

# Comparison between specific IgE levels and skin prick test results of local and imported American cockroach, dog, cat, dust mites and mold allergen extracts

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## Abstract

**Background:** Skin prick test (SPT) and specific IgE (sIgE) are approved for evaluation of allergen sensitization. Local allergen extracts are less expensive and more available but need to be standardized.

**Objective:** To compare SPT results of local and imported allergen extracts and sIgE levels in response to the American cockroach, dog, cat, *Dermatophagoides pteronyssinus* (*Dp*), *Dermatophagoides farinae* (*Df*) and *Cladosporium* spp. allergens.

**Method:** This prospective, randomized, double-blind, self-controlled study was performed in respiratory allergic volunteers. Each subject was pricked with local and imported allergen extracts and sIgE levels were measured.

**Results:** The agreement between SPT results from each local and imported allergen extract was statistically significant, and the level of agreement for dog allergens was very good ( $\kappa > 0.8$ ). All patients with a positive SPT in response to imported *Dp* allergen extract had positive SPT in response to local *Dp* allergen extract. Mean wheal diameter of each allergen in both groups showed significant correlation with sIgE levels. The correlation coefficient (CC) for cat allergens showed a very good-to-excellent relationship ( $CC > 0.75$ ). When compared with sIgE levels, SPT results for imported and local allergen extracts showed comparable sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio (LR)<sup>+</sup> and LR<sup>-</sup>.

**Conclusions:** When the SPT results are compared between local and imported allergen extracts, all were found to have significant agreement, with very good agreement for cat allergens. When the SPT results are compared with specific IgE levels, both local and imported allergen extract provided comparable validity.

**Keywords:** American cockroach, cat, *Cladosporium* spp., dog, dust mites, skin prick test, specific IgE

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## Introduction

The International Study of Asthma and Allergy in Childhood (ISAAC)<sup>1</sup> showed that the prevalence of respiratory allergy has increased all over the world, including Thailand. In Thailand, allergy is classified as having a moderately-high prevalence. The prevalence of childhood asthma has increased from 4% to 15% over the past 10 years.<sup>2</sup> In Bangkok, the prevalence of allergic rhinitis is 32.6% in children aged 6-7 years and 43.4% in children aged 13-14 years.<sup>3</sup> In adults, the prevalence of asthma is 10%<sup>4</sup> and allergic rhinitis is 26%.<sup>5</sup>

A previous study showed that asthma and allergic rhinitis were usually caused by aeroallergens such as those from dust mites [*Dermatophagoides pteronyssinus* (*Dp*) and *Dermatophagoides farinae* (*Df*)], the American cockroach, cats, dogs, grasses and mold (especially *Cladosporium* spp.).<sup>6</sup> In Thai patients aged 10–59 years, the prevalence of sensitization is as follows: dust mites, 76–79%; American cockroach, 60%; cat, 29%; dog, 28%; mold, 26%; Johnson grass, 21%; Bermuda grass, 17%; and weeds, 16%.<sup>7</sup>

In the management of allergic diseases, medications are useful to treat symptoms and allergen avoidance is important in the prevention of symptoms and further sensitization. Finding the causative allergens is necessary for allergen avoidance. The type of sensitization also affects the recovery rate of allergic patients. A previous 8-year study in allergic rhinitis patients showed that the recovery rate of patients who were allergic to mites, animal furs and grasses was 38, 19 and 12%, respectively.<sup>8</sup>

The skin prick test (SPT) is an approved and validated tool for evaluating allergic sensitization in atopic diseases. The SPT is simple, accurate, safe and less expensive compared with specific IgE (sIgE) measurement.<sup>9</sup> The incidence of adverse reactions related to SPTs is usually not more than 0.04 %<sup>10</sup> and the reaction is usually mild. Severe side effects caused by SPTs are rare.<sup>11</sup> There have been no fatal cases reported that were related to the aeroallergen SPT.<sup>9-12</sup> A previous study in 5,879 Thai patients showed no adverse systemic reactions from 82,306 SPTs.<sup>13</sup>

Imported allergen extracts are expensive and they are not always available, and the pollen species used to prepare the imported allergen extracts may not be the same as those present in the local area. Local allergen extracts of *Dp*, *Df*, American cockroach, cat, dog, grasses and *Cladosporium* spp. were prepared by Mahidol University and the technique has been transferred to the private sector to prepare the extract for commercial use. After *in vitro* evaluation of the antigen levels, *in vivo* evaluation of the potency of the extract is necessary. Besides comparing the SPT results between local and imported allergen extracts, serum sIgE levels will give more information on the *in vivo* potency of the local extracts.

