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# NMJ Original Research: Reproducibility and Reliability of Two Food Allergy Testing Methods

By Wendy Hodsdon, ND, and Heather Zwickey, PhD


## Abstract

The ability to identify and eliminate food allergens in the diet affects an individual's health. Thus, clinicians need a reliable and reproducible way to identify foods allergies or sensitivities for their patients. **Objective**: To compare and test the reliability and consistency of 2 different food allergy testing methods: cell size allergy testing versus IgG ELISA food allergy testing within the same donor. **Design**: Blood samples from a single donor were sent to 2 different food allergy testing labs under different names. Both laboratories used different food allergy testing methods. Two samples were sent to each lab on the first day (split sample), and 2 more samples were sent to each lab over the course of the following week (4 samples sent to each lab in the same week). The results from these tests were evaluated 3 ways: 1) within test repeatability on a split sample; 2) within test variability over the course of a week; and 3) interlaboratory variability between the 2 testing methods. **Outcomes**: Reaction results from both testing methods were reported as no reaction, low reaction, moderate reaction, or high reaction. Reactions to individual foods were evaluated and compared statistically between different time points. **Results**: The IgG ELISA food allergy testing method showed consistency both in a split sample on a single day and over the course of a week in the reported results. The cell size testing method generated random results for split samples in both time periods in both time periods (split sample and over a week). **Conclusion:** This study calls into question the reliability of blood cell size testing as a method for identifying food allergies. While the sample size was small, these tests are completed for individual patients in a clinical setting and thus, variability must be minimal for the test to be clinically valid. IgG food allergy testing was reproducible and reliable in this study.


## Introduction

The consumption of food should result in oral tolerance in a healthy individual. If tolerance occurs, the person will not develop physical symptoms as a result of ingesting the food. In contrast, food allergies and hypersensitivities result in wide variety of symptoms in otherwise healthy individuals.[1,2,3,4]

Food allergies or sensitivities are often underreported because many people don't recognize the signs and symptoms of a food allergy and are never tested. Common food allergy symptoms include diarrhea, constipation, abdominal bloating, gas, rashes (including eczema), tinnitus, nasal congestion, chronic sinus infections, joint pain, and headaches.[5,6,7,8] These symptoms are caused by the immune response to the food allergen. This immune response is measured by looking at antibodies made to particular foods, either directly or indirectly. Food antigens may elicit different classes of antibodies, designated as IgM, IgA IgE, and IgG (subtypes). These antibodies may trigger different adverse reactions depending on the person.

The greater medical community examines classic food allergies through identification of an IgE response to food antigens. When an allergenic food is ingested, it is taken up by antigen-presenting cells in the Peyer's patches of the intestine. CD4 T cells specific for the food make Th2 cytokines (IL-4, IL-5, and IL-13), causing B cells specific for the food allergen to make IgE (from IL-4) or secretory IgA (sIgA from IL-5). IgG antibodies may also be made in response to food allergens. IgE antibodies attach to Fc receptors on mast cells and eosinophils. When these cells encounter triggering foods, they degranulate, which may include the release of histamine. It is this degranulation that causes the more severe allergic reactions such as hives, diarrhea, and anaphylaxis.[9]

Food hypersensitivity is common, but the symptoms may be difficult to distinguish from other chronic diseases or conditions. In contrast to an IgE allergic response which is faster and more severe, food hypersensitivity is a delayed type cell-mediated response. It often is confused with other chronic diseases or conditions. Delayed type hypersensitivity (DTH) responses begin when ingested food is taken up by antigen-presenting cells and presented to CD4 T cells that make Th1 cytokines (IFNg, TNFa, and TNFb). B cells specific for the food antigen respond by class switching to IgG3. In DTH reactions, the macrophages, basophils, and CD8 T cells are responsible for the symptoms rather than the antibodies. The cell-mediated response causes the production of reactive oxygen species, prostaglandins, and leukotrienes, leading to a variety of symptoms in the body. This increases overall inflammation, and therefore can be confused with other diseases that have inflammatory components.[10]

