ALLERGY AND INTOLERANCE REGARDING IgG4 IMMUNOGLOBULIN

- ***HYPOTHESIS – IgG4 levels will not change throughout testing, due to IgG4 behaving similarly to IgE Level.***
- ***NULL HYPOTHESIS – IgG4 will constantly change throughout testing because IgG4 is linked to exposure.***
- ***Abstract***

"Allergy and Intolerance Regarding IgG4 Immunoglobulin" explores the complex interplay of Immunoglobulin G4 (IgG4) in the context of food allergies and intolerances. The authors present hypotheses regarding IgG4 levels and their stability during testing, and they investigate the relationship between IgG4, IgE, and immune responses to various food antigens. This review provides a concise summary and evaluation of the article's key findings and research methods. In their study, the authors emphasize the anti-inflammatory role of IgG4, highlighting its capacity to inhibit IgE activity and protect against type 1 hypersensitivity reactions. They discuss the prevalence of food reactions in Europe, differentiating between IgE-mediated allergies and non-IgE-mediated food intolerances. The immune mechanisms involving specific IgG antibodies in food intolerance development are elucidated, shedding light on the formation and accumulation of food protein complexes and resulting inflammatory processes.

The article discusses the production of both IgE and IgG antibodies in response to interleukins (ILs), with particular emphasis on IL4 triggering IgE-mediated reactions and IFNg and TNFa influencing IgG3 production. IgG4 like IgE is also induced by IL-4 and IL-3. The authors argue that IgG1, IgG2, and IgG3 antibody production, while contributing to the formation of immune complexes, lacks concrete evidence of a direct connection to specific symptoms, particularly in intolerance testing. The use of IgG4 antibodies in the study is rationalized, as they serve to explore immune responses to food allergens before allergic reactions develop. IgG4 is characterized as a blocking antibody against IgE, preventing IgE from accessing allergens. This preventive screening approach is underscored by the high concentration of IgG4 in comparison to IgE, facilitating faster and more frequent binding to allergens. IgG4 antibodies are noted for their minimal histamine release, making them predominant when allergies remain asymptomatic. The primary function of IgG4 in influencing immune inflammatory responses without histamine release holds the potential for understanding patient symptoms such as bloating, abdominal cramps, and headaches. The article outlines the materials and methods used in the study, including the collection of blood samples from volunteers over 10 weeks. The methodology involves the use of auto blot/automated western blot, nitrocellulose strips, and various reagents and equipment to measure IgG4 levels in response to food antigens. Allergy and Intolerance Regarding IgG4 Immunoglobulin" provides valuable insights into the role of IgG4 in food allergies and intolerances. The article's focus on preventive screening using IgG4 antibodies presents an intriguing avenue for further research in understanding and managing adverse reactions to food. However, it is essential to consider the limitations and potential biases in the study's methodology and interpretation of results. Further research and clinical validation are warranted to establish the clinical utility of IgG4 testing in the context of food-related health issues.

- ***Keywords***

IgG4 Immunoglobulin, Food Allergy, Food Intolerance, Immune Response, IgE Inhibition, Preventive Screening

- ***INTRODUCTION***

Allergy and Intolerance Regarding IgG4 Immunoglobulin presents a comprehensive exploration of the intricate relationship between Immunoglobulin G4 (IgG4) and various facets of food allergies and intolerances. In recent years, the significance of IgG4 antibodies in the realm of immunology and its potential impact on human health has gained substantial attention (Ortolani, C., Ispano, M., Pastorello, E., Bigi, A. and Ansaloni, R. (1988). This review aims to provide a thorough examination of the key concepts and findings within the article.

Immunoglobulin G4 (IgG4) is the focal point of this article, and it is portrayed as a pivotal player in modulating immune responses, particularly in the context of adverse reactions to dietary components. The central premise of the article revolves around the dual roles of IgG4: its ability to inhibit the activity of Immunoglobulin E (IgE) and its potential to serve as an indicator of exposure to food antigens. IgG4 makes up to 5% of IgG when measured in serum. It also accounts for non-microbial allergens (Qin et al., 2022). IgE is traditionally associated with immediate allergic responses, commonly referred to as Type 1 hypersensitivity reactions, characterized by rapid onset of symptoms following the consumption of specific foods. In contrast, IgG4 is linked to a more delayed immune response, denoted as Type 3 hypersensitivity, where symptoms may manifest hours to days after exposure to triggering food components.

The article introduces two opposing hypotheses. The first hypothesis posits that IgG4 levels remain relatively stable during testing, akin to the behaviour of IgE levels (Sampson, H.A. (2006). The second hypothesis, the null hypothesis, suggests that IgG4 levels fluctuate throughout testing due to their association with exposure to food antigens. These hypotheses form the basis for the research conducted in the article, ultimately seeking to elucidate the dynamics of IgG4 in the context of food-related immune reactions.

Moreover, the article underscores the critical distinction between IgE-mediated food allergies and non-IgE-mediated food intolerances. It emphasizes that while the prevalence of IgE-mediated allergies is estimated at 3-4% in young children and adults in Europe, food intolerance affects a considerably larger portion of the population, affecting approximately 60%. IgE-mediated reactions typically result in immediate symptoms, such as hives or anaphylaxis, whereas IgG antibodies, including IgG4, are implicated in delayed immune responses. These delayed responses often contribute to chronic conditions like atopic dermatitis, enterocolitis, and oesophagitis (Smit, W. and Barnes, E. (2014).

The immune mechanisms underpinning food intolerance are intricately linked to the formation and accumulation of specific IgG antibodies against food proteins. These antibodies can lead to inflammatory processes, contributing to a range of symptoms. The article cites scientific literature indicating that up to 50% of patients suffering from chronic diseases may possess IgG-delayed mediated food intolerance. Consequently, IgG antibodies, and specifically IgG4, play a pivotal role in the immune response to dietary antigens and may hold the key to understanding underlying health issues (Trampert, D.C., Hubers, L.M., van de Graaf, S.F.J. and Beuers, U. (2018).

"Allergy and Intolerance Regarding IgG4 Immunoglobulin" offers a comprehensive exploration of the pivotal role of IgG4 in the context of food allergies and intolerances. It introduces hypotheses, distinguishes between different immune responses, and delves into the mechanisms underlying these reactions. The following sections of this review will delve deeper into the article's key findings, methodologies, and implications in further detail. The intricate interplay between the human immune system and dietary components has long captivated the realms of immunology and clinical nutrition. Among the antibodies garnering attention in this intricate dance, Immunoglobulin G4 (IgG4) stands as a sentinel. Traditionally deemed a pivotal player in immune responses, particularly in the context of food allergies and intolerances, IgG4 antibodies have become a focal point of research (Michailidou, D., Schwartz, D.M., Mustelin, T. and Hughes, G.C. (2021). This extended introduction seeks to provide a comprehensive overview of the multifaceted relationship between IgG4 immunoglobulins, dietary reactions, and their implications for human health.

Immunoglobulin G4 (IgG4), a subclass of IgG antibodies, has earned a reputation for its paradoxical nature. Unlike its more notorious counterpart, Immunoglobulin E (IgE), which is synonymous with rapid and often severe allergic reactions, IgG4 has been traditionally viewed as a mediator of immune tolerance. It has been considered as an immunoglobulin class associated with dampening immune responses, inhibiting inflammation, and fostering a state of immune equilibrium. However, recent research has unveiled a more complex narrative.

In recent years, IgG4 has emerged as a subject of intense scientific scrutiny due to its potential role in food intolerances. Unlike immediate allergic reactions typified by IgE-mediated responses, food intolerances often manifest with delayed symptoms, making them challenging to diagnose and manage. IgG4 antibodies have been implicated in these delayed immune reactions, leading to questions about their precise function in the context of dietary antigens (Wasserman, S. and Watson, W. (2011). This article embarks on a journey to explore the multifaceted nature of IgG4 immunoglobulins concerning food allergies and intolerances. It delves into the theories, research, and hypotheses surrounding IgG4, attempting to decipher the enigma of why these antibodies persist even when specific foods are eliminated from the diet. The study employs specialized testing techniques designed to differentiate between true antibody responses and potential false detections, adding a layer of precision to the investigation.

Moreover, this article underscores the importance of distinguishing between IgE-mediated food allergies and IgG4-mediated food intolerances. While the prevalence of IgE-mediated allergies is well-documented, affecting a significant but comparatively small percentage of the population, food intolerances cast a wider net, impacting a substantial portion of individuals worldwide. Recognizing the unique immune mechanisms governing these two categories of reactions is essential for accurate diagnosis and tailored management strategies (Velikova, T. and Peruhova, M. (2018).

Intriguingly, the study's findings challenge conventional wisdom. Participants who had diligently excluded specific food items from their diets continued to exhibit notable IgG4 and IgE antibody levels. This unexpected persistence raises fundamental questions about the factors driving these immune responses and their clinical significance. As

we venture further into this exploration, the article unfolds not only the enigma of IgG4 immunoglobulins but also the implications of these findings for clinical practice. Recommendations are provided for future research directions, including the imperative need for clinical validation studies, standardized testing protocols, and evidence-based clinical guidelines. Additionally, the article advocates for public awareness campaigns, longitudinal research efforts, therapeutic interventions, and collaborative healthcare approaches to enhance our understanding of IgG4-mediated food intolerances (Mullin, G.E., Swift, K.M., Lipski, L., Turnbull, L.K. and Rampertab, S.D. (2010). This comprehensive introduction sets the stage for a deep dive into the intricate world of IgG4 immunoglobulins, dietary reactions, and their profound impact on human health. As we navigate this complex landscape, we aim to unravel the mysteries surrounding IgG4 and its role in food intolerances, ultimately contributing to improved diagnostics and patient care in the ever-evolving field of immunology and nutrition.

- ***LITERATURE***

Immunoglobulin G4 (IgG4) are antibodies considered to be anti-inflammatory by inhibiting IgE activity IgG4 actively protects against type 1 hypersensitivity (Legatowicz-Koprowska (2018)). Immunoglobulin is known to be antibodies that are structured by the white blood cells (plasma cells). In the last decade, conversations about the relationship between abnormal nutritional reactions and health issues have gained a lot of interest (Canan et al., 2014). The prevalence of food reactions (IgE-mediated allergy) in Europe has been estimated at 3-4% in young children and adults (Macchia et al., 2015), while food intolerance affects about 60% of the population (Johansson et al., 2008). Abnormal reactions to food antigens can be classified as immune-mediated (IgE, with clinical manifestations, anaphylactic) and non-IgE mediated (atopic dermatitis, enterocolitis, oesophagitis) (Ortolani et al., 1988) (Wasserman and Watson, 2011). IgE antibodies are involved in Type 1 hypersensitivity, and they mediate rapid type immune reactions, with symptoms occurring within a few minutes to two hours of eating a specific food, while IgG antibodies are responsible for delayed immune reaction in food (Type 3 hypersensitivity) with symptoms occurring within few hours to few days. Both immune-mediated and non-immune-mediated reactions are involved in food intolerance development. The immune mechanism involved in food intolerance is associated with the formation of specific IgG antibodies. IgG antibodies are involved in the formation and accumulation in various organs of food protein complexes, resulting in inflammatory processes (Sampson, 2006) (Canan et al., 2014). In scientific literature is reported that 50% of patients affected by chronic diseases may possess IgG-delayed mediated food intolerance (Type 3 hypersensitivity) (Wachholz and Durham, 2004).

Both IgE and IgG antibodies are near chromosome 14 and they are read in sequence. Their production depends on Interleukins (ILs) in fact when IL4 is released it can be assumed that an IgE (Type 1 hypersensitivity) response will occur, with an immediate food reaction (Velikova and Peruhova, 2018) (Mullin et al., 2010). The activation of inflammatory response in terms of the IgE mediated response is due to the release of histamine compounds from the mast cells after the binding of the IgE antibody to the mast cell receptor, as response to the antigen (with antigen we are including all those external agents that can be recognized as foreign and activate an immune response) attack. In the case of sensitization, when IL10 is present, the production of IgG3 antibodies will be involved (Velikova and Peruhova, 2018), while if other ILs are synthesized IgG1, IgG2, IgG3 are mainly produced. The production of IgG1, IgG2, and IgG3 antibodies when a specific food is consumed activates the formation of immune complexes with deposition in the body where the problem is (Aalberse et al., 2009). The lack of scientific studies showing evidence that the deposition of immune complexes on tissues is connected to specific symptoms makes the test of IgG1, IgG2, and IgG3 antibodies not attractive from an intolerance testing point of view. On the contrary, it was demonstrated that those antibodies play a role in the identification of allergic reactions or non-specific systemic reactions in which inflammatory processes play a major role (Stapel et al., 2008).

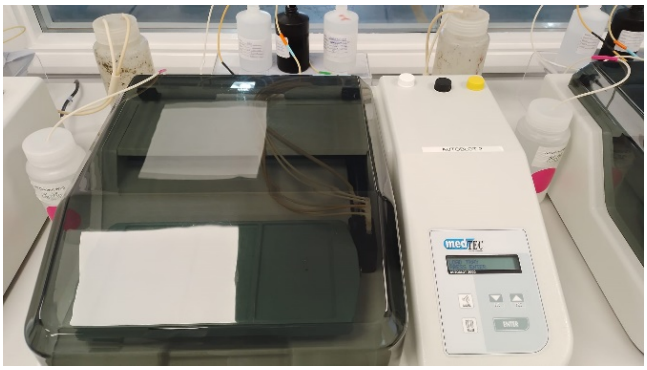
We are using IgG4 antibodies because when we are testing intolerance, we are not interested in the immediate allergic reaction (Type 1 hypersensitivity) or inflammation related to chronic disease (IgG1, IgG2, and IgG3). IgG4 is considered the blocking antibody concerning IgE and it blocks access of the IgE to the allergen, helping us to understand how your immune system reacts to food antigens before an allergic reaction (Type 1 hypersensitivity) is developed (preventive screening). The concentration of IgG4 is about 10,000 times higher than the IgE concentration. Therefore, IgG4 can bind faster and with greater frequency than IgE (Aalberse et al., 2009) mapping your immune response to food allergens. IgG4 antibodies result in only 1% of the histamine released by IgE, with few patients experiencing allergic symptoms therefore, IgG4 antibodies are mostly produced when the allergy is asymptomatic. The primary function of IgG4 is to influence the immune inflammatory response without the release

of histamine factors (the main cause of inflammatory reactions), having the possibility to explore how the amount of IgG4 antibodies could be related to some of the patient's symptoms (bloating, abdominal cramps, headaches, to mention some). This will be the first step in your journey to find a better version of yourself.

- ***MATERIAL REQUIRED***
  - Auto blot/Automated western blot
  - Nitrocellulose Strips
  - 10 weeks of volunteer blood samples
  - Wash buffer
  - Pipettes
  - B4C strip reader
  - AK1 G4 (anti-digoxigenin-labelled) Conjugate
  - TMB (tetramethylbenzidine) Colour substrate
  - Eppendorf tubes
  - Laminar air flow cabinet
  - EBF 903 Dried Blood Spot cards

- ***METHODS***
  - Initial sample collection

    The test kits were sent to volunteers every week for a span of 12 weeks. Six spots of blood were requested from the volunteers and sent to the laboratory inside an envelope included in the test kit alongside instructions.



