

Committee report

Variability in the measurement of specific immunoglobulin E antibody by the RAST procedure

Richard Evans III, M.D. *Committee on In Vitro Tests, American Academy of Allergy*

This is a report of a study from the Committee on In Vitro Tests of the American Academy of Allergy, which evaluated variance of the RAST procedure when different allergic sera were tested by 12 Committee member laboratories using the Phadebas RAST kit (Pharmacia Diagnostics, Piscataway, N. J.) and when serial dilutions of a ragweed allergic sera were sent to nine different commercial laboratories in the U.S.A. The Phadebas RAST was found to differentiate high-, medium-, and low-titer allergic sera. The test was not useful in measuring smaller changes (less than 61%) in specific IgE antibody that might result from treatment with immunotherapy. Alternative methods of reporting the data, percent total activity and percent negative control, were not superior to the Phadebas RAST unit. Four of the commercial laboratories surveyed did not differentiate by their own measure of significance medium- from low-titer ragweed allergic sera. (J ALLERGY CLIN IMMUNOL 69:245, 1982.)

The Committee on In Vitro Tests, a member committee of the Research Council of the American Academy of Allergy consisting of 14 member laboratories (Table I) experienced in immunologic techniques, was established in 1977 to evaluate current in vitro tests used in the care of allergic patients and to advise the physician about the development and application of these tests. The Committee consists of the following members:

Richard Evans III, M.D., Chairman 1977-1980

Douglas B. Tang, Ph.D.

Sonny Londono, M.A., M.T.

Laurie J. Smith, M.D.

Walter Reed Army Medical Center, Washington D.C.

N. Franklin Adkinson, Jr., M.D., Chairman 1980-1983

Richard R. Rosenthal, M.D.

Johns Hopkins University School of Medicine, Baltimore, Md.

From Allergy-Immunology Service, Walter Reed Army Medical Center, Washington, D. C.

Received for publication Sept. 14, 1981.

Accepted for publication Oct. 30, 1981.

Reprint requests to: Richard Evans III, M.D., Col. USA MC, Chief, Allergy-Clinical Immunology Service, Walter Reed Army Medical Center, Washington, D. C. 20012.

Harold Baer, Ph.D.

M. Christine Anderson, M.S.

Food and Drug Administration, Bethesda, Md.

I. Leonard Bernstein, M.D.

Joan S. Gallagher, Ph.D.

University of Cincinnati Medical Center, Cincinnati, Ohio

Daniel I. Mullally, M.D.

National Institutes of Health, Bethesda, Md.

Ilda De Filippi, M.S.

Pharmacia Diagnostics, Piscataway, N. J.

Gerald J. Gleich, M.D.

John W. Yunginger, M.D.

Mayo Medical School, Rochester, Minn.

Robert N. Hamburger, M.D.

University of California, San Diego

Donald R. Hoffman, Ph.D.

East Carolina University School of Medicine, Greenville, N. C.

S. G. O. Johansson, M.D.

Uppsala University Hospital, Uppsala, Sweden

Robert A. Krumholz, M.D.

Midwestern Diagnostic Laboratories, Dayton, Ohio

Lewis L. Perelmutter, Ph.D.

Department of National Health and Welfare, Ottawa, Canada

Paul Steinberg, M.D.

St. Louis Park Medical Center, Minneapolis, Minn.

Abbreviations used

RAST: Radioallergosorbent test
PRU: Phadebas RAST unit

Robert G. Townley, M.D.

Creighton University School of Medicine, Omaha, Neb.

John I. Wypych, Ph.D.

Buffalo General Hospital, Buffalo, N. Y.

Sam S. Yanari, Ph.D.

Henry Ford Hospital, Detroit, Mich.

Tai-June Yoo, M.D.

