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Editorial

Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver; it is a type of viral hepatitis. During the initial infection people often have mild or no symptoms. Occasionally a fever, dark urine, abdominal pain, and yellow tinged skin occurs. The virus persists in the liver in about 75% to 85% of those initially infected. Early on, chronic infection typically has no symptoms. Over many years however, it often leads to liver disease and occasionally cirrhosis. In some cases, those with cirrhosis will develop serious complications such as liver failure, liver cancer, or dilated blood vessels in the esophagus and stomach.

HCV is spread primarily by blood-to-blood contact associated with injection drug use, poorly sterilized medical equipment, needlestick injuries in healthcare, and transfusions. Using blood screening, the risk from a transfusion is less than one per two million. It may also be spread from an infected mother to her baby during birth. It is not spread by superficial contact. It is one of five known hepatitis viruses: A, B, C, D, and E.

Diagnosis is by blood testing to look for either antibodies to the virus or viral RNA. In the United States, screening for HCV infection is recommended in all adults age 18 to 79 years old.

There is no vaccine against hepatitis C. Prevention includes harm reduction efforts among people who inject drugs, testing donated blood, and treatment of people with chronic infection. The **“DISEASE DIAGNOSIS”** section highlights all clinic-diagnostic aspects of Hepatitis C.

“INTERPRETATION” portion outlines understanding Hepatitis in general while **“TROUBLE SHOOTING”** segments delves deep in to Hepatitis B markers.

“BOUQUET” and TULIP NEWS as usual stand out in their own right.

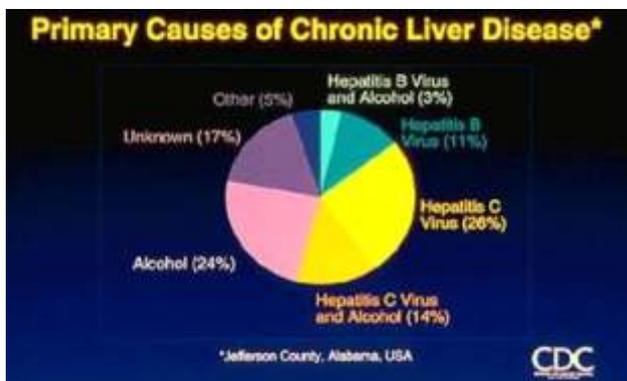


DISEASE DIAGNOSIS

HEPATITIS C

Background

Hepatitis C is a worldwide problem. The hepatitis C virus (HCV) is a major cause of both acute and chronic hepatitis. The World Health Organization (WHO) estimates about 71 million people globally have chronic hepatitis C, with approximately 399,000 dying from this infection, primarily due to cirrhosis and hepatocellular carcinoma (HCC). **The prevalence of HCV infection varies throughout the world.** For example, Frank et al reported in 2000 that Egypt had the highest number of reported infections, largely attributed to the use of contaminated parenteral antischistosomal therapy. This led to a mean prevalence of 22% of HCV antibodies in persons living in Egypt. **In the United States, the incidence of acute HCV infection has sharply decreased during the past decade,** but its prevalence remains high. According to US Centers for Disease Control and Prevention (CDC) estimates, 2.7-3.9 million people (most of whom were born from 1945 through 1965) in the United States have chronic hepatitis C which develops in approximately 75% of patients after acute infection. This virus is the most common blood-borne pathogen in the United States and a leading cause of morbidity and mortality, primarily through the development of liver fibrosis and cirrhosis; persons with chronic infection live an average of 2 decades less than healthy persons. **Infection due to HCV accounts for 20% of all cases of acute hepatitis,** an estimated 30,000 new acute infections, and 8,000-10,000 deaths each year in the United States. HCV has rapidly surpassed human immunodeficiency virus (HIV) as a cause of death in the United States. An examination of nearly 22 million death records over 9 years revealed an HCV mortality rate of 4.58 deaths per 100,000 people per year and an HIV mortality rate of 4.16 deaths per 100,000 people. Almost 75% of HCV deaths occurred among adults between the ages of 45 and 64 years. **Medical care costs associated with the treatment of HCV infection in the United States are high.** Treatment with a 12-week regimen of HCV antiviral agents can cost up to \$95,000. The incremental cost-effectiveness ratio (ICER) for direct-acting antiviral agents (DAAs) remains up to \$100,000 across all HCV genotypes and fibrosis stages. With an estimated 2.7-3.9 million people having chronic infection, the potential medication costs alone could range from \$257 billion to \$371 billion per year. **Because most patients infected with HCV have chronic liver disease,** which can progress to cirrhosis and HCC, chronic infection with HCV is one of the most important causes of chronic liver disease (see the image below) and, according to a report by Davis et al, the most common indication for orthotopic liver transplantation (OLT) in the United States.

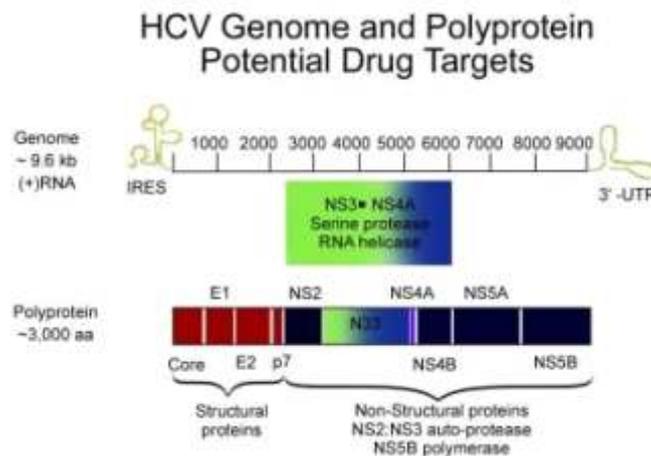


Hepatitis C. Causes of chronic liver disease

Most patients with acute and chronic infection are asymptomatic. Patients and healthcare providers may detect no indications of these conditions for long periods; however, chronic hepatitis C infection and chronic active hepatitis are slowly progressive diseases and result in severe morbidity in 20-30% of infected persons. Astute observation and integration of findings of extrahepatic symptoms, signs, and disease are often the first clues to the underlying HCV infection. **Although acute HCV infection is usually mild, chronic hepatitis develops in at least 75% of patients.** Although liver enzyme levels may be in the reference range, the presence of persistent HCV-RNA levels discloses chronic infection. Biopsy samples of the liver may reveal chronic liver disease. Cirrhosis develops in 20-50% of patients with chronic hepatitis C infection. Liver failure and HCC (11%-19%) can eventually result.

Pathophysiology

Hepatitis C virus (HCV) is a spherical, enveloped, single-stranded RNA virus belonging to the family Flaviviridae, genus *Flavivirus*. Lauer and Walker reported that HCV is closely related to hepatitis G, dengue, and yellow fever viruses. HCV can produce at least 10 trillion new viral particles each day. **The HCV genome consists of a single, open reading frame and two untranslated, highly conserved regions,** 5'-UTR and 3'-UTR, at both ends of the genome. The genome has approximately 9500 base pairs and encodes a single polyprotein of 3011 amino acids that are processed into 10 structural and regulatory proteins (see the image below).



Hepatitis C viral genome.

The natural targets of HCV are hepatocytes and, possibly, B lymphocytes. Viral clearance is associated with the development and persistence of strong virus-specific responses by cytotoxic T lymphocytes and helper T cells. **In most infected people, viremia persists and is accompanied by variable degrees of hepatic inflammation and fibrosis.** Findings from studies suggest that at least 50% of hepatocytes may be infected with HCV in patients with chronic hepatitis C. **The proteolytic cleavage of the virus results in two structural envelope glycoproteins (E1 and E2) and a core protein.** Two regions of the E2 protein, designated hypervariable regions 1 and 2, have an extremely high rate of mutation, believed to result from selective pressure by virus-specific antibodies. The envelope protein E2 also contains the binding site for CD-81, a tetraspanin receptor expressed on hepatocytes and B lymphocytes that acts as a receptor or coreceptor for HCV. HCV core protein is an important risk factor in the development of liver disease; it can modulate several signaling pathways affecting cell cycle regulation,

cell growth promotion, cell proliferation, apoptosis, oxidative stress, and lipid metabolism. Other viral components are nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B, and p7), whose proteins function as helicase-, protease-, and RNA-dependent RNA polymerase, although the exact function of p7 is unknown. These nonstructural proteins are necessary for viral propagation and have been the targets for newer antiviral therapies, such as the direct-acting antiviral agents (DAAs). NS2/3 and NS3/4A are proteases responsible for cleaving the HCV polyprotein. NS5A is critical for the assembly of the cytoplasmic membrane-bound replication complex; one region within NS5A is linked to an interferon (IFN) response and is called the IFN sensitivity-determining region. NS5B is an RNA dependent RNA polymerase required for viral replication; it lacks proofreading capabilities and generates a large number of mutant viruses known as quasispecies. These represent minor molecular variations with only 1%-2% nucleotide heterogeneity. HCV quasispecies pose a major challenge to immune-mediated control of HCV and may explain the variable clinical course and the difficulties in vaccine development.

Genotypes

HCV genomic analysis by means of an arduous gene sequencing of many viruses has led to the division of HCV into six genotypes based on homology. Numerous subtypes have also been identified. Arabic numerals denote the genotype, and lower-case letters denote the subtypes for lesser homology within each genotype. Molecular differences between genotypes are relatively large, and they have a difference of at least 30% at the nucleotide level. The major HCV genotype worldwide is genotype 1, which accounts for 40%-80% of all isolates. Genotype 1 also may be associated with more severe liver disease and a higher risk of hepatocellular carcinoma. Genotypes 1a and 1b are prevalent in the United States, whereas in other countries, genotype 1a is less frequent. Genotype details are as follows:

- Genotype 1a occurs in 50%-60% of patients in the United States.
- Genotype 1b occurs in 15%-20% of patients in the United States; this type is most prevalent in Europe, Turkey, and Japan.
- Genotype 1c occurs in less than 1% of patients in the United States.
- Genotypes 2a, 2b, and 2c occur in 10%-15% of patients in the United States; these subtypes are widely distributed and are most responsive to medication.
- Genotypes 3a and 3b occur in 4%-6% of patients in the United States; these subtypes are most prevalent in India, Pakistan, Thailand, Australia, and Scotland.
- Genotype 4 occurs in less than 5% of patients in the United States; it is most prevalent in the Middle East and Africa.
- Genotype 5 occurs in less than 5% of patients in the United States; it is most prevalent in South Africa.
- Genotype 6 occurs in less than 5% of patients in the United States; it is most prevalent in Southeast Asia, particularly Hong Kong and Macao.

