



Validation of a Multiplex Assay for Measuring Specific IgG4

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Purpose: Specific IgG4 (sIgG4) increases with allergen specific immunotherapy and may reflect a state of immune tolerance in food allergy. While ImmunoCAP[®] has been widely used to measure sIgG4 to a single allergen, PROTIA[™] Specific IgG4[®] has been designed as a multiplex assay for measuring sIgG4. This study sought to validate this assay in comparison to ImmunoCAP[®].

Materials and Methods: Measurements of sIgG4 were compared between PROTIA[™] Specific IgG4[®] and ImmunoCAP[®] using sera from 519 allergy patients (asthma: 114, allergic rhinitis: 318, food allergy: 146) with 731 paired tests. sIgG4 was measured against nine inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat dander, dog dander, birch pollen, oak pollen, ragweed pollen, mugwort pollen, and *Alternaria alternata* spores) and nine food allergens (egg white, casein, wheat, peanut, walnut, crab, shrimp, apple, and peach).

Results: PROTIA[™] Specific IgG4[®] showed 95.6% agreement rate with ImmunoCAP[®] in the positivity comparison. For sIgG4 positivity to each individual allergen, an agreement rate of more than 84.8% was observed. In Cohen's kappa analysis, these assays displayed substantial correlations [Cohen's kappa coefficient (κ) ≥ 0.699], except for shrimp ($\kappa=0.448$). Furthermore, both assays displayed strong correlations in quantitative comparisons [correlation coefficients value (ρ) ≥ 0.8014], except for apple ($\rho=0.6571$, $p=0.175$). Serial dilution tests also showed consistency between the assays.

Conclusion: PROTIA[™] Specific IgG4[®] showed high consistency with ImmunoCAP[®] in measuring sIgG4. This assay is applicable to various clinical fields, including allergen immunotherapy and food allergy.

Key Words: Immunoglobulin G, immunoassay, allergens

INTRODUCTION

Immunoglobulin G (IgG) is the most common immunoglobulin, accounting for approximately 75% of all immunoglobulins in the blood.¹ IgG is further subdivided into the subtypes IgG1, IgG2, IgG3, and IgG4,² among which IgG4 accounts

for approximately 2–5% of all IgG.² Unlike the other IgG subtypes, IgG4 is known to have non-inflammatory properties.³ It forms a bispecific monovalent antibody through Fab arm exchange,⁴ exhibits a limited ability to cross-link with allergens, and cannot form immune complexes.^{3,5} Further, it has a disrupted C1q-binding site and, thus, cannot activate complement pathways.⁶ It also has reduced effector function relative to other IgG subtypes.⁴ Furthermore, specific IgG4 (sIgG4) competes with specific IgE (sIgE) to prevent mast cell and basophil degranulation and is known as a blocking antibody.³ These unique characteristics of IgG4 make it favorable for the assessment of immunotherapy efficacy: sIgG4 levels are increased following immunotherapy and then decrease after termination of immunotherapy.^{3,7} In addition, sIgE/sIgG4 ratios for food allergens, such as egg, milk, and peanut, are associated with the prediction of immune tolerance to these aller-

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gens.^{8,9} Therefore, sIgG4 is considered as a monitoring tool of immunotherapy¹⁰ and an alternative to the double-blind placebo-controlled food challenge test (DBPCFC) for predict remission.¹¹

Meanwhile, IgG4 can be harmful in some diseases. IgG4 is closely associated with eosinophilic esophagitis¹² and is involved in the immune response of factor VIII and factor IX.^{13,14} These factors can be inhibited by IgG4, and this immune reaction is associated with hemophilia. Moreover, IgG4 can reduce clinical responses to recombinant antibodies, such as adalimumab.¹⁵ Additionally, IgG4 is associated with parasite infection and IgG4-related disease.^{16,17} Therefore, IgG4 may be used as biomarkers for such pathologic conditions.

The ImmunoCAP[®] assay (Thermo Fisher Scientific, Uppsala, Sweden) has been widely used to detect sIgG4. However, ImmunoCAP[®] is a singleplex assay, and the cost per allergen is higher than that of a multiplex assay. Recently, PROTIA[™] Specific IgG4[®] (ProteomeTech Inc., Seoul, Korea), a multiplex sIgG4 assay, was developed to solve this issue. It is based on the enzyme immunoassay technique that uses nitrocellulose membrane as the solid-phase for allergen immobilization and can quantify sIgG4 against 42 important food and inhalant allergens using only 10 μ L of serum (Table 1). This study aimed to validate PROTIA[™] Specific IgG4[®] in comparison with ImmunoCAP[®].

