

The Evolution of Reactivity Testing: Why Immunoglobulin G or Immunoglobulin A Antibody for Food May Not Be Reproducible from One Lab to Another

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ABSTRACT

The gold standard for identifying food reactions is the elimination-provocation diet. Embarking on this long, tedious journey takes an expert practitioner and a completely dedicated patient, with a whole lot of patience from both. In the contemporary fast lane, microwave, “give me a pill” popping, I-want-satisfaction-now society, many clinicians have turned to laboratory assessments for quick answers to food reactivity. From the introduction of cytotoxic testing for food allergies in 1947 until today, food reactivity testing has evolved and branched; it has been both pseudoimproved and scientifically improved. With multiple available options for methodology,

specimen types, and clinical lab, how is a clinician expected to find the one that fits the requirements of a particular practice? How, indeed, when one self-promoting paper supports a particular methodology, while another criticizes it? In this article, with the benefit of his years of training and experience as a research scientist and test development expert, the author, who is trained in both microbiology and immunology, discusses the history of food testing, analyzes the criticisms of it, reviews the scientific literature, and tours the methodologies. (*Altern Ther Health Med.* 2015;21(suppl 1):##-##.)

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Health care practitioners have a variety of choices available for reactivity testing to detect food intolerance (ie, immunoglobulin E [IgE] or immunoglobulin G [IgG], in saliva or in blood, in vitro or in vivo, and a few or hundreds of antigens). The range of tests seems impressive. However, deciding which test is best

for a given patient can be a daunting task. Adding to the confusion are nonpeer-reviewed, often self-promoting articles implying that the author’s test is better than any other. Multiple such articles have appeared, even in the same issue of a publication. This scenario only adds to the confusion of the reader.

To make an informed decision, the reader must consider the source. Is the author commercially connected to the test being reviewed? Further, the reader must consider the source’s sources. Are the references used to support the test relevant to the test methodology? In the current article, the author provides a guided tour through the labyrinth of food reactivity testing to help readers understand testing methodologies, recognize poorly written articles, and appreciate solid scientific publications and lab protocols, allowing them make informed decisions on testing for food reactivity.

THE CRITICISMS

In the past 3 decades, several articles have been published in scientific or alternative medicine journals and other magazines that question the clinical relevance of non-IgE-mediated testing for food allergies.¹⁻⁶ The titles for some of these articles include “In-vitro Testing for Allergy”¹; “IgG Food-Allergy Testing by ELISA (Enzyme-linked Immunosorbent Assay)/EIA (Enzyme Immunoassays): What Do They Really Tell Us?”²; “Evaluating the Clinical Relevance of Food-sensitivity Tests: A Single-subject Experiment”³; “Reproducibility and Reliability of Two Food-Allergy Testing Methods”⁴; “Testing for Food Reactions: The Good, the Bad, and the Ugly”⁵; and even the extreme title, “Should We Do Blood Tests for Food Allergy?”⁶. This skepticism has led some national clinical societies to write position papers calling for a ban on the use of cytotoxic and ELISA/EIA testing for food reactivity.⁷⁻¹⁰ The 3 main categories of criticisms are (1) the tests lack reproducibility within a single lab and correlation from lab to lab; (2) cell-based measurements of food reaction or food IgG are clinically meaningless; and (3) contamination of the food antigen used in testing may interfere with test results. Each of these criticisms are reviewed and addressed in the following sections.

Reproducibility and Correlation

In the world of laboratory testing, if a test is not reproducible, it is considered invalid. Reproducibility in the lab means that testing gives comparable results when a patient's specimen has been split and run as 2 specimens. Comparable results, according to federal laboratory regulations, should be within a 20% variance. As early as 1998, the reproducibility of food reactivity testing was studied by drawing blood specimens from one patient, splitting it into 3 parts, and sending each to a different laboratory that already routinely performed the test.² Additional specimens were collected from the patient on the same day as the first and immediately frozen. One week later, the frozen specimens were sent to the same 3 labs for food testing. After obtaining the results, it was concluded that 2 of the 3 labs had numerical variances of 49% to 73%,² which was outside the acceptable lab standard of 20%. Further, the clinical interpretation (ie, whether the patient should avoid the food) varied from 7% to 59%.² When a practitioner counsels a patient on the appropriate diet for his or her well-being, the recommendations must be based on reliable results. Clearly, many are not.

More than a decade later, a study was done that compared ELISA and cell size testing methodologies.⁴ Comparing test results from one lab to another lab that was performing the test using the same methodology is like comparing apples to oranges; comparing test results between 2 labs using 2 different methodologies is like comparing apples to sausages. Nevertheless, the study found the results of the IgG testing to be reproducible and to have exhibited results that were between 82% and 95% identical. Unfortunately, the way the data were presented did not allow the reader to determine definitively or accurately the reliability of the test.

From a scientific point of view, at least 10 individuals should have been tested for such a study, and the data should have been presented in the form of bar graphs to allow readers to examine the very reactive foods. For example, assume that a lab tested serum IgG for 100 foods, recording a positive IgG antibody against only 4 foods, with the other 96 foods showing a negative result. The next day, upon a repeated test of the same specimen, assume that only 2 of the 4 foods originally positive were recorded as positive again, and the other 98 foods were reported as negative. Calculation of test results would indicate the results were 98% identical, although in reality, the reproducibility of positive results for IgG foods on the 2 different days was only 50%.

Now, looking at the issue from another perspective, what if the test results were reproducible? Food-testing researchers know that several foods, such as ginger and cabbage, contain enzymes and many other factors that can react nonspecifically with reagents used in ELISA. Unless the food proteins are purified before being used in the assay, false-positive reactions could occur. In such a case, the patient could test positive to the nonpurified antigen each time, indicating reproducibility, but that would be a false-positive. Therefore, reproducibility of the results from day to day should be calculated only with the use of biochemically purified food proteins or peptides. The clinicians who wrote the articles mentioned previously¹⁻⁶ may not have had the required level of understanding, nor may scientists who have not done extensive research with food antigens. Knowing such details requires many years of research experience and hands-on work with lab assays.

All companies that perform IgG or IgA antibody testing use their own in-house ELISA. This fact means that each lab has designed its own process from beginning to end, which is fine as long as all steps in the process are scientifically supported. In-house testing is quite common. These tests are called lab-developed assays (LDAs) or lab-developed tests (LDTs), and their validation processes are subject to federal laboratory regulations.

Remember the following piece of common sense: An ELISA test is only as good as the antigen used to coat the ELISA plate. Having developed more than 300 ELISA assays for the detection of various immune disorders, the author knows this fact very well. The antigen used on the plate results in the binding of circulating antibodies or nonspecific factors from the patient's blood to the coated wells. Many labs buy their food antigens in a lyophilized (freeze-dried) form from a company in the Midwest. Two of the 3 labs in the aforementioned study admitted to buying antigens from an Oklahoma-based business.² Having once been a client of this antigen-producing company, the author is very familiar with the company's process of food antigen preparation. The company obtains foods from the local markets to make antigens. The foods are chopped and first diluted in saline or other solution to make the suspension and then dried. The dried form of food is sold by weight. Other than rinses with acetone, the food antigens are not purified.² Whether food

antigens are obtained directly from the supermarket by the clinical testing lab, which then prepares them for use, or from companies producing them for sale, their purification is the responsibility of the lab developing the assay. This responsibility is why a very tedious and lengthy validation procedure was developed and recommended by the Clinical and Laboratory Standards Institute (CLSI) and the Food and Drug Administration (FDA) for all LDAs. If all required procedures are followed and if purified or synthetic food or tissue antigens are used in assays, undoubtedly the lab test will be specific to the antigen in question and the results will be reproducible.

Clinicians often run into scenarios where the results for a food antigen are positive from one lab but negative from another. Which result should be used? Only one rule must be remembered in this situation. The practitioner cannot compare the results of one lab with the results of another lab. Many reasons exist for no correlation between labs. One factor, antigen purity, has already been discussed. Other aspects to consider include (1) specimen type, (2) the type of immune reaction that is tested, and (3) the time at which the specimens used in each test were collected (eg, on different days).

Specimen Type. If Lab A used saliva and Lab B used serum, the labs are testing 2 different specimen types as well as 2 different immune reactions.

Immune Reaction. If Lab A tested IgE or IgG4 and Lab B measured IgG, the labs are providing results for 2 different immune reactions. Both IgE and IgG can play a role in food immune reactivity.¹¹ IgE functions via its high-affinity receptor, FcεRI, which is highly expressed on mast cells and basophils. IgG has several receptors: the high-affinity FcεRI and FcγRIV, and the low-affinity FcγRIIB and FcγRIII. All of these receptors are expressed on several types of cells involved in anaphylaxis, including mast cells, basophils, neutrophils, and macrophages. Inhibition of FcγRII/III abolished temperature drops associated with shock in IgG-mediated, but not IgE-mediated, anaphylaxis.¹¹

Five pathways are involved in food immune reactivity¹¹:

1. Classical pathway: involving IgE and its receptor FcεRI, mast cells, and histamine.
2. Alternative pathway: mediated by IgG₁, FcεRIII, macrophages, and platelet-activating factor (PAF) pathway.
3. IgG-basophil-PAF pathway.
4. IgG-neutrophil-PAF pathway via FcγRIV.
5. IgG-immune complex neutrophil pathway.

