

## DIAGNOSTIC PARAMETERS OF IN VIVO (SKIN PRICK) AND IN VITRO (ELISA) TESTS FOR EPIDERMAL CAT AND DOG ALLERGENS SENSITIZATION DETERMINATION IN PATIENTS WITH ALLERGIC RHINITIS AND ATOPIC ASTHMA

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Respiratory allergic diseases are one of the most common chronic pathologies in the world [1-3]. Allergic rhinitis is the most common immune disease and one of the most common chronic diseases worldwide—with an ever increasing prevalence. Almost one in three European citizens is affected by allergic rhinitis. Their treatment, in addition to the generally accepted regimens of drug therapy, must necessarily include measures to eliminate contact with the allergen, education of the patient and, if possible, allergen-specific immunotherapy [4-6]. Based on these facts, the specific diagnosis of the causative factor of allergic rhinitis or atopic bronchial asthma is the most important task of the clinician. The variety of diagnostic methods sometimes puts the specialist into the problem of choosing one or another method, based on the results of which the above treatment methods will subsequently be carried out [7-9].

The diagnostic value of an allergen extract can only be assessed with respect to a population consisting of sensitised (true positive) and non-sensitised (true negative) patients. The Guideline on Clinical Evaluation of Diagnostic Agents recommends comparing the results received by the investigational diagnostic agent with the results of the so-called ‘standard of truth’ [10]. For allergen skin prick test (SPT) solutions, no such ‘standard of truth’ is defined [13]. In current medical practice, analyses for circulating specific IgE antibodies in serum (ELISA, ImmunoCap, western-blot) as well as the clinical history and SPT are considered to be standard methods to differentiate sensitised from non-sensitised patients [11, 12], and to confirm the clinical relevance of the allergen in question. In this study, each of these three reference methods was chosen as reference for the assessment of sensitivity and specificity of the SPT solutions.

In this article, we present the data of a part of our study comparing different diagnostic methods with each other.

**Materials and methods.** During this research, 88 patients with allergic rhinitis and / or atopic asthma were examined by three different methods of specific allergic diagnosis (*in vivo* and *in vitro*). The inclusion criteria were allergic rhinitis diagnosis (both intermittent and persistent) or atopic asthma, previously confirmed clinically, anamnestic and laboratory (ImmunoCap or ELISA) diagnosis. Among them, 20 patients had mono- or

polisensitization to epidermal allergens, all other patients were sensitive to another allergens (pollen, dust mite, fungal etc) and formed negative control. According to the objectives of the study, the aim was not to establish / confirm the diagnosis and sensitization, but to determine the diagnostic parameters of the tests with a previously established diagnosis and sensitization. Skin prick test (SPT) was carried out according to the classical testing procedure in accordance with regulatory documents with commercial extracts of allergens (Immunolog, Vinnitsa, Ukraine). SPT results were assessed in 15 min visually using a ruler in mm and were classified according to the existing scale as negative, doubtful, weak (+), strong (++) and very strong (+++).

A standard medical interview and the qualification of patient were performed during an earlier visit, and then, 15 mL of blood for the sIgE test was collected. Western blot testing for specific IgE levels was performed using RIDA qLine test systems (R-Biopharm AG, Darmstadt, Germany) and Euroline (Euroimmun) system. The sIgE concentration was converted to a nominal scale (grades) according to the following rules: < 0.35 IU mL<sup>-1</sup>-level 0 (negative), (0.36-0.69) IU mL<sup>-1</sup>-level 1 (boundary levels), (0.7- 3.49) IU mL<sup>-1</sup>-level 2 (slightly elevated), (3.50-17.4) IU mL<sup>-1</sup>-level 3 (moderately elevated), (17.5-49, 9) IU mL<sup>-1</sup>-level 4 (high levels), (50-100) IU mL<sup>-1</sup>-level 5 (very high levels) and > 100 IU mL<sup>-1</sup>-level 6 (extremely high levels).

**Results and discussion.** Mean age of the patients was 31.4 [95% CI: 29.8; 33.1] years. Among our patients, sensitization to the cat allergen had 13,6 % (12 patients) and to the dog allergen - 9,0 % (8 persons) by skin prick test, specific IgE by Rida AllergyScreen was found in 13,6 % (12 patients) and 11,3 % (10 patients); the presence of specific IgE by Euroline was detected in 13,6 % (12 patients) and 9,0 % (8 patients), respectively.

