

Allergen-specific IgE measurement with the IMMULITE 2000 system: Intermethod comparison of detection performance for allergen-specific IgE antibodies from Korean allergic patients

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ABSTRACT

Background: Intermethod comparison between IMMULITE 2000 chemiluminescent enzyme immunoassay (CLEIA) and the established CAP test for allergen-specific IgE (sIgE) has only been evaluated by a few studies. **Methods:** We performed such an interassay comparison using 283 Korean allergic patients with the following: asthma (18.4%), allergic rhinitis (18.4%), both asthma and allergic rhinitis (14.5%), atopic dermatitis (21.9%), and others (26.8%). We compared the sIgE detection performance of both systems for 10 major inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blattella germanica*, cat dander, dog hair, alder, birch, oak, ragweed, and mugwort) and four food allergens (egg white, cow milk, peanut, and shrimp).

Results: After 645 paired comparison tests, close association and significant correlation were observed between the results of both assays for most of these allergens ($r=0.525\text{--}0.979$, $p<0.05$, respectively), except for shrimp. Intermethod agreement based on sIgE detection was fair to good (74.1–100%, $\kappa=0.514\text{--}1.000$, $p<0.05$, respectively) for most allergens except for *B. germanica*, ragweed, and shrimp. Although both assays showed good accuracy in ROC curve analysis, some minor differences were noted.

Conclusions: IMMULITE 2000 CLEIA for sIgE detection showed fair to good intermethod correlation, association, agreement, and accuracy in comparison to the established CAP assay among Korean allergic patients. However, we should take into account the intermethod differences between both assays for clinical applications.

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1. Introduction

In vitro laboratory tests determining serum allergen-specific IgE (sIgE) antibodies have been routinely used in the clinical diagnosis of allergic diseases, such as asthma, allergic rhinoconjunctivitis, and atopic dermatitis [1,2]. Such *in vitro* procedures have been continuously developed and improved during the last 40 years and became one of the most important diagnostic tools for IgE-mediated allergic disorders [1,3].

Since the first description of the *in vitro* technique for allergen antibodies by Wide et al. in 1967 [4], the radioallergosorbent test (RAST) has been modified and advanced [3,5–7]. Improvements

continued and led to the development of the fluorescent enzyme immunoassay (FEIA) by major manufacturers [8–10]. About a 25-year-long period was required for this evolution from the advent of original RAST technique in 1974 [10]. These methods had striking advantages, including greater speed, higher binding capacity, non-radioisotope labels, and a continuous standardized scale (kU/L, according to WHO International Reference Preparation 75/502 for human IgE) [1,10].

Although there is no gold standard method for *in vitro* sIgE tests, the FEIA, UniCAP[®] system (Phadia, Uppsala, Sweden) has been accepted and validated as a quasi-standard, in consideration of its wide usage, analytical reliability, and its generally adequate (positive/negative response based) correspondence to skin test results [1,7,11,12]. However, the FEIA sIgE measuring technique has showed several limitations, including sample management, testing duration, laboratory integration/personnel requirements, and solid-phase allergen immobilization [1].

Recently, a new chemiluminescent enzyme immunoassay (CLEIA) for allergen-sIgE was developed [13,14]. These new technology systems have considerably shorter turnaround times (approximately 1 h), usage of chemiluminescence, liquid-phase allergens, monoclonal

Abbreviations: sIgE, allergen-specific IgE; CLEIA, chemiluminescent enzyme immunoassay; RAST, radioallergosorbent test; FEIA, fluorescent enzyme immunoassay; SPT, skin prick test; ROC curve, Receiver Operating Characteristic curve; AUC, area under the curve.

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Table 1
Demographic features of study population

Demographic features	Number
Enrolled patients	283
Male	165 (58.3%)
Female	118 (41.7%)
Age, y (mean $\pm$ SD), range	20.8 $\pm$ 18.9, 1–76
Area	Seoul, Korea
Ethnicity	Asian (Korean)
Disease entity	
BA without AR (%)	18.4
AR without BA (%)	18.4
BA and AR (%)	14.5
AD (%)	21.9
Urticaria and angioedema	6.8
Food allergy (%)	3.3
Anaphylaxis (%)	1.5
Others (%)	15.2
Intermethod comparison	
Tested allergens	14 species
Aeroallergens	10
Food allergens	4
Tested serum samples	645
Aeroallergens	483
Food allergens	162
Complete results set (SPT, UniCAP 100, and IMMULITE 2000)	169

BA: Asthma, AR: Allergic rhinitis, AD: Atopic dermatitis, SPT: skin prick test.

detection, and an actual zero calibrator for extended range with a lower detection limit (0.10 kU/L) [1,10].

In consideration of such advantages and the possibility of replacing the FEIA systems, there have been a few clinical studies looking at performance validation of the IMMULITE 2000 CLEIA compared to the established FEIA CAP test for allergic patients [1–3,10,13]. We evaluated the IMMULITE® 2000 CLEIA system (Siemens Healthcare Diagnostics, Deerfield, IL, USA; previously, DPC, LA, USA) by intermethod comparison with the UniCAP® 100 FEIA system (Phadia, Uppsala, Sweden) and skin prick test. Frequently identified aeroallergens and food allergens in Korea were selected for this study.

