REX HEALTHCARE

Laboratory Bulletin...

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Viral Hepatitis Testing Update

Hepatitis refers to a disease characterized by inflammation of the liver. Most cases of hepatitis are due to viral infection. Many cases of viral hepatitis produce an acute self-limited illness, generally treated by supportive care. Viral hepatitis lasting longer than 6 months is termed *chronic hepatitis*. Chronic hepatitis is relatively infrequent with hepatitis B (< 5% in immunocompetent adults) but common with hepatitis C (> 70%)[1] Chronic viral hepatitis can result in cirrhosis, liver failure or hepatocellular carcinoma. Laboratory tests are crucial in the diagnosis and management of viral hepatitis. This management includes prevention of transmission to others and, in some cases, specific therapy (as new antiviral agents for both chronic hepatitis B and chronic hepatitis C evolve). This paper will review the serologic markers available to evaluate viral hepatitis, discuss some of the newer viral genomic assays, and offer suggestions for the rational application of these tests. The management of patients with chronic viral hepatitis is complex and requires evaluation of multiple parameters: liver enzymes, serologic markers, quantitative viral studies and liver histology. A full discussion of these factors is beyond the scope of this review. Consultation with appropriate specialists is encouraged.

First, a brief review of viral hepatitis…

Viral Hepatitis

Type (Nucleic Acid)	Transmisson	Transaminases	Chronicity	Specific Therapy (Chronic Hepatitis)
A (RNA)	Fecal-Oral	↑↑↑↑	No	No
B (DNA)	Blood/Body Fluid	↑ - ↑↑↑ (acute) Variable (chronic)	Yes	Alpha Interferon Lamivudine
C (RNA)	Blood/? Semen	↑ - ↑↑↑	Yes	Alpha Interferon Ribavirin
D (RNA)	Blood/Semen (requires Hep B coinfection)	↑ - ↑↑↑↑	Yes	Alpha Interferon
E (RNA)	Fecal-Oral (predominantly "3rd World" countries)	↑ - ↑↑↑↑	No	No
G (RNA)	Blood/Semen	??? ↑ (? Any disease)	No	No
Other (e.g. EBV, CMV)	Variable	Variable	Rare	No

Next, a review of current laboratory tests for viral hepatitis. The prices listed are "retail", subject to change, and presented primarily to give a relative idea of patient charges depending on test selection.

Tests for Hepatitis A (HA)

- Anti-HAV (IgM): IgM antibody against HA. Marker of acute infection. *($16.00)*
- Anti-HAV (total): Total (IgG and IgM) antibody against HA. Marker of acute or past infection. A (+) anti-HAV (total) with a (-) anti-HAV (IgM) indicates past infection (and immunity). Most laboratories do not test for anti-HAV, IgG directly. *($18.00)*

Tests for Hepatitis B (HB)

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- HBsAg: "surface antigen" (external structural protein of HB). Indicates acute or chronic infection. *($13.00)*
- Anti-HBc (IgM): IgM antibody against internal structural protein of HB. Indicates acute or recurrent infection. Rare patients (< 5%) with acute HB have (-) HBsAg but (+) anti-HBc, IgM. *($17.00)*
- Anti-HBc (total): Total (IgG and IgM) antibody against internal structural protein of HB. Indicates current or past infection. Most laboratories do not test for anti-HBc, IgG directly. *($17.00)*
- HBeAg: immunogenic portion of core protein. Marker of "replicative" infection (i.e. patient remains highly infectious). In acute HB, generally disappears prior to disappearance of HBsAg. As it is invariably present in acute HB, testing for its presence in this setting is generally not indicated. It is most useful in assessing chronic HB. In chronic HB, the presence of HBeAg indicates active infection while its absence suggests nonreplicative infection. A goal of therapy in patients being treated with antivirals is clearance of HbeAg. *($56.10)*
- Anti-HBe: antibody against "e" antigen. Indicates conversion to non-replicative state (reduced infectivity) and signals probable disease resolution (depending on status of HBsAg and anti-HBs) *($56.10)*
- Anti-HBs: antibody against surface antigen. Indicates clearance of HBsAg and disease resolution. HB vaccine should provoke detectable anti-HBs, thus conferring immunity. *($15.00)*
- HBV-DNA: Hepatitis B DNA by chemiluminescent molecular hybridization. A sensitive test for detecting presence of HB and a marker for active viral replication. Capable of detecting 142 x 10^3 copies/mL. Its appearance (and clearance) generally parallels that of HBsAg and HbeAg. Generally not indicated in the diagnosis or management of acute hepatitis B, although the detection of HBV-DNA eight weeks after onset of symptoms may signal progression to chronic hepatitis.[7] May also be helpful in rare cases of fulminant acute HB or chronic HB where HBsAg is negative, or in distinguishing between HB "carrier state" and chronic HB in cases where HbeAg is negative, but ALT is elevated. Useful (in conjunction with ALT level, HBeAg status, and liver biopsy findings) in assessing **chronic** HB patients for antiviral therapy. When used to monitor therapy, it is important that the same test and the same laboratory are used to assure as much standardization of results as possible. (A negative test result does not exclude the presence of HBV DNA.) Quantitative HBV testing is a highly competitive area and refinements in testing are expected to continue (Ultra-sensitive polymerase chain reaction (PCR) assays for HBV-DNA exist that can detect as few as 1-10 virus particles per mL. The clinical significance of this finding is uncertain, so their use is recommended only for research settings and not clinical diagnosis.)[1,6] *($169.20)*

