

Analytic precision and accuracy of commercial immunoassays for specific IgE: Establishing a standard

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Background: Different laboratory assays are used to detect and measure specific IgE antibodies. No standard exists to assess their analytic performance.

Objective: We sought to analyze reported specific IgE results from different laboratories on the same serum samples for their accuracy and precision.

Methods: Blinded serum samples (26) containing variable levels of specific IgE to 17 common aeroallergens were sent on 3 different occasions through normal channels to 6 laboratories that used 5 different test procedures. Six samples were presented as a dilution series. Laboratory-assay performance was assessed by analyzing the reported results ($n = 12,708$) by using ordinary least squares regression with slope coefficients, the t statistic, SEs, confidence intervals, and R^2 values. These were compared with a theoretic ideal assay as the reference. **Results:** Analysis revealed that one system used in two different laboratories performed nearly as well as the ideal standard, with an overall average slope (0.97; range, 0.91-1.01), SE (0.05; range, 0.02-0.16), R^2 value (93%; range, 0.64-0.99), and coefficient of variation (10.3%; range, 6%-14%). Extensive variability was observed in the other 4 laboratory-assay systems with respect to overall average slope (0.76; range, 0.11-1.24), SE (0.19; range, 0.03-0.95), R^2 value (53%; range, 0.00-0.98), and coefficient of variation (19%; range, 5%-49%). For some specific allergens, some laboratories-assays were not able to detect serial dilutions of the same sample.

Conclusions: One commercial system used in two different laboratories performed nearly as well as the ideal standard. Four of the laboratories-assays for specific IgE antibodies demonstrated substandard overall performance with multiple instances of poor precision and accuracy, particularly for certain allergens, such as weeds and molds. (*J Allergy Clin Immunol* 2000;105:1221-30.)

Key words: Specific IgE, laboratory tests, allergy, standardization, precision, accuracy, quantitation, diagnosis

From a clinical perspective, reliable and accurate assessment of specific IgE antibodies is critical for identification of the causative agents in patients with allergic disease.¹ These measurements, combined with results of the clinical history and physical examination, are used to establish a diagnosis of allergic disease. They are also used to make treatment decisions, including advice regarding avoidance of allergen exposure, selection of medications, anticipation of seasonal symptoms, and decisions regarding immunotherapy. Since the original serologic tests for the measurement of IgE antibodies were introduced some 25 years ago, progress through a number of procedural modifications has been steady.² These modifications have resulted in a number of currently available test formats that make use of different chemistries, reagents, tests, and scoring systems to measure specific IgE antibodies.³

Given current procedural options, it is not surprising that studies of the available assays demonstrate discrepancies both with respect to analytic precision and quantitative abilities for the same and different allergens.⁴⁻⁶ These differences are clearly pointed out in laboratory proficiency surveys.⁷ As a result of this variability and the lack of an objective standard, it has been difficult to judge the precise clinical performances of these tests.

Licensing and guideline requirements for manufacturers and laboratories performing these tests have been set forth by the Food and Drug Administration and the Clinical Laboratory Improvement Amendment of 1988.^{8,9} These measures have assisted in ensuring more consistent results but do not address either accuracy or precision parameters in a comprehensive fashion. In summary, one of the major challenges in the development of consistent allergy testing is the need to identify and accept a well-recognized test standard.

An ideal immunoassay for specific IgE antibodies should meet certain analytic performance criteria. These criteria include a slope of unity over the useful measurement range for all allergens, low variation across replicate measurements, statistically determined limits of detection, a practical cut-off point, and defined saturation

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Abbreviations used

A-RAST:	Modified RAST by Allergy Testing Laboratories
A-STAT:	Alastat by Allercare
C-RAST:	Modified RAST by Commonwealth Medical Laboratory
CV:	Coefficient of variation
I-CAP:	ImmunoCAP system by IBT Reference Laboratory
L-RAST:	Modified RAST by Laboratory Corp of America
OLS:	Ordinary least squares
S-CAP:	ImmunoCAP system by SmithKline Beecham Laboratories

points.¹⁰ For an assay to be considered as a standard, it would require close approximation to these criteria. There is currently no established standard in this field.

We report an analysis of 12,708 specific IgE test results on the same 26 serum samples sent through normal channels to 6 different major commercial laboratories that used 5 different assays. Each sample was analyzed for specific IgE antibodies to 17 different allergens. The resulting data are compared with an ideal standard to evaluate each laboratory and assay's analytic performance.

METHODS

Serum samples

Twenty-six random serum samples from consenting individuals containing different levels of specific IgE antibodies to a variety of different allergens were obtained from Pharmacia & Upjohn Diagnostics AB serum bank (Uppsala, Sweden). For 6 of these (selected by their containing significant amounts of specific IgE to a number of common allergens), five 2-fold serial dilutions were prepared by using human serum that was negative for specific IgE antibodies. This dilution process resulted in serum samples containing concentrations of 100%, 50%, 25%, 12.5%, 6.2%, and 3.1%, respectively, for each of the study samples. All samples were placed in aliquots in serum vials containing 2.2 mL, given a coded identification number, randomized for shipment, and then stored at -70°C . Each laboratory assayed each undiluted sample on 3 separate occasions.

Serum samples were submitted to each of 6 laboratories as routine clinical samples. These included shipping a weekly supply of samples to physicians to be picked up by the relevant laboratory on a biweekly basis. Five to 7 samples were included in each shipment. As a result of this process, study samples were sent to each laboratory over a span of 6 weeks.

