

# **WHY TEST FOR FOOD IgA + IgM ANTIBODY IN SALIVA AND IgA AND IgG IN BLOOD?**

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## INTRODUCTION:

Food allergy has become a problem which concerns many clinicians. Adverse reactions to foods in which the pathogenesis involves an immunological response to food components are appropriately called food-hypersensitivity reactions. This term is considered to be synonymous with "food allergy."

According to the International Workshop of Food Supplementation in Food Allergy and Immunity at Olsztyn, Poland, August 30 - September 1, 2002, the top four medical problems in the world are

1. **Cardiovascular diseases**
  2. **Cancer**
  3. **AIDS**
  4. **Allergy**
- General allergy (35%)
- Food allergy (7%)

Immune-mediated adverse reactions to foods can be divided into distinct clinicopathologic entities based on presentation (immediate or delayed), target organ specificity, and pathogenic mechanisms. By far, the most recognized reactions are IgE mediated and dependent on activation of mast cells in specific tissues. Such reactions are immediate and in severe cases may be life-threatening.

Immediate reactions to foods can involve one or more target systems, including the skin, respiratory tract, gastrointestinal, mucosal, and cardiovascular system.

Careful clinical observation has made it possible to document that the signs and symptoms initially follow a pattern reflecting the sites of initial exposure to the incriminated food. Thus, oropharyngeal reactions are frequently reported first, followed by gastrointestinal responses, and then involvement of the skin and respiratory tract.

Unlike the immediate effects of IgE-mediated allergy, the IgG and IgA-mediated food allergy and intolerance reactions can take several days to appear. Levels of IgG and IgA antibodies in the blood against different food antigens have been used for demonstration of delayed food allergy and intolerance reactions. Therefore, raised serum or plasma IgG and IgA levels of food-specific antibodies are often associated with food allergies and intolerance. This classification of immune mechanisms related to dietary proteins and peptides is shown in Table 1.

However, measurement of IgG or IgA in the blood may miss abnormal immune reaction to many food antigens. In one instance, it is known that oral or intragastric administration of dietary soluble proteins such as bovine gammaglobulin (BGG) and ovalbumin or eggalbumin results in salivary IgA production, but not in any antibody production in serum.

**Table 1 - Classification of immune mechanisms to dietary proteins and peptides**

● <b>Gastrointestinal intolerance</b>	<b>IgA and IgM mediated</b>
● <b>Immediate type hypersensitivity</b> Type-I	<b>IgE mediated</b>
● <b>Delayed immune reaction</b> Type-II Cytolytic Type-III	<b>IgG and IgA mediated</b> <b>IgG and IgA immune complex mediated</b>
● <b>Delayed hypersensitivity</b> Type-IV	<b>Cell mediated with involvement of cytokines</b>

### **Manifestation of IgA Antibodies in Secretions**

The deposition of antigens in the gut has been shown to lead to the production of IgA antibodies in secretion at sites distant from the gut, such as colostrums, lacrimal and salivary secretions.

It can be concluded, therefore, that the secretory immune system can be stimulated centrally and that precursors of IgA-producing cells migrate from the gut-associated lymphoid tissue to several secretory sites, as well as to the lamina propria of the gut itself. Therefore, if antigens are injected into the submucosal tissues, they are likely to induce serum IgG antibodies as well as secretory IgA antibodies in saliva. However, if it is applied topically to the skin or to the intraepithelial tissue, the resultant main product is only secretory IgA, which is detected in saliva (see Figure 1).

