GEORGIAN MEDICAL NEWS

ISSN 1512-0112

No 6 (327) Январь 2022

ТБИЛИСИ - NEW YORK

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

No 6 (327) 2022

Published in cooperation with and under the patronage
of the Tbilisi State Medical University

Издается в сотрудничестве и под патронажем
Тбилисского государственного медицинского университета

გამოიცემა თბილისის სახელმწიფო სამედიცინო უნივერსიტეტთან
თანამშრომლობითა და მისი პატრონაჟით

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.) since 1994. **GMN** carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией и Международной академией наук, образования, искусств и естествознания (IASEIA) США с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения.

Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

МЕДИЦИНСКИЕ НОВОСТИ ГРУЗИИ

Ежемесячный совместный грузино-американский научный электронно-печатный журнал
Агентства медицинской информации Ассоциации деловой прессы Грузии,
Международной академии наук, индустрии, образования и искусств США.
Издается с 1994 г., распространяется в СНГ, ЕС и США

Website:
www.geomednews.org

The International Academy of Sciences, Education, Industry & Arts. P.O.Box 390177,
Mountain View, CA, 94039-0177, USA. Tel/Fax: (650) 967-4733

Версия: печатная. **Цена:** свободная.
Условия подписки: подписка принимается на 6 и 12 месяцев.
По вопросам подписки обращаться по тел.: 293 66 78.
Контактный адрес: Грузия, 0177, Тбилиси, ул. Асатиани 7, IV этаж, комната 408
тел.: 995(32) 254 24 91, 5(55) 75 65 99
Fax: +995(32) 253 70 58, e-mail: ninomikaber@geomednews.com; nikopir@geomednews.com

По вопросам размещения рекламы обращаться по тел.: 5(99) 97 95 93

GEORGIAN MEDICAL NEWS
Monthly Georgia-US joint scientific journal published both in electronic and paper formats of the Agency of Medical Information of the Georgian Association of Business Press; International Academy of Sciences, Education, Industry and Arts (USA).
Published since 1994. Distributed in NIS, EU and USA.

CONTACT ADDRESS IN TBILISI

GMN Editorial Board
7 Asatiani Street, 4th Floor
Tbilisi, Georgia 0177

Phone: 995 (32) 254-24-91
995 (32) 253-70-58
Fax: 995 (32) 253-70-58

CONTACT ADDRESS IN NEW YORK

NINITEX INTERNATIONAL, INC.
3 PINE DRIVE SOUTH
ROSLYN, NY 11576 U.S.A.

Phone: +1 (917) 327-7732

WEBSITE
www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

GEORGIAN MEDICAL NEWS
No 6 (327) 2022

Содержание:

GEORGIAN MEDICAL NEWS
No 6 (327) 2022

STATUS OF THE COMPOSITION OF ALLERGENIC EXTRACTS FOR SKIN TESTING IN UKRAINE AND THE WAYS TO OPTIMIZE IT

Gogunskaya I.V[1]., Zaikov S.V[1]., Tkhorovskyi M.A[2]., Plykanchuk O.V[2]., Bogomolov A.Ye[2].

[1]State institution, O. S. Kolomiychenko Institute of otolaryngology of National Academy of Medical Sciences of Ukraine; [2]Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine; [3]National Pirogov memorial medical university, Vinnytsya, Ukraine.

Abstract. Objective was to analyze the protein components of extracts of diagnostic allergens made in Ukraine in order to determine the next steps for their standardization.

Materials and methods. Electrophoresis in polyacrylamide gel was used to obtain electropherograms of Acarus siro, Dermatophagoides farinae, Dermatophagoides pteronyssinus, Alnus glutinosa, Betula pendula, Carpinus betulus, Ambrosia artemisiifolia, Artemisia absinthium, Dactylis glomerata, Festuca pratensis, Helianthus annuus, Lolium perenle, Poa pratensis, Secale cereale, Zea mays, cat hair, dog hair, sheep allergens, and allergen of feather pillows. The official site for the systematic nomenclature of allergens approved by the World Health Organization and the International Union of Immunological Societies was used to identify allergenic components.

Results and discussion. Analysis of the data shows that 14 out of 20 (70%) of our studied allergen extracts contain ballast non-allergenic protein. In some cases (for example, for mite allergens, ragweed), this non-allergenic protein is able to mask the major protein components of allergens. For most of the studied extracts, the major components were clearly detected. Most of the studied extracts, in addition to major, also contained minor components, forming a "cocktail" of each individual allergen. However, as we mentioned earlier, non-allergenic ballast proteins can be a serious problem in the existing scheme of standardization of extracts by protein units of nitrogen, because such standardization actually calculates the total amount of protein per unit volume of extract.

