

COMMUNIQUÉ

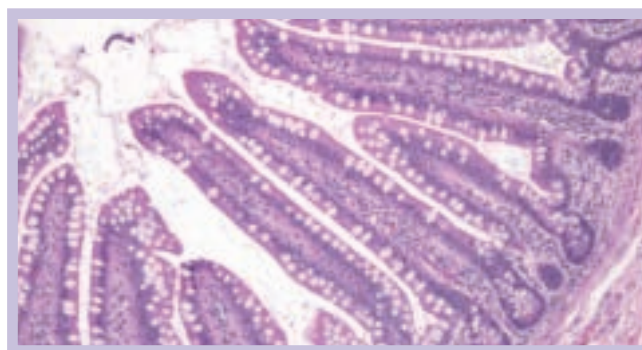
IMPROVING PATIENT CARE THROUGH ESOTERIC LABORATORY TESTING

JUNE 2008

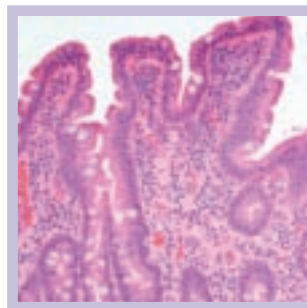
Update on Celiac Disease: New Standards and New Tests

The National Institutes of Health (NIH) has reported that as many as 1% (3,000,000) of all Americans have celiac disease and hundreds of thousands of these patients are undiagnosed.^{1,2} In 2006, the NIH launched an aggressive campaign to heighten physician awareness of the disease. With new diagnostic guidelines and new tests available, this effort should significantly reduce the time to diagnosis, which currently averages 10 years from the onset of symptoms.

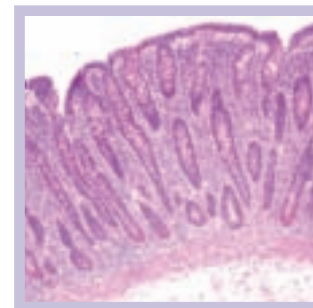
Celiac disease (also known as celiac sprue and gluten-sensitive enteropathy) is an autoimmune disease that affects the small intestine. The trigger, gluten, is a protein found in wheat, rye, and barley. Exposure to gluten triggers an isolated inflammatory response in the small intestinal mucosa, which interferes with the absorption of nutrients and leads to nutritional deficiencies and gastrointestinal and behavioral symptoms including villous atrophy (Photos). For symptomatic patients, celiac disease carries a significantly increased risk of mortality and morbidity.^{3,4} Early detection and treatment can decrease these risks.



Normal appearing small intestine (H&E stain)



Celiac disease: intraepithelial lymphocytosis, partial villous atrophy, crypt hyperplasia and chronic inflammation of the lamina propria



Celiac disease, total villous atrophy

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The only treatment for celiac disease is lifelong avoidance of gluten; even small amounts of gluten cause intestinal damage. Adherence to this diet is difficult, especially since food processing can add gluten to foods that would otherwise be considered safe for patients with celiac disease. For example, potatoes are a safe food but, depending on the processing used to create them, french fries may not be safe. More problematic for avoidance are the less well-known inclusions of gluten in some prescription or over-the-counter medicines and other commonly encountered products like stamp and envelope adhesive.² A diagnosis of celiac disease means a lifelong commitment to reading the labels of any product the patient will consume and avoiding those with gluten.

Dermatitis herpetiformis, a related disease, is an itchy, blistering skin disease that includes the same intestinal damage as celiac disease.⁵ As with celiac disease, dermatitis herpetiformis is a result of gluten intolerance. A gluten-free diet is essential to controlling both the skin and intestinal symptoms. Medication may be necessary to control the rash.

Epidemiology

While celiac disease occurs most commonly in those of European descent, anyone can be affected. Early detection is key to reducing the risk of malnutrition-associated complications in children. The high prevalence of celiac disease in children aged 2.5 to 15 years (estimates based on several studies put the prevalence in this population at 3 to 13 per 1000 children),⁶ makes physician awareness critical for early intervention.

