

# Enzyme-Linked Immunosorbent Assay for Determination of Major Excrement Allergens of House Dust Mite Species *D. pteronyssinus*, *D. farinae* and *D. microceras*

P. LIND

The Protein Laboratory, University of Copenhagen and ALK Research Group, Copenhagen, Denmark

Species-specific enzyme-linked immunosorbent assays (ELISA) for major excrement allergens (Dp42, Df6 and Dm6) of *D. pteronyssinus*, *D. farinae* and *D. microceras* in house dust were established, using immunoabsorbed, monospecific rabbit antibodies, coupled to horse radish peroxidase. The limit of detection was 13, 4 and 38 ng/ml, respectively. The coefficient of variation for the entire procedure, including dust sieving (212  $\mu$ m) and extraction was 5-16% for allergen levels above 1000 ng/g dust. Allergen concentration by ELISA correlated well with the number of mite bodies identified and counted by microscopy in 31 dusts ( $r = 0.88, 0.86$  and  $0.82$  for combined *Dermatophagoides* sp., *D. pteronyssinus* and *D. farinae* group, resp.) *Dermatophagoides* allergen was recorded in 21/22 mattress dusts (median: 26000 ng/g; maximum: 290,000 ng/g). *D. pteronyssinus* allergen occurred in largest amounts (median 7500 ng/g) followed by *D. microceras* (median 650 ng/g) and *D. farinae* (median 240 ng/g).

**Key words:** ELISA; excrement allergens; house dust mite.

Accepted for publication 25 April 1986

House dust mites (fam.: *Pyroglyphidae*) are widespread inhabitants of human dwellings (1), frequently causing asthma and rhinitis in susceptible individuals (26).

Elimination or reduction of allergen exposure through intensified cleaning and regulation of indoor climate is a promising approach, but controlled trials of such elimination programmes have so far given contradictory results (3, 4, 5, 16, 23). There is probably a need for more thorough monitoring of allergen levels during the time course and a better knowledge of the underlying parameters.

Potent allergen preparations of house dust mites are available for diagnosis and immunotherapy, and international standardization is in rapid progress (10, 11). Differences between the species of *Dermatophagoides*, however, exist at the level of the major allergens (Dp42 = P1 and

analogues), which necessitate a proper selection of species (or species mixture) for clinical practice (18).

We report here the development and testing of ELISAs for Dp42, Df6 and Dm6, with a sensitivity and specificity sufficient to quantitate clinically relevant levels of mite allergen and to reveal the species composition in dust.

## MATERIAL AND METHODS

**Dust samples.** Mattress dust from 22 homes of normal (non-allergic) persons in Copenhagen and environs was obtained by vacuum-cleaning during the month of October 1984. Samples were stored refrigerated (5°C) until processing for analysis. Dust extracts were produced by agitation for 3 h at room temperature in 0.125 M ammoniumhydrogencarbonate, containing

0.1% sodiumazide. Samples of material collected by regular cleaning (mixed dust) from allergic patients' homes were obtained from Bente Schwartz, Diagnoselaboratoriet a/s, Copenhagen: 13 samples of bag dust and 45 extracts of vacuum cleaner dust collected in all rooms of the habitation. The dust extracts had been prepared using a 1:2 (w/v) extraction ratio for routine dust examination as described in (20) and were stored at  $-20^{\circ}\text{C}$ .

**Reference antigens** for use in quantitation of allergens were freeze-dried whole mite culture (WMC) extracts of the *Dermatophagoides* mite species, *D. pteronyssinus*, *D. farinae* and *D. microceras*, produced as previously described (19), and with known content of major allergens Dp42 (6.4%), Df (8.6%) and Dm6 (6.9%) in accordance with references (17) and (18).

House dust mite preparations, all labelled as *D. farinae*, were obtained as purified mite bodies (A), culture samples (B, C) or prepared allergen extracts (D, E, and F) from European manufacturers. Preparation of crude allergen extracts form D, E, and F took place as described in (19). Five *D. pteronyssinus* extract (C, F, H, J, and K) from various manufacturers originated from the IUIS collaborative study on allergen standardization (10).

**Reagent antibodies.** Monospecific rabbit antibodies raised against purified allergens Dp42, Df6 and Dm6 were column affinity-purified on CNBr-activated Sepharose 4B (Pharmacia, Uppsala) coupled with the corresponding partially purified allergens (AML-preparations) (18). Equilibration and application of antibodies to the column took place in 0.05 M sodium citrate buffer pH 6.2, and the purified antibodies were eluted with 0.05 M sodium citrate buffer pH 2.6, containing 1.0 M NaCl, followed by dialysis against distilled water and freeze-drying. These antibodies were used for coating the wells in the ELISA.

