

Volume 26  
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## The Serologic Approach to Diagnosing Celiac Disease

Mayo Medical Laboratories (MML) offers a new serologic test to aid in the diagnosis of celiac disease. The test, **Tissue Transglutaminase (tTG) IgA Antibodies, Serum #82587**, identifies the presence of IgA antibodies to tissue transglutaminase (tTG), which is produced in patients with untreated celiac disease.

### Celiac Disease

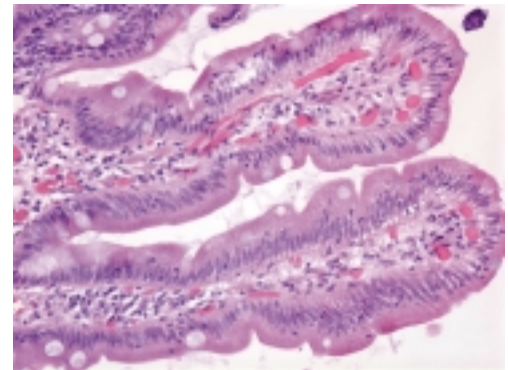
Celiac disease is an autoimmune digestive disease characterized by a permanent intolerance to gluten. When gluten is ingested, the immune system triggers an isolated inflammatory response in the small intestinal mucosa (the lining of the small intestine).

The disease may present at any age, from infant to adult. Infants present only after gluten containing products are added to their diet. It should also be noted that some people with celiac disease do not experience symptoms. Still, the disease causes damage to the small intestine, putting the patient at risk for complications of the disease. Symptoms may include one or more of the following:

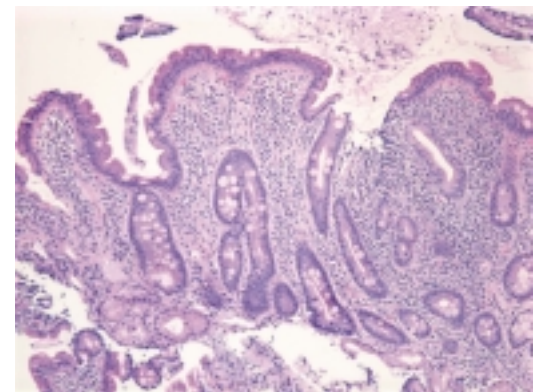
- fatigue due to anemia
- abdominal pain and recurring abdominal bloating
- intermittent diarrhea and/or foul-smelling, loose stools
- weight loss
- bone pain
- chronic nausea
- infertility or fetal loss
- dental anomalies
- in children and infants
  - failure to thrive
  - irritability

Normally, as digested food passes through the small intestine, nutrients are absorbed by the villi that line the walls of the small intestine

(see Image 1). The villi are atrophied in celiac disease (see Image 2) and characteristic inflammatory lesions appear in the mucosal lining of the small intestine. When the villi undergo this pathologic change, they can no longer properly absorb nutrients. The degree of malnourishment will depend on the extent of mucosal damage.



*Image 1 - Normal small intestine villi. Note the elongated shape of the villi, which increases the surface area for absorption.*



*Image 2 - Atrophied small intestine villi due to gluten intolerance. Note the flattened, wide villi, which results in reduced surface area for absorption and an increase in inflammatory cells in the lamina propria and surface layer.*

A lifetime gluten-free diet can completely stop the immune response. Once the patient is on a gluten-free diet, the small intestine begins to repair itself and the antibody levels decline and eventually disappear. However, reintroduction of gluten-containing products stimulates the immune response again. A significant reduction in morbidity and mortality occurs when patients adhere to the gluten-free diet.

## Epidemiology

Celiac disease can affect anyone. However, it is a genetically inherited disease and tends to occur in families of European descent.<sup>1</sup> Celiac disease is one of the most common diseases in Europe, with a prevalence of 1 in 250-300 people. It is so common in Italy that children are routinely screened by age 6 to minimize damage from the disease.