Tests for Hepatitis C (HC)

- Anti-HCV: antibody against HC detected by enzyme immunoassay. Currently use a "3rd generation test" which has shortened the "window" between infection and antibody detection to 11 weeks.[2] The sensitivity is outstanding for past exposure to, or current infection with, HCV (present in 99% of patients with chronic HC).[3] If anti-HCV is negative, it is highly unlikely that a patient has chronic HC. But because of the "window effect", a positive result is found in only 60-70% of cases of acute HC at the onset of symptoms.[3] And while the sensitivity has improved, specificity remains problematic. False positive results in low prevalence populations (e.g. blood donors) are not unusual (up to 50%).[3] While some false positives are the result of recent immunization, rheumatoid factor, or hypergammaglobulinemia, many remain unexplained. Both of these phenomena (lack of sensitivity for acute HC and suboptimal specificity) have implications for test interpretation and selection of follow-up testing. **All reactive anti-HCV EIA specimens require confirmatory testing (either by HCV recombinant immunoblot assay (RIBA) or HCV RNA by PCR) as discussed below.** For blood donors with (+) anti-HCV EIA, HCV RIBA is automatically performed. For patients, the ordering physician must order the appropriate test. *($20.00)*
- HCV RIBA: Recombinant Immunoblot Assay. This is the confirmatory test recommended for low risk patients (e.g. blood donors). It has a high degree of **specificity** for confirming the presence of **antibody** to HCV. It is not a sensitive test in terms of diagnosing acute infection. It cannot distinguish between resolved infection and chronic active HCV infection. A negative HCV RIBA in the setting of a (+) anti-HCV EIA indicates a false (+) EIA result. Indeterminate results should be followed up with repeat testing in 6-12 months. As noted above, this test is automatically performed on blood donors with (+) EIA results. *($154.30)*
- HCV RNA by PCR (qualitative): This is the confirmatory test recommended for high-risk patients (including patients with elevated ALT). A positive test indicates active HC (acute or chronic). A negative test does **not** exclude HCV exposure or infection. If there is a high degree of clinical suspicion, repeat testing in 2-4 weeks should be considered. The qualitative PCR test is the **most sensitive** test for current HCV infection and will detect low levels of viremia (700-1000 copies/mL) missed by quantitative PCR or branched DNA (bDNA) assays. *($241.00)*

- HCV (quantitative) by bDNA: HCV RNA measurement by branched DNA (bDNA) is the **recommended quantitative assay** for HCV because of a high degree of reproducibility. Currently detects 2.0 x 10^5 – 1.2 x 10^8 copies/mL. HCV levels do not correlate with disease severity and are useful primarily to assess response to therapy. This test is **not** recommended for confirming (+) anti-HCV EIA results due to inferior sensitivity compared to HCV RNA by PCR

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(qualitative). For monitoring HCV viral load, the same quantitative procedure should be used for all specimens. (*$277.60)*

- HCV (quantitative) by RT-PCR: An alternate method of quantitation using reverse transcriptase PCR. May not be as reproducible as HCV (quant.) by bDNA, but can detect lower quantities of virus (2.0×10^3 copies/mL). HCV levels do not correlate with disease severity and are useful primarily to assess response to therapy. This test is **not** recommended for confirming (+) anti-HCV EIA results due to inferior sensitivity compared to HCV RNA by PCR (qualitative). For monitoring HCV viral load, the same quantitative procedure should be used for all specimens. *($166.80)*
- HCV Genotyping: RT-PCR w/ DNA probe hybridization. Over 9 genotypes available. Helpful for predicting response to therapy and duration of therapy. Most US cases are genotypes 1a (50-60%) or 1b (15-20%). Therapy for genotypes 2 or 3 is recommended for 24 weeks, while 48 weeks is recommended for genotype 1 (assuming response to therapy, absence of HCV RNA, demonstrable at 24 weeks). *($303.90)*

Tests for Hepatitis D (HD)

- Anti-HDV: antibody to HDV. HD requires coinfection w/ HB. HDV may cause a self-limited coinfection with acute HB or a superinfection of chronic HB. The former may produce a more fulminant presentation, while the latter may accelerate progression to cirrhosis or liver failure. The test should be considered for patients with chronic HB who develop an acute exacerbation of their disease. Drug addicts and hemophiliacs are at particular risk. *($64.70)*

