REX

Laboratory Bulletin

August 2005

UPDATES AND INFORMATION FROM REX PATHOLOGY LABORATORY

Issue Number 106

(Not Quite) As Easy As A B hepC
Hepatitis C Testing at Rex

Hepatitis C virus (HCV) has emerged as a major cause of liver disease. Forty thousand new cases are estimated to occur each year in the United States. 3.5 to 4 million Americans (1.8%) have antibodies to HCV and greater than 70% of these patients have viral DNA detectable in the blood, evidence of chronic infection. The annual infection rate has decreased since the mid 1980s, but the number of patients with cirrhosis and hepatocellular carcinoma secondary to HCV will continue to rise, due to the length of chronic infection.

Acute HCV infection is usually mild and clinically undetected. In contrast to hepatitis B, which has a more prominent acute phase and a higher chance of resolution, the majority of hepatitis C patients develop chronic infection (Figure 1).

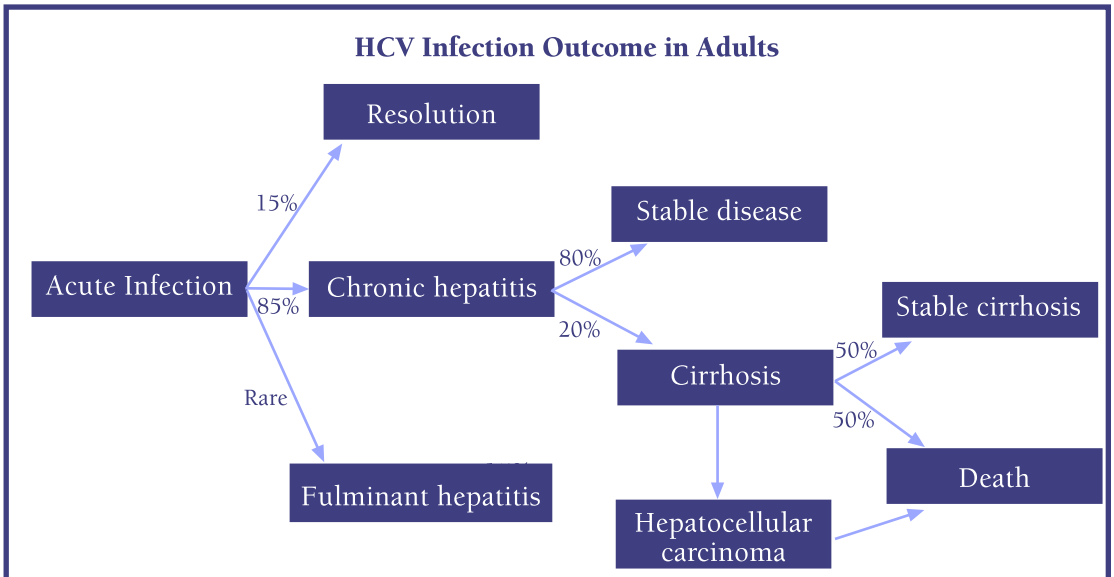

Figure 1 *Adapted from Pathologic Basis of Disease, figure 18-12, p. 894.*

The timing of serologic markers is summarized in Figures 2 and 3. Note the incubation period of 2-26 weeks. In chronic carriers, there can be a period where HCV-RNA is not detectable, and therefore one negative HCV-RNA result does not entirely rule out active disease. Immunocompromised patients as well as acutely infected individuals may not have antibodies to HCV. In these two scenarios, Mayo Medical Laboratories recommend skipping anti-HCV testing and going directly to HCV-RNA by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

HCV is genetically diverse. There are more than six distinct genotypes and multiple subtypes. In the US, genotype 1a is the most common; followed by 1b, 2b, and 3a. Genotypes one and four are associated with high viral titer, male gender, increasing age, and poor response to current therapies. Within a single patient, HCV exists as a distribution of subtypes secondary to HCV RNA polymerase coding errors. This genetic instability means that anti-HCV IgG does not confer immunity and has hampered the development of an HCV vaccine.

The Food and Drug Administration first licensed tests for HCV in 1990. Since that time there have been several new tests available for screening, diagnosis, and follow-up to treatment.