It may be of interest to note that the antigen doses required to trigger each mechanism may be related to the different pathways to systemic anaphylaxis, because the classical pathway or IgE production is activated by small doses, and the alternative pathways or IgG production are activated by large doses.¹¹

Specimen Collection. If Lab A's results are from a blood specimen collected in February and Lab A tested a new blood

specimen from the same person that was collected in May, the tests occurred on 2 different collection dates, during which significant changes in immune responses could have occurred.

This section's review of criticisms has been centered on ELISA testing. The reliability of cytotoxic food testing has also come under fire. The author will discuss reproducibility and other aspects of cytotoxic food testing in a later section.

Clinical Value of IgG Food Testing

The argument that IgG antibodies are an indication of exposure to antigens or a failure in immune tolerance has been made and repeated many times. This notion is based on the virus model, where levels of IgG are indicative of past exposure and high levels of IgG are indicative of superimmune protection.¹² Contemporary immunologists have not applied this concept to other antigen reactivities, but many authors appear to be stuck in the virus model.

Elevated IgG antibodies against food antigens certainly have documented clinical significance. It is very well known that IgG against *Helicobacter pylori* toxin is an indication of *H pylori*-induced ulcers. Patients with systemic lupus erythematosus, who were negative on standard tests, have been shown to have high levels of antiprothrombin IgG.¹³ IgG testing for fibulin is used to differentiate osteoarthritis from rheumatoid arthritis.¹⁴

In general, elevated IgG antibodies against various tissue antigens can be used for the diagnosis of many autoimmune disorders. In fact, in a study on biopsy-confirmed celiac patients, antibodies to endomysial cell antigen, thyroid peroxidase, glutamic acid decarboxylase, insulin, and islet cell were elevated but normalized on a gluten-free diet.¹⁵ The author could cite many more studies supporting his point (ie, that IgG antibody testing holds clinical value in diagnostic medicine and that the removal of the identified antigen from the patient's blood results in clinical improvement).

The human body is a constant reconstruction zone. Tissues are perpetually broken down and replaced. New cells are born every second. When the immune system is exposed to antigens, antibodies are formed. Because of the body's regeneration activities, it is normal for the healthy adult human to produce small amounts of IgA or IgG antibodies to self-tissues.¹⁶⁻¹⁷ Likewise, it is normal for the human who eats potatoes twice a week, every week, to have some antibodies to potatoes. Laboratories are required to follow minimum standards set by the federal agencies that regulate laboratories, which establish reference ranges.¹⁸ A few laboratories, such as those under the author's direction, use the higher standards set by the FDA as the minimum requirements for establishing reference ranges on LDAs.

What is not normal for a healthy individual is to have high levels of antibodies to a given antigen, with the exception of viruses.¹⁶⁻¹⁷ Even that notion is being challenged by emerging research.¹⁹ In fact, a few labs currently base their diagnosis of a herpes virus infection on the levels of the IgG antibody, measured against purified glycoprotein. When a lab assesses antibodies, whether to thyroid peroxidase or

glutenin, it uses the reference ranges established for those specific antigens when reporting the results. If the patient's numeric reading is above the reference range, the lab reflects that fact on the report. The practitioner receiving the report will use the results, together with other pertinent clinical information, to interpret a test's meaning and provide a diagnosis and treatment protocol for the patient.

Is there clinical significance to IgG food testing? The aforementioned researchers of extraintestinal autoimmunity in celiac patients would say "yes."¹⁵ Specific food testing has been used in studies with irritable bowel syndrome (IBS), showing that the IBS symptoms were alleviated by eliminating the IgG-positive foods.²⁰⁻²² An in-house testing of a donor, who experienced a rash in the area where the elastic from undergarments contacted the skin across the abdomen, resulted in extremely elevated IgG + IgA to banana. The individual had neither been suspected of having latex allergy nor tested positive to latex on a skin prick test. Further, she did not react to other foods known to be related to latex allergy, such as avocados, even though she regularly consumed that fruit. After she removed bananas from her diet, the skin condition disappeared.

In a clinical, double-blind, randomized, crossover trial of 30 migraine sufferers, elimination diets based on IgG food testing resulted in the reduction of the frequency of migraine attacks.²³ As Shaw²⁴ said in this quote from a recent *Townsend Letter* article,

In IgG-mediated, food-allergy testing, the goal is to identify foods that can cause inflammation, and thus, trigger a large number of adverse reactions. IgG1, IgG2, and IgG3 can all cause inflammation because these antibodies do not exchange heavy and light chains with other antibodies to form bispecific antibodies. Thus, IgG1, IgG2, and IgG3 antibodies to food antigens can and do form large immune complexes or lattices that fix complement and increase inflammation.

The author agrees with this statement. The formation of the antibody-and-immune complex, however, depends on the type of antigens used, which may vary from one lab to another lab. The author will delve deeper into lab-to-lab antigen variances later in this article.

Despite all of the work that has been done with IgG immune responses, the literature fails to link IgG food reactions to clinical manifestations. In the context of the modalities used by many conventional and alternative practitioners, a well-respected scientist⁵ has written, "immunoglobulin G (IgG)-based testing has shown promise, with clinically meaningful results." Unless the author's definition of clinically meaningful results pertains to a defect, a breach in oral tolerance, or disturbances at different steps in the path to oral tolerance that are known to result in food hypersensitivity,^{25,26} in reality, the direct clinical meaning of food IgG testing is not known. The industry needs more extensive clinical research in this area.

Contamination of Antigen Poses an Interfering Factor

One author has published an article questioning the validity of testing for food antibodies based on a theory that the microorganisms and pesticides on the food could cause false-positives.² Indeed, microorganisms live on food. These organisms include common bacteria and fungi, and, possibly, some parasites and viruses. Pesticides or other chemicals may also be present on the food. Theoretically, if these components are in the food antigen well, the patient who has circulating antibodies to the microorganisms will test positive for the food. This finding would be considered a nonspecific response. The author posing this theory interviewed an unnamed technologist, after which she wrote that microorganisms, pesticides, and organic solvents are not rinsed away during test preparation.²

First, if a lab follows proper antigen purification processes, only a pure food antigen will be used to coat the ELISA plate wells. Second, having been educated and trained as a microbiologist and an immunologist, and thus being armed with an understanding of both the world of microorganisms and the human immune system, the author would have to disagree.

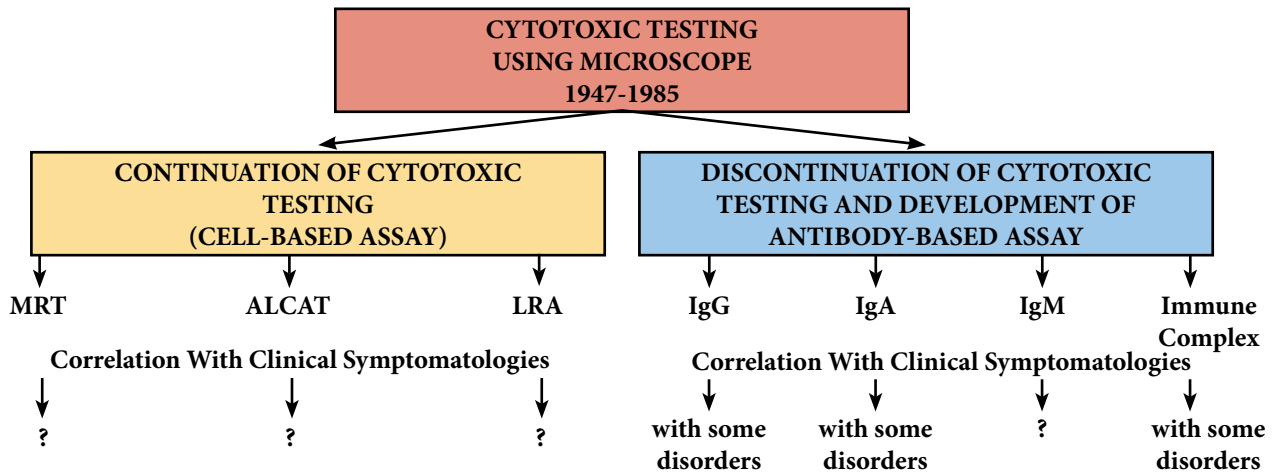
Readers must understand the ratio between certain bacteria, fungi, or parasites in fruits or vegetables and their protein contents to see the error of this statement. This ratio is at least 1000:1, or even in some cases 10000:1, in favor of food protein over microorganism protein. Usually each ELISA plate well is coated with 1 mcg or 1000 ng of antigen. If out of 1000 ng, 1 ng—or, in the worst-case scenario, 10 ng—is originated from bacteria or fungi, then only 0.01% of that immune reaction is going to be nonspecific, and 99.99% is going to be associated with the food antigen in the ELISA plate well. Therefore, a nonspecific reaction to 0.01% of the total antigen proteins is not enough to cause a false-positive result.

