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Short Communication

Diagnostic and analytical performance of a screening panel for allergy

Ileana Herzum[1,*], Nicole Blümer[1], Werner Kersten[2] and Harald Renz[1]

[1] Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, Marburg, Germany
[2] Institute for Environmental Medicine, Moers, Germany

Abstract

Worldwide, allergic diseases are increasing in prevalence and incidence. Early assessment of the immunoglobulin E (IgE) sensitisation status has a major impact on clinical outcome and selection of therapeutic options. Recently, a number of new IgE-detecting test systems have entered the market, including screening tests allowing identification of a wide spectrum of sensitising allergens. We evaluated the analytical and diagnostic performance of the newly developed Allergy Screen test panel for atopy (Mediwiss Analytic, Moers, Germany). The evaluation was performed for four major respiratory and four major nutritional allergens in 142 patients with clinical suspicion of respiratory and/or food allergies. For all allergens, the test showed acceptable concordance to the skin-prick test and the in vitro IgE CAP system (Pharmacia, Freiburg, Germany). The analytical performance was acceptable, with CVs between 2 and 8% in the positive range and good dilution linearity ($R = 0.9735$). Imprecision in the low IgE concentration range dramatically improved by lowering the cut-off value to 0.2 IU/mL IgE. In conclusion, the Allergy Screen panel yields reliable results in the detection of allergic sensitisation to common allergens.

Keywords: allergy; CAP classes; screening; specific IgE.

Immunoglobulin E (IgE)-mediated type-I allergies are amongst the most common causes of chronic inflammatory illnesses in industrialised nations, where they show a steadily increasing prevalence and incidence (1, 2). According to the reports of the Center for Disease Control and Prevention, the estimated prevalence of current asthma in 54 reporting areas in the USA was 7.6%, the prevalence of lifetime asthma ranged at about 11.9% (3). Especially in children, from 1980 to 1996, the prevalence increased from 3.6 to 6.2%. The high socio-economic impact is illustrated by the estimated treating costs of about 3.2 billion USD per year in the population under 18 years of age (4).

A hallmark in the diagnosis of allergic disease is the assessment of allergen-specific IgE antibodies in the blood. Several test systems have been developed for routine diagnostics, but it still remains a great challenge to provide IgE testing for as many allergens as possible, using a sample volume as low as possible. The discrimination between positive and negative test results is of great clinical importance. In this regard, the definition of the cut-off is of relevance. Until recently the cut-off of 0.35 IU/mL IgE was traditionally used, however, the relevance of an IgE value below this level must be considered as well.

It was the aim of this study to evaluate the analytical and diagnostic performance of a novel test system, the Allergy Screen (AS) test panel for atopy (Mediwiss Analytic, Moers, Germany). The test is based on a technology that passively binds 20 common respiratory and nutritional allergens to the nitrocellulose surface of the panel. Four incubation steps were required and the total test duration was 150 min. Patient serum (200 μL) was incubated on the panel under permanent shaking, followed by incubation with a biotin-plated anti-human IgE antibody. Streptavidin-conjugated alkaline phosphatase dying was performed with bromochlor-indolylphosphat/nitro blue tetrazolium. When specific IgE was present, a positive reaction appeared as a band and permitted quantification by optical density measurements. Documentation was performed by photography with a Charged Coupled Device (CCD) camera. The software (1v4, Information Manager, MATEST, Münsingen, Germany) evaluated the optical density of each band as the calculated integral surface of the peaks. The optical density was then plotted on a standard curve to determine the specific IgE concentration in the sample (Figure 1). The curve was determined as a logistical dose-effect function of optical density (Y) vs. specific Pharmacia gx4 grass pollen IgE (X, IU/mL): $Y = (A1 - A2)/(1 + (x/x0)^p) + A2$. Two different versions of the software were used, initially setting the cut-off value for positive results at 0.35 IU/mL. Due to the fact that positive results were also found below 0.35 IU/mL, a second version was developed setting the cut-off at 0.2 IU/mL.