HCV Tests

- ***Hepatitis C Antibody (Anti-HCV)*** ORTHO HCV Version 3.0 ELISA. Offered at Rex M/W/F as a single test ($39.88) or as part of the acute hepatis panel ($124). The test requires a red top tube, which can be sent at ambient temperature or refrigerated at 2- 8 C for up to five days. If the result is reactive (initial reactive tests are retested in duplicate) then a comment is made recommending confirmation by HCV RNA by RT-PCR (next bullet). A separate specimen collection is needed (see below) and will be sent to the Mayo Medical Laboratories for this follow-up testing.

Rex Pathology Associates, P.A.

Stephen V. Chiavetta, MD	(919) 784-3060	Keith V. Nance, MD	(919) 784-3286
Timothy R. Carter, MD	(919) 784-3058	Vincent C. Smith, MD	(919) 784-3056
John D. Benson, MD	(919) 784-3059	Rhonda Humphrey, Practice Manager	(919) 784-3063
John P. Sorge, MD	(919) 784-3062		

REX LABORATORY SERVICES
Healthcare for life. Your life.

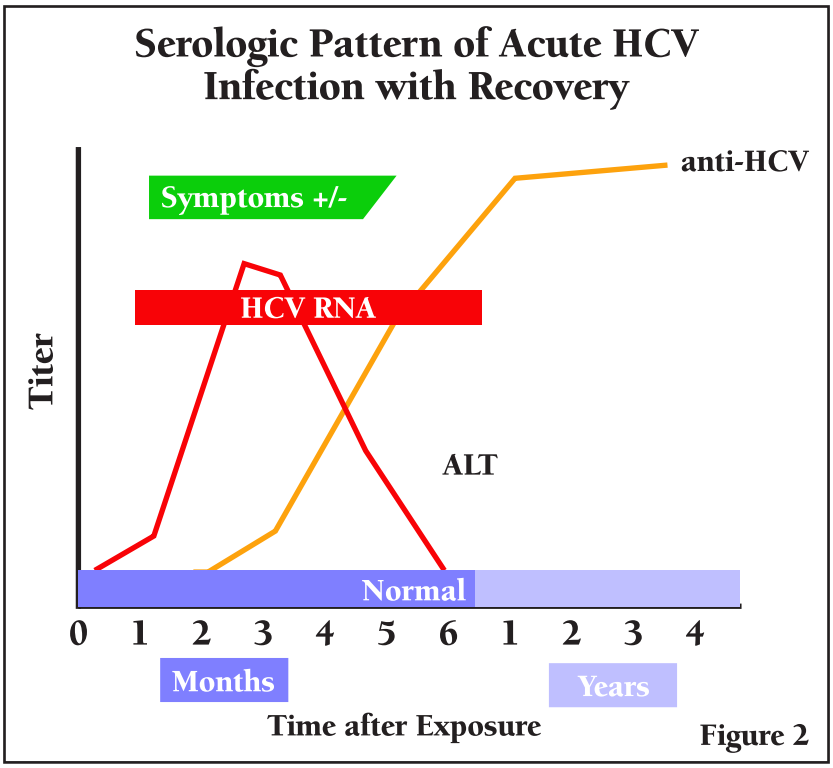

Figure 2

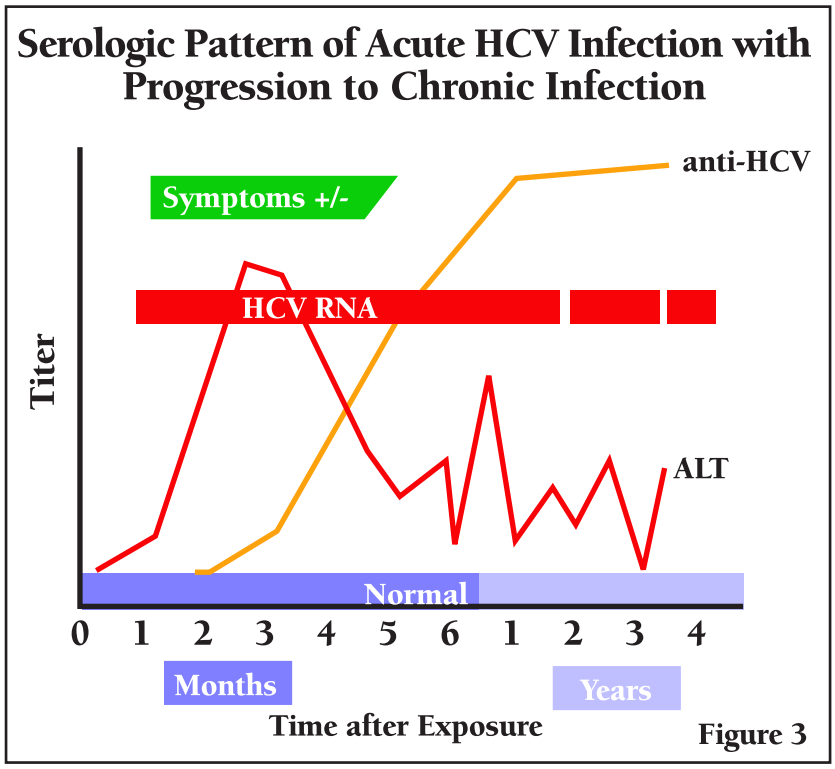

Figure 3

- ***HCV Detection and Quantitation*** *by RT-PCR* (Mayo Medical Laboratories test code 83142, offered daily, $175). This is the most sensitive and specific test offered. It is a new test offered at Mayo and it uses real-time PCR by TaqMan technology. This test replaces the older generation quantitative assays as well as the prior qualitative RT-PCR tests. The quantitative range is 10 IU/ml to 28,800,000 IU/ml. Below 10 IU/ml, the test can provide a qualitative result as well. Because of these features this test can be used for diagnosis of HCV as well as monitoring before, during, and after treatment. Blood is to be drawn in a red top or serum gel tube, spun down and ***frozen within four hours***, with the serum sent in a plastic vial. Because of these requirements, it is best to have blood drawn and sent to Rex immediately, or have the patient come to the Rex Outpatient Laboratory for the blood draw.