MATERIALS AND METHODS

Study participants

Five hundred and nineteen Korean allergy patients who visited the Allergy and Asthma Center of Severance Hospital for the diagnosis and treatment of their allergic diseases from August 2013 to May 2019 were enrolled in this study. The demographic characteristics of the patients are summarized in Table 2. There were 310 males and 209 females. The mean age of the patients was 24.0 \pm 16.9 years (mean \pm standard deviation). Three hundred and eighteen patients (61.3 %) had allergic rhinitis, 198 (38.2%) patients had atopic dermatitis, 146 patients (28.1%) had food allergy, and 114 patients (22.0%) had asthma. Patients did not have other chronic diseases, including autoimmune diseases, cancer, chronic infections, or other immune-related diseases. Blood samples were collected from all patients to perform the two diagnostic tests, PROTIA[™] Specific IgG4[®] and ImmunoCAP[®]. The total number of paired tests was 731, and the most frequently tested allergen was *Dermatophagoides farinae* (235), followed by *Dermatophagoides pteronyssinus* (176), *Alternaria alternata* spores (36), walnut (33), casein (31), cat dander (30), dog dander (26), egg white (24), wheat (24), peanut (23), peach (17), crab (16), shrimp (16), apple (15), birch pollen (10), oak pollen (7), ragweed pollen (7), and mugwort pollen (5). The Severance Hospital Ethical Review Board approved this study (No. 4-2017-1258), and in-

Table 1. Specific IgG4s That Can Be Quantified Using PROTIA[™] Specific IgG4[®]

Number	Allergen	Number	Allergen
1	<i>Dermatophagoides pteronyssinus</i>	22	Milk
2	<i>Dermatophagoides farinae</i>	23	Casein
3	Cat dander	24	α -lactalbumin
4	Dog dander	25	β -lactoglobulin
5	Birch pollen	26	Codfish
6	Oak pollen	27	Mackerel
7	Grass pollen mix	28	Crab
8	Ragweed pollen	29	Shrimp
9	Mugwort pollen	30	Oyster
10	Japanese hop pollen	31	Wheat
11	<i>Aspergillus fumigatus</i> spores	32	Buckwheat
12	<i>Penicillium notatum</i> spores	33	Rice
13	<i>Alternaria alternata</i> spores	34	Soy bean
14	Bee venom	35	Peanut
15	Wasp venom	36	Walnut
16	Cockroach	37	Almond
17	Pork	38	Peach
18	Beef	39	Apple
19	Chicken	40	Kiwi
20	Egg yolk	41	Mango
21	Egg white	42	α -Gal

formed consent was obtained from all 519 patients.

Serum preparation and allergen selection

Five milliliters of whole blood was collected in a vacuum tube (Vacuette[®]; Greiner Bio-One GmbH, Kremsmünster, Austria) for serum separation via centrifugation at 3000 rpm for 5 min, and the supernatant was aliquoted into several round-bottom tubes (5-mL BD Falcon[™] tubes; BD Bioscience Discovery Labware, Bedford, MA, USA), and stored at -76°C until further use.

Eighteen culprit allergens were selected for measuring sIgG4: nine inhalant allergens (*D. pteronyssinus*, *D. farinae*, cat dander, dog dander, birch pollen, oak pollen, ragweed pollen, mugwort pollen, and *Alternaria alternata* spores) and nine food allergens (egg white, casein, wheat, peanut, walnut, crab, shrimp, apple, and peach).

In vitro sIgG4 measurements

Both PROTIA[™] Specific IgG4[®] and ImmunoCAP[®] sIgG4 assays were performed according to the manufacturers' instructions. The researcher performing the PROTIA[™] Specific IgG4[®] assay was blinded to the results of ImmunoCAP[®]. The sIgG4 detection range of PROTIA[™] Specific IgG4[®] was 0.50–30 mg/L, and that of ImmunoCAP[®] was 0.07–30 mg/L. To compare the agreement rate of these two assays, sIgG4 values \geq 0.50 mg/L were regarded as positive.

