# Viral Hepatitis
# Diagnosis and Management

## CLINICAL BACKGROUND

Viral hepatitis is a relatively common disease (25 per 100,000 individuals in the United States) caused by a diverse group of hepatotropic agents that lead to liver inflammation and cell death. Five hepatitis viruses have been well characterized (A, B, C, D and E; Table 1), and others have been identified (F and G). Hepatitis A and E viruses (HAV, HEV) are transmitted through the fecal-oral route and manifest as acute or asymptomatic disease. There is no chronic carrier state and serious sequelae are rare. Hepatitis B, C and D viruses (HBV, HCV, HDV) establish persistent infections with significant morbidity and mortality. All three are transmitted parenterally. HBV and HCV are also transmitted through sexual contact and perinatally. HDV is unique in that it is a "defective" virus that can replicate only in the presence of HBV. HDV coinfection (HDV, HBV) significantly increases the severity of the disease. Acute HCV infection may be asymptomatic, but most infections are chronic. Chronic infection may lead to cirrhosis and hepatocellular carcinoma. In the United States, viral hepatitis is generally caused by HAV, HBV or HCV. Other causative viruses include cytomegalovirus (CMV), Epstein-Barr virus (EBV), and human immunodeficiency virus (HIV). Hepatitis may also be due to other diseases or medications. A variety of immunologic and molecular assays are available for diagnosing viral hepatitis and monitoring treatment response. This guide provides an overview of available tests and indications for their use.

**Table 1 Clinical Spectrum of Viral Hepatitis**

| Hepatitis Virus | Transmission | Incubation Period (weeks) | Mortality (%) | Likelihood of Carrier State | Likelihood of Chronic Disease | Assocation with Hepatocellular Carcinoma |
|---|---|---|---|---|---|---|
| A | Fecal-oral | 2-6 | 1 | None | None | No |
| B | Parenteral, Perinatal, Sexual | 4-26 | 1-2 | 10% Adults 90% Infants | 1-10% | Yes |
| C | Parenteral, Perinatal and possibly sexual | 2-23 | 1-5 | 50-80% | 80-90% | Yes |
| D | Parenteral, sexual, perinatal | 6-26 | 2-20 | Variable | 80% in super-infection | Yes* |
| E | Fecal-oral | 2-9 | 1-2** | None | None | No |

*Requires co-infection with HBV. Simultaneous infection with HBV is associated with severe acute disease and low likelihood of chronic infection (<5%); superinfection of HBV carries high likelihood of fulminant disease (2%-20%), chronic HDV infection (up to 80%), and cirrhosis (60-70%), and may progress to hepatocellular carcinoma.
**15-20% in pregnant women.

## INDIVIDUALS SUITABLE FOR TESTING

- Individuals with clinical symptoms or abnormal liver enzyme levels
- Children born to infected women, especially those who are HBV e antigen (HBeAg)-positive
- Household or sexual contacts of infected persons or carriers
- Individuals participating in high-risk sexual activities (multiple sexual partners, same-sex intercourse)
- Intravenous drug users
- Recipients of organ transplants or multiple blood transfusions
- Hemodialysis patients
- Recent visitors or immigrants from endemic areas
- Health care workers

## TEST AVAILABILITY

Tests for antibodies and antigens are available for hepatitis A-E, including those specific for hepatitis B core, surface, and e proteins. Techniques for antibody detection include enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA). EIA technology is also used for antigen testing. Branched DNA (bDNA), solution hybridization-hybrid capture (Digene), transcription-mediated amplification (TMA), and polymerase chain reaction (PCR) are used for DNA and RNA testing to determine the presence of viremia and measure the viral load. HCV and HBV genotypes can also be determined with hybridization or sequencing-based assays.

## TEST SELECTION

### Diagnose Acute Infection

Four initial tests are generally recommended to diagnose acute hepatitis (see Fig.1): HAV immunoglobulin M (IgM) antibody, HBV core IgM antibody (HBcAb, IgM), HBV surface antigen (HBsAb), and HCV antibody by EIA. HAV IgM antibody is the preferred test for diagnosis of acute hepatitis A infection because it rises early and persists only 3 to 12 months. HBcAb IgM is detectable during acute but not chronic HBV infection. HBsAg, however, is detectable in both stages. Simultaneous use of these two tests therefore not only detects both acute and chronic HBV infection but also helps to differentiate between them. The EIA (antibody test) is used as the initial assay for diagnosing HCV infection because of its high sensitivity, wide availability, and low cost. However, antibody is not detected for many months after infection. RNA tests can detect virus prior to seroconversion and serve to differentiate between active and resolved infection. RIBA may be used in patients with positive EIA results and negative HCV RNA results, although a repeat HCV RNA assay may also be used in this setting. A negative RIBA result is required for re-entry into the blood donor pool.

