CLINICAL APPLICATION GUIDE

ARRAY 3

ARRAY 3 – Antibody

WHEAT/GLUTEN PROTEOME REACTIVITY & AUTOIMMUNITY™

CYREX™ Laboratories

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WHEAT/GLUTEN PROTEOME REACTIVITY AND AUTOIMMUNITY™

OVERVIEW

MEASURING GLUTEN SENSITIVITY

Introduction

Current testing for Gluten Sensitivity and Celiac disease includes serum IgG and IgA against gliadin and tissue transglutaminase. These antibodies are measured against minor components of a wheat protein called alpha-gliadin. However, wheat proteins consist of alpha-gliadin, omega-gliadin, glutenin, gluteomorphin, prodynorphin, and agglutinins, any of which has a capacity to challenge the immune system. Because of this heterogeneity of gluten proteins and peptides, multiple variations in T-cell responses may occur against them. Recent medical research indicates that a large number of gluten epitopes, may be implicated in the development of Gluten Sensitivity, Celiac disease and other associated conditions. The repertoire and hierarchy of gluten peptides stimulate the intestinal T-cells and results in a significant elevation of IgG and IgA production. Measurement of IgA and IgG against multiple gluten epitopes in blood can have important implications in the accurate diagnosis and design of therapy for gluten-sensitive and Celiac disease patients. For a comprehensive approach to this problem, pioneering, patent-pending technologies have been developed to measure IgA and IgG against various wheat components including alpha-gliadins, 17 MER and deamidated 33 MER, gamma-gliadin 15 MER, omega-gliadin, glutenin, prodynorphin, gluteomorphin, gliadin-bound transglutaminase, transglutaminase, and glutamic acid decarboxylase.

Research performed at Cyrex Labs confirms that different gluten-sensitive and Celiac disease patients recognize an array of gluten antigens. For example, one patient reacts to omega-gliadin, but not to alpha-gliadin. The second patient reacts to all gliadin peptides, and the third patient reacts only to the wheat germ agglutinin.

Gluten Sensitivity (GS) is a systemic autoimmune disease with diverse manifestations.[1] Celiac disease (CD), or gluten-sensitive enteropathy, is only one aspect of a range of possible manifestations of sensitivity to gluten. And yet, this enteropathy, *"one of the most common lifelong disorders in both the U.S. and Europe,"* [2] receives the lion's share of focus to the point of ignoring other manifestations. Autoimmune disease, the third leading cause of morbidity and mortality in the industrialized world,[3] is 10 times more common in a gluten-sensitive enteropathy than in the general population.[4] Thus, the burden on society from GS cannot be overestimated. Earlier identification might result in earlier treatment, better quality of life, and an improved prognosis for these patients.[5]

The emphasis on Gluten-Sensitive Enteropathy (Celiac disease) as the main manifestation of GS has been questioned. It is now accepted that GS is a systemic illness that can manifest in a range of organ systems. Such manifestations can occur independently of the presence of the classic small-bowel lesion that defines CD.[6] That GS is regarded as principally a disease of the small bowel is a historical misconception.[7]

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The diagnosis of GS has been proposed to include not only CD, but also gluten-reactive patients without mucosal lesions. From the skin (Dermatitis Herpetiformis,[8] Psoriatic arthritis,[9] Alopecia areata, Dermatomyositis, Cutaneous vasculitis[10]), to the muscles (inflammatory myopathies[11]), to the brain (Gluten Ataxia,[12] altered neurotransmitter production,[13] Schizophrenia,[14] peripheral neuralgias,[15] idiopathic neuropathies,[16] etc.) and beyond, pathology to gluten exposure can occur in multiple systems without evidence of an enteropathy.[17]

Negative serology should not necessarily reassure the clinician[18] of neither negative immune activation nor pathology. Several reports show that in the majority of Celiac patients, antibodies to gliadin and transglutaminase may be negative.[19 20 21 22 23] In particular, seronegative CD seems to be quite frequent in patients with milder intestinal damage (Marsh I-IIIa lesions).[24] And these lesions often present without elevated Celiac markers. Some reports identify the sensitivity as low as 27-31%[25] with lesser degrees of villous atrophy. Patients with non-Villous Atrophy GS (Marsh I, Marsh II) are more likely than others to test negative for tissue transglutaminase and endomysial antibodies.[26] Despite the many published reports on seronegative Celiacs, this subgroup understandably continues to be forgotten or not included in diagnostic workup, unless presenting with Celiac crisis. Why would that be? This seronegativity is due to the measurement of antibody IgG and/or IgA against only one antigen of wheat, α-gliadin.

