

18. Fleisch JH, Rinkema LE, Haisch KD, et al. LY 171883, 1-<2-hydroxy-3-propyl-4-<4-(1H-tetrazol-5-yl) butoxy > phenyl > ethanone, an orally active leukotriene D₄ antagonist. *J Pharmacol Exp Ther* 1985;233:148-57.
19. Krell RD, Kusner EJ, Aharony D, Giles RE. Biochemical and pharmacological characterization of ICI 198,615: a peptide leukotriene receptor antagonist. *Eur J Pharmacol* 1989;159:73-87.
20. Krell RD, Aharony D, Buckner C, et al. The preclinical pharmacology of ICI 204,219, a peptide leukotriene antagonist. *Am Rev Respir Dis* 1990;141(4):978-87.
21. Corey EJ, Clark DA, Goto G, et al. Sterospecific total synthesis of a "slow-reacting substance of anaphylaxis" leukotriene C-1. *J Am Chem Soc* 1980;102:1436-9.
22. Smith LJ, Kern R, Patterson R, Krell RD, Bernstein PR. Mechanism of leukotriene D₄-induced bronchoconstriction in normal subjects. *J ALLERGY CLIN IMMUNOL* 1987;80:340-7.
23. Ueno A, Tanaka K, Katori M, Hayashi M, Arai Y. Species difference in increased vascular permeability by synthetic leukotriene C₄ and D₄. *Prostaglandins* 1981;21:637-48.
24. Greenberger PA, Smith LJ, Patterson R, et al. Comparison of cutaneous and bronchial reactivity to leukotriene D₄ in humans. *J Lab Clin Med* 1986;108:70-5.
25. Brain SD, Camp RDR, Black AK, et al. Leukotrienes C₄ and D₄ in psoriatic skin lesions. *Prostaglandins* 1985;29:611-9.
26. Wong E, Greaves MW, O'Brien T. Increased concentrations of immunoreactive leukotrienes in cutaneous lesions of eosinophilic cellulitis. *Br J Derm* 1984;110:653-6.

Modification of the fluorescent allergosorbent test as an inhibition assay for determination of cross-reactivity among aeroallergens

David Perrick, MD, Chester T. Stafford, MD, Elisa Armstrong, BS, and
Robert H. DuRant, PhD Augusta, Ga.

The fluorescent allergosorbent test was adapted as an inhibition assay to determine cross-reactivity between aeroallergens. With this method, similar antigenic determinants were found between short ragweed and giant ragweed, cocklebur, lamb's-quarter, rough pigweed, marsh elder, and goldenrod. Cocklebur and giant ragweed were highly potent in their ability to competitively bind to short ragweed IgE. The other pollens demonstrated lower potency of cross-reacting antigens. The fluorescent allergosorbent test-inhibition assay appears to be a useful method to determine cross-reactivity among aeroallergens. (J ALLERGY CLIN IMMUNOL 1991;87:98-103.)

Since its development in the 1960s, RAST¹ has become the standard in vitro method for measuring antigen-specific IgE. The adaptation of the RAST as an inhibition assay has been the standard laboratory procedure for comparing potency of allergenic extracts^{2,3} and evaluating cross-reactivity among phylogenetically related allergenic pollens^{4,6} and molds.^{6,7}

FAST (3M Riker, St. Paul, Minn.) has also been demonstrated to be a useful method for measuring

Abbreviations used

FAST:	Fluorescent allergosorbent test
SRW:	Short ragweed
GRW:	Giant ragweed
RRRPW:	Rough red root pigweed
GR:	Goldenrod
CB:	Cocklebur
RME:	Rough marsh elder
LQ:	Lamb's-quarter
% inh:	Percent inhibition
C ₅₀ :	Concentration of soluble antigen resulting in 50% inhibition
r:	Pearson's correlation coefficient
FSU:	Fluorescence signal units

antigen-specific IgE in serum.⁸ Technically, it is simpler to perform than the RAST, and the results are available sooner. The FAST can be performed within a few hours, whereas the RAST is an overnight assay.