Table 2. Demographic Characteristics of the 519 Studied Subjects in Accordance with the Allergens

Allergens	Number of samples	Male: female	Age, yr (mean±SD)	ImmunoCAP sIgE			Underlying disease			
				Positive	Negative	Not performed	AR	Asthma	AD	Food allergy
<i>Dermatophagoides pteronyssinus</i>	176	112:64	25.8±10.8	158 (89.8)	4 (2.3)	14 (8.0)	164 (93.2)	32 (18.2)	105 (59.7)	12 (6.8)
<i>Dermatophagoides farinae</i>	235	146:89	29.1±13.8	222 (94.5)	4 (1.7)	9 (3.8)	223 (94.9)	52 (22.1)	7 (3.0)	9 (3.8)
Cat dander	30	15:15	32.7±13.4	30 (100)	0 (0)	0 (0)	22 (73.3)	17 (56.7)	9 (30.0)	0 (0)
Dog dander	26	11:15	31.4±13.5	23 (88.5)	1 (3.8)	2 (7.7)	17 (65.4)	16 (61.5)	13 (50.0)	0 (0)
Birch pollen	10	8:2	19.4±7.5	9 (90.0)	1 (10.0)	0 (0)	7 (70.0)	5 (50.0)	1 (10.0)	0 (0)
Oak pollen	7	6:1	21.0±10.8	4 (57.1)	3 (42.9)	0 (0)	4 (57.1)	2 (28.6)	1 (14.3)	0 (0)
Ragweed pollen	7	4:3	45.6±10.0	6 (85.7)	1 (14.3)	0 (0)	7 (100)	3 (42.9)	1 (14.3)	0 (0)
Mugwort pollen	5	2:3	38.2±16.1	2 (40.0)	2 (40.0)	1 (20.0)	5 (100)	0 (0)	0 (0)	0 (0)
<i>Alternaria alternata</i> spores	36	25:11	17.0±14.3	36 (100)	0 (0)	0 (0)	22 (61.1)	14 (38.9)	7 (19.4)	9 (25.0)
Egg white	24	17:7	4.8±3.8	24 (100)	0 (0)	0 (0)	3 (12.5)	3 (12.5)	12 (50.0)	21 (87.5)
Casein	31	19:12	9.3±13.3	24 (77.4)	7 (22.6)	0 (0)	2 (6.5)	5 (16.1)	18 (58.1)	28 (90.3)
Wheat	24	16:8	12.8±16.8	18 (75.0)	6 (25.0)	0 (0)	3 (12.5)	0 (0)	8 (33.3)	14 (58.3)
Peanut	23	12:11	7.5±9.2	20 (87.0)	2 (8.7)	1 (4.3)	3 (13.0)	3 (13.0)	9 (39.1)	15 (65.2)
Walnut	33	17:16	8.4±4.9	32 (97.0)	1 (3.0)	0 (0)	5 (14.7)	4 (11.8)	10 (29.4)	15 (44.1)
Crab	16	9:7	43.4±17.2	14 (87.5)	2 (12.5)	0 (0)	2 (12.5)	0 (0)	1 (6.3)	10 (62.5)
Shrimp	16	11:5	31.9±21.5	14 (87.5)	2 (12.5)	0 (0)	5 (31.3)	1 (6.3)	1 (6.3)	13 (81.3)
Apple	15	9:6	31.9±20.6	15 (100)	0 (0)	0 (0)	10 (66.7)	2 (13.3)	2 (13.3)	12 (80.0)
Peach	17	8:9	23.2±14.9	14 (82.4)	2 (11.8)	1 (5.9)	4 (23.5)	1 (5.9)	4 (23.5)	5 (29.4)
Total	731 (519 patients)	310:209	24.0±16.9	665 (90.8)	38 (5.2)	28 (3.8)	318 (61.3)	114 (22.0)	198 (38.2)	146 (28.1)

AR, allergic rhinitis; AD, atopic dermatitis. Data are presented as number (%) unless otherwise noticed.

Serial dilution test

sIgG4 levels were measured by PROTIA™ Specific IgG4® using serial dilutions of patient sera. Sera with high IgG4 titers against *D. pteronyssinus*, *D. farinae*, cat dander, dog dander, birch pollen, or ragweed pollen were selected for this analysis. Selected serum samples were diluted one-, two-, four-, eight-, 16-, 32-, 64-, and 128-fold with immunoglobulin-depleted serum.

