Entomophagy : what about allergies ?

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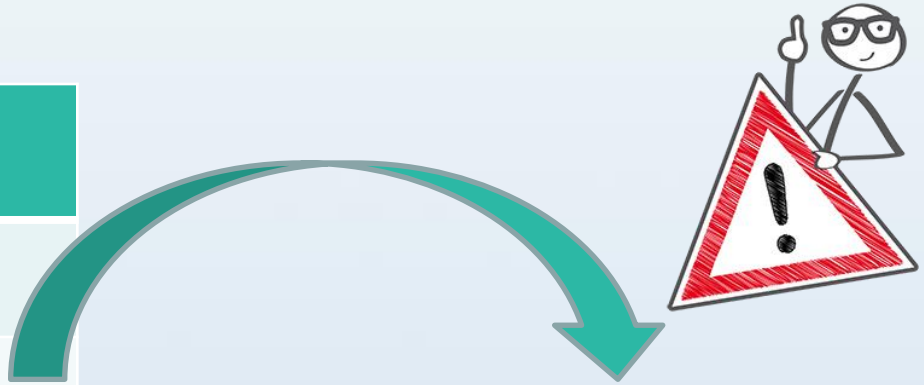
What about entomophagy ?

Entomophagy

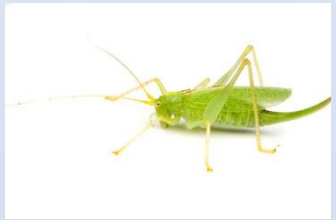
http://www.provivam.com/

Why ?

ADVANTAGES	DISADVANTAGES
Nutritive value	Laws (CE n° 258/97)
Ecology	Allergic risk ? Anaphylaxis ? Angioedema?
Low resources	



Could be FATAL ?



Aims of our study

- Evaluation of the risk of cross-reactions with insect allergens :
  - HDM allergy accounts for 5 to 15% of all allergic patients worldwide
  - Shellfish allergy accounts for 0.5 to 1.5% of all allergic patients worldwide
- Development of new diagnostic tools to point out insect sensitization
- Screening of new foods containing insects ➔ control of labeling

What about allergy ?

Epidemiology of allergy

Food allergy :

- ➔ 2-3% of world population
- ➔ 2-6% of children

Allergic asthma = first chronic disease in childhood

x2 during the last two decades in Europe

Allergy

Definition:

An abnormal reaction of the body to a previously encountered allergen

- Introduced by inhalation, ingestion, injection or by skin contact
- Inadapted and excessive response of our organism
- Reproducible reaction
- Reaction even if there is a low quantity of allergen
- Mainly mediated by IgE (= allergy linked antibody)