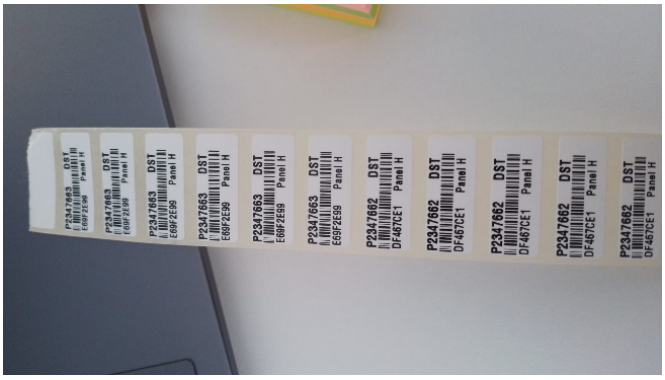
(fig.1)The Autoblot from MedTec was used for processing the eluted samples. They can test 20 samples at once.

For the extraction of chemicals or biomolecules from matrices, elution is a commonly employed procedure in scientific and medical contexts. The Autoblot facilitates this process by allowing up to 20 samples to be evaluated at once. This ability to handle and analyze multiple samples at once makes it easier for scientists and researchers to assess and evaluate a large number of samples.

(fig.2) The samples were spun in a rotator. This helps in the immersion of the cell suspension.

A critical stage in the laboratory process is rotating the samples in a rotator, which is a specialized instrument. Its circular motion performs two functions: it effectively submerges cell suspensions. In general, scientific and research

operations necessitate homogenous cell dispersion and blending. Cell suspensions are made up of cells suspended in a liquid medium. The samples will be placed in the rotator and spun by the researchers to fully and uniformly mix the cells in the suspension.



(fig.3) this barcode was generated unique to each sample. The barcodes were scanned to enter the sample number into the reader to link the strip to the sample number in the reader.
A barcode system is required for sample handling and identification. Throughout the process, each sample is allocated a unique barcode to help differentiate it from other samples. After that, the barcodes are scanned with a specialized barcode reader. This scanning technology extracts data and transfers it to a computer or reader. This data is commonly used to denote the sample size

Sample Type: Intolerance Allergy

No	Barcode	LSV or DBS	Strip Numbers				Strip Type		
1	P2348329	LSV	11	18			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5
2	P2348330	LSV	2	6			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5
3	P2348331	LSV	5	7			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5
4	P2348332	LSV	4	4			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5
5	P2348333	LSV	1	3			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5
6	P2348334	LSV	4	13			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5
7	P2348335	LSV	2	15			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5
8	P2348336	LSV	21	7			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5
9	P2348337	LSV	24	15			E-3	G4-1	G4-2

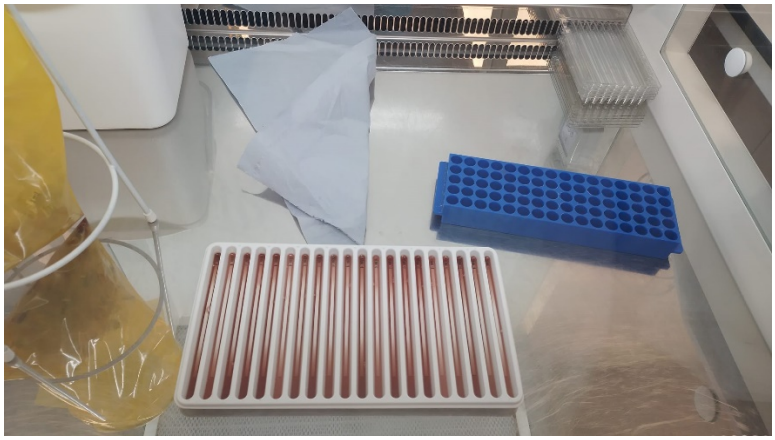
		DBS					G4-3	G4-4	G4-5
10	P2348338	LSV	19	14			E-3	G4-1	G4-2
		DBS					G4-3	G4-4	G4-5

(fig.5) The barcode and the strip numbers were recorded in a log sheet with the respective strip numbers.

The procedure of carefully recording the barcode and strip numbers is an essential part of data management and tracking. For physical identification, each strip has a distinct barcode associated with a specific strip number. The barcode and matching strip number are meticulously written next to each other on this log page.

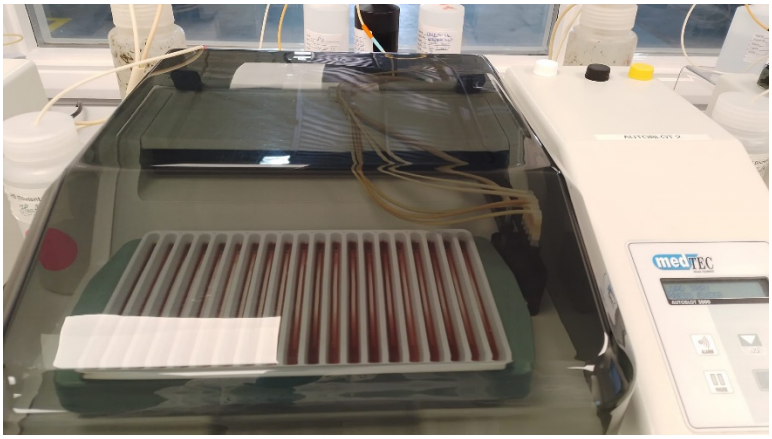
(fig.4) The strips are placed in a tray face up.

Because the side containing critical information or components is facing upward, it is easier to reach the strips for extra jobs or questions. In industrial or laboratory procedures, such attention and strip alignment are frequently necessary to guarantee that the relevant components inside each strip are conveniently available for the following workflow phases.



(fig.6) The samples were pipetted into the tray with the respective strip.

Samples are carefully deposited in trays as part of a continuous critical procedure. This level is distinguished by the degree to which each sample fits its associated strip. These strips, which can be identified by barcodes or strip numbers, are most commonly employed as sample containers or receptacles. To preserve data accuracy and to link each sample to its unique ID, samples must be appropriately linked with matching strips inside the tray.



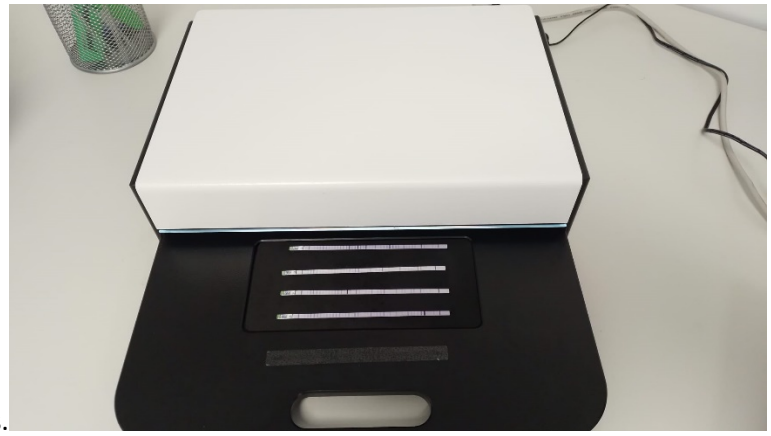
(fig.7) The samples were processed in the autoblot for 2.5 hours. Each of the reagents was added to the samples using the tubes from the bottles.

Samples are processed in the Autoblot for 2.5 hours at this crucial step. The addition of various chemicals to the samples is what sets this stage apart. Via bottles and tubes, the chemicals required for the selected treatment are infused into the samples. Because it guarantees that the intended chemical reactions or treatments are carried out correctly, this precise and controlled reagent input is essential to scientific and laboratory activities. To automate this process and increase its consistency and efficiency, the Autoblot is necessary. Applying the proper reagents to the appropriate samples is another benefit of using tubes from bottles appropriately.

(fig.8) After the samples were processed, the strips appear with purple coloured lines indicating the food antigens present on the strips. The darker the lines the stronger the reactions.

Following sample processing, the strips show purple lines, suggesting the presence of food antigens. The strength of these lines shows the degree of antigen-antibody interactions in the samples; darker hues indicate more responses.

Visually assessing the assay results is crucial; darker lines indicate stronger immune responses to certain dietary antigens.



(fig.9) The strips were read in the B4C reader by placing them sufficiently apart from one another.

Reading the strips using the B4C reader, a device designed expressly for this type of testing, is an important step in the process. To achieve the precision and consistency of this reading method, the strips must be suitably spaced apart. This division is required for several reasons. To begin, it prevents undesirable cross-contamination or interference between neighboring strips, allowing the reader to focus on one strip at a time without worrying about data overlap. Second, maintaining the optimum distance ensures that the reader's sensors properly record data from each strip.

Sample No.	Sample ID	Test	Results			
			Code	**Food**	**Result**	**Class**
1	P2348339	DST ALLERG4Y LINE G4-3 Rev. 021	f 40	Tuna	100	Class 6
			f 930	Trout	63.03	Class 5
			f 802	Pollock	2.16	Class 2
			f 21	Herring	2.43	Class 2
			f 177	Oyster	8.97	Class 3
			f 24	Shrimp	5.32	Class 3
			f 58	Duck	100	Class 6
			f 83	Chicken	69.55	Class 5
			f 143	Turkey	27.35	Class 5
			f 5	Rye	100	Class 6
			f 11	Buckwheat	13.53	Class 3
			f 6	Barley	100	Class 6
			f 159	Durum wheat	100	Class 6
			f 164	Millet	77.79	Class 6
			f 832	Quinoa	100	Class 4
			f 75	Gluten	1.74	Class 6
			f 25	Tomato	0.35	Class 2
			f 48	Onion	0.35	Class 1
			f 197	Zucchini	100	Class 6
			f 812	Olive Green	0	Class 0
			f 65	Lentil	3.49	Class 2
			f 12	Pea-green	7.15	Class 3
			f 950	Bean green	63.03	Class 3
			f 199	Milk	100	Class 5
			f 325	Sheep's milk	100	Class 6
			f 300	Goats milk	43.85	Class 4

			f 29	Banana	25.92	Class 4
			f 84	Kiwi Fruit	0	Class 0
			f 32	Lemon	72.8	Class 6
			f 44	Strawberry	0.35	Class 1
			f 52	Pineapple	1.22	Class 2
			f 156	Sunflower seed	8.07	Class 3
			f 157	Pumpkin seed	9.88	Class 3
			f 89	Mustard	0	Class 0
			s 11	Sweet Basil	100	Class 6
			s 15	Ginger	2.16	Class 2
			f 955	Coffee	0.3	Class 2
			f 97	Cacao	0.19	Class 0
			f 141	Button mushroom	7.58	Class 2
			f 9	Rice	0.33	Class 0

(fig.10) The results were categorized into different classes. The lowest reaction is a class 0 and the highest reaction is a class 5.

Multiple categories are generated based on the outcomes of the preceding procedures. These categories are used to categorize observed reactions based on their severity. Class 0 is the least reactive class in the categorization system, whereas Class 5 is the most reactive or immunological reaction class. This classification presents the various levels of reaction or reactivity regarding the experiment or study in a consistent and accessible manner.

- Sample preparation

  The dried blood samples (DBS) a handled with care by wearing appropriate Personal Protective Equipment (PPE). An online database called Blood Suite and Laboratory Database Software (LDBS) was used to create each Volunteer's sample with their name and contact information. This keeps a record of the Volunteer sample that was used over the past 10 weeks. Once the Volunteer page was made, the sample was linked to Volunteer by generating a personalized QR code on Blood Suite. The QR codes from the Blood suite were scanned using a hand scanner and uploaded onto the LDBS. The QR code was printed out to label the DBS cards and the eluted samples.

  Two blood spots were punched from the cards to prepare the eluted samples. One of each spot was used for IgG4-2 and IgG4-3 strips. Each of the spots was eluted using 1500µL of wash buffer in Eppendorf tubes. Twenty such samples were made; ten for IgG4-2 strips and ten for IgG4-3 strips; and tested at once using the Auto-immunoblot. The barcode was stuck to each Eppendorf tube. The samples were prepared the day before to allow the dried blood spot to be eluted in the wash buffer. The samples were stored in the refrigerator overnight. The next day the samples were mixed well by a rotator for 10mins, 100rpm. One of both IgG4-2 and IgG4-3 strips was properly matched to each sample barcode and maintained in a record. After being mixed in, the sample was poured into an immunoassay tray with assigned strips. The strips are allowed to soak in the sample for 2 minutes.

- Auto blot Reagent preparation

  The reagents used in the auto blot were STOP solution or deionized water, Tetramethylbenzidine Substrate (TMBS) AK1 G4 (anti-digoxigenin) conjugate, and wash buffer. The TMBS and AK1 G4 were diluted. 1 part reagent and 2 parts deionized water. They are stored in the refrigerator at 6˚C.

- Auto blot preparation

  The pump pads were locked into place at the rear end of the equipment. The tubes for STOP solution, TMBS, wash buffer, and the AK1 G4 were primed with the respective solutions. The test should be selected as the ''I-DBS'' intolerance test.

- Strips inoculations
  Each strip number was specifically assigned to each patient's barcode. Each sample was pipetted out and added to its assigned strip number in an auto-blot tray. The strips were allowed to soak for 1-2 minutes. The tray was placed into the auto blot starting the process.

- B4C Reader
  Ten strips were read by the reader at a time. The reader identifies antigen bands and measures the binding intensity of antihuman antibodies attached to each band. The intensity is measured by an inverted sum of RGB values. (Jager, 2017)

❖ *RESULTS*

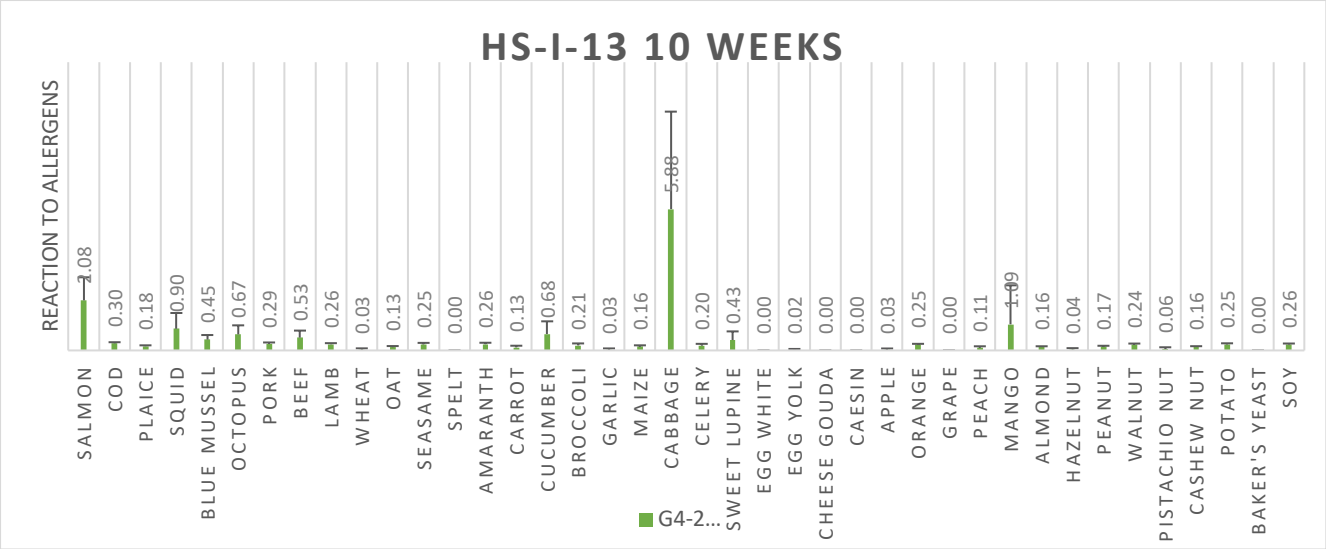
In this study, the data analysis process involved the utilization of a reader to interpret the results obtained from a group of 26 volunteers. The participants, comprising 15 females and 9 males, were subjected to various diets, contributing to the diversity of the dataset. The research spanned a period of 10 weeks, during which data were systematically collected at the commencement of each week.