Considering that a large percentage of the United States population is descended from European ethnic groups, it is logical that the United States prevalence would be similar to European prevalence rates. But, historically, celiac disease has been thought to be less prevalent in the United States. This is may partially be a result of misidentification, due to lack of familiarity with the disease and its symptoms. In fact, a study of blood donors suggests that the prevalence is close to 1 in 250 in the United States.<sup>2</sup>

Family members of people with celiac disease or dermatitis herpetiformis are at increased risk of celiac disease. Other patients may be at increased risk if they have:

- type 1 diabetes mellitus
- thyroid disorders
- Down syndrome
- chronic diarrhea
- unexplained short stature
- infertility
- anemia
- lactose intolerance

## Diagnosis

In countries with high recognition of celiac disease, the diagnosis is typically made quickly. In Italy, for example, diagnosis is usually within 2 to 3 weeks of the onset of symptoms.<sup>3</sup> In the United States, the estimated time from first symptoms to diagnosis averages 10 years.<sup>3</sup>

Serological screening offers a minimally invasive option for rapid identification of those likely to have celiac disease and to select those who should be subjected to biopsy. Patients with celiac disease produce various autoantibodies, including endomysial (EMA), tissue transglutaminase (tTG), gliadin, and reticulin antibodies, as part of the immune response. IgA antibodies usually predominate although patients may also produce IgG autoantibodies.

When patients with IgA deficiency develop celiac disease, these patients only produce IgG autoantibodies. Therefore, as the tTG and EMA tests detect only IgA antibodies, these patients may not test positive in spite of active disease.

Biopsy demonstrating the characteristic small intestine lesion and clinical response to a gluten-free diet is the gold standard for confirming celiac disease. However, the invasive nature and cost of biopsy, and the timeliness and severity of diet changes, are problematic for many patients. Markedly positive (serologically) individuals are highly likely to have celiac disease and should undergo biopsy to confirm the diagnosis. For individuals who test negative, the physician may consider the possibility of IgA deficiency. If the patient is negative and IgA deficiency is not likely, there is a very low probability of the patient having celiac disease and biopsy would not be supported.

## Autoantibodies and Celiac Disease

**Endomysial antibodies (EMA) (#9360)** testing is highly specific (false positives are rare) and sensitive for the diagnosis of celiac disease. However, the test is labor intensive and requires microscopic interpretation of immunofluorescent staining. The subjective interpretation, high cost and limited availability of test components, and 2-day turnaround time make this test less desirable than tTG testing. The levels of these antibodies decline following institution of a gluten-free diet.

Some patient's serum may have strong anti-smooth muscle staining that can make endomysial results difficult to interpret; those cases are interpreted as "Indeterminate." In this situation, the serum is retained for 30 days. A measurement of total IgA may be helpful if no tissue staining at all is seen. If tTG testing is desired, the client must contact MML to order the tTG test, which will be performed at an additional charge on the stored specimen.

**Tissue transglutaminase (tTG) (#82587)** is the primary autoantigen recognized by endomysial antibodies in patients with celiac disease. The tTG test is set up daily, does not require subjective interpretation, is faster to perform, and less labor intensive than the EMA test. Therefore, a tTG enzyme-linked immunosorbent assay (ELISA) may be the most useful first level screening test for celiac disease. The levels of these antibodies decline following institution of a gluten-free diet.

False-positives are rare, but have been reported in patients with other autoimmune syndromes. For example, because the tTG antigen is derived from liver cells, false-positives may be seen in patients with autoimmune liver disease. For these patients, the EMA test is preferred.

**IgA and IgG gliadin antibodies (#81766)** are found in a high percentage of untreated patients with celiac disease. The levels of these antibodies decline following institution of a gluten-free diet. Measurement of anti-gliadin antibodies has a longer track record and has been well standardized as compared to current assays for anti-tTG and anti-endomysial antibodies. Gliadin antibodies may be as useful as the more specific antibody tests for monitoring adherence to a gluten-free diet. However, anti-gliadin antibodies are less specific than anti-tTG and anti-endomysial antibodies and may occur in other intestinal diseases.

**Reticulin antibodies** are no longer considered useful in the diagnosis of celiac disease, particularly compared to the utility of EMA and tTG tests, because they lack the sensitivity and specificity of the EMA and tTG tests.

