

Measurement of IgG blocking antibody in human serum: Comparison of ELISA with monoclonal antibody and fluorogenic substrate and *Staphylococcus* protein A solid-phase RIA

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We compared ELISA with mouse monoclonal antihuman γ -chain antibody and a fluorogenic substrate with the Staphylococcus protein A solid-phase radioimmunoassay (SPRIA) in the measurement of specific IgG antibody to short ragweed pollen. Sera from 51 ragweed-allergic patients undergoing allergen immunotherapy were evaluated for ragweed-specific IgG antibodies with the same ragweed extract in the two assay systems. With optimal conditions, the ELISA and SPRIA displayed comparable positive thresholds (~ 1 ng/ml of ragweed-specific IgG). Both assays also demonstrated consistently parallel dilution curves with 51 sera (mean interdilutional coefficient of variation [CV] $< 8.8\%$ for ELISA and $< 8.6\%$ for SPRIA). Reproducibility was determined by constructing precision profiles for intra- and interassay variations over the working ranges of each assay (ELISA, 0.8 to 100 ng/ml; SPRIA, 1 to 250 ng/ml). ELISA intra-assay CVs ranged from 13% near threshold to $< 5\%$ at higher antibody concentrations; SPRIA intra-assay CVs ranged from 4.3% to 2.8%. Interassay reproducibility was somewhat better for SPRIA (4.6% to 9.6%) than for ELISA (10% to 18%). In direct comparison, 41 (80%) of the 51 sera were concordant in the two assays ($r = 0.91$; $p < 0.001$). Although each assay result was reproducible, 10 (20%) of the sera elicited consistently discrepant results in the two assays. In eight of the 10 discordant sera, the SPRIA results were higher than ELISA, suggesting the possibility that some ragweed allergen may be better represented on the short ragweed-pollen extract agarose than on ELISA plate wells. There is no apparent explanation for the two sera in which ELISA results were consistently higher than SPRIA. The effects of serum dilution on nonspecific binding was minimal ($< 2\%$ in both assays). These two methods provide comparable results for ragweed IgG antibody measurement in most ($> 80\%$) sera. Occasionally, discrepant results would not be expected to be of clinical significance. Performance specifications for the ragweed IgG ELISA were acceptable, and the technical ease of ELISA is superior to the labor-intensive SPRIA. For routine clinical use, the ragweed ELISA is a suitable alternative to SPRIA. (J ALLERGY CLIN IMMUNOL 1988;82:11-19.)

The assessment of specific IgG antibody to relevant allergens has been increasingly used for investigating specific immunologic responses to empirical immunotherapy¹⁻⁵ and for attempting to establish minimal effective dose levels for such immunotherapy.^{6,7} We have previously developed agarose-based SPRIA for quantitative measurement of specific IgG antibodies to complex allergenic mixtures, such as

yellow jacket venom⁸ and pollen extracts.⁹ These SPRIA have not been totally satisfactory with regard to their practical requirements, which include laborious manual procedures, limited half-life of the detection protein, and the need for containment facilities for radioactive wastes.

Accordingly, ELISAs have been of potential interest.¹⁰⁻¹² Those developed to date have had varying performance characteristics, and successful applications have often been limited to homogeneous protein solutions.¹³⁻¹⁷ We have applied a McAb γ -chain antibody to an indirect ELISA assay that uses an enzyme-conjugated second antibody¹⁸⁻²² and a fluorogenic substrate²³⁻²⁶ as an alternative assay system for measurement of human IgG antibody. We compared sensitivity and assay performance of ELISA to that

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Abbreviations used

SPRIA:	<i>Staphylococcus</i> protein A solid-phase radioimmunoassay
RwE:	Short ragweed-pollen extract
Amb a I:	Short ragweed allergen I (formerly antigen E)
McAb:	Mouse monoclonal antihuman IgG
GAM:	Alkaline phosphatase-conjugated goat antimouse IgG
HSA:	Human serum albumin
BSA:	Bovine serum albumin
CV:	Coefficient of variations
B _{max} :	Maximum binding of <i>Staphylococcus</i> protein A to excess IgG (immunoreactivity)

of SPRIA for quantitation of specific IgG to short ragweed pollen. The performance of the ELISAs was highly satisfactory and rendered the test an attractive alternative to SPRIA for specific-IgG antibody determinations.

