Clinical evidence of a Reversed Enzyme Immuno Assay and an Allergy Lateral Flow Assay for the quantitative determination of specific IgE to different allergens

Nadine Offermann[1], Sven Quist[2], Margrit Fooke[1]

[1]*Dr. Fooke-Achterrath Laboratorien GmbH, Neuss, Germany*

[2]*Dermatologie Quist, Haifa-Allee 20, 55128 Mainz, Germany*

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Background: Type I reactions are caused by allergen specific immunoglobulin E (sIgE) and thus sIgE represents a marker for modern allergy diagnosis. The specific IgE REAST (Reversed Enzyme Allergo Sorbent Test) and ALFA (Allergy Lateral Flow Assay) are intended for the quantitative determination of sIgE in human serum or plasma. The use of a special scanner system provides the opportunity of quantitative interpretation of ALFA results within 20 minutes. Consequently, the REAST and ALFA systems are able to detect sIgE concentrations between 0.35 IU/mL and 100 IU/mL.
The objective of the study is the evaluation of REAST and ALFA for the quantitative interpretation of sIgE compared with the clinical documentation regarding type I hypersensitivity.

Methods: Agreement between REAST/ALFA (Dr. Fooke-Achterrath Laboratorien GmbH) and Skin Prick Test (SPT) / anamnesis was investigated using 100 serum samples tested for specific IgE to Dermatophagoides pteronyssinus (d1), Dermatophagoides farinae (d2), cat dander (e1), dog dander (e5), peanut (f13), hazelnut (f17), Alternaria alternata (m6), mugwort (w6) and different grass and tree pollens. Receiver Operating Characteristic (ROC) analysis were performed for every single allergen separately and for all allergens together.
Additionally, recombinant allergens from Timothy Grass and Birch were tested and compared to the results of the corresponding allergen extracts.

A)

Allergen code	Allergen name
d1	Dermatophagoides pteronyssinus
d2	Dermatophagoides farinae
e1	Cat dander
e5	Dog dander
f13	Peanut
f17	Hazelnut
m6	Alternaria alternata
g6	Timothy Grass
RG607	Timothy Grass (Phl p 7)
RG620	Timothy Grass (Phl p 1 / Phl p 5)
g12	Rye
t2	Alder
t3	Birch
RT301	Birch (Bet v 1)
RT302	Birch (Bet v 2)
RT304	Birch (Bet v 4)
t4	Hazelnut (pollens)
w6	Mugwort

B)

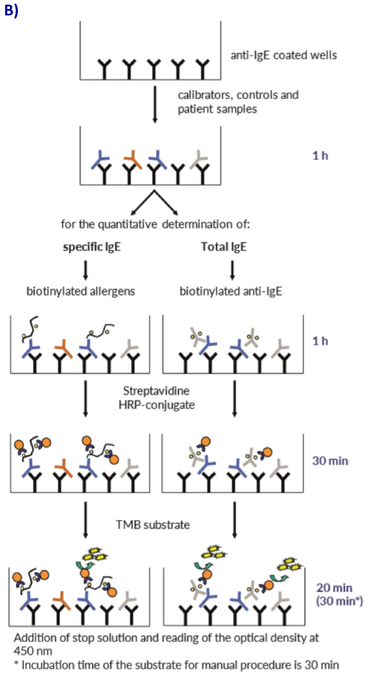
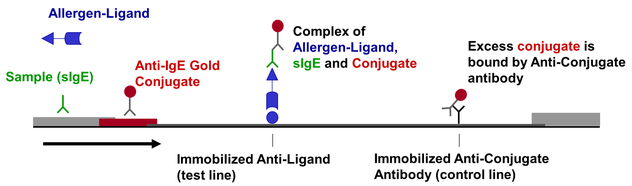


Figure 1 A) List of allergens
b) Test principle REAST

A



B

C

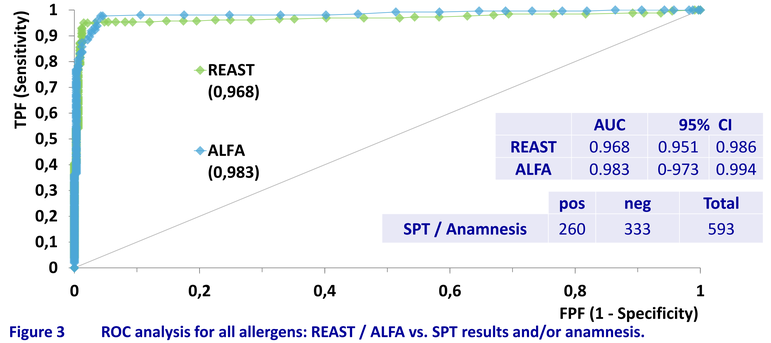
Figure 2 A) Test principle ALFA
B) Single-strip cassette (positive result)
C) Lateral flow assay reader

Results and findings: Excellent agreements were observed between REAST/ALFA results and SPT results and/or anamnesis. Area under the curve (AUC) values of both in-vitro systems compared to SPT and/or anamnesis were found > 0.90. Compared to SPT and/or anamnesis REAST and ALFA show a sensitivity and specificity > 0.8 for all tested allergens. The number of positive results was not sufficient to perform ROC analysis for f13 and f17. Only two positive SPT results were available for f13 and f17. A patient has a confirmed hazelnut allergy.

