RESEARCH ARTICLE

European Journal of Clinical Medicine
www.ej-clinicmed.org

Intrinsic Atopic Dermatitis: Titration of Precipitins in the Screening of Food Allergens for Prescription of Elimination Diets and Desensitization Strategies

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ABSTRACT

Background: **The diagnosis of non-IgE mediated food allergies may be a complex puzzle when there is no start point to establish an elimination diet to allow a clear clinical field to initiate diagnostic Oral Food Challenges tests.**

Objective: **To evaluate the opportunity of the tube titration of precipitins to select food allergens to proceed with elimination diets to assist the diagnosis and management of adult patients with Food Allergy manifested as Intrinsic Atopic Dermatitis (IAD).**

Methods: **The tube titration of specific precipitins against anamnesis-chosen food allergens were performed in 64 IAD patients and their titers were associated with an Improvement Verbal Scale Rate (IVSR) of the patient's perception of the benefits of the Precipitins-based Elimination Diet (PED) performed with these specific food allergens, as well correlated with their positive or negative perception of the impairment of symptoms after the reintroduction of the Symptom-Related Food Allergen (SRFA).**

Results: **In most cases, the PED contributed to a significant clinical improvement that allowed the patients to evaluate the individual effect of the reintroduction of each food allergen on their diets. There was a significant positive correlation coefficient between the titers of the food-specific titration of precipitins and the percentage of positive SRFA (Pearson r = 0.91; p-value = 0.0004).**

Conclusion: **The semiquantitative titration of specific precipitins against food allergens is a promising triage test to select food allergens to proceed with elimination diets to support the diagnosis and management of non-IgE mediated Food Allergy in patients with Intrinsic Atopic Dermatitis.**

Keywords: Allergy, antigen-antibody complex, atopic dermatitis, diet therapy, immune complex diseases, immunoassay, hypersensitivity, precipitins, precipitins tests.

Published Online: November 16, 2021

ISSN: 2736-5476

DOI:10.24018/ejclinicmed.2021.2.6.129

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I. Introduction

Atopic Dermatitis (AD) is a broad diagnostic designation for a group of heterogeneous diseases whose common denominator is a chronic dermal inflammation produced by some kind of immune hypersensitivity against at least one exogenous agent [1]. Dermal inflammation is not an exclusivity of AD, since it may be produced by a myriad of pathogenic conditions (auto-immune diseases, tumoral diseases, infectious diseases, chemical contact aggressors, et cetera). When associating the term "atopic" to the diagnosis of "dermatitis", the assistant physician must have in mind that there is, at least, one mechanism of hypersensitivity against, at least, one external cause, which ideally should be demonstrated [2]. AD is a multifaceted condition with multifactorial pathophysiology involving diversified innate and adaptive immune mechanisms, employing humoral- and cell-mediated inflammatory hypersensitivity, triggered by food allergens, aeroallergens, contact allergens, and toxins produced by skin microbiome, parasitic fungi, and house dust mites [3], [4]. Also called atopic eczema, the AD syndrome covers different phenotypes and endotypes, according to the clinical presentation and underlying molecular mechanisms, some of them linked to psoriasis, that, now a day has also been considered an endotype presentation of AD [5], [6]. Genetic susceptibility determines the diversified expression of AD, as pure or mixed with concomitant respiratory or digestive allergy, which also advocates against the simplified dermatologic management still largely used, justifying a causal investigation and a tailored personalized treatment derived from Precision Medicine [7]-[10]. Instead of one distinct skin disease, AD is better appreciated as the skin compartmentalization of a systemic hypersensitivity