From the Allergy/Immunology Section, Medical College of Georgia, Augusta, Ga.

Received for publication June 22, 1989.

Revised June 29, 1990.

Accepted for publication July 29, 1990.

Reprint requests: Chester T. Stafford, MD, Professor of Medicine and Pediatrics, Allergy-Immunology Section, Medical College of Georgia, Augusta, GA 30912-3790.

1/1/24692

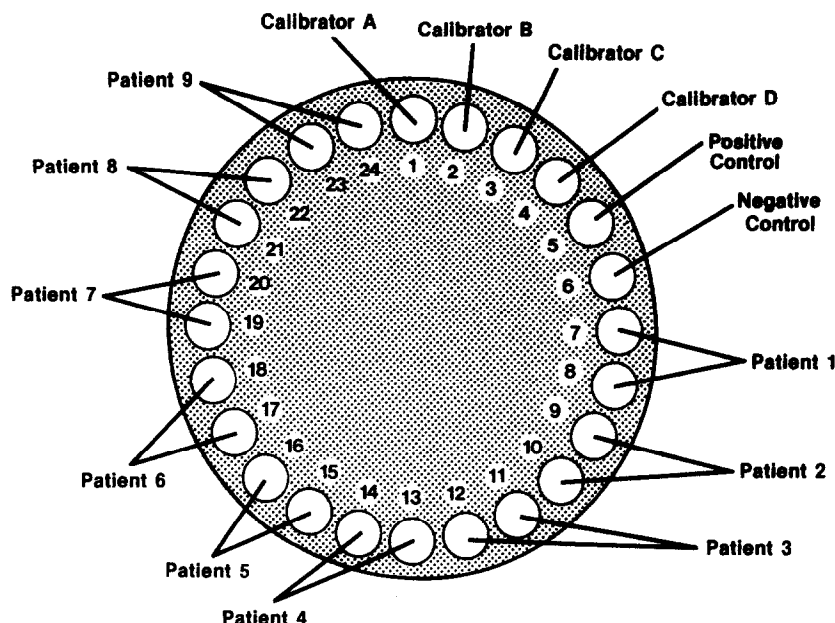


Fig. 1. FAST assay. Microwells 1 through 4 coated with perennial ryegrass allergen, and 7 through 24, coated with SRW. In determining the concentration of serum specific to SRW, each patient's serum was evaluated in duplicate.

We modified the FAST procedure for use as an inhibition assay for determining cross-reactivity between various weed pollens and SRW.

MATERIAL AND METHODS

Study subjects

Patients of at least 12 years of age with fall seasonal hay fever symptoms were recruited for the study from the allergy clinics at the Medical College of Georgia. After obtaining informed consent, each patient was prick skin tested with 1:20 wt/vol of SRW extract purchased from Hollister-Stier Laboratories, Spokane, Wash., and with 0.03% albumin saline and 1.0 mg/ml of histamine as controls. Ten patients who reacted strongly to SRW with development of a wheal with pseudopod formation and who exhibited normal responses to the controls, contributed 15 ml of blood for in vitro assay. The blood specimens were centrifuged within 3 hours of collection, and the individual sera were stored at -70°C .

FAST procedure

The SRW-specific IgE titer of each serum specimen was determined with the FAST technique as follows: All reagents were allowed to come to room temperature. A FAST disk carousel (plastic disk containing 24 serially numbered circular openings to accommodate proprietary allergen-specific coated microtiter wells) was placed on a flat tabletop (Fig. 1). IgE FAST-Plus reference wells, coated with perennial ryegrass allergen were placed in slots 1 to 4. Microwells coated with SRW were placed in positions 5 to 24. Fifty microliters of IgE FAST-Plus calibrators A, B, C, and D, containing 0.35, 0.8, 4, and 20 IU/ml, respectively, of

specific IgE to perennial ryegrass was pipetted into wells nos. 1, 2, 3, and 4. Fifty microliters of 3M positive control serum (high-titer IgE to SRW) was pipetted into well no. 5; 50 μl of 3M negative control serum (containing no detectable SRW IgE antibody) was pipetted into well no. 6. Into the remaining 18 wells, 50 μl of patient sera was pipetted. Each serum sample was tested in duplicate, allowing sera from up to nine patients to be assayed for specific IgE in each run. The disk was covered with aluminum foil and incubated for 2 hours at room temperature.

