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Comparison of specific IgE detection by immunoblotting and fluorescence enzyme assay with *in vivo* skin prick test

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Abstract

Background: Diagnostic tools to identify allergens that cause allergic symptom is important part in the care of allergic patients. Detection of causative allergen can be performed by *in vivo* skin prick test (SPT) or *in vitro* tests for detection serum specific immunoglobulin E (sIgE). The common methods used are fluorescent enzyme assay and immunoblotting assay.

Objective: We aim to evaluate performance of the two sIgE determination systems, immunoblotting assay (Euroline) and fluorescent enzyme assay (ImmunoCAP) in comparison with SPT.

Methods: Two hundred and two participants with allergic diseases were enrolled. Sensitization to common allergens was identified using skin prick test and serum specific IgE assays with Euroline and ImmunoCAP. Both systems provide the result in the same unit and the same cut-off value (0.35 kUA/L). The specific IgE levels of 4 aeroallergens, 6 food allergens and 3 food allergen components were analyzed to evaluate the performance of both sIgE assays with SPT.

Results: When compared with the result of SPT, ImmunoCAP has 63.9-93.2% agreement and Euroline has 68.4-86.2% agreement for allergen detection. Both sIgE assays have significant correlation in measuring sIgE of almost all allergens ($r = 0.626$-0.901, $p < 0.001$) except for dog. For food allergen components, both sIgE tests have outstanding correlation and agreement ($r = 0.816$-0.952, $p < 0.001$; agreement = 87.0-92.9%, respectively). The receiver-operating characteristic curve analysis indicated slight discrepancy of both sIgE assays.

Conclusions: Both sIgE determination systems demonstrate fair to good performance when compared to SPT depending on type of allergens. The two sIgE determination systems had favorable correlation and agreement.

Key words: Fluorescence enzyme assay, Immunoblotting assay, Specific IgE assay, Skin prick test, allergen.

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Introduction

Allergic diseases have been increasing around the world. To identify causative allergens is the important part of diagnosis and treatment. Detection of causative allergen by skin prick test (SPT) is commonly used because of the high sensitivity,[1,2] rapidity and inexpensiveness. Allergen-specific immunoglobulin E (sIgE) blood assay offers the alternative tool to identify the causative allergen. The advantages of sIgE blood assay are the variety of assays including quantitative or semi-quantitative system, lack of medication/ skin condition interference and no risk of severe allergic reaction occurred during the assay. In general, good agreement has been identified between skin test and sIgE blood assay for the most potent aeroallergens including trees, grasses, weeds, pets (eg, cat and dog), and dust mite allergens.[1,3-6] Nevertheless, some studies revealed the discrepancy between SPT and sIgE blood assay.[4,6,7] Variability in skin test and sIgE results may due to several factors such as patients' factors, method of SPT, quality and stability of the allergen extracts, the biological reagents used in the laboratory assay and the methods of laboratory assay.[1]

Recently, various commercial analytical system for sIgE detection have been developed for example radioimmunoas -say, chemiluminescence, enzyme-immunoassay, fluorescence/ enzyme-immunoassay and immunoblotting assay. Only few sIgE determination methods have been approved by United State Food and Drug Administration (US FDA) such as ImmunoCAP (Phadia, Uppsala, Sweden), Turbo RAST (Agilent Technologies Co, Santa Clara, California) and Immulite (Siemens Medical Solutions Diagnostics, Tarrytown, New York). Despite these systems provided the result in the same unit, the result is not always equivalent.[3,8-10]

Euroline (Euroimmun, Medizinische Labordiagnostika, AG, Germany), an IgE determination system using immunoblotting assay has some advantages such as the requirement of only small amount of sera, no automate machine requirement, minimal hands-on time, appropriate for screening multiple allergen and cost-saving. While ImmunoCAP (Phadia, Uppsala, Sweden), a fluorescence/enzyme-immunoassay, has some advantages such as high through-put with a lot of number of sample load per day but it has some disadvantage such as automate machine requirement and expensive. Nowadays, there has been no study to evaluate the performance of sIgE detection systems using immunoblotting assay compared to the widely-used *in vivo* SPT. Our study aimed to evaluate the performance of an immunoblot (Euroline) and a fluorescence/enzyme-immunoassay (ImmunoCAP) for the detection and quantitation of sIgE in comparison with *in vivo* SPT.

