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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Automated dander dispersal in a cat Naturalistic Exposure Chamber (NEC)

To the Editor,

Allergen exposure chambers (AEC) provide controlled allergen exposure to allergic subjects for the clinical study of asthma and allergy. They should ideally mimic natural allergen exposure, and provide better control than possible in field studies. For AEC exposure to cat allergens, typically, either liquid allergen extract is nebulized,[1,2] or natural cat hair and dander are aerosolized by shaking cat bedding.[3,4] While bedding-shaking is more naturalistic than liquid extract exposure, it has resulted in highly variable allergen levels.[4]

We have developed an automated method of natural dander dispersal that uses robotic vacuum cleaners (Roomba 981; iRobot) with filters removed and modified for variable suction to vent aspirated dander into the air (Figure S1). Controlled remotely, the vacuums move throughout the chamber for the duration of exposure, aerosolizing dander that has collected on a carpet. Large debris is filtered by a coarse mesh upstream of the exhaust, and smaller, dense debris collects by gravity in the vacuum's dust bin. The remote operability of the robot vacuum affords minimal intervention from the operator as compared to blanket shaking.

The system was validated in two rooms of a Naturalistic Exposure Chamber (NEC) (3-person [14.4 m^3] and 8-person [36.7 m^3] capacity) where two cats reside (Figure S1). Dispersion was characterized by measured airborne Fel d 1 during 2-hour tests. Fel d 1 collected from air samples was quantified using ELISA (see online supporting information and Table S2).

The NEC is certified by the Ministry of Agriculture, Food and Rural Affairs of Ontario (OMAFRA). The study protocol was approved by the NEC's Animal Care Committee and Canadian Council on Animal Care guidelines were followed. Dust mite and fungal antigens were below measurable levels in aerosolization test air samples (<3.7 ng/m^3 Der p 1, <0.8 ng/m^3 Der f 1, <1.1 ng/m^3 Alt a 1, and <0.6 ng/m^3 Asp f 1). Maximum endotoxin levels were 9.2 EU/m^3.

The automated method generated much more even particle aerosolization in time than did blanket shaking (Figure 1A). Furthermore, a positive correlation of test-averaged Fel d 1 for increasing exhaust flow rate demonstrated that the added suction control provides a degree of control over the aerosolized allergen levels (Figure 1B). The optimized settings for dander aerosolization in both small and large rooms are listed in Table S2.

Four repeat tests were performed in the small chamber at optimized settings, showing good temporal stability of allergen levels over 2 h and homogeneity throughout the room (Figure 2A, B). Average Fel d 1 was 55 (±11 SD) ng/m^3.

Aerosolization in the larger chamber was scaled using two vacuums simultaneously. To account for the greater size of the chamber, rather than increasing the number of cats, carpet allergen levels were supplemented by shaking cat bedding several days in advance of testing, and by adding 10 or 20 g of additional milled cat hair (Stallergenes Greer) directly to the carpets. In the small room validation, a gradual decline of Fel d 1 and particle concentration in time had been observed (Figures 1A and 2A). To correct for this, in the large room tests the vacuum suction level was gradually increased over the course of aerosolization (Figure S2), resulting in stable Fel d 1 (Figure 2C) with average 79 (±30 SD) ng/m^3.

The NEC exposure model offers an intermediate between natural, uncontrolled field exposure and highly controlled, conventional AEC exposure. While the measured allergen levels are much less variable than those in homes or studies using blanket shaking, some variability was observed, as was expected due to natural variation in the cats' allergen production, changes in human and cat activity, and the effects of cleaning.

This novel method of dander dispersal provides controlled, safe exposure to cat allergens in a clinical setting, while maintaining the naturalistic advantages of a field exposure: allergen levels are representative of those measured in homes with cats,[5,6] and subjects are

