



# Exploring the Role of Leukocyte Adherence Inhibition Test in Assessing Non-IgE Mediated Immunoreactivity to Benzoic Acid in Allergic Patients

Celso Eduardo Olivier <sup>a\*</sup>, Daiana Guedes Pinto <sup>a</sup>,  
Ana Paula Monezzi Teixeira <sup>a</sup>,  
Jhéssica Letícia Santos Santana <sup>a</sup>,  
Raquel Acácia Pereira Gonçalves Santos <sup>a</sup>,  
and Regiane Patussi Santos Lima <sup>b</sup>

<sup>a</sup> Instituto Alergoimuno de Americana, Brazil.

<sup>b</sup> Lavoisier's laboratories, São Paulo, Brazil.

## Authors' contributions

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

## Article Information

DOI: <https://doi.org/10.9734/aji/2024/v7i1130>

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/115657>

**Original Research Article**

**Received: 02/04/2024**

**Accepted: 13/04/2024**

**Published: 17/04/2024**

## ABSTRACT

**Background:** Several publications report benzoic acid as responsible for non-IgE-mediated allergic reactions. No standardized lab exam identifies these reactions besides *in vivo* provocation tests.

**Aim:** To evaluate the potential of the Leukocyte Adherence Inhibition Test (LAIT) to discriminate non-IgE-mediated immunoreactivity against benzoic acid in patients with non-IgE-mediated allergic phenotypes.

\*Corresponding author: E-mail: [celso@alergoimuno.med.br](mailto:celso@alergoimuno.med.br);

**Study Design:** We retrospectively examined the medical charts of 100 patients diagnosed with allergic rhinitis, allergic bronchitis, asthma, sinus headache, atopic dermatitis, and/or urticaria with clinical suspicion of non-IgE-mediated benzoic acid hypersensitivity who were investigated with *ex vivo* challenge test monitored by LAIT against benzoic acid. The cohort counted 30 males; the mean age was 41.9 years, the SD was 20.4 years, and the range was 2 to 90 years.

**Place and Duration of Study:** Instituto Alergoimuno de Americana – São Paulo – Brazil – between January 2018 and March 2024.

**Methodology:** The percentage of Leukocyte Adherence Inhibition (LAI) promoted by the *ex vivo* challenges against 1 mg/mL benzoic acid was distributed in ranges through a cascade distribution chart to outline the variability of the results.

**Results:** The LAI ranged from 0% to 97%; the Mean was 41.1%; the Median was 40.5%; the Standard Deviation was 24.2%; the Mode was 0 and 59 (each appeared four times). The cascade distribution demonstrates a wide distribution of LAI results. This extensive distribution of LAI results suggests that some patients had mild, moderate, or severe non-IgE-mediated immunoreactivity against benzoic acid, while others did not present any immunoreactivity against it.

**Conclusion:** Our preliminary results support that the LAIT performed with benzoic acid may discriminate diverse degrees of *ex vivo* immunoreactivity in patients suffering from diversified allergic phenotypes. It is worth carrying out more in-depth studies to evaluate the usefulness of LAIT in diagnosing non-IgE-mediated benzoic acid allergies.

**Keywords:** Allergy; asthma; atopic dermatitis; bronchitis; diagnosis; exposome-wide association study; hypersensitivity; leukocyte adherence inhibition test; non-ige-mediated immunoreactivity; precision medicine; rhinitis; sinus headache; urticaria.

