CLINICAL APPLICATION GUIDE

ARRAY 3X

**ARRAY 3X – Antibody**

# WHEAT/GLUTEN PROTEOME REACTIVITY & AUTOIMMUNITY™

CYREX™ Laboratories

# TABLE OF CONTENTS

# WHEAT/GLUTEN PROTEOME REACTIVITY AND AUTOIMMUNITY™

## OVERVIEW

Gluten-Reactivity 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 reactivity 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 Gluten-Reactivity cannot be overestimated. Earlier detection might result in earlier treatment, better quality of life, and an improved prognosis for these patients.[5]

The emphasis on Celiac disease as the main manifestation of Gluten-Reactivity has been questioned. It is now accepted that Gluten-Reactivity 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 Gluten-Reactivity is regarded as principally a disease of the small bowel is a historical misconception.[7]

The Gluten-Reactivity has been proposed to include not only CD, but also non-celiac gluten sensitive (NCGS) 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]) and beyond, pathology to gluten exposure can occur in multiple systems without evidence of an enteropathy.[1]

## WHEAT PROTEINS AND PEPTIDES

A kernel of wheat is usual divided into three sections.

1. Bran - The outer layer, or the seed covering, of wheat consists of bran. Bran protects the main part of the kernel. The bran comprises about 15 percent of the kernel weight. The bran is a source of protein, large quantities of the three major B-vitamins, trace minerals, and dietary fiber.
2. Endosperm - The main part of the kernel, the endosperm, accounts for 80 percent of the seed weight. It is the starchy section of wheat. This layer contains the greatest share of protein (including gluten and non-gluten), carbohydrates, and iron, as well as the major B-vitamins, such as riboflavin, niacin, and thiamine.
3. Wheat Germ - The germ, making up the remaining 5%, lies at one end of the kernel. The germ is responsible for seed germination when planted in soil. It is a rich source of B-complex vitamins, oil, vitamin E and natural plant fat.

Wheat is, therefore, packed with a variety of proteins and peptides, some of which haven't yet been defined.

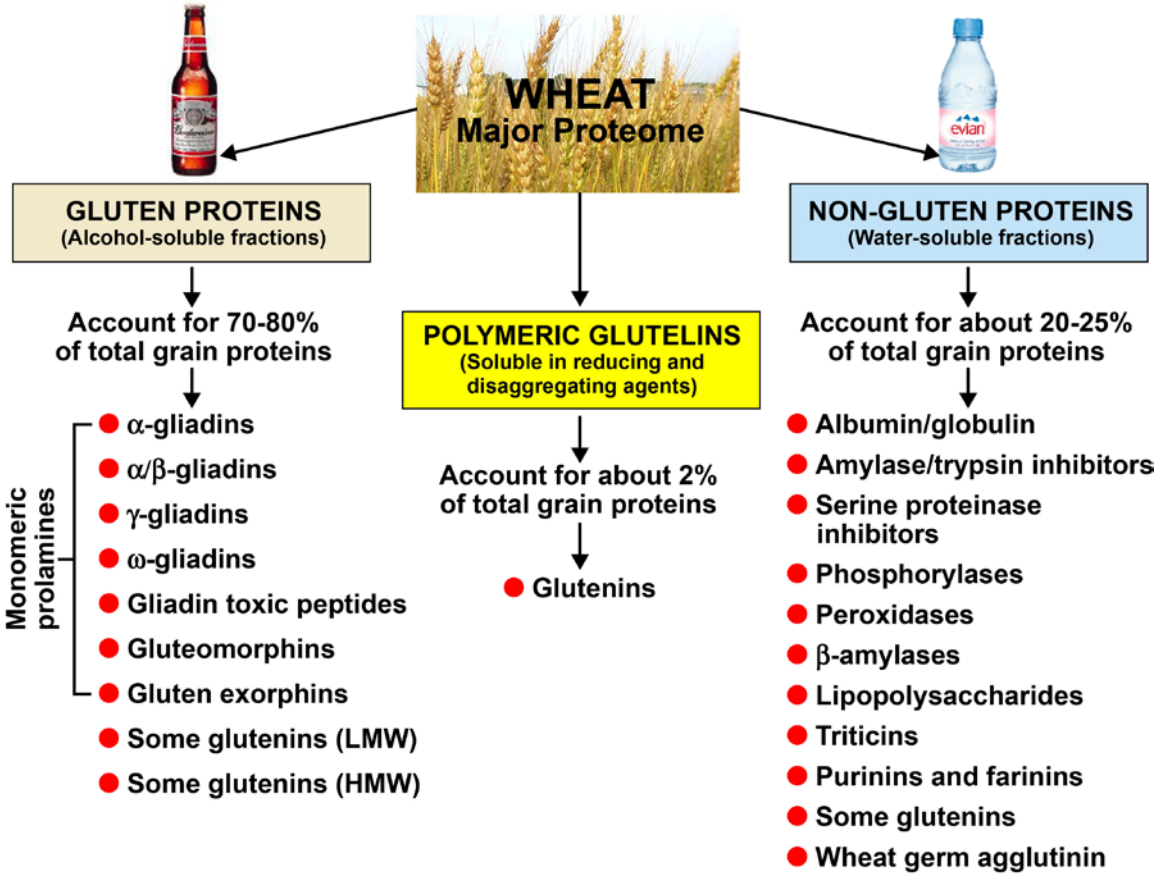


***Figure 1 – The kernel of wheat is comprised of hundreds of proteins****. These molecules can be classified as either water- or alcohol-soluble. Proteins and peptides from each category can potentially be pathogenic to the Gluten-Reactive patient. By assessing both water- and alcohol-soluble fractions of wheat, a clearer picture of Gluten-Reactivity can be obtained.*

The lion's share of wheat proteins are found in the endosperm. This is the home of the gluten family proteins. Gluten family proteins are triggers of CD and non-NCGS. Non-gluten proteins are implicated in non-celiac wheat reactivity (NCWR) and allergies. Non-gluten proteins are also involved in autoimmunity through cross-reactivity, while wheat germ agglutinin triggers autoimmunity, first by molecular mimicry mechanism, and/or by binding to human tissues. Assessment of wheat or gluten reactivity requires an array of antigens to be used for antibody measurements.

### Kernel of Truth

Wheat is made up of multiple proteins and peptides. Therefore, to properly assess wheat/gluten reactivity, a full array of proteins and peptides must be included. Cyrex is a pioneer in the measurements of multiple wheat protein immune reactivity.

