

## Collection of Air Samples to Quantify Exposure to Airborne Allergens

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### Abstract

Under normal conditions, airborne allergens are present at very low concentrations. Allergens may be carried on relatively large identifiable particles such as grains of pollen and mould spores or smaller amorphous particles or both.

The methods that have been applied to quantify animal airborne allergens will be described in this chapter. By careful selection of the air sampling equipment and conditions, samples can be collected which quantify, for example, the personal exposure of an individual when performing a specific task or changes in exposure when allergen control methods are implemented. If as with animal allergens, an airborne allergen is not comprised of identifiable microscopic fragments, it is necessary to extract the soluble allergen for quantification in a specific immunoassay. The basic methods used for the elution of animal allergen from polytetrafluoroethylene (PTFE) filters will be described. The optimization of this method to suit different allergens and the influence of the buffer on extraction efficiency and stability of the allergen during storage will also be discussed.

**Key Words:** Allergen; exposure; air sampling; quantification.

### 1. Introduction

Over the last decade of allergy research, interest has grown in quantifying the amount of airborne allergen inhaled by individuals. Research has focused on describing the factors that affect the intensity of exposure to airborne allergens in both domestic and occupational indoor environments and in assessing the efficacy of allergen avoidance measures. The quantification of airborne allergen has facilitated investigations of the exposure–response relationship to occupational respiratory sensitizers (*1*). There is still very little information available about the intensity of exposure to allergens required to sensitize naïve subjects or provoke symptoms in susceptible individuals.

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The techniques required to make these measurements were first described by Agarwal and co-workers in 1981 (2) and is an adaptation of traditional occupational hygiene practices (3,4). Under normal conditions, airborne allergens are present in very low concentrations ( $\text{ng}/\text{m}^3$  or less). The ambient level of an allergen is affected by the rate of production of the allergen and the rate of its removal. Factors that influence the rate of production will depend on the allergen under investigation, but current or recent disturbance of allergen-containing dust may raise airborne levels 10-fold or more. The airborne concentration of an allergen will be reduced if the allergen source is contained or if the local ventilation is increased.

Allergens may be carried on relatively large identifiable particles such as grains of pollen (10–15  $\mu\text{m}$ ) and mould spores (2–5  $\mu\text{m}$ ) or smaller amorphous particles or both. The size of the carrier particle will influence the site of deposition in the respiratory tract. The total inhalable (or inspirable fraction) is the fraction of dust which enters the nose or mouth. The respirable fraction is the fraction that enters the gas exchange region of the lung (3,4). After a period of disturbance, allergen-containing particles will settle out of the air; the sedimentation rate will be greater for larger particles. Conversely, allergen carried on small particles may remain airborne for long periods of time and get widely dispersed. It is not known for how long allergens retain their allergenic properties in contaminated dust.

The methods that have been applied to quantify animal airborne allergens will be described. Animal allergens are carried on amorphous particles with a wide aerodynamic range ( $>19$  to  $<0.4 \mu\text{m}$ ) and the adaptations of the method to enable accurate measurement of exposure under a wide range of conditions may be applied to the quantification of most airborne allergens. Air containing the aeroallergen is drawn through a filtration medium by means of a pump. The allergen-containing particles impact on the filter and may be eluted for quantification in an immunoassay. As the volume of the air sampled is known, the concentration of the allergen can be reported in  $\text{ng}/\text{m}^3$ . By careful selection of the air sampling equipment and conditions, samples can be collected which quantify the personal exposure of an individual when performing a specific task or decrease in background or static exposure when allergen control methods are implemented.

If the airborne allergen of interest is not comprised of identifiable microscopic fragments (as is the case with animal allergens), it is necessary to extract the soluble allergen from the filter for quantification in a specific immunoassay. As there may be 1 ng of allergen or less impacted on each filter, the optimization of the elution step to maximize allergen recovery is important. Several variations of methods to extract or elute airborne allergens have been described (5), but all involve the wetting or soaking of the filter with an aqueous buffer. Various

lengths of elution time and methods of agitating the filters have been employed. The basic methods used for the elution of animal allergen from polytetrafluoroethylene (PTFE) filters will be described (6). The optimization of this method to suit different allergens and the influence of the buffer on extraction efficiency and stability of the allergen during storage will be discussed.

## 2. Materials

### 2.1. Collection of Air Samples

1. Air sample pumps (mains operated or battery operated, *see* **Notes 1** and **2**).
2. Flow meter.
3. Flexible plastic tubing (internal diameter 6 mm), length approximately 0.7 m.
4. Sampling heads (Institute of Occupational Medicine (IOM), seven-hole, *see* **Note 3**).
5. Filter media (e.g., PTFE, glass fiber, *see* **Note 4**).
6. Forceps.
7. Cassettes for storage of exposed filters (e.g., Millipore PetriSlides).

