IgG Food Allergy Testing by ELISA/EIA
What Do They Really Tell Us?

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Adverse reactions to food may initiate a myriad of physiological effects in the body. These reactions may be immunologically or non-immunologically mediated and can result in signs and symptoms ranging in severity from mild to life threatening anaphylaxis. Although the majority of severe reactions are thought to be immunological and mediated via IgG and IgA may play a role in adverse reactions to food as well.

The clinical laboratory has historically played an important role in the diagnosis and management of patients with allergy. This role has been more clearly defined with the diagnosis of IgE mediated adverse reactions and less well defined with the diagnosis of other immunologic etiologies or adverse reactions of non-immunologic origin. Diagnosis of food allergy, in particular, has classically involved the detection of IgE antibodies with a variety of different methodologies.

Of late, a number of clinical laboratories have set up ELISA/EIA (Enzyme Immunoassays) panels to test the presence of IgG antibodies in patients to numerous food allergens. This is based on the findings that certain subclasses of IgG have been associated with the *in vitro* degranulation of basophils and mast cells, the activation of the complement cascade, (both of which are important mechanisms in allergy and anaphylaxis and the observation that high circulating serum concentrations of some IgG subtypes have been measured in certain atopic individuals. The premise behind this testing is that high circulating levels of IgG antibodies are correlated with clinical food allergy signs and symptoms. These tests, one might extrapolate, would help the physician pinpoint food allergies in their patients so that patients might avoid these foods and their associated signs and symptoms. The ELISA/EIA test itself involves coating a 96 well plate with food antigens, adding a patient's sera and looking for a classic antigen/ antibody interaction. In addition to the IgG antibody detected in most of the newer commercial assays, some companies also detect IgE.

Food allergy panels have found an increasing popularity among physicians who are looking for a reliable method to aid in the diagnosis of an otherwise difficult diagnostic problem. Up until now, the only methods for the detection of food allergy included skin tests, elimination and challenge diets, or double blind placebo controlled oral food challenges. Skin tests, although fairly reliable for the detection of IgE to environmental allergens, are not well correlated with food allergy signs and symptoms. Placebo controlled food challenges and elimination/ challenge diets are extremely time consuming for the patient and practitioner and elimination/ challenge diets require a high degree of patient motivation and compliance.

The detection of food allergies with the use of food allergy panels, in contrast to the previously mentioned methodologies, is easy and convenient for both patient and physician. One need only submit a blood sample from the patient and the laboratory returns not only the foods the patient is "allergic to" but a "rotation" or "elimination" diet for the patient. The cost is moderate to high, running on average between $100 and $400 per panel.

The use of these food allergy panels for the diagnosis and management of food allergies, however, is fraught with problems. These problems include reliability in testing, an arguable theory behind the testing and the prevalence of treatments (food rotations or other diets) prescribed by these testing laboratories based solely on laboratory test results. This article will address these problems and others.

Reliability in Testing

From a laboratory point of view, these are two essential components of any laboratory test. One is the *validity* of a test. In other words, its correlation to a disease state or condition. In laboratory statistics, this is closely related to the *positive predictive value* (PPV) of a laboratory test. This will be discussed later in the article. Before the validity of a laboratory test can be assessed, however, the reproducibility or reliability of the test must be evaluated and confirmed. In the world of laboratory testing, if a test is not reproducible, *it is considered worthless*. The validity of a test or its correlation with disease states is irrelevant if a test is not reliable.

Almost all laboratories do "in-house" reproducibility checks. The majority of good laboratories not only do "in-house" checks but submit to "unknown" reproducibility checks via testing agencies like the CAP (College of American Pathologists). Another option for outside reliability testing, when CAP is not available, is for the testing laboratory to have physicians regularly send in patient "split samples" (with the cost assumed by the testing laboratory). When a sample is split, acceptable variance between the two specimens is 10% or less, according to universal laboratory standards. If more than two split samples are evaluated, there should not be more than 20% variance between the high and low end values. The participation of laboratories in outside reproducibility checks, however, is voluntary. It remains the responsibility of the physician using a particular laboratory to check if their laboratory does reproducibility testing and if so, what type they do.

As part of our ongoing effort to investigate and evaluate *all* laboratory tests done in-house and sent-out, we at Bastyr University Natural Health Clinic Laboratory have recently investigated the reproducibility of food allergy testing panels from the three different laboratories we routinely send samples to. These investigations are part of our normal quality control of laboratories. The testing recently involved sending six specimens apiece (drawn all from the same patient at the same time) to the

three labs. Three specimens were sent at the same time of the draw and three specimens were sent frozen according to outside laboratory processing guidelines a week later. Although all specimens were from the same patient, all specimens were given different names.