Researchers are working to understand the significance of subclasses of IgG during allergic response. IgG1 (induced by TNFa) is found in high levels during infectious disease but is also found in allergic or atopic people.[11,12] Polysaccharide antigens (usually from bacteria or food) stimu-

late IgG2.[13] IgG3 (induced by IFNg and TNFa) is most often elevated during infectious disease. IgG3 is at lower levels in people with allergies and higher levels in people with DTH. Elevated IgG4 antibodies have been found in patients with atopic dermatitis and eczema.[14] IgG4 is thought to be related to prolonged antigen exposure.[15] Similar to IgE, IgG4 requires IL-4 and IL-13 for production.[16] Another cytokine, IL-10 may induce IgG4 secretion.[17] IL-10 may also determine whether B cells continue to produce IgG4 or class switch to IgE.[18] Some allergens do not induce an IgG response at all.[19]

There are reactions to food that are not immune-mediated. These reactions include direct toxic reactions to a food ingredient. There are some food components to which everyone reacts; for example, food poisoning is a reaction to a toxin (most commonly staph enterotoxin A or staph enterotoxin B) made by bacteria. Many people react to monosodium glutamate or other food chemicals that they are unable detoxify. A genetic predisposition can cause susceptible individuals to overreact to a food ingredient, such as histamine contained in foods such as cheeses and smoked meats. Other food components are problematic for people lacking appropriate enzymes, which may lead to lactose intolerance, favism (glucose-6-phosphate deficiency), and other diagnoses.[20]

## Food Allergy Testing

There are a variety of ways to test for food allergies. Allergy skin testing is most common in allopathic medicine. In this test, a suspected food allergen is put into solution and then dropped onto the skin. A small prick is made through the drop of the food allergen with a lancet. If the person is allergic to the food, a hive will appear within 20 minutes. This test identifies IgE mediated food allergies.[21] This test, however, cannot be used if a person has eczema at the site of testing. Also, the test may show no reaction to foods that have low levels of IgE.[22,23] Likewise, a false negative may occur in people taking antihistamines or other immunosuppressive pharmaceuticals. Intradermal skin testing is more sensitive than skin prick testing, but is also more uncomfortable for the patient.[24]

IgE can also be measured from blood using ELISA (enzyme linked immunosorbant assay). Advances in technology over the last 10 years have made these tests more common. They are less invasive for the patient than skin tests. The test can report both the presence of an antibody and the relative quantity of the antibody in the serum. However, it is important to remember that this antibody level will be related to the immediacy of the exposure to the food antigen and to the food antigen that is used by the lab measuring the antibody.

IgG and sIgA ELISA are also becoming popular, but they have the same drawbacks as IgE ELISA. They are dependent on exposure to a food and on the antigen used in the assay. Secretory IgA is most often measured from saliva samples, whereas IgG is measured in blood. IgG subclass may influence the correlation between test results and clinical symptoms and is not always reported by the laboratory completing the test. As discussed above, most often IgG1 and IgG4 are elevated in an allergic response. Thus, while ELISA testing is useful in measuring food allergies, it is unable to measure all the reactions that may cause clinical symptoms.

One of the lab tests available for food allergy and sensitivity testing evaluates the change in white blood cell size and number as a measure of reactivity. It is known that B cells increase in size when they become plasma cells and produce antibodies, T cells increase in size when they produce cytokines, and neutrophils change size when they become activated. However, the lab that uses this method does not report what cells are being measured in their assay, nor do they report the methods of the assay.[25]

This study compares intralaboratory reliability between 2 types of allergy testing: IgG testing and cell size variability. The results reported herein suggest that IgG testing is more reproducible and reliable than cell size variability. While the initial design included an interlaboratory comparison to see how equivalent the results were of these 2 testing methods, the cell size variability data was not consistent enough to use in a comparison with IgG testing.

