

Immunochromatographic direct on sampling filter test for aeroallergens

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Abstract

An immunochromatographic method for qualitative and quantitative determination of aeroallergens direct on sampling (ADOS) filters has been developed. In this method, a porous polytetrafluoroethylene filter carrying adsorbed allergens is fixed by double-coated adhesive tape to a supporting filter paper matrix. Following addition of antibodies specific for the relevant allergens and washing and staining reagents via a reagent applicator an immunochromatogram is developed resulting in a 5–10 mm wide area of the sample filter covered with blue-violet-stained spots appearing on a faintly pink or white background. The method takes 30 to 90 min, depending on the nominal porosity (1.2–5 μm) and the defined reaction area (5–10 mm) of the sample filter. Application experiments with birch and grass pollen, soluble Bet v 1, Phl p 5 and mould allergens as well as cat allergen carried by airborne dust revealed a limit of detection of a few picograms of allergen as stained spots. The specificity of the new method to evaluate the type of allergen is a function of the selected antibodies. The concentrations of the allergen in an air sample are related to the number and intensity of stained spots.

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1. Introduction

A direct on air sampling filter in solution (DOSIS) technique [1,2] for quantification of aeroallergens derived from birch and grass pollen has revealed that indoor air in schools, street level shops and driving compartments of coaches may contain substantial amounts of these allergens [3–5]. A working tool not depending on extraction of the allergens from the sampling filter, which might result in dilution and incomplete and varying recoveries of the analyte, is thus available for mapping and quality tests of the indoor air of exposed environments. The DOSIS technique has recently been adapted to quantification of airborne indoor cat allergen [6]. However, during this work, it became apparent that a simple, rapid and cheap method producing “instantaneous” information of type and concentration of aeroallergens would be favorable for screening tests of indoor air quality. Using immunochromatographic techniques (for review, see Ref. [7]) for visualization of sampled aeroallergens (antigens) firmly bound to a polytetrafluoroethylene sampling filter, a new method, aeroallergens direct on sampling (ADOS) filter, has now been developed that can give levels of allergens 1 h after air sampling, in contrast to previous assays taking days. The details of this method are reported herein.

2. Experimental

2.1. Materials

Water extracts of pollen from birch (*Betula pendula*) and timothy grass (*Phleum pratense*) for skin prick testing, Soluprick SQ[®], each containing 100 000 SQ units/ml (manufacturer's specification), were purchased from ALK-Abelló (Hörsholm, Denmark). 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 from the same company. The birch and grass pollen and the cat hair extracts contained 23 µg Bet v 1, 25 µg Phl p 5 and 14.6 µg Fel d 1 protein/ml, respectively, according to the manufacturer, and were used as reference allergens. Birch pollen, sampled in April 1993 in Sollentuna and stored at –20 °C, and timothy grass pollen, sampled 1979 (Allergon, Ängelholm, Sweden) and stored dark at room temperature, were used for application experiments of the immunochromatographic method. A “100%” pure preparation of the mould *Cladosporium herbarum*, lyophilized and inactivated by gamma irradiation, was a gift from the same company. Microscopic examination revealed that the preparation was composed to the main part of fragmented mycelia with a few intact spores. Polyclonal primary antibodies, rabbit IgG anti-Bet v 1, Phl p 5, Fel d and anti-Cla h were gifts from ALK. The secondary antibodies, alkaline phosphatase-conjugated swine 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 MΩ cm resistivity, produced by a Milli-Q Plus system (Millipore, Bedford, MA). Polytetrafluoroethylene (PTFE, Teflon[™]) sampling filters, 25 mm in diameter with 1.2 and 5 µm nominal pore sizes and polycarbonate

25-mm syringe filter holders (cat. no. 16517E) were purchased from Sartorius (Göttingen, Germany). The sampling filter holders were modified as previously described [1,2]. Polypropylene Nunc-Immuno 11 × 70 mm Minisorp tubes for preparation of allergen standards were obtained from Nunc (Roskilde, Denmark). The BCIP/NBT reagents for specific staining of alkaline phosphatase–allergen complexes on the PTFE sampling filters were obtained from Bio-Rad (Hercules, CA). The substrate was prepared by mixing 25 µl of 5% (w/v) BCIP in dimethylformamide with 50 µl of 5% NBT (w/v) in 70% (v/v) dimethylformamide in water and 5 ml of substrate buffer. This was composed of 0.1 M sodium chloride and 5 mM magnesium dichloride in 100 mM Tris-hydrochloric acid, pH 9.6. AirChek 2000 vacuum pumps for sampling of airborne allergens were from SKC (Eighty Four, PA). Filter paper sheets, 1 mm thick, were from Munktell (Grycksbo, Sweden). Thin double-coated adhesive tape, Syrom 90 (Vinci, Italy) or equivalent, 25 mm broad, and common rubber bands, 1 × 6 mm (th × br) for the immunochromatographic system were purchased from the local office supplies store. Reagent applicators, 1 × 6 × 25 mm, were prepared by cutting 3 × 10 mm rhombic holes with a pair of scissors in the double-folded rubber band glued onto one side of a strip of double-coated tape. Developed and stained immunochromatograms were inspected with a Leica Wild M3Z stereo microscope (Leica Heerbrugg AG, Heerbrugg, Switzerland) equipped with a Sanyo Color CCD model VCC-2972 video camera (Sanyo, Japan).