Allergy

- **Sensitization phase :**

After an initial contact with an allergen ➔ activation of the immune system

➔ NO SYMPTOMS

➔ Specific IgE

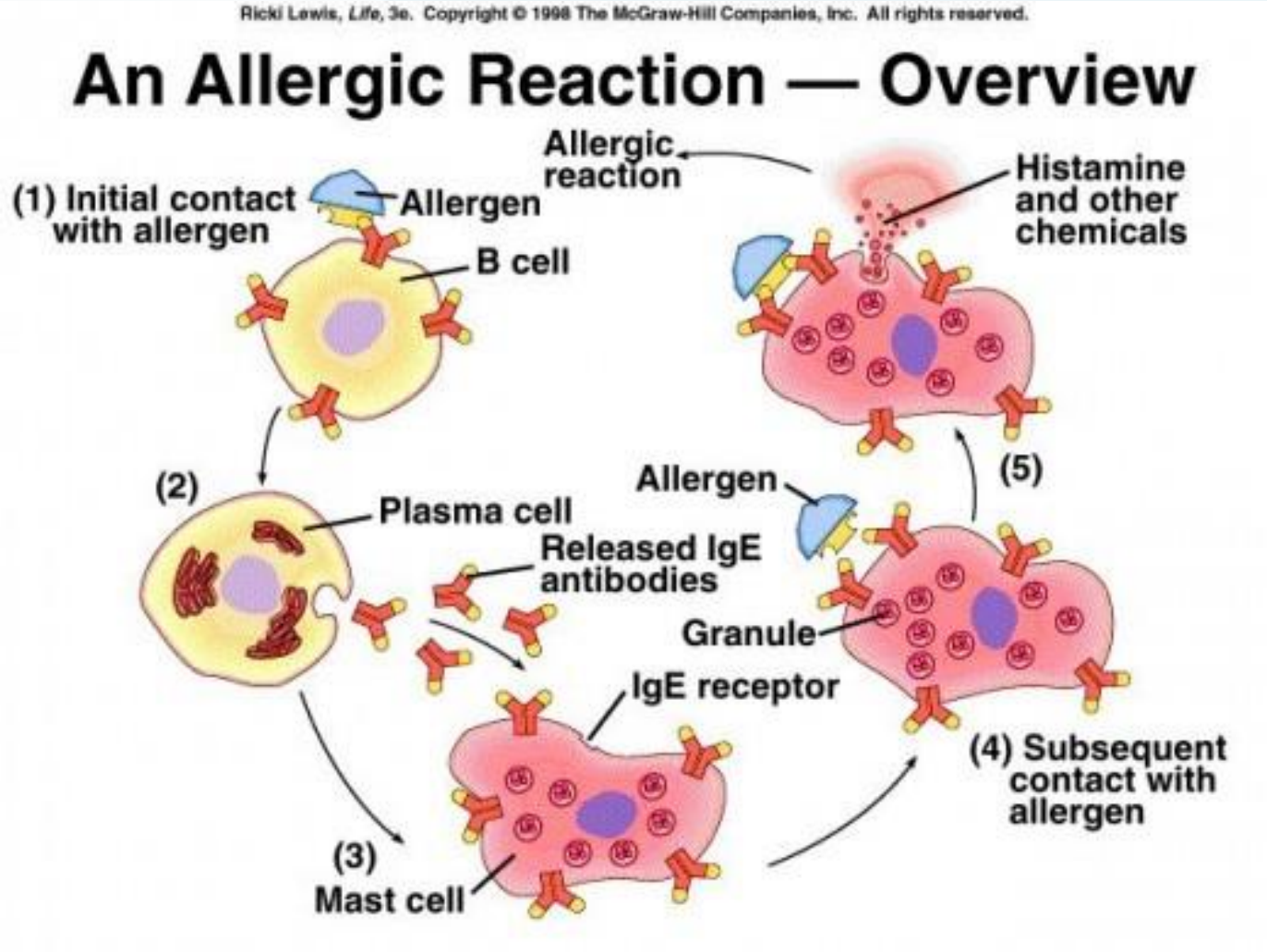
sensitization ≠ allergy

- **Reaction phase :**

Ulterior contact with the same allergen

➔ REACTION (clinical symptoms = allergy)

Allergy



Sensitization phase (1) (2) (3)

Allergic reaction phase (4) (5)

Allergen

Definition:

A substance (mainly protein) that can cause an allergic reaction but is not harmful to most people

https://www.nicelabel.com/fr/blog/2016-11-11/faqs-allergen-ingredients-food-labels/

In our case, we studied shrimp and House Dust Mites allergens that are already described and used for *in vitro* diagnostics:

Shrimp allergens	HDM allergens
sIgE shrimp extract	sIgE *Dermatophagoïdes pteronyssinus*
sIgE tropomyosin (rPen a 1)	sIgE *Dermatophagoïdes farinae*
	sIgE rDer p 1
	sIgE rDer p 2
	sIgE tropomyosin (rDer p 10)

What about cross-reactivity ?

Cross-reactivity

Definition:

Cross-reactivity in allergic reactions occurs when the allergenic proteins in one allergenic source are similar to the proteins found in another source

Example:
If you are allergic to birch tree pollen, you may have an oral allergy symptom reaction when eating an apple

Cross-reactivity

Tropomyosin

Major allergen of invertebrates and minor allergen of House Dust Mites (HDM)

Marker of cross-reaction between shellfish, HDM and insects

Can cause severe allergic reactions

Example :

Some of the HDM allergic patients present an allergy when eating shellfish due to cross-reaction
➔ *only if previously sensitized to tropomyosin !*

In vitro :
rPen m 1
rDer p 10
nBla g 7
rAni s3
rPen a 1
nPen i 1

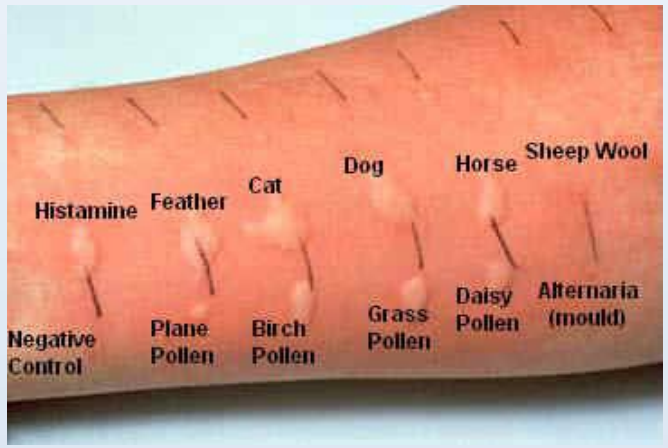
Diagnosis of allergy in a clinical laboratory

Diagnosis

- Clinical history
- Allergic reaction characterization :

  In vivo assays

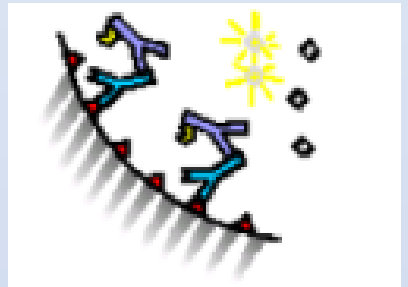
  - Skin prick test
  - Oral food challenge



- Sensitization reaction measurement :