Numerous complications have arisen regarding an accurate identification and diagnosis of GS, with or without the enteropathy CD. Clinicians have been frustrated with the high percentage of false negative serology.[27] For example, CD has been called the "Unforgiving Master of Non-Specificity and Disguise."[28] Therefore, if GS and CD are left undiagnosed for years, the results could be devastating autoimmune conditions. Mechanisms of action are shown in the Figure 1.

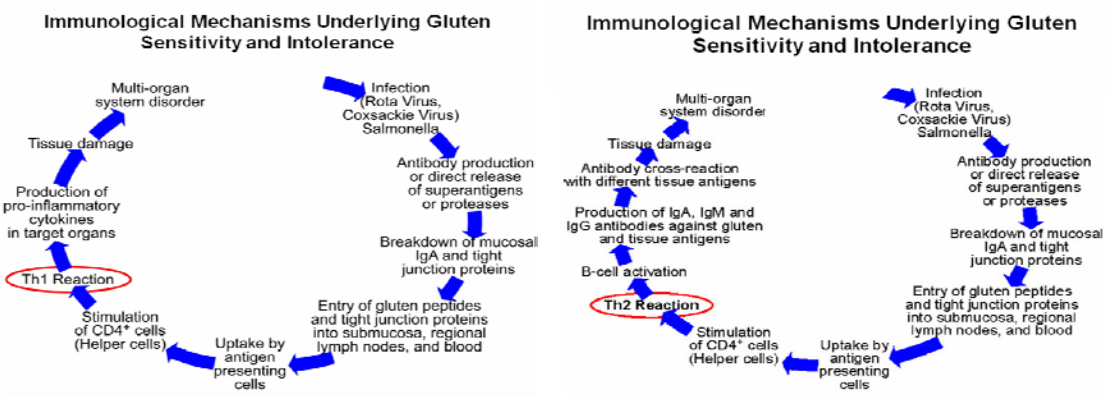

Figure 1 – *The mechanisms behind Gluten Sensitivity can ignite either Th-1 or Th-2 or a combination of both immune reactions.*

Therefore, should Healthcare Practitioners be limiting their diagnostic inquisitiveness solely to the well-referenced indicators of a severe gluten enteropathy (anti-transglutaminase and endomysial antibodies)? Numerous researchers suggest not! [23 13 18]

Current serology testing—although highly sensitive and specific for severe gluten enteropathy—does not address the diversity of gluten peptides and the need for more sensitive markers of GS with or without CD.

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GLUTEN PEPTIDES

During digestion, gluten proteins are enzymatically broken down in the gastrointestinal tract. However, because of the high proline content of gluten, the degradation is not efficient and relatively large gluten peptides can persist[29]. There are thousands of such gluten peptides produced, and multiple peptides can stimulate an immune response in an individual. The protease-resistant 33-amino acid peptide from wheat α-gliadin is the immunodominant antigen in wheat, but little is known about the hierarchy of immunodominance and consistency of recognition of T-cell epitopes in vivo.[30] Although the majority of immune reactions to gluten peptides is due to binding to HLA-DQ2, some gluten peptides have a different antigenic specificity from that of CD and are independent of the action of transglutaminase enzyme and HLA-DQ2/DQ8[31] and therefore are directly presented by antigen presenting cells to T cells. Further cooperation with B cells results in antibody production to the antigens.