The critic goes on to say, "Numerous studies have shown high levels of IgG to pesticides and organic solvents in persons with high exposure rates"² but does not provide the references for these studies. The current author searched scientific journals for such articles and found only 1 study that measured specific IgG against an organic pesticide in farmworkers.²⁷

Chemical pesticides are haptens molecules. Haptens are too small to elicit antibody production by themselves. Pesticides applied to the food must first bind to food proteins to become a neoantigen and, thus, produce an immune response.²⁸ Second, similar to the argument on bacterial and fungal contamination, the pesticide-to-food protein ratio is 1:1000 and, hence, not enough to result in a false-positive for food reactivity.

The author agrees that many false-positive results occur in food testing but not for the reasons reviewed above. The cause is not pesticide, bacterial, or fungal contamination of the food antigen in the well; rather, the truth is that many food proteins act like enzymes. In unpurified food antigens, many components act like substrate to the different enzymes that ELISA testing uses. Examples of these are peroxidase and alkaline phosphatase. Thus, coating the plate with these nonspecific factors plus the food antigens can result in a

Figure 1. Evolution of Cytotoxic and Food Antibody Testing^a



Abbreviations: MRT, mediator release test; ALCAT, antigen leukocyte antibody test; LRA, lymphocyte response assay; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M.

^aQuestion mark indicates that correlation has not been achieved.

nonspecific reaction. In other words, the addition of antibody labeled with enzyme or the substrate to the antigen well during the testing process can cause these reagents to bind to the junk in the food-coated plates and result in color development, thus giving a false-positive. Or in some cases, the factors in nonpurified food antigen can prevent the binding of the antibody in the serum to the real antigens on the plate. In this manner, the use of nonpure food antigens can lead to both false-positive and false-negative results.

The literature that is critical of food IgG testing includes a negative editorial, “Should We Do Blood Tests for Food Allergy?”⁶ This editorial repeats most of the criticisms and conclusions made by the author of the previously mentioned article from the same issue,² including the assertion that the presence of antibodies to food extracts could be indicative of previous exposure to common bacteria or fungi. This conclusion is extremely naïve from the point of view of someone trained in microbiology and immunology. However, the article does contain a very important comment, with which the current author agrees: “Some allergic reactions to food antigen are not due to the major proteins but rather to peptides produced during the process of digestion.”

Examples include (1) cow’s milk versus α -casein, β -casein, or casomorphin peptides or (2) wheat protein versus α -, γ -, or ω -gliadin peptides. If the lab is testing only cows milk and not the peptides, and the patient is actually reacting clinically to the casomorphin peptide after the cows milk is digested, the test for cows milk may result in a negative finding, even though the patient clearly has a clinical response after consuming cows milk. Further, some food reactivity can occur against a raw food, such as a raw peanut, but not against the processed form, such as a roasted peanut. Other food reactivity can occur in the opposite fashion, such as a positive reaction to a hard-boiled egg and a negative one to a raw egg. Indeed, some food immune reactions are caused not by the native food

proteins but by glycosylation products of these proteins that are produced during processing or cooking.²⁸ This issue can result in both false-positive and false-negative results if the testing includes only raw foods rather than raw and processed foods simultaneously.

Conclusion: Criticisms

Of the criticisms reviewed above, the valid one is the one that criticizes the results due to lack of reproducibility within a single lab. Each lab should strictly follow antigen purification processes when preparing the food antigens used in testing procedures. If this purification is done, results will be reliable. Clinicians should challenge labs to ensure that tests are accurate. This challenge may entail secretly splitting a sample and sending it to the lab under 2 different patients’ names. If the results do not correlate by at least 80%, the test from that lab is not valid.

THE PITFALLS

Early attempts to develop serologic reactivity tests for foods were flawed. They lacked double-blind studies and quality control, made outrageous clinical claims, and were completely subjective based on the technician reporting the results. The following section looks at the pitfalls specific to food reactivity testing that have occurred for the last 65 years.

Cytotoxic Testing

When Bryan and Bryan²⁹ widely popularized the food cytotoxicity test in the late 1950s, its existence was exciting and innovative for its time. During decades of use, the test proved to have many problems, and by 1985, it was officially banned in California and other states. An improved version and an automated version of this cytotoxic test are still in use in at least 3 laboratories under the names: ALCAT (antigen leukocyte antibody test); MRT (mediator release testing); and LRA (lymphocyte response assay) (Figure 1).

The process of cytotoxic testing entails the placement of a drop of the patient's blood, including plasma, onto a plate well that is coated with a liquid or dried food extract. The prepared plate is examined microscopically after 10 minutes of incubation and, thereafter, at 30-minute intervals up to 2 hours. The technician looks for observable changes in leukocyte morphology. If the technician observes changes in the cell's size, rounding, inactivity, or vacuolization and/or disintegration of cells, a positive result is recorded.^{9,29} In 1984, the author was involved, on occasion, in immunology research using ELISA testing methods but was really focused on classical cytotoxic testing to measure the effects of toxins on immune cells, specifically natural killer cells. He was approached by the CEO of a lab in west Los Angeles that performed cytotoxic food testing. The lab was challenged by the California Department of Health to prove reproducibility, and the author was hired to refine the cytotoxic food testing method to meet the reproducibility demands of the state agency. After a couple of weeks observing the performance of this method, like Dorothy realizing she was not in Kansas anymore, he saw that cytotoxic testing of natural killer cells and cytotoxic testing of food antigens were worlds apart. The author found many flaws in cytotoxic food testing, including (1) use of lemon extract, (2) unique factors of food extracts, (3) pH of food extracts, (4) lack of consistent results with different technicians, and (5) different results from different times of testing for the same patient.

Lemon Extract. Testing lemon extract activity against a drop of blood was difficult. Because lemon is acidic, the acid naturally causes changes in the white blood cells. The effect is not necessarily due to a patient's immune reactivity but could reflect false-positivity.

Food Extracts. Each food extract has unique factors that can cause false-positives or false-negatives.

pH of Food Extracts. Under the author's direction, the lab changed the pH of all food extracts to human levels at 7.0 to 7.2. By making this change, the number of reactions dropped by more than 200%. For example, a patient originally reacted to 30 food extracts, but when the pH was matched to human levels, the same patient's specimen reacted to only 10 foods. This improved cytotoxic assay was presented in 1985 at the American Academy of Otolaryngic Allergy's annual scientific meeting. The author and his partner won the Best Presentation Award for that refinement of the assay.

Lack of Consistent Results. No matter what kind of improvement was implemented in the preparation of food extracts, the results were still read differently from technician to technician at the lab.

Different Results From Different Times of Testing. A patient's blood drawn once each day for 2 days in a row and tested for cytotoxic food reactivity produced 2 reports that did not correlate with each other.

Even with the author's attempted refinements, reproducibility could not be achieved. The California Department of Health was not satisfied. Throughout

California, labs had to stop performing cytotoxic food sensitivity testing, including the one in west Los Angeles. At that time, this scenario was being played out in other states as well.

Shortly after the banning of cytotoxic food testing, a scientist made a few improvements on the testing method and reintroduced it under a new name. Another scientist took some time and solved the subjective problems with cytotoxic food testing by developing a computerized machine that could observe and record cell size changes. Both of these versions of cytotoxic food testing are currently available. From both the scientific and business points of view, what is being done in these tests?

In contrast to the variety of food-IgG or other antibody-testing publications, there is a noticeable lack of peer-reviewed articles for any version of cytotoxic testing. For an immunologist to measure mediator release, such as cytokines, with 90 to 491 food antigens, a significant amount of blood is needed to release a sufficient amount of mediator. To assess one antigen, the lab needs 1 mL of blood per culture, or approximately 500 000 white blood cells, to obtain the requisite amount of mediators (cytokines, chemokines) to be measured. Thus, to perform this test on 491 food antigens, the lab would need to have 50 tubes of blood. Further, a lab's cost for the reagents used in the determination of these mediators, if the test were done properly, would be more than \$2000 per patient run. In addition, those costs must be combined with the cost of the many hours of labor by skilled technicians to perform the culture of white blood cells. In reality, however, the price of the test to the patient appears to be much lower than a lab's costs could be.

Articles supporting the improved cytotoxic testing were published by a magazine marketed to practitioners and patients. In the article about lymphocyte response assays (LRAs), "Predictive biomarkers in personalized lab diagnosis and evidence-based-practice outcome monitoring,"³⁰ the authors discuss various predictive markers for the assessment of health risk using the LRA. Although most of the biomarkers explained in the article—such as hemoglobin A_{1c} (HbA_{1c}), high-sensitivity C-reactive protein (hs-CRP), or oxidized low-density lipoprotein (LDL)—are scientifically based, the following claims behind the LRA test have not been proven: (1) the tests measure all 3 delayed-allergy pathways; (2) the tests avoid the false-positives common in tests of immunoglobulin reactivity, immune complexes, and direct T-cell activation; and (3) the tests' identification of a patient's specific sensitivities and delayed allergies is a clinical breakthrough. LRA cell cultures are reproducible within less than 3% variability when different readers read split samples on different days.

These claims are bold given that the authors have neglected to support them with peer-reviewed research publications, making readers ask, "Where are the science and/or clinical studies behind the claim that LRA measures all 3 delayed-allergy pathways? Where is the evidence that LRA measures sensitivity to chemicals?"