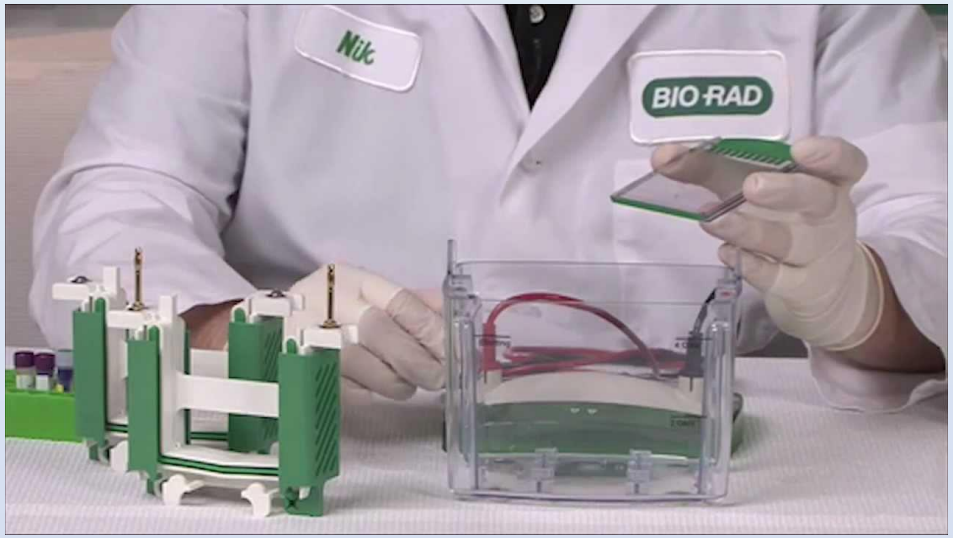
  In vitro assays

  - Measurements of specific IgE in the patient's serum
    - ➔ Traditional method ImmunoCAP250 (ThermoFisher Scientific)
    - ➔ ImmunoCAP ISAC (microarray)



R&D allergy diagnosis

- 1D & 2D Western blot
  - ➔ Detection of the proteins against which the patient has been sensitized
    - ➔ Sensitization profile of one patient !