After incubation the carousel was inverted and flicked to discard the liquid from the wells. All 24 wells were washed with 200 microliters of proprietary 3M wash buffer (0.1 mol/L of phosphate buffer containing detergent) three times. One hundred microliters of IgE FAST-Plus enzyme-antibody conjugate (alkaline-phosphatase-labeled antihuman IgE [monoclonal] conjugate in a Tris buffer, pH 7.5) was pipetted into all microwells. After a 2-hour incubation period, all wells were washed three times with buffer. One hundred microliters of 3M fluorescent substrate (4-methyl-umbelliferyl phosphate in 2-amino-2-methyl propanol buffer at pH 9.5) was pipetted into all 24 empty wells and incubated for 15 to 30 minutes before being analyzed with the 3M FluoroFAST reader.

The reader is a high-speed fluorometer, measuring fluorescence intensity from calibrators A, B, C, and D (each of known specific IgE concentration). With linear regression analysis, the fluorometer determines the best line of log FSUs versus log IgE concentration (international units per milliliter). For each measured FSU from patient samples in wells 5 to 24, the reader extrapolates the corresponding specific IgE concentration in international units per milliliter.

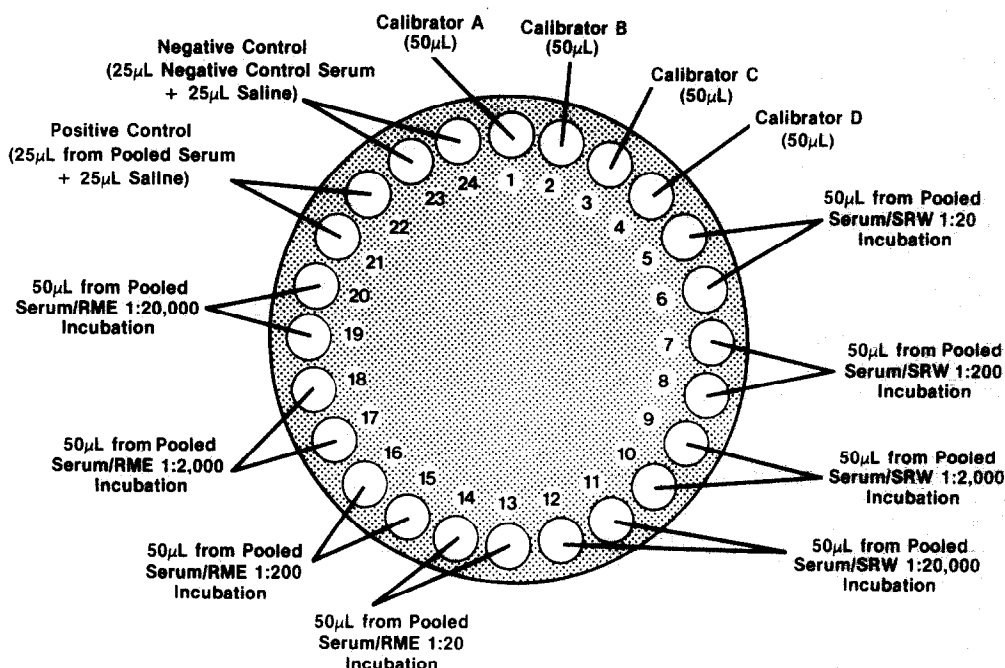


Fig. 2. FAST inhibition. Assay to determine the ability of increasing concentrations of soluble antigens SRW and RME to specifically bind with SRW IgE. Incubations of specific concentrations of fluid-phase antigen with high-titered pooled serum were each performed in duplicate.

