

**"A new development in IgE serum testing.**

**-**

**Its impact on the allergen selection for  
immunotherapy."**

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Switzerland

# Foreword

An unexpected number of multi-positive results on IgE tests have caught our attention.

We tried to understand and identify the potential causes of this phenomenon. The results of our investigations will be presented here.

The reasons for this phenomenon are quite complex. We will try to describe them in the most simple way.

The phenomenon identified is general; **ALL** *in vitro* IgE tests are affected by it.

Our motivation has been to provide clinicians with a reliable approach to allergen selection for ASIT.

# Quick review: why specific IgE is measured ?

Allergen specific IgE in allergic patients is measured with the purpose to identify the allergen(s) the pet is hypersensitive to.

## **Helping in the allergen selection for an ASIT treatment.**

The efficacy of an ASIT treatment is directly related to the precision of the allergen selection.

The selection has to be made based on IDS or in vitro test results and after the clinical diagnosis of atopy has been made.

## A difficulty in *in vitro* IgE testing: the «multi-positive» plant results.

<b>Family</b>	<b>Allergen</b>	<b>Result</b>	<b>Class</b>
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	+++

# Concerns on the «multi-positive» plant results.

## Laboratory side

Are the results technically correct ?

Do they indicate the true amount of allergen specific IgE ?

## Clinical side

Do the results correlate to the clinical findings and sensitization status of the pet ?

How to interpret them ?

How to use them in the allergen selection for an ASIT ?

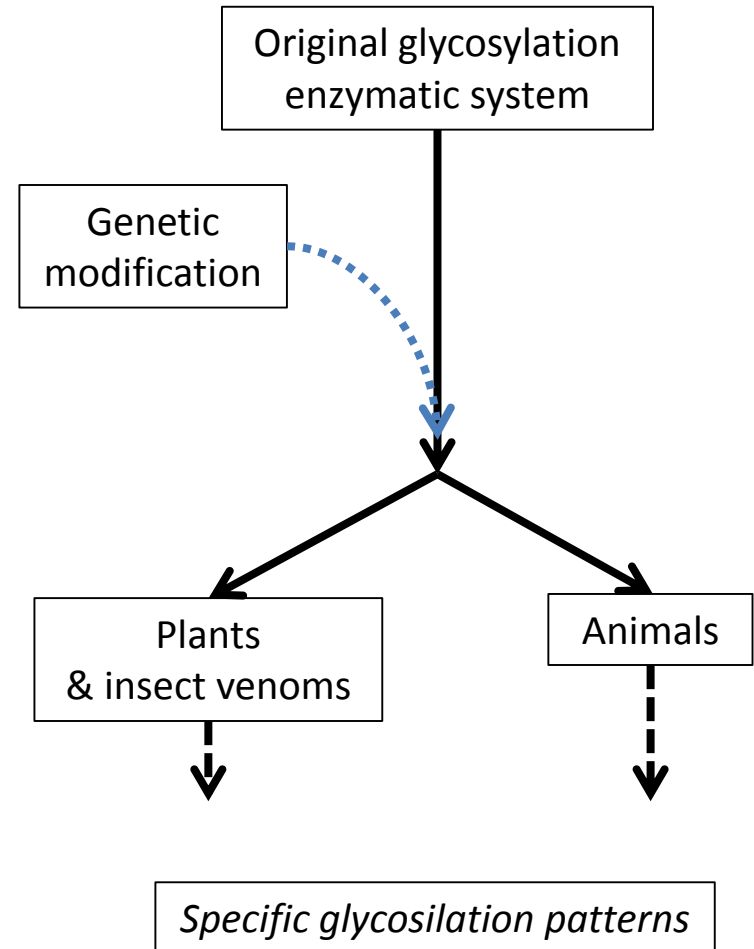
# The legitimate question

Family	Allergen	Result	Class
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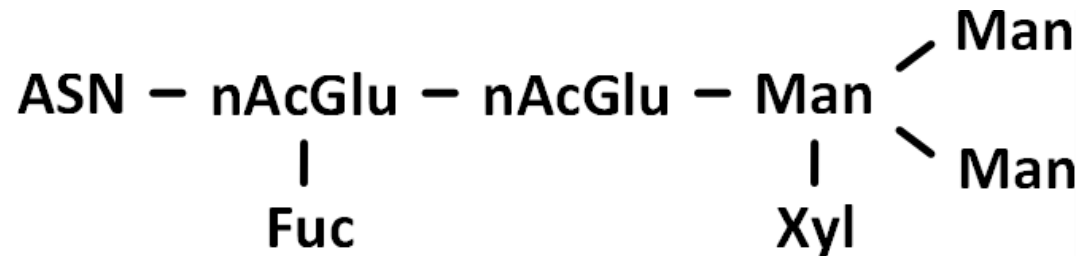
**Is this occurrence understood ?**

