

Silverfish protein in house dust in relation to mite and total arthropod level

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Summary

Two assays have been developed to measure arthropod levels in house dust. The first assay measures silverfish antigens. The second assay measures invertebrate tropomyosin and gives a global assessment of the level of arthropod-derived material. These assays and a Der p 1 and Der p 2 assay were used to analyse 53 dust samples. In most dust samples the ratio of tropomyosin/Der p 2 was higher than in mite body extract, indicating that the assay measures other arthropods besides mites. Silverfish antigen was detectable in most of the dust samples. In many homes in which the inhabitants were unaware of the presence of silverfish, silverfish antigen was detectable. Therefore for information on exposure an immunochemical analysis is superior to a questionnaire.

Keywords: insect, exposure, house dust, arthropod, silverfish, house dust mite, tropomyosin

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Introduction

The presence of specific IgE antibodies to arthropods is common among patients with rhinitis and asthma [1–4]. However, the presence of specific IgE alone is not sufficient to conclude that these IgE antibodies are related to the symptoms, since exposure to arthropods is also necessary for the development of symptoms. Arthropods are present in many households. Several studies have investigated the levels of *D. pteronyssinus* in house dust [5–7]. For *D. pteronyssinus* a threshold level of exposure that increases the risk of sensitization and symptomatic asthma has been proposed [8–11]. Much less is known about exposure levels to insects due to the lack of quantitative detection methods. Only for cockroach, have immunoassays been used to measure exposure levels [12–14].

In the Netherlands the presence of silverfish in households, besides cockroaches, is common. Silverfish are very primitive wingless insects. They are 3–12 mm long, have three tail feelers and are covered with shiny scales. They

shun light and need a humid environment; therefore they prefer kitchens and bathrooms. They live a.o. from paper, crumbs of bread and flour.

In this report we describe two immunoassays we have developed to monitor insects in house dust. The first assay measures silverfish antigens. The second assay, with a monoclonal antibody [15] that reacts both with mite and with insects, gives a global assessment of the level of arthropod-derived material in dust samples.

Materials and methods

Allergens

Dried *D. pteronyssinus* mites were obtained from the Commonwealth Serum Laboratories (CSL; Melbourne, Australia). Mites were extracted for 12 h in PBS, containing 0.1% Tween-20 at pH 7.4 at 4°C (2% w/v). Frozen chironomids (genus *Chironomus*, species unknown, obtained from the local pet shop) were extracted (2% w/v) at room temperature in PBS, containing 0.1% Tween-20 and 0.01% NaN₃ for 4 h. Silverfish (*Lepisma saccharina*) were obtained from our own culture. Cockroaches (*Blattella*

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germanica) were lured from a house with a cockroach infestation. Silverfish and cockroaches were killed by freezing, sonicated and extracted (2% w/v) at room temperature in PBS, containing 0.1% Tween-20 and 0.01% NaN_3 for 12 h. After extraction the solution was defatted with freon and filtered.

Rabbit antibodies and monoclonal antibodies

New Zealand white rabbits were immunized with silverfish extract, 100 μg protein, in Freund's complete adjuvant on day 0, followed by three injections with the same amount in Freund's incomplete adjuvant at monthly intervals. Solid-phase adsorption was used to remove the antibodies directed against flour (on which the silverfishes were cultivated). Ten ml rabbit antiserum was incubated with 300 mg flour-Sepharose for 4 h at room temperature. The supernatant was affinity-purified by incubation for 4 h with 300 mg Sepharose-coupled silverfish extract. Bound antibodies were eluted with 0.1 M glycine HCl, 0.15 M NaCl, pH 2.5. The antibodies were radiolabelled by the chloramine-T method; 30 μg of protein was iodinated with 37 MBequerel.

A monoclonal antibody to tropomyosin (1A6 antibodies) was obtained as described earlier [15]. The antibody was coupled to CNBr-activated Sepharose (4 mg antibody/100 mg Sepharose).