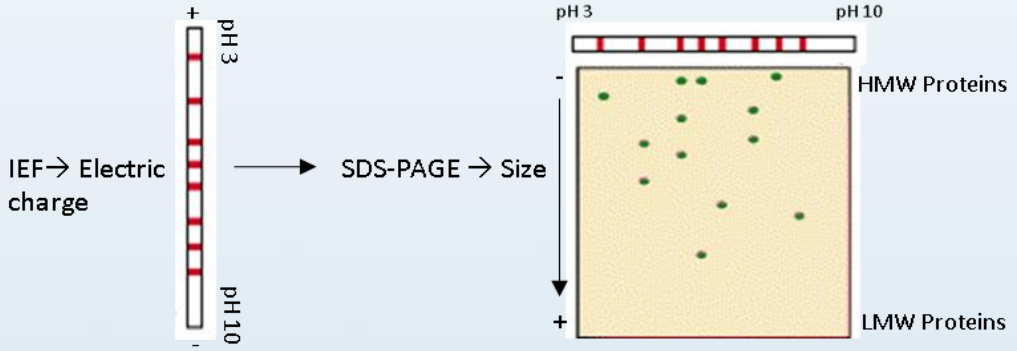
Diagnosis

1. Protein extraction

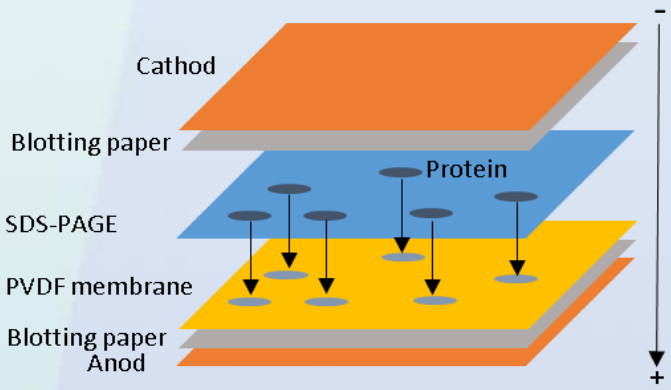
Urea
CHAPS
TBP
Biolytes

→ Total proteins

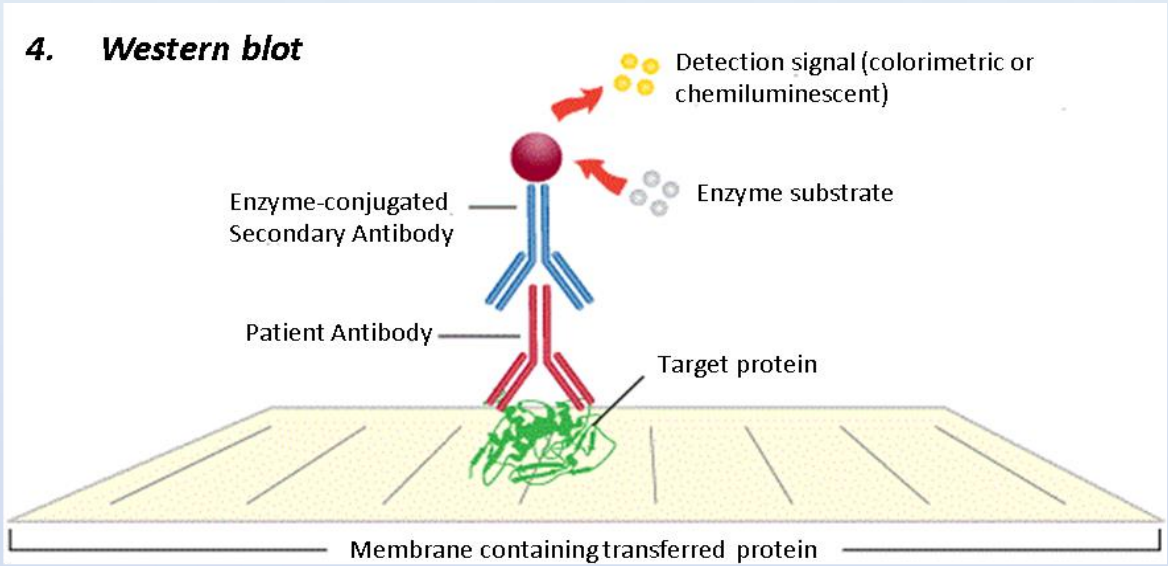
2. 2DE Electrophoresis



3. Protein transfer



4. Western blot



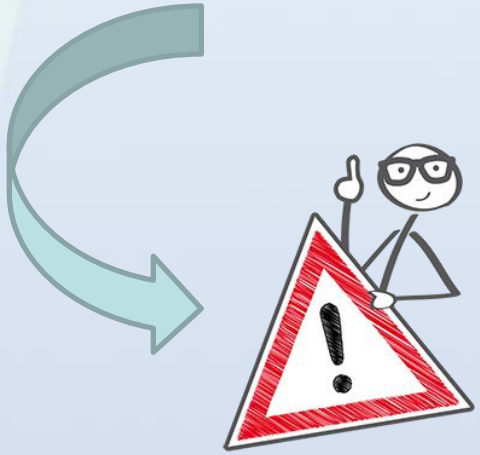
What about insect food allergy ?

Insect Food allergy

Major described allergens :

- Tropomyosin
- Arginine kinase
- Chitin…

Cross-reactions with shellfish and/or HDM allergens ?



Allergens are not always proteins…

How ?

- Total protein extraction from crickets (*Grillodes sigillatus*)

EITHER

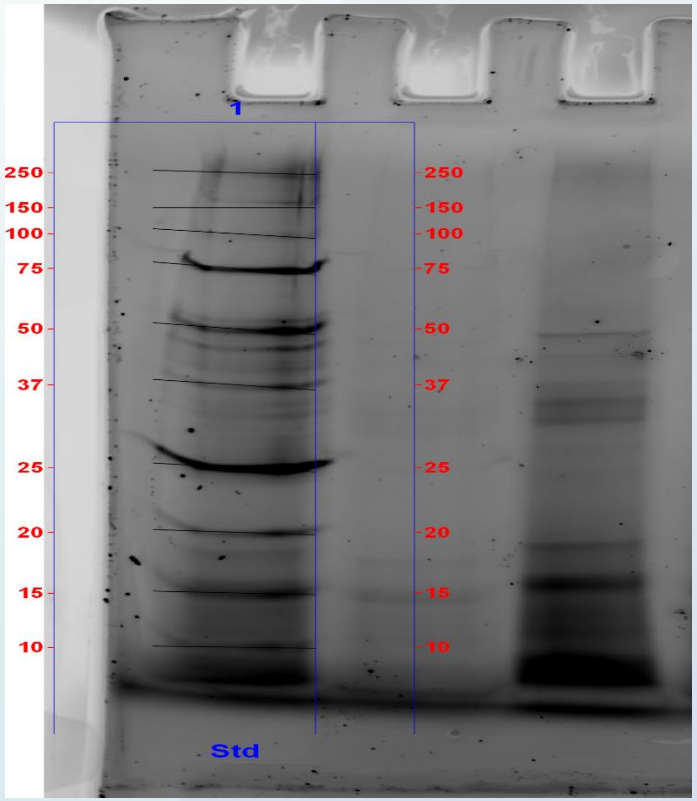
- SDS-PAGE gel (1D)
- Western blot (1D)

OR

- Isoelectric focalization (1D)
- SDS-PAGE gel (2D)
- Western blot 2D

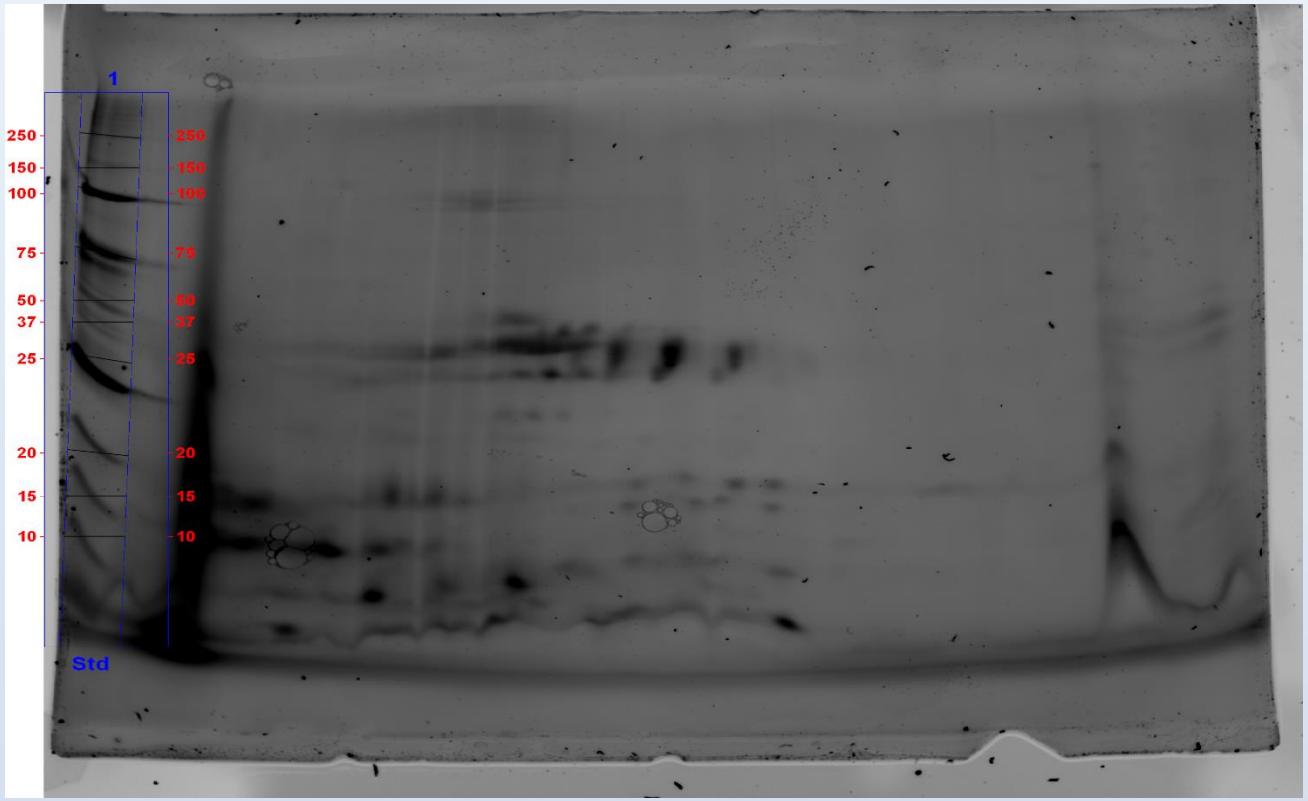
Results : protein extraction from crickets

