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# Disagreement between skin prick test and specific IgE levels in patients with respiratory allergy (literature review and our own research data)

**Key words:** comparison, skin prick test, serum specific IgE, allergy, allergic rhinitis, bronchial asthma.

Skin prick test (SPT) is the most widely used diagnostic test in allergy. The test is simple, quick and is regarded as the gold standard method for allergy testing [1–4].

SPT is a reliable method to diagnose IgE-mediated allergic disease in patients with rhinoconjunctivitis, asthma, urticaria, anaphylaxis, atopic eczema and suspected food and drug allergy. It provides evidence for sensitization and can help to confirm the diagnosis of a suspected type I allergy. It is minimally invasive, inexpensive, results are immediately available and when carried out by trained health professionals, reproducible. Since the first publication about SPT by Helmtraud Ebruster in 1959 [1], who extensively researched this diagnostic test, it has been used as a primary diagnostic tool to detect type I hypersensitivity reactions. Although the principle of SPT still largely resembles the original methods described, a wide array of interpretations and modifications has led to diminished comparability when SPT results are reported. In addition, the different kind of extracts used in various countries makes comparison of data difficult.

In some instances, skin testing may not be possible. Patients who are unable to discontinue antihistamine therapy before skin testing may be candidates for in vitro testing. The main contraindications for SPT are: absolute – inability to discontinue antihistamines, generalized skin disease; relative – pregnancy, adrenergic-receptor

blocking agent therapy, history of anaphylaxis to previous skin tests, dermatographism and unstable angina [5].

However, anaphylaxis is a potential complication of SPT and the emergency resuscitation equipments should always be available at the test vicinity. Serum specific IgE (SSIgE) has now emerged as an alternative test and is gaining popularity in the field of allergy diagnosis as it offers fewer complications and more objective results.

The main indications for in vitro IgE determination shown in picture 1.

While the above-mentioned procedures use allergen mixtures in soluble or coupled form, the specific

**Total serum IgE**

- Atopic diseases (prognosis, atopic diathesis)
- Parasitoses
- Parameters of TH2 reaction (lymphoma, autoimmune disease)

**Specific IgE**

- Allergic (IgE-mediated) disease

**Absolutely indicated in**

- High degree of sensitization
- Life-threatening allergy (e.g., anaphylaxis)
- Impossibility of skin tests (skin lesions, medication, irritating agents)

Picture 1. The main indications for in vitro IgE determination.

reactivity of serum against single proteins within an allergen extract can be visualized after electrophoretic separation on nitrocellulose (Western blotting). Western blotting identifies with specific antibodies proteins that have been separated from one another according to their size by gel electrophoresis. The blot is a membrane, almost always of nitrocellulose or PVDF (polyvinylidene fluoride). The gel is placed next to the membrane and application of an electrical current induces the proteins in the gel to move to the membrane where they adhere. The membrane is then a replica of the gel's protein pattern, and is subsequently stained with an antibody.

Prior studies comparing these diagnostic modalities indicated that SPT is more sensitive than SSiGE [6–8]. These studies used different *in vitro* technologies with varying results and were done in Western population. We found no previous study in Ukraine specifically comparing the SPT and SSiGE in allergy testing.

The knowledge of the correlation between these two diagnostic tests would be important in the scenario where the patient's history is unclear and SPT is equivocal or contraindicated. SSiGE should be considered as an alternative test, particularly before making immunotherapy recommendations. In this scenario, the data on the extent of agreement or disagreement between the two tests would be vital before starting treatment.

Choi I.S. study [9] shown that the sensitivity of the skin test (81 %) was higher than that of the IgE test (67 %), whereas the specificity of the IgE test (71 %) was higher than that of the skin test (52 %). The sensitivity of the skin test was 91 % at 2+ or higher, and the specificity of the IgE test was 95 % at class 6 or higher. However, Schoos A.M. et al. in their new study had found substantial disagreement between SPT and sIgE for diagnosing allergic sensitization [10]. Overall, the agreement between SPT and sIgE levels was poor to moderate (all  $\kappa$ -coefficients  $\leq 0.60$ ) and decreased from moderate to slight for food allergens by increasing age ( $\kappa$ -coefficients: 0.46 to 0.31 to 0.16 to 0.14).