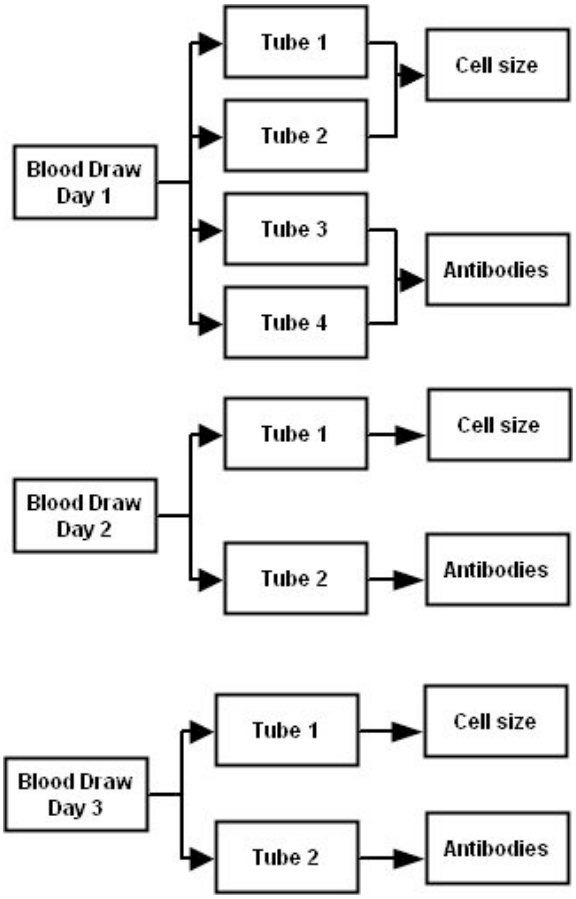


*Figure 1: Design Overview. Samples were collected as described in the Methods section. Antibodies refer to the IgG ELISA method. Cell size refers to the cell size variability testing method.*

## Design and Methods

### Design overview

Blood draws were performed with the same donor for 3 different evaluations: 1) within test repeatability for 2 different diagnostic tests on a single sample, 2) within participant repeatability over time, and 3) interlaboratory reliability between diagnostic food allergy testing methods (see Figure 1).

### Blood collection

Blood was collected using collection tubes or strips provided by each allergy testing company used in the study. The companies use different methods to determine allergic reactions. Alcat (hereafter referred to as cell size variability method) in Deerfield Beach, FL, uses an assay that measures cell size. US Biotek (hereafter referred to as the IgG ELISA method) in Seattle, WA, uses an assay that measures antibody (IgG) concentration.

For the cell size assay, blood was collected into 2 blue-top vials provided (3.8% sodium citrate, 4.5 ml draw). Blood was shipped overnight at room temperature. Peripheral blood collected for the IgG antibody assay was collected on filter paper strips from a finger stick using a lancet. Blood was allowed to dry on the filter strips at room temperature and shipped via the US Postal Service.

### Cell size testing

Cell size variability is measured to determine reactivity to food antigens. Cells from the blood sample are incubated with potential food allergens. If the individual is sensitive to the food, the cell size changes, most likely due to activation or degranulation. A modified Coulter counter is used to measure cell size.

### Antibody testing (ELISA)

Antibody (IgG) specific for a food allergen is measured via ELISA. Food antigens are bound to the surface of an ELISA plate and an individual's whole blood is added. If the individual has antibodies to the food, the antibodies will bind to the antigen. These antibodies can then be detected with an enzymatic or colorimetric reaction.

### Within test repeatability

The first part of the protocol was designed to evaluate repeatability within a testing method as a measure of reliability and reproducibility. Blood was collected from the same donor for all time points. On day 1, the several tubes of blood were collected during the same blood draw; the tubes were blinded and sent to both companies for analysis (see Figure 1).

### Within participant repeatability over time

Two days after the initial draw (day 2), 2 blood samples were collected during the same blood draw. The tubes were blinded and sent to both companies for analysis. The next day (day 3), 2 tubes of blood were collected during the same blood draw. The tubes were coded and sent to both companies for analysis. The samples from days 1, 2, and 3 were compared to see if results were consistent over the course of 4 days. A coefficient of variance (CV) and an intraclass correlation coefficient (ICC) were calculated for each test methodology. For the ICC, a value approaching 1 confirms consistency between the samples. Results were compared between companies in order to determine interlaboratory correlation.

## Results

### Reliability of food allergy testing on a single split sample.

In this study, blood from 1 person was sent to 2 different labs to test for food allergies. On the first day, the blood was split into 4 samples, and 2 samples were sent to each lab. The 2 samples analyzed by each individual lab were compared for similarity. This allowed us to test the internal reliability of each lab. The results were reported as no reaction (0), low reaction (1), moderate reaction (2) or high reaction (3). If the food reactivity levels differed between the samples, the difference was calculated and reported in Table 1. The difference between reactivity levels was 0 if a food showed up in the same category in both samples. Cabbage had 1 test reporting a moderate reaction (2), and 1 test reporting a high reaction (3), so the difference in reactivity level was 1 since the results differed by 1 category. To receive a score of 3, 1 sample had a high reaction (3) and 1 sample had no reaction (0).

