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Letter to the Editor

A dot-blot and immuno-blot assay for the detection of mosquito saliva specific IgE in mosquito bite allergic subjects

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Allergic reactions to mosquito bites are common problems worldwide. The reactions are often cutaneous and difficult to distinguish from non-specific histaminic phenomena. Moreover, the presentation might be similar to be a potential cutaneous sign of mantle cell lymphoma or would be characterized in some cases of natural killer cell lymphocytosis associated with Epstein-Barr virus infection [1]. Therefore, a reliable laboratory test for mosquito bite allergy is required.

This study is the first report of a dot-blot assay using purified saliva allergens of *Culex quinquefasciatus*. The potential clinical relevance of the test as an *in vitro* diagnostic tool for mosquito bite allergy was evaluated and compared to the previously established immuno-blot assay [2].

Forty nine patients with history of mosquito bite allergy and skin lesions which clinically suggested mosquito bite reactions (19 with immediate reactions, 10 with immediate and delayed reactions and 20 with delayed reactions) were included in this study. A wheal or flare lesion occurred within several minutes and peaked at twenty minutes after an exposure indicates an immediate reaction while an indurated pruritic papule developed within a few hours, peaked at 24–36 h and diminished over several days or weeks indicates a delayed response [3]. Negative controls consist of 11 subjects with a history of other insect bite allergies and 24 healthy non-allergic subjects with no history of allergy who had normal IgE levels (0–0.1 $\mu\text{g/ml}$) [4] at the time of blood collection. For total IgE detection, 29 patients with history of mosquito bite had elevated total IgE while 6 out of 11 patients with history of other insect bite allergy were found high total IgE level. There was no significant difference between diagnosis of mosquito bite allergy and total IgE (χ^2 , $P = 1.0$).

Performing the dot-blot assay, salivary proteins were collected from females *C. quinquefasciatus* using the Boorman method [5] and separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Three allergens with molecular weight of 35.5, 32.5 and 22.5 kDa previously identified as specific markers for diagnosis of mosquito bite allergy [2] were excised from the gel at the appropriate position with a clean razor blade and minced into smaller gel pieces, which were then transferred

into an eppendorf tube contained 400 μl of SDS elution buffer. After they had been mixed by vortexing for 10 min, the protein trapped in the gel pieces was eluted by centrifugation at $10,000 \times g$ for 20 min. Then, the eluant containing the purified allergens was recovered and stored. These purified saliva proteins were used as allergens in dot-blot assay. The optimal condition of the assay was determined by checker board titration. The purified saliva allergens measured as 0.5 $\mu\text{g/dot}$ and a serum dilution of 1:40 were used throughout the study. The purified saliva allergens were dotted on a nitrocellulose membrane (Bio-Rad Laboratories, USA) and blocked with blocking buffer. The strips were incubated for 60 min at room temperature with sera of the study subjects. After three washings, the strips were incubated for 60 min at room temperature with 1:1000 diluted rabbit anti-human IgE-Horse Radish Peroxidase-conjugated (Zymed). The strips were treated with a luminal substrate (Bio-Rad Laboratories, USA) and further exposed to an X-ray film. The immuno-blot assay revealed a mosquito specific IgE in sera of study subjects.

Fig. 1a revealed SDS-PAGE and Coomassie Brilliant Blue staining patterns of the mosquito saliva and the purified saliva allergens. The dot-blot reaction was shown in Fig. 1b. Fig. 2 showed the reaction of the immuno-blot assay. By dot-blot assay, the specific IgE was detected in 40 out of 49 (81.6%) mosquito bite allergic patients, in 4 out of 11 (36.36%) subjects diagnosed with other insect bite allergy and in 6 out of 24 (25%) healthy non allergic subjects. By another method, the immuno-blot assay, the specific IgE was detected in 39 out of 49 (79.6%) mosquito bite allergic patients, in 5 out of 11 (45.45%) patients with other insect bite allergy and none of the healthy non allergic subjects (0%). There was a moderate agreement (Kappa = 0.533; 95% CI = 0.347–0.713) between clinical diagnosis and the specific IgE detected by the dot-blot assay and a good agreement (Kappa = 0.640; 95% CI = 0.474–0.805) between clinical diagnosis and the specific IgE detected by the immuno-blot assay. The positive results found in some allergic patients with a history of other insect bite allergy indicates that the mosquito saliva may contain shared allergens with other insects. Cross-reactivity among mosquito saliva allergens, allergens from other biting insects and crustaceans has been reported [6,7]. Although purified saliva allergens were used in dot-blot technique, false positive rate was detected. It may be due to the high sensitivity of the assay. In addition, a serum component deriving from the presence of up to 10^9 -fold excess of other immunoglobulin might present some weak cross-reactivity [8]. There was also no correlation between the type of skin reactions and specific IgE to *C. quinquefasciatus* (χ^2 , $P = 0.45$).