Methods

Material and Methods

The study has been carried out at out-patient clinic, King Chulalongkorn Hostpital during June 2012 to October 2015. Two hundred and two subjects with allergic diseases: asthma, allergic rhinitis, atopic dermatitis, food allergy and anaphylaxis, age from 1 month to 60 years, were enrolled. Subjects with uncontrolled cardiovascular and respiratory diseases other than allergic asthma, pregnant or lactating women, or patient who received allergen immunotherapy, systemic immunosuppressive drugs, beta-blocking agents were excluded. All subjects were free from anti-histamine for at least 7 days prior to the procedure. The study was approved by Ethics Committee. Informed consent was obtained.

Skin prick test method

SPT was performed by the prick technique using a metal lancet (Vitrex® steel, Vitrex Medical A/S, Herlev, Denmark) on the volar side of the forearm of the subjects. All extracts were supplied by ALK-Abello (Hørsholm, Denmark). Histamine Dihydrochloride in 50% glycerin (1 mg/mL) and 50% glycerosaline were served as the positive and negative control accordingly. Commercially available allergens were used as the following; house dust mites extract (*Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides faerinae* (Der f)); cat hair extract; dog epithelial extract; shrimp extract; crab extract; egg white extract; egg yolk extract; and wheat extract. Skin prick test was read at fifteen minutes for allergens and ten minutes for positive control. A positive skin response was defined as the presence of a wheal with a mean wheal diameter of at least 3 millimeters (mm) greater than that elicited by the negative control accompanied by erythema. The mean of the largest and midpoint orthogonal diameters was designated as the mean wheal diameter (MWD).

Serum specific IgE assays

Serum samples were collected from the participants at the same visit as SPT, then divided into 2 aliquots and store at -20°C until use. The level of sIgE was quantified using fluorescence enzyme immunoassay (PharmaciaCAP, Pharmacia) and immunoblotting assay (Euroline, Medizinische Labordiagnostika AG) according to the manufacturer's instructions. SIgE for Der p, Der f, cat, dog, egg white, egg yolk, cow's milk, wheat, shrimp and food allergen components (gluten/omega-5 gliadin, ovalbumin, ovomucoid) were measured. Level of specific IgE ≥ 0.35 kUA/L (Class 1) was considered to be positive result for both assays.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of Euroline and ImmunoCAP were analyzed according to SPT for each allergen. The correlation of sIgE level and SPT as well as between Euroline and ImmunoCAP were analyzed using Spearman's rho correlation. A receiver-operating characteristic (ROC) curve analysis was performed to verify the performance of Euroline and ImmunoCAP with SPT. Agreement between sIgE and SPT was assessed by kappa statistics.

Results

Study population

There were 202 participants, age from 1 month to 60 years (mean ± SD = 10.57 ± 14.13), in the study. Fifty-seven were male and 145 were female. One hundred and fifty nine participants age ≤ 17 years. The underlying allergic diseases were asthma (n = 9, 4.46%), allergic rhinitis and allergic rhino-conjunctivitis (n = 58, 28.71%), atopic dermatitis (n = 28, 13.86%), food allergy (n = 86, 42.57%), urticaria (n = 18, 8.91%) and anaphylaxis (n = 3, 1.49%).

Performance of Immunoblotting assay (Euroline) and Fluorescence enzyme assay (ImmunoCAP) compared with skin prick test

The performance of two *vitro* sIgE assays for each allergen was compared to the skin prick test as shown in **Table 1**. Using the SPT cut-off mean wheal diameter of at least 3 millimeters and sIgE cut-off value (≥ 0.35 kUA/L) (class I), overall agreement of ImmunoCAP and SPT were 63.9-93.2% while the agreement of Euroline and SPT was 68.4-86.2%. When compared to SPT, both Euroline and ImmunoCAP displayed high sensitivity, specificity, PPV and agreement for Der p, Der f and crab sIgE detection. However, Euroline had lower sensitivity but higher specificity than ImmunoCAP for cat, dog and wheat sIgE detection (sensitivity 48.0%, 33.3%, 31.4 % and specificity 96.6%, 96.7%, 90.0%, respectively).