### 2.2. Elution of Air Samples

1. Two pairs of forceps.
2. Plastic tube (75 mm × 12 mm) and cap – 1 per filter.
3. Plastic screw cap vial – 2 per filter.
4. Extraction buffer made up fresh such as 0.1 M PBS or ammonium bicarbonate buffer: 1.58 g Ammonium hydrogen carbonate dissolved in 200 mL distilled water and pH adjusted to 7.0–7.5 using 6 M hydrochloric acid. One milliliter of Tween 20 is added after adjusting pH (*see* **Note 5**). Additional protein and preservatives can be added to the buffer (*see* **Note 6**).

## 3. Methods

### 3.1. Collection of Air Samples

1. Attach the pump to the sampling head using the tubing.
2. If the concentration of the airborne dust is required (gravimetric analysis), the filter must be weighed on a sensitive balance before and after sampling (*see* **Note 7**).
3. Using clean forceps, transfer filter to sampling head and secure by fastening to 'finger tightness' (*see* **Note 8**).
4. Switch on pump and leave running for approximately 1 min.
5. Adjust flow rate to desired level (e.g., 2 L/min) using flow meter for accurate calibration (*see* **Note 9**) or if level cannot be adjusted, record the flow rate (*see* **Note 10**).
6. Position air sampling unit equipment in situ (*see* **Note 11**) and record the time switched on and off (some pumps have integral timers). The flow rate should be checked and recorded at the beginning and end of each sampling period as a minimum. For prolonged sampling periods or if sampling in a dusty environment, more frequent checks of the flow rate are recommended.
7. At the end of the sampling period, transfer the exposed filter to its own individually labeled cassette and store at –20°C.

8. Appropriate blank samples should also be collected and analyzed in parallel. 'Media blanks' are filters that have been taken 'straight from the box' whereas 'field blanks' are filters that have been loaded into the sampling equipment and taken into the sampling area, but the pump has not been turned on. As a guide one 'field blank' should be collected for every 10 samples.
9. The volume of air sampled is calculated in m<sup>3</sup> (*see Note 12*).
10. Wash the sampling heads in warm soapy water, rinse, and leave to air dry.
11. Wipe over casing of the sampling pumps and recharge as required.

### 3.2. Elution of Air Samples

1. In a ventilated safety cabinet, remove filter from the sampling head or storage cassette using clean forceps. Fold the filter loosely in half, exposed side inwards, and transfer to a labeled tube (*see Note 13*).
2. Wash the forceps in distilled water and wipe dry (*see Note 14*).
3. Repeat steps 1 and 2 until all the filters have been transferred to appropriately labeled tubes for extraction.
4. Add 2 mL of extraction buffer to each tube and cap.
5. Agitate tubes, for example, vortex mix each tube for 3 × 1-s bursts and then leave to stand for 1 h at room temperature (*see Note 15*).
6. Repeat step 5 once more.
7. Vortex mix each tube for 3 × 1-s bursts.
8. With clean forceps, remove the filter from the extraction buffer by slowly drawing the filter up the side of the tube. Discard the filter and recap the tube.
9. Remove all the filters as described in step 8, making sure to wash and wipe the forceps in between each tube as described in step 2.
10. Centrifuge all tubes containing the eluate for 10 min at 1300g.
11. For each filter eluate, transfer 0.9 mL of the supernatant to each of the labeled screw cap vials, taking care not to disturb the pellet of dust debris.
12. Store all vials sealed at -70°C (*see Note 16*).

### 4. Notes

1. A wide range of pumps is available from manufacturers such as Casella (<http://www.casella.co.uk>), SKC Inc. (<http://www.skinc.com>), etc. For measuring the exposure of an individual, a small portable pump is required which is battery operated and can sample air at a smooth flow rate of 2 L/min. For approved methods for quantifying personal exposure for compliance with exposure thresholds, please refer to published guidance such as in **refs. 3 and 4**. These small pumps may also be used as 'static' or 'area' monitors if the aeroallergen concentration is expected to be high. Static samples collected may provide additional useful information for the clinician or researcher. If the intensity of exposure is expected to be low (e.g., appropriate control measures are being used or monitoring for contamination of 'clean' areas), larger mains-operated pumps can be purchased, which operate at flow rates of 15 L/min or more. Caution must be exercised when operating pumps at high flow rates in a confined area because if the rate of removal of allergen by

the pump exceeds the rate of production, a falsely low level of allergen will be recorded. Under such circumstances, a pump that operates at a lower flow rate should be employed and longer sampling times performed.

