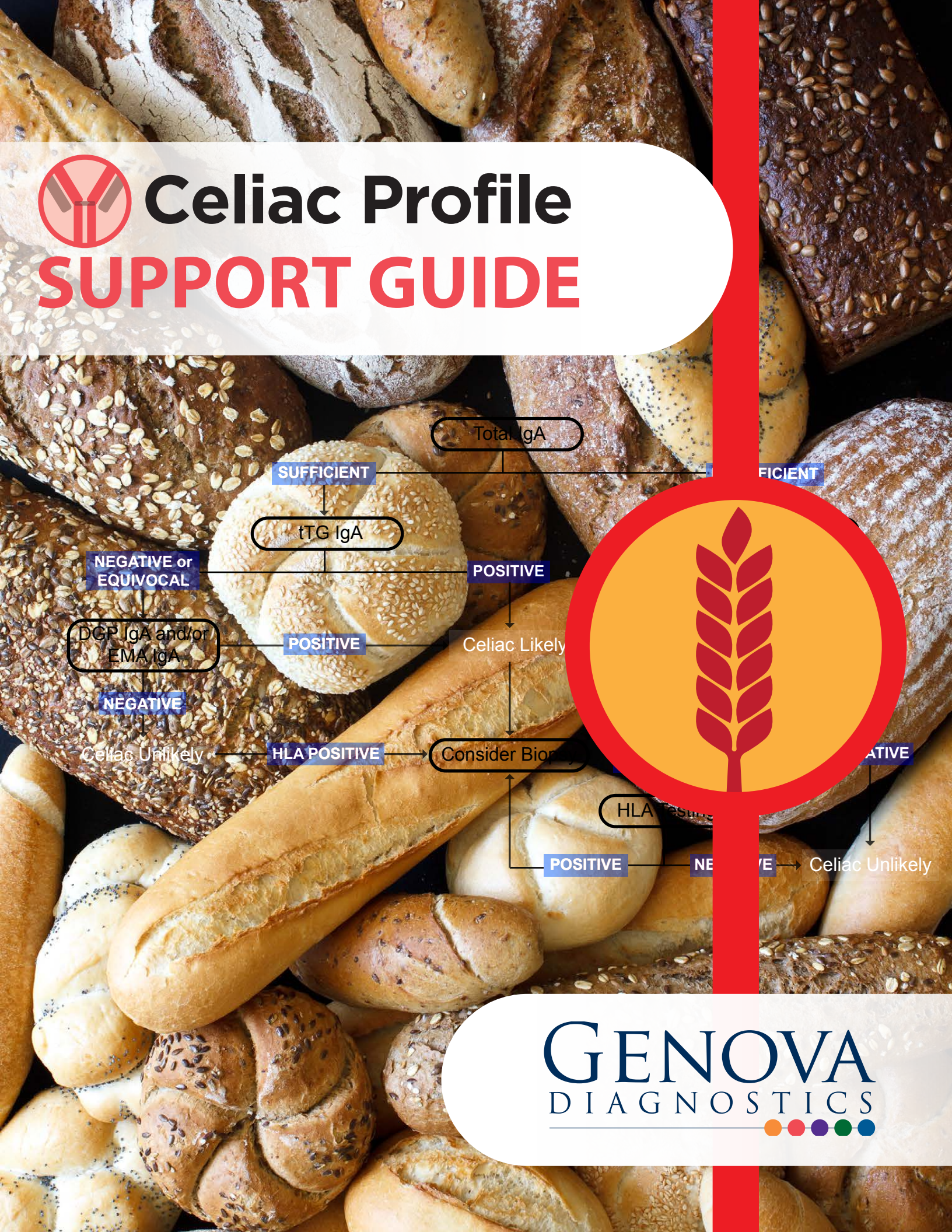
Celiac Profile
SUPPORT GUIDE



GENOVA DIAGNOSTICS

Contents

Celiac Profile 3

What is Celiac Disease? 4

Gluten 4

Pathophysiology of Celiac Disease 5

Laboratory Diagnosis of Celiac Disease 5

When to Test for Celiac Disease 5

Total Immunoglobulin A (IgA) 6

Anti-Tissue Transglutaminase

Antibodies (tTG IgA and tTG IgG) 7

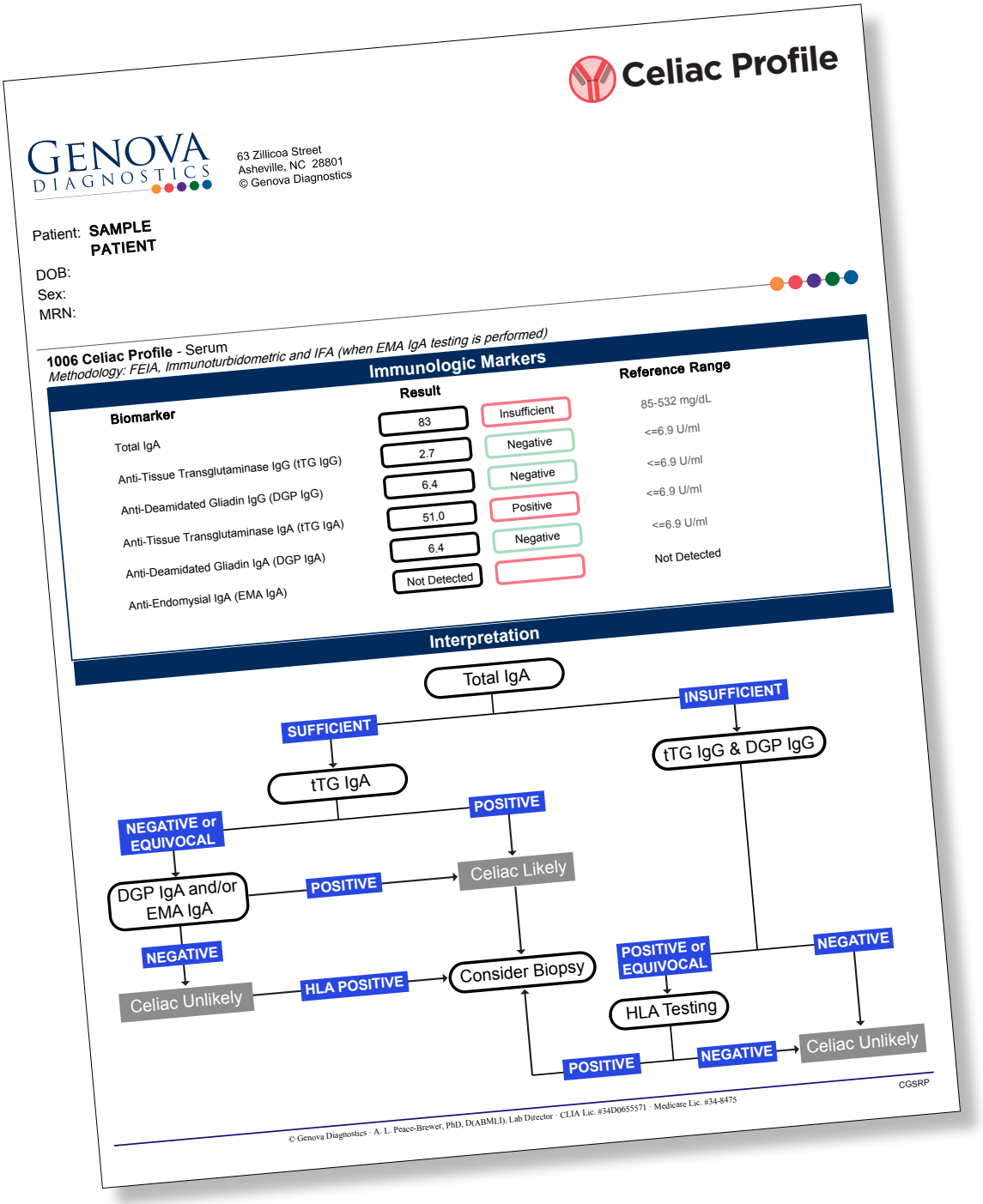
Anti-Deamidated Gliadin (DGP IgA and DGP IgG) 7

Anti-Endomysial IgA (EMA IgA) 7

References 8

Celiac Profile

Genova Diagnostics' Celiac Profile is a serum assessment of celiac-specific antibodies. Due to the increased prevalence of selective Immunoglobulin A (IgA) deficiency in celiac patients, total IgA is first measured to inform interpretation of both IgA and IgG antibody levels against tissue transglutaminase and deamidated gliadin. Reflex anti-endomysial IgA antibodies may offer further insight when anti-tissue transglutaminase and anti-deamidated gliadin antibody levels are positive or equivocal. The Celiac Profile report also offers a graphic schematic to aid in the interpretation of results and guide further diagnostic options.



