Literature Compendium: Volume I

Allergy Testing

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Introduction

An allergy is a bodily reaction to a variety of otherwise harmless substances, resulting in the production of specific IgE antibodies. Allergic reactions can range from mild to severe. Anaphylaxis is the most severe form of an allergic reaction—one that may even result in death. Allergic reactions can manifest themselves as allergic asthma, allergic rhinitis, allergic conjunctivitis, atopic eczema/atopic dermatitis, or anaphylaxis, and these manifestations may vary between adults and children. Allergies are common, with the incidence rising over the past several years. The WHO estimates that 20% of the global population suffers from IgE-mediated allergic diseases, one-tenth of the population suffers from drug allergies, and 400 million from rhinitis.[1] Timely diagnosis and treatment are necessary to manage and control allergies to improve long-term patient outcomes and quality of life. Confirmation of allergy diagnosis and identification of causative allergens are crucial in correctly managing and treating allergic diseases. Precise diagnosis allows the implementation of therapies oriented to the etiologic factors of allergic diseases, such as environmental measures and immunotherapy.

Food allergy affects an estimated 8% of children under age 5 and approximately 4% of adults.[2] Over the last several decades, the prevalence of food allergies has increased significantly. Children are especially at risk, and 90% of allergies in children are to milk, eggs, wheat, and peanuts. Peanut allergy in particular can be life-threatening due to higher incidence of severe reactions, such as anaphylaxis.

Allergy testing can be performed by in vivo or in vitro methods. There are benefits and limitations to both methods, and it is recommended that the testing be performed in conjunction with a clinical history and physical examination. The clinical scenario should determine the appropriate test for each patient.[3] In vivo testing involves a skin prick test (SPT), which is often the first-line approach to determine the release of allergen-specific IgE antibodies. However, in vitro testing with highly purified allergens or recombinants can be used as an alternative or complementary diagnostic tool.[4]*

There is no "gold standard" for making the diagnosis of IgE-mediated allergic diseases. The diagnosis of allergic disease must be made with a combination of an allergy-specific history, physical examination, and a diagnostic test, e.g., specific IgE testing, SPT, or the oral food challenge (OFC) test. It is well-established that the presence of IgE antibodies is necessary but not sufficient for symptomatic allergic disease. The presence of IgE antibody signifies sensitization to an allergen that can be viewed as a risk factor. It does not, however, ensure that the patient will manifest allergic symptoms on allergen exposure. In vitro testing is especially useful when skin test results do not correlate with the history or cannot be performed. In vitro tests can be applied to "probability of disease" prediction in food allergy.[1]*

**Not included in the Siemens Healthineers IMMULITE 3gAllergy IFU.*

The Siemens Healthineers IMMULITE® 3gAllergy™ allergen-specific IgE method provides a quantitative measurement of specific IgE for the purpose of identifying offending allergens, supporting precise and timely therapeutic intervention and as an aid in diagnosis and management. The use of liquid allergens on polymer solid phase, unique to 3gAllergy, allows higher testing accuracy compared to the cellulose solid-phase method and provides reliable results as an aid in the clinical diagnosis of IgE-mediated allergic disorders. "This methodology is characterized by a broader working range, ability to report results quantitatively and categorized, use of liquid allergens, and automation features which reduce labor requirements, total assay time, and possibility of error."[5]

Allergy prevalence, morbidity, and mortality continue to rise globally, and while traditional in vivo testing remains common, in vitro testing has been proven to empower clinicians and elevate the standard of care. Despite the challenging nature of allergy diagnosis and management, 3gAllergy testing remains a simple and intuitive approach to allergy diagnosis. IMMULITE 3gAllergy offers a broad range of tests to address various clinical needs related to quantitative determination of allergen-specific IgE. The assay provides reliable results as an aid in the clinical diagnosis of IgE-mediated allergic disorders.

Allergy Testing Literature Compendium (Part I)

This compendium presents a selection of published studies on the clinical utility of allergy diagnostic testing with a focus on IMMULITE 3gAllergy allergen-specific IgE assays, including comparison to other in vivo and in vitro allergy tests.

Each article is presented as an abstract from the published paper followed by Siemens Healthineers interpretation of the significance of each work. We hope that these synopses encourage you to read each article in its entirety for a more complete understanding of these highly relevant works in the field.

Glossary of Terms[7]

Allergen extract: The part of allergen sources that is soluble in water or other specific solvents. Allergen extracts from different sources and batches may vary, and the allergen contents can be both qualitatively and quantitatively different. Many proteins or other kinds of molecules without allergenic properties are contained in an allergen extract. The main problem is the presence of multiple allergens in the mixture, some of which may be clinically relevant and others irrelevant, causing cross-sensitization patterns in subjects with sensitization to common components.

Allergen source: The raw material from which the allergen extract is obtained, such as pollens, animal furs, or cultures of molds. Allergen sources vary from producers and over time. Significant batch-to-batch heterogeneity has also been observed. Thus, standardization of allergen sources, and of allergen extracts, is important.

Allergen: The molecule that expresses epitopes recognized by an sIgE.

Allergy: The presence of sensitization to one or more allergens and the presence of clinical symptoms that can be associated with that sensitization. Laboratory tests can only identify sensitization, not an allergy. The diagnosis of allergy is the responsibility of the allergist.

Component: See Allergen.

Cross-binding: The ability of IgE to bind to allergens with significant sequence homology.

Cross-reactivity: Allergy caused by an allergen to which an individual is sensitized via cross-binding to the allergen that caused the primary sensitization.

Cross-reactive carbohydrate determinants (CCDs): CCDs are protein-linked carbohydrate structures. CCDs with wide homogeneity to many allergens are considered pan-allergens. CCD can be found only in natural allergens and not in recombinant molecules produced in *E. coli*. In patients sensitized to CCD, sIgE tests on allergen extracts may show false-positive results.

Cross-sensitization: Sensitization caused not through primary exposure, but due to cross-binding of IgE to allergens with significant sequence homology. Cross-sensitization may be clinically irrelevant. If it causes symptoms, it may be referred to as cross-reactivity.

Molecular allergen: See Allergen.

Primary sensitization: Sensitization caused by the individual allergen itself rather than through cross-sensitization to a homologous allergen.