To comprehensively illustrate the trends and variations, an average of the 10-week data set was computed for each volunteer. This extensive dataset was subsequently graphically represented to facilitate a visual understanding of the participants' reactions to different food items.

The criteria for categorizing the reactions were defined by specific threshold values. Any measurement falling below 0.35 U/ml was classified as indicative of no reaction, while a value of 3.50 U/ml signified a medium reaction. Notably, a reading of 50,000 U/ml was designated as a high reaction. These thresholds served as crucial benchmarks in evaluating the responses of the participants to the various dietary stimuli.

To present the findings with precision, the data were graphically organized using bar graphs in Microsoft Excel. For each food item, an average value across the 10 weeks was calculated, providing a consolidated representation of the participants' reactions. Additionally, the standard deviation was computed and incorporated into the graphs as error bars, offering insights into the variability and reliability of the obtained results.

This meticulous approach not only allowed for a comprehensive analysis of individual reactions to different diets but also provided a visually compelling presentation of the data, enhancing the interpretability of the study outcomes.

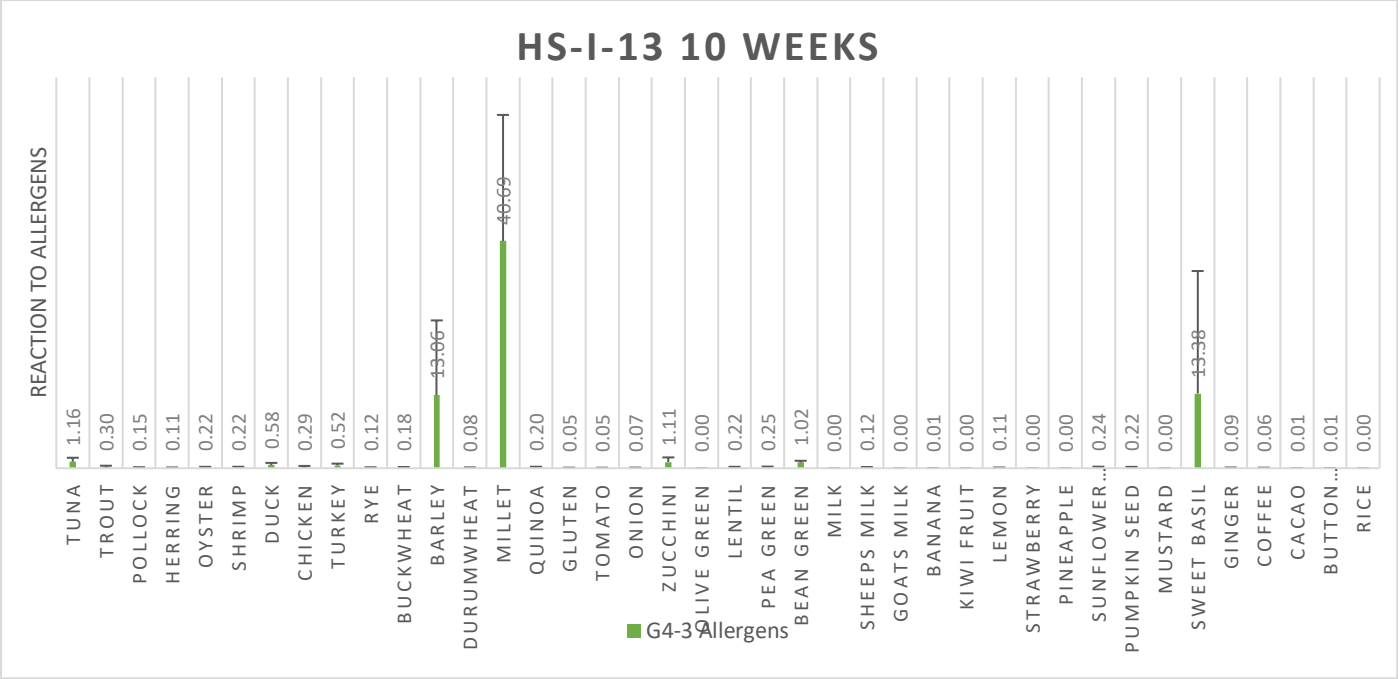


The significance level, often denoted as the p-value, plays a critical role in hypothesis testing. A p-value of 0.01 indicates a relatively low probability of observing the obtained results (or more extreme results) if the null hypothesis is true. In statistical terms, it suggests strong evidence against the null hypothesis.

When conducting hypothesis tests, researchers compare the calculated p-value to a predetermined significance level (often denoted as α). If the p-value is less than or equal to α, the null hypothesis is typically rejected in favor of the alternative hypothesis. In the context of a p-value of 0.01, researchers would commonly use a significance level (α) of 0.05, although the specific significance level chosen depends on the study design and conventions within the field.

If the p-value is less than or equal to 0.01, it implies that there is strong evidence to reject the null hypothesis, supporting the idea that the observed results are statistically significant. Researchers would typically interpret this as an indication that the observed effects are unlikely to have occurred by random chance alone.

It's important to note that the choice of the significance level is a decision made by the researcher and is influenced by factors such as the nature of the study, the consequences of Type I and Type II errors, and disciplinary standards. A lower significance level, such as 0.01, suggests a more conservative approach, requiring stronger evidence to reject the null hypothesis.

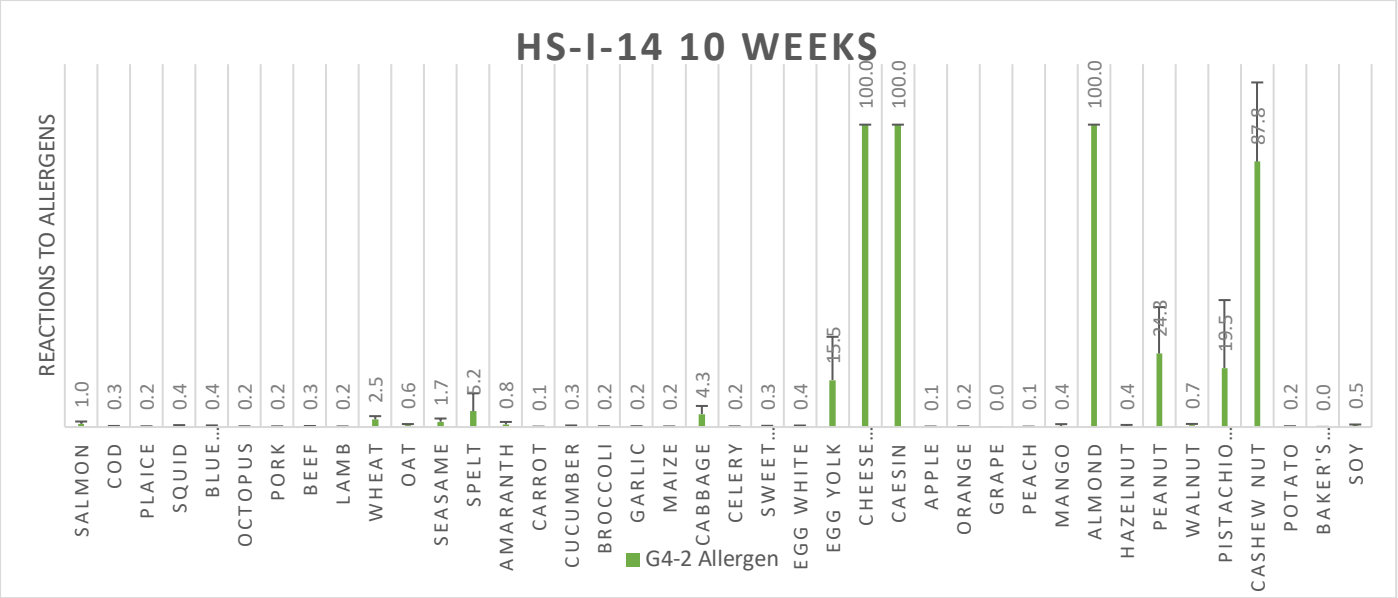


A p-value of 0.03 in the context of hypothesis testing indicates that there is a 3% probability (or 3 in 100) of observing the obtained results, or more extreme results if the null hypothesis is true. In statistical hypothesis testing, this p-value is compared to a predetermined significance level (often denoted as α) to make decisions about the null hypothesis.

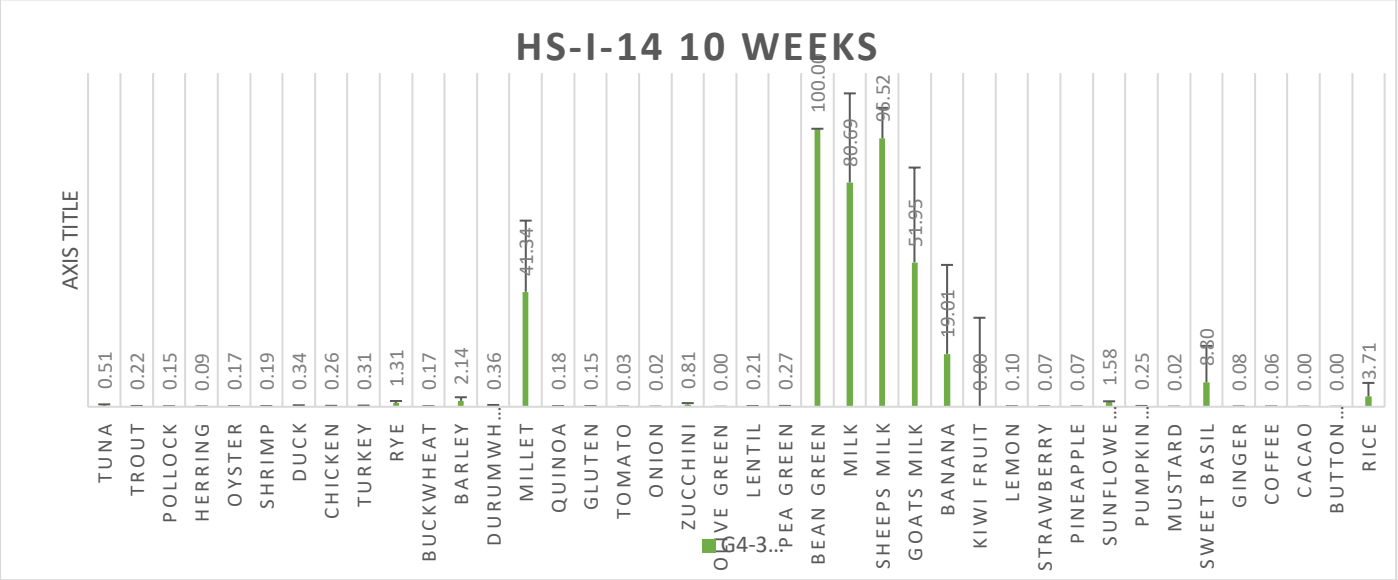
Typically, a significance level of 0.05 is commonly used in many scientific studies. If the calculated p-value is less than or equal to the chosen significance level (e.g., 0.05), researchers would reject the null hypothesis in favour of the alternative hypothesis. In the case of a p-value of 0.03, it would suggest that the observed results are statistically significant at the 0.05 significance level.

The interpretation would be that the evidence against the null hypothesis is strong enough to warrant rejecting it in favour of the alternative hypothesis. In practical terms, this means that the observed effects are unlikely to have occurred by random chance alone, and there is statistical support for the presence of a real effect or relationship.

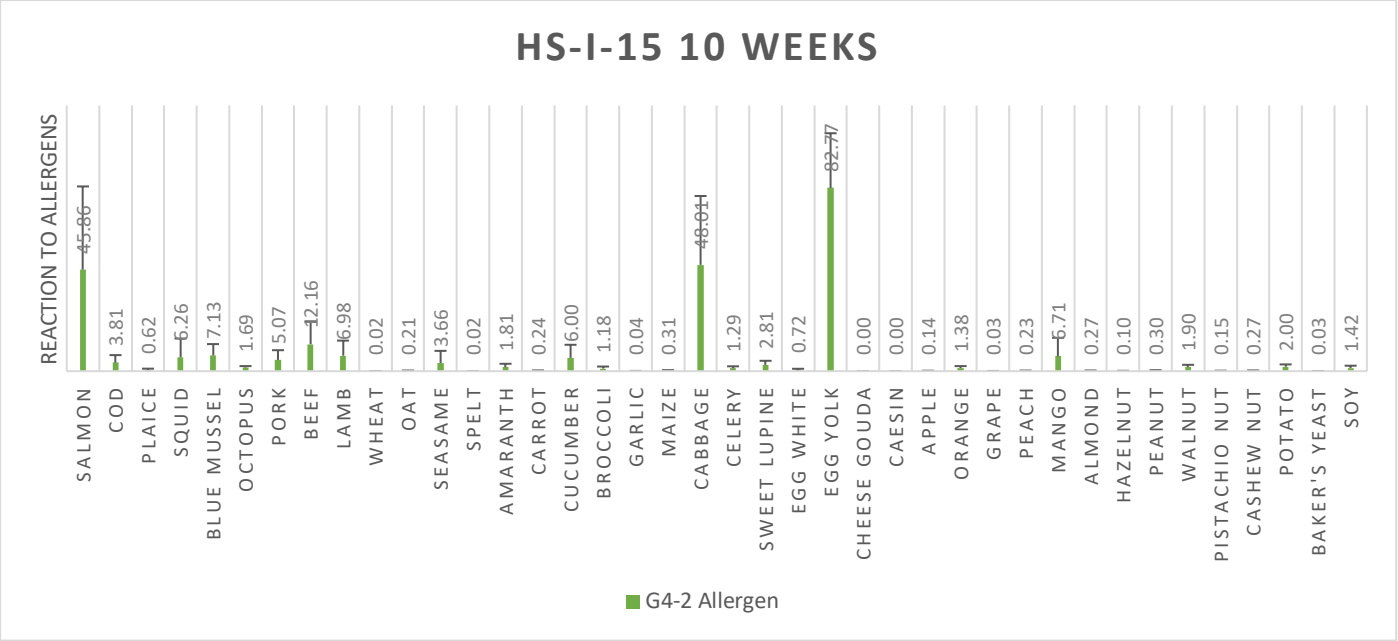
It's important to note that the choice of the significance level is a decision made by the researcher and should be based on the specific requirements of the study and the field of research. Additionally, while p-values provide a measure of statistical significance, they should be considered in conjunction with other factors, such as effect size and study design, for a comprehensive interpretation of the results.