Key words. Skin prick testing, allergen extract, standardization.

Introduction. The prevalence of allergic diseases (AD) is growing rapidly worldwide and is one of the main concerns of world health professionals, as well as the subject of study in various regional and international epidemiological, immunological and clinical studies. More and more public and official organizations are drawing the attention of governments to the situation with allergopathology, pointing out the problems with the diagnosis and treatment of people with AD and predicting the estimated number of such people at one billion [WHO, EAACI, WAO, 2018].

Skin prick test (SPT) is a simple and reliable method of specific diagnosis in patients with allergic rhinoconjunctivitis, asthma, urticaria, anaphylaxis, atopic eczema (dermatitis) and suspected Ig-mediated food and drug allergies [1-4]. A significant disadvantage of SPT in routine clinical practice is the quality and standardization of extracts. The fact that the exact standardization of extracts is of great importance for their quality has led manufacturers to implement their own standardization protocols. Each company uses its own reference standard and unique methods to determine the quality of the extract. The above approaches lead to different component composition of allergen extracts from different manufacturers and inability to compare different products and test results [1,5,6]. However, since the most of the major components of the allergens have been identified over the last few decades, the currently implemented concept is to quantify the major components in each of the allergenic extracts.

The main aim of the study was to analyze the protein components of extracts of diagnostic allergens made in Ukraine in order to determine the next steps for their standardization.

Materials and methods. Electrophoresis in polyacrylamide gel was used to obtain electrophoregrams of the following allergens: mites Acarus siro, Dermatophagoides farinae, Dermatophagoides pteronyssinus, tree pollen - European alder (Alnus glutinosa), birch (Betula pendula), hornbeam (Carpinus betulus), ragweed (Ambrosia artemisiifolia), wormwood (Artemisia absinthium), orchard grass (Dactylis glomerata), meadow fire (Festuca pratensis), sunflower (Helianthus annuus), perennial fenugreek (Lolium perenle), thyme (Poa pratensis), rye (Secale cereale), corn (Zea mays), epidermal allergens of animals - cat hair (Lana felis), dog hair (Lana canis), sheep (Lana ovis), allergen of feather pillows (Pluma pulvini).

Electrophoresis of allergen diagnostic solutions was performed by SDS-PAGE in columns recommended by Rockland Immunochemicals, Inc. (https://rockland-inc.com/sds-page.aspx). The results were scanned and processed using Image Studio Lite Ver. 5.2 with the subsequent construction and analysis of spectrograms using Gel Analyzer 1.0 software. The database of www.allergen.org, the official site for the systematic nomenclature of allergens approved by the World Health Organization and the International Union of Immunological Societies (WHO / IUIS), was used to identify allergenic components.

Results. Electrophoregrams and control molecular masses are shown in Figure 1 and Figure 2.

Using photometric analysis, a spectrogram of the protein composition of the control masses of protein fractions was formed and all spectrograms of allergens were further compared with it in order to determine their component composition (Figure 3).

Analysis of the component composition of extracts showed that they are quite diverse in the protein composition. The generalized results of determination of protein composition of domestic allergenic extracts are shown in Table. 1.

Analysis of the data in the table shows that 14 out of 20 (70%) of our studied allergen extracts contain ballast non-allergenic protein. In some cases (for example, for mite allergens, ragweed), this non-allergenic protein is able to mask the major protein components of allergens (Figure 4).

Table 1: *Analysis of protein composition allergenic extracts.*

Group	Allergen	Allergenic protein components	Ballast proteins
Mites	Dermatophagoides farinae	Der f1	Yes
	Dermatophagoides pteronyssinus	Der p1	Yes
	Acarus siro	Aca s13	Yes
Pollens	Birch	Bet v1, Bet v3	No
	Alder	Aln g1	Yes
	Rye	Sec c38, Sec c5	Yes
	Tarragon	Art ab1	No
	Meadow fire	Fes p4	Yes
	Sunflower	Hel a1, Hel a2, Hel a6	Yes
	Fenugreek	Lol p1, Lol p4, Lol p11, Lol p2-Lol p3	Yes
	Timothy grass	Phl p5, Phl p4, Phl p2	No
	Meadow bluegrass	--	Yes
	Corn	Zea m1, Zea m12	Yes
	Ragweed	Amb a3	No
	Hornbeam	Car b1	No
	Cocksfoot	Dac g1, Dac g4, Dac g2, Dac g3	Yes
Epidermal	Dog	Can f1, Can f2, Can f3	Yes
	Cat	Fel d1, Fel d2, Fel d3, Fel d4	No
	Sheep	--	Yes
	Feather pillows	--	Yes