Within a region, a specific genotype may also be associated with a specific mode of transmission, such as genotype 3 among persons in Scotland who abuse injection drugs.

Etiology

Transmission

Transfusion of blood contaminated with hepatitis C virus (HCV) was once a leading means of HCV transmission. Since 1992, however, the screening of donated blood for HCV antibody sharply reduced the risk of transfusion-associated HCV infection. With the advent of more advanced screening tests for HCV such as polymerase chain reaction

(PCR), the risk is considered to be less than 1 per 2 million units transfused. The newer assays have decreased the window after infection to 1-2 weeks. Persons who inject illicit drugs with nonsterile needles are at the highest risk for HCV infection. In developed countries, most of the new HCV infections are reported in injection drug users (IDUs). The most recent surveys of active IDUs in the United States indicate that approximately one third of young (aged 18-30 years) IDUs are HCV-infected. Older and former IDUs typically have a much higher prevalence (approximately 70%-90%) of HCV infection, attributable to needle sharing during the 1970s and 1980s, before greater understanding of the risks of blood-borne viruses and the implementation of public educational strategies. The additional risk of acquiring hepatitis C infection from noninjection (snorted or smoked) cocaine use is difficult to differentiate from that associated with injection drug use and sex with HCV-infected partners. Transmission of HCV to healthcare workers may occur via needle-stick injuries or other occupational exposures. Needle-stick injuries in the healthcare setting result in a 3% risk of HCV transmission. According to Rischitelli et al, however, the prevalence of HCV infection among healthcare workers is similar to that of the general population. Nosocomial patient-to-patient transmission may occur by means of a contaminated colonoscope, via dialysis, or during surgery, including organ transplantation before 1992. HCV may be transmitted via sexual transmission. However, studies of heterosexual couples with discordant serostatus have shown that such transmission is extremely inefficient. A higher rate of HCV transmission is noted in men who have sex with men (MSM), particularly those who practice unprotected anal intercourse and have infection with the human immunodeficiency virus (HIV). HCV may also be transmitted via tattooing, sharing razors, and acupuncture. The use of disposable needles for acupuncture, now the standard practice in the United States, should eliminate this transmission route. Maternal-fetal HCV transmission may occur at a rate of approximately 4%-5%. Breastfeeding is not associated with transmission. Casual household contact and contact with the saliva of those infected are inefficient modes of transmission. No risk factors are identified in approximately 10% of cases.

Epidemiology

International statistics

Worldwide, more than 170 million persons have hepatitis C virus (HCV) infection, of whom 71 million have chronic infection. The Eastern Mediterranean region and Europe have the highest prevalence (2.3% and 1.5%, respectively), with other regions having an estimated prevalence of 0.5%-1.0%. Jeddah City, Saudi Arabia, has a reported HCV prevalence of 0.38%. The prevalence rates in healthy blood donors are 0.01%-0.02% in the United Kingdom and northern Europe, 1%-1.5% in southern Europe, and 6.5% in parts of equatorial Africa. Prevalence rates as high as 22% are reported in Egypt and are attributed to the use of parenteral antischistosomal therapy.

Prognosis

Infection with hepatitis C virus (HCV) is self-limited in 15% to 50% of patients. In a review of HCV infection, it was reported that chronic infection developed in 70%-80% of patients. Cirrhosis develops within 20 years of disease onset in 20% of persons with chronic infection. The onset of chronic hepatitis C infection early in life often leads to less serious consequences. Hepatitis B virus (HBV) coinfection, iron overload, and alpha 1-antitrypsin deficiency may promote the progression of chronic HCV infection to HCV-related cirrhosis. Two studies of compensated cirrhosis in the United States and Europe

showed that decompensation occurred in 20% of patients and that hepatocellular carcinoma (HCC) occurred in approximately 10% of patients. The survival rate at 5 and 10 years was 89% and 79%, respectively. HCC develops in 1-4% of patients with cirrhosis each year, after an average of 30 years. [The risk of cirrhosis and HCC doubles in patients](#) who acquired HCV infection via transfusion. Progression to HCC is more common in the presence of cirrhosis, alcoholism, and HBV coinfection. [In an observational study of Veterans Affairs \(VA\) HCV clinical registry data on 128,769 patients](#), McCombs et al found that those who achieved an undetectable HCV viral load had a decreased risk of subsequent liver morbidity and death. Viral load suppression reduced the risk for future liver events by 27% (eg, compensated/decompensated cirrhosis, HCC, or liver-related hospitalization), as well as reduced the risk of death by 45%, relative to patients who did not achieve viral load suppression. Additionally, patient race/ethnicity and HCV genotypes affected the risk of future liver events and death. The risk for all liver events and death was higher in white patients relative to black patients, and those with HCV genotype 3 had a higher risk for all study outcomes compared to patients who had HCV genotype 2 (lowest risk) or genotype 1.

Patient Education

Patients with hepatitis C virus (HCV) infection should be advised to abstain from alcohol use, as it accelerates the onset of cirrhosis and end-stage liver disease. Patients should be informed about the low but present risk for transmission to sex partners. Optimally, patients should use barrier protection during sexual intercourse. Sharing personal items that might have blood on them, such as toothbrushes or razors, should be avoided. [Patients with hepatitis C should not donate blood or organs](#). One exception is in patients with HCV who require liver transplantation. Arenas et al showed that liver transplant recipients who received liver grafts from HCV-positive donors had 5-year survival rates comparable to recipients who received grafts from HCV-negative donors. Given the shortage of organs and the long waiting list, this strategy has proven safe and effective. [Patients should also check with a healthcare professional before](#) taking any new prescription pills, over-the-counter drugs, or supplements, as these can potentially damage the liver.

CLINICAL PRESENTATION

History

Acute hepatitis C virus (HCV) infection becomes chronic in 70% of patients, which represents a high rate of chronicity for a viral infection. Most patients with chronic hepatitis C are asymptomatic or may have nonspecific symptoms such as fatigue or malaise in the absence of hepatic synthetic dysfunction. Patients with decompensated cirrhosis from HCV infection frequently have symptoms typically observed in other patients with decompensated liver disease, such as sleep inversion and pruritus. [Symptoms characteristic of complications from advanced or decompensated liver disease](#) are related to synthetic dysfunction and portal hypertension. These include mental status changes (hepatic encephalopathy), ankle edema and abdominal distention (ascites), and hematemesis or melena (variceal bleeding). [Symptoms often first develop as clinical findings](#) of extrahepatic manifestations of HCV and most commonly involve the joints, muscle, and skin. In a large study of the extrahepatic manifestations of HCV, 74% of medical workers with HCV infection demonstrated extrahepatic manifestations, of which the following were the most common

- Arthralgias (23%)
- Paresthesias (17%)
- Myalgias (15%)

- Pruritus (15%)
- Sicca syndrome (11%)

In addition, sensory neuropathy has been reported as an extrahepatic manifestation in 9% of patients with HCV infection. Risk factors for manifestations of extrahepatic chronic hepatitis C infection include advanced age, female sex, and liver fibrosis. [Patients also present with symptoms that are less specific](#) and are often unaccompanied by discrete dermatologic findings. Pruritus and urticaria are examples of less specific clues to underlying HCV infection in the appropriate setting (eg, posttransfusion, organ transplantation, surgery, injection drug use, injury of the nasal mucosa from snorting cocaine through shared straws). [Patients with ongoing pathology associated with chronic hepatitis C](#) that eventually results in organ failure can present with symptoms and signs in the skin. Pruritus, dryness, palmar erythema, and yellowing of the eyes and skin are examples of less specific findings in patients with end-stage liver disease with cirrhosis; these findings provide clues that lead to further evaluation of the underlying causes. [Chronic hepatitis C has a strong association with pruritus](#). Indeed, some authorities believe that all patients with unexplained pruritus should be investigated for HCV infection.

Physical Examination

Most patients with hepatitis C virus (HCV) infection do not have abnormal physical examination findings until they develop portal hypertension or decompensated liver disease. One exception is patients with extrahepatic manifestations of HCV infection, such as porphyria cutanea tarda or necrotizing vasculitis. Signs in patients with decompensated liver disease include the following:

- Hand signs: Palmar erythema, Dupuytren contracture, asterixis, leukonychia, clubbing
- Head signs: Icteric sclera, temporal muscle wasting, enlarged parotid, cyanosis
- Fetor hepaticus
- Gynecomastia, small testes
- Abdominal signs: Paraumbilical hernia, ascites, caput medusae, hepatosplenomegaly, abdominal bruit
- Ankle edema
- Scant body hair
- Skin signs: Spider nevi, petechiae, excoriations due to pruritus

Other common extrahepatic manifestations include the following:

- Cryoglobulinemia
- Membranoproliferative glomerulonephritis
- Idiopathic thrombocytopenic purpura
- Lichen planus (see the images below)



Lichen planus.



Lichen planus (oral lesions).



Lichen planus (volar wrist).

- Keratoconjunctivitis sicca
- Raynaud syndrome
- Sjögren syndrome
- Porphyria cutanea tarda
- Necrotizing cutaneous vasculitis

Approximately 10%-15% of affected patients have symptoms/signs such as weakness, arthralgias, and purpura; these are often related to vasculitis. The precise pathogenesis of these extrahepatic complications has not been determined, although most are the clinical expression of autoimmune phenomena.

DIFFERENTIAL DIAGNOSIS

Differential Diagnoses

- Autoimmune Hepatitis
- Cholangitis
- Viral Hepatitis

WORKUP

Approach Considerations

AASLD/ISDA guidelines

The AASLD/ISDA recommend the following for initial HCV testing and followup :

- Initial HCV testing: HCV-antibody test; if the result is positive, confirm current infection with a sensitive HCV-RNA test.
- Negative HCV-antibody test but clinical suspicion of liver disease: Test for HCV RNA or followup testing for HCV antibody if HCV exposure occurred within the past 6 months; consider testing for HCV RNA in immunocompromised individuals.
- Reinfection after previous spontaneous or treatment-related viral clearance: Obtain initial HCV-RNA testing (because an HCV-antibody test is expected to be positive).
- Before initiation of antiviral therapy: Obtain quantitative HCV-RNA testing to document baseline viral load.
- Selection of the most appropriate antiviral regimen: Use HCV genotype testing for guidance.
- Positive HCV-antibody test with negative HCV RNA by polymerase chain reaction (PCR): Inform patients they do not have evidence of current (active) HCV infection.