2. Materials and methods

2.1. Patients

Allergic patients ranging from infants to the elderly who were suspected of having IgE-mediated or related allergy to aeroallergens and/or food allergens were enrolled according to their clinical history and medical record review from June 2005 to May 2006. All of them were outpatients who underwent allergen-sIgE CAP test and/or skin prick test (SPT) of Allergy and Asthma Clinic, Severance Hospital, Yonsei University Health System. We obtained their own and/or guardian's written or verbal informed consents for collecting additional sera and using those samples in the intermethod comparison study of sIgE measurement. This study was approved by the Institutional Review Board of Yonsei University College of Medicine.

The inclusion criteria were as follows: age between 1 and 75 years; allergic patients suspected of having IgE-mediated or related allergy to aeroallergens and/or food allergens according to clinical history; no medication which could influence SPT results; and no grave comorbidities including cancer, infectious disease, autoimmune disease, or other immunologic disease. SPT results were also evaluated, if possible.

A total of 283 allergic patients (mean 20.8 $\pm$ 18.9 years (range: 1–76 years), 161 males, and 122 females) met these inclusion criteria and agreed to participate in this study. The enrolled patients' demographic features are shown in Table 1. Among this Korean allergic population, respiratory allergic diseases such as asthma and/or

allergic rhinitis were the most common disorders (51.3%), followed by atopic dermatitis (21.9%), urticaria with/without angioedema (6.8%), and food allergy (3.3%).

2.2. Serum samples and selection of allergens

Blood samples were drawn up to 4 mL from every subject among the above-mentioned 283 suspected allergic patients. Serum separation vacuum tubes (Vacuette®; Greiner Bio-One GmbH, Kremsmünster, Austria) containing blood samples were sent to the laboratory. After centrifugation at 3000 rpm for 5 min, each serum specimen was divided into several round-bottom tubes (5 mL BD Falcon™; BD Biosciences Discovery Labware, Bedford, MA, USA) and frozen at -28°C for short-term (less than 24 h) storage or -80°C for long-term (more than 24 h) storage until sIgE measurement. A total of 645 serum aliquots were obtained. We classified these serum samples into several groups according to the allergen species for which the patients underwent CAP and/or skin prick tests. All serum specimens were submitted to the laboratory as blind clinical samples.

Fourteen representative allergen sources were selected for intermethod comparison between the IMMULITE 2000 CLEIA and the CAP FEIA test. Those allergen species included 10 inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat dander, dog hair, *Blattella germanica*, alder, birch, oak, mugwort, and ragweed) and four food allergens (egg white, cow milk, peanut, and shrimp). We selected the most frequent and well known aeroallergens, such as mugwort pollen, which is known to be the most common sensitizer among weeds, followed by ragweed and Japanese hop in Korea [15]. Major food allergens were also selected based upon clinical importance [2].

2.3. Skin prick tests

Skin prick tests (SPT) were performed with commercially available allergen extracts (Prick-Test Solutions; Allergopharma, Reinbek, Germany). Histamine dihydrochloride (1.7 mg/mL) and physiologic saline served as controls (Prick-Test Solution No. 902 and 901; Allergopharma). All SPTs were performed on the back by fully trained and experienced personnel. Wheal sizes were measured as the mean of the two most extreme diameters 15 to 20 min after the prick. Reactions were considered positive if the wheal was larger than the negative control by 3 mm or more [16].

2.4. In vitro allergen-specific IgE measurement

Aliquoted serum samples without patient identifiers were submitted to the laboratories, each using a different allergen-sIgE assay system (UniCAP® 100; Phadia, Uppsala, Sweden, and IMMULITE® 2000; Siemens Health Diagnostics, Deerfield, IL, USA). Serum specimens were evaluated by both sIgE assay methods for the 14 above mentioned allergens (10 inhalant allergens and 4 food allergens). The UniCAP® 100 FEIA system was used according to the manufacturer's procedure manual for the intermethod comparison. The detection range was from 0.35 to 100 kU/L.

The IMMULITE® 2000 CLEIA system was also operated following the manufacturer's instruction. It uses an enzyme-enhanced chemiluminescent detection system and has been validated for a broad spectrum of assays; it also has a very low detection limit (0.10–100 kU/L) [1,3]. The chemiluminescent signal is generated by decomposition of destabilized dioxetane due to hydrolysis of a dioxetane-phosphate ester [3].

2.5. Intermethod comparison and statistical analysis

A total of 645 paired allergen-sIgE assays were done for intermethod comparison of the IMMULITE® 2000 and the UniCAP® 100 systems with 14 major allergens (Table 1). The results of allergen-sIgE measurement from the UniCAP® 100 and the IMMULITE® 2000 systems were analyzed to determine intermethod correlation, association, and agreement. Scatter plots, simple and Spearman correlation analysis, gamma analysis for association, and Cohen's kappa analysis for agreement were used.

The UniCAP® 100 was used as the comparison method in this analysis. Allergen-sIgE class systems of both assays were recruited for association and agreement analysis (Table 3). Then, we performed gamma and kappa analysis for association and agreement according to these similar class criteria with only a slight modification: matching class I (0.35–0.7 kU/L) of the UniCAP® 100 system to class 0/I (0.1–0.35 kU/L) and I (0.35–0.7 kU/L) of the IMMULITE® 2000 system in consideration of different lower detection limits between the two systems.

