

# Specific Immunoglobulin (Ig) G Reference Intervals for Common Food, Insect and Mold Allergens

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**Abstract. Background.** The clinical utility of serum IgG measurement in the diagnosis of allergy and food-induced hypersensitivity has been largely discredited. Recent studies, however, have shown that specific IgG can inhibit IgE mediated allergies, and may play a role in allergen specific desensitization. Accurate reference intervals for IgG specific allergens have not been widely established and are needed for better interpretation of serum antibody concentrations. In this study we established 64 IgG reference intervals for 48 common food allergens, 5 venoms, and 11 molds. **Design.** Specific IgG concentrations were determined employing an automated fluorescent enzyme immunoassay on serum samples from 130 normal adults (65 males and 65 females), age range 18-69 y, mean 37.3 y. **Results.** The lower reference interval limit for all allergens tested (n=64) was <2 mcg/mL. The median upper reference interval value for all 64 allergens was 12.9 mcg/mL, with Tuna (f40) having the lowest upper interval limit at 3.8 mcg/mL, and the mold *Setomelanomma rostrata* (m8) demonstrating the highest upper interval limit at 131 mcg/L. **Conclusions.** The considerable variation observed among the upper reference interval limits emphasizes the need for the establishment of allergen specific ranges for IgG. These newly established ranges should be a useful aid for clinicians in the interpretation of laboratory serum IgG results.

**Key Words:** Allergens; IgG; reference intervals; adult; immunoassay.

## Introduction

There are a growing number of laboratories that offer specific allergy immunoglobulin G (IgG) serological testing as an aid in the diagnosis of allergy and food-induced hypersensitivity or intolerance. Serological tests may provide a more timely result for clinicians when compared to food elimination and challenge diets [1]. IgG antibodies play a bigger role in Type III delayed hypersensitivity reactions and adverse symptoms may take several hours or days to develop, making food challenge interpretations difficult, and IgG serology testing an attractive alternative. Still, the clinical utility of such testing remains controversial with several scientific advisory societies speaking out against IgG testing for determining food sensitivities or allergies [2-4]. Further studies have also shown a number of incidences of patients having elevated IgG concentrations to a specific food, with no correlating clinical

symptoms [5]. Opponents of IgG allergen testing also note that most IgG lacks histamine release inducing properties and there are few controlled studies showing diagnostic value to warrant specific IgG allergen testing.

More recent studies, however, indicate that IgG allergen specific antibodies may actually play a role in desensitization and promote tolerance to IgE mediated allergies. Using peanut allergy as a model, studies have also shown that the ratio of specific IgG to IgE is significantly greater in patients with peanut sensitivity compared to those with actual peanut allergy, suggesting that IgG has an inhibitory effect on IgE. [6, 7]. With either scenario, as an aid in determining food intolerance or allergy, or for the measurement of concentrations and/or ratios of potential IgG allergen desensitizing antibodies, the clinical utility for the quantitation of IgG allergy antibodies is gaining acceptance. In this study we determined IgG reference intervals for 130 subjects for 64 allergens including 48 common foods, 5 venoms, and 11 molds.

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**Table 1.** Demographics of Healthy Adult Controls.

Characteristic	
Female (n)	65
Mean age (y) ± SD	36.4 ± 11.3
Age range (y)	18-62
Male (n)	65
Mean age (y) ± SD	38.1 ± 10.5
Age range (y)	22-69
Total (n)	130
Mean age (y) ± SD	37.3 ± 10.8
Age range (y)	18-69

## Materials and Methods

**Clinical samples.** Sera were collected from 130 self-assessed healthy adults (65 males and 65 females) ranging in age from 18 to 69 years. Exclusion criteria included patients taking prescription medication or with significant clinical history of a pathological condition. None of the subjects reported severe food allergies or were suffering from asthma, rhinitis, urticaria or atopic dermatitis at the time of sample collection, and none were on medication for these disorders. It is possible that some may have had seasonal allergies that were not active at the time of enrollment. All studies performed with human subjects were approved by the Institutional Review Board of the University of Utah. Patient demographics are shown in **Table 1**.