Statistical analysis

The positivity of sIgG4 values was compared using Cohen’s kappa analysis. Cohen’s kappa values (κ) were categorized as almost perfect (0.8–1.0), substantial (0.6–0.8), moderate (0.4–0.6), fair (0.2–0.4), or poor correlations (below 0.2).¹⁸ Pearson’s correlation coefficients were used for quantitative comparisons of house dust mite sIgG4, and Spearman’s rank coefficient was used for quantitative comparisons of sIgG4 for other allergens. The degrees of correlation were expressed as a Pearson’s correlation coefficient value (r) or Spearman’s correlation coefficient value (ρ). Correlation coefficient values were categorized as very strong (0.90–1.00), strong (0.70–0.89), moderate (0.40–0.69), weak (0.10–0.39), or negligible correlations (0.00–0.09).¹⁹ Because negative values cannot be compared, only sIgG4 values ≥0.50 mg/L were used for quantitative comparison. SPSS 25.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis, and p<0.05 was considered statis-

tically significant.

RESULTS

Qualitative comparison between the two assays

Comparing positivity with the cut-off level of 0.50 mg/L, the agreement rate for all 731 paired tests was 95.6%, with at least equal or more than 84.8% agreement rate (walnut) being observed between the two assays for each allergen (Table 3). Inhalant allergens showed high agreement rates of more than 90%. Food allergens also showed high agreement rates of more than 90%, except walnut (84.8%) and shrimp (87.5%). In the Cohen’s kappa analysis, most inhalant allergens showed almost perfect correlation (κ>0.8). Dog dander showed substantial correlation (κ=0.752). Most food allergens also showed almost perfect correlation, and walnut showed substantial correlation (κ=0.699). However, shrimp showed only moderate correlation (κ=0.448). The κ value could not be determined for crab because all sIgG4 titers against crab were negative.

Quantitative comparison between the two assays

The measured sIgG4 values of 12 allergens (*D. pteronyssinus*, *D. farinae*, cat dander, dog dander, birch pollen, ragweed pollen, egg white, casein, wheat, peanut, walnut, and peach) that

Table 3. Qualitative Comparison of ImmunoCAP® and PROTIA™ Specific IgG4®

Allergens	ImmunoCAP®		PROTIA™ Specific IgG4®		Agreement rate (%)	κ value
	Positive (n)	Negative (n)	Positive (n)	Negative (n)		
<i>Dermatophagoides pteronyssinus</i>	69	107	79	97	94.3	0.884 [†]
<i>Dermatophagoides farinae</i>	91	144	93	142	97.4	0.946 [†]
Cat dander	18	12	16	14	93.3	0.865 [†]
Dog dander	21	5	21	5	92.3	0.752 [†]
Birch pollen	5	5	5	5	100.0	1*
Oak pollen	2	5	2	5	100.0	1*
Ragweed pollen	6	1	6	1	100.0	1*
Mugwort pollen	1	4	1	4	100.0	1*
<i>Alternaria alternata</i> spore	1	35	1	35	100.0	1 [†]
Egg white	12	12	12	12	100.0	1 [†]
Casein	21	10	22	9	96.8	0.924 [†]
Wheat	15	9	15	9	100.0	1 [†]
Peanut	7	16	9	14	91.3	0.810 [†]
Walnut	12	21	17	16	84.8	0.699 [†]
Crab	0	16	0	16	100.0	N/A
Shrimp	1	15	3	13	87.5	0.448*
Apple	6	9	7	8	93.3	0.865 [†]
Peach	11	6	10	7	94.1	0.876 [†]
Total	299	432	319	412	95.6	0.910 [†]

N/A, not applicable.

κ=Cohen's kappa coefficient, κ values were categorized as almost perfect (0.8–1.0), substantial (0.6–0.8), moderate (0.4–0.6), fair (0.2–0.4), or poor correlations (<0.2).

* $p < 0.05$, [†] $p < 0.001$.

were positive in both assays are shown in Fig. 1. The sIgG4 values of other allergens that had only a few cases (oak pollen, mugwort pollen, *Alternaria alternata* spores, shrimp, and apple) are shown in Supplementary Fig. 1 (only online). The values of the two assays showed consistent tendencies, and correlation analysis between the two assays also showed a significant correlation (Table 4). Inhalant allergens showed more than strong correlation (r or $\rho > 0.7$). Food allergens also showed more than strong correlation, except for apple (moderate, $\rho = 0.6571$, $p = 0.175$). Oak pollen, mugwort pollen, *Alternaria alternata* spores, crab, and shrimp could not be assessed owing to very few positive pairs of results.

