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DETECTION OF AIRBORNE ALLERGENS

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ABSTRACT

Detection of both viable and non-viable allergens is necessary when investigating the causes of occupational asthma. Viable microorganisms can be counted and identified using filter aerosol monitors, liquid impingers, Andersen cascade impactors and other samplers but non-viable allergens can only be detected and quantified using immunological methods to assay samples collected by filtration, impingement or electrostatic precipitation. These have been compared, using RAST inhibition and ELISA immunoassays, to determine the most satisfactory sampling method. In field tests, yields of airborne scampi protein were almost 20 times greater in samples collected by electrostatic precipitation than by filtration. This result was confirmed in a model system using egg albumin aerosols and a commercial antibody in ELISA assays. Better recovery of egg albumin from filter samples was obtained using ammonium bicarbonate than using Coca's solution. Again, high-volume filtration samplers yielded less allergen than a large-volume Litton-type electrostatic sampler which collected into liquid. Recovery on dry filters decreased with time of sampling suggesting adsorption of allergen onto the filter or its denaturation through dehydration.

INTRODUCTION

The study of airborne allergens has been chiefly been concerned with pollens and microbial antigens. Pollens and fungal spores can be counted on spore trap slides although their identification can usually be no better than to genus. For specific identification, culture is necessary but time-discriminated continuous assessment is then rarely possible and

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identification may be limited if species do not produce the airborne spore form in culture. For instance, airborne *Leptosphaeria* spores may produce the imperfect *Phoma* stage in culture (Ganderton, 1968) while *Didymella exitialis* produces *Ascochyta*. Actinomycetes may be important in occupational lung disease, with *Saccharopolyspora rectivirgula* (*Micropolyspora faeni*) and *Thermoactinomyces* spp. implicated in farmer's lung disease and *Thermoactionomyces sacchari* in bagassosis. Fungi may be similarly implicated in some forms of allergic alveolitis as with *penicillium glabrum (P. frequentans)* in suberosis. Recently, the role of non-viable and non-microbial allergens and antigens has been recognized. Examples include bacterial enzymes used in biological washing powders, mites in domestic and agricultural buildings, fly and locust protein in insect rearing rooms, beet protein in sugar beet factories and proteins from sea foods in processing plants, all implicated in occupational asthma (Lacey and Dutkiewicz, 1994).

Methods have long been established for the detection, isolation, identification and enumeration of viable microbial antigens but methods for non-viable and non-microbial antigens are still evolving. A wide range of methods has been used for the isolation and enumeration of microbial allergens and antigens. Methods for collecting non-viable antigens operate on the same principles but individual methods differ greatly in their suitability, particularly in the assay methods they allow and in their collection efficiencies (Table 1). Usually, high volume filtration samplers have been used to collect non-viable antigens (Agarwal et al., 1981, 1983) but sometimes aerosol monitors have been used with membrane filters (Price et al., 1990). Antigens have been extracted by elution and assayed using radio-allergosorbent test (RAST) and RAST inhibition assays. RAST inhibition assays allow detection of specific IgE antibodies and ELISA generally of IgG. Allergens from domestic and laboratory animals, insects, mites, plant and animal proteins and from enzymes have now been assayed in samples collected by filtration (Edwards et al., 1983; Swanson et al., 1985; Tee et. al. 1985, 1985, Griffin et al., 1988; Forster et al., 1989; Price et al., 1990).

Table 1: Recovery of egg albumin from glass fibre filters after exposure to air flow on high volume filtration sampler for different periods.

Sampling Period (min)	Egg albumin recovered (%)				
	Control[1,2]	10[3]	60[2]	120[2]	180[3]
Mean ± SEM	74.78±7.58	22.71±2.35	1751±0.49	19.25±0.25	20.65±0.82

[1] Egg albumin added directly to ammonium bicarbonate, dialysed, lyophilised and assayed.
[2] Mean of two determinations
[3] Mean of three determinations

Other methods besides filtration can also be used to collect airborne antigens and in a sea food factory where water jets were used to remove scampi meat from their shells, a Litton-type large-volume electrostatic air sampler (Sci-Med Inc., Eden Prairie, MN) allowed more efficient recovery of airborne antigen in liquid than filtration through glass fibre filters. The reasons for this difference could include adsorption of protein to the filter fibres or denaturation of the protein perhaps through dehydration. Cyclones could also be used to collect airborne antigens but their usefulness for this has not been assessed.

This paper describes the use of a model system utilising egg albumin aerosols in exposure chambers and enzyme-linked immunosorbent assay (ELISA) with an egg albumin polyclonal antibody to compare the collection efficiencies of different samplers.

