639 Quantitation of Aspergillus versicolor Antigen Using ELISA and Fluorescent Multiplex Array Technology

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RATIONALE: *Aspergillus versicolor* is an allergenic and potentially toxigenic mold found in damp indoor environments and water-damaged buildings. The health implications associated with mold exposure are not well understood. Reliable methods for exposure assessment are required to determine associated risks. Our aim was to develop sensitive immunoassays for the detection of *A. versicolor* antigen.

METHODS: Five monoclonal anti-*A. versicolor* antibodies were used in ELISA to screen extracts of 10 different mold species commonly found in water-damaged buildings. The extracts were also tested by fluorescent multiplex array using monoclonal antibodies covalently coupled to the surface of internally labeled microspheres. Extracts prepared from mold-contaminated wallboard material were tested by mAb ELISA as well as by polyclonal ELISA for *A. versicolor* antigen.

RESULTS: The ELISA and multiplex array were shown to be specific for *A. versicolor* and recognized an antigen designated AveX. Both assays exhibited high levels of sensitivity: dynamic ranges were 1000-1.9ng/ml for ELISA and 500-0.24ng/ml for the fluorescent multiplex array. The polyclonal ELISA had a dynamic range of 10-0.04ng/mL. Measurements of AveX in wallboard samples (n=11) correlated with the polyclonal ELISA, particularly for samples with relatively high antigen content.

CONCLUSIONS: We have developed species-specific and highly sensitive assays for *Aspergillus versicolor* antigen using monoclonal antibody-based ELISA and fluorescent multiplex array. The data suggest that murine antibody responses to *A. versicolor* tend to be species specific and/or that *A.versicolor* produces unique antigens. These assays may be useful for *A.versicolor* exposure assessment and to determine associated health risks.

640 House Dust Mites Are Unable to Penetrate or Colonize Suede

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RATIONALE: Although smooth leather furnishings are recommended for patients with dust mite allergy, there is no information about the suitability of suede objects for mite allergen avoidance.

METHODS: Dense cultures of *D. pteronyssinus* mites were sprinkled onto pieces of suede (Royal Suede, Edelman Leather Co.) which were then observed under low power microscopy. The inoculated suede samples were then cleaned with a vacuum cleaner, and reexamined for the presence of mites. Finally, Bischoff's mobility test was performed, covering the suede samples with clear Contact adhesive sheets, placing them in the dark for 2 days, and then examining the adhesive sheets to see if mites were present.

RESULTS: Previous observations of mites placed on carpeting or other fabrics had revealed that all mites burrowed into the depth of the fabric within less than a minute. Similar observations with non-woven encasings had revealed a slower progression of the photophobic mites into the material. In contrast, repeated observations of mites on suede revealed numerous apparent attempts of the mites to penetrate the substrate, but with the mites invariably terminating the attempt and backing out of the space they had attempted to penetrate. No mites were visible on the suede after vacuuming, nor were any present on the mobility test.

CONCLUSIONS: Despite its rough surface, mites are unable to penetrate or colonize suede, and are easily removed by vacuuming. Although this study did not examine mite allergen, it does suggest that suede furnishings, particularly if vacuumed regularly, are suitable for mite-allergic patients.

641 Isoallergen Analysis of Pectate Lyases (Amb a 1 and Amb a 2) from Commercial Short Ragweed Pollen

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RATIONALE: To provide the basis for the decision if a single recombinant isoallergen of Amb a 1/2 is sufficient or if a mixture of isoallergens should necessarily be included in a recombinant immunotherapeutic vaccine for the treatment of ragweed allergy.

METHODS: Different oligonucleotide primer were designed based on the 5'- and 3'-UTRs of known Amb a 1 and Amba 2 isoallergen genes and were used to amplify Amb a 1 and Amb a 2 genes from cDNA generated from commercial short (common) ragweed pollen (Allergon, Sweden) originated from the United States. Additionally, spots of Amb a 1/2 proteins separated by 2D-gel electrophoresis of ragweed pollen extract were analyzed by MSMS-sequencing.

RESULTS: With the PCR approach we analyzed 78 sequences. The majority (49%) were Amb a 1.03 sequences, followed by Amb a 1.01, Amb a 1.02, Amb a 2.01 and Amb a 1.04. Ten new isoallergen variants were found, but additional isoallergens could not be detected. The MSMS-analysis of protein spots confirmed the existence of the 5 isoallergens, however the most abundant isoallergen was Amb a 1.01.

CONCLUSIONS: On a first sight it seems that Amb a 1.01 is the most important isoallergen of short ragweed Amb a 1/2. This is in line with published data showing a high IgE-binding capacity of Amb a 1.01. However, this needs to be confirmed with a large number of sera from ragweed allergic subjects and a set of natively folded recombinant isoallergens of Amb a 1.

642 Quantification of Group 5 Allergens in Grass Extracts

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RATIONALE: The quantification of major allergens is a significant improvement in the characterization and standardization of allergenic extracts. The aim of this study was to determine the content of group 5 allergens in pollen extracts of *Dactylis glomerata*, *Festuca elatior*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, using the same monoclonal antibody kit.

METHODS: Group 5 was measured in three extracts from each species, using monoclonal antibodies and rPhl p 5a (Indoor Biotechnologies, VA, USA) as standard. The allergen content was calculated by interpolation of the OD of the extracts on the linear portion of the standard curve. The accuracy and linearity of the method was calculated. The presence of group 5 was confirmed by 2D-immunoblot and immunoblot inhibition, using rPhl p 5 in solid phase and inhibiting the extracts with a specific pool of sera from allergic sensitized individuals.

RESULTS: The curves obtained (OD vs log ng/ml) showed a high degree of parallelism respect to the standard. The average content of group 5 was: 29.5 mg/mg (SD:9) for *D. glomerata*, 23 mg/mg (SD:8.2) for *F. elatior*, 12 mg/mg (SD:5.7) for *L. perenne*, 47 mg/mg (SD:15) for *P. pratense* and 37 mg/mg (SD:9.1) for *P. pratensis*. Grass extracts were capable to inhibit rPhl p 5. Monoclonal antibodies anti-Phl p5 recognized the Phl p 5 isoforms present in the extracts.

CONCLUSIONS: The method is suitable for the quantification of group 5 allergens in different grass species. The results can be used for the improvement of the standardization of grass extracts.