condition, which fundaments its treatment with systemic approaches such as oral/sublingual allergen-specific immunotherapy, currently used to treat allergic patients presenting respiratory, gastrointestinal or dermatologic symptoms [11]-[13]. The main subdivision of AD is established according to the IgE status i.e., the "intrinsic" (not mediated by IgE) and "extrinsic" (IgE-mediated), however, patients with IgE-mediated mechanisms may also present, concomitantly, a non-IgE mediated hypersensitivity mechanism [14]-[16]. The non-IgE mediated IAD represents just 20% of the AD cases but introduces a diagnostic dilemma for the physician [17], [18]. Intrinsic Atopic Dermatitis (IAD) presents relatively late-onset and milder severity than the extrinsic phenotype. Perturbation of the skin barrier and filaggrin mutations are features of Extrinsic AD, but not IAD, which is better immunological characterized by the lower expression of IL-4; IL-5, and IL-13 and higher expression of interferon-gamma [14]. The sensitivity of the skin is relatively lower in IAD than in the extrinsic endotype, which correlates with systemic disease severity markers, but not with skin barrier impairment [19]. Hypersensitivity reactions are usually recognized to be elicited by adaptive immune responses i.e., mediated by lymphocytes and antibodies, but the innate system is an essential part of the inflammatory process. In the course of the clinical investigation of AD patients, usually, the immunologist works to identify, according to the four types of hypersensitivity reactions reported by Gell and Coombs, the particular mechanism(s) responsible for the production of the symptoms. The undetectability of specific IgE against a comprehensive battery of most common allergens usually discards the Gell and Coombs' type I hypersensitivity reaction and defines the IAD. The so-called Gell and Coombs' type II hypersensitivity reactions include a wide variety of immune mechanisms that possess in common the participation of immune cells and IgG and/or IgM antibodies. The Gell and Coombs' type III hypersensitivity reaction may produce lymphocytic infiltrates and circulating immune complexes. The Gell and Coombs' type IV hypersensitivity reaction is a contact dermatitis produced by the interaction of cytotoxic T lymphocytes and macrophages and may produce dermal lymphohistiocytic infiltrates [20], [21]. Hypersensitivity to food allergens, food additives, and/or food contaminants is one of the main causes of IAD [22]-[24]. The high diversity of possibilities of causative agents able to produce IAD turns the definition of the etiologic diagnosis into a real challenge to the assistant physician. Mostly, in non-immediate, non-IgE mediated allergic reactions, it is hard or almost impossible to indict culprits through anamnesis, especially in polysensitized patients [25], [26]. In these cases, the diagnosis of non-IgE mediated food allergies depends on in vivo Oral Food Challenge tests (OFC) [27]. However, before initiating the OFC, the patients must be engaged in an efficient exclusion diet that eliminates their symptoms [28]-[31]. The OFC is also impracticable when the patient is unable to discontinue the use of antihistamines and steroids. Additionally, the OFC is, traditionally, a suitable technique to discriminate immediate reactions, such as the occurring in patients with acute urticaria, and technically inoperable when the symptoms are insidious, dependent of high thresholds, and/or take several days to appear and weeks to disappear. So, it is a common medical practice to suggest empirically based elimination diets during 4 to 8 weeks to the management of patients with suspected non-IgE mediated food allergies [32], [33]. However, some colleagues, instead of using an empirically based strategy, reported an IgG-based approach to propose elimination diets to manage, with variable success, non-IgE mediated hypersensitivity syndromes [34]. Motivated by these perspectives, and by our own previous experience with precipitin's titration for the screening of non-IgE mediated acute urticaria, we begun to study the opportunity of the use of food-specific precipitins as a tool to the management of patients with IAD. Since it is hard to differentiate the particular participation of the individual measures in the long-term management of non-immediate hypersensitivity disease; adding the fact that the management of IAD involves a combination of personalized therapeutic prescriptions (such as the skin moistening, the treatment of occasional infections, the avoidance of concomitant chemical and biological irritants, for instance), we proposed a proof-of-concept study to evaluate the feasibility of the food-specific precipitation titration in a medium-term experience.

II. Methods

A. Subjects

The study was designed to characterize the utility of the titration of food-specific precipitins as an aid to indicate suspected food allergens, to proceed with diagnostic and therapeutic elimination diets for management of non-IgE mediated food allergies in adults with clinical features of IAD [35]. After receiving institutional review board approval, we selected a group of patients with these characteristics:

A) Clinical diagnosis of moderate to severe IAD, with more than 10% of affected body surface, with at least three years of evolution.

B) Non-reagent allergic skin scrape tests done with airborne, microbial, and food allergens [36].

C) Normal-range total IgE and non-detectable specific-IgE against airborne, microbial, and food allergens.

D) Negative history of anaphylactic reactions to food allergens.

We invited, with informed consent formularies, 64 outpatients (17 male; 20-90 years; mean age 52,7 years; SD 19,1 years) with the above-mentioned characteristics, to voluntarily provide blood samples to perform in vitro food-specific precipitation titrations, according to the principles of the World Medical Association Declaration of Helsinki and the International Committee of Medical Journals Editors requirements of privacy [37]. After a throughout nutritional anamnesis, we choose, with the patient's accordance, a personalized group of three to eight suspected food allergens, which were regularly consumed by them. The patients' blood was collected to perform the precipitin's titration against these food allergens and the patients were stimulated to engage an elimination diet based on the precipitin's titers: the Precipitin-based Elimination Diet (PED). After at least four weeks of effective PED, the patients were invited to return and describe, in their own words, their perception of the influence of the PED on the improvement of the symptoms, especially the pruritus. During the evaluation, they were

stimulated to graduate according to an Improvement Verbal Rating Scale (IVSR) a tenfold percentage (0–100%) describing their subjective perception of the benefits of the PED on the improvement of their symptoms, especially pruritus [38], [39]. After this, the unmedicated patients were oriented to re-introduce during a whole week, successively and non-concomitantly, each suspected food allergen that had participated in the PED. The patients were oriented to return for re-evaluation and describe, to each re-introduced food, a positive or negative association with the subjective impairment of their symptoms. The food allergens were recorded as "positive" or "negative" Symptom-Related Food Allergen (SRFA) and their percentages were correlated with the corresponding precipitins' titer: the highest dilution factor that yielded a positive precipitation reading.

B. *Food Antigens Preparation*

Food antigens for the cutaneous and in vitro challenge tests were bought at a local marketplace, extracted, and purified at our laboratory. The raw food allergens were extracted in Coca's solution at 4 °C for 24 hours, before centrifugation and separation of the water-soluble fraction from solid particles and oily fraction [40]. The protein quantification of the allergen extracts was done according to Bradford's protein-dye binding methodology [41]. All allergen extracts were diluted to an estimated protein concentration of 1 mg/mL and stored at 4 °C. The food allergens studied by allergic skin scrape tests and precipitin's titration were Brazil nut, banana, bovine meat, chicken meat, cocoa, cow's milk, egg's white, egg's yolk, tilapia, garlic, gluten, latex-related food (or Hevea brasiliensis latex for skin and blood tests), nut, onion, orange, peanut, pecan nut, red bean, red pepper, rice, shrimp, soybean, swine meat, tomato, and bread/beer Saccharomyces cerevisiae yeast. The total number of pairs of tests was 312. When the anamnesis revealed a suspicion of allergy against more than one latex-related food, the precipitin's tests were done with the Hevea brasiliensis latex. When the suspicion was against only one food allergen related to latex, the tests were done with this specific food. All relevant and mandatory laboratory health and safety measures have been complied with in the complete course of the experiments.

