

PROTEIN MICROARRAYS TECHNOLOGY APPLIED TO THE ENVIRONMENT ALLERGEN QUANTIFICATION

*Técnica de microarrays de proteína aplicada a la cuantificación
de alérgenos medioambientales*

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INTRODUCTION

Allergic disorders caused by air-borne pollen and spores constitute an important public health problem, which is increasing dramatically since the last decades. The allergenic particles causing these disorders are not only present inside the pollen grains and the spores but they are also free

in the atmosphere or associated to particles. The quantification of these external allergens together with the classic aerobiological measurements is necessary to build a more precise knowledge of the biological quality of the air.

Protein microarrays is a biotechnological method based on molecular biology and used to detect and quantify

proteins in biological fluids, allowing the analysis of more than one hundred allergens simultaneously. Basically, a protein microarray is a solid-phase immunoassay where the allergens are immobilised on a solid substrate and incubated in presence of human serum. Specific binding of primary antibodies is detected by the addition of a secondary fluorescence-labelled antibody emitting the signal that is digitized.

The aim of this study is to show the usefulness of the microarrays for the detection and quantification of airborne allergens and that they can be used to measure several airborne allergens in a single analysis.

To achieve these goals, we have to analyze: a) the protein threshold that microarrays study can detect (we used *Phleum pratense* pollen as example) and, b) the relationship between the allergen load measured in the air and the pollen counts (we used Phl p 1 - Poaceae).

METHODS

Airborne pollens were sampled using a Hirst collector and following the standard methods of the Spanish Aerobiology Network (REA) (GALÁN *et al.*, *Manual de Calidad y Gestión de la REA*. 2007). Allergenic airborne particles were sampled with a Multi-vial Cyclone Sampler. Both sampling traps are placed at the Universitat Autònoma de Barcelona. Classical aerobiological results were used to decide the dates that will provide the

allergen samples to analyze. The chosen 10 allergen samples corresponded to days with high, medium and, low Poaceae pollen concentrations. The measurement of the allergenic load was realized with the combination of a competitive inhibition followed by a microarrays analysis (Immune-Solid Phase Allergen Chip ISAC, Austria) using the serum of a patient diagnosed by Skin Prick Test and specific IgE to be allergic to Poaceae, *Olea* and *Cupressus*. The study comprised three steps where different elements were used as inhibitors and were mixed with the serum: in the first step authors used different concentrations of the Group-1 grass pollen allergen (from 0.1 to 10.000 pg/mL) to establish the standard curve (see results in Figure 1); in the second phase different concentrations of fresh *Phleum pratense* pollen (1,50,500,5000 grains/mL) were used to validate the sensitivity of the methodology; and in the last part the samples obtained from the Cyclone allergen trap were used to measure the airborne allergenic load on Phl p 1.

RESULTS AND CONCLUSION

The main results obtained let the authors conclude that:

- 1) ISAC inhibition is a highly sensitive methodology, able to detect the allergen content released from 1 grain of natural Poaceae pollen (minimum quantifiable in classic Aerobiology) and 2.1 pg/mL of Phl p 1.

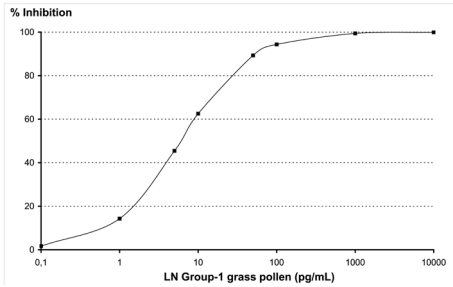


FIGURE 1. Limit detection assay. Group-1 grass pollen allergen in solid-phase (ISAC platform) and complex rPhl p 1-human serum as inhibitor.

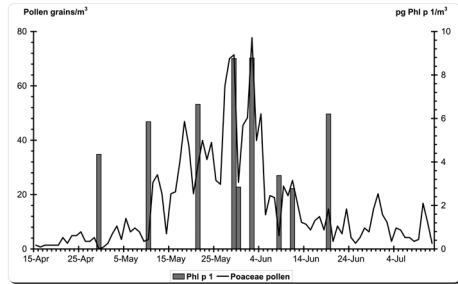


FIGURE 2. Comparison of airborne Poaceae pollen (pollen/m³) and airborne Phl p 1, allergen (pg/m³) analysed by microarrays technology. Data from Bellaterra (Barcelona) year 2009.

- 2) Airborne allergen samples from Phl p 1 showed a rather good correspondence with Poaceae pollen counts (see Poaceae pollen and Phl p 1 concentrations in Figure 2) .
- 3) Microarrays are good tools to detect the airborne allergenic molecules in the environment and should facilitate the simultaneous measurement of several allergens in the air, improving airborne allergen quantification and helping allergy diagnosis and prevention.

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