All patients with HCV infection are recommended to have an evaluation for advanced fibrosis with the use of liver biopsy, imaging, and/or noninvasive markers to aid in decision making regarding treatment strategies and to determine whether additional measures for the management of cirrhosis should be initiated (eg, screening for hepatocellular carcinoma). Patients in whom therapy is deferred should undergo repeat liver assessment on an ongoing basis.

WHO guidelines

The World Health Organization (WHO) recommends nucleic acid testing for qualitative or quantitative HCV RNA detection as well as for test of cure at 12 or 24 weeks following antiviral treatment completion. In areas with limited resources, the WHO suggests using the aminotransferase/platelet ratio index (APRI) or the fibrosis-4 (FIB-4) score for evaluating hepatic fibrosis rather than other noninvasive tests that require more resources (eg, elastography, FibroTest), as follows :

- $APRI = [(AST (IU/L)/AST_{ULN} (IU/L)) \times 100] / \text{platelet count} (10^9 /L)$
- $FIB-4 = \text{age (years)} \times AST (IU/L) / \text{platelet count} (10^9 /L) \times [ALT (IU/L)]^{1/2}$

where ALT is alanine aminotransferase, AST is aspartate aminotransferase, IU is international unit, and ULN is the upper limit of normal. [Serologic screening for HCV involves an enzyme immunoassay \(EIA\)](#). These assays are 97% specific but cannot distinguish acute from chronic infection. A rapid antibody test for HCV is available. The recombinant immunoblot assay is used to confirm HCV infection. [A meta-analysis comparing point-of-care screening tests \(POCTs\)](#) with rapid diagnostic tests (RDTs) indicated that POCTs are highly accurate for diagnosing hepatitis C. POCTs do not require special equipment or electricity and are more robust than RDTs at high temperatures; thus, they may enable expanded screening. [Healthcare personnel who sustain a needle-stick injury](#) involving an HCV-infected patient should undergo PCR testing for HCV immediately and then every 2 months for 6 months. If HCV infection is diagnosed, therapy can be instituted.

Other baseline studies include the following :

- Complete blood cell (CBC) count with differential
- International normalized ratio (INR)
- Liver function tests, including levels of ALT and AST, alkaline phosphatase, albumin, and total and direct bilirubin

- Calculated glomerular filtration rate (eGFR)
- Thyroid function studies
- Screening tests for coinfection with human immunodeficiency virus (HIV) or hepatitis B virus (HBV)
- Screening for alcohol abuse, drug abuse, and/or depression
- Hepatitis B virus (HBV) testing with hepatitis B surface antigen (HBsAg) (to identify coinfection), as well as hepatitis B surface antibody (anti-HBs) and antibody against hepatitis B core antigen (anti-HBc) (for evidence of previous infection)
- Serum pregnancy testing in women of childbearing age before initiating a treatment regimen that includes ribavirin or that includes direct-acting antiviral agents (DAAs) without ribavirin

The CBC demonstrates thrombocytopenia in approximately 10% of patients. Low thyroxine levels are found in approximately 10% of patients, as well. Stress testing may be necessary in appropriate patients. An ophthalmologic examination may also be necessary. [In August 2012, the Centers for Disease Control and Prevention \(CDC\) expanded their existing, risk-based testing guidelines to recommend a one-time blood test for HCV infection in baby boomers, the generation born between 1945 and 1965, who account for approximately three fourths of all chronic HCV infections in the United States. \[Screening for HCV in the emergency department \\(ED\\)\]\(#\) has been found to be feasible, albeit costly. All individuals identified with HCV should be screened and/or managed for alcohol abuse, followed by referral to preventive and/or treatment services, as appropriate.](#)

Hepatitis C Antibody Test

Hepatitis C virus (HCV) infection is diagnosed through the detection of antibodies to recombinant HCV polypeptides. However, antibody assays do not distinguish past from current HCV infection. For this reason, follow-up testing for HCV RNA is necessary to distinguish between ongoing or prior infection in persons with HCV antibodies. [Several generations of US Food and Drug Administration \(FDA\)-approved enzyme immunoassays \(EIAs\) to measure antibodies against NS4, core, NS3, and NS5 sequences are commercially available. The third-generation assay is 97% sensitive. It can detect HCV antibody at an average of 8 weeks after the onset of infection. The recombinant immunoblot assay, previously used to confirm HCV infection, is not necessary owing to the improved sensitivity of the positive EIA tests with currently recommended higher cutoff values. \[False-negative results for the presence of HCV antibody\]\(#\) can occur in persons with compromised immune systems, such as those with human immunodeficiency virus \(HIV\) infection, renal failure, or HCV-associated essential mixed cryoglobulinemia. False-positive EIA results can also occur; the likelihood of a false-positive result is greater in persons without risk factors and in those without signs of liver disease, such as blood donors or healthcare workers. \[In 2010, the FDA approved the OraQuick HCV Rapid Antibody Test\]\(#\), which can be used for persons at risk for hepatitis or for those with signs or symptoms of hepatitis. The test strip can be used with a sample collected from a fingerstick or venipuncture whole blood.](#)

Qualitative and Quantitative Assays for HCV RNA

Qualitative assays

Qualitative assays can be used to test for hepatitis C virus (HCV) RNA. HCV RNA can be detected in blood using amplification techniques such as polymerase chain reaction (PCR) or transcription-mediated amplification (TMA). The following are a few of the FDA-approved PCR-based tests for qualitative HCV RNA detection :

- COBAS AmpliScreen HCV Test, version 2.0: PCR with a lower limit

of detection of 50 IU/mL

- Hepatitis C Virus Reverse-Transcriptase (RT) PCR Assay
- UltraQual HCV-RT PCR Assay
- Versant HCV RNA Qualitative Assay: TMA with a lower limit of detection of 9.6 IU/mL

Quantitative assays

Quantitative assays ascertain HCV RNA quantity in blood, using signal amplification (branched DNA [bDNA] assay) or target amplification techniques (PCR, TMA). RT-PCR is more sensitive than bDNA testing. The HCV RNA level in blood helps predict the likelihood of a response to treatment, and the change in HCV RNA level can also be used to monitor the therapeutic response. [The same quantitative test should be used throughout therapy to avoid confusion](#), and results should be reported in international units (IU) to standardize data. The Versant HCV RNA Assay, version 3.0, is based on bDNA technology and has a dynamic range of 615-7,700,000 IU/mL. Another FDA-approved HCV quantitative test is the Aptima HCV Quant Dx Assay; its limit of detection is 3.9 IU/mL in plasma and 3.4 IU/mL in serum.

The following are the best laboratory evidence of acute HCV infection:

- A positive HCV RNA test in the setting of a negative HCV antibody test (seronegative “window” period)
- A positive HCV antibody test after a prior negative HCV antibody test (seroconversion)

It should be noted that impaired antibody production in immunosuppressed individuals may result in misleading information.

HCV Genotyping

Hepatitis C virus (HCV) genotyping is helpful for predicting the likelihood of response and duration of treatment. Genotyping can be performed by direct sequence analysis, reverse hybridization to genotype-specific oligonucleotide probes, or restriction fragment length polymorphisms (RFLPs). [In June 2013, the FDA approved the Abbott RealTime HCV Genotype II test](#), which, by analyzing a sample of an infected patient's blood plasma or serum, can differentiate HCV genotypes 1, 1a, 1b, 2, 3, 4, and 5. This test is approved for use in adult, non-immunocompromised patients with known chronic HCV infection but has not been approved for diagnostic use or as a screening test for HCV genetic material. FDA approval was based partly on a comparison of the test's accuracy with that of a validated gene-sequencing method.

Other genotype tests are available, including the following, although none have been approved by the FDA :

- Trugene HCV 5'NC Genotyping Kit: Based on direct sequencing followed by comparison with a reference sequence database
- Line Probe Assay (Inno LiPA HCV II): Based on reverse hybridization of PCR amplicons on a nitrocellulose strip coated with genotype-specific oligonucleotide probes
- Versant HCV Genotyping Assay (INNO-LiPA) 2.0: Next-generation line-probe assay

In addition to HCV genotype, a growing body of research indicates that patient genetics play a role in the response to treatment. The single-nucleotide polymorphism (SNP) rs12979860, located near the *IL28B* gene on chromosome 19, which encodes type III interferon, is associated with more than a two-fold difference in the rate of sustained virologic response (SVR) to antiviral treatment with pegylated interferon and ribavirin. This SNP can be detected by PCR and is an independent predictor of SVR regardless of HCV genotype.

Other Testing

Della Rossa et al reported that cryoglobulins are found in as many as 50% of persons with hepatitis C viral infection. Hepatitis C virus (HCV) is the primary cause of essential mixed cryoglobulinemia (ie, type 2

cryoglobulinemia); as many as 90% of affected persons have HCV viremia. Cryoprecipitates usually contain large amounts of HCV antigens and antibodies. Vasculitis, arterial hypertension, purpura, lichen planus, arthralgias, and low thyroxine levels were associated with titers positive for cryoglobulin.

Other common serologic findings in patients with chronic HCV infection include one or more of the following :

- Antinuclear antibody (ANA)
- Rheumatoid factor
- Anticardiolipin antibody
- Antithyroid antibody
- Anti-smooth muscle antibody

Additional evaluations may include testing for infection with human immunodeficiency virus (HIV), screening for susceptibility to hepatitis A and hepatitis B virus infections, and for other underlying causes of liver disease (eg, autoimmune liver disease, hemochromatosis, Wilson disease, α 1-antitrypsin).

