

Serologic Diagnosis of Acute and Chronic Viral Hepatitis

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There are at least five forms of viral hepatitis caused by five different viral agents. All five viruses can induce acute hepatitis, but only three can lead to chronic infection. General features of the five forms of viral hepatitis are shown in Table 1.

The five viruses of hepatitis are distinct and show no homology of structure, virus family, or replicative cycle. Yet the five diseases are all very similar and cannot be distinguished reliably by clinical features, routine laboratory tests, or even liver biopsy results. In the past 25 years, several serologic assays have been developed for specific forms of viral hepatitis. However, the number of viral agents, the multiplicity of their antigens, the variability of antibody responses, and the complexity and differing sensitivities and specificities of serologic assays makes interpretation of these tests complex. In this review, we will summarize the major assay available for each form of viral hepatitis and then try to place them in the context of their routine use in serologic diagnosis.

HEPATITIS A

The hepatitis A virus (HAV) is a small RNA virus that causes an acute hepatitis only.¹ Hepatitis A is common worldwide: it is almost a universal disease of childhood in underdeveloped countries and is a frequent cause of both sporadic and epidemic disease in more industrialized nations. Hepatitis A is spread largely by the fecal-oral route. A typical serologic course of acute hepatitis A is shown in Figure 1. Commercial immunoassays are available for anti-HAV and immunoglobulin M (IgM) anti-HAV. Tests for neutralizing anti-HAV as well as for HAV antigen and HAV RNA are research tests.

Anti-HAV is detected at the onset of acute hepatitis A and persists for life.^{1,2} A sensitive and specific radioimmunoassay (RIA) and enzyme-linked immunoassay (EIA) are available that measure total antibody: IgG, IgM, and IgA. The presence of anti-HAV indicates previous or ongoing infection with HAV and is a reliable marker for immunity to hepatitis A. Patients who receive

gamma globulin will have low titers of anti-HAV for a few weeks after inoculation. Patients who receive HAV vaccines (which are now experimental) will have anti-HAV for variable periods. Tests for neutralizing anti-HAV have been useful in evaluating HAV vaccines and gamma globulin efficacy but are not helpful in routine clinical situations.³

IgM anti-HAV is detected at the onset of acute hepatitis A and persists for 3 to 12 months only.⁴ Sensitive RIAs and EIAs for IgM anti-HAV are commercially available. The presence of IgM anti-HAV indicates ongoing (or very recent) infection with HAV and is a reliable diagnostic marker for acute hepatitis A. False-positive reactions are rare but do occur and should be suspected when IgM anti-HAV is found to persist for more than a year.

HAV antigen is detected in the stool of patients with acute hepatitis A, especially during the late incubation period and early symptomatic phase.¹ RIAs for HAV antigen have been used in research laboratories to document the duration of viral shedding. Tests for HAV antigen in liver by immunoperoxidase or immunofluorescence assays provide a means of confirming the diagnosis, but they require a liver biopsy and are rarely necessary.⁵

HAV RNA is detectable in the liver, serum, and stool during the late incubation period and early symptomatic phase of acute hepatitis A. Northern or dot hybridization tests for HAV RNA have been described, but their reliability and sensitivity remain incompletely evaluated.⁶ Testing for HAV RNA by the polymerase chain reaction (PCR) is potentially a powerful means for confirming the diagnosis of this disease and documenting continued infectivity or the source of infection⁷ (such as contaminated water, food, or shellfish).

Diagnosis of acute hepatitis A can be made in a patient with clinical features of acute hepatitis and IgM anti-HAV in serum.

HEPATITIS B

The hepatitis B virus (HBV) is a medium-sized DNA virus that is unique among human viruses in its genomic and antigenic structure and replicative cycle.⁸ Hepatitis B can lead to acute or chronic hepatitis, and it causes much of the morbidity and mortality from acute

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TABLE 1. Five Forms of Viral Hepatitis

	Hepatitis				
	A	B	C	D	E
Virus	HAV	HBV	HCV	HDV	HEV
Family	Picornavirus	Hepadnavirus	Flavivirus	Satellite	Calicivirus
Size	27 nm	42 nm	30–60 nm	40 nm	32 nm
Genome	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA
Length	7.8 kb	3.2 kb	10.5 kb	1.7 kb	8.2 kb
Acute mortality	0.2%	0.2–1%	0.2%	2–20%	0.2%
Chronicity	None	2–7%	50–70%	2–70%	None
Spread	Fecal-oral	Parenteral Sexual Perinatal	Parenteral ?sexual	Parenteral ?sexual	Fecal-oral
Antigens	HAV Ag	HBsAg HBcAg HBeAg	HCV Ag	HDV Ag	HEV Ag
Antibodies	Anti-HAV	Anti-HBs Anti-HBc Anti-HBe	Anti-HCV	Anti-HDV	Anti-HEV
Viral markers	HAV RNA	HBV DNA DNA polymerase	HCV RNA	HDV RNA	Viruslike particles

and chronic liver disease worldwide. Hepatitis B is spread by the parenteral route as well as by intimate contact. The typical serologic course of acute, self-limited hepatitis B is shown in Figure 2; a representative course for chronic hepatitis B is shown in Figure 3. Serologic assays for HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, IgM anti-HBc, and HBV DNA are commercially available. Tests for pre-S antigens and antibodies, for HBV DNA polymerase in serum, and for HBV DNA by PCR in serum and liver are experimental assays that provide interesting insights into this disease but are not often useful in clinical situations.