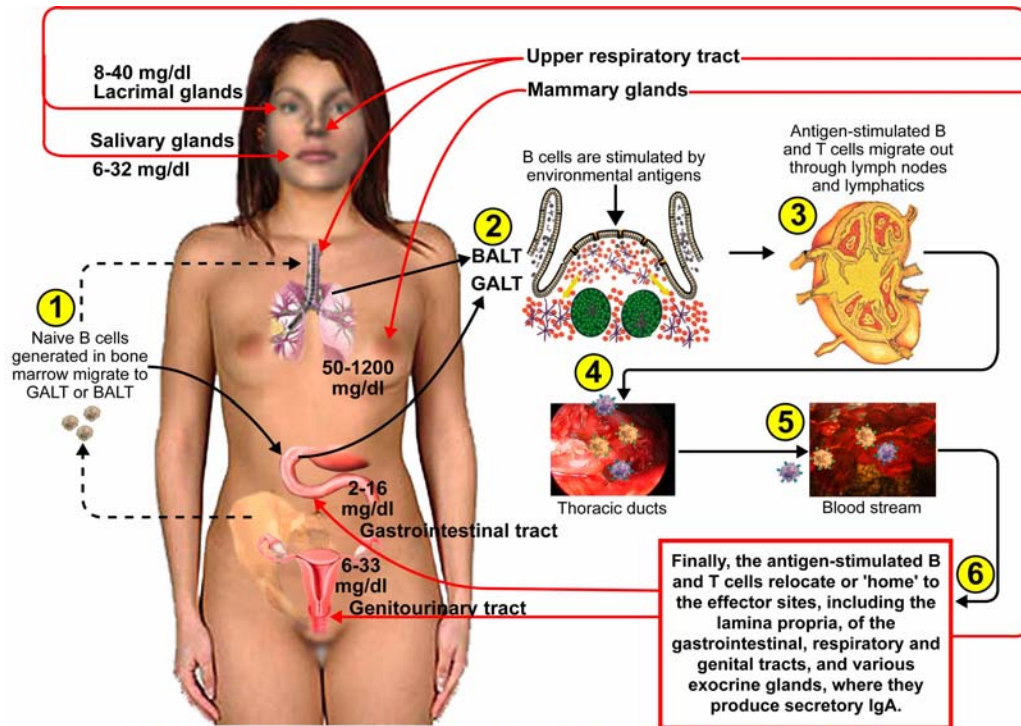
Based on this mechanism of action, saliva is a source of body fluid for detection of an immune response to bacterial, food, and other antigens present in the oral cavity and gastrointestinal tract. Indeed, salivary antibody induction has been widely used as a model system to study secretory responses to ingested material, primarily because saliva is an easy secretion to collect and analyze. Indeed, saliva, as a diagnostic fluid, is certainly preferable to blood in the pharmacological monitoring of patients with chronic therapies

Besides its protective and lubricating properties, saliva meets the demand for inexpensive and easy-to-use diagnostic aids for oral and systemic diseases.

As mentioned previously, food allergy has become a problem that concerns many clinicians. Adverse reactions to foods in which the pathogenesis involves an immunological response to food components are appropriately called food-hypersensitivity reactions. Therefore, using saliva, a single test that will accurately inform a physician of clinical conditions and diagnose patients who may suffer from food

allergies or food intolerance has been developed. The test measures antibody levels to dietary antigens or peptides.

The antibodies present in saliva are IgA (90%) and IgM (10%). For detection of these antibodies, saliva can be a source of body fluid for the measurement of immune response to dietary antigens present in the nasopharyngeal cavity and gastrointestinal tract.



**Figure 1 - The common mucosal immune system and its involvement in the production of IgA antibody in secretion.** Naïve B cells generated in the bone marrow migrate to the inductive sites of mucosal immunity represented by the gut-associated lymphoid tissues (GALT; Peyer's patches and lymphoid follicles of the large bowel) or bronchus-associated lymphoid tissues (BALT) (1), where they are stimulated by antigens taken up and presented by antigen-presenting cells and cognate T-helper cells (2). Antigen-stimulated B and T cells migrate out through the draining lymph nodes and lymphatics (3), enter the blood stream (4) (5), and finally relocate or 'home' to the effector sites, including the lamina propria, of the gastrointestinal, respiratory and genital tracts, and various exocrine glands, where they secrete a significant amount of IgA antibody (6).

### Why are purification and characterization of dietary proteins and peptides essential for reproducibility of IgA + IgM in saliva and IgG and IgA in blood?

