Adverse effect of CCD reactions on IgE in vitro testing.

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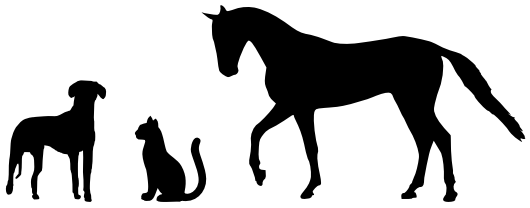
Its impact on the allergen selection for immunotherapy.

Heska AG
Switzerland



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The case of interpreting multi positive pollen results in *in vitro* tests

Family	Allergen	Result	Class
Grasses	Phleum pratense	880	++++
	Dactylis glomerata	898	++++
	Poa annua	635	++++
	Lolium perenne	879	++++
	Festuca pratensis	922	++++
Weeds	Rumex acetosella	897	++++
	Plantago lanceolata	631	++++
	Artemisia vulgaris	139	+++
	Ambrosia artemisifolia	516	++++
Trees	Alnus sp.	131	+++
	Quercus sp.	204	++++
	Betula populifolia	87	+++

High frequency of multi-positive pollen results on *in vitro* tests which are not verified in intradermal tests.

Multi-positive pollen results complicate interpretation and in most cases lead to an incorrect allergen selection for immunotherapy.

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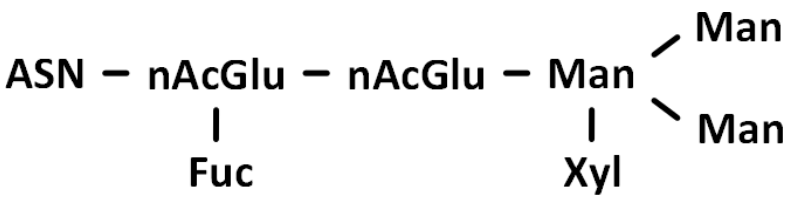
Plant glycosilation (CCD's)



The common glycosylation pattern of plant proteins produces carbohydrate structures, commonly known as CCD's which are responsible for plant multi-positive results in *in vitro* tests.

Animals react against the CCD structures

CCD's elicit specific IgE in allergic and non-allergic patients. Their incidence varies between 15 to 40% depending on the animal species. Plant carbohydrates are the most abundant allergens found in nature.

It has not been proven that the presence of anti-CCD specific IgE gives rise to clinical symptoms.

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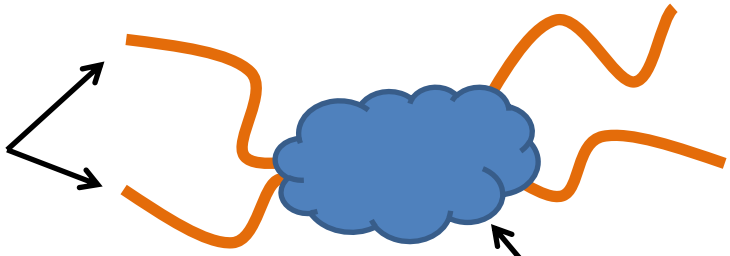
Pollen allergens used in ELISA based tests carry the CCD structures

The pollen allergen extracts used to manufacture *in vitro* IgE tests, are extracts from natural sources and therefore they all contain the generic plant n-linked carbohydrates (CCDs).

Allergen structure (protein + carbohydrate)

Common CCD motives are found in different plant pollens (N-glycans)



Allergen (proteins) are structures specific to each allergen

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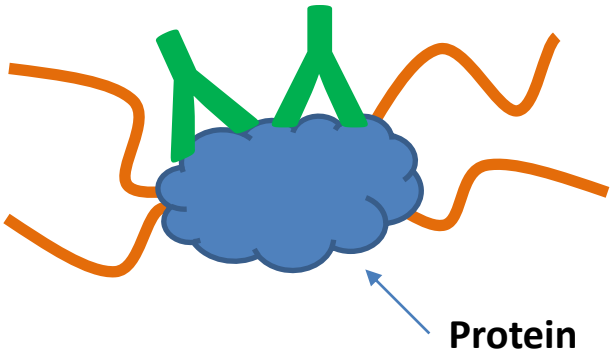
Allergen Specific IgE REACTION
represented by the green antibody

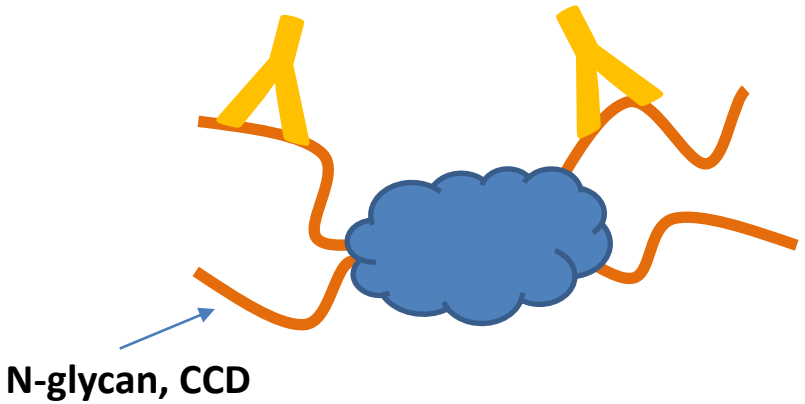


Allergen specific IgE binds to the protein chain of the allergen.