In Table 1 the results of the comparison of Rida AllergyScreen to the cat allergen with the data prick test method are presented. Comparing two different types of specific allergic diagnosis by the method of establishing the correlation relations with cat, the dominance of the elements of the main diagonal is noted, indicating a close coincidence of the results of two different methods (validity coincidence of results was 100,0 % - 88 cases).

Table 1. Sensitization to cat by the results of skin testing and the detection of specific IgE by Rida AllergyScreen

Prick test	Specific IgE (ku/l)			Total
	< 0.35 (negative)	0.35–0.7 (questionable)	> 0.7 (positive)	
Papula 0 mm (negative result)	76	0	0	76
Papula 1-2 mm (questionable result)	0	0	0	0
Papule ≥ 3 mm (positive result)	0	0	12	12
Total	76	0	12	88

Table 2. The results of statistical estimation of the consistency of results on the results of skin testing and the detection of specific IgE by the method of Rida AllergyScreen to determine sensitization to cat

Kappa coefficient	1,0
Asymptotic kappa error	0
Lower border 95 % confidence interval	1,0
Upper boundary 95 % confidence interval	1,0

Table 3. Sensitization to dog by the results of skin testing and the detection of specific IgE by Rida AllergyScreen

Prick test	Specific IgE (ku/l)			Total
	< 0.35 (negative)	0.35–0.7 (questionable)	> 0.7 (positive)	
Papula 0 mm (negative result)	76	2	2	80
Papula 1-2 mm (questionable result)	0	0	0	0
Papule ≥ 3 mm (positive result)	0	0	8	8
Total	76	2	10	88

Table 4. The results of statistical estimation of the consistency of results on the results of skin testing and the detection of specific IgE by the method of Rida AllergyScreen to determine sensitization to dog

Kappa coefficient	0,778
Asymptotic kappa error	0,105
Lower border 95 % confidence interval	0,486
Upper boundary 95 % confidence interval	0,959

Table 5. The results of statistical estimation of the null hypothesis of the lack of consistency of results on the results of skin testing and the detection of specific IgE by the method of Rida AllergyScreen for definition sensitization to cat

Asymptotic kappa error for $H_0$	0,0
Z	9,381
One-way testing $Pr > Z$	< 0,0001
Two-sided testing $Pr >  Z $	< 0,0001

The results of two different methods of specific diagnostics to determine allergic sensitization to cat are almost similar, but there is a certain asymmetry of the differences in the results of skin testing by the blind test method and the determination of specific IgE when one test gives negative results and the other one is positive or questionable.

To obtain conclusions about the reliability of this asymmetry, we conducted an in-depth statistical analysis of the correlation of laboratory allergic and skin tests. The analysis of harmony results of two different methods to determine the sensitization to cat through the construction of the confidence interval (Table 2) showed that the coefficient suggests a perfect agreement ( $r = 1.00$ ) of this two different tests. The limits of the 95 % confidence interval (1,0–1,0) exclude zero, which indicates the accuracy of the match. The lower limit is in the range of good coherence, and the upper one is in the area of excellent coherence.

In Table 3 the results of the comparison of Rida AllergyScreen to the dog allergen with the data prick test method are presented. Comparing two different types of specific allergic diagnosis by the method of establishing the correlation relations with dog allergen, the dominance of the elements of the main diagonal is noted, indicating a close coincidence of the results of two different methods (validity coincidence of results was 95,5 % - 84 cases).

The results of two different methods of specific diagnostics to determine allergic sensitization to dog are almost similar, but

there is a certain asymmetry of the differences in the results of skin testing by the blind test method and the determination of specific IgE when one test gives negative results and the other one is positive or questionable.

To obtain conclusions about the reliability of this asymmetry, we conducted an in-depth statistical analysis of the correlation of laboratory allergic and skin tests. The analysis of harmony results of two different methods to determine the sensitization to dog through the construction of the confidence interval (Table 4) showed that the coefficient suggests a perfect agreement ( $r = 0,778$ ) of this two different tests. The limits of the 95 % confidence interval (0,486 – 0,959) exclude zero, which indicates the accuracy of the match. The lower limit is in the range of good coherence, and the upper one is in the area of excellent coherence.