TABLE I. Calculated slope, C_{50} , and correlation coefficient (r) of regression line of inhibition by soluble-phase weed-pollen allergens

Fluid-phase aeroallergen	Slope	C_{50}^*	r
SRW	27.6	0.01	1.00
GRW	24.7	0.51	0.99
RRRPW	57.1	13.5	0.99
GR	43.6	10.0	0.99
CB	13.2	0.08	0.98
RME	47.0	10.4	1.00
LQ	32.8	2.6	0.99

*Values extrapolated from weight per volume concentrations.

Sera were obtained from five individuals with elevated titers of serum IgE to SRW (>1.0 IU/ml) as determined by the FAST assay. The five donors ranged in age from 15 to 53 years. All patients were healthy except for having fall seasonal allergic rhinitis. None of the patients had received allergen immunotherapy.

Preparation of high-titer pooled serum

An equal volume of serum from each donor was combined to form a pooled serum from which 1 ml aliquots were prepared and subsequently stored at -70°C . This pooled serum was demonstrated to have a high concentration (greater than class 2) of SRW-specific IgE by Allercoat

EAST assay (Kallstad Diagnostics, Austin, Texas). Parallel testing of this pool by RAST revealed an SRW-specific IgE concentration of 4.7 IU/ml.

Fluid-phase antigens

The fluid-phase aeroallergens evaluated for cross-reactivity with SRW IgE were SRW, GRW, RRRPW, GR, CB, RME, and LQ. Extracts of these allergens were purchased from Hollister-Stier Laboratories because they were not available from 3M Riker. Hollister-Stier quality control procedures include counts of at least 1000 pollen grains from each batch of raw pollen. The maximum contamination of any of the seven extracts used in this study with a differing pollen was 0.9% by volume of raw material. Two soluble extracts were contaminated with *Ambrosia* in concentrations less than two pollen grains per 1000 counted. The allergens were purchased as 50% glycerinated extracts in 1:20 wt/vol concentrations, representing 1 gr of defatted pollen source material extracted in 20 ml of buffer. Additional fluid-phase antigens studied were red oak, nonglycerinated SRW, and nonglycerinated GRW.

FAST inhibition

The FAST-inhibition procedure was a modification of the RAST-inhibition method of Gleich et al.³ Various concentrations of competing fluid-phase antigens, diluted with 0.03% human serum albumin saline in 0.4% phenol, were incubated with an equal volume of pooled serum for 2 hours. After incubation, 50 μL aliquots were pipetted into SRW-coated microwells for measurement of SRW-specific IgE concentrations determined by the FAST procedure.

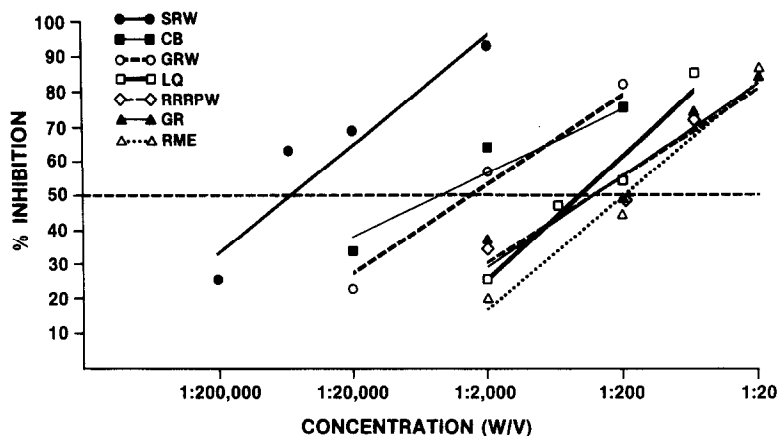


Fig. 3. Regression lines of inhibition by fluid-phase weed pollen allergens.