Fig. 1. Scatterplots of intermethod comparison between IMMULITE® 2000 CLEIA and UniCAP® 100 FEIA systems for each of the 10 inhalant allergens. Blue gridlines represent the implied class boundaries (0.1, 0.35, 0.7, 3.5, 17.5, 50 or 52.5, and 100 kU/L). The major diagonal represents the line of identity on these log-scaled scatterplots. The working ranges of both assays were different (0.10–100 kU/L for IMMULITE® 2000 and 0.35–100 kU/L for UniCAP® 100), and are marked with red lines on each graph. The largest rectangular area surrounded by red lines demonstrates working ranges detected by both assays. Out-of-range results by either system were arbitrarily rearranged and are shown in the gutter area of the above-mentioned rectangle. The number of results out-of-range low and out-of-range high by both methods are recorded parenthetically on the lower left and upper right of the plotting area, respectively. The scatterplot header includes the total number of tested samples (explicit results within detection ranges + out-of-range low or high results by both methods + the other results). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

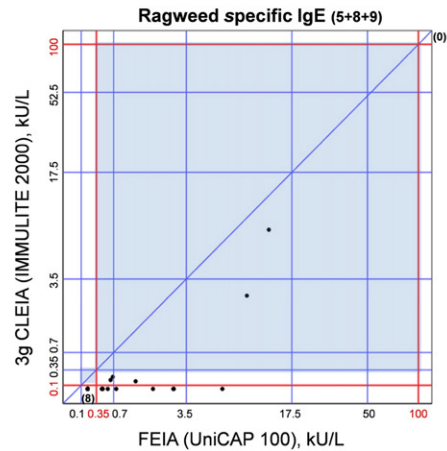
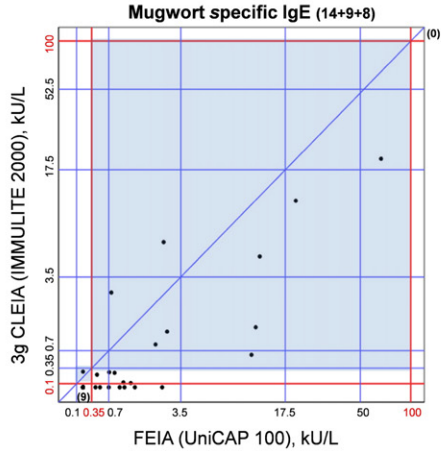
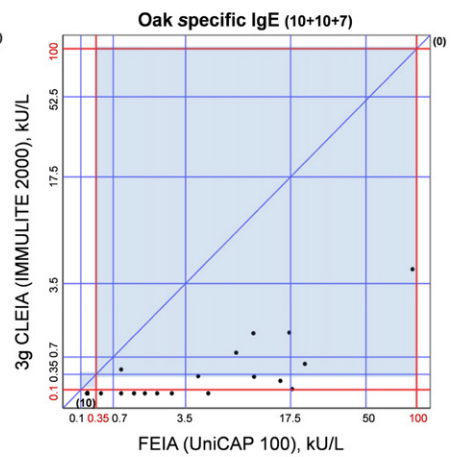
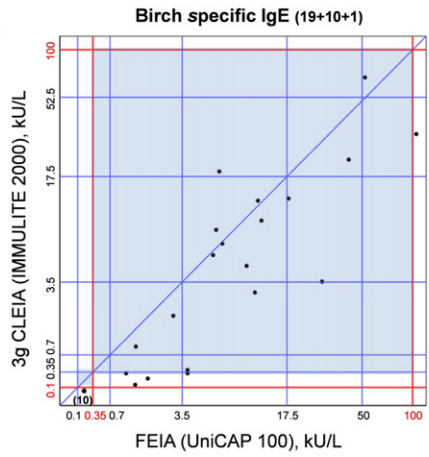
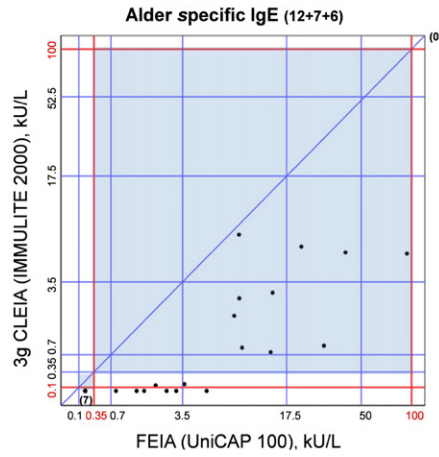
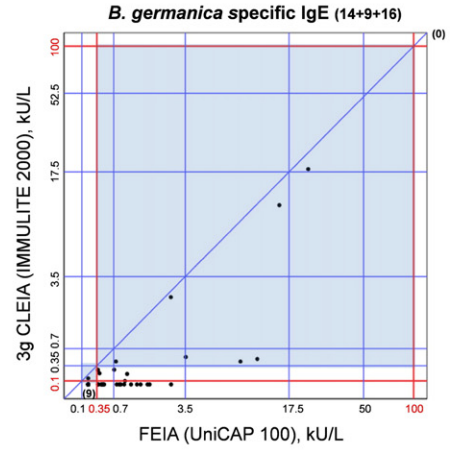
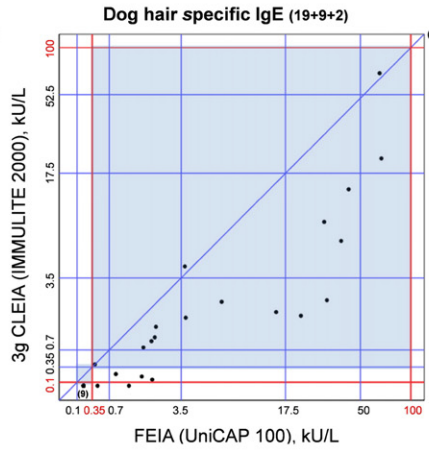
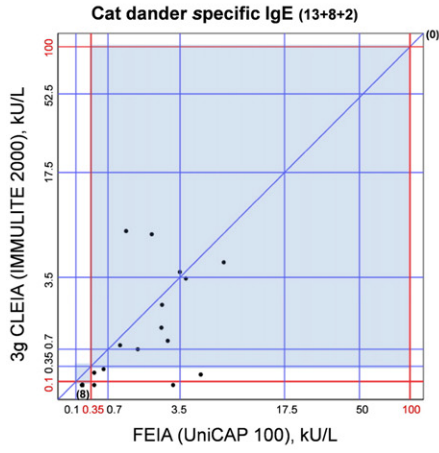
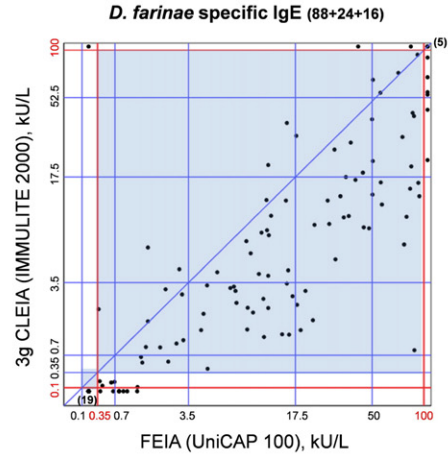
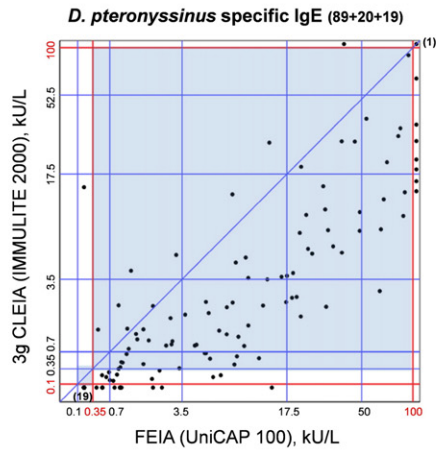


