

Supplementary methods related to the assay of IgE antibodies in dilutions of sera

When sera are assayed for IgE antibodies using either ImmunoCAP or other solid phase methods, the assumption is that results in the range 0.35 IU/ml up to at least 80 IU/ml are not limited by shortage of relevant allergen on the solid phase. Thus dilution of sera is only recommended if the result approaches ≥ 100 IU/ml. In fact there are situations where specific IgE is directed against a minor component of the extract and the recorded result using undiluted serum will underestimate the correct value for IgE antibodies to that allergen extract. A typical example would be a serum with high titer IgE antibodies to the peanut allergen Ara h 8 where assay of the serum with the solid phase for peanut extract (f13) can underestimate the value because there is only a small amount of Ara h 8 in the extract.

Given the results reported here for IgE antibodies to food extracts in patients with EoE we asked whether the low quantities and generally negative results for molecular allergens could be explained if the IgE was directed against a protein that was only present in small quantities in the extract. We have carried out extensive dilution studies on those sera where sufficient IgE was available (See Table V and Tables E2 and E3). The results show clearly that many of the sera which had positive results for egg, milk, wheat, or soy gave higher calculated results when the sera were diluted (See Table E2 for examples of the calculation). By contrast most, but not all, of the values for IgE to the primary inhalant allergens, i.e. dust mite and cat, gave very similar calculated results when diluted (See Table V and Table E2). The results suggest very strongly that many of the low titer IgE antibody results to food are specific for a protein that is only present in small quantities in the extract. Furthermore, the negative results with ISAC suggest that the relevant proteins are not one of the allergens that are currently well recognized.

Legend to Repository Figure

Fig E1. The relationship between quantity of IgE antibody specific for soy and IgE antibody specific for peanut with each dot representing an individual patient. The regression line is shown.

Table E1A. Serum IgE measurements were performed to allergens and components.

Foods	Aeroallergens	Other allergens	Components
milk	dust mite	galactose- α -1,3-galactose	Bos d 4
egg	cat	bromelain	Bos d 5
wheat	dog	<i>Candida albicans</i>	Bos d 8
soy	mold mix (mx1)	Staphylococcal enterotoxin A	Bos d 6
peanut	birch	Staphylococcal enterotoxin B	Ara h 1
cashew	rye grass		Ara h 2
beef	weed mix (wx1) ragweed		Ara h 3
			Ara h 8
			Ara h 9
			Gluten
			Gliadin
			Omega-5
			Tri a 14

Table E1B. Prevalence of sensitization to allergens not mentioned in the text.

	Prevalence		Children	
	Adults			
	n/number tested	%	n/number tested	%
Allergens				
Alpha-gal	0/23	0	1/27	3.7
bromelain	2/23	8.7	1/5	20
<i>Candida albicans</i>	3/23	13	2/5	40
Staphylococcal enterotoxin A	2/23	8.7	0/5	0
Staphylococcal enterotoxin B	3/23	13	0/5	0

Table E2. Comparison of the prevalence of having specific IgE antibody in EoE patients and unselected pediatric populations.

	Dust mite*	Cat (e1)	Cow's milk (f2)	Wheat (f4)	Peanut (f13)	Grass*
EoE & IgE positive	26/87 (29.9%)	31/87 (35.6%)	42/89 (47.2%)	39/89 (43.8%)	33/89 (37.1%)	33/87 (37.9%)
Control & IgE positive	73/220 (33.2%)	53/220 (24.1%)	33/220 (15.0%)	31/220 (14.1%)	36/220 (16.4%)	36/220 (16.4%)
p value	0.6	0.04	<0.001	<0.001	<0.001	<0.001
OR (95% CI)	0.86 (0.5-1.5)	1.7 (1.0-3.0)	5.1 (2.9-8.8)	4.8 (2.7-8.4)	3.0 (1.7-5.3)	3.1 (1.8-5.5)

* Specific IgE assays for dust mite and grasses were chosen based on the species that are the most relevant allergen source in the respective geographic areas for both the EoE (Ohio) and Control (Ohio and East Coast) populations.

† OR (95% CI) is odds ratio and 95% confidence interval.

Table E3: EoE samples (adults and children) assayed for IgE to egg, milk, wheat, or soy on dilutions of sera (1:4 and 1:8)*

