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AllergyScreen™

Circular letter 3

Knowledge and know how in allergy diagnosis

Food allergies caused by immunological cross-reactions

Cross-reactions of IgE antibodies are based on the reactivity to homologous structures, which are similar to the allergen inducing the IgE response.
Structures of proteins with the same function and from different organisms can be highly conserved and cross-reactive, even if they show a low phylogenetic relationship. Examples are the profilines, which are involved in the polymerization of actin in many eukaryotic cells, or the tropomyosines, the regulators of myocontraction.
In many cases clinical relevant cross-reactions of proteins with an aminoacid sequence identity of less than 50% cannot be expected. But this can be disproved by the finding that, for example, the main allergens Api g 1 of celery and Dau c 1 of carrot show less than 40% sequence identity with Bet v 1 of birch pollen.
Therefore, it is assumed that in individual cases clinical relevant cross-reactions between such lowly related proteins can occur.

Characteristics of the in-vitro diagnosis

On the one hand, the sensitivity of plant allergens is very different because of the allergen instability. On the other hand, positive antibody values due to cross-sensitization are often not clinical relevant.
In case of a systemic reaction against foods, the specific IgE antibodies should be determined.

In the evaluation of sensitizations against cross-reacting allergens a clinical relevant cross-reaction and a sensitization can be distinguished. It is assumed that sensitizations against cross-reacting allergens using skin tests or/and antibody detections are more frequently as they show clinical symptoms. Also from the degree of skin reaction and level of antibodies you can only draw limited conclusions about an apparent food allergy.

The immunological detection of a cross-reaction doesn't need to be associated with a clinical manifestation. One important reason for this is could be the presence of specific IgE against carbohydrate components in the allergen extracts.

Crossreactive Carbohydrate determinants (according Aalberse *et al.* (1997)):

Non-specific binding of IgE in the in-vitrotest is particularly relevant when the total IgE is very high. On the other hand a multireactivity could also be the impression of independent sensitization to many different allergens.

Some cases of cross-reactivity are predictable because the antigens are closely related (f.e. animal epithelia). Other cases of cross-reactivity were unexpected f.e. the cross-reaction between profilin in pollen and vegetable foods. Other studies suggested that IgE in these cases lacks the specificity - normally associated with antibody-antigen reaction. Extensive investigations of this type of cross-reactivity indicated that the specific IgE has it's specificity, but the antigenic determinant is not specific. This ubiquitous antigenic determinant consists of sugars and glycoproteins.

In food (esp. vegetables) there will be three possibilities of interpretations:
1.) Food: with reaction to specific IgE antibodies
2.) Food: with induction of allergic reactions
3.) Food: with induction of an allergic sensitization

For a "correct" allergen all of these 3 things have to be fulfilled. If pos. 2 and 3 is not fulfilled we can describe the allergen as a "nonelicitor".

A "nonelicitor" (usually, but not necessarily, non-sensitizing) reacts with specific IgE on the mast cell, but this interaction does not trigger the degranulation. The reason for this lack of mast cell stimulation is unclear, but it might be related to monovalency of the allergen. One of the dogmas in allergy is that an allergen has to bind via at least two epitopes in order to crosslink two specific IgE antibodies at the mast cell surface. A monovalent allergen will bind only a single IgE antibody and so this will not result to a IgE receptor crosslinking and mast cell stimulation.

Many foods have carbohydrate determinants (CCD) which can react with specific IgE. The CCD's are more stable in relation to heat or gastrointestinal enzymes. In in-vitro tests the CCD structure stands out prominently and may mask clinical more relevant peptide structures.
Anti-CCD IgE tend to be highly cross-reactive; consequently a serum that has these antibodies will be positive in in-vitro tests for a very wide range of vegetables. Most of these vegetables are usually well-tolerated by patients with specific-IgE against CCD's.

To analyse if CCD's are responsible for the positive in-vitro test we need CCD's without any relevant protein backbone.

Therefore we will present a membrane with CCD's in the near future with the possibility to check the specific binding against these glycoproteins if there are f.e. food positive results in the in-vitro test without any clinical manifestation.