### Serology Testing Strategy

It is very important that the patient be on a normal gluten-containing diet before testing unless the patient is known to have celiac disease. The presence of endomysial and/or tTG antibodies in a peripheral blood specimen, combined with clinical symptoms of celiac disease is very suggestive of celiac disease. However, these findings are considered inadequate to commit the patient to a diagnosis of celiac disease. Most clinicians believe that a positive biopsy is necessary before placing the patient on a lifelong treatment

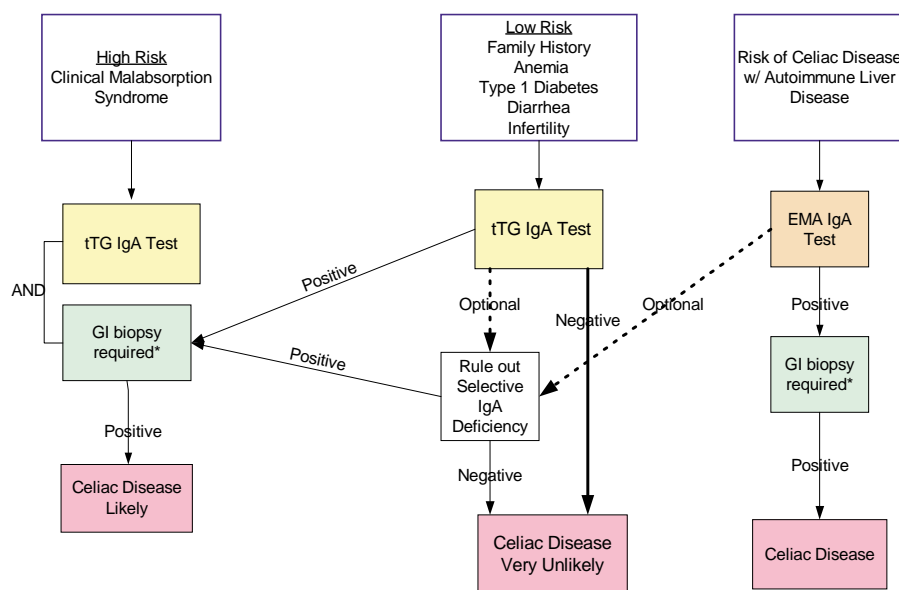
regimen. A suggested algorithm is shown in Figure 1. A negative tTG or EMA test does not rule out celiac disease. Therefore, IgA deficiency also should be considered for symptomatic patients. IgA deficiency is uncommon, but if serology tests are negative for celiac disease and the patient is symptomatic, a total IgA serum test should be performed to rule out IgA deficiency confounding the serologic diagnosis of celiac disease.

If serology is negative and/or there is substantial clinical doubt remaining, then further investigation should be performed with endoscopy and bowel biopsy. This is especially important in patients with frank malabsorptive symptoms since many syndromes can mimic celiac disease. For the patient with frank malabsorptive symptoms, bowel biopsy should be performed regardless of serologic test results.

### Patient Management

In the treatment of celiac disease, EMA, tTG, and gliadin antibody levels decrease with adherence to a gluten-free diet and with normalization of intestinal pathology. Therefore, compliance with dietary gluten avoidance can be monitored by tTG, EMA, or gliadin levels.

## Celiac Disease Algorithm



\*GI biopsy is required for a confirmed diagnosis of celiac disease

Figure 1 - This algorithm provides guidelines for the evaluation of the patient suspected of having celiac disease, as approached by Mayo gastroenterologist, Joseph A. Murray MD. Dr. Murray is a specialist in the diagnosis and treatment of celiac disease. This algorithm is designed to be used in conjunction with clinical judgment based on the clinical presentation of the patient.

## Dermatitis Herpetiformis

Patients with dermatitis herpetiformis (DH) also have an autoimmune response to gluten intake. DH presents as a severely pruritic rash with blistering skin. However, patients with DH develop the same intestinal damage as patients with celiac disease, but they may or may not have intestinal symptoms. Patients with DH usually are diagnosed by skin biopsy. A punch biopsy of perilesional skin from patients with DH will show IgA concentrated in dermal papillae and/or in a granular pattern.

