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Detection of allergen-specific IgE using the ALLERG-O-LIQ System based on the Reversed-enzyme-allergo-sorbent-test

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Background: Allergen-specific IgE (sIgE) causes histamine release of mast cells and basophils and thus plays an important role in the pathogenesis of type I allergies. Beside patient's case history, physical examination and skin prick testing, *in-vitro* tests for the detection of IgE are mandatory for allergy diagnosis. For the determination of the sIgE and total IgE concentration in human serum a high number of commercial test systems is available. Most commercial assay systems use immobilized allergens in combination with an anti-IgE enzyme conjugate as reporter system. In contrast, the ALLERG-O-LIQ System follows the reversed-enzyme allergo-sorbent test (REAST) protocol using anti-IgE coated microtiter plates in combination with biotinylated allergens and streptavidin HRP as detection method. This assay architecture results in two purification steps during the assay procedure, namely the purification of the IgE fraction from the patient's samples and the enrichment of IgE binding proteins from the extract (see Figure 1).

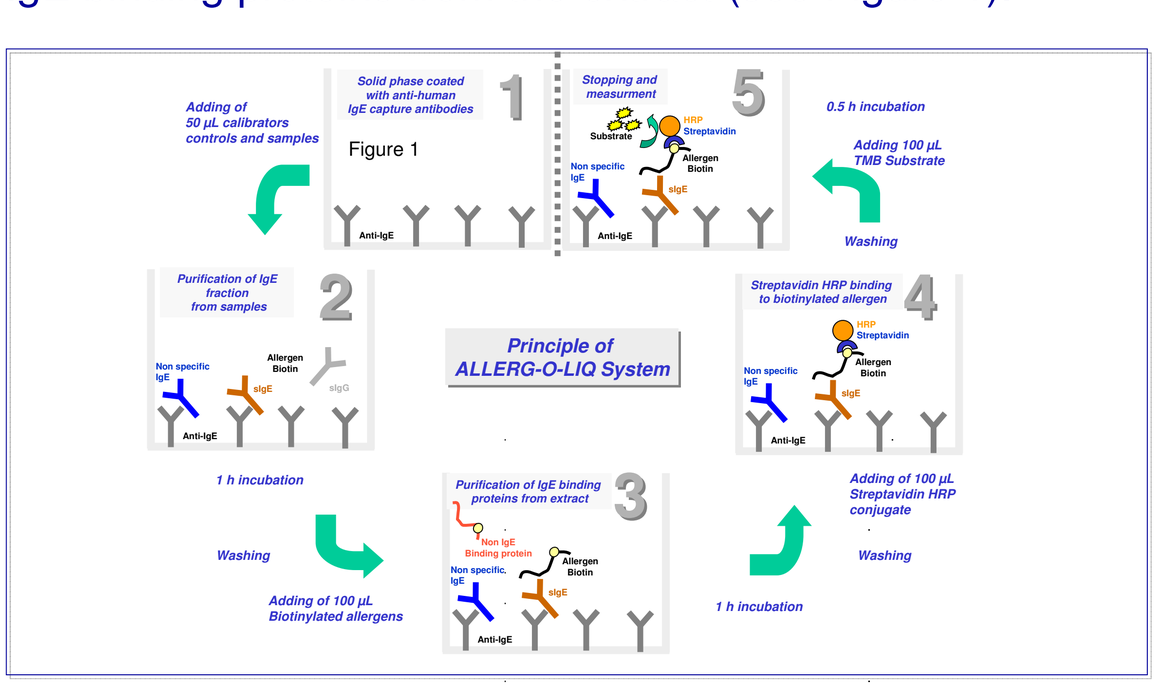

Figure 1
Principle of the ALLERG-O-LIQ System. First, patient samples, calibrators and controls (50 µL each) are added to wells of an anti-IgE coated microtiterplate. During an incubation time of 1 h, the IgE fraction of the samples is immobilized on the surface. The subsequent washing step removes all other serum components including IgG which may interfere with the specific IgE-allergen interaction. Afterwards biotinylated allergens are added and incubated for 1 h. IgE binding proteins are immobilized in the presence of allergen specific IgE on the surface. Unbound allergens are removed by washing followed by adding of the streptavidin HRP conjugate and another washing step. Finally, substrate is added to the wells and after 30 min of incubation the reaction is stopped and optical densities are photometrically determined at 450 nm wavelength (reference filter 620 nm).

Recent studies have compared the ALLERG-O-LIQ System with Skin Prick Test (SPT) and with ImmunoCAP® (Phadia) showing a high degree of concordance for most allergens. Several factors such as the presence of specific IgG, the affinity of the specific IgE and the total IgE content may influence the results of certain specific IgE assays.

Moreover, the affinity of specific IgE and the density of specific IgE in relation to the total IgE concentration have been discussed as important factors which play an important role in the pathogenesis of type I allergy. This presentation summarizes all important studies on REAST compared to assays based on the classical protocol for the detection of specific IgE.