In 2013, Kim YH et al. try to confirm diagnostic value of MAST-immunoblot assay comparing it with SPT. The sensitivity, specificity, and efficiency of the MAST assay were 63.16 %, 65.57 %, and 63.92 %, respectively. Sensitivity, specificity and efficacy for common allergens were not high enough for MAST to replace skin prick test in detecting causative allergens [11].

Xiao-Dan Jiang et al. made correlation analysis of two serum-specific Immunoglobulin E test systems and skin-prick test in allergic rhinitis patients from northeast China in 2011 and show that compared with the SPT, the diagnostic indexes (accuracy, sensitivity and specificity) of the AllergyScreen system and the ImmunoCAP system (methods of ssIgE testing) were 0.819 versus 0.810, 0.780 versus 0.872, and 0.862 versus 0.741, respectively. The accuracy was similar between the two systems ( $p < 0.05$ ) [11].

Our previous study [12] shows, that the specificity of the method was high for some pollen allergens (oak – 97.6 %, birch – 96.4 %) and epidermal allergens (cat – 97.3 %), the average for some fungi (*Penicillium* – 95.4 %, *Aspergillus* – 95.2 %), epidermal (95.0 % coat dogs), but for most pollen allergens and dust mites allergens it is low (84.4 – 93.0 %). The accuracy of the method was near 100 % only for certain allergens, while most pollen allergens accuracy was at least 95 %.

In this study our aim was to check diagnostic parameters of two western-blot assays and compare them.

## Materials and Methods

88 patients with allergic rhinitis and / or asthma were included in the study to investigate the sensitivity of western-blot methods. The study was open, prospective, comparative. All patients included in the supervision group, were interviewed and signed a letter of the study participation. All the women were interviewed for possible pregnancy.

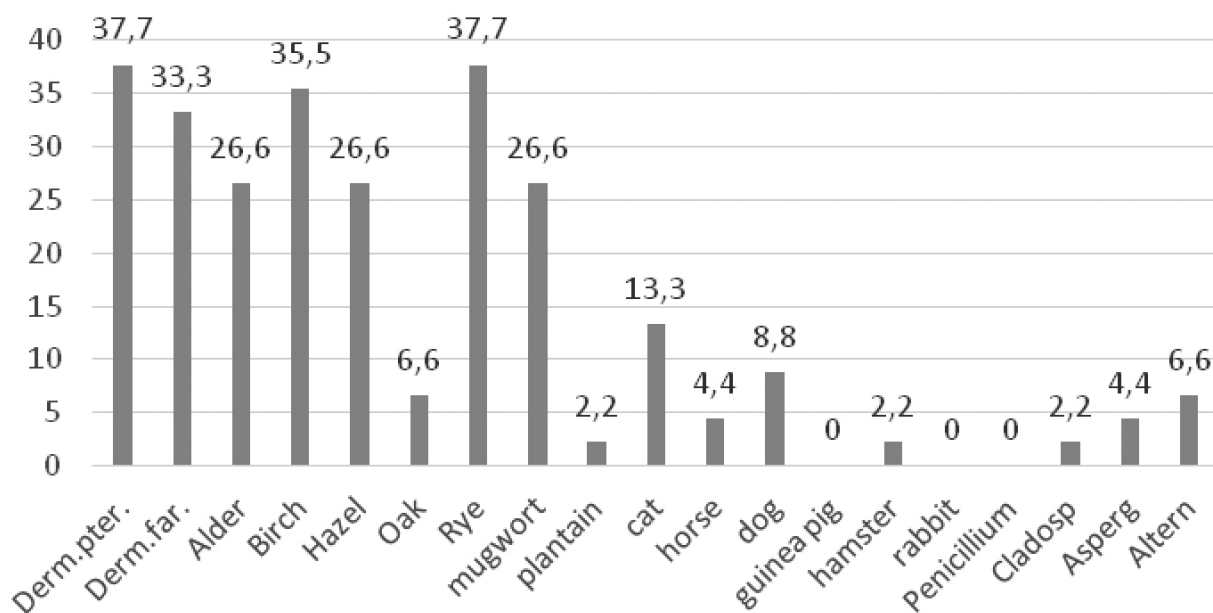
The main criteria for patient inclusion in the study were:

- age – 18 to 60 years;
- controlled intermittent asthma and mild persistent asthma according to criteria specified in GINA 2010 and Adopted clinical guidelines based on evidence “Bronchial asthma”, approved by the Ministry of Health of Ukraine № 868 of 08.10.2013;
- allergic origin of asthma (increased total IgE level in the patient's blood);
- seasonal or perennial allergic rhinitis;
- clinically significant mono- or polysensitization to allergens by results of skin prick testing with these allergens.
- Exclusion criteria were:
- age less than 18 and older than 60 years;
- uncontrolled asthma, including persistent moderate and severe;
- the presence of concomitant severe allergic disease and severe allergic reactions in history;
- the presence of concomitant severe acute and chronic somatic pathologies;
- pregnancy and breastfeeding;
- taking simultaneous participation in another clinical trial;
- patient's refusal to participate in this study.

The group of patients who were participated in this study consist of 88 patients. Among them were 46 patients (53.3 %) with allergic rhinitis and 42 patient (46.7 %) with asthma, 35.5 % of them were men and 64.5 % were women aged 18–53 years (mean age  $35.3 \pm 6.1$  years).

Complaints and medical history collection, physical examination, functional tests and patients skin tests were performed in the office of an allergist and functional dia in the Private clinics «Allergoimmunocentr KPP».

Collection of allergic history was conducted by the standard scheme predicted by determining the presence of a history of atopic dermatitis, allergic rhinitis, including «atopic march» and other possible clinical manifestations of allergic disease, details of complaints for preliminary determination of cause and significant allergen establishment of preliminary diagnosis for further laboratory and functional test. There were also collecting and detailing of hereditary anamnesis of allergic patients.



Picture 2. Sensitization frequency by skin prick testing, %.

Quantitative determination of specific IgE in serum was carried out by immunoblot RIDA® AllergyScreen (R-Biopharm AG, Germany) and Euroline (Euroimmun, Germany) on the basis of private laboratory in Private clinics Allergoimmunocentr KPP.

Standards of specific IgE concentrations ranged from 0.35 to 100 kU / l. Two hundred and fifty uL of patient serum were added to reaction wells of inhalant panel which contain 20 kinds of allergens («Respiratory allergens» – positive control, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, alder, birch, hazel, oak (pollens), mixed grass, rye (pollen), mugwort, plantain, cat, horse, dog, guinea pig, golden hamster, rabbit, *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*). After 45 minutes of incubation at room temperature and wash, 250 uL of Biotin tagged anti-IgE were added. After 45 minutes of incubation at room temperature and wash, 250 uL of streptavidin conjugate were added. Twenty minutes of incubation at room temperature and wash, 250 uL of luminescent reagent were added. After 20 minutes of incubation, results were scanned with CCD camera and interpreted as class 0 – 6. Class  $\geq 1$  was interpreted as positive. In clinical practice, allergens with results greater than that of class 2 (sIgE  $\geq 0.7$  kU/L) were considered positive.

For all comparisons between the panels Bland-Altman plot was made to evaluate correlation and systematic errors of tests. Statistical analysis was done using Statistica base 12.0 (Dell Software Company; Aliso Viejo, CA, USA).

