

Direct on air sampling filter quantification of cat allergen

Leif Holmquist*, Olof Vesterberg

*Respiratory Health and Climate Programme, National Institute for Working Life, Ekelundsvägen 16,
Solna, S-11279 Stockholm, Sweden*

Received 23 August 2001; received in revised form 4 October 2001; accepted 4 October 2001

Abstract

A direct on sampling filter in solution (DOSIS) method for quantification of airborne cat allergens has been developed. In this method, the allergens firmly adsorbed to a porous polytetrafluoroethylene filter are reacted with specific antibodies conjugated to alkaline phosphatase, generating a matrix-bound allergen–antibody–phosphatase complex. The treated filter is subsequently floated on a commercially available chemiluminescent phosphatase substrate solution. Aliquots of this solution are removed and analyzed luminometrically. The light intensity of the product is linearly related to the amount of allergen over a large mass range, 0–100 SQ units (1 SQ unit is about 146 pg of the allergenic protein Fel d 1). DOSIS demonstrated intra- and interassay precisions of 9% and 8% and 14% and 21% for the levels 4 and 20 SQ units per filter, respectively. The limit of quantification was estimated to 0.4 SQ units (58 pg Fel d 1) of cat allergen per filter. Application of DOSIS to analysis of cat allergen concentrations of indoor air in homes with and without cats revealed, on average, a six times higher concentration in the former (142 SQ units/m³) as compared to the latter (24 SQ units/m³). The recorded concentrations for airborne cat allergen in homes with cats are in accordance with previously reported figures. Allergen-specifically stained sampling filters revealed the particulate nature of airborne cat allergen which seemed predominantly to be carried by numerous large dust particles. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Cat allergy; Allergen; Airborne sampling; PTFE filter; Direct quantification; Chemiluminescence

1. Introduction

Allergic symptoms from the human nose and eye, as well as asthma, often are caused by airborne particles carrying allergens from furred animals. The prevalence of asthma has,

* Corresponding author. Tel.: +46-8-730-9601; fax: +46-8-730-9897.

E-mail address: olof.vesterberg@niwl.se (L. Holmquist).

in the later years, shown an increasing trend among children [1]. In some countries, more than 50% of children with asthma are sensitized to furred pets [2]. Family members keeping cats may transfer clothes-bound allergen from their homes to others without cats and to school or work rooms where it can be released into the indoor air. The occurrence of cat allergens in many schools has been confirmed [3,4]. This is a big public health problem in most western countries where more than 10% of the pupils are sensitized to cat allergens. In general, extensive efforts should be taken to eliminate airborne indoor allergens. A first step in this process has to be mapping of the concentration of the allergens in the air in the different work premises. For that purpose, we recently reported a “direct on-sampling filter in solution” technique (DOSIS) for quantification of airborne pollen allergens [5]. The principle of this method is that the allergens are firmly adsorbed to a porous polytetrafluoroethylene air sampling filter and subsequently directly reacted with specific antibodies conjugated to alkaline phosphatase. This generates a filter-bound allergen–antibody–phosphatase complex which is allowed to float on a chemiluminescent enzyme substrate solution. Light emitted by the reaction product in aliquots removed from the substrate solution was linearly related to the amount of allergen over a very large mass range of major allergenic protein. A further advantage of this method is that it excludes extraction of allergen collected on the sampling matrix with detergent-containing buffers. This may result in losses related to dilution and incomplete and varying yields of sampled allergens. Using DOSIS, we have in previous studies mapped the airborne pollen allergen concentrations in schoolrooms [6], street level shops [7] and driving compartments of coaches [8]. In the present study, we report the development of DOSIS for assessment of airborne cat allergens in different indoor environments to monitor and control air cleaning measures.

2. Experimental

2.1. Materials

Lyophilized water extracts of cat hair (*Felis domesticus*), Aquagen SQ®, yielding 100,000 SQ units/ml after reconstitution with 4.50 ml of 0.15 M sodium chloride containing 20 mM Tris–hydrochloric acid, pH 7.2 (TBS), were purchased from ALK-Abelló (Hørsholm, Denmark) and used as reference allergen for calibration curves. The extracts contained 14.6 µg of Fel d 1 protein/ml according to the manufacturer. Polyclonal primary antibodies, rabbit IgG anti-Fel d were from the same company. The secondary antibodies, alkaline phosphatase-conjugated swine IgG anti-rabbit IgG were obtained from Dakopatts (Gentofte, Denmark).