Allergens

Tests for specific IgE antibodies were requested on each submitted sample to the following 17 allergens commonly assessed during diagnosis of allergic problems: *Dermatophagoides farinae* (d2), cat dander (e1), dog dander (e5), Bermuda grass (*Cynodon dactylon*, g2), timothy grass (*Phleum pratense*, g6), white oak (*Quercus alba*, t7), short ragweed (*Ambrosia elatior*, w1), giant ragweed (*Ambrosia trifida*, w3), mugwort sage (*Artemisia vulgaris*, w6), Russian thistle (*Salsola kali*, w11), rough pigweed (*Amaranthus retroflexus*, w14), *Penicillium notatum* (m1), *Cladosporium herbarum* (m2), *Aspergillus fumigatus* (m3), *Mucor racemosus* (m4), *Candida albicans* (m5), and *Alternaria tenuis/Alternaria alternata* (m6).

Assay procedures

Assay procedures and laboratories in the present study included the following: Allergy Testing Laboratories used an in-house developed modified RAST (A-RAST) with license from Hycor Biomedical to produce allergen paper discs in their laboratory; Allercare used the Diagnostic Products Corporation-AlaSTAT (A-STAT); Commonwealth Medical Laboratory used a modified RAST manufactured by Hycor Biomedical (C-RAST); IBT Reference Laboratory used the Pharmacia CAP System-Standard scoring (I-CAP); Laboratory Corporation of America used an in-house developed modified RAST with a license from Hycor Biomedical to produce allergen paper discs in their own laboratory (L-RAST); and SmithKline Beecham Clinical Laboratories used the Pharmacia CAP System-Standard scoring (S-CAP).

Data analysis

Individual laboratory reports for each of the serum samples were collected and forwarded to an independent statistical team who broke the code and performed the statistical analysis. The standard for comparison used in this study is the ideal dilution value with a slope of 1.00, which is based on serial dilution procedure (semilog plot of 100%, 50%, 25%, 12.5%, 6.25%, and 3.12% with a slope of 1.00). After the first 1:2 dilution, the expected value of the assay reading should be 50% of the average of triplicate results from the nondiluted sample. After the second 1:2 dilution, the expected value should be 25% and so on through the five 1:2 dilutions.

To test the accuracy of the observed slope coefficients for the diluted samples, ordinary least squares (OLS) regression analysis was used.¹¹ To test differences among distributions, ANOVA was used, followed by a Bonferroni post hoc test of pairwise differences.¹²

For the nondiluted samples, results for one system showing the closest correspondence with the ideal standard was used as the independent variable (x-axis) and regressed against the results of the other methods as the dependent variable (y-axis). Because of the differences in scaling values for the different assay procedures, the standardized slope coefficients were analyzed. If the assay results from different assay procedures are interchangeable, the standardized slope coefficient would have a value of 1.00.¹⁰ Thus an increase in 1 SD of the selected system would produce 1 SD in the value of the other laboratory. The ability of these slopes to capture the range of observations was compared by determining the R^2 values for each. The coefficient of variation (CV) for reported positive values was determined by calculating the SD of the 3 replicates on undiluted samples and dividing that value by their mean.

RESULTS

Scale conversion

Because different assay procedures use different scaling values (Table I), it was first necessary to calculate an observed value to compare against the ideal value for each assay system.

The observed values used the actual reported readings to calculate the dilution value percentages.

The ranges of specific IgE measurements in the nondiluted samples reported by the different assay procedures are shown in Table II. This demonstrates that a broad spectrum of possible results for the 17 individual allergens was used in this study.

Statistical test of differences among assay procedures

Inspection of the results indicated that there were major differences among the results of these assay pro-

TABLE I. Assay scoring systems

Class	I-CAP and S-CAP (kU _A /L)	A-STAT (IU/mL)	A-RAST and C-RAST (count)	L-RAST (count)
0	<0.35	<0.35	0-500	0-500
0/I			501-750	501-750
I	0.35-0.69	0.35-0.69	751-1600	751-1600
II	0.70-3.49	0.70-3.49	1601-3600	1601-3600
III	3.50-17.49	3.50-17.49	3601-8000	3601-8000
IV	17.50-52.49	17.5-52.49	8001-18,000	8001-18,000
V	52.5-99.99	52.5-99.99	18,001-40,000	>18,000
VI	>100	>100	>40,000	

TABLE II. Range of reported specific IgE antibody readings by allergen

Reported values	Count		IU/mL*		kU _A /L	
	Lowest	Highest	Lowest	Highest	Lowest	Highest
d2 <i>Dermatophagoides farinae</i>	180	47,846	0.00	>100	<0.35	29.13
e1 Cat dander	4886	78,419	2.27	100.00	0.87	34.33
e5 Dog dander	255	69,226	0.07	>100	0.49	44.93
g2 Bermuda grass (<i>Cynodon dactylon</i>)	405	20,316	0.04	5.82	0.52	12.16
g6 Timothy grass (<i>Phleum pratense</i>)	634	55,986	0.10	>100	4.00	>100
t7 White oak (<i>Quercus alba</i>)	888	34,675	0.00	1.79	0.49	5.37
w1 Short ragweed (<i>Ambrosia elatior</i>)	294	58,441	0.13	>100	0.52	21.60
w3 Giant ragweed (<i>Ambrosia trifida</i>)	484	53,774	0.03	46.62	1.24	27.13
w6 Mugwort sage (<i>Artemisia vulgaris</i>)	717	23,906	0.00	20.14	1.61	5.80
w11 Russian thistle (<i>Salsola kali</i>)	444	6960	0.01	5.13	1.31	4.64
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)	428	4903	0.00	5.38	0.81	4.27
m1 <i>Penicillium notatum</i>	312	8109	0.00	2.87	0.47	5.45
m2 <i>Cladosporium herbarum</i>	303	30,299	0.00	15.96	0.89	28.10
m3 <i>Aspergillus fumigatus</i>	328	6425	0.00	12.65	2.64	11.17
m4 <i>Mucor racemosus</i>	230	17,519	0.00	4.17	0.17	7.09
m5 <i>Candida albicans</i>	447	16,143	0.00	5.03	1.97	15.57
m6 <i>Alternaria tenuis/Alternaria alternata</i>	1830	36,779	0.02	12.91	0.77	23.53

*A-STAT reported readings by using two different scales, sometimes on the same assay report. One scale ranged from less than 0.35 to greater than 100 (standard system), and the other ranged from 0.00 to greater than 25 (extended system). The values shown above reflect the total reported range for both systems. We assume values of less than 0.35 were determined by their extended system.