SDS-PAGE gel 1D



SDS-PAGE gel 1D with *Grillodes* extract

SDS-PAGE gel 2D



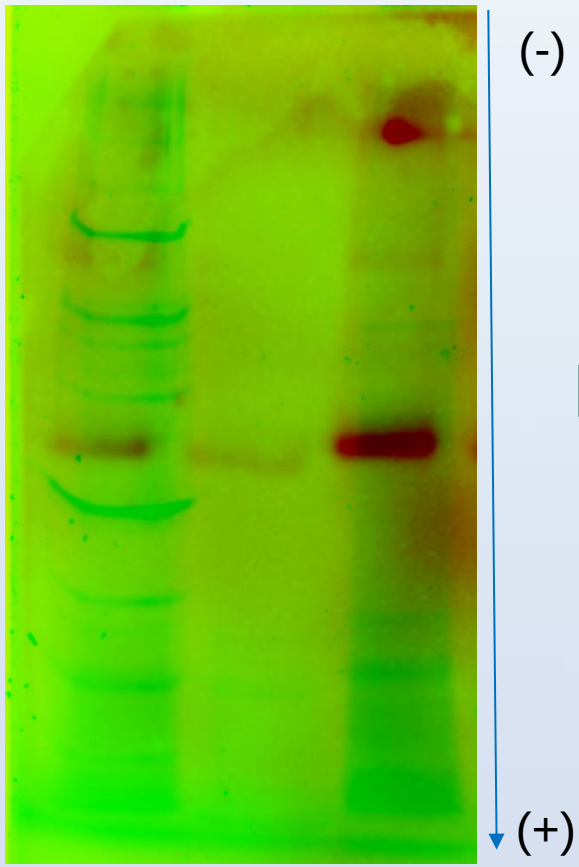
SDS-PAGE gel 2D with *Grillodes* extract

First case analyzed : 1D

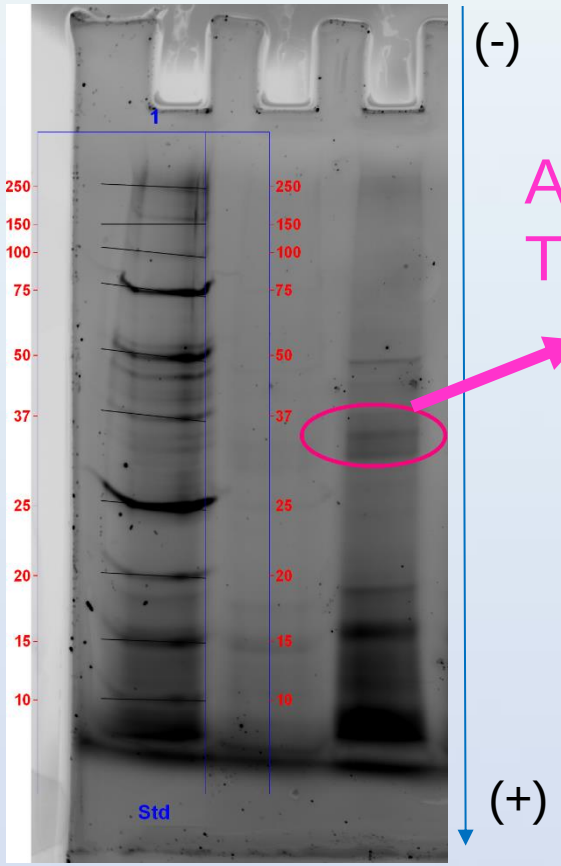
27 y.o. woman with shellfish and HDM allergies

Systemic reactions

sIgE Shrimp = 9.89 kUA/L
sIgE Shrimp tropomyosin (rPen a 1) = 14.3 kUA/L
sIgE HDM (rDer p1) = 92.4 kUA/L
sIgE HDM (rDer p2) >100 kUA/L
sIgE HDM tropomyosin (rDer p10) = 16.9 kUA/L



Western blot 1D with *Grillodes* extract



Arginine kinase ?
Tropomyosin ?