Two of the three laboratories (Lab A and Lab B) to which we send our specimens report numerical values and interpretations for these values. High numerical values represent high circulating levels of IgG (according to the laboratory) and are associated with foods that should be avoided. Low values represent lower circulating levels and are associated with foods that may be eaten. The third laboratory (Lab C) reports semi-quantitative numerical values (1+, 2+, etc) but interprets all positives the same. In other words, all foods that give even the slightest reaction (1+) should be avoided, according to this laboratory.

Two laboratories (Lab A and B) had numerical variances that were incredibly high. Lab A had an average numerical variance of 73%. What that means is for any one food (eg. American cheese), there was an average of 73% between the high and low numerical values. Lab B had an average numerical variance of 49%. The numerical variances, however, mean very little to the average physician. What most doctors care about is the interpretations. Therefore, we examined the interpretations (clinical recommendations) from the labs as well. Lab A had a 59% average variance in clinical interpretation. What that means is that for any one food, the recommendations to "eat or not to eat" were contradicted in 59% of the foods tested in at least two of the six samples. Lab B had an acceptable clinical variance of 7%. Only in 7% of the foods tested were clinical interpretations contradicted. Of special note is that Lab A, upon learning of the results of our split samples requested to be "tested" again. We complied several weeks later with three split samples (drawn from the same patient at the same time and sent to the lab immediately). This time there was a clinical variance of 46% but with only three samples!

Lab C had more reasonable variances in its testing results. There was only an average 9% numerical variance between all the samples. This correlated to a 9% clinical variance because all positives by this lab were considered significant. Both of the variances from Lab C numerical and clinical interpretation were well within accepted laboratory standards.

In conclusion, two of three labs tested had numerical variances outside acceptable laboratory standards and are not considered reliable. In addition, one of these labs had clinical interpretations outside these limits as well. It is important to note that these results have no relation at all to the *accuracy* of this testing or the closeness to the "real" value. Accuracy is impossible to measure for food allergy IgG ELISA/ EIA because there is no acceptable "gold standard" in food allergy testing to measure this against. This leads us to the question of validity of food allergy testing via IgG ELISAs.

Theory behind testing

Second to reliability is validity when it comes to evaluating laboratory testing. Part of the validity evaluation is to either compare a new test to currently accepted "gold standards" for the particular substance being measured or to initiate studies that show the positive predictive value (PPV) of the new test. In other words, what percentage of the population with an abnormal or positive test will have a particular disease/ condition/ set of defined signs and symptoms? A simple mathematical formula exists for PPV that takes into consideration the true positives (those correctly classified with a positive test) and false positives (those incorrectly classified with a positive test). This PPV is of extreme importance when no "gold standard" exists for a newly measured substance like IgG for food allergy.

At this time, after extensive literature searches and interviews with various companies offering this test, we at Bastyr are unaware of any peer-reviewed published study examining the positive predictive values of IgG for the diagnosis of food allergy or the association of this test with food allergy signs and symptoms. Only one company (in Florida) of all we interviewed, reports that a study examining correlation of "food" IgG levels and elimination diets is currently underway (n=50). Therefore, with regard to high serum levels of IgG and the aforementioned in vitro work on basophils, mast cells and complement, it is a large extrapolation that IgG to food antigens is correlated to signs and symptoms of food allergy. Furthermore, the clinical meaning of elevated IgG levels in atopic individuals has caused much debate of late, including the premise of IgG as a blocking antibody.

What is Really Being Measured in the ELISA/ EIA?

In addition to the lack of documented correlation between IgG and food allergy,, it is uncertain if numerous companies doing this assay are even measuring what they think they are. Upon interviewing the companies that we send our patient samples to, we learned that all of these companies do their own "in-house ELISA/ EIA". What that means is they designed their own EIA/ ELISA tests from scratch. The questions that arise concerning "in-house" ELISAs is how and where the companies obtained the food antigens that coat the 96 well ELISA plates. In other words, what are the circulating antibodies in patient sera binding to?

One of the labs that we evaluated claimed "proprietary information" as to the manufacture of their antigens but the other two labs both bought the food antigens for their ELISA panels from a company in Oklahoma. Interviewing the chief technologist from this Oklahoma Company gave some surprising insight into their food antigen preparation. The foods to make the antigens were obtained from a local Oklahoma market they "tried to buy organic foods whenever they could"). The foods were then chopped finely and diluted to make the antigens. Other than several rinses with an organic solvent (acetone), the food antigens were not purified.