# The cause of this occurrence lies in the particular carbohydrate structures of plants

- During evolution, the plant and insect venoms enzymatic system responsible for attaching sugars to proteins underwent a genetic modification.
- This change resulted in the production of distinct glycosylation enzymes for plants and animals.
- In 1970 the structure of the plant carbohydrates was elucidated.
- It was confirmed that plant specific carbohydrate structures were not found in animals.



# Plant carbohydrate structure (CHO's)



On N-linked oligosaccharides, sugars are attached to the aminoacid asparagine (ASN).

These CHO structures, have in common a 5 sugar core consisting of two *N*-acetylglucosamine and three mannose residues.

Additional sugars could be attached to this core to form a great variety of oligosaccharide patterns found in plant glycoproteins.



# Plant carbohydrates may produce a special reaction in animals

Plant CHO structures may **elicit a Type-I reaction** (CHO specific IgE) in a certain proportion of allergic and non-allergic animals.

The presence of CHO specific IgE in serum of the animals does not give rise to observable clinical symptoms.

They appear to be clinically irrelevant.

# Prevalence of positive CHO specific IgE in animals

**It is estimated that 1/3 of clinically diagnosed allergic dogs show CHO specific IgE.**

In terms of potential sensitization, plant carbohydrates are present everywhere in very high amounts.

It is the most abundant allergen found in nature. They are unavoidable, animals are permanently exposed to them.

These allergens are present airborne, in food and are part of the living environment.

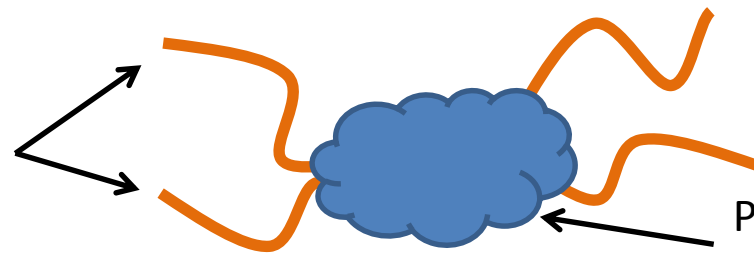
# Plant allergen extracts used in *in vitro* tests contain CHO's

Plant allergenic extracts are crude extracts prepared from natural sources.

They are heterogeneous preparations which contain a variety of different proteins.

Plant proteins are glycosylated (glycoproteins) and carry the CHO structures.

Similar CHO chains are found in different plant glycoproteins.

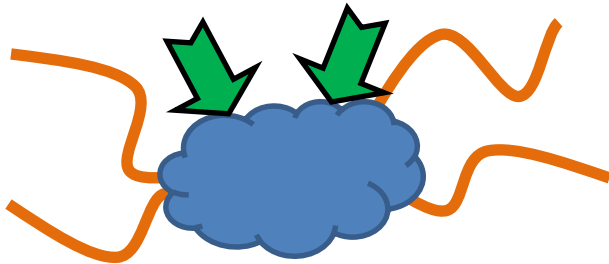


Protein are allergen-specific structures.

# Animals can produce IgE (Type-I reaction) against allergen proteins and carbohydrate structures

## ALLERGIC REACTION

Allergen specific IgE (green arrows)



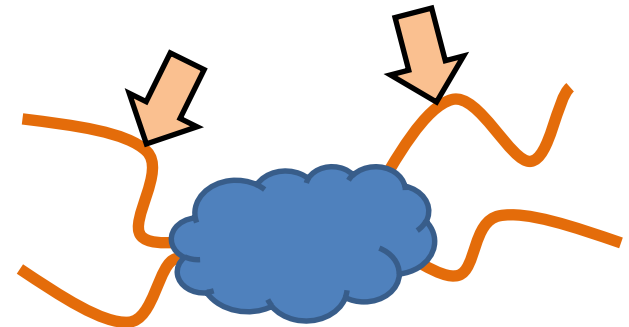
Each allergen is composed by unique proteins.

Each allergen specific IgE molecule will react to a single protein = single allergen.

