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

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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 levels⁴.

We have developed an automated method of natural dander dispersal that uses robotic vacuum cleaners (Roomba 981; iRobot, Bedford, Massachusetts, USA) 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 m³] and 8-person [36.7 m³] capacity) where two cats reside (**Figure S1**). Dispersion was characterized by measured airborne Fel d1 during 2-hour tests. Fel d1 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/m³ Der p1, <0.8 ng/m³ Der f1, <1.1 ng/m³ Alt a1, and <0.6 ng/m³ Asp f1). Maximum endotoxin levels were 9.2 EU/m³.

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 d1 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-hours and homogeneity throughout the room (**Figure 2A, B**). Average Fel d1 was 55 (± 11 SD) ng/m³.

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, London, UK) directly to the carpets. In the small room validation, a gradual decline of Fel d1 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 d1 (**Figure 2C**) with average 79 (± 30 SD) ng/m³.

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 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.

FIGURES (FOR REFERENCE ONLY, SEE PROVIDED PDFS FOR PRINT)

(A)

(B)

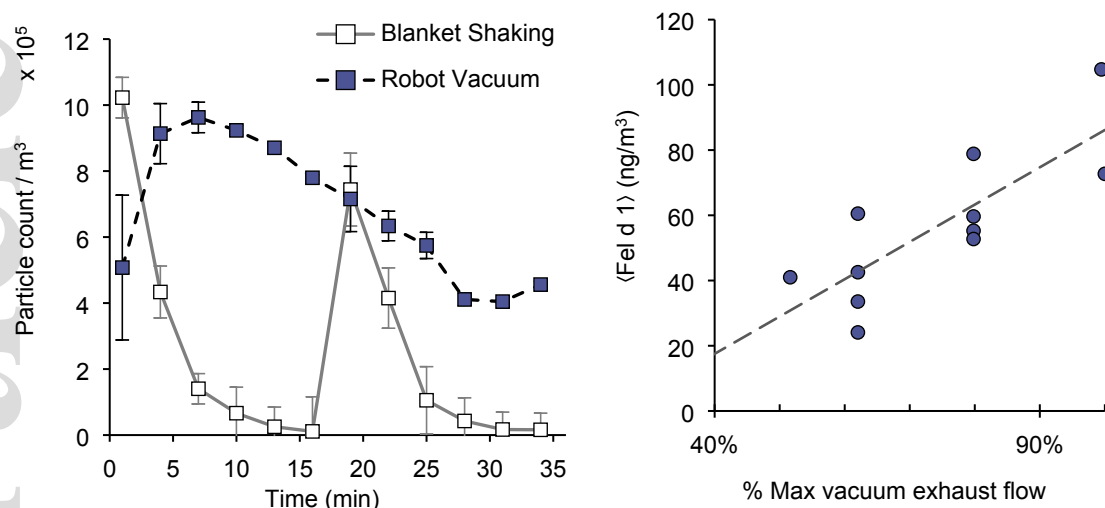
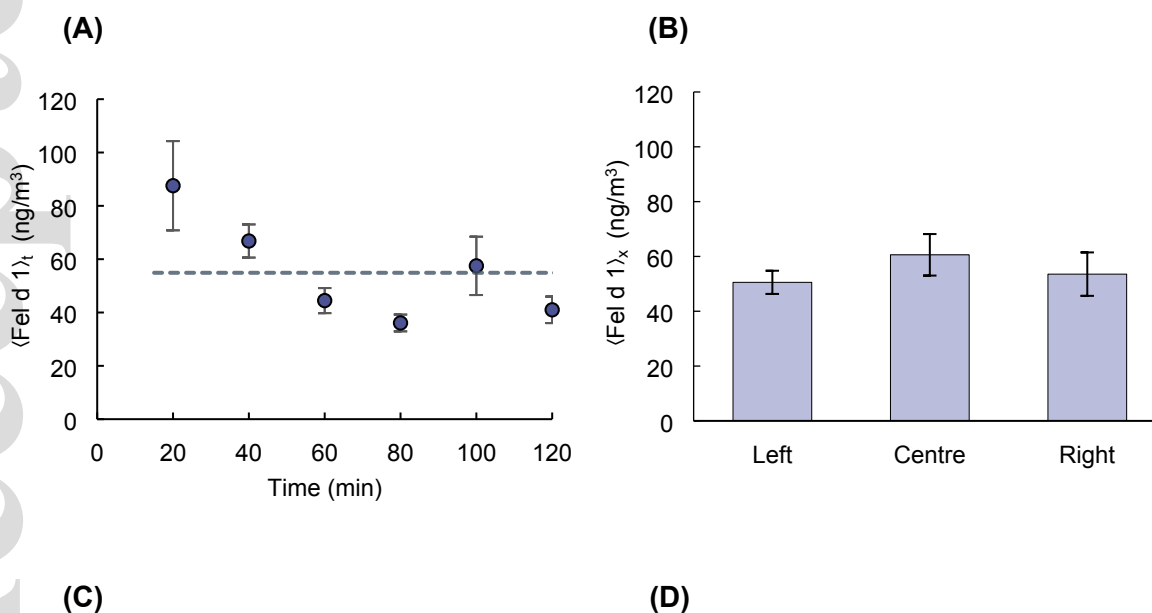


