



Letters

Detection of American cockroach allergens as environmental markers using rapid competitive lateral flow tests



Sensitization to cockroach allergens is associated not only with the development of allergic diseases, but also with increasing severity and mortality of asthma. Additionally, the levels of cockroach allergens in settled household dust are associated with airway sensitization and asthma morbidity,^{1,2} making allergen avoidance a crucial step in the management of allergies.³ However, patients are usually unaware of the degree of cockroach infestation in their homes. Previous surveys of house dust samples found that 20% to 62% of the homes tested had detectable levels of cockroach allergens, despite there being no obvious evidence of an infestation.^{4,5}

The American cockroach (*Periplaneta americana*) is the predominant species present in tropical and subtropical regions and is the second leading source of inhalant allergens in Taiwan.⁶ Previous studies have identified 12 major allergen components from the American cockroach designated as Per a 1 to Per a 12.^{7,8} Sensitization to Per a 2 correlates to more severe airway allergic inflammation compared with the other allergen components, suggesting that the allergen components of the American cockroach have different allergenic potentials.⁷ Recent research by the authors found that Per a 2 and Per a 10 can be used as markers of long-term environmental cockroach control, whereas Per a 9 is a marker of recent control in patients' houses.⁹ The aim of the current study was to develop a simple and rapid detection kit to help patients monitor the effectiveness of environmental cockroach allergen control at home.

The Institutional Review Board of Taichung Veterans General Hospital approved the study protocol (Approval No: CE13089A-2). Anti-Per a 2 and anti-Per a 9 polyclonal antibodies were generated by immunizing young adult New Zealand white rabbits using recombinant Per a 2 and Per a 9 proteins expressed in *Escherichia coli* vectors pET30 and pQE30. Colloidal gold conjugated antibodies were used as both the detector and capture antibodies on the testing strips to detect Per a 2 and Per a 9 cockroach allergen components in the environment. The lateral flow strips were prepared by Bio Smart Co. Ltd. (Taiwan), as shown on the left panel of Figure 1. Rabbit anti-Per a 2 or anti-Per a 9 polyclonal antibodies were conjugated to colloidal gold and sprayed onto a glass fiber pad at 10 $\mu\text{L}/\text{cm}$. E-rPer a 2 or E-rPer a 9 proteins were micro-sprayed onto a nitrocellulose membrane at 1 $\mu\text{L}/\text{cm}$ at the test line position on the completed strip. Protein A in phosphate-buffered saline was micro-sprayed onto the same nitrocellulose membrane at the control line position. The assembly was cut into 4-mm-wide strips, housed in aluminum foil bags and stored at room temperature until

their use. To demonstrate the competition test format, a PBS–Tween 20 buffer containing the target allergen was applied to the sample pad, which was subsequently migrated to the conjugate pad of the strip. At the conjugate pad, the target allergen was captured by the immobilized labeled antibodies, which resulted in the formation of gold antibody/specific allergen complexes. These complexes subsequently reached the test line because of capillary action. To test the designed format, a specific recombinant protein was sprayed at the test line position. As the analyte and recombinant protein compete the binding sites of the gold antibody, only left-over or unreacted antibodies were captured at the test line. Therefore, less color at the test line indicates the higher degree of target allergen level.

The sensitivity of the test strips was determined by testing a series of diluted recombinant proteins as known standards. The limit of detection with the naked eye was set at the amount of rPer a 2 or rPer a 9 producing a clearly visible difference in the intensity of the test strip color. The signal color on the test line changed from strong to weak and then disappeared completely at 0.312 to 0.625 $\mu\text{g}/\text{mL}$ for Per a 2, as shown on the right panel of Figure 1 and 1.25–2.5 $\mu\text{g}/\text{mL}$ for Per a 9 (data not shown). We therefore set the cutoff of the prototype strips at 0.312 $\mu\text{g}/\text{mL}$ and 1.25 $\mu\text{g}/\text{mL}$ for Per a 2 and Per a 9, respectively. Each test takes 10 minutes and can be evaluated by the naked eye.