## ABBREVIATIONS

LAI : Leukocyte Adherence Inhibition

LAIT : Leukocyte Adherence Inhibition Test

## 1. INTRODUCTION

Benzoic acid is an organic compound formed by an aromatic ring and a carboxyl group [1]. Benzoic acid is a parent and a metabolic common pathway of a large group of structurally related substances (aromatic salts, alcohols, aldehydes, esters, and acetals) legally regulated to be used as additives to industrialized foods, cosmetics, and medicaments [2]. Used as an antimicrobial, the legally acceptable daily intake uppermost limit for benzoic acid (or the benzyl/benzoic moiety) is 5.0 mg/kg body weight [3]. "There is evidence that benzyl benzoate is hydrolyzed to benzyl alcohol and benzoic acid; as well, benzyl alcohol and benzaldehyde suffer *in vivo* oxidation to benzoic acid" [4]. "In plants and animals, benzoic acid is produced endogenously through the phenylalanine-tyrosine pathway" [5]. "Several of its derivatives occur naturally in foods, such as fruits (apple, avocado, blackberry, blueberry, cherry, cranberry, melon, papaya, plum, raspberry, strawberry, tomato), vegetables (artichokes, asparagus, beans, cabbage, corn, leek, mushroom, potatoes), meats (beef, chicken, pork, shellfish), cheeses, teas and wines" [6].

Food additives (among them benzoic acid) have long been described as sensitizer agents responsible for human allergic reactions [7]. A double-blinded provocation study done with benzoic acid produced objective reactions in 7% of patients with urticaria [8]. There is a report of a child who developed chronic cheilitis when ingesting daily benzoates-preserved industrialized foods [9]. There are reports of asthmatic patients who had a crisis of bronchospasm after the intake of benzoate-containing antiasthmatic medicines [10]. Reports of cross-reactivity among benzoates, azo dyes, and aspirin in patients with urticaria are also common [11].

Most provocation tests performed with benzoic acid do not elicit immediate reactions; instead, the reactions appear within 14 hours after the challenge [12]. The immunoreactivity elicited against benzoic acid is non-IgE-mediated and is not yet acknowledged explicitly among the recently classified hypersensitivity mechanisms [13]. The main knowledge about the mechanism of hypersensitivity against benzoate (and similar food additives) was brought by an *ex vivo* leukocyte challenge test determining the

increasing sulfidoleukotriene production [14]. Since leukotrienes are known mediators of the leukocyte adherence inhibition phenomenon, we hypothesize that the employ of the Leukocyte Adherence Inhibition Test (LAIT) could help identify the endotype responsible for benzoic acid hypersensitivity [15-17].

“We routinely employ the LAIT in our facilities to evaluate non-IgE-mediated immunoreactivity against suspected allergens, previously engaging in exhaustive provocation tests” [18-24]. To evaluate the potential of the LAIT to discriminate non-IgE-mediated immunoreactivity against benzoic acid, we retrospectively compiled the electronic medical charts of patients with non-IgE-mediated allergic rhinitis, allergic bronchitis, asthma, sinus headache, atopic dermatitis, and/or urticaria who were investigated with this procedure.

The present study hypothesizes that the LAIT may differentiate diverse degrees of immunoreactivity against benzoic acid among patients suffering from allergic phenotypes.

## 2. MATERIALS AND METHODS

### 2.1 Subjects

After receiving Institutional Review Board approval from the Instituto Alergoimuno de Americana (Brazil; 03/2024), we proceeded with the electronic chart review of 8,500 outpatients who attended our facility from January 2018 to March 2024. A cohort of 100 outside patients had been submitted to an *ex vivo* allergen challenge test with benzoic acid 1mg/mL monitored with LAIT for presenting non-IgE-mediated allergic rhinitis, allergic bronchitis, asthma, sinus headache, atopic dermatitis, and/or urticaria.

This study did not include pregnant women, breastfeeding, and patients under biological and/or systemic anti-inflammatory therapy (corticoids, cyclosporin). “The cohort counted 30 males; mean age 41.9 years; SD 20.4 years; range 2 to 90 years; median 43 years; modes = 26; 28; 43; 43 and 53 (each appeared four times); geometric mean = 34.4 years. This procedure was offered to patients with clinical suspicion of benzoate hypersensitivity who demonstrated a non-reactive or inconclusive skin test against benzoic acid” [25].