Figure 1. Characterization of dander aerosolization in the small NEC: **(A)** Particles (aerodynamic diameter $D \geq 2 \mu\text{m}$) measured using a time-of-flight particle size distribution (PSD) analyzer (PSD 3603, TSI Incorporated, Minnesota, USA) during aerosolization with robot vacuum (constant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



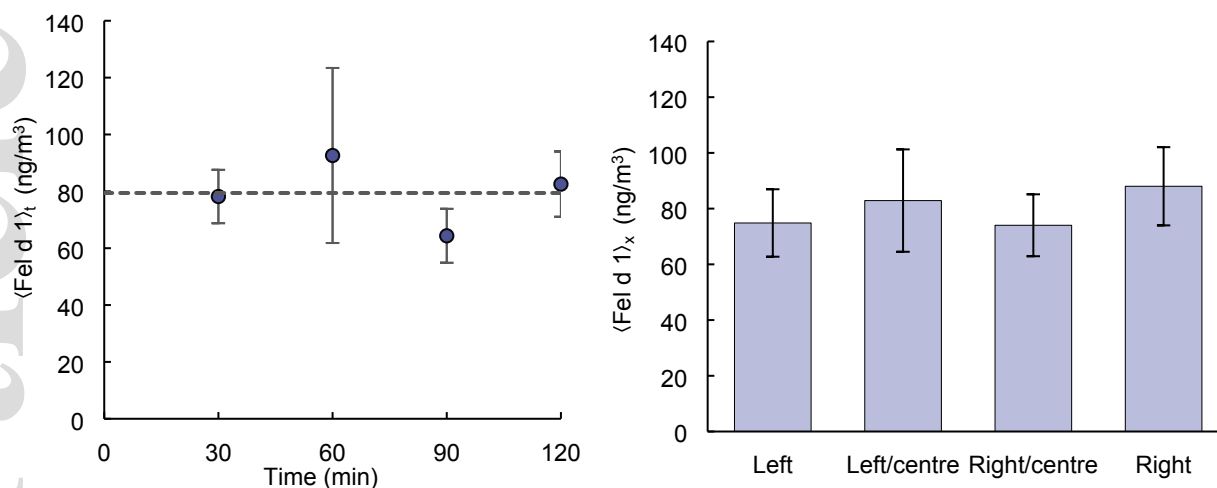


Figure 2. Fel d 1 distributions in the NEC at optimized settings: Small room (A) time distribution and (B) spatial distribution from 4 repeat tests; Large NEC (C) time distribution and (D) spatial distribution averaged from 5 repeat tests. Error bars indicate standard error, and dashed line indicates time-average (55 ng/m³ in small room and 79 ng/m³ 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**.

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The authors were involved in the study design and collection, analysis, and interpretation of data. All authors had full access to all the data in this study and take complete responsibility for the integrity of the data and accuracy of the data analysis.