## THE CYREX DIFFERENCE

Current testing for Gluten-Reactivity and (CD) includes serum IgG and IgA against gliadin and tissue transglutaminase-2 (tTG2). These antibodies are measured against minor components of a wheat protein called alpha-gliadin. However, as noted above, wheat consists of multiple proteins and peptides including, alpha-gliadin, alpha-beta-gliadin, omega-gliadin, low- and high-molecular weight glutenins, gluteomorphin, prodynorphin, farinin, serpin, amylases, globulins, albumins, agglutinins and more. Any of these antigens 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-Reactivity, CD and other associated conditions such as NCWR.

Why, if there are several proteins in wheat, do we assess reactivity to only one? The repertoire and hierarchy of gluten peptides stimulate the intestinal T-cells and results in a significant elevation of IgG and IgA production. Publications regarding T cell reactions stimulated our concept for the development of the original Array 3 – Wheat/Gluten Proteome Reactivity and Autoimmunity (launched in 2011).

Comprehensive quantitative mapping of T-cell epitopes was determined in CD.[17] 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 Gluten-Reactivity and CD.

We extended this heterogeneity in T-cell responses to gluten and other proteins or peptides originated from wheat to humoral immune responses by measuring IgG and IgA antibodies against multiple wheat antigens and peptides as well as enzymes associated with wheat/gluten autoimmunities. Heterogeneity in IgG and IgA antibodies against these antigens was confirmed by variation in antibody response against different wheat associated antigens on individual basis.

Research, performed by Aristo Vojdani,[18] confirms that different Gluten-Reactive and CD patients recognize an array of gluten and non-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.

Up to 86 % of patients recognize a different array of peptides.[17] And yet, commercially, the only peptide that is tested is alpha-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,[19] and increase the sensitivity of the test to identify Gluten-Reactivity (with or without the enteropathy CD).

### *ELISA technology*

Enzyme-Linked ImmunoSorbent Assay (ELISA) is a testing methodology that was first used for the detection of antibodies in the late 1970s. The use of ELISA grew substantially in the 1980s

and continues to be a hallmark test method against which newer testing methodologies measure the quality of the new technology.[20-24] While newer testing methods haven't gone through the full process of strengthening the weak spots of the process, these tests are being offered as clinical assessments. The unreliability of these tests are being done at the expense of patients, whose lives are subject to the results of the test. Cyrex continues to utilize ELISA because it has a proven track record for reproducibility and accuracy. The standard of a clinical assay is its reproducibility. If a lab cannot run the same specimen in duplicate and get comparable results, the testing process is flawed.

### *Cyrex Pillars*

Every single test developed by Cyrex and every single patient specimen tested by Cyrex Laboratories, LLC has the benefit of Cyrex's Four Pillars of Excellence.

1. Antigen Purification System™
   Cyrex uses advanced proprietary extraction and purification techniques to ensure that it assesses reactivity to the un-adulterated antigen, resulting in greater specificity. Without this critical step, the risk of reporting false negative or false positive results is exponentially increased.
2. Optimized Antigen Concentration™
   As antigens contain different levels of protein. Cyrex technology uses proprietary protein concentrations for each antigen to ensure that optimum protein ratios are achieved to detect immune reactivity. This is most evident in assessing food immune reactions as foods such as Almonds and Beef are much higher in protein per unit of weight than Mint or Oranges. Thus, one cannot simply put the same amount of antigen extract on the plate.
3. Antigen Specific Validation™
   To achieve Cyrex quality, we validate each antigen individually, rather than applying one arbitrarily chosen reference curve to an entire group of different antigens. The application of one chosen reference curve to a group of antigens is often done in the food testing industry. At Cyrex, it is clear with our individual reference ranges for every food protein, that we have performed individual antigen validations.
4. Parallel Testing Technology™
   Cyrex runs each patient specimen in side-by-side duplicate. Correlation between the parallel tests must be met before reporting results. If correlation is not seen, the patient specimen is pulled and rerun in quadruplicate. If correlation is still not found, Cyrex requests a new specimen. No result is released without first making sure duplicate testing is accurate on each patient specimen for each analyte tested.

Together, the Four Pillars of Excellence ensure accurate results.

**Kernel of Truth**

ELISA testing methodology for the detection of antibodies against a variety of antigens has an excellent track record. Cyrex's additional safeguards ensure the accuracy of each test result. Thus, Cyrex's ELISA results are the highest quality available.

### *Measuring antibodies*

For a comprehensive approach to prevent false negatives resulting on the traditional gluten/celiac test, pioneering, patent-pending technologies have been developed to measure IgA and IgG against wheat as the combination of gluten and non-gluten proteins/peptides as well as the components: non-gluten proteins including the lectin, wheat germ agglutinin (WGA), and antigens farinin, serpin, globulin and alpha-amylase; gliadin toxic peptides; gluten family proteins/peptides (alpha-gliadins, -17-mer and native + deamidated -33-mer, gamma-gliadin-15-mer, omega-gliadin-17-mer, glutenin-21-mer); opioid peptides (prodynorphin + gluteomorphin); gliadin-transglutaminase complex, and enzymes (tissue transglutaminases -2, -3, -6 and non-tissue transglutaminase).

**Wheat Proteome**

The full wheat kernel contains both gluten and non-gluten proteins, which are discussed below. It is the combination of multiple proteins and peptides involved in both autoimmunity and allergy. This quality of wheat antigen makes it a commonly reactive analyte. Indeed, patients with CD and/or Dermatitis Herpetiformis typically have high levels of IgG antibodies against wheat.[25] IgA antibodies against wheat are found in patients with CD.[25] Genetically susceptible people, prone to diabetes, have higher incidence of spontaneous Type 1 Diabetes when exposed to wheat antigens in association with a pro-inflammatory gastrointestinal environment.[26]

**Wheat Germ Agglutinin**

Wheat germ agglutinins (WGAs) are lectins or carbohydrate-binding proteins with a capacity to bind to many cells and tissue antigens (see WGA Binding to Human Tissues section below). The binding of WGA to human tissue receptors sets the stage for autoimmunity. The bound tissue is no longer recognized as self, thus it ignites an antibody response, which can lead to the formation of autoantibodies. Our study revealed a strong correlation between WGA antibodies and human tissue antibodies.[27] This small sampling showed 76% of patients with WGA antibodies only, gluten-family protein reactors were excluded, made antibodies to self-tissue. This percentage was higher than the non-celiac gluten sensitive group, 63% of which made antibodies to self-tissue. The number one targeted tissue was neurological.

