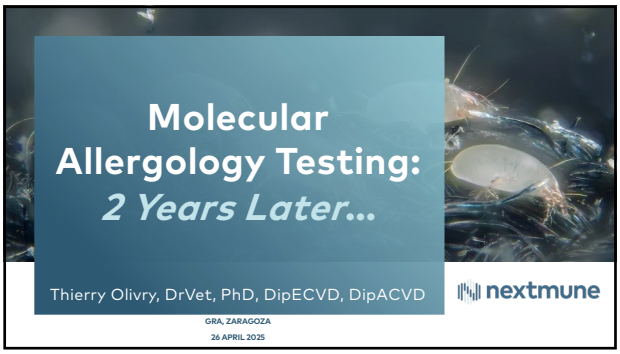
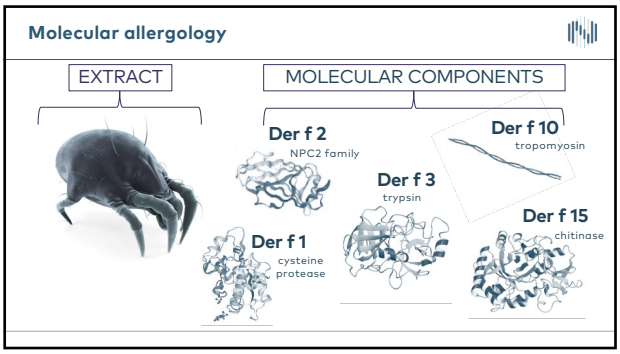

Molecular
Allergology Testing:
2 Years Later...
Thierry Olivry, DrVet, PhD, DipECVD, DipACVD
nextmune
GRA, ZARAGOZA
26 APRIL 2025

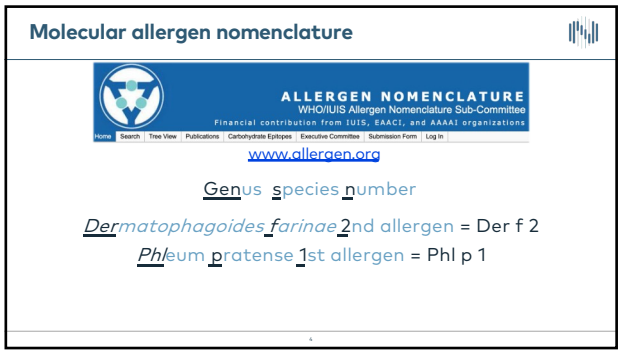
1


Disclosure
I am the Head of R&D
at
nextmune
Driven by Science
Powered by Passion

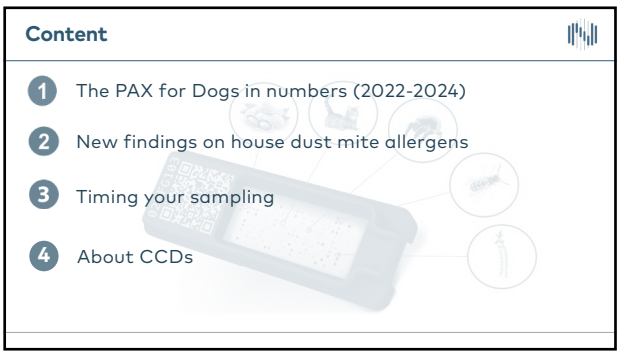
2


Molecular allergology
EXTRACT
MOLECULAR COMPONENTS
Der f 2
NPC2 family
Der f 10
tropomyosin
Der f 3
trypsin
Der f 15
chitinase
Der f 1
cysteine
protease

3


Molecular allergen nomenclature
ALLERGEN NOMENCLATURE
WHO/IUIS Allergen Nomenclature Sub-Committee
www.allergen.org
Genus species number
Dermatophagoides farinae 2nd allergen = Der f 2
Phleum pratense 1st allergen = Phl p 1

4


Content
1 The PAX for Dogs in numbers (2022-2024)
2 New findings on house dust mite allergens
3 Timing your sampling
4 About CCDs

5


The PAX for Dogs in Numbers
(2022-2024)