Liver Biopsy

Liver biopsy is not considered mandatory before the initiation of treatment for hepatitis C, but it may be helpful for assessing the activity and severity of hepatitis C virus-related liver disease. However, some experts recommend biopsy only in the following situations:

- The diagnosis is uncertain
- Other coinfections or disease may be present
- The patient being considered for treatment has normal liver enzyme levels and no extrahepatic manifestations
- The patient is immunocompromised

Histologic Findings

Lymphocytic infiltration, moderate degrees of inflammation and necrosis, and portal or bridging fibrosis are noted in hepatitis C. Regenerative nodules are seen in patients with cirrhosis. Some patients also may have findings indicative of hepatocellular carcinoma (HCC). [Most pathologists provide separate measurements of disease activity \(grade\) and fibrosis \(stage\).](#) Many scoring systems are used, including the Ishak (6-point scale) and the Knodell histologic activity index (18-point score); although both scoring systems are useful for assessing improvements in histologic findings in studies, they are impractical for clinical use because of interobserver disagreement. [The METAVIR score was developed by the French METAVIR Cooperative Study Group and reported by Bedossa and Poynard in 1996; it is frequently used in European trials. This score consists of a 3-point activity scale and 4-point fibrosis score, with good agreement among pathologists. In the United States, many pathologists use a scale described by Batts and Ludwig \(Batts-Ludwig score\) in 1995, which consists of an activity grade \(0-4\) and a fibrosis stage \(0-4\). HCC may occur rapidly following treatment with direct-acting antiviral agents \(DAAs\) in HCV-related cirrhosis;](#) thus, patients with cirrhosis should be closely monitored after DAA therapy. Most of the neoplastic HCC nodules appear to have aggressive imaging features of microvascular invasion in this setting. [Noninvasive methods of assessing hepatic fibrosis are in development.](#) Current serum assays are directed at measuring breakdown products of extracellular matrix constituents (eg, glycoproteins, propeptides) and their regulatory enzymes (eg, lysyl oxidase, lysyl hydroxylase, propyl hydroxylase).

Radiologic Studies

A liver stiffness test (FibroScan) is available as a noninvasive method of staging liver disease in persons with chronic hepatitis C. Obesity, female sex, operator inexperience, and age older than 52 may give invalid results. Falsely high estimates of liver fibrosis have also been reported with acute inflammation and recent food intake. [On December 17, 2014,](#)

[the FDA gave marketing approval for the Hepatiq radiologic image processing system.](#) The software application uses quantitative analysis of nuclear medicine liver-spleen images to determine the severity of liver disease and to predict clinical outcomes. The developer noted that Hepatiq "automates the Quantitative Liver Spleen Scan (QLSS) that has been proven to be an accurate predictor of clinical outcomes in the recently concluded HALT-C [Hepatitis C Antiviral Long-term Treatment against Cirrhosis] trial." The HALT-C trial was a multicenter, randomized controlled study that evaluated whether long-term interferon would suppress HCV, prevent progression to cirrhosis, prevent liver cancer, and reduce the need for liver transplantation. [In a study that compared abdominal computed tomography \(CT\) and laboratory data from 469 HCV-infected patients with those of histopathologic METAVIR fibrosis scores, investigators found that the use of multiparametric CT evaluation of HCV-associated liver fibrosis further improved its diagnostic performance over that of individual parameters. These parameters included hepatosplenic volumetrics, texture features, liver surface nodularity \(LSN\) score, and linear CT measurements, as well as the fibrosis-4 \(FIB-4\) score and aspartate transaminase-to-platelets ratio index \(APRI\). The diagnostic performance of LSN plus FIB-4 scores approached that of panels with more parameters and compared favorably with elastography.](#)

TREATMENT AND MANAGEMENT

Approach Considerations

Please note that the guidelines for the current diagnostic workup and management of hepatitis C (HCV) infection continue to rapidly evolve. Clinicians are advised to refer frequently to HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C, the most recent recommendations of the American Association for the Study of Liver Diseases (AASLD) and Infectious Diseases Society of America (IDSA). [Spontaneous resolution of acute HCV infection may occur in 15% to 50% of patients.](#) Monitoring for spontaneous clearance for a minimum of 6 months before initiating treatment is therefore recommended. Patients with acute HCV infection appear to have an excellent chance of responding to 6 months of standard therapy with interferon (IFN). However, IFN-sparing regimens are safer and are currently recommended for the treatment of acute HCV infection as with chronic HCV infection. [Hepatitis C has become a curable disease with the use of antiviral agents \(>95%\).](#) Treatment for chronic HCV is based on guidelines from the Infectious Diseases Society of America (IDSA) and the American Associations for the Study of Liver Diseases (AASLD), in collaboration with the International Antiviral Society-USA (IAS-USA). These guidelines are updated often. [The AASLD/IDSA guidelines previously proposed that because all patients cannot receive treatment immediately upon the approval of new agents, priority should be given to those with the most urgent need. The recommendations included the following:](#)

- Patients with advanced fibrosis, those with compensated cirrhosis, liver transplant recipients, and those with severe extrahepatic complications are to be given the highest priority for treatment.
- Based on available resources, patients at high risk for liver-related complications and severe extrahepatic hepatitis C complications should be given high priority for treatment.
- Treatment decisions should balance the anticipated reduction in transmission versus the likelihood of reinfection in patients whose risk of HCV transmission is high and in whom HCV treatment may result in a reduction in transmission (eg, men who have high-risk sex with men, active injection drug users, incarcerated persons, and those on hemodialysis).

More recently, the AASLD/ISDA guidelines have removed their prioritization tables but continue to strongly recommend treatment for all patients with chronic HCV infection, barring those with shortened life expectancies that cannot be resolved by HCV treatment, liver transplantation, or another directed therapy. [With the exception of pregnant women, the World Health Organization](#) recommends treatment be offered to all individuals aged 12 years or older diagnosed with HCV, regardless of their disease stage. [Initiating treatment earlier for patients with lower stage fibrosis](#) may extend the benefits of sustained virologic response (SVR). In a long-term follow-up study, 820 patients with METAVIR stage F0 or F1 fibrosis confirmed by biopsy were followed for up to 20 years. The 15-year survival rate was statistically significantly better for those who experienced SVR (93%) compared to those whose treatment had failed (82%) or for those who remained untreated (88%) ($P = .003$). [Treatment of chronic HCV infection has two goals](#). The first is to achieve sustained eradication of HCV (ie, SVR), which is defined as the persistent absence of HCV RNA in serum 12 weeks after completing antiviral treatment. The second goal is to prevent progression to cirrhosis, hepatocellular carcinoma (HCC), and decompensated liver disease requiring liver transplantation. [In a prospective study of 158 patients with chronic HCV infection](#) and liver cirrhosis who received interferon-free therapies with direct-acting antiviral agents (DAAs) and 184 control HCV patients with untreated liver cirrhosis, the short-term risk (1.5 years) for de novo HCC did not change. [Antiviral therapy for chronic hepatitis C should be determined on a case-by-case basis](#). However, treatment is widely recommended for patients with elevated serum alanine aminotransferase (ALT) levels who meet the following criteria :

- Age older than 18 years
- Positive HCV antibody and serum HCV RNA test results

- Compensated liver disease (eg, no hepatic encephalopathy or ascites)
- Acceptable hematologic and biochemical indices (hemoglobin at least 13 g/dL for men and 12 g/dL for women; neutrophil count $>1500/mm$, serum creatinine $<1.5 mg/dL$)
- Willingness to be treated and to adhere to treatment requirements
- No contraindications for treatment

A further criterion is liver biopsy findings consistent with a diagnosis of chronic hepatitis. However, a pretreatment liver biopsy is not mandatory. It may be helpful in certain situations, such as in patients with normal transaminase levels, particularly those with a history of alcohol dependence, in whom little correlation may exist between liver enzyme levels and histologic findings. [Viral load suppression reduces the risk of hepatitis C liver morbidity and mortality](#). In an observational study of Veterans Affairs (VA) HCV clinical registry data from 128,769 patients that spanned more than a decade, researchers found that those who achieved an undetectable HCV viral load had a decreased risk of subsequent liver morbidity and death. Viral load suppression reduced the risk for future liver events by 27% (eg, compensated/decompensated cirrhosis, HCC, or liver-related hospitalization) as well as reduced the risk of death by 45%, relative to patients who did not achieve viral load suppression. Among the entire study population, only 24% had been treated previously for HCV; of these patients, only 16% (4% of all patients) achieved an undetectable viral load. [Patient race/ethnicity and HCV genotypes also affected the risk of future liver events and death](#). The risk for all liver events and death was higher in white patients relative to black patients, and those with HCV genotype 3 had a higher risk for all study outcomes compared to patients who had HCV genotype 2 (lowest risk) or genotype 1.

INTERPRETATION

Understanding Hepatitis -- Diagnosis and Treatment (HEPA, B & C)

How Do I Know If I Have Hepatitis?

Viral hepatitis, such as hepatitis A (HAV), hepatitis B (HBV) and hepatitis C (HCV), is diagnosed by your symptoms, a physical exam and blood tests. Sometimes imaging studies such as a sonogram or CAT scan and a liver biopsy are also used.

What are the types of Hepatitis?

There are several types of hepatitis, but the three most common in the U.S. are:

- **Hepatitis A** – It is considered highly contagious but is not a long-term infection and usually has no complications. Your liver usually heals within two months. Preventable with a vaccination, it can be spread by eating or drinking something that has been contaminated with the stool of a person who has the virus.
- **Hepatitis B** – While it can lead to long-term liver damage, most children and adults recover within 6 months. You can spread the virus even though you show no symptoms. Pregnant women who are infected by the virus can pass it along to their newborn. Also, preventable through vaccine, hepatitis B is spread by:
 - Having sex with someone who's infected
 - Sharing dirty needles
 - Having direct contact with infected blood or the body fluids of someone who's got the disease
- **Hepatitis C** – Usually a long-term infection, those infected often don't show symptoms. It can lead to scarring of the liver or cirrhosis. There is no vaccine to prevent it. It is spread by:
 - Sharing dirty needles
 - Having direct contact with infected blood or the body fluids of someone who's got the disease
 - Have had a blood transfusion prior to screenings put in place in 1992
 - It is possible but less common to contract it through sex with someone who is infected.

Who's at Risk for Hepatitis Infection?