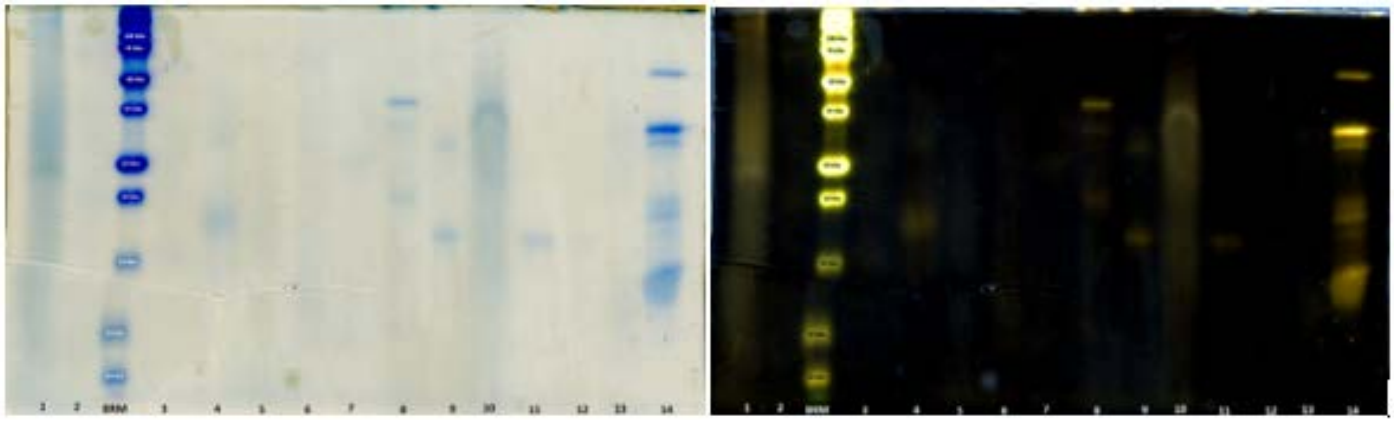

Figure.1. Electrophoregrams of diagnostic allergens and control masses of protein fractions before (left) and after (right) computer processing for analysis (part 1).

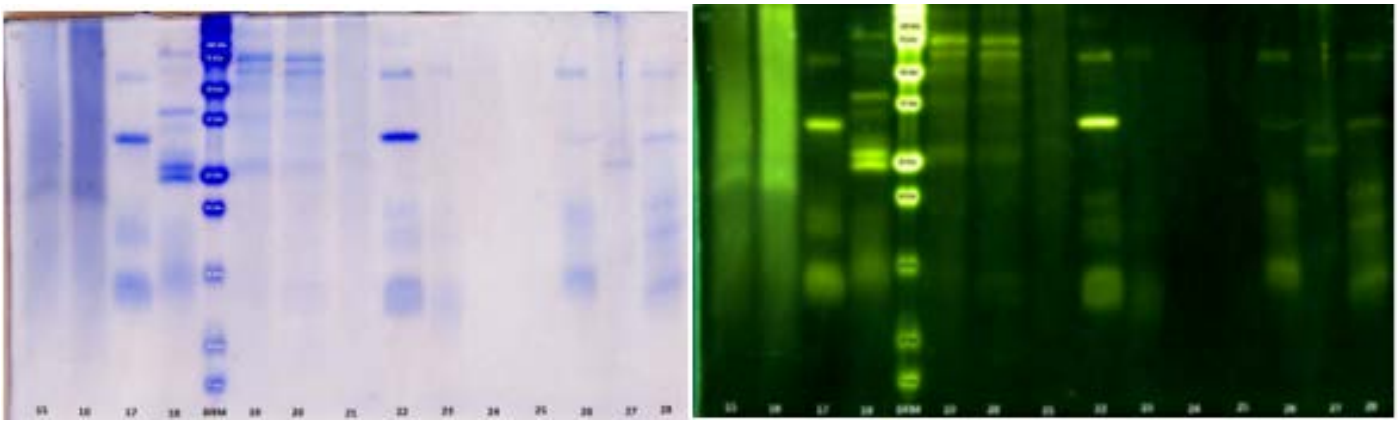

Figure. 2. Electrophoregrams of diagnostic allergens and control masses of protein fractions before (left) and after (right) computer processing for analysis (part 2).