**CLINICALLY RELEVANT**

## CHO SPECIAL REACTION

CHO specific IgE (orange arrows)

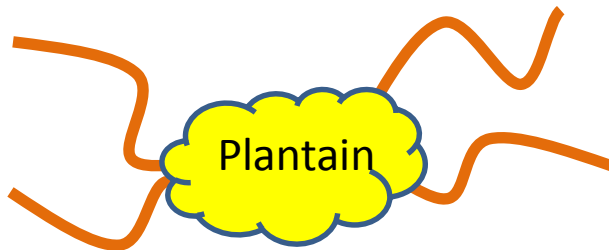
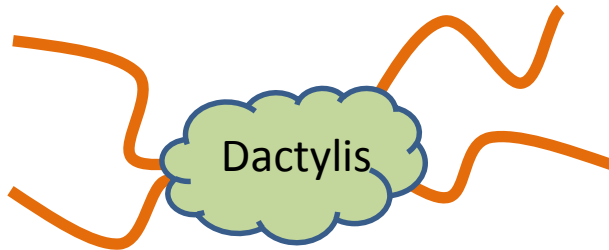
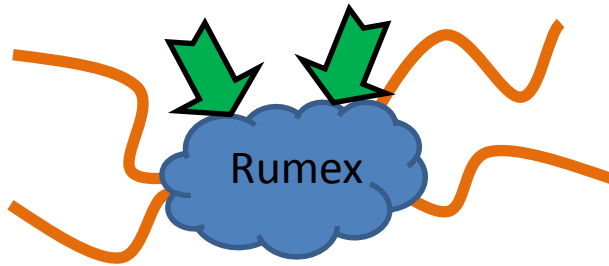


CHO's are common structures of plant glycoproteins.

Each CHO specific IgE will react to most plant allergens.

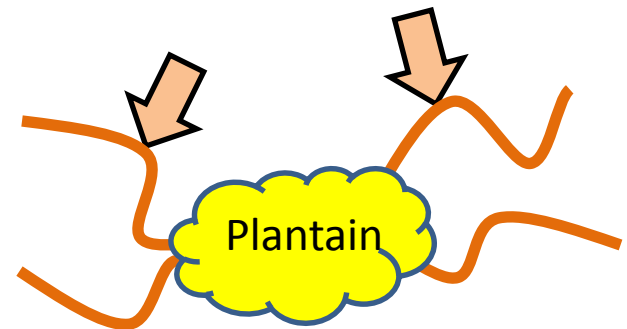
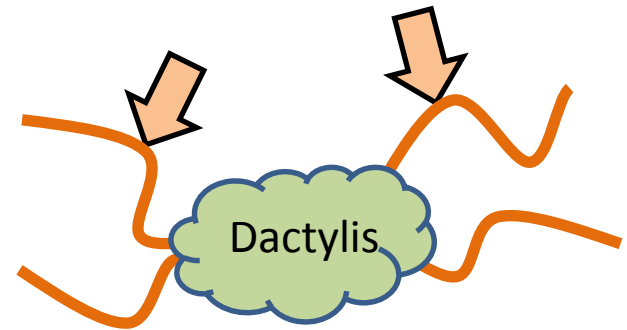
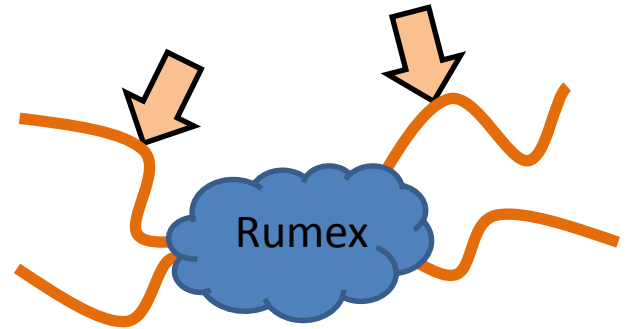
**CLINICALLY IRRELEVANT**

