

Short communication

Comparison of quantitative ELISA and semiquantitative Dustscreen[™] for determination of Der p 1, Der f 1, and Fel d 1 in domestic dust samples

Background: Quantitative ELISA kits used for the measurement of indoor allergens are time-consuming. Therefore, an immunodot assay (Dustscreen[™]) has been introduced, so that results can be obtained within 4 h. In order to assess the potential applications of the new method, we compared quantitative ELISA methods and Dustscreen.

Methods: We compared the measurements of the mite allergens Der p 1 and Der f 1, and Fel d 1 by both Dustscreen and one brand of ELISA in 82 dust samples, and we also compared the measurement of the cat allergen Fel d 1 by Dustscreen and a different brand of ELISA in 77 dust samples. Optimum dilutions of dust extracts were compared between the ELISA and the immunodot assay. The nonparametric coefficient of correlation (Spearman's rank) was calculated for each allergen.

Results: The immunodot assay was easy to perform. There was a good correlation between the results of the ELISA and Dustscreen (Der p 1: $r=0.98$, Der f 1: $r=0.93$, and Fel d 1: $r=0.98$). However, the coefficient of variation varied between 50% and 100% for Fel d 1 above 100 000 ng/g in Dustscreen. No cockroach allergen (Bla g 2) was detected in domestic dust samples in Berlin, and lower concentrations of group 2 allergen of mite (from not detectable to 19 500 ng/g; median concentration 2000 ng/g) were found than mite group 1 allergens (sum of Der p 1 plus Der f 1: median 12 350 ng/g, minimum n.d., maximum 153 120 ng/g).

Conclusions: Our results indicate that quantification of indoor allergens in house-dust samples can be performed easily with Dustscreen, and both the technology and methodology are satisfactory.

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Key words: domestic dust samples; ELISA; house-dust mite and cat allergen analysis; immunodot.

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Accepted for publication 27 April 2001

Indoor allergens of the house-dust mite genus *Dermatophagoides*, cat, and cockroach all play a major role in the development of specific sensitization and allergic airway disease in susceptible individuals (1–4). Quantitative ELISA kits have been used for the measurement of indoor allergens in solid dust for years (1, 5, 6). The assays require expertise in laboratory techniques, and 24 h is needed to follow the various steps. Recently, the immunodot assay Dustscreen[™] has been introduced; it is a combined strip for simultaneous determination of the allergens Der p 1, Der f 1, mite group 2, Fel d 1, and Bla g 2 in house dust (7). Results

can be obtained after 4 h semiquantitatively by evaluation with the naked eye and a color chart, or quantitatively with a densitometer.

We compared the measurements of the mite allergens Der p 1 and Der f 1 and mite group 2 by both Dustscreen and one brand of ELISA in 82 dust samples, and we also compared the measurement of cat allergen Fel d 1 by Dustscreen and a different brand of ELISA in 77 dust samples.

Material and methods

ELISA for determination of allergens of house-dust mite, Der p 1 and Der f 1, and cat allergen Fel d 1

Forty-two households of patients of the pediatric outpatient clinic were selected in autumn 1998, as well as 40 farms from the multicenter ALEX study in 2000, conducted in Bavaria, Germany; Switzerland; and Austria by von Mutius and coworkers. Eighty-two

Abbreviations. Der p 1: major allergen of *Dermatophagoides pteronyssinus*; Der f 1: major allergen of *D. farinae*; Fel d 1: major allergen of cat (*Felis domesticus*); Bla g 2: major allergen of cockroach (*Blattella germanica*); ELISA: enzyme-linked immunoassay; mAb: monoclonal antibody; n.d.: not detectable.