### 2.2 *Ex vivo* Investigation: Leukocyte Adherence Inhibition Test

“We performed the LAIT as previously described” [26-34]. Shortly, each donor's fresh plasma was divided into two parts and used in paralleled *ex vivo* challenging tests with benzoic acid 1 mg/mL and the unchallenged plasma assay. We collected the plasma with high leukocyte content (buffy coat) from the heparinized tube after one hour of sedimentation at 37 °C. Then we distributed aliquots of 100 µL into Eppendorf tubes kept under agitation for 30 minutes (200 rpm at 37 °C) with benzoic acid (10µL of a solution with 1mg/mL and pH 7.5) or without benzoic acid (when used as control). After incubation, the plasma was allocated into a standard Neubauer hemocytometer counting chamber with a plain, non-metallic glass surface and left to stand for 2 hours at 37 °C in the humidified atmosphere of the covered water bath to allow leukocytes to adhere to the glass. Next, we counted the leukocytes, removed the coverslip, and washed the chamber by immersion in a beaker with PBS at 37 °C. Then, we added a drop of PBS to the hemocytometer's chamber and allocated a clean coverslip over it. The remaining cells were counted in the same squares as previously examined. The percentage of Leukocyte Adherence (LA) of each assay was estimated as: (the number of leukocytes observed on the hemocytometry chamber after washing divided by the number of leukocytes observed on the hemocytometry chamber before washing) and multiplied by 100 (%). The Leukocyte Adherence Ratio (LAR) was estimated based on the ratio between the LA from the antigen-specific challenged plasma and the LA from the unchallenged control plasma:  $LAR = LA \text{ of the challenged sample} / LA \text{ of unchallenged control plasma} \times 100$  (%). To further calculate the Leukocyte Adherence Inhibition (LAI), we subtracted the LAR from 100 (%). We employed the LAI results for the cascade distribution chart and the statistics calculations, both performed with the help of the Microsoft Excel® statistical package.

## 3. RESULTS

As a retrospective survey, there was no research protocol; therefore, we report the incidentally immune investigation as registered in the digital medical charts. The LAI ranged from 0% to 97%; the Mean was 41.1%; the Median was 40.5%; the Standard Deviation was 24.2%; the Mode was 0 and 59 (each appeared four times).

The cascade distribution demonstrates a wide range of distribution of LAI results (Fig.1). Four patients ignored the presence of the allergen on the plasma and presented no inhibition of leukocyte adherence (LAI = 0%) after contact with benzoic acid (4% of the tests). Some patients showed low or moderate immunoreactivity during the *ex vivo* challenge test. In contrast, others displayed strong immunoreactivity, which could possibly reflect the participation of benzoic acid in a theoretical non-IgE-mediated hypersensitivity condition to be further corroborated by *in vivo* provocation tests.

#### 4. DISCUSSION

The non-IgE-mediated hypersensitivities are characterized by a challenging technical diagnosis due to the lack of standardized immunoassays [35]. Diagnosing these conditions among allergic patients is based on individual laborious medical work accomplishing anamnesis, cutaneous tests, and challenge *in vivo* provocation tests performed after meticulous exclusion diets [36].

“As an increaser of the sulfidoleukotriene production, hypersensitivity to benzoic acid may be, in a certain way, similar (and potentially an enhancer) of the hypersensitivity produced by non-steroidal anti-inflammatory drugs (NSAIDs).

The principal pharmacological action of NSAIDs is the inhibition of the cyclooxygenase enzymes, which catalyze the synthesis of prostaglandins and thromboxanes” [37]. “Cyclooxygenase enzymes catalyze the conversion of arachidonic acid released from the cellular membrane by cytosolic phospholipases activated by nociceptive mechanisms” [38]. “The cyclooxygenases and the lipoxygenases oxidize the arachidonic acid liberated into the cytosol. The cyclooxygenases pathway generates pro-inflammatory autacoids such as prostaglandins and thromboxanes. The lipoxygenase pathway generates leukotrienes” [39]. The pharmacologic inhibition of the cyclooxygenases increases the lipoxygenases’ activity, increasing the leukotrienes’ production. Any substance that (pharmacologically or immunologically) increases the production of leukotrienes affects the autacid balance, adversely producing allergic symptoms.

