NEW BETA-HYDROXYBUTYRATE ASSAY
Annu Khajuria, PhD; Chemistry 24 Hour Services

Effective May 12, 2014, a new beta-hydroxybutyrate assay will be used at Marshfield Labs.

BACKGROUND

The American Diabetes Association recommends beta-hydroxybutyrate (BOH) as the preferred method for diagnosing and monitoring the treatment of diabetic ketoacidosis.

BOH is the predominant ketone body in the blood. It is the most sensitive marker for detecting ketosis. Ketone bodies are catabolic products of free fatty acids. Beta-hydroxybutyrate accounts for 78% of ketone bodies in the blood; the other two are acetoacetate (20%) and acetone (2%).

BOH is increased in alcoholic ketoacidosis, lactic acidosis (shock, renal failure), liver disease, infections, and salicylate poisoning. BOH has been shown to be better than urinary ketones in managing seizure reduction in patients on a ketogenic diet with refractory epilepsy.

METHOD

The new assay is an enzymatic quantitation by beta-hydroxybutyrate dehydrogenase. Laboratory method evaluation has shown improved precision and accuracy in comparison to the current assay. Reference intervals have been evaluated for the new beta-hydroxybutyrate assay in normal subjects.
## Test Information

**Test Name:**
Beta-Hydroxybutyrate

**Test Code:**
BOH

**Specimen Requirements:**
- **Fasting Required:** No
- **Specimen Type:** Plasma
- **Container/Tube:** Lithium heparin

**Reference Values:**
Adult & Pediatric: \( \leq 0.3 \text{ mmol/L} \)

## Questions
For more test information refer to the online Marshfield Labs Test Reference Manual.

For clinical & technical information contact:
- **Clinical questions:** Annu Khajuria, PhD, Chemistry - 24 Hour Services, at 1-6311 or 715-221-6311.
- **Technical questions:** Bryan Robeson, Chemistry - 24 Hour Services, at 1-6334 or 715-221-6334.

## References

## New Liver-Specific Antinuclear Antibody Indirect Immunofluorescence Assay

**Joyce J. Flanagan, PhD, Clinical Chemist; Jeffrey M. Resnick, MD, Pathologist**

Effective May 21, 2014, an antinuclear antibody (ANA), liver-specific, immunofluorescence assay (IFA), (test code: ANALIV) will be available. The initial titer of this ANA IFA starts at 1:40 and is used as one of the scoring criteria to aid in the diagnosis of liver-specific autoimmune diseases such as autoimmune hepatitis (AIH). Titer and pattern will be reported for positives. Regular ANA IFA for the evaluation of connective tissue-related diseases (test code: ANA) starting titer remains at 1:80.

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QUESTIONS
For more test information refer to the online Marshfield Labs Test Reference Manual as of 5/21/2014.

Interpretive questions: Jeffrey M. Resnick, MD, Pathologist, at ext. 1-6112 or 715-221-6112.
Technical contact: Joyce Flanagan, PhD, Immunodiagnostics, at ext. 1-6310 or 715-221-6310.
Assistant manager: Greg Simon, Immunodiagnostics, at ext. 1-6343 or 715-221-6343.

REFERENCE

CHANGES IN CELIAC DISEASE SCREENING PANELS
Joyce L. Flanagan, PhD, Clinical Chemist; Jeffrey M. Resnick, MD, Pathologist

Effective June 1st, 2014, tests performed in the Celiac Screening panels, Celiac Panel, Adult (ACELPAN) and Celiac Panel, Pediatric (PCELPAN), will be updated per 2013 American College of Gastroenterologists practice guidelines.

SEROLOGIC SCREENING FOR CELIAC DISEASE
Maintaining a gluten-containing diet prior to testing is recommended in order to minimize the risk of false-negative lab results.

There is no methodology change in the celiac disease-specific serology tests, and all tests may be ordered separately.

