## Measurements of allergen specific antibodies during allergen specific immunotherapy using the evanescent field method: A comparison

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Currently, allergen specific immunotherapy (SIT) represents the only curative treatment approach for allergic diseases. Increasing doses of an allergen are applied to an allergic patient with the aim to induce peripheral T-cell tolerance and a shift from a TH2 to a TH1/Treg-biased immune response. The aim of this study is the development of diagnostic assays for the monitoring of changes in levels of allergen-specific antibodies during SIT using the evanescent field technology.

The EVA-biosensor is a near patient testing device that allows fast and quantitative measurements of minute amounts of biomolecules with reduced wash and incubation steps, compared with standard ELISA methods.

In an EVA-Assay, the major allergen responsible for cat allergy, Fel d1, was immobilized on the surface of a polysterene EVA-biosensor chip. The detection of Fel d1 specific IgE in human serum was achieved by a human anti IgE antibody labeled with fluorescent Allophycocyanine (APC). The detection limit of this assay is 0.17 kUA/L Fel d1 specific IgE and as sensitive as an ELISA (LOD=0.14 kUA/L) which was performed with the same components. Serums from allergic patients were measured by EVA, ELISA, and ImmunoCapTM and the results obtained with the three detection systems showed significant correlations.

In a second example of SIT, serum samples from 8 different Mugwort pollen allergic patients undergoing SIT were also measured in a direct assay, using the major allergen Art v1. APC labeled anti human IgE or anti human IgG4 served as detection antibodies. During the first months of SIT an increase and in a later stage a decrease of Art v1 specific IgE were observed. The levels of Art v1 specific IgG4 starts to increase after the first month of SIT. These data results in a decrease of the Art v1 specific IgE/IgG4 ratio, commonly accepted as one indicator of successful SIT.