C. *Precipitin's Titration*

A simplified serum dilution method for the semi-quantitative tube titration of precipitins against soluble food allergens in a pure antigen-antibody system was performed. The patient's blood was collected in a clot-activator collecting tube. After serum separation, the tube was centrifugated at 2,000 rpm for 10 minutes. The allergen extracts were allocated in sets of nine glass tubes at progressive duplicated serum dilutions. The progressive dilutions were combined with the 15 µL of the antigen (1 mg/mL) with 250 µL of the patient's serum, progressively diluted into physiological saline solution (NaCl 0,9%) in the dilution ratios of 1:1; 1:2; 1:4; 1:8; 1:16; 1:32; 1:64 and 1:128. The ninth tube was a blank control done just with the serum to observe occasional spontaneous precipitation (cryoglobulins). After 24 hours, the tubes were examined by one of us and the titers (the highest dilution factor that yields a positive reading) were recorded [42].

D. *Tabular and Graphic Presentation of Data*

A total-data distribution table was constructed to allow an overview of the contribution of each precipitation test inside the context of each patient's perception (Appendix 1). A contingency table was elaborated according to the patient's perception of the effect of the Precipitin-based Elimination Diet (PED), evaluated by the Improvement Verbal Scale Rate (IVSR), and associated with the possibilities of combination between the positive/negative results of the precipitins' research and the patient's positive/negative perception after the reintroduction of each Symptom-Related Food Allergen (SRFA). The lowest precipitin titer, associated with each IVSR category row, was indicated in the positive precipitin/positive SRFA column. The highest precipitin titer, associated with each IVSR category row, was indicated in the positive precipitin/negative SRFA column (Table I). Another contingency table was elaborated distributing the precipitin's titration and the frequencies (%) of patient's positive or negative SRFA (Table II). Based on this table, the correlation coefficient between the precipitin's titers and the percentages of positive reports was performed using GraphPad Prism software (version 5.0; GraphPad Software, Inc., San Diego, CA, USA). Also based on Table III, a graphic distribution of the frequency of the positive Symptom-Related Food Allergen (SRFA) was plotted according to each precipitin's titer (Fig. 1).

III. Results

There was no spontaneous precipitation (cryoglobulins) on the control samples. There was also no suspended flocculation, all the positive reactions were observed as bottom precipitates. All recorded data of the patients: age, sex, suspected food allergens, IVSR, titers of the precipitation titrations, and positive or negative SRFA are presented in Appendix 1. The proportion of positive precipitins for each tested allergen were (positive tests/total tested): Brazil nut (1/1), banana (1/3), bovine meat (2/3) chicken meat (1/4), cocoa (7/44), cow's milk (24/37), egg's white (5/13), egg's yolk (2/2), tilapia (0/2), garlic (1/1), gluten (8/22), latex-related food (or Hevea brasiliensis latex for skin and blood tests) (19/56), nut (1/1), onion (1/1), orange (3/5), peanut (28/45), pecan nut (1/1), red bean (0/1), red pepper (0/1), rice (0/1), shrimp (1/1), soybean (3/4), swine meat (34/42), tomato (0/10), bread/beer Saccharomyces cerevisiae yeast (6/21). Table II shows that 42 (65,6%) of the 64 patients presented at least a 70% improvement of the symptoms after the PED, allowing a clear field to facilitate the perception of a positive/negative SRFA. Table I also shows that the highest titer associated with a negative SRFA is 1:32; meaning that greater titers will be more probably associated with a positive SRFA. Table 1 also shows that even low titers such as 1:2 may be associated with positive SRFA. Therefore, the titer's range interval from 1:2 to 1:32 is not predictive of a positive or negative SRFA. Table II shows to each precipitin's titer, the probabilities of a positive or negative SRFA. From this data, if one considers the positive SRFA as a true indicator of hypersensitivity, we may infer that when the precipitin's titer is 1:64 or 1:128 the sensibility of the test is 100% (all tests with these titers were associated with a positive SRFA).

Likewise, if one considers the negative SRFA as a true indicator of the inexistence of hypersensitivity, we may infer that the specificity of the test is 100% (no negative precipitin was associated with a positive SRFA). The correlation coefficient calculated between the titers of the food-specific precipitins and the corresponding percentages of positive SRFA showed a significant correlation (alpha=0.05) Pearson r = 0.91; 95% confidence interval (0.61 to 0.98); p-value = 0.0004; R square = 0.82.