You are at increases risk hepatitis A if you meet one or more of these criteria:

- You knowingly have had direct contact with persons who have hepatitis A
- Have traveled to a country that is known to have a high incidence of HAV infection
- Have been in close contact with someone who has traveled to a country with a high rate of infection
- Are a male in a sexual relation with another male
- Use drugs
- Have a clotting factor disorder
- You work with primates

The following groups of people should be screened for hepatitis B virus:

- People born in areas where HBV is endemic
- Men who have sex with men
- Intravenous drug users (both present and former users)

- Anyone with chronic kidney disease
- HIV-infected people
- Pregnant women
- Family members, household members, and sex partners of HBV-infected people (even if sex occurred on only one occasion)
- People who have had more than one sex partner within 6 months
- People who will need to be on medicines that will weaken their immune system.
- People with hepatitis C
- Children born to mothers who have HBV
- People with certain high liver function blood tests

For hepatitis C, the CDC recommends that you have a blood test if any of the following is true:

- You have received an organ transplant or transfusion before July 1972.
- You have been notified that you received blood or an organ transplant from a donor who later tested positive for the disease
- You have ever injected drugs, even once many years ago
- You received a blood transfusion or an organ transplant before July 1992
- You received a blood product used to treat clotting problems that was made before 1987
- You were born between 1945 and 1965
- You have had long-term kidney dialysis
- You have signs or symptoms of liver disease
- You have HIV
- You have a known exposure to HCV
- You have persistent elevations of a liver blood test called ALT (alanine aminotransferase levels)
- Children born to HCV-positive mothers

Otherwise, routine screening for hepatitis typically is not recommended unless you have symptoms or signs (such as abnormal liver-related blood tests) of the condition.

What are the Symptoms of Viral Hepatitis?

Many people with hepatitis will not have any symptoms at all. When they do occur, symptoms of all three of the most common types of hepatitis are very similar and may include:

- Dark urine
- Stomach pain
- Yellowing of skin or eyes
- Pale or clay-colored stool
- Low-grade fever
- Loss of appetite
- Fatigue
- Feeling sick to the stomach

If you have hepatitis A or B, you may also have achy joints.

What If I Have Symptoms of Viral Hepatitis?

If you have symptoms or signs of viral hepatitis, your health care provider can perform a blood test to check for the presence of an antibody. If you have hepatitis B or C, more blood samples may be necessary later -- even if the symptoms have vanished -- to check for complications and determine if you have progressed from acute (infected within the past six months) to chronic (having the virus for greater than six months) disease. Most people have vague or no symptoms at all; hence, viral hepatitis is often referred to as a silent disease. **Your healthcare provider may also require a liver biopsy**, or tissue sample, in order to determine

the extent of the damage. A biopsy is commonly performed by inserting a needle into the liver and drawing out a fragment of tissue, which is then sent to a lab to be analyzed.

What Are the Treatments for Viral Hepatitis?

The treatment for viral hepatitis depends on the type and stage of the infection. Over the last several years, excellent treatments for both hepatitis B and C have become available. More and improved treatments are being evaluated all the time. **Your primary care doctor should be able to provide adequate care of your hepatitis.** However, if you have severe hepatitis, you may require treatment by a hepatologist or gastroenterologist -- specialists in diseases of the liver. Hospitalization is normally unnecessary unless you cannot eat or drink or are vomiting. **Hepatitis A usually requires minimal treatment** and your liver usually heals within 2 months. Make sure you stay hydrated and well-nourished. While a vaccination can prevent you from getting hepatitis A, once you have had it, you cannot be re-infected. Doctors sometimes recommend drug therapy for people with hepatitis B and C. Antiviral medication for hepatitis B includes adefovir (Hepsera), entecavir (Baraclude), interferon, lamivudine (Epivir), peginterferon (Pegasys), telbivudine (Sebivo or Tyzeka), and tenofovir (Viread). **Until recently, the standard treatment for chronic hepatitis C** was a course of peginterferon plus ribavirin for people with genotype 2 and 3, and peginterferon plus ribavirin plus a protease inhibitor for people with genotype 1. These treatments had been shown to be effective in from 50% to 80% of those infected with hepatitis C but the side effects were very difficult for people to tolerate. **Treatment now centers around direct acting antiviral drugs (DAAs).** These medicines are highly effective for most people with hepatitis C and are interferon-free and often ribavirin-free. This means they typically have fewer side effects. The treatments

are often simpler- consisting of fewer pills for a shorter amount of time. DAAs are available as either single drugs or combined with other medicines in one pill. Elbasvir-grazoprevir (Zepatier), ledipasvir-sofosbuvir (Harvoni), and sofosbuvir-velpatasvir (Epclusa) and are once daily combination pills. Depending on the type of hepatitis C infection, these can often cure the disease in 8 to 12 weeks. Other treatment options include: ombitasvir-paritaprevir-ritonavir plus dasabuvir (Viekira Pak, Viekira XR); ombitasvir-paritaprevir-ritonavir (Technivie), or some combinations of daclatasvir (Daklinza), peginterferon, ribavirin, or sofosbuvir (Sovaldi). Ask your doctor what's best for you, based on your medical needs.

Hepatitis in Pregnant Women

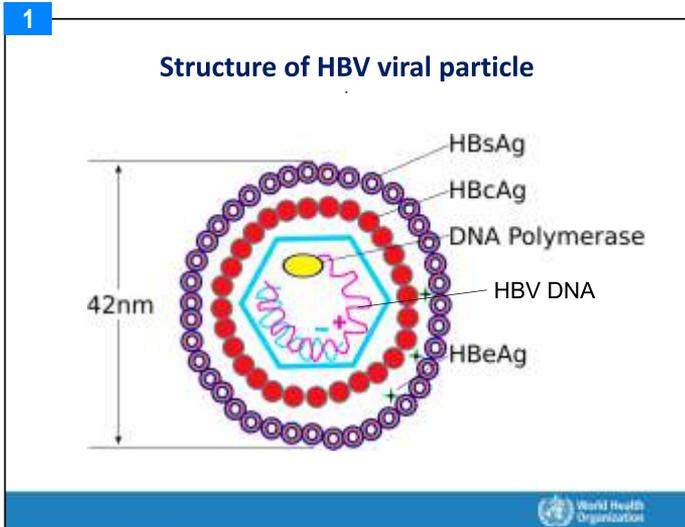
If you are pregnant, your doctor will test you for hepatitis B; if you are infected with the virus, your baby will be given immune globulin shots and a hepatitis vaccination. This will help protect your baby from contracting the virus. In addition, it may be recommended that a mother with active HBV receive treatment with an antiviral medication during the third trimester of pregnancy. **Hepatitis E can be fatal to pregnant women during their third trimester,** and if the mother has hepatitis B, the baby is likely to contract the disease at birth.

Other Points to Consider

If your hepatitis, either viral or nonviral, is in the acute stage (occurred within the last six months), avoid alcoholic beverages, as your body's efforts to process alcohol puts an added strain on an already injured liver. Also, be aware that your sexual partners, especially if you have hepatitis B, are at risk of contracting the disease. Hepatitis C is difficult to pass through sexual contact, unless there is blood-to-blood contact. **Most adults recover completely** from acute hepatitis A and B within six months.

Hepatitis	A	B	C	D	E
Transmission	-Fecal-Oral -Contaminated Food/Water	-Blood to blood -Sex	-Blood to blood -Sex (less common)	-Blood to blood -Sex -Only occurs when HBV is present	-Fecal-Oral -Contaminated Food/Water
Chronic Infection	No	Yes	Yes	Yes	Genotype 3 only
Prevention	-Pre-exposure vaccine	-Pre-exposure vaccine	-Screening donor blood -Harm reduction	-Pre-exposure vaccine	-Ensure safe drinking water, -Avoid undercooked pork & shellfish
Treatment	-Management of symptoms	-Treatment for management of chronic infection	-Treatment/cure for chronic infection	-No approved treatments	-Management of symptoms

TROUBLESHOOTING



- This slide shows the structure of the HBV viral particle:
- HBsAg, HBV surface antigen is on surface of virus.
 - There is nucleocapsid, core, in inside of viral particle.
 - HBcAg, HBV core antigen is on surface of nucleocapsid.
 - HBV DNA is inside of nucleocapside.
 - HBeAg, HBV envelope antigen, is located between HBV surface and core.

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Types of serological markers

Antigen-antibody		Nucleic acid
Antigen	Antibody	
HBsAg (Hepatitis B surface Antigen)	Anti-HBs (HB surface antibody)	HBV DNA (qualitative or quantitative)
HBcAg (Hepatitis B core Antigen) Does not appears in blood	IgM anti-HBc (HB core antibody IgM)	
	Total anti-HBc (IgM plus IgG)	
HBeAg (Hepatitis B e Antigen)	Anti-HBe (Hepatitis B e antibody)	

This table shows types of serological markers:

- HBsAg means Hepatitis B surface antigen, Anti-HBs means HB surface antibody
- HBcAg means Hepatitis B core antigen, IgM anti-HBc means HB core antibody IgM
- Total anti-HBc means IgM and IgG
- HBeAg means Hepatitis B envelope antigen, Anti-HBe means Hepatitis B envelope antibody.

3

HBV serological markers

Test	Clinical interpretation
HBsAg (hepatitis B surface antigen)	Hallmark of infection Positive in the early phase of acute infection and persists in chronic infection Quantification of HBsAg is a potential alternative marker of viraemia and it is also used to monitor the response to antiviral treatment
Anti-HBc IgM (hepatitis B core antibody)	IgM subclass of anti-HBc and observed during acute infection (used to differentiate between acute and chronic HBV infection) Might become positive during severe exacerbation of chronic infection
Anti-HBc (total)	Develops around 3 months after infection (most constant marker of infection) Total anti-HBc (IgM, IgA and IgG) indicates resolved infection
HBeAg (hepatitis B e antigen)	Viral protein usually associated with high viral load and high infectivity
Anti-HBe (hepatitis B e antibody)	Antibody to HBeAg usually indicates decreasing HBV DNA But present in the immune-control and immune-escape phases
Anti-HBs (hepatitis B surface antibody)	Neutralizing antibody that confers protection from infection Recovery from acute infection (with anti-HBc IgG) Immunity from vaccination

Here, we introduce the various serological markers of HBV infection, which will help us to understand the various phases of acute and chronic hepatitis B.

HBsAg (hepatitis B surface antigen) is the hallmark of HBV infection.

Anti-HBc IgM (hepatitis B core antibody) is observed during acute infection.

Anti-HBc (total antibody against HBV core antigen) indicates the presence of IgM and/or IgG against the core antigen. A positive total anti-HBc with negative anti-HBc IgM antibodies indicates resolved infection.

HBeAg (hepatitis B envelope antigen) is viral protein associated usually with a high viral load and high infectivity.

Anti-HBe (antibody to HBeAg) usually indicates decreasing HBV DNA.

Anti-HBs is a neutralizing antibody.

