

Lab Updates

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May 2010

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Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus (HCV)

The HCV is a major public health problem and a leading cause of chronic liver disease. Hepatitis C is the principal cause of death from liver disease and the leading indication for liver transplantation in the USA. Some studies suggest that mortality revealed to HCV infection will continue to increase over the next two decades.

The optimal approach to detecting HCV infection is to screen persons for history of risk exposure to the virus and to test selected individuals who have identifiable risk factors, including:

- Persons who have ever injected illegal drugs, including those who injected only once many years ago
- Recipients of clotting factor concentrates made before 1987
- Recipients of blood transfusions or solid organ transplants before July 1992
- Patients who have ever received long-term hemodialysis treatment
- Persons with known exposures to HCV, such as
- Healthcare workers after needle sticks involving HCV-positive blood
- Recipients of blood or organs from a donor who later tested HCV-positive



- All persons with HIV infection
- Patients with signs or symptoms of liver disease (e.g., abnormal liver enzyme tests)
- Children born to HCV-positive mothers (to avoid detecting maternal antibody, these children should not be tested before age 18 months)



Photo: Kevin Vance

UMass Memorial Medical Center Laboratories

One Biotech Park, Suite 200
365 Plantation Street Worcester, MA 01605-2376
800-476-4431 or 508-334-2863 FAX: 508-334-4210
Email: LabsCS@ummhc.org

L. Michael Snyder, M.D.
*Chairman, Dept. of Hospital Laboratories
Professor of Medicine and Pathology
University of Massachusetts Medical School*

Guy M. Vallaro, Ph.D.
*Vice President, Lab Operations
Medical Center customer liaison*

Betsy Harder
*Sr. Director, Lab Outreach Program
Non-Medical Center customer liaison*

In clinical laboratories, two classes of assays are used in the diagnosis and management of HCV infection: serological assays that detect specific antibody to HCV and molecular assays that detect viral nucleic acid. At UMMHC laboratories, antibodies to HCV are detected in the serum using highly specific (99%) FDA approved chemiluminescent immunoassay (CIA).

False-positive anti-HCV results are rare in certain clinical settings, because the majority of persons being tested have evidence of liver disease and the sensitivity and specificity of the screening assays are high. However, among populations with a low prevalence of HCV infection, false-positive results do occur. This is of concern when testing is performed on asymptomatic persons for whom no clinical information is available, when persons are being tested for HCV infection for the first time, and when testing is being used to determine the need for post exposure follow-up.

CDC has recommended that a person be considered to have serologic evidence of HCV infection only after an anti-HCV screening-test-positive result has been verified by a more specific serologic test (e.g., RIBA) or a nucleic acid test (NAT). This more specific, supplemental testing is necessary, particularly in populations with a lower prevalence of disease, to identify and exclude false positive screening test results.

To facilitate practice of reflex supplemental testing, the recommended anti-HCV testing algorithm has been expanded to include an option that uses the signal-to-cut-off (s/co) ratios of screening-test-positive results to minimize the number of specimens that require supplemental testing and provide a result that has a high probability of reflecting the person's true antibody status.

The relationship between s/co ratios and a confirmatory RIBA test results was evaluated by FDA and for specimens that were screening-test-positive by CIA from two groups; one group with a low prevalence (range 2 -5% -persons in the general population, health care workers, and consecutive CIA anti-HCV positives), and one with a high prevalence (range $\geq 12.5\%$ -hemophiliacs, dialysis patients, persons identified with high risk sexual behavior, patients with signs and symptoms, transfusion/transplant recipients and injection drug users). For both groups, an s/co ratio of ≥ 11.0 predicted RIBA positivity in 97% or more of the screening-test-positive samples. These results indicate that for the FDA-approved CIA, reflex supplemental testing of screening-test-positive samples can be limited to those with s/co ratios < 11 . When screening test positive results with s/co ratios ≥ 11 can be reported without confirmation with explanatory comments.



