

Original Article

Dot-blot assay for the semiquantitative detection of major dust mite allergens Derp1 and Derf1 in house dust

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ABSTRACT

Mite allergens Derp1 and Derf1 were tested in 200 house dust samples using a nitrocellulose membrane-based dot-blot assay. The assay enables the mite allergens in a dust extract to be detected with results that can be visually read semiquantitatively within 30 min. Positive results obtained with this method were easily observed as brown dots. The Derp1 and Derf1 allergens were also precisely quantitated by a two-site monoclonal antibody based enzyme-linked immunosorbent assay. Results of Derp1 and Derf1 mite allergens measured from the two methods were compared. The semiquantitative analysis using Spearman's correlation yielded a correlation coefficient of 0.892 ($P < 0.01$) for Derp1 and 0.872 ($P < 0.01$) for Derf1. The sensitivity, specificity and accuracy were 94.4%, 76.9% and 86.5% for Derp1 whereas the sensitivity, specificity and accuracy for Derf1 were 95%, 77% and 86%, respectively.

Key words: dot blot, enzyme-linked immunosorbent assay, house dust mite, mite allergen.

INTRODUCTION

Hypersensitivity to house dust mites has been shown to be the most important risk factor for the development of

asthma worldwide.¹ The two species of house dust mites that have been shown to be important in Thailand are *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*.² Complex protein allergens produced from mites can be grouped into at least 11 important distinctive categories.³ It has been previously estimated that specific IgE against group 1 mite allergens accounts for over 85% of total IgE among these mite-sensitive individuals.⁴ Levels of group 1 mite allergens (Derp1 and Derf1) in homes of asthmatics have been shown to correlate well with the severity of their symptoms. From the three International Workshops on allergy to house dust mites held within the past 10 years, arbitrary cut-off levels for group 1 mite allergens have been proposed. A level of 2 µg/g dust or over has been found to be associated with the development of mite sensitization and a level of 10 µg/g dust or over is the level associated with the development of asthmatic attacks.¹ Hence, monitoring of group 1 mite allergen levels is extremely important in the management of asthma and in the prevention of mite sensitization among atopic prone patients. Such measurement could aid in the following: (i) identifying those who are at risk for the development of asthmatic and allergic symptoms; (ii) implementing adequate and effective environmental control measures to eliminate house dust mites; and (iii) assuring patient's compliance in adhering with strict environmental control measures. Such environmental control measures, largely ignored by most Thai physicians and patients alike, have been clearly shown to be some of the most important treatment measures leading to a successful outcome of the therapy for asthma and the other allergic diseases. The most widely used assay for group 1 mite allergen determination is the two-site monoclonal antibody based

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enzyme-linked immunoassay (mAb-ELISA),⁵ which is not applicable for clinical use because it requires a long time to perform. The availability of a more rapid method for the estimation of the total or specific allergen content from house dust mites would therefore be desirable. Furthermore, if made possible in a simple manner, such a test could be performed in clinical situations, which would be extremely useful in everyday management of allergic patients. The immunoassay for proteins blotted on a support of nitrocellulose membrane has been developed by Towbin and Gordon⁶ and further development of this procedure for direct application of the protein to the nitrocellulose and combining it with biosynthetic labelling of antibody provides a direct measurement of antigen-antibody reaction.⁶ The dot-blot assay has been successfully applied to many fields for the diagnosis of various diseases,⁷⁻⁹ but it still has not been applied for the detection of house dust mite allergen. Thus, the aim of the present study is to apply this dot blot, a rapid and simple assay for the detection of species-specific *Dermatophagoides* group 1 allergen contents in house dust, and to assess its reliability by comparing it with the standard method, two-site mAb-ELISA.

METHODS

Specimens

Two hundred house dust samples, collected from homes of volunteers, were used in the study. The samples were 10% (w/v) sieved dust in phosphate-buffered saline (PBS), pH 7.2 (100 mg sieved dust in 1 mL PBS).

These dust samples were extracted following the method of Chapman *et al.*¹⁰ Extracted samples were then centrifuged at 500 g at 4°C for 20 min with clear supernatants separated and kept frozen at -20°C until the time of allergen analysis.

Detection of group 1 mite allergens using the standard two-site monoclonal antibody based ELISA method

Detection of Derp1 or Derf1 was performed as described by Luczynska *et al.*⁵ In brief, monoclonal antibodies to common group 1 mite allergens (4C1) and to species-specific allergens (5A8 for Derp1 and 6A8 for Derf1) were purchased from Indoor Biotechnology (Chester, UK). Twofold dilutions of dust extracts from 1:10 to 1:80 w/v in 1% bovine serum albumin (BSA)-PBS-Tween (PBS-T) were assayed and group 1 allergens were

determined by interpolating onto standard curves constructed with dilutions of purified Derp1 and Derf1. Group 1 mite allergen content in dust samples was expressed as sums of Derp1 and Derf1 in µg/g dust.