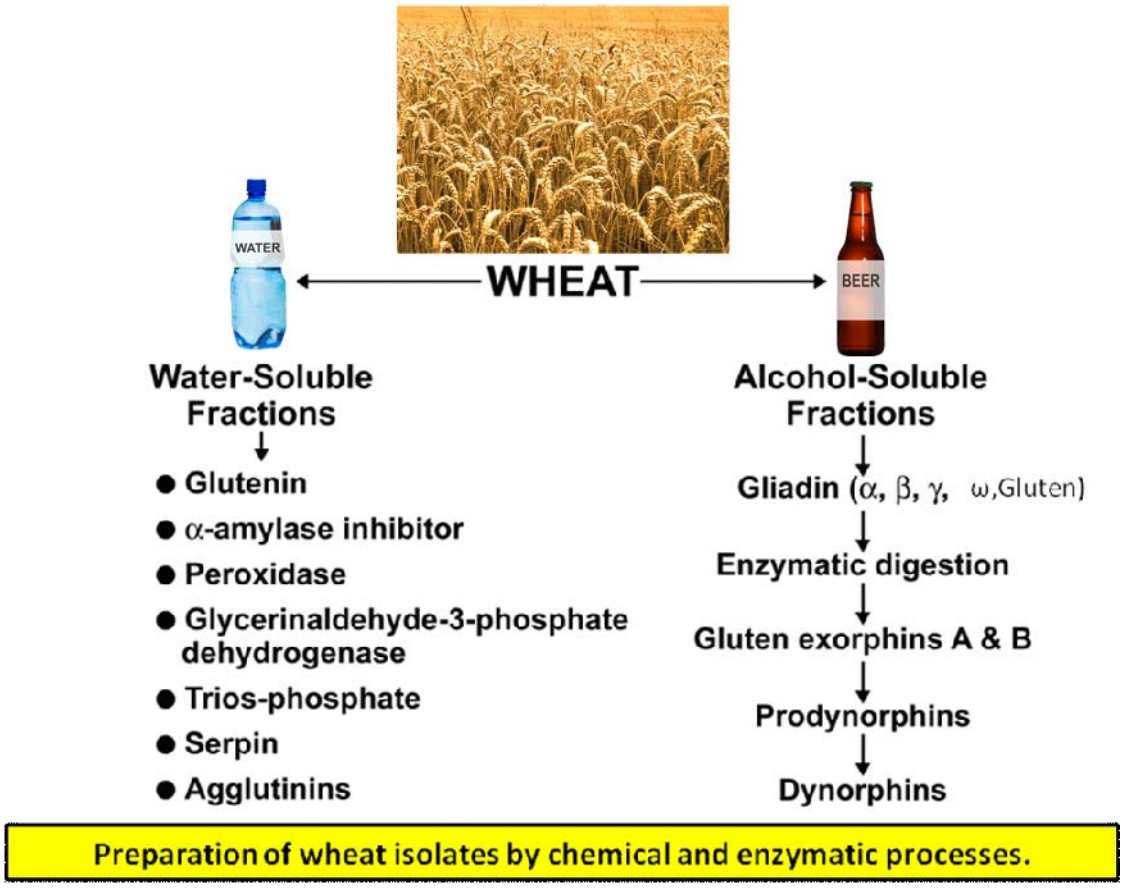

Figure 2 – *The kernel of wheat is comprised of hundreds of proteins. These molecules can be classified as either water- or alcohol-soluble. Proteins and peptides from each category can potentially be pathogenic to the gluten-sensitive patient. By assessing both water- and alcohol-soluble fractions of wheat, a clearer picture of Gluten Sensitivity can be obtained.*

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Up to 86 % of patients recognize a different array of peptides.[32] And yet, commercially, the only peptide that is tested is α-gliadin 33 MER. A panel of gluten peptides, which includes a number of the more common immunodominant antigens, would provide new opportunities to screen, prevent disease development in individuals at risk,[33] and increase the sensitivity of the test to identify GS (with or without the enteropathy CD).

Antibody Array 3 includes testing for antibodies to the gluten peptides deamidated α-Gliadin 33 MER, γ-Gliadin 15 MER, α-Gliadin 17 MER, ω-Gliadin and glutenin.

EXORPHINS

Exorphins are peptides which may have activity similar to that of morphine and other opioids.[34] Five distinct exorphins have been identified in the pepsin-digest of gluten.[35] [36] The inhibitory action of the exorphins in wheat has a specific opiate effect.[37] This morphine-like psychoactive nature of the peptides results from the incomplete digestion of these dietary proteins binding to the opiate receptors in the brain, and offers a possible explanation for some of the reported psychiatric reactions to these gluten proteins, including the sense of 'brain fog' that often accompanies immune reactions to these foods[38] [39] [40] and which may follow with panic attacks, depression, or other neurological complaints.