TABLE III. Matrix of pairwise mean differences

	Expected	A-STAT	A-RAST	C-RAST	I-CAP	L-RAST	S-CAP
Expected	0.000						
A-STAT	0.234*	0.000					
A-RAST	0.338*	-0.105†	0.000				
C-RAST	0.279*	-0.046‡	-0.059‡	0.000			
I-CAP	0.066‡	0.167*	0.272*	0.213*	0.000		
L-RAST	0.257*	0.023‡	-0.082‡	-0.022‡	0.190*	0.000	
S-CAP	0.075‡	0.159*	0.263*	0.204*	0.075‡	0.182*	0.000

*P = .01.

†P = .05.

‡Not significant.

cedures on the same diluted samples. ANOVA was used to test possible significance of this observation. There was indeed a statistically significant difference ($F_{6,3148} = 36.08$, $P < .01$) among the different assay procedures. To determine the specific pattern of these differences, a Bonferroni post hoc test was performed by comparing each assay's results with those of an ideal assay. The results of this test are shown in Table III.

Examination of the matrix of pairwise mean differences in Table III reveals that results from only two laboratories (I-CAP and S-CAP) did not differ significantly from the ideal distribution with a slope of 1.00. All of the other results were significantly different from the ideal. In terms of statistical groupings, it can be said that one homogenous group is composed of the ideal, I-CAP, and S-CAP (group 1); a second group is composed of A-RAST, C-RAST, and

TABLE IV. OLS regression analysis of dilution data by assay procedure

Allergen	Assay	Slope	SE	t Statistic	95% Confidence Interval		R ²
					Lower	Upper	
d2 <i>Dermatophagoides farinae</i>	A-STAT	0.61	0.49	1.24 [‡]	−0.40	1.62	0.05
e1 Cat dander		1.01	0.03	38.73 [‡]	0.95	1.06	0.98
e5 Dog dander		0.87	0.27	3.27 [†]	0.33	1.41	0.26
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.85	0.23	3.72 [†]	0.38	1.31	0.32
g6 Timothy grass (<i>Phleum pratense</i>)		1.00	0.14	7.02 [†]	0.71	1.29	0.63
t7 White oak (<i>Quercus alba</i>)		0.68	0.47	1.46 [†]	−0.27	1.64	0.07
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.91	0.07	12.56 [†]	0.76	1.06	0.84
w3 Giant ragweed (<i>Ambrosia trifida</i>)		1.00	0.23	4.43 [†]	0.54	1.47	0.40
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.72	0.25	2.82 [†]	0.20	1.24	0.21
w11 Russian thistle (<i>Salsola kali</i>)		1.06	0.29	3.63 [†]	0.46	1.66	0.30
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		0.92	0.25	3.74 [†]	0.42	1.43	0.33
m1 <i>Penicillium notatum</i>		0.66	0.36	1.86 [‡]	−0.07	1.39	0.10
m2 <i>Cladosporium herbarum</i>		1.03	0.07	14.01 [†]	0.88	1.19	0.92
m3 <i>Aspergillus fumigatus</i>		1.00	0.12	8.59 [†]	0.76	1.24	0.71
m4 <i>Mucor racemosus</i>		0.81	0.19	4.17 [†]	0.41	1.21	0.43
m5 <i>Candida albicans</i>		0.99	0.22	4.56 [†]	0.55	1.43	0.41
m6 <i>Alternaria tenuis/Alternaria alternata</i>		1.00	0.10	9.61 [†]	0.79	1.21	0.75
d2 <i>Dermatophagoides farinae</i>	A-RAST	0.61	0.26	2.32 [*]	0.08	1.15	0.14
e1 Cat dander		0.91	0.05	18.03 [†]	0.81	1.01	0.91
e5 Dog dander		0.85	0.07	12.65 [†]	0.72	0.99	0.82
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.30	0.61	0.50 [‡]	−0.93	1.54	0.01
g6 Timothy grass (<i>Phleum pratense</i>)		0.76	0.23	3.31 [†]	0.29	1.22	0.24
t7 White oak (<i>Quercus alba</i>)		0.48	0.44	1.10 [‡]	−0.41	1.36	0.03
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.45	0.50	0.90 [‡]	−0.57	1.47	0.02
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.84	0.28	2.95 [†]	0.26	1.42	0.21
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.79	0.35	2.29 [*]	0.09	1.50	0.15
w11 Russian thistle (<i>Salsola kali</i>)		0.11	0.77	0.14 [‡]	−1.46	1.68	0.00
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		0.64	0.25	2.60 [*]	0.14	1.14	0.17
m1 <i>Penicillium notatum</i>		0.82	0.06	14.00 [†]	0.70	0.94	0.85
m2 <i>Cladosporium herbarum</i>		0.73	0.10	7.41 [†]	0.53	0.93	0.62
m3 <i>Aspergillus fumigatus</i>		0.76	0.08	9.24 [†]	0.59	0.93	0.72
m4 <i>Mucor racemosus</i>		0.35	0.33	1.04 [‡]	−0.33	1.03	0.03
m5 <i>Candida albicans</i>		0.85	0.05	18.19 [†]	0.75	0.94	0.91
m6 <i>Alternaria tenuis/Alternaria alternata</i>		0.77	0.09	8.40 [†]	0.59	0.96	0.67
d2 <i>Dermatophagoides farinae</i>	C-RAST	0.87	0.20	4.31 [†]	0.46	1.28	0.35
e1 Cat dander		0.75	0.10	7.76 [†]	0.55	0.94	0.64
e5 Dog dander		0.81	0.06	14.25 [†]	0.70	0.93	0.86
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.85	0.13	6.57 [†]	0.59	1.12	0.56
g6 Timothy grass (<i>Phleum pratense</i>)		0.60	0.10	5.92 [†]	0.39	0.80	0.51
t7 White oak (<i>Quercus alba</i>)		0.85	0.07	11.40 [†]	0.70	1.00	0.79
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.72	0.10	7.27 [†]	0.52	0.92	0.61
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.78	0.09	8.91 [†]	0.60	0.95	0.70
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.77	0.09	8.35 [†]	0.58	0.96	0.68
w11 Russian thistle (<i>Salsola kali</i>)		0.87	0.12	7.26 [†]	0.62	1.11	0.62
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		0.89	0.33	2.75 [†]	0.23	1.55	0.19
m1 <i>Penicillium notatum</i>		0.85	0.12	7.38 [†]	0.62	1.09	0.62
m2 <i>Cladosporium herbarum</i>		0.81	0.10	8.44 [†]	0.62	1.01	0.68
m3 <i>Aspergillus fumigatus</i>		0.85	0.09	9.42 [†]	0.67	1.03	0.72
m4 <i>Mucor racemosus</i>		0.84	0.09	9.55 [†]	0.66	1.02	0.73
m5 <i>Candida albicans</i>		0.78	0.08	9.38 [†]	0.61	0.95	0.72
m6 <i>Alternaria tenuis/Alternaria alternata</i>		0.75	0.10	7.42 [†]	0.54	0.96	0.62
d2 <i>Dermatophagoides farinae</i>	I-CAP	0.98	0.06	15.11 [†]	0.84	1.11	0.89
e1 Cat dander		1.00	0.02	61.69 [†]	0.97	1.03	0.99
e5 Dog dander		1.00	0.02	45.60 [†]	0.95	1.04	0.99
g2 Bermuda grass (<i>Cynodon dactylon</i>)		1.00	0.10	9.80 [†]	0.79	1.21	0.77
g6 Timothy grass (<i>Phleum pratense</i>)		1.00	0.04	24.15 [†]	0.92	1.09	0.94
t7 White oak (<i>Quercus alba</i>)		0.97	0.04	25.13 [†]	0.89	1.05	0.96
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.98	0.09	11.05 [†]	0.80	1.16	0.81
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.99	0.05	19.76 [†]	0.89	1.09	0.93
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.96	0.05	18.43 [†]	0.86	1.07	0.92