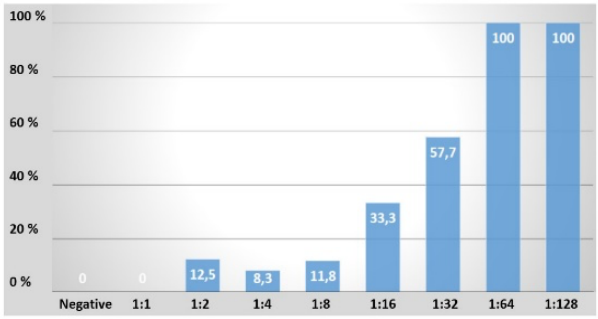

Fig. 1. Graphic distribution of the frequency (%) of the positive Symptom-Related Food Allergen (SRFA) according to each precipitin's titer.

TABLE I: CONTINGENCY TABLE DISTRIBUTED ACCORDING TO THE TENFOLD GRADUATED PATIENT'S PERCEPTION OF THE EFFECT OF THE PRECIPITIN-BASED ELIMINATION DIET (PED), EVALUATED BY THE IMPROVEMENT VERBAL SCALE RATE (IVSR), ASSOCIATED WITH THE FOUR POSSIBILITIES OF COMBINATIONS BETWEEN THE POSITIVE/NEGATIVE RESULTS OF THE PRECIPITINS' RESEARCH AND THE POSITIVE/NEGATIVE PATIENT'S PERCEPTION FOR THE SYMPTOM-RELATED FOOD ALLERGENS (SRFA)

IVRS (%) (Number of patients)	Number of Tests	Positive Precipitin Positive SRFA (Lowest titer)	Positive Precipitin Negative SRFA (Highest titer)	Negative Precipitin Positive SRFA	Negative Precipitin Negative SRFA
10% (3)	12	1 (1:8)	5 (1:8)	0	6
20% (2)	9	2 (1:8)	4 (1:16)	0	3
30% (5)	26	4 (1:4)	17 (1:32)	0	5
40% (3)	15	7 (1:16)	7 (1:16)	0	1
50% (3)	16	4 (1:16)	10 (1:16)	0	2
60% (6)	26	13 (1:16)	12 (1:32)	0	1
70% (14)	67	31 (1:8)	32 (1:32)	0	4
80% (16)	78	46 (1:2)	21 (1:32)	0	11
90% (7)	39	26 (1:4)	6 (1:16)	0	7
100% (5)	24	15 (1:64)	7 (1:32)	0	2
Total (64)	312	149	121	0	42

The lowest precipitin titer associated with each IVSR Category Row is Indicated in the positive precipitin/positive SRFA column. The highest precipitin titer associated with each IVSR category row is indicated in the positive precipitin/negative SRFA column.

TABLE II: CONTINGENCY TABLE DISTRIBUTION OF PRECIPITIN'S TITERS RELATED WITH THE NUMBER OF REPORTS OF POSITIVE AND NEGATIVE SYMPTOM-RELATED FOOD ALLERGENS (SRFA)

Precipitin's Titer	Number of Tests	Positive SRFA	Negative SRFA
Negative	42	0 (0,0 %)	42 (100,0 %)
1:1	3	0 (0,0 %)	3 (100,0 %)
1:2	8	1 (12,5 %)	7 (87,5 %)
1:4	36	3 (8,3 %)	33 (91,7 %)
1:8	51	6 (11,8 %)	45 (88,2 %)
1:16	33	11 (33,3 %)	22 (66,7 %)
1:32	26	15 (57,7 %)	11 (42,3%)
1:64	19	19 (100,0%)	0 (0,0 %)
1:128	94	94 (100,0 %)	0 (0,0 %)
TOTAL	312	149 (47,8%)	163 (52,2%)

IV. DISCUSSION

One of the main physiologic defenses against food allergy is the gastrointestinal digestion of food proteins [43], [44]. Any disturbance of the digestion processes (even a simple antacid or an alteration on mucosal microbiota) may constitute a predisposition factor to the immune sensitization against food antigens [45]-[47]. The main purpose of the gastrointestinal barriers is to block the undesirable entrance of immunoreactive food proteins into the bloodstream [48]. When foreign proteins gain access to the internal milieu, specific antibodies are produced to inactivate these proteins, allowing their clearing from circulation by the reticuloendothelial system [49]. When the allergic patient develops an IgE-mediated food allergy, it is relatively easy to prove a causal relationship between the specific allergen(s) and the clinical symptoms. Now a day, the medical community has access to very comprehensive reviews, algorithms, and guidelines to manage IgE-mediated food allergies [50], [51]. However, when the patient develops a non-IgE mediated hypersensitivity, such as in the case of the IAD, the demonstration of the causal agents is not an easy task. The contribution of food-specific IgG antibodies to the pathogenesis of food allergy is now a day a controversial field [52]-[54]. Before the association of IgE with immediate reactions, in 1967, the research of precipitins and soluble immune-complexes against food allergens were historically used to study allergy [55]-[58]. The association of the IgE to the reaginic reactions, somehow dodged the attention from the non-IgE mediated hypersensitivity [59]. Nevertheless, even after this insight, some colleagues were yet able to report and associate food-specific non-IgE mediated mechanisms to their patients' hypersensitivities [60], [61]. Our results, in similarity with these previous reports, also suggest that an excessive immune response produced against inappropriately blood-acquired undigested food allergens may participate in the production of IAD symptoms. The specific antibodies remarked by the precipitin's titration maybe not, necessarily, the etiologic agents responsible for the production of the IAD inflammation. They can simply be considered a marker of intestinal persorption of undigested food antigens and/or of occasional opportunistic microbial toxins that may add participation to the inflammatory reactions [62]-[64]. The assistant physician that read this paper must be assured that the research of food-specific precipitins is not here defined as a confirmatory assay able to diagnose, solely by itself, any kind of disease, intolerance, hypersensitivity, or allergy [65]. It just proposes the use of the precipitin's titration as a predictive screening pre-test to elaborate a justified