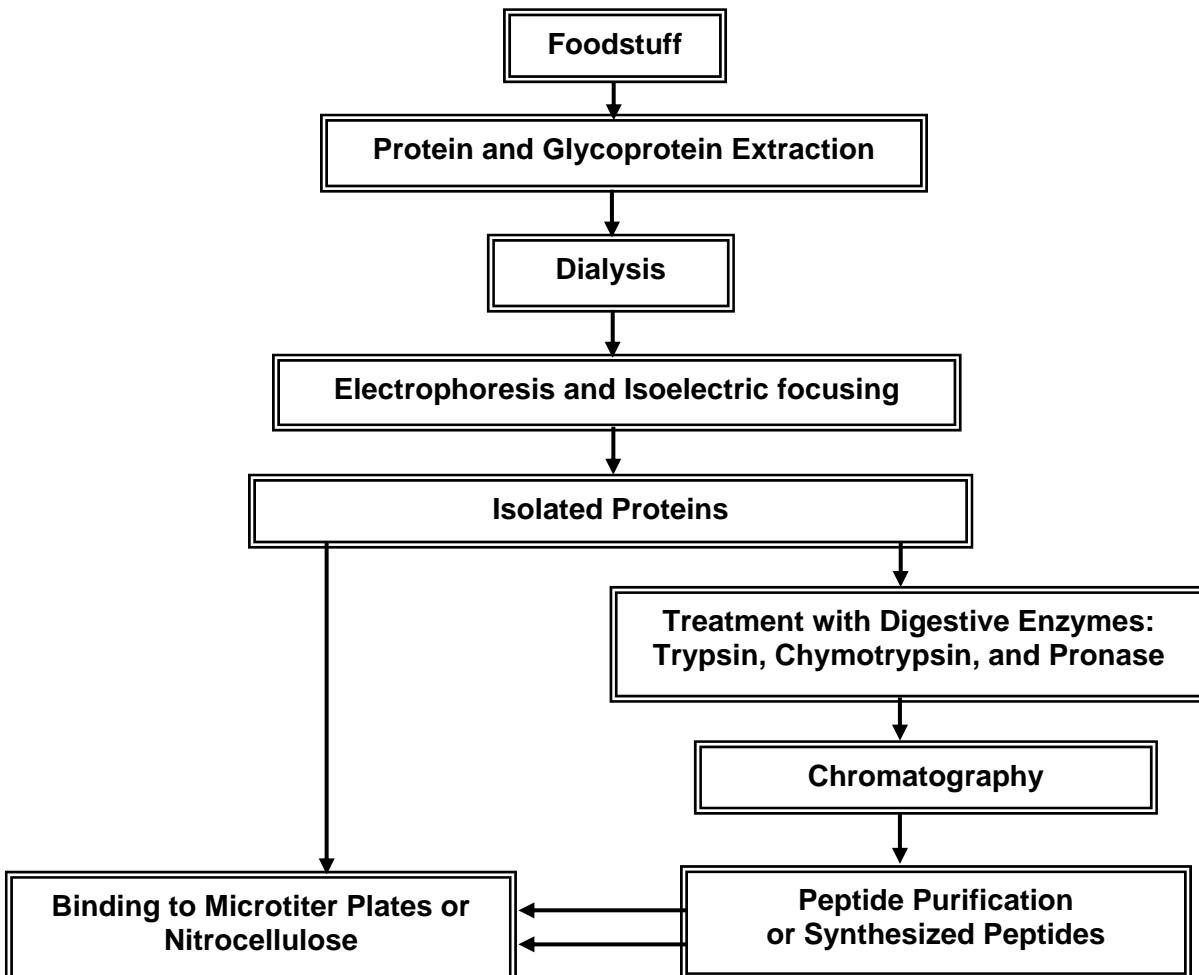
After 20 years of research we came to the conclusion that reproducibility of any antibody assay depends on the purity of antigens used to coat the plates. During the purification process all interfering substances are removed from the mixture and only antigens such as proteins, glycoproteins and lipoproteins are left. The isolation of proteins and glycoproteins in a native form is the prerequisite for the extraction from foodstuffs. The foodstuffs are homogenized with cold acetone at  $-40^{\circ}\text{C}$ . The precipitated proteins are washed, lyophilized, and extracted with phosphate buffered saline. The contents of the protein extracts are measured. Under these controlled conditions, the exact amount of

food antigens used for coating microplates is the same in each preparation. Therefore, there is no variation from preparation to preparation. Binding each of these food antigens to the wells of the microtiter plates in duplicate not only increases the reproducibility of the IgG and IgA assay but results in greater specificity and sensitivity, producing a better clinical outcome.