Serial dilution test

Each serum sample with positive sIgG4 against *D. pteronyssinus* (12.3 mg/L), *D. farinae* (12.0 mg/L), cat dander (19.4 mg/L), dog dander (7.3 mg/L), birch pollen (14.5 mg/L), and ragweed pollen (10.87 mg/L) was serially diluted with immunoglobulin-depleted serum. The decreasing concentration-dilution curves for sIgG4 values are illustrated in Fig. 2. The average reduction rates based on the two-fold dilution for sIgG4 were around 50%: *D. pteronyssinus* (51%), *D. farinae* (48%), cat dander (51%), dog dander (54%), birch pollen (53%), and ragweed pollen (50%). When values were lower than the measurable value, tests showing negative results were repeated.

DISCUSSION

This study is the first, to our knowledge, to validate the sIgG4 measurement ability of PROTIA™ Specific IgG4®. This assay showed a high agreement with ImmunoCAP® for the qualitative and quantitative measurements of sIgG4. Furthermore, the serial dilution test confirmed the measurement consistency of this assay. The PROTIA™ Specific IgG4® assay can simultaneously measure sIgG4 against 42 important allergens, and this assay can be performed with very small amounts of serum.

sIgG4 is widely used as a biomarker for immunotherapy,¹⁰ and its level increases during immunotherapy. Therefore, it is considered as a compliance marker of immunotherapy,¹⁰ and because an increase in sIgG4 is associated with a beneficial effect in immunotherapy, a low sIgG4 level is a predictor of a poor immunotherapeutic effect.¹⁰ However, in immunotherapy with multiple allergens, multiple ImmunoCAP® tests are needed to measure the sIgG4 levels of each allergen, making the cost of the assay relatively high. On the contrary, a single test with PROTIA™ Specific IgG4® can simultaneously quantify the sIgG4 levels of multiple allergens. This assay can measure sIgG4 levels against allergens commonly used for immunotherapy, including house dust mites, birch pollen, oak pollen, ragweed pollen, mugwort pollen, cat dander, dog dander, *Alternaria alternata* spores, bee venom, egg, milk, and peanut. Therefore, PROTIA™ Specific IgG4® is appropriate for observing the progress of immunotherapy for multiple allergens. Multiple allergen