Celiac Profile

GENOVA DIAGNOSTICS

63 Zillicoa Street
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Patient: SAMPLE PATIENT
DOB:
Sex:
MRN:

1006 Celiac Profile - Serum
Methodology: FEIA, Immunoturbidometric and IFA (when EMA IgA testing is performed)

Immunologic Markers

Biomarker	Result		Reference Range
Total IgA	83	Insufficient	85-532 mg/dL
Anti-Tissue Transglutaminase IgG (tTG IgG)	2.7	Negative	<=6.9 U/ml
Anti-Deamidated Gliadin IgG (DGP IgG)	6.4	Negative	<=6.9 U/ml
Anti-Tissue Transglutaminase IgA (tTG IgA)	51.0	Positive	<=6.9 U/ml
Anti-Deamidated Gliadin IgA (DGP IgA)	6.4	Negative	<=6.9 U/ml
Anti-Endomysial IgA (EMA IgA)	Not Detected		Not Detected

Interpretation

CGSRP

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What is Celiac Disease?

Celiac disease (CD) is defined as an autoimmune enteropathy of the small intestine caused by exposure to dietary gluten in genetically pre-disposed individuals. In susceptible individuals, gluten ingestion generates an inflammatory reaction predominantly centered in the upper parts of the small intestine. The mucosal injury caused by this reaction will eventually reduce the intestinal absorptive area and interfere with uptake of nutrients.[1] Epidemiologic studies estimate a worldwide prevalence of CD of approximately 1:100 individuals, with a considerable proportion of patients remaining undiagnosed and untreated.[2]

Celiac disease can be asymptomatic. When present, celiac disease has many clinical symptoms and is associated with many conditions such as:[2-5]

- Chronic diarrhea with weight loss
- Steatorrhea
- Postprandial abdominal pain
- Bloating
- Malabsorption with nutrient deficiencies (iron, B12, calcium)
- Osteoporosis
- Irritable bowel syndrome
- Elevated liver enzymes
- Cerebellar ataxia
- Peripheral neuropathy
- Type I Diabetes
- Autoimmune hepatitis
- Autoimmune thyroiditis
- Infertility
- Dermatitis herpetiformis
- Sjogren syndrome
- Addison's disease
- Parathyroid disorders
- Growth hormone deficiency
- Primary biliary cirrhosis
- Primary sclerosing cholangitis

Gluten

Gluten is a complex mixture of many related but distinct proteins commonly found in wheat grains (durum, einkorn, emmer, kamut, spelt), barley, rye, oats, and triticale. The primary components are gliadin and glutenin, though similar proteins such as secalin and hordein, can be found in barley, rye, and oats. These proteins are collectively referred to as 'gluten.' Gluten acts as a 'glue' to help food maintain its shape and consistency.[6,7]

The gluten molecule contains a significant amount of two amino acids - glutamine and proline. This bonded structure makes them relatively resistant to protease and peptidase enzymes resulting in incomplete digestion. Although some gluten can be subsequently digested by commensal bacteria, most undigested gluten fragments are excreted without immune activation.[8,9]

However, some undigested gluten is actively transported across the intestinal endothelium and makes its way into the lamina propria where an immune response occurs. Gliadin and its various gluten fragments induce zonulin release which opens the tight junctions of the intestinal epithelium. These tight junctions are dynamic structures involved in coordinated responses to regulate the passage of molecules under physiologic and pathologic circumstances. Gut-associated lymphoid tissue (GALT) then works to safeguard potentially harmful antigens, such as gluten, from reaching the systemic circulation, and it induces systemic immune tolerance using secretory IgA and T-regulatory-cell activity. In addition to GALT, the major histocompatibility complex called 'Human leukocyte antigen' (HLA) class I and II also contribute to gastrointestinal immunologic responsiveness.[10] The interplay between innate and adaptive immunity involves the recognition of antigens and activation of toll-like receptors (TLRs) to signal cytokine release and recruit immune and inflammatory cells. In healthy individuals, this may have little consequence. In genetically predisposed patients, this can be clinically significant.