HBsAg is produced by the envelope or surface (S) gene of HBV and can be detected in high levels (generally 0.1 to 100 $\mu\text{g/ml}$) in serum during acute or chronic

hepatitis.⁹ Sensitive RIAs and EIAs that can detect HBsAg in concentrations of ≥ 0.1 ng/ml are available commercially. The presence of HBsAg is indicative of ongoing HBV infection; however, the amount of this antigen does not correlate with the level of active virus in serum, nor does it indicate whether the disease is acute or chronic, mild or severe. Some patients circulate HBsAg in high concentrations and are completely healthy.¹⁰ False-positive reactions for HBsAg are uncommon, especially with RIA, most false-positive reactions being due to technical errors and are suggested by the finding of borderline or low positive results. HBsAg can also be detected in liver tissue, generally in a cytoplasmic or membranous pattern, by immunofluorescence or immunoperoxidase techniques. However, tests for

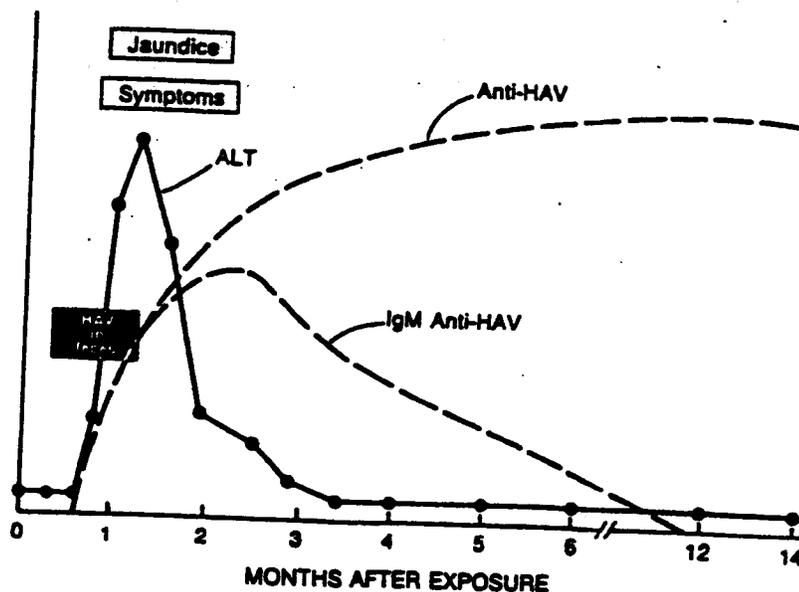


FIG. 1. Typical course of a case of acute hepatitis A. ALT: alanine aminotransferase; HAV: hepatitis A virus; anti-HAV: antibody to HAV.

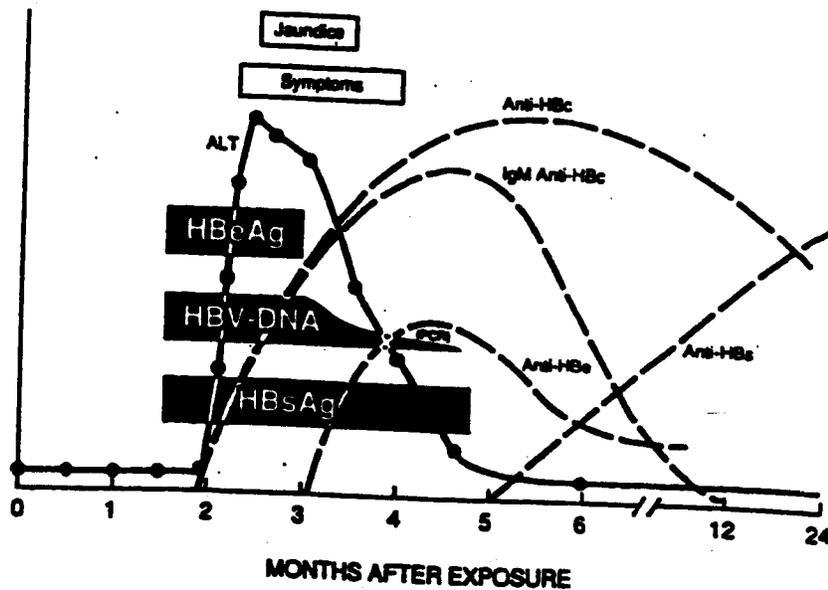


FIG. 2. Typical course of a case of acute hepatitis B. Initially, HBV DNA can be detected by blot hybridization, but as the disease resolves only low levels detectable by polymerase chain reaction can be detected. ALT: alanine aminotransferase; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis B e antigen; HBV DNA: hepatitis B virus deoxyribonucleic acid; anti-HBc: antibody to hepatitis B core antigen; anti-HBe: antibody to HBeAg; anti-HBs: antibody to HBsAg; PCR: polymerase chain reaction.

HBsAg in serum are more sensitive and reliable than staining tests in liver.³ Thus, immunostaining for HBsAg in liver adds information only when serum test results are not available.

The pre-S(1) and pre-S(2) antigens are synthesized in variable amounts with HBsAg and represent antigens that are particularly rich on the surface of virus particles, as opposed to major HBsAg which is found both on the surface of virions as well as subviral particles.¹¹ Tests

for pre-S antigens are generally difficult and remain research reagents. The presence of pre-S antigen in serum has been claimed to indicate high levels of HBV. However, when sensitive methods are used, pre-S antigens are found in virtually all HBsAg-positive sera, although in lower titer in patients who have low levels of actual virus.^{12,13}

HBeAg represents a secreted product of the nucleocapsid gene of HBV¹⁴ and is found in serum early during