# ALLERGIC REACTION



***CLINICALLY RELEVANT***

# CHO SPECIAL REACTION



***CLINICALLY IRRELEVANT***

# The «multi-positive» plant results...

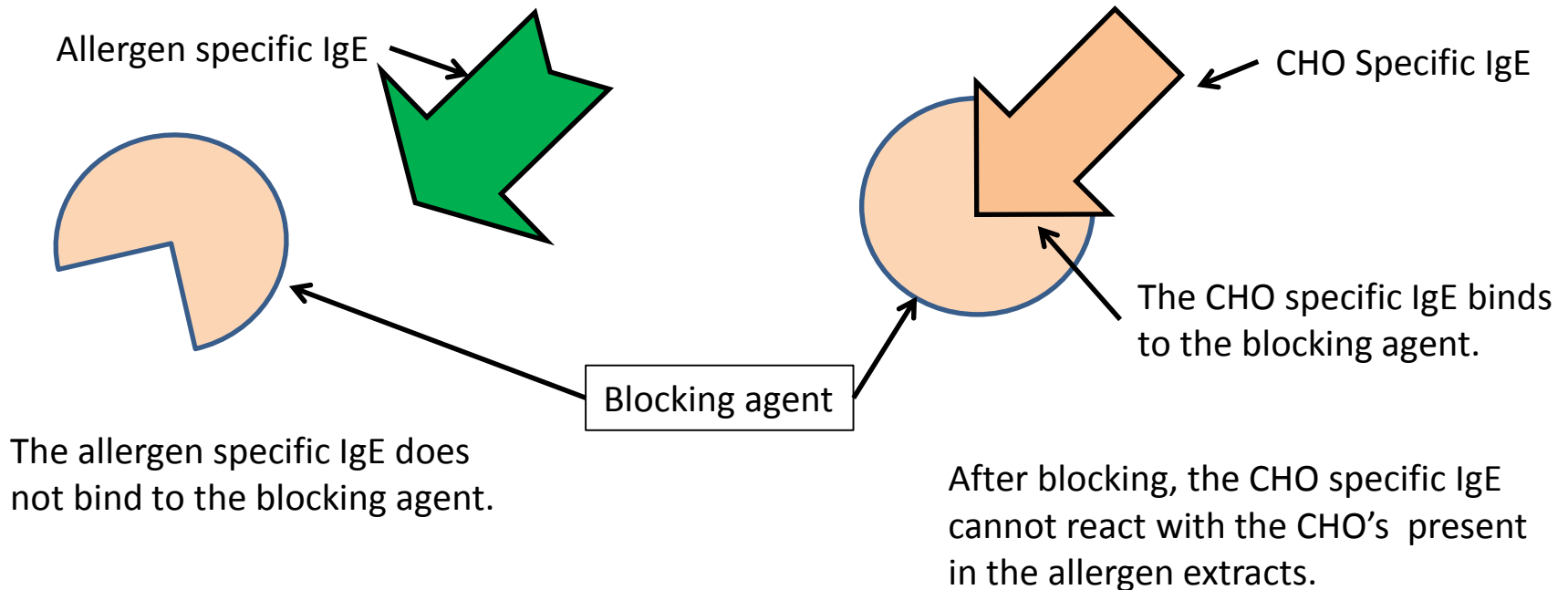
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Weeds	Rumex acetosella	897	++++
	Plantago lanceolata	631	++++
	Artemisia vulgaris	139	+++
	Ambrosia artemisifolia	516	++++
Trees	Alnus sp.	131	+++
	Quercus sp.	204	++++
	Betula populifolia	87	+++

**Is this occurrence controllable ?**

# The control of «multi-positive» plant reactions. The blocking agent.

A proprietary blocking agent, carrying the CHO structures, has been developed.

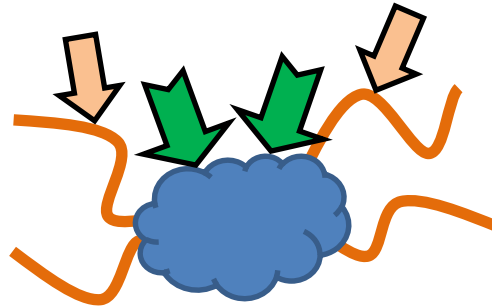
The blocking agent is a complex mix of molecules that contain the CHO structures.



# The effect of the blocking procedure.

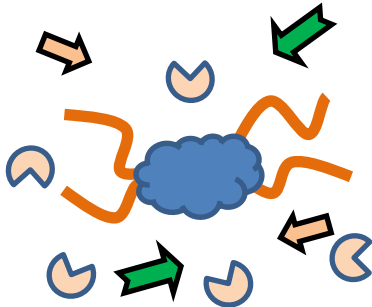
1st case:

- Allergic pet
- CHO reacting

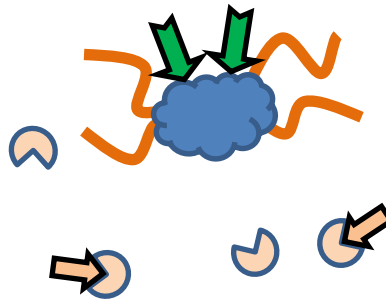


Giving rise to « all across the board » reaction.