Elimination Diet as a starting point to initiate the already established golden-standard procedure to diagnose food allergy: the OFC. Here we report a preliminary feasibility study performed in real life, evaluating the subjective perception of a small group of patients with different characteristics and that were treated according to their particular symptoms and comorbidities. There was no control group and the unblinded patients may have been suggested by the results of the exams, focusing their attention on the appointed food allergens that had already been conjointly selected by anamnesis. More multi-center studies are needed, with double-blind placebo-controlled strategies, evaluating more food allergens, as well with a greater range of serum dilutions to better appreciated the equivalence zone between the antigen-excess/antibody-excess areas. Anyway, the titration of food-specific precipitins, prescribed according to the patient's perceptions and dietary habits, helped to provide a clean field to initiate the clinical management; added confidence and adherence to the elimination diet; as well showed a good correlation with the clinical diagnosis [66]. The study's design does not allow a strong level of evidence to take definitive conclusions but opens an avenue of investigation in the direction of the titration of precipitins, as an easy and unexpensive strategy to clarify the non-IgE mediated hypersensitivities reactions that haunt the IAD patients.

ABBREVIATIONS

AD: Atopic Dermatitis
IAD: Intrinsic Atopic Dermatitis
PED: Precipitins-based Elimination Diet
IVSR: Improvement Verbal Scale Rate (related with the PED)
SRFA: Symptom-Related Food Allergen (positive or negative)
OFC: Oral Food Challenge test

APPENDIX

Total data distribution table of 312 allergen-specific precipitation titers done with 64 IAD patients according to their improvement Verbal Scale Rate (IVSR) perception of the benefits of a Precipitin-based Elimination Diet (PED) and their positive or negative perception of the impairment of the symptoms after the reintroduction of each excluded Symptom-Related Food Allergen (SRFA)

Patient	Sex	Age	IVRS (%)	Food Allergen	Precipitin Titer	SRFA	Patient	Sex	Age	IVRS (%)	Food Allergen	Precipitin Titer	SRFA
AA	M	36	10	Egg's white	Negative	Negative	AS	M	39	70	Cow's milk	1:8	Positive
AA	M	36	10	Gluten	Negative	Negative	AS	M	39	70	Egg's white	1:128	Positive
AA	M	36	10	Latex	Negative	Negative	AS	M	39	70	Egg's yolk	1:128	Positive
AB	F	53	80	Cocoa	1:16	Negative	AS	M	39	70	Yeast	1:8	Positive
AB	F	53	80	Egg's white	1:16	Negative	AT	M	90	90	Cocoa	1:8	Negative
AB	F	53	80	Latex	1:32	Positive	AT	M	90	90	Cow's milk	1:128	Positive
AB	F	53	80	Peanut	1:128	Positive	AT	M	90	90	Gluten	1:128	Positive
AB	F	53	80	Swine meat	1:128	Positive	AT	M	90	90	Latex	1:128	Positive
AC	M	26	70	Cocoa	1:8	Negative	AT	M	90	90	Peanut	1:128	Positive
AC	M	26	70	Latex	1:8	Negative	AT	M	90	90	Swine meat	1:128	Positive
AC	M	26	70	Peanut	1:8	Negative	AV	F	49	20	Cocoa	1:8	Positive
AC	M	26	50	Swine meat	1:128	Positive	AV	F	49	20	Cow's milk	1:4	Negative
AC	M	54	50	Yeast	1:8	Negative	AV	F	49	20	Gluten	1:8	Negative
AD	M	54	50	Cow's milk	1:16	Positive	AV	F	49	20	Latex	1:16	Negative
AD	M	54	50	Egg's white	1:4	Negative	AV	F	49	20	Swine meat	Negative	Negative
AD	M	54	50	Latex	Negative	Negative	AZ	M	73	70	Cocoa	1:4	Negative
AD	M	54	50	Red pepper	1:1	Negative	AZ	M	73	70	Cow's milk	1:128	Positive
AD	M	54	50	Yeast	1:4	Negative	AZ	M	73	70	Latex	1:16	Negative
AL	F	58	60	Brazil nut	1:16	Positive	AZ	M	73	70	Peanut	1:128	Negative
AL	F	58	60	Latex	1:4	Negative	AZ	M	73	70	Swine meat	1:128	Positive
AL	F	58	60	Nuts	1:16	Positive	BS	F	31	70	Cocoa	1:8	Negative
AL	F	58	60	Peanut	1:4	Negative	BS	F	31	70	Cow's milk	1:128	Positive
AL	F	58	60	Pecan nuts	1:16	Positive	BS	F	31	70	Latex	1:8	Negative
AM	F	87	90	Bovine meat	1:64	Positive	BS	F	31	70	Peanut	1:128	Positive
AM	F	87	90	Chicken meat	1:4	Negative	BS	F	31	70	Soybean	1:8	Negative
AM	F	87	90	Cocoa	1:8	Negative	CC	F	63	90	Cocoa	1:128	Positive
AM	F	87	90	Cow's milk	Negative	Negative	CC	F	63	90	Cow's milk	1:128	Positive
AM	F	87	90	Egg's yolk	1:128	Positive	CC	F	63	90	Latex	1:128	Positive
AM	F	87	90	Gluten	Negative	Negative	CC	F	63	90	Peanut	1:128	Positive
AM	F	87	90	Latex	Negative	Negative	CC	F	63	90	Swine meat	1:128	Positive
AM	F	87	90	Swine meat	1:128	Positive	CF	F	63	80	Cow's milk	1:64	Positive
AN	F	84	70	Cocoa	1:4	Negative	CF	F	63	80	Egg's white	1:64	Positive
AN	F	84	70	Latex	1:2	Negative	CF	F	63	80	Gluten	1:32	Negative
AN	F	84	70	Peanut	1:16	Positive	CF	F	63	80	Latex	1:128	Positive
AN	F	84	70	Swine meat	1:128	Positive	CF	F	63	80	Swine meat	1:8	Negative
AN	F	84	70	Yeast	1:64	Positive	DD	M	61	60	Cocoa	1:16	Negative
AP	F	51	10	Cocoa	1:4	Negative	DD	M	61	60	Cow's milk	1:32	Negative
AP	F	51	10	Gluten	Negative	Negative	DD	M	61	60	Latex	1:8	Negative
AP	F	51	10	Latex	1:4	Negative	DD	M	61	60	Peanut	1:32	Positive
AP	F	51	10	Peanut	Negative	Negative	DD	M	61	60	Swine meat	1:32	Positive
AP	F	51	10	Swine meat	1:2	Negative	DL	F	73	80	Cocoa	1:4	Positive
AR	F	23	80	Bovine meat	1:16	Positive	DL	F	73	80	Cow's milk	1:128	Positive
AR	F	23	80	Garlic	1:128	Positive	DL	F	73	80	Gluten	1:64	Negative
AR	F	23	80	Garlic	1:128	Positive	DL	F	73	80	Latex	1:8	Positive
AR	F	23	80	Onion	1:8	Positive	DL	F	73	80	Peanut	1:16	Positive
AR	F	23	80	Red bean	1:8	Negative	CF	F	63	80	Cow's milk	1:64	
AS	M	39	70	Banana	1:8	Negative	CF	F	63	80	Egg's white	1:64	