Table 1. Performance of ImmunoCAP and Euroline compared with SPT for each allergen

a) The analysis at SPT cut-off 3mm and class I sIgE

Allergen	System (n)	% of positive test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Agreement (%)	Kappa (95%CI.)
Der p	ImmunoCAP (88)	78.41	97.1	78.9	94.4	88.2	93.2	0.791 (0.631-0.951)
	Euroline (161)	56.52	82.4	88.6	90.4	79.5	85.1	0.701 (0.591-0.811)
Der f	ImmunoCAP (64)	68.75	97.7	80.0	91.5	94.1	92.2	0.81 (0.652-0.969)
	Euroline (87)	71.26	85.5	88.0	94.6	71.0	86.2	0.686 (0.524-0.848)
Cat	ImmunoCAP (26)	57.69	66.7	72.7	76.9	61.5	69.2	0.385 (0.034-0.735)
	Euroline (112)	22.32	48.0	96.6	80.0	86.6	85.7	0.52 (0.319-0.72)
Dog	ImmunoCAP (36)	16.67	100.0	66.7	37.5	100.0	72.2	0.4 (0.147-0.653)
	Euroline (100)	9.0	33.3	96.7	50.0	93.6	91.0	0.353 (0.026-0.681)
Egg white	ImmunoCAP (76)	61.84	83.0	55.2	75.0	66.7	72.4	0.395 (0.181-0.608)
	Euroline (106)	54.72	81.0	75.0	79.7	76.6	78.3	0.561 (0.403- 0.72)
Egg yolk	ImmunoCAP (31)	74.19	52.2	100.0	100.0	42.1	64.5	0.36 (0.127-0.594)
	Euroline (61)	59.02	61.1	92.0	91.7	62.2	73.8	0.495 (0.297- 0.692)
Cow's milk	ImmunoCAP (78)	39.74	77.4	68.1	61.5	82.1	71.8	0.436 (0.24-0.631)
	Euroline (113)	35.40	52.5	83.6	63.6	76.3	72.6	0.375 (0.196- 0.555)
Wheat	ImmunoCAP (72)	44.44	59.4	67.5	59.4	67.5	63.9	0.269 (0.045- 0.492)
	Euroline (95)	36.84	31.4	90.0	64.7	69.2	68.4	0.24 (0.052-0.428)
Shrimp	ImmunoCAP (72)	65.28	87.2	64.0	82.0	72.7	79.2	0.527 (0.319- 0.736)
	Euroline (154)	35.06	63.0	94.0	85.0	82.5	83.1	0.606 (0.472- 0.739)
Crab	ImmunoCAP (17)	47.06	87.5	66.7	70.0	85.7	76.5	0.534 (0.146-0.923)
	Euroline (19)	47.37	88.9	70.0	72.7	87.5	78.9	0.582 (0.227-0.938)

b) The analysis at SPT cut-off 5 mm and class III sIgE

Allergen	System (n)	% of positive test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Agreement (%)	Kappa (95%CI.)
Der p	ImmunoCAP (88)	71.59	85.7	96.0	98.2	72.7	88.6	0.75 (0.6-0.89)
	Euroline (161)	50.31	74.1	97.5	96.8	78.8	85.7	0.72 (0.61-0.82)
Der f	ImmunoCAP (64)	59.38	100	88.5	92.7	100	95.3	0.9 (0.79-1)
	Euroline (87)	60.92	84.9	91.2	93.8	79.5	87.4	0.74 (0.6-0.88)
Cat	ImmunoCAP (26)	34.62	22	100	100	70.8	73.1	0.27 (-0.04-(-0.59))
	Euroline (112)	14.29	68.8	99.0	91.7	95.0	94.6	0.76 (0.57-0.94)
Dog	ImmunoCAP (36)	11.11	100.0	96.9	80.0	100.0	97.2	0.87(0.63-1)
	Euroline (100)	5.0	60.0	100	100	97.9	98.0	0.74 (0.4-1)
Egg white	ImmunoCAP (76)	47.37	61.1	90.0	84.6	72.0	76.3	0.52 (0.33-0.71)
	Euroline (106)	44.34	63.8	91.5	85.7	76.1	79.2	0.57 (0.41- 0.72)
Egg yolk	ImmunoCAP (31)	61.29	15.8	100.0	100.0	42.9	48.4	0.13 (-0.02-0.27)
	Euroline (61)	49.18	33.3	93.5	83.3	59.2	63.9	0.27 (0.08- 0.47)
Cow's milk	ImmunoCAP (78)	24.36	68.4	91.5	72.2	90.0	85.9	0.61 (0.4-0.82)
	Euroline (113)	21.24	25	93.3	50.0	82.2	78.8	0.22 (0.01- 0.44)
Wheat	ImmunoCAP (72)	22.22	43.8	92.9	63.6	85.2	81.9	0.41 (0.15- 0.67)
	Euroline(95)	20.0	26.3	97.4	71.4	84.1	83.2	0.31 (0.07-0.55)
Shrimp	ImmunoCAP (72)	45.83	51.5	84.6	73.9	67.3	69.4	0.37 (0.16- 0.58)
	Euroline (154)	24.03	35.1	94.0	65.0	82.1	79.9	0.35 (0.17- 0.52)
Crab	ImmunoCAP (17)	35.29	66.7	81.8	66.7	81.8	76.5	0.49 (0.05-0.92)
	Euroline (19)	36.84	71.4	91.7	83.3	84.6	84.2	0.65 (0.29-1)