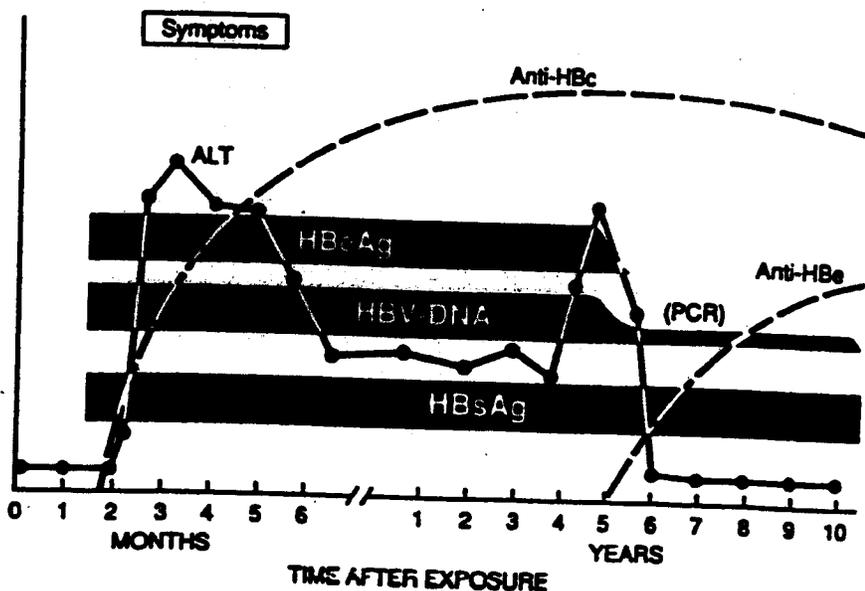


FIG. 3. Representative course of a case of chronic hepatitis B in which acute infection is followed by chronic infection. Ultimately, there is a remission in disease when seroconversion from HBeAg to anti-HBe occurs. See Figure 2 for abbreviations.

acute and chronic hepatitis B.¹⁵⁻¹⁷ Except in very rare instances, HBeAg is not detected in serum without HBsAg. Reasonably sensitive and specific RIAs and EIAs for HBeAg are commercially available. The finding of HBeAg in conjunction with HBsAg indicates a high level of viral replication, with a likelihood of active liver disease and high levels of infectivity. Testing for HBeAg is clinically useful in assessing the degree of infectivity and whether active viral replication is present. Most patients with HBeAg have active liver disease; the majority of patients without HBeAg have resolving, minimal, or no active liver disease.

HBcAg, the major product of the nucleocapsid gene, is not detectable in serum by conventional techniques. However, HBcAg is readily detected in liver in patients with acute or chronic hepatitis.^{5,18,19} Immunoperoxidase techniques for detecting HBcAg as well as HBsAg in liver are easily established using standard techniques and commercial reagents; such tests should be available in most pathology departments. Testing for HBcAg in liver tissue may be the most sensitive means of detecting ongoing viral replication in chronic hepatitis B.¹⁹

HBV DNA is detectable in serum during acute and chronic hepatitis B infections.¹⁸⁻²¹ Using direct "blot" or liquid hybridization assays, HBV DNA can be detected in levels of 10 to 500 pg/ml (which represents approximately 1,000,000 genome-equivalents/ml) early during the course of acute hepatitis B and for prolonged periods during chronic hepatitis B while the liver disease is active. Research assays for HBV DNA are commercially available from some clinical pathology laboratories.²¹ Testing for HBV DNA can be helpful clinically, since loss of HBV DNA from the serum during both acute and chronic hepatitis B indicates resolution of the active viral replication and usually precedes resolution of the disease activity.¹⁶ HBV DNA testing is also used to assess whether treatment with antiviral agents is needed and to assess the outcome of treatment, the primary endpoint of successful antiviral therapy being loss of HBV DNA as detected by hybridization.²² Recently, the PCR technique has been applied to detecting HBV DNA in serum.²³ HBV DNA testing results obtained by PCR provides information that has greatly different clinical significance. PCR is 10,000-fold more sensitive than direct hybridization in detecting nucleic acids and will detect HBV DNA in levels of 10 pg/ml (approximately 10 to 50 genomes/ml). Using PCR, HBV DNA can be detected in most HBsAg-positive sera, and occasionally for a short period before HBsAg arises or after it disappears. The major problem with PCR for HBV DNA is that it is so sensitive that it is prone to false-positive reactions, largely based on minute contamination of the sample being tested or the reagents used in the test. Thus, if strict methods are not used to avoid contamination, PCR for HBV DNA can be very unreliable. Furthermore, the clinical significance of HBV DNA as detected by PCR is quite different from that of finding HBV DNA by hybridization. Detection of HBV DNA by PCR has the same clinical significance as detection of HBsAg, indicating ongoing HBV infection. In contrast, detecting HBV DNA by hybridization is similar in clinical significance to detecting

HBeAg, indicating high levels of viral replication and the high probability of active liver disease and infectivity.¹⁶⁻¹⁹

Anti-HBs develops during convalescence from hepatitis B and is usually not detectable when HBsAg is present.^{15,24} Both RIAs and EIAs for anti-HBs are available commercially. The presence of anti-HBs is generally interpreted as indicating recovery and immunity from HBV infection. Successful vaccination against HBV induces anti-HBs and is generally protective against infection.²⁵ However, the level of protection is somewhat variable. In general, the level of anti-HBs is expressed as mIU or as a ratio of sample results to negative control results on immunoassay, with a ratio of 10 or greater being considered minimal for protection against exposure. Commercial laboratories should express the anti-HBs result as mIU or a ratio or at least indicate whether a borderline result was obtained (ratio less than 10). It should also be stressed that some chronic HBsAg carriers have both HBsAg and anti-HBs, the antibody being directed against HBsAg epitopes not shared by the circulating antigen and thus not protective.²⁶

Anti-pre-S antibodies generally arise before anti-HBs during the course of acute hepatitis B, at a time that HBsAg is detectable.^{12,13} The development of anti-pre-S (and loss of pre-S antigen) has been claimed to be a favorable serologic sign, indicating clearance of HBV replication. However, tests for anti-pre-S have not become standardized and there is a lack of agreement in the literature on the pattern of development of these antibodies and their ultimate significance. Patients with chronic hepatitis B also develop anti-pre-S, suggesting that these antibodies are not responsible for viral clearance.