- ***HCV Recombinant Immunoblot Assay (RIBA).*** (Mayo Medical Laboratories test code 80181, Monday through Saturday, $66). This test also requires a red top or serum gel tube, which is spun down within 24 hours. The serum is shipped frozen in a plastic vial. The HCV RIBA is a confirmatory test for the presence of anti-HCV. Like the anti-HCV ELISA, it does not distinguish active from past disease. See the flowchart below (Figure 4).

- ***HCV Genotype after amplification*** (Mayo Medical Laboratories test code 84434, Monday through Friday, $170 for genotype if amplification is positive). This test can aid in determining the duration of therapy and predicting the chance of treatment success in active HCV infection. The specimen requirements are the same as *HCV Detection and Quantitation by RT-PCR* above.

Because of the confusion surrounding HCV testing, the CDC published new guidelines in 2003. In this document they give different options for testing, all of which start with an anti-HCV screening test. The screening anti-HCV test is over 99% specific but the CDC reports that in low prevalence groups this can mean a false positive rate of 15%. The CDC also recommends that all positive HCV screening tests be followed up with confirmatory testing (similar to HIV testing), either the HCV-RIBA or the HCV-RNA by RT-PCR test. One option includes using a signal to cut-off ratio to determine the next test. Based on this document and recommendations from the Mayo Medical Laboratories, all positive anti-HCV screens (regardless of the signal to cut-off ratio) will contain a comment, recommending follow-up testing for HCV-RNA by RT-PCR. The ordering physician is responsible for follow up with appropriate additional tests or treatment if necessary. A testing algorithm is provided below (figure 4) and is adapted from the CDC document and the Mayo Clinic Interpretive Handbook. Table 1 summarizes the interpretation of HCV testing.