All chemicals were of analytical grades. Incubation media and reagents were prepared with ultrapure water of 18.2 MΩcm resistivity, produced by a Milli-Q Plus system (Millipore, Bedford, MA, USA). Polytetrafluoroethylene (PTFE, Teflon™) filters, 25 mm in diameter with 1.2-µm nominal pore sizes, and polycarbonate 25-mm syringe filter holders (Cat. No. 16517E) were purchased from Sartorius (Göttingen, Germany). The sampling filter holder was modified by removing the Luer lock outlet plastic part, the attached filter support and the silicon gasket, leaving an opening of 23 mm in diameter.

Polystyrene Multidish Six-Well Nunclone Delta SI vials and polypropylene Nunc-Immuno 70 × 11 mm Minisorp tubes for preparation of allergen standards were obtained from Nunc (Roskilde, Denmark). Luminometric measurements were performed using a Luminoskan instrument and White Cliniplates from Labsystems OY (Helsinki, Finland). The chemiluminescent alkaline phosphatase substrate 4-methoxy-4-(3-phosphatephenyl)spiro[1,2-dioxetane-3,2'-adamantane] disodium salt (Lumi-Phos® Plus) was from Lumigen (Southfield, MI, USA).

The chemiluminescent substrate for detection of low amounts of allergen was composed of concentrated CDP-Star™ solution (34 µl) and Sapphire II™ enhancer (100 µl) from Tropix (Bedford, MA, USA) per ml of 0.1 M sodium chloride and 5 mM magnesium chloride in 100 mM Tris–hydrochloric acid buffer, pH 9.60. AirChek® vacuum pumps for air sampling of cat allergen were purchased from SKC (Eighty Four, PA, USA).

2.2. Methods

2.2.1. General methods

The PTFE filters were handled with a sharp pointed pair of forceps. The wells of the multidish vials were emptied by aspiration with a Pasteur pipette connected to a water pump. Incubation and washing media were gently pipetted onto the walls of the wells to keep the PTFE filter to float on the surface of the relevant incubation or washing medium, ensuring that no air bubbles were entrapped under the filters. If not otherwise stated, all incubations and washings were performed at 22 ± 1 °C with the vials covered with their matching lids. Incubation of the PTFE filters with Lumi-Phos® Plus was made in the dark by circular shaking with 300 rpm on an IKA-Schüttler MTS 2 apparatus (Junke & Kunkel, Staufen i. Br., Germany). All other incubations and washings were performed with moderate longitudinal shaking. Luminometric endpoint readings were made for 3 s with no lag time. If not otherwise stated, the values for standards and samples represent single determinations of individual filters.

2.2.2. Application of method

Airborne cat allergens from four Swedish homes without cats and four with cats were collected on the PTFE filters placed in the filter holders using one sampling filter in each home and an air flow sampling rate of 2 l/min for 4 h. The total sampling air volume at each occasion was recorded by the pump. After completed sampling, each filter was collected and stored for about 1 month in a sealed polystyrene plastic box at -70 °C prior to analysis. Staining of sampling filters after luminometry was performed with the BCIP/NBT reagents from Bio-Rad laboratories (Hercules, CA, USA). Data scanning of stained filters was made with an Agfa Duoscan T1200 scanner (Dustin, Stockholm, Sweden).

2.2.3. Preparation of cat allergen calibration curves

2.2.3.1. Step 1. To each of the six wells of the multidish polystyrene vial, 300 µl of 2-propanol was pipetted followed by addition of the PTFE filter which was placed on the surface of the alcohol. After equilibration for 15 min, 5 ml of 0.15 M sodium chloride containing 20 mM Tris–hydrochloric acid, pH 7.2 (TBS), was gently layered under each

filter allowing them to float on the surface of the liquid during the addition. The vial was subsequently shaken moderately for 15 min.