**Total IgG determination.** Specific IgG allergens were measured using the Thermo Scientific ImmunoCAP test system (Thermo Scientific, Phadia USA, Portage MI). This assay employs an FEIA (Fluorescent enzyme immunoassay) methodology in which patient serum (diluted 1:100) is incubated with the specific allergen that has been covalently coupled to an ImmunoCAP (a spongy, hydrophilic activated cellulose carrier). During this incubation phase, patient IgG allergen specific antibody binds to the ImmunoCAP. Non-specific IgG antibodies are then washed away leaving the patient IgG-allergen complex. A  $\beta$ -Galactosidase labeled mouse anti-IgG monoclonal antibody is then added forming an allergen/IgG/anti-IgG enzyme complex upon incubation. After incubation, unbound anti-IgG enzyme is washed away. The remaining bound complex is then incubated with a developing agent (4-Methylumbelliferyl- $\beta$ -D-galactoside). The reaction is stopped by the addition of 4% sodium carbonate and the fluorescence of the eluate is measured. To quantify test results, fluorescence of the patient sample is compared directly with fluorescence of standards assayed in parallel. The higher

the fluorescence value, the greater the concentration of allergen specific IgG present in the patient serum. The calibrators are assayed in duplicate and are traceable to the International Reference Preparation 67/86 for Human Serum Immunoglobulins A, G, and M from the World Health Organization. Lower limit of detection of the assay was 2.0 mcg/mL. The list of allergens for which reference intervals were established is shown in **Table 2**.

The allergen specific ImmunoCAPs used in the IgG assay are identical to those used in the IgE assays. Comparison of the calibrator counts, curve data, and quality control calculated values show that the runs were acceptable and essentially equivalent.

**Statistical Analysis.** Data analyses were performed using the EP Evaluator program (Data Innovations, LLC: Release 10; Copyright 1991-2013). Outliers excluded from the reference interval analysis were determined as follows: outliers were determined based on Dixon's Test or Dixon-Q Statistics and eliminated if a point exceeded a third of the total range of values above the next highest point. SD ratios were then calculated by EP Evaluator software to determine if sex partitioning was required. Non-parametric analysis was used according to the CLSI C28-A guidelines for all allergens with sufficient patient number (>120), and transformed parametric analysis was used for groups less than 120 [8].

## Results

Allergen specific IgG reference intervals were established for 48 common foods, 6 insect venoms, and 11 molds using 130 normal adult samples with equal male/female distribution ranging in age from 18 to 69 y (**Table 1**). IgG reference intervals for the food-associated allergens are shown in **Table 2**. There was considerable variation between the upper reference interval limits with whey (rf236) being the highest at 88.6 mcg/mL and tuna (f40) the lowest at 3.8 mcg/mL. Mean upper reference interval was 19.5 mcg/mL for all the food allergens. Reference intervals for the 5 insect venoms (**Table 2**) were more closely grouped with white-faced hornet (i2) having the highest upper limit at 8.5 mcg/mL, and honey bee (i1) the lowest 5.5 mcg/mL. The molds showed the highest overall measurable IgG responses with the mean upper range of 53.0 mcg/mL for the 11 allergens tested in this group. *Setomelanomma rostrata* (m8) had the highest upper range at 131 mcg/mL, and *Rhizopus nigricans* (m11) the lowest at 8.4 mcg/mL.