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Hepatitis B surface antigen and antibody

Test	Clinical interpretation
HBsAg	<ul style="list-style-type: none"> • First marker to appear following HBV infection • Positivity indicates presence of virus in a person's body • Acute infection: Disappears within 6 months • Chronic infection: Persists for several years (lifelong in most) <p>• Measurement of HBsAg concentration is being tried as a potential alternative marker of viremia and to monitor response to treatment, but still not well accepted</p>
Anti-HBs	<ul style="list-style-type: none"> • Antibody to HBsAg • Is a neutralizing antibody and confers protection from infection • Appears following clearance of acute infection • Does not develop in those who have chronic infection • Also develops in response to hepatitis B vaccine • Presence indicates immunity following acute infection or vaccination • Anti-HBs titre >10 mIU/mL is considered to be protective • Persists for several years (often lifelong) after infection, but disappears in a few years after immunization

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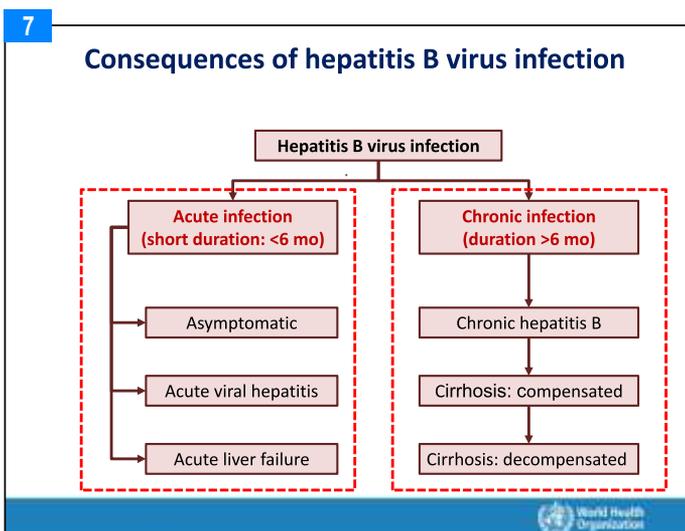
Hepatitis B core antigen and antibody

Test	Clinical interpretation
HBeAg	<ul style="list-style-type: none"> An internal component of the virus Present in the nucleus of infected cells But, does not appear in infected person's blood Not tested in clinical settings Hepatitis B vaccine does not contain this antigen
Anti-HBc	<ul style="list-style-type: none"> Develops in all those who get HBV infection, whether acute or chronic Does not develop after immunization Two types IgM and IgG
IgM anti-HBc	<ul style="list-style-type: none"> Appears following acute infection, and persists for up to ~6 months Hence: presence indicates recent (acute) infection Occasionally, detectable (in low amount) during severe exacerbation of chronic infection
IgG (or Total) anti-HBc	<ul style="list-style-type: none"> Develops soon after IgM anti-HBc Most constant marker of exposure (current or past infection) Positive total anti-HBc (IgG, IgM) with negative IgM anti-HBc in HBsAg negative indicates resolved infection

6

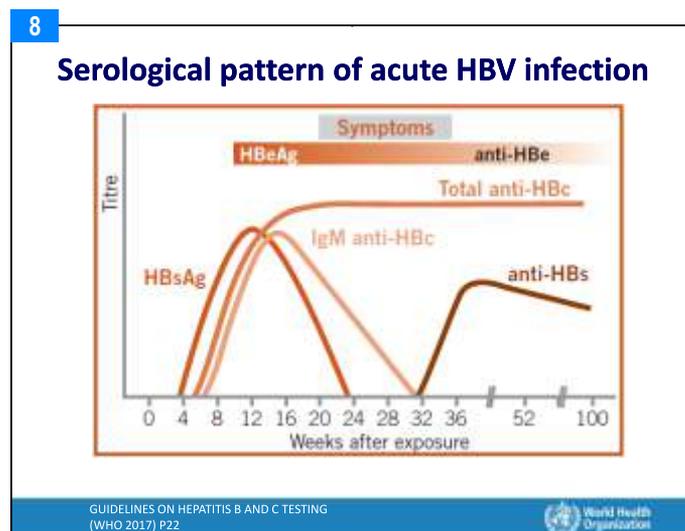
Hepatitis B e-antigen and antibody

Test	Clinical interpretation
HBeAg	<ul style="list-style-type: none"> Produced in cells where virus is actively replicating, and is secreted into the plasma Usually its presence indicates high viral load and high infectivity Its absence indicates lower viral load, lower HBV DNA level. But, in some, may be absent despite high viral load (due to viral mutation) Associated with high risk of HBV transmission following exposure, such as needle-stick injury, mother-to-child transmission, etc
Anti-HBe	<ul style="list-style-type: none"> Indicates host immune response against HBeAg Usually associated with reduced viral replication, lower HBV DNA titer and reduced infectivity But also present in those in HBeAg-negative viral mutation
HBV DNA	<ul style="list-style-type: none"> Direct and accurate marker of HBV replication Serum level seems to correlate with the risk of disease progression Used to decide need for anti-viral drugs Also used to monitor efficacy of anti-viral drug treatment Unit: almost 5 copies = 1 IU



The consequences of hepatitis B virus infection are divided into two clinical courses:

- After hepatitis B virus infection, the individual may have acute infection, which is defined as infection duration less than 6 months, OR chronic infection, where the infection duration lasts more than 6 months.
- In acute infection, the patient may be/have:
 - (a) Asymptomatic: the infected persons have no clinical symptoms and they do not notice the infection.
 - (b) Acute viral hepatitis: the infected persons have clinical symptoms such as general malaise, appetite loss or flu-like symptoms and usually they resolve with no treatment or only needing supportive care.
 - (c) In acute liver failure: the infected persons have severe clinical symptoms related to liver failure, such as jaundice, ascites and hepatic encephalopathy. In this stage, patients generally will not be able to recover without liver transplantation (i.e. mortality is high).
- In chronic infection, hepatitis B virus infection causes chronic hepatitis and the chronic inflammation over the next 20 – 30 years, after which may result in development of cirrhosis.



This figure shows the serological pattern of acute HBV infection:

- After infection, first, HBsAg appears and increase within 2-10 weeks.
- Next, IgM anti-HBc and total anti-HBc increases after 2 weeks
- IgM anti-HBc is a specific marker for acute HBV infection and it decrease and disappears after 32 weeks.
- Total anti-HBc, mainly IgG anti-HBc continues to be positive for life. Thus, total anti-HBc is the marker for post-infection.
- HBsAg decrease and disappears within 6 months, with acute infection.
- After that, the neutralizing antibody, anti-HBs, appears. In this phase, the person is considered as cured.

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Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-			-	Never exposed

We will now look at interpreting the panel of HBV serological markers. This is important in clinical practice when you receive results back from the laboratory.

HBsAg negative and Anti-HBs negative means “never exposed”.

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Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-	+	-	+	Past natural infection, cleared, immunity achieved

Total anti-HBc positive and anti-HBs positive means “past natural infection, cleared and immunity achieved”.

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Serological pattern of acute HBV infection

GUIDELINES ON HEPATITIS B AND C TESTING (WHO 2017) P22

In this figure, the red dash box shows “past natural infection, cleared and immunity achieved”
 - where total anti-HBc and anti-HBs tests are positive

12

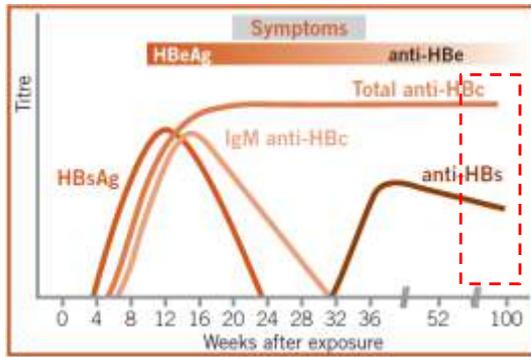
Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-	+	-	-	Past natural infection, cleared, anti-HBs has waned over time

Total anti-HBc only positive also means past natural infection, cleared and anti-HBs levels have waned over time.

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Serological pattern of acute HBV infection



GUIDELINES ON HEPATITIS B AND C TESTING (WHO 2017) P22



This figure illustrates waning of the anti-HBs levels, which have dropped and disappeared over time, and where total anti-HBc remains positive.

Interpretation of the test: “past natural infection, infection cleared and anti-HBs levels have waned over time”.

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Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-	-	-	+	Immunity due to vaccination



Anti-HBs only positive means “immunity due to vaccination”.

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Interpretation of serological markers

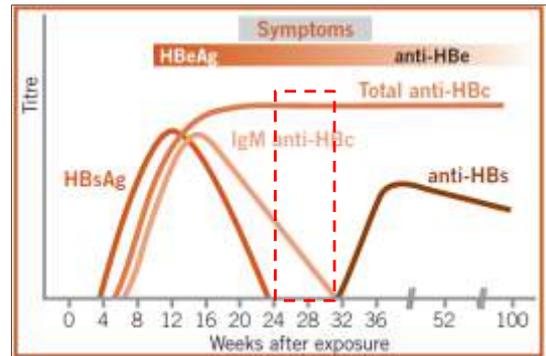
HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-	+	+	+	Recent infection, recovered, immunity achieved



HBs negative, total anti-HBc positive, IgM anti-HBc positive and anti-HBs positive means “recent infection, recovered, immunity achieved”.

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Serological pattern of acute HBV infection



GUIDELINES ON HEPATITIS B AND C TESTING (WHO 2017) P22



In this figure illustrate the above slide on “recent infection, recovered, immunity achieved”.

Noted the red dash box: Anti-HBs levels have dropped and disappeared.

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Interpretation of serological markers

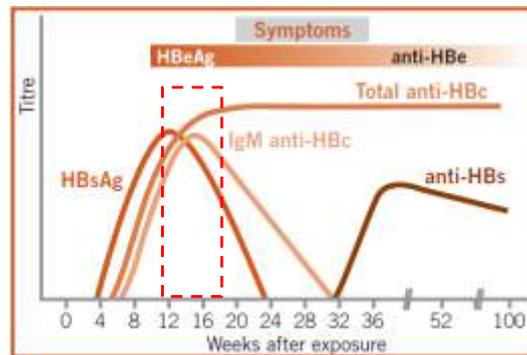
HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
+	+	+	-	Acute infection, ongoing



HBs positive, total anti-HBc positive, IgM anti-HBc positive and anti-HBc negative means “acute infection, ongoing”.