University of Iowa, Iowa City

Recently the committee undertook an evaluation of the variability of in vitro measurements of specific serum IgE antibody by the Phadebas RAST procedure (Pharmacia Diagnostics, Piscataway, N. J.). Two approaches in the evaluation were used. In phase I of the study, a number of different pooled sera from allergic individuals were sent to each of 12 participating Committee laboratories for measurement of specific IgE antibody by RAST; the primary purpose was to quantitate the magnitude of variability associated with replicate measurements of allergic sera (using the same kit) made on the same day or on different days. In addition, the study was to assess the total additional variation associated with the use of different kits from different lots and different laboratories. Alternative methods of expression of IgE antibody titers were considered. In phase II of the study, serial dilutions of pooled ragweed allergic sera were sent to nine commercial laboratories across the U.S.A. for similar in vitro testing. This is a report on the phase I and phase II studies in both research and commercial laboratories. On the basis of these results, the committee has made several recommendations that should reduce the variability in the in vitro measurement of serum specific IgE antibody by RAST.

MATERIALS AND METHODS

A coordinating laboratory, Walter Reed Army Medical Center, was responsible for preparation and distribution of serum pools from allergic and nonallergic individuals, for distribution of Phadebas RAST kits, and for data analysis.

Phase I (Committee laboratories)

Serum pools. Eight pools of sera were prepared for testing, which consisted of six pools drawn from individuals whose sera contained IgE antibody to allergens of ragweed, cat dander, honeybee venom, house dust mite (*Dermatophagoides farinae*), and *Alternaria*. Two different ragweed sera were selected; one contained IgE antibody to heat-stable antigens of ragweed (Ra5), and one contained IgE antibody to heat-labile antigens of ragweed, AgE, AgK,

TABLE I. Member laboratories of Committee on In Vitro Tests

Good Samaritan Hospital, Johns Hopkins—Baltimore
East Carolina University—Greenville
State University of New York—Buffalo
Allergic Diseases Research Laboratory, Mayo Clinic—Rochester
University of Cincinnati—Cincinnati
University of California—San Diego
Walter Reed Army Medical Center—Washington
Midwestern Diagnostics—Dayton
Department of Maternal Health and Welfare—Ottawa
Henry Ford Hospital—Detroit
St. Louis Park Medical Center—Minneapolis
Bureau of Biologics, Food and Drug Administration—Bethesda
VA Hospital—Iowa City*
Creighton University—Omaha*

*Did not participate in study.

and antigen Ra3. In addition, two negative control preparations containing no IgE antibodies were submitted. These were 5% human serum albumin and an AB pooled serum that consisted of nondefibrinated, nondiluted plasma obtained from 34 donors.

Phadebas RAST kits. RAST test kits were provided by Pharmacia Diagnostics. Each kit contained appropriate antigen discs, radiolabeled anti-IgE (Fc) antisera, and the Phadebas reference material, which is a birch allergen-IgE antibody system. Four separate concentrations of IgE antibody were provided in the kit in vials labeled A, B, C, and D. Measurements of radioactive counts bound (in counts per minute) from each of these reference sera were assigned values of 17.5, 3.5, 0.7, and 0.35 PRU/ml, respectively. These points were used to construct a reference curve for determining PRU/ml from radioactivity measurements from unknown sera, a determination usually made by visual interpolation.

Study design. The study design is schematically shown in Fig. 1. Twelve participating laboratories were provided twice over a 1-wk interval with: (1) a single RAST kit (all kit reagents were the same lot number for each laboratory but a different lot number each week); (2) two aliquots (sera 1 and 2) of each of the six pools of allergic sera; and (3) one aliquot from each of the two negative control sera. Although antigen discs appropriate for testing were indicated, the laboratories did not know or were not told that each of the two sera to be tested with a specified disc was an aliquot of the same serum pool. The laboratories were instructed to test each serum with the same lot of reagents from the kit provided on two different days. Each serum was tested in triplicate, yielding three replicate measurements of radioactive counts bound. The reference curve data from vials A, B, C, and D of the reference system were obtained in triplicate separately on each day.