Patient	Sex	Age	IVRS (%)	Food Allergen	Precipitin Titer	SRFA	Patient	Sex	Age	IVRS (%)	Food Allergen	Precipitin Titer	SRFA
CF	F	63	80	Gluten	1:32	Negative	GR	M	64	70	Egg's white	1:128	Positive
CF	F	63	80	Latex	1:128	Positive	GR	M	64	70	Latex	1:32	Negative
CF	F	63	80	Swine meat	1:8	Negative	GR	M	64	70	Peanut	1:32	Positive
DD	M	61	60	Cocoa	1:16	Negative	GR	M	64	30	Swine meat	1:128	Positive
DD	M	61	60	Cow's milk	1:32	Negative	IL	F	38	30	Cow's milk	Negative	Negative
DD	M	61	60	Latex	1:8	Negative	IL	F	38	30	Gluten	Negative	Negative
DD	M	61	60	Peanut	1:32	Positive	IL	F	38	30	Peanut	Negative	Negative
DD	M	61	60	Swine meat	1:32	Positive	IL	F	38	30	Swine meat	1:32	Positive
DL	F	73	80	Cocoa	1:4	Negative	IL	F	38	30	Yeast	1:4	Negative
DL	F	73	80	Cow's milk	1:128	Positive	IM	F	31	70	Cocoa	1:1	Negative
DL	F	73	80	Gluten	1:64	Positive	IM	F	31	70	Latex	1:2	Negative
DL	F	73	80	Latex	1:8	Positive	IM	F	31	70	Peanut	1:64	Positive
DL	F	73	80	Peanut	1:16	Negative	IM	F	31	70	Swine meat	1:128	Positive
EA	F	56	60	Chicken meat	1:16	Negative	IM	F	31	70	Yeast	Negative	Negative
EA	F	56	60	Cocoa	1:8	Negative	JF	M	70	60	Cow's milk	1:32	Positive
EA	F	56	60	Cow's milk	1:128	Positive	JF	M	70	60	Gluten	1:64	Positive
EA	F	56	60	Eggs's white	1:16	Negative	JF	M	70	60	Latex	1:2	Negative
EA	F	56	60	Swine meat	1:32	Positive	JM	F	24	30	Chicken meat	1:32	Positive
EF	F	42	10	Cow's milk	1:8	Negative	JM	F	24	30	Fish (Tilapia)	1:2	Negative
EF	F	42	10	Egg's white	1:4	Negative	JM	F	24	30	Gluten	Negative	Negative
EF	F	42	10	Latex	1:8	Positive	JM	F	24	30	Latex	1:8	Negative
EF	F	42	10	Orange	Negative	Negative	JM	F	24	30	Rice	1:4	Negative
EG	F	80	70	Latex	1:4	Negative	JM	F	24	30	Soybean	1:4	Positive
EG	F	80	70	Peanut	1:64	Positive	JO	F	32	80	Cocoa	1:128	Positive
EG	F	80	70	Swine meat	1:128	Positive	JO	F	32	80	Orange	1:8	Negative
EM	F	56	80	Cocoa	Negative	Negative	JO	F	32	80	Peanut	1:64	Positive
EM	F	56	80	Cow's milk	Negative	Negative	JO	F	32	80	Soybean	1:32	Positive
EM	F	56	80	Egg's white	Negative	Negative	JO	F	32	80	Swine meat	1:32	Positive
EM	F	56	80	Latex	1:128	Positive	JP	M	60	40	Bovine meat	1:1	Negative
EM	F	56	80	Orange	1:128	Positive	JP	M	60	40	Cocoa	1:8	Negative
EO	F	78	90	Cocoa	1:128	Positive	JP	M	60	40	Cow's milk	1:16	Negative
EO	F	78	90	Cow's milk	1:4	Negative	JP	M	60	40	Peanut	1:128	Positive
EO	F	78	90	Gluten	1:4	Negative	JP	M	60	40	Swine meat	1:32	Positive
EO	F	78	90	Latex	1:4	Positive	JR	F	31	90	Cocoa	1:16	Negative
EO	F	78	90	Peanut	1:128	Positive	JR	F	31	90	Latex	1:128	Positive
EO	F	78	90	Swine meat	1:128	Positive	JR	F	31	90	Peanut	1:128	Positive
ER	F	39	80	Cocoa	Negative	Negative	KC	M	24	80	Swine meat	1:128	Positive
ER	F	39	80	Latex	1:128	Positive	KC	M	24	80	Cow's milk	1:128	Positive
ER	F	39	80	Peanut	Negative	Negative	KC	M	24	80	Egg's white	1:4	Positive
ER	F	39	80	Swine meat	1:128	Positive	KC	M	24	80	Latex	1:16	Negative
ER	F	39	80	Yeast	1:8	Negative	KC	M	24	80	Peanut	1:128	Positive
ES	F	25	80	Cow's milk	1:64	Positive	KR	F	42	90	Yeast	1:2	Negative
ES	F	25	80	Latex	1:16	Positive	KR	F	42	90	Cocoa	Negative	Negative
ES	F	25	80	Peanut	1:128	Positive	KR	F	42	90	Cow's milk	1:128	Positive
ES	F	25	80	Swine meat	1:128	Positive	KR	F	42	90	Latex	1:128	Positive
ES	F	25	80	Yeast	1:2	Negative	KR	F	42	90	Peanut	1:128	Positive
GR	M	64	70	Cocoa	1:8	Negative	KR	F	42	90	Swine meat	1:128	Positive
GR	M	64	70	Cow's milk	1:128	Positive	LB	F	69	80	Cocoa	Negative	Negative