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Serological pattern of acute HBV infection



GUIDELINES ON HEPATITIS B AND C TESTING (WHO 2017) P22



In this figure (red dash box) illustrates that “acute infection, is ongoing”, and where HBs is positive, total anti-HBc is positive, IgM anti-HBc is positive and anti-HBc is negative

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Interpretation of serological markers

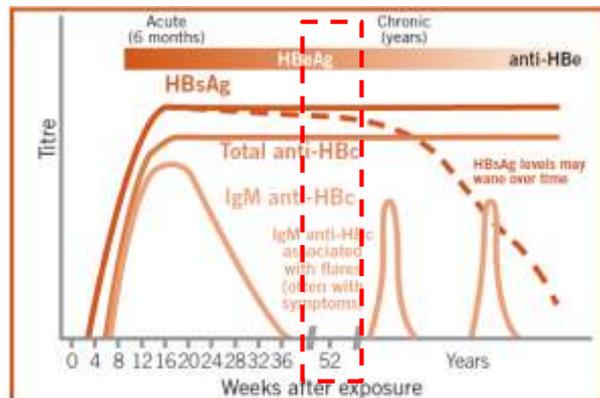
HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
+	+	-	-	Chronic infection (ongoing)



HBsAg positive, total anti-HBc positive, IgM anti-HBc negative and anti-HBs negative means chronic infection is ongoing.

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Serological pattern of chronic HBV infection



GUIDELINES ON HEPATITIS B AND C TESTING (WHO 2017) P22



Let’s have a look at the serological pattern of CHRONIC infection
This is the part of the red dash box – where chronicity is being established, and the person moves into a chronic phase.

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Interpretation of serological markers

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
-			-	Never exposed
-	+	-	+	Past natural infection, cleared, immunity achieved
-	+	-	-	Past natural infection, cleared, anti-HBs has waned over time
-	-	-	+	Immunity due to vaccination
-	+	+	+	Recent infection, recovered, immunity achieved
+	+	+	-	Acute infection, ongoing
+	+	-	-	Chronic infection (ongoing)



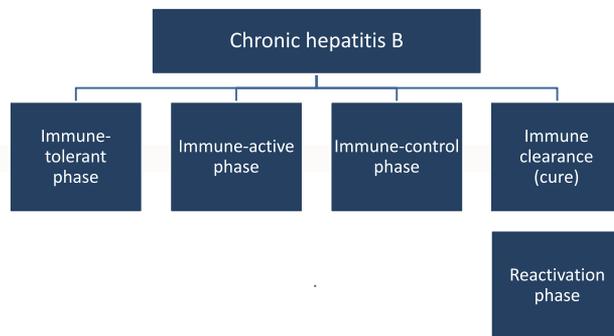
This is summary table of interpretation of serological markers.

Take some time to understand this:

- From the point of screening for HBV infection, the lower two (boxed part) is important.
- If HBsAg is positive after screening, IgM anti-HBc is useful to differentiate between acute and chronic infection. However, IgM anti-HBc may not be affordable or available in resource-limiting settings.
- In such cases, clinical symptoms related to acute hepatitis or chronic infection are useful.

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Natural history of chronic hepatitis B

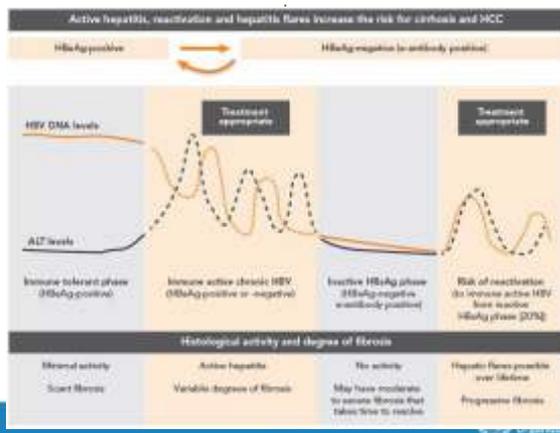


The natural history of chronic hepatitis is shown in this slide.

- There are basically 4 phases, immune-tolerant phase, immune-active phase, immune-control phase and immune clearance, cure phase.
- Another is reactivation phase, this is specific situation.

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Natural history of chronic HBV infection



This is graph of ALT level and HBV DNA level during natural history of chronic HBV infection.

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Natural history of chronic HBV infection



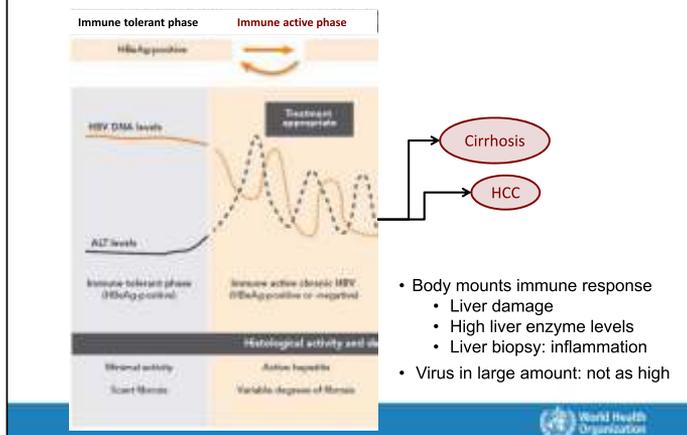
- Virus in large amount
- No host immune response
 - No liver damage
 - Normal liver enzyme levels in serum
 - Normal liver biopsy



- In the immune tolerant phase, large amount of viruses in blood, but there is no host immune response.
- Therefore, there is no liver damage, and liver enzyme levels in serum is normal.
- Liver biopsy shows little inflammation.

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Natural history of chronic HBV infection

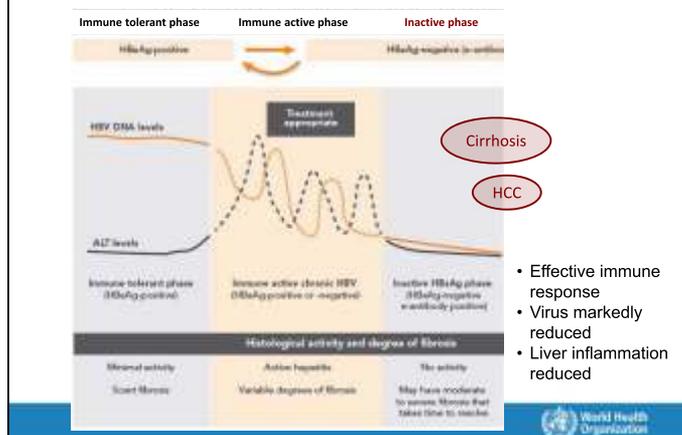


In the immune active phase, the body mounts an immune response.

- The liver is damaged by host immunity.
- Liver enzyme levels are elevated and fluctuate.
- Liver biopsy shows various grades inflammation.
- But, virus levels (i.e. viral load) which is the HBV DNA, is not high compared to the immune tolerant phase and viral levels often fluctuate.
- If this phase where the viral levels remain relatively high, liver cirrhosis or HCC can develop.

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Natural history of chronic HBV infection

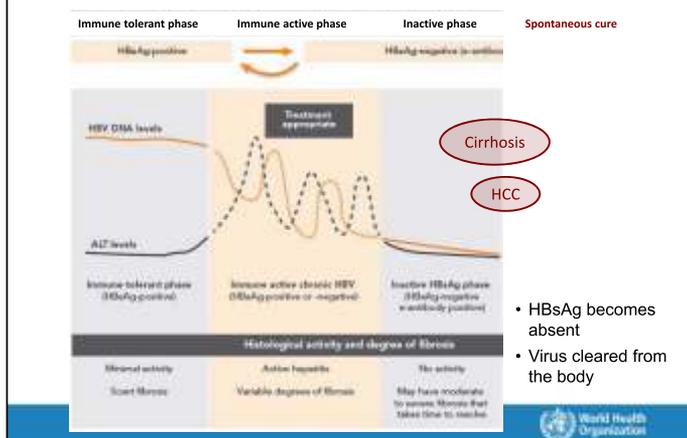


In the inactive HBsAg phase, HBsAg positive becomes negative.

- The host immune response is effective in controlling the hepatitis B virus.
- ALT levels markedly decrease and viral load markedly reduced.
- Liver biopsy shows reduced inflammation.
- However, even in this phase, the risk for cirrhosis or HCC remain.

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Natural history of chronic HBV infection

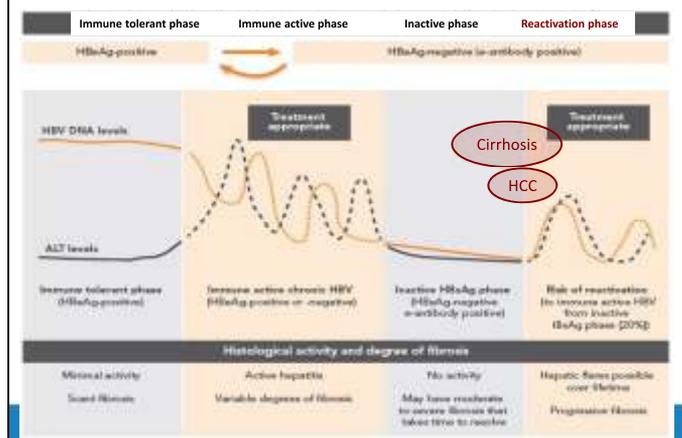


In some cases, HBsAg becomes absent and the HBV virus is cleared from the body.

- This is immune clearance, that is, the "functional cure" phase (where HBsAg is negative, in a previously documented chronically infected individual).

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Natural history of chronic HBV infection

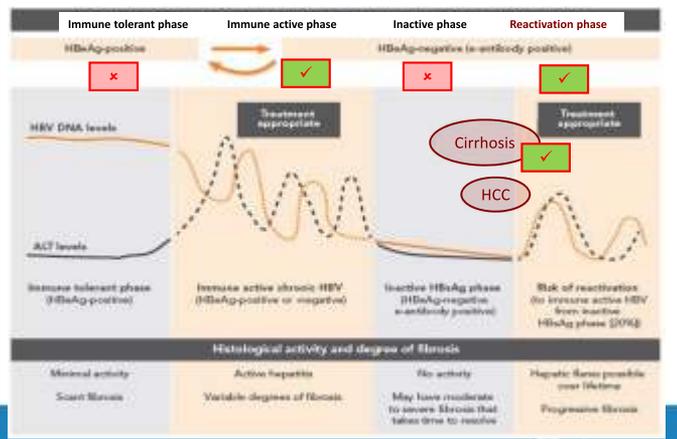


In the reactivation phase, there is a sudden increase in HBV replication (where the viral load increases) in a patient with previously immune inactive stage. Reactivation can happen spontaneously, but is typically triggered by immunosuppressive therapy of cancer, autoimmune disease, or organ transplantation.

