

Comparison of the AllergyScreen (R-Biopharm AG) with the Skin Test (HAL, Düsseldorf -in-vivo) and the CAP-System (Pharmacia, Freiburg -in-vitro).

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Summary:

Screening tests with the aid of multiallergen combinations facilitate the clarification of a wide range of allergens in the serum. Depending on the test mixture, with this method proof of sensitisation is possible not only for a single allergen, but also for a complete allergen group. These tests are also helpful in doubtful anamnestic situations, and where it is necessary to ascertain results with comparatively small quantities of serum.

Comparison of the AllergyScreen concept with the established single system of Pharmacia and the Skin Test has shown that this is a convincing method for determining a comprehensive specific sensitisation pattern in the patient. This can be achieved quickly at low cost, and with minimum material expenditure, which is not possible with single allergen determinations. The sensitivity and specificity of the system is very closely approximate to skin testing, and corresponds to a conventional single allergen system.

Data of the mean sensitivity: AllergyScreen/Skin prick test: 95.1%; AllergyScreen/CAP: 84.3%, CAP/Skin prick test: 95.8%; data of the mean specificity: AllergyScreen/Skin prick test: 80.2%, AllergyScreen/CAP: 95%; CAP/Skin prick test: 76.1%; data of the mean agreement: AllergyScreen/Skin-Prick test: 88.3%, AllergyScreen/CAP: 90.6%; CAP/Skin prick test: 87.5%

Introduction and method of procedure:

Allergy-specific laboratory tests are an indispensable part of allergiological diagnostics. In contrast to clinical test methods, they have the advantage of more exact control of sensitivity and specificity, as well as of the correctness and precision of the results. They also cause the patient less stress and, in cases of severe sensitisation, avoid any kind of risk (Kersten et al. 2000). In this connection screening concepts are becoming more and more important in allergy diagnostic methods (von Wahl et al. 1999; Kersten et al. 1998).

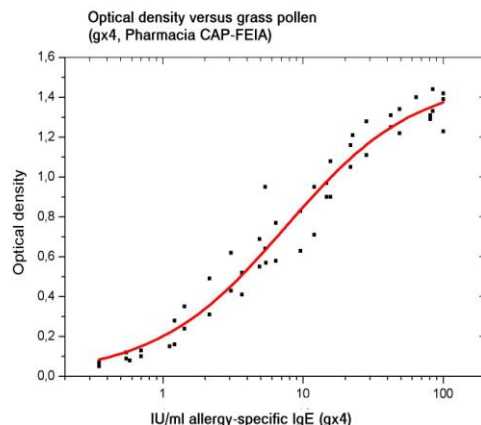
The AllergyScreen Test of the firm R-Biopharm Ag (Darmstadt) is an immunoblot for the semi-quantitative determination of circulating allergen-specific immunoglobulin E (IgE) in human serum.

The system is based on the following principle: Special allergens, which have been prepared specifically for the in-vitro diagnostic method, are bound onto the surface of nitrocellulose membranes. The allergens are applied to the nitrocellulose by means of a newly developed contact plot system. Unlike most of the other test methods, in which single allergens are covalently coupled to a matrix, here the allergens are bound passively to the nitrocellulose as an unmodified, concentrated extract. A

technique of this type is also used in the Western Blot analytical method. In the AllergyScreen System the nitrocellulose strips can take up a maximum of 20 allergens. The advantage compared with determinations in single systems is that here simple analysis of a whole range of allergens is possible in one operation, and only 250 µl serum is required for 20 allergens.