MATERIAL AND METHODS**Sera**

Sera were obtained from 51 patients undergoing immunotherapy for allergy to ragweed pollen and analyzed by both ELISA and SPRIA. A standard reference serum was prepared by pooling human sera with high levels of ragweed pollen-specific IgG antibody. The amount of specific-IgG antibody present in the reference serum pool was estimated by means of the SPRIA elution technique, as previously described.²⁷ Normal sera were collected from healthy laboratory volunteers who had no history of allergy to ragweed pollen.

Antigen

Lyophilized RwE (lot No. L56-85) (217 µg of Amb a I per milliliter, formerly antigen E) and purified Amb a I (catalog No. A601-902-185) were supplied by Research Resources Branch of National Institute of Allergy and Infectious Diseases (National Institutes of Health, Bethesda, Md.). Ragweed Amb a III, Amb a V, and Amb a VI were kindly provided by Dr. David Marsh (Baltimore, Md.).

Detection proteins and reagents

McAb IgG antibody (lot No. 3598R, clone HP 6064) was purchased from Unipath Inc. (U.K.). Affinity-purified GAM (lot No. 50-15-02) was purchased from Tago Inc. (Burlingame, Calif.); 4-methylumbelliferyl phosphate, adenosine 2-monophosphate buffer, BSA, Tween 20, Triton X-405, and human IgG were purchased from Sigma Chemical Co. (St. Louis, Mo.). Purified protein A from *Staphy-*

lococcus aureus was purchased from Pharmacia (Piscataway, N.J.).

Allergosorbent

RwE was insolubilized to Sepharose CL-4B (Pharmacia) with cyanogen bromide by a procedure described previously.⁸

Buffers

For plastic microtiter plate adsorption of RwE, a 0.1 mol/L of acetate buffer containing 1.0 mol/L of NaCl, pH 6.5, was used. A diluent containing 0.05 mol/L of Na₂HPO₄, 0.5 mol/L of NaCl, 0.5% NaN₃, 0.5% Tween 20, and 0.2% BSA, pH 7.5, was used for both ELISA and SPRIA. Washing buffer for the microtiter plate was a 0.05 mol/L of PBS containing 0.02% Triton X-405 and 0.01% of BSA, pH 7.4. Substrate buffer was 1.0 mol/L of 2-amino-2-methyl-1-propanol buffer, pH 9.5. Coupling buffer, containing 0.1 mol/L of Na₂CO₃ and 0.1 mol/L of NaCl, pH 8.5, was used for agarose insolubilization of RwE.

Radiolabeled detection proteins

Staphylococcus protein A (2 mg) was iodinated with 2 mCi of ¹²⁵I (Amersham Corp., Arlington Heights, Ill.) with the chloramine-T method.²⁸ ¹²⁵I-labeled protein A was then separated from unbound ¹²⁵I by gel filtration with a Sephadex G-25 column and, at the first radioactive peak, was pooled, aliquoted, and stored at -20° C until use.⁸

Optimization of RwE-specific IgG ELISA

The method used was a modification of an indirect ELISA as described by Kemeny et al.²⁹ Polystyrene microtiter plates (MicroFluoro B, Dynatech Laboratories Inc., Alexandria, Va.) were coated 150 µl per well of 200 ng Amb a I per ml of RwE in an acetate buffer. The plates were incubated overnight at 4° C and then washed three times with wash buffer. After drying, the plates could be safely kept at 4° C for at least 4 weeks without discernible loss of antigenic activity (data not presented).