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**HOW TO ORDER**

<table>
<thead>
<tr>
<th>Test Description</th>
<th>Order Code</th>
<th>CPT code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celiac panel for children age 2 years or under</td>
<td>PCELPAN</td>
<td>IGA:82784, TTG IGA:83516, DGP IGA:83516, DGP IGG:83516</td>
</tr>
<tr>
<td>Celiac panel for patients older than 2 years</td>
<td>ACELPAN</td>
<td>Initial IGA:82784, TTG IGA:83516, Reflex TTG IGG:83516, DGP IGG:83516</td>
</tr>
<tr>
<td>Tissue Transglutaminase IgA</td>
<td>TTG-IGA</td>
<td>83516</td>
</tr>
<tr>
<td>Tissue Transglutaminase IgG</td>
<td>TTG-IGG</td>
<td>83516</td>
</tr>
<tr>
<td>Deamidated Gliadin IgA</td>
<td>GLIGA</td>
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</tr>
<tr>
<td>Deamidated Gliadin IgG</td>
<td>GLIGG</td>
<td>83516</td>
</tr>
</tbody>
</table>

**QUESTIONS**

For more test information refer to the online Marshfield Labs Test Reference Manual as of 6/1/2014.

Interpretive questions:  Jeffrey M. Resnick, MD, Pathologist, at ext. 1-6112 or 715-221-6112. Joyce Flanagan, PhD, Immunodiagnostics, at ext. 1-6310 or 715-221-6310. Assistant manager:  Greg Simon, Immunodiagnostics, at ext. 1-6343 or 715-221-6343.

**BACKGROUND**

Celiac disease (CD) is one of the most common causes of chronic malabsorption and remains underdiagnosed in the United States. Celiac disease can present with many symptoms, including typical gastrointestinal symptoms: diarrhea, steatorrhea, weight loss, bloating, flatulence, abdominal pain; and also nongastrointestinal abnormalities: abnormal serum liver-associated enzyme levels, iron deficiency anemia, bone disease, skin disorders, and many other protean manifestations. Some CD patients are asymptomatic. Celiac disease is usually detected by serologic testing (e.g., tissue transglutaminase IgA antibody). The diagnosis is confirmed by duodenal mucosal biopsies. Both serology and biopsy should be performed on patients whose diet has included gluten-containing foods.

The 2013 American College of Gastroenterologists (ACG) guidelines make recommendations based on the current literature and present a summary of the evidence supporting those recommendations.

**Recommendations of when to test for CD**

1. Patients with symptoms, signs, or laboratory evidence suggestive of malabsorption, such as chronic diarrhea with weight loss, steatorrhea, postprandial abdominal pain, and bloating, should be tested for CD. (Strong recommendation, high level of evidence)
2. Patients with symptoms, signs, or laboratory evidence for which CD is a treatable cause should be considered for testing for CD. (Strong recommendation, moderate level of evidence)
3. Patients with a first-degree family member who has a confirmed diagnosis of CD should be tested if they...
show possible signs or symptoms or laboratory evidence of CD. (Strong recommendation, high level of evidence)

4. Consider testing of asymptomatic relatives with a first-degree family member who has a confirmed diagnosis of CD. (Conditional recommendation, high level of evidence)

**Recommendations on diagnosis of CD**

1. Immunoglobulin A (IgA) anti-tissue transglutaminase (TTG) antibody is the preferred single test for detection of CD in individuals over the age of 2 years. (Strong recommendation, high level of evidence)

2. When there exists a high probability of CD wherein the possibility of IgA deficiency is considered, total IgA should be measured. An alternative approach is to include both IgA and IgG-based testing, such as IgG-deamidated gliadin peptides (DGPs), in these high-probability patients. (Strong recommendation, moderate level of evidence)

3. In patients in whom low IgA or selective IgA deficiency is identified, IgG-based testing (IgG DGPs and IgG TTG) should be performed. (Strong recommendation, moderate level of evidence)

4. If the suspicion of CD is high, intestinal biopsy should be pursued even if serologies are negative. (Strong recommendation, moderate level of evidence)

5. All diagnostic serologic testing should be done with patients on a gluten-containing diet. (Strong recommendation, high level of evidence)

6. Antibodies directed against native gliadin are not recommended for the primary detection of CD. (Strong recommendation, high level of evidence)

7. Combining several tests for CD in lieu of TTG IgA alone may marginally increase the sensitivity for CD but reduces specificity and therefore is not recommended in low-risk populations. (Conditional recommendation, moderate level of evidence)

8. When screening children younger than 2 years of age for CD, the IgA TTG test should be combined with DGP (IgA and IgG). (Strong recommendation, moderate level of evidence)

**Recommendation on the role of ancillary testing in CD**

1. HLA-DQ2/DQ8 testing should not be used routinely in the initial diagnosis of CD. (Strong recommendation, moderate level of evidence)

2. HLA-DQ2/DQ8 genotyping testing should be used to effectively rule out the disease in selected clinical situations. (Strong recommendation, moderate level of evidence). Examples of such clinical situations include but are not limited to:
   a. Equivocal small-bowel histological finding (Marsh I – II) in seronegative patients.
   b. Evaluation of patients on a gluten-free diet (GFD) in whom no testing for CD was done before GFD.
   c. Patients with discrepant celiac-specific serology and histology.
   d. Patients with suspicion of refractory CD where the original diagnosis of celiac remains in question.
   e. Patients with Down’s syndrome.