Patient	Sex	Age	IVRS (%)	Food Allergen	Precipitin Titer	SRFA	Patient	Sex	Age	IVRS (%)	Food Allergen	Precipitin Titer	SRFA
LB	F	69	80	Latex	1:128	Positive	MC	F	54	50	Peanut	1:8	Negative
LB	F	69	80	Peanut	1:16	Positive	MC	F	54	50	Swine meat	1:32	Positive
LB	F	69	80	Swine meat	1:64	Positive	MC	F	54	50	Yeast	1:4	Negative
LJ	M	36	30	Cocoa	1:16	Negative	MG	F	79	40	Cocoa	1:64	Positive
LJ	M	36	30	Gluten	1:32	Negative	MG	F	79	40	Cow's milk	1:8	Negative
LJ	M	36	30	Latex	1:8	Negative	MG	F	79	40	Gluten	1:16	Positive
LJ	M	36	30	Peanut	1:16	Negative	MG	F	79	40	Latex	1:16	Negative
LJ	M	36	30	Swine meat	1:16	Negative	MG	F	79	40	Swine meat	1:64	Positive
LP	F	35	20	Cocoa	Negative	Negative	MH	F	77	80	Cocoa	1:8	Negative
LP	F	35	20	Latex	1:4	Negative	MH	F	77	80	Orange	1:2	Positive
LP	F	35	20	Peanut	Negative	Negative	MH	F	77	80	Peanut	Negative	Negative
LP	F	35	20	Swine meat	1:32	Positive	MH	F	77	80	Swine meat	1:128	Positive
LT	F	23	30	Cocoa	1:4	Negative	MH	F	77	80	Yeast	1:32	Negative
LT	F	23	30	Latex	1:8	Negative	ML	F	73	60	Cow's milk	1:4	Negative
LT	F	23	30	Peanut	1:128	Positive	ML	F	73	60	Egg's white	1:64	Positive
LT	F	23	30	Swine meat	1:32	Negative	ML	F	73	60	Gluten	1:8	Negative
LT	F	23	30	Yeast	1:8	Negative	ML	F	73	60	Latex	1:16	Positive
MA	F	49	70	Cocoa	1:32	Negative	ML	F	73	60	Swine meat	1:16	Positive
MZ	F	58	100	Latex	Negative	Negative	MM	F	55	90	Egg's white	Negative	Negative
MZ	F	58	100	Peanut	1:128	Positive	MM	F	55	90	Latex	Negative	Negative
MZ	F	58	100	Soybean	1:128	Positive	MM	F	55	90	Peanut	Negative	Negative
MZ	F	58	100	Yeast	Negative	Negative	MM	F	55	90	Swine meat	1:128	Positive
NJ	M	50	80	Cocoa	1:128	Positive	MM	F	55	90	Yeast	1:128	Positive
NJ	M	50	80	Latex	1:128	Positive	MP	F	53	70	Chicken meat	1:8	Negative
NJ	M	50	80	Peanut	1:8	Negative	MP	F	53	70	Cow's milk	1:128	Positive
NJ	M	50	80	Swine meat	1:8	Negative	MP	F	53	70	Latex	1:16	Negative