A statistical evaluation of the null hypothesis lack of consistency of the results of two different methods of specific diagnostics to determine allergic sensitization to cat shown in Table 5.

The hypothesis is rejected both in one-sided and bilateral tests, which testifies to the true consistency of both allergic tests.

That is to say, according to the data of skin testing with cat allergen and the detection of specific IgE by the Rida AllergyScreen has a perfect consistency between the results.

A statistical evaluation of the null hypothesis lack of consistency of the results of two different methods of specific diagnostics to determine allergic sensitization to dog shown in Table 6.

**Table 6. The results of statistical estimation of the null hypothesis of the lack of consistency of results on the results of skin testing and the detection of specific IgE by the method of Rida AllergyScreen for definition sensitization to dog**

Asymptotic kappa error for $H_0$ ,	0,001
Z	8,061
One-way testing $Pr > Z$	< 0,0001
Two-sided testing $Pr >  Z $	< 0,0001

*Table 7. Sensitization to cat by the results of skin testing and the detection of specific IgE by Euroline*

Prick test	Specific IgE ( ku / l )			Total
	< 0.35 (negative)	0.35-0.7 (questionable)	> 0.7 (positive)	
Papula 0 mm (negative result)	74	2	0	76
Papula 1-2 mm (questionable result)	0	0	0	0
Papule $\geq$ 3 mm (positive result)	0	0	12	12
Total	74	2	12	88

*Table 8. The results of statistical estimation of the consistency of results on the results of skin testing and the detection of specific IgE by the Euroline method to determine the sensitization to the cat allergen*

Kappa coefficient	0,911
Asymptomatic kappa error	0,061
Lower border 95 % confidence interval	0,755
Upper boundary 95 % confidence interval	1,0

*Table 9. The results of statistical estimation of the null hypothesis of the lack of consistency of results on the results of skin testing and the detection of specific IgE by the Euroline method for determination sensitization to the cat allergen*

Asymptotic kappa error for $H_0$ ,	0,022
Z	9,162
One-way testing $Pr > Z$	< 0,0001
Two-sided testing $Pr >  Z $	< 0,0001

The hypothesis is rejected both in one-sided and bilateral tests, which testifies to the true consistency of both allergic tests.

That is to say, according to the data of skin testing with dog allergen and the detection of specific IgE by the Rida AllergyScreen has a good consistency between the results.

In Table 7 we showed the comparison of the presence of specific IgE to the cat by Euroline with skin prick testing test. Comparing two different types of specific diagnostics by setting correlative relationships to cat is noted the domination of the elements of the main diagonal, indicating a high degree of coincidence of the results of two different methods (validity of the results was 97,7% - 80 cases).

The results of two different methods of specific allergic diagnosis to determine the sensitization to the cat allergen are closely identical, but there is a certain asymmetry of the differences in the results of skin testing by the blind test method and the determination of specific IgE blood when one test gives negative results and the other one is positive or doubtful.

To obtain conclusions about the reliability of this asymmetry, we conducted an in-depth statistical analysis of the correlation of laboratory allergic and skin tests. The analysis of harmony results of two different methods to determine the diagnosis of allergic sensitization to cat through the construction of the

confidence interval (Table 8) showed that the coefficient suggests great agreement ( $r = 0,911$ ) of the findings of the two different tests. The limits of the 95 % confidence interval (0,755–1,0) exclude 0, which indicates the accuracy of the match. The lower limit lies in the range of poor consistency, and the upper one is in the area of moderate coherence.

A statistical evaluation of the null hypothesis lack of consistency of the results of two different methods of specific diagnostics to determine allergic sensitization to cat shown in table 9.

The hypothesis is not accepted either by one-sided, or by double-sided testing the loyalty to the consistency of the tests among themselves.

That is to say, according to the data of skin testing with cat allergens and the detection of specific IgE by the Euroline method, there is a great agreement between the research results.

In Table 10 we showed the comparison of the presence of specific IgE to the dog by Euroline with skin prick testing test. Comparing two different types of specific diagnostics by setting correlative relationships to dog is noted the domination of the elements of the main diagonal, indicating a high degree of coincidence of the results of two different methods (validity of the results was 86,4% - 76 cases).