For performing the assay, 100 µl of a 1:100 or greater dilution of either the standard reference pool or unknown sera were added in triplicate to antigen-coated wells. All dilutions of serum greater than 1:100 were made in normal serum diluted 1:100 in dilution buffer. After an overnight incubation at 4° C, the plates were washed three times with buffer. Then, 100 µl of McAb, diluted 1:10,000 in dilution buffer, was added to each well for a 4-hour incubation at room temperature, followed by three washes. One hundred microliters of GAM, diluted 1:2000 in dilution buffer, was placed into each well for a 2-hour incubation at room temperature, followed by three washes. The optimal dilution of McAb and GAM was predetermined by checkerboard titrations. Finally, 100 µl of 10⁻⁴ mol/L of 4-methylumbelliferyl phosphate was allowed to react at room temperature for 30 to 60 minutes until the top concentration of reference serum pool accumulated 3000 to

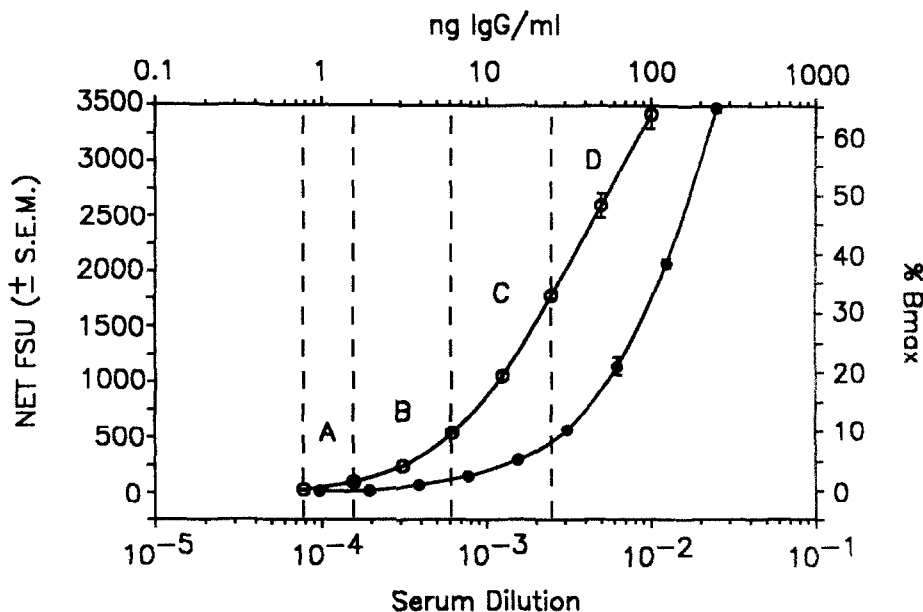


FIG. 1. Optimized standard curves of IgG ELISA (open circles) and SPRIA (closed circles) with standard reference serum pool. The net fluorescent signal units at 1:100 serum dilution for ELISA and the percent B_{max} from net counts per minute at 1:40 serum dilution for SPRIA were taken as top points, respectively. Positive thresholds were 0.78 ng/ml for ELISA and 0.98 ng/ml for SPRIA. Upper 95% confidence limits for negative sera (nonspecific binding) consistently remained at 85% of lowest reference curve point in ELISA and 91% of lowest reference curve point in SPRIA. Each point illustrated is mean $\pm$ SEM for three replicates. The standard curves were divided into four regions (labeled A-D) for precision profile analysis (Fig. 2).

4000 fluorescent signal units, as read by a microtiter fluorometer (3M Diagnostics Inc., Mountain View, Calif.) with 405 nm for excitation, and reading emission at 450 nm. In preliminary experiments, the fluorescence product generation was linear with time from 20 to 60 minutes.²² The specific-IgG levels were calculated by interpolation from dilution of a standardized reference pool containing 10 μ g/ml of ragweed IgG.