NM	F	64	70	Cocoa	1:16	Negative	MP	F	53	70	Tomato	1:4	Negative
NM	F	64	70	Cow's milk	1:128	Positive	MR	F	56	100	Cocoa	1:32	Negative
NM	F	64	70	Latex	1:4	Negative	MR	F	56	100	Latex	1:4	Negative
NM	F	64	70	Peanut	1:128	Positive	MR	F	5	100	Peanut	1:32	Negative
NR	F	57	50	Cocoa	1:16	Negative	MR	F	56	100	Swine meat	1:128	Positive
NR	F	57	50	Cow's milk	1:8	Negative	MR	F	56	100	Yeast	1:4	Negative
NR	F	57	50	Latex	Negative	Negative	MT	F	36	80	Cocoa	1:32	Negative
NR	F	57	50	Swine meat	1:4	Negative	MT	F	36	80	Gluten	Negative	Negative
NR	F	57	50	Yeast	1:128	Positive	MT	F	36	80	Latex	1:8	Negative
OA	M	79	80	Banana	1:4	Negative	MT	F	36	80	Peanut	1:32	Positive
OA	M	79	80	Cow's milk	1:128	Positive	MT	F	36	80	Swine meat	1:128	Positive
OA	M	79	80	Orange	1:128	Positive	MV	F	30	100	Cocoa	1:16	Negative
OA	M	79	80	Peanut	1:128	Positive	MV	F	30	100	Latex	1:16	Negative
OA	M	79	80	Swine meat	1:128	Positive	MV	F	30	100	Peanut	1:128	Positive
MA	F	49	70	Latex	1:64	Positive	MV	F	30	100	Yeast	1:128	Positive
MA	F	49	70	Peanut	1:128	Positive	MZ	F	58	100	Cow's milk	1:128	Positive
MA	F	49	70	Shrimp	1:64	Positive	PR	F	20	100	Cocoa	1:8	Negative
MA	F	49	70	Yeast	1:128	Positive	PR	M	20	100	Cow's milk	1:128	Positive
MC	F	54	50	Cocoa	1:4	Negative	PR	M	20	100	Gluten	1:128	Positive
MC	F	54	50	Cow's milk	1:64	Positive	PR	M	20	100	Latex	1:128	Positive
MC	F	54	50	Latex	1:16	Negative	PR	M	20	100	Peanut	1:128	Positive

Patient	Sex	Age	IVRS (%)	Food Allergen	Precipitin Titer	SRFA	Patient	Sex	Age	IVRS (%)	Food Allergen	Precipitin Titer	SRFA
PR	M	20	100	Swine meat	1:128	Positive	TM	F	87	80	Swine meat	1:128	Positive
RL	F	32	70	Cocoa	1:18	Negative	TP	F	62	40	Cow's milk	Negative	Negative
RL	F	32	70	Cow's milk	1:128	Positive	TP	F	62	40	Latex	1:4	Negative
RL	F	32	70	Latex	1:4	Negative	TP	F	62	40	Peanut	1:128	Positive
RL	F	32	70	Peanut	Negative	Negative	TP	F	62	40	Swine meat	1:64	Positive
RL	F	32	70	Swine meat	1:8	Negative	TP	F	62	40	Yeast	1:8	Negative
RM	F	53	100	Cocoa	1:64	Positive	WS	M	57	80	Banana	1:128	Positive
RM	F	53	100	Cow's milk	1:128	Positive	WS	M	57	80	Cocoa	Negative	Negative
RM	F	53	100	Gluten	1:128	Positive	WS	M	57	80	Cocoa	Negative	Negative
RM	F	53	100	Latex	1:128	Positive	WS	M	57	80	Latex	Negative	Negative
SF	F	47	30	Cocoa	1:4	Negative	WS	M	57	80	Peanut	1:8	Negative
SF	F	47	30	Cow's milk	1:32	Negative	WV	M	59	70	Cow's milk	1:128	Positive
SF	F	47	30	Gluten	1:8	Negative	WV	M	59	70	Gluten	Negative	Negative
SF	F	47	30	Latex	Negative	Negative	WV	M	59	70	Latex	1:8	Negative
SF	F	47	30	Peanut	1:4	Negative	WV	M	59	70	Peanut	1:8	Negative
TA	F	32	60	Egg's white	Negative	Negative	WV	M	59	70	Yeast	1:8	Negative
TA	F	32	60	Gluten	1:128	Positive	ZP	F	85	70	Cocoa	1:4	Negative
TA	F	32	60	Latex	1:8	Negative	ZP	F	85	70	Gluten	Negative	Negative
TM	F	87	80	Cocoa	1:16	Negative	ZP	F	85	70	Latex	1:8	Negative
TM	F	87	80	Cow's milk	1:128	Positive	ZP	F	85	70	Swine meat	1:128	Positive
TM	F	87	80	Latex	1:32	Positive	ZP	F	85	70	Yeast	1:8	Negative
TM	F	87	80	Peanut	1:128	Positive							

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