Recombinant allergen: Allergens produced through genetic engineering and often expressed in *E. coli*.

Recombinant component: See Recombinant allergen.

Sensitization: The presence of sIgE to one or more allergens in serum tests. In skin prick tests, sensitization is the presence of a cutaneous reaction in the presence of a given allergen. Sensitization cannot be considered an allergy.

SIgE: The antibody secreted in sensitized patients and specific for a given allergen. The detection of these antibodies suggests that the patient is sensitized to the allergen. The presence of signs and symptoms compatible with the IgE profile allows the allergist to identify the patient as allergic.

Total IgE: The concentration of IgE circulating in the serum. It includes allergens that do not generally cause a severe or systemic reaction in sensitized patients, although exceptions are possible. Includes profilins, polcalcins, PR-10, and CCD.

Performance Evaluation of IMMULITE 3gAllergy: Comparison to Phadia IMMUNOCAP and Skin Prick Test (SPT) Methods

Allergen-specific IgE measured by a continuous random-access immunoanalyzer: interassay comparison and agreement with skin testing

Ollert M, Weissenbacher S, Rakoski J, Ring J. Clin Chem. 2005 Jul;51(7):1241-9.

Objectives

- Evaluate the performance of the IMMULITE 2000 Allergy system for measuring circulating allergen-specific IgE (sIgE) against an established in vitro assay on IMMUNOCAP.
- Assess the system's diagnostic accuracy against objective clinical criteria for identifying sensitization to specific allergens.

Methods

- Prospective evaluation of patients with suspected allergies to airborne or insect venom allergens.
- Measured sIgE in serum samples from 169 persons with these suspected allergies to airborne or insect venom allergens on the IMMULITE 2000 and on IMMUNOCAP.
- SPT outcome served as the clinical comparison method.

Results

- Inter-assay classification agreement between the IMMULITE and IMMUNOCAP, relative to the usual allergen-specific IgE cutoff of 0.35 kIU/L, ranged from 76% (yellow jacket venom) to 95% (orchard grass).
- Overall agreement between IMMULITE and IMMUNOCAP was 88.3% for all 9 allergens combined (766 results).
- Compared with skin testing, for each of the 9 allergens studied, the area under the ROC curve was at least as large for the IMMULITE as for the IMMUNOCAP, reflecting in part the more extensive working range of the IMMULITE (0.10–100 kIU/L vs. 0.35–100 kIU/L for IMMUNOCAP).

Author's Conclusion

"Laboratory testing for sIgE can be performed on a fully automated, random-access system with an extended working range and with diagnostic accuracy for representative allergens equivalent to or better than that of the semiautomated IMMUNOCAP technology."

Significance

- The study protocol was applied to a spectrum of aeroallergens and insect venom allergens representative of the core high-volume workload in this laboratory.
- The study demonstrated good quantitative agreement between the two methods—IMMULITE and IMMUNOCAP—with the SPT method being considered the gold standard for determining IgE in allergy testing.
- Automated laboratory technologies can provide serum IgE tests with diagnostic accuracy similar to SPT.

Table 1. ROC curve statistics for IMMULITE and IMMUNOCAP assays vs. skin testing.

		AUC			Maximum Sensitivity (%)[b]			SYM[c]		
Allergen[a]	n	IMMULITE	IMMUNOCAP	Difference	IMMULITE	IMMUNOCAP	Difference	IMMULITE	IMMUNOCAP	Difference
D1	99	0.917	0.851	0.066	97.9	93.5	4.4	85.6	79.6	6.0
D2	75	0.897	0.773	0.124	94.9	79.5	15.4	82.4	78.7	3.7
E1	99	0.897	0.774	0.123	95.3	81.4	13.9	86.2	NA[d]	NA
G3	78	0.889	0.807	0.082	92.6	83.3	9.3	85.1	79.2	5.9
G6	99	0.858	0.804	0.054	89.7	82.8	6.9	82.0	80.5	1.5
T3	94	0.967	0.908	0.059	97.9	91.5	6.4	92.4	89.7	2.7
W6	96	0.730	0.635	0.095	76.7	67.4	9.3	75.7	NA	NA
I1	63	0.867	0.719	0.148	97.1	85.3	11.8	79.3	72.4	6.9
I3	63	0.946	0.724	0.222	100	75.9	24.1	83.9	74.0	9.9

[a]*See Table 2 of the online Data Supplement for allergen codes.*
[b]*Maximum sensitivity is the highest clinical sensitivity achievable by the assay for that allergen.*
[c]*Also known as Q,* the highest value for that sensitivity achievable by the assay for that allergen.*
[d]*NA: not applicable (below the 0.35 kIU/L lower limit of the IMMUNOCAP assay's working range).*

Allergen component specific IgE measurement with the IMMULITE 2000 System: diagnostic accuracy and intermethod comparison

Villalta D, Da Re M, Conte M, Martelli P, Uasuf C, Barrale M, La Chiusa S, Brusca I. J Clin Lab Anal. 2015;29:135-41.

Objectives

- Evaluate the diagnostic accuracy of different allergen components using the fully automated singleplex quantitative platform IMMULITE 2000.

Methods

- 195 allergic outpatients (35 to olive pollen, 35 to birch pollen, 35 to profilin, 35 to house dust mites, 35 to peach, 20 to shrimp) and 20 negative controls were enrolled for the study.
- The inclusion criteria were as follows: clinical history consistent with IgE-mediated allergy to the aeroallergens or food allergens considered in the study, both positive SPT and sIgE to allergen extracts, and (in cases of food allergens) positive challenge test with the relevant allergen when necessary.
- SPT was performed using commercially available allergen extracts. Only wheals showing a mean diameter exceeding 3 mm at 15 were considered as a positive response.
- Component recombinant allergens Bet v 1, Bet v 2, Ole e 1, Der p 1, Der p 2, Der f 1, Der f 2, Pru p 3, and tropomyosin, a major allergen of shrimp (Pen m1 on IMMULITE and Pen a 1 on IMMUNOCAP) were tested both with IMMULITE 2000 and IMMUNOCAP 250. A positive result for the quantitative allergen-specific IgE tests was defined as a concentration ≥0.35 kU/L.