Tests for Other Types of Hepatitis

- Hepatitis E is similar to Hepatitis A, although clinical symptoms may be more intense. The virus is found predominantly in Southeast Asia, India, the Middle East, and Mexico. No tests available for Hepatitis E (outside of research labs or the CDC where experimentally infected marmosets are held captive to permit antibody blocking assays with their diseased livers).[5] (We will alert the media with the name of any physician who actually requests this test.) EIA assays are under development.
- Hepatitis G is a recently discovered virus of questionable clinical significance. The prevalence is 1-2% in blood donors with a higher prevalence in IV drug abusers, and patients who have received hemodialysis, transplants or blood transfusions.[2] Most people harboring this virus are asymptomatic and have normal LFTs. No serologic tests are available, but an HGV-RNA (qualitative) by RT-PCR is available. *($245.70)*
- Other viruses capable of producing hepatitis include Epstein-Barr virus (infectious mononucleosis), cytomegalovirus, adenovirus, and Herpes virus. In the appropriate clinical setting, serologic tests for these viruses may be helpful in identifying the cause of an apparent infectious hepatitis.
- Other causes of hepatitis (ethanol toxicity, drug effect, autoimmune hepatitis, alpha-1-antitrypsin deficiency, hemochromatosis, Wilson disease, etc.) must certainly be considered in the evaluation of patients with abnormal LFTs.

Testing Strategy for Acute Hepatitis

For evaluation of viral hepatitis of less than 6 months duration, the following tests are recommended: anti-HAV (IgM), HBsAg, anti-HBc (IgM), and anti-HCV. Fortunately these tests constitute the "HCFA approved" hepatitis panel (the only hepatitis panel offered by Rex - *$65.00*). A guide to interpretation is offered in the **Acute Viral Hepatitis** table (insert).

Testing Strategy for Chronic Hepatitis

Anti-HBc (total), anti-HBc (IgM), and anti-HCV. A guide to interpretation is offered in the **Chronic Viral Hepatitis** table (insert)

Closing Comments

- Due to HCFA mandates (discussed in previous issues of the *Bulletin*), only one hepatitis panel (perfect for evaluation of acute hepatitis) is available at Rex. Any request for "hepatitis panel" will result in the default selection of this panel.

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- For other situations, the tests must be ordered individually. The following test groupings are presented to assist in test selection for various clinical situations where viral hepatitis testing is desirable.

Chronic Hepatitis (Unknown Type): HBsAg, Anti-HBs, Anti-HBc (total), anti-HBc (IgM), and anti-HCV
Chronic Hepatitis B: HBsAg, HbeAg, anti-HBe
Hepatitis B Immunity screen: anti-HBs
Hepatitis A Immunity screen: anti-HAV (total)
Prenatal Hepatitis screen: HBsAg, HbeAg, anti-HBe, (in high risk groups consider adding anti-HCV)
Previous Hepatitis Exposure screen: anti-HAV (total), HBsAg, anti-HBc (total), anti-HBs, anti-HCV

- When ordering hepatitis C RNA tests, please specify whether **qualitative** or **quantitative** tests are desired.
- There is considerable variability in the performance characteristics of viral load testing, with a great deal of ongoing competitive research to enhance sensitivity and reproducibility. Standardization is still a goal, rather than a reality. When ordering **quantitative** tests to measure viral load, it is critical to use the same laboratory and the same methodology to optimize standardization of results. All specimens collected at Rex are currently forwarded to Mayo Medical Laboratories. In view of this, viral load testing on inpatients is discouraged unless previous or subsequent testing has been or will be sent to Mayo Medical Laboratories.

John D. Benson, MD

References

1. Schiff ER *et al*. Schiff's Diseases of the Liver, 8th ed., Lippincott, Willams & Wilkins, Phila. 1999, pp 762-812.
2. Sacher RA *et al*. Testing for Viral Hepatitis. *Am J Clin Pathol* 113:12-17, 2000.
3. Hoofnagle JH. Chronic Viral Hepatitis (lecture notes). Armed Forces Institute of Pathology, 2000 Hepatic Pathology Course. Sept. 2000.
4. *Mayo References Services Communiqué*, v. 25 (4), April 2000.
5. Sherman KE. Interpretation of Liver Tests (lecture notes). Armed Forces Institute of Pathology, 2000 Hepatic Pathology Course. Sept. 2000.
6. Pao CC *et al*. Serum Hepatitis B Virus DNA in Hepatitis B Virus Seropositive and Seronegative Patients with Normal Liver Function. *Am J Clin Pathol* 95:591-596, 1991.
7. *Mayo Services Communiqué*, v. 25 (11), November 2000.

A Final Note

It is our sad task to report that Dr. Rusty Ball has accepted a position with the dermatopathology service at Moses Cone Hospital in Greensboro effective January 2001. His expertise and enthusiasm will be missed by all of us. He leaves with our best wishes for the future. We have retained the services of the UNC Athletic Dept. to assist in the search for a successor.

Rex Healthcare Pathology Department

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