Microarray – Food - Screen

Microarray for the qualitative determination of specific IgG4 to food antigens in humanem Serum or Plasma

Microarray Specifications

- High sample throughput
  - 96 patients with up to 96 allergens in one ELISA Plate
- Reduced reagent and sample consumption
  - 96 times reduced reagents
  - only 10µL sample material necessary
- Reduced working time due to parallel handling of 96 samples

Immunoglobulins (Ig) of the IgG4-subclass seem to play an important role in the humoral immune reaction to food antigens. Symptoms similar to those mediated by IgE but no IgE antibodies were found have been shown for instance in IBS (Irritable Bowel Syndrome). Instead, specific IgG4 antibodies to food antigens could be detected. It was shown that IBS patients experience a significant improvement in symptoms in response to a "food-specific IgG4"-guided exclusion diet. Serum IgG4 antibody testing may provide an objective and quick method for selecting an exclusion diet for treating such patients.
In so far the measurement of food specific IgG4 antibodies seems to get more importance.

The principle of the test is based on an Enzyme Linked Immuno Sorbent Assay (ELISA) combined with a Microarray technique.
Each well of a microplate is spotted with 96 food antigens. Diluted patient's serum or plasma is incubated in the antigen-spotted well. The specific IgG4 from the patient's sample bind to the solid phase coupled antigens. After addition of anti-IgG4-Enzyme-Conjugate a solid phase bound antigen/IgG4/anti-IgG4-HRP-complex is formed. The amount of bound antibody corresponds to the amount of peroxidase in the respective well and is detected by incubation with precipitating Tetra-methyl-benzidine (TMB) Substrate resulting in the development of blue coloured antigen-spots.

Microarray	REF	10106-M	96

After a washing step with distilled water the optical density of each spot is measured by a special Microarray Scanner. A Calibration curve is run simultaneously with the serum samples and the concentration of the food-specific IgG4 antibodies is read by a special Microarray Reader from the standard curve. The concentrations are directly proportional to the intensity of the coloured spot within the measuring range.

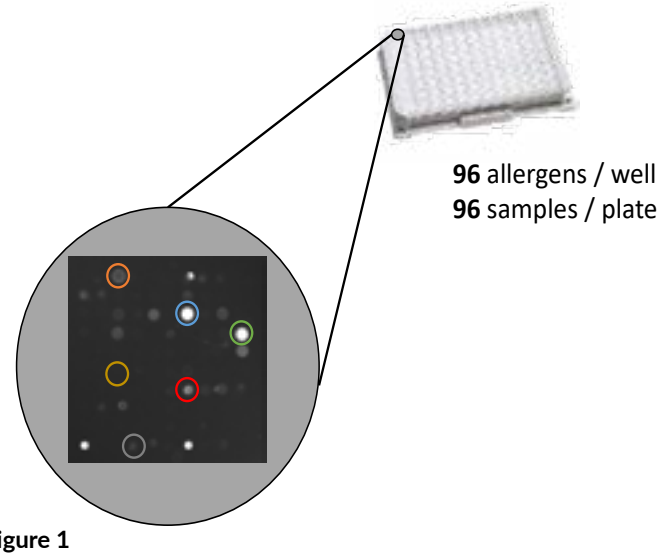

Figure 1
Microarray System

Figure 2
Spotted microtiterplate

Dr. Fooke-Achterrath Laboratorien GmbH - Habichtweg 16 - 41468 Neuss - Germany
Phone: + 49 2131 2984-0 - Fax: + 49 2131 2984-184 - email: information@fooke-labs.de

Performance data

The intra-assay, inter-assay and lot-to-lot variations were found within the following specifications:

Intra-Assay Variation	< 15%
Inter-Assay Variation	< 25%
Inter-Batch Variation	< 30%

Evaluation of the results

Using the calibration curve the concentrations of samples are calculated and evaluated as follows:

- < 2000 U/mL IgG4 Foods can be eaten without limitation
- > 2000 < 5000 U/mL IgG4 Foods allowed once or twice per week, alternating
- > 5000 U/mL IgG4 Avoid Foods for at least 3 months

The interpretation of the results has to be done individually for each patient and in consultancy with a medical doctor or professional nutritionist!

Diagnostical sensitivity and specificity

The results of the assay have always to be considered in the context of the clinical situation of the patient and his anamnesis.
In an evaluation, the overall sensitivity and specificity (compared to a commercial available ELISA for the determination of food specific IgG4) were each determined as 94%.

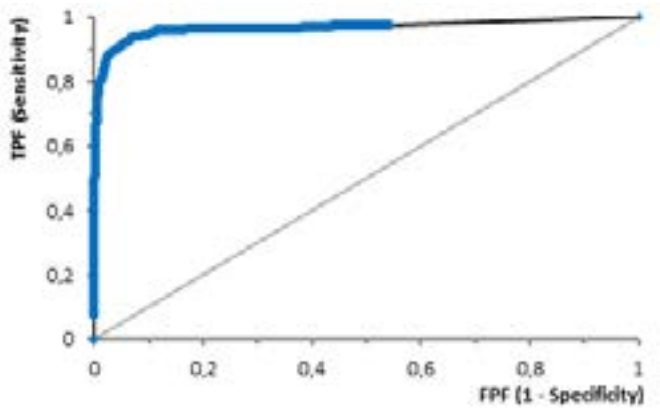

Figure 3
ROC analysis for Microarray Food Screen (Dr. Fooke Laboratorien GmbH) vs. a commercial available ELISA with n=3009 results.

Literature

1. Jarvinen KM, Chatchatee P, Bardina L, Beyer K, Sampson HA: IgE and IgG Binding Epitopes on alpha-Lactalbumin and beta-Lactoglobulin in Cow´s Milk Allergy. Int Arch Allergy Immunol 2001, 126:111-118.
2. Chatchatee P, Jarvinen KM, Bardina L, Vila L, Beyer K, Sampson HA: Identification of IgE- and IgG-binding epitopes on alpha(s1)-casein: Differences in patients with persistent and transient cow´s milk allergy. J Allergy Clin Immunol 2001, 107:379-383.
3. Kruszewski J, Raczka A, Klos M, Wiktor-Jedrzejczak W: High Serum Levels of Allergen Specific IgG-4 (asIgG-4) for Common Food Allergens in Healthy Blood Donors. Archivum Immunologiae et Therapiae Experimentalis 1994, 42:259-261.
4. Zar S, Mincher L, Benson MJ, Kumar D: Food-specific IgG4 antibody-guided exclusion diet improves symptoms and rectal compliance in irritable bowel syndrome. Scand J Gastroenterol 2005, 40:800-807.
5. Zar S, Benson MJ, Kumar D: Food-specific serum IgG4 and IgE titers to common food antigens in irritable bowel syndrome. Am J Gastroenterol 2005, 100:1550-1557.
6. Noh G, Ahn HS, Cho NY, Lee S, Oh JW: The clinical significance of food specific IgE/OgG4 in food specific atopic dermatitis. Pediatr Allergy Immunol 2007, 18: 63-70

DR FOOKE

Dr. Fooke-Achterrath Laboratorien GmbH - Habichtweg 16 - 41468 Neuss - Germany
Phone: + 49 2131 2984-0 - Fax: + 49 2131 2984-184 - email: information@fooke-labs.de