### Results and conclusions

According to the results of skin prick testing monosensitization was found in 12 patients (26.6 % of cases). The most frequently observed sensitization was to pollen allergens (rye – 34 patients (37.7 % of cases), birch – 32 patients (35.5 % of cases), alder – 25 patients

(26.6 % of cases)) and household mites allergens (*Dermatophagoides pteronyssinus* – 33 patients (37.7 % of cases) and *Dermatophagoides farinae* – 30 patients (33.3 % of cases)). The results of skin testing of patients are shown in picture 2.

By comparing results of the immunoblot methods, some disagreement was found. To check the level of agreement, Bland-Altman plots was performed (shown on the pictures above).

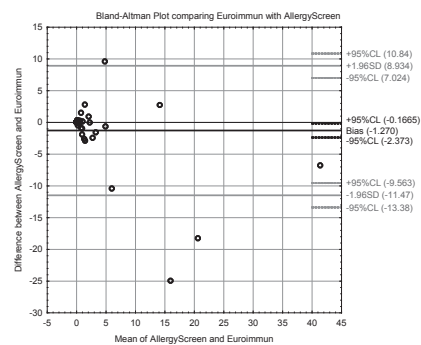
First, the systematic error of measurement results was from 0.10 ku / l to 5.6 ku / l, which indicates the presence of a small to high systematic differences between the methods results. This graphs charts the distribution of the type of absolute bias. Second, the standard deviation of the difference was from 0.51 to 2.52, which is insignificant compared to the same values. Thirdly, there is no difference depending on the number of measurements of specific IgE in the blood. Not all values are put confidence limits of  $\pm 95$  %.

By the data of this analysis, two immunoblots not always had a good agreement.

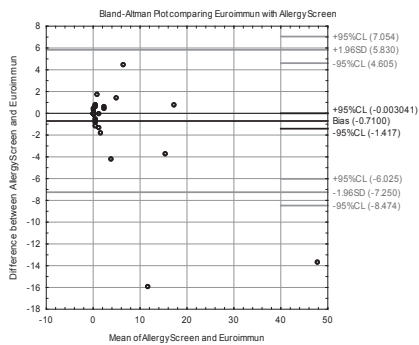
### Conclusions

The allergens that cause the allergy are the most important factors in selecting SIT as the main treatment method. The standard method for allergy diagnosis is the skin prick test (SPT), which has high sensitivity and good reproducibility. However, it has some contraindications. Various methods to measure serum-specific IgE (sIgE) have been developed to overcome these limitations, and upon development were confirmed to have good reliability and correlation with SPT. But, agreement between the data of skin prick test and immunoblot is not always good and we found some disagreement comparing results of two immunoblots. Further research this issue with the possible development of panels from Ukrainian allergens required.