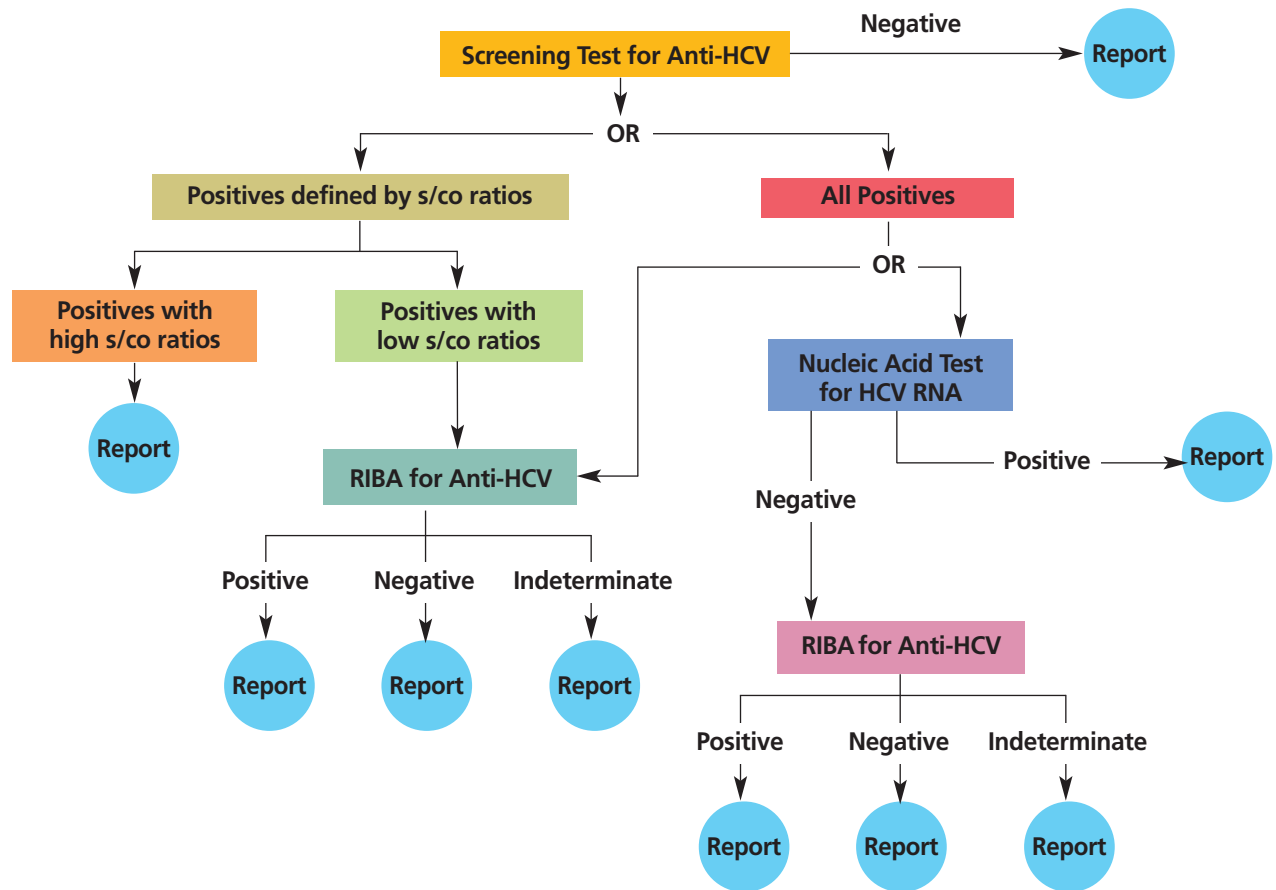
Implementation of these recommendations will provide more reliable results for physicians and their patients, so that further counseling and clinical evaluation are limited to those confirmed to have been infected with HCV. This is especially critical for persons being tested for HCV infection for the first time, for persons being tested in non-clinical settings, and for those being tested to determine the need for medical referral. Implementation of these recommendations also will improve public health surveillance systems.

Effective May 10, 2010

UMass Memorial Laboratories will implement the following CDC recommendations on all positive HCV antibody test results:

- All Positive HCV antibody test results with S/CO ratio's < 11 will be flagged as "**Low Positive**" and will be automatically reflexed to a confirmatory RIBA testing.
- All Positive HCV antibody test results with S/CO ratio's ≥ 11 will be flagged as "**High Positive**" and will be released with a canned comment suggesting follow up with nucleic acid testing (NAT).