Purification of tropomyosin

Shrimp-extract was prepared by extraction in water at 100°C for 15 min, filtered, concentrated and dialyzed against distilled water. The shrimp extract (30 ml 850 $\mu\text{g}/\text{ml}$ protein) was incubated for 4 h with 600 mg 1A6 Sepharose. Bound

components were eluted with 0.1 M glycine HCl, 0.15 M NaCl, pH 2.5. In this way we collected 3 ml tropomyosin, with 180 μg protein per ml by Pierce protein assay. Tropomyosin was radiolabelled by the chloramine-T method; 45 μg of protein was iodinated with 37 MBequerel.

House dust

In 53 households dust was collected by vacuum cleaning of the entire house. Unsieved dust was extracted (20% w/v) for 4 h in 0.35 M NaBenzoate.

Silverfish assay

To 1 ml silverfish Sepharose (0.33 mg/ml), 50 μl ^{125}I -labelled rabbit-anti-silverfish and 50 μl of the test extract was added. The mixture was incubated overnight under vertical rotation at room temperature. After centrifugation and removal of non-bound components, the radioactivity bound to the solid phase was counted. The concentration was read from a standard curve of silverfish extract. The detection limit was 230 ng total silverfish protein/ml.

Tropomyosin assay

To 0.5 ml goat-anti-mouse Sepharose (1 mg/ml), 50 μl diluted 1A6 antibodies, 50 μl ^{125}I -labelled tropomyosin and 50 μl of the test extract was added. The mixture was incubated overnight under vertical rotation at room temperature. After centrifugation and removal of non-bound components, the radioactivity bound to the solid phase was counted. The concentration was read from a standard curve. The detection limit was 20 ng/ml.

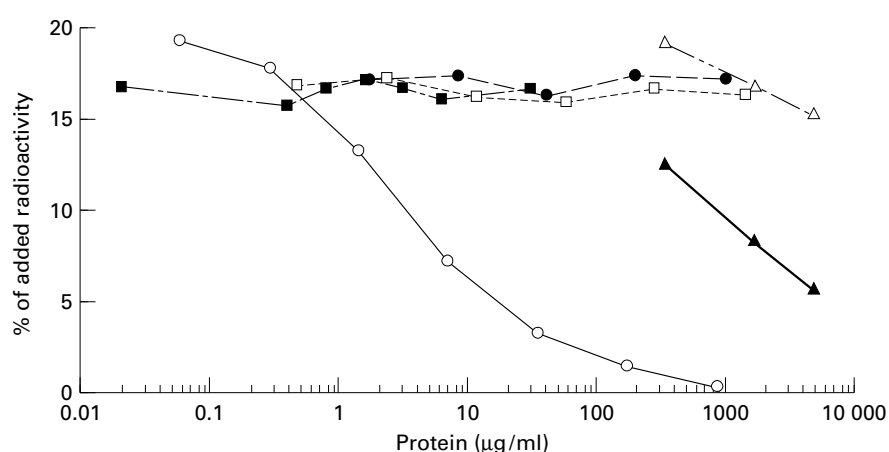


Fig. 1. The reference-curve of the silverfish assay. No inhibition occurred with mite, cockroach or shrimp extract. House dust 1 is a sample with a low level of silverfish antigen, house dust 2 contains a high level of silverfish antigens. ○ = silverfish; □ = cockroach; △ = house dust 1; ● = mite; ■ = shrimp; ▲ = house dust 2.

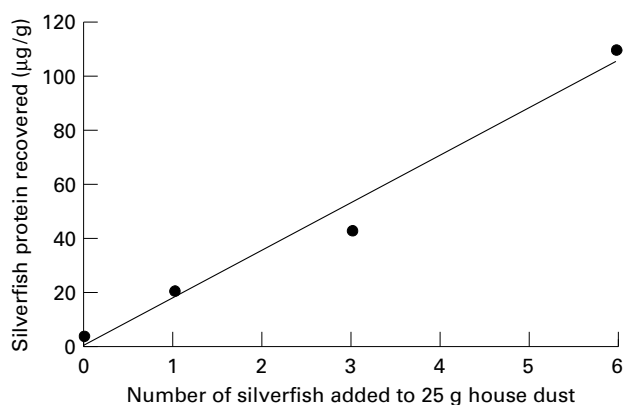


Fig. 2. The recovery of silverfish added to house dust.

Der p II Elisa

Purified mAb against *Der p* II (mAb 10E11) was coated overnight at room temperature (1 µg/ml in PBS, 100 µl/well) on microtitre plates (Nunc, Maxisorb). All subsequent incubations were in 100 µl volumes at room temperature.

