

Successful Standardization of Purified Natural and Recombinant Mite Allergens as part of the European Union (EU) CREATE Project.

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Rationale: To develop purified Group1 and Group2 mite allergens with verifiable allergen and protein content, as part of the EU CREATE program, which will serve as international standards for *in vitro* assays and for research use.

Methods: Natural allergens, or recombinant allergens from *Ecoli* or *Ppastoris* were purified by affinity chromatography or HPLC and analyzed by SDS-PAGE, protein assay, amino acid analysis, mass spectrometry and by ELISA. Purified allergens were compared by ELISA with three existing allergen standards and with six commercial Dpt or Df extracts.

Results: Amino acid analysis showed good agreement between the protein content, with recombinant:natural protein ratios of 2.4-2.7 and 0.51-1.4 for Group1 and Group2. Q-TOF MS showed major peaks at MW 14027-14096 for purified Group2 allergens. Multiple isoforms were detected in natural allergens. ELISA showed parallel dose response curves and consistent quantitative relationships between all five allergen standards with detection limits ranging from 4-13ng/ml (Group1) and 0.5-1ng/ml (Group 2). Inter-assay ELISA CV's were 12-23% Group 1 (IBI), 37-45% Group 1 (ALK) and 26-36% Group 2. Allergen levels in Dpt or Df extracts ranged from 8-1000µg/vial (Group 1) and 3-170µg/vial (Group2).

Conclusions: Purified mite allergen standards have been developed and the quantitative relationship between current mite references and CREATE reference materials has been established. Immunoassays for both major mite allergens have been validated. The purified CREATE references are suitable for international comparisons of allergen levels by immunoassay and should have applications in standardizing allergenic products and environmental exposure measurements.