**Non-Gluten Proteins**

Non-gluten proteins are generally soluble in water or aqueous salt solutions and function as storage or enzyme proteins. Immune reactivity and clinical manifestations of non-gluten proteins are most often associated with non-celiac wheat reactivity and hypersensitivities/allergies.[28-35] More recently, they have been associated with NCWR. Non-gluten proteins are suspected of playing a role in irritable bowel syndrome.[35] Immune reactivity to non-gluten proteins may appear in gluten-reactive patients as there is an apparent relationship with gluten family proteins. IgG and IgA antibodies to non-gluten proteins may be present due to cross-reactivity between non-gluten and gluten proteins.[36-37] Homology between γ-gliadin and non-gluten proteins has been shown.[36]

**Gliadin Toxic Peptides**

Gliadin Toxic Peptides (GTPs) are a group of peptides highly resistant to digestion. Cyrex's Chief Scientific Advisor conducted research on GTP.[37] This publication inspired Cyrex to conduct extensive validations to bring the assessment of this antigen into the clinical lab world. Depending on an individual's digestive enzymes, some may breakdown gliadins into GTPs, while others may not. GTPs bind to specific receptors on intestinal epithelial cells. The outcome of GTPs varies from individual to individual because different ligands for the same receptor induce very different biological effects.[38-39] GTPs have a tendency to bind to receptors on the epithelial cells. When GTP binds with this specific chemokine receptor induces inflammation and causes the release of zonulin.[40] A release of zonulin signals intestinal tight junctions to open. If there is multiple daily intake of gluten, these will be a continuous opening of intestinal tight junctions, which puts the body at risk for autoimmunity. The ability of GTPs to restructure cytoskeletal proteins,[40-41] breaks the intestinal barrier allowing for the infiltration of dietary proteins, gut bacterial toxins and other environmental antigens into the submucosa and into circulation. Here, immune responses against alien antigens can lead to autoimmunities. Thus, if antibodies are made against the GTP, it is advised to follow up with Array 2 – Intestinal Antigenic Permeability Screen. If actomyosin and/or occludin/zonulin are elevated, the GTP has contributed to the breakdown of the intestinal barrier. This patient would be at greater risk for autoimmunity. If the tissue antigens in Array 2 are not elevated, the GTPs have not yet broken the intestinal barrier. By removing gluten from the diet of the patient, the risk for autoimmunity is decreased.

**Gluten Proteins/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.[42] There are thousands of such gluten peptides produced during the digestive process, which are dependent on the individual's digestive enzyme function. Multiple peptides can stimulate an immune response in an individual. The protease-resistant 33-amino acid peptide from wheat α-gliadin is the major immunodominant antigen in wheat, but little is known about the hierarchy of immunodominance and consistency of recognition of T-cell epitopes *in vivo*.[43]

**Opioid Peptides**

Exorphins are peptides which may have activity similar to that of morphine and other opioids.[44] Five distinct exorphins have been identified in the pepsin-digest of gluten.[45-46] The inhibitory action of the exorphins in wheat has a specific opiate effect.[47] 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,' behavioral problems, or mood swings that often accompany immune reactions to these foods[48-50] and which may follow with panic attacks, depression, or other neurological complaints.

**Neo-Antigen**

The gliadin-transglutaminase complex is a neo (new) antigen formed when transglutaminase-2 and deamidated gliadin covalently bind to each other. The complex can adhere to intestinal walls.[51] This positioning allows the Gliadin-tTG Complex, particularly in HLA DQ2/DQ8 positive patients, to be recognized by antigen-presenting cells, which produces an immune

response cascade that results in autoantibodies against gliadin peptide, transglutaminase and Gliadin-tTG Complex.[51-53] The production of these autoantibodies may perpetuate a pro-inflammatory gastrointestinal destructive cycle.[54] Complexes may be formed between gliadin and other transglutaminases.

**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.

Microbial transglutaminase (mTg) is an enzyme produced by different bacteria that is used in the food industry. Microbial transglutaminase is not required to be included in an ingredients list. Experiments using mTg in wheat bread indicate a prevention of the deamidation of gliadin, thus making the bread less immunogenic to patients with Celiac disease.[55] Microbial transglutaminase may also be used in some medications to make them more water soluble, non-aggregating, non-immunogenic and more stable against digestion.[56] The combination of mTg with other foods can significantly alter the native food protein,[57] making the food more antigenic to a person who may not react to the native food protein. Antibodies against this ingested product can cross-react with the gliadin-transglutaminase complex.[58]

Since humans have transglutaminase (tTG) in many other tissues, including bone, antibodies produced against epithelial cell tTG2 can cross-react with other tTGs in tissues 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. tTG2, the predominant enzyme in intestinal villi, very similar to mTG, has been shown to form complexes with gliadin.[52]

Tissue Transglutaminase-3 (tTG3) is expressed mainly in the epidermis, and to a lesser extent in the placenta and the brain. tTG3 has been shown to be up-regulated in a variety of degenerative diseases.[59-60] In certain patients, gluten-sensitive enteropathy manifests as a disorder of the skin called dermatitis herpetiformis (DH).[61] DH is characterized by granular IgA deposits in the papillary dermis, which contribute to polymorphic papules and blisters often located over extensor surfaces of the major joints.[61] Patients with Huntington's disease have been shown to make elevated antibody levels to Transglutaminase-2 and -3.[60] Transglutaminase is activated by oxidative stress, during which inflammatory cytokine production increases, specifically tumor necrosis factor-alpha and interferon-gamma.[59-61] Huntington patients have been shown to produce more interferon-gamma and interleukin-2 than healthy controls.[60] Elevated tTG3 expression is seen in esophageal cancer.[62]

Tissue Transglutaminase-6 (tTG6) is expressed in neural tissue.[6] The tTG6 enzyme is not commonly expressed in the small intestine but can be found in mucosal antigen-presenting cells.[63] Due to its close homology to tTG2 and tTG3, it provides a clear possibility that tTG6 could be involved in the pathogenesis of gluten reactivity–related neurological dysfunction.[6] Researchers speculate that autoimmunity against tTG6 may result from early brain damage and associated inflammation.[64] Patients with high levels of antibodies against tTG6 are suspected of having autoimmunity against neuronal tissue. Neuronal clinical conditions may manifest as Cerebral Palsy,[64] Gluten Ataxia[6] or Peripheral Neuropathy.[6]

Antibodies against transglutaminases may appear in serum years before the clinical onset of symptoms, which may be associated with CD or other wheat-related disorders such as dermatitis herpetiformis or gluten ataxia.