Vincent C. Smith MD

References:
Alter MJ et al. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. MMWR. February 7, 2003, 52, p 1-16.
Mayo Medical Laboratories Interpretive Handbook and Test Catalog. 2005
Mayo Reference Services Communique Test Update. Feb. 2005 p 13-15.
Kumar V. Pathologic Basis of Disease. ElSevier, Philadelphia, Seventh Edition, 2005, p 894-5.
www.cdc.gov/ncidod/diseases/hepatitis/c/

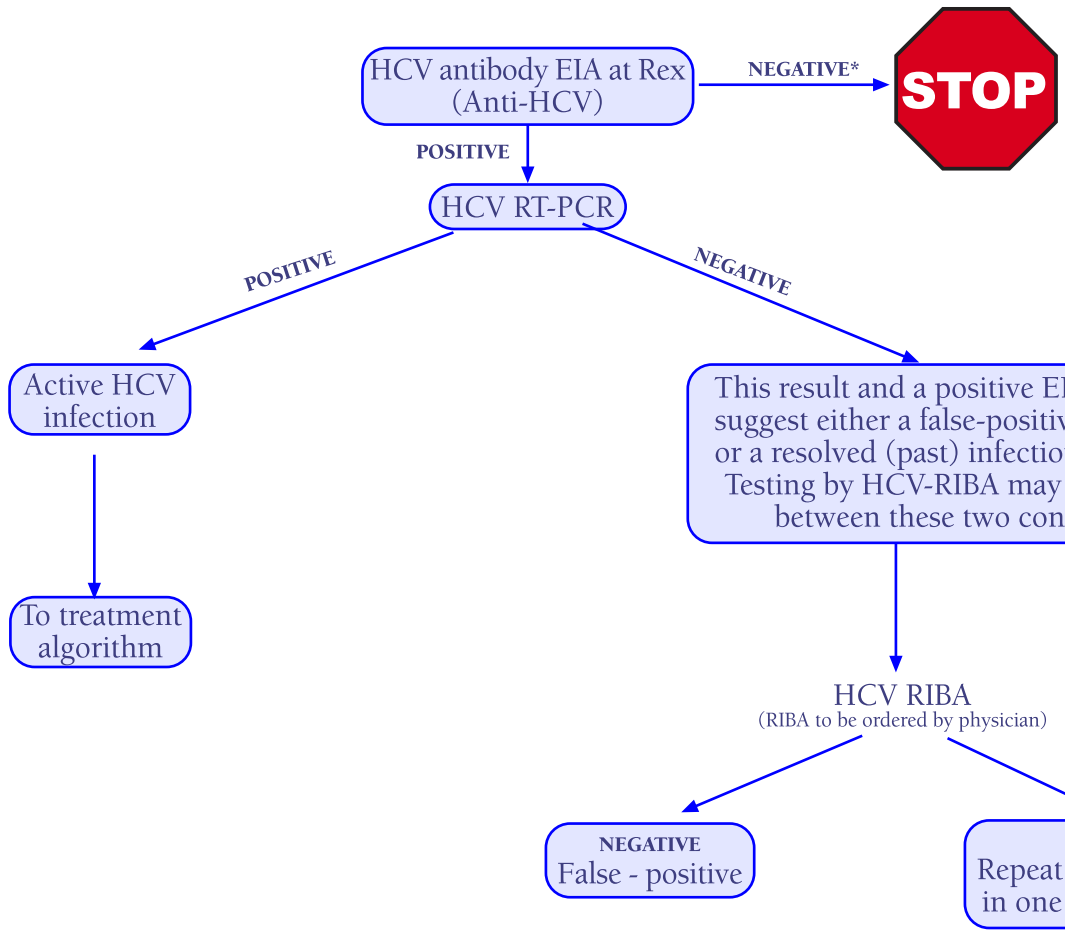

Figure 4

**Mayo Medical Laboratories recommends beginning with HCV PCR for recently exposed patients and the immunocompromised.*

Table 1: HCV test result interpretation (adapted from CDC and Mayo Medical Laboratories)

Anti-HCV ELISA	HCV RNA by PCR *	Anti HCV RIBA	Presence of anti HCV	HCV Infection	Next step
Negative	Not Done	Not Done	Negative	None	None
Positive	Positive	Not Done	Positive	Current	Treatment algorithm
Positive	Negative	Negative	Negative	None	None (false positive ELISA)
Positive	Negative	Not done	Possible	None or past infection	Consider RIBA testing. If negative then false pos. ELISA, if positive then past infection and Consider repeat PCR in 1-3 months.
Positive	Negative	Positive	Positive	Resolved	Consider repeat PCR in 1-3 months.

** A single negative HCV RNA cannot rule out intermittent viremia.*
 # Mayo Medical Laboratories recommends beginning with HCV PCR for recently exposed patients and the immunocompromised.