2.2.3.2. Step 2. For calibration curves ranging from 0 to 100 SQ units, cat hair extracts (allergen) with a defined single concentration of major allergen were pipetted as multiples of 10- μ l droplets on the surfaces of six different floating PTFE filters leaving the sixth filter as a blank. Alternatively, the standard PTFE filters were prepared from equal volumes of different dilutions of a fixed concentration of the cat allergen. The vial with the PTFE filters with adsorbed reference allergens was stored at 5 °C overnight.

2.2.3.3. Step 3. The following day, each standard PTFE filter was turned upside down and incubated floating on the 2-propanol–TBS solution for 60 min.

2.2.3.4. Step 4. The propanol–TBS solution in each well of the vial was removed and substituted with 5 ml of 0.5 M sodium chloride in 20 mM Tris–hydrochloric acid, pH 7.2, containing 0.2% (w/v) Tween 20 (0.2% TBST). The vial with the floating filters was moderately shaken for 90 min for blocking of residual protein-binding sites.

2.2.3.5. Step 5. The 0.2% TBST was removed and replaced by 0.5 M sodium chloride containing 0.05% (w/v) Tween 20 in 20 mM Tris–hydrochloric acid, pH 7.2 (0.05% TBST). The vial was incubated with shaking as in step 4 for 15 min.

2.2.3.6. Step 6. The floating standard filters were each incubated with shaking for 60 min in 5 ml of a 1:1000 dilution of the rabbit IgG anti-Fel d in 0.05% TBST followed by three successive washings with 5 ml of 0.05% TBST for 15 min.

2.2.3.7. Step 7. After completion of step 6, the standard filters were incubated with shaking on the surface of 5 ml of a 1:1000 dilution of swine IgG anti-rabbit IgG conjugated with alkaline phosphatase in 0.05% TBST for 60 min followed by three successive 5 ml washings with 0.05% TBST and a final one with 5 ml of TBS for 15 min each. The multidish vial containing the six standard filters was stored overnight at 5 °C.

2.2.3.8. Step 8. The following day, the filters were transferred to a new, third multidish six-well vial containing 5 ml of 0.1 M sodium chloride and 5 mM magnesium dichloride in 100 mM Tris–hydrochloric acid, pH 9.6, in each well. After equilibration for 15 min with moderate shaking, the buffer was removed and 1.0 ml of Lumi-Phos® Plus was layered under each filter. The multidish vial with the set of six filters were incubated in the dark under kinetic control with shaking (see Section 2.2.1) for 15–30 min followed by removal of 50–200- μ l aliquots of the substrate solution of each well being rapidly transferred to wells of the microplate for luminometric reading. This started 30 s after removal of the last aliquot.

2.2.4. Treatment of sample filters

The sample filters containing the adsorbed allergen were wetted, with the filter surface containing the allergen facing the air, with 2-propanol and TBS in a multidish six-well vial

as described in step 1 for the preparation of standard curves (Section 2.2.3.1). After 15-min incubation with moderate shaking, the filters were turned upside down and incubated for 60 min. Finally, the filters were treated by the same procedures as described for preparation of calibration curves, starting with step 4 (Section 2.2.3.4) where the filters are floating on the surfaces of the 2-propanol–TBS solutions.

3. Results

The present method of analysis of PTFE filters charged with 0–100 SQ units of allergens of cat hair extract yielded a linear relation between amounts of allergen and luminometric response with correlation coefficient and intercept close to 1 and 0, respectively, when the allergen was pipetted onto the filters as multiples of 10- μ l droplets of a solution of 1000 SQ units (146 ng Fel d 1) cat allergen per ml. However, using serial dilutions of the same solution and applying 10 $\times$ 10 μ l of allergen solutions onto each filter, a tendency to non-linear relation was observed as shown in Fig. 1. Starting the serial dilutions with standard allergen solutions of 20 or 200 SQ units per ml yielded linear calibration curves up to 20 SQ units per filter. (Figs. 2 and 3). The intra-assay precision of the method was estimated to 9% and 8% for levels 4 and 20 SQ units of cat allergen, respectively, using six different filters at each level. Analysis of single filters each carrying 4 or 20 SQ units on two runs each on 3 days gave inter-assay precisions of 14% and 21%,