MATERIALS AND METHODS

Samplers and Collection Media

A range of different samplers and collection media were used as detailed below:

High volume filtration sampler. A 30 cm × 20 cm filter holder mounted on a multi-stage turbine suction pump operating at 6501 min^{-1} loaded with EPM 200 glass fibre filters (Whatman).

Large volume air sampler (Sci-Med, Eden Prairie, MN, USA). A Litton-Type electrostatic precipitator, operating at 650 min^{-1} and collecting into 200 ml liquid (0.02M ammonium bicarbonate) which was recycled continuously during sampling.

Porton raised impinger or A-30. Aerosols are drawn in through a curved inlet tube and a capillary jet 30 mm above the collecting fluid (0.02 M ammonium bicarbonate) which controls the airflow at 111 min^{-1}. A pre-impinger, containing 5 ml collecting fluid, was attached to the inlet tube to collect particles > 5 μm and allow only particles 1–5 μm to be collected in the impinger which contained 20 ml fluid. In these tests preimpinger and impinger samplers were bulked for assay.

Multi-stage liquid impinger (May, 1966). This is a three-stage sampler with collection characteristics simulating deposition in the human lung. A 20l min^{-1} version was used containing 4 ml collection fluid in each stage. An hemispherical baffle was used in moving air to allow stagnation point sampling.

Personal monitor filters. Polypropylene, glass fibre or polycarbonate (Nuclepore) or cellulose ester (Millipore) membrane filters were mounted in 47 mm, 37 mm (closed face) or 25 mm filter holders connected to personal sampler pumps (L2SF; Rotheroe and Mitchell, Aylesbury) operating at 21 min^{-1}.

Sierra Marple personal cascade impactor. This is an eight-stage cascade impactor operated by a personal sampler pump at 21 min^{-1}. Samples are collected on perforated "Mylar" substrates, 34 mm diam.

Antigen

Egg white was freeze dried for 24 h and the resultant powder was stored at –20°C until required. Egg albumin for aerosolisation was prepared by reconstituting the powder in coating buffer (0.02 M Tris containing 0.15 M NaCl, adjusted to pH 9.0 with HCl) at 1.5 mg ml^{-1}. A stock solution of 0.1 mg ml^{-1} was similarly prepared in coating buffer ad stored at <5° C until required to prepare standards of 0.1–1000 ng ml^{-1} on the day of assay.

Exposure Chambers

A wind tunnel, 1 m square in cross section and with a working length of 10 m was used as an exposure chamber. In some tests, egg albumin was aerosolised, using a spinning disk over a 30 min period, 6 m upwind of the samplers which sampled simultaneously side by side, in a turbulent airstream of 1 m s^{-1}. The samplers were then allowed to run for a further 4 h to simulate use in practice before samples were processed and assayed. In other tests, a 2 m section of the wind tunnel was isolated using polyethylene sheet and the samplers were exposed to egg albumin aerosol for 15 min, in still air conditions, and then run for a further 60 min before processing and assay.

Extraction Methods

Egg albumin was extracted from glass fibre filters either by descending elution or by cutting and soaking the exposed filters. Exposed filter papers were eluted in 23 cm × 14 cm × 32 cm chromatography tank for 8 h at 4°C using 200 ml 0.02 m ammonium bicarbonate. One end of the filter paper was mounted in the buffer reservoir and the other folded and stapled to form a tapered tip to allow the eluate to be collected in a beaker. The eluate was dialysed (Visking size 12 tubing) for 24 h in running tap water and then in three changes of distilled water, freeze dried, weighed and stored in sterile bottles at –20°C until assayed. Other filters were cut into pieces and soaked in 200 ml of 0.02 M ammonium bicarbonate at 4°C. Filter debris was then removed by filtration, first through 1.2 μm and then through 0.2 μm pores. The filtrate was dialysed and freeze dried as before. Some exposed filters were cut up and extracted in Coca's solution (5 g NaCl, 2.75 g $NaHCO_3$, 4 g phenol in 11 distilled water) for 6 h at 4°C and then treated as before. Exposed membrane filters and "Mylar" substrates from the Sierra-Marple cascade impactor were extracted in 5 ml 0.02 M ammonium bicarbonate. Liquid samples were dialysed against 0.02 M ammonium bicarbonate, freeze dried and again stored at –20°C.