Results

- Diagnostic accuracy of different allergen components was measured using the IMMULITE 2000 3gAllergy sIgE assay in serum samples of 19 allergic patients. Very similar data for sensitivity and specificity were obtained using the IMMULITE 2000 and IMMUNOCAP 250. Table 2 illustrates sensitivity, specificity, and agreement relative to SPT on both systems.
- The intra-method (IMMULITE 2000) comparison between two profilin (nBet v and nMal d 4) and two nsLTP of the Rosaceae family (nPru p 3 and nPru av 1) showed a perfect agreement and a very high correlation. Thus, for the detection of sIgE to profilin in patients with fruit-pollen syndrome and of sIgE to nsLTP in those with allergy to fruits of the Rosaceae family, the two different molecules of each allergenic class may be used interchangeably.
- Sensitivity of allergen-specific Immunoglobulin E (sIgE) to Ole e 1, Bet v 1, Der p 1, Der p 2, Der f 1, Der f 2, Pen m 1, and Pru p 3 with IMMULITE 2000 was 100%, 100%, 77.1%, 94.3%, 71.4%, 94%, 75%, and 97.1%, respectively, and the specificity was 100% for all the allergens. The overall agreement between IMMULITE 2000 and IMMUNOCAP platforms was 98.6% (Cohen's kappa = 0.979; confidence interval [CI] 95%: 0.960–0.997). From moderate to strong, positive linear correlations between the assays (r(2) from 0.322 to 0.860, and Spearman's rho from 0.824 to 0.971) were showed.

Author's Conclusions

"A high diagnostic accuracy of the sIgE to allergen components measurement with IMMULITE 2000 and a high agreement with IMMUNOCAP platforms were shown in this study."

Significance

- This is one of the first studies demonstrating the clinical performance of the IMMULITE 2000 XPi system for recombinant molecules used in the new concept in allergy diagnosis employing component-resolved diagnosis (CRD).
- The study showed high diagnostic accuracy of the sIgE to allergen components measurement with the IMMULITE 2000 XPi system performed in patients with both positive SPT and sIgE to allergen extracts or with positive food challenge test in case of food allergens.
- The study showed high agreement of the IMMULITE 2000 system with the IMMUNOCAP system for the measurement of IgE levels for the component allergens.
- For the detection of sIgE on the IMMULITE 2000 system to profilin in patients with fruit-pollen syndrome and of sIgE to nsLTP in those with allergy to fruits of the Rosaceae family, the two different molecules of each allergenic class may be used interchangeably as determined by the intra-method comparison.

Table 2. Sensitivity and specificity of sIgE measurement to the individual molecules evaluated.

Sensitizer	Allergen	IMMULITE 2000 System		IMMUNOCAP System	
		Sensitivity	Specificity	Sensitivity	Specificity
Olive	Ole e 1	35/35 (100%)	0/20 (100%)	34/35 (97.1%)	0/20 (100%)
Birch	Bet v 1	35/35 (100%)	0/20 (100%)	35/35 (100%)	0/20 (100%)
HDM	Der p 1[a]	27/35 (77.1%)	0/20 (100%)	27/35 (77.1%)	0/20 (100%)
	Der p 2[a]	33/35 (94.3%)	0/20 (100%)	32/35 (91.4%)	0/20 (100%)
	Der f 1[a]	25/35 (71.4%)	0/20 (100%)	25/35 (71.4%)	0/20 (100%)
	Der f 2[a]	33/35 (94.3%)	0/20 (100%)	31/35 (88.5%)	0/20 (100%)
Shrimp	Pen m 1/Pen a 1[b]	15/20 (75.0%)	0/20 (100%)	16/20 (80.0%)	0/20 (100%)
Peach	Pru p 3 (nsLTP)	34/35 (97.1%)	0/20 (100%)	34/35 (97.1%)	0/20 (100%)
	Pru av 3 (nsLTP)	34/35 (97.1%)	0/20 (100%)	—	—
	Pru av 1 (PR-10)	0/35 (0%)	0/20 (100%)	—	—
	Pru av 4 (profilin)	0/35 (0%)	0/20 (100%)	—	—

The specificity has been calculated on the 20 negative controls.
[a]Since Der f 1 and Der f 2 molecules are not available on the IMMUNOCAP 250 platform, for detection of sIgE to house dust mites (HDM) the IMMUNOCAP ISAC platform has been used.
[b]For detection of sIgE to tropomyosin IMMULITE 2000 uses Pen m 1 and IMMUNOCAP pen a 1 as allergen.

Table 3. Agreement between IMMULITE 2000 and IMMUNOCAP (IMMUNOCAP ISAC for HDM allergens[a]) systems.

Tested Allergens	Number of Tests	Agreement (%)	Kappa (95% CI)
Bet v 1	55	100	1.000 (1.000–1.000)
Ole e 1	55	98.1	0.961 (0.885–1.036)
Der p 1[a]	55	98.1	0.963 (0.893–1.034)
Der p 2[a]	55	98.1	0.962 (0.889–1.035)
Der f 1[a]	55	100	1.000 (1.000–1.000)
Der f 2[a]	55	98.1	0.963 (0.891–1.034)
Bet v 2	55	100	1.000 (1.000–1.000)
Pen m 1/Pen a 1[b]	40	97.5	0.947 (0.846–1.049)
Pru p 3	55	100	1.000 (1.000–1.000)
Overall	480	98.6	0.979 (0.960–0.997)

Patients selected for each specific-allergen group and negative controls were considered.
The specificity has been calculated on the 20 negative controls.
[a]Since Der f 1 and Der f 2 molecules are not available on the IMMUNOCAP 250 platform, for detection of sIgE to house dust mites (HDM) the IMMUNOCAP ISAC platform has been used.
[b]For detection of sIgE to tropomyosin IMMULITE 2000 uses Pen m 1 and IMMUNOCAP pen a 1 as allergen.

Allergen-specific IgE measurement: intermethod comparison of two assay systems in diagnosing clinical allergy

Bulat Lokas S, Plavec D, Rikić Pišković J, Živković J, Nogalo B, Turkalj M. J Clin Lab Anal. 2017;31:e22047.