2.2. Design of the immunochromatographic system

The system as shown in Fig. 1 is composed of an 1 mm thick filter paper strip, about 3 × 7 cm, as support for the wetted sample PTFE filter. The paper strip is fitted with an about 5 cm length of double-coated tape. An about 2 × 3 cm liquid absorbing filter pad cut from the same filter paper sheet as the sample filter-support is oriented to cover the upper segment of the sample filter. The reagent applicator with a 3 × 10 mm rhombic aperture is

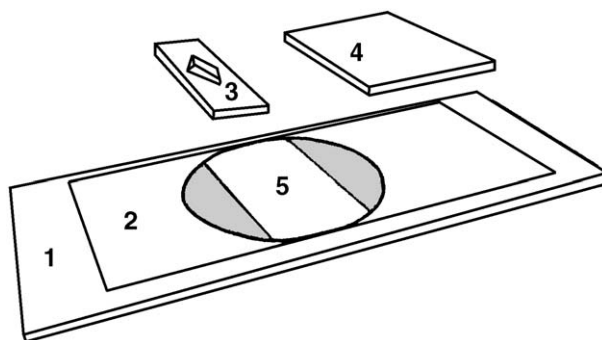


Fig. 1. ‘Exploded’ diagram of the immunochromatographic system demonstrating the vertical relative orientations of the parts (1) filter paper support for the sample filter; (2) double-coated tape; (3) reagent applicator with double-coated tape on the filter side; (4) liquid absorbing pad and (5) sample filter (for further details, see Sections 2.1 and 2.2). The shadowed areas of the sampling filter indicate those in contact with the parts 3 and 4.

oriented over the lower segment of the sample filter (for further details of the individual parts, see Section 2.1).

2.3. Methods

2.3.1. Immunochromatographic procedure

The calibration or sample PTFE filter wetted and floating on the surface of a propanol–TBS wetting solution as described in Ref. [1] was removed and, within 2 min, tightly placed on the uncovered double-coated tape on the sample filter support (Fig. 1). The sample filter must not dry. Without delay, the reagent applicator was tightly glued on the lower segment of the PTFE filter followed by the addition of 20 μ l of TBS containing 0.2% (w/v) Tween 20 (0.2% TBST) to the reagent applicator well, for blocking of residual protein-binding sites. The liquid-absorbing filter paper pad was then immediately glued on the sample filter support to cover the upper segment of the PTFE filter, leaving a 5 to 10 mm open area of the sample filter between the pad and the reagent applicator. When the added volume of 0.2% TBST had disappeared from the reagent applicator well, a second 20 μ l portion of this reagent was added, followed in sequence by a number of 20 μ l volumes of: 1 $\times$ 0.05% (w/v) Tween 20 in TBS (0.05% TBST); 2 $\times$ primary antibody diluted 1:1000 in 0.05% TBST; 2 $\times$ 0.05% TBST; 2 $\times$ secondary antibody diluted 1:1000 in 0.05% TBST and 2 $\times$ freshly prepared BCIP/NBT reagent. Each reagent was allowed almost completely to disappear from the applicator well before the following one was added. The immunochromatogram was finally stabilized with 2 $\times$ 20 μ l of distilled water. It could immediately be evaluated by visual inspection of number and intensity of blue-violet-colored spots representing specifically stained allergen on a faintly pink or white background.

2.3.2. Application of method

Sample filters carrying birch or grass pollen allergens were prepared by seeding pollen grains over a filter mounted in its holder connected to an SKC vacuum pump running with an air flow rate of 2 l/min. Filters were seeded with the mould mycelia preparation in the same way. Airborne cat allergens from a Swedish home with cat were collected on PTFE filters placed in filter holders using an air flow sampling rate of 2 l/min for 4 h. The sample filters with adsorbed birch and grass pollen and cat allergens were wetted, with the filter surface containing the allergen facing the air, with 2-propanol and TBS as described in Step 1 of DOSIS [1] and shaken moderately for 15 min.