Pathophysiology of Celiac Disease

The development of celiac disease usually involves the triad of genetic predisposition, intestinal permeability, and an environmental trigger – in this case gluten.

Most commonly, celiac disease-relative risk involves genetic variants of major histocompatibility complex genes HLA-DQ2 and HLA-DQ8. These are cell surface receptor proteins that act as antigen presenting cells allowing the immune system to bind and recognize antigens and itself. HLA DQ2/DQ8 are present in greater than 98% of all celiac patients. However, they are also present in a high percentage of their relatives, and nearly 50% of the general population without the disease. A positive genetic variant of HLA-DQ2 and/or HLA-DQ8 is indicative of susceptibility but does not necessarily translate to disease development. A negative test has more significant value because gluten intolerance rarely occurs in the absence of these predisposing alleles.[5,11]

When these genetically predisposed patients eat gluten, it signals and enhances the release of zonulin allowing some degree of intestinal permeability and causes some luminal damage. Once gluten reaches the inner layer of the intestine, called the lamina propria, it activates an enzyme called tissue transglutaminase (tTG). This enzyme modifies the gluten peptide by removing an amide group and converting glutamine residues to glutamic acid. The deamidated peptides bind very well to the HLA-DQ2 and HLA-DQ8 molecules as part of both the adaptive and innate immune response. Also, the intestinal T cells of celiac patients preferentially recognize these deamidated gluten peptides.[12] The innate immune response occurs within the intestinal epithelial component of the mucosa. Cytokine production is proliferated, and intraepithelial lymphocytes differentiate into cytotoxic T cells. The cumulative damage to the mucosa from this inflammatory cascade causes villous atrophy and crypt hyperplasia.[5,13]

Laboratory Diagnosis of Celiac Disease

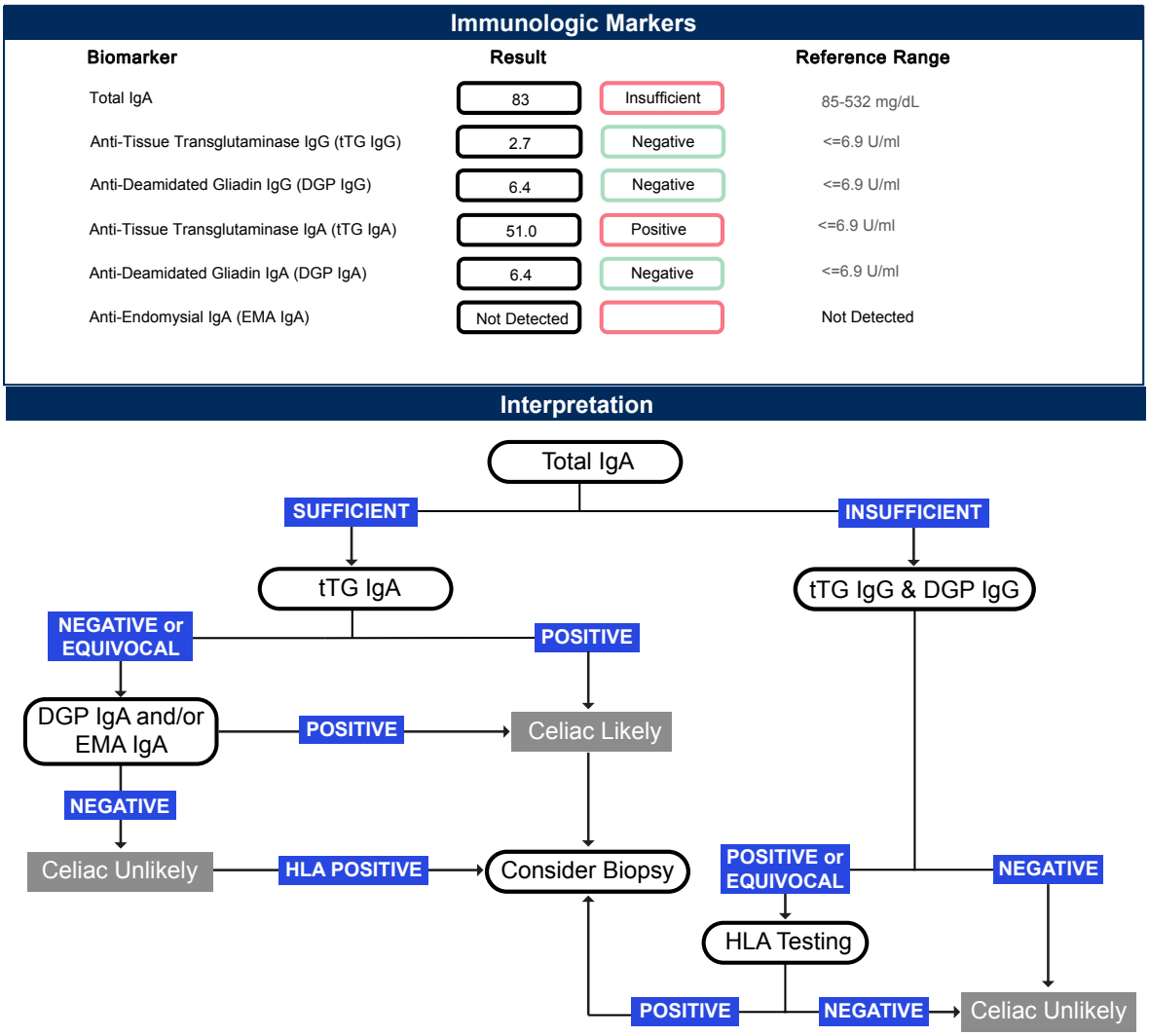
Although celiac disease is usually detected by serologic testing of celiac-specific antibodies, both serology and intestinal biopsy should be performed, preferably on a gluten-containing diet. In fact, if the suspicion of celiac disease is high, intestinal biopsy should be pursued in spite of serologies being negative.[14]