Objective

- Compare the clinical performance of the IMMULITE 2000 assay for specific IgE (sIgE) to IMMUNOCAP technology in light of clinical background.

Methods

- Prospective evaluation of a selected patient group (n = 569; varied sample size for each allergen) and in a random sample group (n = 100; 8 allergens).
- Inclusion criteria for the selected patient group (n = 569) were clinical history consistent with IgE-mediated allergy to aeroallergens and insect venom or food allergy with a positive SPT to allergen extracts.
- The nonselected patient group (n = 100) consisted of banked serum samples from randomly selected patients.
- Measurements of sIgE were performed on IMMULITE 2000 and IMMUNOCAP technology.
- The automated sIgE results were correlated with SPT results in selected patients and with medical history in nonselected patients.

Results

- Observed fair to excellent correlation and agreement between the results of both assays in both selected and nonselected patient group (ρc = 0.431–0.976; ρc = 0.390–0.972, respectively).
- Associations of sIgE levels with SPT levels and medical history have shown significant correlation for both assays for the majority of tested allergens.

Author's Conclusion

"Laboratory testing for sIgE can be successfully accomplished by IMMULITE 2000 immunoanalyzer at a diagnostic accuracy relative to SPT, comparable to the results acquired by CAP technology, but not fully comparable to the level of an individual patient."

Significance

- Excellent correlation and agreement between the sIgE results were obtained on IMMULITE 2000 and IMMUNOCAP technology, despite their methodological differences.
- The association of IgE levels with subcutaneous SPT levels and clinical history showed significant correlations for both methods tested, when applicable (cat hair, egg white, common ragweed, etc.; $P < 0.05$ for all).

Table 4. Agreement between IMMULITE 2000 and IMMUNOCAP 100 systems in selected group of patients.

Tested Parameters	n	Agreement	Lin's Concordance Test Values (95% CI)
Total IgE	121	Almost perfect	0.976 (0.970–0.980)
Aeroallergens			
Alternaria tenuis	76	Substantial	0.651 (0.548–0.735)
Birch	75	Substantial	0.610 (0.498–0.702)
Cat dander	71	Substantial	0.761 (0.668–0.831)
Common ragweed	74	Almost perfect	0.931 (0.893–0.956)
D. pteronyssinus	66	Almost perfect	0.918 (0.872–0.948)
Orchard grass	74	Substantial	0.720 (0.638–0.786)
Food allergens			
Egg white	77	Substantial	0.654 (0.559–0.731)
Milk	68	Almost perfect	0.927 (0.889–0.952)
Peanut	79	Almost perfect	0.895 (0.845–0.930)
Insect venom			
Honey bee venom	40	Almost perfect	0.951 (0.910–0.974)
Yellow jacket venom	36	Almost perfect	0.828 (0.711–0.900)
rApi m 1—*Apis Melifera*	33	Almost perfect	0.859 (0.747–0.923)
rVes v 5—*Vespula vulgaris*	28	Moderate	0.431 (0.331–0.521)

0–0.20 as slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1 as almost perfect agreement.

Comparison of singleplex specific IgE detection immunoassays: IMMUNOCAP PHADIA 250 and IMMULITE 2000 3gAllergy

Park KH, Lee J, Sim DW, Lee SC. Ann Lab Med. 2018 Jan;38(1):23-31. https://doi.org/10.3343/alm.2018.38.1.23

Objectives

- Compare the diagnostic agreement of two singleplex sIgE detection assays: IMMULITE 2000 and IMMUNOCAP 250.
- Assess inter-method comparison and detection performance of IMMUNOCAP and IMMULITE 2000 using 14 inhalant and food allergens.

Methods

- Retrospective study using serum samples from 209 Korean patients with allergic disease to the inhalant and food allergens.
- Comparison of sIgE levels to the inhalant and food allergens on the IMMUNOCAP and IMMULITE 2000 assay systems.
- Data from 902 paired comparison tests were included for comparisons.
- Qualitative (positivity/negativity), semi-quantitative (Class correlation 0–6), and quantitative (sIgE titers) comparisons between the two systems were performed using statistical analyses.

Results

- In qualitative comparisons between IMMULITE 2000 and IMMUNOCAP 250, the positivity and negativity agreements ranged from 75% (wheat, shrimp) to 96% (Alternaria).
- In semi-quantitative analysis, the Class consistency (classes 0–6) was well matched between the two methods.
- Spearman's rank correlation coefficients for all allergens except shrimp were over 0.7.
- In quantitative comparisons, all allergens excluding shrimp showed >0.7 intra-class correlation coefficients.

Author's Conclusion

"The IMMUNOCAP and IMMULITE 2000 systems showed similar performances. However, clinicians should consider fundamental methodological differences between the assays."

Significance

- The results of the IMMUNOCAP and IMMULITE 2000 systems showed good correlation with respect to sIgE detection of common inhalants and food allergens.
- Both IMMUNOCAP and IMMULITE 2000 showed good linearity as quantitation assays.

Table 5. Positivity and negativity agreement between the IMMUNOCAP and IMMULITE assays.

Tested Parameters	Total Agreement Ratio[a]	Kappa Index[b] (95% CI)
D. farinae	0.88 (84/95)	0.413 (0.075–0.692)
Cat dander	0.88 (49/56)	0.749 (0.557–0.920)
Dog dander	0.86 (48/56)	0.701 (0.497–0.885)
Oak	0.79 (46/58)	0.593 (0.386–0.793)
Rye grass	0.85 (50/59)	0.686 (0.495–0.860)
Mugwort	0.79 (50/63)	0.521 (0.267–0.723)
Alternaria	0.96 (52/54)	0.923 (0.805–1.000)
German cockroach	0.82 (50/61)	0.647 (0.460–0.805)
Egg	0.89 (72/81)	0.777 (0.626–0.901)
Milk	0.88 (63/72)	0.744 (0.576–0.886)
Wheat	0.75 (40/53)	0.465 (0.250–0.686)
Peanut	0.77 (57/74)	0.557 (0.394–0.731)
Soybean	0.80 (55/69)	0.593 (0.402–0.767)
Shrimp	0.75 (38/51)	0.494 (0.247–0.693)

All P values were <0.001. P values were calculated using [a]Fisher's exact (D. farinae and wheat) or Pearson's chi-square tests (all others). All P values were <0.001 except D. farinae (P = 0.001). P values were calculated using [b]Cohen's kappa analysis.
Abbreviations: D. farinae: Dermatophagoides farinae
CI: confidence interval.