Antibody Array 3 includes testing for antibodies to the gluten opioids Gluteomorphin and Prodynorphin.

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LECTINS

Wheat germ agglutinins (WGAs) are lectins or carbohydrate-binding proteins with a capacity to bind to many cells and tissue antigens. Lectins bind to cells involved in the immune system and induce toxic damage, inflammation, and autoimmunity. Most lectins, including WGAs, are resistant to proteolysis, the degradation of proteins by cellular enzymes. WGAs profoundly interfere with enzyme function and inhibit their digestive function.[41] [42] [43] [44] [45] By the binding of lectins to different tissue antigens, WGA can enhance antibody production against itself as well as against the tissue and cells it binds to. For example, in humans, WGA binds to the same surface receptors to which islet autoantibodies bind. Therefore, an islet cell with bound lectins would be a sitting duck for autoimmune diseases.[46] [47] In humans, the evidence incriminating wheat lectin as a cause of IgA nephropathy (IgAN) is now impressive.[48] Children with IgAN have high blood levels of anti-gliadin and anti-mesangium antibodies, and their IgA is unusually lectin-sensitive.[49] [50] Also, there is circulating WGA in the blood of children with active IgAN, which may suggest that it is, in fact, wheat lectin from the diet that may be an initiator of the autoimmune response. A gluten-free diet in IgAN patients was shown to reduce proteinuria, IgA-immune complexes and IgA food antibodies.[51]

Antibody Array 3 includes testing for antibodies to the lectin, Wheat Germ Agglutinin.

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ENZYMES

Enzymes complete this panel. Transglutaminases are a family of enzymes that form protein polymers, like scaffolding, which are vital in the formation of barriers and stable structures. Since humans have transglutaminase (tTG) in many other tissues, including bone, antibodies produced against epithelial cell tTG can cross-react with other tTGs such as bone, brain and skin. In such cases, this cross-reaction leads to autoimmune responses against other tissues and thus develops into osteoporosis, neuroautoimmunity and skin disorders. Generally, patients with elevated antibodies to tTG are susceptible to autoimmunity. Tissue transglutaminase (tTG) has been shown to form complexes with gliadin. The incubation of tTG with gliadin results in the formation of covalent tTG-peptide complexes, which can adhere to intestinal walls. This positioning allows the gliadin-tTG complex to be recognized by antigen-presenting cells, which produces an immune response cascade that results in autoantibodies. The production of these autoantibodies may perpetuate a pro-inflammatory gastrointestinal destructive cycle. Glutamic Acid Decarboxylase is a major enzyme that catalyzes the conversion of L-glutamate to γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the brain, and a putative paracrine signal molecule in pancreatic islets. The calcium-dependent transglutaminase catalyzes the formation of epsilon-(γ-glutamyl) lysine bonds between protein-bound glutamine and lysine residues. Production of antibody against GAD-65—in addition to wheat proteins and peptides—indicates not only GS beyond the gut, but possible impairment of neurotransmission and induction of cerebellar ataxia (Figure 3).

Antibody Array 3 includes testing for antibodies to enzymes GAD65, Transglutaminase and Transglutaminase bound to Gliadin.