After a celiac diagnosis, serology testing is often used to confirm adherence with a gluten-free diet. Findings may corroborate adherence to gluten restriction, raise the possibility of hidden sources of gluten contamination, or if negative, prompt a re-evaluation of initial diagnosis in patients with ongoing symptoms.

When to Test for Celiac Disease

According to the American College of Gastroenterology clinical guidelines, patients with symptoms, signs, or laboratory evidence consistent with malabsorption, chronic diarrhea with weight loss, steatorrhea, postprandial abdominal pain and bloating should be tested. Additionally, first degree family members of those with confirmed celiac disease should be tested if they show signs, symptoms, or laboratory evidence of the disease, with consideration for also testing asymptomatic first-degree relatives. Patients with unexplained elevations in serum aminotransferase levels as well type I diabetics with digestive symptoms should undergo testing as well.[14]

Genova's Celiac Panel includes total IgA assessment as well as both IgG and IgA antibody levels against tissue transglutaminase and deamidated gliadin. A reflex measurement of anti-endomysial IgA antibodies is performed when anti-tissue transglutaminase or anti-deamidated gliadin IgA levels are positive or equivocal.



Total Immunoglobulin A (IgA)

There are two types of **Immunoglobulin A (IgA).** **Secretory IgA** is made and secreted from organized gut-associated lymphoid tissues and is the predominant antibody class in the external excretions that bathe mucosal surfaces. They play a key role in immune protection and act as a first line of defense against pathogens. The importance of IgA in mucosal secretions is well established, however some IgA is secreted systemically. Additionally, a proportion of serum IgA is made by marginal zone B cells in the spleen and lymph nodes. Serum and secretory IgA have different structures though work together in humoral immunity. Serum IgA antibodies may be playing a backup role to guard against systemic infection from pathogenic invasion across the mucosal epithelium.[15,16]

Selective immunoglobulin A deficiency (SIgAD) is a fairly common primary immunodeficiency and can be defined as undetectable serum IgA in the presence of normal levels of other immunoglobulins. Partial IgA deficiency is present when levels are two standard deviations below normal for age. Selective IgA deficiency is estimated to affect nearly 3% of celiac disease patients making it 10-15 times higher than in the general population.[17] With IgA deficiency being commonly encountered in this patient population, it can create some uncertainty in celiac-related antibody testing interpretation.

Sufficient levels of IgA are necessary to correctly interpret subsequent measurement of anti-tissue transglutaminase, anti-deamidated gliadin, and anti-endomysial IgA levels.

