

Comparison of Component Resolved
Diagnosis (CRD) with skin prick test in
respiratory allergy [36 cases]
and Significance of CRD in
Anaphylaxis [8 cases]
& Chronic Urticaria with/without Angioedema
[16 cases]

Dr Manisha Rai

NATIONAL ALLERGY CENTRE 1/3 EAST PATEL NAGAR DELHI

Mob. : 09312285947

Email : pc_kathuria@yahoo.com Web : www.nationalallergycentre.in

Objective

- a) **In 36 cases of respiratory allergy**, we examined the qualitative concordance between SPT and sIgE as measured on the CRD (ALEX2) platform for the following allergen components: Der p 1, Der p 2 & Der p 23 (Dermatophagoides pteronyssinus) Dermatophagoides farinae (Der f 1 and Der f 2), Cynodon dactylon, Artemisia vulgaris, Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarium.

(Comparison of kappa coefficient and p-value test for CRD Vs SPT)

(Correlation; $r = 0$ to 0.25 indicates poor or no correlation; $r = 0.26$ to 0.50 indicates fair correlation; $r = 0.51$ to 0.75 indicates good correlation and $r = 0.71$ to 1.0 indicates excellent or perfect correlation.)

- b) **In 8 cases of anaphylaxis**, CRD was performed. SPT is indicated only after 6 weeks of anaphylaxis, hence it could not be done.
- c) **In 16 cases of Chronic spontaneous urticaria with/without angioedema**, CRD was performed to detect species-specific and cross reactive allergen.

•

Demographic Characteristics of the Patients

Sl. No	Parameter	n=60
1	Age (yr, mean±SD)	34.08 ± 16.49
2	Gender, n	
	Male	39
	Female	21
3	Diagnosis, n	
	a) Respiratory allergy [AR+AA]	36
	b) Anaphylaxis	8
	c) CSU w/s angioedema	16

Material and Methods

CRD

- The different allergens and components coupled onto the **ALEX chip [117-Allergen Extract / 178- Components]** were incubated with 0.5 mL of 1:5 diluted serum under shaking condition. Notably, the serum diluent contains a **Cross-Reactive Carbohydrate Determinants (CCD) inhibitor**.
- After incubation for 2 h, the chips are extensively washed, and anti-human IgE detection antibody (labeled with alkaline phosphatase) is added and incubated for 30 min.
- Following another cycle of extensive washing, the **enzyme–substrate** is added, and after a few minutes, the reaction is complete. The membranes are dried, and the intensity of the color reaction for each allergen spot is measured by a Charge Coupled Device (CCD) camera. The Raptor software digitalizes the images and prepares a report that lists the allergens and **components and their score in kUA/L**.

SPTs

Were performed with a **commercial standardized extract** panel (Stallergenes, Greer) which included Dermatophagoides pteronyssinus, Dermatophagoides farinae, Cynodon dactylon, Artemisia vulgaris, Alternaria alternata, Aspergillus fumigatus, Cladosporium. Alternaria was also included for being a very frequent co-sensitisation in pollen patients of the studied area. Histamine (10 mg/ml) was used as positive control and saline solution as negative control. Any ≥ 4 mm mean wheal diameter was considered positive.

Cross reactive group of allergens

> 50%



Cross
reactive
allergens



**Dermatophagoides
pteronyssinus**

**Dermatophagoides
farinae**

Male size-250 um
Female size-350 um
Weight- 3.1 ug
Lifetime: 60-120 days
Reproduction: about 100 eggs per female
Allergen nomenclature: Der p