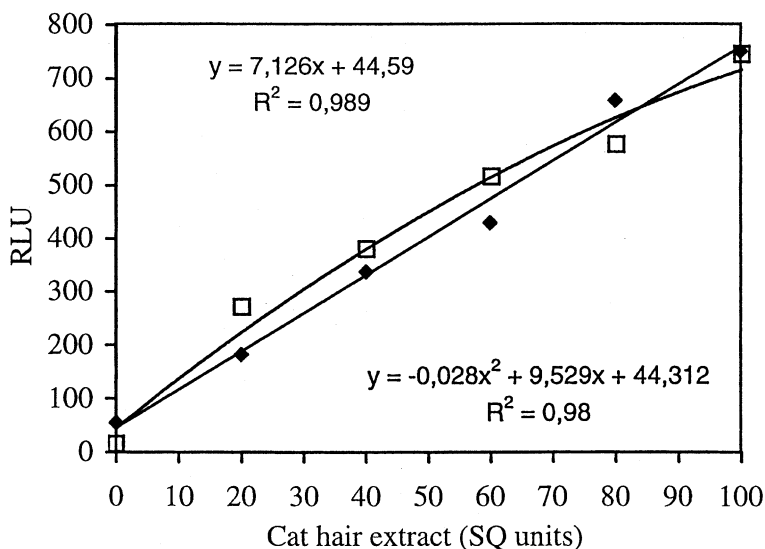


Fig. 1. Plot of amount of cat hair extract allergen standard against luminescence intensities (RLU) obtained with DOSIS for the 0–100 SQ units range. The points of the calibration curves represent single determinations of a solution of standard cat allergen pipetted on individual 1.2- μ m PTFE filters as multiples of 10- μ l droplets of 10 SQ units (filled squares) or as 10 $\times$ 10- μ l volumes (open squares) of serial dilutions of a 1000-SQ units/ml standard allergen solution. The substrate, substrate sample size and incubation time were Lumi-Phos® Plus, 200 μ l and 30 min, respectively.

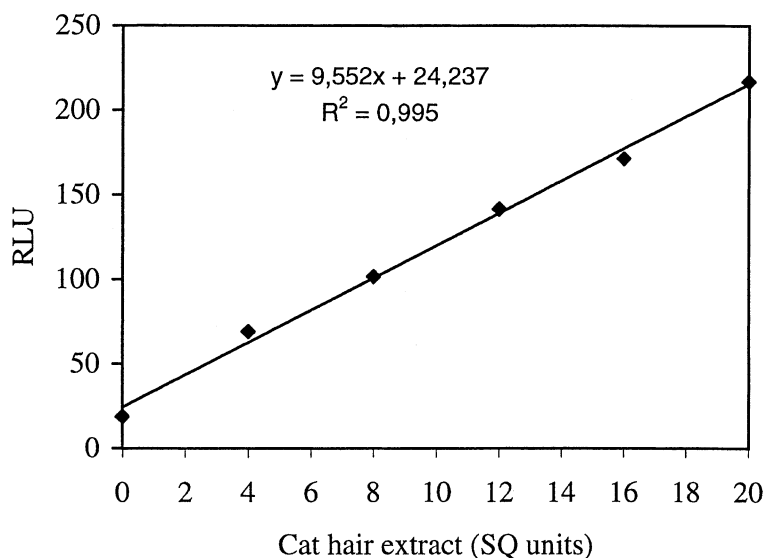


Fig. 2. Plot of amount of cat hair extract allergen standard against luminescence intensities (RLU) obtained with DOSIS for the 0–20 SQ units range. The points of the calibration curves represent single determinations of a solution of standard cat allergen pipetted on individual 1.2- μ m PTFE filters as 10×10 - μ l volumes of serial dilutions of a 200 SQ units/ml standard allergen solution. The substrate, substrate sample size and incubation time were Lumi-Phos® Plus, 200 μ l and 30 min, respectively.