Table 6. Correlation analysis of sIgE titers between the IMMUNOCAP and IMMULITE assays.

Tested Parameters	sIgE Titer Correlation[a] (95% CI)	Intra-class Correlation (95% CI)
D. farinae	0.882[c] (0.764–0.955)	0.910 (0.865–0.940)
Cat dander	0.875[c] (0.754–0.950)	0.918 (0.861–0.952)
Dog dander	0.948[c] (0.909–0.965)	0.962 (0.861–0.952)
Oak	0.788[c] (0.630–0.892)	0.324 (-0.139–0.599)
Rye grass	0.871[c] (0.866–0.951)	0.787 (0.634–0.876)
Mugwort	0.888[c] (0.790–0.944)	0.750 (0.587–0.848)
Alternaria	0.924[c] (0.765–0.917)	0.921 (0.867–0.953)
German cockroach	0.869[c] (0.790–0.915)	0.864 (0.774–0.918)
Egg	0.890[c] (0.804–0.946)	0.873 (0.803–0.918)
Milk	0.897[c] (0.825–0.936)	0.782 (0.652–0.863)
Wheat	0.874[c] (0.752–0.936)	0.865 (0.767–0.922)
Peanut	0.910[c] (0.859–0.938)	0.941 (0.906–0.963)
Soybean	0.847[c] (0.739–0.916)	0.892 (0.826–0.933)
Shrimp	0.643[c] (0.463–0.766)	0.620 (0.337–0.783)

[c]P < 0.001 (P values were calculated using [a]Spearman's correlation coefficient).
Abbreviations: See Table 5.

IMMULITE 3gAllergy and Food Allergies

The diagnosis of food allergy poses many challenges. Current methods of testing include patient history and physical exam, skin-prick test (SPT), oral food challenge (OFC), and in vitro testing, which assesses the specific IgE levels to allergens. The diagnosis must be made with a combination of an allergy-specific history and physical and a diagnostic test, e.g., specific IgE testing, SPT, or the oral food challenge (OFC) test.

The methodology to determine the serum IgE levels is different, and it can vary from system to system. Thus, it is very important to establish so-called cut points, so physicians can make a clinical decision on whether to proceed with the open food challenge (OFC).[9]*

Sampson HA, et al. published diagnostic levels of food-specific IgE for a variety of foods established with Thermo Fisher (Phadia) technology. "When a patient has a food-specific IgE level exceeding any of the established cut-off values, they are greater than 95% likely to experience an allergic reaction if they ingest the specific food."[8] There is a direct correlation between the food-specific IgE level and the probability that an individual will react to food if ingested. Consequently, when the medical history is taken into account, a clinician might conclude that an allergen-specific IgE level that is 60% predictive of reactivity is sufficient to make the diagnosis of clinical food allergy.

**Not included in the Siemens Healthineers IMMULITE 3gAllergy IFU.*

Agreement between predictive, allergen-specific IgE values assessed by IMMUNOCAP and IMMULITE 2000 3gAllergy assay systems for milk and wheat allergies

Al Hawi Y, Nagao M, Furuya K, Sato Y, Ito S, Hori H, Hirayama M, Fujisawa T: IPAD3g Investigators. Allergy Asthma Immunol Res. 2021 Jan;13(1):141-53.

Objectives

- Determine and correlate the predictive values of sIgE in the diagnosis of milk and wheat allergies in children.
- Compare the quantitation of sIgE by two different technologies: IMMULITE 2000 system and IMMUNOCAP.
- Multi-center study design: The patient populations tested in this study were those most likely to be encountered at general pediatric clinics.
- OFC (oral food challenge) as a gold standard was performed as a requisite diagnostic procedure being part of food allergy management.

Methods

- Prospective and observational study of children who had undergone oral food challenge (OFC) for the diagnosis of milk and wheat allergies. A total of 395 patients were recruited from 7 primary care clinics and 19 hospitals in Japan.
- The OFCs were performed to diagnose either true allergy in the 1-year-old group (A) or tolerance in the 2- to 6-year-old group (B).
- sIgE values for milk, casein and β-lactoglobulin, and wheat and ω-5 gliadin were measured on IMMULITE 2000 and IMMUNOCAP 250 systems.
- The predictive accuracy of each sIgE for the OFC outcome was assessed using receiver operating characteristic (ROC) curves. The probability of a positive OFC outcome was estimated by logistic regression analysis.

Results

- Oral food challenges (OFC) to milk were performed for 87 patients in group A and 124 in group B patients. OFCs to wheat were performed in 102 group A patients and in 82 group B patients. The ROC analysis yielded similar areas under the curve (AUC) for the 2 assays (0.7–0.9).
- For milk sIgE, IMMUNOCAP and IMMULITE 3gAllergy showed similar AUCs in both A and B groups, with slightly higher values for IMMULITE 3gAllergy.
- For wheat sIgE values, the AUCs with IMMUNOCAP and IMMULITE 3gAllergy for the wheat OFC outcomes were similar.
- The log-transformed sIgE data showed a strong linear correlation with the estimated probabilities (R >0.9). This signifies that the results of the 2 assay methods are comparable and may be interchangeable for the studied allergens.

Author's Conclusion

"The 2 tested systems, IMMULITE 2000 and IMMUNOCAP, may be interchangeable for diagnosis of milk and wheat allergies in young children."