Species-specific antibodies for enzyme-conjugation were produced by passing monospecific rabbit antibodies through CNBr-activated Sepharose 4B columns coupled with appropriate mixtures of heterologous antigens. In a typical

immunoabsorption, 3 ml anti-Df6 antibodies (immunoglobulinfraction) was passed through a column with 60 mg Dp-AML + 12.3 mg Dm-AML coupled to a total volume of 12.8 ml Sepharose-gel, and fractions of 3 ml collected. Fractions 3-9, eluted with the 0.5 M sodium citrate-buffer, pH 6.2 and shown by fused rocket immunoelectrophoresis (22) to contain the main part of rabbit immunoglobulin, were pooled. These antibodies were conjugated with horse radish peroxidase (HRP) (Sigma, type VI) using the two-step glutaraldehyde procedure (7). In a typical conjugation, 2000 units HRP (approx. 6.7 mg) was activated in 0.2 ml 1.25% glutaraldehyde in phosphate buffered saline (PBS, pH 7.4) and left overnight at  $20^{\circ}\text{C}$ . After additions of 0.8 ml 0.1 M sodium-carbonate buffer pH 9.2, dialysis took place for  $4 \times 1.5$  h against the same buffer. To 0.275 ml of the activated peroxidase was added 350  $\mu\text{l}$  species-specific antibodies (approx. 5 mg/ml) and conjugation proceeded overnight at  $20^{\circ}\text{C}$ . Excess active groups were blocked by addition of 35  $\mu\text{l}$  0.2 M lysine and, finally glycerol was added at 50% (v/v) for preservation.

#### Assay procedure:

1. Polystyrene microtiter trays (96-well NUNC Immunoplate I) were coated with 10  $\mu\text{g/ml}$  affinity-purified anti-Dp42, anti-Df6 and anti-Dm6 (0.1 ml/well). Reaction took place overnight at  $20^{\circ}\text{C}$  in 0.1 M sodiumcarbonate buffer, (pH 9.6).

2. Excess adsorption sites were blocked for 1 h in 0.1 ml bovine serum albumin (BSA), 10 mg/ml in PBS. Coated trays were stored up to 1 week at  $5^{\circ}\text{C}$ , in which case 15 mM sodiumazide was added to the blocking solution.

3. Antigen incubation took place with 0.1 ml reference antigen solutions, sample dilution, and controls (heterologous species in BSA (5 mg/ml in PBS) overnight at  $20^{\circ}\text{C}$ , after which the content of each well was removed by suction to avoid cross-contamination. Shorter incubation periods (3 h) were possible but produced lower readings of absorbance.

4. Incubation with conjugated antibodies (0.1 ml in 5 mg/ml BSA solution in PBS) was carried out for 1 h at 20°C. The dilution of conjugated antibodies (between 1:100 and 1:500) was determined for each production lot by assaying a dilution series of standard antigen, and selecting the most favourable ratio between maximal response and blanks.

Each incubation step (1-4) was followed by 3 × 3 min washing in PBS containing 0.05% Tween-20.

5. Substrate (0.1 ml 3.0 mM hydrogenperoxide/5.0 mM ortho-phenylenediamine in 0.1 M sodium-citrate buffer, pH 5.0) was prepared immediately before use and reaction took place for 15 min at 20°C in darkness, after which 0.1 ml 1 M sulphuric acid was added. The absorbance at 490 nm was read on the Dynatech minireader MR590.

### Microscopy

Dust portions of 0.1 g were transferred to 10 cm petri dishes containing 10 ml 90% lactic acid with a few crystals of lignin pink added and incubated for at least 48 h at 60°C. All mites

observed by stereomicroscopy at 20 × magnification were transferred to drops of lactophenol solution on microscope slides and sought, identified at 500 and 1250 × magnification using a Leitz transmission microscope. Identification followed Hughes (14), Fain (8, 9) and Griffiths & Cunnington (12). For some of the comparisons of microscope data with ELISA the following calculations were performed: For each dust sample the number of specimens (mainly adults) identified to one of the two categories, *D. pteronyssinus* or *D. farinae* group, was divided by the total number of identified *Dermatophagoides* specimens. This fraction was then multiplied by the total number of recorded *Dermatophagoides* specimens (including adults and all juvenile stages), thus giving an estimate of the total number of specimens of a given species. The calculation assumes an equal stage distribution of the species.

### Control experiments

#### a) Dust processing

The raw dust content of four vacuum cleaner bags (room dust) was divided into two equal

Table 1  
Assay performance

| Assay           | Absorbance      |                           |                          |                         |                             |                            |                            | Limit of detect. ng/ml* |
|-----------------|-----------------|---------------------------|--------------------------|-------------------------|-----------------------------|----------------------------|----------------------------|-------------------------|
|                 | Blank (BSA-PBS) | <i>D. pter.</i> 320 ng/ml | <i>D. far.</i> 430 ng/ml | <i>D. mic</i> 690 ng/ml | <i>D. pter.</i> 64000 ng/ml | <i>D. far.</i> 86000 ng/ml | <i>D. mic.</i> 69000 ng/ml |                         |
| <i>D. pter.</i> | 0.16            | 0.82                      | -                        | -                       | ND                          | 0.16                       | 0.21                       | 13                      |
| <i>D. far.</i>  | 0.14            | -                         | 1.55                     | -                       | 0.22                        | ND                         | 0.20                       | 4                       |
| <i>D. mic.</i>  | 0.17            | -                         | -                        | 0.72                    | 0.14                        | 0.19                       | ND                         | 38                      |

\*Lowest concentration giving response twice that of blanks.