Expression of antibody content. The basic data from the RAST test were expressed in three ways: (1) percent total activity (% TA = counts bound/counts added $\times$ 100), (2)

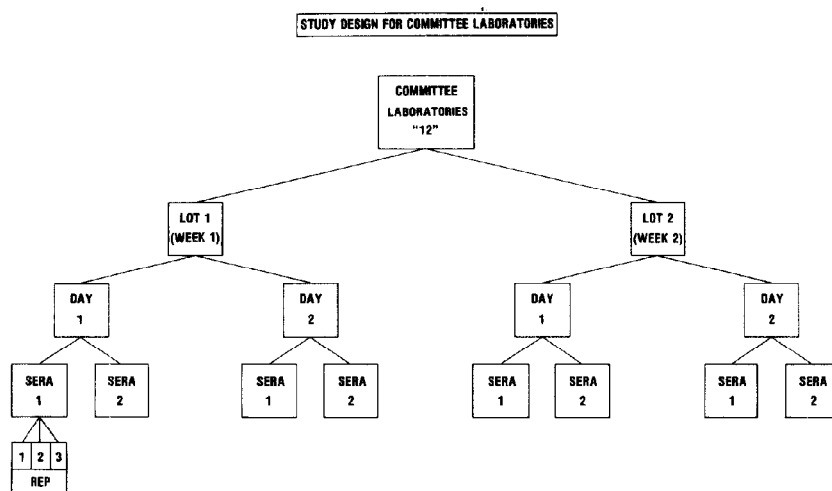


FIG. 1. Twelve committee laboratories were provided twice over a 1-wk interval with: a single RAST kit of the same lot number, two aliquots of six pools of allergic sera, and antigen discs appropriate for these sera.

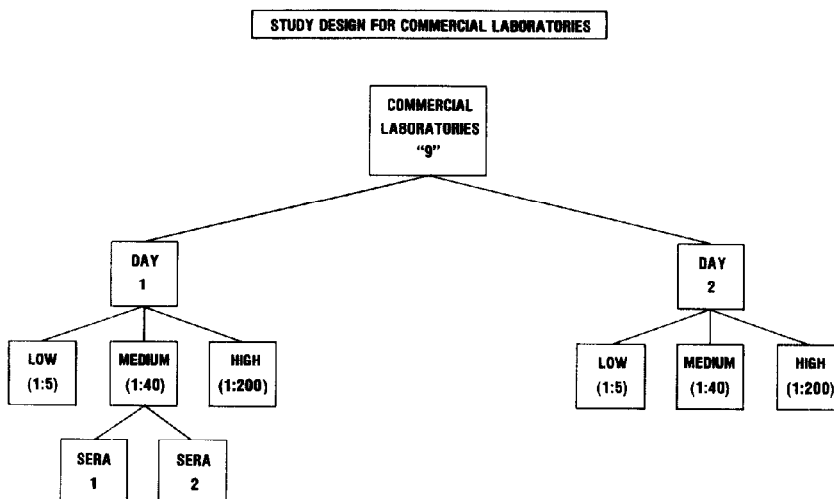


FIG. 2. Nine commercial laboratories were provided high-, medium-, and low-titer ragweed allergic sera. Two aliquots of each of these dilutions were sent at a 2-wk interval.

percent of the negative control sera (% NC = counts bound/counts bound in negative sera $\times 100$), and (3) PRU/ml. The PRU was determined by referring the number of bound counts per milliliter to the curve obtained for the four reference materials provided with the RAST kits and tested each day.

Data analysis. For each expression of antibody content (% TA, % NC, PRU/ml), variance components (expressed as standard deviations and coefficients of variation) associated with replicates, sera, days, lots, and laboratories were

estimated (see Statistical Appendix). No attempt was made to eliminate what might be judged "outlying values."

Phase II (Commercial laboratories)

The second phase of this study was an evaluation of IgE antibody measurements performed by American commercial laboratories. A high-titer serum from a ragweed-allergic individual was diluted 1/5, 1/40, and 1/200 with serum from a nonallergic individual. Two aliquots of each of these dilutions were labeled as patient samples and sent to nine

TABLE II. Estimates of component standard deviations for titers expressed as percent total activity, PRU/ml, and ratio to negative control