Significance

- The strength of the study was the enrollment of patients at various ages from multiple centers across Japan who were likely to be examined at pediatric clinics, where a physician must determine (1) whether or not a toddler has true milk or wheat allergy, and (2) whether or not a preschool-aged child has outgrown an allergy.
- The predictive values (to milk and wheat allergies) established in this study fit those clinical needs.
- Based on strong correlations between the log-transformed values and predicted probabilities, the two systems may be interchangeable for the diagnosis of milk and wheat allergies in young children.*

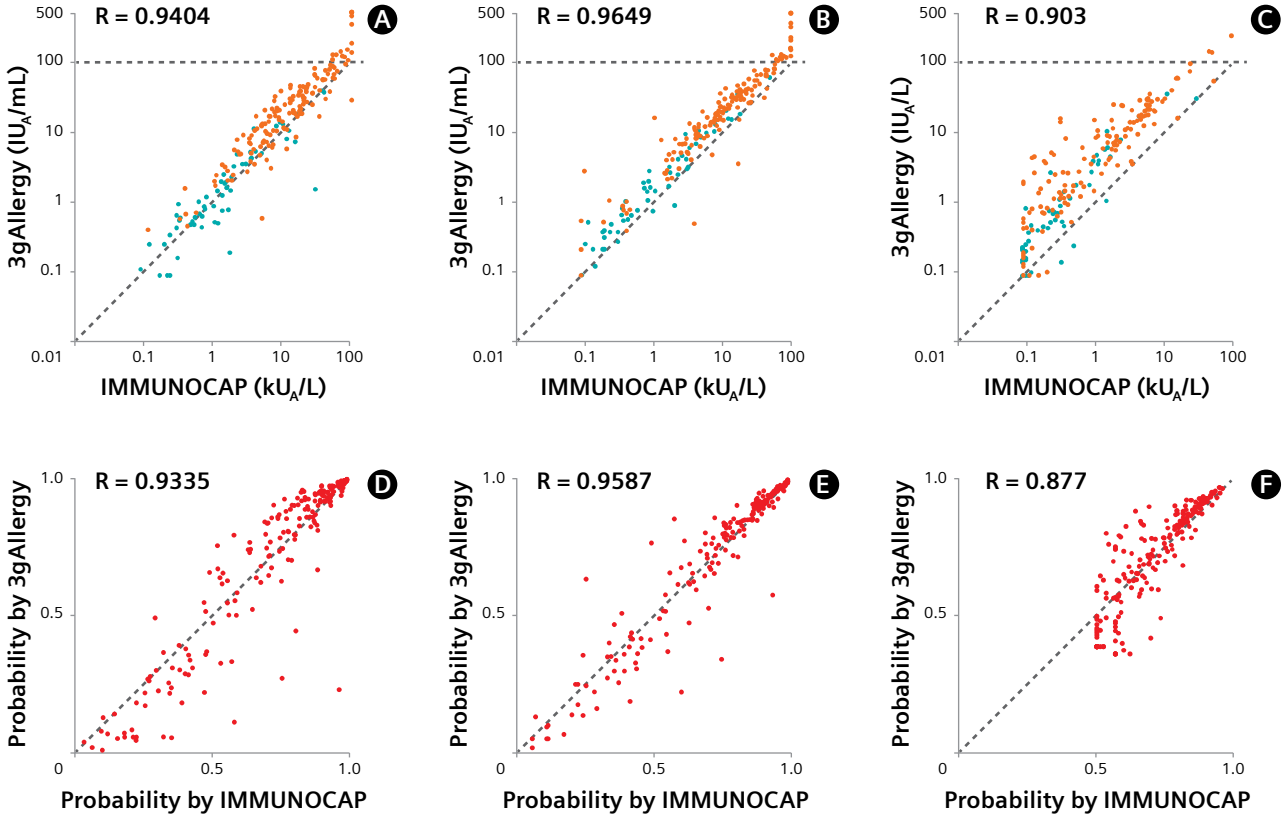

Figure 1. Correlation between the log-transformed values for milk sIgE (A), casein sIgE (B), and BLG sIgE (C) by IMMUNOCAP and 3gAllergy. Pearson's Rs were 0.9404 (95% CI, 0.9225–0.9542; P < 0.0001) (A), 0.9649 (95% CI, 0.9542–0.9731; P < 0.0001) and 0.9030 (95% CI, 0.8746–0.9252; P < 0.0001) (C). Red dots indicate patients with a positive milk OFC (Failed), and blue dots indicate patients with a negative (Passed) milk OFC. Correlation between probabilities predicted by IMMUNOCAP and 3gAllergy (D, E, F). Estimated probabilities by IMMUNOCAP and 3gAllergy for a positive milk OFC at given milk-sIgE (D), casein-sIgE (E) and BLG-sIgE (F) levels. Pearson's Rs were 0.9335 (95% CI, 0.9136–0.9489); P < 0.0001) (D), 0.9587 (95% CI, 0.9462–0.9684; P < 0.0001) (E) and 0.8770 (95% CI, 0.8416–0.9048; P < 0.0001) (F). Dotted diagonal lines connect from the origin of the axis at 0.01 to the point of 100 (A, B, and C) and from the origin of the axis at 0 to the point of 1 (D, E, and F). sIgE, specific immunoglobulin E; BLG, β-lactoglobulin; OFC, oral food challenge; CI, confidence interval.

**Not included in the Siemens Healthineers IMMULITE 3gAllergy IFU.*

Extension of food allergen-specific IgE ranges from the IMMUNOCAP to the IMMULITE systems

Hamilton RA, Mudd K, White MA, Wood RA. Ann Allergy Asthma Immunol. 2011. 107:139-44.

Objective

- Define factors that could be used to relate IMMULITE-measured IgE antibody levels for chicken egg white, cow's milk, and peanut into IMMUNOCAP-comparable quantities that could then be correlated with published levels that have been generated with the IMMUNOCAP system.

Methods

- Retrospective evaluation of serum samples from 328 patients (median age 5.4 years; age range 1–18 years; 32% female) who were known to be IgE-positive (>0.1 kU/L) to chicken egg white (n = 120), cow's milk (n = 135), and/or peanut (n = 304).
- The IgE levels were measured and analyzed in both the IMMUNOCAP and IMMULITE 2000 analyzers.
- Patient positivity to food allergens was determined by oral food challenge test and/or patient history.
- Correlation and agreement were used to compare the two assays.

Results

- IgE antibody levels from both assays for each of the 3 food specificities were highly correlated: r(2) = 0.95 for egg white, r(2) = 0.93 for milk, and r(2) = 0.95 for peanut (with $P < 0.001$ for all three allergens).
- Empirically determined IMMULITE/IMMUNOCAP ratios (mean ±1 SD) were 4.85 ±1.79 kU/L (egg), 2.33 ±1.0 kU/L (milk), and 1.86 ±0.98 kU/L (peanut).
- For milk and peanut, the IgE antibody levels for individuals who either passed or failed a food challenge were not significantly different between the assay methods. Because of the small sample size of egg white-challenged patients, no statistical analysis was performed.