Culturing lymphocytes in chemical media and then measuring changes in cell size to determine if a specific chemical has a toxic effect on the lymphocyte, does not measure any type of allergy pathway. The same statement applies to reactions of lymphocytes to foods. A change in the cell size could be associated with hundreds of factors in the food extracts that are toxic to white blood cells, which is why this test was originally called a *cytotoxic assay*.²⁹ In fact, according to an article published in *Allergy*,³¹ adverse reactions to food can be classified as toxic or nontoxic; however, many reactions to foods could be toxic to white blood cells. Nontoxic reactions that involve immune mechanisms resulting in IgE production are collectively termed *allergy*. If antibodies other than IgE are involved, the reaction is termed *intolerance* due to the breakdown in immunological tolerance. Patients with intolerance produce IgG, immunoglobulin A (IgA), or immunoglobulin M (IgM) antibodies against these foods. An industry-wide proposed term for food intolerance could be *non-IgE food immune reactivity*.

In the same issue of the magazine in which the LRA article appeared, another article, "Mediator Release Test (MRT): A Comprehensive Blood Test for Inflammation Caused by Food and Food-Chemical Sensitivities,"³¹ was published. The message of the second article to the readers was that antibodies, such as IgG, IgM, or IgA, cannot identify reactivity to foods and food chemicals, and that antibody testing has not demonstrated an acceptable correlation with inflammation or clinical symptomatology in adverse food reactions. Thus, according to the author, the information provided by most commercially available food sensitivity tests was of limited clinical value. The author discussed the importance of white blood cells in food-induced inflammation, which he called an *immunologic endpoint*.³¹ To support this theory, a very nice graphic was presented describing an *in vivo* reaction of cells involved in the innate inflammatory reactions:

When sensitive food antigens cross the tight junctions, neutrophils and macrophages are typically the first cells to react. They engage in the destruction of offending pathogens or antigens, ultimately releasing various cytokines and other pro-inflammatory mediators.³²

The way the quote was presented would have led the reader to believe that the logical end action after exposure of white blood cells to different food chemical solutions was to measure the aforementioned cytokines and proinflammatory mediators. But on the next page, the author indicated that the described test measured volumetric changes in white blood cells, assumingly caused by food- or chemically induced release of mediators. The clinical utility of this test as it relates to food and, in particular, to chemical sensitivity, however, has yet to be determined by scientific studies.

Although these 2 articles described tests that are based on volumetric changes in white blood cells (cytotoxic testing), each made the claim that the information provided by non-IgG antibody testing for food reaction has limited clinical value.^{30,32} In the same issue of the magazine, however, the clinical usefulness of IgG food allergy testing was discussed by another author.²⁴

Even with improvements in cytotoxic testing, recent reproducibility studies still have shown a lack of correlation of results for the method.^{4,5} One set of authors concluded,

... well-designed clinical trials should be published before patients are subjected to expensive testing for delayed hypersensitivities; eg, ALCAT and MRT testing, that offer little evidence of effectiveness. Disclosing the basis for food reactions continues to present a diagnostic challenge, and testing for food allergies in the context of an appropriate clinical history is paramount to making the correct diagnosis.⁵

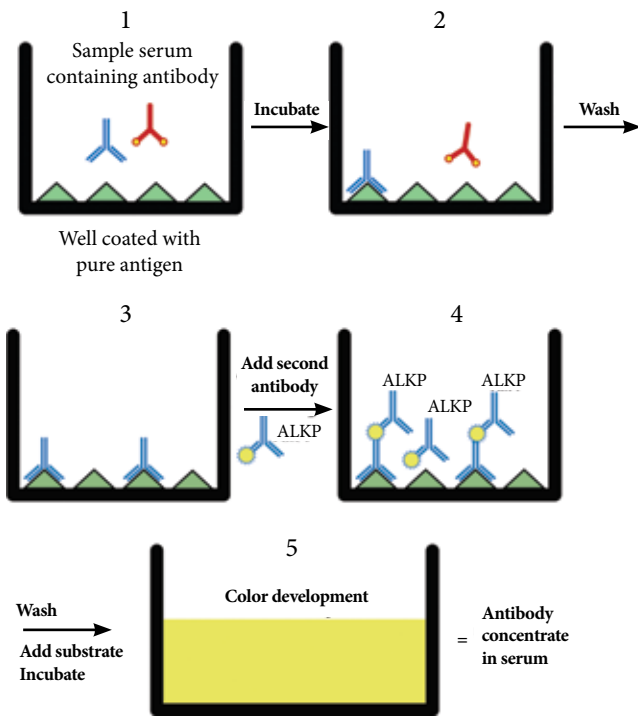
In a well-written paper by Hodsdon and Zwickley,⁴ the results of a small study on the repeatability of food-testing methodologies were revealed. The authors challenged both cell size and IgG ELISA labs with split specimens and specimens collected in the course of days. The study showed that cell size testing was not reproducible by split samples or by samples provided in the course of a week. In the split-sample challenge, only 17 of 50 foods were identical when using the cell size method (34%) versus 91 of 96 (95%) using IgG ELISA. Further, the split-samples challenge tested the internal reliability of the 2 labs compared the levels of reactivity between the cell size lab and the IgG ELISA lab (0, no reaction and 3, high reaction). The cell size method resulted in 14 cell-size foods of 50 (28%) showing a 3-level reactivity difference, whereas the IgG ELISA method showed 0%.

Compiling the results of blood samples collected on each of 3 days within the same week revealed an even more clinically disturbing result. Only 1 of 50 cell-size foods reported identical results. The corresponding IgG time challenge produced identical results of 82%. The difference in time-challenged, 3-level reactivity for cell size was a staggering 60%, or 30 of 50 foods; again, the difference for the IgG tests was 0.⁴ The study's conclusion called into question the reliability of cell size testing for identification of food allergies but found that IgG testing was reproducible and reliable.⁴

Lack of Quality Assurance

In 1986, the author developed the ELISA IgG food test after cytotoxicity food testing was banned in California. He opened the first clinical lab that offered IgG reactivity testing for food by ELISA based on the principles summarized in Figure 2. Soon other labs that had first used the author's original lab as a reference lab copied the method and added it to their test menus. Unfortunately, those other labs did not then or even today fully understand the theories behind the test. One telltale is a lab reporting immune reactivity to sugar or sweeteners. The author uses the sugar well as quality control for ELISA testing. If the sugar well produces color, he knows that something has not gone right, and he needs to repeat the test. Humans do not make antibodies against sugars. Sugar is a very small molecule approximately the size of 200 to 300 Da, like the chemical haptens described earlier. The human immune system does not recognize molecules smaller than 5000 Da, unless the hapten, in this case the sugar molecule, binds to food proteins during its processing

Figure 2. Steps involved in the enzyme-linked immunosorbent assay (ELISA) procedure. A patient's serum is added to a plate well that is coated with pure antigen. If antibodies are present in the sample after incubation, they will bind to the antigen. After washing an enzyme, a second antibody is added to the plate to make the blood and antigen react. After washing, the addition of the substrate, and incubation, the plate well's degree of color development determines the presence and amount of antibody.



Abbreviation: ALKP, alkaline phosphatase.

or cooking, and thus forms glycosylation products. It is only after this process that the immune system recognizes sugar-bound protein (glycosylated protein) or advanced-glycation end products, but it will never react to unbound sugar or other sweetener molecules.³³⁻³⁵

Recently a new test, the dried bloodspot test, has been promoted as an easy way to test for food allergies, even directly to patients on Amazon.com.³⁶ For this assessment, the patient uses a lancet to prick a finger and place drops of blood on a special bloodspot collection card. The card is air dried and then shipped to the lab for the performance of either IgG or IgG4 ELISA testing of a varied number of food antigens. The report, depending on the lab, ranks the foods' allergenicity either as borderline to mild, moderate, or severe, or as safe, moderately safe, or avoid.^{37,38} Without any unbiased, double-blind, published studies comparing bloodspot food testing with venipuncture food testing, how does anyone know it is a reliable or reproducible improvement on the venipuncture IgG ELISA testing?

Scientifically, the promotion of this type of test is misleading. Measurement of IgG is not used for classical allergy testing. Rather, IgG measures food immune reactivities due to a breakdown in immunological tolerance. Serum specimens that can be accurately measured are the best for use in antibody measurement. In bloodspot testing, no lab can control how much blood is collected and, therefore, used in the testing process. IgG4 comprises only approximately 4% of total blood IgG, making the tests questionable if they are supposed to be measuring IgG4. A lab would need 2 to 4 mL of blood to accurately assess IgG4 reactivity to 45 to 95 foods. A couple of drops of blood do not equal 2 to 4 mL. Therefore, exactly what the tests that use the bloodspot method are actually measuring is seriously in question. Unless comparisons by scientific method are done between the serum and bloodspot methods and a standard operating procedure for bloodspot is established and published in peer-reviewed journals, clinicians cannot be encouraged to use this method.