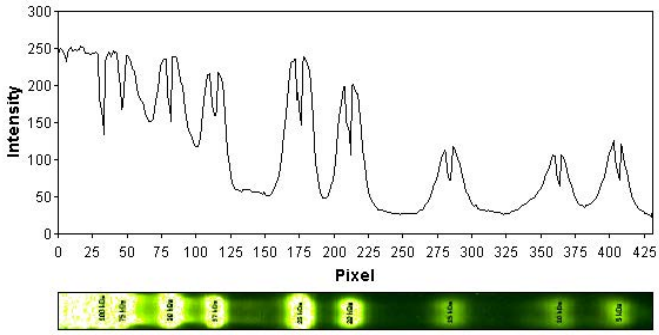

Figure. 3. Spectrogram of the control masses protein composition of protein fractions.

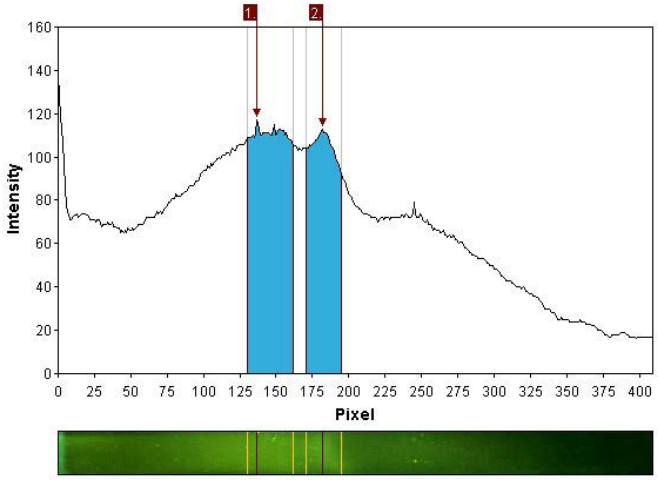

Figure. 4. Spectrogram of Dermatophagoides farinae diagnostic allergen composition.

Analysis of the spectrogram showed that in the protein composition of the allergen there is a major component of Der f1 with a molecular weight of 27 kDa - cysteine protease (mark 2). In addition, the spectrogram does not have a clearly separated molecular mass of 15 kDa, which corresponds to the second important major component of the mite Dermatophagoides farinae - Der f2 and there are non-allergenic ballast protein components with a molecular weight of 29-33 kDa (mark 1).

For most of the studied extracts, the major components were clearly detected. Most of the studied extracts, in addition to major, also contained minor components, forming a "cocktail" of each individual allergen. However, as we mentioned earlier, non-allergenic ballast proteins can be a serious problem in the existing scheme of standardization of extracts by protein units of nitrogen, because such standardization actually calculates the total amount of protein per unit volume of extract.

Discussion. For a long time, expert opinion was sufficient to place allergen products for in vivo diagnosis and therapy on the market, and clinical trials that meet Good Clinical Practice (GCP) standards have never been conducted. In many countries, especially in the European Union, the legal situation has changed dramatically over the last two decades. Safety and efficacy now need to be demonstrated for therapeutic allergen products (for allergen-specific immunotherapy) as well as for allergen products used for in vivo use, such as diagnostic allergen extracts for provocative testing, including skin testing, bronchial, nasal, conjunctive and food provocative testing [5-8]. Although there are differences in regulation on different continents and in different countries, the overall goal is that according to the "International Council for the Harmonization of Technical Requirements for Pharmaceuticals for Human Use" (http://www.ich.org/home.html), medicines that also include allergenic products for in vivo diagnosis and treatment should be evaluated in clinical trials and carefully evaluated to be registered for use in patients. The main prerequisite for clinical trials and subsequent use in humans is that the medicinal product is manufactured in accordance with good manufacturing practice (GMP) and has been shown to have the same characteristics and quality. This requirement is already a major barrier to allergen extracts produced from natural allergen sources [7,8].

Standardization for a specific allergen product is the main prerequisite for consistency in different parameters (composition, allergenic activity in vivo and in vitro) between batches of allergen product. The use of proven reference standards ensures the application of analytical methods to control the quality parameters of different batches produced at different times, which leads to consistent quality, safety and efficiency. This is necessary, but very difficult due to the complex structure of allergen extracts in combination with the high variability of the relevant natural substances in the raw material. In recent years, the level of standardization of allergenic products, and hence knowledge about their component composition has increased significantly. Not only regulatory requirements, but also the pressure exerted by society, academia and clinicians are advancing this development. This is also reflected in the position of the expert panel on allergen-specific immunotherapy, which emphasizes that only standardized extracts should be used in clinical practice, as the effectiveness and safety of allergen-specific immunotherapy directly depends on the quality of the extract.

In 2001, the EU-funded CREATE project was launched to encourage the standardization of allergen extracts based on the main allergen content. The mass units of the main allergens were intended to be used to quantify the active ingredients of the allergen, allowing comparisons between manufacturers [9].