The company using the cell size variability method tested 50 foods in its food allergy panel. Table 1 demonstrates that only 34% of the foods (17 foods) generated identical results between the split samples. Twenty-eight percent of the foods (14 foods) differed by 1 reactivity level, 10% of the foods (5 foods) differed by 2 reactivity levels, and 28% of the foods (14 foods) differed by 3 reactivity levels. If this were a reliable test for food allergies, we would expect the majority of the foods tested to have identical results. In this case, 66% of the foods tested differed by 1 or more reactivity levels. The scatterplot for cell size testing method, Figure 2A, depicts a large variability in the results for the split sample.

The company using the IgG ELISA method tested 96 foods in its food allergy panel. In contrast to the cell size variability method, 95% of the foods (91 foods) were identical between the split samples. Five percent of the foods (5 foods) differed by 1 reactivity level. No foods differed by more than one reactivity level between the split samples. The scatterplot for the IgG ELISA method, figure 2B, has a more linear pattern.

| **Between Split Samples** | **Cell Size Method** (# of foods out of 50 foods tested) | **IgG ELISA Method** (# of foods out of 96 foods tested) |
|---|---|---|
| **Identical results** | 34% (17) | 95% (91) |
| **1 reactivity level difference** | 28% (14) | 5% (5) |
| **2 reactivity level difference** | 10% (5) | 0% |
| **3 reactivity level difference** | 28% (14) | 0% |

*Table 1: This table compares results from a split sample. The reactivity level differences refer to the percentage of samples that have identical results, or results that are off by a number of categories. For this purpose, no reaction = 0, low = 1, moderate = 2, and high = 3. The reactivity level difference is calculated by taking the absolute difference between the split sample scores.*

### Consistency of food allergy testing over time

On 3 different days, samples were collected and sent to both labs to test the consistency of test results over the course of a week. A total of 4 time points were compared: 2 samples from Monday, 1 sample from Wednesday, and 1 sample from Thursday. The difference in reactivity levels was recorded as the greatest difference in the 4 time points compared. For example, if lamb had no reaction (0) for 3 time points, and high (3) for 1 time point, it would receive a difference in reactivity of 3 (the difference between 0 and 3). If all 4 time points were identical for a food, the difference was scored as 0. If 1 food had a low reaction (1) for 1 time point, moderate reactions (2) for 2 time points and high reaction (3) for 1 time point, the largest difference between reactivity levels was calculated at 2 (the difference between 1 and 3).

As shown in Table 2, 2% of the samples (1 of 50 foods) tested by the cell size variability method yielded identical results over the 4 time points. Twelve percent (6 foods) differed by 1 reactivity level. Twenty-six percent of foods (13 foods) differed by 2 reactivity levels, and 60% of foods (30 foods) differed by 3 reactivity levels. This means that 60% of the foods had at least 1 sample that scored no reaction at 1 time point and scored high reaction for the same food at a different time point during the same week. When comparing all 4 time points, the coefficient of variance for the cell size variability method was calculated to be 0.55, with an ICC of 0.01.

Comparatively, 82% of the foods tested (79 of 96 foods) by the IgG ELISA method produced identical results over the 4 time points. Seventeen percent (16 foods) differed by 1 reactivity level. One percent (1 food) had a reactivity level difference of 2 (one sample, no reaction and three

| **Over the Course of a Week** | **Cell Size Method** (# of foods out of 50 foods tested) | **IgG ELISA Method** (# of foods out of 96 foods tested) |
|---|---|---|
| **Identical results** | 2% (1) | 82% (79) |
| **1 reactivity level difference** | 12% (6) | 17% (16) |
| **2 reactivity level difference** | 26% (13) | 1% (1) |
| **3 reactivity level difference** | 60% (30) | 0% |
| **Coefficient of variance (CV)** | 0.55 | 0.05 |
| **Intraclass correlation coefficient (ICC)** | 0.01 | 0.99 |

*Table 2: Results in table 2 show the differences in 4 samples all taken over the course of 4 days. See Procedure section for more details on the samples taken during 1 week. The reactivity level difference in this table reflects the greatest absolute difference between the 4 samples.*