6

nextmune

MADX MACRO ARRAY DIAGNOSTICS

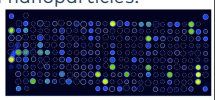
PAX
pet allergy xplorer

7

Nextmune PAX with MADX technology

PAX pet allergy xplorer

- automated **quantitative multiplex macroarray** with cartridges containing up to 300 allergen-covered nanoparticles:
  - one-third of "classical" extracts
  - two-thirds of molecular components
- **dogs:** January 2023
- **horses:** September 2023
- **cats:** January 2024



8

Sensitization rates (2022 – 2024)

PAX pet allergy xplorer (versions 22.2 and 23.1)

anti-IgE:	5.91
≠ environmental spots:	125 (60 ≠ species)
≠ venom spots:	13 (5 ≠ species)
≠ food spots:	98 (30 ≠ species)
# possible results:	236 (extracts + components)

9

Sensitization rates (2022 – 2024)

PAX pet allergy xplorer (version 22.2)

anti-IgE:	5.91
≠ environmental spots:	125 (60 ≠ species)
≠ venom spots:	13 (5 ≠ species)
≠ food spots:	98 (30 ≠ species)
# possible results:	236 (extracts + components)
# dogs tested:	60 130
% positive:	92%
% IT-treatable:	85%
avg allergens IT:	3,8 – 9,7

10

Sensitization rates in Nextmune laboratories

Avacta | HESKA | alergovet

	5.91 mAb	FcεRIα	4H4 mAb
anti-IgE:			
≠ environmental allergens:	38	24	34
≠ venom allergens:	0	0	0
≠ food allergens:	0	0	-
# possible results:	38	24	34
# dogs tested:	2 460	1 165	4 972
% positives:	74%	99%	87%
% IT treatable:	74%	99%	87%
avg allergens IT:	4,0	7,1	4,8

11

Sensitization rates between PAX and MAC test

PAX pet allergy xplorer | Biogenix

	5.91 mAb (2022-2024)	mAb cocktail (MAC) (2016-2022)
anti-IgE:		
# dogs tested:	60 130	25 451
% with ≥ 1 positive:	85.1%	75.6%
% with ≥ 1 mite positive:	81.7%*	67.9%
% Der f positive:	73.5%	63.6%
% with ≥ 1 pollen positive:	36.9%	39.8%

* 2024 only

(Drouet, Vet Dermatol 2024)

12

Top 20 sensitizations (2022-2024) (97 841)

versions 22.2 23.1 24.1

1.	Der f	66.9%	11.	Api m 10	10.5%
2.	Api m 1	20.8%	12.	Amb a	10.4%
3.	Api m	18.1%	13.	Bos d 6	9.9%
4.	Tyr p	15.8%	14.	Par j 2	9.4%
5.	Ves v 5	15.7%	14.	Ves v	9.4%
6.	Ves v 1	14.2%	16.	Ovi a_meat	9.2%
7.	Api m 3	14.1%	17.	Cte f 1	8.6%
8.	Tyr p 2	13.8%	18.	Pol d 5	7.1%
9.	Api m 2	10.7%	19.	Par j	7.0%
10.	Aca s	10.6%	20.	Ave s	6.5%

13

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14

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15

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16

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17

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9.	Api m 2	10.7%	19.	Par j	7.0%
10.	Aca s	10.6%	20.	Ave s	6.5%

18

19

20

Mite extracts and components

Der f
Der f 2 Der f 1
Der f 15* Der f 18*
** without natural glycans*

Aca s

Tyr p **Lep d**
Tyr p 2 Lep d 2

Gly d 2

Blo t
Blo t 5 Blo t 21
Blo t 10

Der p
Der p 1 Der p 2
Der p 5 Der p 7 Der p 10
Der p 11 Der p 21
Der p 23

21

Main IgE reactivity to mite components

60 130 sera from European dogs suspected of allergic diseases

Der f 73.5 %
Der p 6.8 %

1. Der f 2	5.7 %	6. Der f 21	2.3 %	11. Der p 20	1.8 %
2. Der p 2	5.4 %	7. Der p 10	2.2 %	12. Der p 7	0.7 %
3. Der f 1	4.3 %	8. Der p 5	1.7 %	13. Der f 18	0.6 %
4. Der p 1	3.3 %	9. Der p 23	1.3 %		
5. Der p 11	2.5 %	10. Der f 15	1.0 %		