Optimization of RWE-specific IgG SPRIA

The RWE-specific SPRIA was developed and standardized in a manner similar to that previously reported.^{8,9} Briefly, 500 μ l of RWE-agarose sorbent (3% vol/vol) was added to 100 μ l of a 1:40 or greater dilution of either reference or unknown serum preadsorbed overnight with 5% (vol/vol) of agarose to remove carbohydrate-specific IgG.³⁰ This mixture was rotated orbitally for 4 hours at room temperature, after which, the sorbent was washed three times with 2.5 ml of dilution buffer to remove unbound IgG. Approximately 125,000 disintegrations per minute of ¹²⁵I-labeled *Staphylococcus* protein A was added in 0.5 ml to the washed IgG-RWE sorbent complexes. After an overnight incubation at room temperature, the sorbent was washed four times with 2.5 ml of dilution buffer. Radioactivity bound was then quantitated in a gamma counter. Results were expressed as percent B_{max} , calculated by dividing the net counts per minutes bound by B_{max} , the im-

munoreactive counts per minute of ¹²⁵I-labeled *Staphylococcus* protein A added.⁸

Analysis of data

The standard pool dilution curve was mathematically fit by a 3-parameter spline function as previously described.³¹ Doses of unknown sera were calculated from the curve function. For performance assessment, the ELISA and SPRIA precision profiles were calculated according to the method of Ekins.³² Statistical computations were performed with CLINFO software at the Johns Hopkins Clinical Research Center.

RESULTS

Sensitivity and nonspecific binding

The same high-titered reference pool was used in both assay systems and produced the standard curves illustrated in Fig. 1. Nonspecific binding was studied by analyzing 1:100 pooled normal sera. The upper 95% confidence limits for negative sera consistently remained <85% of the lowest reference curve point (0.78 ng/ml) in ELISA and <91% of the lowest reference curve point (0.98 ng/ml) in SPRIA. This indicated that assay thresholds could be conservatively estimated at 0.8 ng/ml for ELISA and 1.0 ng/ml in SPRIA (Fig. 1).

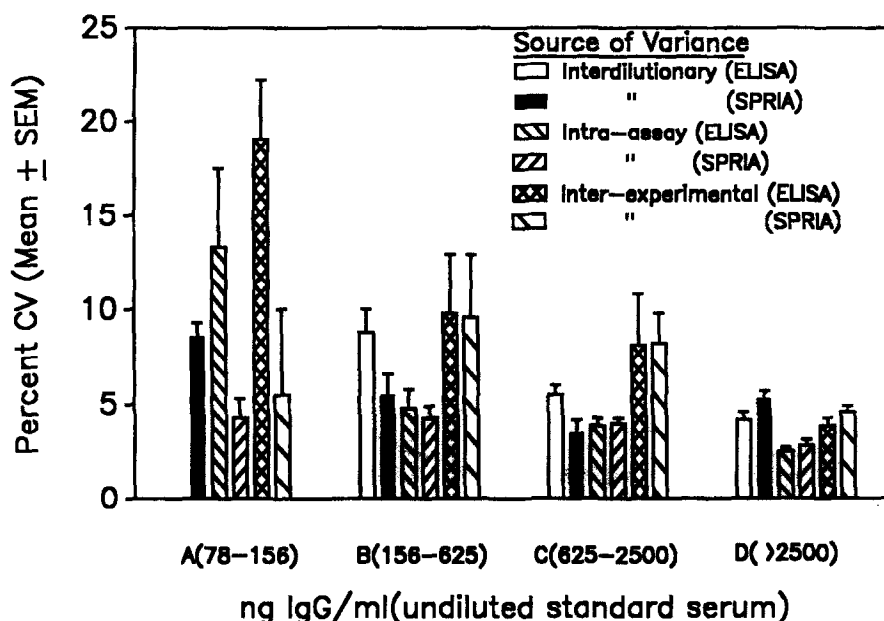


FIG. 2. Precision profiles for IgG ELISA and SPRIA. Parallelism, precision, and reproducibility were analyzed according to the method of Ekins.³² For parallelism analysis, dose estimates from two or three dilutions of serum were calculated from dose-response curves and corrected for dilution before calculation of mean CV $\pm$ SEM. Analyses with intra-assay precision profiles and interexperimental variation were made similarly. The zones of the standard curve (A-D) are derived as illustrated in Fig. 1. Ranges of sample sizes are A, 2 to 9; B, 6 to 18; C, 8 to 49; and D, 93 to 97.