The plates were washed (3 ×) with PBS, 0.02% Tween and test samples were added to the plates for 1 h. Again the plates were washed 3 times with PBS-Tween and biotinylated purified monoclonal antibody against *Der p* 2 (mAb αDpx [16]), diluted in PBS-Tween, 0.2% gelatin (final concentration 3 µg/ml) was added for 1 h. After washing (3 times with PBS-Tween), the plates were incubated with

streptavidin-horseradish peroxidase (strep-HRP, Amersham, UK), 1/1000 diluted in PBS-Tween, for 0.5 h, washed and developed with a solution of 100 µg/ml of 3, 5, 3', 5'-tetramethylbenzidine (Merck, FRG) with 0.003% H₂O₂ in 0.11 M sodium acetate, pH 5.5. The reaction was stopped by the addition of 2 M H₂SO₄ to the wells. Plates were read at 450 nm in a Titertek Multiskan reader. Control values read at 450 nm were subtracted. The concentration was read from a WHO standard curve, assuming that the WHO standard of 100 000 kU contains 500 ng *Der p* 2 [8,17]. The detection limit was 1 ng/ml.

Der p I assay

An inhibition assay analogous to the assay described by Chapman [18] was applied to measure the *Der p* I concentration in the samples. To 0.5 ml protein A-Sepharose 0.05 ml diluted rabbit antibodies, 0.05 ml 125 I-labelled *Der p* I [16] and 0.05 of the test extract was added. After centrifugation and washing of the Sepharose, radioactivity bound to the solid phase was counted. The concentration was read from a WHO standard curve, assuming that the WHO standard of 100 000 kU contains 12.5 µg *Der p* 1 [8,17]. The detection limit was 1.6 ng/ml.

Results

The silverfish assay is shown in Fig. 1. As can be seen there

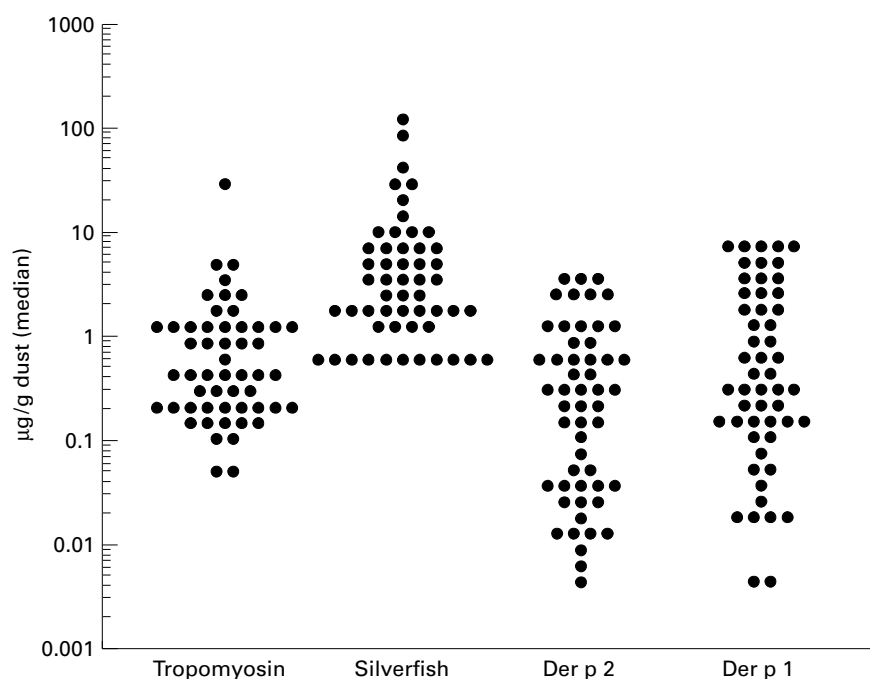


Fig. 3. The levels of the different arthropod-antigens in 53 dust samples. The silverfish level is expressed as total silverfish protein.