(continued)

TABLE IV. Cont'd

Allergen	Assay	Slope	SE	t Statistic	95% Confidence Interval		R ²
					Lower	Upper	
w11 Russian thistle (<i>Salsola kali</i>)	L-RAST	0.96	0.05	20.74†	0.86	1.05	0.93
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		0.93	0.06	14.80†	0.80	1.06	0.88
m1 <i>Penicillium notatum</i>		0.99	0.04	23.40†	0.90	1.07	0.95
m2 <i>Cladosporium herbarum</i>		0.99	0.04	24.06†	0.91	1.08	0.95
m3 <i>Aspergillus fumigatus</i>		0.98	0.03	28.70†	0.91	1.05	0.96
m4 <i>Mucor racemosus</i>		0.98	0.05	20.75†	0.88	1.07	0.95
m5 <i>Candida albicans</i>		0.98	0.02	51.24†	0.94	1.02	0.99
m6 <i>Alternaria tenuis/Alternaria alternata</i>		1.01	0.02	49.92†	0.97	1.05	0.99
d2 <i>Dermatophagoides farinae</i>		0.81	0.06	13.15†	0.69	0.94	0.84
e1 Cat dander		0.94	0.04	22.20†	0.85	1.02	0.94
e5 Dog dander		0.85	0.05	16.56†	0.74	0.95	0.89
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.80	0.12	6.71†	0.56	1.04	0.57
g6 Timothy grass (<i>Phleum pratense</i>)		0.74	0.08	9.05†	0.57	0.91	0.73
t7 White oak (<i>Quercus alba</i>)		0.80	0.06	14.03†	0.68	0.92	0.85
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.73	0.18	4.01†	0.36	1.11	0.32
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.81	0.17	4.65†	0.46	1.17	0.40
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.62	0.33	1.88‡	-0.05	1.29	0.10
w11 Russian thistle (<i>Salsola kali</i>)		0.63	0.36	1.74‡	-0.12	1.38	0.10
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		0.68	0.06	10.85†	0.55	0.81	0.78
m1 <i>Penicillium notatum</i>	S-CAP	0.71	0.10	7.22†	0.51	0.91	0.61
m2 <i>Cladosporium herbarum</i>		0.81	0.05	16.21†	0.71	0.91	0.89
m3 <i>Aspergillus fumigatus</i>		1.24	0.95	1.31‡	-0.69	3.16	0.05
m4 <i>Mucor racemosus</i>		0.83	0.08	10.71†	0.68	0.99	0.77
m5 <i>Candida albicans</i>		0.78	0.06	12.27†	0.65	0.91	0.82
m6 <i>Alternaria tenuis/Alternaria alternata</i>		0.89	0.04	22.04†	0.81	0.98	0.93
d2 <i>Dermatophagoides farinae</i>		0.91	0.16	5.86†	0.59	1.24	0.64
e1 Cat dander		0.99	0.03	35.06†	0.93	1.05	0.98
e5 Dog dander		0.99	0.04	27.25†	0.91	1.06	0.96
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.95	0.07	14.17†	0.81	1.09	0.91
g6 Timothy grass (<i>Phleum pratense</i>)		0.99	0.04	24.28†	0.91	1.07	0.95
t7 White oak (<i>Quercus alba</i>)		0.95	0.05	18.87†	0.84	1.05	0.95
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.97	0.05	18.71†	0.87	1.08	0.95
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.96	0.06	14.93†	0.83	1.09	0.91
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.95	0.05	17.43†	0.83	1.06	0.94
w11 Russian thistle (<i>Salsola kali</i>)		0.90	0.07	12.55†	0.75	1.06	0.90
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		1.04	0.08	12.70†	0.87	1.20	0.86
m1 <i>Penicillium notatum</i>		0.96	0.05	20.25†	0.86	1.05	0.95
m2 <i>Cladosporium herbarum</i>		0.97	0.03	27.72†	0.90	1.04	0.97
m3 <i>Aspergillus fumigatus</i>		0.95	0.03	34.15†	0.89	1.01	0.98
m4 <i>Mucor racemosus</i>		0.94	0.03	27.18†	0.87	1.02	0.98
m5 <i>Candida albicans</i>		0.97	0.03	37.07†	0.91	1.02	0.98
m6 <i>Alternaria tenuis/Alternaria alternata</i>		0.98	0.03	32.08†	0.92	1.05	0.97