To validate that the detection limits of the strips are of clinical relevance, we performed skin prick tests (SPT) on 5 cockroach-allergic patients. A mixture of cockroach allergens (30 $\mu\text{g}/\text{mL}$) was prepared in PBS containing 50% glycerol; they were then used for skin testing with serial 2-fold dilutions. Histamine 1 mg/mL and 50% PBS–glycerol were used as the positive and negative controls, respectively. All SPT results were read 20 minutes after the placement. The grading of the skin test results was as described previously.¹⁰ Dose 1 (30 $\mu\text{g}/\text{mL}$) and dose 2 (15 $\mu\text{g}/\text{mL}$) of the cockroach allergens elicited wheal and flare reactions in all 5 subjects. Dose 6 (0.9375 $\mu\text{g}/\text{mL}$) did not elicit any skin reactions in the patients (data not shown). The data indicated that the lowest clinically relevant threshold of cockroach sensitization was approximately 1.0 $\mu\text{g}/\text{mL}$.

Although the lateral flow assay method was approximately 300-fold less sensitive than the competitive enzyme-linked immunosorbent assay method, the benefits of the method are that rapid and simple results can be obtained without the need for special equipment and skills. To the best of our knowledge, this is the first report of detecting cockroach allergen components using one single polyclonal antibody conjugated with colloidal gold as both the detector and capture antibody. The authors propose that this idea can be extended to any allergen with available polyclonal antibodies with good specificity. The developed competitive lateral flow assay has potential because it is low in cost, and fast and

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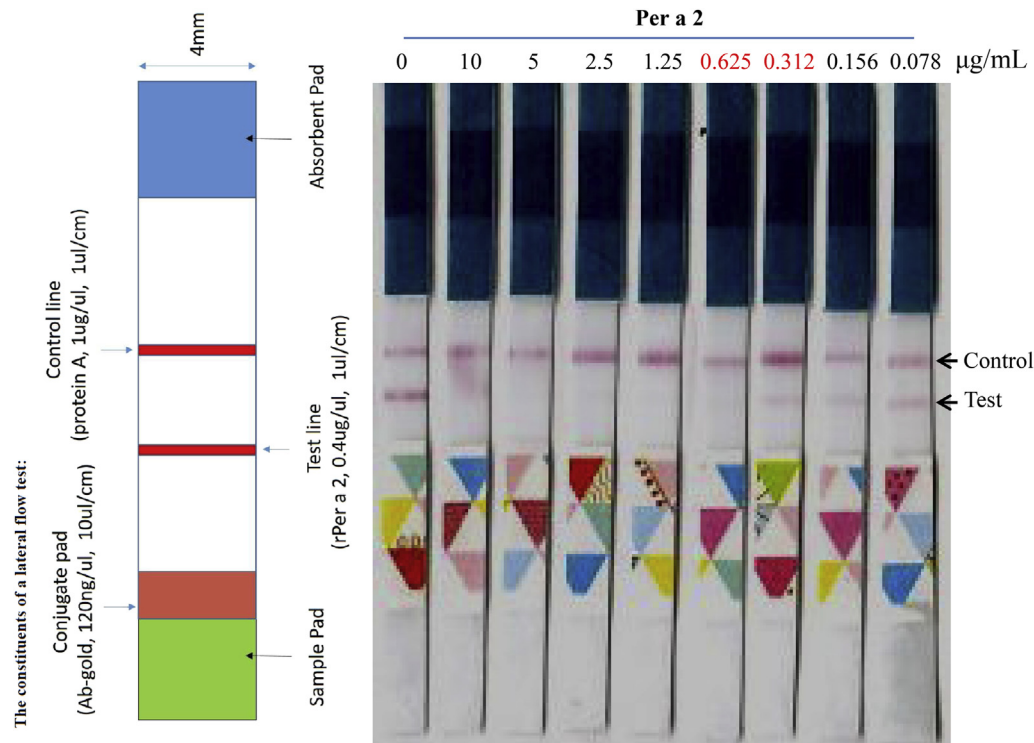


Figure 1. Strip design (left panel) and representative results of test strips detecting Per a 2 in house dust samples (right panel).

simple to use. Using Per a 2, which is a marker allergen for long-term American cockroach control and Per a 9, which is a marker allergen for recent cockroach control, also may give patients and their families more precise information regarding the cockroach levels in their environment, and the effect of any control measures.

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Anaphylaxis due to macrogol in a laxative solution with a positive basophil activation test



Polyethylene glycol (otherwise known as macrogol or PEG) belongs to a family of synthetic nonionic hydrophilic polymers achieved as

condensation products of glycols with ethylene oxide. Different types of macrogol exist, each with a specific molecular weight (from 200 to 20,000 g/mol) and length, which gives them specific pharmacological qualities (eg, viscosity, density). As a consequence, PEG has wide applications as an excipient, solvent, dispersing, and

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