

# Extracts freeze dried and stable allergens from House Dust Mites for diagnostic, research and immunotherapy purposes

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## Introduction

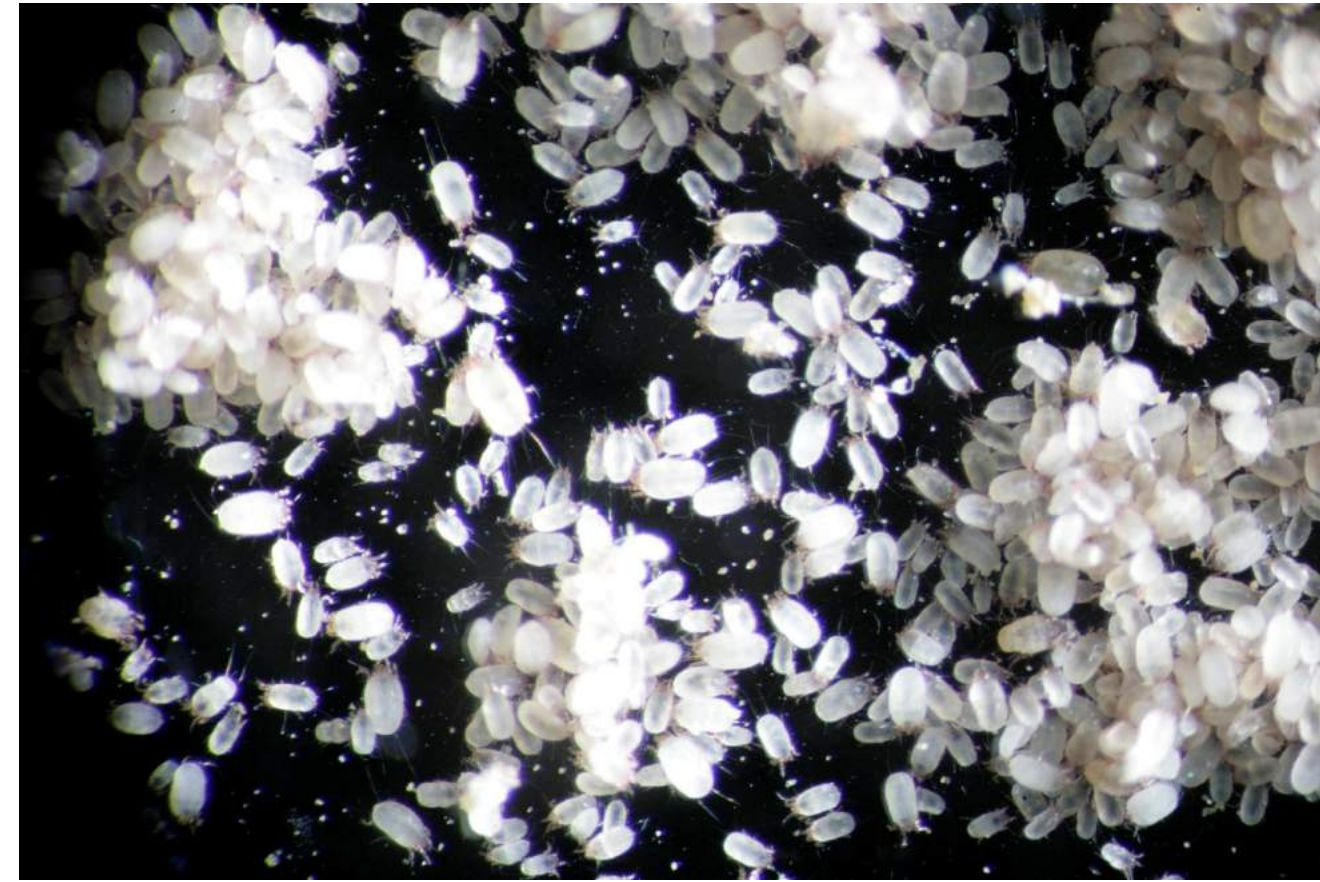
House dust mite allergy is one of the most widespread allergic diseases. Most patients allergic to house dust mites react to group 1 and 2 allergens.