Lab Involvement in Therapeutics

Labs have come under fire for suggesting that a patient must consume a wide range of foods in the 3 weeks prior to specimen collection to obtain accurate results.³⁹ When a test is intended for allergy assessments (IgE or IgG4), why would anyone risk a patient's life by making such an irresponsible recommendation? Allergists do not require their patients to consume potentially dangerous foods before performing an allergy test for IgE. The stated requirement is not only irresponsible on the part of the lab, but also immunologically inaccurate.

If the patient has a fully functioning, intact intestinal barrier, 1 meal of a specific food is not enough to elicit a serologic antibody response. For these patients, dendritic cell sampling, endocytosis, transcytosis, and retrotransport of secretory IgA are the routes that food antigens take to reach the blood stream where serologic antibodies are produced.⁴⁰ Thus, it takes multiple exposures, over a period of time, for the immune system to produce antibodies to a food protein. Food challenges should never be taken lightly. Dangers are involved, and the patient needs to be forewarned. Some foods are known to cause anaphylaxis⁴¹; others can exacerbate autoimmune conditions in some people.⁴²⁻⁴⁴ Rather than force a patient to consume foods he or she does not normally eat, why not find the most appropriate food test panel that closely reflects the foods that the patient consumes regularly?

As if a lab making clinical suggestions is not enough of a critical problem in itself, some labs provide diet protocols based solely on test results. Miller² states,

Included in these problems [associated with food allergy testing] are the distribution of therapeutics by a laboratory, the prescription of therapeutics based solely on the basis of laboratory testing, and the possibility that therapy recommendations are based on a lab test that may not be correct.

A single lab report is only one piece of the complicated puzzle of today's multifaceted disorders. It can be interpreted one way when put in context with another lab report and

another way when combined with the patient's medical history. The lab is not involved with the patient, has not seen the patient, and does not have the patient's other test results and clinical history. How, then, can a lab put together a therapeutic protocol for an individual patient? Are labs trying to replace health care practitioners and the vital patient-practitioner relationship?

Conclusions: Pitfalls

Over the years, lab testing has found itself in a few pitfalls. The cytotoxic testing for foods has time and time again failed reproducibility challenges. A lack of understanding of even the theories behind testing methodologies has led to false-positive results. The introduction of improvements has been done without unbiased control studies. And finally, the evidence that labs are crossing the line into the clinical setting, as if to replace the practitioner, leaves some critics cold. Laboratories should focus on providing the best, proven, reproducible results for each and every specimen coming into the lab. Nothing is more important than that responsibility.

THE IMPROVEMENTS

As long as critics and visionaries exist, clinical lab tests will continue to be tweaked, improved, or replaced as new technologies are born and old mysteries are solved. The evolution of lab testing has led to lactulose/mannitol currently being replaced by intestinal-tissue antibody testing as the gold standard for antigenic intestinal permeability. Salivary antibody testing of IgA alone produced too many false-negative results, but by adding the measurement of IgM, salivary antibody testing has become more sensitive and accurate. The traditional serologic, celiac test panel, which had been proven to give 50% false-negatives for patients with Celiac disease,^{45,46,47} has been improved significantly by measuring multiple proteins and peptides from wheat, together with multiple transglutaminases. Valid scientific improvements to food testing methods have been achieved in recent years.

ELISA Replaces Cytotoxic Testing

The article by Engvall and Perlmann⁴⁸ in the *Journal of Immunology*, "Enzyme-linked Immunosorbent Assay, Elisa: III. Quantitation of specific antibodies by Enzyme-Labeled Anti-Immunoglobulin in Antigen-Coated Tubes," inspired the author during his postdoctoral studies. Earlier, when he was working on both his master's and doctoral degrees, he and his classmates used the tedious lab test method called *agglutination*. Today this method is used in blood banks for typing blood. In the past for immunological testing, a separate tube was used for each determination of reactivity. The old method relied on the human eye to determine the level of agglutination, and the process was done manually, one sample at a time. By contrast, in the ELISA method, 96 to 384 wells are used simultaneously, and the color is generated due to an antigen-antibody reaction that can be measured by automated spectrophotometers called *ELISA*

readers. For the author, this change was revolutionary because the aforementioned agglutination method would have taken months to measure antibodies against only 50 antigens. However, it took another 5 to 8 years before the scientific world realized how ELISA could make work easier by measuring 96 antigens simultaneously.

The author couldn't use the new technology in his master's and doctoral studies. An announcement was made, however, while he was doing postdoctorate studies in immunology at the University of California, Los Angeles in 1979 that a 2-day workshop on ELISA was being taught. The author immediately signed up for the expensive course. The cost of \$350 then was the equivalent of more than \$1100 today, a hefty sum for a postdoc researcher. He learned the ins and outs of ELISA testing, and to this day, he feels it was his best investment, not only for his own work, but also for the clinical world as well. Since the invention of ELISA, the method has seen a myriad of analytical and clinical applications.

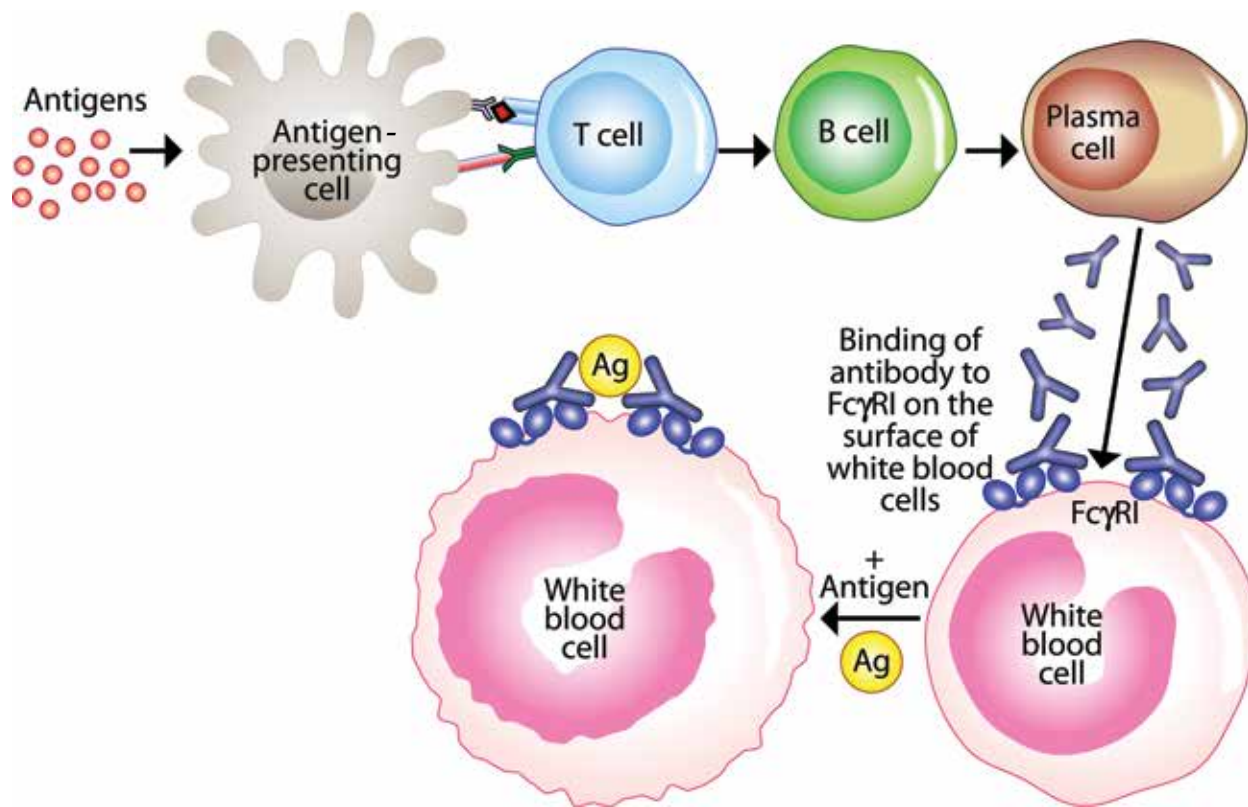
When cytotoxic food testing was banned in California, the author began reading as many articles as he could on the immune mechanisms behind the type 2 immune reaction associated with cytotoxicity and found that antibodies in the blood could bind to food antigens and that the antigen + antibody could bind to a receptor on the surface of white blood cells, causing changes in the cell size. This receptor, which has since been named FcγRI, has a high affinity for the Fc region of IgG, as is shown in Figure 3.^{49,50} Therefore, the question that the author asked was, "If the antibody is IgG, which is responsible for the receptor's binding to the white blood cells, then why aren't clinicians measuring the IgG itself against specific foods rather than looking for changes in cell size, the tests for which have not been proven to be reproducible?" The ELISA workshop became the key to the answers.

The author made a scientific guess, because he did not know if IgG would be produced against every food. He did not know to which white blood cell receptor the IgG could bind. But he took a chance. The IgG ELISA test for food immune reactivity was produced, admittedly without doing clinical studies and associating it with allergies or sensitivities.