## CLINICAL SIGNIFICANCE

Numerous complications, rampant in laboratory testing for gluten reactivity and its associated chronic disorders, have arisen regarding an accurate identification and diagnosis of Gluten-Reactivity, with, or without, enteropathy. Clinicians have been frustrated with the high percentage of false negative serology.[1] For example, CD has been called the "Unforgiving Master of Non-Specificity and Disguise."[65] Therefore, if Gluten-Reactivity and CD go undetected for years, the results could be devastating autoimmune conditions. Mechanisms of action are shown in the Figures 2a and 2b.

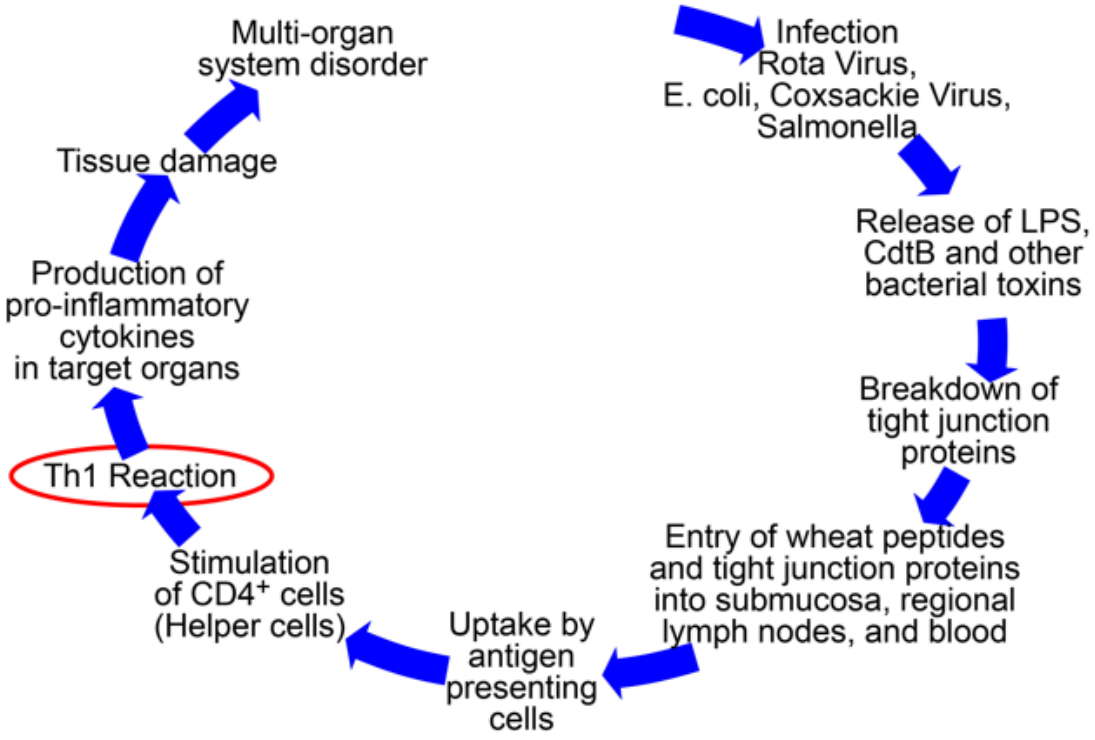


***Figure 2a – Th1 Immunological Mechanisms.*** *The mechanisms behind Gluten-Reactivity can ignite either Th-1 or Th-2 or a combination of both immune reactions. In Th1 reactions the infection is followed by release of bacterial toxins and the breakdown in intestinal tight junctions. With a broken intestinal barrier, antigen presenting cells can take antigens to helper cells igniting a Th1 reaction. Th1 pathways produce proinflammatory cytokines targeting self-tissue, this results in tissue damage or autoimmunity.*

Therefore, should Healthcare Practitioners limit their diagnostic inquisitiveness solely to the well-referenced indicators of a severe gluten enteropathy (anti-transglutaminase and endomysial antibodies)? Numerous researchers suggest not. [13 66-67]

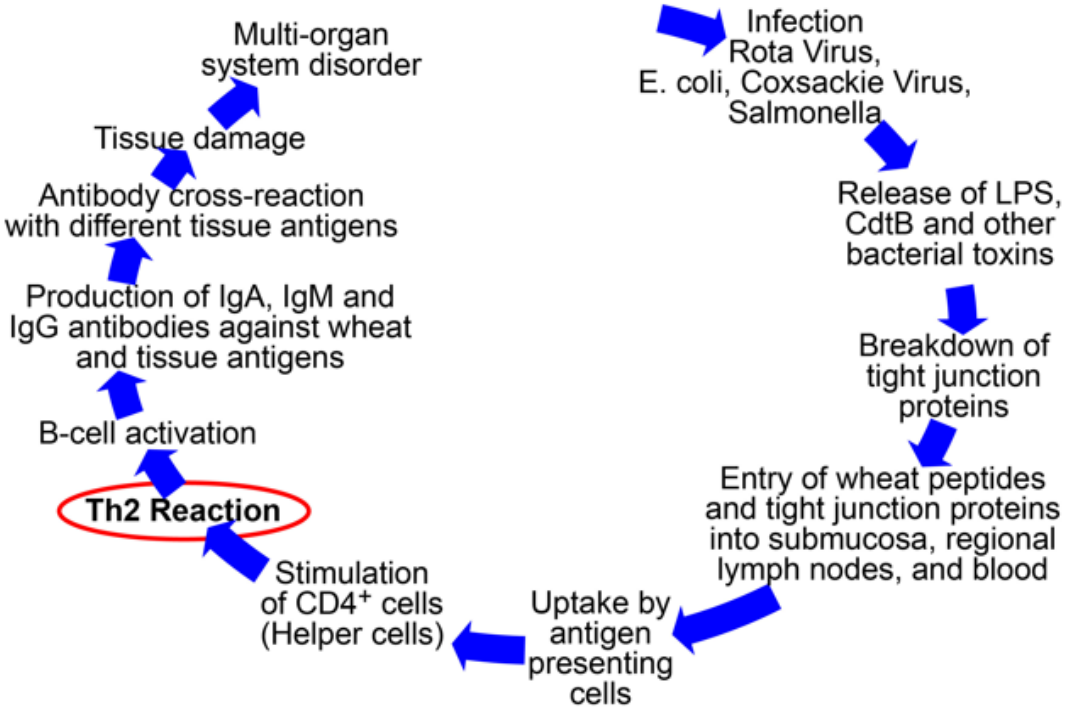


***Figure 2b – Th2 Immunological Mechanisms.*** *The mechanisms behind Gluten-Reactivity can ignite either Th-1 or Th-2 or a combination of both immune reactions. In Th2 reactions the infection is followed by release of bacterial toxins and the breakdown in intestinal tight junctions. With a broken intestinal barrier, antigen presenting cells can take antigens to helper cells igniting a Th2 reaction. Th2 pathways activate B cells, which results in antibody production. Antibodies can be made targeting wheat proteome antigens and self-tissues. Autoantibodies result in tissue damage or autoimmune disease.*

**Kernel of Truth**

Wheat reactivity can manifest as a gastrointestinal disorder such as Celiac disease. It can also manifest as an extra-intestinal condition such as ataxia, inflamed joints or skin disorders. Wheat contains both cross-reactive and binding proteins, which play roles in autoimmunity.