In cases of fulminant hepatitis, the possibility of coninfection of superinfection (HBV with HCV or HDV) should be explored (see Fig.1).

### Diagnose Chronic Infection

When screening for chronic hepatitis, two laboratory tests are generally recommended (see Fig.2): HBsAg and HCV antibody by EIA. Positive HBsAg results obtained six months or more after the initial diagnosis indicate chronic infection. Either HBV DNA or the HBeAg assay can be used for confirmation. HCV RNA testing is used to confirm positive HCV antibody results, but when clinical suspicion is high and results from the two tests are discrepant, RIBA can be helpful.

HDV infection should be considered in chronic HBV carriers who experience a further acute attack and/or rapidly progressive liver disease. The HDV antibody assay is recommended for the initial test; positive results may be followed by the HDV antigen test for confirmation. HDV antigen testing may also be useful in HBsAg-positive/HDVAb-negative individuals if coinfection or superinfection is strongly suspected.

**Demonstrate Carrier Status**

Following disappearance of clinical symptoms, carrier status can be demonstrated by testing for persistence of antigen, DNA, or RNA. For HBV, the presence of the following conditions indicate inactive carrier state: HBsAg positive for greater than or equal to six months, HBeAg-negative, HBeAb-positive, serum HBV DNA less than $10^5$ copies/mL, and persistently normal ALT/AST levels. For HCV, use a quantitative or qualitative RNA test. For HDV, use the HCV antigen test.

**Demonstrate Recovery or Differentiate Between Active and Resolved Infection**

Differentiating current vs. past infection is possible using HBV DNA, HBsAg, and HBeAg testing for HBV; highly sensitive quantitative or qualitative HCV RNA testing for HCV; and antigen testing for HDV. Recovery from hepatitis B (in patients with known history of acute or chronic HBV or the presence of HBcAb and/or HBsAb) is signaled by the disappearance of HBsAg and HBV DNA along with persistently normal ALT levels. Some individuals have detectable DNA after disappearance of HBsAg. While this may not be associated with active disease in immunocompetent individuals, it may represent treatment failure or failure of natural immunity when HBs antibody is absent. Additionally, HBV DNA-positive individuals may be at risk for recurrent HBV disease and HBV DNA-positive organ donors could potentially transmit the infection to organ transplant recipients. HCV recovery is indicated by repeatedly negative HCV RNA test results. HDVAg disappears within months after recovery.

**Assist with Treatment Decision-Making and Therapeutic Monitoring**

The alanine aminotransferase (ALT) assay is important when assessing liver function. In chronic HBV infection, the baseline ALT level is associated with the likelikhood of treatment response; elevated ALT in the presence of a positive HBV DNA assay is an indication for treatment initiation. Quantitative HBV DNA assays can help assess the likelihood of response to to therapy, and predict the emergence of resistance to antiviral agents. The HBV genotyping assay is used to determine the HBV genotype, which is important for epidemiologic studies and may be associated with the clinical course and response to therapy. The HBV genotype can also detect the emergence of mutations associated with resistance to antiviral drugs.

In HCV infection, elevated ALT in the presence of a positive HCV RNA assay is an indication for treatment initiation. Highly sensitive qualitative and quantitative HCV RNA rests are useful to monitor response to therapy, and document resolved infection. Quantitative RNA tests can also help predict the likelihood of response and select the appropriate duration of therapy. The HCV genotype assay is important for selecting the appropriate duration of therapy and determining the likelihood for treatment response.

**Screen for Immunity or Successful Vaccination**

HAV total antibody, HBsAb, or HBc total antibody assays are generally recommended to determine immune status following infection, pre- or post-vaccination or post-administration of immune globulin.