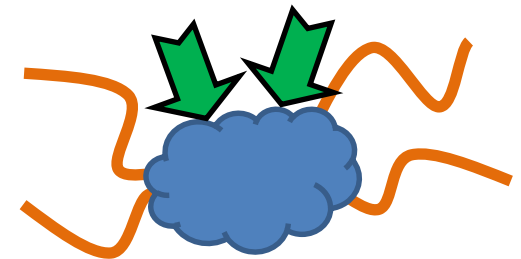
1- Adding the blocking agent



2- Removal of blocked complexes



3- Specific allergen IgE reaction is measurable.

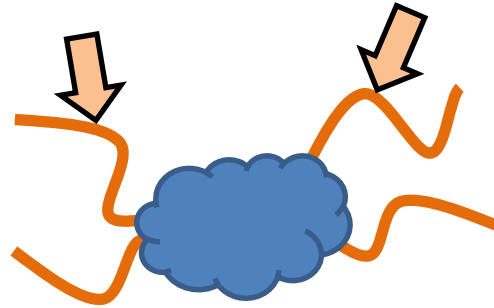




# The effect of the blocking procedure.

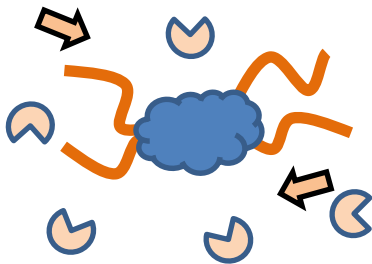
2nd case:

- CHO reacting only

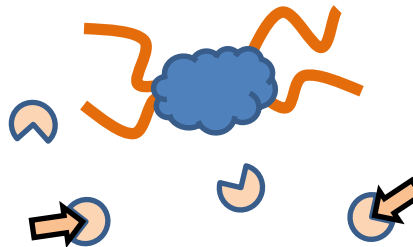


Giving rise to « all across the board » reaction.

1- Adding the blocking agent



2- Washing blocked complexes away



3- Allergen specific reaction can be read (this case being negative).



# Example: Patient-1.

Family	Allergen	Result	Class	ASIT	Blocked	Class	ASIT
Grasses	Phleum pratense	880	++++	Yes	10	-	
	Dactylis glomerata	898	++++	Yes	13	+	Yes
	Poa annua	635	++++	Yes	6	-	
	Lolium perenne	879	++++	Yes	16	+	Yes
	Festuca pratensis	922	++++	Yes	0	-	
Weeds	Rumex acetosella	897	++++	Yes	8	-	
	Plantago lanceolata	631	++++	Yes	0	-	
	Artemisia vulgaris	139	+++	Yes	0	-	
	Ambrosia artemisifolia	516	++++	Yes	2	-	
Trees	Alnus sp.	131	+++	Yes	0	-	
	Quercus sp.	204	++++	Yes	0	-	
	Betula populifolia	87	+++	Yes	0	-	

# Example: Patient-2.

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 artemisifolia	188	++++	Yes	2	-	
Trees	Alnus sp.	22	+	Yes	8	-	
	Quercus sp.	66	+++	Yes	4	-	
	Betula populifolia	60	+++	Yes	59	+++	Yes

# Example: Patient-3.

Family	Allergen	Result	Class	ASIT	Blocked	Class	ASIT
Grasses	Phleum pratense	149	+++	Yes	10	-	
	Dactylis glomerata	266	++++	Yes	8	-	
	Poa annua	283	++++	Yes	7	-	
	Lolium perenne	280	++++	Yes	6	-	
	Festuca pratensis	227	++++	Yes	4	-	
			-				
Weeds	Rumex acetosella	344	++++	Yes	1	-	
	Plantago lanceolata	231	++++	Yes	0	-	
	Artemisia vulgaris	40	++	Yes	2	-	
	Ambrosia artemisifolia	30	++	Yes	10	-	
Trees	Alnus sp.	46	++	Yes	2	-	
	Quercus sp.	140	+++	Yes	0	-	
	Betula populifolia	24	+	Yes	3	-	

# Can “multi-positive” samples be anticipated ?

Yes, with the **new Heska CHO-test**.