Der p, *Dermatophagoides pteronyssinus*; Der f, *Dermatophagoides farinae*
PPV, positive predictive value; NPV, negative predictive value
n, number of participants
The value of kappa index was interpreted according to the following scale: < 0: poor agreement, 0–0.2: slight agreement,0.21–0.40: fair agreement, 0.41–0.60: moderate agreement, 0.61–0.80: substantial agreement, 0.81–1.00: perfect agreement.

Table 2. The correlaton analysis between sIgE level (kU/L) measured by ImmunoCAP and Euroline and the result of SPT (diameter of wheal in mm.)

Allergen	ImmunoCAP and SPT			Euroline and SPT			ImmunoCAP and Euroline		
	Spearman's rho	p-value	n	Spearman's rho	p-value	n	Spearman's rho	p-value	n
Der p	0.788	< 0.001	88	0.840	< 0.001	161	0.843	< 0.001	107
Der f	0.852	< 0.001	64	0.755	< 0.001	87	0.813	< 0.001	68
Cat	0.640	< 0.001	26	0.574	< 0.001	112	0.626	< 0.001	38
Dog	0.579	< 0.001	36	0.454	< 0.001	100	0.428	0.002	52
Egg white	0.606	< 0.001	76	0.684	< 0.001	106	0.901	< 0.001	96
Egg yolk	0.534	0.002	31	0.614	< 0.001	61	0.779	< 0.001	33
Cow's milk	0.574	< 0.001	78	0.418	< 0.001	113	0.619	< 0.001	103
Wheat	0.390	0.001	72	0.395	< 0.001	95	0.686	< 0.001	96
Shrimp	0.623	< 0.001	72	0.664	< 0.001	154	0.707	< 0.001	101
Crab	0.573	0.016	17	0.667	0.002	19	0.735	< 0.001	44

Values presented as correlation. P-value corresponds to Spearman's rho correlation; Der p, *Dermatophagoides pteronyssinus*; Der f, *Dermatophagoides farinae;* n, number of participants; NA, not applicable

Correlation between specific IgE measurement using ImmunoCAP and Euroline with SPT

The correlation of both sIgE assays and SPT was analyzed. For most of the allergens, both Euroline and ImmunoCAP revealed good correlation with SPT (r = 0.395-0.840, p ≤ 0.002 and r = 0.390-0.852, p ≤ 0.016, respectively). Both sIgE assays have significant correlation for almost all allergens (r = 0.626-0.901, p < 0.001) except for dog. For food allergen components, both sIgE assays have good correlation and agreement (r = 0.816-0.952, p < 0.001; agreement = 87.0-92.9%, respectively) (**Table 2 and 3, Figure 1**).