REX Laboratory Bulletin 4

Lyme Disease Lab Test Alert

The Centers for Disease Control (CDC) and Food and Drug Administration (FDA) recently issued an alert regarding commercial laboratories using tests for Lyme disease that are of dubious quality.[1] These tests include urine antigen tests, immunofluorescence staining for cell wall-deficient forms of *Borrelia burgdorferi* and lymphocyte transformation assays. They also note that some laboratories are performing polymerase chain reaction (PCR) on inappropriate specimens, such as blood or urine. In addition, some laboratories use unvalidated criteria for Western blot interpretation.

Photo provided by Dr. Robert D. Gilmore, CDC. Borrelia burgdorferi is the bacterium that causes Lyme disease.

Laboratory evaluation for Lyme disease should follow after careful evaluation of the patient's clinical illness and risk for exposure to infected ticks as both false positive and false negative results are common.[2-5] Only validated tests should be used. The FDA has approved 70 serologic assays to assist in the diagnosis of Lyme disease. Initial testing with an enzyme immunoassay (EIA) or immunofluorescent assay (IFA) is recommended. IgM antibodies peak three - six weeks after infection, while IgG antibodies are detectable several weeks after infection and may persist for years. Specimens that are EIA/IFA (+) should be referred for Western blot (WB) confirmation. False positive EIA/IFA results may due cross-reactivity observed with to other infections (Epstein-Barr virus, HIV, syphilis), autoimmune disease (rheumatoid arthritis, scleroderma, Sjögren's syndrome, SLE), chronic nephritis, or indigenous microflora.[2] A positive Western blot provides serologic support for past or current infection, but clinical correlation is necessary. If negative results are obtained on a specimen collected within the first two weeks of symptom development, and there is a strong clinical suspicion of Lyme disease, repeat testing on a second specimen collected two - four weeks after the first specimen is recommended.

Rex Laboratory uses a sensitive EIA capable of detecting either IgM or IgG antibodies to Lyme disease. Positive specimens are reflexed to Mayo Medical Laboratories for WB evaluation.

John D. Benson, MD

References

1. *Centers for Disease Control. Notice to readers: Caution regarding testing for Lyme disease. MMWR 54:125, 2005.*
2. *Tierno PM, Cadet-Legros J. Methods comparison for diagnosis of Lyme disease. Lab Med 27:542-6, 1996.*
3. *Duffy J et al. Diagnosing Lyme disease: the contribution of serologic testing. Mayo Clin Proc 63:1116-21, 1988.*
4. *Steere AC et al. The overdiagnosis of Lyme disease. JAMA 269:1812-6, 1998.*
5. *Fix AD et al. Tick bites and Lyme disease in an endemic setting. Problematic use of serologic testing and prophylactic antibiotic therapy. JAMA 279:206-10, 1998.*
6. *Kleeman K. Diagnosing Lyme disease using serology. REX Healthcare Laboratory Bulletin Issue 23, August 1997.*

New Laboratory Director - Brian D. Smith

We are pleased to welcome Brian D. Smith as the new Laboratory Director beginning September 26, 2005. Brian comes to us after serving as the Administrator of Rex Nursing Care Center of Apex since 2000. He has an A.B. in Biology and a Masters in Healthcare Administration from UNC-CH. He is a member of the NC Board of Examiners for Nursing Home Administrators, the Board of Directors for the NC Health Care Facilities Association, and served as a delegate to the American Health Care Association in 2004. His interests include sports, travel and automobiles. Brian will assume responsibility for the day-to-day operations of the Laboratory and look to expand outreach services. We are grateful to Elaine Patterson MT(ASCP) for serving admirably as interim Laboratory Director, in addition to her duties as Core Lab Manager, during the search period. Dr. Tim Carter will continue to serve as Medical Director.

Rex Healthcare Laboratory (919) 784-3040. Telephone extensions are: Pathologists' Direct Line (3063), Robin Ivosic (Customer Service and Outreach Manager 3053), Elaine Patterson (Core Lab, Blood Bank and Microbiology Manager 3054), Jackie Okoth (2nd and 3rd Shift Manager 4248), Diane Young (Anatomic Pathology Manager 3888).

Client Response Center (919) 784-6000 (phone) (919) 784-6299 (fax)