Anti-HBc appears at the onset of symptoms or liver test abnormalities in acute hepatitis B, rapidly rises to high levels, and persists for life.^{15,24} Tests for anti-HBc are commercially available.²⁷ The presence of anti-HBc indicates previous or ongoing infection with HBV. Testing for anti-HBc is therefore a valuable epidemiologic tool in assessing patterns of HBV spread. Patients who receive HBV vaccine do not develop anti-HBc; so that the presence of this antibody is helpful in distinguishing between successful vaccination and HBV infection. Rare instances of HBV infection without the development of anti-HBc have been described.¹⁷

IgM anti-HBc is detected at the onset of acute hepatitis B and persists for only 3 to 12 months if the disease resolves.²⁸ In patients who develop chronic hepatitis B, IgM anti-HBc persists at low levels as long as viral replication persists.²⁹ A commercial RIA and EIA for IgM anti-HBc have been developed that detect this antibody only when present in high titer.²⁸ This commercial IgM anti-HBc test is helpful as a diagnostic assay for acute hepatitis B (it does not detect the low levels present in chronic hepatitis B cases).

Anti-HBe becomes detectable when HBeAg is lost in acute or chronic hepatitis B.^{15,24} The commercial RIAs and EIAs for anti-HBe are not very sensitive and many patients do not develop detectable levels of anti-HBe. In patients with acute hepatitis B, the appearance of anti-HBe indicates that the infection is resolving even though HBsAg may still be present.¹⁶ In chronic hepatitis B,

most patients with anti-HBe have resolving, minimal, or no associated liver disease. Exceptions to this occur.¹⁹ Particularly striking are cases of hepatitis B infection with an HBV mutant that has an A-to-T single base-pair mutation in the precore region of the genome that creates a stop codon and thus prevents the synthesis of HBeAg.^{30,31} Patients with this "e-minus" mutant have HBV DNA with anti-HBe (and no HBeAg) in serum. The associated disease can be mild or severe. These e-minus mutants are most common in Mediterranean countries but occur elsewhere and may arise during the course of typical chronic hepatitis B. Indeed, it has been suggested that the persistence of HBsAg after seroconversion from HBeAg to anti-HBe may be largely due to the persistence of e-minus mutants that are relatively poor in producing virions but relatively efficient in synthesizing HBsAg. The only current technique for detecting these HBV mutants is amplification of the HBV DNA using PCR followed by nucleotide sequencing of the precore region. Using similar techniques to amplify other regions of the genome, new mutants have been described;³² their clinical significance remains to be demonstrated.

Another exception to the correlation between presence of anti-HBe and absence of significant liver disease occurs with reactivation of chronic hepatitis B.³³ Some patients who have lost HBV DNA from serum and seroconverted from HBeAg to anti-HBe later have a return of these viral markers in association with a flare in the hepatitis. Reactivation can be transient or sustained. On occasion, reactivation can be severe and life threatening and can mimic acute viral hepatitis.³⁴ In a proportion of patients, reactivation can be recurrent and associated with severe and progressive liver disease. Reactivation is diagnosed based on the return of HBV DNA as detected by direct hybridization (or DNA polymerase activity) in a patient previously nonreactive for HBeAg and HBV DNA. Some patients also become HBeAg positive during reactivation.

The HBV harbors an endogenous *DNA polymerase activity* that has reverse transcriptase activity as well.⁴ Tests for DNA polymerase activity in serum are often used to assess viral replication in studies of antiviral therapy.^{16,22} By and large, levels of DNA polymerase correlate with those of HBV DNA as detected by direct hybridization.¹⁸ Assays for DNA polymerase activity are simple and inexpensive; however, measuring HBV DNA in serum is more quantitative and more reliable as a marker of active, high levels of viral replication.^{21,22} Recently, reports of finding antibody to DNA polymerase activity have appeared.³⁵ This antibody arises commonly during convalescence and its clinical significance is not yet clear.

The HBV has four potential gene regions: the S or envelope region (HBsAg), the C or nucleocapsid region (HBcAg and HBeAg), the P region (DNA polymerase), and, fourth, a less well-characterized region known as X. The X gene product is known as hepatitis B x antigen (HBxAg) and has been detected in liver in patients and experimental animals with acute or chronic hepatitis.³⁶ Antibody to HBxAg (anti-HBx) can occasionally be detected in serum. However, the assays for the HBxAg and anti-HBx are not commercially available and the clinical

significance of the presence or absence of this antigen and antibody is not clear.

Diagnosis of acute hepatitis B can be made in a patient with clinical features of acute hepatitis and HBsAg or IgM anti-HBc, or both, in serum. If IgM anti-HBc is absent, the patient is likely to have an underlying chronic hepatitis B or HBsAg carrier state. A tentative diagnosis of chronic hepatitis B can be made in a patient with the clinical features of chronic hepatitis and HBsAg in serum for at least 6 months; confirmation of the diagnosis requires the finding of serologic evidence for active viral replication such as HBeAg or HBV DNA in serum or HBeAg in liver. In both acute and chronic hepatitis B, the possibility of delta hepatitis co- or superinfection should be considered and appropriate tests done for anti-HDV (see later).

HEPATITIS C

The hepatitis C virus (HCV) is a recently discovered RNA virus that appears to be responsible for most cases of parenterally transmitted non-A, non-B hepatitis.^{37,38} HCV infection becomes chronic in approximately 50% of cases and can lead insidiously to cirrhosis in as many as 25% of patients with chronic infection and to hepatocellular carcinoma in a proportion of those with cirrhosis.³⁸⁻⁴⁰ The epidemiology of hepatitis C has not been completely defined. This disease is found worldwide and appears to be almost as common in developed as underdeveloped countries. Hepatitis C can be spread parenterally, but appears to be transmitted only rarely by familial, sexual, or maternal-infant exposure.⁴⁰

Serologic tests for HCV infection have been developed recently and are in a stage of rapid evolution. At present, the only assay for HCV infection that is commercially available and widely used is an EIA for anti-HCV.⁴¹ These immunoassays as well as the original RIA for anti-HCV have used a recombinant yeast polypeptide referred to as C100-3, which was derived from a non-structural region of the HCV genome and produced by extending the original, smaller HCV cDNA clone (named 5-1-1). Other tests for HCV infection that are available on a research basis include immunoblot assays for anti-HCV, immunostaining for HCV antigen in liver, and PCR for HCV RNA in serum and liver. The course of a representative case of hepatitis C that progressed from an acute to chronic infection is shown in Figure 4.