Allergen	No. of R/S/D/K/L*	Mean % TA	% TA				
			SD due to:				
			REPS	SERA	DAYS	LOTS	LABS
House dust mite	3/2/2/2/12	25.8	2.86	0	2.75	2.09	2.15
Ragweed (heat-stable)	3/2/2/2/12	29.3	2.57	0	2.22	2.36	3.00
Cat dander	3/2/2/2/12	30.4	2.40	0.82	2.44	2.92	3.20
Honeybee venom	2/1/2/2/12	28.3	1.88	—	1.74	2.54	2.84
<i>Alternaria</i>	2/2/2/2/12	18.5	2.70	0	0.87	3.68	1.50
Ragweed (heat-labile)	3/2/2/2/12	9.7	1.01	0.23	1.04	1.48	1.94
Reference sera							
A	3/1/2/2/12	28.6	2.97	—	1.94	3.60	2.18
B	3/1/2/2/12	13.8	1.09	—	1.43	3.09	0
C	3/1/2/2/12	4.8	0.48	—	0.68	1.06	0.33
D	3/1/2/2/12	3.2	0.33	—	0.54	0.33	0.50

— = not estimated when only one serum was tested.

*Number of replicates/sera/days/kits/labs providing data for estimating variance components.

TABLE III. Estimates of component coefficients of variation (CV)* for titers expressed as percent total activity, PRU/ml, and percent negative control

Allergen	% TA					
	CV due to:					
	REPS	SERA	DAYS	LOTS	LABS	Total
House dust mite	11.1	0	10.6	8.1	8.3	19
Ragweed (heat-stable)	8.8	0	7.6	8.1	10.2	17
Cat dander	7.9	2.7	8.0	9.6	10.5	18
Honeybee venom	6.6	—	6.1	9.0	10.0	16
<i>Alternaria</i>	14.6	0	4.7	19.9	8.1	26
Ragweed (heat-labile)	10.4	2.4	10.7	15.2	20.1	29
Reference sera						
A	10.4	—	6.8	12.6	7.6	19
B	7.9	—	10.3	22.4	0	26
C	10.0	—	14.1	22.0	6.9	29
D	10.1	—	16.9	10.3	15.3	27

*CV expressed as percent.

TABLE IV. Expected variation* in the difference between two measurements (made on the same sera in the same laboratory) for titers expressed as PRU/ml

Allergen	Mean	Same day— same kit	% Mean	Different day— different kit	% Mean
<i>Alternaria</i>	7.16	±5.91	82.6	±8.54	121.1
Ragweed (heat-labile)	2.21	±1.34	60.5	±2.61	118.1
Reference sera					
A	17.25	±3.94	22.9	±3.94	22.9
B	3.77	±1.80	47.2	±1.88	49.2
C	0.75	±0.42	55.0	±0.42	56.1
D	0.42	±0.22	53.3	±0.31	74.9

*95% Confidence limits for difference of two measurements = $1.96\sqrt{2S^2}$ where S^2 is total variance estimate depending on contributing sources.

PRU/ml						Ratio to negative control					
Mean PRU/ml	SD due to:					Mean ratio	SD due to:				
	REPS	SERA	DAYS	LOTS	LABS		REPS	SERA	DAYS	LOTS	LABS
						17.7	2.04	0.42	2.84	2.76	3.66
						16.9	1.55	0	1.97	3.07	3.47
						19.5	1.63	0.48	3.35	2.67	3.58
						12.4	0.99	—	3.25	0.39	2.77
7.2	2.13	0	1.7	1.44	0	9.3	1.23	0	0.95	0.64	2.32
2.2	0.48	0	0.50	0.64	0	5.6	0.61	0	0.65	0.79	1.82
17.2	1.42	—	0	0	0.21						
3.8	0.64	—	0	0.19	0.13						
0.76	0.15	—	0	0.03	0.073						
0.42	0.08	—	0.03	0.07	0.05						

PRU/ml						% NC					
CV due to:						CV due to:					
REPS	SERA	DAYS	LOTS	LABS	Total	REPS	SERA	DAYS	LOTS	LABS	Total
						11.5	2.4	16.0	15.6	20.6	32
						9.2	0	11.7	18.2	20.5	31
						8.4	2.5	17.2	13.7	18.4	30
						8.0	—	26.2	3.1	22.3	35
29.8	0	23.6	20.1	0	44	13.2	0	10.2	6.9	24.9	30
21.8	0	22.7	29.09	0	43	11.0	0	11.6	14.1	32.5	39
8.3	—	0	0	1.2	8						
16.8	—	0	5.0	3.4	18						
19.7	—	0	3.9	9.2	22						
19.0	—	7.1	16.6	11.9	30						

commercial laboratories located in different geographic areas. Two weeks later, samples of the same two aliquots were again sent to the same laboratories (Fig. 2). Replicate samples are usually not performed by commercial laboratories.