Detection of group 1 mite allergens using a dot-blot assay

Optimization of the developed rapid enzymatic technique

The optimal dilutions of the reagents and house dust extract were determined by checkerboard titration.

Preparation of a dot-blot strip

Monoclonal antibody (20 µL) to each species-specific allergen (5A8 for Derp1 and 6A8 for Derf1, each with a dilution of 1:500) was blotted separately onto the nitrocellulose paper using the Bio-Rad dot-blot apparatus. After it was dried, the blotted membrane was blocked with 1% BSA in PBS-T for 20 min. Then the paper was cut into strips, each with four dot positions designated by four windows on a plastic strip. The first and second dots were blotted with 50 µL of positive controls at concentrations of 10 µg/g dust for Derp1 and 2 µg/g dust for Derf1. The positive controls were prepared from spent mite cultures of *D. pteronyssinus* and *D. farinae*, which had been standardized against the Derp1 and Derf1 standards (93/03 and 93/02) from Indoor Biotechnology. The third and fourth dot positions were used as test dots for Derp1 and Derf1 allergens, respectively. The prepared strips were kept at -20°C until used.

Performance of the dot-blot assay

The procedure for the dot-blot assay is briefly described. The two tested dots were immersed in 0.5 mL dust extracts for 10 min and then the strip was washed in PBS-T for 10 s. The strip was then immersed in mAb solution to group 1 allergen (4C1) at a dilution of 1:200 for 10 min prior to washing in PBS-T for another 10 s. Streptavidin-horseradish peroxidase (HRP) conjugate was then applied to the strip for 10 min and washed with PBS-T. Finally, the strip was placed in a substrate solution (2-6 dichlorophenol and 2% H₂O₂) for 2 min before rinsing in running tap water. The developed color on the strip was then visually read by comparing with the colors of the two controls.

Table 1 Comparison of semiquantitative results between a dot-blot assay and gold standard mAb-ELISA for Derp1 detection

mAb-ELISA for Derp1 ($\mu\text{g/g}$ house dust)	Rapid test for Derp1 ($\mu\text{g/g}$ house dust)			Total
	< 2	2–9.9	> 10	
< 2	70	4	–	74
2–9.9	6	38	17	61
> 10	–	–	65	65
Total	76	42	82	200

Comparison of semiquantitative results between a dot-blot assay and gold standard two-site monoclonal antibody based enzyme-linked immunosorbent assay (mAb-ELISA) for Derp1 detection in 200 house dust samples.

Table 2 Comparison of semiquantitative results between a dot-blot assay and gold standard mAb-ELISA for Derf1 detection

mAb-ELISA for Derf1 ($\mu\text{g/g}$ house dust)	Rapid test for Derf1 ($\mu\text{g/g}$ house dust)			Total
	< 2	2–9.9	> 10	
< 2	77	10	–	87
2–9.9	3	22	13	38
> 10	–	2	73	75
Total	80	34	86	200

Comparison of semiquantitative results between a dot-blot assay and gold standard two-site monoclonal antibody based enzyme-linked immunosorbent assay (mAb-ELISA) for Derf1 detection in 200 house dust samples.

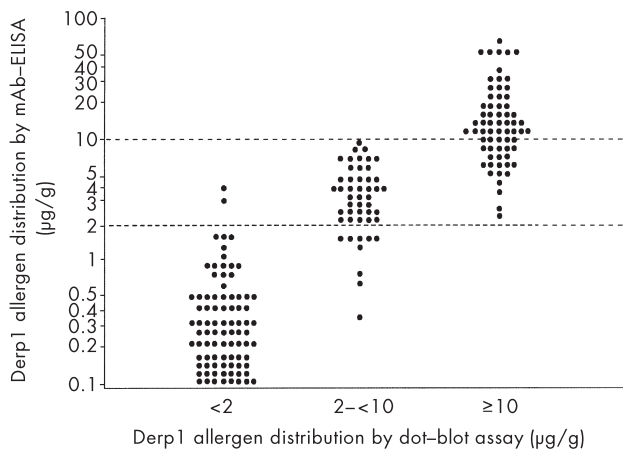


Fig. 1 Comparison of the distribution of Derp1 allergens measured by dot-blot assay and with the standard monoclonal antibody enzyme-linked immunosorbent assay (mAb-ELISA) in 200 house dust samples. Each dot represents a single measurement of each sample.