Table 2
Correlation and association analysis between IMMULITE® 2000 CLEIA and UniCAP® 100 FEIA systems

Tested Allergens (n)	Correlation of sIgE levels (kU/L)	Association of class for sIgE
	r (p-value)	gamma (p-value)
<i>D. pteronyssinus</i> (128)	$r_p = 0.639$ (<0.001)	0.857 (<0.001)
<i>D. farinae</i> (128)	$r_p = 0.714$ (<0.001)	0.868 (<0.001)
Cat dander (23)	$r_s = 0.732$ (<0.001)	0.833 (<0.001)
Dog hair (30)	$r_p = 0.768$ (<0.001)	0.980 (<0.001)
<i>B. germanica</i> (39)	$r_p = 0.894$ (0.001)	0.675 (0.001)
Alder (25)	$r_s = 0.856$ (<0.001)	0.977 (<0.001)
Birch (30)	$r_p = 0.718$ (<0.001)	0.955 (<0.001)
Oak (27)	$r_s = 0.781$ (<0.001)	0.921 (<0.001)
Ragweed (22)	$r_s = 0.525$ (0.012)	0.714 (0.029)
Mugwort (31)	$r_p = 0.929$ (<0.001)	0.860 (<0.001)
Egg white (56)	$r_p = 0.845$ (<0.001)	0.917 (<0.001)
Cow milk (54)	$r_p = 0.979$ (<0.001)	0.862 (<0.001)
Peanut (36)	$r_p = 0.849$ (<0.001)	0.868 (<0.001)
Shrimp (16)	$r_s = 0.337$ (0.201)	0.469 (0.209)

r_p = Pearson's correlation coefficient, r_s = Spearman's correlation coefficient.

Gamma (γ) analysis is a symmetric measure of association between two ordinal variables that range between -1 and 1 [17]. Values close to an absolute value of 1 indicate a strong relationship between the two variables and values close to zero indicate little or no relationship [17].

Agreement (%) was calculated as follows: (total number of results–number of discrepancies) × 100 / total number of results, where discrepancies are positive results with one method but negative results with the other [10]. Cohen's kappa (κ) is the agreement between the evaluations of two raters when both are rating the same object and is only available for tables in which both variables use the same category values and both variables have the same number of categories [17,18]. A value of 1 indicates perfect agreement and a value of 0 indicates that agreement is no better than random chance [17,18]. A p-value of less than 0.05 was considered statistically significant.

Available SPT results (positive and negative) were adopted as the objective clinical reference value for comparison of diagnostic accuracy between the two systems. Such analysis was done only for cases with complete result sets from all three tests (SPT, CAP, and 3 g CLEIA). Receiver Operating Characteristic (ROC) curves for accuracy and relevant statistical analyses including sensitivity [19,20] were used for this interassay comparison. Statistical analyses, data processing, and graphic works for tables and figures were performed using SPSS 12.0 (SPSS Inc., Chicago, USA) and Microsoft® Office Excel 2003 (Microsoft Corporation, Seattle, USA).

3. Results

3.1. Intermethod comparisons for allergen specific IgE concentrations

After a total of 645 paired tests, we observed that identical profiles between the results of the two *in vitro* allergen-sIgE measuring

systems were fair to good on scatter plots (Figs. 1, 2). Such observations were more prominent among the 10 inhalant allergens, especially house dust mites (*D. farinae* and *D. pteronyssinus*) and birch pollen (Fig. 1). In the 4 food allergens, egg-white and peanut showed relatively better identical profiles between the two assays (Fig. 2). Though most of the results were scattered somewhat randomly but nearly around the line of identity for both systems, several results out of the detection range for either one system or the other were also noted in the gutter area (see Fig. 1 legend).