As an inherited disease, the risks are higher for those with an affected family member; 4% to 15% of first-degree relatives also will have the disease.^{2,5} It also has been established that there is an increased prevalence of celiac disease in certain disease populations. Celiac disease is seen in 3% to 8% of type 1 diabetics, and 5% to 12% of people with Down syndrome.^{2,5} Approximately 3% to 5% of patients with celiac disease are IgA deficient, and 5% to 10% of patients with selective IgA deficiency have celiac disease. Celiac disease also has been associated with Turner syndrome, Williams syndrome, and other autoimmune disorders.⁵ Both celiac disease and dermatitis herpetiformis may be associated with thyroiditis.⁵

While rare, certain cancers such as enteropathy-associated T-cell lymphoma and adenocarcinoma of the small intestine are more common in patients with long-term celiac disease. It is also possible that there is an increased risk for carcinoma elsewhere in the gastrointestinal tract.⁵

Presentation

Celiac disease may present at any age and, as an autoimmune disorder, it affects multiple organ systems.⁵ There are no typical celiac disease-specific symptoms; most people with the disease have general complaints such as intermittent diarrhea, abdominal pain, and bloating. The signs and symptoms of celiac disease are highly varied and can be attributed to many other conditions and diseases such as irritable bowel syndrome, gastric ulcers, Crohns disease, parasite infections, anemia, skin disorders, or nervous conditions (see Table).^{2,5}

Childhood presentation is the most common, but some individuals remain asymptomatic (no gastrointestinal symptoms at all), even as adults. Even asymptomatic patients will experience villous atrophy. Latent disease, which is characterized by the absence of villous atrophy, can become active disease when the patient undergoes physical (surgery, pregnancy or childbirth, viral infections) or emotional stress.²

Laboratory Diagnosis of Celiac Disease

A presumptive diagnosis of celiac disease, according to the 2004 NIH consensus statement, is dependent upon demonstration of positive serology and characteristic biopsy results. Autoantibody testing offers the least invasive option for identifying those at risk of celiac disease and, therefore, those patients for whom biopsy should be performed. Once serology has been performed, it is possible to segregate patients into high-risk and low-risk groups based on their results. Those at high risk should have a small intestine biopsy performed. Those individuals at low risk may need to be studied for other diseases, but biopsy would not normally be justified for celiac disease diagnosis.

A definitive diagnosis of celiac disease is ultimately confirmed when symptoms resolve after institution of a gluten-free diet. While older diagnostic guidelines required demonstration of a reversal of histological changes in a second biopsy, the newer (2004)

Gastrointestinal	Nongastrointestinal
Anorexia	Failure to thrive in infants
Chronic diarrhea	Anemia
Bloating	Thyroid disorders
Vomiting	Chronic fatigue
Constipation	Unexplained short stature
Pale, foul-smelling stool	Depression
Lactose intolerance	Irritability
Abdominal pain	Seizures/neuropathy
Ulcers	Recurrent fetal loss/infertility
	Delayed menarche
	Early menopause
	Osteoporosis, osteopenia
	Oral sores
	Tooth discoloration or loss of enamel
	Vitamin deficiencies
	Myocarditis
	Hyposplenism
	Pulmonary hemosiderosis

Table. Signs and symptoms of celiac disease

guidelines no longer require the second biopsy for confirmation in most patients.⁵

The relatively recent introduction of DNA-based tests for HLA markers DQ2 and DQ8, which are associated with celiac disease susceptibility, improves the ability to identify those patients at risk for celiac disease. Nearly 100% of patients with celiac disease carry the HLA markers DQ2 or DQ8 (>90% are DQ2; <10% are DQ8).⁷ For this reason, it is possible to use HLA DQ typing to help segregate the patient population and virtually exclude celiac disease from the diagnosis in those who do not carry these HLA types. This test is available from Mayo Medical Laboratories as [#88906 Celiac-Associated HLA-DQ Alpha 1 and DQ Beta 1 High-Resolution DNA Typing, Blood](#). However, as up to 40% of the general population also carry these markers, HLA typing cannot be used as a screening test.⁵

In response to recent testing advances, Mayo Medical Laboratories has developed a new diagnostic testing algorithm for celiac disease (Figure 1). This algorithm identifies the tests (individually orderable) that are involved in the diagnosis. Because several of these tests require significant expertise to perform and interpret, Mayo Medical Laboratories will soon introduce several new testing options.

For a complete evaluation, including HLA DQ typing, Mayo Medical Laboratories has developed [#89201 Celiac Disease Comprehensive Cascade](#) (Figure 2). This test provides the fastest, most complete assessment that can be ordered, and can virtually rule out celiac disease in most patients. Mayo's Comprehensive Cascade begins with concurrent IgA serology testing and HLA DQ typing. The HLA DQ typing provides additional support for the final diagnosis, in the case of indeterminate serology