The results were expressed as score classes identical with the CAP classes. Class 0 includes all results

*Corresponding author: Ileana Herzum, MD, Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, 35033 Marburg, Germany
Phone: +49-6421-286-5031,
E-mail: herzumi@med.uni-marburg.de

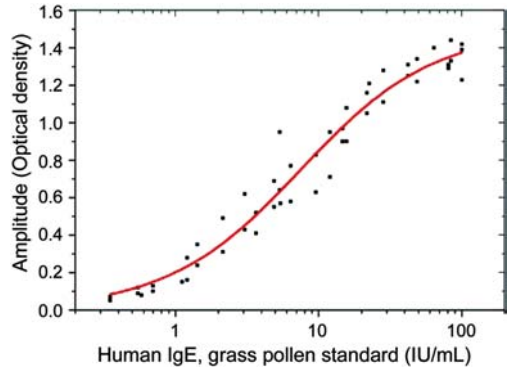

Figure 1 Standard curve of the Allergy Screen system: optical densities of the reaction bands plotted against the specific IgE concentration (CAP) in patients allergic to grass pollen.

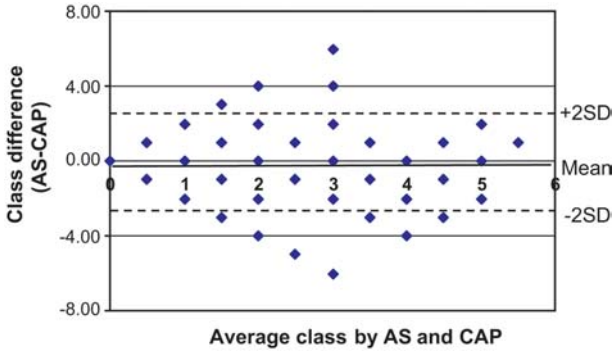

Figure 2 Bland-Altman plot showing the overall class comparison between the Allergy Screen and CAP systems.

below 0.35 IU/mL; results above 0.35 IU/mL were expressed in classes ranging from 1 to 6. For better interpretation of the results (5, 6), each class of the AS panel was further split on a linear basis into 10 subclasses, for instance class 2 ranges from 2.0 up to 2.9. Results were compared with the established Pharmacia CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden); the assay was handled as recommended by the manufacturer (7).

A total of n = 142 patients (95 men and 47 women, aged between 3 and 80 years) were referred to the pulmonary out-patient clinic with suspicion of respiratory allergies with or without accompanying food hypersensitivities. All patients were submitted to diagnostic skin-prick testing (SPT) with standard allergen solutions (HAL Allergy, Düsseldorf, Germany). A negative control with sodium chloride and a positive control with histamine were run for each patient. Wheal reactions with a diameter larger than 3 mm were considered positive.

Sufficient data to allow reliable statistical analysis were obtained for four respiratory (dermatophagoides pteronyssinus D1, dermatophagoides farinae D2, birch pollen T3 and grass pollen GX) and four nutritional allergens (whole egg F1, cow's milk F2, soybean F14 and hazelnut F17).

Overall concordance between AS and CAP was calculated as the ratio between the number of positive and negative results in both tests and the total number of samples tested (8). Overall concordance, sensitivity and specificity data are shown in Table 1. Both tests performed well, with a mean concordance of 92.15% and 87.79% as compared to the SPT, for the AS and CAP systems, respectively. The AS system showed better sensitivity than CAP for cow's milk and whole egg IgE. Therefore, there were some AS positive, CAP negative results that were positive by SPT. To compare the methods, Bland and Altman plotting (9) was used for class comparison; the results are shown in Figure 2. Dilution linearity was performed with a positive serum pool and evaluated as the regression line between measured and calculated values. Linearity, reported here for grass pollen as an example, was excellent: 13 serial dilutions, ranging from 0 to 40 IU/mL, slope 1.0487, intercept 0.13 IU/mL, R = 0.9735.