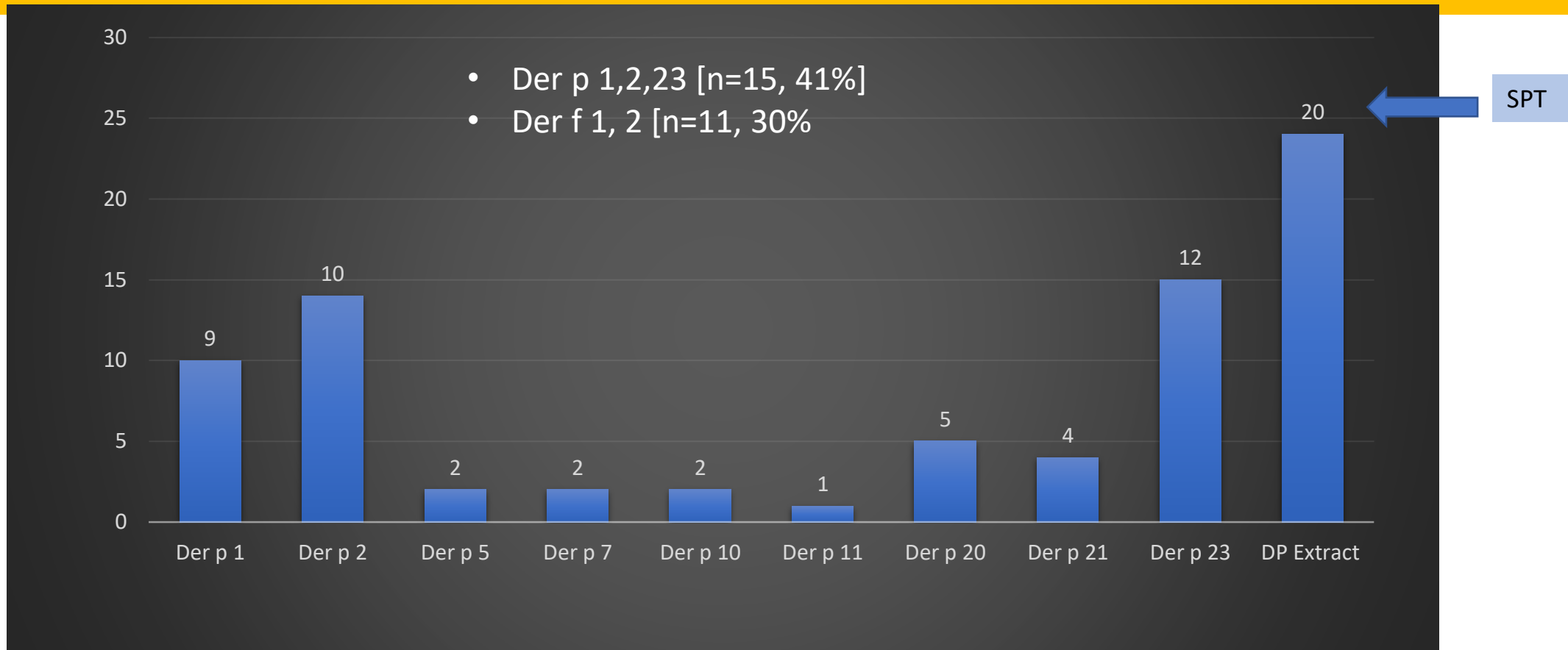
Male size-310um
Female size-390 um
Weight- 3.4 ug
Lifetime: 100-120 days
Reproduction: about 30-60 eggs per female
Allergen nomenclature: Der f

House Dust Mites

Total Sample = 36

Total Sample = 36	CRD	SPT	CRD Vs SPT Correlation Test			
			Kappa Test	Kappa Interpretation	p value	Correlation Interpretation
Dermatophagoides pteronyssinus	15 (41%)	20 (55%)	K = 0.291	Slight Agreement	p < 0.0958	Not Significant
Der p 1	9 (25%)					
Der p 2	10 (27%)					
Der p 23	12 (33%)					
Dermatophagoides farinae	11 (30%)	20 (55%)	K = 0.103	No Agreement	p < 0.481	Not Significant
Der f 1	8 (22%)					
Der f 2	11 (30%)					