Serologic tests for celiac disease may be used to confirm a diagnosis of DH and to monitor compliance with dietary gluten restriction. The antibody pattern in DH may be more variable than in celiac disease; therefore, both EMA and tTG antibody determinations are recommended to maximize the sensitivity of the serologic tests.

### Summary

Celiac disease is believed to be an underdiagnosed disease in the United States. Serology testing for tTG is highly sensitive and specific for celiac disease and may provide a rapid means of identifying those patients who should be biopsied.

For more information about the Immunodermatology Laboratory at Mayo Clinic, please visit our web site at <http://www.mayo.edu/lab-iderm>.

### References

1. Murray JA: The widening spectrum of celiac disease. *Am J Clin Nutr* 1999;69:354-65
2. National Digestive Diseases Information Clearinghouse: Celiac Disease. National Institutes of Health. NIH Publication No. 01-4269, April 1998, Updated: August 2001. Retrieved 6/14/01. Available from URL: <http://www.niddk.nih.gov/health/digest/pubs/ceciac>
3. Tidwell J: How is celiac disease diagnosed. About® The Human Internet™ Allergies. Retrieved 6/14/01. Available from URL: <http://allergies.about.com/library>

### Changes to Cortisol Testing

The Endocrine laboratory has converted cortisol testing to a high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Testing for urine cortisol was previously performed as **Cortisol, Free, Urine #8546** by an immunoassay chemiluminescent (ICMA) method. When there was a suspicion of interfering substances, **Cortisol, Free, Urine (By HPLC) #9367** was the recommended test. With the conversion to LC-MS/MS, **test #9367 has been discontinued.**

The new LC-MS/MS method uses less volume of urine specimen and also eliminates the effect of interfering substances including synthetic corticosteroids. Additionally, analysis of cortisone (not hydrocortisone), a downstream metabolite of cortisol has been added to the cortisol test and provides an additional information about the *in vivo* metabolism of cortisol to cortisone. **Cortisol, Urine #8546** will be performed Monday through Saturday and has a 1 day turnaround time.

#### New Test Name

Cortisol, Urine #8546

#### Previous Test Name

Cortisol, Free, Urine #8546

#### New Specimen Requirement

5 mL (pediatric: 3.0 mL) from a 24-hour collection. Add 10g of boric acid as preservative at start of collection. See "Urine Preservatives" in Special Instructions in *2001 Test Catalog* or MayoAccess for multiple collections. Send specimen *refrigerated* in a plastic, 13-mL urine tube.

**NOTE: 24-HOUR VOLUME IS REQUIRED ON REQUEST FORM FOR PROCESSING.**

#### Previous Specimen Requirement (abbreviated)

Cortisol, Free, Urine - 10 mL (pediatric: 5.0 mL) from a 24-hour collection, with boric acid preservative

Cortisol, Free, Urine (By HPLC) - 50 mL (pediatric: 20 mL) from a 24-hour collection, with boric acid preservative

#### New Reference Values

Pediatric: Not established

Adult:

Cortisol: 5-55 µg/24 hour

Cortisone: 16-128 µg/24 hour

#### Previous Reference Values

0-17 years: Reference values are not well established for children. Reported upper limit of normal is 91 µg/24 hours  
≥18 years: 24-108 µg/24 hours

#### New CPT Code

82530

83789

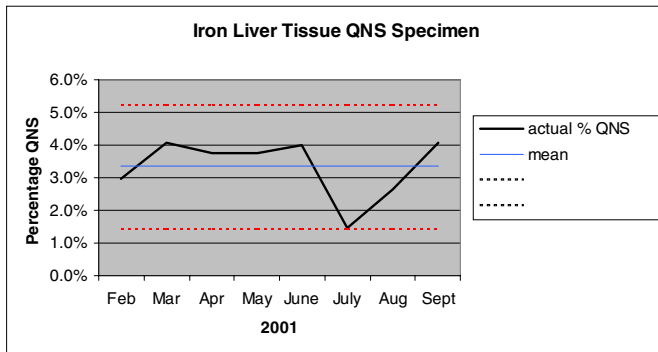
#### Previous CPT Code

82530

# Improving Test Success in Iron Liver Tissue Specimens

Quantitative tissue analyses for iron or copper are key laboratory tests in the diagnosis of hemochromatosis resulting from iron overload and hepatolenticular degeneration resulting from copper overload (Wilson's disease). Further, the hepatic iron concentration is considered the "gold standard" for diagnosis of hemochromatosis and can be indexed to correct for the natural, benign accumulation of iron in the liver with age.