Accurate diagnosis of Gluten Reactivity is vital, as clinical manifestations of Gluten Reactivity can reach far beyond the gut. As discussed above, wheat or gluten reactivity, whether by antibody cross-reactivity with human tissue, or by covalent binding of wheat protein to self-tissue, (as described in the next section) has been shown to play a role in multiple autoimmune, or chronic, conditions. See Figure 3.

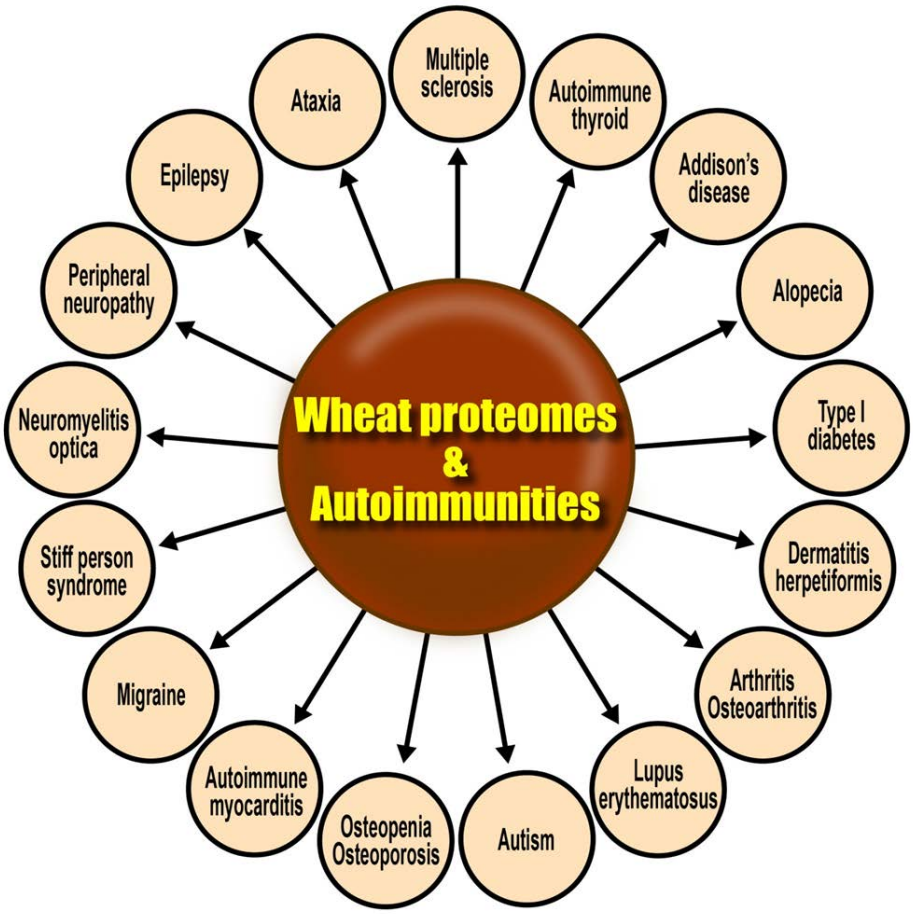


***Figure 3. Spectrum of autoimmune disorders that are associated with wheat proteomes.*** *Wheat/Gluten reactivities have been shown to play a role in multiple disorders. Many of these disorders are beyond the gut.*

When wheat/gluten reactivity is identified, the best practice is to implement a lifestyle change. By removing wheat/gluten from the diet, the pathogenic role of wheat/gluten is stopped, which in turn, may arrest the pathogenesis of autoimmunity. The strict, life-long, adherence to a gluten-free diet is essential for the prevention or exacerbation of these disorders.

Traditionally, IgA reactivity to gliadin along with tTG2 has been accepted as a serological biomarker of possible CD. Indeed, patterns arise on Array 3. IgA predominant results, with tTG2 and/or the gliadin-transglutaminase complex positive, point to possible CD, while IgG predominant results in possible NCGS.[68] Some scientists argue that IgG immune reactivity to gliadin and tTG2 in IgA suppressed individuals should be used as a serological biomarker of possible CD.[69-70]

## PATHOPHYSIOLOGY (MECHANISMS OF TISSUE DAMAGE)

Although some immune reactions to gluten peptides are due to HLA-DQ2/DQ8 genetic predisposition for CD, in those lacking this genetic composition, gluten reactivity can occur. The main mechanisms involved in food protein-induced autoimmunity, whether CD, NCGS or NCWR, are antibody cross-reactivity and the biding of food and human tissue proteins.

### Wheat Proteome and Human Tissue Cross-reactivity

Shared amino acid homology between gliadin and human tissues and human tissues has been illustrated.[72] Due to this similarity, if antibodies are produced against gliadin, in some individuals, those gliadin antibodies could potentially mistake cerebellar tissue, or thyroid peroxidase for gliadin, and thus, attack self-tissue as if it were a foreign antigen. Figure 4 illustrates the mechanism of food contributing to autoimmunity via antibody cross-reactivity. In an earlier study, Vojdani and Tarash[73] found significant cross-reactivity between gliadin antibody and multiple human tissues including: asialoganglioside, cytochrome P450, glutamic acid decarboxylase 65, 21 hydroxylase, myelin basic protein, cerebellar, osteocyte, synapsin, myocardial peptide, ovary, thyroid peroxidase. Indeed, we found a significant number of gluten-reactive patients made autoantibodies.[27] In these study, we selected patients who reacted only IgG to gluten family proteins. Those with IgA reactions were excluded. Of the IgG positive patients, 64% of them reacted to one or more of his/her tissues.[27] The number one targeted tissue was neurological followed by adrenal and a close third was heart.