Calibration filters with Soluprick SQ[®] and Aquagen SQ[®] reference allergens were prepared by pipetting allergen solutions as multiples of 1- μ l droplets directly onto the surfaces of the wetted PTFE filters floating on the 2-propanol–TBS solution [2].

The sample filters carrying the allergens were then submitted to the immunochromatographic procedure (Section 2.3.1). The time for completed chromatography using primary and secondary antibodies varied from about 30 to 90 min, depending on the nominal porosity of the filters, 1.2 or 5 μ m, and the width, about 5 to 10 mm of the area accessible for analysis.

3. Results

Sample filters for application of the method were obtained by pipetting birch allergen dilutions as multiplets of 1- μ l droplets of 1 SQ unit or by seeding birch pollen grains onto the surfaces of the PTFE filters. They yielded easily visible blue-violet spots originating from the defined allergen (antigen) after being developed and specifically stained in the immunochromatographic procedure, depicted in black and white in Fig. 2. Similar results were obtained with soluble grass pollen allergen and grains. Viewing the stained spots produced by pollen via stereo microscope showed that single and aggregated grains were centered in the middle of circularly stained zones. Individual birch pollen grains seemed to have fully exhausted their allergen contents as they appeared as colorless remnants in contrast to the grass pollen grains which were strongly stained (Fig. 3A and B). The method could detect the amount of allergen released from a single pollen grain corresponding to a few picograms of protein [8] and 1 SQ of soluble allergen (about 250 pg of protein) distributed over the area resulting from 1 μ l of a reference solution pipetted onto the filter.

Analysis of cat allergen in air samples from a home with cat showed spots on the sample filters after immunochromatography, confirming a heterogeneous population of dust particles carrying different amounts of allergenic protein [6]. Particles of varying forms and sizes from small stained fragments and fibers to invisible material, all surrounded by blue-violet-stained zones of different areas, could be observed as shown in black and white in Fig. 3C. The limit of detection of cat allergen carried by dust particles was of the same order of magnitude as that of the pollen allergens. Sample filters with mould allergen yielded spots of particles corresponding to fragments of mycelia, but they did not show spots originating from soluble proteins as shown in Fig. 3D. The final

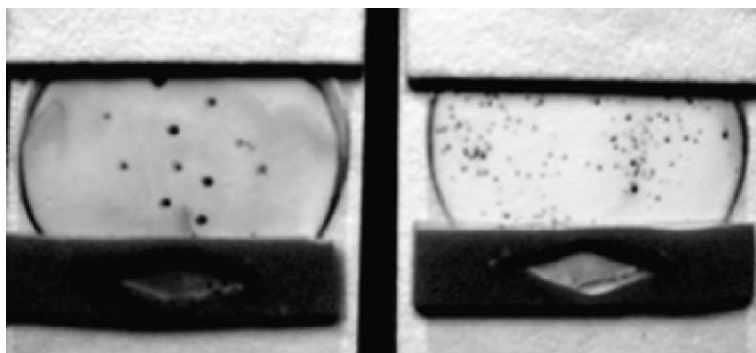


Fig. 2. Photographs of immunochromatograms from analysis of soluble birch reference allergen (left) and of birch pollen grains (right). The stained spots obtained with the reference allergen originate from 1- μ l droplets, each containing 1 SQ unit of allergen, directly pipetted on a sampling filter floating on the wetting solution as described in Section 2.3.2. The black lines at the liquid absorbing pads at the tops of the free filter areas are photographic shadow artefacts and are not seen on the authentic chromatograms. The structures below the filter areas are the reagent applicators.

