

# How immunoblotting and mass spectrometry can help to diagnose kiwi fruit allergy

J. Courtois<sup>1</sup>, C. Bertholet<sup>2</sup>, E. Cavalier<sup>2</sup>, N. Gillard<sup>3</sup>, B. Quinting<sup>4</sup>, R. Gadisseur<sup>2</sup>

1: CRIG Liège, Belgium, 2: CHU Liège, Belgium, 3: CER Group Marloie, Belgium, 4: HELMo Liège, Belgium

## Introduction :

Allergy to kiwi fruit is often associated with severe reactions in addition to oral allergy syndrome (OAS). The kiwi fruit matrix is very complex as it contains many allergenic proteins. We describe a clinical case of allergy to kiwi fruit (*Actinidia deliciosa*) in a woman presenting birch pollen allergy and recurrent urticaria.

## Objectives :

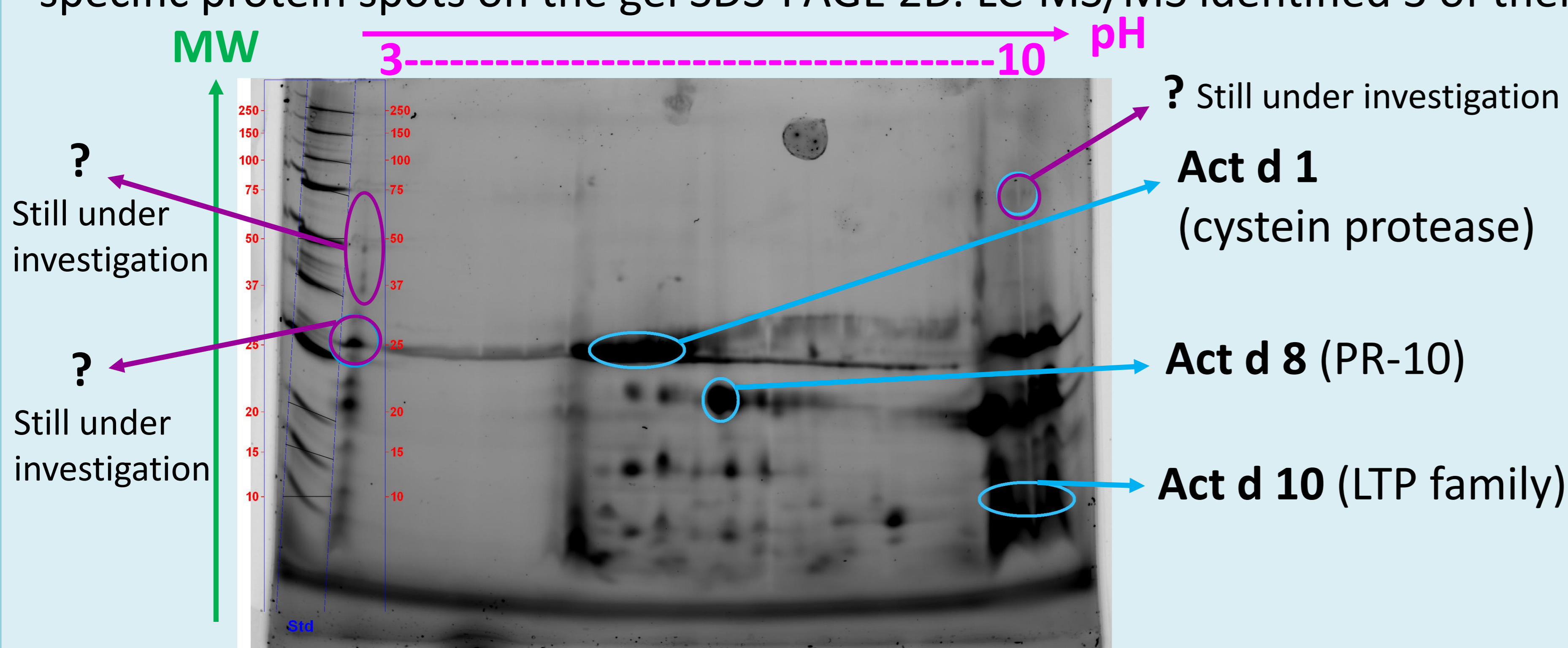
The diagnosis of kiwi fruit allergy is based on a clinical anamnesis, skin prick test (SPT) and specific IgE (sIgE) measurement to total kiwi fruit extract. Currently the *in vitro* diagnostic tools cannot help the physician to define the precise kiwi allergen involved in the allergic reaction. Indeed, only one molecular allergen component is commercially available for the traditional method : Act d 8 (PR-10 protein, Birch Bet v 1-homologous). The allergens Act d 1, Act d 2 and Act d 5 are available for use in the microarray method. This study aimed to adapt a 2D Western blot (WB) to determine the molecular allergen sensitization profile of the patient. Afterwards, mass spectrometry (LC-MS/MS) was used to accurately identify the allergens recognized by the patient sIgE.