FOODS												
	f1 (chicken egg)			f2 (cow's milk)			f4 (wheat)			f14 (soy)		
	undiluted	1:4	1:8	undiluted	1:4	1:8	undiluted	1:4	1:8	undiluted	1:4	1:8
<u>Adults</u>	<0.35	nd [†]	nd	7.29	7.16	7.52	0.72	1.08	1.28	<0.35	nd	nd
	0.69	0.88	1.12	6.81	21.8	27.0	11.9	14.9	16.6	0.67	0.92	1.20
	<0.35	nd	nd	<0.35	nd	nd	2.16	nes [‡]	nes	<0.35	nd	nd
	<0.35	nd	nd	1.10	nes	nes	0.73	nes	nes	<0.35	nd	nd
	<0.35	nd	nd	<0.35	nd	nd	9.54	18.5	20.8	2.05	nes	nes
	2.02	nes	nes	2.33	7.00	8.96	3.76	5.44	6.56	2.93	nes	nes
	0.58	0.76	0.96	<0.35	nd	nd	0.66	1.16	1.84	6.32	6.80	7.68
	0.48	nes	nes	<0.35	nd	nd	0.49	1.00	1.36	0.60	0.80	1.12
<u>Children</u>	<0.35	nd	nd	8.82	12.8	13.4	<0.35	nd	nd	<0.35	nd	nd
	7.09	7.40	8.32	<0.35	nd	nd	<0.35	nd	nd	1.47	1.48	1.60
	<0.35	nd	nd	<0.35	nd	nd	<0.35	nd	nd	11.5	12.1	13.0
	0.99	nd	nd	6.12	20.6	37.7	0.93	nd	nd	1.06	nd	nd
	17.8	24.5	27.8	12.5	nes	nes	28.3	nes	nes	9.99	14.3	16.4
	0.40	nd	nd	0.67	1.36	1.68	2.01	4.96	5.92	1.35	4.16	4.32
	0.51	nes	nes	<0.35	nd	nd	<0.35	nd	nd	<0.35	nd	nd
	0.74	nd	nd	0.36	nd	nd	7.22	13.1	17.4	4.67	nes	nes
	5.74	11.0	11.8	3.06	nes	nes	1.28	nes	nes	2.07	nes	nes
	nes	nes	nes	nes	nes	nes	nes	nes	nes	6.89	8.32	9.36
	2.11	3.20	3.44	6.78	8.08	6.56	6.47	nes	nes	2.68	nes	nes
	0.90	0.96	1.20	15.8	29.6	48.9	0.43	0.60	0.88	<0.35	nd	nd
	23.0	23.2	23.2	12.3	12.5	13.2	5.39	8.88	10.5	4.89	7.64	9.44

* Values shown for dilutions 1:4 and 1:8 were calculated as shown in table E2.

[†] nd is not determined.

[‡] nes is not enough serum remaining for measurement.

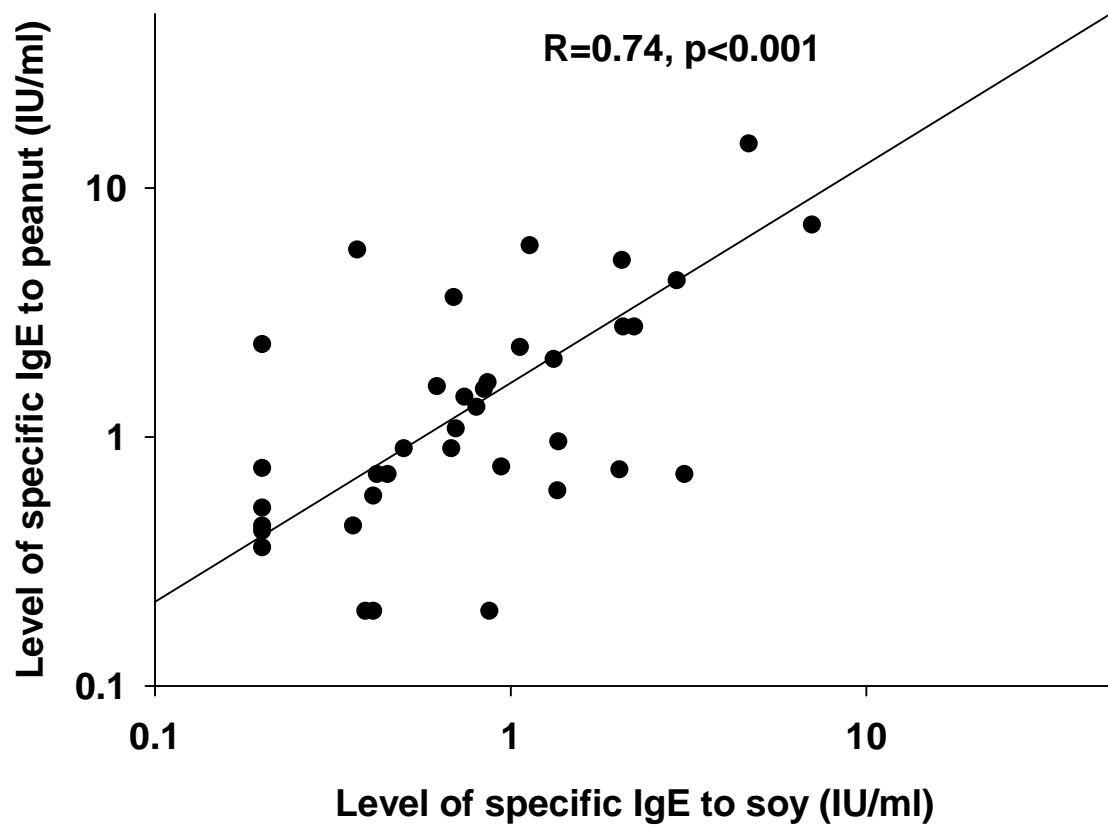


Fig E1