*P ≤ .05.

†P ≤ .01.

‡Not significant.

L-RAST (group 2); and a third group is composed of C-RAST, L-RAST, and A-STAT (group 3). The overlap between groups 2 and 3 results from a statistically significance difference between A-RAST and A-STAT, and both C-RAST and L-RAST lie in between. Collectively, these results indicate that only the assays in group 1 were comparable with the performance of the ideal standard.

Differences across allergens

The ANOVA analysis of the diluted samples not only revealed significant differences between laboratories and methods but also a significant difference ($F_{6,3148} = 2.59$,

$P < .01$) across different allergens. To examine these discrepancies, the reported results from each laboratory on each individual allergen were compared against the ideal value by using OLS regression analysis. These OLS results are shown in Table IV.

The average overall performance of an assay, laboratory, or both can be judged by assessing the average slope coefficient across all allergens.¹² An ideal assay would have a slope of 1.00. The average slope coefficient for all allergens in order of best to worst was 0.98 for I-CAP, 0.96 for S-CAP, 0.82 for A-STAT, 0.80 for C-RAST, 0.77 for L-RAST, and 0.65 for A-RAST.

TABLE V. OLS regression of nondilution samples*

	Assay	Standard slope†	R ² ‡	CV§
d2 <i>Dermatophagoides farinae</i>	S-CAP	0.98	0.96	11.2%
e1 Cat dander		0.90	0.81	14.1%
e5 Dog dander		0.98	0.96	7.4%
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.97	0.94	11.1%
g6 Timothy grass (<i>Phleum pratense</i>)		0.97	0.94	13.9%
t7 White oak (<i>Quercus alba</i>)		0.98	0.97	11.6%
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.98	0.97	6.5%
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.97	0.94	12.0%
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.99	0.98	10.5%
w11 Russian thistle (<i>Salsola kali</i>)		0.97	0.95	8.5%
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		0.99	0.97	13.1%
m1 <i>Penicillium notatum</i>		0.99	0.97	11.0%
m2 <i>Cladosporium herbarum</i>		0.98	0.97	10.3%
m3 <i>Aspergillus fumigatus</i>		0.99	0.98	10.7%
m4 <i>Mucor racemosus</i>		0.98	0.97	9.4%
m5 <i>Candida albicans</i>		0.98	0.97	12.5%
m6 <i>Alternaria tenuis/Alternaria alternata</i>		0.97	0.94	10.6%
d2 <i>Dermatophagoides farinae</i>	A-RAST	0.93	0.86	17.7%
e1 Cat dander		0.73	0.54	16.8%
e5 Dog dander		0.80	0.64	17.6%
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.78	0.60	19.7%
g6 Timothy grass (<i>Phleum pratense</i>)		0.78	0.61	16.9%
t7 White oak (<i>Quercus alba</i>)		0.77	0.59	17.4%
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.91	0.83	18.2%
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.92	0.85	24.6%
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.86	0.74	22.0%
w11 Russian thistle (<i>Salsola kali</i>)		0.72	0.52	16.3%
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		0.30	0.09	16.3%
m1 <i>Penicillium notatum</i>		0.77	0.59	20.8%
m2 <i>Cladosporium herbarum</i>		0.59	0.34	26.1%
m3 <i>Aspergillus fumigatus</i>		0.25	0.06	20.7%
m4 <i>Mucor racemosus</i>		0.50	0.25	21.7%
m5 <i>Candida albicans</i>		0.65	0.42	20.4%
m6 <i>Alternaria tenuis/Alternaria alternata</i>		0.88	0.77	23.4%
d2 <i>Dermatophagoides farinae</i>	L-RAST	0.92	0.85	9.9%
e1 Cat dander		0.73	0.54	9.7%
e5 Dog dander		0.79	0.62	15.4%
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.79	0.63	14.2%
g6 Timothy grass (<i>Phleum pratense</i>)		0.81	0.66	14.0%
t7 White oak (<i>Quercus alba</i>)		0.82	0.67	12.9%
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.90	0.80	14.1%
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.94	0.89	9.9%
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.84	0.70	12.9%
w11 Russian thistle (<i>Salsola kali</i>)		0.66	0.44	14.8%
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)		0.42	0.17	15%
m1 <i>Penicillium notatum</i>		0.58	0.34	17.2%
m2 <i>Cladosporium herbarum</i>		0.47	0.22	11.9%
m3 <i>Aspergillus fumigatus</i>		0.35	0.12	11.5%
m4 <i>Mucor racemosus</i>		0.58	0.33	31.3%
m5 <i>Candida albicans</i>		0.54	0.30	17.0%
m6 <i>Alternaria tenuis/Alternaria alternata</i>		0.80	0.64	8.5%
d2 <i>Dermatophagoides farinae</i>	A-STAT	0.96	0.92	17.7%
e1 Cat dander		0.80	0.64	18.8%
e5 Dog dander		0.88	0.77	19.7%
g2 Bermuda grass (<i>Cynodon dactylon</i>)		0.70	0.49	28.7%
g6 Timothy grass (<i>Phleum pratense</i>)		0.77	0.59	22.8%
t7 White oak (<i>Quercus alba</i>)		0.16	0.03	16.5%
w1 Short ragweed (<i>Ambrosia elatior</i>)		0.57	0.32	24.0%
w3 Giant ragweed (<i>Ambrosia trifida</i>)		0.80	0.63	28.2%
w6 Mugwort sage (<i>Artemisia vulgaris</i>)		0.76	0.58	49.1%
w11 Russian thistle (<i>Salsola kali</i>)		0.30	0.09	27.0%