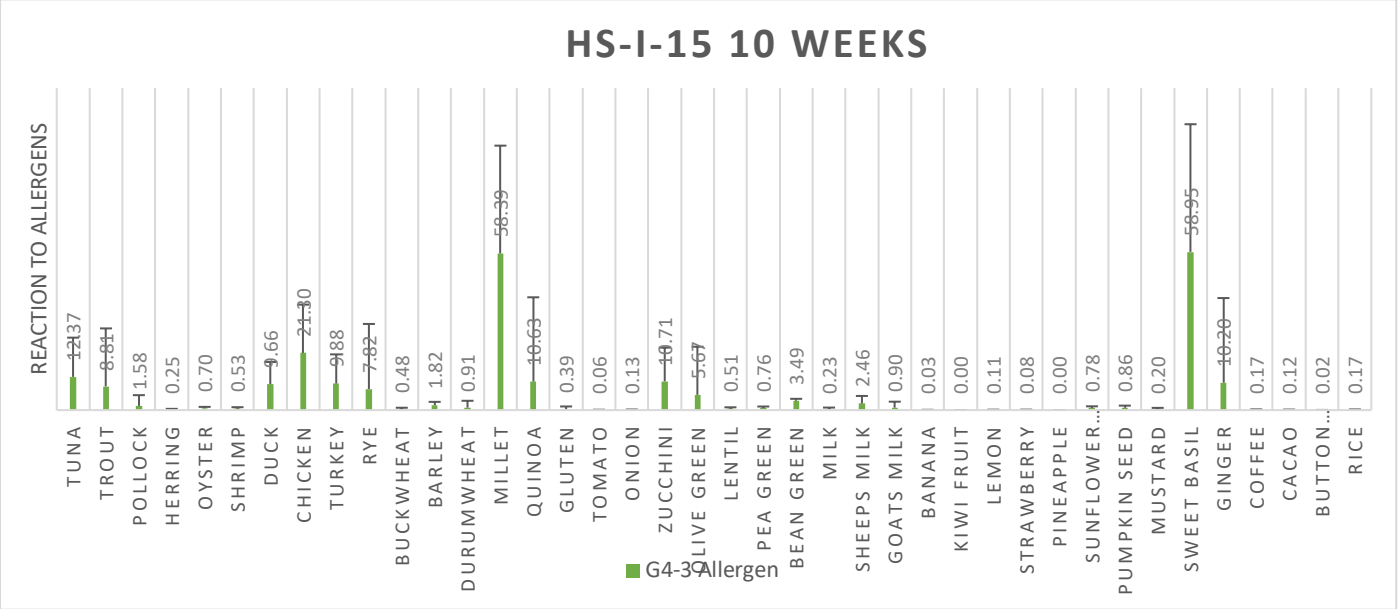
P-value is 0.04.



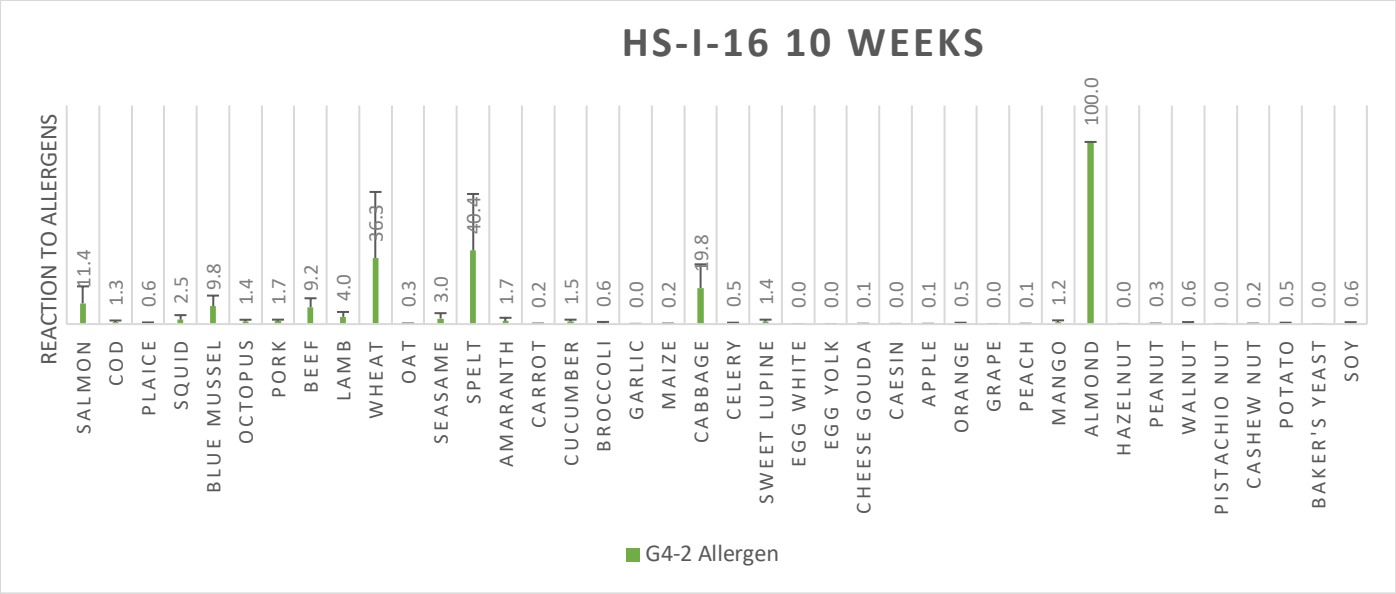
A reported p-value of 0.00 signifies an exceedingly low probability of observing the obtained results, or more extreme results, under the assumption that the null hypothesis is true. While technically not exactly zero, it is rounded as such for reporting purposes. This minuscule p-value suggests exceptionally strong evidence against the null hypothesis, leading to the rejection of the null hypothesis in favour of the alternative hypothesis. Researchers should interpret this result cautiously, considering its context alongside other factors like effect size and study design to draw robust conclusions about the significance and practical relevance of the observed effects.



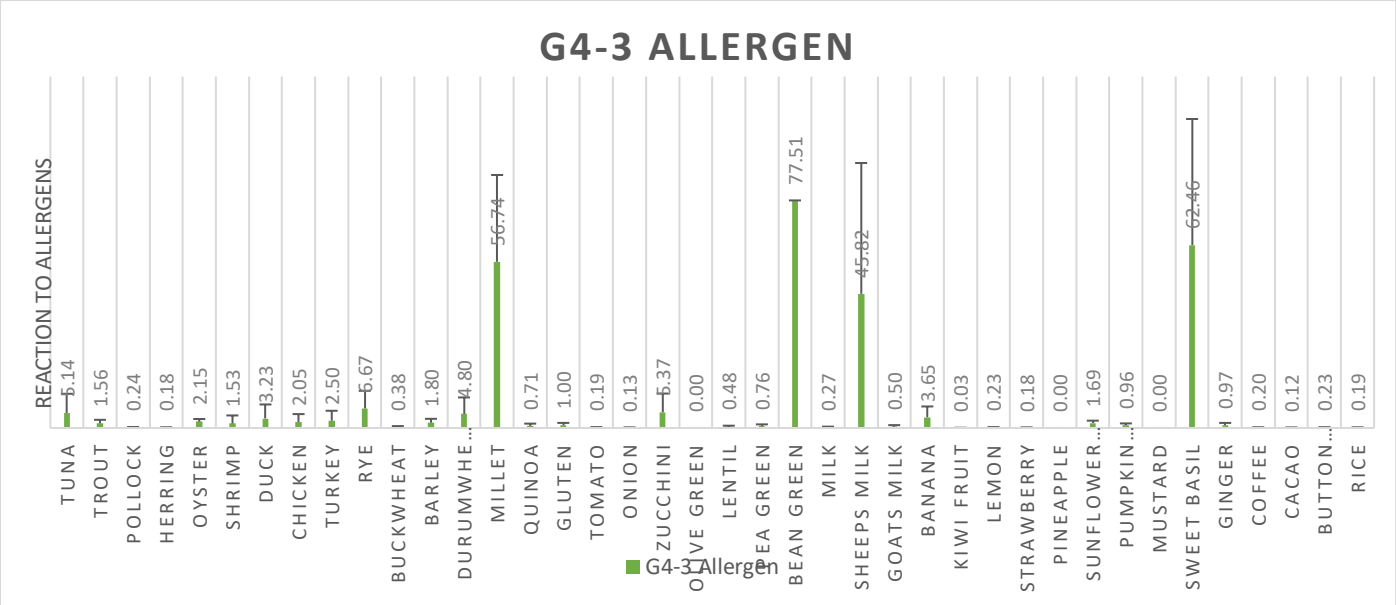
A reported p-value of 0.00 indicates an extremely low probability of observing the obtained results, or more extreme results, under the assumption that the null hypothesis is true. While p-values are not precisely zero but are often rounded for reporting, a value of 0.00 is used to convey that the probability is negligible. This outcome implies robust evidence against the null hypothesis, leading to the rejection of the null hypothesis in favour of the alternative hypothesis. Researchers should interpret this result cautiously, considering other factors such as effect size, study design, and the broader context to ensure a comprehensive understanding of the statistical and practical significance of the findings.



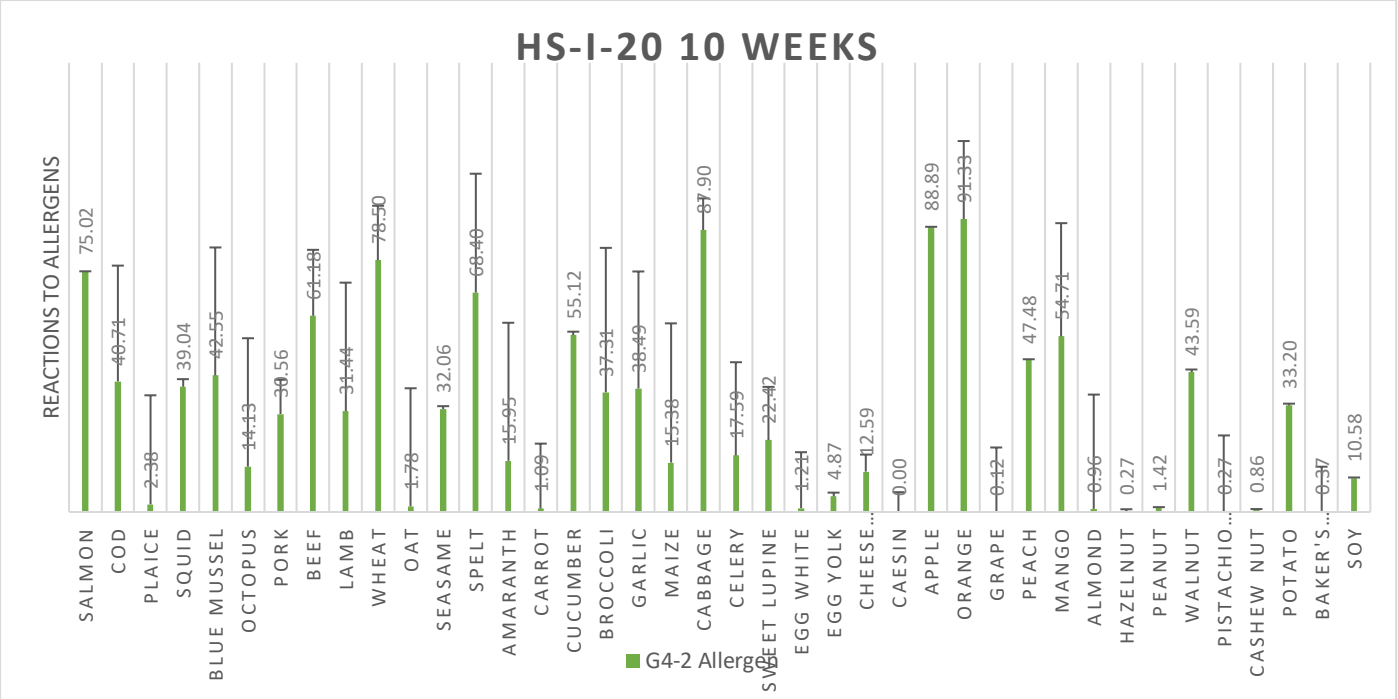
The P-value is still 0.00. It means an extremely low possibility of the results.



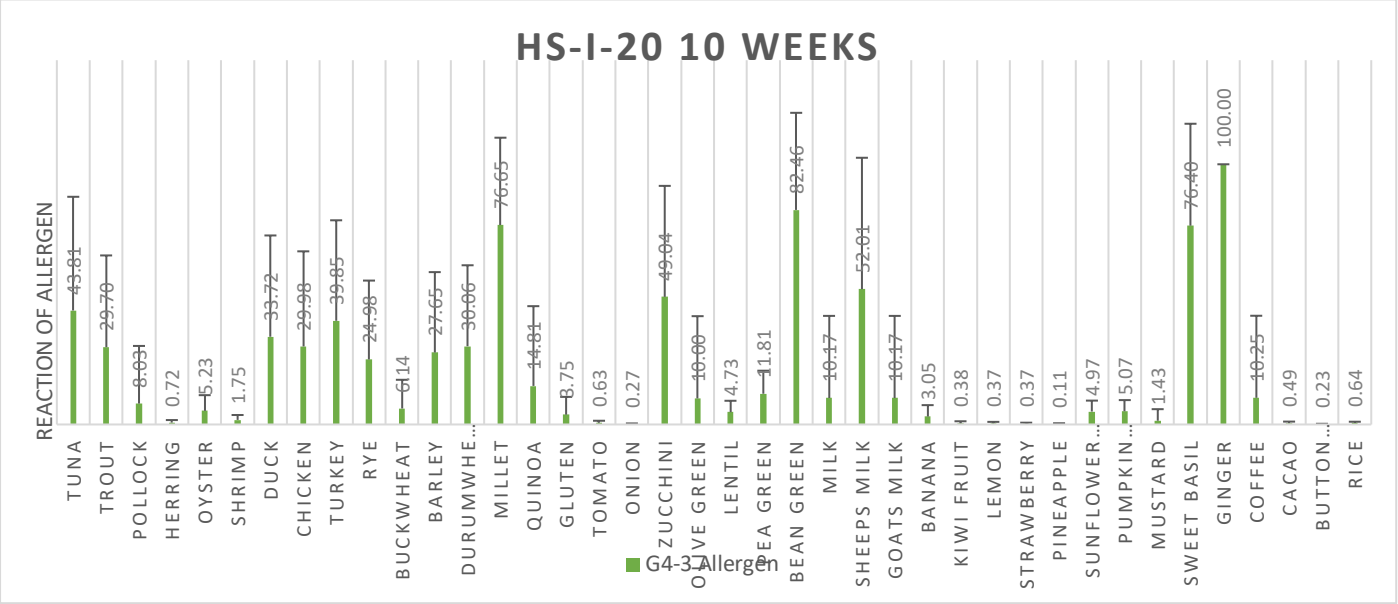
A reported p-value of 0.04 indicates that there is a 4% probability of observing the obtained results, or more extreme results, under the assumption that the null hypothesis is true. In the context of hypothesis testing, where a significance level (often denoted as α) of 0.05 is commonly used, a p-value of 0.04 suggests that the results are statistically significant at the 0.05 significance level. This implies that there is evidence to reject the null hypothesis in favour of the alternative hypothesis. Researchers would typically interpret this result as indicating that the observed effects are unlikely to have occurred by random chance alone and are statistically meaningful. However, it's important to consider other factors, such as effect size and study design, for a comprehensive understanding of the practical significance of the findings.