Proteins are allergen specific, the reaction is individual.

clinically RELEVANT

IgE anti-CCD REACTION
represented by the orange antibody



In the CCD reaction, IgE binds to the carbohydrates of the allergen.

Since the same carbohydrates are shared by most plant allergens, the result is a multi positive reaction.

clinically IRRELEVANT

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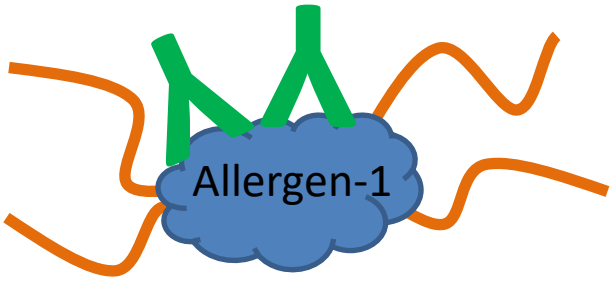
Allergen specific reaction
One antibody recognizes one protein (allergen specific)

CCD specific reactions
One antibody recognizes the CCD present in multiple allergens

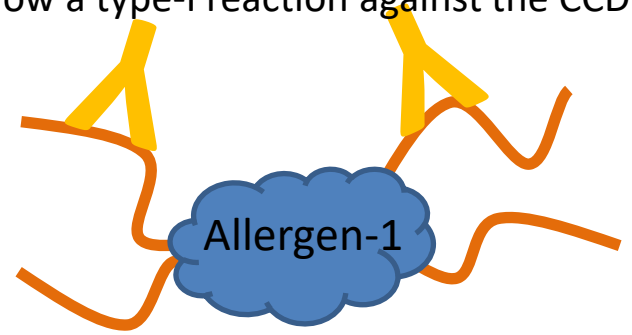
The Heska CHO test, identifies the patients that show a type-I reaction against the CCD motives.



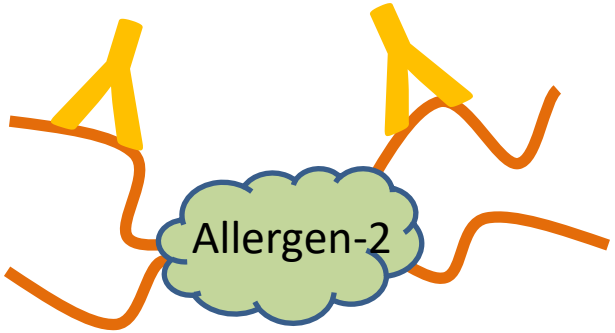
Positive

Negative

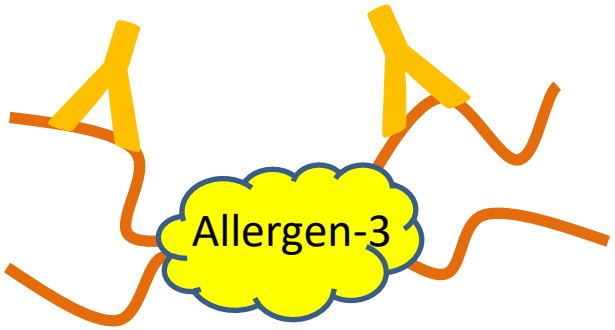
Negative



Positive



Positive



Positive

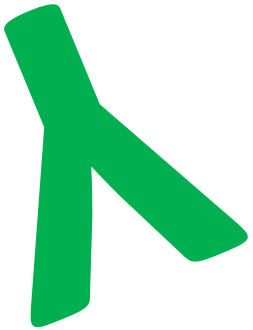
Is the CCD reaction predictable?

Yes. Before the ALLERCEPT panel test is made, the Heska CHO test allows identifying the samples presenting significant levels of IgE anti-CCD motives.

Is the CCD effect controllable?

A Heska proprietary IgE anti-CCD blocker, prevents the binding to CCD-carbohydrates in the ALLERCEPT test.

Allergen specific IgE does not recognize the CCD blocker molecule



CCD blocker molecule

IgE anti-CCD is neutralized by the CCD blocker

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Example of blocked and unblocked test result on an IgE anti-CCD positive patient

		UNBLOCKED			BLOCKED		
Family	Allergen	Result	Class	ASIT	Blocked	Class	ASIT
Grasses	Phleum pratense	595	++++	Yes	570	++++	Yes
	Dactylis glomerata	1010	++++	Yes	1105	++++	Yes
	Poa annua	398	++++	Yes	119	+++	Yes
	Lolium perenne	939	++++	Yes	942	++++	Yes
	Festuca pratensis	1027	++++	Yes	1054	++++	Yes
Weeds	Rumex acetosella	984	++++	Yes	8	-	
	Plantago lanceolata	460	++++	Yes	0	-	
	Artemisia vulgaris	65	+++	Yes	0	-	
	Ambrosia artemisif.	188	++++	Yes	2	-	
Trees	Alnus sp.	22	+	Yes	8	-	
	Quercus sp.	66	+++	Yes	4	-	
	Betula populifolia	60	+++	Yes	59	+++	Yes

The reactions against grasses are confirmed to be true allergen reactions. In the weed pollen family the results are CCD reactions.

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The use of the Heska's proprietary CCD blocker addresses the multi positive reactivity commonly observed on IgE tests.

Reliable allergen specific IgE measurements can thus be made.

In immunotherapy, accurate allergen selection is an essential element required for the successful control of the allergic patient.

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