## Method :

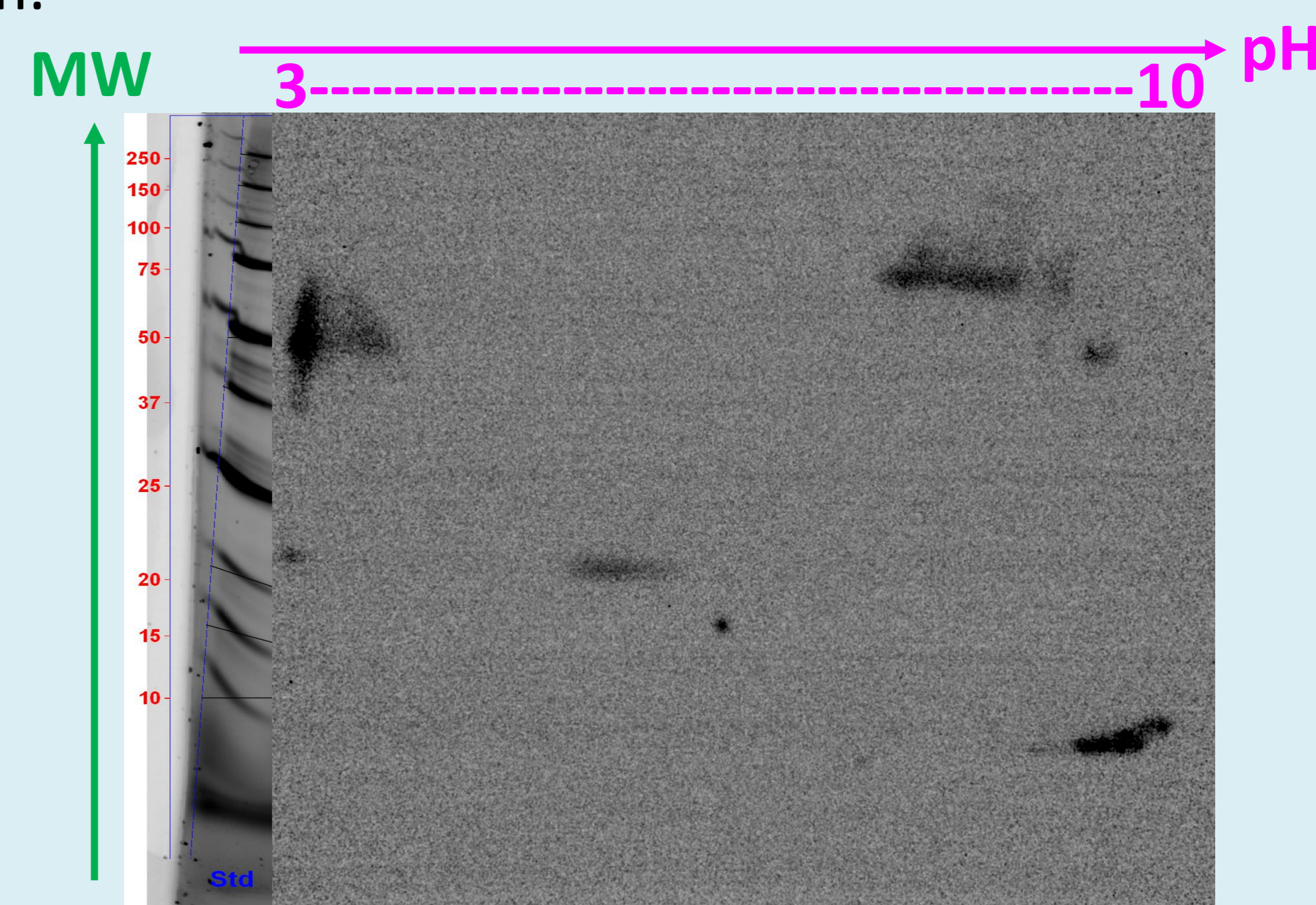
The serum of a 23 y.o. woman presenting a positive SPT and a clinical anamnesis of kiwi fruit allergy was analyzed. The laboratory performed several sIgE measurements against kiwi fruit and against CCD markers (ImmunoCAP 250, ThermoFisher Scientific, Uppsala, Sweden). An ImmunoCAP ISAC (ThermoFisher Scientific, Uppsala, Sweden) allergen microarray was also performed. Total *Actinidia deliciosa* proteins were extracted and the proteins separated on the basis of their isoelectric point and molecular weight. The patient serum was analyzed by 2D WB in order to evaluate its sIgE reactivity against the different protein spots. Finally, the protein spots recognized by the patient sIgE were identified by LC-MS/MS (Xevo TQS-micro, Waters).

## Results :

The sIgE measurement for kiwi fruit extract was low (0.11kUA/L). In contrast, the sIgE for Act d 8 measurement was positive (6.66 kUA/L) whereas the measurement for CCD markers was negative (< 0.10 kUA/L). The ImmunoCAP ISAC showed a positive reaction against Act d 8 but no reaction against Act d 1, Act d 2 and Act d 5. The patient sIgE sensitization profile showed 6 specific protein spots on the gel SDS-PAGE 2D. LC-MS/MS identified 3 of them.



**Figure 1 :** 2D electrophoresis of kiwi fruit proteins extract with specific reacting areas highlighted that were analyzed by LC-MS/MS.



**Figure 2 :** 2D Western blot with the patient serum allergic to kiwi fruit. Six reacting areas were identified.

## Conclusion :

This study describes the case of a birch pollen-allergic woman presenting recurrent urticaria with low sIgE to kiwi extract but positive SPT to kiwi. The 2D WB provided a sIgE profile showing multiple sensitization to kiwi fruit allergens. Amongst them, we confirmed a sensitization to Act d 8 associated with OAS in birch pollen allergic patients (PR-10, Bet-v 1 like) and identified Act d 1 and Act d 10, both frequently associated with severe reactions to kiwi fruit. We pointed out a potential role of Act d 1 and Act d 10 in the clinical symptoms of urticaria in this patient. Furthermore, we demonstrated the additional benefit of 2D WB compared to the traditional diagnostic methods which are unable to reach the same precision.

In relation to this presentation, I declare that there are no conflicts of interest.

### Contacts

- COURTOIS Justine; j.courtois@crig.be
- GADISSEUR Romy; romy.gadisseur@chu.ulg.ac.be

### Partners

- GADISSEUR Romy, University of Liège, Clinical Chemistry Department of CHU of Liège
- GILLARD Nathalie, CER Group Health Department, Marloie