Parallelism

Agreement between dilutions in both IgG ELISA and SPRIA systems was studied by triplicate determinations of 51 serum samples at two or more dilutions. Interdilutional CVs were calculated as an indication of parallel dilution curves. In both assay systems, all 51 sera diluted within acceptable limits ($<15\%$ CV), ranging from 4.2 to 5.3 for low doses (zone D) to 8.2 to 8.6 for higher doses (zone B) (Fig. 2).

Precision and reproducibility

The intra-assay precision and interexperimental reproducibility were compared by sera evaluated in triplicate in three or more runs of ELISA and two or more runs of SPRIA. The intra-assay CVs of triplicate measurements were compiled and sorted, based on four regions of the ELISA standard curve (Fig. 1). The mean intra-assay percent CV varied from 13.3% for ELISA to 4.3% for SPRIA at low doses (zone A), and 2.5% for ELISA and 2.8% for SPRIA at high antibody concentration (zone D). The interexperimental mean CVs of interpolated doses in nanograms per milliliter ranged from 3.9% (zone D) to 19.1% (zone A) for ELISA and 4.6% to 9.6% for SPRIA. In general, there is tendency for higher interexperi-

mental variation with lower antibody levels, especially <156 ng/ml (undiluted serum value) in both ELISA and SPRIA systems (Fig. 2).

Comparison of IgG, ELISA, and SPRIA results

Fifty-one serum samples were assayed for RWE-specific IgG antibodies with ELISA and SPRIA. Forty-one of the 51 sera (80.4%) elicited concordant results that agree with an average deviation of $\leq 35\%$ in two assay systems (Fig. 3). Ten of the sera, however, displayed discordant results indicated by *triangles* as differences $>35\%$ in both analyses of linear regression and bias plotting (Figs. 3 and 4). Although eight of the discrepant IgG antibody levels by ELISA were less than levels by SPRIA, two sera were consistently higher by ELISA (Fig. 3). By bias plot there is a tendency for good agreements at lower IgG levels, with generally higher values observed with SPRIA at higher serum IgG levels (Fig. 4).

Some discrepant sera were further analyzed in both assay systems by performing complete serum dilution curves. Complete dilution curves obtained from four discrepant sera and from four concordant sera demonstrated acceptable parallelism to the reference serum in both ELISA and SPRIA systems (data not presented). These discrepancies were further inves-