Note: among people with HBV/HCV coinfection – HBV reactivation can occur during treatment of the HCV infection, and thus may need to be provided HBV drugs during this period.

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Chronic HBV infection: Need for treatment



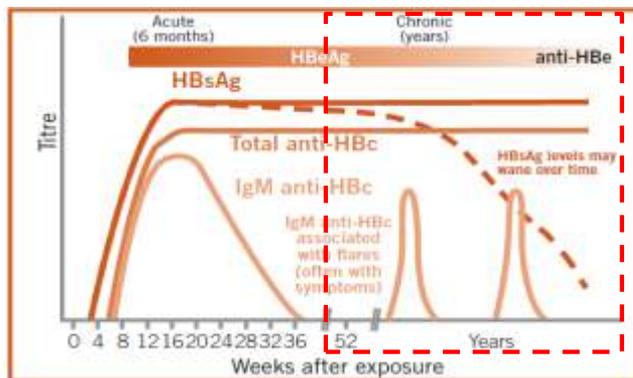
Treatment is needed for immune active phase and reactivation phase [green boxes with ticks]

Cirrhosis with any phase also need provision of treatment [green box with ticks]

On the other hand, in immune tolerant phase and inactive phase, there is no need for treatment [red boxes]

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Serological pattern of chronic HBV infection



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To review: this slide shows the serological pattern of chronic HBV infection.

- Basically HBsAg and anti-HBc continue to be positive.
- HBeAg gradually decreases and finally anti-HBe become positive (this is often called "minor seroconversion")
- In some case, IgM anti-HBc become positive with low level associated with hepatitis flare.
- HBsAg levels may wane over time as age increases (especially in elderly people).

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Approaches for detecting HBV infection

- General population testing, i.e., mass screening
- Focused or targeted testing of specific high-risk groups
- Blood donor screening
- Screening of pregnant women



Next, let's talk about approaches for detecting HBV infection.

There are four approaches for testing:

- General population testing, i.e., mass screening
- Focused or targeted testing of specific high-risk groups e.g. people living with HIV, prisoners, people who inject drugs, other at-risk groups, older people more than 40 years of age (testing by birth cohort), people who received unscreened/unsafe blood and blood products etc.
- Blood donor screening (usually compulsory for blood banks)
- Screening of pregnant women (as part of integrated antenatal services towards triple elimination of mother to child transmission of HIV, syphilis and viral hepatitis).

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Approach for testing for HBV infection

Testing approach and population	Recommendations
General population testing	In a setting with $\geq 2\%$ or $\geq 5\%$ HBV seroprevalence, all adults have access to HBV serological testing and linkage to care
Focused testing in most affected populations	In all settings, serological testing for HBV antibody be offered to the following individuals <ul style="list-style-type: none"> ● Adults and adolescents from populations most affected by HBV infection ● High prevalence: migrants, high/intermediate prevalence, tribes ● History of exposure ● High-risk behaviors ● Adults and children with a clinical suspicion of chronic viral hepatitis ● Sexual partners, children and other family members, and close household contacts of those with HBV infection ● Health care workers

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Approach for testing for HBV infection

Testing approach and population	Recommendations
Pregnant women screening	In a setting with $\geq 2\%$ or $\geq 5\%$ HBV seroprevalence, routine testing of pregnant women for HBV infection is recommended
Blood donors screening	In all setting, all donors have to be screened for HBV infection

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Country adaptation of the WHO guidelines for testing for hepatitis B and C is needed. Testing for target groups and people at risk should be determined

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How to test for chronic HBV infection

Topic	Recommendations
Which serological assays to use	For the diagnosis of chronic HBV infection, a serological assay (in either RDT or laboratory-based immunoassay format) is recommended to detect hepatitis B surface antigen (HBsAg) . - In settings where existing laboratory testing is already available and accessible, laboratory-based immunoassays are recommended as the preferred assay format. - In settings where there is limited access to laboratory testing and/or in populations where access to rapid testing would facilitate linkage to care and treatment, use of RDTs is recommended.
Serological testing strategies	<ul style="list-style-type: none"> In settings or populations with an HBsAg seroprevalence of $\geq 0.4\%$, a single serological assay for detection of HBsAg is recommended, prior to further evaluation for HBV DNA and staging of liver disease. In settings or populations with a low HBsAg seroprevalence of $< 0.4\%$, confirmation of HBsAg positivity on the same immunoassay with a neutralization step or a second different RDT assay for detection of HBsAg may be considered.

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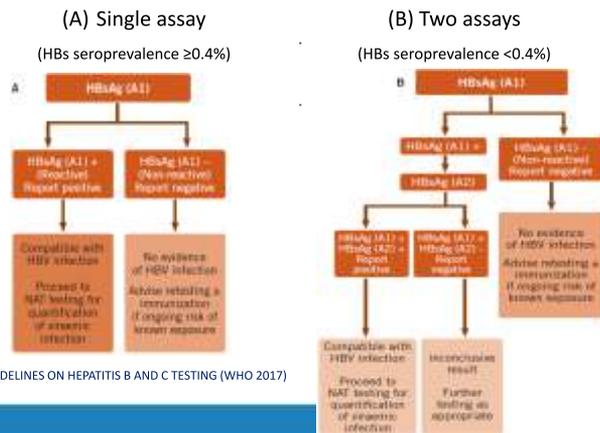
For the diagnosis of chronic HBV infection, a serological assay (in either RDT or laboratory-based immunoassay format) is recommended to detect hepatitis B surface antigen (HBsAg).

WHO outlines two strategies for HBV serological testing in setting with HBsAg seroprevalence.

- In high HBsAg seroprevalence of more than 0.4%, single serological assay.
- In low HBsAg seroprevalence of less than 0.4%, two assays with confirmation test is recommended.

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How to test for chronic HBV infection?



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In high HBsAg seroprevalence, you should follow (A) single assay:
- After a positive result on the single HBsAg assay, patients can be diagnosed as having HBV infection and can proceed to NAT testing for their viral load (HBV DNA) testing

In low HBsAg seroprevalence, algorithm (B) with two assays is preferred:
- After positive on the first HBsAg assay, a second HBsAg assay is used for confirm infection status.
- When both tests are positive, patients are then diagnosed as having HBV infection and can proceed to NAT testing for their viral load (HBV DNA) testing

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Summary: Serological markers of HBV infection

- HBsAg positivity indicates current HBV infection
- If HBsAg remains positive for > 6 months: chronic infection
- Presence of IgM anti-HBc implies recent (acute) infection
- Presence of anti-HBc (total) indicates
 - If HBsAg-negative: Prior exposure to HBV with clearance
 - If HBsAg-positive: Current HBV infection
- Anti-HBs indicates immunity against HBV infection, either because of prior cleared infection (anti-HBc +) or immunization (anti-HBc -)
- HBeAg, anti-HBe and HBV DNA helps in identifying the various phases in a patients with chronic HBV
- For HBV screening, 1-assay or 2-assay approach may be used, depending on disease prevalence



BOUQUET

In Lighter Vein

Teacher : why are you sleeping in the class ?? >.<

Student : Your voice is so sweet that is why Im sleeping :P

Teacher : Then why others are not sleeping ? :0

Student : they aren't listening to you mam :D ♡



Teacher : who will tell the chemical formula of water?

One student : Its "h.i.j.k.l.m.n.o."

Teacher : What is this?

Student : Mam, yesterday you told us that it is H to O !!

Wisdom Whispers

Most of the problems in life are because of two reasons: we act without thinking or we keep thinking without acting.



LOOK FOR SOMETHING POSITIVE IN EACH DAY, EVEN IF SOME DAYS YOU HAVE TO LOOK A LITTLE HARDER.

Brain Teasers

- According to the CDC, which HCV-infected persons should be treated for HCV?
 - Only those with advanced fibrosis (eg, Stages 3-4)
 - Only those with minimal-to-moderate fibrosis (eg, Stages 0-2)
 - Everyone who is HCV antibody-positive, regardless of the degree of fibrosis
 - Everyone who has a detectable HCV viral load, except for those with a "short" life expectancy
- What is the initial test that is most commonly used for HCV screening?
 - HCV RNA by PCR
 - Anti-HCV (ELISA)
 - Liver biopsy
 - Liver function tests.
- Which of the following classes of FDA-approved HCV drugs has a high intrinsic barrier to the development of resistance?
 - NS5B non-nucleoside polymerase inhibitors
 - NS5A inhibitors
 - NS3/4A protease inhibitors
 - NS5B nucleoside polymerase inhibitors
- What is the most common symptom associated with chronic HCV infection?
 - Jaundice
 - Fever with or without jaundice
 - Abdominal pain with or without ascites
 - Asymptomatic

ANSWER: 1: D; 2: B; 3: C; 4: D

CounCell™ PENTA 2.0

5 - PART AUTO HEMATOLOGY INSTRUMENT

Technology for Accurate Analysis



▶ Principle:

Flow cytometry + Tri-angle laser scatter method for differential analysis for and WBC counting.
Electrical impedance for RBC and PLT.
Colorimetric method for estimation of hgb.

▶ Total 36 parameters having 28 reportable and 8 ruo parameters:

- ▶ 3-diff scattergrams and 1 baso scattergram.
- ▶ 2 histogram for RBC & PLT.
- ▶ In built thermal printer & supports HP printer.
- ▶ 3 – Reagent system.
- ▶ Throughput : 60 samples/hour.
- ▶ Sample Volume : 20 μ L (CBC + Diff).
- ▶ 10.4 Inches colour touch screen.
- ▶ Calibration: 3 Types of Calibration - Manual Calibration, Auto Calibration with calibrators and Auto Calibration with fresh blood.
- ▶ Control: LJ & X-B QC with appropriate QC Chart.
- ▶ Wide Linearity Range.

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