domestic dust samples (carpet or mattress) were collected with patients' vacuum cleaners (>1000 W) in a standardized procedure by a family member. Solid dust samples were immediately brought into the laboratory and extracted. Dust extracts were prepared by agitation for 3 h at room temperature 1:10–1:30 (w/v) in 0.125 M of ammonium hydrogen carbonate. Supernatants were filtered through a 220-nm pore size filter and stored at -20°C . Eighty-two samples were analyzed for house-dust mite allergens, and 77/82 samples were analyzed for cat allergen. Der p 1 and Der f 1 were determined with a sandwich ELISA obtained from ALK, Copenhagen, Denmark, using monoclonal non-cross-reacting antibodies for Der p 1 (Nhp5) and polyclonal antibodies for determination of Der f 1, coated on polystyrene microtiter trays. Volumes of 100 μl of dust extracts and dilutions thereof (1:10, 1:100, and 1:1000) were added to the precoated wells, and after overnight incubation and washings, the second horseradish peroxidase-conjugated antibody was added and finally the substrate (hydrogen peroxide/*o*-phenyldiamine). The absorption at 492 nm was read 5 min after addition of 1 mol/l sulfuric acid. Response curves for Der p 1 were plotted with the standard related to NIBSC 82/518. Assay sensitivity was 5 ng/ml or 15 ng/gm of dust.

Fel d 1 was measured by ELISA delivered by INDOOR biotechnologies, Chester, UK, with the monoclonal capture antibody 6F9, provided as HPLC-purified mAb. Assays were conducted according to the manufacturer's directions. Fel d 1 reference 94/01 was standardized from the CBER cat dander reference, E5, which contains 9.7 U/ml Fel d 1 (1 unit = 4 μg protein). Assay sensitivity was 0.4 mU/ml or about 16 ng/gm of dust. The intra-assay coefficient of variation for all three allergens was 5–12% (1).

Results are expressed in ng/gm of dust.

Dustscreen

The immunodot assay uses a nitrocellulose/PVC strip with a positive and negative control and dot areas for the following five major allergens: *D. pteronyssinus* (Der p 1), *D. farinae* (Der f 1), group 2 of *Dermatophagoides*, cat (Fel d 1), and cockroach (Bla g 2). The monoclonal antibodies (mAb) described by Chapman and colleagues (5, 6) were used as first and second antibodies (Table 1). Kits were kindly provided by Trimedal, Switzerland, and ADL Wallac, Freiburg, Germany. The supernatants of dust extraction prepared for the ELISA were used (1:10–1:30 (w/v) in 0.125 M of ammonium hydrogen carbonate). The test strip was incubated with 1 ml of the supernatant or a dilution thereof (undiluted, 1:10, 1:100, 1:1000, and 1:10 000) in an incubation chamber for 1 h. After a washing procedure, 1 ml of the second horseradish-peroxidase labeled antibody was added, and the mixture was incubated for 2 h. After washing, 1 ml of chromogen (4-chloro-1-naphthol) plus substrate (3% H_2O_2) was added, and the mixture was incubated for 15 min. After a washing procedure and 30 min of drying, optical density was read as arbitrary units with a densitometer. Corresponding concentrations were obtained by using a standard curve for each allergen, which was calculated (batch no. DS 980518/07). For Der p 1, an optical density of 55 was equivalent to 50 ng/ml; for Der f 1, an optical density of 43.5 was equivalent to 50 ng/ml; for mite group 2, an optical density of 37 was equivalent to 10 ng/ml; and for Fel d 1, an optical density of 60 was equivalent to 20 ng/ml. Furthermore, batch nos. DS 991117/10 and DS 000718/11 were used. The assay sensitivity was 1 ng/ml for Der p 1, Der f 1, and group 2; for Fel d 1, the sensitivities were 0.05 mU/ml and 0.1 ng/ml, respectively (7). According to the dilution factor, results are expressed as ng/ml or ng/g of dust.

Statistical evaluation

For data analysis, we used SPSS 8.0. Coefficients of correlation were calculated with a parametric test (Pearson) and a nonparametric test (Spearman's rho). Only results of nonparametric testing are shown, because values were not normally distributed. The interassay

Table 1. Monoclonal antibodies (mAbs) used for Dustscreen

Allergen	Capture mAb on solid phase	Horseradish peroxidase-conjugated mAb
Der p 1	5H8	4C1
Der f 1	6A8	4C1
Mite group 2	1D8	7A1
Fel d 1	6F9	3F4
Bla g 2	7C11	8F4

coefficient of variation was calculated by repeating the allergen measurements of three samples with low, median, and high allergen concentrations five times in order to assess reproducibility. Statistical significance was assumed at $P < 0.05$.

