



House dust mite trapping kit

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Abstract

Examination of dust collected from the homes of asthmatic patients showed there were significant numbers of house dust mites (HDM). The allergens generated from HDM are considered to be among the risk factors for asthma development. HDM can be identified in mattresses, carpets, quilts and pillows. HDM are about 0.2 x 0.3 mm in size, so they are only clearly visible under microscopic magnification. Therefore, it is important to develop a trapping kit to capture and stain them. We devised a trapping kit that is comprised of an adhesive pad and a staining agent. The adhesive pad is made of cotton cloth and is coated with glue, which can attract and trap HDM to prevent them from further spreading. The staining agent contains nanogold-coupled monoclonal antibodies, which can react with HDM through binding with group 2 allergens on the body surface of HDM. After staining, both *Dermatophagoides pteronyssinus* (Dp) and *Tyrophagus putrescentiae* (Tp) become visible to the naked eye. Our study showed that the HDM trapping pad can attract and trap HDM. The staining agent makes them visible so they can be counted. This kit can help us to identify the predominant HDM infestation area in the household environment. By identifying the infestation area, we can reduce HDM exposure and prevent the development of allergic disease.

Keywords

Dermatophagoides pteronyssinus; *Tyrophagus putrescentiae*; mite trapping kit

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Introduction

Domestic mites are prevalent in tropical and subtropical regions worldwide. Two groups of domestic mites, house dust mites (HDM) and storage mites (SM), can be found in the household environment. At least four species of mites have been identified in Taiwan [1], including *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Df), the most common HDM, and *Blomia tropicalis* (Bt) and *Tyrophagus putrescentiae*, the most common SM.

HDMs are the most common indoor allergens, and sensitization to mite allergen is an important risk factor for the development of allergic diseases including allergic rhinitis, bronchial asthma and atopic dermatitis [2-4]. More than 80% of allergic patients are allergic to Dp and Df as determined by skin test and specific IgE assay [5- 7]. Recently, *T. putrescentiae* has been reported to be a clinically important allergic component of house dust [8, 9] and more elderly COPD patients have been found to be sensitized to *T. putrescentiae* than *D. pteronyssinus* [10].

Our previous study showed that the group 2 allergens (Dp2) of dust mites are the major allergens in the dust mite extract [11]. More than 80% of allergic asthmatic patients tested positive for Dp2. Most group 2 allergens of dust mites are cross-reactive and have been reported to cause cross-reactions between Dp, Df and Tp [12]. Therefore, these 3 species of dust mite can be identified simultaneously with Dp2 monoclonal antibody.

Since dust mites are nearly invisible to the naked eye, the most common method used to identify them is to vacuum up house dust and count the mites under a microscope. The purpose of this study was to make a trapping kit to capture HDM in the household environment, then stain them using Dp2 monoclonal antibody, and count them.

Material and method

C1 monoclonal antibody preparation

Monoclonal antibodies (C1 MoAb) against Dp 2 were prepared as previously described [11,13]. Briefly, spleen cells obtained from BALB/c mice immunized with rDp 2 were fused with murine plasmacytoma NS-1 cells in the presence of polyethylene glycol (molecular weight 1, 500 daltons; Merck, Hohenbrunn, Germany). Antibody-producing hybrid cells were screened using an enzyme-linked immunosorbent assay (ELISA) and recombinant GSTDp 2 and GST alone. Briefly, hybridomas-producing MoAbs were expanded and purified by protein G agarose (Millipore, Temecula, CA) according to the manufacturers' instructions. The Dp 2 specificity of the MoAbs was determined by ELISA with rDp 2.

Colloidal gold -MoAb conjugation

Colloidal gold (Nano gold-40, 25~35 nm, Evernew) was purchased from Yu-shing Biotech (Taipei, Taiwan). The pH of the colloidal gold solution was adjusted to 8.2 by using 1% potassium carbonate (wt/vol), then C1 MoAb (75ul, 1 mg/ml) was added to 10 ml of colloidal gold solution. The mixture was stirred for 5 min, then added to 1 ml of 10% (wt/vol) BSA to block excess reactivity of the gold colloid, and stirred for an additional 5 min at room temperature. After incubation for 30 min, the mixture solution was centrifuged at 10,000g for 30 min to remove unconjugated protein molecules. The resulting conjugate pellets were re-suspended in 1 mL of PBS solution (containing 1% BSA) and stored at 4 °C for later use.

Trapping pad staining

The adhesive trapping pad with captured mites (Dp/Tp) was placed in the colloidal gold – C1 MoAb-conjugated solution and incubated for 30 min at room temperature. Then the adhesive trapping pad was removed from the colloidal gold – C1 MoAb-conjugated solution and the stained HDMs were counted with the naked eye.

Results

When the adhesive trapping pads with captured Dp with/without C1 MoAb staining were examined with the naked eye (A & B) and then under a microscope (a & b), the mites could be observed more clearly on the pads stained with C1 MoAb (B). When the trapping pads with captured Tp with/without C1 MoAb staining were examined with the naked eye (C & D) and then under a microscope (c & d), the results were similar to those observed with captured Dp.