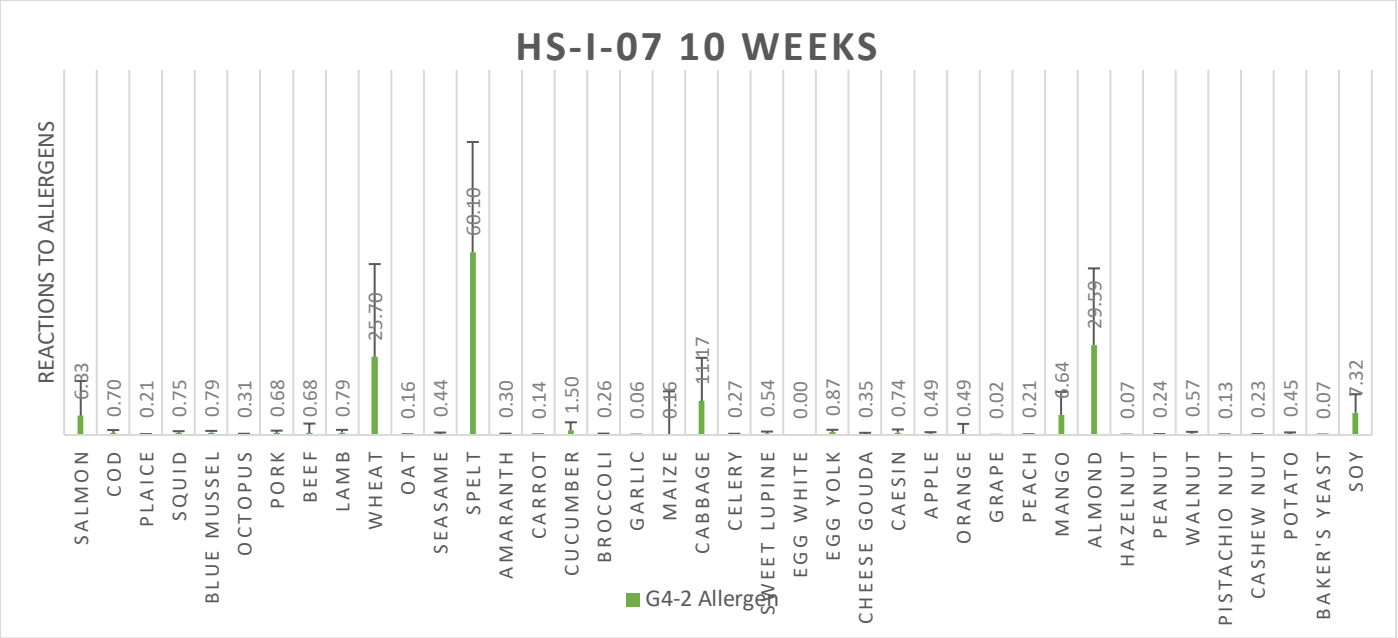
Again, the p-value is low having a very low chance of obtaining the results.



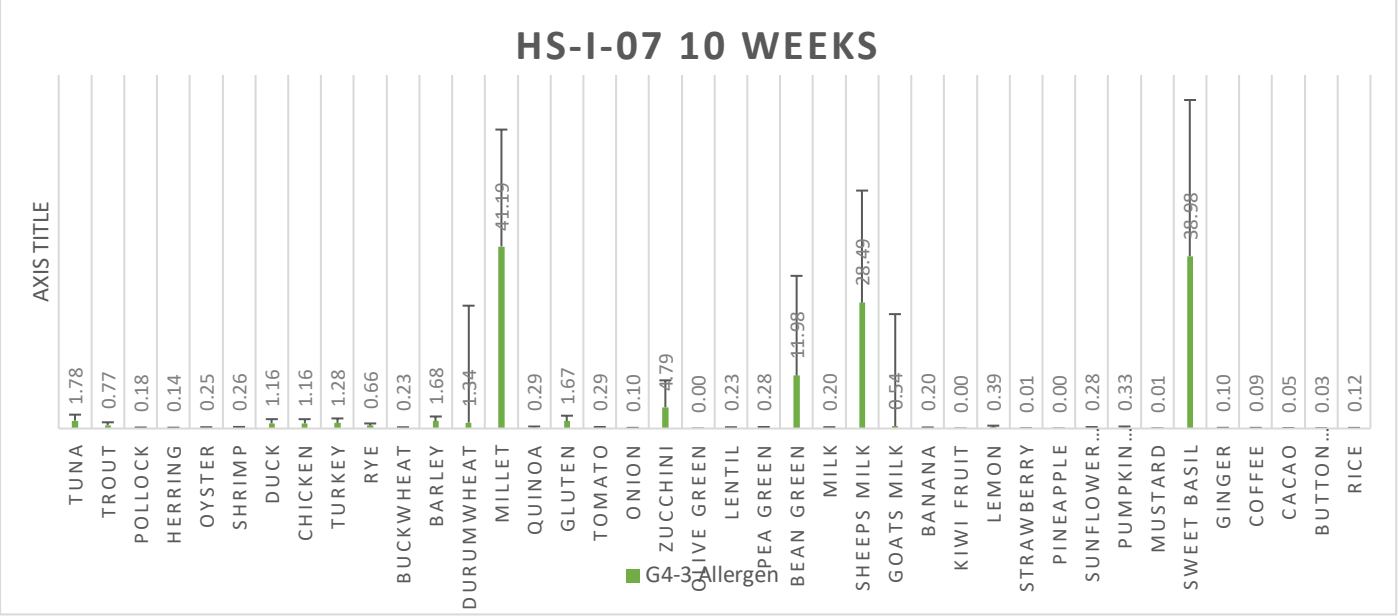
The P-value is 0.00. The probability is still low.



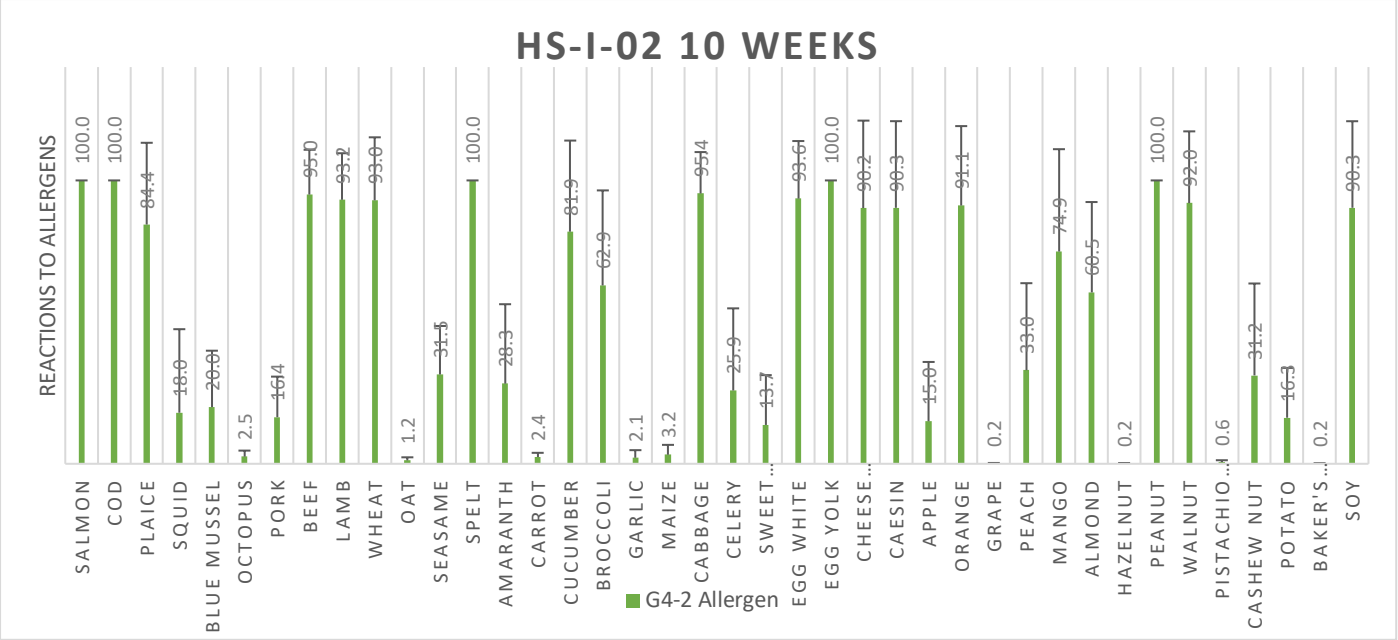
The P-value is 0.00 the values have a very low result index.



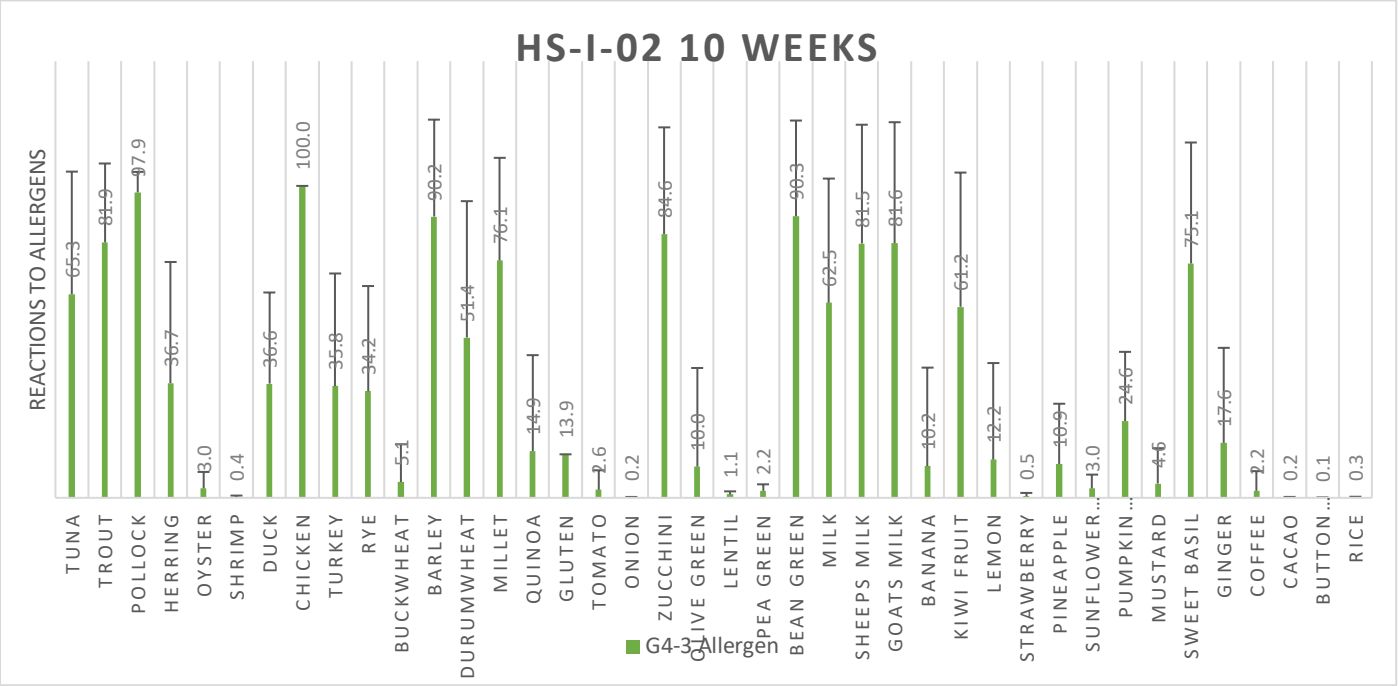
The P-value is 0.00. At this stage, the chances of obtaining the results remain the same.



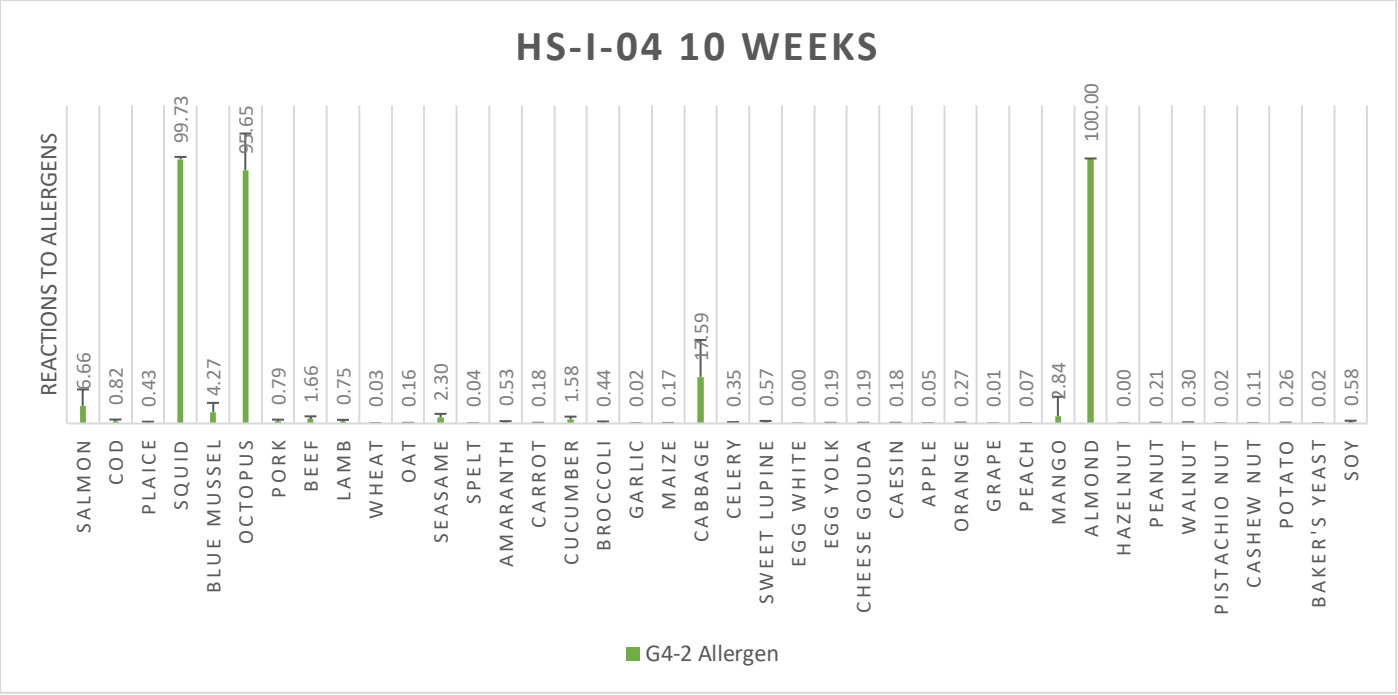
The P-value is 0.00. The chances are still the same. A reported p-value of 0.00 signifies an exceedingly rare probability of observing the obtained results or more extreme results if the null hypothesis is true. While not precisely zero, this notation is used to convey an almost negligible probability. In the realm of hypothesis testing, a p-value of 0.00 typically leads to the rejection of the null hypothesis, indicating strong statistical evidence in favour of the alternative hypothesis. This suggests that the observed effects are highly unlikely to be the result of random chance alone. Researchers should approach this result judiciously, considering additional factors like effect size, study design, and the broader context to gain a comprehensive understanding of both the statistical and practical implications of the findings.



