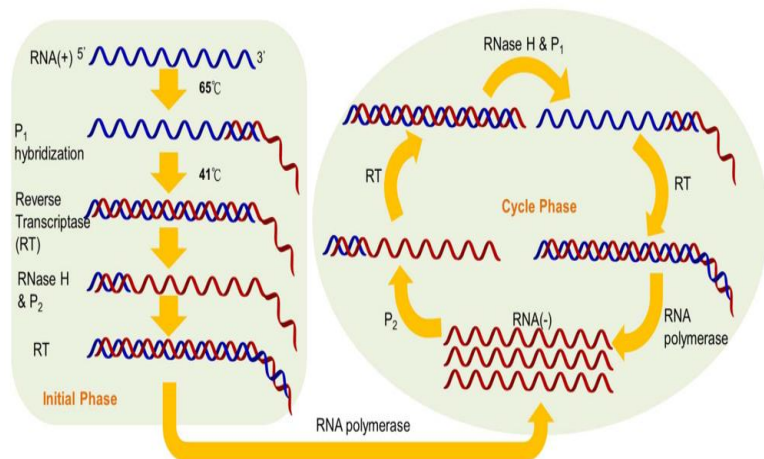


Fig. 3. Schematic representation of the NASBA amplification.

CONCLUSION

There is no completely satisfactory therapy for chronic delta hepatitis. Early studies indicated that a 3- to 4-month course of IFN- α suppressed HDV replication and reduced liver disease in some patients, but almost all patients relapsed after therapy was discontinued [29]. Subsequent studies with longer courses of IFN- α have shown more promising results. Approximately one-half of patients with chronic delta hepatitis who were treated with 9 million units of IFN- α three times a week for 48 weeks had a complete response to treatment [30]. A positive response includes a return of serum alanine aminotransferase levels to normal, clearance of HDV RNA from the serum by hybridization assay, and histologic improvement on liver biopsy. Half of the patients who had a biochemical response to treatment still had normal alanine aminotransferase levels more than. However, none of the treated patients had a sustained clearance of HDV RNA from the serum. While IFN therapy may be helpful in many patients with chronic delta hepatitis, liver transplantation may be the only treatment option for patients with fulminant delta hepatitis or with advanced disease and decompensated cirrhosis. Because HDV can establish infection and cause disease only in individuals who are also infected with HBV, prevention of HBV infection is the most effective way to prevent HDV infection. Persons who are immune to HBV as the result of past infection or vaccination with the hepatitis B vaccine are also protected against HDV infection. Individuals at high risk for HDV infection who are susceptible to HBV infection should receive the hepatitis B vaccine to prevent coinfection with HBV and HDV. Postexposure prophylaxis following percutaneous or permucosal exposure to both HBV and HDV is the same as that for exposure to HBV alone [31]. Prevention of HDV superinfection in persons with chronic HBV infection is more difficult because it must depend on avoiding percutaneous or permucosal exposure to HDV [32].

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FINANCIAL DISCLOSURE

None.

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