3.2. Correlation and association analysis between 3 g CLEIA and CAP FEIA

Simple correlation and Spearman correlation analysis showed moderate to strong positive linear correlation between the two assays (Table 2). The implied class boundaries of sIgE concentrations (see footnote of Table 3) are essentially the same for both sIgE assays [1,10]. All of the tested allergen species, except for shrimp, demonstrated good correlation and strong association between both sIgE assay systems (Table 2) with the gamma analysis.

3.3. Intermethod agreement analysis

We also considered the similar classification between the two sIgE assays and the fact that all assay results were paired. Detection of sIgE (positive vs. negative) could be used as a status variable for each assay (cutoff value: 0.1 kU/L for IMMULITE, and 0.35 kU/L for UniCAP). Table 3 shows the agreement (%) and kappa value for sIgE detection and class of both assays. Detection of sIgE demonstrated fair to good agreement for all of the tested allergens except for *B. germanica*, ragweed, and shrimp. However, degrees of agreement between the sIgE classes of the two assays were relatively low.

3.4. Receiver operating characteristic (ROC) curve analysis

With 169 complete result sets from all three tests (SPT, CAP, and 3 g CLEIA), ROC curves and relevant statistical analyses, including sensitivity and specificity, were performed for all of the allergens combined and individually for each of the nine major inhalant allergens (Fig. 3, and Table 4). All of the ROC curves were plotted for a given allergen and sIgE assay based upon skin prick test results (positive vs. negative) (Fig. 3). Area under the curve (AUC), maximum sensitivity, index Q* (the highest value that sensitivity and specificity

Table 3
Agreement between Immulite® 2000 CLEIA and UniCAP® 100 FEIA systems

Tested Allergens (n)	Specific IgE detection		Specific IgE class ^a	
	Agreement (%)	kappa (95%CI)	Agreement (%)	kappa (95%CI)
<i>D. pteronyssinus</i> (128)	91.4	0.725 (0.574–0.875)	34.4	0.203 (0.103–0.303)
<i>D. farinae</i> (128)	93.8	0.789 (0.649–0.928)	44.5	0.336 (0.232–0.440)
Cat dander (23)	91.3	0.819 (0.583–1.000)	69.6	0.570 (0.319–0.820)
Dog hair (30)	93.3	0.851 (0.653–1.000)	53.3	0.398 (0.190–0.607)
<i>B. germanica</i> (39)	56.4	0.234 (0.026–0.441)	38.5	0.188 (0.000–0.448)
Alder (25)	76.0	0.528 (0.238–0.818)	32.0	0.088 (0.000–0.326)
Birch (30)	100.0	1.000 (uncalculable)	66.7	0.568 (0.372–0.764)
Oak (27)	74.1	0.514 (0.243–0.785)	37.0	0.131 (0.000–0.347)
Ragweed (22)	59.1	0.288 (0.037–0.539)	50.0	0.248 (0.000–0.607)
Mugwort (31)	74.2	0.490 (0.208–0.771)	45.2	0.266 (0.033–0.499)
Egg white (56)	94.6	0.825 (0.635–1.000)	64.3	0.544 (0.384–0.704)
Cow milk (54)	88.9	0.664 (0.425–0.903)	64.8	0.488 (0.314–0.662)
Peanut (36)	75.0	0.529 (0.294–0.764)	58.3	0.395 (0.171–0.618)
Shrimp (16)	56.3	0.200 (–0.179–0.579)	37.5	0.064 (0.000–0.437)

IMMULITE (0: <0.1 kU/L, I: 0.1–0.7 kU/L [O/I: 0.1–0.35, I: 0.35–0.7], II: 0.7–0.35 kU/L, III: 3.5–17.5 kU/L, IV: 17.5–52.5 kU/L, V: 52.5–100 kU/L, VI: > 100 kU/L.

95% CI: 95% confidence interval.

^a Allergen specific IgE Class: Unicap (0: <0.35 kU/L, I: 0.35–0.7 kU/L, II: 0.7–3.5 kU/L, III: 3.5–17.5 kU/L, IV: 17.5–50 kU/L, V: 50–100 kU/L, VI: >100 kU/L).