Insufficient or undetected IgA levels should prompt attention toward the IgG levels against tissue transglutaminase and deamidated gliadin for directing celiac diagnostic options.

Anti-Tissue Transglutaminase Antibodies (tTG IgA and tTG IgG)

Tissue transglutaminase is an enzyme released by damaged intestinal epithelial cells which removes an amide group from gluten thereby enhancing its immunostimulatory effect and resulting in a higher affinity for the HLA-DQ2 and HLA-DQ8 molecules – a key step in the pathogenesis of celiac disease.

In addition to its effects on gluten and subsequent immune response, tissue transglutaminase itself is a target autoantigen. IgA and IgG antibodies are mounted against tTG which can be detected in serum and used in the diagnosis of celiac disease. These antibodies can also be seen in other autoimmune diseases as well as GI diseases such as liver disease and inflammatory bowel disease. This may be multi-factorial and due to hypergammaglobulinemia, hyperactive immune responses, dual diagnosis with celiac disease, cross-reactivity with other protein antigens, variations within assay epitopes, or latent gluten intolerance.[18-21]

The sensitivity of tTG IgA for untreated celiac disease is approximately 95% and the specificity is also 95% or greater. The higher the titer, the greater the likelihood of a true positive celiac result.[14] The European Society of Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for Diagnosing Celiac Disease (2020) outlines that for initial testing in pediatric patients, the combination of total IgA and tTG IgA is more accurate than other test combinations in pediatrics and biopsy may not be needed in these patients.[22] The no-biopsy approach is confirmed to be safe in children with anti-tissue transglutaminase IgA values ≥ 10 times upper limit and other positive serologies. The American College of Gastroenterology (ACG) notes that prospective data to validate the ESPGHAN recommendations in children are lacking. ACG recommends combining IgA tTG with anti-deamidated peptide antibody levels. Current guidelines by the ACG for the adult population remain a combination of serologies, clinical history, physical exam, and upper endoscopy with biopsy.[14]

Anti-Deamidated Gliadin (DGP IgA and DGP IgG)

When tissue transglutaminase is released from the intestinal epithelial lining, it removes an amide group from the gluten structure. This deamidated gluten peptide (DGP) binds easily to the HLA major histocompatibility complexes genetically found in celiac patients. DGP then stimulates the adaptive and innate immune system cascades which subsequently result in villous atrophy and crypt hyperplasia. Autoantibodies can also be directed at this deamidated gliadin molecule.

Because DGP is the resultant molecule of the enzyme tTG, autoantibodies to both DGP and tTG are often used to evaluate patients for celiac disease. Elevations of DGP antibodies alone may not have a significant positive predictive value for celiac disease, though titers can correlate with the severity of histologic changes.[23,24] Studies suggest that combining serologies of anti-DGP and anti-tTG show a 98-99% sensitivity and a 100% specificity for celiac diagnosis.[25,26]

On Genova's Celiac Profile, if DGP IgA or tTG IgA are elevated, a reflex measurement of anti-endomysial antibodies is performed.

Anti-Endomysial IgA (EMA IgA)

As noted previously, the enzyme tissue transglutaminase is released with intestinal epithelial damage and itself becomes an autoantigen. IgA and IgG antibodies are mounted against tTG. Another class of antibodies also mounts an immune response directly to tTG – anti-endomysial antibodies.[13]

In the gastrointestinal tract, the 'mucosa' is comprised of three layers: epithelium, lamina propria, and muscularis mucosae. Mucosal damage from gluten results in tTG activation in the lamina propria and endomysial damage to the muscularis mucosae. The damage to the endomysial layer stimulates an autoantigenic immune response. Anti-endomysial antibodies directly target tTG, in addition to the IgA/IgG response against tTG.

On Genova's Celiac Profile, when positive or equivocal levels of anti-tTG IgA (and/or anti-DGP antibodies) are detected, a reflex measurement of anti-endomysial antibodies to tTG is performed to increase sensitivity and specificity of celiac diagnosis.[25]

Elevations of anti-endomysial antibodies, in the presence of positive anti-tTG antibodies, show high specificity (at times greater than 95%) for celiac disease, as well as equally high sensitivity though percentages vary within literature.[27-29]

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