(continued)

TABLE V. Cont'd

Assay	Standard slope†	R ² ‡	CV§
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)	0.66	0.44	17.8%
m1 <i>Penicillium notatum</i>	0.65	0.42	41.4%
m2 <i>Cladosporium herbarum</i>	0.39	0.15	32.1%
m3 <i>Aspergillus fumigatus</i>	0.85	0.72	26.8%
m4 <i>Mucor racemosus</i>	0.24	0.06	27.2%
m5 <i>Candida albicans</i>	0.70	0.48	25.7%
m6 <i>Alternaria tenuis/Alternaria alternata</i>	0.83	0.70	18.3%
d2 <i>Dermatophagoides farinae</i>	C-RAST	0.66	23.7%
e1 Cat dander	0.61	0.37	5.3%
e5 Dog dander	0.74	0.55	11.0%
g2 Bermuda grass (<i>Cynodon dactylon</i>)	0.79	0.63	8.2%
g6 Timothy grass (<i>Phleum pratense</i>)	0.70	0.50	5.4%
t7 White oak (<i>Quercus alba</i>)	0.81	0.66	10.5%
w1 Short ragweed (<i>Ambrosia elatior</i>)	0.83	0.68	7.5%
w3 Giant ragweed (<i>Ambrosia trifida</i>)	0.85	0.72	10.0%
w6 Mugwort sage (<i>Artemisia vulgaris</i>)	0.64	0.41	7.8%
w11 Russian thistle (<i>Salsola kali</i>)	0.76	0.58	16.0%
w14 Rough pigweed (<i>Amaranthus retroflexus</i>)	0.28	0.08	23.5%
m1 <i>Penicillium notatum</i>	0.77	0.60	15.1%
m2 <i>Cladosporium herbarum</i>	0.60	0.36	15.3%
m3 <i>Aspergillus fumigatus</i>	0.34	0.12	14.9%
m4 <i>Mucor racemosus</i>	0.52	0.27	28.4%
m5 <i>Candida albicans</i>	0.87	0.75	15.7%
m6 <i>Alternaria tenuis/Alternaria alternata</i>	0.71	0.51	9.6%

*Each of the regression results shown above was determined by setting I-CAP as the independent variable (x-axis) and regressing it against each of the other assay methods as the dependent variable (y-axis). To accomplish the regressions, a series of data transformations were necessary.

In the case of missing observations for any laboratory, the missing value was replaced by the average reading for the other two replicates, or in the case of the two missing observations, the one reported value was assigned to the two missing observations. If it was not possible to extrapolate a value by any of the above methods, the value was left as missing and thus excluded from the regression calculations.

†Because of the differences in scaling values across the different assay procedures, we report the standardized slope coefficient in column 3. If IgE readings from different assay procedures are interchangeable, the standardized slope coefficient would have a value of 1.00, which would mean that an increase of 1 SD in the value of x (I-CAP) would produce an increase of 1 SD in the value of y.

‡The coefficient of determination (R^2) is shown in column 4. The coefficient of determination is the portion of variation in the data that is explained by the regression line. If each x-y combination of observations lie along the regression line, then R^2 would equal 1.00.

§Values shown above in column 5 are the CVs for reported positive values by assay. The CV is a measure of the variability of reported values across the 3 replicates. It is determined by calculating the SD of the 3 replicates and then dividing that value by their mean.

1. For I-CAP values reported as less than 0.10, this value was set to 0. For values reported as greater than 100, a value of 101 was assigned.
2. For S-CAP values reported as less than 0.35, the equivalent I-CAP value was substituted if the I-CAP value was less than 0.35. If the I-CAP value was greater than 0.35 and the S-CAP value was less than 0.35, the S-CAP value was set to 0.34. For values reported as greater than 100, a value of 101 was assigned.
3. For A-STAT values reported as less than 0.10, a value of zero was assigned. In several cases A-STAT reported values as greater than 25.0. In these cases a close examination was undertaken to see whether the meaning was the same as greater than 100 (101 assigned) or simply some value greater than 25. If the meaning was simply greater than 25, then readings from the other replicates for the same sample were used where possible. If this was not possible, then a value of 26 was assigned.