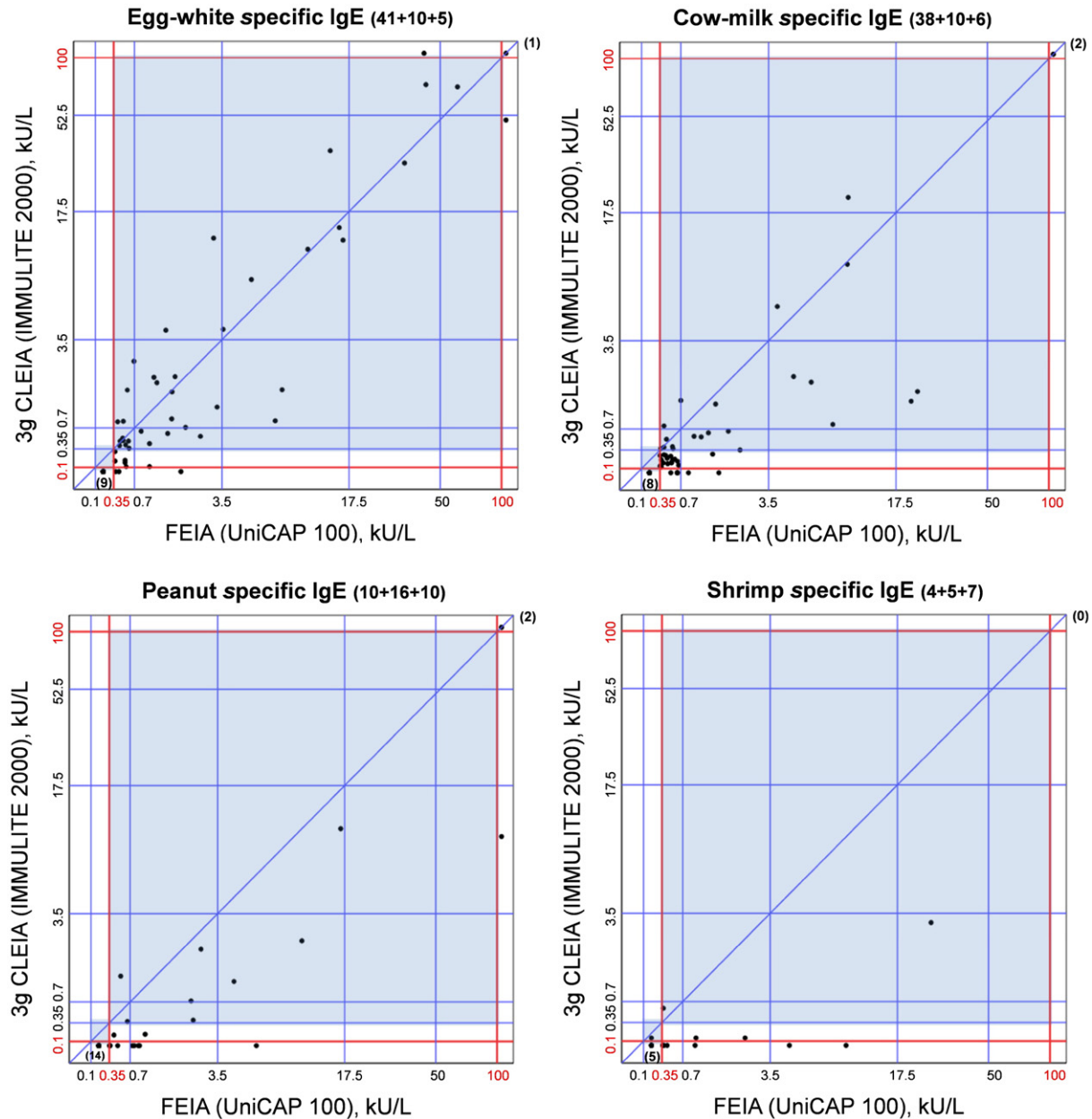


Fig. 2. Scatterplots of intermethod comparison between IMMULITE® 2000 CLEIA and UniCAP® 100 FEIA systems for each of the 4 food allergens (See Fig. 1 legend for additional explanation).

can be jointly attained), and cutoff values at Q^* for total/respective allergen and each assay were also calculated (Table 4). Both sIgE assays showed good accuracy and had similar AUC (0.870 for CAP vs. 0.834 for IMMULITE) and index Q^* for SPT results in the total results analysis. Excellent accuracy was obtained for *D. farinae* by both methods. Although there were only minor differences of AUC, maximum sensitivity, and index Q^* between the two assays, the UniCAP® 100 system was more accurate and useful for SPT results in house dust mites, cat dander, and oak pollen. In contrast, the IMMULITE® 2000 system was more accurate and useful for dog hair (Table 4).

4. Discussion

IMMULITE CLEIA sIgE system was already introduced to Asian countries including Korea. Although there had been several studies for

validating and comparing this system with the established CAP FEIA, most data were mainly based on Caucasian populations [1–3,10,13]. Considering influences of gene–environmental interactions on allergic diseases, such overrepresentation of limited biogeographic population might result in incomplete data [22]. We performed an intermethod comparison between IMMULITE CLEIA sIgE and the established CAP FEIA in Korean allergic patients, and also found several clinical tips for using and interpreting the newer sIgE assay. This study is one of the earliest data based on Asian populations.

There were close association, a fair to good correlation, and agreement between the two sIgE assays, but we also found interassay discordance in some allergens. Such observations are consistent with previous Caucasian-based studies [1–3,23]. Scatterplots showed good agreement between the two assays. We also found good and strongly-positive interassay correlation and association for all of the tested allergens, except for shrimp.

Table 4
ROC curve statistics including sensitivity and specificity for Immulite® 2000 CLIEA and UniCAP® 100 FEIA systems according to skin prick testing for total explicit result sets ($n = 169$ on 14 major inhalant and food allergens) and available respective data for 9 major inhalant allergens (see also Fig. 2)