The objectives of the CREATE project were to evaluate the potential of purified recombinant allergens as certified reference materials and to evaluate available enzyme-linked immunosorbent assays to measure major allergens using certified reference materials as a standard. Eight major allergens were selected from the four most important sources of inhalation allergens: Bet v 1 from birch pollen, Phl p 1 and Phl p 5 from grass pollen, Ole e 1 from olive pollen and Der p 1 and 2 and Der f 1 and Der f 2 of house dust mites. It was found that three of them are suitable as biological reference materials; others, except rPhl p 1a, indicate the potential for optimization by modifying aspects of the processes of their

protein expression. As a result of this study, recombinant Bet v 1 and Phl p 5 were manufactured as part of "Good Manufacturing Practice" and evaluated by the European Directorate for Drug Quality as biological reference products to be included in the European Pharmacopoeia as international standards. Therefore, theoretically, the standardization of these allergen products will be global, which will allow comparisons between different production sources of Ole e 1 from olive pollen and Der p 1 and 2 and Der f 1 and Der f 2 house dust mites.

Project BSP090, sponsored by the EDQM Biological Standardization Program (European Directorate for Medicinal Products and Health), was a continuation of the CREATE project and aimed to develop biological reference drugs for Bet v 1 and Phl p 5, as well as to test the standard methods of enzyme-linked immunosorbent assay to quantify them [9]. According to the results of the work, recombinant Bet v 1 was obtained, intended for use as a standard for calibration of secondary standards and / or in internal reference preparations for determination of Bet v 1 content in birch allergen extracts (Betula verrucosa) and recombinant Bet v1 preparations by ELISA. The ELISA reference method for its quantification was also chosen after testing in multicenter studies. According to physicochemical properties, recombinant Phl p 5.0109 is produced as highly stable, monomeric and immunologically equivalent to its natural counterpart, and is currently available as a reference standard of the European Pharmacopoeia for pollen allergens.

Another attempt to standardize the extracts involved the development of extracts of recombinant allergens. Although some of these recombinant allergens show comparability with allergen extracts derived from the source material, they cover only a limited number of allergens and are still under investigation.

The first big problem is the source of the allergen, which is used to produce allergen extracts. It is reported that the content, concentrations and ratios of individual allergens vary greatly depending on a large number of factors. Thus, in the study of Focke and co-authors in the evaluation of birch allergen extracts found a difference of more than 10 times the total protein content (23.1 - 314 μg / ml) and the amount of major allergen in birch pollen Bet v1 (1.62 - 19 , 6 μg / ml) [10]. Highly cross-reactive allergen Bet v4 was absent in three of the five extracts tested. In addition, various skin test results have been obtained in patients with birch pollen allergy with allergen extracts. In another study, the content of the major dog hair allergen Can f 1 and Can f 2 varied significantly between extracts [11]. In one of the extracts, neither Can f 1 nor Can f 2 could be detected by immunoblotting. The content of the minor allergen Can f 3, albumin, also showed great variability. A similar situation was shown with extracts of allergens of house dust mites - in all extracts were found only Der p 1 and Der p 2, but their concentrations and ratios showed high variability (Der p1: 6.0 - 40.8 μg / ml; Der p2: 1.7 - 45.0 μg / ml) [12]. At least 1 of 4 allergens (ie Der p5, 7, 10 and 21) was not detected in 8 of the studied extracts. Individuals with sensitization to tick allergens have shown different profiles of IgE reactivity to individual tick allergens, and the extracts have shown different allergenic activity in skin tests and false-negative results.

In this context, only a few examples should be mentioned: for example, the content of allergens in pollen varies depending on environmental factors, such as ozone exposure, pollution, and several plant species. The content of allergens from house dust mites and their ratio depend on the conditions of mite growth, the method of their feeding and cultivation and what mite material (feces, body parts) is used as a raw material for the production of extracts. With regard to animal allergens, the type and content of allergens may differ depending on sex. In addition, allergens have been shown to occur in pollen as different isoforms with different allergenic activity and immunological properties in different amounts, meaning that no homogeneous natural allergen preparation can be obtained from a natural allergen source. Therefore, only recombinant expression of a particular isoform based on the corresponding gene can overcome this problem.

Extracts should not contain preservatives that can cause false positive reactions, such as Sodium ethylmercurithiosalicylate. They should also not be mixed with other allergens, such as house dust mites with dog dandruff extract. When testing for non-commercial allergens, there is a real need to use control tests in people who do not have allergies to compare the results with subjects who suffer from allergies. For certain plant allergens, especially for fresh foods and vegetables, the injection method is more reliable than the use of manufactured extracts.