Table 2

Interference from heterologous species. Figures denote the mean per cent reduction for two readings within the range of measurement

| Assay           | <i>D. pteronyssinus</i> |             | <i>D. farinae</i> |             | <i>D. microceras</i> |             |
|-----------------|-------------------------|-------------|-------------------|-------------|----------------------|-------------|
|                 | 6400 ng/ml              | 64000 ng/ml | 8600 ng/ml        | 86000 ng/ml | 6900 ng/ml           | 69000 ng/ml |
| <i>D. pter.</i> | -                       | -           | 34                | 49          | 42                   | 51          |
| <i>D. far.</i>  | 0                       | 13          | -                 | -           | 18                   | 30          |
| <i>D. mic.</i>  | 30                      | 55          | 15                | 23          | -                    | -           |

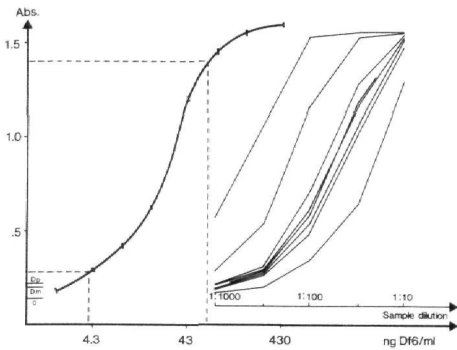


Fig. 1. Dose response curves for *D. farinae* standard extract (—) and mattress dust samples (---). Horizontal axis: ng Df6/ml (standard) and sample dilutions of 1:5 (w/v) extract. Vertical axis: Absorption units. Response to blanks (0) and heterologous extracts (Dp and Df) denoted on the vertical axis. The limits of the range of measurement (4–70 ng/ml) are denoted by broken lines.

parts, from one of which  $5 \times 1$  g portions were taken as uniformly as possible, extracted 1:10 (w/v) and analysed by ELISA, whereas the other part was sieved through  $212 \mu\text{m}$  to obtain fine dust, from which  $5 \times 0.5$  g portions were extracted 1:10 (w/v). All fractions weights were recorded. The coefficient of variation for the reported determinations was calculated as the standard deviation/mean. Further,  $4 \times 0.5$  g

portions of a fine dust were extracted in relations 1:2, 1:5, and 1:20 (w/v), quantitated by ELISA, and recovered antigen calculated relative to the 1:20 (w/v) extraction.

#### b) Interference by other dust components

Dilution series of reference antigen Dp42: 13–130  $\mu\text{g/ml}$ ; Df6: 17–170  $\mu\text{g/ml}$  and Dm6: 14–140  $\mu\text{g/ml}$  was produced in BSA-PBS containing antigen of the heterologous species and human dander (the main organic constituent of house dust) in increasing amounts up to 1 mg WMC/ml (Dp42: 64000 ng/ml Df6: 86000 ng/ml and Dm6: 69000 ng/ml) and 10 mg/ml human dander, respectively. The percent depression of the antigen amount assayed by ELISA was calculated by comparison with the results obtained with BSA-PBS diluent without heterologous species.

## RESULTS

### Assay performance

Typical performance of the ELISA for the three species is shown in Table 1. The *D. farinae* assay yielded the largest maximal response and the lowest limit of antigen detection. The *D. microceras* assay was approximately 10 times less sensitive, with the *D. pteronyssinus* ELISA taking an intermediate position. The response to heterologous species, measured in large excess, was

Table 3

Subsample variation (SD/mean,  $n = 5$ ) and recovery for raw mixed dust (R) and fine dust (F, sieved at  $212 \mu\text{m}$ )

| Sample | wt. F/R (%) | <i>D. pteronyssinus</i> |      | <i>D. microceras</i> |      | Recovery* (%) |
|--------|-------------|-------------------------|------|----------------------|------|---------------|
|        |             | Mean, ng/g              | CV % | Mean, ng/g           | CV % |               |
| M5     | 61          | R 10800                 | 28   | 1780                 | 11   | 85            |
|        |             | F 14300                 | 16   | 2580                 | 5    |               |
| M6     | 25          | R 1280                  | 27   | 610                  | 38   | 95            |
|        |             | F 5600                  | 15   | 1950                 | 9    |               |
| M7     | 20          | R < 100                 | –    | 6090                 | 34   | 97            |
|        |             | F 435                   | 51   | 29500                | 11   |               |
| M8     | 63          | R < 100                 | –    | < 200                | –    | –             |
|        |             | F 140                   | 25   | < 200                | –    |               |

\* Mean recovery for two species in per cent of concentration in raw dust.