22

Main IgE reactivity to mite components

60 130 sera from European dogs suspected of allergic diseases

Der f 73.5 %
Der p 6.8 %

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2. Der p 2	**5.4 %**	7. Der p 10	2.2 %	12. Der p 7	0.7 %
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4. Der p 1	**3.3 %**	9. Der p 23	1.3 %		
5. Der p 11	2.5 %	10. Der f 15	1.0 %		

most prevalent sensitizations to mite group 1 and 2 allergens

23

Main IgE reactivity to mite components

60 130 sera from European dogs suspected of allergic diseases

Der f 73.5 %
Der p 6.8 %

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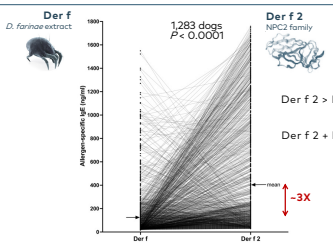
3. Der f 1	**4.3 %**	8. Der p 5	1.7 %	**13. Der f 18**	**0.6 %**
4. Der p 1	**3.3 %**	9. Der p 23	1.3 %		
5. Der p 11	2.5 %	**10. Der f 15**	**1.0 %**		

most prevalent sensitizations to mite group 1 and 2 allergens
rare sensitizations to high-molecular-weight allergens without natural glycans

24

Extract versus molecular component testing



Der f 2 > Der f: 78% of dogs

Der f 2 + but Der f -: 30%!

weaker IgE recognition of extract compared to major component

(Olivry, Vet Sci 2024)

25

Sensitization to mite group 2 allergens

60 130 sera from European dogs suspected of allergic diseases

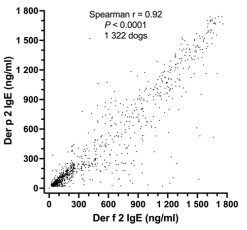
⇢ Der f 2	5.7 %
⇢ Der p 2	5.4 %
⇢ Gly d 2	2.7 %
⇢ Lep d 2	0.8 %
⇢ Tyr p 2	11.3 %

mite group 2
NPC2 family

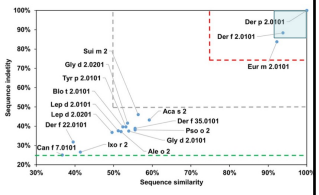
☞ *Do these allergens cross-react with each other?*

26

Are mite group 2 allergens cross-reactive?



A-RISC Der f 2 <-> Der p 2 = 0,91

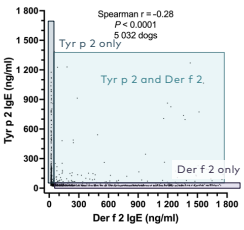


very high risk of cross-reactivity between Der f 2 and Der p 2

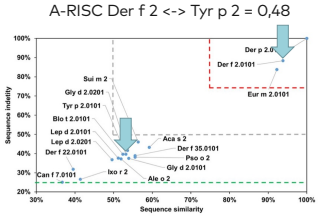
(Olivry, Vet Sciences 2024) (Chruszcz, PLOS One 2018)

27

Are mite group 2 allergens cross-reactive?



A-RISC Der f 2 <-> Tyr p 2 = 0,48



low risk of cross-reactivity between Der f 2 and Tyr p 2

(Olivry, Vet Sciences 2024) (Chruszcz, PLOS One 2018)

28

Sensitization to mite group 2 components

While there is **cross-reactivity between extracts of house dust and storage mites**, this does not apply to all homologous mite allergens!

→ *Pay attention to which group 2 mite allergen(s) your pet is sensitized, and take this into account when performing immunotherapy!*

29

Main IgE reactivity to mite components

60 130 sera from European dogs suspected of allergic diseases

Der f	73.5 %
Der p	6.8 %

~ 11X

1.	Der f 2	5.7 %
2.	Der p 2	5.4 %
3.	Der f 1	4.3 %
4.	Der p 1	3.3 %

→ Der f extract-to-Der f 2 ratio = ~13X

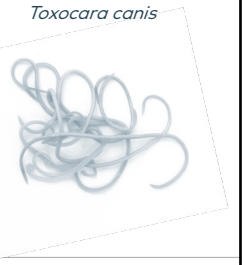
→ Der p extract-to-Der p 2 ratio = ~ 1X

30

Why such a difference between Der f and Der p?