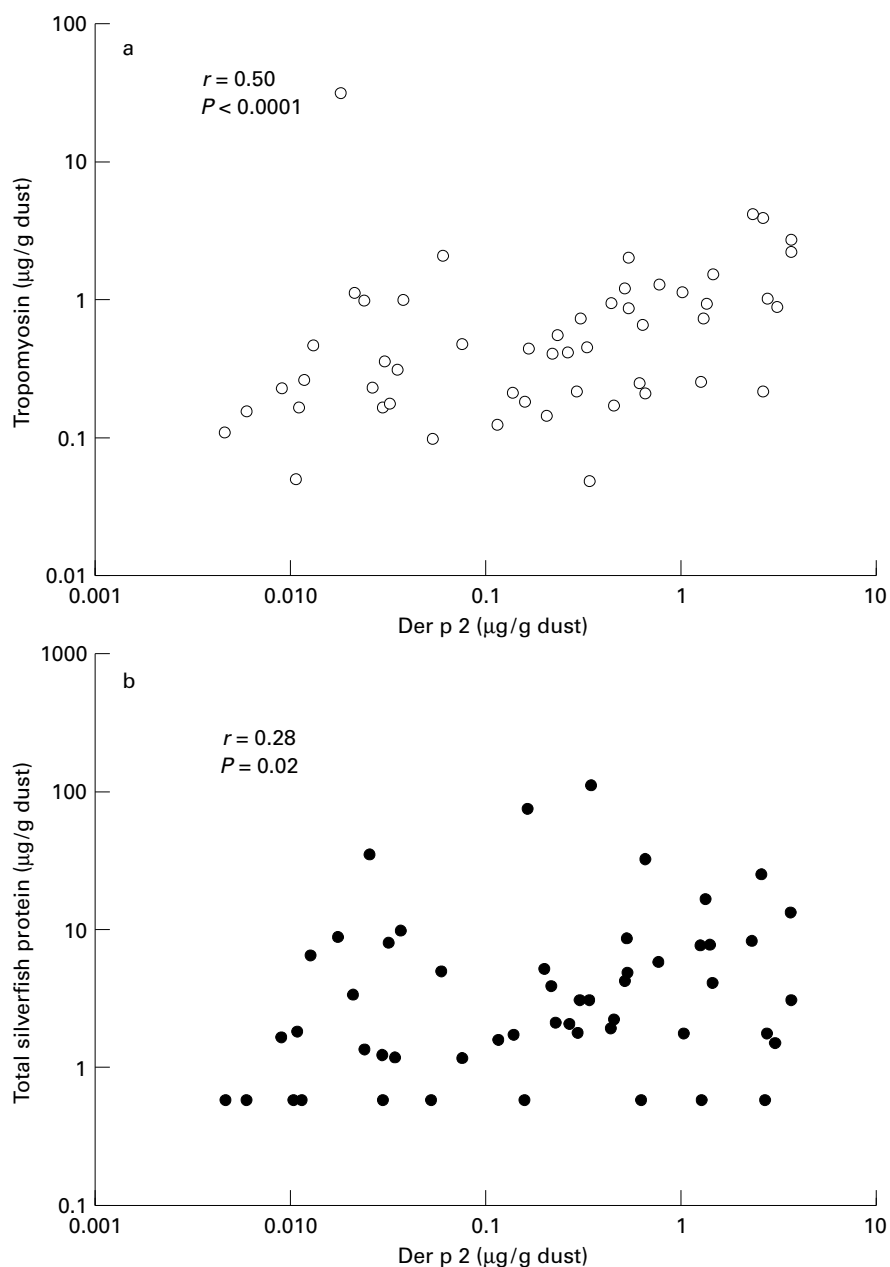


Fig. 4. (a) The relation between Der p 2 and tropomyosin in 53 dust samples. (b) The relation between Der p 2 and silverfish protein in 53 dust samples.

is no inhibition with house dust mite-, cockroach- or shrimp extract (a particularly rich source of tropomyosin).

We tested the recovery of silverfish protein in house dust. Different numbers of silverfish (1 silverfish is approximately 20 mg) were added to 25 g house dust (Fig. 2). The slope of the regression line indicates that a single silverfish corresponds to 450 µg extractable protein.

We were able to detect tropomyosin and silverfish proteins in house dust. For tropomyosin only two, and for silverfish 11 of the 53 dust samples were below the detection

limit. Fig. 3 shows the concentration of the antigens in house dust. The correlations between Der p 2 and tropomyosin (Spearman $r = 0.5$, $P < 0.001$) or silverfish (Spearman $r = 0.28$, $P = 0.02$) are shown in Fig. 4 a and b.