After his presentation in 1985 at the American Academy of Otolaryngic Allergy's annual scientific meeting, many visitors came to him to study the refinement of cytotoxic assays. Those individuals also became interested in ELISA. By 1988, many negative articles were being published about cytotoxic testing. In 1987, an article in the *Journal of the American Medical Association (JAMA)*, "In Vitro Testing for Allergy,"⁵¹ condemned cytotoxic testing, stating, "The National Center for Health Care Technology reviewed the evidence and concluded that this test is unproved, unreliable, and without scientific basis." It further stated,

The Food and Drug Administration concurs and has developed a regulatory position that cytotoxic test kits marketed for use in the diagnosis of allergic diseases are adulterated and misbranded devices under the Federal Food, Drug, and Cosmetic Act."⁵¹

Figure 3. The formation of IgG antibodies against various antigens can result first in the binding of antibodies to FcγRI on white blood cells and, then, to the formation of the antigen-antibody complex. The combination of antibody-antigen complexes with the functional receptor (FcγRI) leads to structural changes in the cell membrane and cell size.



Abbreviations: IgG, immunoglobulin G.

The same article also included 3 sentences on IgG testing, one of which brought out the argument that IgG antibodies to food antigens do not correlate with food hypersensitivity.⁵¹ The author agrees with this statement. IgG is not a measurement of hypersensitivity; it is a measurement of loss of immune tolerance. Because of these publications, some labs abandoned cytotoxic testing and offered ELISA IgG instead. Eventually, many labs sprang up offering ELISA IgG food sensitivity testing across the United States.

Since its introduction, the ELISA method for IgG food reactivity has seen improvements. Despite a 2003 statement published by the American Academy of Allergy, Asthma, and Immunology that “Measurement of specific IgG antibodies to foods is also unproven as a diagnostic tool,”⁵² most of the IgG testing on food reactivity throughout the world is done using the same ELISA technique.²⁴ Indeed, many laboratories around the globe use the ELISA methodology to detect IgG reactivity to various food proteins and peptides; the labs must use food antigens that represent the diet of the population being tested, which must be purified to avoid nonspecific reactivity.

The author of the American Academy’s statement goes on to say,

The clinical usefulness of IgG testing in an array of illnesses was illustrated in an early article published by an otolaryngologist who reported that the majority of his patients had substantial health improvements after an elimination of foods positive by IgG food allergy tests. The overall results demonstrated a 71% success rate for all symptoms, achieving at least a 75% improvement level.²⁴

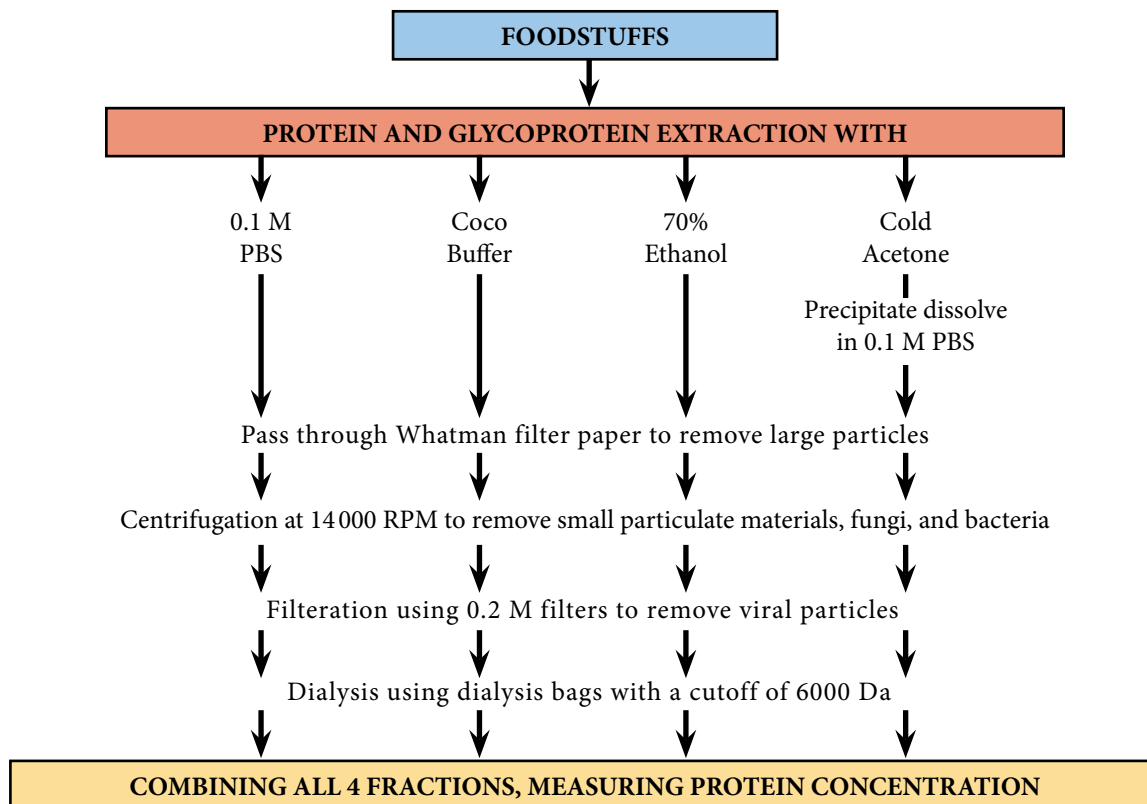
This statement is correct, but the provided reference⁵³ is about a completely different method (RAST), and different IgG subclasses were measured. This scenario represents a case in which the reader needs to check the sources to be able to determine the validity of the information.

Antigen Selection and Purity Guidelines

Some patients are vegans. Some patients are on Paleo diets. Some children have never knowingly consumed a vegetable. But the average patient tested for food immune reactivity consumes a variety of foods, both raw and cooked. If our diets are varied, why are the tests for food reactivities not? Few labs offer testing of food proteins that have been heated, yet only a few patients are completely raw foodists.

In a large-scale study by Zeng et al,⁵⁴ variable serum levels of 14 different food-specific IgGs were assessed among

Figure 4. Summary of analytical methods for the preparation of food antigens.



Coat ELISA plates with optimal amount of antigens to obtain the most specific antigen-antibody reaction.

Abbreviations: PBS, peripheral blood smear; ELISA, enzyme-linked immunosorbent assay.

healthy and symptomatic Chinese adults, and the possible association of the foods with chronic symptoms was investigated. The researchers indicated,

Although testing for the presence of food-specific IgGs has been regarded as a potential tool for the diagnosis of food allergy/intolerance, it's the accuracy and clinical utility of such testing that remains unclear.⁵³

Interestingly, this study reflected that women had higher concentrations of food-specific IgG reactivity than men for most of the foods tested, which had been shown by previous research publications.⁵⁵⁻⁵⁷

The food-group correlations with self-reported symptoms were shown. Participants who reported having eczema had higher concentrations of crab- and rice-specific IgG than subjects with no eczema. Another example was a participant with no gastrointestinal symptoms, who had higher concentrations of IgG against codfish, rice, mushroom, tomato, and wheat compared with an individual with chronic symptoms. Overall, these findings do not make sense and only add additional variables to many existing confusions associated with food-specific IgG testing.

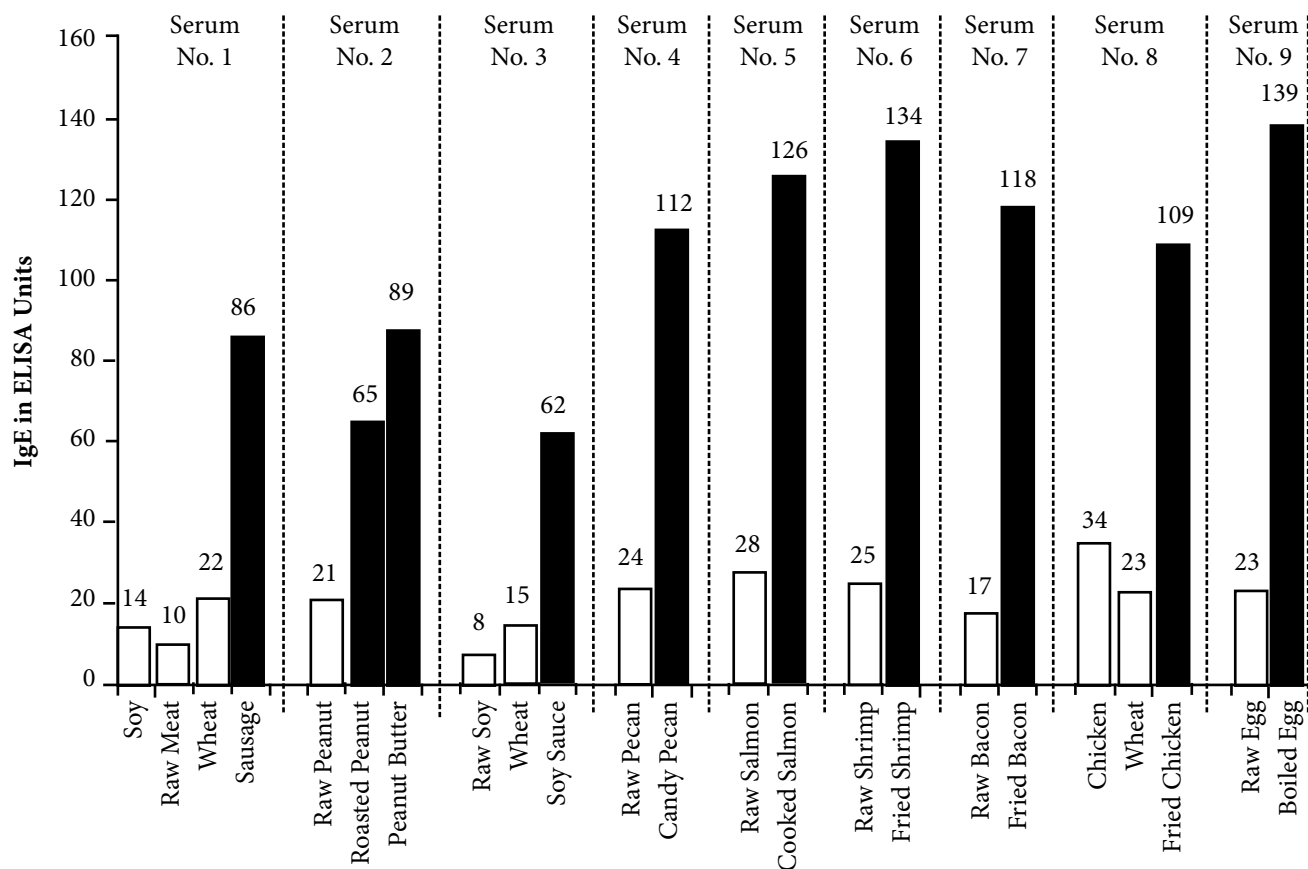
Indeed, the authors admitted to several limitations in the study. First, IgG antibodies were tested against only 14