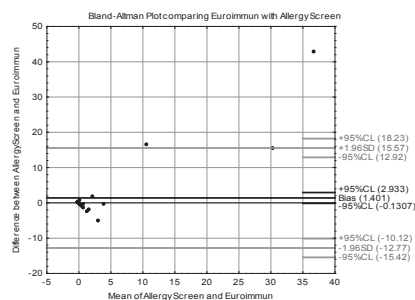
## D. Pteronissinus



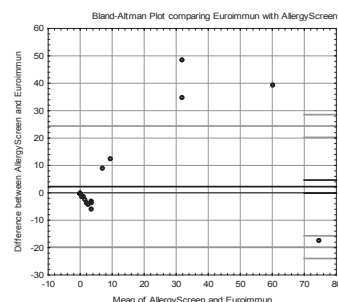
## D. Farinae



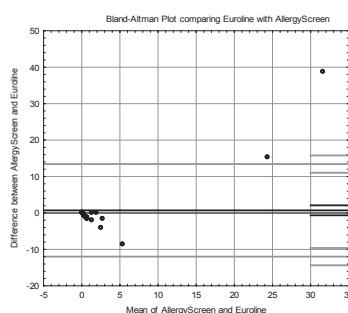
## D. Farinae



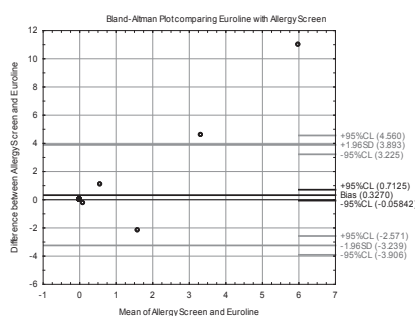
## Birch



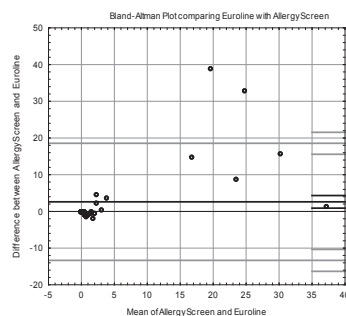
## Hazel



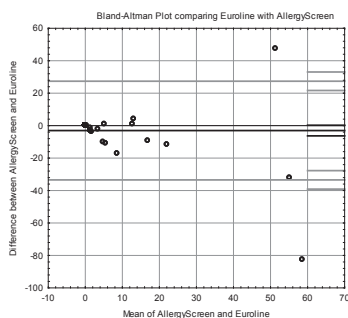
## Oak



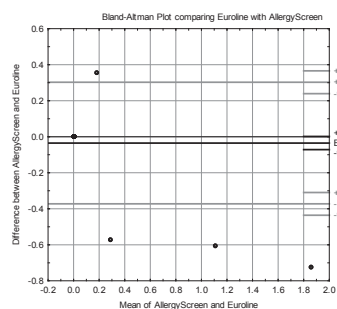
## Rye (pollen)