Protein of interest (allergen) detected by Western blot 1D

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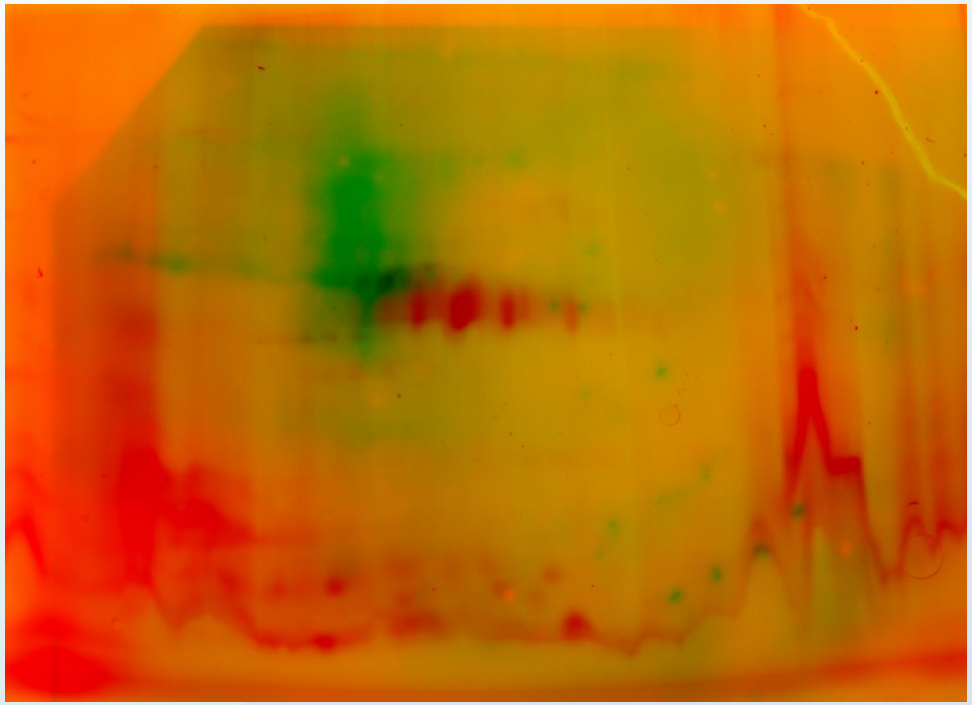
First case analyzed : 2D

pH 3 ⟶ 10

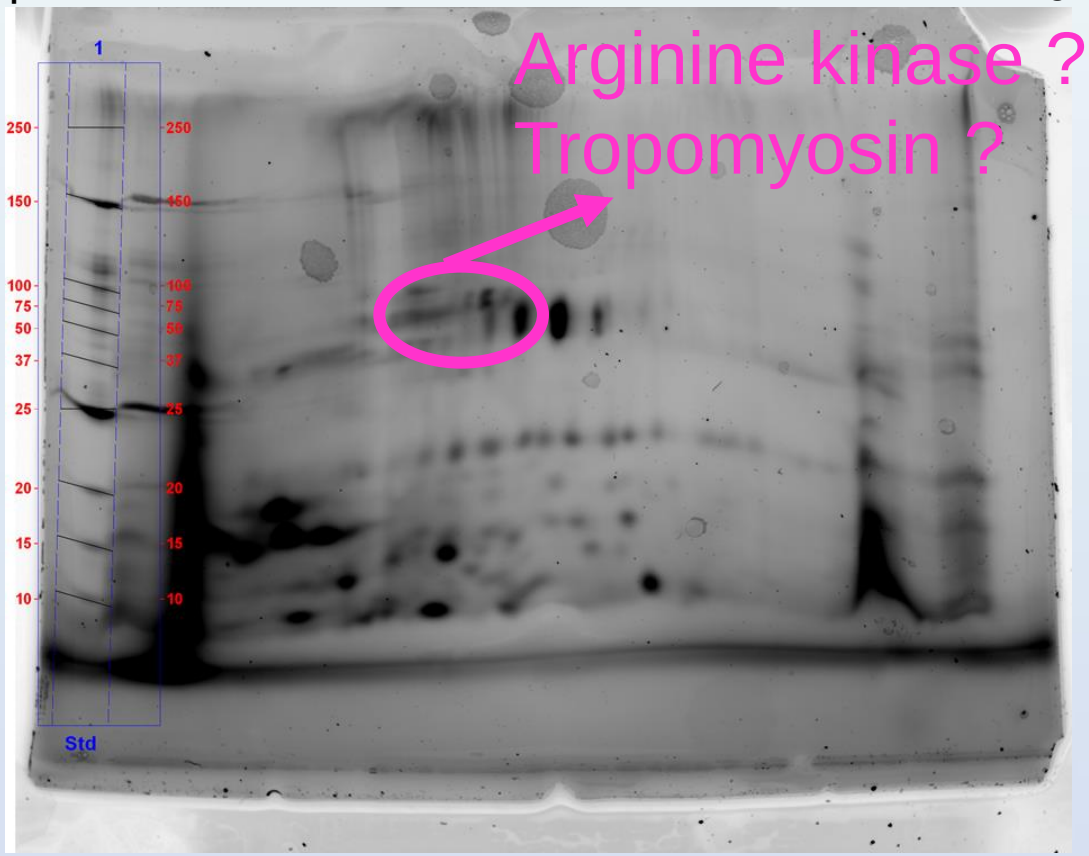


(-)

(+)

Western blot 2D with *Grillodes* extract

pH 3 ⟶ 10



(-)

(+)