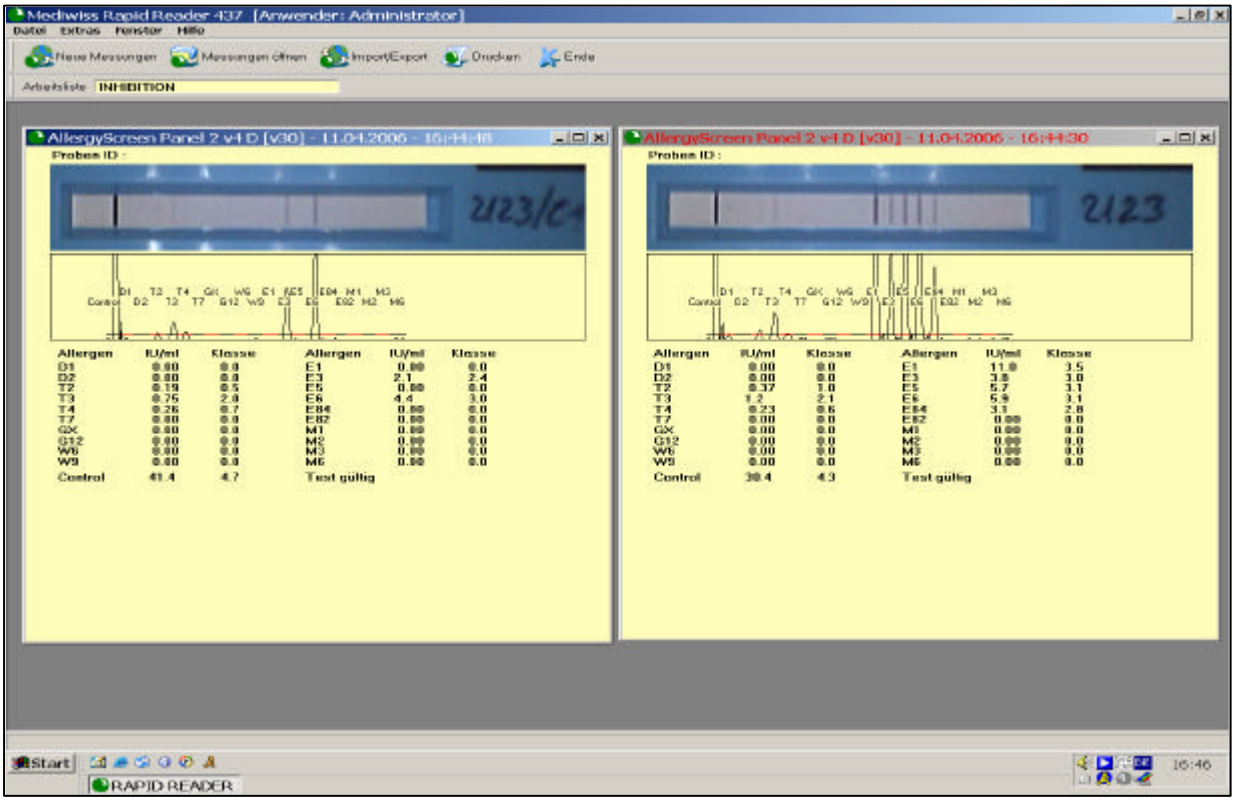
This membrane will have Bromelain, horseradish peroxidase and ascorbate oxidase – glycoproteins with a different amount of carboyhydrate chains. An elevated result for these glycoproteins can indicate the presence of a high titer of anti-CCD IgE in the serum.

Cross-reaction between mammalian proteins:

Investigations showed that serum albumin is a common allergen in pet allergy. It can also be demonstrated that these albumins from mammalian animals have an extremely high cross-reaction to each other. So it is suggested that patients sensitized by one pet species are likely to react to several other different animals epithelia. Furthermore it was demonstrated that serum albumin is also in the milk and this could lead to a positive reaction with milk if there is an animal sensitization and also inverted.

To verify a cross-reaction you can perform an inhibition test.

As an example we performed an inhibition with a cat sensitized patient with several other reactions to animals in the AllergyScreen (right picture) with a reported clinical manifestation against cat and guinea pig. For this 200 µl serum and 50 µl cat extract (concentration: 2 mg/ml cat extract) were incubated for 12 hours at room temperature.
After incubation the AllergyScreen test was performed according to the instructions of use. Both results (before and after inhibition) were measured in the RapidReader.



Result after inhibition with cat extract Result before inhibition with cat extract

You can see that the inhibition process has no influence to the light sensitization against tree pollen. But the inhibition with cat extract block 100% the cat specific antibodies in the serum – but also the dog and the hamster specific IgE-antibodies –

as a result of the cross reaction to the cat allergen. Also the horse specific antibodies are blocked but not 100%

Only horse and guinea pig specific IgE antibodies are present after the inhibition – expressed as a non cross-reactive, separate sensitization.