The LAIT theoretically explores every immune pathway as an *ex vivo* challenge test with a viable leukocyte buffy coat, allowing the interaction of all immune-circulating participants [40]. However, as an observant of the final phenomenon, the LAIT did not indicate which pathways were involved in inhibiting the adherence (or increasing the production

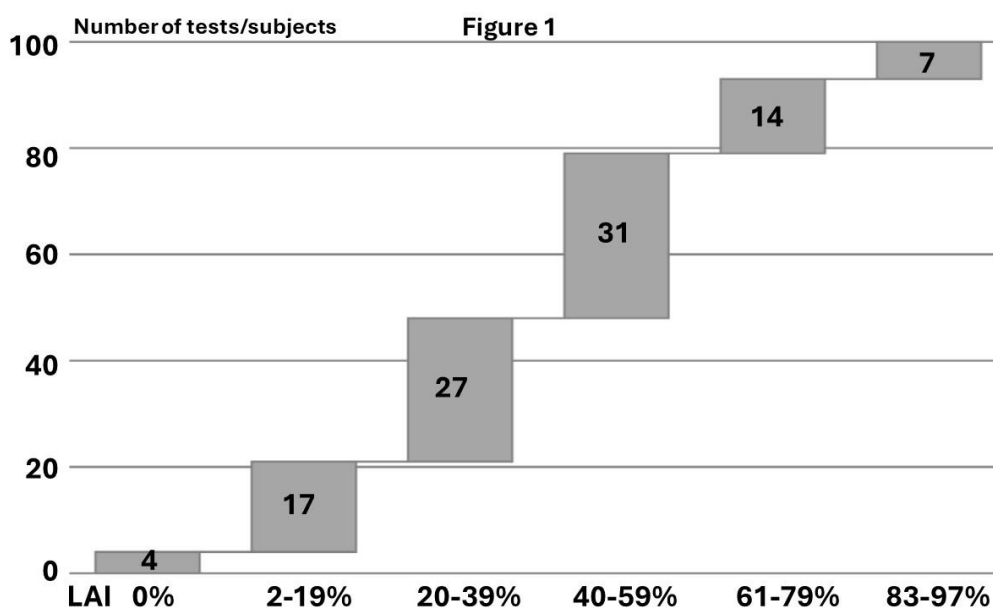


Fig. 1. Cascade distribution chart of the range groups of Leukocyte Adherence Inhibition (LAI) results (x-axis %) of *ex vivo* benzoic acid challenges monitored by the Leukocyte Adherence Inhibition Test (LAIT), according to the respective number of outcomes over 100 tests/subjects (y-axis)

of leukotrienes), whether pharmacological or immunological [41-44]. As proposed by the exposome-wide association study, the LAIT also configures itself as an exposome measurement, qualifying itself as an immune marker of the contact and the response to a specific antigen instead of being associated with a specific phenotype [45].

This preliminary retrospective survey demonstrated an extensive range of results from the *ex vivo* challenge test with benzoic acid monitored by LAIT in a cohort of patients with various allergic phenotypes. We routinely employ the LAIT as a complementary triage test to select worthwhile antigens to proceed with more laborious *in vivo* provocation tests when the specific IgE is undetectable. None of our patients presented an exclusive reaction to benzoic acid. Every patient was simultaneously tested with protein allergens (mites, fungi, food allergens), demonstrating positive results for some of them [46]. Our results may suggest that allergic patients may impair their symptoms by a pharmacological or an immune additional action of benzoic acid over the hypersensitivity response.