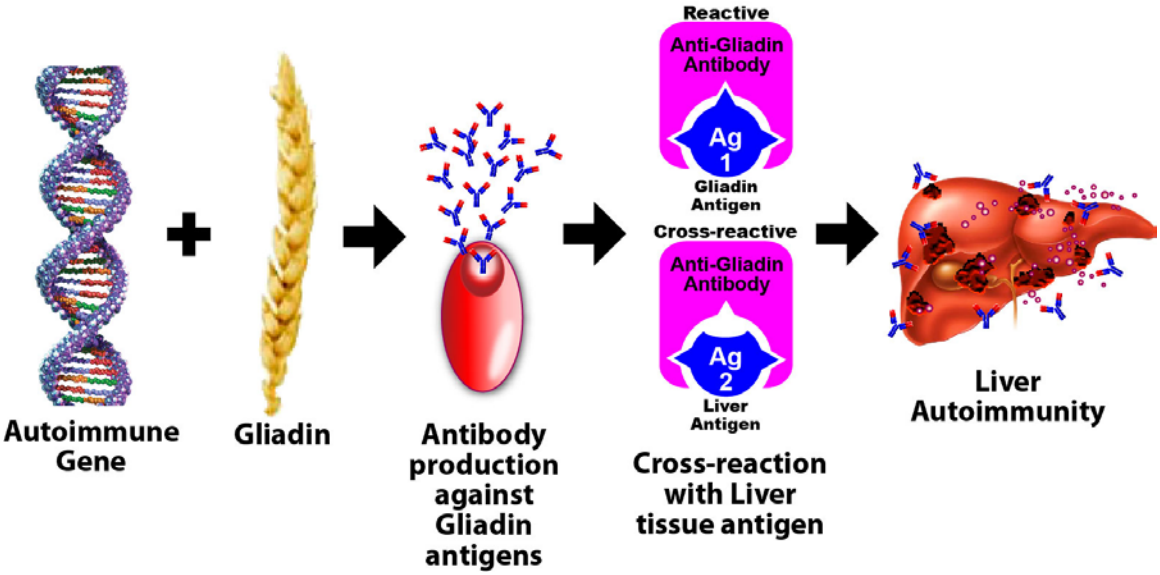


***Figure 4. Mechanism of cross-reactivity.*** *In a genetically-susceptible individual, the ingestion of wheat may produce antibodies against gliadin. Those antibodies made to target gliadin may mistake liver enzyme cytochrome P450 for gliadin and attack it. This attack on self-tissue may result in liver autoimmunity.*

## WGA Binding to Human Tissues

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 capabilities.[73-77] WGA has an affinity for binding to skin, buccal mucosa, stomach, parietal cells, intestinal brush border, colonic mucosa, connective tissue, thyroid, cartilage, liver, pancreas, kidney, prostate, skeletal muscle, cardiac muscle, breast, pituitary, eye and myelin.[78] By binding to different tissues, WGA can enhance antibody production against itself, as well as, against the tissue, and cells, to which it binds. 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.[79-80] In humans, the evidence incriminating WGA as a cause of IgA nephropathy (IgAN) is now impressive.[81] Children with IgAN have high blood levels of anti-gliadin antibodies, and their IgA is unusually lectin-sensitive.[82-83] Also, there is circulating WGA in the blood of children with active IgAN, which may suggest that it is, in fact, WGA 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.[82] Therefore, WGA reactivity is no less serious than gluten reactivity. We found that patients who reacted to WGA, but not gluten proteins, reacted strongly against self-tissues.[27] Neuronal tissues were the favored target. In addition to WGA binding to receptors on a variety of tissues, very recent experiment in our lab showed that antibody made against WGA reacted from moderate to strongly with 18 out of 30 different tested tissue antigens. This include: thyroid peroxidase, ASCA+ANCA, alpha-myosin, ovary/testis, insulin and islet cell, myelin basic protein, and dopamine receptor. The results of this research indicate that if immune reaction to WGA, is left undetected, can result in multiple autoimmunities years down the road.

## 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-Reactivity.[84-85] Since only 1%-3% of those carriers of the D22/D28 genes, may develop celiac disease during their lifetime, screening for these genes is not recommended.

### Environmental

Numerous environmental factors have been hypothesized as being catalysts for the development of not only the gluten enteropathy CD,[86] but also systemic manifestations of Gluten-Reactivity with or without the enteropathy. Some of these catalysts include bacteria,[87] viruses,[56] dysbiosis,[88] and cross-reactive foods.[84]

Environmental factors that have an important role in the development of CD have been suggested by epidemiologic studies. These include a protective effect of breast-feeding[89] and the introduction of gluten in relation to weaning.[90-91]

### Family History

CD and Gluten-Reactivity 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).[6, 92-93]

Clinical manifestations of Gluten-Reactivity and CD 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).[94-96]

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.

**Kernel of Truth**

Between genetic susceptibility, broken intestinal barriers, and environmental triggers, wheat, due to its structural similarity to a variety of human tissues, plays a major role in autoimmunities.

## MEASURING WHEAT/GLUTEN REACTIVITY AND AUTOIMMUNITY

We measured IgG and IgA antibodies against various wheat proteins/peptides/microbial transglutaminase and various tissue transglutaminases in 784 blood samples representing the general population. The results summarized in Fig 5, show that between 16%-36% of individuals produced significant levels of IgG antibodies against various wheat proteins. The IgG immune reactivity against non-gluten proteins was the lowest in 16%, compared to wheat proteins in 36%. The reaction of IgA antibody was the lowest against glutenin at 8%, against wheat protein at 26.8%. In relation to transglutaminases, IgG antibody was elevated in 14.8%, 18.6%, 20%, and 26.8% against tTG-6, mTG, tTG-3 and tTG-2 respectively. While the IgA antibody was elevated against tTG-6 in 7.4%, mTG in 10.5%, tTG-3 in 10.7%, and against tTG-2 in 15% of the general population. These results indicate that up to 36% of the general population are reactive to various wheat proteins and hence, may suffer from NCGS or NWGR. Furthermore, up to 27% of the general population produces IgG antibodies against various wheat proteins. If these IgA

antibodies are produced simultaneously against mTG, tTG-2 or tTG-3 may result, not only in celiac disease, but also in other associated autoimmunities including neuro-autoimmunity (Figure 5).

The measurement of antibodies to the wheat proteome requires adequate and timely exposure to wheat. Those who have been on a gluten-free diet for months may no longer carry antibodies to the food and thus, may produce false negative results. Patients on immune suppressing medications may also produce false negative results. For the most accurate results, ask the patient about his/her consumption of wheat products and any medications, including over the counter products used during the past months.