Allergens	Assay system	AUC	p-value	95% CI	SEmax (%)	Q*	Cutoff value (kU/L) at Q*
Total ($n = 169$)	CAP	0.870	<0.001	0.816–0.925	92.9	0.79	1.20
	IML	0.834	<0.001	0.770–0.899	78.8	?0.80	<0.10
<i>D. pteronyssinus</i> ($n = 26$)	CAP	0.971	0.001	0.910–1.033	100.0	0.95	1.51
	IML	0.824	0.027	0.626–1.021	95.2	0.67	1.35
<i>D. farinae</i> ($n = 23$)	CAP	1.000	0.001	1.000–1.000	100.0	1.00	1.62
	IML	0.956	0.002	0.862–1.049	100.0	0.80	1.78
Cat dander ($n = 17$)	CAP	0.905	0.079	0.749–1.060	92.9	0.79	0.61
	IML	0.810	0.113	0.589–1.030	78.6	0.67	0.25
Dog hair ($n = 17$)	CAP	0.779	0.101	0.548–1.010	92.3	0.75	1.56
	IML	0.856	0.036	0.638–1.073	92.3	0.75	0.38
Alder ($n = 12$)	CAP	0.844	0.062	0.615–1.072	75.0	0.75	0.71
	IML	0.813	0.089	0.567–1.058	62.5	?0.73	<0.10
Birch ($n = 17$)	CAP	0.842	0.031	0.636–1.048	91.7	0.80	1.72
	IML	0.808	0.051	0.537–1.080	91.7	0.80	0.47
Oak ($n = 12$)	CAP	1.000	0.007	1.000–1.000	100.0	1.00	0.80
	IML	0.813	0.089	0.567–1.058	62.5	?0.72	<0.10
Ragweed ($n = 9$)	CAP	0.611	0.606	0.228–0.994	100.0	0.50	0.67
	IML	0.667	0.439	0.240–1.093	33.3	?0.60	<0.10
Mugwort ($n = 17$)	CAP	0.629	0.380	0.337–0.920	80.0	0.60	0.65
	IML	0.700	0.172	0.432–0.968	60.0	?0.68	<0.10

CAP: UniCAP 100 FEIA. IML: IMMULITE 2000 3 g CLEIA. 95% CI: 95% confidence interval. SEmax: Maximum sensitivity is the highest sensitivity attainable by that assay with in the working range. Q*: Index Q* is the highest value that sensitivity and specificity can be jointly attained. ?: undetermined value due to out-of-range-low cutoff (<0.10 kU/L of IMMULITE 2000).

This cross-sectional study has some strong points, such as a large sample size covering nearly all ages, various allergic diseases, and major inhalant/food allergens that are predominant in Korea. Because Far East Asia including China, Japan, and Korea has similar genetic and geographical backgrounds including allergen distributions, this study can be clinically useful in this large populated area and gives complementary data for previous studies. However, several limitations also exist in this study. For example, data from single institution might induce biased results, and data analysis on some allergen species were not enough, for we could get only small number of samples for some allergens.

Recently, Wang et al. reported that there were significant differences among three commercial assays (Phadia ImmunoCAP® Agilent Turbo-MP® and Siemens IMMULITE® 2000) in the measurement of allergen-specific IgE levels that would affect the clinical decision-making process [2]. They also warned that clinicians, who rely on published decision points for certain food allergies (milk, egg, and peanut) or try to monitor IgE levels in individual patients over time, should not substitute one commercial assay for another, because such decision points were established by the ImmunoCAP assay [2].

In this study, such interassay discrepancies were also observed by the low agreement between class-based sIgE results of both systems. Technical differences between the two systems could result in these interassay discrepancies [1–3,23]. We should also consider differences in allergen sources and constituents for each assay [23]. For example, ImmunoCAP uses birch pollen allergens from *Betula verrucosa*, but IMMULITE uses those from *Betula nigra* [2]. Although these two birch species have a similar geographic distribution, the minor differences may cause interassay discrepancies. Ethnic background and geographic environment of patients should be taken into account for different allergen sources. However, if we only consider sIgE detection (positive, or negative), agreement between both systems increases (Table 3). This approach might indicate how to address such discrepancies. Previous studies suggested several useful features of the IMMULITE® 2000 CLEIA for allergen-sIgE detection, including a lower detection limit (0.1 kU/L), functional sensitivity (0.2 kU/L), liquid allergens, and an enzyme-enhanced chemiluminescence technique which reduces test time to about 1 h [3]. In our scatterplot analysis for intermethod comparison, the

advantage of lower detection limits of the IMMULITE® 2000 CLEIA system was not obvious (Figs. 1 and 2). Technical problems including calibration, system settings, different allergen sources, processing procedures, serum storage conditions, and interassay time-gaps might influence such results.

Despite many potential discrepancies between the *in vivo* and *in vitro* procedures [21], sIgE assay systems have been expected to demonstrate acceptable concordance with objective clinical tests, such as skin testing [1]. In our ROC analysis based on SPT, we compared accuracy between the two *in vitro* sIgE assays and observed incomplete concordance between the two methods and skin prick testing (Fig. 3). Such incomplete concordance could be due to the different sIgE-measuring mechanisms (circulating sIgE for *in vitro* assays vs. sIgE-bound mast cell reactivity in skin) [1]. Well-known major allergens (house dust mites, cat dander, and dog hair) showed relatively higher AUC for both systems than other allergens. Maximum sensitivity and index Q* by ROC analysis based on SPT were good for both sIgE assays for house dust mites, dog hair, and birch pollen (Table 4). Such higher accuracy for well-documented and clinically important major allergens may be associated with the well-established standardization and regulations for these allergens. For example, international standards for major allergens including house dust mite, dog hair, birch, timothy, and ragweed pollen were already established and have been coordinated by the World Health Organization (WHO)/International Union of Immunological Societies (IUIS) Allergen Standardization Sub-committee since the late 1980s [24]. And U.S. Food and Drug Administration (FDA) have been regulating the integrity of the manufacturing process, including consistency in source materials, production methods, and have been requiring manufacturers to meet the established U.S. reference standards for 19 allergen extracts [25,26].