## 5. CONCLUSIONS

Our preliminary results show that the LAIT may differentiate diverse degrees of *ex vivo* immunoreactivity against benzoic acid in patients clinically diagnosed with non-IgE-mediated allergies. The propaedeutic meaning of these results, however, must be established. More studies with prospective larger double-blind cohorts need to evaluate the potential contribution of LAIT for the etiologic diagnosis of patients suspected of symptomatic hypersensitivity against benzoic acid and other similar food, cosmetic, and pharmaceutical additives.

## 6. LIMITATIONS

This study is a retrospective analysis of data collected over six years. There was no protocol research, and the subject's data were limited to the essentials available on our electronic sheets. The number of subjects is appropriate for a preliminary study; however, future studies must be more comprehensive. The lack of a research protocol implies the possibility of a bias produced by the point of view of the physician who indicated the exam (CEO) based on a clinical suspicion led purely by the anamnesis. The study

lost many of these patients to follow-up, so assessing the relationship between LAIT outcome and the patient's subsequent clinical outcome was impossible.

## CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES

1. Nair B. Final report on the safety assessment of Benzyl Alcohol, Benzoic Acid, and Sodium Benzoate. *Int J Toxicol.* 2001;20(suppl 3):23-50.
2. Adams TB, Cohen SM, Doull J, Feron VJ, Goodman JI, Marnett LJ. Et al. The FEMA GRAS assessment of benzyl derivatives used as flavor ingredients. *Food Chem Toxicol.* 2005;43(8):1207-40.
3. FAO-WHO Expert Committee. Toxicological evaluation of certain food additives with a review of general principles and of specifications. Seventeenth report of the joint FAO-WHO Expert Committee on Food Additives. World Health Organization technical report series. 1974;539:1-40.
4. FAO-WHO Expert Committee. Evaluation of certain food additives. Twenty-third Report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization technical report series. 1980;648:1-45.
5. Jia W, Wang X, Shi L. Endogenous benzoic acid interferes with the signatures of amino acids and thiol compounds through perturbing N-methyltransferase, glutamate-cysteine ligase, and glutathione S-transferase activity in dairy products.