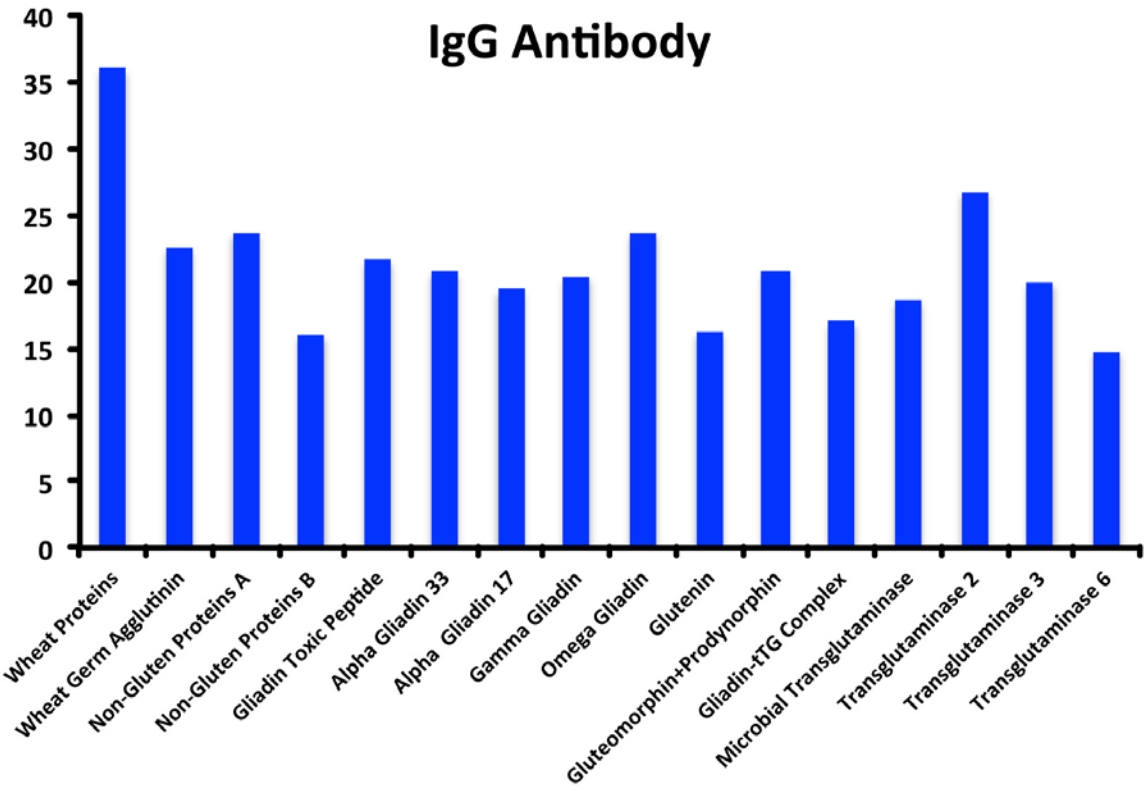


***Figure 5. Percent elevation of IgG against wheat proteomes and transglutaminases****. 784 blood donors specimens were assessed using the Array 3X. This chart shows the percent elevation of antibodies for each antigen.*

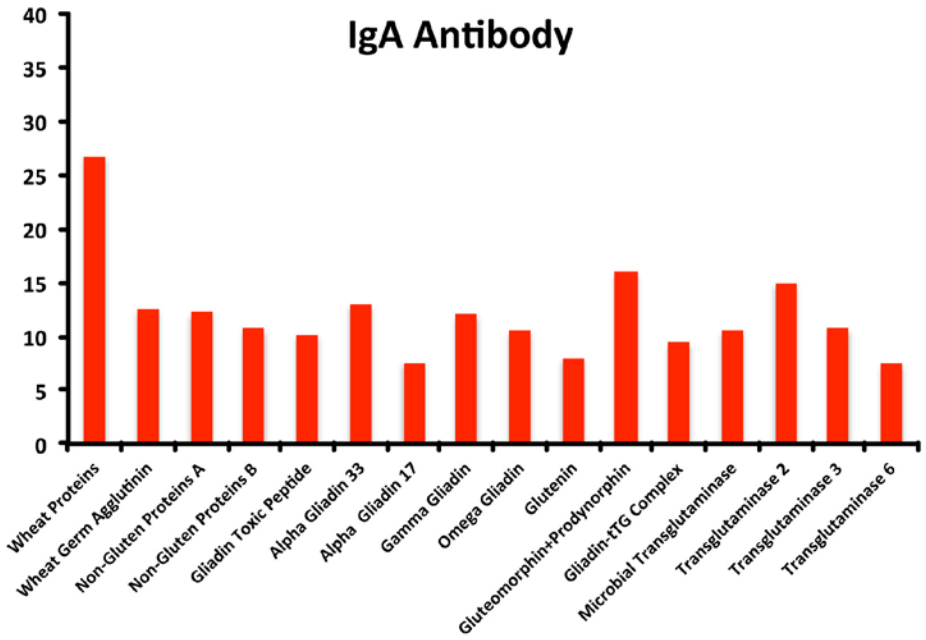


***Figure 6. Percent elevation of IgA against wheat proteomes and transglutaminases****. 784 blood donors specimens were assessed using the Array 3X. This chart shows the percent elevation of antibodies for each antigen.*

## CLINICAL USE OF ANTIBODY ARRAY 3X

Measuring a patient's immune response to an array of the wheat proteome 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. Array 3X – Wheat/Gluten Proteome Reactivity and Autoimmunity can be used to:

- Identify possible Celiac disease, non-celiac gluten sensitivity, non-celiac wheat sensitivity, dermatitis herpetiformis, gluten ataxia or other wheat/gluten-related disorder.
- Assess autoimmune reactivity associated with wheat proteins and peptides.

Assessing wheat/gluten reactivity and intestinal autoimmunity is recommended for patients who:

- Have non-responsive GI symptoms.
- Present multiple-symptom complaints (including Chronic Fatigue Syndrome and Fibromyalgia).
- Suffer from early symptoms of autoimmunities including neuro-autoimmunity.

Consider Array 3X for patients who suffer from wheat-related disorders:

- Thyroiditis
- Arthritis
- Myocarditis
- Dermatitis
- Endocrinopathy

- Polyendocrinopathy
- Osteoarthritis
- Pernicious anemia
- Irritable bowel syndrome
- Crohn's disease
- Ulcerative colitis
- Others

## CLINICAL INTERPRETATION OF ANTIBODY ARRAY 3X

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.

Please review the Array 3X interpretation webinar available on our website's "Education Library" for interpretation details and a review of test results.