Table 7. Sensitivity and specificity of sIgE measurement to the individual molecules evaluated.

Allergen	IMMUNOCAP Cutpoint[1]		Ratio		IMMULITE 2000 Calculated Cutpoint
Milk	15	x	2.33	=	35 kU/L
Egg White	7	x	4.85	=	34 kU/L
Peanut	14	x	1.86	=	26 kU/L

1. Sampson HA. J Allergy Clin Immunol. 2001;107:891-6.

Author's Conclusion

"These data indicate that specific IgE levels to egg white, milk, and peanut measured by the IMMULITE and IMMUNOCAP systems are highly correlated and that differences between the systems are circumscribed and modest (IMMULITE was a mean of 2- to 5-fold higher than IMMUNOCAP)."

Significance

- Quantitative measures of food-specific IgE antibodies using the Phadia IMMUNOCAP method have documented value in the diagnosis of food allergy.
- The data in this report suggest that egg white, milk, and peanut-specific IgE antibody measurements generated by the IMMULITE system most often are higher than those determined by IMMUNOCAP, but they can be used with confidence to make decisions about whether an oral food challenge is needed to confirm a symptomatic food allergy.
- The clinician may choose to translate published IMMUNOCAP predictive data into comparable IMMULITE levels or generate new predictive criteria based on their own experience with prospectively collected IMMULITE results.
- The clinician must ultimately follow the National Institute of Allergy and Infectious Diseases (NIAID) guidelines that state that IgE antibody measurements generated by any serologic assay method alone are not diagnostic of food allergy. They must be ultimately interpreted within the context of the patient's clinical history.

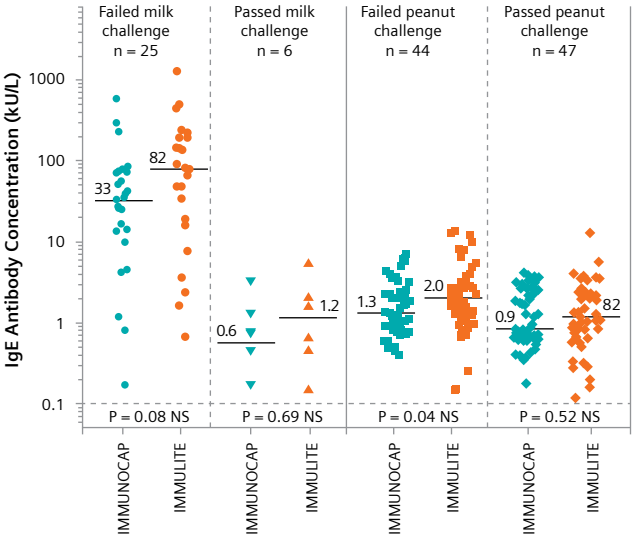

Figure 2. Levels of milk IgE antibody (left 2 panels) and peanut IgE antibody (right 2 panels) as measured by IMMUNOCAP and IMMULITE in the serum samples of patients who failed and passed a milk or peanut challenge, respectively. The horizontal lines in each data column indicate the median value of IgE antibody for that group. The only data that displayed a significant intermethod difference (e.g., P = 0.04) was in the peanut group that failed their peanut challenge.

Predicting peanut allergy in an unbiased allergy clinic population using peanut-specific IgE levels measured in two independent assays: IMMUNOCAP and IMMULITE 2000

Santos CB, Lanser BJ, Strand MJ, Gelfand EW. J Allergy Clin Immunol. 2018 Feb;L23.

Objectives

- To compare the performance of IMMULITE 2000 and IMMUNOCAP specific IgE testing to predict peanut allergy as was determined by the gold standard double blinded placebo-controlled clinical test (DBPC OFC).
- To compare the accuracy of the tests in an unbiased allergy clinic population.

Methods

- The study enrolled 51 children coming to the clinic, who either had physician-diagnosed peanut allergy (PA) or detectable levels of peanut-specific IgE. The children that had severe atopic dermatitis or asthma were excluded from the study.
- The following tests were performed on all subjects:
  - Specific IgE values to peanut extract on IMMULITE 2000
  - Specific IgE to peanut extract and to peanut component Ara h 2 IgE on IMMUNOCAP
  - Skin-prick test (SPT)
  - The DBPOFC oral food challenge
- Fitted logistic regression model expressed the probability of an allergic reaction, and 95% PPV and 50% NPV were calculated using SAS v9.4. Receiver operating curves (ROC) were constructed and area under the curve (AUC) was computed to compare each test's ability to predict clinical peanut allergy.

Results

- 51 subjects, ages 3–20 years (median = 8) underwent peanut DBPC OFC; 30 subjects failed (58.8%).
- IMMULITE peanut sIgE and IMMUNOCAP Ara h 2 component testing performed similarly and was superior to the IMMUNOCAP crude peanut sIgE assay in predicting peanut allergy.
- The area under the curve (AUC) was calculated for each test.
  - The skin prick test had the highest AUC of 0.93.
  - IMMUNOCAP component Ara h 2 and IMMULITE specific IgE had a comparable AUC of 0.87 and 0.85.
  - IMMUNOCAP specific IgE to peanut extract had the lowest AUC: 0.76.
- The 95% PPV for peanut allergy via IMMUNOCAP assay (80.3 kUA/L) is higher than previously published values.