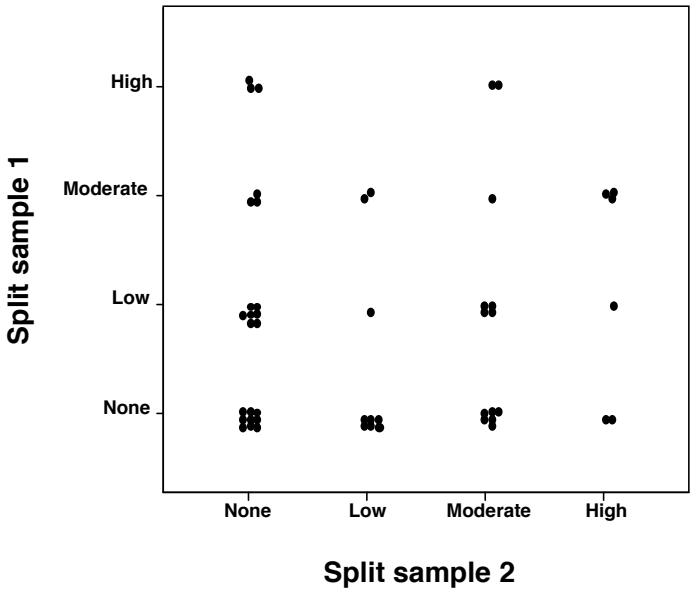


*Figure 2A: Scatterplot of split sample for the cell size variability method. Blood was split into 2 samples and measured for reactivity to foods. Each point on the scatterplot represents the reactivity to a single food in the split sample.*

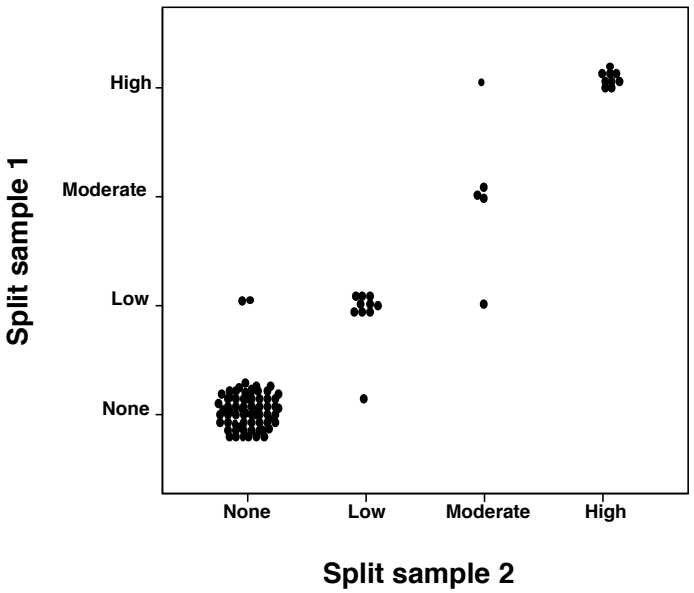


*Figure 2B: Scatterplot of split sample for the IgG ELISA method. Blood was split into 2 samples and measured for reactivity to foods. Each point on the scatterplot represents the reactivity to a single food in the split sample.*

samples, moderate reaction). There were no foods that differed by 3 reactivity levels over the 4 time points. The coefficient of variance was 0.05 for the IgG ELISA method, with an ICC of 0.99, which demonstrates a much more consistent reactivity pattern.

## Discussion

An ideal response to food antigen is tolerance. Yet, some patients develop allergic responses to seemingly innocuous food antigens. Clinicians commonly recommend lab testing when a patient has a suspected food allergy. It is possible that some types of food reactivity will show up in 1 type of testing method and not others. Clinicians and patients rely on the lab tests to be both accurate and reproducible. When a physician reported that 2 types of food allergy tests reported different results for a single patient, we tested the reliability of food allergy testing for the 2 types of food allergy tests in question. Although both of these labs are Clinical Laboratory Improvement Amendments (CLIA)– certified, certification does not ensure the consistency and reproducibility of laboratory tests.