Results

In the ELISA, concentrations of Der p 1 ranged from below the detection limit to 148 455 ng/g (median 4100 ng/g). Der f 1 concentrations ranged from below the detection limit to 117 360 ng/g (median 765 ng/g). Fel d 1 concentrations ranged from n.d. to 1 521 600 ng/g (median 3600 ng/g). The results of the Dustscreen assay were in the following ranges: Der p 1: from n.d. to 153 000 ng/g (median 4975); Der f 1: from n.d. to 112 500 ng/g (median 1000); and Fel d 1: from n.d. to 1 200 000 ng/g (median 3000).

Optimum dilutions of dust extracts were undiluted or 1:10 for the ELISA and 1:10 or 1:100 for Dustscreen in most cases. For dust samples below a major allergen concentration of 1000 ng/g, undiluted extracts were used in the ELISA, and extracts in a dilution of 1:10 were used in Dustscreen. For allergen concentrations of 1000–10 000 ng/g, a dilution of 1:10 was best for the ELISA and 1:100 for Dustscreen. For allergen concentrations of 10 000–50 000 ng/g, a dilution of 1:100 was best for ELISA and 1:1000 for Dustscreen. A dilution of 1:1000 was rarely used in the ELISA, but was used in Dustscreen for concentrations above 100 000 ng/g; above 500 000 ng/g, a dilution of 1:10 000 was favorable for Dustscreen. The correlation for ELISA and Dustscreen was 0.98 for Der p 1, and 0.93 for Der f 1 ($P < 0.0001$). The correlation for cat allergen was 0.98 ($P < 0.0001$). However, the coefficient of variation was 50–100% for Fel d 1 concentrations above 100 000 ng/g measured in by Dustscreen. There was no difference between fresh or frozen samples (data not shown). However, repeated freezing and thawing of

Table 2. Interassay coefficients of variation (%) for measurement of indoor allergens in ELISA and Dustscreen

Allergen	ELISA	Dustscreen
Der p 1	7–20	15–25
Der f 1	7–25	16–28
Fel d 1	10–25	15–50

Samples with low (200 ng/g), median (2000 ng/g), and high (10 000 ng/g) allergen concentrations measured five times on different days.

samples (more than three times) resulted in a 10% decrease of measured allergen concentration in the ELISA and Dustscreen. No cockroach allergen Bla g 2 and lower concentrations of group 2 allergen of mites (from not detectable to 19 500 ng/gm, median concentration 2000 ng/g) were detected in the 82 house-dust samples. The coefficient of correlation for the sum of mite group 1 and mite group 2 measurements was $r=0.83$. Coefficients of variation of both assays are shown in Table 2.

Discussion

The determination of major indoor allergens in solid dust samples is useful for patients with sensitization to these allergens and allergic airway disease, in order to introduce and monitor allergen-avoidance procedures in households with significant allergen exposure (1).

Dustscreen can be easily performed and is less time-consuming than ELISA, but some knowledge of laboratory techniques is required. The interassay coefficient of variation is 15–50%; therefore, the reproducibility is slightly different but comparable with the conventional ELISA techniques (1, 5, 6). Only for samples with a Fel d 1 allergen concentration above 100 000 ng/ was the coefficient of variation higher than for the ELISA.

In our hands, the correlation of results compared to the conventional ELISA systems was very satisfactory. The fact that measurements of very high Fel d 1 concentrations showed larger variations

is not a major problem in clinical practice. Although the same capture antibodies are used in both assays, there may be some differences in the procedure of coating the monoclonal antibodies on the solid phase, but median concentrations were comparable in both assays. Evaluation of results by the naked eye was not satisfactory because of greater inaccuracy; thus, densitometric measurement is advisable.

Exposure to mite group 2 allergens was fairly low in the Berlin dust samples. Concentrations of Der p 2 were previously reported to be an order of magnitude lower than group 1 concentrations in mattress dust samples of the Berlin area (8). Other studies have shown mite group 1 and group 2 allergens in house dust to be similar in airborne samples (9).

Cockroach allergen could not be found in the dust samples. Other authors have also reported that exposure to Bla g 2 is not of major importance in Germany (10).

In summary, Dustscreen is an efficient tool for the quantification of indoor allergens by specialized private practices and hospital departments in order to plan and monitor allergen-reducing measures. The costs of both assays are comparable.

Acknowledgments

We thank the researchers of the ALEX study, C. Braun-Fahrlander, Switzerland; E. von Mutius and D. Nowak, Germany; and J. Riedel, Austria, for providing dust samples from farms.

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