For diagnosis and immunotherapy of allergies, and for research purposes, it is necessary to use house dust mite (HDM) extracts and specific major allergens of reliable and consistent quality.

Currently, commercially available group 2 ELISA kits do not distinguish consistently between group 2 allergens of different species. Large deviations from actual concentrations of group 2 can be observed. Therefore, individual antibodies for Der p 2 and Der f 2 are necessary.

## Aim

Our aim is to deliver defined extracts, stable major allergens and species-specific group 2 antibodies.



## Approach

Whole Cultures (WC), extracts and specific major allergens from *Dermatophagoides pteronyssinus* (DP) and *Dermatophagoides farinae* (DF) are produced by Citeq.

Mite WC's are extracted in a suitable buffer, concentrated by ultrafiltration and freeze dried.

Major allergens of group 1 and group 2 from DP and DF are purified as described in literature, with modifications.

Rabbit-polyclonal antibodies are obtained from rabbits immunized with purified allergens. Monoclonal antibodies are obtained from a hybrid between BALB/c spleen cells and a myeloma cell line.

## Results

### Extract vs Whole Culture

DP extracts compared to Whole Culture: 10 times higher Der p 1 levels, 5 times higher Der p 2 levels and a slight increase in endotoxin levels.

Batch	Bradford	BCA	Der p 1	Der p 2	Endotoxin	Protease activity	
	mg/g	mg/g	mg/g	mg/g	EU/mg	Serine*	Cysteine**
<b>Extract from Whole Culture</b>							
15J01	68.6	283.9	46.32	4.54	1.05E+06	0.07	68656
15J02	63.7	198.2	29.86	4.49	1.07E+04	0.11	102423
15G10	68.5	291.1	31.41	4.53	4.59E+04	0.05	55188
<b>Whole culture</b>							
14C30	12.7	127	3.64	0.58	1.06E+02	0.05	13874
16H14	15.6	150.7	6.52	1.04	9.71E+02	0.06	15968