The statistical significance of the slope coefficients (t statistic) for most allergens was a *P* value of less than .01, indicating that for the diluted samples most assay readings did indeed decrease as the level of dilution increased. A nonsignificant slope coefficient indicates that the assay could not detect differences among the diluted samples. Nonsignificant slope coefficients were seen for *Dermatophagoides farinae*, *Quercus alba*, and *Penicillium notatum* by A-STAT; *Cynodon dactylon*, *Quercus alba*, *Ambrosia elatior*, *Amaranthus retroflexus*, and *Mucor racemosus* by A-RAST; and *Artemisia vulgaris*, *Salsola kali*, and *Aspergillus fumigatus* by L-RAST (Table IV). A-RAST had 3 other allergens that only reached significance at the .05 level (*Dermatophagoides farinae*, *Artemisia vulgaris*, and *Amaran-*

thus retroflexus). All slope coefficients for, I-CAP, S-CAP, and C-RAST were significant at a *P* value of .01.

The standard error of the slope coefficients for each assay and allergen for the diluted samples can also be seen in column 3 of Table IV. This is a measure of the average variability of the reported data and can be viewed as a measure of assay precision.¹² The average SEs from lowest to largest were 0.05 (range, 0.02-0.10) for I-CAP, 0.05 (range, 0.03-0.16) for S-CAP, 0.12 (range, 0.07-0.33) for C-RAST, 0.16 (range, 0.04-0.36) for L-RAST, 0.22 (range, 0.03-0.47) for A-STAT, and 0.26 (range, 0.05-0.77) for A-RAST. Of the assay procedures examined in the current study, only the results reported by I-CAP and S-CAP had consistently small SEs across all of the different allergens.

The slope data and the SE for each individual allergen were used to calculate the 95% confidence intervals for each allergen dilution series. The confidence interval represents the range of values covering the true slope of the determined values.¹² More specifically, an assay performing ideally should not only have a small range of values, but the confidence interval should encompass the value of 1.0. A-RAST, C-RAST, and L-RAST had 6 (35.3%), 8 (47.1%), and 10 (58.8%) of 17 allergens, respectively, that did not fulfill this requirement. A-STAT confidence intervals all encompassed the value of 1.0 but had very wide confidence intervals for all but one allergen (cat dander). I-CAP and S-CAP confidence intervals all encompassed the value of 1.0 and had small confidence intervals for all allergens, with the exception of *Dermatophagoides farinae* for S-CAP.

An additional measure of assay performance is the R^2 value shown in the last column of Table IV. R^2 is the proportion of variance in a set of data that is explained by the regression line.¹² Given the dilution range used in the present study, R^2 values below approximately 0.80 generally reflect poor performance in the ability to correctly detect levels of specific IgE antibodies. In this regard, I-CAP and S-CAP had one R^2 value below 0.80 (1/17 [6%]), whereas L-RAST had 7 (7/17 [41%]), A-RAST had 13 (13/17 [76%]), A-STAT had 14 (14/17 [82%]), and C-RAST had 16 (16/17 [94%]) below this value. The average R^2 values from best to worst were 93% for I-CAP, 93% for S-CAP, 62% for C-RAST, 62% for L-RAST, 45% for A-STAT, and 43% for A-RAST. Only I-CAP and S-CAP had consistently acceptable R^2 values, suggesting good performance in their ability to correctly detect the concentrations of specific IgE antibodies across the different samples and allergens.

To examine the performance of these laboratories and systems, 26 undiluted samples were assayed for specific IgE directed against each allergen on 3 different occasions. The standardized slope coefficients, their R^2 values, and the CVs for each assay and each allergen are shown in Table V.

The average standardized slope coefficients from best to worst were 0.98 for S-CAP, 0.71 for A-RAST, 0.70 for L-RAST, 0.68 for C-RAST, and 0.65 for A-STAT. These data indicate that the S-CAP was very close to the standardized slope of the I-CAP, which in turn closely approximated the ideal slope, as shown above.

The R^2 values determine the proportion of variance that do not fall on the regression line and can be taken as a measure of assay variability.¹² The average R^2 values for all allergens were 0.95 for S-CAP, 0.55 for A-RAST, 0.52 for L-RAST, 0.50 for C-RAST, and 0.47 for A-STAT. These data demonstrate large deviations from the regression lines for all laboratories-procedures, except for the S-CAP. The assay variability, as determined by R^2 values, differed for different allergens in different assays, as noted in Table V.

It is possible that much of this variation is due to imprecision in these assays. Therefore the CV (SD/mean as a percentage) for each allergen in each laboratory-assay on the repeated samples was determined. The average CV for all undiluted samples that

were positive was 9.8% for I-CAP (range, 7%-13%), 10.8% for S-CAP (range, 7%-14%), 13.4% for C-RAST (range, 5%-28%), 14.7% for L-RAST (range, 9%-31%), 20.3% for A-RAST (range, 16%-26%), and 27.5% for A-STAT (range, 17%-49%). The imprecision varied among different allergens and laboratories but was seen most often with specific IgE measurements to mold and weed allergens.

DISCUSSION

Test results combined with the clinical history and physical examination are often all a clinician has to make important diagnostic and treatment decisions. This is particularly important in the field of allergy, where symptoms derived from IgE-mediated inflammation are not easily differentiated from other causes by clinical review or skin testing with largely uncharacterized extracts and procedures.¹⁴ These factors make it important that laboratory results be accurate, consistent, and reliable.

Immunoassays for specific IgE antibodies are complex. They require the measurement of polyclonal antibodies of differing affinity to multiple antigens. An ideal assay for measuring these antibodies would have a logarithmic dose response of unity over the useful measuring range for each allergen, low variation across replicate measurements, statistically determined limits of detection, and a usefully defined cut-off point.¹⁰

Several varieties of these immunoassays are commercially available to clinicians in the United States. The majority of the available tests performed include different varieties of the modified RAST (A-RAST, L-RAST, and C-RAST), the Pharmacia ImmunoCAP system (I-CAP and S-CAP), and the Diagnostic Products Alastat test (A-STAT). Because there is no reported standard to judge the analytic performance of these tests, we have compared their performance with that of an ideal assay by using diluted and nondiluted blinded samples.

