ARRAY 1

ARRAY 1 - Antibody

MUCOSAL GLUTEN REACTIVITY SCREEN™

Cyrex Laboratories
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OVERVIEW

MUCOSAL IMMUNITY

The mucosal immune system acts as the primary host defense against the physical environmental factors (food, airborne molecules, viruses and commensal antigens), and plays a significant role in barrier functions. The intestinal mucosal interface is a complex system that must integrate interactions among the microbiota, biofilms, mucus layer, associated protective compounds, defensins, enzymes, secretory IgA, epithelial physiological interconnections, and underlying immune cells of the lamina propria. Notably, it has become clear that both the state of the microbial community and underlying immune cells contribute to the health or disease of the host. Secretory immunoglobulins IgA and IgM are important components of the first line of defense that operates at all mucosal sites.
CLINICAL IMPORTANCE OF SECRETORY IgA IN ORAL FLUID

Secretory Immunoglobulin A (SIgA) is a major immunoglobulin in saliva, and is essential in the protection of mucosal surfaces against dietary proteins and infections. Compared to IgG, IgM and IgA in the blood, IgA in secretion is the most abundant antibody. Between 5-7 grams/day are produced and distributed in the secretory components, including saliva. By covering the epithelial cell layers of mucosal surfaces, SIgA protects against the colonization, and possible invasion, of pathogenic microorganisms (see Figure 1). Any deficiencies or increase in secretion of secretory IgA may result in or be associated with a pathology and eventual progression to disease.

**Figure 1 – The major antibody isotype present in the lumen of the gut is secretory polymeric IgA. Secretory IgA is synthesized by plasma cells in the lamina propria and transported into the lumen of the gut through epithelial cells at the base of the crypts. Polymeric IgA binds to the mucus layer overlying the gut epithelium and acts as an antigen-specific barrier to the pathogens and toxins in the gut lumen.**

FUNCTION OF SALIVARY IgA

The remarkable stability of SIgA makes it well suited to function in protease-containing secretions produced by many pathogens. Several oral bacteria such as *streptococcus sanguis*, *porphyromonas gingivalis*, bacteroides, and capnocytophaga, which are involved in periodontal disease, can produce proteases to selectively cleave SIgA, enhance epithelial colonization, and invade the mucosal tissues. Secretory IgA production is increased during enhanced colonization in order to prevent bacterial invasion and tissue pathology. In the mucous membrane, diseases such as cutaneous candidiasis, due to mucosal immune deficiency, may cause salivary IgA production to *Candida albicans* to be low. This is similar to patients with low capacity for IgA antibody production against herpes infection, who are predisposed to recurrent infection and complications. Conversely, heavy smokers and/or chronic alcoholics have increased IgA levels in whole saliva, and the same is true for patients with oropharyngeal carcinoma. It
Antibody Array 1 – Mucosal Gluten Reactivity Screen

has been reported that total and specific salivary IgA tends to be reduced in infection-prone children with no overt immunodeficiency. The result could be explained by degradation of SIgA by microbial proteases.\(^4\) Moreover, salivary IgA may be decreased in children with recurrent tonsillitis or adenoid hyperplasia, and also in asthmatic children when wheezing is precipitated mainly by recurrent respiratory tract infections.\(^5\)\(^6\) Conditions that may change the level of SIgA\(^7\)\(^8\) in oral fluid are shown in Table 1. The measurement of secretory IgA in oral fluid is recommended for the early detection of these conditions or triggers.

Table 1

<table>
<thead>
<tr>
<th>Increased Secretory IgA Level</th>
<th>Decreased Secretory IgA Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute stress</td>
<td>Chronic stress</td>
</tr>
<tr>
<td>Some medications</td>
<td>Some medications</td>
</tr>
<tr>
<td>Oropharyngeal carcinoma</td>
<td>Adrenal insufficiencies</td>
</tr>
<tr>
<td>Chronic oral infection</td>
<td>Bacterial colonization on molar surfaces</td>
</tr>
<tr>
<td>Chronic GI infection</td>
<td>Recurrent tonsillitis</td>
</tr>
<tr>
<td>Heavy smoking</td>
<td>Adenoid hyperplasia</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>Cutaneous candidiasis</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Asthmatic with recurrent respiratory tract infection</td>
</tr>
<tr>
<td>Dental plaque accumulation</td>
<td>Intestinal barrier dysfunction</td>
</tr>
<tr>
<td>Intestinal barrier dysfunction</td>
<td>Nutritional deficiencies</td>
</tr>
<tr>
<td>Celiac disease, Crohn’s disease</td>
<td>Recurrent herpes infection</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Celiac disease, Crohn’s disease, Ulcerative colitis</td>
</tr>
</tbody>
</table>