Multiple tests may be required to completely characterize an individual patient's infection. To further clarify the role of the available viral hepatitis tests, specific clinical applications for each are addressed in Table 2. Figures 1,2, provide algorithms for the use of diagnostic assays in the diagnosis and management of viral hepatitis.

**Table 2 Clinical Application of Laboratory Tests for Hepatitis**

| Test | Clinical Application |
|---|---|
| Alanine Aminotransferase (ALT) | Indicate hepatic disease; Monitor response to antiviral therapy; Indicate resolution of HAV or HEV infection |
| HAV Antibody (IgM) | First-line diagnostic test for acute hepatitis A |
| HAV Total Antibody | Screen for immunity prior to vaccination |
| HBV Core Antibody (IgM) | First-line diagnostic test for acute hepatitis B; Indicate recent infection (within preceding 4-6 months) |
| HBV Core Total Antibody (IgG + IgM) | Indicate current or prior infection |
| HBV DNA, Qualitative | Determine need to treat chronic HBV infection; Indicator of chronic hepatitis when still positive 6 months after diagnosis of acute HBV infection; Monitor response to therapy; Demonstrate viral replication in patients with mutant HBV (eg, HBeAg- and HBeAb+ individuals) |
| HBV DNA, Quantitative | All indications listed for qualitative HBV DNA assay above; Predict likelihood of response to therapy; Indicate emergence of resistant variants during antiviral therapy |
| HBV e Antibody | Indicate treatment response |
| HBV e Antigen | Indicate active viral replication and high infectivity |
| HBV Genotype | Detect hepatitis B virus (HBV) mutations associated with resistance to antiviral agents; predict and monitor response to therapy; identify HBV genotype (A-G) for epidemiologic and prognostic purposes; detect mutations in precore and basal core promoter regions, which may influence immune response and outcome. |
| HBV Surface Antibody, Qualitative | Indicate immunity post-infection, vaccination, or HBIG* |
| HBV Surface Antibody, Quantitative | Indicate immunity post-infection, vaccination, or HBIG*; high levels are suggestive of a protective response. |
| HBV Surface Antigen | First-line diagnostic test for acute hepatitis B; Indicates chronic hepatitis when still positive 6 months after diagnosis of acute HBV infection. |
| HBV Surface Antigen Confirmation | Routinely used to confirm positive HBV surface antigen tests |
| HCV Antibody, EIA | First-line screening test for detection of acute and chronic hepatitis C |
| HCV Antibody, RIBA | Confirm infection when the antibody and RNA tests are discrepant and clinical suspicion is high; negative result required for re-entry to blood donor pool. |
| HCV Genotyping | Predict likelihood of therapeutic response<br>Determine the duration of treatment |
| HCV RNA, Qualitative | Detect acute infection prior to seroconversion (ie, within 1-2 weeks post-exposure); Confirm EIA diagnosis of acute or chronic infection (LOD <50 IU/mL); Differentiate between resolved and active infection; Demonstrate resolution of infection |

| | |
|---|---|
| HCV RNA, Quantitative | Highly sensitive quantitative assays (at least as sensitive as relevant qualitative assay) only; all indications listed for qualitative HCV RNA assay above; Predict response to antiviral therapy; Differentiate lack of therapeutic response from partial therapeutic response |
| HDV Antibody | Diagnose HDV infection in patients with fulminant hepatic failure or known previous HBV infection |
| HDV Antigen | Diagnose HDV infection in symptomatic, HBsAg-positive, HDV antibody-negative individuals |
| HEV Antibody (IgG) | Demonstrate recent or past hepatitis E infection |
| HEV Antibody (IgM) | Diagnose acute HEV infection |

*Hepatitis B immune globulin
LOD = limit of detection

**Figure 1 Diagnosis of Acute Hepatitis**
**For Symptomatic or High Risk Individual Test for HAV IgM Ab, HBc IgM Ab, HBs Ag, and HCV Ab (EIA)**

| | | | | | |
|---|---|---|---|---|---|
| HAV IgM Ab – | HAV infection unlikely | | | | |
| HAV IgM Ab + | HAV infection | | | | |
| HBc IgM Ab – | HBV infection unlikely | | | | |
| HBc IgM Ab + or – HBsAg + | HBV infection | Consider test for HDV Ab | HDV Ab – | HDV infection unlikely | |
| | | | HBc IgM Ab + | HBV with HDV coinfection | |
| | | | HDV Ab + | HBV with HDV superinfection | |
| HCV Ab (EIA) – | HCV infection unlikely | | | | |
| HCV Ab (EIA) + | Test for HCV Ab (RIBA™) | Positive | HCV infection | | |
| | | Pos/Neg | Test for HCV RNA | HCV RNA + | HCV infection |
| | | | | HCV RNA – | HCV infection unlikely |
| | | Negative | HCV infection unlikely | | |