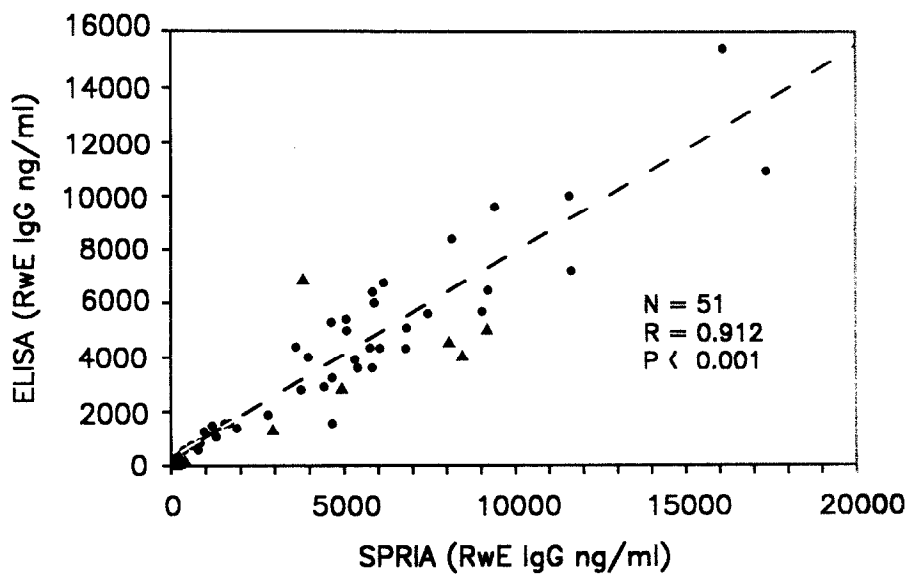


FIG. 3. Correlation of RWE IgG antibodies determined by ELISA (ordinate) and SPRIA (abscissa). The values illustrated are means of results with 51 sera in three or more experiments. A common reference serum pool provided a standard curve for each assay. Discrepant results (CV >25%) are illustrated in *triangles* (n = 10).

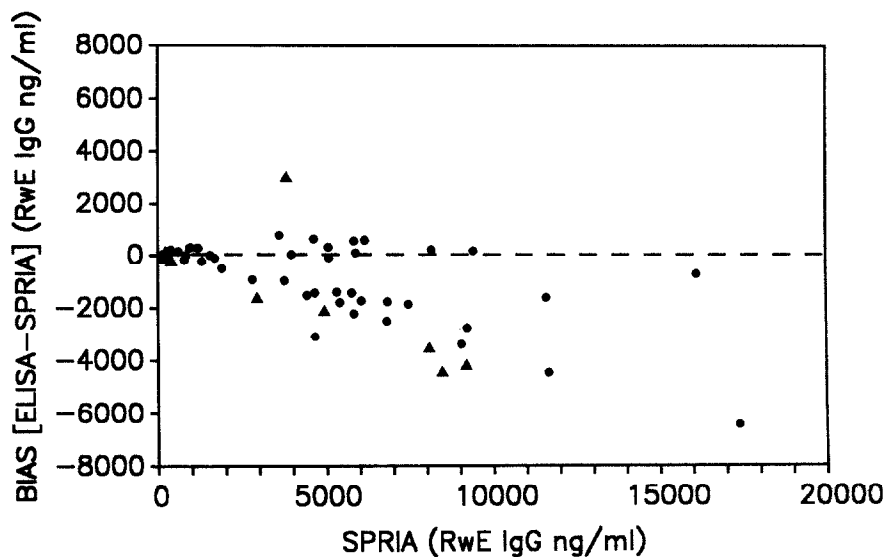


FIG. 4. Bias plotting of RWE IgG antibodies measured by ELISA and SPRIA. Forty-one of the 51 sera elicited concordant results (*circles*), and 10 of them displayed discrepancies (*triangles*) (percent CV <25%). All discrepant sera had dilution curves parallel to reference serum pool (data not presented).

tigated for the possibility that one system might adsorb more RWE-IgG antibodies than the other system.

To compare the difference of antibody depletion by the RWE-coated wells in ELISA system and RWE-agarose sorbent in SPRIA, the eight positive sera (four concordant and four discordant) were serially transferred three times into comparable wells for overnight incubations in ELISA and then transferred for a final

incubation with RWE-agarose in a SPRIA assay. Each plate was processed, as described above, and the RWE-IgG concentration of the supernatants evaluated with the standard ELISA assay. Replicate supernatants were also transferred directly to the SPRIA sorbent.

As presented in Table I, the adsorption of 4 to 8 ng of RWE-specific IgG antibody to the first RWE-coated plate was $74.4 \pm 8.3\%$ complete, as deter-

TABLE I. Specific RWE IgG antibody binding of RWE-coated polystyrene microtiter plates and RWE-agarose sorbent*