The nine commercial laboratories used different reporting methods for results: Phadebas RAST (Pharmacia Diagnostics),¹ modified RAST assay,² percent of total counts bound in sample as compared with that in nonallergic sera (Mayo Clinic), and the RASP assay (Lapapa Institute, Inc.).*

*Pamphlet: Allergy-Immuno-Diagnostics, Lapapa Institute, Inc., Los Angeles, Calif. 90057.

RESULTS

Phase I (Committee laboratories)

Tables II and III summarize the estimates of the variance components or antibody titers expressed as percent total activity, PRU/ml, and percent negative control. The variance components identified in Tables II and III are defined as follows:

REPS: Variation associated with replicate analyses (same sera-day-kit-lab).

SERA: Variation associated with different aliquots of the same sera.

DAYS: Variation associated with measurements made on different days (same sera-kit-lab).

TABLE V. Ragweed sera tested by commercial RAST—significance of titer according to laboratories' scales

Laboratory	Cost (\$)	High titer (1:5)			
		Day 1		Day 2	
		Serum 1	Serum 2	Serum 1	Serum 2
A.M.L., Fairfax	12.00	VS	VS	VS	VS
ICN, Portland	7.45	VS	VS	VS	VS
Mayo Clinic,* Rochester	8.00	S	S	S	S
Pharmacia, Piscataway	12.00	VS	VS	VS	VS
AID, Princeton	8.00	VS	VS	VS	VS
Barry, Pompano Beach	7.95	VS	VS	VS	VS
Diagnostics, Falls Church	8.00	VS	VS	VS	VS
Serolab, Waco	8.00	VS	VS	VS	VS
Lapapa, Los Angeles	7.25	VS	S	S	S

VS = very significant; S = significant; B = borderline; N = not significant.

*Regional Medical Laboratory, Mayo Clinic, Rochester; this laboratory does not use the VS designation.

LOTS: Total variation associated with differences between lots (kits within same lot, lot by laboratory interaction, and weeks).

LABS: Differences between laboratories.

Table II entries are the analysis of variance estimates of the component standard deviations. The corresponding coefficients of variation in Table III were obtained by dividing the standard deviations by the corresponding mean levels and are expressed as percent. In Table II, for example, the standard deviation for replicate measurements (REPS) of percent total activity is 2.86 for house dust mite. The corresponding coefficient of variation (Table III) is 11.1%, $2.86/25.8 \times 100$.

The first four pooled allergic sera contained antibody titers greater than the reference A provided with the RAST kits, and therefore the corresponding PRU/ml concentration was greater than 17.5. For this reason, PRU/ml values for these four sera could not be included in the tables. Negative control sera were not tested with the Phadebas reference sera, and these data were therefore not included in tables for percent of the negative control.

Testing each serum in triplicate with the RAST kit allowed for estimation of the amount of variation in replicate measurements of a single serum sample (columns labeled REPS). This source is indicative of the variability that would be expected with a single determination when the same kit is used on the same day. Among the three methods of expressing antibody titers, the relative variation in repeat analyses of the same sample appeared to be greater with the PRU/ml system (Table III). It should be noted that of these

methods, the PRU/ml method uses a logarithmic scale in determination of values and this relative variation in PRU/ml is more apparent than real.

To ensure that the sources of variation of interest (REPS, DAYS, LOTS, LABS) could not be ascribed to differences between the serum specimens, the two sera sent (sera 1 and 2) were aliquots of the same serum samples. As one would expect, and as Tables II and III demonstrate, there are negligible variations attributable to sera differences. From Tables II and III it is also evident that the variance components (DAYS and LOTS) made significant contributions to the total variability in all three means of expressing IgE antibody titer. These sources of variance were present at both high and low antibody titers.