Percent inhibition (% inh) by specific concentrations of competing soluble antigen was calculated as follows

$$\% \text{ Inh} = \frac{[(\text{IU/ml of } + \text{ Control}) - (\text{IU/ml of sample})]}{[(\text{IU/ml of } + \text{ Control}) - (\text{IU/ml of } - \text{ Control})]}$$

For example, to calculate the % inh of SRW IgE by RME at the 1:20 concentration (Fig. 2):

$$\% \text{ Inh} = \frac{[(\text{Average of wells no. 21 \& 22}) - (\text{Average of wells no. 13 \& 14})]}{[(\text{Average of wells no. 21 \& 22}) - (\text{Average of wells no. 23 and 24})]}$$

Percent inhibition of SRW IgE by each soluble antigen was plotted on semilog graph paper, with Y axis as % inh and X axis as log concentration (milligrams per milliliters) of competing fluid-phase antigen. As demonstrated by Gleich et al.,³ by increasing the amount of antigenically related competing soluble antigen, inhibition curves plotted on semilog paper approximate a linear relationship, particularly in the region between 30% to 70% inhibition. As suggested by Schröder and Yman,⁹ two points on either side of the 50% inhibition level are used for calculation of the slope. In the case of a very steep curve, three points can be used for the calculation. The Pearson's correlation coefficient of the line determined from such points should be >0.90. For each competing fluid-phase antigen evaluated, the graph of inhibition was determined by linear regression. Similarity in slopes were a reflection of antigenic similarity to SRW, whereas comparison of concentration of competing antigen required to produce C_{50} determined relative potency.

RESULTS

The correlation coefficients (r) from calibrators A, B, C, and D for all assays were ≥ 0.99 . For assays determining % inh of SRW IgE by fluid-phase SRW, SB, GRW, LQ, GR, RME, and RRRPW antigens, correlation coefficients (r), slopes and C_{50} values are

presented in Table I. The regression lines of all aeroallergens studied are illustrated in Fig. 3. Based on a Student's t test designed to compare slopes, none of the weed pollens studied had slopes of inhibition that differed significantly from the slope of SRW ($p \leq 0.01$).

Assays comparing 2-hour versus overnight (16 hours) incubations of high-titer serum with competing fluid-phase antigen generated very similar inhibition curves, as did use of 50% glycerinated versus non-glycerinated soluble antigens. Incubation of pooled high-titer SRW IgE serum with red oak allergen demonstrated no resultant inhibition. Last, the FAST assay was performed on dilutions of the high-titer pooled serum itself. The slope of the best fit line through individual experimental points (corresponding to log FSU [Y axis] and log concentration SRW IgE [in international units per milliliter] X axis) did not differ significantly from that derived from calibrators A, B, C, and D (Fig. 4).

DISCUSSION

The principle of RAST inhibition is based on competition between solid-phase and soluble antigen for specific IgE antibody binding sites.³ As increasing amounts of antigenically related fluid-phase antigen combine with high-titer serum IgE, fewer IgE molecules are available to bind with the solid-phase antigen. Gleich et al.³ have demonstrated that derived inhibition curves plotted on semilog paper (Y axis as % inh and X axis as log amount of fluid-phase antigen) approximate a linear relationship, particularly in the region between 30% to 70% inhibition. Similarity between slopes of inhibition lines reflects antigenic relatedness, whereas the quantity of soluble antigen required to produce C_{50} indicates the relative potency of allergenic extracts.¹⁰