Table 4

Extraction efficiency at different extraction ratios. Figures denote recovered antigen in ng/g and (in parentheses) per cent recovery relative to the 1:20 extraction. Sample M5, fine dust fraction

| Extraction ratio (w/v) | <i>D. pteronyssinus</i> | <i>D. microceras</i> |
|------------------------|-------------------------|----------------------|
| 1:2                    | 13400 (61)              | 1470 (67)            |
| 1:5                    | 20200 (91)              | 1920 (88)            |
| 1:10                   | 20200 (91)              | 1660 (76)            |
| 1:20                   | 22100 (100)             | 2180 (100)           |

larger than the blanks in several cases (Table 1), but did not approach the value of twice the blanks, which was set as the lower limit of readings.

The upper limit of the range of measurement (corresponding to readings at 85% of maximal response) was approx. 10 times the lower limit (Fig. 1). Generally, each sample extract was assayed undiluted and diluted 1:10 and 1:100 to obtain readings within measurement limits.

The dose-response curves for samples with large amounts of mite allergens were approximately parallel to the standard curve (Fig. 1), indicating only low levels of interfering substances, at least from sample extract dilution 1:10 and downwards.

Negative interference caused by large concentrations of heterologous antigens was separately investigated (Table 2). At concentrations above 50,000 ng/ml the antigen concentration, as read from the standard curve, was depressed up to 55%, the *D. pteronyssinus* and *D. microceras* assay being most affected. At 10 times lower concentration the interference was reduced, although still of considerable size. No interference was induced by large concentrations of human dander extract.

The coefficient of variation (CV) for the total procedure (including extraction) was investigated by assaying five subsamples of raw and fine (sieved) dust from each four samples of mixed dust (Table 3). Assay on fine dusts gave CV-values below 20% (5–16%), when the allergen content exceeded 1000 ng/g dust, but variation was larger when lower antigen concentrations were measured (sample M7). Assays on raw dusts produced large CVs (11–38%). The re-

Table 5

Maximal ELISA response and slope of dose-response curve for mite preparations originating from various manufacturers

| Lable               | <i>D. farinae</i> ELISA |        | <i>D. microceras</i> ELISA |        |
|---------------------|-------------------------|--------|----------------------------|--------|
|                     | Max.                    | Slope* | Max.                       | Slope  |
| <i>D. far.</i> A    | 1.74                    | 0.87   | 0.30                       | < 0.05 |
| <i>D. far.</i> B    | 0.32                    | < 0.1  | 0.79                       | 0.36   |
| <i>D. far.</i> C    | 0.27                    | < 0.1  | 0.83                       | 0.27   |
| <i>D. far.</i> D    | 1.72                    | 0.89   | 0.31                       | < 0.05 |
| <i>D. far.</i> E    | 0.28                    | < 0.1  | 0.79                       | 0.33   |
| <i>D. far.</i> F    | 1.74                    | 0.82   | 0.31                       | < 0.05 |
| <i>D. far.</i> Std. | 1.75                    | 0.97   | 0.36                       | < 0.05 |
| <i>D. mic.</i> Std. | 0.33                    | < 0.1  | 0.79                       | 0.36   |

\* Calculated as the largest difference in absorption units between two 10-fold dilutions.

covery of allergen in the fine dust fraction approached unity (85–97%)

The relative extraction efficiency was largest at a dust/buffer (w/v) ratio of 1:20 (Table 4). Reducing the volume of extraction fluid to 5 ml, thus theoretically improving sensitivity by a factor of 4, led to a 10% average fall in recovered antigen. Reducing the volume further led to considerably lower recoveries.

#### *Response of allergen extracts from various sources*

Five *D. pteronyssinus* extracts from various allergen manufacturers produced maximal responses (0.81–0.83) and slopes (0.32–0.47) in the *D. Pteronyssinus* ELISA similar to the standard extract (0.88–0.37, resp.).

The dose-response relationships of six extracts, all labelled *D. farinae*, were investigated in both *D. farinae* and *D. microceras* ELISA (Table 5). The extracts clearly fell into two discrete groups, three extracts (A, D and F)) responding similarly to the *D. farinae* standard and the three others (B, C and E) responding like the *D. microceras* standard.