**Figure 2 Diagnosis of Chronic Hepatitis**
**Test for HBs Ag and HCV Ab**

| | | | | | | |
|---|---|---|---|---|---|---|
| HBsAg – No clinical symptoms | HBV infection unlikely | | | | | |
| HBsAg – Clinical symptoms | Test for HBV DNA | Negative | HBV infection unlikely | | | |
| | | Positive | HBV infection | | | |
| HBs Ag + | HBV infection | Consider test for HDV Ab | HDV Ab – | HDV infection unlikely | | |
| | | | HDV Ab + | HBV/HDB infection | | |
| HCV Ab – | HCV infection unlikely | | | | | |
| HCV Ab + | HCV active or resolved infection | HCV Ab (RIBA™) – | HCV infection unlikely | | | |
| | | HCV Ab (RIBA™) +/- | Test for qualitative HCV RNA | Negative | HCV infection unlikely | |
| | | | | Positive | HCV infection | |
| | | HCV Ab (RIBA™) + | HCV active or resolved infection | Test for qualitative HCV RNA | Negative | Resolved HCV infection |
| | | | | | Positive | Active HCV infection |

## TEST INTERPRETATION

A negative antibody test result indicates lack of immunologic response to that type of hepatitis virus. False-negative results may occur in early-stage acute disease (prior to seroconversion) or in patients with a suppressed or non-functioning immune system. If clinical suspicion is high, negative results can be verified by testing for type-specific antigen, DNA, or RNA as appropriate.

If an antibody test is positive, the patient has generated an immune response to that type of hepatitis virus and should be evaluated further with type-specific supplemental testing (eg, antigen, DNA, or RNA testing). In an infant less than 18 months of age, a positive antibody test result may indicate passive transfer of maternal antibody. Testing with a type-specific antigen or nucleic acid-based assay may reveal active infection. Additional antibody-specific interpretive information follows:

- Presence of total HAV antibody, in the absence of HAV IgM antibody, indicates immunity against HAV infection.
- HBc IgM antibody positivity usually indicates HBV infection within the preceding 4 to 6 months.
- HBc IgG antibody positivity indicates prior HBV infection and may be associated with chronic or resolved infection.
- HBeAb presence indicates resolving infection or response to therapy.
- HBsAb presence indicates immunity against HBV infection.
- A positive HDVAb test, coincident with the presence of HBsAg, indicates HBV/HCV coinfection.
- False-positive HCV antibody results may be generated by the EIA in cases of alcoholic liver disease, autoimmune chronic active hepatitis, or improper sample storage and testing conditions. A positive HCV RNA test result confirms a positive EIA result. In the presence of a positive EIA result, a negative HCV RNA result should be confirmed by repeat RNA assay or RIBA.
- A negative RIBA excludes HCV as the cause of a positive EIA result and is required for re-entry into the blood donor pool.

A negative HBsAg, HBV DNA, or HCV RNA test result indicates lack of current infection. In rare cases, a negative result reflects a viral load below the assay's limit of detection. Thus, a negative result does not exclude the possibility of infection. Repeatedly positive antigen, DNA, or RNA test results, on the other hand, indicate active infection. A return to a negative test result following therapy indicates resolution of the infection. After resolution, reappearance of a marker may indicate a relapse.

HBsAg presence does not reflect the level of active virus, nor does it differentiate between acute and chronic infection or between mild and severe disease. False-positive results are uncommon, but when they occur they are generally due to technical limitations of the test. In low-risk populations, the HBsAg confirmation test can help verify a repeatedly reactive result as a true positive.