The problems that may be associated with this preparation are enormous. For one, all food (organic and non-organic) is coated with microorganisms. The most common of these include bacteria and fungi but viruses and parasites may also be found on fruits, vegetables, grains, milk and meat products. Microorganisms have many antigens that are highly immunogenic. It is common knowledge that most people have high circulating levels of IgG to a number of common microorganisms. To this likely wealth of microorganisms in the testing wells, there is the presence of possible pesticides and organic

solvents that are not (according to the technologist interviewed) rinsed away during preparation.

Therefore, what is really being measured in these panels? Is it an immune reaction to certain foods or is it a person's exposure to common bacteria and fungi? What about a person's previous exposure to pesticides and organic solvents? Numerous studies have shown high levels of IgG to pesticides and organic solvents in persons with high exposure rates. It is possible that there are many antigens in each well. If that is true, then one would see a high number of false positives in these tests.

Are there a high amount of nonspecific binding and false positives occuring in these tests? There is no way to test this easily, at the present time. However, what was seen in our small study correlates with this hypothesis. The patient whose blood was drawn for our reproducibility studies is in very good health with no current signs and symptoms of food allergy. This person, however, tested reactive in 76% of Lab A's test (73 positive/ 96 foods), in 29% (28 positive/ 95 foods) of Lab B's test, and reactive in 22% (22 positive/ 102 foods0 of Lab C's test.

Therapeutic Diets

Last, but certainly not least, of the problems associated with food allergy testing are the therapeutic "elimination" or "rotation" diets that are sent back with the test results from most of the laboratories performing IgG food allergy testing. Although these diets that are usually sent to the physician ordering the test, they may be sent directly to patients by certain labs via physician requests. There are several problems with this practice. Included in these problems 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.

The first of these problems mentioned above is that laboratories do not have a license to practice medicine by prescribing treatments or therapeutics. Licensed laboratories have the right to perform quality laboratory testing and to provide consultation on interpretation of these lab tests to physicians when necessary. This stops short of prescribing therapeutics. This also applies to NDs or MDs working for the testing laboratory. Although the laboratories that perform food allergy testing may argue that diets are not treatments, one may vehemently disagree with this due to the fact that most Naturopaths and some Allopaths use dietary changes (including elimination or rotation diets) as a major constituent of many treatment plans. These treatment plans are made by the doctor, often in consult with a qualified nutritionist, after very thorough histories and physical exams are performed with the patients. This brings me to the second problem.

At Bastyr University, a very important part of the ND student's clinical education is the emphasis on the history of the patient. Medical students are taught that the majority of diagnoses can be made from listening to patients and asking the right questions. Another major constituent of diagnosis is a complete physical exam of the patient. Laboratory testing is taught to be used only as required. That is, to confirm or rule out a diagnosis. An important guideline taught by our chief medical officer about laboratory test may change the way you treat a patient, then it is valid to order. If not, then don't order it and waste your patient's time and money. In this author's opinion, prescribing therapy based solely on the results of laboratory testing is as far away from holistic medical practice as one can get.

Another problem to consider in the practice of therapy based solely on lab tests is what if the test is incorrect? Although it is unlikely serious harm will come to patients if they avoid certain foods, one has to consider the effort, anguish, time, and cost involved in removing many foods from a diet that may not be causing harm to patients. In addition, there is the potentiality of promoting nutritional deficiencies if certain food groups are removed from the diet for long periods of time. There is also the possibility that an allergic food may be missed from an inaccurate test. This, however, is less likely due to the extremely high number of foods an "average" person is "allergic to" in the typical test reports we have received. An additional point is the cost to the patient of a laboratory test that is neither reliable nor valid. If a test is not reliable or valid, this cost is excessive no matter how much it is.

Conclusion

In conclusion, food allergy testing by IgG ELISA/ EIA panels is a convenient and easy way to diagnose food allergies in a patient. It is, however, a testing method that is questionable in both its theory and validity. It is also costly and may not be reliable, depending on which laboratory you use.

An argument in its favor by certain physicians is that it isa extremely popular with patients because it gives "printed proof" to the patient that the patient is "allergic" to certain foods. This makes it easier for the doctor to convince the patient that they need to change their diet. Is this "printed proof" however, a very costly substitute for discussion with and education of patients? Would patients insist on this test if they knew they may not be reliable?

After preliminary investigation of food allergy-testing panels offered by three different laboratories, it is this author's suggestion that physicians give serious consideration to the aforementioned issues before ordering these panels for the diagnosis and management of patients with food allergies. If one does order these tests, it is highly recommended that reproducibility of these tests be investigated. At the very least, physicians should consider the possibility of sending split samples to their testing lab (at the cost to the lab) on a regular basis.

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