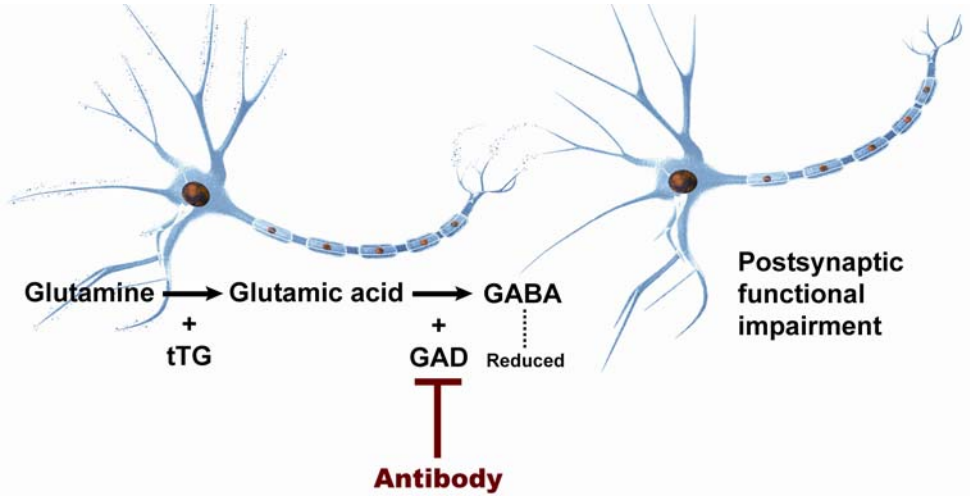

Figure 3 – *by inhibiting neurotransmission, functional impairment of the nervous system may result in cerebellar ataxia.*

Measurements of antibodies against various wheat protein peptides and enzymes associated with autoimmunities are shown in Figure 4.

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Figure 4 – *Antibody Array 3 measures IgG and IgA antibodies against wheat proteins, peptides and associated enzymes.*

INFLUENCING FACTORS:

GENETIC

Of the general population, 40-50% are carriers of DQ2/DQ8 genes, however close to 90% of Celiac disease patients carry the gene DQ2 (*DQA1*05/DQB1*02*), and a minority (10%) of the Celiac disease patients carry DQ8 (*DQA1*03/DQB1*0302*). Typically, gluten peptides bind to the DQ2 and DQ8 molecules. Recent research however, has identified at least eight new genomic regions with robust levels of disease association to Gluten Sensitivity.[52] [53]

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ENVIRONMENTAL (CHEMICALS, FOODS, BIOTOXINS, DRUGS…)

Environmental factors that have an important role in the development of Celiac disease have been suggested by epidemiologic studies. These include a protective effect of breast-feeding[54] and the introduction of gluten in relation to weaning.[55] [56]

Numerous environmental factors have been hypothesized as being catalysts for the development of not only the gluten enteropathy Celiac disease,[57] but also systemic manifestations of Gluten Sensitivity with or without the enteropathy. Some of these catalysts include bacteria,[58] viruses,[59] dysbiosis,[60] and cross-reactive foods.[61]

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HISTORY (FAMILY, MEDICAL)

Celiac disease and gluten sensitivity are characterized by a variety of clinical manifestations. These include the typical malabsorption syndrome (classic symptoms) and a spectrum of symptoms potentially affecting any organ or body system (non-classic symptoms).[62] [63] [64]

Clinical manifestations of gluten sensitivity and Celiac disease can present at any age:

- **Infancy** (less than 2 years old) – diarrhea, abdominal distention, failure to thrive (low weight, lack of fat, hair thinning), anorexia, vomiting, psychomotor impairment (muscle wasting)

- **Childhood** – diarrhea, constipation, anemia, loss of appetite, short stature, osteoporosis

- **Adulthood** – diarrhea, constipation, anemia, aphthous ulcers, sore tongue & mouth (mouth ulcers, glossitis, stomatitis), dyspepsia, abdominal pain, bloating (weight loss), fatigue, infertility, neuropsychiatric symptoms (anxiety, depression, etc.), bone pain (osteoporosis), weakness (myopathy, neuropathy).[65] [66] [67]

Reviewing current medications (antibiotics, steroids, NSAID's, etc.), supplements, diets, and a detailed medical history are critically important in determining who may have gluten sensitivity. The correlation between food ingestion and symptom onset is of great clinical importance.

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CLINICAL – SYTEMIC IMMUNE EFFECTS