different foods consumed in China, but the kits were purchased from the United States. The kit company extracted food antigens from food products available in the United States and did not measure levels of specific IgGs against fruits, nuts, many vegetables, seeds, food additives, and more.⁵⁸ Second, chemical symptoms were determined by self-reporting and were not confirmed by health care professionals. This procedure may have led to misclassification of chronic symptoms. Third, the study did not connect identified mechanisms of IgG food reactivity to clinical manifestations.

Additional limitations of the study were not mentioned by the authors. First, the researchers purchased kits for measuring antibodies against various extracts made from raw and unmodified foods. Any food reactivity testing performed must reflect the test subject's current diet, and the Chinese are not particularly known for eating solely raw food. Therefore, a study on the same topic should be repeated using modified food extracts. Second, in this extensive study on 5394 adult participants from different regions of China, only IgG was measured but not IgA or IgE antibodies.

It is a very well-established scientific fact that due to breakdowns in immune tolerance mechanisms, the first isotype antibody produced against food antigens is IgA, followed by IgG. Since 1985, when the author replaced cytotoxic testing with

Figure 5. Serum levels of IgE antibodies against raw (white bars) vs processed (black bars) food antigens, expressed by ELISA units. The graphic shows the measurement of IgE antibodies against different raw or crude ingredients vs the processed or cooked version of the foods in the sera of 9 individuals with food allergy.⁵⁸



IgG testing, researchers have continued to study food-specific IgG or its subclasses, but not IgA, or even IgM.⁵⁹⁻⁶² Further elucidation of the role played by food-specific IgA in food immune reactivity (not food allergy) should be the first priority of future studies, followed by IgG and IgM.

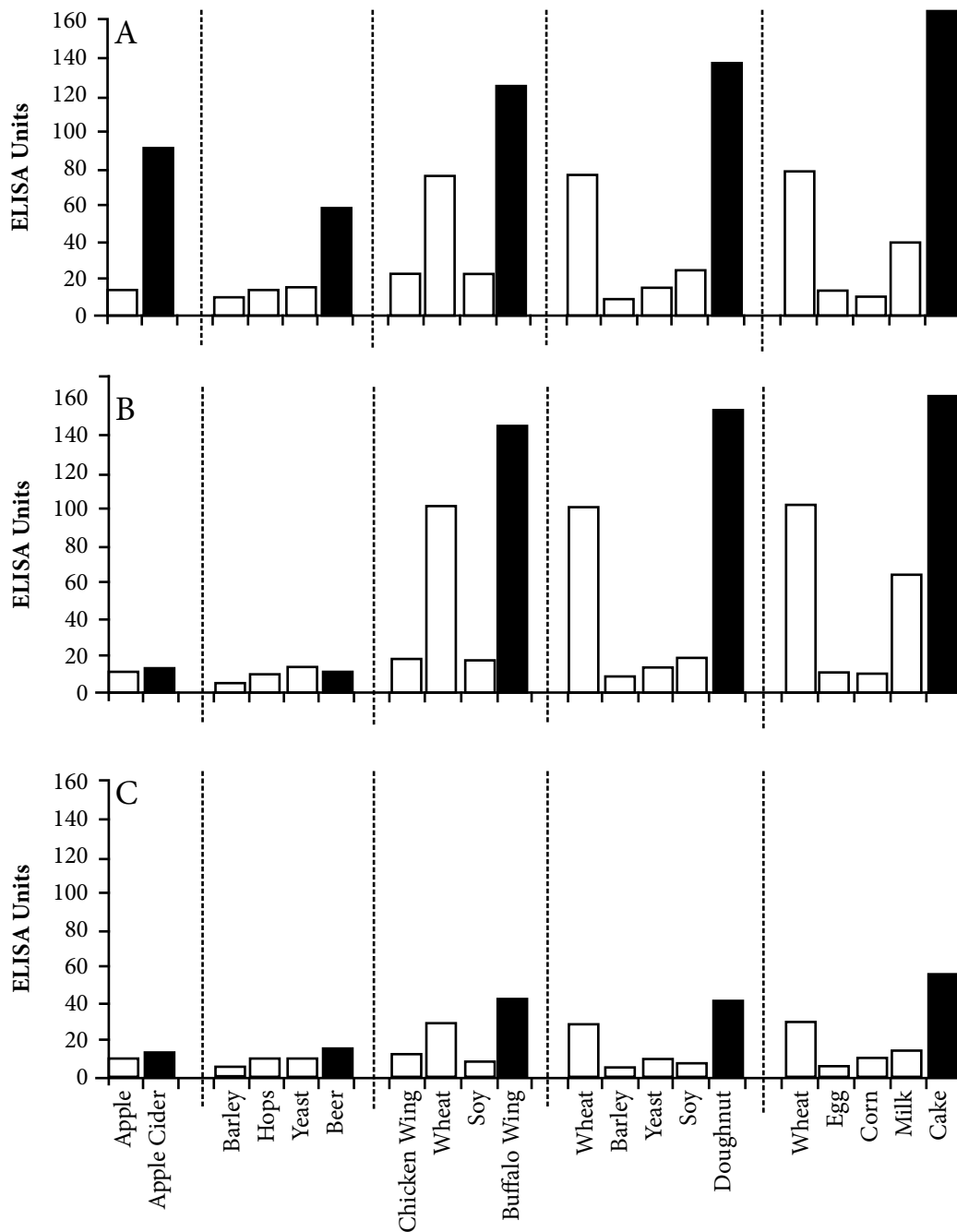
The ELISA test, whether for food, bacteria, or human tissues, is only as good as the purity of the antigen on the plate. Both the degree of purity obtained in the extraction of different food proteins and other components of the foods tested, and the relative efficiency of the coupling of different extracted food components and antigens to the ELISA plate, are known to influence the accuracy of food-specific IgG measurements.⁵⁴ When preparing an antigen that will be used to coat an ELISA plate, all interfering substances must be removed, leaving only the food-specific glycoproteins and lipoproteins. The process has been well described⁶³ and is shown in Figure 4. Clements first developed a low-temperature, food extraction method for vegetables in 1965, which the author has modified.²⁸ Centrifuge machines and 0.2 M filters are used to remove debris, bacteria, fungi, and viral particles. The remaining proteins are then dialyzed multiple times to remove molecules smaller than 6000 Da. Then the food antigens are fingerprinted using electrophoresis with sodium dodecylsulfate gel. This process for the

preparation of quality food antigens is summarized in Figure 5. In a majority of cases, various researchers have determined the exact epitope—a combination of many amino acids—that is responsible for allergenicity of food antigens. These peptides can be synthesized to a purity of more than 90% and can be used in IgE- and non-IgE-mediated food reactivities.^{28,64,65}

As mentioned above, most people eat a mixture of both raw and cooked foods. The first documented case of immune reactivity to cooked food was reported in 1921.⁶⁶ Commercial labs use food antigens prepared only from raw food. Research has shown that food can become more antigenic or less antigenic when it is heated.²⁸ When a lab tests only raw-food antigens, it can fail the patient who reacts to the cooked or processed versions of the food rather than the raw form.