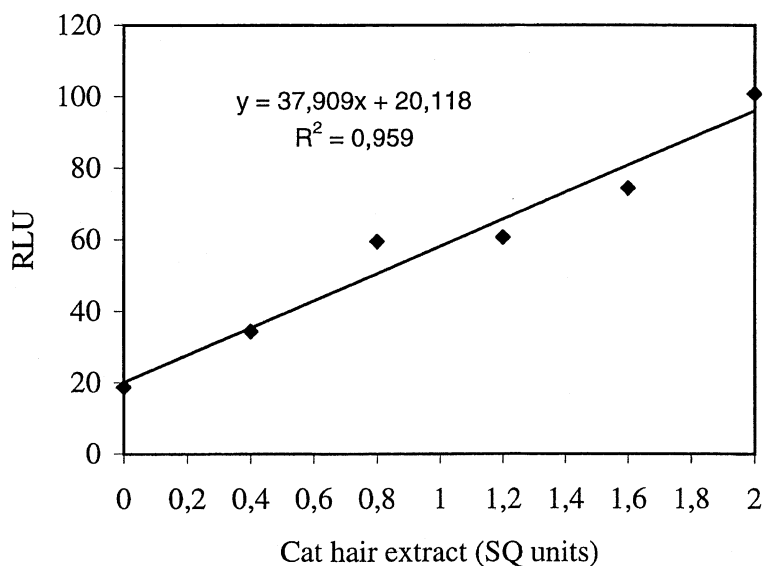


Fig. 3. Plot of amount of cat hair extract allergen standard against luminescence intensities (RLU) obtained with DOSIS for the 0–2 SQ units range. The points of the calibration curves represent single determinations of a solution of standard cat allergen pipetted on individual 1.2- μ m PTFE filters as 10×10 - μ l volumes of serial dilutions of a 20 SQ units/ml standard allergen solution. The substrate, substrate sample size and incubation time were CDP-Star™, 50 μ l and 15 min, respectively.

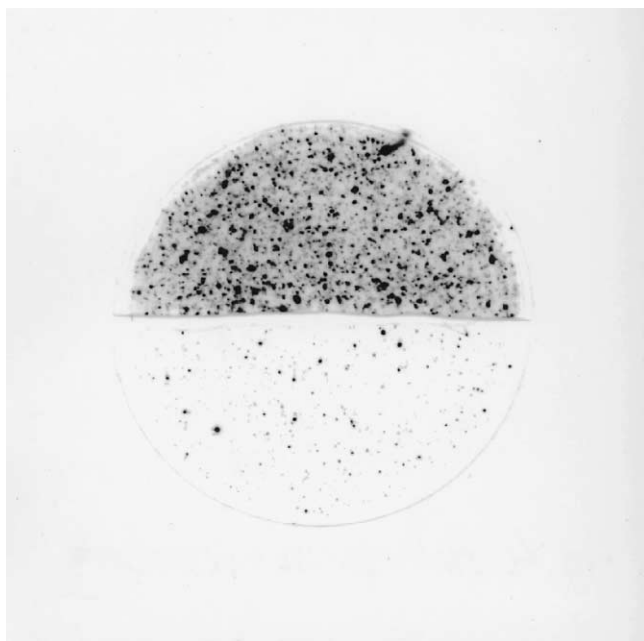


Fig. 4. Reflection scanning data images of halves of antigen-specifically stained sampling filters from simultaneous analysis of airborne cat allergen in home with (upper half, 153 SQ units/m³) and without cat (lower half, 18 SQ units/m³). Note the even distribution of stained dots originating from numerous large particles over the whole filter area. Minor particles are also present but they are not as small as those of pollen allergens (Ref. [5], Fig. 6).

respectively. The limit of quantification was set to 0.4 SQ units per filter corresponding to 58 pg of Fel d 1.

Analysis of cat allergen in air samples from four homes without cats and four with cats yielded on average (mean $\pm$ S.D.), 24 ± 14 (range: 18–45) SQ units/m³ or 3.6 ± 2.0 (range: 2.0–6.5) ng Fel d 1/m³ and 142 ± 71 (range: 74–237) SQ units/m³ or 20.8 ± 10.4 (range: 10.8–34.6) ng Fel d 1/m³, respectively. This corresponds to a statistically significant ($p=0.02$) six times higher concentration of cat allergen in the air of homes with cats as compared to those without, as analyzed by one-sided *t*-test. Staining of filters by the reagents BCIP/NBT specific for alkaline phosphatase-conjugated antibody–allergen complexes after luminometry revealed the particulate nature of the air samples as illustrated in Fig. 4.