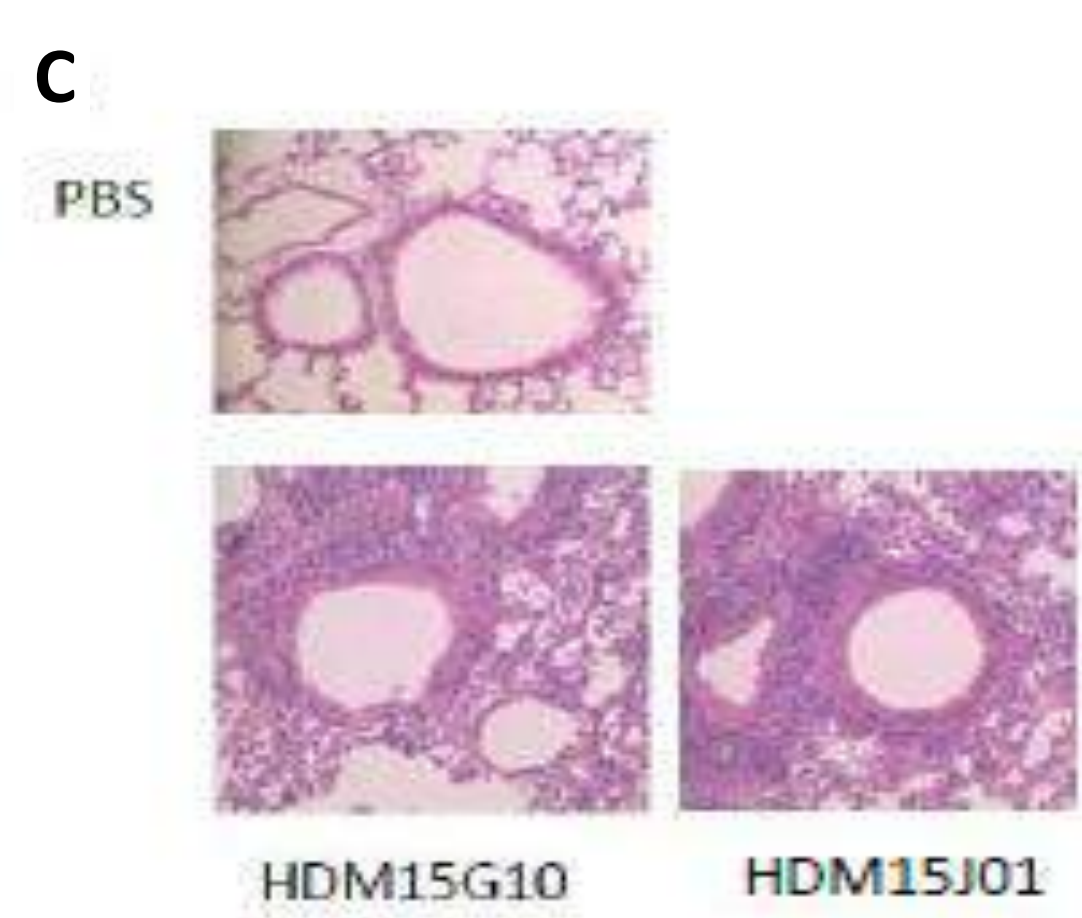
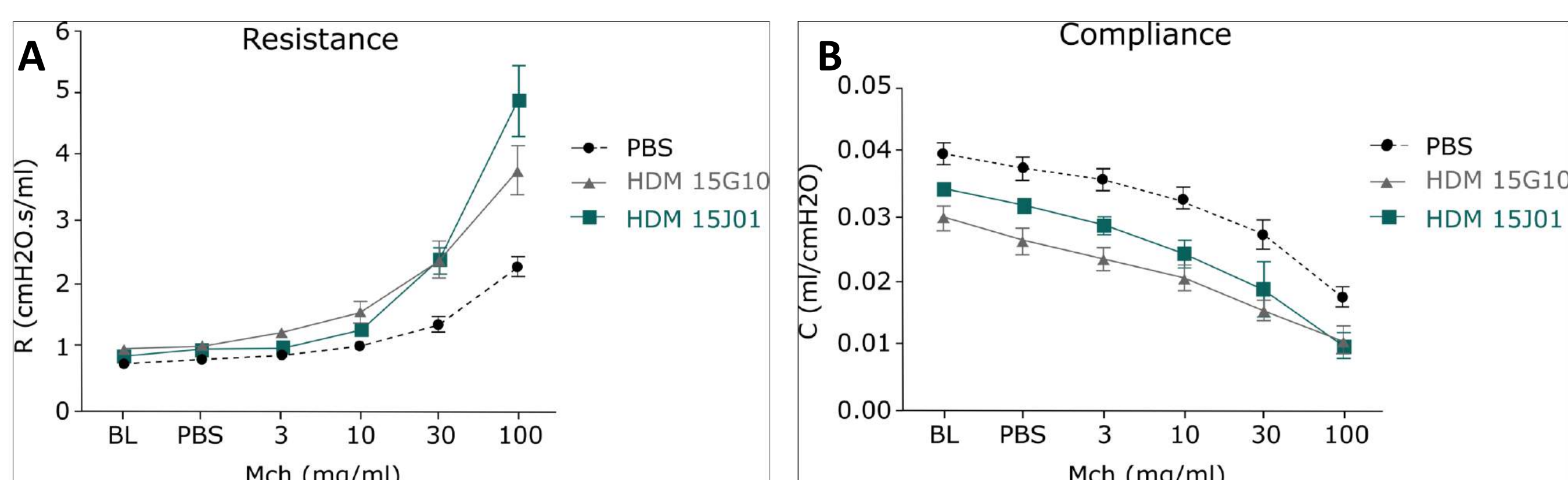
\* U trypsin/mg protein BCA

\*\* U papain/mg protein BCA

### Citeq DP extracts induce an inflammatory response<sup>1</sup>

A&B) Increase in airway resistance and decrease in airway compliance is equal across different batches of Citeq HDM extracts.