It has been shown that patients with untreated Celiac disease (CD) or gluten-sensitive enteropathy have elevated levels of SIgA antibodies against wheat gluten.\(^9\)\(^10\) These antibodies almost disappear after weeks of treatment with a gluten-free diet. In an attempt for finding a non-invasive method to screen for CD, gliadin antibodies were measured in both saliva (unstimulated whole saliva) and serum. Interestingly, measurements of IgA antibodies in saliva were equally efficient or even better in discriminating between healthy individuals versus patients.\(^11\)\(^12\) Findings strongly recommend measurement of salivary antibodies for the assessment of mucosal immune function and screening for Gluten Sensitivity (GS) and CD.
GLUTEN SENSITIVITY AND CELIAC DISEASE SCREEN

Gluten Sensitivity is a systemic clinical condition with diverse manifestations. Celiac disease, or gluten-sensitive enteropathy, is only one aspect of a range of possible manifestations of gluten sensitivity. And yet, this enteropathy is "one of the most common lifelong disorders in both the U.S. and Europe." Auto-immune disease, the third leading cause of Morbidity and Mortality in the industrialized world, is 10 times more common in Celiac disease than in the general population. Thus, the burden on society from Gluten Sensitivity cannot be overestimated. Earlier identification might result in earlier treatment, better quality of life, and an improved prognosis for these patients.

Indeed, in several studies it has been shown that salivary IgA antigliadin and anti-transglutaminase antibodies could predict forthcoming gluten sensitivity and Celiac disease. These findings are based on the fact that under normal conditions mucosal surfaces do not react to many dietary proteins and infectious agent antigens. The immune reactions to these antigens occur due to a breakdown in immune tolerance, which may lead to the production of IgA and IgM antibodies against gliadin and transglutaminase, pro-inflammatory cytokines in oral fluid, and subsequent inflammation and tissue damage or autoimmunity.

A recent observation outlines the capacity of secretory IgA immune complexes to promote the retrotransport of intact gliadin peptides across the intestinal epithelium in patients with active CD. The role of a defective epithelial barrier may be to promote the entrance of gluten peptides through...
It seems that dietary antigens, including gluten peptides, are complexed to intraluminal secretory IgA that is produced against them. The gliadin peptides now complexed with secretory IgA bind to the IgA receptor, which then transports and protects them from lysosomal degradation through a specific transcytosis pathway, thereby perpetuating the immune inflammatory responses, which result in the production of IgA, IgM and cytokines in oral fluid.

Another possibility is that antigen-sensitized cells from the gut enter the circulation and then populate within secretory tissues, where final differentiation into IgA-secreting plasma cells occurs. During this process, a subset of these cells in the form of memory cells that recognize food antigens remains in circulation. Upon the entry of food antigens into the circulation, this population of memory cells will respond to these antigens and produce IgA and IgG antibodies against dietary proteins in blood. Studying the pattern of antibody production in IgA-deficient individuals provides supporting evidence for the existence of memory lymphocytes reacting to bacterial or food antigens. In these IgA-deficient individuals, ingestion of the bacterial antigen led to the appearance of IgM-producing cells in peripheral blood and secretory IgM antibodies in saliva. A different mechanism for the production of IgA antibodies in blood is spillover from increased mucosal IgA production. This is very well established in patients with Celiac disease, where the number of jejunal IgA immunocytes and the level of IgA gliadin antibodies in saliva correlate with circulating IgA gliadin antibodies. Conceivably, an intestinal immune reaction involving IgA immune complexes and proinflammatory cytokines may lead to enhanced intestinal permeability, increased antigen exposition, and intensified production of IgA and IgG activities in serum. Indeed, untreated Celiac patients compared to control groups show significantly higher IgG and IgA activity against gliadin and transglutaminase.

**Clinical Indication of IgA and IgM Antibody in Saliva**

IgA and IgM antibody production against dietary proteins, such as gliadin, and the building enzyme tissue transglutaminase, in saliva indicate a defect in oral tolerance which can result in inflammatory conditions in the bowel. In fact, defects in the mechanism of oral tolerance have been reported as being responsible for several diseases of the gastrointestinal and respiratory tract in particular gastric autoimmunity.