The nitrocellulose membranes are situated in a plastic reaction trough, in which all working operations are carried out one after the other. A horizontal or tilting shaker is also necessary for the operation. The patient's serum is pipetted into the reaction trough, and this is incubated at room temperature. Here the allergen-specific IgE antibodies react with the allergen, and are thus bound to the nitrocellulose membranes through the allergens. Non-bound substrate is removed by washing. This is followed by the addition of an anti-human IgE antibody coupled with biotin. This binds onto the respective specific IgE from the first incubation in the test fields. Non-bound detector antibodies are removed by washing. A streptavidin conjugated with alkaline phosphatase is then added. This binds onto the biotin from the second incubation in the test fields and onto the positive control. Non-bound streptavidin conjugate is removed by washing. After the addition of BCIP dye, an enzymatic colour reaction of the alkaline phosphatase takes place, with the formation of precipitates on the test strips in accordance with a specific reaction. The colouration is directly proportional to the content of specific antibody in the serum sample (fig. 1).

Fig. 1: Optical density of allergy lines for persons allergic to grass pollen, with known specific IgE with respect to grass pollen



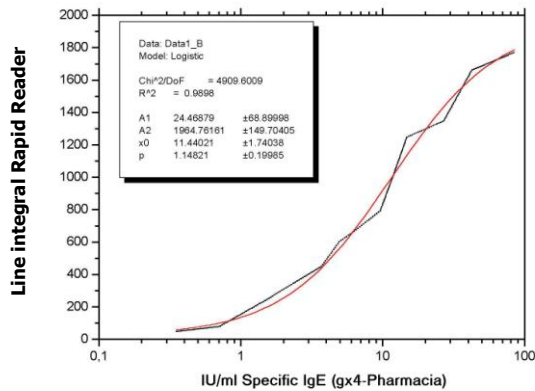
After complete drying of the test strip, evaluation is carried out by means of a camera system (Information Manager, MATEC, Münsingen). The basis of

development of the software is the digital image evaluation of Western Blot lines. For this purpose the test strips are photographed by means of a CCD camera in the system. A software programme evaluates the colouration of the allergy lines in certain expectation fields, which are fixed by the allergy lines. In addition the calculated area integral of each line of the membrane is compared with a mathematical curve fixed in the software (fig. 2). This is a logistic dose-response function from pharmacology, which is used for calculating the concentration of the specific IgE's. This function (fig. 3) serves as a calculation basis for grouping the determined density values into classes from 1 to 6.

Fig. 2: Logistic function

$$Y = - \frac{A_1 - A_2}{1 + (x/x_0)^p} + A_2$$

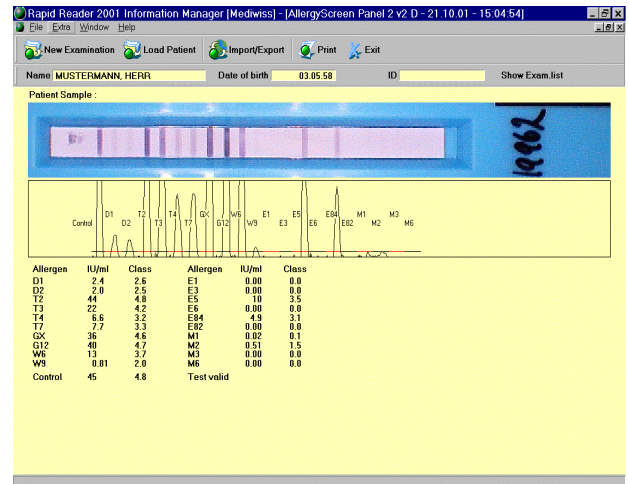
Fig. 3: Fit parameters of the logistic dose-response function for the concentration of specific IgE's with respect to grass pollen.



Interferences which are not based on an immunological reaction on the strip, are taken into consideration in the calculation by means of a "rolling disc", such as is typical for the blot analysis, and a "cut-off" value.

After measurement, a print-out gives the operator a photo of the strips, a densitometer curve of the membranes, the classes and the concentration data for each allergen band in IU/ml. Patient-specific data are stored in the system, these being documented and recallable (see fig. 4).