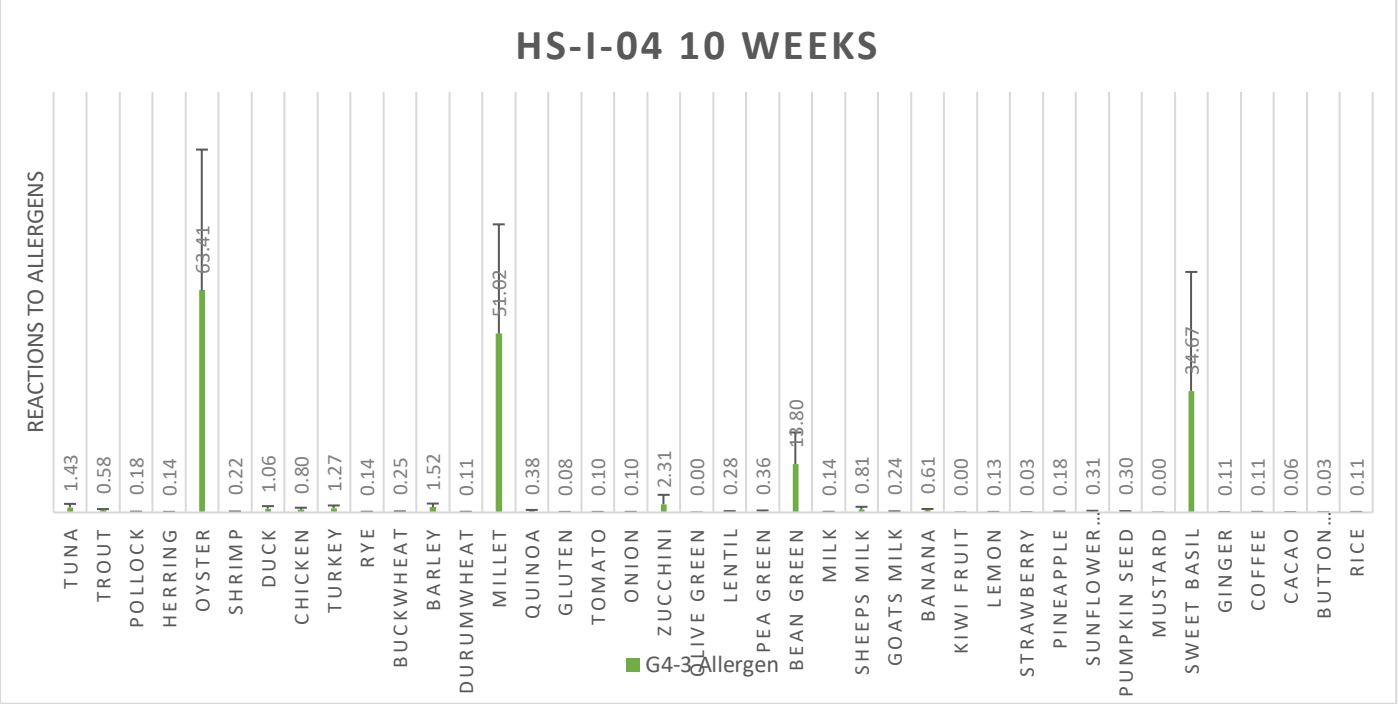
The P-value is 0.00. A p-value of 0.00 reflects an extraordinarily low likelihood of observing the obtained results or more extreme outcomes under the assumption that the null hypothesis is true. While not precisely zero, this notation emphasizes an extremely rare probability, leading to the rejection of the null hypothesis in favour of the alternative hypothesis.



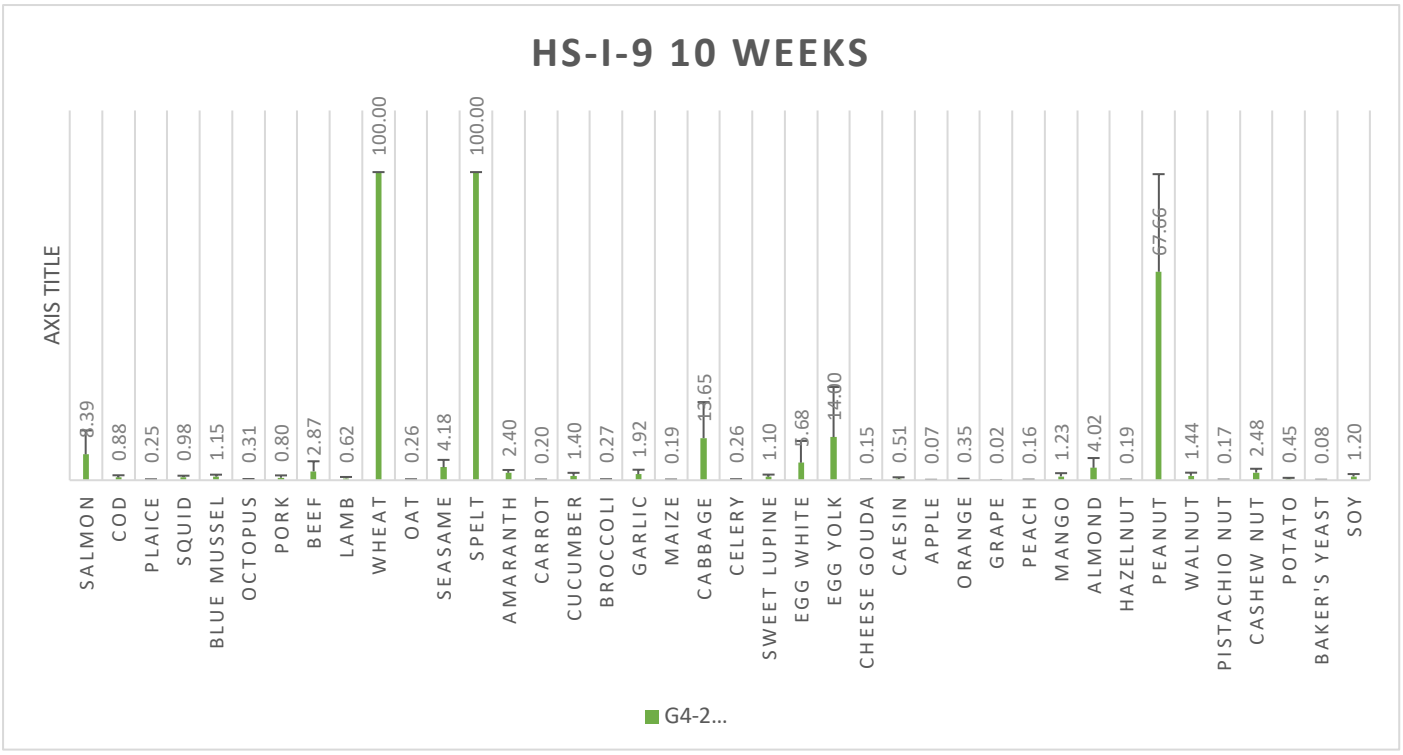
The P-value is 0.00. The reported p-value of 0.00 indicates an almost infinitesimal probability of obtaining the observed results by random chance alone, providing compelling evidence against the null hypothesis. This underscores the statistical significance of the findings.



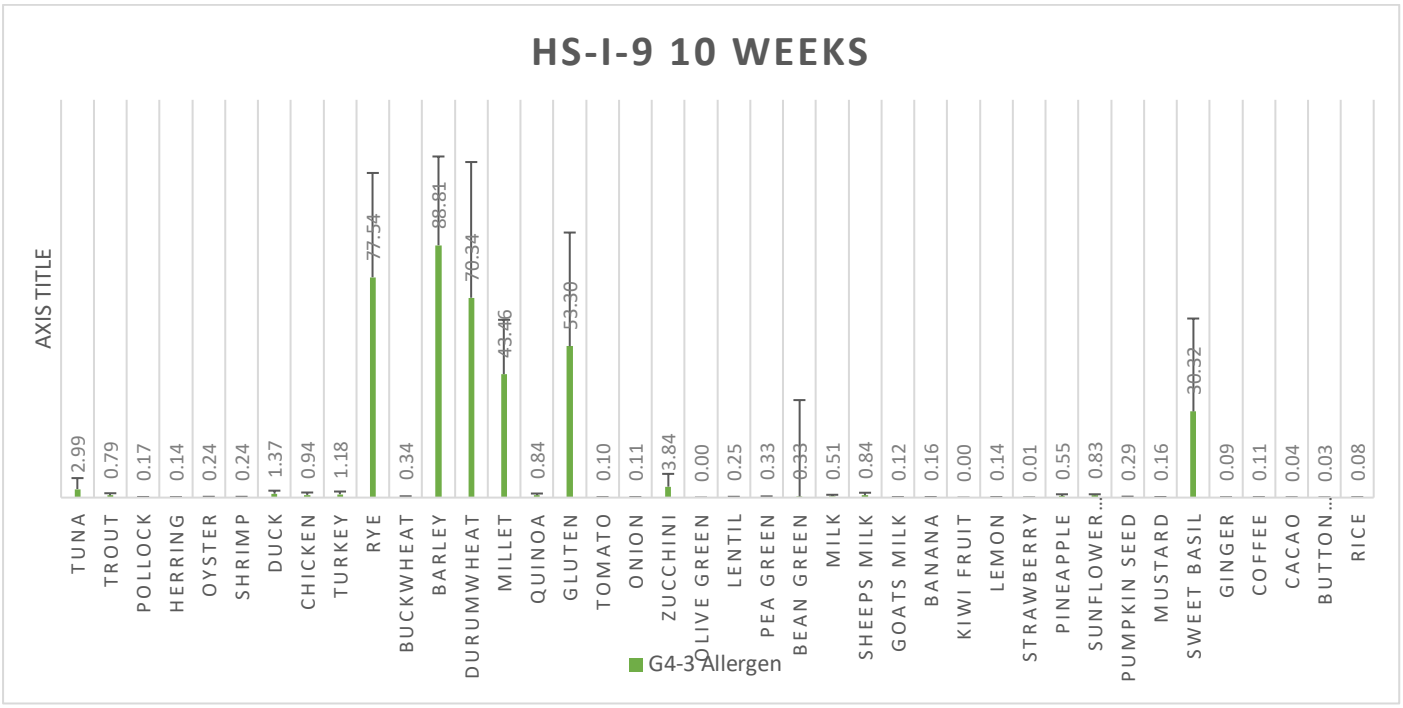
P-value is 0.01. A reported p-value of 0.01 signifies a 1% chance of observing the obtained results, or more extreme outcomes if the null hypothesis is true. This level of probability falls below the commonly used significance level of 0.05, suggesting statistical significance and providing grounds to reject the null hypothesis in favour of the alternative. In practical terms, this indicates that the observed effects are likely not due to random chance alone, enhancing confidence in the meaningfulness of the results. Researchers should, however, consider additional factors such as effect size and study design to ensure a comprehensive interpretation of the statistical and practical implications of the findings.



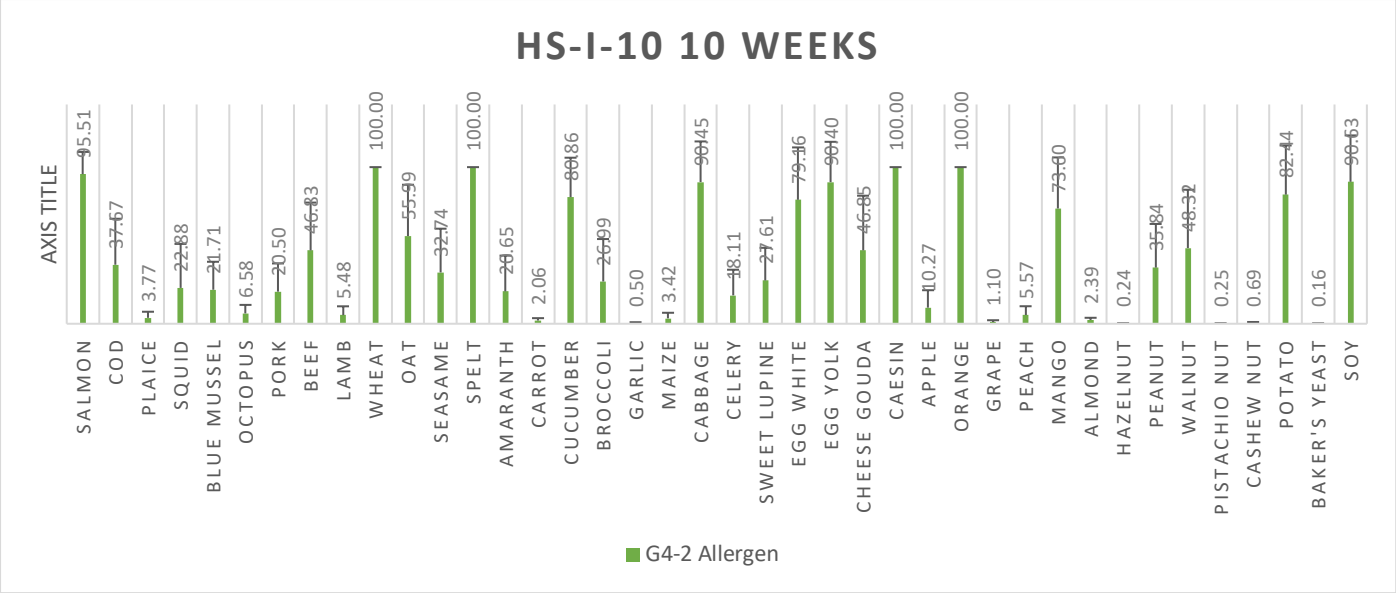
The P-value is 0.00. With a p-value effectively at zero, the statistical analysis demonstrates a minute probability of the results occurring under the null hypothesis. This robust evidence supports rejecting the null hypothesis and accepting the alternative, indicating substantial confidence in the observed effects.