**Table 2.** Specific IgG reference intervals for common food allergens, insect venoms and molds.

Food Allergens	CAP ID	Reference interval mcg/mL	n	Analysis
Almond	f20	<2-15.2	125	Nonparametric
Avocado	f96	<2-9.1	97	Transformed parametric
Baker's Yeast	f45	<2-11.4	129	Nonparametric
Banana	f92	<2-46.1	129	Nonparametric
Barley	f6	<2-20.3	125	Nonparametric
Beef	f27	<2-22.0	128	Nonparametric
Black Olive R	f342	<2-21.6	99	Transformed parametric
Broccoli	f260	<2-9.3	98	Transformed parametric
Casein	f78	<2-38.7	88	Transformed parametric
Cashew	f202	<2-7.2	99	Transformed parametric
Cheese, Cheddar	f81	<2-46.2	97	Transformed parametric
Cheese, Mold	f82	<2-85.6	99	Transformed parametric
Chicken	f83	<2-6.2	129	Nonparametric
Chocolate	f93	<2-20.4	129	Nonparametric
Clam	f207	<2-12.8	97	Transformed parametric
Coconut	f36	<2-6.3	99	Transformed parametric
Coffee R	f221	<2-13.5	128	Nonparametric
Corn	f8	<2-10.5	88	Transformed parametric
Crab	f23	<2-9.1	99	Transformed parametric
Egg, White	f1	<2-15.7	88	Transformed parametric
Egg, Whole	f245	<2-30.2	98	Transformed parametric
Egg, Yolk	f75	<2-19.6	128	Nonparametric
Garlic	f47	<2-12.7	129	Nonparametric
Gluten	f79	<2-47.4	125	Nonparametric
Lettuce	f215	<2-11.3	129	Nonparametric
Lobster	f80	<2-7.3	99	Transformed parametric
Malt	f90	<2-22.3	125	Nonparametric
Mushroom (champignon)	Rf212	<2-15.6	129	Nonparametric
Oat	f7	<2-13.3	85	Transformed parametric
Onion	f48	<2-17.0	129	Nonparametric
Orange	f33	<2-8.6	129	Nonparametric
Oyster	f290	<2-13.4	99	Transformed parametric
Peanut	f13	<2-6.8	86	Transformed parametric
Pineapple	f210	<2-12.5	98	Transformed parametric
Pork	f26	<2-7.9	128	Nonparametric
Potato	f35	<2-6.1	129	Nonparametric
Rice	f9	<2-16.7	127	Nonparametric
Rye	f5	<2-26.7	125	Nonparametric
Scallop	f338	<2-11.0	99	Transformed parametric
Shrimp	f24	<2-10.4	99	Transformed parametric
Soybean	f14	<2-5.3	85	Transformed parametric
Strawberry	f44	<2-12.1	99	Transformed parametric
Tomato	f25	<2-7.2	127	Nonparametric
Tuna	f40	<2-3.8	99	Transformed parametric
Turkey meat	f284	<2-6.1	99	Transformed parametric
Walnut	f256	<2-6.8	99	Transformed parametric
Wheat	f4	<2-60.2	86	Transformed parametric
Whey, Cow	Rf236	<2-88.6	129	Nonparametric

**Table 2 (continued).** Specific IgG reference intervals for common food allergens, insect venoms and molds.

Venoms	CAP ID	Reference		Analysis
		interval mcg/mL	n	
Common Wasp	i3	<2-6.3	127	Nonparametric
Honey Bee	i1	<2-5.5	127	Nonparametric
Paper Wasp	i4	<2-7.5	129	Nonparametric
White-Faced Hornet	i2	<2-8.5	129	Nonparametric
Yellow Hornet	i5	<2-6.9	129	Nonparametric

  

Molds	CAP ID	Reference		Analysis
		interval mcg/mL	n	
Alternaria tenuis/alternata	m6	<2-16.7	129	Nonparametric
Aureobasidium pullulans	m12	<2-17.5	126	Nonparametric
Candida albicans (yeast)	m5	<2-84.0	128	Nonparametric
Cladosporium herbarum	m2	<2-86.1	129	Nonparametric
Fusarium proliferatum	m9	<2-46.4	128	Nonparametric
Mucor racemosus	m4	<2-9.8	126	Nonparametric
Penicillium chrysogenum/notatum	m1	<2-55.5	129	Nonparametric
Phoma betae	m13	<2-12.9	128	Nonparametric
Rhizopus nigricans	m11	<2-8.4	125	Nonparametric
Setomelanomma rostrata	m8	<2-131	129	Nonparametric
Stemphylium herbarum	m10	<2-115	129	Nonparametric

## Discussion

The large variation in reference intervals observed between the different allergens included in this study emphasize the need for the establishment of allergen specific ranges for IgG. This is not the case for IgE allergens where a standard reference range is typically used for all allergens based on seven classes of IgE concentrations. Class 0 and 1 are the lowest, indicating a negative or equivocal IgE result respectively, with classes 2-6 indicating an increased likelihood of allergic disease. Laboratories that perform IgG allergen testing, for the most part, do not give specific IgG reference intervals but rather list the lower limit of quantitation of the assay and let the ordering physician interpret the result.

While the clinical utility of serum IgG testing as an aid in the diagnosis of allergy and food-induced hypersensitivity remains controversial, there is growing evidence it can provide beneficial information in certain cases. We believe these proposed reference intervals accurately reflect values found in today's population, and should benefit clinicians in the interpretation serum IgG allergen results.

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