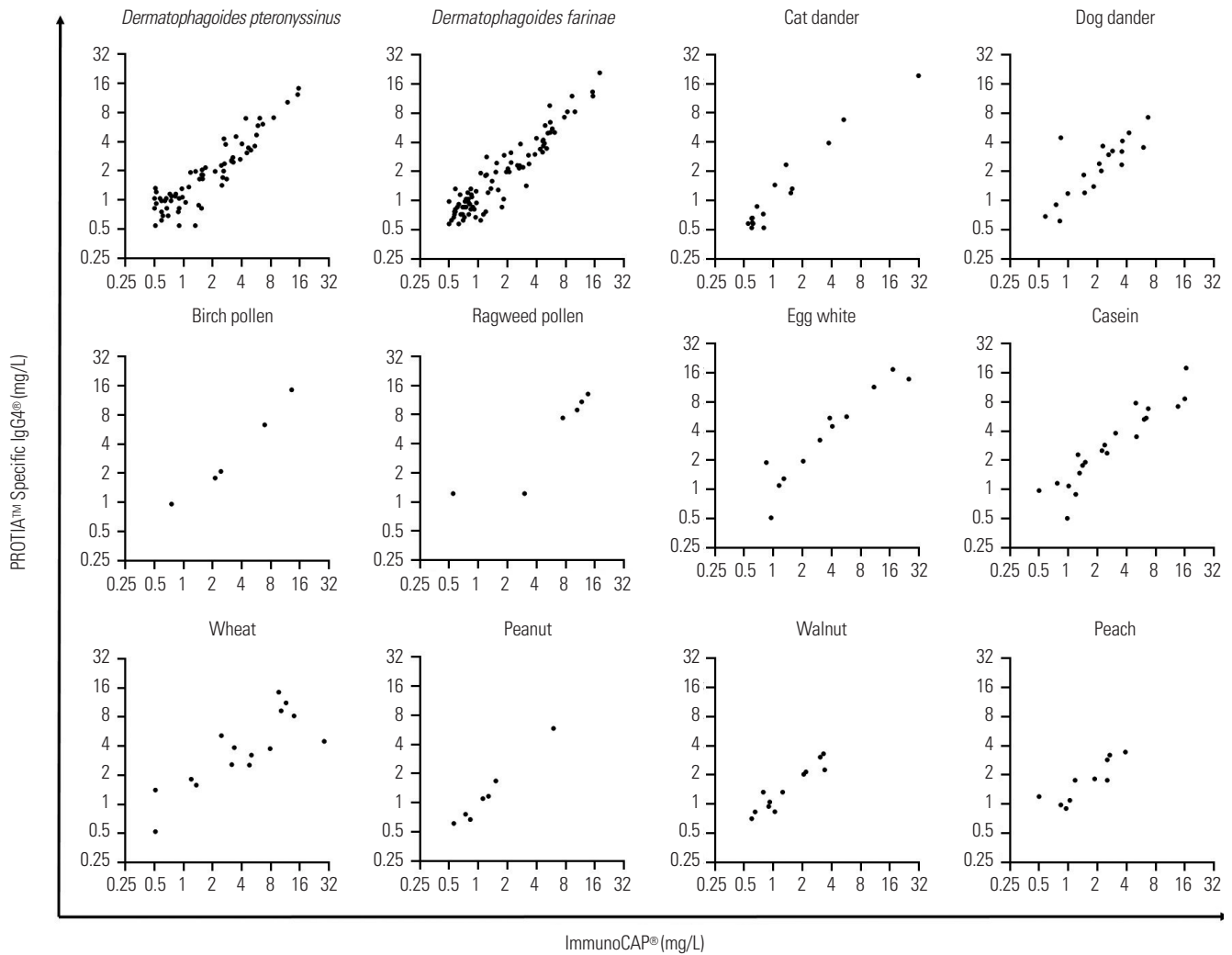


Fig. 1. Measured sIgG4 values for 12 allergens that were positive in both ImmunoCAP® and PROTIA™ Specific IgG4®.

Table 4. Quantitative Comparison between ImmunoCAP® and PROTIA™ Specific IgG4®

Allergens	Number of cases	Correlation coefficient value	Degree of correlation	p value
<i>Dermatoptogoides pteronyssinus</i>	69	0.9651	Very strong	<0.001
<i>Dermatoptogoides farinae</i>	89	0.9582	Very strong	<0.001
Cat dander	16	0.8400	Strong	<0.001
Dog dander	20	0.8014	Strong	<0.001
Birch pollen	5	1.0000	Very strong	0.017
Ragweed pollen	6	0.9856	Very strong	0.006
Egg white	12	0.9441	Very strong	<0.001
Casein	21	0.9494	Very strong	<0.001
Wheat	15	0.8097	Strong	<0.001
Peanut	7	0.9643	Very strong	0.003
Walnut	12	0.9037	Very strong	<0.001
Apple	6	0.6571	Moderate	0.175
Peach	10	0.8811	Strong	0.001

Positive results in both tests were compared through correlation analysis. A sIgG4 value ≥ 0.50 mg/L was considered a positive result. Correlation coefficient values were categorized as very strong (0.90–1.00), strong (0.70–0.89), moderate (0.40–0.69), weak (0.10–0.39), or negligible correlations (0.00–0.09).

immunotherapy is especially popular in the US where the mean number of prescribed allergens is about 8.²⁰

sIgG4 is an important marker for food allergy immunotherapy.²¹ Although it is unclear whether IgG4 is directly linked to the development of tolerance to food allergens,²² food allergen immunotherapy is often associated with an increase in sIgG4 and a reduction in sIgE,²³ and high sIgG4 and low sIgE levels have been reported to be associated with food allergy tolerance.²¹ Furthermore, patients with food allergy who test positive have a

higher sIgE/sIgG4 than those who test negative during DBP-CFC.⁸ Therefore, sIgG4 is used as an immune tolerance biomarker for food allergy.²¹ However, patients with food allergy are often sensitized to multiple allergens. Lee, et al.²⁴ reported that adult patients with food allergy are allergic to more than two culprit food allergen, and Wang²⁵ reported that pediatric patients are allergic to three to four culprit food allergens. In addition, because pollen-food allergy syndrome is often associated with pollinosis patients,^{26,27} sIgG4 levels of several food aller-