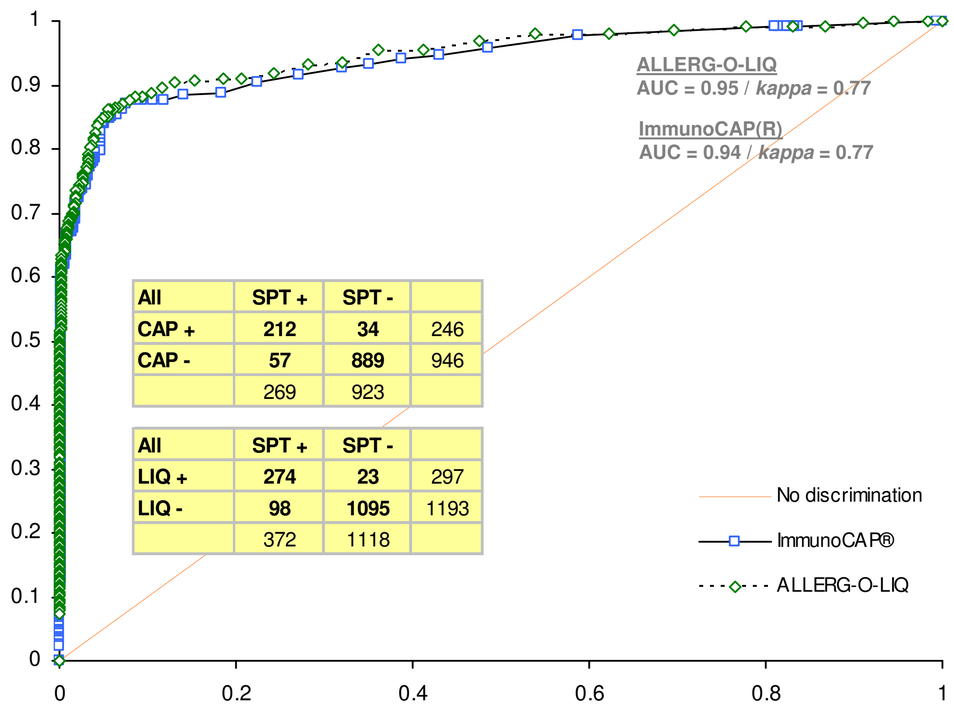

Figure 2
Comparative Receiver Operating (ROC) analysis ALLERG-O-LIQ and ImmunoCAP® vs. skin prick test (SPT).

Table 1 Qualitative agreement between three methods according to Chi-square and *kappa* test (including confidence interval, CI)

	SPT no. pos (%)	ALLERG-O-LIQ no. pos (%)	ImmunoCAP no. pos (%)	ALLERG-O-LIQ vs. SPT		ImmunoCAP vs. SPT	
	(n=149)	(n=150)	(n=150)	%	Kappa	%	Kappa
g6	68 (46.0)	62 (41.3)	61 (40.7)	92.0	0.84	94.6	0.89
g12	61 (41.0)	55 (36.7)	n.d.	92.0	0.83	n.d.	n.d.
t3	59 (40.0)	48 (32.0)	49 (32.7)	89.3	0.81	93.3	0.86
m6	12 (8.1)	11 (7.3)	12 (8.0)	96.7	0.76	96.0	0.73
w6	29 (19.0)	17 (11.3)	32 (21.3)	91.3	0.59	86.6	0.58
d1	45 (30.0)	34 (22.7)	43 (28.7)	87.3	0.68	87.2	0.69
d2	42 (28.0)	33 (22.0)	n.d.	91.3	0.77	n.d.	n.d.
e1	41 (27.5)	20 (13.3)	31 (20.7)	86.0	0.58	91.9	0.79
e2	14 (9.4)	15 (10.0)	10 (6.7)	94.0	0.66	91.9	0.46
m3	1 (0.7)	2 (1.3)	5 (3.3)	99.3	n.d.	97.4	0.73

Table 2 Overview of technical and clinical studies on the ALLERG-O-LIQ System

No.	Title	Result / conclusion	Reference
1	Allergen-sepcific IgE-values to inhalant and food allergens – Comparison between two commercial immunoassays: Dr. Fooke ALLERG-O-LIQ versus Pharmacia CAP-System.	Good to excellent correlation between ALLERG-O-LIQ and ImmunoCAP®; *Spearman's rho* values: t3 (0.95), g6 (0.90), w6 (0.82), d1 (0.85), e1 (0.87), e5 (0.82), f1 (0.84), f2 (0.60), f4 (0.60), f14 (0.31), f17 (0.68), f49 (0.82)	Kleine-Tebbe et al.
2	Technical and clinical evaluation of a fully automated Reversed Enzyme Allergo Sorbent Test (REAST) using liquid biotinylated allergens.	Clinical sensitivity of ALLERO-O-LIQ/ImmunoCAP(R) for i1 (70%/80%) and i3 (98%/80%)	Duyan et al.
3	Comparison between skin prick test and a reversed enzyme allergo-sorbent test	Good to excellent correlation between ALLERG-O-LIQ and SPT	Mahler et al.
4	Allergen-Specifi c IgE to Inhalant and Food Allergens and Total IgE Values in China: Comparison of 2 Commercial Immunoassays	Good to excellent correlation between ALLERG-O-LIQ and ImmunoCAP®; *kappa* values: d1 (0.9), d2 (1.0), d5 (0.8), e1 (0.9), e5 (0.6), i6 (0.79), m3 (0.89), f1 (0.6), f2 (0.9), f23 (n.d.), f24 (n.d.)	Sun et al.

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