#### *Comparison between ELISA and microscopical findings*

Species of the family *Pyroglyphidae* predominated among the mite fauna observed by microscopy in

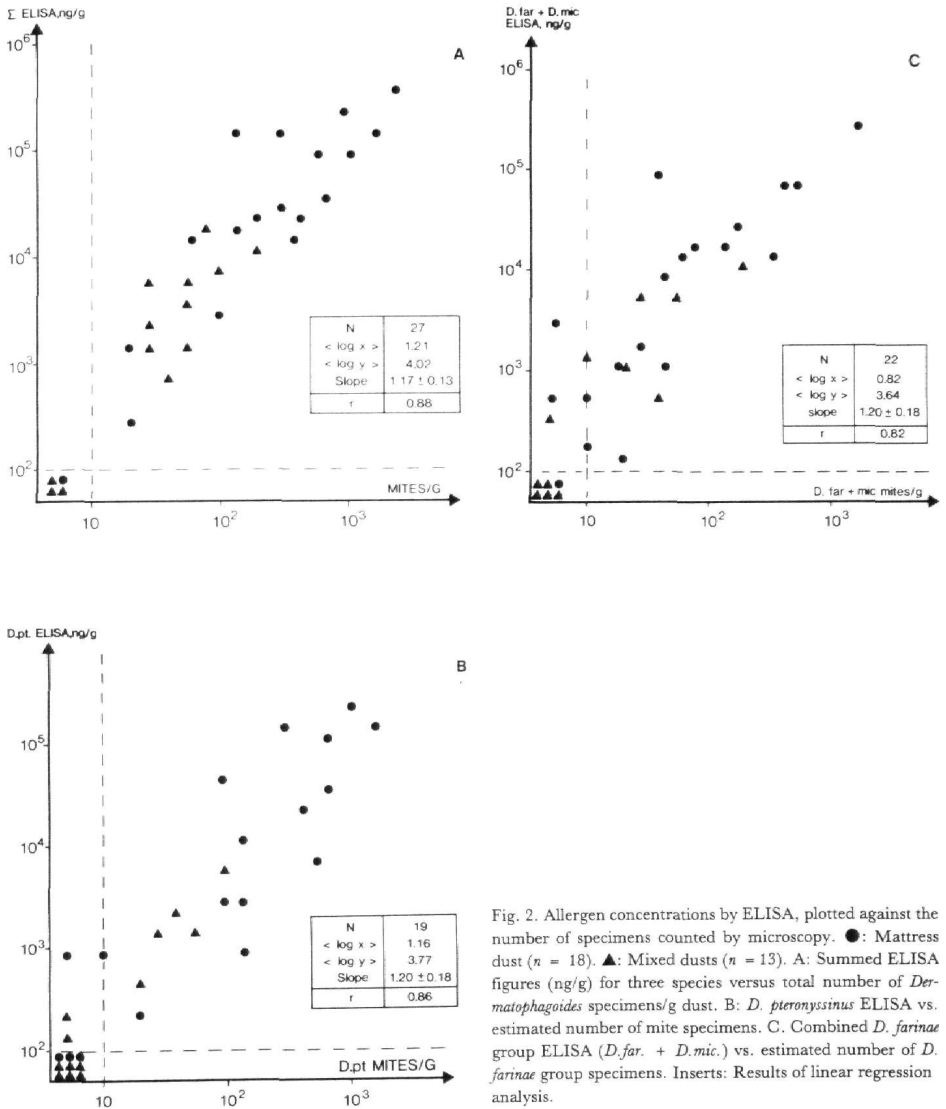


Fig. 2. Allergen concentrations by ELISA, plotted against the number of specimens counted by microscopy. ●: Mattress dust ( $n = 18$ ). ▲: Mixed dusts ( $n = 13$ ). A: Summed ELISA figures (ng/g) for three species versus total number of *Dermatophagoides* specimens/g dust. B: *D. pteronyssinus* ELISA vs. estimated number of mite specimens. C: Combined *D. farinae* group ELISA (*D. far.* + *D. mic.*) vs. estimated number of *D. farinae* group specimens. Inserts: Results of linear regression analysis.

18 mattress dusts and 13 mixed dusts (96% and 75%, resp., of collected specimens). Identified non-pyroglyphid mites belonged to the mite families *Glycyphagidae* and *Cheyletidae*. In each of two samples one mite of the subfamily *Pyroglyphinae* was found. A total of 753 specimens of the genus *Dermatophagoides* were collected (67% *D. pter-*

*onyssinus*, 33% *D. farinae* group (*D. farinae* + *D. microceras*)). Both *D. farinae* and *D. microceras* occurred frequently: In six and seven samples, resp., the quality of the specimens was good enough to allow identification at the species level.