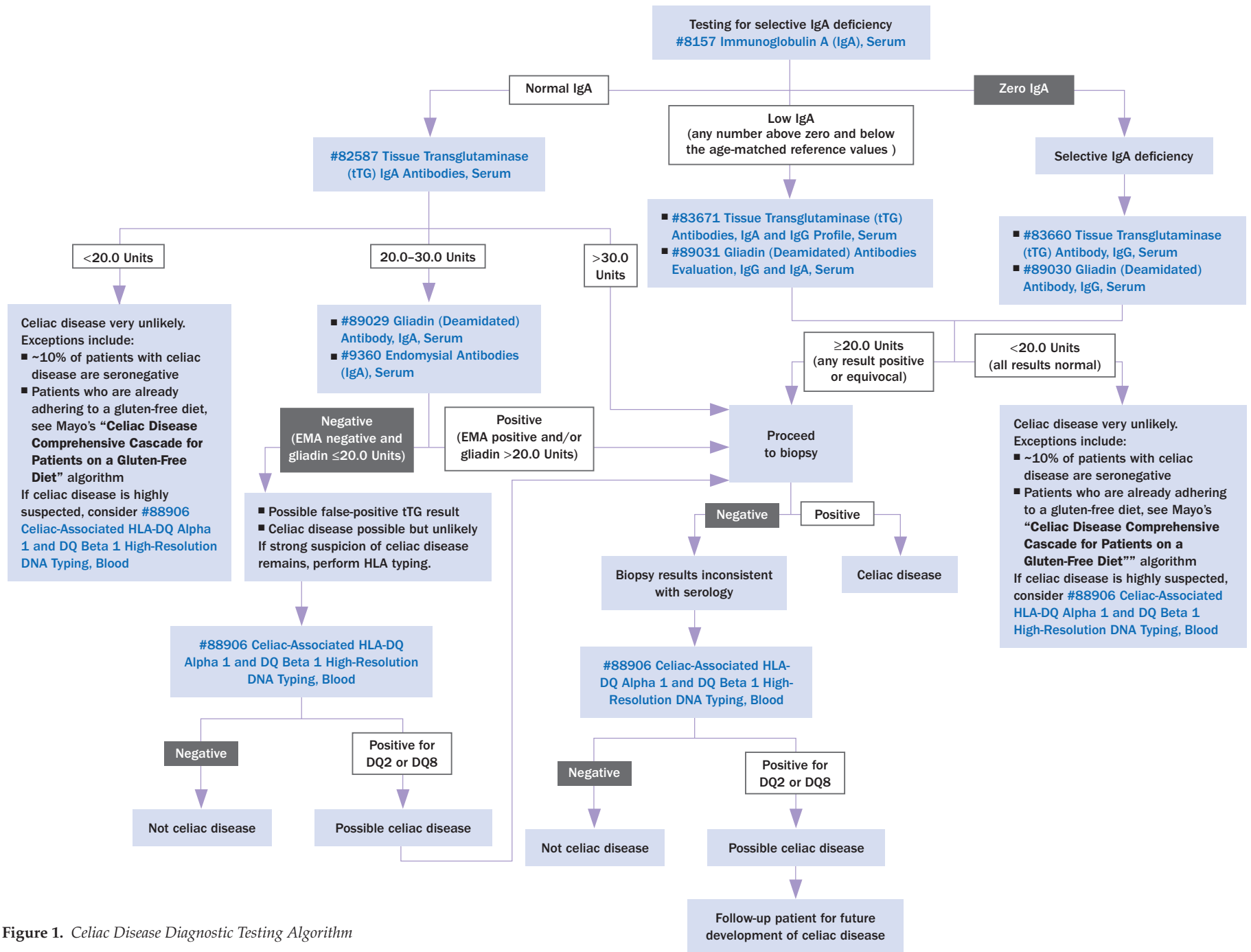


Figure 1. Celiac Disease Diagnostic Testing Algorithm

results. IgA serology is performed to evaluate whether the patient is IgA deficient. Increased IgA antibodies against tissue transglutaminase (tTG IgA) are highly specific for celiac disease; however, IgA-deficient patients with celiac disease may not have elevated tTG IgA results. For this reason, when patients are IgA deficient, the evaluation will progress to the assay that detects IgG antibodies against tissue transglutaminase (tTG IgG). When patients have below normal IgA levels, both tTG IgA and tTG IgG will be performed. The evaluation also includes deamidated gliadin IgA, IgG, or IgA and IgG, again depending on the level of IgA the patient is capable of producing. When the patient has normal IgA production and equivocal tTG IgA levels, in addition to deamidated gliadin IgA testing, endomysial IgA antibodies are evaluated. The endomysial antibodies also are highly sensitive and specific for celiac disease, and this assay provides another method to help resolve the equivocal tTG result.

The Comprehensive Evaluation's interpretive result includes the individual test results and a clinical interpretation with recommendations on whether to proceed to biopsy or pursue another diagnosis. The evaluation includes the most specific and sensitive tests available for celiac disease.

Another new panel, [#89199 Celiac Disease Serology Cascade](#), will be available when HLA DQ typing is not desired. This test follows the same approach to rapidly evaluate the patient, but does not include the HLA DQ typing. The interpretive report includes recommendations on the need for confirmatory biopsy.