After repeated exposure of mucosal immune cells to dietary proteins and production of IgA + IgM in the mucosal secretions, these antibodies then interact with many dietary proteins (antigens), resulting in immune complex formation, which further contributes to the inflammatory reaction in the gastrointestinal tract.

Based on this mechanism of action, saliva is a source of body fluid for detection of an immune response (90% IgA and 10% IgM) to bacterial, food, and other antigens present in the oral cavity and gastrointestinal tract. Indeed, salivary antibody induction has been widely used as a model system to study secretory responses to ingested material, primarily because saliva is easy to collect and analyze. Therefore, its analysis is used to measure a wide range of antibodies against gliadin, other dietary proteins and transglutaminase. Therefore, the protective and lubricating properties of saliva meet the demand for inexpensive and easy-to-use diagnostic aids for oral and systemic diseases, including gluten intolerance and Celiac disease.

Gluten intolerance and Celiac disease have increasingly become a problem that concerns many patients and clinicians. Although the percentage of individuals with IgA deficiency is less than 5, our IgA + IgM
antibody in saliva against gliadin and transglutaminase was developed in order to cover the mucosal immune reaction against these antigens in non-IgA-deficient (95%) and the IgA-deficient (about 5%) individuals. Therefore, using this patented saliva test will accurately inform a physician of clinical conditions and aid him or her in the detection of a breakdown in immune tolerance and in the diagnosis of patients with gluten intolerance and possible Celiac disease.44

**SALIVA ANTIBODIES AS EARLY BIOMARKERS OF AUTOIMMUNITY**

Since the mucosal immune system is a central component of host defense, as a whole, any dysregulation and inflammatory reaction in the GI tissue results in intestinal barrier dysfunction and the entry of digested dietary proteins into the circulation. Dietary proteins in the circulation result in systemic immune response and the production of very high levels of IgG and IgA against dietary proteins and peptides.

This breach of the intestinal barrier by dietary proteins due to loss of tolerance not only can lead to IgG and IgA production in blood, but also might lead to an immune response to different target organs and the induction of autoimmune diseases.45 46 47 48 49 50 51 52 53 54 55 56 57 58

Therefore, for a complete picture of gluten intolerance and Celiac disease, both IgA + IgM antibodies in saliva and IgG and IgA in blood, must be examined before therapeutic interventions can be implemented. We concluded that diseases of the GI tract and autoimmune disease cannot be fully understood and treated without determining the coordination of the mucosal and systemic immune response against dietary proteins and peptides, which includes IgA + IgM in saliva, and IgG and IgA in blood against gliadin and its target antigen transglutaminase. This comprehensive approach was borne out of 25 years of research experience.

Measurement of IgA + IgM against gliadin and transglutaminase in oral fluid for screening, and IgG and IgA in blood as a confirmatory test can be used to help clinicians evaluate their patients for inflammation and autoimmunity. This evaluation can then be used to design new therapeutic strategies that may include elimination diets, reestablishing the intestinal barrier function, the use of pre-and pro-biotics, glutathione, glutamine, lipoic acid EPA/DHA, and medication or nutritional supplements with anti-inflammatory characteristics.59 60 61 62 63 64 65 66 67 68

**INFLUENCING FACTORS:**

**GENETIC**

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.69 70 Between 40 – 50% of the population is a DQ2/DQ8 gene carrier, thus with a 50% probability for being a gene carrier, genetic testing is not recommended as a diagnostic tool.
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 and the introduction of gluten in relation to weaning.

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

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).

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)

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.

CLINICAL – SYSTEMIC IMMUNE EFFECTS

The range of autoimmunities associated with Gluten Sensitivity includes the enteropathy Celiac disease, initiated by the autoimmune response to one or more of the peptides of gluten, and many other antibodies.

The enigma of the Gluten Sensitivity-associated autoantibodies being an epiphenomenon or playing a pathogenic role remains unresolved and presents a challenging area for future research. However, the "take-home" messages include:

- Numerous autoantibodies are prevalent in CD patients and their first-degree relatives.
• Longer exposure to gluten increases the risk for developing autoimmune diseases in patients with CD.\textsuperscript{86}

• Maintaining a gluten-free diet and cross-reactive food antigens reduces the risk of developing antibodies and eventual autoimmune diseases.\textsuperscript{87}

• Several autoantibodies reflect the degree of damage to the intestinal mucosa and resolve completely on a gluten-free diet.