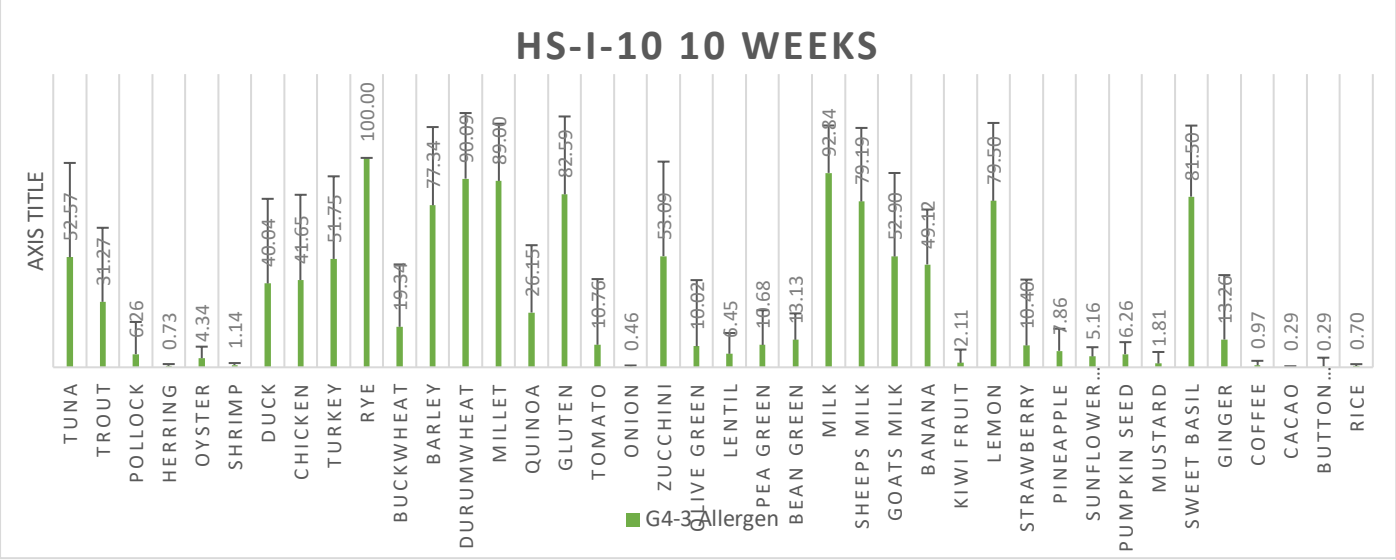
The P-value is 0.00. The notation of a p-value as 0.00 underscores the highly improbable nature of the observed results if the null hypothesis were true. This rarity in probability solidifies the conclusion that the observed effects are statistically significant.



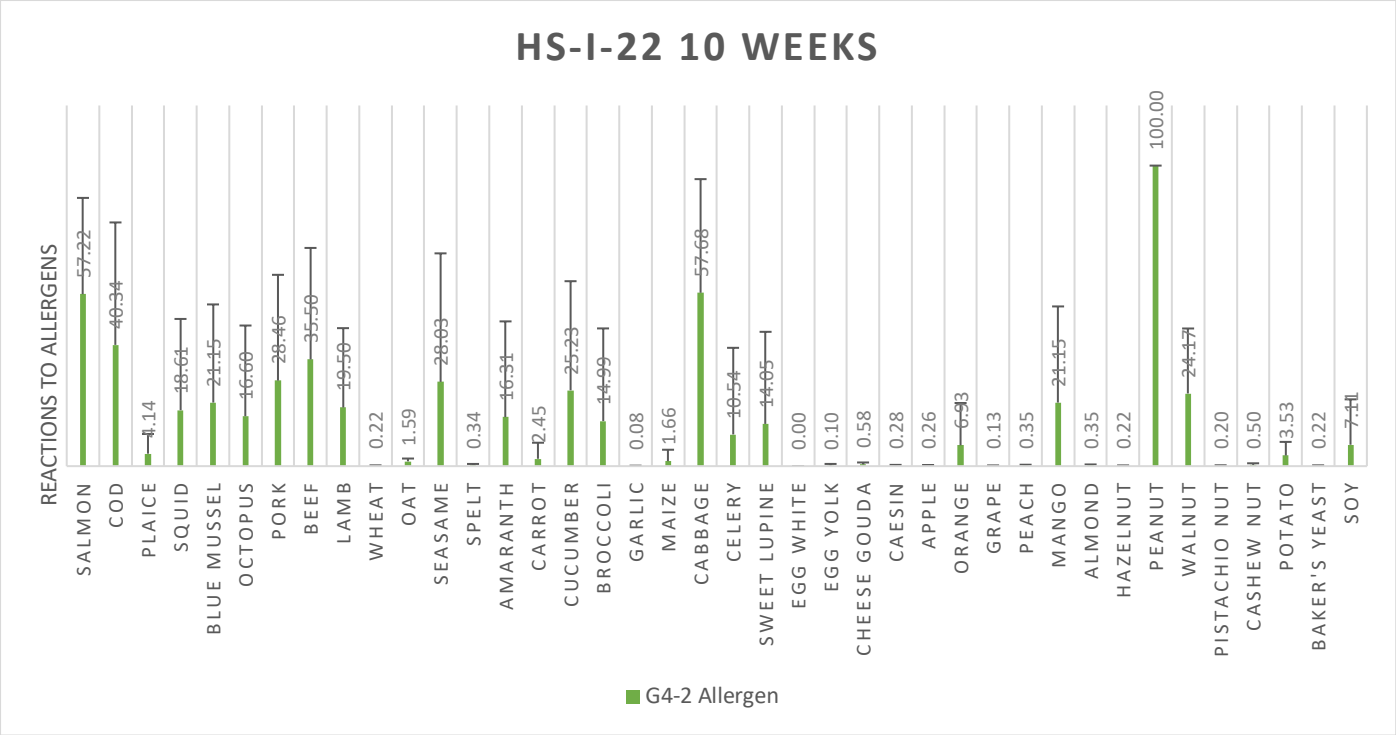
The P-value is 0.00. A p-value approaching zero signifies an extremely low chance of the results occurring by random chance, leading to the rejection of the null hypothesis. This statistical rarity reinforces the strength of the evidence supporting the alternative hypothesis.



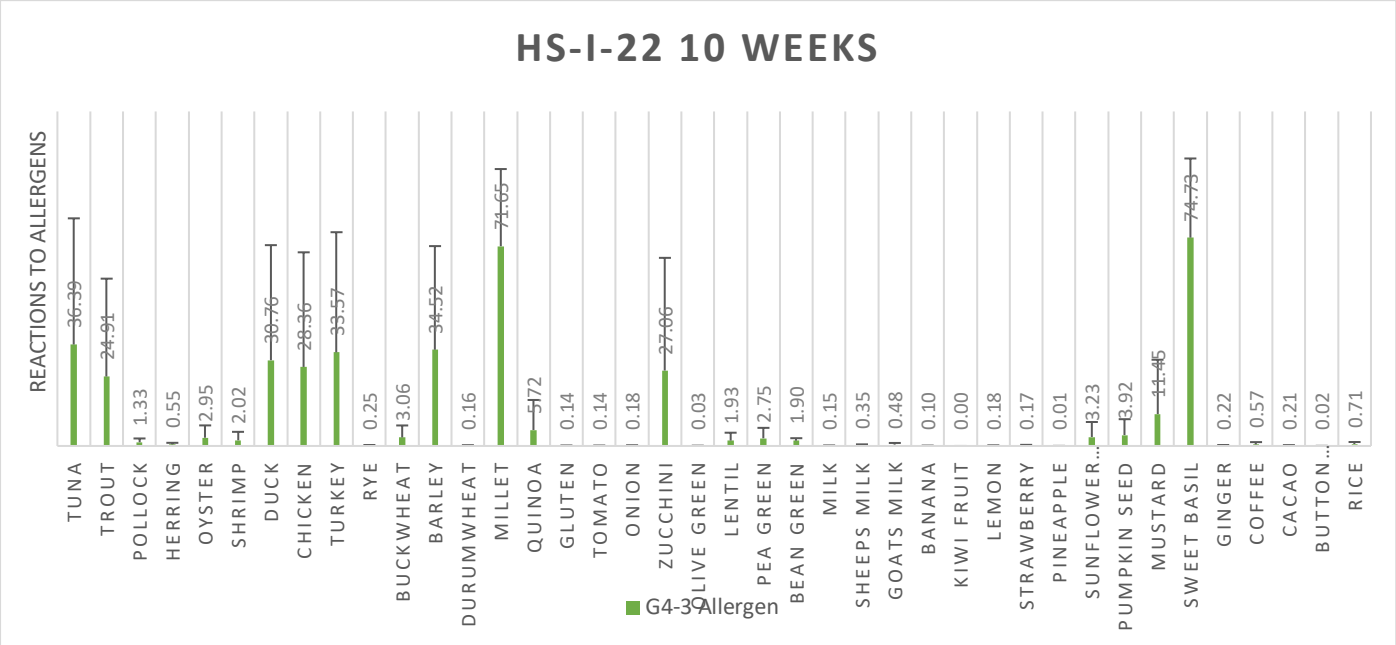
The P-value is 0.00. The reported p-value of 0.00 emphasizes an extraordinarily small probability, indicating that the observed results are highly unlikely under the null hypothesis. This compelling evidence supports the rejection of the null hypothesis in favour of the alternative.



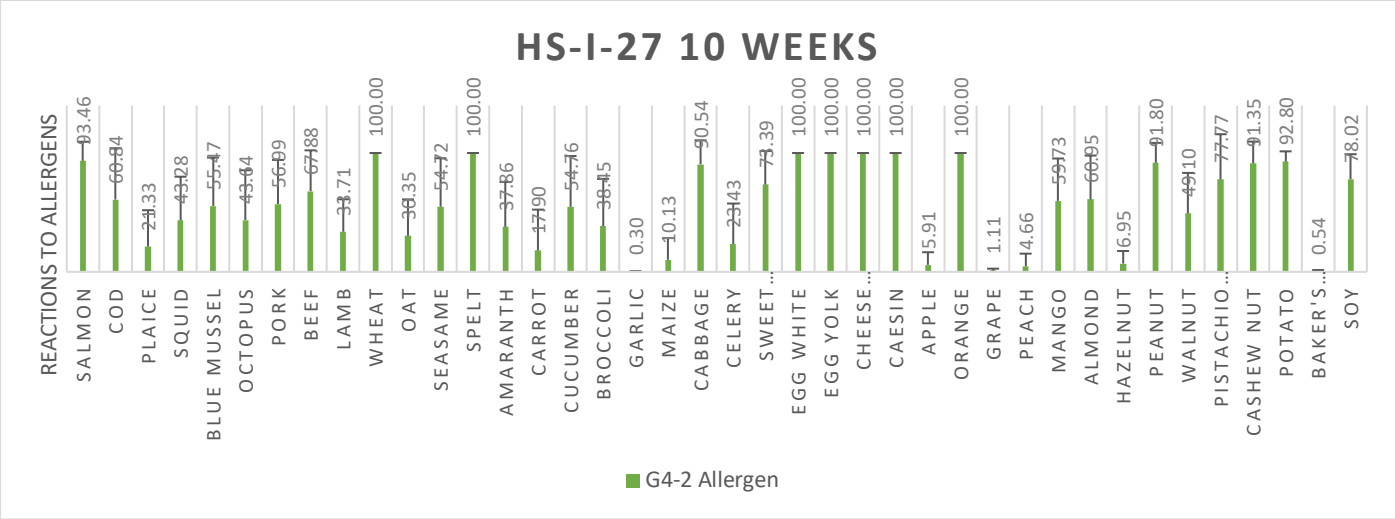
The P-value is 0.00. With a p-value effectively at zero, the statistical analysis suggests a vanishingly small likelihood of the observed results occurring by chance alone. This supports the decision to reject the null hypothesis, pointing towards the presence of a genuine effect.



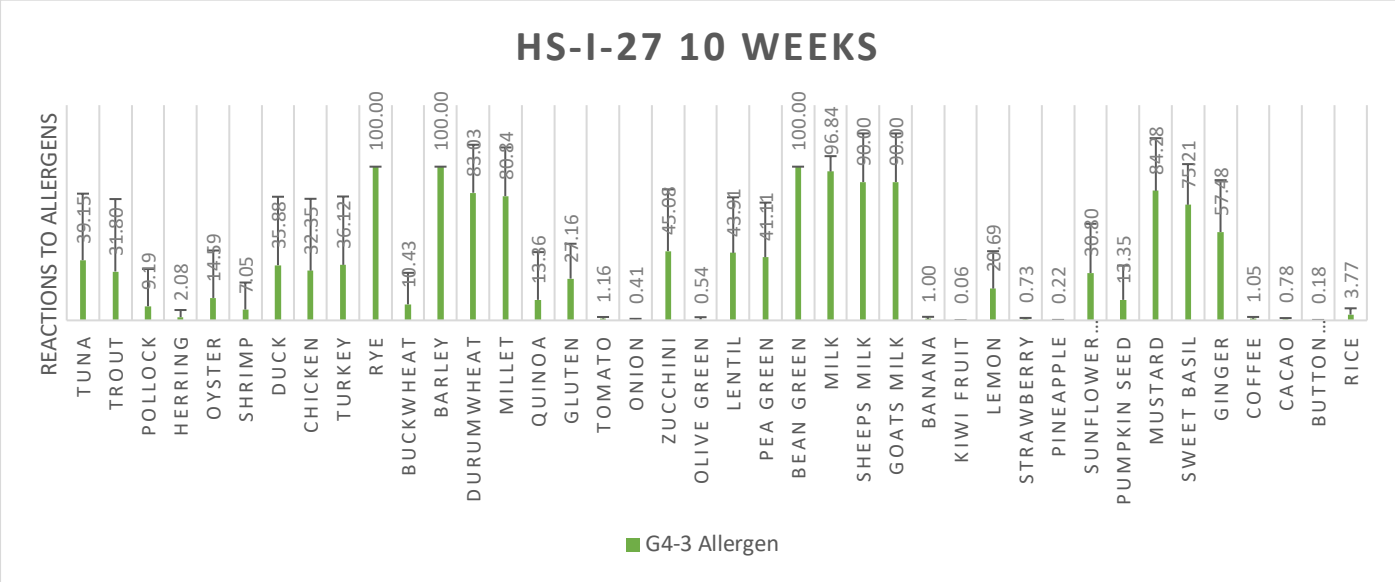
The P-value is 0.00. A p-value approaching zero underscores the extreme rarity of the observed results under the null hypothesis, providing strong statistical support for rejecting the null hypothesis and accepting the alternative.



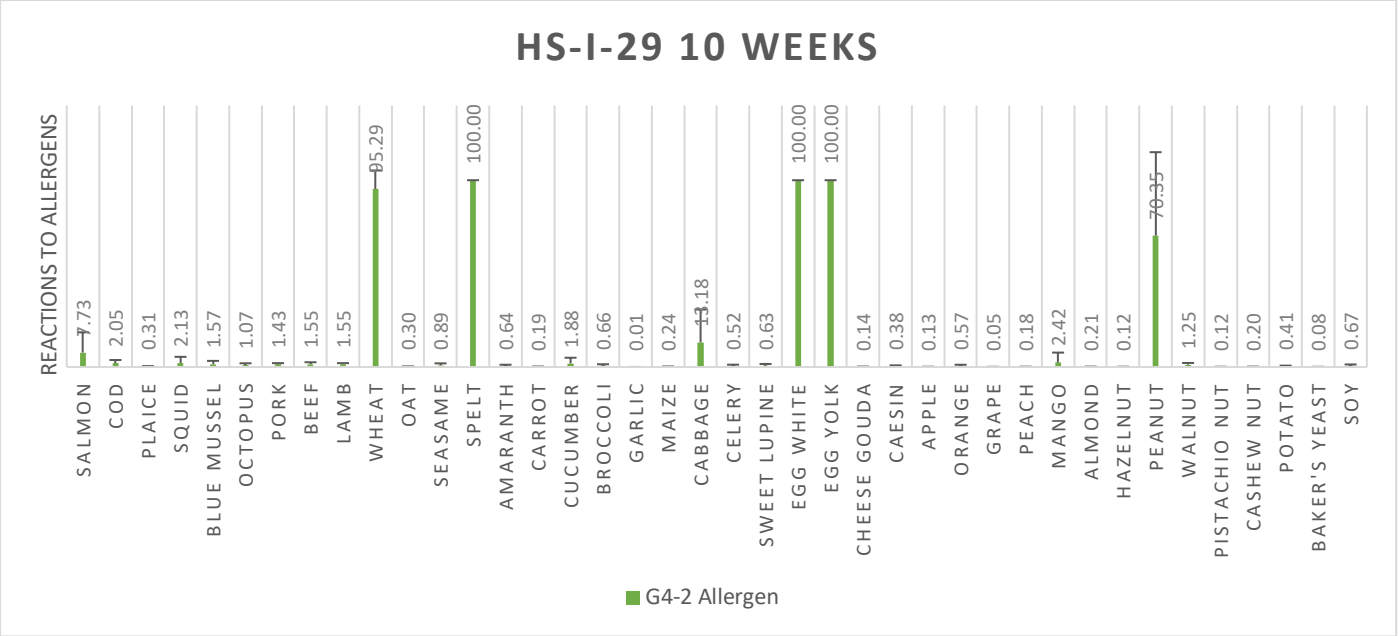
The P-value is 0.00. The notation of a p-value as 0.00 signifies an exceedingly rare occurrence of the observed results if the null hypothesis were true, reinforcing the conclusion that the findings are statistically significant.