The IMMULITE peanut sIgE and IMMUNOCAP Ara h 2 IgE assays performed similarly and were superior to the IMMUNOCAP peanut sIgE assay in predicting peanut allergy. SPT using commercial peanut extract was the most accurate test:

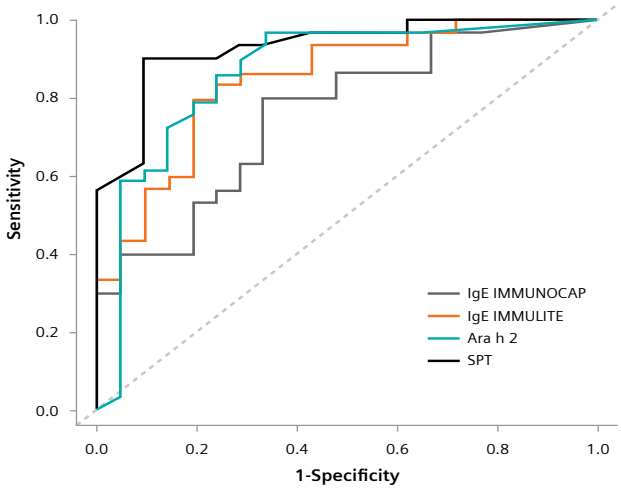

Figure 3. Receiver operating curves (ROC) to compare each test's ability to predict clinical peanut allergy.

Authors' Conclusions

"These results, generated from a unique population, proved valuable for the diagnosis of PA in a general allergy clinic population. This suggests that sIgE to Ara h 2 by IMMUNOCAP or peanut sIgE by IMMULITE may be the most accurate tests for diagnosing and predicting PA."

Significance

- The major strengths of the National Jewish Health study was that (1) this was a prospective clinical study evaluating patients who were moderately atopic, which was a more representative population seen in the allergy clinic, and (2) all subjects underwent double-blinded placebo-controlled oral food challenge regardless of the level of specific IgE results for peanuts.
- They calculated cutoffs for food allergens that may guide physicians in further diagnostic testing. This study established the IMMULITE 2000 cutoff value of 33.5 kU/L for peanut allergy. The Hamilton et al.[9] study reported a similar cutoff of 26 kU/L.
- The results of in vitro allergy testing may guide clinicians in making decisions about whether or not to perform the oral challenge for foods that triggers immediate type I hypersensitivity.

The diagnostic value of component resolved diagnostics in peanut allergy in children attending a regional pediatric allergology clinic

van Veen L, Heron M, Batstra M, van Haard P, de Groot H. BMC Pediatrics. 2016;16:74. doi: 10.1186/s12887-016-0609-7.

Objectives

- Double-blind food challenges are considered the gold standard, but they are time-consuming as well as potentially hazardous.
- To investigate the predictive value of component-resolved diagnostics for a clinically relevant allergy in a group of children suspected of a peanut allergy.
- Three objectives were of special interest:
  - Can we predict the positive or negative outcome of the DBPCFC with peanut by measuring the levels of specific IgE to different recombinant peanut allergens?
  - Can we predict the eliciting dose (ED) by using CRD?
  - Can we predict the severity of the allergic reaction occurring at the DBPCFC with peanut?

Methods

- 62 out of 72 children with suspected peanut allergy were analyzed using serum-specific IgE and/or skin prick tests and specific IgE to several components of peanut (Ara h 1, 2, 3, 6, 8, 9).
- Double-blind food challenges were performed.
- Measurement of peanut-specific IgE (sIgE) was performed in all children using the 3gAllergy assay on an IMMULITE 2000 XPi system
- Specific IgE directed against peanut protein components Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9 was determined using the Immuno Solid-phase Allergen Chip (ISAC) assay.
- The correlation between the various diagnostic tests and the overall outcome of the double-blind food challenges were studied, in particular the severity of the reaction and the eliciting dose.

Results

- The double-blind provocation with peanut was positive in 33 children (53%).
- There was no relationship between the eliciting dose and the severity of the reaction.
- A statistically significant relationship was found between the skin prick test, specific IgE directed to peanut, Ara h 1, Ara h 2, or Ara h 6, and the outcome of the food challenge test.
- Found no relationship between sensitization to peanut extract or the different allergen components and the severity of the reaction or the eliciting dose.
- No correlation between IgE directed to Ara h 3, Ara h 8, Ara h 9 and the clinical outcome of the food challenge.

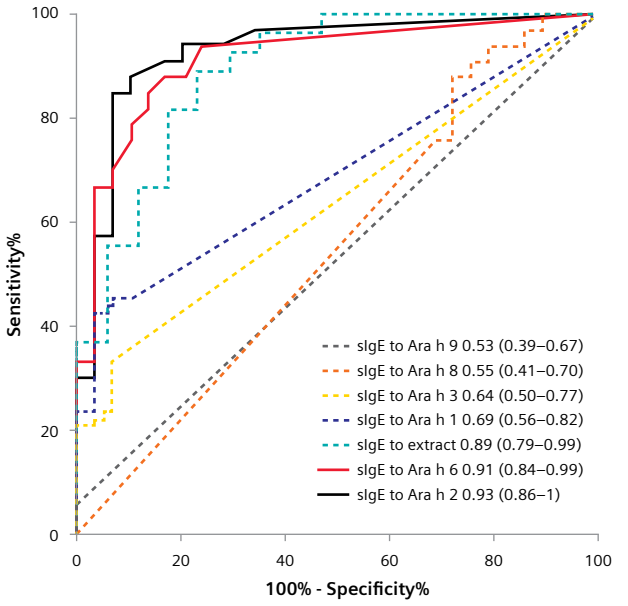

Figure 4. Receiver operating curves (ROC) data are presented for peanut components Ara h 1, 2, 3, 6, 8, 9, and peanut extract as area under the curve (95% CI).

Authors' Conclusions

The author concluded the following about IMMULITE 2000/2000 XPi 3gAllergy Specific IgE assay performance as compared to SPT: "This study shows that component-resolved diagnostics is not superior to specific IgE to peanut extract or to skin prick testing. At present, it cannot replace double-blind placebo-controlled food challenges for determination of the eliciting dose or the severity of the peanut allergy in our patient group."

Significance

- The study found a high negative predictive value for sIgE to peanut extract (100%), similar to SPT and superior to the negative predictive value of specific IgE to individual peanut components. This means that with a negative test (sIgE to peanut or SPT), a food challenge will not be necessary anymore, and peanut should be reintroduced into the diet.
- The positive predictive value of all the diagnostic tests (sIgE, SPT, recombinant allergens) was low in this patient group. This means that in sensitized patients, there is a considerable chance that the oral provocation will turn out to be negative.

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Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestr. 127
91052 Erlangen, Germany
Phone: +49 9131 84-0
siemens-healthineers.com

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Siemens Healthcare Diagnostics Inc.
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