Fig. 4: Display of results



Study:

In an application study, 142 patients with an allergic respiratory tract disease attended the practice. These patients, for whom results had already been ascertained using the Skin Test and/or the CAP Test, were tested retrospectively using the AllergyScreen SQ Test System. In the Skin (Prick)Test the criteria of Werner & Ruppert (1979) were used as a basis, and the CAP-FEIA results with ≥ 1 were assessed as positive.

Not all allergens of the AllergyScreen System were ascertained for all patients – neither in the CAP Test nor the Skin Test. Therefore only the results corresponding to those determined either in the Skin Test and/or the CAP System were used. For this reason the numerical values determined differ from allergen to allergen.

The results for the most important inhalative allergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, birch pollen, grass pollen, mugwort, cat, horse, dog and alternaria) were then statistically compared with respect to sensitivity, specificity and precision in 4-field panels (AS= AllergyScreen, CAP= Pharmacia CAP-FEIA).

Results

Tab. 1: Dermatophagoides pteronyssinus

	Skin Test neg	Skin Test pos
AS neg	66	1
AS pos	13	44

	Skin Test neg	Skin Test pos
CAP neg	61	2
CAP pos	10	45

	CAP neg	CAP pos
AS neg	76	8
AS pos	1	47

	Skin Test-AS	Skin Test-CAP	AS-CAP
Sensitivity:	97.8	95.7	83.9
Specificity:	83.5	85.9	98.7
Precision:	88.7	89.8	93.2

Tab. 2: Dermatophagoides farinae

	Skin Test neg	Skin Test pos
AS neg	63	4
AS pos	12	45

	Skin Test neg	Skin Test pos
CAP neg	34	2
CAP pos	7	44

	CAP neg	CAP pos
AS neg	39	7
AS pos	4	45

	Skin Test AS	Skin Test-CAP	AS-CAP
Sensitivity:	91.8	95.6	86.5
Specificity:	84.0	82.9	90.7
Precision:	87.0	89.6	88.4

Tab. 3: Birch pollen

	Skin Test neg	Skin Test pos
AS neg	54	1
AS pos	8	60

	Skin Test neg	Skin Test pos
CAP neg	50	2
CAP pos	5	61

	CAP neg	CAP pos
AS neg	64	8
AS pos	2	60

	Skin Test-AS	Skin Test-CAP	AS-CAP
Sensitivity:	98.3	96.8	88.2
Specificity:	87.1	90.9	96.9
Precision:	92.7	94.1	92.5

Tab. 4: Grass pollen mixture

	Skin Test neg	Sin Test pos
AS neg	38	1
AS pos	8	72

	Skin Test neg	Skin Test pos
CAP neg	33	2
CAP pos	11	66

	CAP neg	CAP pos
AS neg	47	7
AS pos	6	72

	Skin Test-AS	Skin Test-CAP	AS-CAP
Sensitivity:	98.6	97.0	91.1
Specificity:	82.6	75.0	88.7
Precision:	92.4	88.4	90.1

Tab. 5: Mugwort pollen

	Skin Test neg	Skin Test pos
AS neg	46	3
AS pos	10	23

	Skin Test neg	Skin Test pos
CAP neg	22	0
CAP pos	8	15

	CAP neg	CAP pos
AS neg	53	1
AS pos	3	17

	Skin Test-AS	Skin Test-CAP	AS-CAP
Sensitivity:	88.5	100.0	94.4
Specificity:	82.1	73.3	94.6
Precision:	84.1	82.2	94.6

Tab. 6: Cat

	Skin Test neg	Skin Test pos
AS neg	8	1
AS pos	8	21

	Skin Test neg	Skin Test pos
CAP neg	6	1
CAP pos	5	22

	CAP neg	CAP pos
AS neg	77	8
AS pos	1	23

	Skin Test-AS	Skin Test-CAP	AS-CAP
Sensitivity	95.4	95.6	74.2
Specificity	50.0	54.5	98.7
Precision	76.3	82.3	91.7