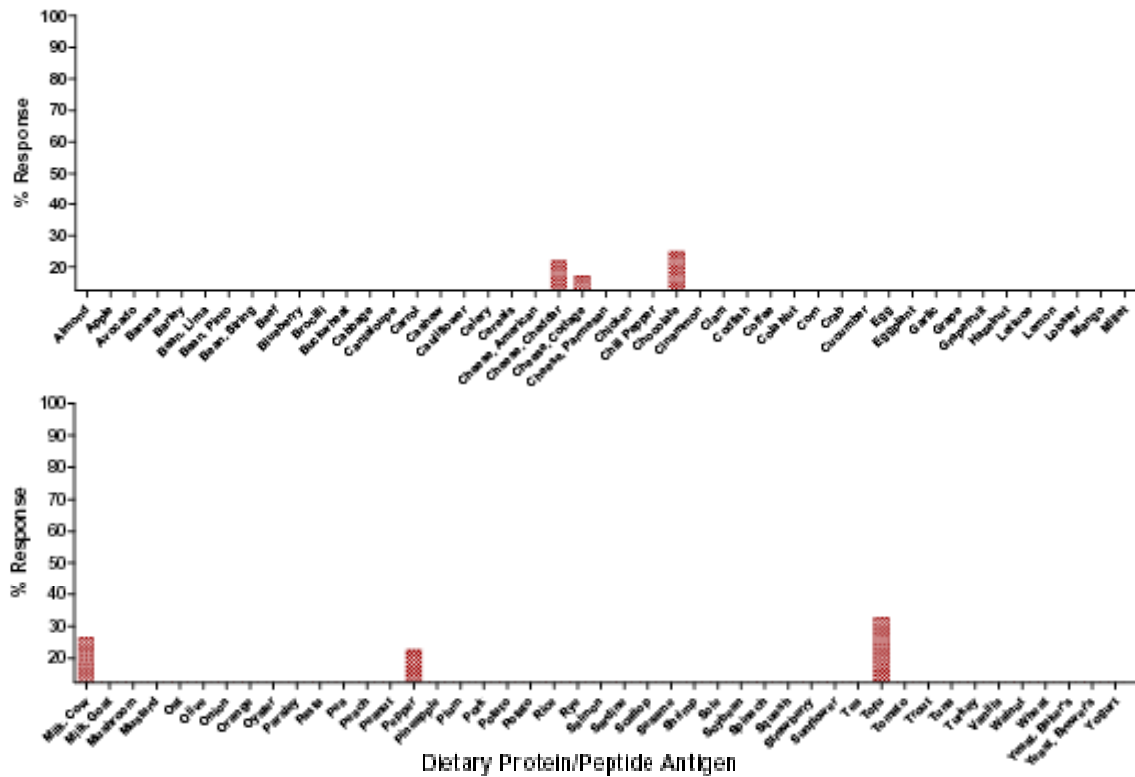
The separation of the different proteins from food is carried out by applying chromatographic and electrophoretic methods. The electrophoretic methods include sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF). In the case of SDS-PAGE, the separation of the proteins is carried out according to their molecular weight. On the other hand, IEF is used to separate proteins and peptides by their isoelectric points. The electrophoretically-separated proteins in polyacrylamide gels are visualized by silver staining or Coomassie brilliant blue staining.

Apart from electrophoretic techniques, immunological methods are used with regard to the identification and characterization of allergens. The specific determination of food allergens can be carried out by immunoblotting and enzyme linked immunosorbent assays (ELISA). Figure 2 shows a diagram of a representative procedure for preparation of dietary antigens and peptides used in our patent #6,689,569.