The aim of this study was to compare SPT results between local allergen extracts, imported allergen extracts and sIgE levels for American cockroach, dog, cat, dust mites and mold allergens.

## Methods

This double-blind, self-controlled study was approved by the Institutional Ethics Committee of Siriraj Hospital, Mahidol University. The study was performed in volunteers with respiratory allergy. Patients with acute asthma exacerbation, severe skin diseases, chronic diseases (such as autoimmune disease, immune deficiency and cancer) or who were pregnant were excluded. The use of antihistamines, systemic corticosteroids ( $\geq 20$  mg/day) and topical corticosteroids was discontinued for at least 7 days before testing. Written informed consent and informed assent were obtained from the patients (and parents if the child was <18 years old) before enrollment in the study. The study was registered with ClinicalTrials.gov (NCT02561390). Local allergen extracts (Greater Pharma Manufacturing Co., Ltd. Bangkok, Thailand) were prepared from *Dermatophagoides pteronyssinus* (*Dp*), *Dermatophagoides farinae* (*Df*), *Periplaneta americana* (American cockroach), *Canis familiaris* (dog), *Felis catus* or *Felis domesticus* (domestic cat), and *Cladosporium cladosporioides* extracts. The processes of preparation were sonification and dialysis, and the protein content, protein profiles, endotoxin level and sterility were verified.

## Skin prick test

A blood lancet (Vitrex® steel, Vitrex Medical A/S, Herlev, Denmark) was used for the SPT in this study. SPT was performed on the upper back of patients by one experienced technician in a room equipped with resuscitation equipment. Each patient was pricked with local allergen extracts and imported allergen extracts (ALK Laboratories, Port Washington, NY) from the American cockroach, cat, dog, *Cladosporium* spp., *Dp* and *Df*. Histamine dihydrochloride (10 mg base/ml) and sterile 50% v/v glycerine were used as positive and negative controls, respectively. Wheals and flares induced by the allergens were recorded 10 minutes after histamine testing and 15 minutes after allergen extract testing. The mean wheal diameter (MWD, the longest diameter + the perpendicular diameter / 2) was calculated. Reactions at least 3 mm larger than the negative control were considered positive. All patients in the study were observed for adverse reactions for at least 30 minutes after SPT. If MWD was more than 10 mm, the patient received less-sedating antihistamine immediately and the observation time was increased to 2 hours or until MWD decreased. Serum sIgE levels in response to the American cockroach, dog, cat, dust mites and mold antigens were measured using ImmunoCAP (UniCAP 250, Instrument Pharmacia Diagnostic AB, Uppsala, Sweden). The sIgE results were considered to be positive when the level was  $>0.35$  kUA/L.

## Statistical analysis

The data were analyzed using SPSS software version 18 (SPSS Inc., Chicago, IL, USA). Characteristics data were presented using the median (range) for continuous data or the number and percentage for categorical data. Agreement between the SPT results of local and imported allergen extracts was evaluated using Kappa and intraclass correlation (ICC).<sup>14</sup> Correlation between sIgE and MWD of allergen extracts was evaluated using Spearman's rho correlation. A p-value of  $<0.05$  was considered statistically significant. The negative predictive value (NPV), positive predictive value (PPV), sensitivity, specificity, likelihood ratio (LR)+ and LR- were used to evaluate the validity of the results of SPT from local and imported allergen extracts and sIgE, using sIgE as a reference standard.

## Results

Eighty-four respiratory allergy patients (mean age, 22.1 years; range, 10.5–60.5 years; male:female, 50:50) were enrolled into the study. Demographic data are shown in **Table 1**. Allergic rhinitis was diagnosed in 81 patients (96.4%) followed by asthma (28.6%) and food allergy (14.3%). Oral antihistamine and intranasal corticosteroids were used by 63.1% and 50% of patients, respectively. Two patients (2.4%) were cigarette smokers and 8 (9.5%) drank alcohol.

