Laboratory animal allergy is preventable in modern research facilities

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Online supplement

Aeroallergen sampling methods:

Using convenience sampling, we invited employees (animal carers, scientific researchers, support staff) to wear a Casella Apex air-sampling pump (2L/min) for the collection of inhalable particulates on to 25mm fluoropore membrane (1µm) filters, using IOM sampling heads placed in the breathing zone. Workers collected samples over full-shifts (of duration between three and ten hours) keeping a written record of their activities. We also used static monitors to collect background aeroallergen concentrations in different locations within the animal units. In parallel, on each sampling day we placed 'blank' filters in the facility to control for contamination during their placement and processing.

After sampling, we analysed the filters for Mus m 1 using a commercial Sandwich Enzyme-Linked Immunoassay (Indoor Biotechnologies; see below). We converted the values obtained (concentration in ng/ml) to mass (ng/m^3) by adjusting for the volume of sampled air. We assigned a value of 0.2ng/ml to samples where the concentration of Mus m 1 was below the assay's limit of detection (LOD: 0.2ng/ml). On days where the 'blank' samples suggested contamination (defined as at least one blank >1 ng/ml) all measurements for that day were discarded; where aeroallergen levels for blanks were between 0.1 and 1ng/ml, the other measurements for the day were adjusted accordingly. Airborne concentrations were

then simply calculated by dividing the blank-corrected mass of Mus m 1 on the filter by the volume of air sampled (flow-rate multiplied by the duration of sampling for that filter).

Measurement of Mus m 1 in aeroallergen filters using ELISA:

Air sampling filters were eluted in 1ml of elution buffer (1% BSA-PBS/0.05% Tween-20) and rotated at room temperature for 2 hours. Filters were removed from the eluate and stored at -20°C. Air samples were analysed using a Mus m 1 ELISA kit (EL-MM1, Indoor Biotechnologies, Cardiff) according to manufacturer's instructions. In brief, MaxiSorp 96 well-plates (Thermo Scientific, Nunc) were coated with 100ul of pAb Rabbit anti nMus m 1 (1:1000) in 50mM carbonate-bicarbonate buffer (pH 9.6) and incubated overnight in a humid box at 4°C. Plates were washed 3x with PBS/0.05% Tween-20 (PBS-T) and blocked with 100ul of 1% BSA-PBS/0.05% Tween-20 PBS-BSA for 30 minutes at room temperature. Plates were washed 3x with PBS-T and 100ul of either air sampling eluate or standards added in duplicate to the plates and incubated for 1 hour at room temperature. Plates were washed 3x with PBS-T and 100ul of Biotin Rabbit anti nMus m 1 (1:1000) in PBS-BSA was added and incubated for 1 hour at room temperature. Following a further wash 100ul of ExtrAvidin-Peroxidase (Sigma) (1:1000) in PBS-BSA was added to the plate and incubated for 30 minutes at room temperature. Plates were washed with PBS-T and 100ul of ABTS substrate solution was added. Plates were read when the optical density of the high control reached between 2.0 and 2.4 at 405nm on a Dynex Technologies MRX Microplate Absorbance Reader (Labmode, Borehamwood). Results were analysed using GraphPad Prism 6 software.

PBS-Phosphate Buffered Saline

BSA- Bovine Serum Albumin (Sigma)

Substrate solution- (0.0137g of 1Mm ABTS (Sigma) in 25ml of 70Mm citrate-phosphate buffer (pH 4.2) and 25ul Hydrogen Peroxide (Sigma) (1:1000)