- Food Res Int (Ottawa, Ont.) 2022; 161:111857.
6. Boelens MH, Visscher CA, Willemsens LC, Maarse H. Volatile compounds in food: qualitative and quantitative data. 6. Ed. Zeist Food Analysis Institute. Zeist. 1989.
  7. Simon RA. Adverse reactions to drug additives. J Allergy Clin Immunol. 1984; 74(4 Pt 2):623-30.
  8. Lahti A, Hannuksela M. Is benzoic acid really harmful in cases of atopy and urticaria? Lancet. 1981;2(8254):1055.
  9. Jacob SE, Hill H, Lucero H, Nedorost S. Benzoate Allergy in Children - From Foods to Personal Hygiene Products. Pediatr Dermatol. 2016;33(2):213-5.
  10. Balatsinou L, Di Gioacchino G, Sabatino G, Cavallucci E, Caruso R, Gabriele E. Asthma worsened by benzoate contained in some antiasthmatic drugs. Int J Immunopathol Pharmacol. 2004;17(2):225-6.
  11. Ros AM, Juhlin L, Michaëlsson G. A follow-up study of patients with recurrent urticaria and hypersensitivity to aspirin, benzoates and azo dyes. Br J Dermatol. 1976;95 (1):19-24.
  12. Michaëlsson, G.; Juhlin, L., Urticaria induced by preservatives and dye additives in food and drugs. Br J Dermatol. 1973;88 (6), 525-32.
  13. Jutel M, Agache I, Zemelka-Wiacek M, Akdis M, Chivato, T, del Giacco S, et al. Nomenclature of allergic diseases and hypersensitivity reactions: Adapted to modern needs: An EAACI position paper. Allergy. 2023;78(11):2851-2874.
  14. Worm M, Vieth W, Ehlers I, Sterry W, Zuberbier T. Increased leukotriene production by food additives in patients with atopic dermatitis and proven food intolerance. Clin Exp Allergy. 2001;31(2): 265-273.
  15. Fink A, Bibi H, Eliraz A, Tabachnik E, Bentwich Z. Leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>) confer glass non-adherence on leukocytes of asthmatic individuals. Dependency on cyclooxygenase products and calcium ion. Immunol Lett. 1985;10(6):319-23.
  16. Fink A, Bibi H, Eliraz A, Schlesinger M, Bentwich Z. Ketotifen, disodium cromoglycate, and verapamil inhibit leukotriene activity: determination by tube leukocyte adherence inhibition assay. Ann Allergy. 1986;57(2):103-6.
  17. Fink A, Shahin R, Eliraz A, Bibi H, Berkenstadt H, Levin S, et al. Interferon modulates the leukotriene C<sub>4</sub>-induced non-adherence properties of leukocytes: acquisition of an asthmatic phenotype. Immunol Lett. 1985;10(3-4):159-63.
  18. Kuratsuji T. Studies on leukocyte adherence inhibition test. Part II. Clinical applications of LAI test to detect delayed type hypersensitivity in infants and children. Keio J Med. 1981;30(2):65-9.
  19. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Evaluating Non-IgE-mediated Allergens' Immunoreactivity in Patients with "Intrinsic" Persistent Rhinitis with Help of the Leukocyte Adherence Inhibition Test. Eur J Med Health Sci. 2023;5(1):17-22.
  20. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Evaluating Non-IgE-Mediated Allergens' Immunoreactivity in Patients Formerly Classified as "Intrinsic" Asthmatics with Help of the Leukocyte Adherence Inhibition Test. Eur J Clin Med. 2023;4(2):1-7.
  21. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Innate Non-IgE-mediated Immunoreactivity against *Alternaria alternata*. Asian J Immunol. 2023;6(1):243-251.
  22. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Innate Non-IgE-mediated Immunoreactivity against *Saccharomyces cerevisiae*. Asian J Immunol. 2023;6(1):185-195.
  23. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Non-IgE-mediated Immunoreactivity against *Candida albicans* in Patients with Atopic Dermatitis. Asian J Immunol. 2023;6(1):268-276.
  24. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test in Diagnosing Non-IgE-Mediated Immunoreactivity against *Aspergillus fumigatus* in Patients with Allergic Rhinitis and Asthma. Asian J Immunol. 2024;7(1):12-20.