Assay System

Egg albumin in extracts was quantified by enzyme linked immunosorbent assay using an egg albumin polyclonal antibody obtained from Sigma

Chemical Co. (Poole, Dorset, UK). Microtiter plates (Nunc Immunoplate Maxisorp) were coated overnight (16 h) with sample extracts, reconstituted in Tris HCl, pH 9.0, or with standard solutions containing different concentrations of egg albumin (100 μl/Well) then washed four times with wash buffer (0.02M Tris containing 0.05% Tween-20 and 0.15 M NaCl, adjusted to pH 7.4 with HCl). The coated plates were then incubated with rabbit anti-chicken egg albumin antibody (1;10,000 or 1:1,000; 100 μl/well) for 30 min at 30°C, again washed four times with Tris HCl wash buffer, and then incubated for 1 h with goat anti-rabbit IgG-horse radish peroxidase conjugate (1:5,000 100 μl/well) for 30 min at 30°C. After washing four times with Tris HCl wash buffer, 3,3'5, '5'-tetramethylbenzidine (150 μl of a 10 μg ml^{-1} solution in dimethyl sulphoxide and 5 ml H_2O_2 in 15 ml substrate buffer, 0.01 M sodium acetate adjusted to pH 5.0 with citric acid; 100 ml/well) was added as substrate and the enzyme reaction was allowed to proceed for 15 min before stopping with 10% H_2SO_4 (50 ml/well) and measuring absorbance at 450 nm using a Dynatech 5000 plate reader. This procedure allowed detection of 10–250 ng albumin ml^{-1} with antibody diluted 1:10,000 and 1–25 ng ml^{-1} with 1:1,000 dilution. Coefficients of variation within assays ranged from 2.67 to 8.63% and between assays from 4.09 to 12.3%

RESULTS

Extraction Methods

Egg albumin (2 mg in distilled water at 1 mg ml^{-1}) was atomised directly onto the glass fibre filter of a high volume filtration sampler while it was running for 10 min to simulate sampling. The filters were then extracted either by descending elution in chromatography tanks, using either 0.02 M ammonium bicarbonate or Coca's solution, or by cutting up the filters and soaking in 0.02 M ammonium bicarbonate. In four such tests, cutting and soaking filters allowed a mean recovery of 23.4 ± 3.64% of the albumin applied while descending elution allowed 16.05 ± 7.07% recovery. The difference was thus not significant. Extraction with 0.02 M ammonium bicarbonate extraction buffer allowed recovery of almost twice as much antigen (Mean of four determinations 19.68 ± 0.33%) as Coca's solution (Mean 10.17 ± 0.86%)

COMPARISON OF SAMPLERS

Comparison of High Volume Filtration and Electrostatic Samplers

The relative efficiencies of a Litton-type electrostatic air sampler and a high-volume filtration sampler in recovering egg albumin antigen were determined for comparison with earlier results obtained in field trials sampling scampi protein (Griffin et al., 1988). In each test, 2 mg egg albumin, as a 1 mg ml^{-1} solution, was atomised into the sampling inlet of the electrostatic sampler or onto the filter paper while the samplers

were running and then for 10 min afterwards. The filters were extracted by cutting and soaking, dialysed and freeze dried and the collecting fluid from the electrostatic sampler was dialysed and freeze dried as described above. More than twice as much antigen was recovered using the electrostatic sampler (Mean of three determinations 65.51 ± 5.19%) as was recovered from filter extracts (Mean 30.22 ± 2.27%)

In an attempt to explain this difference, the high volume filtration sampler was run for periods of 10, 60, 120 and 180 min after adding the egg albumin and compared with recovery from ammonium bicarbonate to which the egg albumin was added directly, before dialysis, freeze drying and reconstitution for assay. Recovery from the control ammonium bicarbonate solution, was almost 75% (Table 1) while mean recovery from the filters ranged between 17.51 and 22.71%, with no significant change with increasing sampling period. Thus, any mechanism that prevents recovents recovery of the antigen must act within 10 min of the start of sampling.

Comparisons of High Volume Samplers with Other Sampler Types

The samplers under test were arrayed at one end of the wind tunnel so that all were equally exposed to the egg albumin aerosol that was atomised about 5 m upstream in an airflow averaging 1 m s^{-1}, made turbulent to ensure good mixing. Egg albumin was atomised over a 30 min period. The results are shown in Table 2. Again, the large volume electrostatic sampler recovered more antigen than the high volume filtration sampler but both were better than all other samplers. However, the Sierra Marple personal cascade impactor allowed recovery of almost as much egg albumin as the high volume filtration sampler. Recoveries from the Porton raised jet impinger, the multi stage liquid impinger and from personal monitor filters were all much smaller, with polycarbonate filters allowing the best recovery from this group

Because of the unexpected results, the Porton raised jet impinger, the multi-stage liquid impinger and the Sierra Marple personal cascade impactor were tested further, again in a moving air stream. In two determinations, the mean recoveries from the Porton impinger was 0.98 ± 0.08 mg m^{-3} air, from the multi-stage liquid impinger was 0.96 ± 0.70 mg m^{-3} and from the cascade impactor was 1.89 ± 0.42 mg m^{-3}, confirming the earlier findings although the large difference in recovery from the personal cascade impactor between the two tests meant that the differences were not significant.