***Array 3X, by itself, is not diagnostic for any condition or disease. Array 3X results can be used in conjunction with other pertinent clinical information in the formation of a diagnosis.**

| IgG | IgA | Wheat |
|---|---|---|
| + | + | Wheat reactivity. |
| - | + | Wheat reactivity. |
| + | - | Wheat reactivity. |
| - | - | No detectable Wheat reactivity. |
| **IgG** | **IgA** | **Wheat Germ Agglutinin (WGA)** |
| + | + | Lectin reactivity. |
| - | + | Lectin reactivity. |
| + | - | Lectin reactivity. |
| - | - | No detectable WGA reactivity. |
| **IgG** | **IgA** | **Non-Gluten Proteins - A** |
| + | + | Wheat reactivity. |
| - | + | Wheat reactivity. |
| + | - | Wheat reactivity. |
| - | - | No detectable Non-Gluten Proteins-A reactivity. |
| **IgG** | **IgA** | **Non-Gluten Proteins - B** |
| + | + | Wheat reactivity. |
| - | + | Wheat reactivity. |
| + | - | Wheat reactivity. |
| - | - | No detectable Non-Gluten Proteins-B reactivity. |

| IgG | IgA | Gliadin Toxic Peptides (GTPs) |
|---|---|---|
| + | + | GTPs can lead to release of zonulin and breakdown of the intestinal barrier. |
| - | + | GTPs can lead to release of zonulin and breakdown of the intestinal barrier. |
| + | - | GTPs can lead to release of zonulin and breakdown of the intestinal barrier. |
| - | - | No detectable Gliadin Toxic Peptides reactivity. |
| **IgG** | **IgA** | **Native + Deamidated Alpha Gliadin 33** |
| + | + | Gluten reactivity |
| - | + | If tTG2 IgA positive, possible case of Celiac disease.* |
| + | - | Indication of non-Celiac gluten sensitivity.* |
| - | - | No detectable Native + Deamidated Alpha Gliadin 33 mer reactivity. |
| **IgG** | **IgA** | **Alpha Gliadin 17 mer** |
| + | + | Gluten reactivity |
| - | + | If tTG2 IgA positive, possible case of Celiac disease.* |
| + | - | Indication of non-Celiac gluten sensitivity.* |
| - | - | No detectable Alpha Gliadin 17 mer reactivity. |
| **IgG** | **IgA** | **Gamma Gliadin 15 mer** |
| + | + | Gluten reactivity |
| - | + | If tTG2 IgA positive, possible case of Celiac disease.* |
| + | - | Indication of non-Celiac gluten sensitivity.* |
| - | - | No detectable Gamma Gliadin 15 mer reactivity. |
| **IgG** | **IgA** | **Omega Gliadin 17 mer** |
| + | + | Gluten reactivity |
| - | + | If tTG2 IgA positive, possible case of Celiac disease.* |
| + | - | Indication of non-Celiac gluten sensitivity.* |
| - | - | No detectable Omega Gliadin 17 mer reactivity. |
| **IgG** | **IgA** | **Glutenin 21 mer** |
| + | + | Gluten reactivity |
| - | + | If tTG2 IgA positive, possible case of Celiac disease.* |
| + | - | Indication of non-Celiac gluten sensitivity.* |
| - | - | No detectable Glutenin 21 mer reactivity. |
| **IgG** | **IgA** | **Gluteomorphin + Prodynorphin** |
| + | + | Immune reactivity to wheat/gluten-derived opioids.<br>This patient may be addicted to wheat/gluten and may experience withdrawals on a gluten-free diet. |
| - | + | Immune reactivity to wheat/gluten-derived opioids.<br>This patient may be addicted to wheat/gluten and may experience withdrawals on a gluten-free diet. |
| + | - | Immune reactivity to wheat/gluten-derived opioids.<br>This patient may be addicted to wheat/gluten and may experience withdrawals on a gluten-free diet. |
| - | - | No detectable Gluteomorphin + Prodynorphin reactivity. |
| **IgG** | **IgA** | **Gliadin-Transglutaminase Complex** |
| + | + | Indication of severe gut damage. Commonly seen in Celiac disease. |
| - | + | Indication of severe gut damage. Commonly seen in Celiac disease. |
| + | - | Indication of severe gut damage. Commonly seen in Celiac disease. |
| - | - | No detectable Gluteomorphin + Prodynorphin reactivity. |

| IgG | IgA | Microbial Transglutaminase |
|---|---|---|
| + | + | Immune reactivity to mTg. |
| - | + | Immune reactivity to mTg. |
| + | - | Immune reactivity to mTg. |
| - | - | No detectable mTg reactivity. |
| **IgG** | **IgA** | **Tissue Transglutaminase-2** |
| + | + | Autoimmune reactivity to tTG2 |
| - | + | Indication of intestinal villi damage. |
| + | - | Reactivity to tTG2 – may be extra-intestinal. |
| - | - | No detectable Tissue Transglutaminase-2 reactivity. |
| **IgG** | **IgA** | **Tissue Transglutaminase-3** |
| + | + | Indicates autoimmune reactivity to skin transglutaminase. |
| - | + | Indicates autoimmune reactivity to skin transglutaminase. |
| + | - | Indicates autoimmune reactivity to skin transglutaminase. |
| - | - | No detectable Tissue Transglutaminase-3 reactivity. |
| **IgG** | **IgA** | **Tissue Transglutaminase-6** |
| + | + | Indicates autoimmune reactivity to neurological transglutaminase. |
| - | + | Indicates autoimmune reactivity to neurological transglutaminase. |
| + | - | Indicates autoimmune reactivity to neurological transglutaminase. |
| - | - | No detectable Tissue Transglutaminase-3 reactivity. |

## SPECIMEN REQUIREMENT

2 mL Serum
Ambient

## RELATED TESTING

- **Antibody Array 4 - Gluten-Associated Cross-Reactive Foods and Food Sensitivity**
- **Antibody Array 2 - Intestinal Antigenic Permeability Screen (Serum)**
- **Antibody Array 22 - Irritable Bowel / SIBO Screen (Serum)**
- **Antibody Array 20 - Blood-Brain Barrier Permeability Screen (Serum)**
- **Antibody Array 5 - Multiple Autoimmune Reactivity Screen (Serum)**
- **Antibody Array 6 - Diabetes Autoimmune Reactivity Screen (Serum)**
- **Antibody Array 7/7X - Neurological Autoimmune Reactivity Screen (Serum)**
- **Antibody Array 8 - Joint Autoimmune Reactivity Screen (Serum)**

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