Before we could examine interlaboratory results of food allergy testing, intralaboratory reliability needed to be evaluated. Good intralaboratory reproducibility means that when a sample is compared with itself (as in a split sample), the results are expected to be identical. Similarly, when a person maintains a normal dietary routine over the course of a week, the results of a food allergy test would be expected to be the same. In this study, the cell size variability method delivered very different results for all the samples submitted, and therefore had no internal reproducibility or accuracy. The IgG ELISA method had excellent intralaboratory correlation for the split sample and the samples analyzed over the course of a week. The unreliability of the cell size variability method results prevented an interlaboratory analysis comparing the results of the cell size variability method to the IgG ELISA method.

Other researchers have compared allergy testing methods, although most studies focus on IgE-related allergies as opposed to IgG-mediated responses. Double-blind placebo-controlled food challenge (DBPCFC) is considered the gold standard in food allergy testing and is strongly correlated with IgE testing.[26,27] In this type of testing, the reaction of a suspected allergenic food is compared to a placebo food, known to not evoke a response. Foods that are known to induce anaphylaxis are not generally tested. The DBPCFC identifies foods that evoke immediate food allergy symptoms.[28] Skin tests can also be used to identify food allergens. These tests are more sensitive than IgE blood tests.[29]

IgE to food allergens demonstrates an immediate phase immune response. Delayed type responses, however, are not mediated by IgE antibodies and will not show up with this type of testing.[30] For symptoms of food allergies caused by delayed type hypersensitivity reactions such as headaches, mood swings, intestinal upset, pain, and attention problems, the DBPCFC or skin tests may present a false negative.

In this study, IgG ELISA testing was more reproducible than cell-size testing. In general ELISA is known to be consistent and is routinely used for scientific testing.[31] The sensitivity of ELISA as a method for food allergy testing is dependent upon the food antigen used as well as the amount of antibody present. IgE food antigens used for ELISA assays have been standardized and are consistent between different laboratories. IgG food antigens have not been standardized, which accounts for some of the variation between laboratories. All commercial food antigens for ELISA testing are made from raw foods (both IgE and IgG antigens). Cooking food exposes different antigens and epitopes which may affect ELISA test results.[32] For example, pecans, wheat flour, roasted peanuts, lentils, almonds, cashews, walnuts, soy beans, shrimp, scallop, tuna, egg, apple, plum, milk, and potatoes have been shown to have antigens that differ between raw and cooked forms.[33,34] Another researcher suggests that cooked egg (baked egg especially) produces less of a reaction than raw egg.[35]

The participant of this study had IgG reactions to milk and soy. The most common IgE mediated food allergens in the general population are milk, soy, egg, peanut, wheat, tree nuts (walnuts and cashews), fish, and shellfish. These foods account for 85% of the commonly recognized food allergens.[36] The other foods with high reactions for this participant included almonds, corn, lima beans, bananas, and blueberries. All of these foods were regularly included in the participant's diet before the food allergy tests.

Cell size testing as a measure of food reactivity is not well studied in the literature. Consistent with the data reported herein, most studies suggest that it is unreliable.[37,38,39] The company that performed the cell size variability method was told of the results in a phone call a month after the testing was complete and said that there were no irregularities during that week and that they stood by their results. While we can hypothesize a mechanism for identifying food allergies from cell size differentials, the data clearly demon-

strate this method is not specific, not reproducible, and was not related to food reactions in this participant. As the scientific community continues to understand the importance of the antigen being used and the accuracy of different tests in providing relevant clinical guidance, the consistency between laboratories and the method they employ must improve.

## Conclusion and Future Directions

Cell size variability testing for food allergies proved to be completely random in all tests, and therefore has no clinical relevance. The IgG ELISA method proved to be a consistent, reproducible, and specific test for food allergies in this small study. The clinical relevance of these results were not examined in this study. The results presented here verify that IgG ELISA testing is more reliable and consistent than cell size testing for identifying food sensitivities.

In order to further improve clinical practice, future research should demonstrate how long IgG antibodies remain active after food elimination. Future studies could also evaluate how long it takes to resolve physical symptoms after food elimination. In addition, identifying specific disease states exacerbated by IgG food reactivity would help clinicians identify the patients who would benefit the most from IgG testing.

Food is medicine, in some very fundamental ways. Eliminating harmful foods and encouraging healthful, nutritious foods can have a big impact on health. In order to eliminate foods, accurate identification of potentially harmful foods is essential. In this study, we have identified a food allergy test that is specific and reproducible. This can help clinicians have more confidence in the food allergy test they recommend to patients.


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