The log-transformed number of mites per 100 mg in each sample correlated well with the

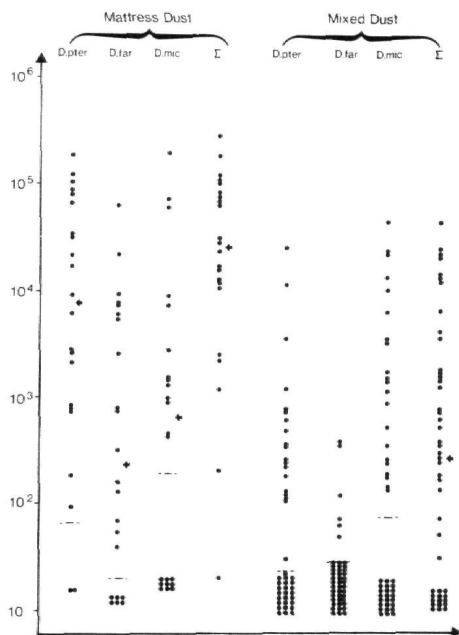


Fig. 3. Levels of *Dermatophagoides* allergen measured by ELISA in 22 mattress dust samples (columns 1-4) and 45 mixed dust samples (columns 5-8). Summed figures for three species appear in columns 4 and 8. Figures on vertical axis denote ng Dp-42/Df-6/Dm-6/g dust. Arrows in columns 1-4 and 8 point to median value. Observations positioned under the broken lines were below the limit of detection.

log-transformed major allergen concentration by ELISA. This was the case when the *Dermatophagoides* mites in total was compared with the summed ELISA values for the three species (Fig. 2A) or when the estimated number of *D. pteronyssinus* mites (Fig. 2B) or *D. farinae* group mites (Fig. 2C) were compared with the corresponding ELISA values. The slopes of the regression lines were in no case significantly different from unity, indicating a simple linear relationship between microscopy and ELISA. The average amount of major allergen per mite body was 65 ng for combined pyroglyphids, 40 ng for *D. pteronyssinus* and 66 ng for *D. farinae* group. These values were not statistically different at the 5% level as judged from the standard deviations on the Y-intercept.

The numbers of specimens of *D. farinae* and *D. microceras*, identified to species level, were too small to allow a statistical comparison with the ELISA.

#### *Species distribution in mattress and mixed dusts*

Major allergens of the three *Dermatophagoides* species were found with varying frequency and within a broad range of concentrations in the 22 mattress dusts and the 45 mixed dusts (Fig. 3). In the mattress dusts, *D. pteronyssinus* was the dominant species, 20/22 homes being positive (above the detection limit of 65 ng/g) and with a median value of 75000 ng/g. The frequency of detection of *D. farinae* and *D. microceras* was smaller (16/22 and 13/22, resp.). The median value for *D. microceras* (650 ng/g), however, exceeded that of *D. farinae* (250 ng/g). The median value for the summed *Dermatophagoides* major allergen content was 26,000 ng/g. No correlation existed between measured allergen concentrations of the three species (Sperman,  $r_s$ : *D.pt/D.far*: -0.11; *D.pt/D.mic*: 0.22; *D.far/D.mic*: 0.01;  $n = 22$ ,  $P > 0.05$  in all cases).

In the mixed dust (collected from other homes and at a different time), *D. pteronyssinus* and *D. microceras* were recorded with approximately equal frequency (19/45 and 21/45, resp.). The proportion of homes with allergen concentrations above 1000 ng/g was significantly larger for *D. microceras* (12/45) than for *D. pteronyssinus* (4/45). *D. farinae* displayed only sporadic occurrence (6/45 homes positive, all below 1000 ng/g). The median value for combined *Dermatophagoides* allergen was 260 ng/g. A weak correlation existed between *D. pteronyssinus* and *D. microceras* concentrations ( $r_s = 0.31$ ,  $n = 45$ ,  $P < 0.05$ ). However, when only dusts recorded positive to both species were considered, no correlation existed ( $r_s = -0.15$ ,  $n = 18$ ,  $P > 0.05$ ).

## DISCUSSION

The purpose of the present study was to develop an assay for mite allergen content in dust samples, for future use in the monitoring of patients' allergen

exposure in the habitation, regarding both allergen concentration and species composition.

The presented ELISAs for measurement of major excrement allergens (Dp42, Df6 and Dm6) of three *Dermatophagoides* species were found to be of adequate specificity and sensitivity for dust analysis. Although minor responses (above blanks) were detected in each assay towards the other species, only small effects within the range of measurement resulted. This was demonstrated by the approximate parallelism of sample dose-response curves with the standard curve (Fig. 1), by the frequent observations of large figures for a singly occurring species, and by the lack of correlation between allergen levels of the three species.

However, in the developed assay, antigens of heterologous species, present in large amounts in the extract, will compete with homologous antigens for 1st layer (coating) antibodies, which were not rendered species-specific. Readings of sample absorbance may thus be reduced compared to the standard extract, causing erroneously low values. The effect is largest for undiluted samples, where maximum interference values, corresponding to an approx. 50% reduction, may be anticipated in extreme cases. The problem may be solved by using species-specific antibodies in the 1st layer, a change which is now being introduced.