4. Discussion

The purpose of the present study was to adapt the previously reported direct on sampling filter technique (DOSIS) for quantification of airborne birch and grass pollen allergens to those of cat. By contrast to what was observed in quantification experiments

with birch and grass pollen allergens a tendency to non-linear relations between luminometric response and amount of allergen were recorded when serial dilutions of a relatively high concentration of reference cat allergen solution (1000 SQ units /ml) were seeded onto the PTFE filters (see Section 3). This concentration gives a surface density of 0.22 ng/mm² of soluble Fel d 1 when the standard solution is seeded as a 10- μ l droplet. A reason for this non-linearity might be that aggregation of the purified cat allergen protein occurs at high concentrations leading to multiple layers on the solid surface resulting in loss of antigen–antibody complexes during the incubation and washing procedures. Other reasons might be related to steric hindrance phenomena.

Cat allergen represents a very heterogeneous population of dust particles carrying different amounts of allergenic protein. It is not possible to obtain information about the true nature and composition of the allergen of all these individual particles [9]. Thus, only artificial standards are available. We have selected Aquagen SQ[®] because it is standardized and commercially available. The calibration curves of any immunological method of analysis of cat allergen, thus primarily serve to reveal precision and reproducibility parameters and to standardize the recording instrument (luminometer) against SQ units, but can never give absolute values of total amount of cat allergen. However, airborne cat allergen particles collected on the PTFE sampling filter are expected to give surface densities of allergen below critical aggregation at the treatment of the filter in the DOSIS procedure as the total amount of protein allergen carried by the large number of trapped dust particles is allowed to freely dissolve and diffuse over a large filter surface in an *in situ* elution procedure (see Section 2.2.4). A single 10- μ m large protein particle has a calculated weight of approximately 0.5 ng. Calibration curves may thus be optionally prepared by loading reference filters with equal volumes of serial dilutions of a standard allergen solution or simpler, with multiples of a volume of a single concentration of the standard. In the DOSIS procedure adsorbed allergens does not need to be extracted into detergent-containing buffer prior to their quantification. This results in dilution of the target antigens and small amounts of allergens collected on conventionally used air sampling filters may be difficult to extract. In addition, significant losses of allergen by adsorption to glass and plastic surfaces may occur in diluted extracts even in the presence of detergents.

Fel d 1 levels in homes with cats have been reported to range from 2 to 468.5 ng/m³ of air which should correspond to about 14–3200 SQ units/m³, and predominantly to be present on particles larger than 17 μ m [10]. The results for homes with cats of the present study, significantly six times higher than in homes without cats, are in accordance with these figures. Antigen-specifically stained filters from samples of air in homes with cats showed the presence of numerous large spots originating from large particles similar to those obtained from stained filters with birch pollen grains (about 20 μ m) of outdoor air [5,6]. The fine-granularly stained area representing small allergenic particles observed on-sampling filters at analysis of indoor air birch pollen allergen [6] was not seen on filters with cat allergen (Fig. 4). In homes without cats, we recorded airborne cat allergen concentrations similar to the lowest concentrations earlier reported for homes with cats, 2 ng of Fel d 1 [10]. However, cat allergen is ubiquitous, even being reported to occur in camps on the South Pole [11]. Thus, the present method should be valid for mapping and elimination studies of airborne allergen in indoor environments.

5. Conclusions

The DOSIS procedure seems to have adequate sensitivity, precision and limit of quantification for assessment of concentrations of airborne cat allergen in indoor environments. Many possibilities to vary amount of sampled air, enzyme substrate, reaction time and temperature and sample volume of reaction product will make it easy to find ideal analytical conditions. Visual comparison of allergen-specifically stained air sampling filters with coloured dots originating from dust particles sampled from contaminated and reference environments offers rapid semiquantitative estimation of cat allergen concentrations without using expensive analytical instruments.

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