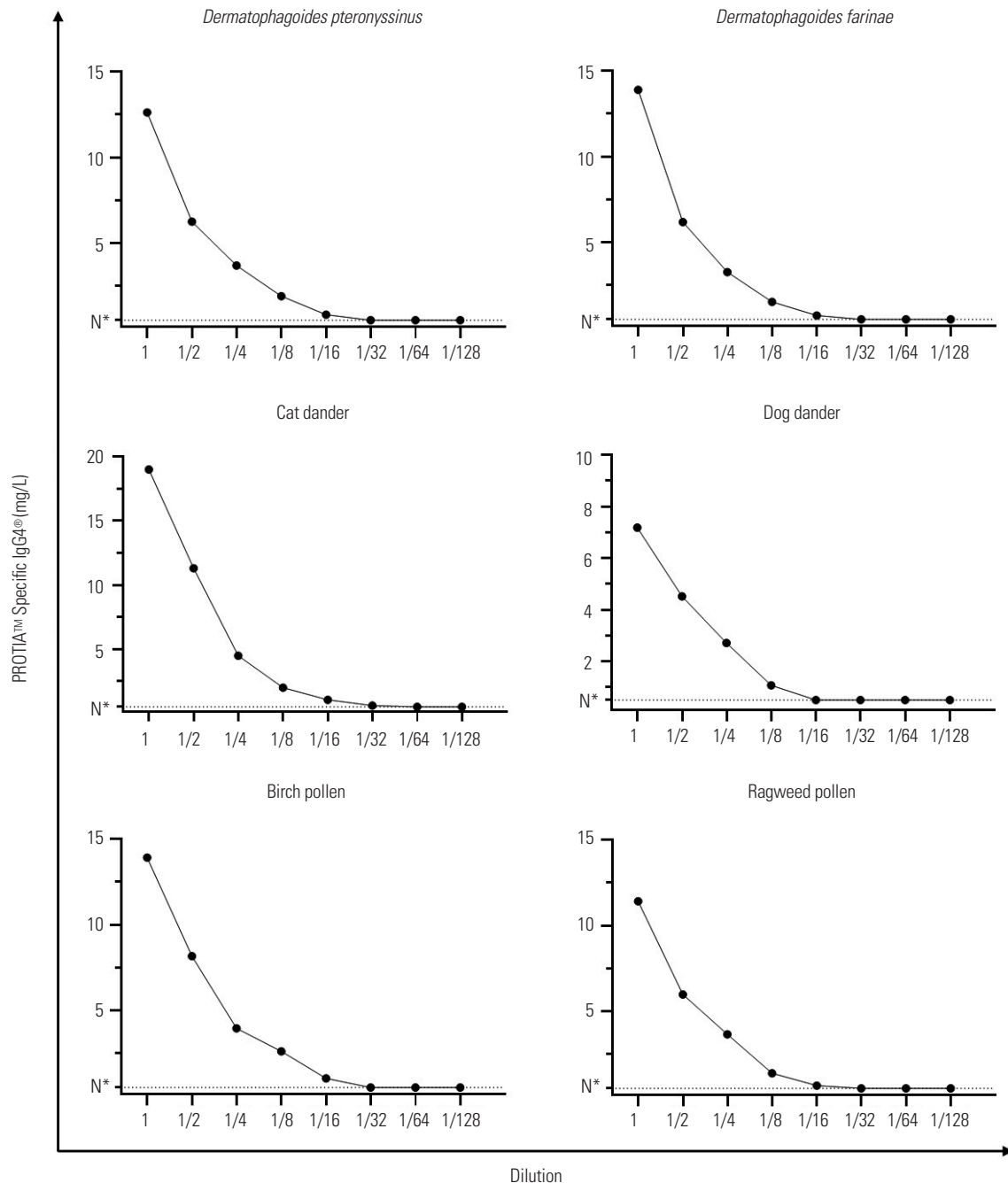


Fig. 2. Serial dilution tests performed using PROTIA™ Specific IgG4®. Patient sera were diluted with immunoglobulin-depleted serum. N*: negative. Specific IgG4 <0.50 mg/L is considered a negative result.

gens might need to be tested during pollen immunotherapy. Therefore, there is a limit in measuring sIgG4 with a singleplex assay. Because PROTIA™ Specific IgG4® can measure 26 food allergens, this assay can be applied to monitor immunotherapy for food allergy patients with multiple culprit allergens or to predict the tolerance status of those patients.

Meanwhile, sIgG4 may play a vicious role in other diseases. sIgG4 is associated with eosinophilic esophagitis, and the level of tissue IgG4 correlates with the severity of eosinophilic esophagitis and tissue eosinophilia, suggesting the harmful role of sIgG4 in this disease.^{28,29} Identification of the culprit allergen by measuring sIgE and quantifying sIgG4 levels may help diagnose and treat patients with eosinophilic esophagitis.³⁰ Culprit food allergens for eosinophilic esophagitis are usually many, including common food allergens, such as milk, egg, wheat, soy, and seafood.³¹ Identification of culprit inhalant allergens may also be important in eosinophilic esophagitis, because accompanying sensitization to inhalant allergens is common.³² Oral allergy syndrome can also induce eosinophilic esophagitis,³³ and oral immunotherapy for food and sublingual immunotherapy for inhalant allergens may also cause eosinophilic esophagitis.^{34,35} sIgG4 concentrations against these food and inhalant allergens can simultaneously be determined using PROTIA™ Specific IgG4®. Therefore, this assay is potentially applicable to patients with eosinophilic esophagitis.

