QUANTIFICATION OF Phl p5 AEROALLERGEN FROM OUTDOOR AIR SAMPLES USING AN IMPROVED ELISA METHOD

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Introduction: The allergen exposure is nowadays determined from pollen counts, in air samples. However, there is not yet enough evidence to establish this technique as a reliable indicator of allergen exposure. Presently, there are only a few reliable and sensitive ELISA methods that allow the quantification of allergen from environmental air samples but none is known to work well with Poaceae allergen, mostly due to its very low concentration.

In this work, we developed a strategy that allowed the quantification of the one of the main allergen from Phleum pratense Phl p5 using a modified ELISA method.
Methods: The samples of airborne pollen were collected on a meteorology platform at the city of Évora using a "cyclone" technology collector (Coriolis ® δ by Bertin Technologies, France) and a modified ELISA method, using a Kit obtained from Indoor Biotechnologies, in order to determine its content in Phl p5. This ELISA method was manipulated in order to improve its detection limits toward lower antigen concentration; several steps were engaged, especially antibody dilution and incubation periods at different steps of the method. The sampling had a daily frequency which totally overlapped the pollen season 2007.
Results: The sensitivity limit of the standard curve was diminished from 10ng/mL to ≈6ng/mL, as a result of the modifications introduced. However, even after the development of an improved method the samples were found too diluted; although detectable, antigen quantification was not possible. At this stage two different strategies were followed: 1) Sample concentration by ultracentrifugation and 2) Addition of a constant amount of antigen to every sample in order to render it quantifiable. Parallel to the samples, a blank with the same amount of antigen was also prepared. It was found that the sample volume (1-2mL) was not sufficient to allow a significant concentration. In contrast, the second strategy allowed the quantification of all the analysed samples. The Phl p5 concentration found in the samples collected from March to June varied between less than 1ng/mL to 20ng/mL, values impossible to detect with the standard methodology.
Conclusions: In this work, an amenable methodology that enabled the quantification of airborne Phl p5 antigen was developed. Inter-laboratorial assays and the quantification of a blank with known amount of antigen would be useful in order to standardize this methodology and allow the comparison of results from different laboratories. Correlation of these data with the pollen counts and clinical data may contribute to a better understanding of the allergen exposure and its consequences.