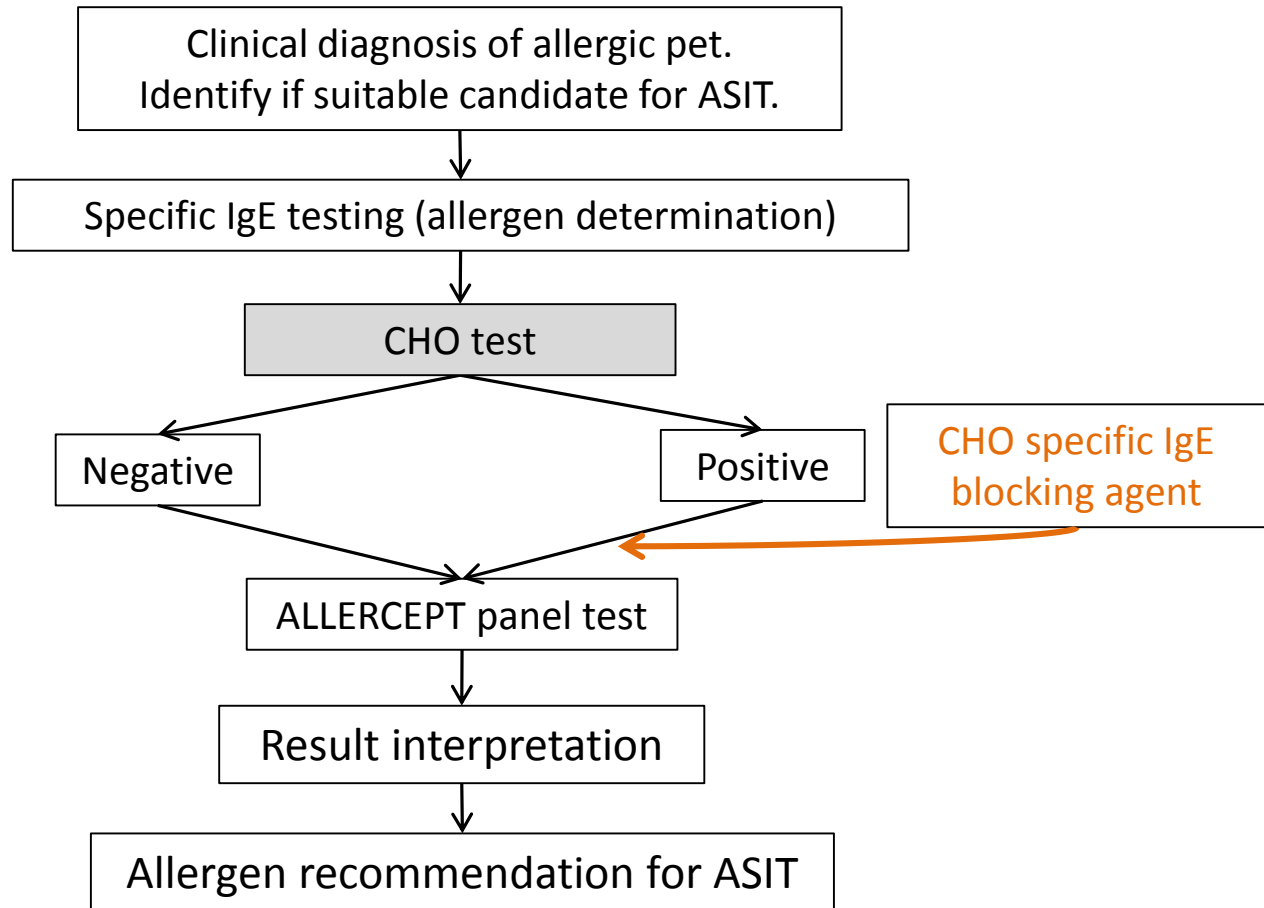
The CHO-test detects CHO specific IgE in the sample of dogs, cats and horses.

The test is to be performed before an allergen panel is run.

The sample will be processed according to the CHO-test result:

- When negative = standard IgE testing procedure
- When positive = blocking CHO specific IgE's required

# The ALLERCEPT-CHO combined system



Heska partner laboratories assist clinicians in the identification of these special cases to come up with the most precise ASIT recommendation for each patient.

**The introduction of the new CHO system solves the “multi-positive” plant results problem.**

**Therefore, specific IgE measurements become thus highly reliable.**

**In ASIT, precise allergen selection is one of the essential elements required for the successful control of the allergic patient.**