Disclosures/Conflicts of Interest

Laura Haya is an employee of Red Maple Trials, Inc. Rym Mehri and Shawn Somers-Neal received grant contributions from Mitacs and Red Maple Trials, Inc. Stefan Van de Mosselaer and Ashley Spence are employees of Red Maple Trials, Inc, and Red Maple Trials received wage subsidies for their positions through the Biotalent Student Work Placement Program. Suzanne Kelly is a stockholder and employee of Red Maple Trials, Inc. Edgar Matida was the academic supervisor for Rym Mehri and Shawn Somers-Neal. William H. Yang is a stockholder and employee of Red Maple Trials, Inc. He has received consultant and speaker fees from CSL Behring, Shire/Takeda, Novartis, Sanofi, Merck. Also, he has received research grants from CSL Behring, Shire/Takeda, BioCryst, Pharvaris, Sanofi, Regeneron, GSK, AstraZeneca, Amgen, Genentec/Roche, Pfizer, ALK, Stallergenes, Providence, Galderma, Glenmark, Dermira/Eli Lilly, AnaptysBio, Celgene, Johnson & Johnson, and Pharming.

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

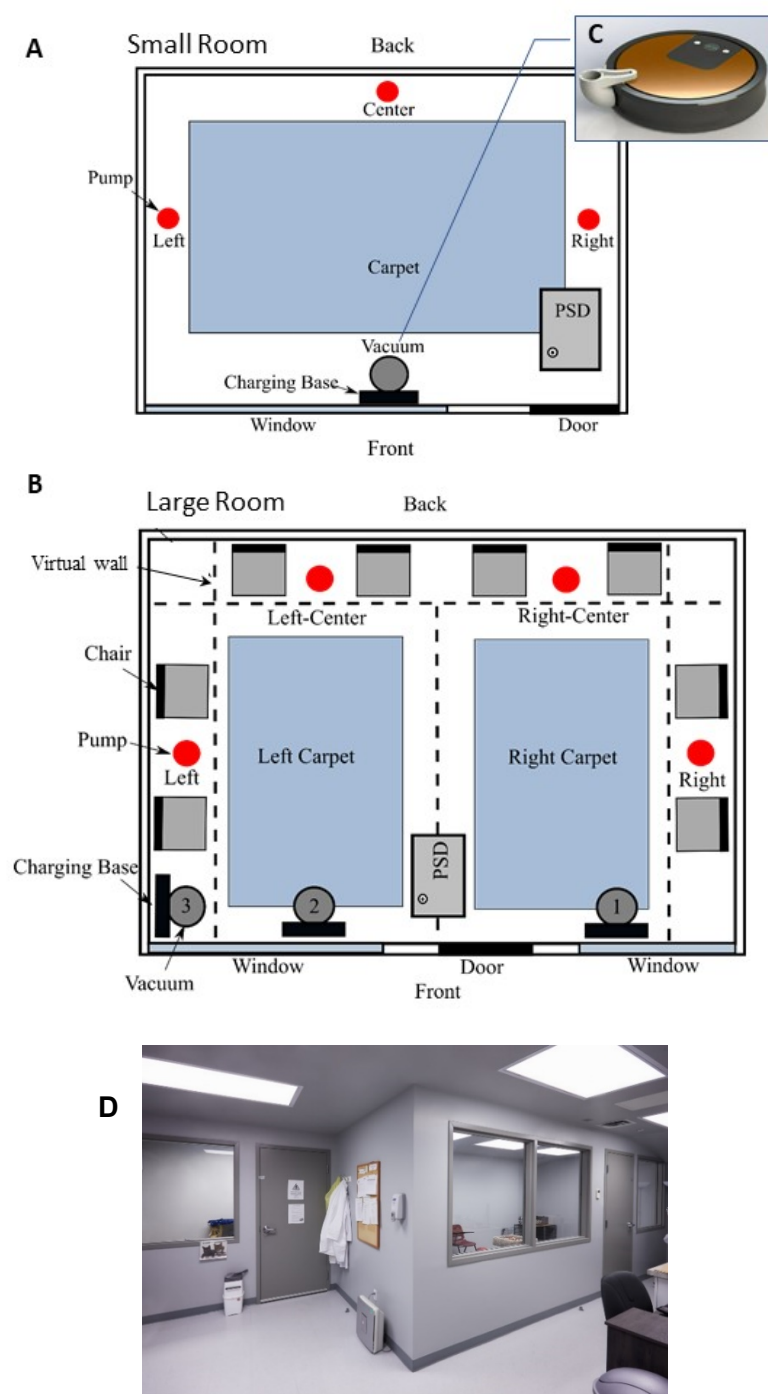


Figure S1. The NEC testing layouts in **(A)** the small room and **(B)** the large room. Pumps (shown as red circles) and particle size distribution analyzer (PSD) sampled at 1.2 m, and 0.87 m above the floor, respectively. Inset **(C)** shows the modified robotic vacuum cleaner with custom exhaust. **(D)** The NEC viewed from the subject monitoring room. The small chamber is on the left and the large chamber is on the right.