A comparison of SPT results between local and imported allergen extracts is shown in **Table 2**. Agreement between local and imported allergen extracts was statistically significant ( $p < 0.05$ ). The level of agreement between local and imported allergen extracts of American cockroach, cat, *Cladosporium* spp. and *Df* were good. The level of agreement between dog

local and imported allergen extracts was very good (kappa >0.8). The agreement for *Dp* was 100% but kappa was unable to be evaluated because all 84 patients had a positive SPT in response to local *Dp* allergen extract. There was a statistically significant correlation for MWD of each allergen in both groups. ICCs for the American cockroach, cat and *Df* showed a very good-to-excellent degree of relationship (ICC >0.75), while the ICC of dog, *Cladosporium* spp. and *Dp* showed a moderate-to-good degree of relationship (ICC 0.5–0.75).

Correlation between the results of SPT for local and imported allergen extracts and serum sIgE is shown in Table 3. The agreement between sIgE level and MWD of allergen extract varied among allergens. The level of agreement between cat sIgE and local and imported allergen extracts was good (kappa 0.695 and 0.629, respectively). The level of agreement between dog sIgE and local and imported allergen extracts was fair (kappa 0.491 and 0.563, respectively). Little or no agreement was found between other allergens. A significant correlation was found when MWDs of each allergen in both groups were compared with sIgE levels (*Cladosporium* spp., p<0.05, other allergens p<0.0001). The correlation coefficient (CC) of cat allergen showed a very good-to-excellent degree of relationship (CC >0.75) while the correlation coefficient of dog, *Dp*, *Df* and commercial American cockroach allergens showed a moderate-to-good degree of relationship (CC 0.5–0.75).

**Table 1. Demographic data**

Characteristics	N (%)
Sex: male	42 (50.0)
Age (yr): mean (SD, min-max)	22.1 (10.5, 10.6-60.5)
<b>Diagnosis*</b>	
Asthma	24 (28.6)
Allergic rhinitis	81 (96.4)
Atopic dermatitis	8 (9.5)
Urticaria	7 (8.3)
Food allergy	12 (14.3)
Drug allergy	6 (7.1)
<b>Medication**</b>	
Antihistamine	53 (63.1)
Intranasal corticosteroid	42 (50.0)
Leukotriene antagonist	19 (22.6)
Smoker	2 (2.4)
Drink alcohol	8 (9.5)

Note: N=84

\*One patient may have more than one diagnosis

\*\*One patient may take more than one medication

**Table 2. Comparison of SPT results between local and imported allergen extracts**

	Patients with positive SPT (n)		Mean wheal diameter mm (maximum)		kappa	ICC*
	Imported	Local	Imported	Local		
American cockroach	58 (69.0)	57 (67.9)	3 (17)	3 (15)	0.642	0.859
Cat	45 (53.6)	48 (57.1)	10 (30)	5 (20)	0.784	0.869
Dog	37 (44.0)	36 (42.9)	3 (14)	3 (23)	0.879	0.694
<i>Cladosporium</i> spp.	20 (23.8)	19 (22.6)	3 (6)	3 (15)	0.700	0.729
<i>D. pteronyssinus</i>	83 (98.8)	84 (100.0)	10 (37)	10 (60)	NA**	0.668
<i>D. farinae</i>	82 (97.6)	83 (98.8)	3 (50)	10 (25)	0.661	0.756

\*ICC, intraclass correlation coefficient

\*\*NA, not applicable

**Table 3. Correlation between SPT results for local and imported allergen extracts and specific IgE**

Allergens	Patients with >0.35 kAU/L		kappa*		Correlation coefficient**	
	n	Maximum level (kAU/L)	Imported	Local	Imported	Local
American cockroach	45	14.8	0.388	0.365	0.518	0.488
Cat	34	100	0.695	0.629	0.809	0.810
Dog	36	50.7	0.491	0.563	0.645	0.646
<i>Cladosporium</i> spp.	3	5.87	0.185	0.198	0.230	0.261
<i>D. pteronyssinus</i>	80	100	-0.022	0.00	0.683	0.671
<i>D. farinae</i>	79	100	0.168	0.205	0.628	0.682

\* kappa between positive sIgE and SPT; \*\* Correlation coefficient between level of sIgE and mean wheal diameter of SPT