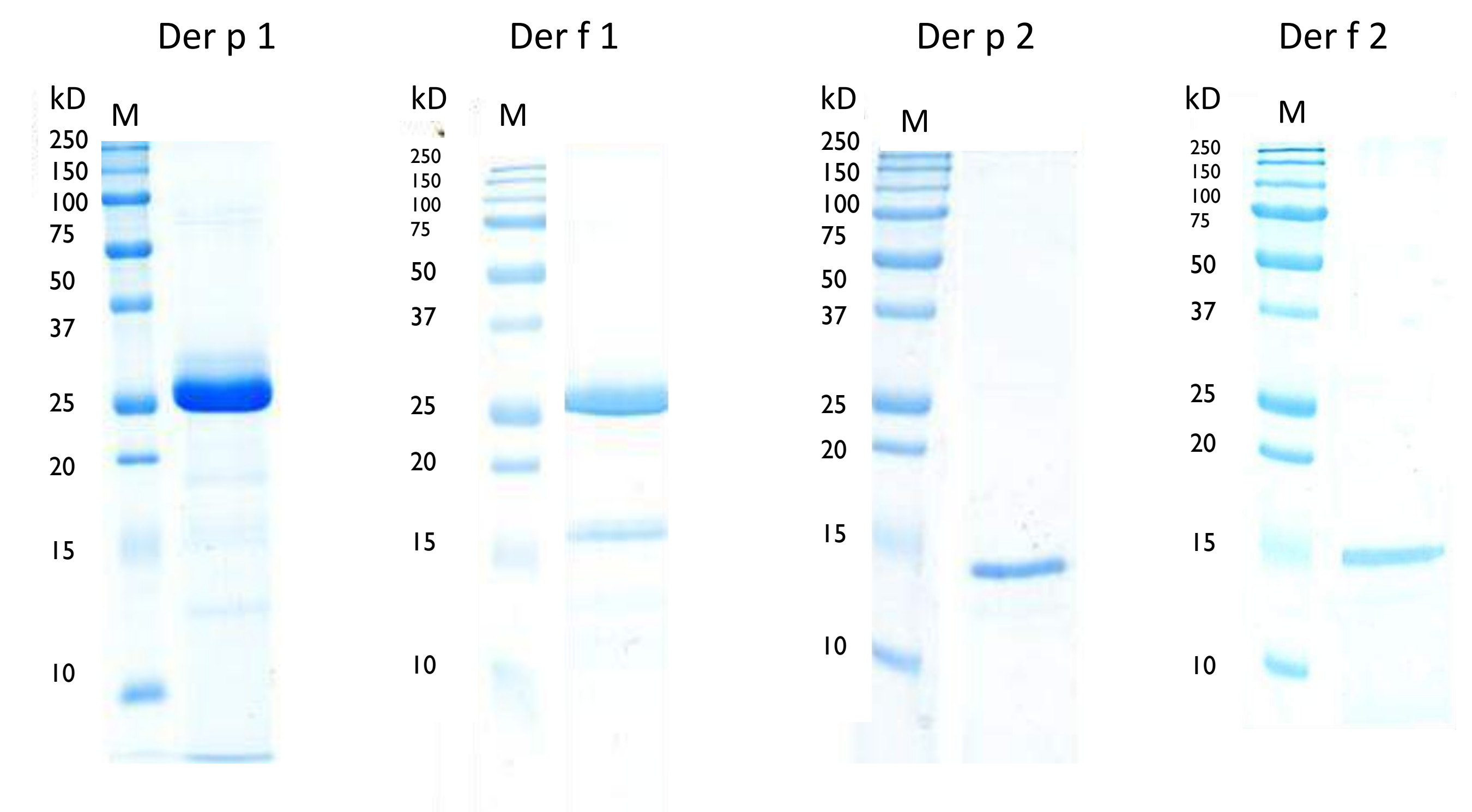
C&D) Citeq products cause inflammation of the airway.



<sup>1</sup> Gregory, L. (2016). *Citeq HDM comparison*. Imperial College, UK: Unpublished data.