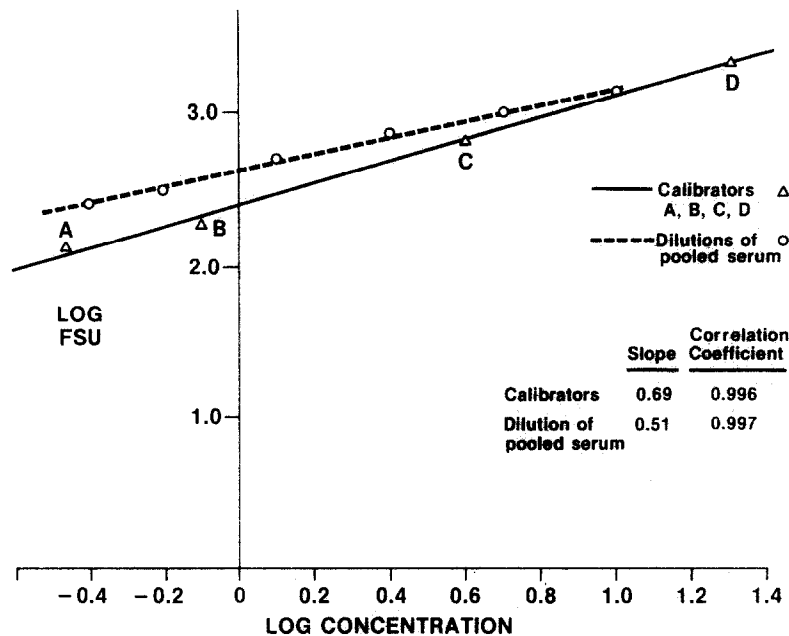


Fig. 4. Comparison of regression lines of dilutions of high-titered pooled serum with calibrators A through D. The slopes are not statistically different, allowing for the valid determination of concentration of IgE (international units per milliliters) from measured FSU.

Leiferman et al.¹¹ used RAST inhibition to demonstrate cross-reactivity among several species of ragweed. Stafford et al.¹² used Phadezym RAST inhibition to measure cross-reactivity of weed pollens with ragweed. In this pilot study, we were interested in evaluating the use of FAST inhibition to investigate cross-reactivity among various genera of fall pollens with SRW. Other than differences relating to the solid-phase antigen, the principle of FAST inhibition is similar to the principle described for RAST inhibition.³

The FAST-inhibition assay is technically simple. The entire procedure can be performed in approximately 7 hours and does not require the use of radioactive materials. Reproducible inhibition curves were obtained for each fluid-phase antigen studied. The intra-assay coefficient of variation was $5.75\% \pm 5.73\%$.

The inhibition slopes of all the fall pollens studied demonstrate some degree of cross-reactivity with SRW, although the potencies vary widely, as indicated by the broad range of C_{50} values measured as determined by dilutions of the 1:20 wt/vol concentration. Among the pollens studied, CB exhibited the most dissimilar inhibition slope compared to that of SRW; however, this apparent difference was not statistically significant. CB also demonstrated the greatest potency in terms of its ability to competitively bind with SRW IgE. GRW exhibited an intermediate degree of cross-reactivity, whereas LQ, GR, RME, and RRRPW ex-

hibited only low levels of cross-reactivity with SRW, as evidenced by their relatively high C_{50} values. The maximum allowable contamination of two pollen grains per thousand could account for up to half the cross-reactivity noted between marsh elder and SRW in our assay.

Neither the length of incubation with competing fluid-phase antigen (2 hours versus 16 hours) nor the use of 50% glycerinated versus nonglycerinated extracts had any significant effect on the regression lines. Incubation of pooled high-titer SRW IgE serum with red oak allergen, for comparison of unrelated antigens, demonstrated no resultant inhibition. These data suggest that the inhibition was not solely due to non-specific binding. The slopes of the regression lines for dilutions of individual and pooled high-titer sera did not differ significantly from the slope for calibrators A through D (log FSU versus log concentration of soluble antigen). These results appear to reinforce the validity of the FAST-inhibition assay.

Experience with performance of the FAST-inhibition assay suggests that the results may be affected by use of (1) sera from patients receiving allergen immunotherapy and (2) wells fixed with a low density of IgE binding sites.

Individual dose-response curves from SRW-immunized patients appeared to differ from those of nonimmunized patients (data not presented). This difference may be due to the presence of IgG blocking

antibody that may have an inhibitory effect on the FAST and possibly other inhibition assays. Additional study will be necessary to define better the magnitude and immunologic mechanisms involved in this inhibitory effect.

Initial determinations in the FAST-inhibition assay yielded falsely low IgE antibody titers, even on sera demonstrated by RAST to contain high-titer IgE specific for SRW (data not presented). This problem was overcome by use of high-titer wells fixed with a higher density of SRW antigen. Only high-density wells are recommended for performance of the FAST-inhibition assay.