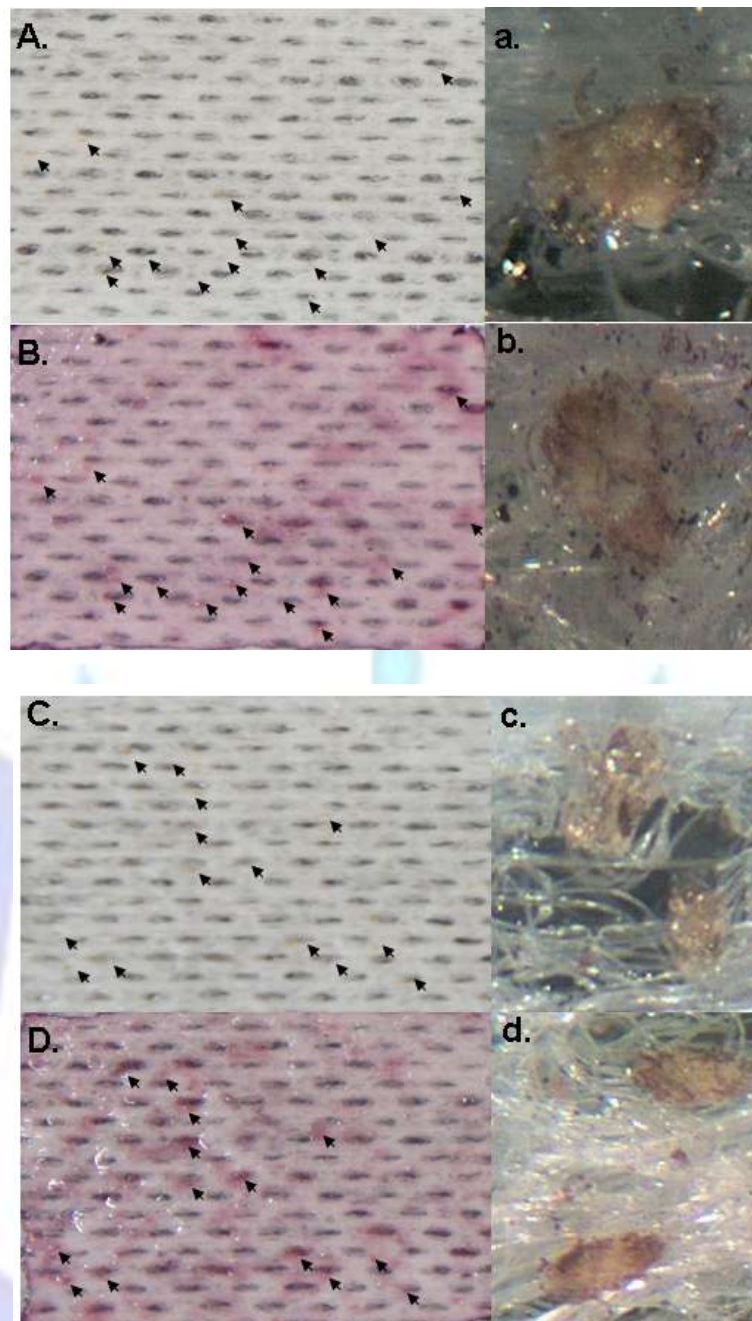


Fig 1: Detection of HDM using the adhesive trapping pad with immunocolloidal gold staining.

(A, a) Adhesive trapping pad with *D. pteronyssinus* but without staining. (B, b) Adhesive trapping pad with *D. pteronyssinus* stained with C1 MoAb. (C, c) Adhesive trapping pad with *T. putrescentiae* but without staining. (D, d). Adhesive trapping pad with *T. putrescentiae* stained with C1 MoAb. Mites are indicated with arrows (↑).

Discussion

In this study, we trapped dust mites in an adhesive trapping pad and stained them with Dp2 MoAb. Both Dp and Tp were stained using the same MoAb. Although we could not differentiate between Dp and Tp without microscopic magnification, both species of mite could be identified and enumerated with the naked eye.

Our previous reports showed that Dp2 is the major allergen of Dp and it causes the cross-reactivity between Dp and Tp [14]. The IgE binding epitope of Dp can be inhibited by crude extract of Tp and vice versa. Therefore, both Dp and Tp can be detected with Dp2 MoAb.

Other reports have shown that allergens of Dp2 and Df2 are cross-reactive [12], and their molecular structure and sequence homology are around 90%. Therefore, the binding epitopes of Dp2 MoAb are similar and can be used to identify both Dp2 and Df2.

Since the presence of HDM is closely related to the development of airway allergy, it is important to identify the predominant infestation areas of HDM in the home environment so they can be trapped and removed. In our study, we detected the presence of dust mites, but we could not confirm the species of dust mite without microscopic magnification. It is important to identify all species of mite for diagnosis and treatment. However, this goal can not be achieved until all species of specific antigen components have been identified and monoclonal antibodies have been



produced.

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