The results of an ANOVA analysis demonstrate statistically significant differences among the results of these different laboratories-assays. The pattern of these differences determined by using a Bonferroni post hoc test revealed that results from two laboratories (I-CAP and S-CAP) could be grouped with results expected from an ideal immunoassay. The results from the other laboratory-assays were statistically different from this. The ANOVA results also demonstrate a significant difference across different allergens for these assays. This indicates that A-RAST, L-RAST, C-RAST, and A-STAT produced overall results that were significantly ($P = .01$) different from those of the ideal standard.

By using samples in a dilution series, we examined the reported results by means of regression analysis. The slopes of the reported results for each allergen and laboratory-assay were compared by determining their SEs, significance, 95% confidence intervals, and R^2 values. From this analysis, it is clear that large deviations from the ideal exist in most of these assays. Nonsignificant slopes determined by means of the t statistic occurred

with several of these assays, indicating that in these cases a particular laboratory-assay could not recognize different concentrations of specific IgE for those allergens.

The average overall slope for most of these laboratories-assays did not compare favorably with the ideal standard. The exceptions to this were the I-CAP and S-CAP, where the average slopes were greater than 0.96, with comparatively small average SEs and 95% confidence intervals. Large variation leads to wide confidence intervals, which can also be viewed as the particular laboratory-assay in question having a lack of precision or reproducibility.¹² That is, although a particular assay was able to detect that different concentrations of specific IgE existed, it was particularly sporadic in measuring them correctly. Several laboratories-assays did exhibit some reasonable comparisons with the ideal slope for some allergens, but this was the exception and not the rule. For example, the A-STAT results revealed a reasonable comparison with the ideal for 6 allergens, although not the rest. The modified RAST procedures exhibited notably poor overall comparisons with the ideal assay, and this probably indicates a saturation effect reflecting the assay's inability to differentiate higher levels of specific IgE for most allergens.¹³ These deviations from the ideal reflect the inability of these assays to accurately quantitate different concentrations of specific IgE antibodies.

If a particular assay reported the dilution values exactly, the R^2 value would equal 1.00, which would indicate that all of their reported values fell exactly on the ideal dilution line. The wide variability in the R^2 values and assays with wide 95% confidence levels represent an indication of their imprecision. In multiple cases the R^2 value was less than 0.10, indicating that the reported results were most closely represented by a random scattering of values. Collectively, these data indicate that these laboratories-assays do not report reproducible results for a number of allergens and had rather poor overall performance with respect to quantitative abilities.

Another relevant observation is the lack of precision in some of these assays in their analysis of triplicates. Here some assays demonstrated unacceptably large CVs for some allergens. This also demonstrates a lack of precision in laboratory-assay procedures that could be responsible for the large 95% confidence intervals of the slopes observed.

Three of these laboratories used a version of the modified RAST. It has been previously suggested that this procedure is likely to be affinity dependent and unlikely as such to be quantitative.¹³ Anomalies with various allergens, such as hook effects, saturation at low levels of antibody, and low slopes, were seen with different allergens in these tests. Our results suggest that the inability to correctly measure IgE antibodies for different allergens is not necessarily intrinsic to the modified RAST procedure because the different modified RAST procedures differed in their performance with different allergens. The A-STAT procedure also deviated from the ideal having the largest CVs and 95% confidence intervals. Collectively, these data indicate that these assays give

different results for different allergens and have multiple instances of poor precision and accuracy.

Strengths of this study include the large number of tests compared, that all the sera were sent through normal channels, that these samples were all run and analyzed in a blinded fashion, and that assay performance was compared with an ideal standard. Limitations include the fact that although the samples used for this analysis were chosen randomly, they contained different levels of specific IgE to the various allergens tested. This could have resulted in the amplification of some of the differences seen between these laboratories-assays. We consider this unlikely in view of the number of sera tested (reduces random errors) and the fact that the results from the diluted sera were consistent with the results of the undiluted samples.

The samples for this study were placed in aliquots, frozen, and sent to physicians who then sent them to the respective laboratories. One might argue that the variability seen in this study was a result of the procedures used to blind this study. However, this is unlikely for 3 reasons. First, the serum IgE antibodies have been demonstrated to be quite stable for determination by assays, such as those used in the present study.¹⁵ Second, the fact that I-CAP and S-CAP results were so close in both accuracy and precision suggest that the sample distribution procedure used in this study did not bias the results. Third, the fact that the different laboratories were consistent in their discordant results and that they were discordant for different allergens would strongly suggest that factors other than sample integrity were the basis for the discordant results.

The I-CAP and S-CAP compared favorably with the ideal assay with respect to slope values, SEs, 95% confidence intervals, and R^2 values for most allergens. This was true for both the diluted and undiluted samples assayed in two different laboratories. The CV data obtained from repeated measurements of the same samples demonstrate that the I-CAP and S-CAP had very acceptable CVs for all allergens and were performing with precision. These findings indicate that this assay is capable of measuring specific IgE antibodies over a large range with precision and accuracy for the allergens studied and support its use as a current standard for quantitative measurements of specific IgE antibodies.

There has been a longstanding debate regarding the clinical value of serologic tests for specific IgE antibodies, such as those analyzed in this study. The results of this study indicate that data from different assay systems are not interchangeable. The Pharmacia & Upjohn CAP system performed well when compared with the standard of an ideal assay, although other assays often did not perform up to this standard. Because clinical studies have used these different systems, discrepant interpretations of their efficiencies were inevitable. Our data provide insight into the reasons for these deviations from an ideal assay and indicate where studies should be focused to improve these assays. Systematic improvements in some assay systems and ongoing monitoring with blind clinical samples are needed to assure that all commercial measurements of specific IgE are accurate and reliable.

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