2. In some environments such as animal houses and food preparation areas, it is of paramount importance not to spread contamination. It is therefore good practice to ensure that all the air sampling equipment is cleaned with mild detergent, then alcohol. If sterilization is required,  $H_2O_2$  may be used safely with some air sampling equipment but the manufacturer's instructions (both for the air sampling equipment and sterilization equipment) should be followed.
3. The main types of sampling heads which are available and most practical are: (a) open faced (25- and 37-mm diameter), (b) IOM head (25-mm diameter, has one orifice, measures 'inhalable' fraction), (c) the Seven-Hole sampling head (25-mm diameter, seven equally spaced 4-mm diameter holes, measures 'inhalable' fraction), (d) cyclone sampling head (25-mm diameter, measures 'respirable' fraction). Preloaded cassettes (which may be more practicable if sterile filters are required, for example, if measuring exposure to endotoxin) are also commercially available. Foams which selectively sample different particle sizes are also usefully and may be successfully used for size-selective allergen quantification (7).
4. A wide variety of filters are available from companies such as Millipore (<http://www.millipore.com>), Sartorius (<http://www.sartorius.com>), etc. When selecting the filter type, consideration needs to be given to several factors. The diameter of the filter must be compatible with the diameter of the sampling head. The pore size of the filter will determine the size of particle captured, and a pore size of 1.0  $\mu m$  is recommended for most applications. However, if the allergen of interest is carried on smaller particles, a smaller pore size may be required. The ability of the filter media to absorb moisture during the sampling period may influence the stability of the allergen and gravimetric analysis. Allergen has been shown to be more efficiently eluted from hydrophobic filter media such as PTFE.
5. The addition of a small amount of detergent such as Tween 20 (0.05–0.5% v/v) has been shown to increase the efficiency of a number of aeroallergens up to 10-fold (6).
6. The addition of protein (e.g., human serum albumin 0.3% w/v), stabilizers (e.g., glycerine 50% v/v), preservatives (Kathon 0.15% v/v), or enzyme inhibitors should be considered if the allergen is unstable and/or the filter eluates are to be stored for prolonged periods. The compatibility of these additives with the immunoassay used to quantify the aeroallergen should be determined.
7. Gravimetric analysis: Before the filter is weighed prior to sampling, the filter should be 'conditioned'. This means that any moisture in the filter should be allowed to equilibrate with the atmosphere in the weighing room. This can be done by leaving the filter overnight in its individual, labeled container with the lid slightly ajar. The balance used to weigh the filters should measure to at least five decimal places. If the balance is to be transported to the location where the fieldwork is to be conducted, the instructions from the manufacturer should be followed with respect to a suitable surface for the balance to be sited, an adequate warm-up period, and calibration checks. After the sampling period, the filters should be 'conditioned'

again before being weighed. The mass of dust captured is obtained by subtracting the weight before sampling from that obtained after sampling. Corrections are then made for the volume of air sampled (*see Note 11*) and dust expressed as  $\text{mg}/\text{m}^3$ .

8. The front of the sampling head should not be overtightened as this may dislodge the filter underneath.
9. Some pumps have an integral flow meter but, if not, a variety of equipment are available to calibrate the flow rate of the pump. These range from basic flow meters to more sophisticated electronic devices. Contact the manufacturer (*see Note 1*) for further information about the best device to suit your needs.
10. If the resistance to the flow of air is high, the operating noise of the pump will increase and/or the pump may cut out. This can be reduced by one or more of the following: (a) operate the pump at a lower flow rate, (b) increase the pore size of the filter, (c) select a different filter media, (d) increase the diameter of the sampling head/filter.
11. The position of air sampling equipment is of paramount importance and will greatly influence the quality of the data obtained. If personal exposure of an individual is being measured, the sampling head must be situated within the breathing zone of the person, i.e., as close as possible (i.e., <30 cm) to their nose and mouth. In practice, it is most convenient to place the sampling head securely on the subject's lapel. For collection of static samples, attention must be paid to the airflow in the room; sampling close to the inlet or exhaust air supplies may decrease or increase the aeroallergen concentrations recorded. In practice, the collection of several samples may be needed. For static samples, the sampling head should be positioned at least 1 m from the ground and preferably at head height. The collection time is another factor that can influence the data obtained. The shorter is the sampling time, the more information will be available about the 'peaks' of exposure. However, in practice, this needs to be balanced against obtaining enough allergen on the filter for detection in the immunoassay.
12. The volume of air sampled is obtained by calculating the total sampling time in minutes, multiplying this by the average flow rate in L and then converting from L to  $\text{m}^3$  ( $10,000 \text{ L} = 1 \text{ m}^3$ ).  
Example: Pump switched on at 09.15 (2 L/min) and off at 10.45 (1.9 L/min).  
Total volume of air sampled –  $90 \times 1.95 \times 0.001 = 0.175 \text{ m}^3$ .
13. Under optimal conditions, the dust on the filter should not be excessive. If the filter is overloaded and the material falls off, then this can be quantitatively transferred to the elution tube (and any extra volume of buffer taken into account). Investigators who use disposable sample heads may elute the filter in situ (7).
14. Cleanliness in the handling of the filters is essential. Crosscontamination between filters is very easy and immunoassays are very sensitive! Thorough cleaning of the forceps is required.
15. Methods of elution other than vortexing have been employed, namely 'end over end' rotation and sonication. For more details see reference (8). All are satisfactory, provided the elution buffer contains Tween 20, although prolonged sonication should be avoided.

16. The concentration of aeroallergen in the filter eluate can be measured by immunoassay directly or after storage at  $-20^{\circ}\text{C}$ . If the concentration of the analyte is below the limit of detection of the assay, then the filter eluate can be concentrated by lyophilization and reconstituted in a smaller volume. However, it is advisable to check the stability of the analyte before using this method routinely. If the eluates are consistently below the level of detection of the immunoassay, then the sensitivity of the method can be increased by (a) increasing the volume of air sampled and (b) pooling extracts from several filters and concentrating prior to assay.

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