Recently, the UniCap test, using mosquito whole body extract and a mosquito saliva-capture ELISA were developed [9]. Mosquito whole-body extracts contain various extraneous proteins that might interfere to the diagnostic tests [10]. Regardless, ELISA is a time consuming method and required laboratory equipments such as ELISA washer and ELISA reader. Dot-blot assay is simple to

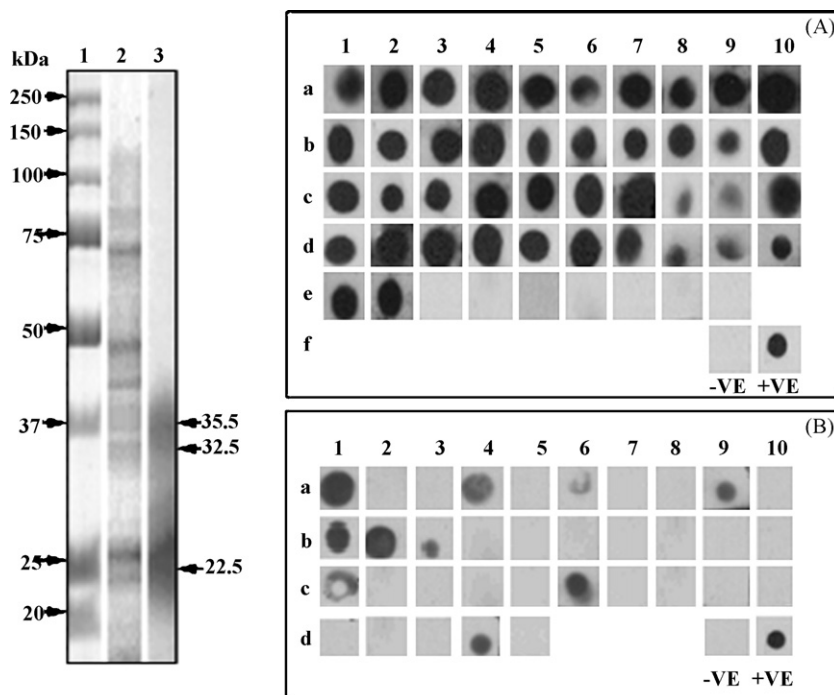


Fig. 1. (a) Protein profiles of *C. quinquefasciatus* mosquito saliva. Lane 1: standard molecular weight marker (kDa); lane 2: mosquito saliva; lane 3: purified saliva allergens. (b) Detection of *C. quinquefasciatus* mosquito saliva specific IgE by the dot-blot assay. A: mosquito bite allergic subjects, B: control subjects.

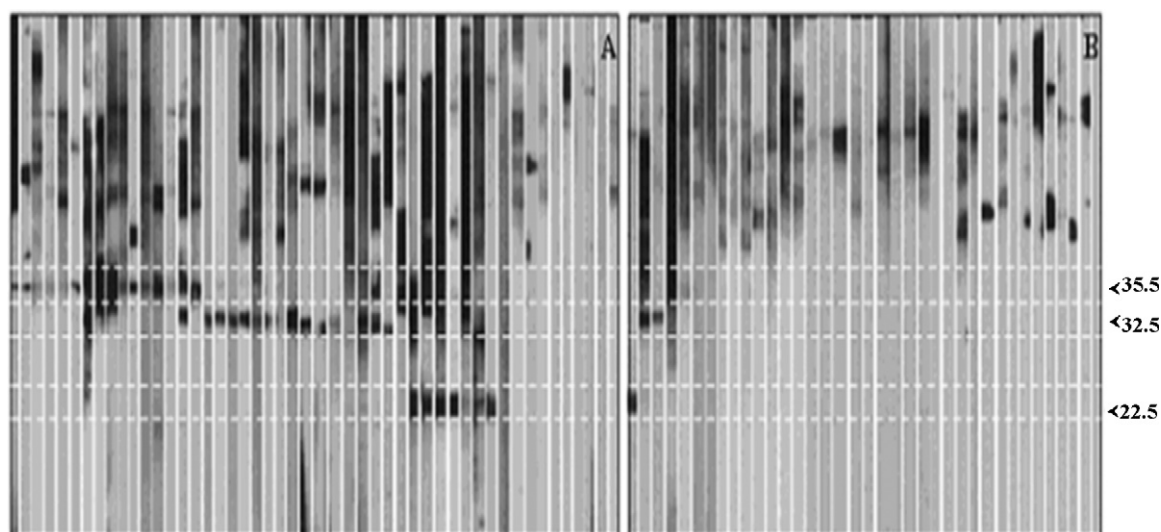


Fig. 2. Detection of *C. quinquefasciatus* mosquito saliva specific IgE in mosquito bite allergic subjects by immuno-blot assay. A: mosquito bite allergic patients, B: control subjects. (The specific allergens in kDa as markers for diagnosis of mosquito bite allergy are indicated by right arrows.)

perform and cheap since only a small amount of serum and reagents are required. A screening picture of specific IgE responses to mosquito saliva could be obtained by the dot-blot assay. Moreover, it could be useful for epidemiological study of mosquito bite allergy in a population. On the other hand, the immuno-blot assay provides a unique opportunity for analyzing responsive patterns of multiple allergens. Due to its high specificity, it can be a confirmatory test for the presence of the specific IgE in mosquito bite allergic subjects.

In conclusion, the developed assays have potential as a supportive diagnostic method of choice for the clinical diagnosis of mosquito bite allergy.

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Sirichit Wongkamchai*

Premjit Vaiyavatjamai

Darawan Wanachiwanawin

*Department of Parasitology, Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok 10700, Thailand*

Wanee Wisuthsarewong
*Department of Pediatrics,
Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok 10700, Thailand*

Kanokvalai Kulthanan
*Department of Dermatology,
Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok 10700, Thailand*

Patama Eakpo
*Department of Immunology,
Faculty of Medicine Siriraj Hospital,
Mahidol University, Bangkok 10700, Thailand*

*Corresponding author. Tel.: + 66 24196468;
fax: + 66 24112084
E-mail address: siswk@mahidol.ac.th
(S. Wongkamchai)

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