Dust Mites Allergy



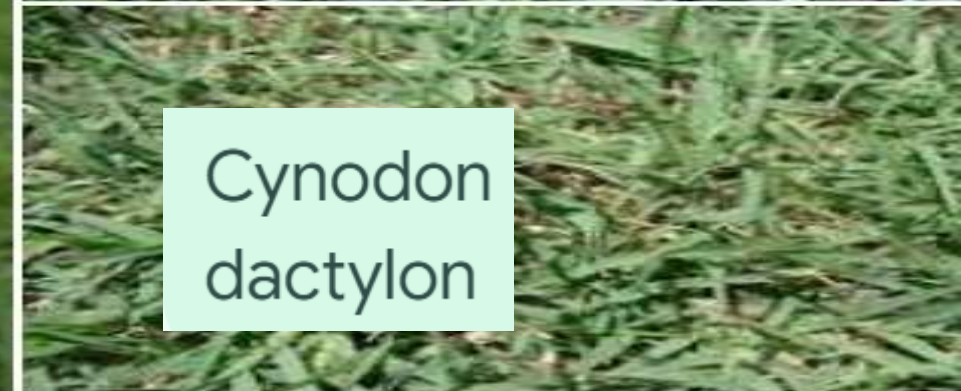
The poor agreement percentage between the two methods is because of different source of preparation and different methods of testing for allergenic components and extracts.

➤ **SPT positive, CRD positive with Der p 1 & 2- AIT INDICATED**

➤ **SPT positive, CRD negative with Der p 1 & 2- AIT NOT INDICATED**

Respiratory Allergy

Pollen Allergy	CRD	SPT	Kappa Test	Kappa Interpretation	p value	Correlation Interpretation
Cynodon dactylon	7 (19%)	6 (16%)	K = 0.531	Moderate Agreement	p< 0.0076	Significant
Artemisia vulgaris	3 (8%)	3 (8%)	K = 0.273	Slight Agreement	p< 0.235	Not Significant
Mould Allergy						
Alternaria alternata	5 (13%)	6 (16%)	K = 0.464	Moderate Agreement	p< 0.0243	Significant
Aspergillus fumigatus	5 (13%)	2 (5%)	Kappa= 0	No Agreement	p<1	Not Significant
Cladosporium herbarium	4 (11%)	1 (2%)	K= 0.372	Fair Agreement	p<0.111	Not Significant