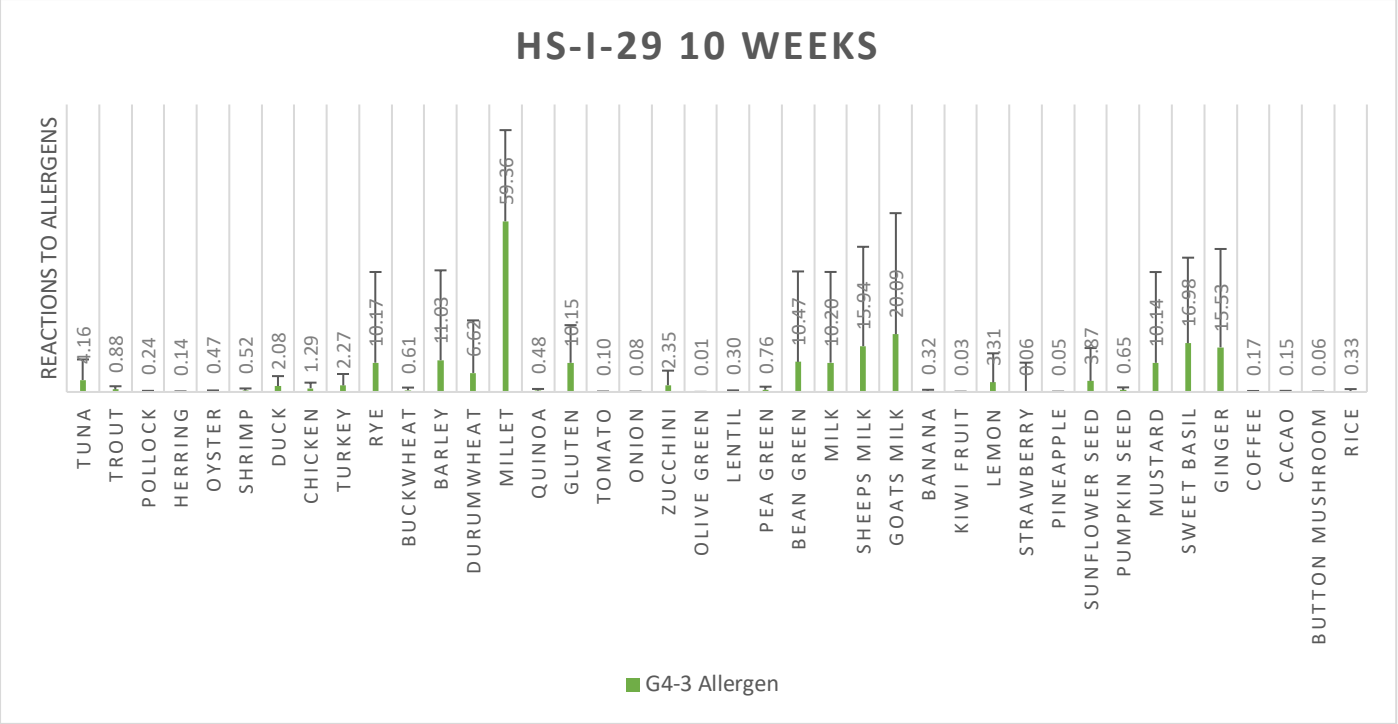
The P-value is 0.00. A reported p-value of 0.00 highlights the near-impossibility of the observed results happening by random chance alone, supporting the robust rejection of the null hypothesis and favouring the alternative hypothesis.



The P-value is 0.00. With a p-value effectively at zero, the statistical analysis suggests an extraordinarily rare occurrence of the observed results if the null hypothesis were true. This rarity in probability provides strong support for the rejection of the null hypothesis, underscoring the reliability of the observed effects.



P-value is 0.01. A reported p-value of 0.01 suggests a 1% probability of observing the obtained results, or more extreme results, under the assumption that the null hypothesis is true. In the context of hypothesis testing, where a significance level (often denoted as α) of 0.05 is commonly used, a p-value of 0.01 indicates that the results are statistically significant at the 0.05 significance level. This implies that there is evidence to reject the null hypothesis in favour of the alternative hypothesis. Researchers would typically interpret this result as indicating that the observed effects are unlikely to have occurred by random chance alone and are statistically meaningful. However, as always, it's important to consider other factors such as effect size, study design, and the broader context for a comprehensive understanding of the practical significance of the findings.



The P-value is 0.00. The reported p-value of 0.00 indicates an incredibly remote probability of observing the obtained results under the null hypothesis. This virtually zero probability solidifies the decision to reject the null hypothesis in favour of the alternative, emphasizing the statistical significance of the findings.

- ***DISCUSSION***

  Despite the theory that the appearance of IgE and Igg4 is only due to the consumption of a particular food item, there were still significant levels of IgE and Igg4 detected in some volunteers even after eliminating the food item for years. This gives rise to the question, why are the antibodies still produced when the food item was not consumed? The strips used were specifically made to identify the antibodies produced in the blood. This helps avoid cross-reaction between false detection. The anti-human IgG4 antibodies from the strips bind to the antibodies produced in the blood to detect accurate levels of intolerance. The general trend of the results shows that the presence of IgG4 levels is steadily present over the ten weeks. It simply does not increase when consuming different food items, the levels don't decrease when a food item is temporarily eliminated from the diet for the week. The reported P-values across all the graphs were consistently found to be less than 0.05, reinforcing the validity of our hypothesis. The statistical analysis was conducted using the ANOVA method in Microsoft Excel, providing a robust indication that there are significant differences among the groups being compared. However, it's crucial to acknowledge the presence of potential confounding factors that may influence the results. Variables such as illness due to cold or flu, technical errors in the experimental procedure, and environmental factors during the transport of samples could introduce variability and contribute to the observed patterns.

  Recognizing these potential sources of variation is imperative in ensuring a comprehensive understanding of the study outcomes. To establish a more precise and targeted understanding of intolerance levels, it is recommended to conduct further confirmatory tests. Specifically, these tests should focus on isolating the contributions made by IgG4 immunity, a key factor in immune responses associated with food intolerance. By honing in on this specific aspect, researchers can enhance the accuracy and reliability of their findings, thereby strengthening the scientific basis for any subsequent conclusions drawn from the study. Another noticeable trend was proven in cases of volunteer graphs of HS-i-27 with a restricted diet like vegans or vegetarians. Their blood results showed high levels of antibodies against seafood like Salmon and Cod along with egg and meat, which they have strictly eliminated from their diet.

- ***Recommendation***

  It is imperative to prioritize the conduct of clinical validation studies to rigorously assess the reliability and clinical utility of IgG4 testing in diagnosing food intolerances. Such studies should involve diverse patient populations and healthcare settings to ensure the accuracy and generalizability of results. Simultaneously, the development of standardized testing protocols is essential to establish consistent measurement methods for IgG4 antibodies. These protocols should be widely adopted to eliminate variations in test outcomes across different laboratories, thereby enhancing the reliability of diagnostic results. The creation of evidence-based clinical guidelines is paramount, providing healthcare practitioners with clear and standardized criteria for interpreting IgG4 test results. These guidelines should offer guidance on patient care and dietary recommendations, ensuring that IgG4 testing is integrated into comprehensive diagnostic processes. Public awareness campaigns should be initiated to educate individuals about the distinctions between IgE-mediated allergies and IgG4-mediated intolerances, empowering them to make informed decisions about testing and treatment options. Longitudinal research efforts should be supported to investigate the long-term health effects of IgG4-mediated food intolerances, particularly their potential associations with chronic diseases. Exploration of therapeutic interventions targeting IgG4-mediated food intolerances is crucial, including investigations into the modification of IgG4 responses to alleviate symptoms and enhance patient well-being. Additionally, further research into the role of interleukins (ILs) in regulating IgG4 responses could pave the way for IL-modulating therapies. Wendy Hodsdon, ND along with Dr. Heather Zwickey researched cell size allergy testing and IgG ELIZA food allergy testing. The results of the IgG ELISA method had repeatable results using a split sample on the same day along with results across one week. The coefficient of variance was proven to be 0.05. To further prove the ICC, the Intraclass Correlation Coefficient was 0.99 (Hodson

and Zwickey, 2014) To ensure effective patient care, comprehensive educational materials should be developed for individuals with IgG4-mediated food intolerances, covering dietary modifications, symptom management, and strategies for improving overall health. Collaboration among healthcare professionals from various disciplines, including immunologists, allergists, dietitians, and gastroenterologists, should be encouraged to provide holistic care for affected individuals. Ethical considerations, such as informed consent and patient data privacy, must be addressed to maintain the highest ethical standards in IgG4 testing and treatment. Finally, international research initiatives should be supported to gather data on IgG4-mediated food intolerances across diverse populations, facilitating a more comprehensive understanding of these conditions on a global scale. These recommendations collectively aim to enhance the diagnosis, management, and patient outcomes associated with IgG4-mediated food intolerances, ultimately improving the well-being of affected individuals.

Further Research on IgG4 and Food Reactions:

Encourage in-depth research into the mechanisms by which IgG4 antibodies interact with food antigens and the immune system. Investigate whether these interactions play a causal role in the development of food intolerances.

Clinical Validation:

Advocate for rigorous clinical validation studies to assess the reliability and accuracy of IgG4 testing in predicting and managing adverse reactions to dietary components.

Promote research that involves diverse patient populations to ensure the generalizability of findings.

Standardized Testing Protocols:

Call for the establishment of standardized testing protocols and methodologies for measuring IgG4 antibodies in clinical settings. Standardization will help eliminate variability in test results between different laboratories and improve diagnostic consistency.

Clinical Guidelines:

Encourage the development of evidence-based clinical guidelines that provide clear recommendations for healthcare practitioners regarding the interpretation of IgG4 test results.

Highlight the importance of using IgG4 test results as part of a comprehensive diagnostic process, considering clinical symptoms and patient history.

Public Awareness:

Promote public awareness campaigns to educate individuals about the differences between IgE-mediated allergies and IgG4-mediated intolerances. Explain that IgG4 testing is not a substitute for allergy testing but can offer valuable insights into food-related health issues.

Longitudinal Studies:

Support longitudinal research projects to follow individuals with IgG4-mediated food intolerances over extended periods. This could help identify any associations between these intolerances and the development of chronic diseases, offering insights into prevention and management strategies.

Explore Treatment Options:

Encourage investigations into potential therapies or interventions that specifically target IgG4-mediated food intolerances. Explore whether modifying IgG4 responses can alleviate symptoms and enhance the quality of life for affected individuals.

Interleukin Research:

Promote research into the role of interleukins (ILs) in regulating IgG4 responses. Investigate whether modulating IL production or activity could serve as a viable therapeutic approach for managing IgG4-mediated food intolerances.

Patient Education:

Develop comprehensive educational materials for individuals with IgG4-mediated food intolerances. These resources should cover dietary modifications, symptom management strategies, and tips for improving overall well-being.

Collaborative Research:

Encourage collaboration between healthcare professionals, including immunologists, allergists, dietitians, and gastroenterologists, to develop a holistic approach to managing IgG4-mediated food intolerances. Combining expertise from various fields can lead to more effective patient care.

Ethical Considerations:

Address ethical concerns related to IgG4 testing, such as ensuring that patients provide informed consent for testing and treatment. Emphasize the importance of patient privacy and data security in handling sensitive medical information.

Global Studies:

Support international research initiatives that collect data on IgG4-mediated food intolerances across diverse populations and geographic regions. This global perspective can reveal patterns and insights that might not be apparent in smaller, localized studies.

❖ *CONCLUSION*

Using our tests, you can test for IgE and IgG4 antibodies at the same time. Testing for IgE and IgG4 antibodies you have the opportunity to test not only the immediate allergic reactions associated with an immune response (IgE) but also those reactions that are not IgE-mediated antibodies are known to be the blocking antibodies preventing the release of histamine factors and the activation of immediate allergic reaction (Type 1 hypersensitivity). IgG4 antibodies can influence an immune response but not activate that directly. Our Prime110 test can help you to have a better overview of the specific reactions (immune response or not) in the presence of specific allergens. In this way we will be able to draw you a map of the reactions that you can take with you, to start your journey to find a better version of yourself. Our tests are developed for the identification of specific IgE-mediated allergies and IgG4 antibody reactions. Unfortunately, there are also no IgE-mediated allergic reactions, and our tests are not able to detect those allergies. IgG4 antibodies are only used for the identification of intolerance and not allergies. The study on IgG4 immunoglobulins and their relationship with food intolerances and allergies underscores the complexity of the human immune response to dietary components. While IgG4 antibodies have been considered as potential markers for food intolerances, intriguing findings have emerged. Notably, some participants exhibited persistent IgG4 and IgE antibody levels even after long-term elimination of specific food items from their diets, challenging conventional assumptions about the direct link between antibody presence and food consumption. The study's methodology, involving specialized testing strips designed to prevent false detections and cross-reactions, provides a robust foundation for further research in this field. The consistent trend of IgG4 levels remaining relatively stable over ten weeks, regardless of dietary variations, suggests a need for deeper investigations into the mechanisms governing these antibody responses. As the science of food intolerance evolves, it is imperative to prioritize clinical validation studies, standardized testing protocols, and evidence-based clinical guidelines. These measures will enhance the reliability of IgG4 testing, aid healthcare practitioners in interpreting results accurately, and guide patient care effectively. Public awareness campaigns can empower individuals to distinguish between IgE-mediated allergies and IgG4-mediated intolerances, enabling them to make informed decisions about their health.

Longitudinal research efforts are essential to uncover potential long-term health implications of IgG4-mediated food intolerances, particularly their associations with chronic diseases. Furthermore, therapeutic interventions targeting IgG4-mediated responses and investigations into the role of interleukins (ILs) in modulating IgG4 responses hold promise for future advancements in food intolerance management. Comprehensive educational materials, collaborative care among healthcare professionals, ethical considerations, and international research

initiatives will collectively contribute to a deeper understanding of IgG4-mediated food intolerances on a global scale. In sum, this study marks a pivotal step in unraveling the complexities of IgG4 immunoglobulins, food intolerances, and allergies. It offers a foundation upon which further research and advancements in diagnostics and patient care can be built, ultimately improving the well-being of individuals navigating the intricate landscape of dietary-related immune responses.

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