In summary, the measurement of salivary IgA + IgM antibody to gliadin and transglutaminase is potentially useful for screening patients who may have GS or CD. The assay of salivary IgA + IgM antibodies to gliadin and transglutaminase offers a non-invasive test, which would be particularly useful in:

- The investigation of gluten sensitivity and Celiac disease (gluten sensitive enteropathy)
- Monitoring of patients with a family history of autoimmunity, especially CD
- Tracking compliance with a gluten-free diet
- Detecting potential CD before villous atrophy occurs

**CLINICAL USE OF ANTIBODY ARRAY 1**

This Array is a very cost effective, easy, and non-invasive method, which measures autoantibodies against gliadin and tTG in oral fluid (IgA+ IgM). It is estimated that the prevalence of gluten sensitivity is higher than current epidemiological statistics due to greater sensitivity of this test and earlier detection of antibodies. Antibody Array 1 also measures total Secretory IgA, which can assist Healthcare Practitioners in obtaining a better clinical picture of background conditions, such as stress, infections, alcohol, etc.

Screening for Gluten Sensitivity and Celiac disease through this array will have the following advantages:

• Identification and replacement of hidden nutritional deficiencies
• Relieving mild intestinal and many unreasonable extraintestinal symptoms and signs
• Decreasing the risk for malignancy
• Decreasing the chance for autoimmunity
• Decreasing the chance for neurological issues related to gluten sensitivity
**CLINICAL INTERPRETATION OF ANTIBODY ARRAY-1**
**MUCOSAL GLUTEN REACTIVITY SCREEN**

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>High</th>
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</thead>
<tbody>
<tr>
<td><strong>Secretory IgA</strong></td>
<td>- Chronically Stress</td>
<td>- Acute Stress</td>
</tr>
<tr>
<td></td>
<td>- Adrenal Insufficiencies</td>
<td>- Chronic Oral and GI Dysbiosis</td>
</tr>
<tr>
<td></td>
<td>- Intestinal Barrier Dysfunction</td>
<td>- Intestinal Barrier Dysfunction</td>
</tr>
<tr>
<td><strong>Gliadin IgA + IgM</strong> combined in Oral Fluid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><strong>Transglutaminase IgA + IgM</strong> combined</td>
<td>-</td>
<td>+</td>
</tr>
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<td></td>
<td></td>
<td>+</td>
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<tr>
<td><strong>Indication</strong></td>
<td>- Breakdown in immune tolerance</td>
<td>- Enzyme Deficiencies</td>
</tr>
<tr>
<td></td>
<td>- An early event in gluten sensitivity</td>
<td>- Gastro-Auto-Immunities</td>
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<tr>
<td></td>
<td>- Other food sensitivities</td>
<td>- Breakdown in immune tolerance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- An early and late event in GS and possible CD</td>
</tr>
<tr>
<td><strong>Clinical Approach</strong></td>
<td>- Gluten-free diet</td>
<td>- Digestive enzymes</td>
</tr>
<tr>
<td></td>
<td>- Measure adrenal function</td>
<td>- Anti-inflammatory supplements</td>
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<tr>
<td></td>
<td>- Digestive enzymes</td>
<td>- Gluten-free diet</td>
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<td></td>
<td>- Pre- &amp; Pro-biotics</td>
<td>- Pre- &amp; Pro-biotics</td>
</tr>
<tr>
<td></td>
<td>- Elimination diet</td>
<td></td>
</tr>
</tbody>
</table>

*See Table 1: Conditions or triggers that can change the level of Secretory IgA in oral fluid*

Measurement of IgA + IgM against gliadin and transglutaminase in oral fluid for screening, and IgG and IgA in blood as a confirmatory test can be used to help clinicians evaluate their patients for inflammation and autoimmunity. This evaluation can then be used to design new therapeutic strategies that may include elimination diets, reestablishing the intestinal barrier function, and medication or nutritional supplements with anti-inflammatory characteristics.

**SPECIMEN REQUIREMENT**

Minimum 2 mL oral fluid
Ambient
RELATED TESTING

- Antibody Array 2 – Intestinal Antigenic Permeability Screen (Serum)
- Antibody Array 3 – Wheat/Gluten Proteome Reactivity and Autoimmunity (Serum)
- Antibody Array 4 – Gluten-Associated Cross-Reactive Foods & Foods Sensitivity (Serum)
REFERENCES

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