**Figure 2 - Summary of Analytical Methods for Preparation of Food Antigens and Their Peptides**







**Figure 4 – IgG antibodies against dietary proteins in negative individual**

### **Clinical Significance of Elevated IgA + IgM Antibody in Saliva and IgG and IgA in Blood.**

The mucosal immune system is vastly different from the systemic immune system in that mucosal cells are constantly exposed to antigenic substances found in food and released from endogenous microbial flora. Therefore, this system must have a means of regulating responses to these substances in order to avoid harmful reactions to common mucosal antigens. This homeostasis between the host and antigenic stimulus is maintained by the mucosal immune system's induction of immunologic ignorance (oral tolerance) against dietary proteins and commensal bacteria.

Oral tolerance is a state of immunological unresponsiveness that follows oral administration of most protein antigens. This active suppression of immune response to protein antigens is done by regulatory T cells secreting an immunoregulatory cytokine called transforming growth factor- $\beta$  (TGF- $\beta$ ) or by activation of suppressor T cells. Any disruption of this immunologic balance can lead to mucosal-associated immune diseases, including inflammatory bowel disease and food allergies. In the absence of oral tolerance, specific antibody-dependent protection is induced by secretory IgA and IgM, the predominant isotypes in human external secretions, including saliva. This IgA and IgM is synthesized by plasma cells in the lamina propria and transported into the lumen, where it acts as an antigen-specific barrier to pathogens and toxins in the gut lumen.

Many studies have indicated that a site-restricted secretory IgA and IgM response can be induced either by the application of antigens to mucosal surfaces or ingestion of antigens. This means that in the absence of oral tolerance, ingestion of antigens results in the production of IgA and IgM in saliva but not in blood. However, if food antigens are injected into a gland, systemic responses are manifested by production of IgA and IgG antibodies in circulation.

Another possibility is that antigen-sensitized cells from the gut enter the circulation and then populate 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 recognizing 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 or IgG antibodies against dietary proteins in blood. Evidence for the existence of memory lymphocytes reacting to bacterial or food antigens is supported by studying the pattern of antibody production in IgA-deficient individuals. 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.

Although the percentage of individuals with IgA deficiency is less than 5, our IgA + IgM antibody in saliva was developed in order to cover the mucosal immune reaction against dietary proteins of the non-IgA-deficient (95%) and the IgA-deficient (about 5%) individuals.

A different mechanism for the production of IgA antibody 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 antibody 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 celiacs compared to controls showed significantly higher IgG and IgA activities against gliadin.