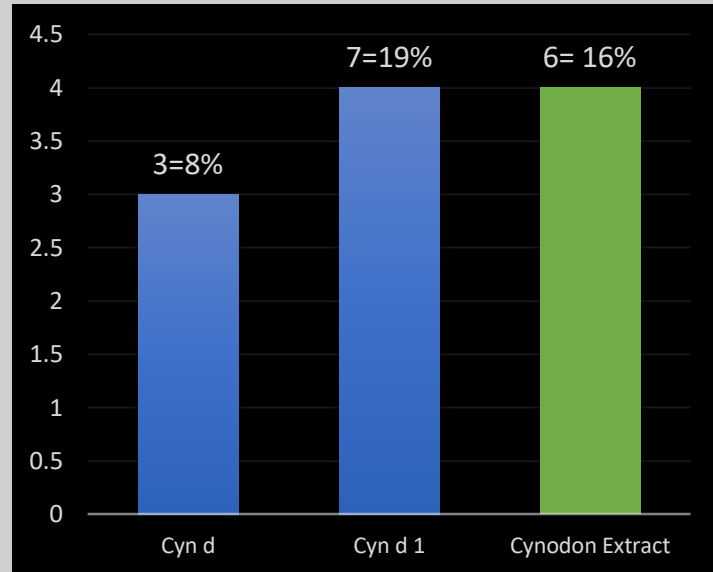


Artemisia vulgaris



CRD showed fair agreement compared with SPT

Cynodon dactylon

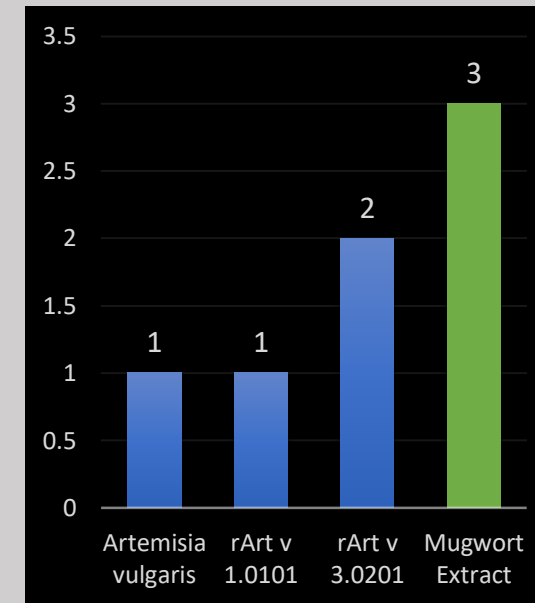


Specific IgE
Extract based

CRD

SPT

Artemisia vulgaris



Specific IgE
Extract based

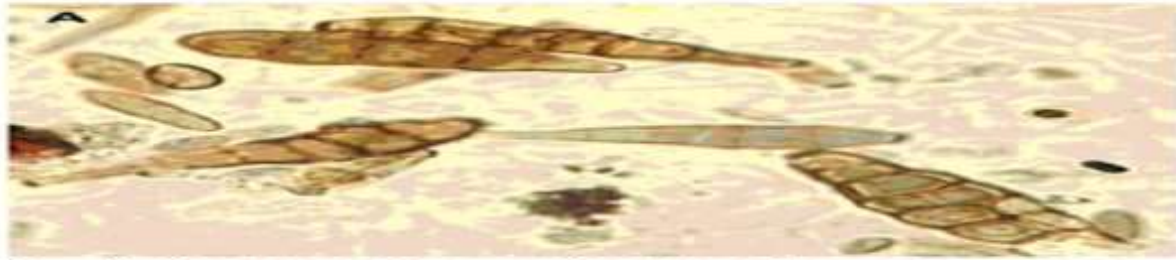
CRD

CRD

SPT

Pollen Allergy	CRD	SPT	Kappa Test	Kappa Interpretation	p value	Correlation Interpretation
Cynodon dactylon	7 (19%)	6 (16%)	K = 0.531	Moderate Agreement	p < 0.0076	Significant
Artemisia vulgaris	3 (8%)	3 (8%)	K = 0.273	Slight Agreement	p < 0.235	Not Significant