Serum No.	Conc (ng/ml)	ELISA (ng/well)								SPRIA (ng/tube)				
		Added		Removed by						Conc (ng/ml)	Added		Removed by	
		I	I (%)	II	III	Total	(%)	I	I (%)		II	III		
5022	11883	6.25	5.28 (84.5)	0.10	0.01	5.39	(86.2)	12494	15.62	13.58 (86.9)				
5593	7579	4.71	3.59 (76.2)	0.13	0.01	3.73	(79.2)	9416	11.77	10.69 (90.8)				
5164	4558	7.81	6.36 (81.4)	0.25	0.21	6.82	(87.3)	7809	19.52	17.48 (89.6)				
5072	4130	6.46	5.35 (82.8)	0.28	0.05	5.68	(87.9)	6460	16.15	14.59 (90.3)				
6630	13404	6.57	4.90 (74.6)	0.51	0.21	5.62	(85.5)	13133	16.42	14.80 (90.1)				
4681	7958	5.12	3.42 (66.8)	0.86	0.21	4.49	(87.7)	10245	12.81	11.85 (92.5)				
4480	7451	4.07	2.56 (62.9)	0.65	0.23	3.44	(84.5)	8141	10.18	9.28 (91.2)				
5153	9570	7.66	5.05 (65.9)	1.17	0.16	6.38	(83.3)	7659	19.15	17.53 (91.5)				
Mean (%)	(SEM)		74.4 ± 8.3			85.2 ± 2.9				90.4 ± 1.7				

Conc = concentration.

*IgG ELISA values were obtained by serial transfer of supernatants of 100 μ l of 1:200 serum dilution into comparable RWE-coated wells of another plate and into another 500 μ l RWE of agarose sorbent of SPRIA for evaluation of IgG depletion. SPRIA values were obtained in the same manner. Means presented are $\pm$ SEM depletion for eight positive sera with I, II, III serial transfer experiments. Nonspecific binding was $1.2 \pm 3.3\%$ for the microtiter plates and $2.9 \pm 3.5\%$ for SPRIA, determined by transferring supernatants of eight positive sera into HSA-coated wells for IgG ELISA and onto penicilloyl-HSA agarose sorbent for SPRIA (data not presented).

mined by SPRIA, and the sum of IgG-antibody serial depletion by three serial RWE-coated plates was $85.2 \pm 2.9\%$. Comparatively, in SPRIA, the adsorption of 11 to 20 ng of RWE-specific IgG antibody to the first RWE-Sepharose sorbent was $90.4 \pm 1.7\%$ complete. There were no consistent differences in the fractional adsorption between concordant and discordant sera. Nonspecific adsorptions of RWE-specific IgG antibody to HSA-coated wells for ELISA and to penicilloylated-HSA agarose sorbent for SPRIA were negligible ($1.2 \pm 3.3\%$ and $2.9 \pm 3.5\%$, respectively).

Unfortunately, it was not feasible to perform the reciprocal transfer experiments because each SPRIA transfer results in sixfold IgG dilution. Taken together, these results suggest somewhat higher capacities for IgG-antibody depletion in the SPRIA systems, but it is doubtful that this small difference is of diagnostic significance in most sera.

Specificity and inhibition studies

In general, SPRIA is more readily inhibited by soluble allergen than ELISA, as illustrated in Fig. 5. RWE and *Amb a I* inhibition curves were very similar in both systems, confirming the paramount importance of *Amb A I* in RWE-IgG antibody responses. The substantial inhibition by *Amb a III* and *Amb a VI* at high concentrations can be attributed to trace contamination

with *Amb a I* in both allergen preparations.* In general, the specificity of ELISA and SPRIA systems with the same RWE appears quite similar.

DISCUSSION

Recent research in the field of specific IgG antibody measurements has focused on methods for improving technical performance, efficiency of cost and labor, and further definition of the clinical utility of IgG measurements. Because of limited efficiency of our *Staphylococcus* protein A SPRIA,³³ we have evaluated a fluorescent ligand ELISA as a possible substitute. Many ELISA assays involving complex protein mixtures do not maintain parallelism. However, our results with relatively low concentration of RWE (200 ng of *Amb a I* per milliliter), according to the method of Kemeny et al.³⁴ demonstrated good parallelism (mean interdilutional percent CV <8.8%) that was comparable to the <8.6% for mean interdilutional percent CV for SPRIA.