Tab. 7: Horse

	Skin Test neg	Skin Test pos
AS neg	7	0
AS pos	2	16

	Skin Test neg	Skin Test pos
CAP neg	3	1
CAP pos	3	13

	CAP neg	CAP pos
AS neg	28	5
AS pos	4	14

	Skin Test-AS	Skin Test-CAP	AS-CAP
Sensitivity	100.0	92.8	73.7
Specificity	77.8	50.0	87.5
Precision	92.0	80.0	82.3

Tab. 8: Dog

	Skin Test neg	Skin Test pos
AS neg	15	1
AS pos	4	17

	Skin Test neg	Skin Test pos
CAP neg	12	2
CAP pos	3	17

	CAP neg	CAP pos
AS neg	68	8
AS pos	8	22

	Skin Test-AS	Skin Test-CAP	AS-CAP
Sensitivity	94.4	89.5	73.3
Specificity	78.9	80.0	89.5
Precision	86.5	85.3	84.9

Tab. 9: Alternaria alternata

	Skin Test neg	Skin Test pos
AS neg	52	1
AS pos	2	10

	Skin Test neg	Skin Test pos
CAP neg	13	0
CAP pos	1	10

	CAP neg	CAP pos
AS neg	33	1
AS pos	0	14

	Skin Test-AS	Skin Test-CAP	AS-CAP
Sensitivity	90.9	100.0	93.3
Specificity	96.3	92.8	100.0
Precision	95.4	95.8	97.9

Discussion:

In an application study of the AllergyScreen System the results of tests on 142 sera of allergic persons and non-allergic persons were determined retrospectively, and compared with results previously obtained using the Skin Test and the CAP System.

In this study rarer allergens, such as guinea pig, rabbit, hamster and moulds were not included in the evaluation, since the existing data did not give the required statistical significance for a meaningful comparison (less than 30 determinations and less than 5 proven sensitisations). For the animal epithelia only a few negative/negative determinations were recorded in a comparison of the two in-vitro systems with the Skin Test. The reason for this was that, within the scope of routine diagnosis, the individual animal epithelia had been examined using the Skin Prick Test only if this was suspected from the anamnesis.

A total of 737 data comparisons of Skin Test versus AllergyScreen, 592 comparisons of Skin Test versus CAP and 881 comparisons of CAP versus AllergyScreen was ascertained for the allergens described here.

In comparison the allergens of the AllergyScreen System showed very good sensitivity. This turned out to be even somewhat better than the CAP System in comparison with the SkinTest for the animal epithelia. The high sensitivity can certainly be attributed to the fact that the allergens on the membrane are not subject to any covalent coupling, and therefore – as in the Skin Test – are used in the unmodified form. In addition the system undergoes a reinforcing effect for each specific reaction on the membrane through the biotin-streptavidin system. This means that even the smallest traces of specific IgE's are detected, as is typical for the Western Blot in research.

Tab. 10: Sensitivity (rounded off):

	Skin Test-AS	Skin Test CAP	CAP-AS
Der. pteronyssinus	97.8	95.7	83.9
Der. farinae	91.8	95.6	86.5
Birch pollen	98.3	96.8	88.2
Grass pollen	98.6	97	91.1
Mugwort	88.5	100	94.4
Cat	95.4	95.6	74.2
Horse	100	92.8	73.7
Dog	94.4	89.5	73.3
Alternaria alternata	90.9	100	93.3
Mean value	95.1	95.8	84.3

In considering the specificity, both systems are found to be similar again in comparison. However, compared with skin testing the specificity is lower than the sensitivity. The frequent occurrence of "false" positive results for the cat is conspicuous in both systems. Since only Skin Prick Tests were carried out in the allergy diagnosis, the values for the specificity of both systems would certainly have increased through the use of the more sensitive intracutaneous test, particularly since there is very good agreement between both in-vitro methods for the cat. The same applies to the horse, for which the AllergyScreen System correlates significantly better with the Skin Test than the CAP system. It is important here to consider the frequent cross reactions of cat, horse and dog, and also between the other epithelia. These are attributable to the albumiins in the epithelia (Cabanas et al. 2000), and are more frequently detected in the AllergyScreen System than in the CAP.