Table 3. Agreement and correlation between Euroline and ImmunoCAP for food components

Food component (n)	Agreement	Kappa (95%CI.)	Spearman's rho	p-value
Gluten / Omega-5 Gliadin (23)	87.0%	0.725 (0.446-1)	0.816	< 0.001
Ovalbumin (28)	92.9%	0.757 (0.436-1)	0.883	< 0.001
Ovomucoid (28)	92.9%	0.851 (0.654-1)	0.952	< 0.001

n, number of participants

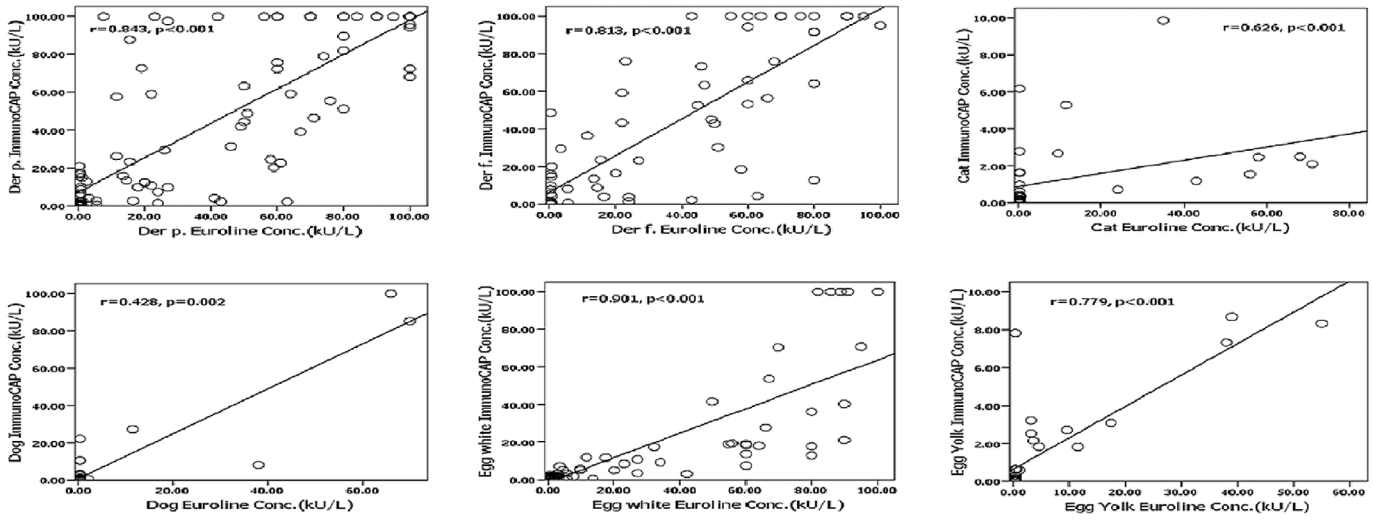

Figure 1. Scatterplots of correlation analysis of sIgE levels (kU/L) between Euroline and ImmunoCAP for 4 aeroallergens, 6 food allergens and 3 food components. Each dot represents the sIgE of one patient.