Mayo's Metals Laboratory prefers fresh tissue for analysis, but will accept formalin fixed or paraffin blocks if fresh tissue is not available. Currently, over 3% of all specimens are of inadequate mass to perform the analysis and reports are subsequently generated stating: "Quantity Not Sufficient to Perform Analysis". Data collected and analyzed this year indicates a consistent rate of quantity not sufficient (QNS) specimens for [Iron, Liver Tissue \(#8350\)](#) and [Copper, Liver Tissue \(#8687\)](#). See graph below.



Due to the invasive nature of specimen collection, the Metals Laboratory has focused on ways to reduce the number of QNS specimens. Fresh tissue is the best specimen type for metals analysis. The vast majority of QNS cases occur when a paraffin block has been submitted. In general, if more than two cuts for slides have been taken, there is not enough tissue remaining for analysis. (See photos-paraffin block specimens.) Consequently, the Metals Laboratory strongly recommends a second biopsy be sent as fresh tissue in cases where a paraffin block is being submitted and iron or copper overload is highly suspected.

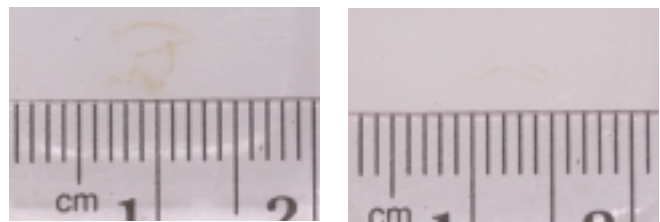
The following table provides the **minimum** specimen size requirements for tissue analysis.

Type of Biopsy	Minimum Specimen Size
18 gauge needle	0.3 x 10-20 mm (~1/2 inch in length)
14 gauge needle	1.0 x 5-10 mm (~1/4 inch in length)
Wedge biopsy	10 mg

Specimens that do not meet these minimum requirements cannot be analyzed for a variety of reasons. First, the weight is used in calculating the amount of iron or copper present and the possibility of introducing and multiplying error increases dramatically below the minimum specimen requirement. Second, any analysis is only as good as the quality of the sample. Interpretation of quantitative iron and copper is complicated by the presence of cirrhotic tissue, fibrosis, and fat. The laboratory has found that small pieces of biopsy specimens are often not representative of the metal distribution in the entire liver. Many times all that remains is a piece of fat, fibrotic tissue, a pocket of cirrhotic tissue, blood clot, or localized concentration of iron or copper. Finally, for specimens whose results do not correlate with clinical expectations, the laboratory will have insufficient specimen to redigest and reanalyze.

Careful attention to these guidelines can minimize the probability that your patient will be required to undergo a second biopsy for liver metals analysis. The laboratory is happy to assist with any questions or concerns regarding these tests. The laboratory can be contacted by calling 1-800-533-1710 and requesting the Metals Laboratory.

## Paraffin Block Specimens



Photos 1a and 1b. Inadequate Specimens. These blocks were used to make slides. The remaining specimen is completely inadequate for metals analysis

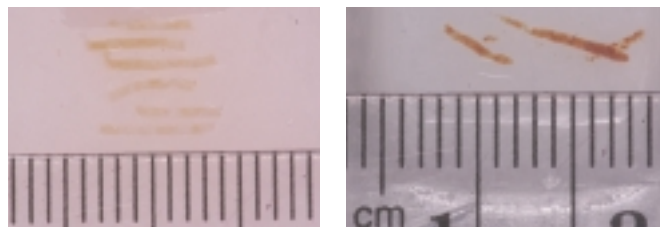


Photo 2. Optimal specimen including several segments from a punch biopsy.