Intra-assay imprecision studies were performed for the eight allergens with human pooled sera at two different concentration classes (Table 2). A relatively high imprecision was observed in the close to cut-off range, that dramatically improved by lowering the decision cut-off to 0.2 IU/mL, using an adapted version of the software (1v14, Information Manager, MATEST, Münsingen, Germany). Imprecision improved from 38%, 32% and 22% to 11%, 20% and 13%, for hazelnut, soybean and cow milk allergens, respectively. The overall agreement between the two software versions was evaluated for n = 860 bands (20 × 43 panels tested) by regression analysis as described by Passing and Bablok (10). The results were acceptable:

Table 1 Diagnostic performance and overall concordance of the Allergy Screen atopy panel.

Allergen	Sensitivity, % (referred to SPT)	Specificity, % (referred to SPT)	Overall concordance AS–SPT, %	Overall concordance AS–CAP, %	Overall concordance CAP–SPT, %
D1 (n = 124)	97.8	83.5	88.7	93.2	89.8
D2 (n = 124)	91.8	84	87	88.4	89.6
T3 (n = 123)	98.3	87.1	92.7	92.5	94.1
GX (n = 119)	98.6	82.6	92.4	90.1	88.4
F1 (n = 30)	100	90	93.3	76.6	83.3
F2 (n = 35)	85.7	96.4	94.3	77.2	77.1
F14 (n = 19)	100	87.5	94.7	100	94.7
F17 (n = 34)	91.3	100	94.1	97	85.3
Mean	95.43	88.88	92.15	89.36	87.79

SPT, skin-prick test; AS, Allergy Screen.

Table 2 Intra-assay imprecision (n = 10).

Allergen	CV (mean class)			
	Level 1		Level 2	
	SW-1v4	SW-1v14	SW-1v4	SW-1v14
D1	3% (3.8)	4% (3.8)	8% (2.8)	9% (2.8)
D2	3% (4.1)	5% (4.1)	5% (2.1)	6% (2.1)
T3	2% (3.2)	3% (3.2)	5% (5.7)	2% (5.7)
GX	6% (4.8)	6% (4.8)	8% (5.3)	7% (5.4)
F1	3% (3.06)	4% (3.06)		
F2	33% (0.8)	7% (1.3)	22% (1.3)	13% (1.5)
F14	32% (1.1)	20% (1.3)	8% (2.5)	5% (2.6)
F17	38% (1.2)	11% (1.5)	7% (3.4)	3% (3.4)

SW, software version. Figures in parentheses: mean values.

slope 0.98, intercept 0.01 IU/mL, R = 0.9845. For class 0, the agreement was poor (R = 0.07), as many samples with class 0 by 1v4 were classified in class 1 by the more sensitive 1v14 software.

Handling of the AS assay was comfortable; assessment of 20 common allergens is obtained with only 200 μL of serum, whilst for the CAP system, a 50-μL sample volume is needed for each allergen. The definition of the classical RAST classes, with class 1 beginning at 0.35 kU/L, was set in the early 1970s and has traditionally been used since then. The presence of specific IgE antibodies reflects the sensitisation status of the patient against the respective allergen. There is currently an increasing debate about the likelihood that such IgE antibodies may also be indicative for the presence of clinically relevant allergic reactions. Along this line, it has been shown that the concentrations of specific IgE antibodies are, particularly in cases of food hypersensitivities, predictive for the presence of allergic symptoms (11–20). Further data also suggest a possible predictive value of specific IgE in cord blood for the development of atopic disease (12, 21–23). The postnatal period is normally characterised by negative IgE values, since IgE does not pass the placental barrier. To detect IgE responses to allergens at this early period in life, it is, therefore, necessary that the test system is effective to pick up even extremely low concentrations of IgE antibodies, maybe even below the traditional cut-off value of 0.35 IU/mL. To explore this possibility, we adapted the software AS system to a cut-off value of 0.2 IU/mL and found a better test performance at this lower concentration range. These results indicate that the AS test panel is a useful test system with acceptable performance in comparison to the CAP system, as well as in comparison to SPT results. Particularly in children, this test has some advantages due to the requirement of only small sample volumes and good performance in the low-IgE concentration range.

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