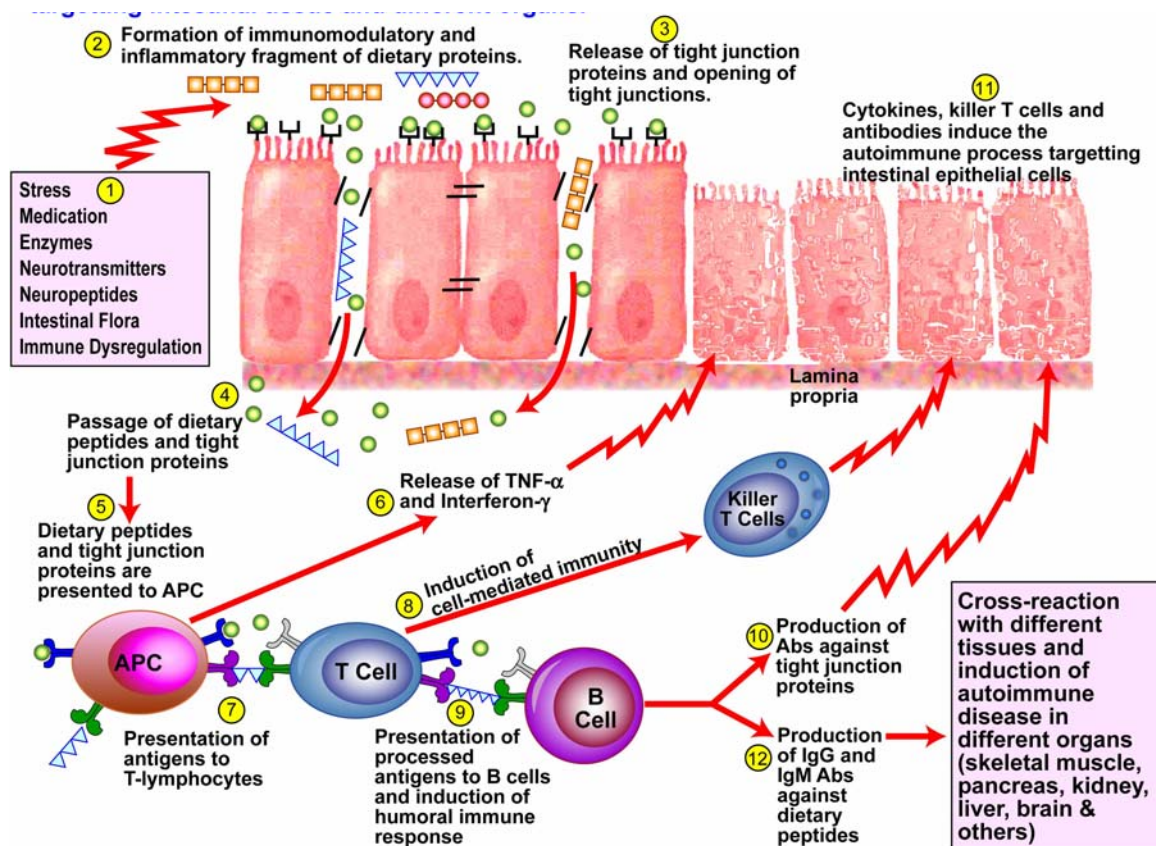
IgA and IgM antibody production against dietary proteins and endogenous bacteria in saliva indicate a defect in oral tolerance and inflammation of the mucosal tissue resulting in inflammatory conditions in the bowel. In fact, defects in the mechanism of oral tolerance have been reported as being responsible for several diseases of the gastrointestinal and respiratory tract.

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 protein antigens, resulting in immune complex formation, which further contributes to the inflammatory reaction in the gastrointestinal tract.

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 undigested dietary proteins into the circulation. The proposed mechanism by which various factors induce intestinal permeability and antigen entry into the circulation is shown in Figure 5.

The entry of dietary proteins in the circulation results in systemic immune response and the production of very high levels of IgG and IgA against dietary proteins and peptides. This systemic immune reaction against dietary proteins and peptides depends on the antigenic structure protein antigen, particulate antigens, polysaccharides, lipoproteins or enzymes and their molecular sizes, and of course, the genetic makeup of exposed individuals. One may produce IgG antibodies against dietary proteins, while others may produce IgA antibodies, or IgG and IgA antibodies against different dietary proteins and peptides.

**Figure 5 – Proposed role of abnormal intestinal permeability in the pathogenesis of autoimmune disease targeting intestinal tissue and different organs.**



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 might lead to an immune response to different target organs and the induction of autoimmune diseases.

Therefore, for a complete picture of food sensitivity, both IgA + IgM antibodies in saliva and IgG and IgA in blood must be examined before therapeutic interventions. This comprehensive approach was developed because our 25 years of research experience determined that diseases of the GI tract and autoimmune disease cannot be fully understood and treated without a coordination of the mucosal and systemic immune response against dietary proteins and peptides that include IgA + IgM in saliva, and IgG and IgA in blood against 90 different food antigens.

Measurement of IgA + IgM in oral fluid, IgG and IgA in blood against dietary proteins and peptides helps clinicians to evaluate their patients for inflammation and autoimmunity, based on which they can design new therapeutic strategies that may include elimination diets, reestablishing the intestinal barrier function, the use of pre- and probiotics, glutathione, lipoic acid EPA/DHA and medication or nutritional supplements with anti-inflammatory characteristics.

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