Anti-HCV is detected late during the course of acute hepatitis C, generally between 4 and 24 weeks (mean, 15 weeks) after onset of symptoms.⁴¹ In patients who develop chronic hepatitis C, anti-HCV appears to persist indefinitely, whereas in cases in which the infection resolves, anti-HCV reactivity often disappears over the ensuing few years. The anti-HCV assay does not distinguish between IgM and IgG antibody. Thus, current tests for anti-HCV are not particularly reliable for diagnosis. In acute hepatitis C, the antibody reactivity often arises late so that both acute and convalescence phase samples must be tested. In chronic disease, 10 to 20% of patients infected with virus do not test positive. Cases of apparent chronic non-A, non-B hepatitis lacking anti-HCV may

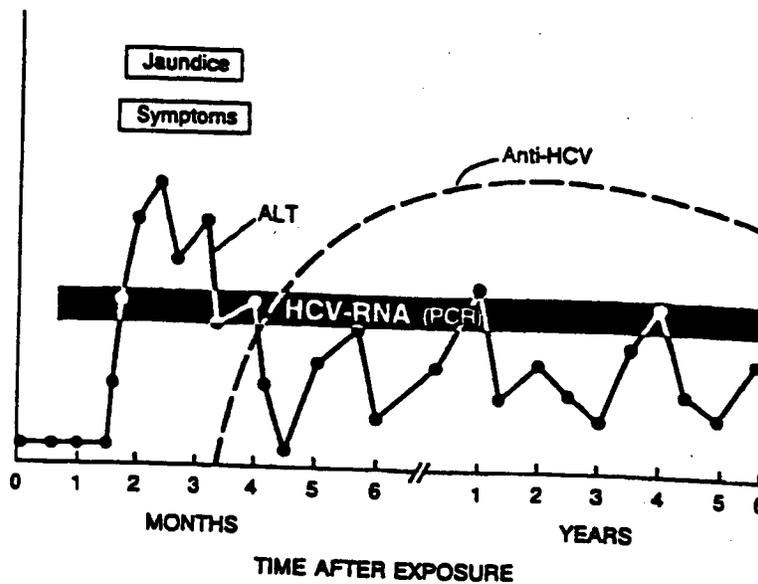


FIG. 4. Typical course of a case of acute hepatitis C that progresses to chronic infection and disease. ALT: alanine aminotransferase; HCV RNA: hepatitis C virus ribonucleic acid; anti-HCV: antibody to hepatitis C virus; PCR: polymerase chain reaction.

have another form of chronic hepatitis (viral or nonviral) but may also be patients with chronic hepatitis C who do not generate a detectable anti-HCV response.

Recently, concerns regarding the specificity of current anti-HCV tests have arisen. A high rate of apparently false-positive anti-HCV reactions have been detected in patients with alcoholic liver disease and autoimmune chronic active hepatitis.⁴² In these patients, anti-HCV positivity has correlated with the degree of hyperglobulinemia. The conditions of serum sample storage and testing may also account for false-positive reactions. However, false-positive anti-HCV reactivity can occur even in populations at low risk for HCV infection, such as normal volunteer blood donors. Most but not all false-positive test results for anti-HCV have yielded borderline or low level EIA absorbancy reactions.

Because of concerns about false-positivity, a recombinant immunoblot assay (RIBA) has been developed as a supplemental test.⁴³ In this assay, serum is applied to a strip of nitrocellulose to which has been blotted yeast recombinant HCV polypeptide (C100-3), a bacterial recombinant HCV protein (from clone 5-1-1), and yeast recombinant superoxide dismutase (to which the C100-3 protein is linked for efficient expression in yeast). After staining with a second antibody, samples are interpreted as positive, nonreactive, or indeterminate. A positive test for anti-HCV by RIBA provides confirmation of an EIA result, but a negative or indeterminate reaction cannot be interpreted as proof of a false-positive EIA reaction.

Newer EIA assays for anti-HCV are being developed that utilize several recombinant proteins from various regions of the HCV genome. By broadening the spectrum of antibodies detected, these assays may overcome the shortcomings of the current anti-HCV tests that lack optimal sensitivity and specificity.

HCV antigen has been detected by immunofluorescence microscopy in a spotty and sparse cytoplasmic pattern in hepatocytes from cases of acute and chronic hepatitis C.⁴⁴ The HCV antigen becomes detectable before onset of disease and appearance of anti-HCV in serum. The HCV antigen detected in this assay represents epitopes not present in the C100-3 clone used in the EIA for anti-HCV, but its exact location on the HCV genome is not yet known. The immunofluorescence test for HCV antigen is still a research reagent, the sensitivity and specificity of which has not been fully evaluated. Because HCV antigen can be detected in acute and chronic hepatitis C, detection may play an important role in confirming anti-HCV results and in documenting the presence of active HCV infection.