The inhabitants of the houses in which the dust was sampled were asked ($n = 50$) if they ever had seen silverfish in their homes (Fig. 5). Although significant differences (median 'yes', 7.4; median 'no', 1.86; Mann-Whitney U -test, $P < 0.001$) exist in silverfish content between the two groups, many homes in which the inhabitants are unaware of

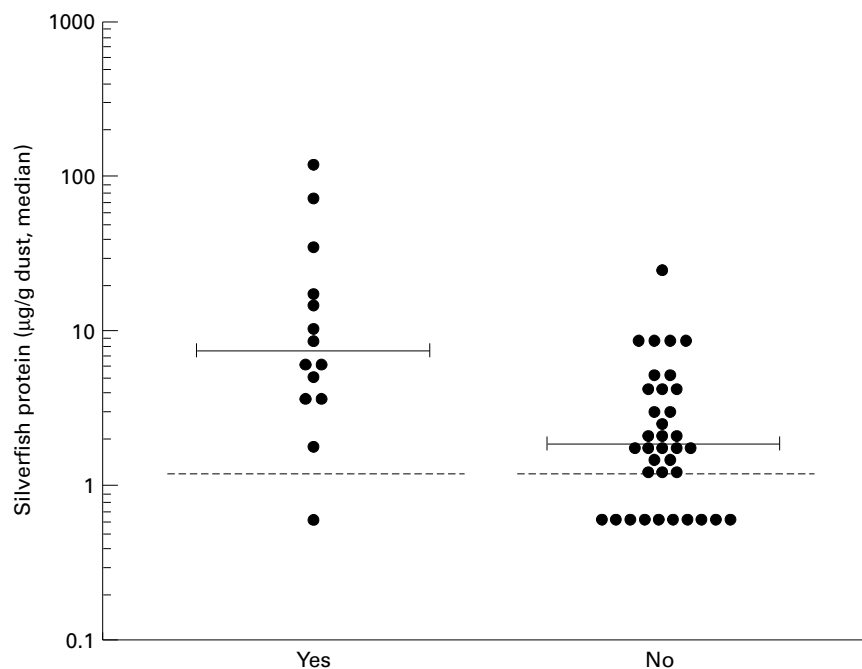


Fig. 5. Differences in silverfish protein in house dust from houses in which the inhabitants gave a positive answer to the question 'Do you have silverfish in your home?' and in house dust from houses in which the inhabitants gave a negative answer to that question (Mann–Whitney *U*-test, $P = 0.0007$).

the presence of silverfish contain silverfish antigen concentrations indicative of silverfish exposure.

Discussion

The purpose of this study was to determine levels of insect antigens in house dust. Therefore we developed two immunoassays. In the assay to measure silverfish antigens we used polyclonal rabbit antibodies against silverfish extract. This was done because no major allergens of silverfish are known as yet. With the silverfish assay we were able to measure silverfish antigens in 42 of the 53 dust samples. The results are expressed in μg total silverfish protein. Per adult silverfish we recovered $450 \mu\text{g}$ protein from dust.

With the tropomyosin-assay it was possible to measure tropomyosin in 51 of the 53 dust samples. In addition to the levels of *D. pteronyssinus*, the amount of tropomyosin gives information about exposure to insects. Der p 2 as well as tropomyosin are mite body proteins. The ratio of tropomyosin/Der p 2 in mite body extract (CSL) is 0.7. When house dust only contains mites and no insects, the ratio of tropomyosin/Der p 2 is expected to be 0.7. As can be seen in Fig. 4a this is not the case. Most dust samples contain more tropomyosin than Der p 2 and some dust samples have a high concentration of tropomyosin but low levels of Der p 2, indicating the presence of other arthropods than mites as a source for tropomyosin. The correlation between Der p 2 and silverfish protein (Fig. 4b) can probably be explained by

the fact that both arthropods need a humid environment.