3. Capsule endoscopy should not be used for initial diagnosis, except for patients with positive-celiac specific serology who are unwilling or unable to undergo upper endoscopy with biopsy. (Strong recommendation, moderate level of evidence)

4. Capsule endoscopy should be considered for the evaluation of small-bowel mucosa in patients with complicated CD. (Strong recommendation, moderate level of evidence)

5. Intestinal permeability tests, D-xylose, and small-bowel follow-through are neither specific nor sensitive
and are not recommended for CD diagnosis. (Strong recommendation, moderate level of evidence)

6. Stool studies or salivary tests are neither validated nor recommended for use in the diagnosis of CD. (Strong recommendation, weak level of evidence)

References:

**NEW TESTING METHODS EXPAND THE REPORTABLE RANGE FOR HEPATITIS C VIRUS QUANTITATIVE ASSAY**

Timothy S. Uphoff, PhD, DABMG, Molecular Pathology Laboratory

Marshfield Labs is using a new Hepatitis C Viral Load (HCVQT) assay which replaces the previous method and expands the dynamic range for detection and quantitation of hepatitis C virus (HCV). The hepatitis C virus quantitative assay is highly sensitive and specific utilizing real time PCR technology to provide a wide linear analytic reporting range. The implementation of this new HCVQT test should be transparent to providers (the test name does not change) with the exception of two enhancements:

- The lower limit of detection and quantitation is now 15 IU/mL. This new lower limit of detection and quantitation enhances the assay’s utility at critical treatment decision points for newer direct acting antivirals such as boceprevir, telaprevir, sofosbuvir, and simeprevir. The new reportable range for the assay is 15-100,000,000 IU/mL. Note: the previous reportable range was 43-69,000,000 IU/mL.

- The serum requirement is reduced from 2.5 mL to1.5 mL.

**REPORTING**

The expanded linear reportable range for Hepatitis C Virus RNA, Quantitative (test code HCVQT) is 15-100,000,000 IU/mL.

- If no HCV was detected, the result is reported as “HCV RNA Not Detected”.
- If HCV was detected but the titer was less than 15 IU/mL, the result will be reported as “HCV RNA is detected, less than 15 IU/mL HCV RNA”.
- If the result is 15-100,000,000 IU/mL, the numerical result will be reported.
- Results greater than 100,000,000 IU/mL will be reported as “HCV RNA Detected, but was above the analytical limit of 100,000,000 IU/mL”.

**RECOMMENDED LABORATORY TESTING FOR HEPATITIS C VIRUS (HCV)**

Timothy S. Uphoff, PhD, DABMG, Molecular Pathology Laboratory

**HCV TESTING BASICS**

Four questions can typically be answered by HCV testing.

1. Has the patient been exposed to HCV?
   - Test with Hepatitis C Antibody Test (HCVAB)
2. Does the patient have an active HCV infection?
   - Test with HCV RNA Quantitative PCR (HCVQT)
3. What is the recommended therapy for patients with an active infection?
   - Test with HCV Genotyping (HCVGEN)
4. Is the current antiviral therapy effective?
   - Test with HCV RNA Quantitative PCR (HCVQT)

To establish if the patient has ever been exposed to HCV the first step is to determine their immune status against HCV. If a patient demonstrates an antibody response to HCV, the next step is to determine if they have an active infection. Since HCV culture is not routinely performed in clinical laboratories, active infections are identified by performing a quantitative viral RNA test to establish the viral titer. If an active infection is found, there are now a number of factors that will determine whether or not to treat the infection and what is the best therapeutic option. Some genotypes of HCV respond much more favorably to current therapies than others. HCV is highly variable genetically and there are six different HCV genotypes. Treatment options and duration vary depending on the infecting HCV genotype. Of the four most common genotypes in the U.S., genotype 1 accounts for about 77% of cases, genotype 2 for 14%, genotype 3 for 7%, and genotype 4 for 1%. HCV does not replicate clonally but as a quasispecies; even within an infected host there can be up to 2% nucleotide sequence variation among RNA genomes. Once treatment has been initiated, periodic testing for viral RNA levels is used to determine the effectiveness and duration of therapy.