	Test	Sensitivity	Specificity	AUC	Number positive results	Number negative results
d1	ALFA	0.85	0.93	0.94	26	29
	REAST	0.89	0.90	0.90		
d2	ALFA	1.00	0.89	0.99	28	26
	REAST	0.93	0.96	0.97		
e1	ALFA	1.00	0.92	0.98	14	36
	REAST	0.86	1.00	0.95		
e5	ALFA	1.00	0.93	0.99	8	29
	REAST	0.85	1.00	0.90		
m6	ALFA	1.00	0.98	0.99	6	47
	REAST	1.00	1.00	1.00		
g6	ALFA	1.00	0.97	0.99	37	38
	REAST	1.00	1.00	1.00		
g12	ALFA	1.00	0.93	0.99	39	15
	REAST	0.95	1.00	0.99		
t2	ALFA	1.00	0.95	0.99	27	21
	REAST	1.00	1.00	1.00		
t3	ALFA	1.00	1.00	1.00	39	21
	REAST	0.97	1.00	0.99		
t4	ALFA	0.96	1.00	0.98	26	25
	REAST	1.00	1.00	1.00		
w6	ALFA	1.00	1.00	1.00	5	41
	REAST	1.00	1.00	1.00		

Table 1 AUC values, sensitivity and specificity for all allergens

Area under the curve (AUC) values for all allergens, including f13 and f17, were found at 0.968 for REAST and 0.983 for ALFA compared to SPT results and/or anamnesis. A sensitivity and specificity of 0.93 and 0.99 for REAST and 0.98 and 0.96 for ALFA were observed.



	AUC	95% CI	
REAST	0.968	0.951	0.986
ALFA	0.983	0-973	0.994

	pos	neg	Total
SPT / Anamnesis	260	333	593

Figure 3 ROC analysis for all allergens: REAST / ALFA vs. SPT results and/or anamnesis.

Figure 4 shows the distribution of reactions against recombinant allergens from Timothy Grass (g6) and birch pollens (t3). Samples from patients with a confirmed diagnosis were tested to different recombinant allergens. Samples from patients with a Timothy Grass allergy were also tested to Phl p 7 (RG607, minor allergen) and to a mixture of Phl p 1 und Phl p 5 (RG620, major allergens). Samples from patients with a Birch allergy were also tested to Bet v 1 (RT301, major allergen) and to two minor allergens (Bet v 2: RT302 and Bet v 4:RT304).
Figure 4 indicates that most of the serum samples react with the major allergens of Timothy Grass and Birch pollens.

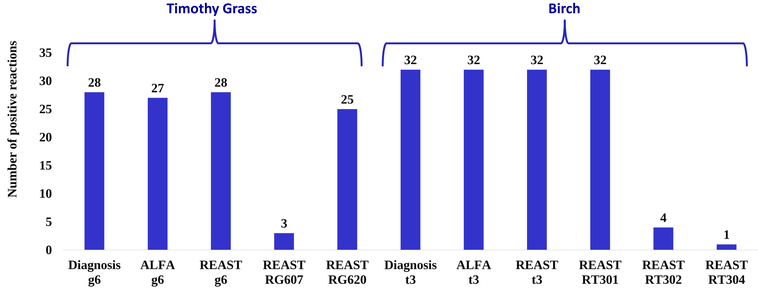


Figure 4 Distribution of reactions against recombinant allergens from Timothy Grass and birch pollens

Conclusion: For the detection of sIgE, REAST and ALFA show high sensitivity and specificity when compared to the clinical documentation regarding allergy. AUCs of >0.90 indicate an excellent agreement between REAST/ALFA and the anamnesis for different allergens. The correlation for REAST versus ALFA is also comparable with spearman's rho > 0.85 for all tested allergens.
Additional testing of recombinant allergens aids to identify possible cross reactions.

In relation to this presentation, we declare that there are no conflicts of interest

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Dr. Fooke-Achterrath Laboratorien GmbH – Habichtweg 16 – 41468 Neuss – Germany
Phone: +49-2131-29840 – Fax: +49-2131-2984-184 – Internet: www.fooke-labs.de – email: information@fooke-labs.de