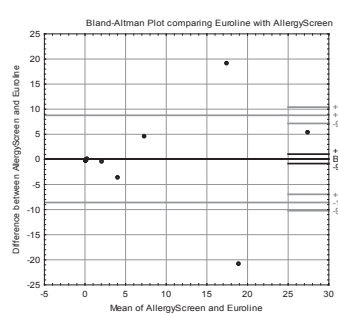
## Mugwort



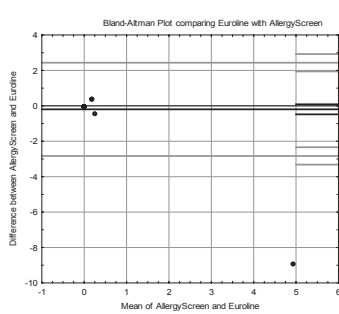
## Plantain



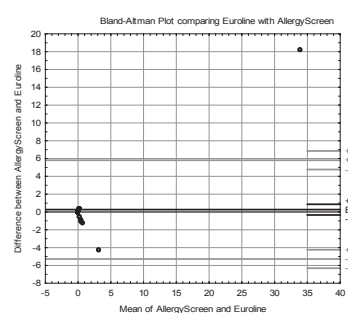
## Cat



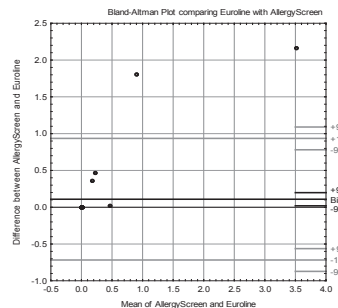
## Horse



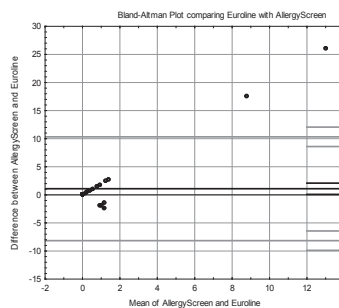
## Dog



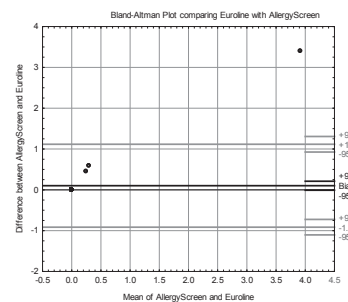
## Guinea pig



## Golden hamster



## Rabbit



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- (жито — 34 пацієнти (37,7 % випадків), береза — 32 пацієнти (35,5 % випадків) та вільха — 25 пацієнтів (26,6 % випадків) та алергенів побутових кліщів (*Dermatophagoides pteronissynus* — 33 пацієнти (37,7 % випадків) та *Dermatophagoides farinae* — 30 пацієнтів (33,3 % випадків)). При порівняльному аналізі результатів визначення специфічного IgE методом імуноблоттингу двох різних виробників визначено, що частина результатів не співпадають між собою; систематичне розходження склало 0,10 ku/l — 5,6 ku/l, що є суттєвим.

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# РАЗЛИЧИЯ В КОЖНОМ ТЕСТИРОВАНИИ МЕТОДОМ ПРИК-ТЕСТА И УРОВНЯМИ СПЕЦИФИЧЕСКОГО IGE У ПАЦИЕНТОВ С РЕСПИРАТОРНЫМИ АЛЛЕРГИЧЕСКИМИ ЗАБОЛЕВАНИЯМИ (ОБЗОР ЛИТЕРАТУРЫ И СОБСТВЕННЫЕ ДАННЫЕ)

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## Резюме

**Цель исследования** — сравнительный анализ точности количественного определения уровня специфических иммуноглобулинов E с помощью иммуноблоттинга двух разных производителей у пациентов с аллергическими заболеваниями дыхательных путей.

**Объект исследования:** 88 пациентов с аллергическим ринитом и/или атопической бронхиальной астмой с клинически значимой и подтвержденной результатами прик-тестов сенсibilизацией к исследуемым аллергенам в возрасте от 18 до 60 лет.

**Методы исследования:** клинико-anamnestический, физикальный, функциональный, кожное тестирование методом постановки прик-тестов, лабораторный, статистический.

## Результаты и их обсуждение

По результатам кожного тестирования методом прик-теста наиболее часто наблюдалась сенсibilизация к пыльцевым аллергенам (рожь — 34 пациента (37,7 % случаев), береза — 32 пациента (35,5 % случаев) и ольха — 25 пациентов (26,6 % случаев) и аллергенов бытовых клещей (*Dermatophagoides pteronissynus* — 33 пациента (37,7 % случаев) и *Deortmatophagoides farinae* — 30 пациентов (33,3 % случаев)). При сравнительном анализе результатов определения специфического IgE методом иммуноблоттинга двух разных производителей установлено, что часть результатов не совпадают между собой; систематическое расхождение составило 0,10 ku/l — 5,6 ku/l, что является существенным.

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# РОЗБІЖНІСТЬ МІЖ ШКІРНИМ ТЕСТУВАННЯМ МЕТОДОМ ПРИК-ТЕСТУ ТА РІВНЯМИ СПЕЦИФІЧНОГО IGE У ПАЦІЄНТІВ З РЕСПИРАТОРНИМИ АЛЕРГІЧНИМИ ЗАХВОРЮВАННЯМИ (ОГЛЯД ЛІТЕРАТУРИ ТА ВЛАСНІ ДАНІ)

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## Резюме

**Ціль дослідження** — порівняльний аналіз точності кількісного визначення рівня специфічних імуноглобулінів E за допомогою імуноблоттингу двох різних виробників у пацієнтів з алергічними захворюваннями дихальних шляхів.

**Об'єкт дослідження:** 88 пацієнтів з алергічним ринітом та / або атопічною бронхіальною астмою з клінічно значимою та підтвердженою результатами прик-тестів сенсibilізацією до досліджуваних алергенів, віком від 18 до 60 років.

**Методи дослідження:** клініко—anamnestичний, фізикальний, функціональний, шкірне тестування методом постановки прик-тестів, лабораторний, статистичний.

## Результати та їх обговорення

За результатами шкірного тестування методом прик-тесту найбільш часто спостерігалась сенсibilізація до пилоквих алергенів