Dermatophagoides farinae
(but not to D. pteronyssinus!)

Toxocara canis



31

Which allergens cross-react between Der f and Toxo c?

Der f 15
Zen-1
(Der f 18)

via glycans

larval
mucins
(TES)

Der f 10
Der f 11

via peptides

adults
Toxo c 3
Toxo c 2

PAX is usually negative for Der f 15/18 as they don't have the natural glycans!

(Olivry, Vet Dermatol 2024)

32

Conclusions: mite allergens

PAX pet allergy xplorer

- House dust and storage mites are **important sources of allergens in dogs and cats**, as in humans
- **Mite group 1 and 2 allergens** are those **most often detected** in the PAX
- Pay attention to **which mite group 2 allergen** your pet is sensitized!
- **Sensitization to the Der f extract** mostly reflects **cross-reaction with *Toxocara canis***
- *What is the clinical relevance of this cross-reactivity?*

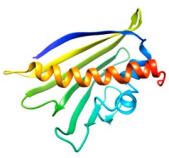
33

34

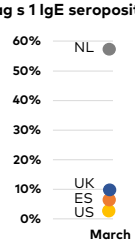
Common Beech (*Fagus sylvatica*)

PAX pet allergy xplorer

Fag s 1
PR-10 family



Fag s 1 IgE seropositivity



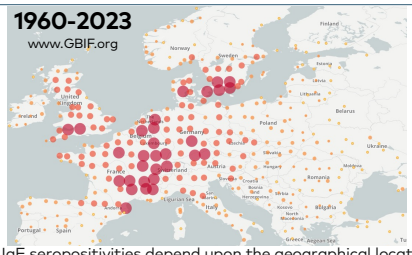
35

Common Beech (*Fagus sylvatica*)

IgE seropositivities depend upon the geographical location as they reflect exposure to the allergen source

36

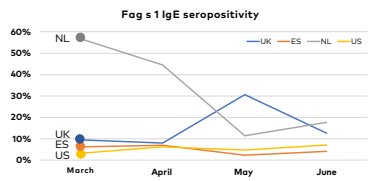
Common Beech (*Fagus sylvatica*)



IgE seropositivities depend upon the geographical location as they reflect exposure to the allergen source

37

Common Beech (*Fagus sylvatica*)

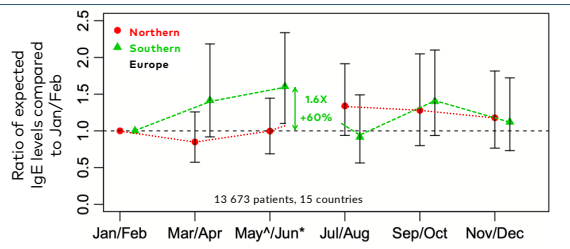


Fag s 1
PR-10 family

differences in IgE seropositivity depend upon the pollination month!

38

Seasonal variation of Timothy grass sIgE levels



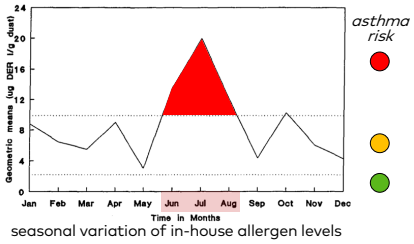
seasonal differences in specific IgE levels based on geography and season

(Lam, Clin Exp Allergy 2020)

39

Seasonal variation of mite allergens in homes

Der f 1 + Der p 1 levels in 424 US homes (1989 to 1991)



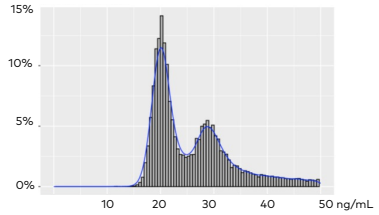
seasonal variation of in-house allergen levels