Table 10. Sensitization to dog by the results of skin testing and the detection of specific IgE by Euroline

Prick test	Specific IgE ( ku / l )			Total
	< 0.35 (negative)	0.35-0.7 (questionable)	> 0.7 (positive)	
Papula 0 mm (negative result)	72	8	0	80
Papula 1-2 mm (questionable result)	0	0	0	0
Papule ≥ 3 mm (positive result)	4	0	4	8
Total	76	8	4	88

Table 11. The results of statistical estimation of the consistency of results on the results of skin testing and the detection of specific IgE by the Euroline method to determine the sensitization to the dog allergen

Kappa coefficient	0,353
Asymptomatic kappa error	0,140
Lower border 95 % confidence interval	0,058
Upper boundary 95 % confidence interval	0,617

Table 12. The results of statistical estimation of the null hypothesis of the lack of consistency of results on the results of skin testing and the detection of specific IgE by the Euroline method for determination sensitization to the dog allergen

Asymptomatic kappa error for $H_0$ ,	0,571
Z	3,025
One-way testing $Pr > Z$	< 0,001
Two-sided testing $Pr >  Z $	< 0,001

The results of two different methods of specific allergic diagnosis to determine the sensitization to the dog allergen are closely identical, but there is a certain asymmetry of the differences in the results of skin testing by the blind test method and the determination of specific IgE blood when one test gives negative results and the other one is positive or doubtful.

To obtain conclusions about the reliability of this asymmetry, we conducted an in-depth statistical analysis of the correlation of laboratory allergic and skin tests. The analysis of harmony results of two different methods to determine the diagnosis of allergic sensitization to dog through the construction of the confidence interval (Table 11) showed that the coefficient suggests moderate agreement ( $r = 0,353$ ) of the findings of the two different tests. The limits of the 95 % confidence interval (0,058–0,617) exclude 0, which indicates the accuracy of the match. The lower limit lies in the range of poor consistency, and the upper one is in the area of moderate coherence.

A statistical evaluation of the null hypothesis lack of consistency of the results of two different methods of specific diagnostics to determine allergic sensitization to dog shown in table 12.

The hypothesis is not accepted either by one-sided, or by double-sided testing the loyalty to the consistency of the tests among themselves.

That is to say, according to the data of skin testing with dog allergens and the detection of specific IgE by the Euroline method, there is a moderate agreement between the research results.

**Conclusions.** Thus, the results of the two systems for the determination of specific IgE for dog allergen by the Rida AllergyScreen and Euroline methods do not agree very well due to the systematic divergence of indicators; the results of the two systems for the determination of specific IgE for cat allergen by the Rida AllergyScreen and Euroline methods agree very well.

There is excellent agreement between the skin test with cat allergen and the detection of specific IgE by the Rida AllergyScreen test, between the skin test with cat allergen and the detection of specific IgE by the Euroline method.

There is good agreement between the skin test with dog wool allergens and the detection of specific IgE by the Rida AllergyScreen test, between the skin test with dog hair allergen and the detection of specific IgE by the Euroline method there is satisfactory agreement.

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the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery (DGHNO-KHC), the German Society of Pediatrics and Adolescent Medicine (DGKJ), the Society for Pediatric Pneumology (GPP), the German Respiratory Society (DGP), the German Association of ENT Surgeons (BV-HNO), the Professional Federation of Paediatricians and Youth Doctors (BVKJ), the Federal Association of Pulmonologists (BDP) and the German Dermatologists Association (BVDD). // *Allergo J Int.* 2014;23(8):282-319.

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## SUMMARY

### DIAGNOSTIC PARAMETERS OF IN VIVO (SKIN PRICK) AND IN VITRO (ELISA) TESTS FOR EPIDERMAL CAT AND DOG ALLERGENS SENSITIZATION DETERMINATION IN PATIENTS WITH ALLERGIC RHINITIS AND ATOPIC ASTHMA

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Objective was to study and compare the parameters of the specificity and sensitivity of skin testing and serologic determination of specific cat and dog IgE.