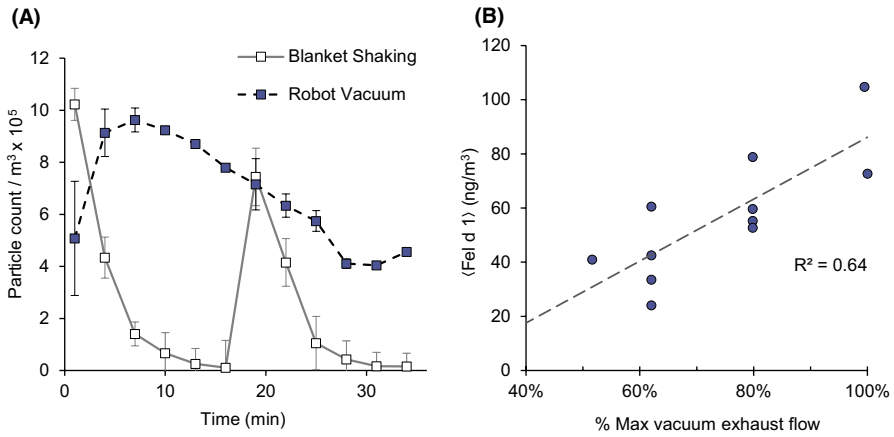

FIGURE 1 Characterization of dander aerosolization in the small NEC: (A) Particles (aerodynamic diameter D ≥ 2 μm) measured using a time-of-flight particle size distribution (PSD) analyzer (PSD 3603, TSI Incorporated, Minnesota, USA) during aerosolization with robot vacuum (constant suction setting = 80% maximum flow) compared to blanket shaking ($N = 4$ for each method). Error bars: standard error. Blanket shaking was done for 1 min each at times 0 and 15 min, after which particle concentrations rose and fell rapidly. Particle concentrations were maintained more evenly with the robot vacuum method, although declined gradually after 10 min while vacuum suction was held constant. (B) Test average Fel d 1 rises linearly with increasing vacuum suction levels

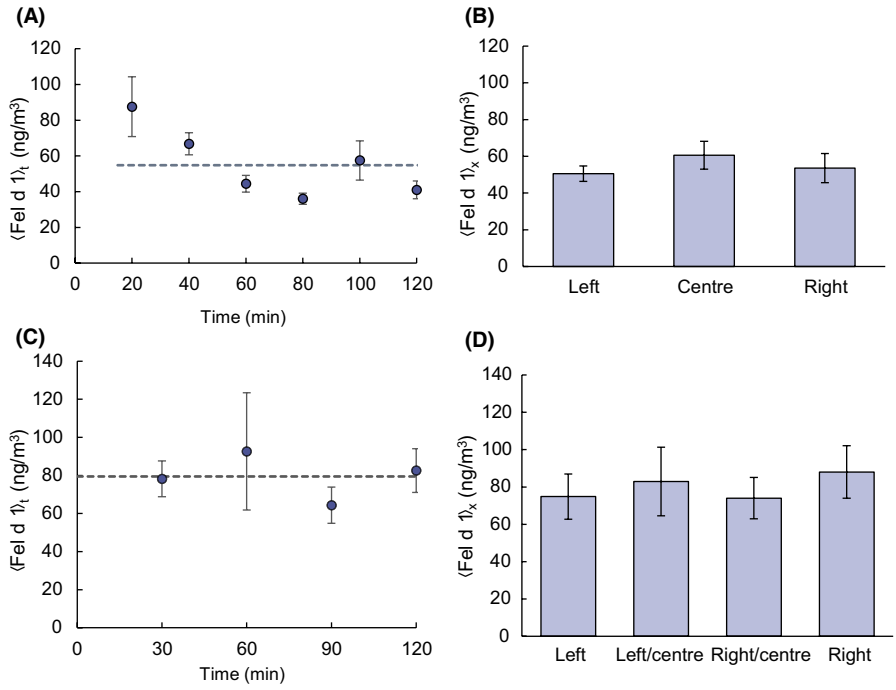

FIGURE 2 Fel d 1 distributions in the NEC at optimized settings: Small room (A) time distribution and (B) spatial distribution from 4 repeat tests; Large NEC (C) time distribution and (D) spatial distribution averaged from 5 repeat tests. Error bars indicate standard error, and dashed line indicates time-average (55 ng/m^3 in small room and 79 ng/m^3 in large room). There was no systematic spatial gradient observed within the rooms and the maximum spatial deviation from the average of five tests was 10% and 11% from the mean in the small, and large rooms, respectively. Sampling locations are illustrated in Figure S1

exposed to all types of cat allergen in its natural form, achieving a more realistic representation of the subject's experience during an allergic reaction. Such a model may help overcome the limitations of field studies and standardize the assessment of anti-allergen immunotherapies.

KEYWORDS

aerobiology, animal models, asthma, challenge tests, clinical immunology

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Prospective studies are needed to elucidate the clinical impact of predominant Api m 10 sensitization

To the Editor,

In 2016, it was published that a predominant sensitization to Api m 10 may be a risk factor for therapy failure in patients treated with bee venom immunotherapy (bee VIT).[1] The authors also reported that some bee venom preparations, two non-purified and one purified preparation, contain little to no Api m 10. Although these data were interesting, the scientific discussion went in a strange direction. Moreover, some companies claimed (partly with no available data on safety and effectiveness) that their venom preparations were superior because of the higher Api m 10 content compared to commonly used products. Within a few years, this unproven narrative left the impression that patients with predominant sensitization to Api m 10 should be treated with non-purified venoms. However, this was not supported by any published data or endorsed by the EAACI guidelines.[2]