Alternaria alternata

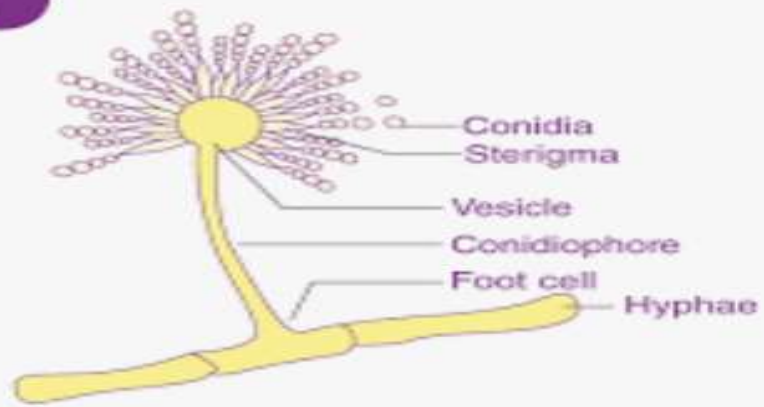


A. *Alternaria alternata* spores

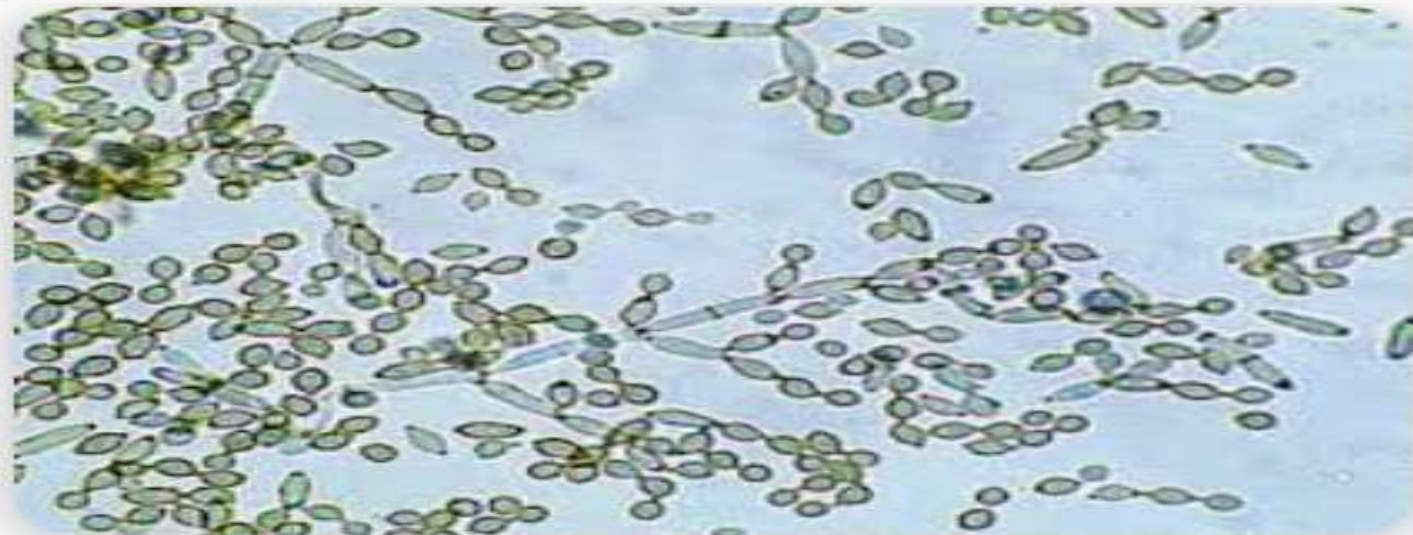


B. Broken *Alternaria* spore adjacent to an intact spore

ASPERGILLUS

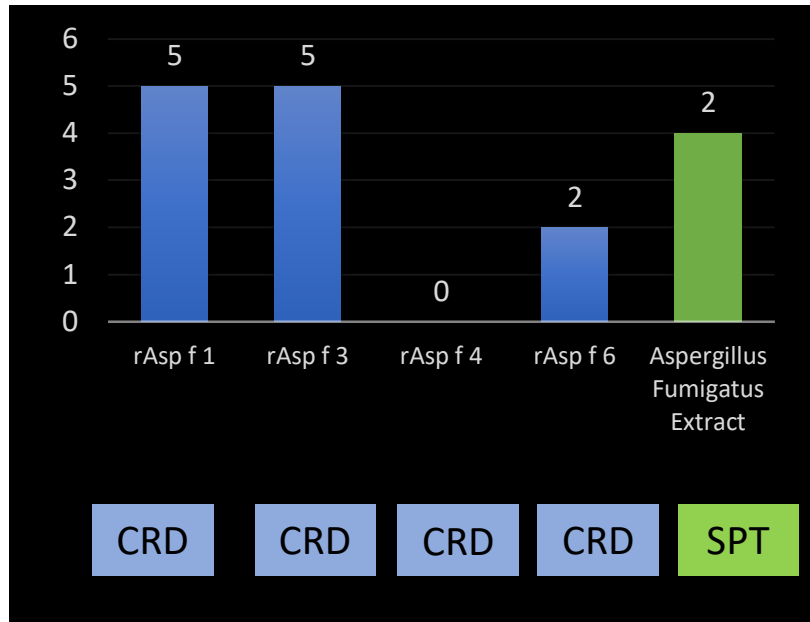


Cladosporium

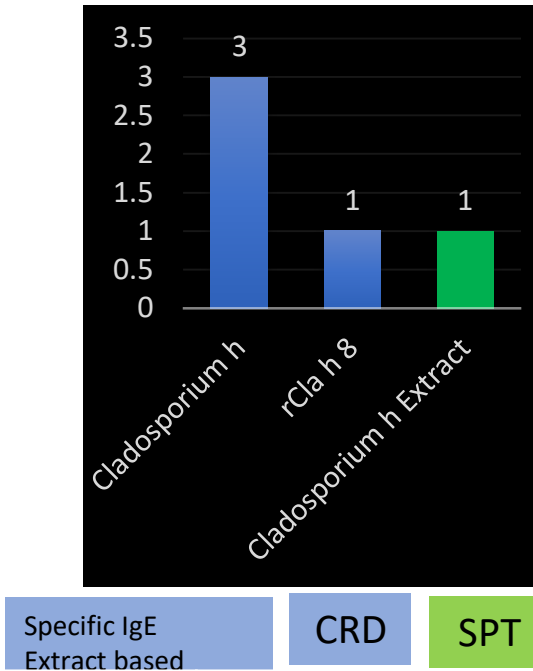


Correlation co-efficient significant for *Alternaria alternata*- moderate Agreement

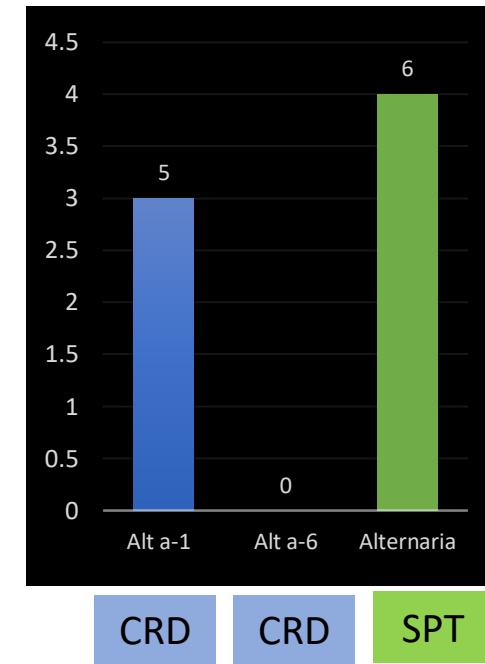
Aspergillus fumigatus



Cladosporium herbarum



Alternaria alternata



	CRD	SPT	Kappa Test	Kappa Interpretation	p value	Correlation Interpretation
Alternaria alternata	5 (13%)	6 (16%)	K = 0.464	Moderate Agreement	p< 0.0243	Significant
Aspergillus fumigatus	5 (13%)	2 (5%)	Kappa= 0	No Agreement	p<1	Not Significant
Cladosporium herbarium	4 (11%)	1 (2%)	K= 0.372	Fair Agreement	p<0.111	Not Significant

Why CRD- Anaphylaxis ?