88 patients with allergic rhinitis and/or asthma were examined

by three different methods of specific allergic diagnosis (in vivo and in vitro) in accordance with the guidelines of the ethics committee of the National Pirogov memorial medical university, all were beyond the acute period. The inclusion criteria were allergic rhinitis diagnosis (both intermittent and persistent) and \ or asthma. Skin prick test was carried out according to the classical testing procedure in accordance with regulatory documents with commercial extracts of allergens. Western blot testing for specific IgE levels was performed using RIDA qLine test systems (R-Biopharm AG, Darmstadt, Germany) and Euroline (Euroimmun). The sIgE concentration was converted to a nominal scale (grades) according to the following rules: <0.35 IU mL<sup>-1</sup>-level 0 (negative), (0.36-0.69) IU mL<sup>-1</sup>-level 1 (boundary levels), (0.7- 3.49) IU mL<sup>-1</sup>-level 2 (slightly elevated), (3.50-17.4) IU mL<sup>-1</sup>-level 3 (moderately elevated), (17.5-49, 9) IU mL<sup>-1</sup>-level 4 (high levels), (50-100) IU mL<sup>-1</sup>-level 5 (very high levels) and > 100 IU mL<sup>-1</sup>-level 6 (extremely high levels).

Thus, the results of the two systems for the determination of specific IgE for dog allergen by the Rida AllergyScreen and Euroline methods do not agree very well due to the systematic divergence of indicators; the results of the two systems for the determination of specific IgE for cat allergen by the Rida AllergyScreen and Euroline methods agree very well.

There is excellent agreement between the skin test with cat allergen and the detection of specific IgE by the Rida AllergyScreen test, between the skin test with cat allergen and the detection of specific IgE by the Euroline method. There is good agreement between the skin test with dog wool allergens and the detection of specific IgE by the Rida AllergyScreen test, between the skin test with dog hair allergen and the detection of specific IgE by the Euroline method there is satisfactory agreement.

The systematic error of the measurement results between two in vitro tests for cat allergen was 0.1 ku/l, which indicates the presence of a small systematic difference, the systematic error of the measurement results between two in vitro tests for dog allergen was 0,26 ku/l, which indicates the presence of a moderate systematic difference.

**Keywords:** skin prick testing, allergy, western-blotting, IgE.

## РЕЗЮМЕ

### ДИАГНОСТИЧЕСКИЕ ПАРАМЕТРЫ IN VIVO (УКОЛ КОЖИ) И IN VITRO (ELISA) ТЕСТОВ ДЛЯ ОПРЕДЕЛЕНИЯ СЕНСИБИЛИЗАЦИИ К ЭПИДЕРМАЛЬНЫМ АЛЛЕРГЕНАМ КОШЕК И СОБАК У ПАЦИЕНТОВ С АЛЛЕРГИЧЕСКИМ РИНИТОМ И АТОПИЧЕСКОЙ АСТМОЙ

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Цель исследования – сравнение и оценка параметров специфичности и чувствительности кожного теста и серологического определения специфического IgE кошки и собаки.

88 пациентов с аллергическим ринитом и/или астмой были обследованы тремя различными методами специфической аллергической диагностики (in vivo и in vitro) в соответствии с рекомендациями комитета по этике Нацио-



ნაწილის მემორიალური მედიცინის უნივერსიტეტი იმ. პიროგოვა, და ყველა იმის გარეშე. კრიტერიუმები ჩართვისა და გამოსარიცხვისა იყვნენ: ალერგიული რინიტის და ასთმის დასაბამისად. ტესტირება უკეთესად იქნა ჩატარებული, როდესაც კლინიკური მონაცემები და კანის ტესტირების შედეგები ერთმანეთს ემთხვევა.

ამგვარად, ორივე სისტემის შედეგები კარგად შეესაბამება ერთმანეთს და კლინიკურ მონაცემებს.