Serology Testing

The development of new tests and technologies has improved the testing options for celiac disease. Serology is an essential part of the diagnostic evaluation; however, biopsy is still required for a diagnosis. As part of the immune response, patients with celiac disease produce gliadin antibodies and

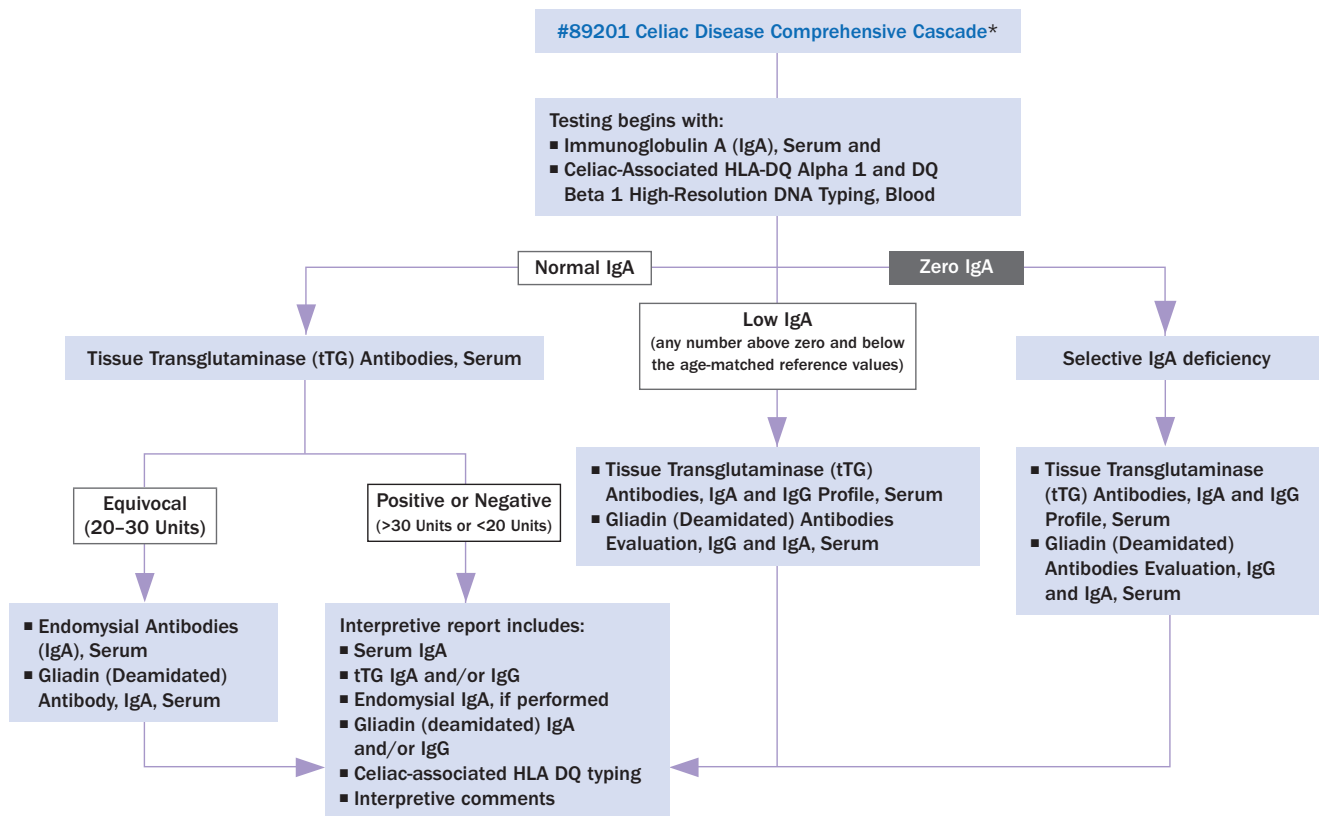


Figure 2. Celiac Disease Comprehensive Cascade Algorithm*

*For [#89199 Celiac Disease Serology Cascade](#), this same approach is followed, but HLA DQ typing is not performed.

various autoantibodies (antibodies against self), including tissue transglutaminase (tTG), endomysial, and reticulin. IgA antibodies usually predominate, although patients may also produce IgG antibodies.

IgA Deficiency

IgA deficiency is a rare disorder in which patients are unable to produce IgA antibodies. When ordering [#89201 Celiac Disease Comprehensive Cascade](#) or [#89199 Celiac Disease Serology Cascade](#), IgA testing is performed first for 2 reasons. First, any negative (normal) tTG IgA result requires IgA testing to demonstrate that the normal tTG IgA result is not a result of IgA deficiency. Second, by performing IgA testing first, the appropriate IgA or IgG assays can be utilized for the determination of deamidated gliadin and tTG antibody levels.

