

366 Correlations between Alt a 1 concentrations, *Alternaria alternata* and Pleosporaceae measured by molecular methods in New York City house dust



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RATIONALE: Alt a 1 is a major allergen produced by *A. alternata* and other species of the *Pleosporaceae* family. Recent advances in immunoassays and DNA sequencing of environmental samples have improved our ability to detect fungi and their allergens. We compared Alt a 1 concentrations measured by enzyme-linked immunosorbent assay (ELISA) with *A. alternata* and other species assessed by quantitative polymerase chain reaction (qPCR) and next generation sequencing (NGS).

METHODS: Bedroom floor dust was collected from participants in the NYC Neighborhood Asthma and Allergy Study. In a subset of samples (n=50), Alt a 1 was measured by ELISA (Indoor Biotechnologies) with amplification utilizing a streptavidin poly-HRP80 conjugate (Fitzgerald Industries International), which improved the sensitivity of the assay by ~2 orders of magnitude. *A. alternata* and other species were measured by qPCR and NGS. Relative humidity was measured in homes for the week after dust collection.

RESULTS: Alt a 1 measured by ELISA correlated modestly with *A. alternata* measured by qPCR ($r=0.41$, $P=0.003$) and *A. alternata* measured by NGS ($r=0.43$, $P=0.0018$). There was a weaker correlation between Alt a 1 and total *Alternaria* species ($r=0.38$, $P=0.007$) and total *Pleosporaceae* family ($r=0.21$, $P=0.13$). The correlations between Alt a 1 and qPCR assessed *A. alternata* was observed in the homes that had higher (above median) relative humidity ($r=0.60$, $P=0.002$), but not those with lower humidity ($r=-0.12$, $P=0.59$).

CONCLUSIONS: Modest correlations between allergen and sequence assessed exposure and modification by relative humidity suggest the importance of multiple methods when evaluating health impacts of domestic exposure to fungi.

367 Aerosolization of Cat Dander in a Naturalistic Exposure Chamber



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RATIONALE: Aerosolization of cat dander in natural exposure rooms is typically done by shaking the bedding. Fel d 1 levels in the room are measured later by ELISA. The purpose of this study was to compare three methods of dander aerosolization in the RMT Natural Exposure Chamber: bedding shaking, an iRobot vacuum cleaner without filter and the iRobot with fans.

METHODS: Particle counts were measured using a particle size distribution analyser (PSD) and a laser particle counter (LPC). Dander samples were collected using portable air sampling pumps (Gilian 5000) at 4L/min with 2 μ m glass fiber filters. Fel d 1 was quantified using ELISA (Indoor Biotechnologies). Measurements were made for 30 minutes after shaking cat bedding for 2 minutes in a central area of the room or after running the iRobot +/- fans.

RESULTS: Particle counts from the PSD and the LPC were found to provide similar trends. During blanket shaking, large particles (2-40 μ m)

were prominent, while using the vacuum cleaner with and without fans provided similar particle counts for particles ranging from 1-20 μ m. The vacuum cleaner and fan method appeared to provide a higher particle count for the smaller particles (<1 μ m). After blanket shaking and turning off the vacuum, the larger particles decreased rapidly and were not detected after 15 minutes, while the smaller particles increased.

CONCLUSIONS: Particle size distribution differs based on the aerosolization method. Compared to the established method of bedding shaking, the vacuum cleaner method seems promising when combined with fans.

368 Development of a Method to Evaluate Cat Dander Levels by Safranin-O Staining



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RATIONALE: Fel d 1 is primarily responsible for allergic symptoms. However in cat exposure rooms replicating home exposure, Fel d 1 levels can vary greatly over time making it difficult to predict patient exposure. ELISA assays delay the ability to regulate allergen levels in a timely fashion. The purpose of this study is to investigate dander counting by staining, light microscopy, and image processing and correlate counts to Fel d 1 levels. This could allow prompt measurements and tighter allergen level control.

METHODS: Dander samples were obtained after shaking cat bedding for 1 minute every 15 minutes over 1 hour. Dander was collected by gravity for 15 minutes on isopore and glass fiber filters at 30, 45 and 60 minutes. Fel d 1 collected on glass fiber filters was quantified using ELISA (Indoor Biotechnologies). Isopore filters were stained with Safranin-O (0.01%) for a minimum of 60 minutes and visualized by light microscopy. Ten images were captured on each quadrant of the filter. Particle count, area and perimeter of detected particles were obtained by computer analysis using ImageJ Software. Particle size distribution, the area ratio of detected particles, and particles/mm² were then calculated.

RESULTS: The area ratio of particles detected as well as the particle count per mm² increased from 15 minutes to 1 hour. A linear correlation was found between Fel d 1 levels and area ratio ($R^2=0.810$).

CONCLUSIONS: The results of this study could be useful for monitoring allergen concentrations in cat challenge chambers.