Inclusion of the specific antigens comprising Antibody Array 3, or Comprehensive GS and Autoimmunity is based on recent medical research studies. Comprehensive quantitative mapping of T-cell epitopes was determined in CD.[32] Results demonstrated that patients respond to a heterogeneous array of peptides; some recognized many peptides from single or multiple gliadin families, while others reacted to only one peptide. These results confirmed that a large number of gluten epitopes may be implicated in the development of CD and associated diseases. Indeed, a T-cell line from one Celiac patient failed to recognize any of the 21 tested peptides, which confirmed that a large number of gluten and other wheat protein epitopes are implicated in development of CD and associated disorders. This suggests that other gliadin peptides and proteins are involved in the pathogenesis of GS and CD. We extended this heterogeneity in T-cell responses to gluten and other peptides originated from wheat to humoral immune responses by measuring IgG and IgA antibodies against nine different wheat antigens and peptides as well as enzymes associated with autoimmunities. Heterogeneity in IgG and IgA antibodies against these twelve antigens was confirmed by variation in antibody response against various wheat associated antigens on individual bases. Therefore, Antibody Array-3, with its measurement of IgG and IgA antibodies against a repertoire of proteins, enzymes and peptides, originated not only from α-gliadin but also from γ- and ω-gliadin, glutenins, agglutinins, exorphins, and specific enzymes involved in the pathogenesis of CD and GS, enhances the clinical sensitivity and specificity for the detection of CD and GS.[68] [69]

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CLINICAL USE OF ANTIBODY ARRAY 3

Measuring a patient's immune response to an array of wheat antigens increases the sensitivity and specificity, and will provide greater confidence in formulation of a diagnosis that allows for better patient compliance with a gluten-free diet. Assessing wheat/gluten reactivity and intestinal autoimmunity is recommended for patients who:

- Have gut dysbiosis, which appears to be resistant to standard therapy

- Are suspected of having intestinal mucosal damage
- Complain of food allergy and intolerance
- Complain of chemical hypersensitivity
- Present multiple-symptom complaints (including Chronic Fatigue Syndrome and Fibromyalgia)
- Suffer from abnormal immune cell count and function
- May suffer from blood-brain barrier permeability, depression, or neuroautoimmunity
- Neuroautoimmune patients to consider:
    - Thyroiditis
    - Arthritis
    - Myocarditis
    - Dermatitis
    - Endocrinopathy
    - Polyendocrinopathy
    - Osteoarthritis
    - Pernicious Anemia
    - Other

CLINICAL INTERPRETATION OF ANTIBODY ARRAY 3

When IgA reactions are predominant, it is an indication of possible Celiac disease and other autoimmunities.

When IgG reactions are predominant, it is an indication of wheat/gluten immune response and possible autoimmunity due to lack of digestive enzymes and/or other factors.

When both IgA and IgG reactions occur, it is an indication of wheat/gluten immune response and its progression to Celiac disease and/or other autoimmune disorders.

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INTERPRETATION OF ANTIBODIES AGAINST WHEAT, GLUTEN, ENZYME ANTIGENS							
POSITIVE REACTION TO:	GLUTEN-SENSITIVITY	WHEAT & GLUTEN-SENSITIVITY	WHEAT-SENSITIVITY	LECTIN-SENSITIVITY	AUTOIMMUNE REACTION	INTER-PRETATION	CLINICAL APPROACH
Wheat						Wheat sensitivity due to lack of digestive enzymes	Wheat-free diet Heal gut
WGA						Sensitivity to wheat germ and sprouted wheat	Check for other lectin sensitivity
γ-Gliadin 15 MER						One or any combination of positives means sensitivity to specific gluten epitopes due to lack of enzymes, in particular DPPIV	Gluten-free diet Heal gut Check for extra-intestinal autoimmunity
α-Gliadin 17 MER							
Deamidated α-Gliadin 33 MER							
ω-Gliadin							
Glutenin							
Pro-Dynorphin						Immune reaction to opioid peptides. Due to lack of digestive enzymes, in particular DPPIV	Patient is "addicted" to wheat
Gluteo-morphin							
Gliadin bound to tTG						Autoimmunity associated with wheat sensitivity	Remove trigger Use anti-inflammatories
tTG						Possible GI autoimmunity not associated with wheat sensitivity, unless wheat antigens are also positive	
GAD65						Possible autoimmunity not associated with wheat sensitivity	Remove trigger Check for Type I diabetes or other autoimmune disorders

SPECIMEN REQUIREMENT

2 mL Serum
Ambient

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RELATED TESTING

- **Antibody Array 2 - Intestinal Antigenic Permeability Screen (Serum)**
- **Antibody Array 4 - Gluten-Associated Cross-Reactive Foods and Foods Sensitivity (Serum)**
- **Antibody Array 5 - Neuroautoimmunity Panel (Serum)**

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