

### 374 Antibody-Based Detection and Quantification of Dog Allergens Can f 1 and Can f 3 in Dog Allergen Extracts.



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**RATIONALE:** Dog allergen extracts are non-standardized products for which no potency measures are currently available. Previous work in our laboratory has identified candidate monoclonal antibodies for sandwich ELISA assays to measure the dog allergens Can f 1 and Can f 3.

**METHODS:** Monoclonal antibodies were generated in mice against the dog allergens Can f 1 and Can f 3 (GenScript). For Can f 1, screening of the hybridoma supernatants and purified antibodies led to the selection of clone 6G1 as the capture antibody and biotinylated 4C3 as the primary antibody. For Can f 3, screening resulted in the selection of biotinylated 2H5 as a capture antibody. However, candidate monoclonal detection antibodies were poorly reactive with untreated Can f 3, so anti-canine albumin polyclonal sera produced in goat (AbCam) was selected as the primary detection antibody instead.

**RESULTS:** Using optimized conditions for both assays, estimated Can f 1 content of commercially-prepared dog allergen extracts ranged from 1.5 to 30  $\mu\text{g}/\text{mL}$  and estimated Can f 3 content ranged from 1 to 300  $\mu\text{g}/\text{mL}$ . Epithelial-based dog allergen extracts had higher overall Can f 3 content. Both assays were specific to the allergens of interest and did not demonstrate cross-reactivity with homologous proteins in other species.

**CONCLUSIONS:** Monoclonal antibody-based sandwich ELISA assays have been developed to reliably and specifically quantify Can f 1 and Can f 3 in dog allergen extracts.

### 375 High Sensitivity Measurements of Airborne Allergens Using a Patient-Operated Sampling Device: A New Technology Reveals Indoor Aerobiome



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**RATIONALE:** We developed a powerful airborne allergen-sampling device for people with allergic asthma or rhinitis to easily operate in their own homes. Current standards rely on concentrations in settled dust as surrogates for inhalable allergens. We establish reference levels based on median values of allergens from air samples, as a direct measure of inhalable allergens.

**METHODS:** Patients from 5 allergist's practices in the greater Chicago region were provided with instructions, informed consent forms and samplers to run for 5 days in their bedrooms. They recorded demographic, temperature, humidity and other environmental information. Air samples were assayed for 12 common household allergens.

**RESULTS:** Unique allergen profiles were obtained for 92 patient homes. The percentage of samples with values above the limit of detection were: total dustmite: 25%; Fel d 1, 63%; Can f 1, 64%; Mus m 1, 13%; Rat n 1, 0%; Bla g 2, 4%; Alt a 1, 6%; Bet v 1, 1%; Asp f 1, 23%; Phlp 5, 9%; Amb a 1, 4%.

**CONCLUSIONS:** The high volume of air sampled permitted the detection of airborne allergens not previously measurable by other air sampling devices. This includes dust mite, cockroach, and cat or dog in homes without cat or dogs. The device may provide a more relevant measurement for indoor pollen and mold allergens than counts received from remote, outdoor stations. Median values provide a reference frame for individual reports, and with further data can be adapted for other regions.

### 376 Reduction of Re-Aerosolized Household Allergens during Vacuuming with a Product Regime in an Environmental Exposure Chamber (EEC) Model



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**RATIONALE:** Although remediation is the recommended method to reduce household allergens, mechanical methods such as vacuuming can

actually increase airborne concentrations. Allergens such as house dust mite (HDM) are extremely small and can be re-aerosolized for hours. Thus, there is an unmet need to assist mechanical removal of allergen. We conducted a study to examine the effect of airborne allergens after treatment with a foam cleaner designed to bind allergens in carpet.

**METHODS:** An EEC with wall-to-wall carpeting simulated a natural home environment. Cat, HDM, and grass pollen allergens were aerosolized and then naturally distributed by naturalistic walking for 60 min. The carpet was then cleaned with a foam cleaner and vacuuming or with vacuuming alone (control). Air samples (4.00 $\pm$ 0.1L/min) were taken for 60 min. from the start of vacuuming. Pollen samples for 5 min. at the start of vacuuming with impaction methods. Samples were extracted in 1% BSA-PBS-T. Samples were quantified for Feld1 (cat), Phlp 5 (grass), or Derp1 (HDM) with a MARIA<sup>TM</sup> assay (Indoor Biotech). Pollen samples were stained and counted with microscopy.

**RESULTS:** The HDM Derp1 control concentration was 14.6X higher (1.17 $\pm$ 1.36ng/m<sup>3</sup>) than foam (0.08 $\pm$ 0.14ng/m<sup>3</sup>). The cat Feld1 concentration was 3.5X higher for the control (21.65 $\pm$ 10.78ng/m<sup>3</sup>) than the control (6.1 $\pm$ 2.78ng/m<sup>3</sup>). The control had a 3.3X higher concentration of pollen (234 $\pm$ 126grains/m<sup>3</sup>) than foam (70 $\pm$ 14grains/m<sup>3</sup>).

**CONCLUSIONS:** For all three allergens, the foam treatment was more effective than the control at reducing the airborne concentration. Compounds that help bind allergens in soft surfaces can prevent the re-aerosolization of allergen.

### 377 Methods for Validating a Cat Allergen Exposure Chamber



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**RATIONALE:** Exposure chambers can assess allergic responses within a controlled environment. Despite cat allergies being common, limited facilities exist to expose subjects to controlled levels of the major cat allergen Feld1. The purpose of this study is to perform technical validation of our cat chamber and ensure stable and consistent levels of Feld1 to facilitate future cat allergen exposure studies.

**METHODS:** The chamber, volume 520 ft<sup>3</sup> (14.7 m<sup>3</sup>) was constructed to accommodate two neutered cats and 1-2 subjects. Samples will be obtained at 3 locations in the chamber using portable air sampling pumps (Gilliam 5000) with glass fiber filters (Millipore), flow rate 4 L/min. Feld1 is quantified using ELISA (Indoor Biotechnologies). After introduction of cats into the chamber, 15-minute samples will be collected daily, and then weekly, to follow evolution of Feld1 levels and their similarity at different points in the room. Chamber cleaning and air circulation as a means to control and homogenize allergen levels will be evaluated.

**RESULTS:** Preliminary data from one sampling pump obtained after 3 days for intervals of 15 minutes, after shaking the cat's blanket, showed a decrease in Feld1 levels from 39.7 to 12.3 to 9.2 to 4.4 ng/m<sup>3</sup> after 15, 30, 45, and 60 minutes, respectively suggesting this is suboptimal to aerosolize cat dander.

**CONCLUSIONS:** The results of validating the chamber should allow controlled levels of Feld1 to be maintained in future cat allergy studies. Further sampling methods will be performed in the chamber to enable more accurate evaluations of efficacy of pharmaceutical interventions in cat allergy.