When physicians elect to order the individual tests, it is recommended that if there is a possibility that the patient is IgA deficient, IgA and IgG tTG tests should both be ordered ([#83671 Tissue Transglutaminase \[tTG\] Antibodies, IgA and IgG Profile, Serum](#)). This assay detects both the IgA and IgG tTG antibodies and is sensitive and specific for celiac disease. If a physician elects to order only a tTG IgA evaluation, all normal results should be followed by ordering [#8157 Immunoglobulin A \(IgA\), Serum](#) to rule out IgA deficiency.

Tissue Transglutaminase Antibodies

When ordering individual tests, rather than a comprehensive evaluation, Mayo recommends that testing for antibodies to tTG be performed first. This is consistent with the recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition.⁶ The most sensitive and specific test for celiac disease is [#83671 Tissue Transglutaminase \(Ttg\) Antibodies, IgA and IgG Profile, Serum](#). The sensitivity and specificity of this profile for active, biopsy-proven celiac disease exceeds 90%.⁸ The finding of IgA-anti-tTG antibodies is highly specific for celiac disease and possibly for dermatitis herpetiformis. Individuals with positive results are highly likely to have celiac disease, and should undergo biopsy to confirm the diagnosis. Patients whose results are in the equivocal range (20-30 Units) are less likely to have celiac disease. In these patients, testing for endomysial antibodies can help determine if biopsy is indicated.

When only tTG IgA is performed, the patient is symptomatic, and the results fall in the normal range, it is recommended to follow-up with [#8157 Immunoglobulin A \(IgA\), Serum](#) to determine whether the patient has normal production of IgA. Because tTG antibody levels decline following institution of a gluten-free diet, patients must maintain a normal diet prior to testing. If the patient has already instituted a gluten-free diet, a gluten challenge must be performed prior to testing. (A gluten challenge involves reintroduction of gluten-containing foods into the diet for a number of weeks.)

The level of tTG IgA generally correlates with the severity of gluten-sensitive enteropathy. If patients strictly adhere to a gluten-free diet, their level should begin to decrease within 6 to 12 months of onset of dietary therapy.

Deamidated Gliadin Antibody Testing

Testing for IgA and IgG antibodies to *unmodified* gliadin proteins is no longer recommended because of the lower sensitivity and specificity of these tests for celiac disease. However, recent studies have identified specific B-cell epitopes on the gliadin molecule that, when deamidated by the enzyme tissue transglutaminase 2, have increased sensitivity and specificity for celiac disease.^{8,9} New tests for deamidated gliadin IgA and IgG antibodies have replaced the older gliadin antibody tests, which have been discontinued at Mayo Clinic. In a recent study conducted at Mayo Clinic, the sensitivity and specificity of deamidated gliadin antibodies for untreated, biopsy-proven celiac disease were comparable to tTG antibodies.⁸

The deamidated gliadin antibody tests are available as: [#89029 Gliadin \(Deamidated\) Antibody, IgA, Serum](#) [#89030 Gliadin \(Deamidated\) Antibody, IgG, Serum](#)

Endomysial Antibodies

Endomysial antibody (EMA) testing by immunofluorescence targets IgA antibodies, and tTG is the primary autoantigen recognized by the EMA assay. For this reason, the tTG and EMA tests are both highly sensitive and specific. However, the EMA test, [#9360 Endomysial Antibodies \(IgA\), Serum](#), is an expensive labor-intensive process, and one test component (rhesus monkey esophagus substrate) is of limited availability. More importantly, the microscopic

analysis is subjective. For these reasons, in Mayo's algorithms, this test is utilized only when tTG IgA and deamidated gliadin test results are equivocal.

Reticulin Antibody Tests

Mayo Medical Laboratories no longer recommends reticulin testing for the diagnosis of celiac disease. The sensitivity and specificity of this test for celiac disease are inferior to the other tests previously mentioned.

Uninformative or Indeterminate Serology

Dietary changes can cause rapid changes in serology results. Serology may be falsely negative in patients who have already reduced the gluten in their diet. Up to 10% of untreated celiac disease patients may also have negative serology. When serology is uninformative or indeterminate, and there is substantial clinical doubt remaining, HLA typing should be performed to help discriminate those patients for whom a diagnosis of celiac disease can be excluded. HLA typing also may be helpful for patients who have reduced their gluten intake before testing.

If serology and HLA typing are inadequate to resolve the diagnosis, further investigation with endoscopy and small bowel biopsy is necessary to rule out celiac disease or alternative diagnoses. This is especially important in patients with frank malabsorption symptoms since many syndromes can mimic celiac disease. For these patients, biopsy should be performed regardless of serologic test results.