Relatively low levels of HBV DNA or HCV RNA are associated with acute or resolving infection and with the probability of a sustained therapeutic response; high levels predict lack of therapeutic response. In general, a stable or rising viral load is indicative of lack of therapeutic response, while falling levels indicate that the patient is responding to treatment. Persistent detection of HCV RNA after 3 months of interferon

monotherapy or 6 months of interferon/ribavirin combination therapy indicates that sustained response to therapy is unlikely. Recent studies have shown, however, that a small subset of these patients may still benefit from further therapy. DNA and RNA levels need to be interpreted in combination with all available clinical, biochemical, and liver biopsy information.

Even after apparent recovery from HBV infection, viable virus may remain in the liver where it can be detected in biopsy material, can infect a transplant recipient who is HBcAb negative, and may even cause recurrent HBV disease in individuals with profound immunosuppression.

Tables 3, 4 and 5 detail test result patterns and their associated clinical significance.

**Table 3 Diagnosis of Acute Hepatitis Virus**

| Hepatitis Virus | HAV IgM | HBsAg | HBV DNA | HBc IgM | HBc Total | HCV Ab | HCV RNA | HDV Ag | HDV Ab | HEV IgM | HEV IgG |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | + | - | - | - | - | - | - | - | - | - | - |
| B | - | + | + | + | - | - | - | - | - | - | - |
| B with D Coinfection | - | + | + | + | - | - | - | + | + | - | - |
| B with D superinfection | - | + | + | - | - | - | - | + | + | - | - |
| C | - | - | - | - | - | + | + | - | - | - | - |
| E | - | - | - | - | - | - | - | - | - | + | -/+ |

**Table 4 Diagnosis of Chronic Hepatitis**

| Hepatitis Virus | HBsAg | HBsAb | HBc IgM | HBc Total | HBeAg | HBeAb | HBV DNA | HCV Ab | HCV RNA |
|---|---|---|---|---|---|---|---|---|---|
| B | + | - | - | + | + | - | + | - | - |
| C | - | - | - | - | - | - | - | + | + |

**Table 5 Interpretative of Hepatitis B Markers**

| Marker | Acute (Early) | Acute (Resolving) | Chronic (Low Infectivity) | Chronic (High Infectivity) | Inactive HBsAg Carrier State | Resolved | Successful Vaccination |
|---|---|---|---|---|---|---|---|
| HBc IgM Ab | ++ | + | - | - | NA | - | - |
| HBc Total Ab | + | + | ++ | ++ | NA | + | - |
| HBsAb | - | - | - | - | NA | +/- | + |
| HBsAg | + | + | + | + | +/- [a] | - | - |
| HBV DNA, Quant | + | - | - | + | +/- [b] | - [c] | - |
| HBeAb | - | +/- | +/- | - | + | +/- | - |
| HBeAg | + | - | - | +/- | - | - | - |

NA = Not applicable

[a] Present for at least 6 months; should be undetectable 1 year after acute infection.

[b] $<10^5$ copies/mL

[c] Very low levels may be detected with highly sensitive assays.

Information obtained from 2005 Directory of Services Manual Quest Diagnostics Nichols Institute, Chantilly, VA.

**Reflex Hepatitis C Testing Protocol**

<table>
<tr><td>HCV Ab –</td><td colspan="6">Not infected</td></tr>
<tr><td rowspan="5">HCV Ab +</td><td rowspan="3">Low <3.8 S/CO Ratio</td><td rowspan="3">Do RIBA</td><td>If Negative</td><td colspan="3">Not infected with HCV- False positive</td></tr>
<tr><td rowspan="2">If Positive or Indeterminant</td><td rowspan="2">Do PCR quant; if positive do genotype</td><td>Negative</td><td>Positive for HCV Ab</td></tr>
<tr><td>Positive</td><td>Active infection with HCV</td></tr>
<tr><td rowspan="3">High ≥3.8 S/CO ratio</td><td rowspan="3">Do PCR quant; if positive then do genotype</td><td>If Negative</td><td>Do RIBA</td><td>Negative</td><td>Not infected; False positive</td></tr>
<tr><td>Indeterminant</td><td>Pathologist Review</td></tr>
<tr><td>Positive</td><td>Positive for HCV; indicates past or present infection. A single negative PCR result does not rule out active infection. Repeat PCR qualitative in a month</td></tr>
<tr><td colspan="2">If Positive</td><td colspan="2">Active infection with HCV</td><td colspan="2">Recommend evaluation for treatment by a specialist in Hepatitis C disease.</td></tr>
</table>