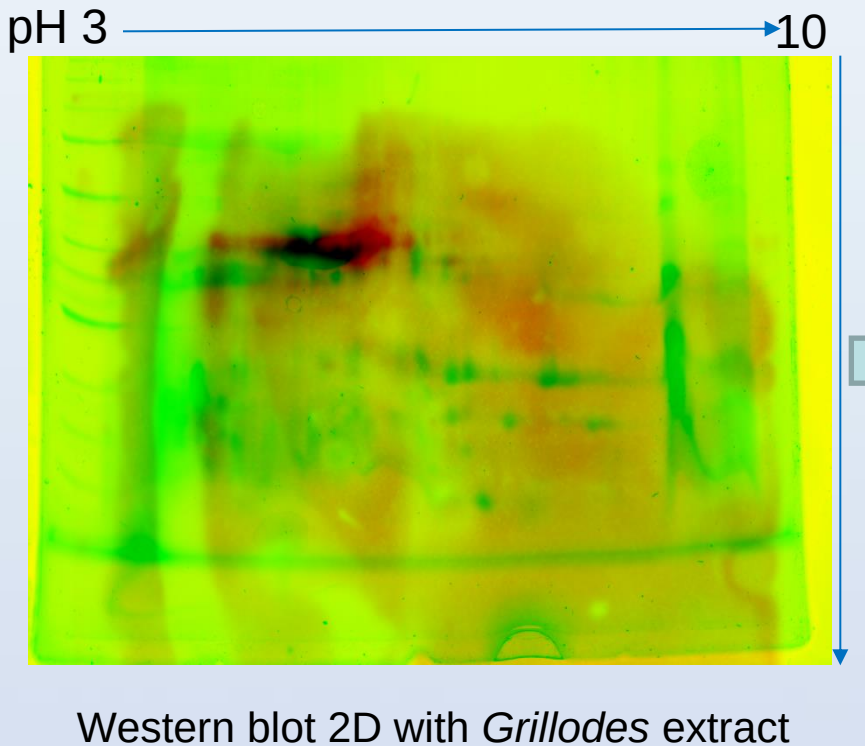
Protein of interest (allergen) detected by Western blot 2D

Second case analyzed : 2D

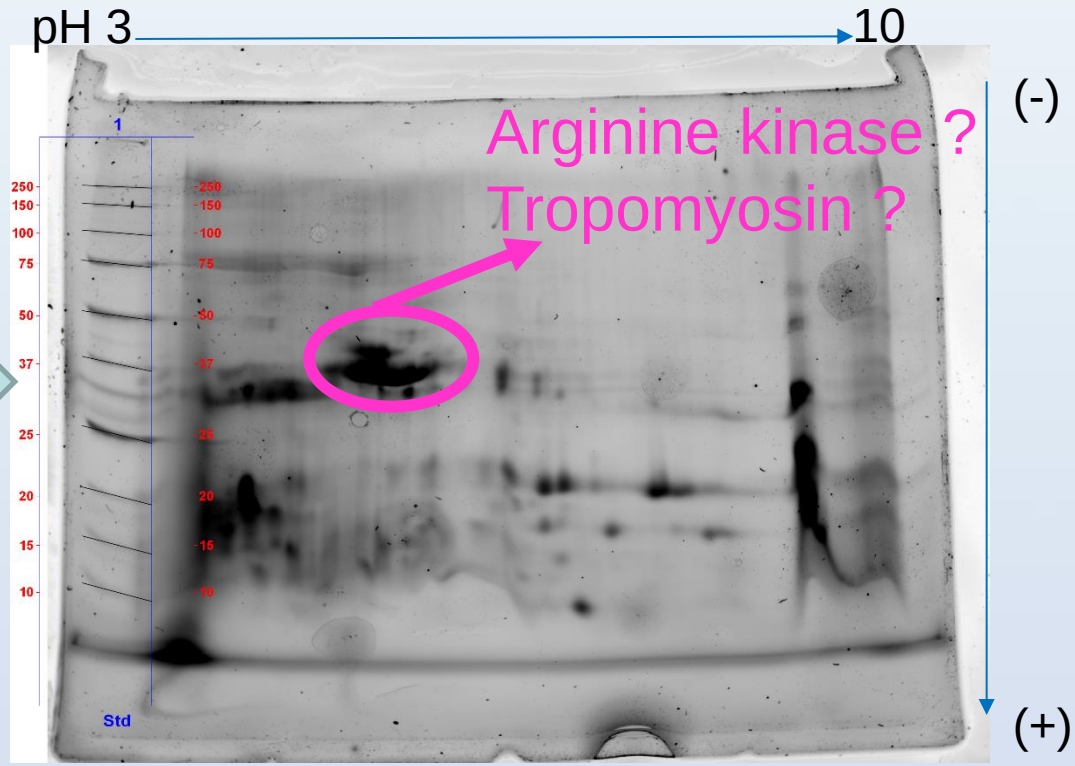
30 y.o. woman with HDM allergy

Atopic dermatitis

sIgE Derm.pteron = 66.3 kUA/L
sIgE Derm.farinae = 45.1 kUA/L
sIgE HDM (rDer p1) = 23.4 kUA/L
sIgE HDM (rDer p2) = 37.9 kUA/L
sIgE HDM tropomyosin (rDer p10) = 0.18 kUA/L