HCV RNA cannot be detected in serum by standard techniques such as northern blot analysis because the virus evidently circulates in low levels only.³⁹ However, the PCR technique, which can detect as few as 1 to 10 molecules of nucleic acid, can be modified for detection of HCV RNA in both serum and liver.⁴⁵ This testing involves a first step of reverse transcription of viral RNA into cDNA, followed by amplification of the specific HCV cDNA by PCR. The amplified products can then be detected by direct molecular hybridization or by ethidium bromide staining after agarose gel electrophoresis. Preliminary studies indicate that most patients with acute hepatitis C circulate HCV RNA during the incubation period and symptomatic phase of disease and that 40 to 70% of patients with chronic hepatitis C have viral genome in serum.⁴⁵⁻⁴⁸ Improvements in the design of the PCR assay (using highly conserved regions of the genome to amplify) should improve the sensitivity and reliability of the PCR assay for HCV RNA. Direct PCR for HCV RNA in serum may well be the best means to confirm the presence of HCV infection, and quantifying

levels of viral genome in serum and liver by PCR may become an important means of assessing antiviral therapy in this disease.

Diagnosis of acute hepatitis C can be made in a patient with the clinical features of acute hepatitis and anti-HCV in acute and convalescent phase serum. The tentative diagnosis of chronic hepatitis C can be made in a patient with the features of chronic hepatitis and anti-HCV serum. In either situation, the diagnosis should be made cautiously and other forms of liver injury should be excluded by appropriate clinical, serum biochemical or serologic features. Confirmatory testing should be performed if possible (RIBA for anti-HCV or PCR for HCV RNA). The history of parenteral exposure to blood or blood products (occupational, accidental, or through blood transfusions or drug abuse) is supportive of the diagnosis of hepatitis C. The diagnosis of non-A, non-B hepatitis should be made in a patient with clinical features of acute or chronic hepatitis who has a clear-cut history of parenteral exposure before the onset of disease but who repeatedly tests negative for anti-HCV. Patients who have no history of potential parenteral exposure and are negative for anti-HCV should be considered to have chronic hepatitis of unknown cause.

HEPATITIS D

The hepatitis delta virus (HDV) is a small defective RNA virus that causes hepatitis only in individuals who are concurrently infected with hepatitis B.⁴⁹ Hepatitis D tends to be a severe disease with a high mortality rate of the acute disease and a propensity of the chronic disease to lead to cirrhosis.⁵⁰ Hepatitis D is found endemically in some areas (Middle East, Amazon Basin), but in most areas of the world it is found largely in certain high-risk

groups, including parenteral drug abusers and recipients of multiple blood transfusions or plasma products (such as patients with thalassemia or hemophilia).

HDV can infect a person who is already a carrier of HBV (superinfection) or can be transmitted simultaneously with HDV (coinfection). These two forms of HDV infection should be separated both because of the differences in prognosis and in patterns of serologic events. The course of a typical case of HDV coinfection is shown in Figure 5 and of HDV superinfection in Figure 6. Both EIA and RIA tests for anti-HDV are widely available; similar immunoassays for IgM anti-HDV and HDV antigen may soon become available. Research assays for detection of HDV infection include immunostaining for HDV antigen, immunoblotting for HDV antigen, and molecular hybridization for HDV RNA.

Anti-HDV arises late during acute delta hepatitis and is usually not detectable in serum at the onset of symptoms.⁵¹⁻⁵³ Indeed, in some cases of self-limited delta hepatitis, anti-HDV is never detectable. For these reasons, the serologic diagnosis of acute delta hepatitis is often unsatisfactory and requires testing of both acute and convalescent phase samples. The majority of patients with acute delta coinfection ultimately clear both HBV and HDV and do not develop chronic disease. In contrast, the majority of patients with acute delta superinfection develop chronic delta hepatitis. In these latter patients, titers of anti-HDV rise to high levels. Thus, the detection of high titers of anti-HDV (>1:100 in dilution series by EIA or RIA) is strongly suggestive of chronic delta hepatitis. These immunoassays detect all forms of antibody and do not distinguish between IgG and IgM anti-HDV.

IgM anti-HDV tests are available in Europe commercially and provide additional helpful information in some cases. The IgM antibody is detected earlier during

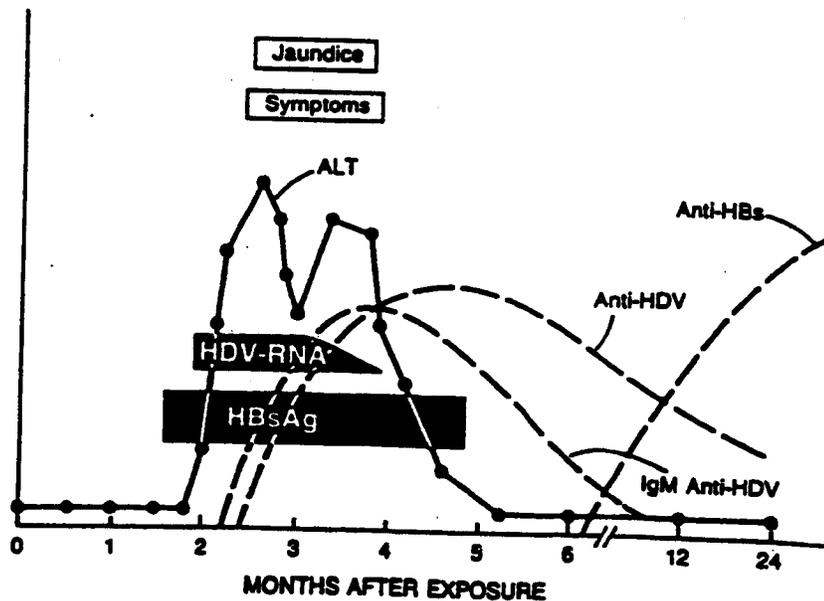


FIG. 5. Typical course of a case of acute delta hepatitis coinfection. ALT: alanine aminotransferase; HBsAg: hepatitis B surface antigen; HDV RNA: hepatitis delta virus ribonucleic acid; anti-HDV: antibody to HDV; anti-HBs: antibody to HBsAg.