**Table 4. Validity of local and imported allergen extracts when compared with specific IgE**

Allergen extracts		Sensitivity %	Specificity %	PPV* %	NPV** %	LR***+ %	LR-	Disease prevalence
American cockroach	Imported	86.67	61.28	67.24	76.92	1.78	0.26	53.57
	Local	84.44	51.28	66.67	74.07	1.73	0.30	53.57
Cat	Imported	97.06	76.00	73.33	97.44	4.04	0.04	40.48
	Local	97.06	70.00	68.75	97.22	3.24	0.04	40.48
Dog	Imported	72.22	77.08	70.27	78.72	3.15	0.36	42.86
	Local	75.00	81.24	75.00	81.25	4.00	0.31	42.86
<i>Cladosporium</i> spp.	Imported	75.00	78.75	15.00	98.44	3.53	0.32	4.76
	Local	75.00	80.00	15.79	98.48	3.75	0.31	4.76
<i>D. pteronyssinus</i>	Imported	98.67	0	89.29	0	0.99	-	89.29
	Local	100.00	0	89.29	0	1	-	89.29
<i>D. farinae</i>	Imported	98.68	12.50	91.46	50.00	1.13	0.11	90.48
	Local	100.00	12.50	91.57	100	1.14	0	90.48

\* PPV, positive predictive value; \*\* NPV, negative predictive value; \*\*\* LR, likelihood ratio

Validity of local and imported allergen extracts compared with specific IgE is shown in **Table 4**. Sensitivity, specificity, PPV, NPV, LR+ and LR- of commercial and local extracts were similar for American cockroach, cat, dog, *Cladosporium* spp., *Dp* and *Df* allergens. Sensitivity of all allergen extracts was good (72-100%), but specificity was good only for cat, dog and *Cladosporium* spp. allergens (70-81.24%). The PPV of *Dp* and *Df* was very good (89% and 91%, respectively) while that of the American cockroach, cat and dog was 66-75%. The NPV of *Cladosporium* spp. and cat allergens was very good (97.22-98.48%). Disease prevalence (prevalence of disease when the test is positive) for *Dp* and *Df* positive SPT results was very good (89.29% and 90.48%, respectively).

No serious adverse reactions related to either local or imported allergen extract SPTs were found.

## Discussion

Our study showed that nearly all of the patients had a positive SPT in response to both local and imported *Dp* and *Df* allergen extracts. The SPT results of local and imported allergen extracts, expressed as the MWD, showed good-to-very good agreement and ICC between them and also showed a moderate to excellent degree of relationship. The level of agreement was very good for dog and good for American cockroach, cat, *Cladosporium* spp. and *Df* allergens. ICCs for the American cockroach, cat and *Df* were very good-to-excellent while the ICC for dog, *Cladosporium* spp. and *Dp* was moderate-to-good.

This good correlation and agreement might be because local and imported allergen extracts are prepared from the same animal and fungal species: *Dp* and *Df* are used to prepare mite allergen extracts, *P. americana* for American cockroach, *C. familiaris* for dog, *F. catus* (or *F. domesticus*) for cat and *C. cladosporioides* for *Cladosporium* spp.

This study shows that the agreement between the sIgE level and MWD of allergen extract varied among allergens. The level of agreement between cat sIgE and local and imported allergen extracts was good but that of other allergens was

fair-to-non-existent. CC for cat allergen also showed a very good-to-excellent degree of relationship. Our results are consistent with those of another study in 120 patients with cat allergy, which showed that SPT and radio allergosorbent test (RAST) values had an excellent efficiency in diagnosing cat allergy.<sup>15</sup> A study in 90 allergic patients in Malaysia also showed that sIgE had better sensitivity but poorer specificity, low PPV and good NPV compared with SPT for all the allergens tested, and there was a significant positive correlation between the wheal and flare diameter for the SPT and sIgE results.<sup>16</sup> Our study also showed significant correlation when MWD of each allergen in both groups was compared with sIgE levels. Another study in 349 allergic rhinitis patients also showed that serum sIgE and SPT were relevant in allergic rhinitis patients who were sensitive to dust mites.<sup>17</sup> However, the positive rates of the two methods were different and they could not substitute for each other.<sup>17</sup> The level of serum sIgE and positive degree of SPT did not reflect the degree of symptoms in allergic rhinitis.<sup>17</sup> SPT was also shown to be more appropriate for the detection of clinically relevant sensitivity to cockroaches than sIgE.<sup>18</sup> The agreement between the SPT and the sIgE results in asthmatic children was weak for *P. americana* ( $\kappa = 0.17$ ).<sup>18</sup> A case-control study in children showed that only a patient with high specific IgE serum levels and a positive SPT to a cockroach species should be truly classified as hypersensitive to *B. germanica* and/or *P. americana*.<sup>19</sup>