HLA Typing

HLA DQ typing is a relatively new option for celiac disease testing and it has an important supporting role. Because nearly 100% of patients with celiac disease carry the HLA markers DQ2 or DQ8, it is possible to use [#88906 Celiac-Associated HLA-DQ Alpha 1 and DQ Beta 1 High-Resolution DNA Typing, Blood](#) to virtually exclude celiac disease. This is important in 3 situations:

- When serologic and biopsy results are indeterminate
- When patients are adhering to a gluten-free diet prior to diagnostic testing and decline to undergo a gluten challenge
- As predictive (exclusion) testing for family members of affected patients and patients with Down syndrome⁷

These HLA markers are present in the general population, but at much lower frequencies than in patients with celiac disease. Nevertheless, the relatively high frequencies of these HLA markers in the general population prohibits use of this test as a screening test for celiac disease.⁵

Treatment Monitoring

The only treatment for celiac disease is a gluten-free diet. At this time, a safe threshold of exposure has not been identified. As a result, patients must be diligent to avoid glutes. Current information suggests that adherence to a gluten-free diet also may reduce the risk of lymphoma.⁵

The other main focus of treatment is care of the various deficiency states that may occur in patients

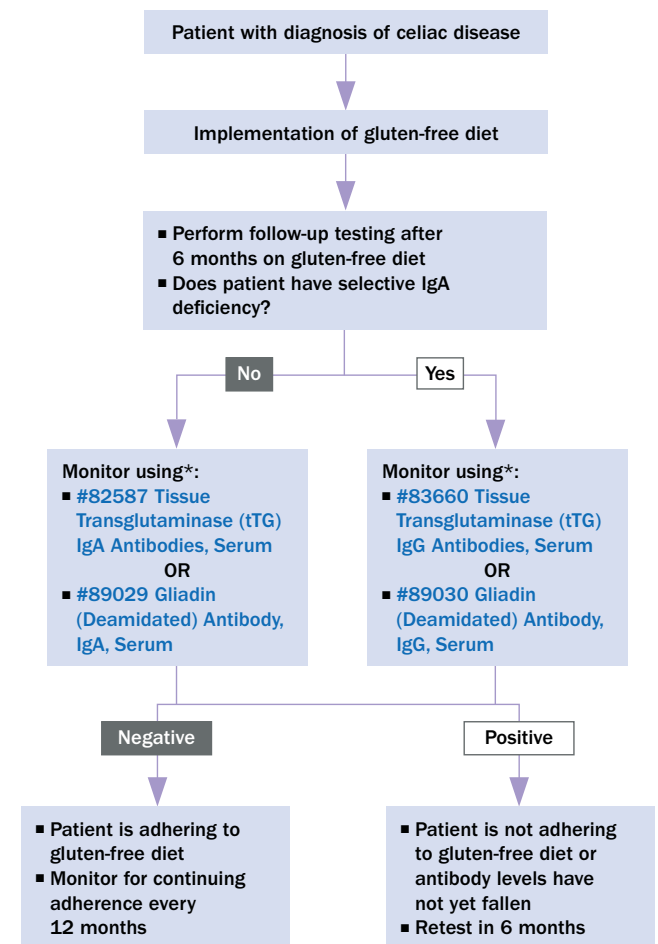


Figure 3. Celiac Disease Routine Treatment Monitoring Algorithm

*When following the patient, use the same assay each time.

with celiac disease. Especially in infants, immediate evaluation for existing deficiency states is critical to reducing celiac disease-associated complications. To assess the degree of malnutrition in affected patients, the following tests should be performed: iron, folate, and B12 levels for anemia, as well as carotene, albumin, prothrombin time, and calcium levels.

Mayo Medical Laboratories has developed a treatment algorithm (Figure 3) to assist in patient care.

In some cases, additional testing may be required to help reach treatment goals or establish a diagnosis. This group may include the unresponsive or refractory patient or those patients (without a diagnosis) who have put themselves on a gluten-free diet. Because these patients may have significantly reduced antibody production in response to the gluten-free diet, tTG or deamidated gliadin IgA testing alone is insufficient. A new panel, [#89200 Celiac Disease Comprehensive Cascade for Patients on a Gluten-Free Diet](#), and algorithm were developed to evaluate these patients (Figure 4).

In this population, HLA typing is the initial test for the purpose of identifying those who do not have the gene and are, therefore, highly unlikely to have celiac disease. If HLA typing is positive for DQ2 or DQ8, serology is performed to evaluate antibody production. Since these patients may have altered their levels of antibody production to normal levels by following gluten-free diet, if serology results are negative and the celiac disease is strongly suspected, a gluten challenge may be necessary to enable accurate serologic testing.