You can do this also in the laboratory routine to be sure that the developed allergen line is really specific to the measured allergen. A positive inhibition test is always of unequivocal evidence for the specificity of a test.

Inhibition allergens can be purchased by us for laboratory use on demand!

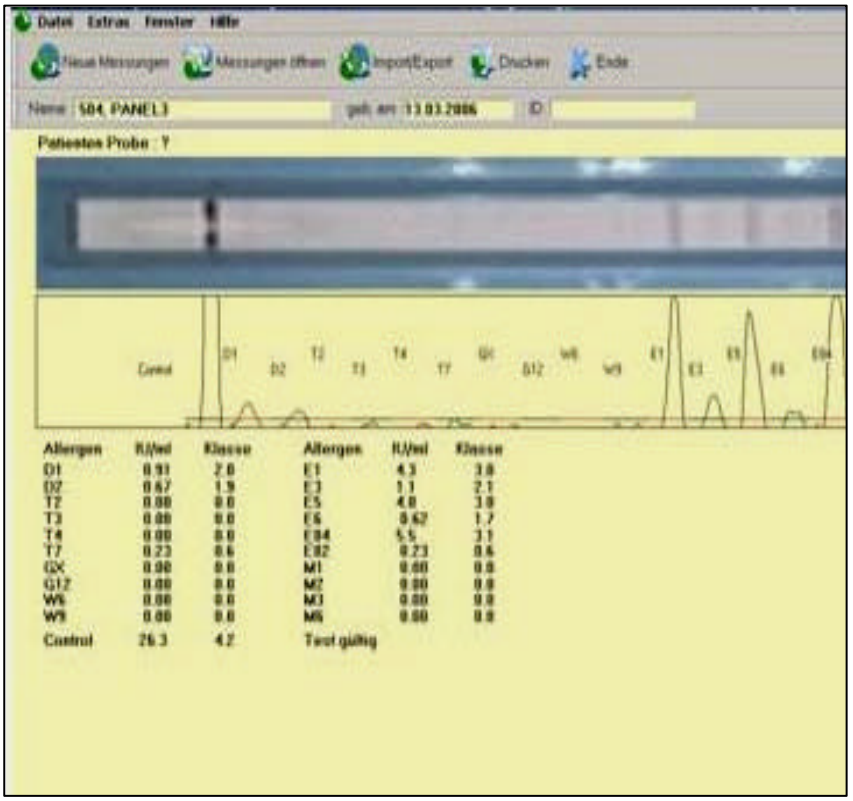
White bubbles on the membrane after processing?

Sometimes in the front or at the end of the plastic trough a long unprocessed area is seen on the membrane which is white (see picture)

What's the reason for this? It's an indicator that there was not enough reagents present during all the incubation times or the liquids could not reach all parts of the membrane.

Then happens following: by adhesion and cohesion the liquids in the trough migrate to the sides of the trough and are responsible for a good development of the lines.

But when the liquids cannot reach the middle of the membrane – there will be a white shade (see picture).



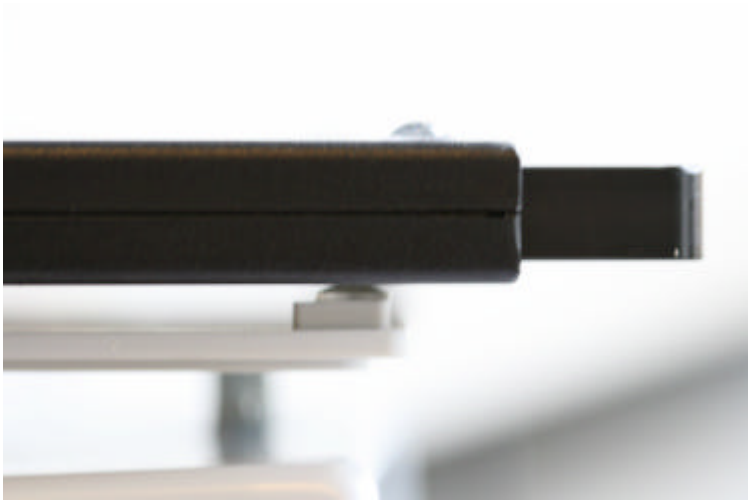
1.) It could happen when there is not enough liquid in the trough (also by spilling out the liquids).

2.) It could happen if the plastic troughs are placed not even in the incubation comb (see picture)

3.) It could happen if the black incubation box is not positioned even on the shaker (the rubber points of the box must be upside – for fixing a second incubation box).

false

correct



So, please always check that the incubation box is positioned correctly on the shaker and that the plastic troughs are placed in the comb in one line.