Figure 1. CDC Recommended Testing Sequence for Identifying Current HCV Infection

* For persons who might have been exposed to HCV within the past 6 months, testing for HCV RNA or follow-up testing for HCV antibody should be performed. For persons who are immunocompromised, testing for HCV RNA should be performed.
† To differentiate past, resolved HCV infection from biologic false positivity for HCV antibody, testing with another HCV antibody assay can be considered. Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.
(Adapted from CDC, 2013. MMWR. 2013;62(18):362-365.)

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BACKGROUND

HCV is a Hepacivirus in the Flaviviridae family. Its positively stranded RNA genome is about 9,600 nucleotides in length and encodes a single large open-reading frame from which 11 proteins are derived. It is estimated that 1.6% of Americans have been infected with HCV and between 3 and 4 million of these are chronically infected. Worldwide, HCV affects an estimated 130 million to 150 million people and results in 350,000 to 500,000 deaths per year. Acute infections are often asymptomatic and chronic HCV infections may eventually lead to liver cirrhosis or carcinoma. Progressive liver damage from chronic HCV infection is a leading indication for liver transplants in the U.S.

Figure 2. Progression of Hepatitis C Infection to Hepatocellular Carcinoma

From: http://islaslab.wikispaces.com/Hepatitis+B+and+Liver+Cancer

Patients at highest risk for HCV include: IV drug users or those with a history of a needle stick injury with HCV infected blood, recipients of clotting factors made before 1987, hemodialysis patients, recipients of blood and/or solid organs before 1992, patients with undiagnosed liver problems, health care workers after possible exposure, and infants age 12-18 months born to HCV infected mothers (approximately 4 of every 100 infants born to HCV-infected mothers become infected with the virus). HCV testing is indicated for all of these high and intermediate risk patients. In August of 2012, the Centers for Disease Control (CDC) issued a recommendation that all persons born in the U.S. between 1945 and 1965 be screened for HCV infection.

NEW TREATMENT OPTIONS AND MANAGEMENT GUIDELINES

Historically, treatment of HCV infected individuals has been very difficult. The introduction of direct-acting agents against HCV in 2011 (boceprevir and telaprevir being first, later sofosbuvir and simeprevir) has rapidly

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changed the treatment of HCV and has improved the outlook for patients; however, the timely diagnosis of infection remains essential. The rapid evolution of HCV therapeutics has prompted the release of newly updated practice guidelines at a very frequent pace. In May of 2013, the CDC released Testing for HCV Infection: An Update of Guidance for Clinicians and Laboratorians (MMWR May 10, 2013 / 62(18);362-365 available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6218a5.htm). The American Association for the Study of Liver Diseases (AASLD), the Infectious Diseases Society of America (IDSA), and the International Antiviral Society-USA released their latest joint treatment recommendations for hepatitis C virus (HCV) infection in March of 2014 which are now available at www.hcvguidelines.org. The World Health Organization (WHO) issued their first guidance on the treatment of HCV in April 2014 and it is available at: http://apps.who.int/iris/bitstream/10665/111747/1/9789241548755_eng.pdf?ua=1.

Response-guided therapy (RGT) is part of all treatment guidelines for the new direct-acting agents. RGT involves the use of HCV viral load monitoring during treatment to guide treatment duration decisions and has become an important part of patient management protocols. Clinicians typically base treatment duration decisions on the viral genotype as well as the rate of change in RNA levels—i.e., shorter treatment if declining rapidly and achieving non-measurable levels at defined time points, or longer if declining slowly, with possible cessation of treatment if declining little or not at all. Despite the complexities that RGT can add to patient management, it represents a personalized approach that can help optimize treatment safety and outcomes while minimizing the duration of periods when patients suffer drug side effects.

HCV treatment decisions are further based on a number of other parameters including the patient’s health, likelihood of compliance, and liver function test results. Current treatment recommendations using the results of all patient information are beyond the scope of this document. We recommend the following references for further testing and treatment guidelines:

REFERENCES