Laboratory Algorithm for Antibody Testing for HCV Antibody Testing



Recommendations for Reporting Results of Testing for HCV Antibody by Type of Reflex Supplemental Testing Performed

Anti-HCV Screening Test Results	Supplemental Test Results	Interpretation	Comments
Screening-test-negative	Not applicable	Anti-HCV-negative	Not infected with HCV, unless recent infection is suspected or other evidence exists to indicate HCV infection
Screening-test-positive with high signal to-cut-off (s/co) ratio	Not done	Anti-HCV-positive	Probably indicates past or present HCV infection: supplemental serologic testing not performed. Samples with high s/co ratios usually ($\leq 95\%$) confirm positive, but <5 of every 100 might represent false-positives: more specific testing can be requested, if indicated
Screening-test-positive	Recombinant immunoblot assay (RIBA®)-positive	Anti-HCV-positive	Indicates past or present HCV infection
Screening-test-positive	RIBA-negative	Anti-HCV-negative	Not infected with HCV, unless recent infection is suspected or other evidence exists to indicate HCV infection
Screening-test-positive	RIBA-indeterminate	Anti-HCV-indeterminate	HCV antibody and infection status cannot be determined: another sample should be collected for repeat anti-HCV testing (>1 month) or for HCV RNA testing
Screening-test-positive	Nucleic acid test (NAT)-positive	Anti-HCV-positive, HCV RNA-positive	Indicates active HCV infection
Screening-test-positive	NAT-negative RIBA-positive	Anti-HCV-positive, HCV RNA-negative	The presence of anti-HCV indicates past or present HCV infection: a single negative HCV RNA result does not rule out active infection
Screening-test-positive	NAT-negative RIBA-negative	Anti-HCV-negative, HCV RNA-negative	Not infected with HCV
Screening-test-positive	NAT-negative RIBA-indeterminate	Anti-HCV-indeterminate-HCV RNA-negative	Screening test anti-HCV result probably a false positive, which indicates no HCV infection

References:

1. "Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C virus". MMWR (CDC) 2003; 52: RR-3. <http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5203a1.htm>
2. "AASLD Practice guidelines: Diagnosis, Management, and Treatment of Hepatitis C: An Update". *Hepatology*, April 2009; 1335-1374.

If you have questions, comments or suggestions, please contact:

Dr. L.V. Rao, Director of Core Laboratories at 774-442-9615 or via email at Lokinendi.Rao@umassmemorial.org

Dr. M. Rabie Al-Turkmani, Associate Director of Immunology & Immunoassay at 774-442-9663 or via email at MRabie.Alturkmani@umassmemorial.org

Ms. Rachel Ambacher, Manager of Immunology & Immunoassay at 774-442-9065 or via email at Rachel.Ambacher@umassmemorial.org

Specimen Handling: *The First Step Towards Excellent Results*

Each specimen that is sent to a laboratory should be sent at the proper temperature and in the correct conditions to ensure the best patient test results.

When bagging specimens for transport to the laboratory, each specimen type should be packaged according to temperature requirements; frozen, room temperature, and refrigerated. Please do not put frozen specimens in the large barcoded bag with room temperature or refrigerated specimens.

► Preparing Frozen Specimens

Centrifuge the specimen and remove the serum or plasma and place in a properly labeled aliquot tube. Place the tube and requisition into the blue frozen specimen bag and put in your on-site freezer.

Please prepare and send a separate frozen specimen for each STAT test.

Just before the courier arrives, package all frozen specimens together and place in a large barcoded specimen transport bag.

► Preparing Refrigerated Specimens

Centrifuge specimen and remove aliquots if necessary. Place specimens and requisitions into separate specimen bags by patient. Be sure that your centrifuged tubes accompany the other tubes to the laboratory.

Just before the courier arrives, package all refrigerated specimens together and place in a large barcoded specimen transport bag.

► Preparing Room Temperature Specimens

Centrifuge specimens and remove aliquots if necessary. Place room temperature specimens into a bag with the requisition.

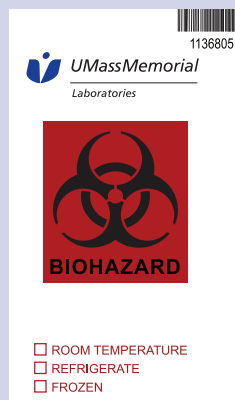
Just before the courier arrives, package all room temperature specimens together and place in a large barcoded specimen transport bag.

If you have any questions about specimen requirements and handling you can visit our online Laboratory Manual at <http://www.umassmemoriallabs.org/LabManual2.asp> or contact Customer Service at 800-476-4431

Use for Shipping ALL Specimens

Please use a separate bag for each storage condition.

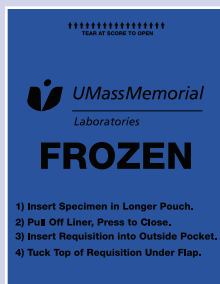
Please check appropriate box.



Use for ALL STAT Specimens



Use for ALL FROZEN Specimens



Use for ALL ROOM TEMPERATURE & REFRIGERATED Specimens

Please check appropriate box.