Another disease where IgG4 may have a pathologic role is IgG4-related disease, which is characterized by a systemic fibroinflammatory condition with an elevated serum total IgG4 level and the accumulation of IgG4-positive plasma cells in the affected organs.¹⁷ In general, total serum IgG4 levels are measured to diagnose IgG4-related disease.³⁶ Culver, et al.³⁷ also reported that food sIgG4 is elevated in IgG4-related disease. However, the association between food sIgG4 and IgG4-related disease remains unknown. Therefore, the applicability of PROTIA™ Specific IgG4® in IgG4-related disease is uncertain.

PROTIA™ Specific IgG4® may be applicable to patients with Crohn's disease, for whom dietary restrictions are recommended. Crohn's disease is an inflammatory bowel disease primarily affecting the ileum and colon.³⁸ Dietary restrictions help treat some patients with this disease.³⁹ However, the complexity of dietary restrictions makes it difficult for patients. A previous study reported an improvement in symptoms and a reduction in the erythrocyte sedimentation rate among patients on a targeted exclusion diet by quantifying sIgG4 levels.⁴⁰ Therefore, PROTIA™ Specific IgG4® might be applied to determine which foods to restrict. Similarly, dietary restrictions are effective in cases of irritable bowel syndrome,⁴¹ wherein sIgG4 levels are increased.⁴² Zar, et al.⁴² reported that dietary restrictions based on food sIgG4 are effective. However, further well designed studies are required to confirm the clinical significance of sIgG4 measurement and sIgG4-based dietary restrictions in these diseases.

There are limitations in this study. First, an sIgG4 level less

than 0.50 mg/L was considered a negative titer in this study. Since IgG production is associated with a normal immune response to allergens,⁴³ it is difficult to determine the clinical negative value, which may be quite different for each food or inhalant allergen. Therefore, 0.50 mg/L was considered as the threshold according to the detection limit of PROTIA™ Specific IgG4®. Further studies are required to determine the negative reference values of sIgG4 in healthy subjects and patients. Second, some allergens, such as walnut and shrimp in qualitative comparison and apple in quantitative comparison, showed relatively low agreement rates. This might be associated with the small sample size. There is also a possibility of kit inaccuracy. Considering that both assays were not compared for all measurable allergens, additional testing is needed. However, the most important inhalant allergens during immunotherapy, including house dust mites, birch, oak, ragweed, mugwort pollens, and *Alternaria alternata* spores, showed high agreement rates, and common culprit food allergens, including egg white, casein, milk, crab, wheat, peanut, and peach, also showed high agreement rates. Therefore, we think that the potential applicability of PROTIA™ Specific IgG4® for measuring sIgG4 in allergic diseases was adequately validated.

In conclusion, PROTIA™ Specific IgG4®, a multiple sIgG4 measurement assay, showed a high consistency with ImmunoCAP® in quantifying sIgG4 levels. This assay is suitable for various clinical applications, especially in multiple allergen immunotherapy and food allergy patients with multiple culprit allergens.

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AUTHOR CONTRIBUTIONS

Conceptualization: Bum Joon Kim, Kook Jin Lim, and Jung-Won Park. **Data curation:** Sung-Ryeol Kim, Kyung Hee Park, and Ji Eun Lee. **Formal analysis:** Sung-Ryeol Kim and Ji Eun Lee. **Funding acquisition:** Bum Joon Kim, Kook Jin Lim, and Jung-Won Park. **Investigation:** Sung-Ryeol Kim and Kyung Hee Park. **Methodology:** Sung-Ryeol Kim and Jung-Won Park. **Project administration:** Jung-Won Park. **Resources:** Kook Jin Lim and Jung-Won Park. **Software:** Kook Jin Lim and Jung-Won Park. **Supervision:** Kook Jin Lim and Jung-Won Park. **Validation:** Sung-Ryeol Kim, Kyung Hee Park, and Ji Eun Lee. **Visualization:** Sung-Ryeol Kim. **Writing—original draft:** Sung-Ryeol Kim. **Writing—review & editing:** Kyung Hee Park and Jung-Won Park. **Approval of final manuscript:** all authors.

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