The range of assay sensitivities reported here (4–38 ng/ml) may seem unsatisfactory from a technical point of view, as a limit of detection below 1 ng P1/ml is possible in radioimmunoassay (24). However, the 3rd layer (detecting) antibodies used here were quite heavily immunoabsorbed, so average affinity may have been reduced. We are presently screening a larger number of rabbit antisera to select reagents with optimal affinity towards species-specific epitopes. Mouse hybridoma antibodies, which are quite readily raised against species-specific epitopes of these antigens (6, 13), may directly yield reagents of suitable specificity without the need for immunoabsorption. Affinity purification and gel-filtration of the conjugated antibodies may further improve sensitivity by reducing unspecific absorption and thus reducing blanks.

With an average major allergen concentration of 40–80 ng/mite body (as calculated from the mean ELISA and microscope figures), the sensitivity of the presented assay corresponds to < 1 mite/g dust for *D. pteronyssinus* and *D. farinae* and < 3 mites/g dust for *D. microceras*. This was sufficient to assign positive values of combined mite allergen content in 21/22 mattress dust samples (Fig. 3, col. 4).

In a population study, involving newly diagnosed mite allergic asthmatics and healthy controls, Korsgaard (15) found that living in habitations with 100 mites/g dust or more contributed a large risk of developing asthma, while significant risk-factors were not obtained with lower cut-off's. Although we believe that mite levels somewhat below this value may sustain symptoms in the very sensitive patients, the presented assay most probably allows representation of all clinically relevant mite allergen concentrations.

The preparation of samples is a source of variability which must be controlled. Sieving (212  $\mu$ m) of mixed dust samples before extraction reduced the coefficient of variation without reducing allergen recovery and resulted in up to 5 times improved sensitivity for dusts with large fractions of coarse particles (Table 3). An extraction ratio of 1:5 (g dust/ml buffer) gave maximal sensitivity (in terms of ng allergen detected/g dust) without significant loss of allergen. These findings are in accordance with the data on the size (10–40  $\mu$ m) of the allergen-bearing excrement particles and the effective dissolution of allergen into saline solutions (25).

ELISA values for major allergen content showed a good correlation with the microscopical counting of mites, whether combined figures for the three species or separate figures for *D. pteronyssinus* or *D. farinae* group were considered (Fig. 2).

The responses of extracts of house dust mite material from various allergen manufacturers could each be classified so as to be approximately identical to one of the three standard preparations. This indicates no further subspecies or strain variation on the major allergen level of these species. However, we cannot exclude the possibility that some of the investi-

gated preparations may be derived from the same primary cultures. Half of those mite preparations labelled *D. farinae* actually contained *D. microceras*, a fact which is probably to be explained by the few and inconspicuous, discriminating characters within the *D. farinae* group (12) and the only recently discovered differences in their antigenic properties (18).

The recorded species distribution in mattresses is similar to that previously published for Aarhus, Denmark (16, 20), where *D. pteronyssinus* and *D. farinae* group dominated, and *Euroglyphus* sp. occurred only sporadically. In Denmark the combined *Dermatophagoides* allergen level will generally represent the total load of house dust mites, but this will only partly be the case in geographic areas, where *Euroglyphus* species occur with significant frequency.

We attempt no direct explanation for different species distribution in the mixed dusts and mattress dusts (Fig. 3), as these were collected from different homes, under different conditions and using different sample preparations. We speculate, however, that this variation may be due to species differences regarding microclimate requirements (21) and exploitation of various substrates (1, 27), factors which might change during elimination measures.

The presented assay should prove useful as a measurement of patients' mite allergen exposure, as a fast and sensitive tool for controlling the effect of allergen elimination programmes, and providing the necessary data in various geographic areas for selection of mite species used in diagnosis and immunotherapy.

## ACKNOWLEDGEMENTS

The skilful technical assistance of Gitte Nordskov Hansen and Lone Fink is gratefully acknowledged.