Not surprisingly, the standardization process focuses on the quality parameters that are components of this product. From the point of view of standardization of allergenic extracts, it is possible to distinguish between standardized approaches focused on the general allergenic activity, on the one hand, and approaches based on the content of allergenic molecules on the other hand. However, the vast majority of allergenic products permitted in the EU are standardized for total allergenic activity by measuring IgE binding potential. In accordance with the current regulatory requirements in the EU, this parameter is expressed in specific units, which are determined in manufacturer-specific in vitro analyzes for the reference product for a particular product. Similarly, the control of individual allergen protein molecules in ASIT products should be performed according to protocol procedures but performed in manufacturer-specific immunoassays or other methods.

The second step of standardization is the degree of comparability between allergenic products, which implies the comparability of products from the same allergenic source from different manufacturers. Although both the total allergenic activity and the content of individual allergen molecules can provide a basis for comparability between allergen extracts from different manufacturers, the current state of the extract specification does not allow performing this next level of allergen extract standardization. The reason is that the prerequisite for comparability is the comprehensive use of a standardized analytical method for a standard reference drug.

In the United States, allergen standardization is based on intradermal testing of allergy sufferers, and sequential batch potency is determined by appropriate in vitro surrogate assays that are based on inhibiting IgE binding to a complex of allergic sera with solid phase allergen extracts [1,5,6]. Extracts in

the United States are more homogeneous in terms of overall allergenic activity than extracts produced in Europe, mainly because the FDA provides the same standardized reagent for internal use by all manufacturing companies. However, these standards are limited to only 19 sources, including the most clinically relevant in the country (membranous venoms, cat fur and skin, house dust mites, pollen from 8 species of grass and ragweed). These drugs are licensed by the Center for Evaluation of Biological Research and Studies, and their number does not increase for more than 10 years.

In the case of non-standardized extracts, the calculation of extract doses for ASIT is more complicated, as the measurement of bulk density or units of protein nitrogen (PNU) may not correlate with biological efficacy. In an official FDA communiqué, an expert panel found that while most of the available allergen extract products are safe for clinical use, about half of them had no evidence of their effectiveness [13]. Accordingly, experts expected a sharp decrease in the number of available non-standard allergen extracts.

Strict regulation of skin tests of extracts has made their production and registration problematic and expensive for the pharmaceutical industry. This has led to gaps in the registration of specific allergens in certain European countries, including Ukraine (eg fungal allergens).

Determining the sensitization profile of a patient with IgE-mediated AD is an integral part of both the diagnosis and the appointment of subsequent treatment of such a patient. This is of great importance in understanding the likelihood of emergencies (anaphylaxis and acute angioneurotic edema), cross-reactions to other allergens, elimination measures (without which any treatment will not be effective enough) and, of course, for a single existing etiotropic treatment - ASIT.

In conclusion, it should be noted that the extracts of diagnostic allergens produced in Ukraine need further standardization. However, most countries in the world have similar problems and unambiguous requirements and methods of standardization are still being studied and discussed.

REFERENCES

1. Heinzerling L, Mari A, Bergmann K-C, Bresciani M, Burbach G, Darsow U, et al. The skin prick test - European standards. Clin Transl Allergy. 2013;3:3.
2. Nicholson PJ. When to patch test and when to prick test. Occupational medicine (Oxford, England). England 2019; 69:521.
3. Patel G, Saltoun C. Skin testing in allergy. Allergy asthma Proc. 2019;40:366-368.
4. Mostafa H, Qotb M, Hussein M, Hussein A. Allergic rhinitis diagnosis: skin-prick test versus laboratory diagnostic methods. Egypt J Otolaryngol. 2019;35:262.
5. Zimmer J, Vieths S, Kaul S. Standardization and Regulation of Allergen Products in the European Union. Curr Allergy Asthma Rep. 2016;16:21.
6. Larsen JN, Dreborg S. Standardization of Allergen Extracts. Methods Mol Biol. 2019;2020:63-76.
7. Himly M, Nandy A, Kahlert H, Thilker M, Steiner M, Briza P, et al. Standardization of allergen products: 2. Detailed characterization of GMP-produced recombinant Phl p 5.0109 as European Pharmacopoeia reference standard. Allergy. 2016;71:495-504.
8. Himly M, Nony E, Chabre H, Van Overtvelt L, Neubauer A, van Ree R, et al. Standardization of allergen products: 1. Detailed characterization of GMP-produced recombinant Bet v 1.0101 as biological reference preparation. Allergy. 2009;64:1038-1045.
9. van Ree R, Chapman MD, Ferreira F, Vieths S, Bryan D, Cromwell O, et al. The CREATE project: development of certified reference materials for allergenic products and validation of methods for their quantification. Allergy. 2008;63:310-326.
10. Focke M, Marth K, Valenta R. Molecular composition and biological activity of commercial birch pollen allergen extracts. Eur J Clin Invest. 2009;39:429-436.
11. Curin M, Reininger R, Swoboda I, Focke M, Valenta R, Spitzauer S. Skin prick test extracts for dog allergy diagnosis show considerable variations regarding the content of major and minor dog allergens. Int Arch Allergy Immunol. 2011;154:258-263.
12. Casset A, Mari A, Purohit A, Resch Y, Weghofer M, Ferrara R, et al. Varying allergen composition and content affects the in vivo allergenic activity of commercial Dermatophagoides pteronyssinus extracts. Int Arch Allergy Immunol. 2012;159:253-262.
13. Larenas-Linnemann D, Rodríguez-Pérez N, Luna-Pech JA, Rodríguez-González M, Blandón-Vijil MV, Del-Río-Navarro BE, et al. Compromising between European and US allergen immunotherapy schools: Discussions from GUIMIT, the Mexican immunotherapy guidelines. World Allergy Organ J. 2020;13:100444.