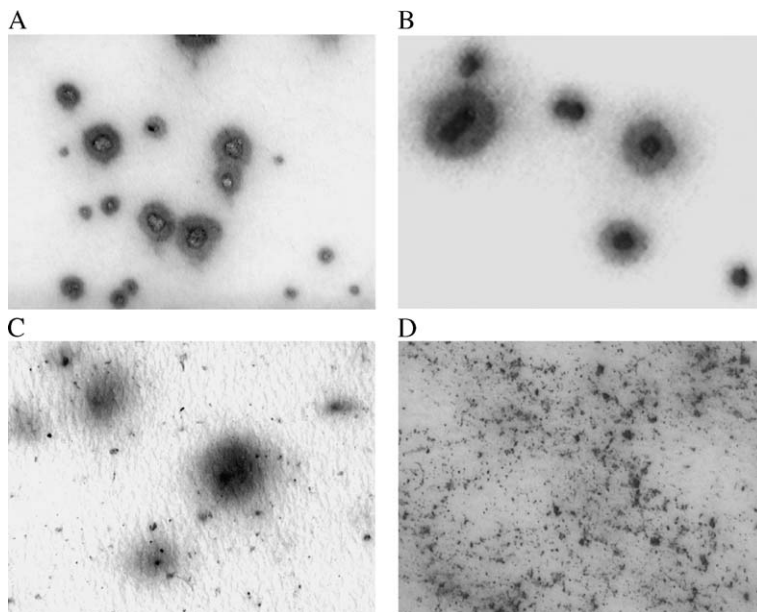


Fig. 3. (A) Photograph of a forty times magnification of an area of the birch pollen grain chromatogram depicted in Fig. 2. The picture shows spots derived from specifically stained allergen released from single and aggregated pollen grains. Note the absence of stain in the grain remnants in the middle of the circular zones. (B) Photograph of a $40\times$ magnification of an area of a grass pollen grain chromatogram. The picture shows spots derived from specifically stained allergen released from single and aggregated pollen grains. Note the stain in the grains in the middle of the circular zones. (C) Photograph of a $40\times$ magnification of an area of a cat allergen chromatogram. The picture shows spots derived from specifically stained allergens from the air in a Swedish home with cat. Note the presence of stained 'particles' with and without stained surrounding zones, the former of which might represent soluble allergens released from sampled dust particles. (D) Photograph of a $40\times$ magnification of an area of a *C. herbarum* chromatogram. The picture shows spots derived from specifically stained fragments of mycelia, which can appear as airborne dust particles after drying and mechanical agitation of mould in an infected environment.

immunochromatograms from all experiments could be stored in the dark at room temperature for at least 2 months (longer times not tested) without losing their inherent information.

4. Discussion

The hallmark of the chromatographic system is the double-coated tape, stabilizing and fixing the sample filter and reagent applicator to a supporting thick filter paper matrix. A liquid film stabilized by capillary forces between the sample filter and the tape inhibits drying out of the PTFE filter between the additions of the different reagents during the chromatographic procedure. The method presented in this report for detection of aeroallergens represents a simple and rapid means for their qualitative and quantitative

determination. The former is a function of the specificity of the chosen antibodies, whereas the latter is a function of the intensity and the number of the blue-violet spots appearing as a result of the procedure. A common magnifying glass further aids the evaluation and the interpretation of the immunochromatograms (Fig. 4). Allergen concentration levels obviously can be obtained by comparison of the number and the intensity of stained spots of sample chromatograms with those obtained with air samples of known concentrations because there is little or no background staining of the sample filters. This can be done by the naked eye or with relevant analytical instruments and computer programs. The very low consumption of antibodies, 40 μ l of a 1:1000 dilution, and reagents allows the use of expensive monoclonal antibodies for an allergen analysis (not reported herein). Experiments aiming at a further reduction of the time of analysis using single-antibody and or visibly labeled antibody techniques are in progress. The immunochromatograms are stable and of low weight and can be sent by normal mail.

4.1. A simplified description of the method and its (future) applications

The method consists of a system for handling of porous sample filters with adsorbed allergens originating from defined volumes of air. The sample filters are fixed to a supporting matrix carrying a strip of double-coated tape. After addition of a reagent applicator and a liquid absorbing pad, immunological reagents are allowed to diffuse through the sample filter, revealing the bound aeroallergens as stained spots on the filter.

This new technique might be adapted to porous filters of materials other than PTFE for the analysis of antigenic proteins in air and liquid samples. Filters of different sizes and geometries, such as strips, in varying designs of sampling filter holders may also be possible to combine with differently labeled single antibodies to get still shorter reaction

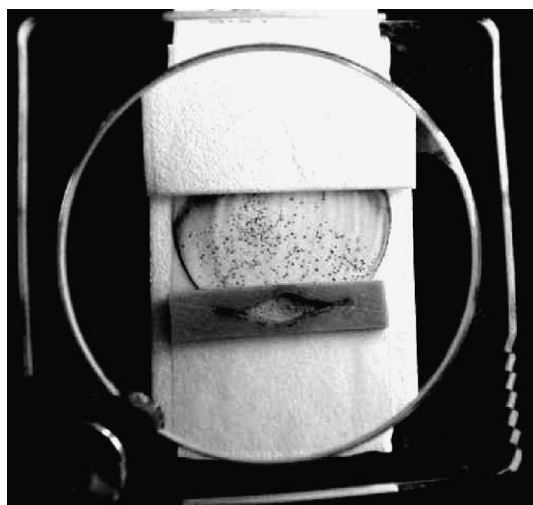


Fig. 4. Photograph of an immunochromatogram showing multiple spots representing allergen originating from sampled grass pollen grains, viewed by a common magnifying glass.

times of the procedure. Readings of the chromatograms with simple lumino- or colorimeters should also be possible. The method will have its future applications for the screening of type and concentration of aeroallergens in outdoor and indoor environments.

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