(Lintner, J Allergy Clin Immunol 1993)

40

Is there a seasonality of mite sensitization levels?

histogram of Der f IgE levels in ~ 24,000 dogs in Europe in 2023

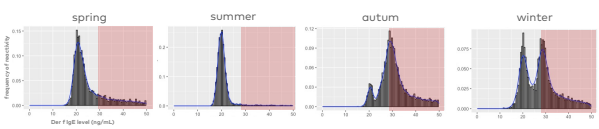


double peak of IgE reactivity to Der f

41

Is there a seasonality of mite sensitization levels?

histogram of Der f IgE levels in ~ 24,000 dogs in Europe in 2023



seasonal variation in Der f IgE levels with higher levels in the autumn

42

Conclusions: influence of seasons on test results

☞ **Serum levels of specific IgE** and **sensitization rates** may **vary between seasons**

☞ This fluctuation can be seen **not only with sensitizations to pollens but also with mites** whose reproduction rates vary with heat and humidity!

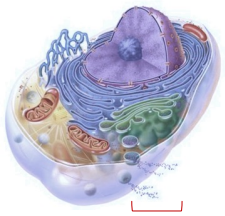
⚠ *Do not only pay attention to pollen levels in the air; **allergen exposure also likely occurs on the ground with deposited pollen***!

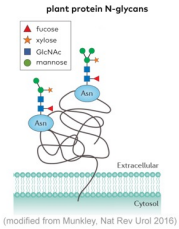
→ **Test within 2-4 weeks after the beginning of a clinical flare**!

43

44

Protein glycosylation

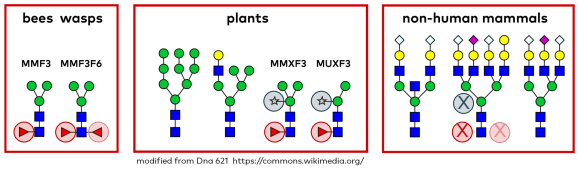




many proteins (and allergens) are glycosylated = **glycoproteins**

45

Complex N-glycans



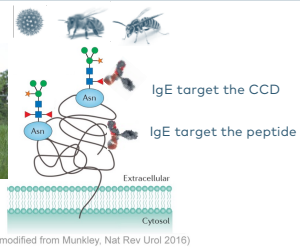
some plants and venom allergens carry complex N-glycans with **fucose** ± **xylose**

mammals have **lost the enzymes to add these fucose/xylose**!

→ these glycans can be recognized as foreign and **targeted by IgE/IgG**!

46

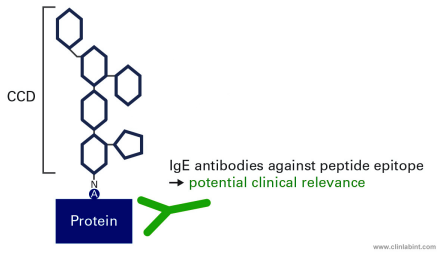
IgE sensitization to pollens or *Hymenoptera* stings



CCD-IgE arise in ~30-90% of dogs sensitized to pollen and venom allergens

47

IgE sensitization to pollens or *Hymenoptera* stings



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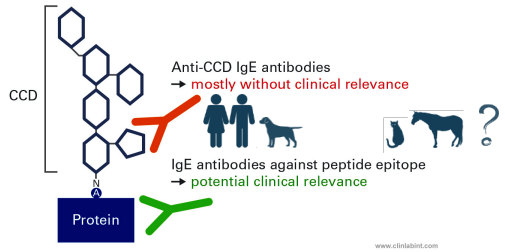
IgE sensitization to pollens or *Hymenoptera* stings



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Limited biological activity of CCD-IgE

animals MDPI

Article

Evaluating the Pathogenic Potential of IgE Targeting Cross-Reactive Carbohydrate Determinants in Dogs

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https://www.mdpi.com/2076-2615/14/22/3275 OPEN ACCESS

(Olivry, Animals 2024)