STATUS OF THE COMPOSITION OF ALLERGENIC EXTRACTS FOR SKIN TESTING IN UKRAINE AND THE WAYS TO OPTIMIZE IT

Gogunskaya I.V[1]., Zaikov S.V[1]., Tkhorovskyi M.A[2]., Plykanchuk O.V[2]., Bogomolov A.Ye[2].

[1]State institution, O. S. Kolomiychenko Institute of otolaryngology of National Academy of Medical Sciences of Ukraine.

[2]Shupyk National Medical Academy of Postgraduate Education, Kyiv, Ukraine.

[3]National Pirogov memorial medical university, Vinnytsya, Ukraine.

Abstract. Objective was to to analyze the protein components of extracts of diagnostic allergens made in Ukraine in order to determine the next steps for their standardization.

Materials and methods. Electrophoresis in polyacrylamide gel was used to obtain electrophoregrams of Acarus siro, Dermatophagoides farinae, Dermatophagoides pteronyssinus, Alnus glutinosa, Betula pendula, Carpinus betulus, Ambrosia artemisiifolia, Artemisia absinthium, Dactylis glomerata, Festuca pratensis, Helianthus annuus, Lolium perenle, Poa pratensis, Secale cereale, Zea mays, cat hair, dog hair, sheep allergens, and allergen of feather pillows. The official site for the systematic nomenclature of allergens approved by the World Health Organization and the International Union of Immunological Societies was used to identify allergenic components.

Results and discussion. Analysis of the data shows that 14 out of 20 (70%) of our studied allergen extracts contain ballast non-allergenic protein. In some cases (for example, for mite allergens, ragweed), this non-allergenic protein is able to mask the major protein components of allergens. For most of the studied extracts, the major components were clearly detected. Most of the studied extracts, in addition to major, also contained minor components, forming a "cocktail" of each individual allergen. However, as we mentioned earlier, non-allergenic ballast proteins can be a serious problem in the existing scheme of standardization of extracts by protein units of nitrogen, because such standardization actually calculates the total amount of protein per unit volume of extract.

Keywords. skin prick testing, allergen extract, standardization

СОСТОЯНИЕ СОСТАВА АЛЛЕРГЕННЫХ ЭКСТРАКТОВ ДЛЯ КОЖНОГО ТЕСТИРОВАНИЯ В УКРАИНЕ И СПОСОБЫ ЕГО ОПТИМИЗАЦИИ

И.В. Гогунская[1], С.В. Зайков[2], А.Е. Богомолов[3]

[1]Государственное учреждение «Институт отоларингологии им. О.С. Коломийченко НАМН Украины

[2]Национальная медицинская академия последипломного образования им. П.Л. Шупика

[3]Винницкий национальный медицинский университет им. Н.И. Пирогова

Резюме

Цель исследования заключалась в анализе белковых компонентов экстрактов диагностических аллергенов, производимых в Украине, с целью определения дальнейших шагов по их стандартизации.

Материалы и методы. Электрофорез в полиакриламидном геле был использован для получения электрофореграмм аллергенов Acarus siro, Dermatophagoides farinae, Dermatophagoides pteronyssinus, Alnus glutinosa, Betula pendula, Carpinus betulus, Ambrosia artemisiifolia, Artemisia absinthium, Dactylis glomerata, Festuca pratensis, Helianthus annuus, Lolium perenle, Poa pratensis, Secale cereale, Zea mays, шерсти кошек, шерсти собак, овец и аллергена перьевых подушек. Официальный сайт систематической номенклатуры аллергенов, одобренной Всемирной организацией здравоохранения и Международным союзом иммунологических обществ, использовался для идентификации аллергенных компонентов.