Western blot 2D with *Grillodes* extract

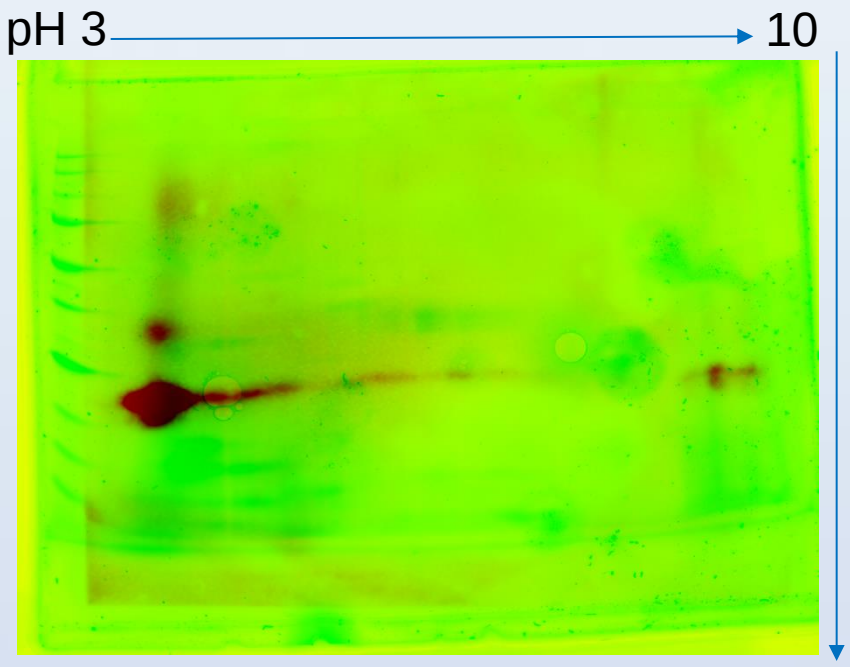


Protein of interest (allergen) detected by Western blot 2D

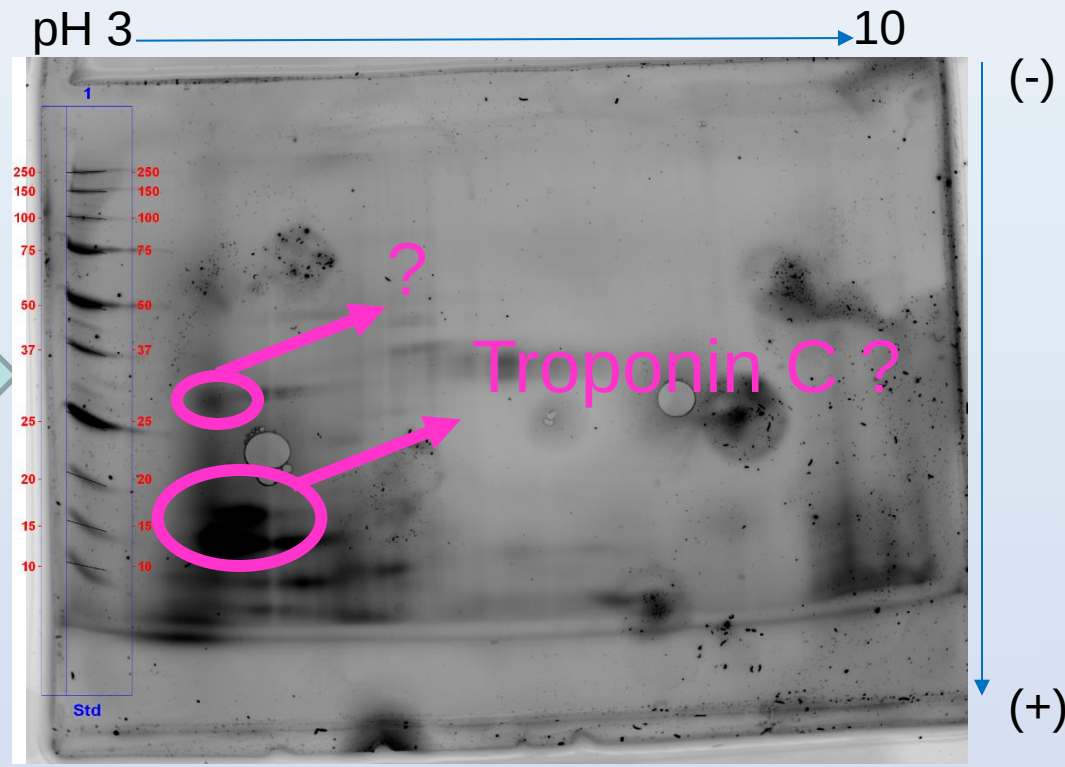
Third case analyzed : 2D

- 38 y.o. man with shrimp allergy
- Oral allergy syndrome

sIgE shrimps = 5.60 kUA/L
sIgE HDM (rDer p1) < 0.1 kUA/L
sIgE HDM tropomyosin (rDer p10) < 0.1 kUA/L



Western blot 2D with *Grillodes* extract



Proteins of interest (allergens) detected by Western blot 2D

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In conclusion

Conclusion : preliminary results

- IgE Cross-reactivities have been shown between shrimp, HDM and crickets
  - ➔ Tropomyosin or Arginine Kinase cross-sensitization
  - ➔ Sensitization to the *Gryllodes*' troponin C

New allergens identified by WB 2D

- The identification of these proteins should be confirmed by mass spectrometry (LC-MS/MS)
- Should be aware of potential allergic reaction in sensitized populations… Should be investigated !

Thank you

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