25. Olivier CE, Argentão DGP, Santos RAPG, Silva MD, Lima RPS, Zollner RL. Skin scrape test: an inexpensive and painless skin test for recognition of immediate hypersensitivity in children and adults. *Open Allergy J.* 2013;6:9-17.
26. Olivier CE, Lima RPS, Pinto DG, Santos RAPG, Silva GKM, Lorena SLS, et al. In search of a tolerance-induction strategy for cow's milk allergies: significant reduction of beta-lactoglobulin allergenicity via transglutaminase/cysteine polymerization. *Clinics.* 2012;67(10):1171-1179.
27. Olivier CE, Santos RAPG, Lima RPS, Argentão DGP, Silva GKM, Silva MD. A Novel Utility for an Old Method: The Leukocyte Adherence Inhibition Test Is an Easy Way to Detect the Immunoreactive Interference of the Collection Tube Anticoagulant on Cellular Immunoassays. *J Cell Adhesion.* 2014:1-6 Article ID 860427 Available:<http://dx.doi.org/10.1155/2014/860427>
28. Olivier CE, Pinto DG, Lima RPS, Silva MD, Santos RAPG, Teixeira, et al. Assessment of Immunoreactivity against Therapeutic Options Employing the Leukocyte Adherence Inhibition Test as a Tool for Precision Medicine. *Eur J Clin Med.* 2021;2(3):40-45.
29. Olivier CE, Pinto DG, Santos RAPG, Lima RPS. Dextran's interference over the Leukocyte Adherence Inhibition Test. *Academia Letter* 2021, Article (number), 3792.
30. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS. Immunoreactivity against Dermatophagoides pteronyssinus Assessed by the Leukocyte Adherence Inhibition Test in Patients with Intrinsic Atopic Dermatitis and Correlated "Intrinsic" Non-IgE-mediated Allergic Conditions. *Eur J Clin Med.* 2021;2(6):45-50.
31. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test to the Evaluation of Cellular Immunoreactivity against Latex-Fruit-Pollen Syndrome in Allergic Candidates to Exclusion Diets and Allergic Desensitization. *Eur J Clin Med.* 2022;3(1):11-17.
32. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test for the evaluation of immunoreactivity against gluten extracts in non-IgE-mediated / non-autoimmune Gluten-Related Disorders. *Eur J Clin Med.* 2022;3(2):1-7.
33. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Leukocyte Adherence Inhibition Test to the Assessment of Immunoreactivity Against Cow's Milk Proteins in Non-IgE-Mediated Gastrointestinal Food Allergy. *Eur J Clin Med.* 2022;3(2):38-43.
34. Olivier CE, Pinto DG, Teixeira APM, Santana JLS, Santos RAPGS, Lima RPS, et al. Contribution of the Leukocyte Adherence Inhibition Test to the Diagnosis of Immunoreactivity against Cobalt. *Asian J Immunol.* 2023;6(1):174-184.
35. Venter C, Vieira MC, Fleischer D. Tolerance development in non-IgE mediated food allergies: lessons from Brazil. *Jornal de Pediatria.* 2023;2(30):1-4.
36. Asero R. Sodium benzoate-induced pruritus. *Allergy.* 2006;61(10):1240-1.
37. Smith WL, Garavito RM, DeWitt DL. Prostaglandin Endoperoxide H Synthases (Cyclooxygenases) 1 and 2. *J Biol Chem.* 1996;271(52):33157-33160.
38. Shimizu T. Lipid Mediators in Health and Disease: Enzymes and Receptors as Therapeutic Targets for the Regulation of Immunity and Inflammation. *Annual Rev Pharmacol Toxicol.* 2009;49(1):123-150.
39. Liu M, Yokomizo T. The role of leukotrienes in allergic diseases. *Allergol Int.* 2015;64(1):17-26.
40. Olivier CE, Lima RPS, Pinto DG, Santos RAPG. The Plasma Preincubation with Papain Before the Assay Suggests that a Gell and Coombs Type II Reaction is Been Demonstrated by the Leukocyte Adherence Inhibition Test. *Biom J Sci Tech Res.* 2021;36(3):28647-28655.
41. Thomson DMP. Assessment of immune status by the leukocyte adherence inhibition test. Academic Press: New York. 1982:380p.
42. Tong AW, Burger DR, Finke P, Barney C, Vandembark AA, Vetto RM. Assessment of the mechanism of the leukocyte adherence inhibition test. *Cancer Res.* 1979;39(2Pt2):597-603.
43. Fink A, Heller L, Eliraz A, Weisman Z, Miskin A, Schlezinger M, et al. Allergen-specific leukocyte adherence inhibition (LAI) assay: sensitivity, specificity and

- mechanism. Immunol Lett. 1987;16(1):65-70.
44. Halliday WJ, Maluish A, Miller S. Blocking and unblocking of cell-mediated anti-tumor immunity in mice, as detected by the leucocyte adherence inhibition test. Cell Immunol. 1974;10(3):467-475.
45. Chung MK, House JS, Akhtari FS, Makris KC, Langston MA Islam, et al. Decoding the exposome: data science methodologies and implications in exposome-wide association studies (ExWASs). Exposome. 2024;4(1):osae001.
46. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<https://www.sdiarticle5.com/review-history/115657>