Photo 3. Borderline specimen. While this specimen has the necessary length, there may be an inadequate volume, depending upon the amount of material that has been "sliced" off the block to make slides. The laboratory would attempt to perform metals analysis on this specimen.

### **Alpha-Fetoprotein Spinal Fluid Method Change**

**Alpha-Fetoprotein (AFP), Spinal Fluid #8876** has been converted from a microparticle enzyme immunoassay to a two-site immunoenzymatic sandwich assay. The methods are equivalent, showing good correlation and similar CV, linearity, and precision. The reference values remain the same. The new method has resulted in a change to the CPT code and a reduction in the specimen requirement. The test will be performed Monday through Saturday.

#### **New Specimen Requirement**

**0.5 mL** of spinal fluid. Send specimen frozen in plastic vial on dry ice.

#### **Previous Specimen Requirement**

1.0 mL of spinal fluid. Send specimen frozen in plastic vial on dry ice.

#### **New CPT Code**

86316

#### **Previous CPT Code**

82105

### **Alpha-Fetoprotein Tumor Marker Method Change**

**Alpha-Fetoprotein (AFP) Tumor Marker, Plasma or Serum #8162** has been converted from a microparticle enzyme immunoassay to a two-site immunoenzymatic sandwich assay. The methods are equivalent, showing good correlation and similar CV, linearity, and precision. The reference values remain the same. The new method has resulted in the addition of heparin as an acceptable anticoagulant for plasma specimens and a reduction in the volume of specimen required for either plasma or serum. The test will be performed Monday through Saturday.

#### **New Specimen Requirement**

##### **Plasma**

Draw blood in a green-top (heparin) tube(s) or a lavender-top (EDTA) tube(s). Spin down and send **0.5 mL** of heparinized or EDTA plasma *frozen* in plastic vial on dry ice.

NOTE: 1. Indicate plasma on request form.  
2. Label specimen appropriately (plasma).

##### **Serum**

Draw blood in a plain, red-top tube(s) or an SST tube(s). Spin down and send **0.5 mL** of serum *frozen* in plastic vial on dry ice.

NOTE: 1. Indicate serum on request form.  
2. Label specimen appropriately (serum).

#### **Previous Specimen Requirement (abbreviated)**

##### **Plasma**

Draw blood in a lavender-top (EDTA) tube(s). Spin down and send 1.0 mL of EDTA plasma *frozen* in plastic vial on dry ice.

##### **Serum**

Draw blood in a plain, red-top tube(s) or an SST tube(s). Spin down and send 1.0 mL of serum *frozen* in plastic vial on dry ice.

### **Hepatitis C Virus Quantitation by bDNA Changes**

Manufacturer modifications to the VERSANT™ HCV RNA 3.0 assay (bDNA) have resulted in changes to the dynamic range and the conversion factor for **Hepatitis C Virus (HCV) Quantitation by bDNA, Serum #81130**. This change to the dynamic range enhances the overall performance at the lower limit of quantitation and ensures a specificity of >95% across all kit lot numbers.

The new dynamic range is: 3,200 - 40,000,000 copies/mL  
or: 615 - 7,692,310 IU/mL

The new conversion factor is: multiply the IU/mL result by 5.2 for copies/mL.

### **Allergen Test Code Changes**

As of November 1st, all individual allergen test codes were changed to a five-digit number beginning with 82. This change was required to eliminate duplication in test codes. Panel tests were not affected by this change. A listing of the new test codes is available by ordering Mayo supply item T470 (allergen test code listing). New test request forms are available for those who order manually by requesting Mayo supply item T236 (request forms).

## **Meeting Calendar**

### **Interactive Satellite Program . . .**

**January 22, 2002**

Supplemental Newborn Screening by Tandem Mass Spectrometry

Presented by: Piero Rinaldo, M.D., Ph.D.,  
Dietrich Matern, M.D.

### **Upcoming Education Conferences . . .**

**May 1-3, 2002**

Integration Through Community Laboratory Insourcing:  
From Mission Statement to Successful Implementation

Hilton in the Walt Disney World®  
Lake Buena Vista, Florida

For additional information regarding the above programs,  
please contact the Mayo Reference Services Education Office  
at 1-800-533-1710.