The expected variation in the differences between two measurements expressed as PRU/ml was also determined (Table IV). This calculation can be used to gauge the usefulness of RAST in detecting changes in IgE antibody titer that might be expected with a form of therapy. This would include the difference in IgE titers between preimmunotherapy and postimmunotherapy serum samples.

For example, for the *Alternaria* allergic sera, a difference between two PRU/ml measurements on the same day with the same kit in the same laboratory could be expected to be within ± 5.9 . This is 83% of the mean value of 7.2. If the two measurements are made on different days with different lots, the expected limits for the difference would be ± 8.5 PRU/ml or 121% of the corresponding mean value of the same *Alternaria* sample. Had these measurements been made in different laboratories, the degree of un-

Medium titer (1:40)				Low titer (1:200)			
Day 1		Day 2		Day 1		Day 2	
Serum 1	Serum 2	Serum 1	Serum 2	Serum 1	Serum 2	Serum 1	Serum 2
S	S	S	S	B	B	N	B
S	B	S	S	N	N	B	B
S	S	S	S	B	B	B	B
S	S	S	S	B	N	N	N
S	S	S	S	B	S	B	B
S	VS	S	S	S	S	B	S
S	S	S	S	S	S	B	S
S	S	S	S	S	S	S	S
VS	S	S	S	VS	VS	S	S

certainty associated with the differences would be even greater.

Phase II (Commercial laboratories)

The results obtained from the survey of nine commercial laboratories are given in Table V. Because the laboratories differed in the method for unit of measurement, these results are recorded in terms of significance derived from each laboratory's enclosure of instructions. The laboratories varied considerably in their assessment of the clinical significance of their results.

Three of the laboratories reported very significant, significant, and borderline (negative) results, respectively, for the three dilutions of ragweed sera. Although three laboratories did not differentiate medium and low titers of ragweed sera, Lapapa Institute, Inc., attributed the same significance to all three titers of ragweed sera, and AID-Princeton furnished its results after initial presentation of these data to the scientific session of the American Academy of Allergy.

DISCUSSION

This study was designed to evaluate replicate and day-to-day reproducibility of the Phadebas RAST procedure when performed by 12 different research laboratories using the same RAST kit reagents and six different allergic sera. The results show that the Phadebas RAST kit will differentiate high, medium, and low titers of IgE antibody in a variety of allergic sera. Antibody titers obtained with the Phadebas RAST kit are reported in PRU/ml, determined from birch-pollen allergic serum provided as a reference

serum with the kit. Because it is not certain that all allergic sera can be compared on the basis of this reference, the Committee was also interested in alternative methods of calculating IgE antibody titers in allergic sera. Two additional methods of calculating titer data were employed: percent of total activity (counts bound/counts added $\times$ 100) and the ratio of the total radioactivity bound by the unknown sample to that bound by a negative control serum (counts bound of sample/counts bound of negative sera $\times$ 100). It should be recognized that the method of reporting results as percent total activity is subject to variation induced by changes in age and immunoreactivity of reagents. Furthermore, these differences of variation between the results from the methods of percent total activity and PRU/ml (Tables II and III) do not indicate that one method is better than another. Since PRU values are obtained by assuming a linear relationship between the logarithms of the two measurement units, percent total activity and PRU, a given difference in absolute or relative terms between two percent activity measurements does not correspond to the same difference in the PRU system. Nevertheless, there is great uncertainty in both methods regarding measurement amounts.

Of particular importance in this study is the observation that there is a very large variability when single determinations are made, an observation that results from testing triplicate samples *in vitro*. Values obtained from single determinations are probably not useful in forming clinical judgment, an observation true of all titers of allergic sera tested. Testing the same serum in replicate would reduce this variability.

For example, if duplicate or triplicate samples were tested and averaged, the standard deviation and coefficient of variation would be reduced by factors of $\sqrt{2}$ and $\sqrt{3}$, respectively.