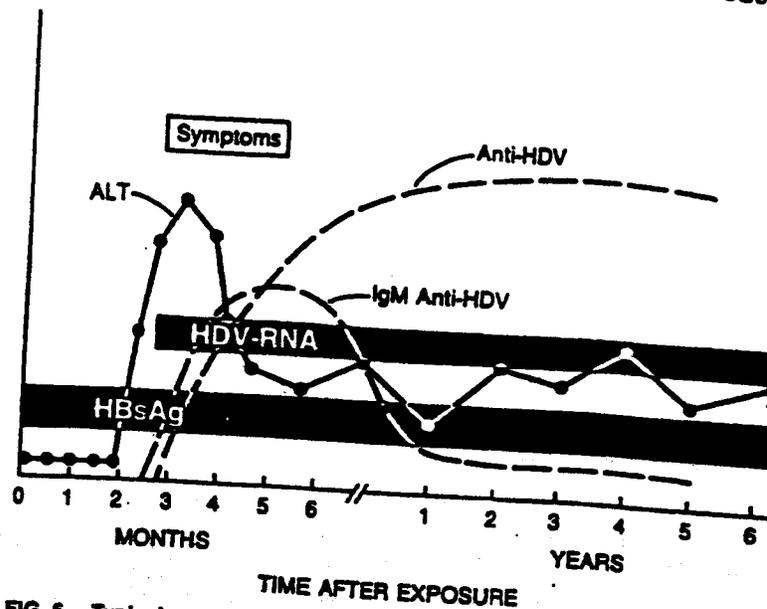


FIG. 6. Typical course of a case of acute delta hepatitis superinfection. See Figure 5 for abbreviations.

the course of acute delta hepatitis and is thus more reliable as a single diagnostic marker for this disease.⁵³ Low titers of IgM anti-HDV continue to be present in patients with chronic delta hepatitis. Indeed, sequential testing for IgM anti-HDV may be helpful in monitoring antiviral therapy because disappearance of this antibody is evidence that the HDV infection has been eradicated.⁵⁴

HDV antigen as detected by EIA or RIA is present in serum transiently during the late incubation period of acute delta hepatitis.⁵⁵ Depending on the sensitivity of the assay, HDV antigen may be present at the onset of symptoms, but it is not reliably detectable by these immunoassays in patients with chronic disease. Thus, this assay can augment serologic diagnosis of acute hepatitis. Interestingly, immunoblotting (Western blot) assays for HDV antigen yield different results from EIA or RIA. Using immunoblotting, a large percentage of patients with chronic delta hepatitis have HDV antigen in serum.⁵⁶ Furthermore, improvements in chronic delta hepatitis, either spontaneously or as a result of antiviral therapy, are usually accompanied by a decrease or loss of HDV antigen from serum.^{49,54} Immunoblotting for HDV antigen is a difficult research technique that is not widely applicable for clinical situations.

The standard for diagnosis of chronic delta hepatitis is the detection of HDV antigen in liver.^{50,57} The antigen is detectable in the nuclei of hepatocytes (and weakly in the cytoplasm of some cells) in almost all patients with chronic delta hepatitis. A variety of immunostaining techniques have been devised, including immunofluorescence and immunoperoxidase (biotin-avidin, and peroxidase-anti-peroxidase [PAP]) for detection of HDV antigen. The PAP test is perhaps the easiest technique and should not be beyond the scope of many pathology laboratories; it employs anti-HDV positive human serum and can be applied to formalin-fixed, paraffin-embedded sections.

HDV RNA can be readily detected by molecular hy-

bridization in the serum of infected individuals.⁵⁸ Either direct spot or Northern blot hybridization will detect the viral genome transiently in patients with acute delta hepatitis and for prolonged periods in the majority of patients with chronic delta hepatitis. Monitoring of alpha-interferon therapy in chronic delta hepatitis is best accomplished by sequential testing for HDV RNA in serum. However, HDV RNA can disappear during antiviral therapy only to be followed by a relapse in disease and reappearance of HDV RNA when treatment is stopped. Testing for HDV RNA in liver by in situ hybridization is probably a more direct and sensitive means of assessing the state of viral replication in this disease.⁵⁹

Diagnosis of acute delta hepatitis can be made in a patient with the clinical features of acute hepatitis and both HBsAg and anti-HDV in serum; both acute and chronic phase sera should be tested for anti-HDV reactivity. The acute delta hepatitis can be referred to as coinfection if IgM anti-HBc is present, since this marker suggests co-occurrence of acute hepatitis B. The acute delta hepatitis can be referred to as superinfection if IgM anti-HBc is absent, since this finding suggests an underlying chronic hepatitis B or HBsAg carrier state. The tentative diagnosis of chronic delta hepatitis can be made in a patient with the clinical features of chronic hepatitis and both HBsAg and high titers of anti-HDV in serum for 6 months or more. The diagnosis can be confirmed by the finding of serologic markers of active HDV replication, such as HDV RNA, IgM anti-HDV, or HDV antigen (by Western blotting) in serum or HDV RNA or HDV antigen in liver.

HEPATITIS E

The hepatitis E virus (HEV) is a small RNA virus that causes acute epidemic or enterally transmitted non-A, non-B hepatitis.^{60,61} HEV infection does not lead to

chronic hepatitis or a carrier state. The virus is transmitted by the fecal-oral route, and transmission is associated with contamination of food or water sources.⁶² Hepatitis E is a common cause of sporadic and epidemic hepatitis in underdeveloped areas of the world, particularly in the Indian subcontinent and Asia. In developed countries, only imported cases of hepatitis E have been reported. Currently, the only tests for HEV infection are the tedious research assays of immune electron microscopy and immunofluorescence, both of which can be used to detect either antigen or antibody. The genome of HEV has recently been cloned and characterized as a 8.2 kb single-stranded RNA, a discovery that should soon lead to reliable, recombinant antigen-based immunoassays.⁶³

HEV antigen can be detected in liver, bile, and stool during the incubation period and symptomatic phase of hepatitis E. The HEV was first identified by immune electron microscopy carried out on acute phase stool specimens using convalescence phase serum.⁶⁴ Small 27 to 32 nm viruslike particles are identifiable in stool⁶¹ and similar structures have been found in bile.⁶⁵ Immune electron microscopy can be used to detect virus particles or antibody. However, the technique is tedious, requires considerable experience, and is not suitable for routine clinical use.