Summary

Historically, the confusing mix of symptoms and the difficulties of reaching a definitive diagnosis have prevented easy recognition of celiac disease. Over the last 10 years, there has been a significant increase in the rate of diagnosis, probably attributable to the improved access to serologic tests and growing awareness of the disease.⁷ The recognition and enumeration of the possible symptoms of celiac disease, along with efforts such as the NIH awareness program, should improve

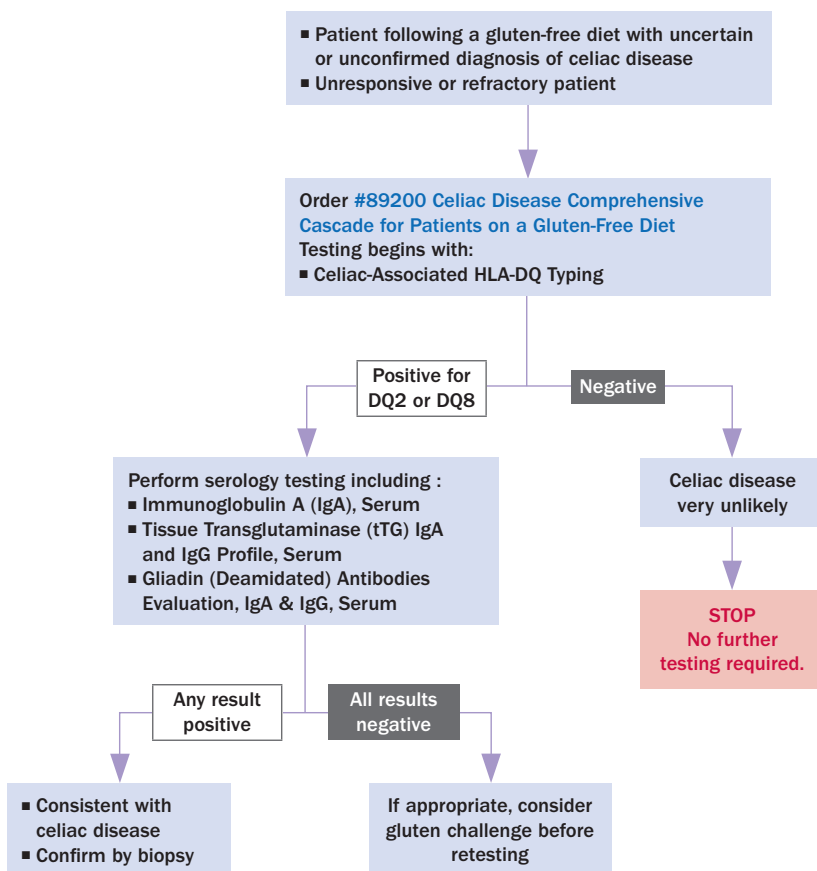


Figure 4. Celiac Disease Comprehensive Cascade for Patients on a Gluten-Free Diet

physician and patient recognition of the disease. With the new testing options offered by Mayo Medical Laboratories, physicians can now utilize simple, algorithmic approaches to evaluate patients at risk for celiac disease and to monitor treatment, improving the lives of these patients.

Watch for the introduction of our new celiac panels this summer:

- #89201 Celiac Disease Comprehensive Cascade
- #89199 Celiac Disease Serology Cascade
- #89200 Celiac Disease Comprehensive Cascade for Patients on a Gluten-Free Diet

Each of the following tests for celiac disease are currently available:

- #88906 Celiac-Associated HLA-DQ Alpha 1 and DQ Beta 1 High-Resolution DNA Typing, Blood
- #82587 Tissue Transglutaminase (tTG) IgA Antibodies, Serum
- #83660 Tissue Transglutaminase (tTG) IgG Antibodies, Serum
- #83671 Tissue Transglutaminase (tTG) Antibodies, IgA and IgG Profile, Serum
- #89031 Gliadin (Deamidated) Antibodies Evaluation, IgG and IgA, Serum
- #89029 Gliadin (Deamidated) Antibody, IgA, Serum
- #89030 Gliadin (Deamidated) Antibody, IgG, Serum
- #9360 Endomysial Antibodies (IgA), Serum
- #8157 Immunoglobulin A (IgA), Serum

This article and the related algorithms are the result of the efforts of the Celiac Disease Consensus Group, which includes the following individuals:

Steven R. DeGoey
Lawrence E. Gibson, MD
Henry A. Homburger, MD
Patricia A. Krause
Denise E. Masoner
S. Brendan Moore, MD
Thomas P. Moyer, PhD
Joseph A. Murray, MD
Thomas C. Smyrk, MD
Melissa R. Snyder, PhD

Authored by: Masoner DE, Dale JC, Homburger HA, Murray JA

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MayoMedicalLaboratories.com— Ongoing Web Site Improvements

If you haven't visited our home page recently, you're in for a big surprise—several, in fact! The most important change to the site is the inclusion of all clinical and interpretive information for Mayo Medical Laboratories' tests. This information was previously only available by client log in to MayoAccess or in our printed *Interpretive Handbook*. We recently also enhanced our Web search tools, enhancing the ability to quickly locate tests and related information, such as algorithms. Hot Topic Videos are also new; these short programs provide information on important topics for laboratorians.

