Quantification of major allergens

Pollens are one source of allergens causing type I allergy, which is IgE-antibody mediated.

For diagnosis and treatment extracts of pollen are used, containing water soluble proteins. These proteins can be further characterized by their amino acid compositions, their molecular weights, their isoelectric points and their ability to react with human IgE-antibodies.

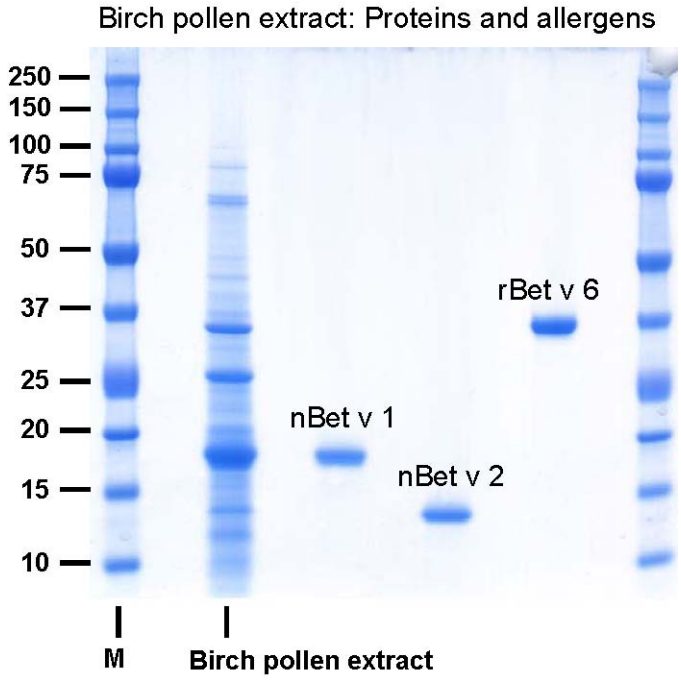
Proteins reacting with human IgE-antibodies are allergens. Allergens are further characterized by the frequency of allergic patients' sera with specific IgE-antibodies. Allergens reacting with more than 50% of allergic patients' sera are denoted as major allergens.

Aqueous extracts of pollen contain mixtures of proteins and glycoproteins, comprising molecules with molecular weights between 5000 and more than 100000. Proteins are characteristic for the pollen they are derived from, so protein pattern can be used to characterize pollen extracts.

One method to generate protein pattern is polyacralamid gelelectrophoresis. By this technique, proteins are separated according to their molecular weights. In figures 1 and 2, protein pattern of birch pollen- and timothy grass pollen extracts are shown. Each band represents at least one protein, characterized by its apparent molecular weight, based on this method and the marker proteins used. Bands representing allergens can be indicated in the mixture by comparison with purified allergens, which is also shown in figures 1 and 2.

In figure 1 the protein pattern of a birch pollen extract is shown, together with purified Bet v 1, the major allergen of birch pollen, Bet v 2 and Bet v 6.

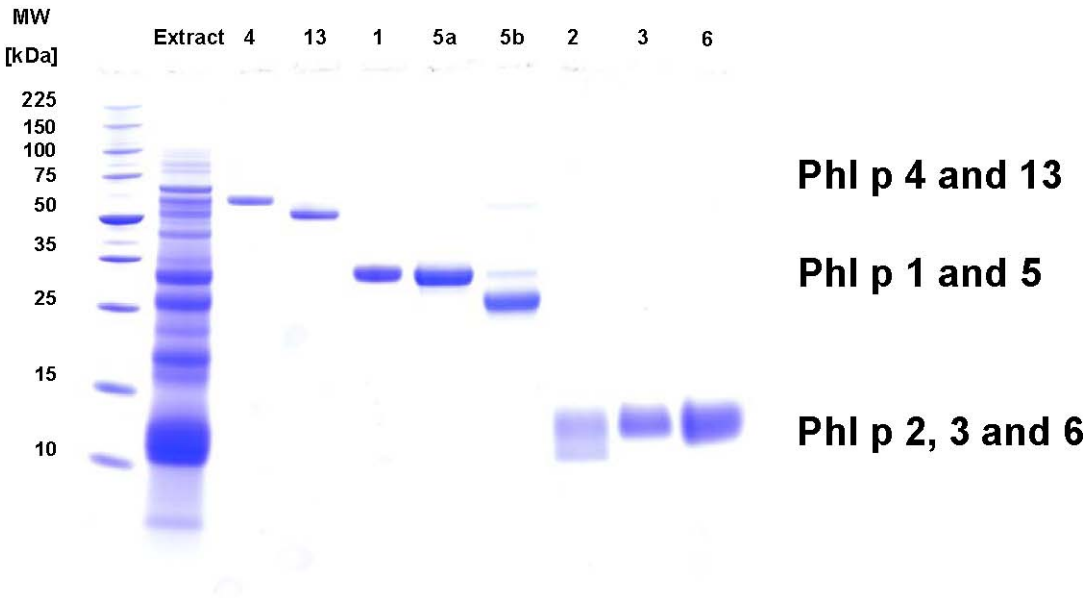
Figure 1. Protein pattern and purified allergens of birch pollen extract.



Bet v 1 has an apparent molecular weight of 17 kDa (between the 15- and 20 kDa marker proteins), which is close to the physical MW.

Detailed information on allergen molecules and bibliographic data are available at:
www.allergome.org.

In figure 2, Timothy grass (*Phleum pratense*) pollen extract and several purified allergens are shown.
Figure 2. Protein pattern and purified allergens of Timothy grass pollen extract



These chromatograms were obtained by gel electrophoresis and subsequent staining of separated proteins by Coomassie blue. M: Molecular weight marker proteins; molecular weights indicated (kDa).
Quality control of pharmaceutical preparations produced from allergen extracts, i.e. skin prick test solutions or preparations for specific immunotherapy comprises several methods aiming to assure batch to batch consistency. One of these methods is the quantification of major allergens by monoclonal antibody based 2-site binding assays. Some of these assays have been developed at Allergopharma since the early 1990s, i.e. the assay for quantification of grass group 5 allergens and the Bet v 1 – quantification assay. Monoclonal antibodies were raised and selected at Allergopharma, and they are produced in cell cultures in Reinbek. These assays are commercially available from Indoor Biotech (www.inbio.com).
As illustrated in figures 1 and 2, allergens are detectable by using gelelectrophoretic techniques. By using quantification assays, also the amount of i.e. Bet v 1 or Phl p 5 can be determined.
These methods are recommended in the current edition of the European Pharmacopoeia. In the European CREATE project [1, 2] references and assays are evaluated, which are currently validated and which will become part of the monograph Allergen Products of the European Pharmacopoeia.
[1] van Ree R et al. The CREATE Project: development of certified reference materials
for allergenic products and validation of methods for their quantification. Allergy 2008: 63: 310–326
[2] Chapman MD et al. The European Union CREATE Project: A model for international standardization of allergy diagnostics and vaccines. J Allergy Clin Immunol 2008;122:882-9.

Dr. Berhnard Weber Project Leade HIALINE for Allergopharma

Allergopharma Joachim Ganzer KG is manufacturer of allergen based products for diagnosis and therapy of IgE mediated allergic diseases. For the HIALINE project Allergopharma provides reagents for monoclonal antibody based 2-site binding assays for the quantification of grass group 5 allergen, a major allergen of grass pollen, and for the quantification of Bet v 1, a major allergen of birch pollen.

Allergopharma developed these assays and is producing the monoclonal antibodies in hybridoma cell cultures. In the company the quantification assays are used for quality control of allergen extracts.