JAK2 Exons 12 and 13 Sequencing Testing:

Reflex for Negative JAK2 V617F Mutation Analysis in Myeloproliferative Disorders

Beginning 05/12/10, an additional molecular assay for the Janus Kinase 2 (*JAK2*) gene will be available through the UMass Memorial Molecular Diagnostic Laboratory. This sequencing assay of exons 12 and 13 will be performed as a reflex in samples from patients with clinically suspected myeloproliferative disorders (MPDs) who have previously tested negative for the common *JAK2* V617F mutation.

Background:

Approximately 90% of patients with polycythemia vera (PV), 50% of patients with primary myelofibrosis (PMF), and 50% of patients with essential thrombocythemia (ET) are positive for the *JAK2* V617F mutation. The absence of this mutation does not rule out a diagnosis of an MPD. Recent studies indicate that mutations other than V617F in the *JAK2* gene may be responsible for the presence of an MPD in a subset of patients.

The 2008 revision of the WHO diagnostic algorithms for PV, PMF, and ET states that analysis for *JAK2* V617F should be performed in patients with a suspected diagnosis of one of these MPDs. If that mutation is absent, testing should be undertaken for other relevant genetic abnormalities, such as those in *JAK2* exons 12 and 13, which may provide proof of clonality of the proliferative process.

Intended Use:

This *JAK2* sequencing test is intended for use as an aid in the diagnosis of PV, PMF, and ET in patients who have previously tested negative for the *JAK2* V617F mutation. In all cases being evaluated for *JAK2* mutation status, the V617F mutation should be tested for initially. *JAK2* sequencing should follow in cases where V617F mutation is absent as the lack of the *JAK2* V617F mutation does not exclude the presence of a myeloproliferative disorder.

Methodology:

Polymerase chain reaction amplification followed by sequence analysis using capillary electrophoresis.

Limitations:

Positive mutation status is highly suggestive of a diagnosis of a myeloproliferative disorder. However, positive mutation status is not specific to a particular diagnosis; therefore, correlation with other clinical and laboratory findings is required in all cases for a definitive diagnosis. A negative result does not exclude the presence of a myeloproliferative disorder. This assay will not detect mutations in other exons of the *JAK2* gene or in other genes. Sequencing may not detect large rearrangements such as duplications or deletions.



Requirements:

The UMass Memorial Molecular Diagnostics Test Requisition should be used and sent with the sample. Copies of this requisition may be obtained from Customer Service at 800-476-4431. Specimen, blood (3 ml) or bone marrow (minimum 1 ml), should be sent in a lavender top (sodium EDTA) tube at room temperature or refrigerated, not frozen. Green top (heparin) tube is acceptable.

References:

1. Kilpivaara O and Levine RL. JAK1 and MPL mutations in myeloproliferative neoplasms: discovery and science. *Leukemia* (2008) 22:1813-1817.
2. Ma W, Kantajarian H, Zhang X, et al. Mutation profile of *JAK2* transcripts in patients with chronic myeloproliferative neoplasms. *J Mol Diag* (2009) 11(1): 49-53.
3. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* (2009) 114(5): 937-951.
4. Wadleigh M and Tefferi A. Classification and diagnosis of myeloproliferative neoplasms according to the 2008 World Health Organization criteria. *Int J Hematol* (2010) 91:174-179.

If you have questions, comments or suggestions, please contact:

Dr. Edward Ginns at 508-856-8134, or via email at Edward.Ginns@umassmed.edu

Dr. Marzena Galdzicka at 508-856-4384 or via email at Marzena.Galdzicka@umassmed.edu

UMass Memorial
Medical Center Laboratories
One Biotech Park, Suite 200
365 Plantation Street
Worcester, MA 01605-2376

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We are one of the largest laboratory providers in New England

UMass Memorial Laboratories has opened a Patient Service Center (phlebotomy draw station) at 1180 Beacon Street, Brookline, Massachusetts.

The vision of UMass Memorial Laboratories is:

- To be a leading provider of laboratory services throughout New England, meeting the needs of patients and providers in the region, and
- To be one of the top ten academic medical center-based laboratories in the United States



Brookline PSC **1180 Beacon Street** **Brookline, Massachusetts**

Brookline PSC is located at 1180 Beacon Street, Brookline, MA. The hours are Monday through Friday 8:00am-5:00pm, closed 12:15pm-1:15pm. The phone number at Brookline PSC is 617-232-1910.