Search Enhancements

Site Search

Refinements to the search tool make it possible to search the entire Mayo Medical Laboratories Web site by using the search box in the upper right corner. When you use this search box, you not only search the Test Catalog, you also search all 2000-plus pages of our Web site, including *Communiqué* articles, algorithms, and test notifications. When searching, try using multiple terms to help refine your results. For example, instead of just "chromosome," try "chromosome 11" or "chromosome 11q".

Test Catalog Search

The purpose of this public Test Catalog is to answer 3 primary questions:

1. Does Mayo Medical Laboratories offer a test?
2. What is the most appropriate test to order?
3. What are the specimen requirements for a test?

Extensive clinical, interpretive, and test methodology information is included to assist in test selection and result interpretation. This catalog is optimized for use by all site visitors.

For clients, your customized view includes proprietary information that is not publicly available (accessible only through your log in to MayoAccess®, MayoLink®, or MayoNet®). Your customized view also maintains your ability to search partial keywords, gives you access to forms, and other information that is not accessible on the Web.

Search Options

If you look to the left side of the home page, you'll find the Test Catalog. The catalog image is a link to a searching strategies document, which offers comprehensive information about the site and how to optimize your search. Review this page to get the most benefit out of the new search capabilities.

As a quick overview, the default search is Test Name, which gives you the most likely test matches. If you're not sure of the test name, but have a disease or condition you want to search on, change the search to Useful For. This will expand your search, but still keep a high degree of relevance. If you are having problems finding what you're looking for, expand your search to Keyword, which will give you the maximum number of results. Of course, if you know the test number, you can select the Test Number search, which will take you directly to the test.

Hot Topic Videos

In addition to our search enhancements, you'll find a new type of information—Hot Topic Videos. In these presentations, Mayo Clinic physicians and scientists discuss topics of current interest. The slides for these programs can be downloaded. Upcoming programs will include seasonal topics such as Lyme disease, specialty topics such as pediatric reference values, and discussions about newly introduced tests. Keep checking, as these videos will change as discussions of new topics become available.

User Input

We are constantly improving our Web services and we want to know what improvements you would like to see. What do you like, or dislike about our redesigned site? We encourage you to contact us with your suggestions for improvements, questions about how the site operates, or difficulties you encounter when searching for specific information. First, we'll help you locate the information, and then we'll use that example to help refine the search capability or improve organization. Any queries or comments can be sent to mml@mayo.edu. If you're unable to find what you're looking for and are under time constraints, you can also call Mayo Laboratory Inquiry at 800-533-1710 and the staff will help you locate the information immediately.



2008 Education Calendar

Upcoming Education Conferences . . .

Bleeding and Thrombosing Diseases Wet Workshop

August 12, 2008
Kahler Hotel
Rochester, Minnesota

Bleeding and Thrombosing Diseases 2008 Mayo Update

August 13–15, 2008
Kahler Hotel
Rochester, Minnesota

Practical Surgical Pathology

September 11–13, 2008
Mayo Clinic
Rochester, Minnesota

Integration Through Community Laboratory Insourcing

October 1–3, 2008
Millennium Knickerbocker Hotel
Chicago, Illinois

Real-Time PCR for the Clinical Microbiology Laboratory

October 23–24, 2008
Mayo Clinic
Rochester, Minnesota

Continuous Process Improvement: Sharing our Lean Journey

November 6–7, 2008
Mayo Clinic
Rochester, Minnesota

Disease Management Strategies . . .

Vitamin D Deficiency: Bones and Beyond

August 5, 2008
Presenter: Kurt A. Kennel, MD

Rheumatoid Arthritis

September 9, 2008
Presenter: Clement J. Michet Jr., MD

Leukemias and Lymphomas

October 14, 2008
Presenter: William G. Morice II, MD, PhD

Alzheimer Update and Treatment

November 11, 2008
Presenters: Neill R. Graff-Radford, MD
and Steven G. Younkin, MD

Cardiovascular Biomarkers—An Update

December 9, 2008
Presenter: Allan S. Jaffe, MD

FOR MORE INFORMATION contact Mayo Medical Laboratories Education Department at 800-533-1710 or visit us at MayoMedicalLaboratories.com/education



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