S1.METHODS

Fel d 1 was measured at locations x , and time intervals t defined in **Table S1**.

The mass of Fel d 1 m_f in ng, collected on each filter and determined by ELISA (Fel d 1 ELISA 2.0 kit, Indoor Biotechnologies, Inc. Charlottesville, USA) was normalized by the volume of sampled air to obtain the air concentration in ng/m³ as follows:

$$Fel\ d\ 1 = m_f / (Q_{pump} \times t_s),$$

where Q_{pump} is the sampling pump suction flow rate, equal to 4L/min for these tests, and t_s is the sampling duration (20 minutes for the small room tests and 30 minutes for the large room tests).

The results are presented as averages and associated standard errors (SE).

Table S1. Locations x (shown in **Figure S1**) and times t of air sampling for Fel d 1 in small and large NEC rooms. The values of times t are the mid-points of the air sampling period, referenced from the start of the aerosolization in minutes.

$x = \begin{cases} [\text{left, centre, right}] & , \text{small room} \\ [\text{left, left-centre, right-centre, right}] & , \text{large room;} \end{cases}$	
$t = \begin{cases} [10, 30, 50, 70, 90, 110] & , \text{small room} \\ [15, 45, 75, 105] & , \text{large room.} \end{cases}$	

S2.RESULTS

The settings and configurations that were identified through iterative testing as optimal for aerosolization in each the small and large room are listed in **Table S2**. For the large room tests, the vacuum suction level was increased gradually over the course of aerosolization. The staged suction levels chosen to achieve stable Fel d 1 levels over two hours, following iterative testing, are illustrated in **Figure S2**.

Table S2. Optimized settings for dander aerosolization in small and large NEC rooms.

	Small NEC	Large NEC
Aerosolisation	One modified robot vacuum circulating about carpeted room.	Two robot vacuums operating simultaneously in adjacent areas of the room.
Allergen source	Two cats	Two cats with additional carpet supplementation with milled cat hair and/ shaking cat bedding in advance of each test
Vacuum duty cycling	15 min on/ 5 min off	15 min on/ 5 min off
Debris extractors	Removed	Removed
Suction power level	80% of maximum exhaust flow	Staged suction power level schedule, starting at 67% of max exhaust flow rate at $t=0$ minutes and gradually increasing to 92% max exhaust flow rate at $t=100$ minutes, according to Figure S2 .

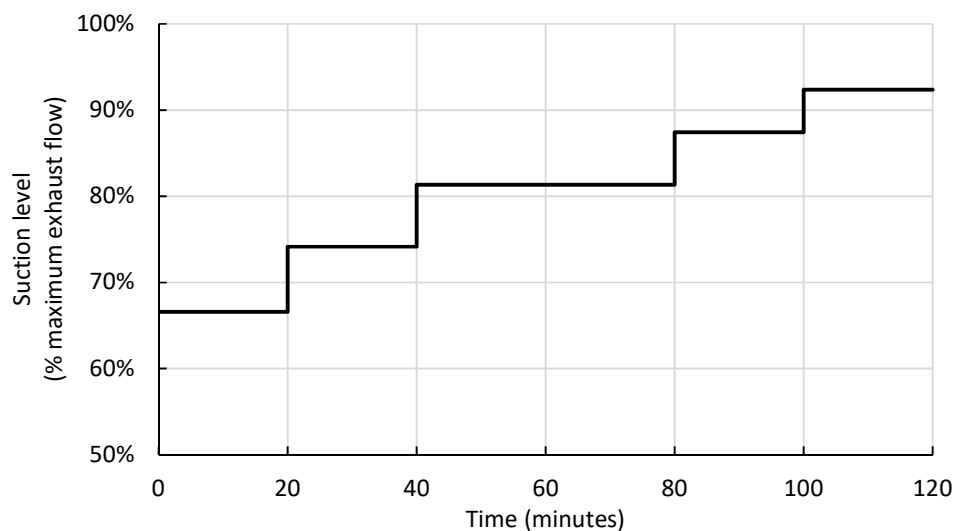


Figure S2. Optimized staged suction level settings during 2-hr dander aerosolization in large NEC.