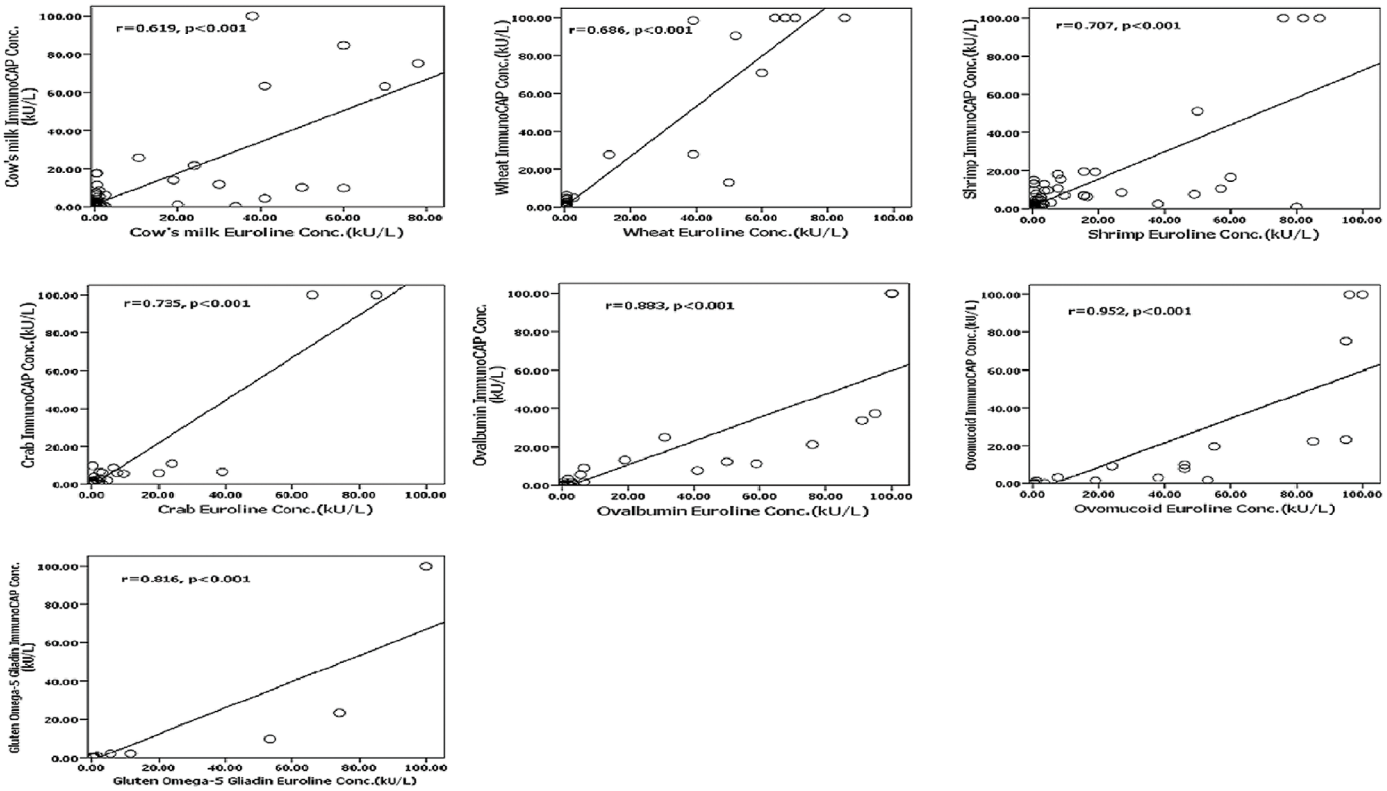

Figure 1. (Continued)

Table 4. ROC curve analysis for ImmunoCAP and Euroline base on the result of SPT

Allergen (n)	System	AUC (95%CI)	p-value	AccuMax$^{\Psi}$	Cutoff valve (kU/L)$^{\theta}$
Der p (79)	ImmunoCAP	0.996 (0.986-1.005)	< 0.001*	97.5	0.39
	Euroline	0.933 (0.875-0.990)	< 0.001*	89.9	0.41
Der f (52)	ImmunoCAP	0.996 (0.984-1.008)	< 0.001*	98.1	0.88
	Euroline	0.929 (0.860-0.998)	< 0.001*	90.4	2.1
Cat (26)	ImmunoCAP	0.745 (0.547-0.943)	0.036*	76.9	0.655
	Euroline	0.767 (0.583-0.950)	0.022*	73.1	0.525
Dog (36)	ImmunoCAP	0.978 (0.929-1.026)	< 0.001*	97.2	5.69
	Euroline	0.750 (0.484-1.016)	0.056	91.7	5.925
Egg white (74)	ImmunoCAP	0.839 (0.748-0.929)	< 0.001*	79.7	1.18
	Euroline	0.862 (0.779-0.945)	< 0.001*	79.7	1.6
Egg yolk (31)	ImmunoCAP	0.834 (0.693-0.976)	0.005*	80.6	0.07
	Euroline	0.731 (0.556-0.906)	0.055	64.5	0.475
Cow's milk (76)	ImmunoCAP	0.804 (0.701-0.907)	< 0.001*	81.6	2.34
	Euroline	0.700 (0.575-0.826)	0.003*	72.4	0.355
Wheat (69)	ImmunoCAP	0.638 (0.500-0.776)	0.051	68.1	1.395
	Euroline	0.618 (0.481-0.754)	0.096	65.2	0.355
Shrimp (69)	ImmunoCAP	0.834 (0.736-0.932)	< 0.001*	81.2	0.605
	Euroline	0.727 (0.605-0.849)	0.002*	69.6	0.585
Crab (17)	ImmunoCAP	0.889 (0.729-1.049)	0.007*	82.4	2.485
	Euroline	0.875 (0.691-1.059)	0.009*	88.2	1.5

n, number of participants; $^{\Psi}$, Maximum accuracy; $^{\theta}$, Cutoff value at maximum accuracy