## Abstracts of Interest

### Endoscopic Palliation of Malignant Dysphagia

Douglas G. Adler, MD, and Todd H. Baron, MD

Esophageal cancer is the primary cause of malignant dysphagia, a major cause of morbidity and mortality. In patients with esophageal cancer that is unresectable at the time of diagnosis, palliation is the major goal. Surgical treatment as well as radiation and chemoradiation therapy are traditional approaches for such patients. Endoscopic therapy is useful for patients with poor performance status, those in whom other treatments have failed, and those with tracheoesophageal fistulas. In recent years, self-expanding metal stents have become an important new endoscopic treatment modality for palliation of malignant dysphagia in a wide range of patients. Appropriate patient selection is paramount when a mode of palliation for malignant dysphagia is being selected. Although various treatment options exist for palliation of malignant dysphagia, comparative studies among these modalities are needed.

Mayo Clinic Proceedings 2001;76:731-738

Ask



US

**Q:** I am planning on sending a fresh tissue specimen for quantitative iron and copper, do I need to send the sample in saline?

**A:** No. In fact, the laboratory prefers the biopsy specimen be placed into a clean, plastic vial containing no liquid of any kind as the specimen must be oven dried early in the process for quantitative iron and copper determination.

**Q:** My iron result is a little confusing. The iron in  $\mu\text{g/g}$  is high, but the index is normal. What does this mean?

**A:** In 1995, MML introduced the Hepatic Iron Index, which relates iron concentration to age and is a useful measure of the progress of iron overload. The index value is included in test results from [Iron, Liver Tissue #8350](#).

The normal range established for iron in  $\mu\text{g/g}$  is for a general, healthy population. Since iron naturally accumulates in the liver with age, a concentration that is considered normal for a 60-year-old patient could be cause for alarm in a 20-year-old patient. In fact, in patients with hemochromatosis the rate of iron accumulation depends on the severity of the disease.

**Q:** What is the correct address for shipping specimens to MML for testing?

**A:** Mayo Medical Laboratories  
Stabile Building  
200 First Street SW  
Rochester, MN 55905

## Parvovirus Method Change

Parvovirus B19 Antibodies, IgG and IgM (Separate Determinations), Serum #80345 has been converted to an enzyme immunoassay (EIA). This change has resulted in changes to the useful for, interpretation, and caution statements for the test and the addition of "Equivocal" to the reference ranges.

### Useful For

Detection of antibody to parvovirus can be used for the diagnosis of recent infection (IgM) or for the assessment of past infection (eg, screening pregnant women) and immunity to this virus infection (IgG).

### Interpretation

Parvovirus B19 IgM Serology	Parvovirus B19 IgG Serology	Interpretation
Negative	Negative	Implies no past infection. Patient may be susceptible to B19V infection.
Negative	Positive	Implies past exposure/infection - minimal risk of B19V infection.
Equivocal	Positive/ Negative	May indicate current or recent B19V infection, retest in 1-2 weeks.
Positive	Positive	Implies current or recent B19V infection.
Positive	Negative/ Equivocal	May indicate current B19V infection, retest in 1-2 weeks.

### New Reference Value

IgG - Negative

IgM - Negative

Results will be reported as Positive, Negative, or Equivocal

### Previous Reference Value

IgG: negative

IgM: negative

The presence of IgM class antibodies indicates recent infection. The presence of IgG antibodies only is suggestive of past exposure.

### Cautions

- Specimens taken prior to seroconversion may yield negative IgM and IgG antibody results, while specimens taken after IgM antibody levels have begun to decline may yield negative IgM antibody results. The results of a single assay or combination of IgM and IgG enzyme immunoassay results specific for parvovirus, should not preclude additional testing, ie, follow-up specimens from the patient 1 to 4 weeks following the initial test.
- Test results of specimens from immunocompromised patients may be difficult to interpret. Testing should not be performed as a screening procedure for the general population.

# Communiqué

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## Mayo Reference Services Communiqué

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