Tab. 11: Specificity (rounded off)

	Skin Test-AS	Skin Test-CAP	CAP-AS
Der. pteronyssinus	83.5	85.9	98.7
Der. farinae	84.0	82.9	90.7
Birch pollen	87.1	90.9	96.9
Grass pollen	82.6	75.0	88.7
Mugwort	82.1	73.3	94.6
Cat	50.0	54.5	98.7
Horse	77.8	50.0	97.5
Dog	78.9	80.0	89.5
Alternaria alternata	96.3	92.8	100.0
Mean value	80.2	76.1	95.0

Consideration of the general agreement (neg/neg and pos/pos results) of both systems with one another and in comparison with the Skin Test again shows very good agreement. While the cat is somewhat "better" in the comparison CAP/Skin Test, for the horse a significantly better agreement in the comparison AllergyScreen/Skin Test is found. The mean values of both systems are in very close agreement with one another at 88%.

Tab. 12.: Precision/agreement (rounded off)

	Skin Test-AS	Skin Test-CAP	CAP-AS
Der. pteronyssinus	88.7	89.8	93.2
Der. farinae	87.0	89.6	88.4
Birch pollen	92.7	94.1	92.5
Grass pollen	92.4	88.4	90.1
Mugwort	84.1	82.2	94.6
Cat	76.3	82.3	91.7
Horse	92.0	80.0	82.3
Dog	86.5	85.3	84.9
Alternaria alternata	95.4	95.8	97.9
Mean value	88.3	87.5	90.6

Quick analysis using a CCD camera and extensive documentation of the results shows the AllergyScreen to be a modern, innovative semi-quantitative in-vitro system, which enables the technology of the scientific blot analysis to be transferred to routine work in the in-vitro laboratory. Through the possibility of constructing special panels for various problems and different priorities, individual solutions are now also possible for the first time using the screening concept.

In spite of the progress in in-vitro medicine, it should always be pointed out that in all test methods detection of specific antibodies in the blood only shows the existence of allergic sensitisation, but does not enable reliable conclusions to be drawn with regard to the clinical relevance of an allergic disease and its need of treatment.

Summary

Screening tests with the aid of multiallergen combinations facilitate the clarification of a wide range of allergens in the serum, and have been available on the market for a long time. Depending on the test mixture, with this method proof of sensitisation is possible not only for a single allergen, but also for a complete allergen group. These tests are also helpful in doubtful anamnestic situations, and where it is necessary to ascertain results with comparatively small quantities of serum.

Comparison of the AllergyScreen concept with the established single system of Pharmacia and the Skin Test has shown that this is a convincing method for determining a comprehensive specific sensitisation pattern in the patient. This can be achieved quickly at low cost and with minimum material expenditure, which is not possible with single allergen determinations. The sensitivity and specificity of the system is very closely approximate to skin testing, and corresponds to a conventional single allergen system.

The following mean values were calculated:

Sensitivity: Prick-Test/AllergyScreen: 95.1%; CAP-FEIA/AllergyScreen: 84.3%; Prick-Test/CAP-FEIA: 95.8%;

Specificity: Prick-Test/AllergyScreen: 80.2%, CAP-FEIA/AllergyScreen: 95%; Prick-Test/CAP: 76.1%;

Agreement: Prick-Test/AllergyScreen: 88.3%, CAP/AllergyScreen: 90.6%; Prick-Test/CAP-FEIA: 87.5%

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Übersetzung der Diagramme:

Fig. 1:

Optical density versus grass pollen (gx4, Pharmacia CAP-
FEIA)

Amplitude
(optical density)

IU/ml allergy-specific IgE (gx4)

Fig. 3:

Line integral Rapid Reader

IU/ml specific IgE (gx4-Pharmacia)