### რეზიუმე

*IN VIVO* (კანის) და *IN VITRO (ELISA)* ტესტების დიაგნოსტიკური პარამეტრები კატის და ძაღლის ეპიდემიური ალერგიების მიმართ სენსიბილიზაციის განსაზღვრისათვის პაციენტებში ალერგიული რინიტით და ასთმით

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<sup>1</sup>ო.კლომიხენკოს სახ. ოტოლარინგოლოგიის ინსტიტუტი; <sup>2</sup>პ.შუპიკის სახ. დიპლომის შემდგომი განათლების ეროვნული აკადემია; <sup>3</sup>ვინიცას ნ.პიროგოვის სახ. ეროვნული სამედიცინო უნივერსიტეტი, უკრაინა

კვლევის მიზანს წარმოადგენდა ძაღლის და კატის სპეციფიკური IgE-ს კანის ტესტის და სეროლოგიური განსაზღვრის სპეციფიკურობის და მგრძობიანობის პარამეტრების შედარება და შეფასება.

88 პაციენტი ალერგიული რინიტით და/ან ასთმით გამოკვლეული იყო სპეციფიკური ალერგიული დიაგნოსტიკის სამი სხვადასხვა მეთოდით (*In vivo* და *In vitro*) ვინიცას ნ.პიროგოვის სახ. ეროვნული სამედიცინო უნივერსიტეტის ეთიკის კომიტეტის რეკომენდაციების შესაბამისად; არც ერთი შემთხვევა არ იყო მწვავე პერიოდში. ჩართვის კრიტერიუმებს წარმოადგენდა ალერგიული რინიტის დიაგნოზი (როგორც წვევტილი, ასევე მუდმივი) და/ან ასთმა. კანის ნემსის ტესტი ტარდებოდა ტესტირების კლასიკური მეთოდით ალერგიების ექსტრაქტების კომერციული ნორმატიული დოზების შესაბამისად. ვესტერნ-ბლოტირება IgE-ს სპეციფიკური დონეებისთვის ჩატარდა ტესტ-სისტემების RIDA qLine (R-Biopharm AG, დარმშტადტი, გერმანია) და Euroline (Euroimmun) გამოყენებით. sIgE-ს კონცენტრაცია გადაჭყავდათ ნომინალურ შკალაში (შეფასებაში) შემდეგი წესების შესაბამისად: <0,35 IU მლ-1 - დონე 0 (უარყოფითი); 0,36-0,69 IU მლ-1 - დონე 1 (მოსაზღვრე); 0,7-3,49 IU მლ-1 - დონე 2 (მცირედ მომატებული); 3,50-17,4 IU მლ-1 - დონე 3 (ზომიერად მომატებული); 17,5-49,9 IU მლ-1 - დონე 4 (მაღალი); 50-100 IU მლ-1 - დონე 5 (ძალიან მაღალი); >100 IU მლ-1 - დონე 6 (ექსტრემალურად მაღალი).

ვხვდებით სისტემატისკო განსხვავებების მაჩვენებლებს ორივე სისტემის შედეგებში Rida Allergy Screen-ის და Euroline-ის მეთოდებით, მაჩვენებლების სისტემატური ცდომილების გამო, ერთმანეთთან კარგად თავსებადი არ არის; კატის ალერგიის სპეციფიკური IgE-ს განსაზღვრის ორი სისტემის შედეგები კი Rida Allergy Screen-ის და Euroline-ის მეთოდებით ძალიან კარგად თავსებადია.

გამოვარჯიშებულია ორი სისტემის შედეგების მიხედვით დადგენილია ძალიან კარგი თავსებადობა კატის ალერგიის კანის ტესტსა და სპეციფიკური IgE-ს აღმოჩენას შორის RidaAllergyScreen-ის ტესტით, ასევე, კატის ალერგიის კანის ტესტსა და სპეციფიკური IgE-ს აღმოჩენას შორის Euroline -მეთოდით. დადგენილია კარგი თავსებადობა ძაღლის ბეწვის ალერგიის კანის ტესტსა და სპეციფიკური IgE-ს აღმოჩენას შორის Euroline მეთოდით - დამაკმაყოფილებელი თავსებადობა.

ორი *In vitro* ტესტით გაზომვის შედეგების სისტემატურმა ცდომილებამ კატის ალერგიის შეფასება 0,1 კU/ლ, რაც მიუთითებს მცირე სისტემატური განსხვავების არსებობაზე; ორი *In vitro* ტესტით გაზომვის შედეგების სისტემატურმა ცდომილებამ ძაღლის ალერგიის შეფასება 0,26 კU/ლ, რაც მიუთითებს ზომიერი სისტემატური განსხვავების არსებობის შესახებ.