Результаты и обсуждение. Анализ данных показывает, что 14 из 20 (70%) исследованных нами экстрактов аллергенов содержали балластный неаллергенный белок. В некоторых случаях (например, для аллергенов клещей, амброзии) этот неаллергенный белок способен маскировать основные белковые компоненты аллергенов. Для большинства изученных экстрактов были четко определены мажорные компоненты. Большинство изученных экстрактов, помимо мажорных, содержали также минорные компоненты, образующие «коктейль» для каждого индивидуального аллергена. Однако, как мы упоминали ранее, неаллергенные балластные белки могут быть серьезной проблемой в существующей схеме стандартизации экстрактов по белковым единицам азота, поскольку такая стандартизация фактически рассчитывает общее количество белка на единицу объема экстракта.

Ключевые слова: кожный прик - тест, экстракт аллергена, стандартизация.

ი.ვ. გოგუნსკაია1, ს.ვ. ზაიკოვი2, ა.ე. ბოგომოლოვი3

კანის ტესტირებისთვის ალერგენის ექსტრაქტების შემადგენლობის მდგომარეობა უკრაინაში და მისი ოპტიმიზაციის მეთოდები

1 სახელმწიფო დაწესებულება „ოტოლარინგოლოგიის ინსტიტუტის სახ ო.ს. კოლომიჩენკო უკრაინის NAMS; 2 დიპლომისშემდგომი განათლების ეროვნული სამედიცინო აკადემია ე.წ პ.ლ. შუპიკა; 3 ვინიცის სახელობის ეროვნული სამედიცინო უნივერსიტეტი ნ.ი. პიროგოვი

□ემაჯამებელი

კვლევის მიზანი იყო უკრაინაში წარმოებული დიაგნოსტიკური ალერგენების ექსტრაქტების ცილოვანი კომპონენტების ანალიზი მათი სტანდარტიზაციის შემდგომი ნაბიჯების დასადგენად.

□ასალა და მეთოდები. პოლიაკრილამიდის გელის ელექტროფორეზი გამოიყენებოდა ალერგენების Acarus siro, Dermatophagoides farinae, Dermatophagoides pteronyssinus, Alnus glutinosa, Betula pendula, Carpinus betulus, Ambrosia artemisiifolia, Artemisia absinthium, Dactylis glomerata, Festuca pratensis, Helianthus annuus, Lolium perenle, Poa pratensis, Secale cereale, Zea mays, თმის, ცხვრის და ბუმბულის ბალიშის ალერგენი. ალერგენების სისტემატური ნომენკლატურის ოფიციალური ვებგვერდი, რომელიც დამტკიცებულია ჯანდაცვის მსოფლიო ორგანიზაციისა და იმუნოლოგიური საზოგადოებების საერთაშორისო კავშირის მიერ, გამოიყენებოდა ალერგენული კომპონენტების იდენტიფიცირებისთვის.

□ედეგები და დისკუსია. მონაცემთა ანალიზი აჩვენებს, რომ ჩვენს მიერ შესწავლილი 20 (70%) ალერგენის ექსტრაქტიდან 14 შეიცავს ბალასტის არაალერგენულ პროტეინს. ზოგიერთ შემთხვევაში (მაგალითად, ტკიპების ალერგენებისთვის, ამბროზიას), ამ არაალერგენულ ცილას შეუძლია დაფაროს ალერგენების ძირითადი ცილოვანი კომპონენტები. შესწავლილი ექსტრაქტების უმეტესობისთვის, ძირითადი კომპონენტები ნათლად იყო იდენტიფიცირებული. შესწავლილი ექსტრაქტების უმეტესობა, გარდა ძირითადისა, ასევე შეიცავდა მცირე კომპონენტებს, რომლებიც ქმნიან "კოქტეილს" თითოეული ინდივიდუალური ალერგენისთვის. თუმცა, როგორც უკვე აღვნიშნეთ, არაალერგენული ბალასტური ცილები შეიძლება იყოს სერიოზული პრობლემა ექსტრაქტების სტანდარტიზების მიმდინარე სქემაში ცილის აზოტის ერთეულებით, რადგან ასეთი სტანდარტიზაცია რეალურად ითვლის ცილის მთლიან რაოდენობას ექსტრაქტის ერთეული მოცულობით.

საკვანძო სიტყვები: კანის ჩირქოვანი ტესტი, ალერგენის ექსტრაქტი, სტანდარტიზაცია.