In our study, *Cladosporium* spp. had less significant correlation than other allergen extracts. This might be because the number of positive tests was too small. Only 20/80 patients had positive SPT and 3/80 patients had positive sIgE (>0.35 kUA/L) to *Cladosporium* spp. A previous study in 69 patients showed that SPT provided more positive reactions than the *in vitro* tests in diagnosing allergic reaction to *D. pteronyssinus*, timothy grass pollen, cat epithelium/dander, *Cladosporium* spp. and *Alternaria*.<sup>20</sup> For the allergens tested, the Pharmacia ImmunoCAP is more sensitive than RAST, and it identifies more positive tests and approximates more closely to the SPT.<sup>20</sup>

Another study showed that in the group of rhinitis patients, skin tests and conjunctival challenge were more sensitive than sIgE determination.<sup>21</sup> In asthmatic patients, the most sensitive techniques were nasal and conjunctival challenges, followed by prick and intradermal skin tests and serum specific IgE determination.<sup>21</sup> When rhinitis and asthma were considered together, the most sensitive test was conjunctival challenge, followed by the skin-prick and intradermal tests. All tests had the same specificity.<sup>21</sup> Skin tests are easy to perform, less expensive and non-traumatic for the patient. The previous study also showed that the skin test was sufficiently specific and sensitive to diagnose *Alternaria* hypersensitivity.<sup>21</sup>

A study in 100 patients with chronic rhinitis and 40 control patients showed good agreement and correlation between SPT with individual specific IgE test and multiple allergens tested simultaneously for the majority of the tested allergens in patients with chronic rhinitis.<sup>22</sup> Comparing the two *in vitro* test results, the individual sIgE test agreed with the SPT better than the multiple simultaneous allergen test.<sup>22</sup> The agreement between specific IgE and the SPT increased as total IgE levels rose.<sup>18</sup> All determinations of cockroach-sIgE were positive for total IgE levels greater than 2500 kU/L, even among asymptomatic patients.<sup>18</sup>

An explanation for the lack of correlation between SPT and sIgE results for *Cladosporium* spp. might be the difference in *Cladosporium* spp. used in the tests. The allergen extract is prepared from *C. cladosporioides* but the species tested using ImmunoCAP was *Cladosporium herbarum*.

When compared with specific IgE, each commercial and local extracts showed comparable sensitivity, specificity, PPV, NPV, LR+ and LR-, and sensitivity of all allergen extracts was good (72-100%). If SPT results for *Dp* and *Df* allergen were positive, the sIgE was likely to be positive (PPV 98-100%). If SPT results for *Cladosporium* spp. and cat allergen were negative, the sIgE was likely to be negative (NPV 98% and 97%, respectively).

SPT is less expensive than sIgE and the test takes less time to complete. However, the patients should not take antihistamines and should not have a serious skin or allergic reaction at the time the test is administered. There were no serious adverse reactions from the SPT for either local or imported allergen extracts; only mild local reactions were found. This is consistent with a previous study that showed no serious adverse reactions from an aeroallergen SPT.<sup>23</sup> However, a generalized reaction was reported in an infant, less than 6 months old, that was administered a fresh-food SPT.<sup>23</sup>

The present study showed that SPT can be used as a screening method to evaluate IgE-mediated allergic reactions and to determine the causative allergens of patients' allergic reactions. Positive SPT results from both local and imported allergen extracts can accurately predict *Dp* and *Df* sensitization, and negative results can rule out *Cladosporium* spp. and cat sensitization. The local allergen extracts were comparable to the imported extracts for determining sensitization to those allergens. The locally-prepared allergen extracts also are less expensive and easier to obtain, so they can substitute for the imported extracts.

## Conclusion

When the SPT results are compared between local and imported allergen extracts (American cockroach, dog, cat, *Dp*, *Df* and *Cladosporium* spp.), all had significant agreement, and cat allergens had very good agreement. When the SPT results in response to specific IgE levels are compared, both local and imported allergen extracts provided comparable validity.

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