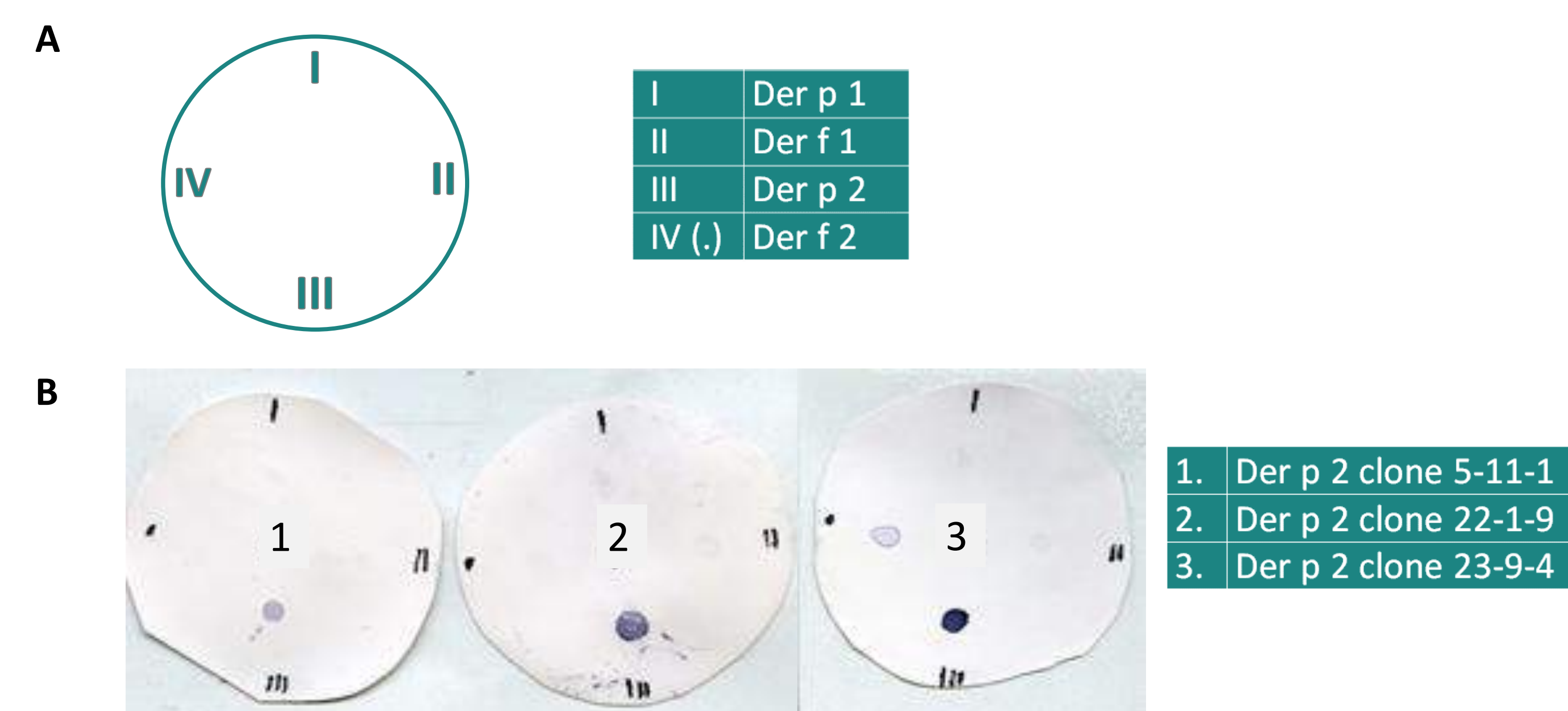
### Major allergens group 1 + 2 of DP and DF

Der p 1, Der f 1, Der p 2 and Der f 2 are 98% pure as determined by SDS-PAGE.