## REFERENCES

1. Bronswijk, J. E. M. H.: Food preference of pyroglyphid house dust mites (Acari). *Netherlands J. Zoology* 22, 335-340, 1972.
2. Bronswijk, J. E. M. H.: Hausstaubmilben, Vorkommen und Bedeutung. *Allergologie* 1, 55-60, 1978.
3. Burr, M. L., St. Leger, A. S. & Neale, E.: Anti-mite measures in mite-sensitive adult asthma: A controlled trial. *Lancet* 1, 333-335, 1976.
4. Burr, M. L., Dean, B. V., Merrett, T. G., Neal, E., St. Leger, A. S. & Verrier-Jones, E. R.: Effects of anti-mite measures on children with mite-sensitive asthma: A controlled trial. *Thorax* 35, 506-512.
5. Burr, M. L., Neale, L., Dean, B. V. & Verrier-Jones, E. R.: Effect of change to mite-free bedding on children with mite-sensitive asthma: A controlled trial. *Thorax* 35, 513-514, 1980.
6. Chapman, M. D., Sutherland, W. M. & Platts-Mills, T. A. E.: Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on antigen P1 by using monoclonal antibodies: Binding to each epitope can be inhibited by serum from dust mite-allergic patients. *J. Immunol.* 133, 2488-2495, 1984.
7. Engvall, E.: Enzyme immunoassay ELISA and EMIT. In Vunakis & Langone (eds.): *methods in enzymology*, Vol. 70: *Immunochemical Techniques*, part A. Academic Press, New York, 419-439, 1980.
8. Fain, A.: Nouvelle description de *Dermatophagoides pteronyssinus* (Trouessart, 1897). Importance de cet acarien en pathologie humaine (Psoroptidae: Sarcoptiformes). *Acarologia* 8, 302-327, 1966.
9. Fain, A.: Le genre *Dermatophagoides* (Bogdanov 1864). Son importance dans les allergies respiratoires et cutanées chez l'homme (Psoroptidae: Sarcoptiformes). *Acarologia* 9, 170-225, 1967.
10. Ford, A. W., Rawle, F. C., Lind, P., Spielsma, F. T. M., Lowenstein, H. & Platts-Mills, T. A. E.: Standardization of *Dermatophagoides pteronyssinus*: Assessment of potency and allergen content in ten coded extracts, *Int. Arch. Allergy Appl. Immunol.* 76, 58-67, 1985.
11. Ford, A., Seagroatt, V., Platts-Mills, T. A. E. & Lowenstein, H.: A collaborative study on the first international standard of *Dermatophagoides pteronyssinus* (house dust mite) extract. *J. Allergy Clin. Immunol.* 75, 676-686, 1985.
12. Griffiths, D. A. & Cunningham, A. M.: *Dermatophagoides microceras* sp.n.: description and comparison with its sibling species, *D. farinae* Hughes, 1961. *J. Stored Prod. Res.* 7 1-14, 1971.
13. Horn, N. & Lind, P.: Selection and characterization of monoclonal antibodies (McAb) against house dust mite. XVI annual meeting of the Scandinavian Society for Immunology, Reykjavik, 1985 (Abstract).
14. Hughes, A. M.: The mites of stored food and houses. *Tech. Bull.* 9, H. M. S. O., London, 1976.
15. Korsgaard, J.: Mite asthma and residency. A case-control study on the impact of exposure to house dust mites in dwellings. *Am. Rev. Respir. Dis.* 128, 231-235, 1983.
16. Korsgaard, J.: Preventive measures in mite asthma - a controlled trial. *Allergy* 38, 93-102, 1983.
17. Lind, P.: Purification and partial characterization of two major allergens from the house dust mite, *Dermatophagoides pteronyssinus*. *J. Allergy Clin. Immunol.* 76, 753-761, 1985.
18. Lind, P.: Demonstration of close physicochemical similarity and partial immunochemical identity between the

- major allergen, Dp42, of the house dust mite, *D. pteronyssinus* and corresponding antigens of *D. farinae* (Df6) and *D. microceras* (Dm6). Int. Arch. Allergy Appl. Immunol. 79, 60-65, 1986.
19. Lind, P., Weeke, B. & Løvenstein, H.: A reference allergen preparation of the house dust mite *D. pteronyssinus*, produced from whole mite culture. A part of the DAS 76 study. Comparison with allergen preparations from other raw materials. Allergy 39, 259-274, 1984.
  20. Lind, P., Korsgaard, J. & Løvenstein, H.: Detection and quantitation of *Dermatophagoides* antigens in house dust by immunochemical techniques. Allergy 34, 319-326, 1979.
  21. Lustgraaf, B., Rijkaert, G. & Linskens, H. F.: Okologie der Hausstaub-Allergene. Allergologie 1, 61-73, 1978.
  22. Løvenstein, H.: Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. Progr. Allergy 25, 1-62, 1978.
  23. Murray, A. B. & Ferguson, A. C.: Dust-free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: A controlled trial. Pediatrics 71, 418-422, 1983.
  24. Tovey, E. R., Chapman, M. D., Wells, C. W. & Platts-Mills, T. A. E.: The distribution of dust mite allergen in the houses of patients with asthma. Am. Rev. Respir. Dis. 124, 630-635, 1981.
  25. Tovey, E. R., Chapman, M. D. & Platts-Mills, T. A. E.: Mite faeces are a major source of house dust allergens. Nature 298, 592-593, 1981.
  26. Voorhoorst, R., Spiekma, F. Th. M. & Varekamp, H.: House dust atopy and the house dust mite *Dermatophagoides pteronyssinus* (Trouessart 1897). Stafleu's Sci., Leiden, 1969.
  27. Wharton, G. W.: Spatial relations of house dust mites. Proceedings of the 3rd International Congress of Acarology, Prague, 1971.

Address:

Peter Lind

The Protein Laboratory

University of Copenhagen

Sigurdsgade 34

DK-2200 Copenhagen N

Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.