The data from this study also provide information concerning the use of the RAST procedure to measure changes in specific IgE antibody that might be expected from treatment such as immunotherapy. Because there is an expected variation in the difference between two measurements, when paired sera are tested and compared they must be tested on the same day in the same laboratory with the same reagents. Under these circumstances, with medium-titer sera (2.2 PRU/ml), changes of 61% or more in the data expressed as PRU/ml would be expected to be a result of the treatment and not a variation in the test. If pretreatment serum is not used and tested at the same time with the same reagents as those used for posttreatment serum, the changes for the same medium-titer serum reported in PRU/ml must be 118%. It is also important to remember that RAST values are subject to the presence of large quantities in the serum of IgG antibody to the allergen tested. High titers of IgG antibody, such as might occur with immunotherapy, have been reported to inhibit RAST for IgE antibody,³ and a falsely low value for the IgE antibody by RAST may result.

The second phase of this study, the survey of commercial laboratories in the U.S.A., demonstrated that not all commercial laboratories could differentiate between high-, medium-, and low-titer ragweed allergic sera. This was particularly true for medium- and low-titer sera. The results from the commercial laboratories were compared by word expression (very significant, significant, borderline, and not significant). Direct comparison by a unit value was not possible because the laboratories varied considerably in their unit-value scoring system. The most common system was not that recommended in the instructions for the RAST kit, but a modification based on a scale of six classes. Laboratories using this scale more frequently failed to differentiate by significance medium- and low-titer sera.

CONCLUSIONS

(1) The RAST procedure is an effective in vitro means of differentiating high-, medium-, and low-titer IgE antibody in allergic sera.

(2) Further characterization of the clinical significance of these in vitro means for determining IgE results is necessary. This is particularly true for clinical laboratories in which clinical significance is attached to the same allergic sera.

(3) There is a large expected variation in RAST results for sera tested on different days, even with the same kit. Sera collected to evaluate changes in IgE antibody titer must be saved and tested in the same laboratory on the same day with the same kit.

(4) Serum samples should be tested in replicate to reduce the inherent test (same kit-day) variation.

REFERENCES

1. Lundkvist U: Research and development of the RAST technology, in Evans R III, editor: *Advances in diagnosis of allergy. RAST*. New York, 1975, Stratton Intercontinental Medical Book Corp., p. 85.
2. Nalebuff DJ, Fadal RG: The modified RAST assay: an aid in the diagnosis and management of allergic disorders. *Continuing Educ Fam Phys* 10:64, 1979.
3. Zeiss CR, Grammer LC, Levitz D: Comparison of the radioallergosorbent test and a quantitative solid-phase radioimmunoassay for the detection of ragweed-specific immunoglobulin E antibody in patients undergoing immunotherapy. *J ALLERGY CLIN IMMUNOL* 67:105, 1981.

STATISTICAL APPENDIX

Estimates of variance components associated with the design in Fig. 1 were based on the following statistical model:

$$Y_{ijklsr} = M + L_l + K_{k(l)} + D_{d(kl)} + S_{s(dkl)} + R_{rs(dkl)}$$

where Y represents a measurement (PRU/ml, %TA, %NC), with subscripts indicating replication (r), sera (s), day (d), kit (k), and laboratory (l). The subscripted terms on the right are random effects representing sources of variation due to differences between (1) laboratories, (2) kits within lots + lots + lot by lab interaction, (3) days using same kit-lab, (4) aliquots of same sera tested on same day-kit-lab, and (5) replicate measurements of same aliquot measured on same sera-day-kit-lab. The component $K_{k(l)}$ represents the total additional variation associated with different kits (possible from different lots and lot by laboratory interaction).

Estimates of the variance components (expressed as standard deviations, Table II) were obtained by equating mean squares (from analysis of variance calculations) to the respective expected values. With only one sera (reference sera A, B, C, or D) no estimate for this component can be made. However, since there was little or no variation between aliquots of same sera, this has no effect on estimates of the remaining components.

Note: No attempt was made to eliminate what might be judged as outlying (aberrant) values or in some cases groups of values (on a particular day or from a particular laboratory). The variance components caused by the various sources of variations (labs, days, etc.) are descriptive of the data from this survey and may not reflect long-term behavior, especially for interlaboratory variation.