Allergen measurements are used extensively in the allergy diagnostics and vaccines, but few purified international allergen standards are available for calibration [24,27]. Recently, the European Union “Development of certified reference materials for allergenic products and validation of methods for their quantification (CREATE)” project has established a model for international allergen standardization and 2 purified recombinant allergens reference [24]. European Union and College of American Pathologists (CAP) have also been

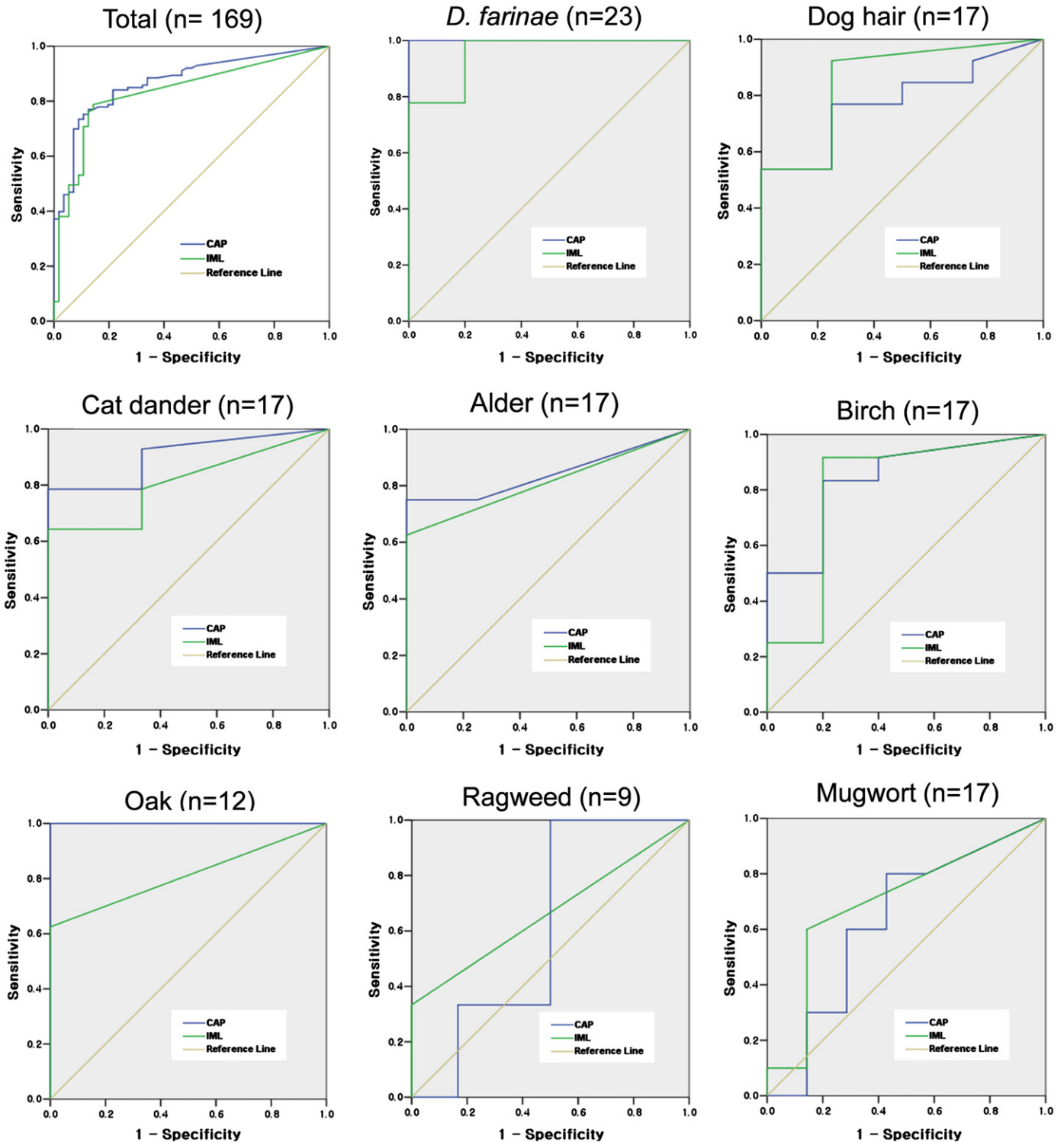


Fig. 3. ROC plots for performance of IMMULITE® 2000 and UniCAP® 100 systems according to SPT results. ROC curve analysis of both assays using sIgE concentrations (kU/L) for 14 allergens from a total of 169 complete results sets with simultaneously explicit data with SPT results is shown in the 1st figure (top left). Nine major inhalant allergens were also analyzed with ROC curves (see Table 4); 8 of these results are also shown. CAP: UniCAP® 100; IML: IMMULITE® 2000.

performing External Quality Assessment schemes (EQA) for evaluating inter-/intra-laboratory variation, intermethod differences, and performance criteria by grouping the laboratories according to types of sIgE assays [23,26]. In Korea, Lim, et al. recently investigated 85 medical laboratories performing sIgE assays to prepare for an EQA according to types of sIgE assays [26]. International allergen standardization and regular EQA would facilitate the development,

validation, and improvement of more accurate allergen-sIgE assay and could decrease the interassay discrepancies. For continuous clinical validation of *in vitro* sIgE assays including CLEIA, further evaluations should be performed in consideration of environmental differences, standardization and expansion of testing allergen sources, reduction of interassay differences, and determination of clinically relevant cutoff values as well as sIgE detection techniques. It was proposed that

future research should investigate mechanisms of the observed interassay differences of specificity and sensitivity with the relevance to ethnic and geographic difference between North East Asian and Caucasian populations.

In conclusion, the IMMULITE® 2000 CLEIA system for allergen-sIgE detection showed fair to good intermethod correlation, association, agreement, and accuracy in comparison to the established CAP assay among Korean allergic patients. However, intermethod differences between both assays should be taken into account for clinical applications.

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