Although we demonstrated differences in silverfish content between homes with and without 'known' presence of silverfish, we also found that in many homes in which the inhabitants were unaware of the presence of silverfish, the dust contained significant silverfish levels. The fact that silverfish avoid light presumably explains why some people are not aware of the presence of silverfish in their homes. Another possibility is that these silverfish levels could be the result of external contact with silverfish allergen as suggested by Wood *et al.* [19] in case of cat allergens.

In conclusion, we have developed two assays to monitor insect exposure. The first assay measures silverfish antigen. The second assay gives a global assessment of the amount of arthropods in the environment. These assays provide the tools for establishing the clinical significance of insect exposure and make it possible to evaluate the effectiveness of methods used to reduce allergen levels in households.

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References

- 1 Kino T, Chihara J, Fukuda K *et al.* Allergy to insects in Japan. III. High-frequency of IgE antibody responses to insects (moth,

- butterfly, caddisfly, and chironomid) in patients with bronchial asthma and immunochemical quantitation of the insect-related airborne particles smaller than 10 µm in diameter. *J Allergy Clin Immunol* 1987; 79:857–66.
- 2 Kay AB, Gad El Rab MO, Stewart J, Erwa HH. Widespread IgE-mediated hypersensitivity in Northern Sudan to the chironomid *Cladotanytarsus lewisi* (green nimitti). *Clin Exp Immunol* 1978; 34:106–10.
 - 3 Kang B, Vellody D, Homburger H, Yunginger JW. Cockroach cause of allergic asthma. Its specificity and immunologic profile. *J Allergy Clin Immunol* 1979; 63:80–6.
 - 4 Baldo BA, Panzani RC: Detection of IgE antibodies to a wide range of insect species in subjects with suspected inhalant allergies to insects. *Int Archs Allergy Appl Immunol* 1988; 85:278–87.
 - 5 Tovey ER, Chapman MD, Wells CW, Platts-Mills TAE. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981; 124:630–5.
 - 6 Sporik R, Holgate S, Platts-Mills TAE, Cogswell JJ. Exposure to house-dust mite allergen and the development of asthma in childhood. *N Engl J Med* 1990; 323:502–7.
 - 7 Price JA, Pollock J, Little SA, Longbottom JL, Warner JO. Measurements of airborne mite allergen in homes of asthmatic children. *Lancet* 1990; 336:895–7.
 - 8 Platts-Mills TAE, de Weck AL. Dust mite allergens and asthma — a worldwide problem. *J Allergy Clin Immunol* 1989; 83:416–27.
 - 9 Lau S, Falkenhorst G, Weber A et al. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989; 84:718–25.
 - 10 Wood RA, Eggleston PA, Mudd KE, Adkinson NF. Indoor allergen levels as a risk factor for allergic sensitization. *J Allergy Clin Immunol* 1989; 83:109.
 - 11 Kuehr J, Frischer T, Meinert R et al. Mite allergen exposure is a risk for the incidence of specific sensitization. *J Allergy Clin Immunol* 1994; 94:44–52.
 - 12 Pollart SM, Smith TF, Morris EC et al. Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays. *J Allergy Clin Immunol* 1991; 87:505–10.
 - 13 Pollart SM, Mullins DE, Vailes LD et al. Identification, quantification, and purification of cockroach allergens using monoclonal antibodies. *J Allergy Clin Immunol* 1991; 87:511–21.
 - 14 Schou C, Fernandez-Caldas E, Lockey RF, Lowenstein H. Environmental assay for cockroach allergens. *J Allergy Clin Immunol* 1991; 87:828–34.
 - 15 Witteman AM, Akkerdaas JH, Van Leeuwen J, Van der Zee JS, Aalberse RC. Identification of a cross-reactive allergen (presumable tropomyosin) in shrimp, mite and insects. *Int Arch Allergy Immunol* 1994; 105:56–61.
 - 16 Van der Zee JS, Van Swieten P, Jansen HM, Aalberse RC. Skin tests and histamine release with P1-depleted *D. pteronyssinus* body extracts and purified P1. *J Allergy Clin Immunol* 1988; 81:884–96.
 - 17 Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE. Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp.). *J Allergy Clin Immunol* 1989; 83:1055–67.
 - 18 Chapman DM, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980; 125:587–92.
 - 19 Wood RA, Eggleston PA, Lind P et al. Antigenic analysis of household dust samples. *Am Rev Respir Dis* 1988; 137:358–63.