Only a limited number of different solid-phase microwells and soluble antigens are presently available from the FAST manufacturer. The availability of a larger selection of antigens would increase the potential use of this assay for evaluation of cross-reactivity among other allergens, for example, molds etc. The solid-phase allergens used in the RAST are remarkably stable, and virtually any allergen can be standardized, provided it can be bound to a solid-phase support.¹⁰ Last, in a recent study comparing RAST and FAST for 11 allergens in 288 patients, concordant results were obtained in 78.7% of the 300 sera analyzed.⁸ When both methods were compared with skin tests in 91 patients, the agreement was 84.6% for RAST and 70.3% for FAST, suggesting that the RAST may be the slightly more sensitive in vitro assay. However, the FAST-inhibition assay also appears to be a useful and efficient method for determining cross-reactivity among aeroallergens.

Additional studies of antigenic relationships among aeroallergens may provide useful information for the practicing allergist. Inhibition assays demonstrating cross-reactivity among aeroallergens may assist in selection of the most clinically relevant allergens for diagnostic testing and immunotherapy.

We thank Dr. Gerald J. Gleich, Mayo Clinic, Rochester,

Minn., and Dr. Dennis R. Ownby, Henry Ford Hospital, Detroit, Mich., for their helpful suggestions in the development of this assay and interpretation of these data. We also thank Linda Impson for her technical assistance and Kathy Rhodes for typing the manuscript.

REFERENCES

1. Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* 1967;2:1105-7.
2. Gleich GJ, Leiferman KM, Jones RT, Hooton ML, Baer H. Analysis of the potency of extracts of June grass pollen by their inhibitory capacities in the radioallergosorbent test. *J ALLERGY CLIN IMMUNOL* 1976;58:31-8.
3. Gleich GJ, Larson JB, Jones RT, Baer H. Measurement of the potency of allergenic extracts by their inhibitory capacities in the radioallergosorbent test. *J ALLERGY CLIN IMMUNOL* 1974;53:158-69.
4. Gleich GJ, Campbell AR, Gleich MC, Swedlund HA. Differences in the reactivity of short and giant ragweed with IgE antibodies. *J ALLERGY CLIN IMMUNOL* 1980;65:110-7.
5. Martin BG, Mansfield LE, Nelson HS. Cross-allergenicity among the grasses. *Ann Allergy* 1985;54:99-104.
6. Agarwal MK, Jones RT, Yunginger JW. Shared allergenic and antigenic determinants in *Alternaria* and *Stemphylium* extracts. *J ALLERGY CLIN IMMUNOL* 1982;70:437-44.
7. Karr RM, Wilson MR, Anicetti VR, Lehrer SB, Butcher BT, Salvaggio JE. An approach to fungal antigen relationships by radioallergosorbent test inhibition. *J ALLERGY CLIN IMMUNOL* 1981;67:194-8.
8. Gueant JL, Moneret-Vautrin DA, De Jardin G, Algalarrondo C, Nicolas JP, Grilliat JP. Comparative evaluation of RAST and FAST for 11 allergens in 288 patients. *Allergy* 1989;44:204-8.
9. Schröder H, Yman L. Standardization of the RAST inhibition assay. *Allergy* 1980;35:234-6.
10. Rose NR, Friedman H, Fahey JL, eds. *Manual of clinical laboratory immunology*. 3rd ed. Washington, D.C.: American Society for Microbiology, 1986:652-9.
11. Leiferman KM, Gleich GJ, Jones RT. The cross-reactivity of IgE antibodies with pollen allergens. II. Analyses of various species of ragweed and other fall weed pollens. *J ALLERGY CLIN IMMUNOL* 1976;58:140-8.
12. Stafford CT, Smith L, Tillirson M, DuRant RH. Cross-reactivity of weed pollens with ragweed by Phadezym RAST inhibition. *Immunol Allergy Pract* 1990;12(1):4-8.