(A) Disease-eliciting components

(B) Epipen/ Inj Adrenaline

Wheat		Omega-5-gliadin
Milk		Bos d 8 (casein)
Egg		Gal d 1 (ovomuroid)

Protein family	nBos d 8	nGal d 1	Tri a 14,	Tri a 19	Act d 10	Act d 2	Jug r 4	Gly m 6	Ses l 1	nMac i 2s Albumin
Milk (Casein)	1	1 patient with a history of anaphylaxis to milk, had a severe sensitization to Bos d 8 belonging to the casein family.								
Egg (Ovomucoid)		1	In 1 case of severe egg allergy, strong sensitization to Gal d 1 (ovomuroid)							
Lipid Transfer Protein (LTP)			1		1					
Wheat (Omega-5-gliadin)				3	Tri a 19 sensitization was frequently demonstrated in these patients, with 3 patients being sensitized to this component.					
Thaumatococcus diglycoside (TDP)						4				
Storage protein (11 S Globulin)							1	1		
Storage protein (2 S Albumin)									1	1

In these 8 cases, 4 patients displayed broad sensitization to storage proteins from different nuts & seeds. This protein family is a high-risk marker and is indicative of systemic reactions.

Why CRD ?

Chronic urticaria with/without angioedema [16 cases]

Allergen	Antigen 5	Arginine kinase	Beta Expensin	Tropomyosin	Cysteine Protease	NPC2	Pectin Methylesterase	Peritophin	Unknown
Pol d 5 (paper wasp venom)	2								
Ves v 5 (wasp venom)	1								
Der p 20 (American HDM)		3							
Pen m 2 (Shrimp)	Markers of cross-reactivity / co-sensitization								
Bla g 9 (German cockroach)		2							
Cyn d 1, Lol p 1 & Phl p 1 (Grass pollen)			1						
Ani s 3, Blo t 10, derp 10, Per a 7, Pen m 1 (mix)				1					
Der p1	Markers of primary sensitization				3				
Der f 1					3				
Der p 2						4			
Der f 2						4			
Gly d 2						3			
Lep d 2						3			
Sal k 1							2		
Der p 23	Markers of cross-reactivity / co-sensitization							5	
Der p 20									2

- Better characterizes patient's sensitization pattern
- Determines the presence of cross-reactivity between allergens
- Primary sensitization with house dust mite and cross-reactivity or co-sensitization with other allergens.

Respiratory allergy -Markers of primary sensitization.

CRD is a reliable new method for AIT

- Der p 1, Der f 1- Cysteine protein
Der p 2, Der f 2- NPC 2 protein
Specific IgE positive in 60-90%. Progressive asthma severity
- Der p 23- Peritrophine-like-protein. 75% of HDM. Asthma development
- Cynodon dactylon (Cyn d 1)
- Artemesia vulgaris (Art v 1)
- Alternaria alternata (Alt a 1)- Specific IgE positive in 80-90%
- Aspergillus fumigatus (Asp f 2/ 4/ 6-ABPA, Asp f 1- Asthma)
- Cladosporium herbarum (Cla h 8): cross-reactive minor allergen

Food Allergy- Markers of primary sensitization

CRD is a reliable new method

- Milk (Casein)
- Egg (Ovomucoid)
- Lipid Transfer Protein (LTP)
- Wheat (Omega-5-gliadin)
- Thaumatococcus like peptide (TLP)
- Storage protein (11 S Globulin)
- Storage protein (2 S Albumin)

Questions

➤ Can CRD-based Specific IgE sensitization in relation to clinical history **solve most of the limitations** of extract-based specific IgE and SPT? -

YES

➤ Can CRD-based specific IgE for food-allergen sensitization **by-pass or replace OFC** and **predict severe systemic reactions?**- **YES / NO**

➤ Can CRD-based specific IgE **improve the selection of allergens for AIT** in polysensitized patients - **YES / NO**

Acknowledgement

- This power point presentation is a collaborative effort with [ALEX² - Allergy, Xplorer](#), Tosoh India Pvt Ltd.
- Special thanks to Dr. Manoj Kumar Singh, Scientific Manager - Product Development for data analysis.



Thanks

NATIONAL ALLERGY CENTRE

Tel : 25884136
25880057
25916170
Mob: 9312285947



e-mail : pc_kathuria@yahoo.com
Website : www.nationalallergycentre.in