ROC curve analysis of each allergen

The data of individuals whom had all 3 tests (SPT, Euroline and ImmunoCAP) performed was analyzed by ROC curve (**Table 4**). The sIgE level measured by Euroline and ImmunoCAP were plotted against the result of SPT. Area under the curve (AUC), maximum sensitivity, maximum specificity, index Q* (the maximum value that sensitivity and specificity can be mutual achieved) and the cutoff value were analyzed. Both Euroline and ImmunoCAP revealed comparable AUC. ImmunoCAP revealed slightly higher accuracy for most allergens except cat and egg white. Although, there were some differences of Q* cutoff value between the 2 systems, most of cutoff values of each allergen of the 2 systems were at the same class.

Discussion

The diagnosis of IgE-mediated allergy is based on clinical symptoms and the evidence of sensitization. SPT is the primary tool for allergist in detection of the causative allergen. It is convenient, rapid, no machine requirement and inexpensive. However, the result of SPT may depend on the performers and can also be affected by medications while *in vitro* sIgE determination can overcome the disadvantage of SPT.

Currently, a number of *in vitro* sIgE determination systems are available. Each of them uses different allergen sources and distinct IgE detection system. Thus, the sIgE results measured by one system might not be interchangeable with the others.[8] Our study evaluated serum sIgE level of common allergens measured by Euroline (Immunoblotting system) and ImmunoCAP (Fluorescence enzyme immune assay) in comparision with SPT.

When compared the two sIgE determination systems to SPT, our results revealed that both Euroline and ImmunoCAP provided good to fair performance depending on types of allergens. For house dust mites (Der p and Der f), the excellent concordance has been identified between SPT and both sIgE determination systems. For other allergens except crab, Euroline performed with lower sensitivity but higher specificity compared to ImmunoCAP. Euroline also provided slightly higher agreement with SPT than ImmunoCAP for egg white and crab allergens. The discordance between sIgE and SPT demonstrated in our study is in line with previous reports demonstrating some discordance between sIgE and SPT. The discordance rates varied depending on type of allergens and patients' factors.[7,11,12] Chauveau et al demonstrated the good agreement between SPT and sIgE measured with Allergy Screen Test Panel (Mediwiss Analytic, Moers,Germany) for house dust mites and a poor agreement for cat, dog, alternaria, and grass pollen.[13] de Vos et al studied the concordance between SPT for 7 common aeroallergens (grass pollen, ragweed pollen, dust mite, cockroach, mouse, cat, and dog) and sIgE testing using Immulite 2000 3gAllergy^{T} system (Siemens AG, Munich, Germany) in 40 atopic inner-city children aged 18 to 48 months. The study revealed a fair correlation for most allergens and no correlation between SPT grade and sIgE level for dog.[14] The discrepancies of the result of SPT and sIgE assays in our study can be explained by the differences in composition of allergens used in SPT and sIgE assays as well as patients' factors.

When the two sIgE determination systems were compared, there was a significant correlation of the sIgE levels for most allergens. The correlation was strong for Der p, Der f and egg white but weak for dog. Interestingly, both Euroline and ImmunoCAP have good concordance in detection of sIgE for food allergen components.

When compared Euroline with ImmunoCAP by ROC analysis, there were some marginal discrepancies between both systems. ImmunoCAP revealed slightly higher accuracy in detection of sIgE to majority of allergens used in this study except cat and egg white. The discrepancies between both systems could be due to the differences in assay technique as well as the composition and concentration of allergens.

It is important to emphasize that a positive serum sIgE or SPT indicates a sensitization to an allergen and is not equivalent to a clinical diagnosis. This limitation highlights the need for the clinician to use medical history together with knowledge of the test characteristics to select and interpret tests properly.

To the best of our knowledge, our study has been the first to evaluate performance of Euroline, the system using immunoblotting technique and another system using fluorescence enzyme-immunoassay (ImmunoCAP) with SPT. Based on SPT, Euroline shows comparable performance with ImmunoCAP for several common allergens. Furthermore, the levels of sIgE detected by Euroline significantly correlated with those measured by ImmunoCAP. Interestingly, there was good concordance between the two systems in detecting sIgE for food allergen components. Therefore, our data suggested that Euroline provided high accuracy for several common allergens and food components. Since system of Euroline has some advantages as prior mentioned, further study with more allergens and the correlation with clinical data should be performed.

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