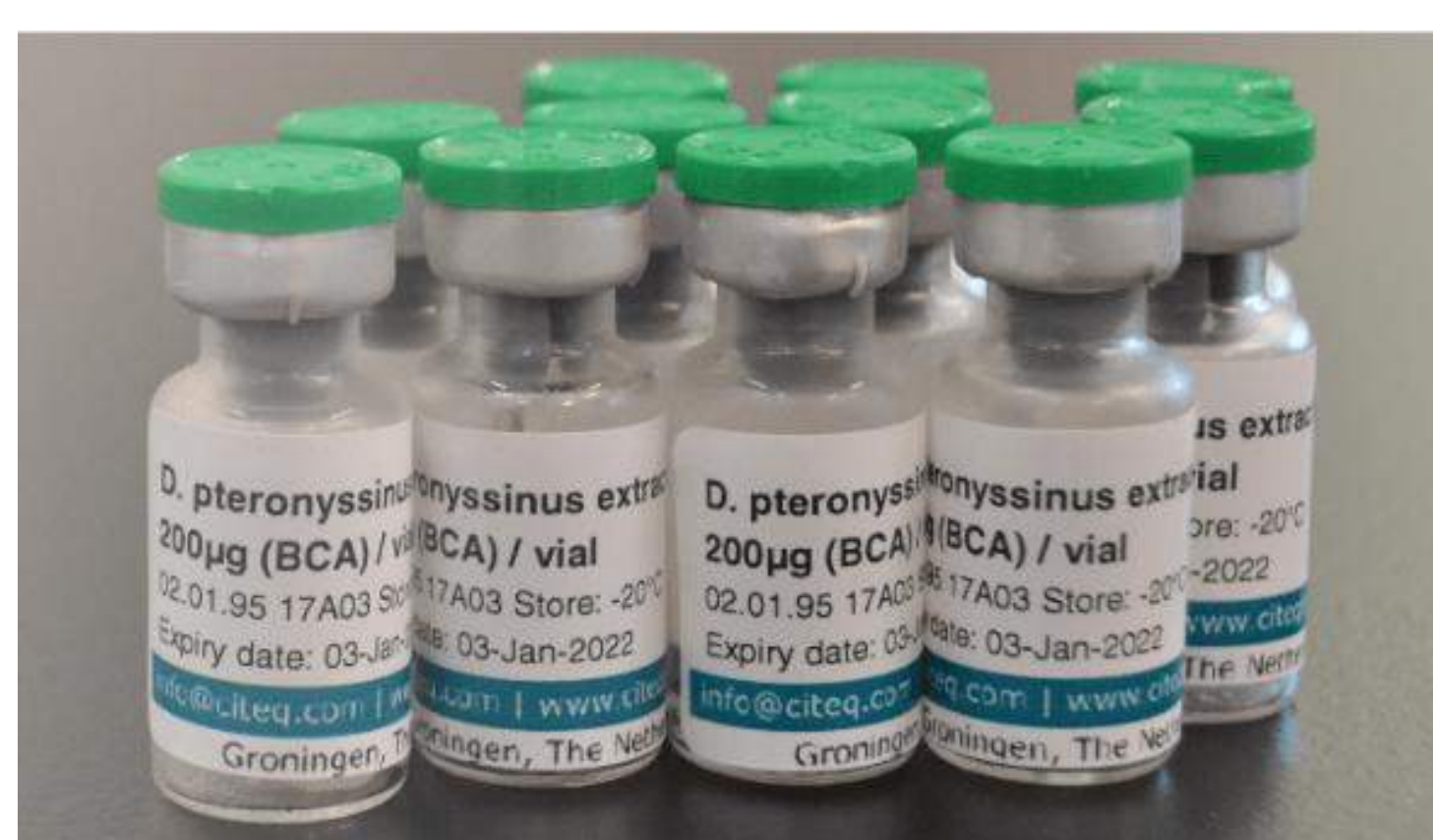
### Der p 2 specific antibodies

A) Shows the arrangement of allergens on the membrane (immunostaining)  
B) Der p 2 clone 5-11-1 and Der p 2 clone 22-1-9 show no cross-reactivity with Der f 2 in comparison with Der p 2 clone 23-9-4.



## Conclusion

1. Extracts produced by Citeq have a consistent quality and can be used in animal models for allergic asthma
2. Major allergens from DP and DF HDM are purified up to 98%
3. The antibodies produced against Der p 2 are specific. There is no cross-reactivity with Der f 2.
4. Future research will be necessary to develop a ready-to-use ELISA for Der p 2
5. Extracts from Whole Culture and specific major allergens produced by Citeq can be used for diagnosis and immunotherapy of allergies and for research purposes.



## Product formulation

- Whole Culture**
- Vials > 1 gr (dry weight)
- Group 1 allergens**
- Vials of 500 µg protein (BCA)
- Group 2 allergens**
- Vials of 250 µg protein (BCA)
- Extract**
- Vials of 10 mg, 100 mg, 1 gr (dry weight)
  - Micro vials of 200 µg protein (BCA)
    - Ready-to-use in animal model testing