Different membrane filter types were further compared by exposure to egg albumin aerosol in an isolated section of the wind tunnel in still air conditions. Aerosol monitors loaded with cellulose ester or polycarbonate fulters were also compared in closed and open faced configurations. The results are shown in Table 3. Unexpectedly large recoveries were given by glass fibre filters under these conditions, even though the samplers were run for 60 min after 15 min aerosolisation of

Table 2. Comparison of samplers for collecting airborne antigen using egg albumin aerosols in a a wind tunnel and an ELISA detection system.

Sampler type	Mean recovery[1] (mg m^{-3} air)	SE_M
High volume filtration sampler	1.47	0.60
Large volume electrostatic air sampler	2.13	0.80
Porton raised jet impinger with per-impinger	0.24	0.08
Multistage liquid impinger	0.33	0.10
Sierra Marple personal cascade impactor	1.26	0.46
Personal monitor filters		
Polypropylene	0.39	0.29
Class fibre	0.42	0.08
Cellulose ester	0.59	0.26
Polycarbonate	0.87	0.14

2, All results are the means of three tests.
SE_M, standard error of the mean

Table 3: Recovery of egg albumim sampled using different membrane filter types

Sample	Egg albumin recovered (µg)[1]					
	Poly-propylene	Glass fibre	Cellulose ester		Polycarbonate	
			open	closed	open	closed
Mean ±SE_M	39.83 ± 4.11	61.50 ± 6.33	48.33 ± 8.33	39.00 ± 4.21	58.00 ± 6.79	49.83 ± 7.03

[1], All results are the means of six determinations.

egg albumin. Glass fibre filters recovered more antigen than any other filter type although recovery differed little from that of polycarbonate filters in open-face aerosol monitors. Least antigen was recovered from polypropylene filters and less was recovered from closed-faced monitors then from open-faced although the difference was not significant.

DISCUSSION

The ability to detect relevant allergens in the environment and to identify their sources is inportant if the causes of outbreaks of occupational asthma are to be determined. A range of samplers has been used to isolate and grow microbial allergens even though their inlet and collection efficiencies have rarely been characterised. The detection of non-viable allergens is more difficult. Recently, immunoassay of samples collected on filters, using RAST inhibition assay, has allowed airborne allergens to be linked to specific IgE antibodies in the sera of asthmatic patients (e.g. Agarwal et al., 1981, 1983; Tee et al., 1985, 1988).

However, the method is relatively insensitive and often requires large air samples. With some protein antigens, recoveries were better from

samples collected into liquid, using large volume electrostatic air samplers, than from those collected dry by filtration (Griffin et al., 1988). The present study sought to determine whether the difference between dry and wet collection occurred with other protein antigens and to measure the efficiencies of other sampling methods in recovering antigen in a model system utilising egg albumin and a specific polyclonal antibody detection system.

The difference between dry collection onto glass fibre filters in a high volume sampler and wet collection in a large volume electrostatic sampler, found initially with scampi protein, was again found with egg albumin. More than twice as much antigen was recovered from the electrostatic sampler as could be recovered from filters. It appeared that the antigen became bound to the filter within 10 minutes and there was little change in the recovery rate after this time.

Few other samplers performed as well as either high volume sampler. Best was the Sierra-Marple personal cascade impactor, in which the antigen is recovered by washing the Mylar plastic collection disks. Surprisingly the two liquid impingers performed poorly. This probably resulted from the poor sampling characteristics of both impingers in a moving air stream rather than from poor retention of antigen in the liquid. Tests of these samplers in stilkl air is still required. Even in still air, the personal samplers all gave poor recoveries of antigen, However, more antigen was recovered from personal samplers loaded with glass fibre filters than with different types of membrane filter, although polycarbonate filters collected only slightly less than glass fibre filters and the difference was not significant. In wind tunnel tests, polycarbonate filters performed much better than glass fibre filters, perhaps reflecting the longer sampling period than in still air.

There was little effect on antigen recovery between elution and cutting and soaking but ammonium bicarbonate appeared preferable to Coca's solution for extraction. Sensitivity still needs to be improved further if personal exposure is to be determined using aerosol monitors or spill-proof micro-impingers. In other studies, improved recovery of some airborne antigens has been obtained by utilising minimal amounts of fluid for extraction and, sometimes, by adding a wetting agent to the extraction fluid. However, it is still not known what proportion of the airborne antigen collected is recovered and further work is required to establish this.

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