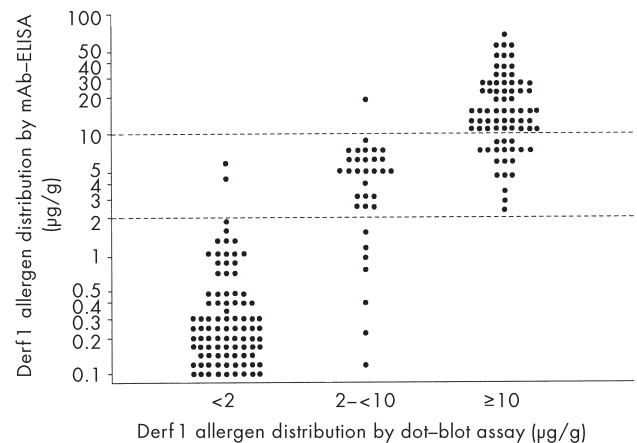


Fig. 2 Comparison of the distribution of Derf1 allergens measured by dot-blot assay and with the standard monoclonal antibody enzyme-linked immunosorbent assay (mAb-ELISA) in 200 house dust samples. Each dot represents a single measurement of each sample.

RESULTS

All 200 house dust samples processed were analyzed both by mAb-ELISA and by a dot-blot assay. The results of the semiquantitative assays of Derp1 and Derf1 as determined by two assays are shown comparatively in Tables 1 and 2. The correlation between the two analyses was good, with correlation coefficients of 0.892 ($P < 0.01$) for Derp1 and 0.872 for Derf1, respectively (Spearman's

rank correlation). The semiquantitative results of the dot-blot assay were plotted with absolute values from the mAb-ELISA assay in Figs 1 and 2 for Derp1 and Derf1 for further clarification. The sensitivity, specificity and accuracy for Derp1 were 94.4%, 76.9% and 86.5%; whereas the sensitivity, specificity and accuracy for Derf1 were 95%, 77% and 86%, respectively.

DISCUSSION

The results from the present study demonstrated that the dot-blot assay can be used as a species-specific assay for the measurement of *D. pteronyssinus* and *D. farinae* group 1 mite allergen contents in house dust. The assay was simple to perform. It required a short performing time and the result could be read visually. The amount of allergen in dust samples could be estimated semiquantitatively by comparing with the color of the controls. When compared with the results of the standard assay, good correlations were demonstrated.

We could not determine the exact amount of total group 1 allergen in house dust samples, due to the semi-quantitative nature of the dot-blot assay. Nevertheless, with a level of less than 2 µg/g dust, either no color developed or very faint color developed and thus quantity was easy to estimate. Any sample that showed a level of less than 2 µg/g dust for Derp1 and/or Derf1 would be an indication that the group 1 allergen level in that sample was sufficiently low. The level of > 2 µg/g dust for Derp1 and Derf1 would be a useful indicator as well, because an amount of allergen from 2 µg/g dust or higher should be regarded as a risk factor for sensitization and development of asthma. This would mean that the allergic level and the risk of development of acute asthma in that sensitive patient was high. Moreover, from the study of Malainual *et al.*,² an epidemiology survey of house dust fauna in Thailand, *D. pteronyssinus* was found to be threefold more common than *D. farinae* (60% vs 24%). In our studied samples, very few homes showed a high level of both species. Mostly either Derp1 or Derf1 were predominant, which made the estimation of group 1 allergen easier.

Some overestimation was observed in levels of > 2 µg/g dust, because the strip used in the assay was prepared from nitrocellulose, which has high binding and absorption capacity.^{6,11} Some non-specific adsorption may occur. Because the purpose of the test was to monitor Derp1 and Derf1 allergens in home of atopic patients, overestimation was considered preferable to underestimation.

Although this dot-blot assay could not be used for determining the exact amount of total group 1 allergens in house dust, it should be used as a useful and accessible tool to identify rapid high or low mite allergen levels in house dust samples and it could be used to indicate the predominant species of major house dust mite allergen in the homes of asthmatic patients.

Despite its speed and simplicity, and no requirement for sophisticated equipment, the test required 30 min to accomplish. Moreover, the cost of the test is expensive, due to the cost of the commercial mAb. Furthermore, the dust extraction was a long process. An improved method for extraction and detection with costs cheaper than the currently reported test is greatly needed. Tests that are simple enough for patients to perform at home would be even more desirable.

In conclusion, the developed dot-blot assay may possibly be used in hospitals and allergy clinics as an additional tool to identify rapidly high or low house dust mite group 1 mite allergen levels. Such a test will be useful in monitoring the effects of avoidance measures as a tool for future investigation.

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