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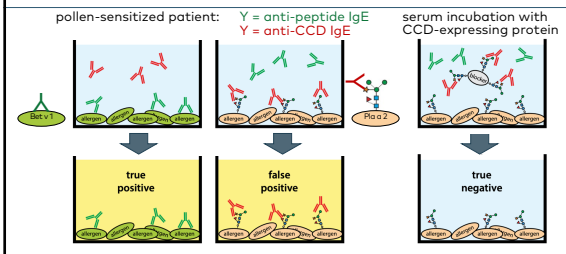
CCD-IgE blocking common strategy



(modified from: Altmann, Allergo J Int 2016)

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PAX: a unique CCD-blocking strategy

PAX pet allergy xplorer

1. blocking of all sera with a **CCD-expressing protein mix**
2. PAX with **2 detectors of CCD blocking efficiency**
3. if one or both CCD detectors > 100.00 ng/mL:
   → 2nd block with a high amount of a different CCD-expressing protein, then 2nd PAX, or
   → machine-learning algorithm
4. if one or both CCD detectors > 28.00 ng/mL:
   → disclosure statement of insufficient blocking of CCD-IgE

(Olivry, Vet Sciences 2024)

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For more details on CCD blocking strategy

PAX pet allergy xplorer

veterinary sciences MDPI

Article

Validation of a Multiplex Molecular Macroarray for the Determination of Allergen-Specific IgE Sensitizations in Dogs

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https://www.mdpi.com/2306-7381/11/10/482 OPEN ACCESS

(Olivry, Vet Sci 2024)

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Importance of an effective blocking of CCD-IgE

PAX pet allergy xplorer

	ng/mL	class		ng/mL	class
• CCD detector:	914	4	• CCD detector:	114	2
• Dac g	76	1	• Dac g	25	0
• Fes p:	59	1	• Fes p:	26	0
• Poa p:	47	1	• Poa p:	23	0
• Sec c_pollen:	110	2	• Sec c_pollen:	25	0
• nCry j 1:	35	1	• nCry j 1:	24	0
• nCup a 1:	59	1	• nCup a 1:	27	0
• Rum a/c	116	2	• Rum a/c	23	0
• Ama r:	103	2	• Ama r:	27	0
• Api m:	190	2	• Api m:	191	2

2nd CCD block

☞ CCD-bearing extracts or components: 16

☞ CCD-bearing extracts or components: 5 (4 venoms)

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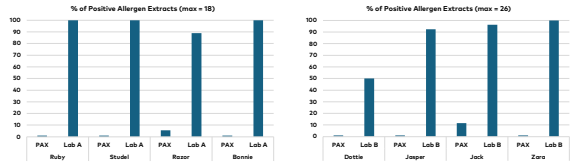
What happens if you don't fully block CCD-IgE?

→ 8 polysensitized dogs with nonseasonal signs and high levels of CCD-IgE
→ tested with the PAX, or ELISA from Laboratories A and B



insufficient blocking of CCD-IgE = many false positives to plant extracts!

(Nextmune, unpublished 2024)

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Conclusions: CCDs

- CCDs are present in **allergens from plants and *Hymenoptera*** venoms and **can trigger the production of CCD-IgE** in humans, dogs, cats, and horses
- **CCD-IgE are not pathogenic in humans and dogs**
- The **PAX has a unique CCD-IgE blocking strategy** with a detection of the efficiency of that blocking
- Four **commercial IgE serological ELISA tests have insufficient CCD-IgE blocking** and report **false positive sensitizations to pollen extracts**

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Conclusions: *What did we cover today?*

- The PAX allows for the **identification of unique sensitization patterns**
- The **main mite allergens (so far) are those of groups 1 and 2**
- **Mite group 2 allergens** could be great **indicators of the primary-sensitizing mites**
- The **high molecular weight allergens cross-react with *Toxocara canis***
- The **timing of sample collection can impact the detection of sensitization** to pollen (and also other) allergens

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Conclusions: *What did we cover today?*

- CCDs are present in **allergens from plants and *Hymenoptera*** venoms and **can trigger the production of CCD-IgE** in a high proportion of humans, dogs, cats, and horses
- **CCD-IgE are not pathogenic in humans and dogs**
- **Insufficient blocking of CCD-IgE** in serological tests **leads to false positive to plant–based extracts and native components**
  - *The chosen serological test must have means to detect the efficiency of CCD-IgE blocking!*

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