Anti-HEV can be detected by immune electron microscopy in serum during the acute illness.⁶⁰⁻⁶² Titers of anti-HEV rise during convalescence. However, it is not clear whether anti-HEV persists for prolonged periods after acute infection, and immunity to reinfection may not be absolute. Recently, a new research assay for anti-HEV has been developed based on the finding of HEV antigen in liver tissue of experimentally infected primates using direct immunofluorescence microscopy.⁶⁶ Blocking of the immunofluorescence reactivity by preincubation with test serum can be used as an assay for anti-HEV. This blocking immunofluorescence has been used to characterize the epidemiology and course of HEV infection, but it is poorly suited to widescale use. The cloning of HEV RNA should soon lead to recombinant immunoassays for both anti-HEV and HEV antigen.⁶³

Diagnosis of acute hepatitis E can be made tentatively in a patient with clinical features of acute hepatitis, recent history of living in or travel to endemic areas of the world (Central and South America, Asia, Africa, and the Middle East), and absence of serologic markers of acute hepatitis A, B, and C. The diagnosis can be confirmed by the finding of anti-HEV in serum, but this diagnosis will remain one of exclusion, supported by epidemiologic features, until serologic tests for anti-HEV become more widely available.

SEROLOGIC DIAGNOSIS

Serologic tests in viral hepatitis are frequently misapplied due to a mistaken impression that all serologic tests can provide helpful information in all situations. Serologic testing can be very expensive; these assays cost between \$20 and \$150 each. In addition, many of the tests do not provide any helpful information either for diagnosis, assessment, or management. The choice

TABLE 2. Serologic Diagnosis of Acute Viral Hepatitis*

Disease	Serologic Results	Comments
Hepatitis A	IgM anti-HAV	Reasonably specific Can be negative (early loss)
Hepatitis B	HBsAg and IgM anti-HBc	
Hepatitis C	Anti-HCV	Indicates acute hepatitis Can appear late in disease
Hepatitis D	HBsAg and Anti-HDV	Can appear late in disease Coinfection
Hepatitis E	IgM anti-HBc present IgM anti-HBc absent	Superinfection History of exposure History of exposure
Hepatitis NANB	All negative All negative	

*Initially four tests should be obtained: IgM anti-HAV, IgM anti-HBc, HBsAg, and anti-HCV. In certain situations, further testing for anti-HDV and anti-HCV are needed (see text).

of serologic tests should be based on the clinical purpose of the testing and interpreted in light of clinical features.

If the purpose of testing is for *diagnosis of acute hepatitis*, four tests are appropriate initially—IgM anti-HAV, IgM anti-HBc, HBsAg, and anti-HCV—the results of which will provide a diagnosis in most cases (Table 2). If HBsAg is present, further testing for anti-HDV is appropriate, especially if there are epidemiologic features that suggest delta hepatitis (intravenous drug abuse or exposure to blood products). If all tests are nonreactive initially, follow-up testing for anti-HCV is appropriate because this antibody can arise late in the disease. Repeat testing for IgM anti-HAV and IgM anti-HBc or initial testing for "total" anti-HAV and anti-HBc or HBeAg, anti-HBc and HBV DNA are not appropriate. If all serologic tests remain negative, the diagnosis of non-A, non-B hepatitis should only be used if other forms of acute hepatic injury are excluded. Important causes of an acute viral hepatitis-like syndrome include acute cholangitis, ischemic liver injury, drug-induced hepatitis, syphilis, mononucleosis, cytomegalovirus infection, and an acute exacerbation of an underlying chronic hepatitis.

If the purpose of serologic testing is *diagnosis of chronic hepatitis*, two tests are appropriate—HBsAg and anti-HCV (Table 3). If HBsAg is present, testing for HBeAg or HBV DNA is appropriate to document whether active viral replication is present and testing for anti-HDV should be performed to assess whether delta hepatitis coexists. It should be stressed that tests for anti-HAV, anti-HBc, anti-HBs are of no help in assessing patients with chronic liver disease; these assays are helpful only in assessing immunity to these infections and in performing epidemiologic surveys. In chronic viral hepatitis, liver biopsy can be helpful in confirming the diagnosis and assessing severity and prognosis. The finding of HBcAg in liver confirms the diagnosis of hepatitis B, and the finding of HDV antigen, the diagnosis of hepatitis D. In the future, tests for HCV antigen may provide confirmatory evidence for hepatitis C. If sophisticated serologic tests are available (such as for HBV DNA,

TABLE 3. Serologic Diagnosis of Chronic Viral Hepatitis*

Disease	Suggested by	Confirmed by
Chronic hepatitis B	HBsAg	HBcAg or HBV DNA or HBcAg in liver
Chronic hepatitis D	HBsAg Anti-HDV (>1:100)	HDV RNA or HDV antigen or HDV antigen in liver
Chronic hepatitis C	Anti-HCV	Exclusion of other diagnoses
Chronic hepatitis NANB	All negative	History of exposure and exclusion of other diagnoses

*The finding of HBsAg without HBV DNA, HBcAg, or HBcAg indicates the inactive HBsAg carrier state.

HCV RNA, or HDV RNA in serum), liver biopsy is not necessary for diagnosis. In any case of chronic hepatitis, but particularly when the diagnosis of chronic non-A, non-B hepatitis is entertained, it is important to exclude other forms of liver disease, such as alcoholic liver disease, autoimmune liver disease, gallstones, hemochromatosis, Wilson's disease, and drug-induced liver injury.

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