Cooking or processing and denaturation of food proteins may cause alterations in immunodominant epitopes, potentially affecting allergenic properties. This processing may destroy existing epitopes on a protein or may cause new ones to be formed (ie, neoallergen formation) as a result of changes in protein conformation. Neoallergen formation has been known for at least 30 years.⁶⁷ The concept of neoallergens may explain why some individuals can tolerate a raw food or a raw-food ingredient but will react to the same food when it

Figure 6. Serum levels of IgG (A), IgA (B), and IgM (C) against raw (white bars) vs processed (black bars) food antigens, expressed by ELISA units. The graphic shows the measurement of IgG, IgA, and IgM antibodies against different raw or crude ingredients vs the processed or cooked versions of specific foods in the serum of a patient with high reactivity.⁵⁸



Abbreviations: IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; ELISA, enzyme-linked immunosorbent assay.

is heated in some way. For example, a person who is reactive to ketchup may not have an immune response to a raw tomato. If this patient is tested using traditional food sensitivity assays, the result would be negative for tomato, and the patient's problem with ketchup or other forms of cooked tomato would go unidentified.⁶⁸⁻⁷⁷ Researchers have found neoallergens from pecans⁶⁷; wheat flour⁶⁹; roasted

peanuts⁷⁸; lentils⁷⁰; almonds, cashews, and walnuts⁷⁹; soybeans^{71,72}; shrimp, scallops, tuna, eggs, apples, plums, milk, and potatoes^{73,74,80-82}; and many other foods that had not been previously tested.

Despite multiple publications in scientific journals demonstrating IgE-mediated reaction to many cooked or processed food antigens without reaction to raw foods,

Table 1. Comparison of Results^a

	Sample No.	No. of Modified Food				
		Antigens With IgG Reactivity > 30 EU	IgG Against AGE-HSA in EU	IgG Against AGE-Hb in EU	IgG Against anti-ox-LDL in mU	IgG Against MBP in EU
8 Patients with high reactivity to many modified food antigens	1	8/45	86	105	1642	118
	2	12/45	43	91	2050	136
	3	7/45	32	26	916	65
	4	16/45	120	111	2431	129
	5	11/45	61	23	1442	84
	6	9/45	25	39	683	27
	7	13/45	97	108	1216	120
	8	15/45	134	82	734	71
	Total	91/360	598	585	11 114	750
8 controls with low reactivity to few modified food antigens	9	1/45	12	7	265	15
	10	0/45	6	13	448	17
	11	2/45	19	22	1025	31
	12	4/45	15	26	683	18
	13	0/45	8	16	343	22
	14	1/45	63	46	205	75
	15	2/45	38	27	412	49
	16	3/45	24	13	157	95
		13/360	185	170	3538	322
P Values		<.000001	<.005	<.005	<.005	<.005

Abbreviations: IgG, immunoglobulin G; EU, equivalent units; AGE-HSA, advanced glycation end products-human serum albumin; AGE-Hb, advanced glycation end products-hemoglobin; anti-ox-LDL, antioxidantized low-density lipoprotein; MBP, myelin basic protein.

^aComparison is for 8 patients with IgG reactivity > 30 EU against many modified food antigens with those for 8 controls with low reactivity to a few modified food antigens, in relation to possible cross-reactivity with AGE-HSA, AGE-Hb, anti-ox-LDL, and MBP.

almost all commercial laboratories measure IgE and IgG antibodies primarily against raw-food antigens. Part of the findings of the author's own studies¹⁰ are reviewed in Figures 5 and 6. Data from only one patient is shown in Figure 6. This and other data published earlier²⁸ clearly indicate that an individual may not show IgG, IgA and IgM antibodies against antigens prepared from raw food, but testing the same patient against antigens prepared from cooked food might result in a severe immune reaction.

One other component to food immune reactivity that is implicated in inflammation and autoimmunities is advanced glycation end products (AGEs). By reacting with AGEs and tissue proteins, antibodies against modified food antigens may cause perturbation in degenerative and autoimmune diseases, such as diabetes, atherosclerosis, inflammation, autoimmunity, neurodegeneration, and neuroautoimmunity. Thus, detection of antibodies against food antigens is not only an indication of immune reactivity to foods but also of

the size of the role that these antibodies play in inflammation, autoimmunity, aging, diabetes, and neuroimmune disorders. The importance of IgG testing against different food antigens and their association with AGE- human serum albumin, AGE-hemoglobin, antioxidantized LDL antibody, and myelin basic protein antibody is shown in Table 1.

Specimen Testing in Duplicates

Many steps are involved in ELISA testing, from adding the patient's specimen to the plate, to washing the plate, incubating the specimen, adding substrate to the plate, and so on. With each step, a potential exists for error. One way to spot these potential errors is to run a patient's specimen in side-by-side duplicate (ie, split the specimen and run the halves side by side). If correlation occurs between the 2 side-by-side wells, then the results can be reported. If the side-by-side wells lack correlation, the patient's specimen must be run again in side-by-side duplicate until correlation is achieved.

The Clinical Laboratory Improvement Amendments (CLIA) of 1988¹⁸ apply to all facilities that perform tests on human specimens. Laboratories employ CLIA guidelines for internal and external quality control or quality assurance. Internal quality testing entails pulling specimens that have already been run, and retesting them to see if reproducibility exists. Some food testing labs run as many as 100 specimens, each specimen on an individual well, and then randomly choose only 2 to 5 wells with a single antigen for quality control. As the inventor of the IgG ELISA test for food, the author feels that this practice provides inadequate quality assurance.

In his opinion, each and every specimen must be run in side-by-side duplicate, thereby guaranteeing quality assurance for each patient. In addition, each food antigen tested should have a negative and positive control on the same plate. Other internal quality-control techniques include splitting a patient's specimen and labeling it as belonging to 2 patients, without the knowledge of the technician running the test. The ideal is that the split specimens should have correlated results. Again, without doing quality control every day that patients' samples are run, labs cannot assure the user of reproducible, quality test results.

Conclusions: Improvements

Throughout the years of laboratories offering IgG ELISA testing, the literature has had a dearth of research papers showing quality controls and testing versus clinical manifestations and outcomes. This lack has led to IgG ELISA testing being discredited by researchers, clinicians, and even countries. For this reason, as the developer of the original assay, the author has kept his distance from IgG food testing. In fact, for many years, he prevented his marketing manager from promoting the food testing offered at his clinical lab. He did not whole-heartedly believe that the labs doing the test were doing the full and necessary steps required for reporting reproducible and reliable food antibody assays. As was discussed earlier, reporting of truly reliable test results is highly dependent on the purity and quality of the antigens used and the steps taken for the validation of the assay, which should be documented in the assay's standard operational procedure. Because of this issue, as the inventor, the author felt a weight on his shoulders and lost much sleep each time the IgG ELISA test for food came under attack.

Despite his misgivings about the implementation of the ELISA IgG food test, the author has continued to research, study, and amend the process to yield better test results. This work entailed perfecting the food extraction method for obtaining only pure food proteins to coat his ELISA plates. The author has insisted that each specimen be run in side-by-side duplicate to ensure correlation and has embraced the concept of assessing not only raw food antigens but also processed ones to develop immune reactivity panels for food that better reflect the diet of the patients being tested. Finally, the testing of each individual antigen should be validated separately and properly documented.

What is really the cause for the low opinion some have for ELISA IgG food testing? Is the issue the test itself or the labs that have failed and continue to fail to implement and follow proper procedures for the validation process before reporting test results?

CLOSING REMARKS

While writing this manuscript, the author received the January 2014 issue of the *Townsend Letter*. It featured 4 different, self-promoting articles about the issue of food allergy and sensitivity. Each article promoted the author's methodology for assessing food immune reactivity. Each article implied, "My test is better than all the other tests." This message may have confused many readers. Although these articles are very well written, none of them dealt with the root causes of the problem, which are as follows: (1) What are labs really measuring?; (2) What is the scientific mechanism behind the testing?; and (3) Where is the clinical correlation of the test results with patients' symptomatology that should have been published in peer-reviewed, scientific journals?

So what do scientists need to do to change this industry trend that began in 1985 after the banning of cytotoxicity testing and the introduction of ELISA food reactivity?

First, the field has to admit that currently it has a huge problem, and unless labs get together to standardize testing methodologies, then thousands of false-positive and false-negative results will continue to be reported by many laboratories.

Second, scientists must classify the lab test simply as being cell-based reactivity or antibody (IgG, IgM, IgA) reactivity assays.

Third, labs that perform the cell-based assay must unite to standardize procedures and try to use the same reagents, in particular the food antigens, that are as close to the reality of human consumption as possible.

Fourth, labs that perform antibody-based assays should standardize procedures and try to use the same food antigens that reflect the dietary trends of the population being assessed.

Fifth, labs performing assays based on volumetric changes in cell size must come up with an acceptable resolution to the documented problems with the methodology and present the clinical significance of their tests.

Sixth, if such clinical significance is not available, the labs must immediately start clinical studies and present the raw data to the industry via publications in peer-reviewed journals.

Seventh, labs performing antibody-based assays need to present the clinical significance of this test to the industry through articles in peer-reviewed journals.

Eighth, scientists must stop referring to these tests as food allergy assessments. None of these tests measure specific IgE against foods, which is the measurement that is associated with food allergy.

Finally, during the validation process, each food antigen should be tested separately according to CLIA and FDA guidelines rather than tested using the shortcut process of validating antibodies to 90 foods simultaneously.

Until standardization of the antigens and procedures for testing occur, no one in the industry can compare one IgG lab to another IgG lab, one cell-based lab to another cell-based lab, or one IgG lab to a cell-based lab. Otherwise, scientists are just talking about comparing apples to oranges or apples to sausages.

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