Monoclonal antibodies have been successfully used in the quantitation of human immunoglobulins and, in general, elicit low nonspecific binding and background.^{7, 19-21} The specificity and immunoreactivity of the McAb γ -chain antibody (clone HP6064) we used in this study were previously documented in an In-

*Marsh DG. Personal communication.

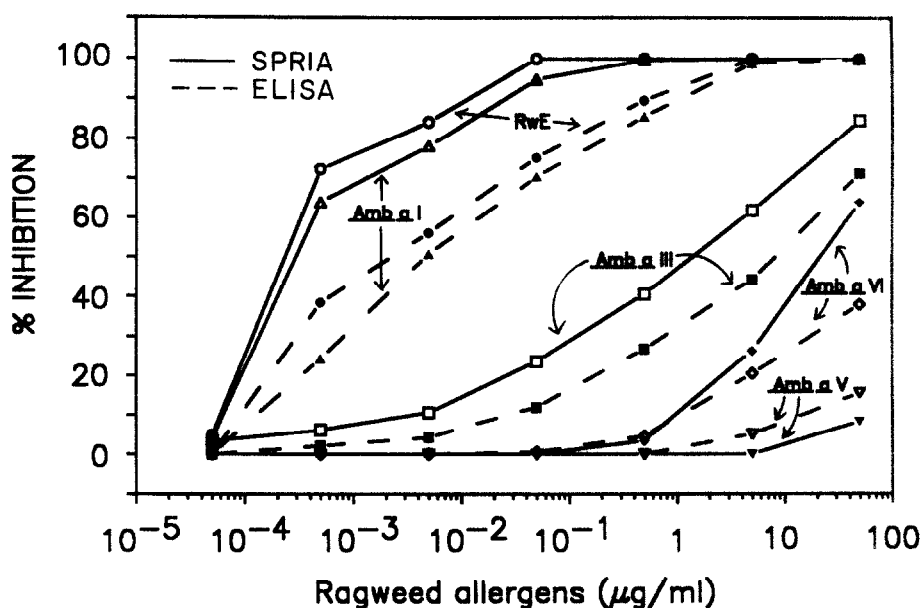


FIG. 5. Inhibition curves of IgG ELISA and SPRIA with ragweed antigens. Percent inhibition of RWE IgG binding caused by preincubation of serum with ragweed antigens is compared with addition of buffer alone. ELISA curves are illustrated in broken lines; SPRIA curves, solid lines. RWE is illustrated in circles, Amb a I in triangles, Amb a III in squares, Amb a VI in diamonds, and Amb a V in inverted triangles.

ternational Union of Immunological Societies/World Health Organization collaborative study.³⁵

Use of a fluorogenic substrate has significant advantages.²²⁻²⁶ The most significant advantages are very low blanks (nonspecific activity) that contribute to greater sensitivity. Our experience with the use of this McAb together with a GAM and a fluorogenic substrate consistently resulted in low nonspecific binding in our ELISA (7.1% of the top standard curve point), quite similar to the 5.1% of $B_{\max}$ background observed in SPRIA. The absolute sensitivity of the two assay systems was also comparable (~ 1 ng/ml) when serum dilutions were ignored. Since the SPRIA uses 1:40 dilution of test sera compared to 1:100 for ELISA, the working sensitivity for undiluted sera is about two times higher for SPRIA.

Based on precision profile analysis, we have defined the acceptable working range of the ELISA to be 0.8 to 150 ng/ml of RWE-IgG antibody, and that of SPRIA to be 1.0 to 250 ng/ml of RWE-IgG antibody. The precision and reproducibility of the IgG ELISA was also similar to that of SPRIA (Fig. 2).

The antibody depletion and cross absorption studies suggested that ragweed antigens were stable on the wells of microtiter plates and capable of adsorbing up to 6.4 ng of IgG antibody per well compared to 17.5 ng of IgG antibody per tube in SPRIA (Table I). Nev-

ertheless, because of the amplifying property of ELISA systems, this RWE ELISA can readily distinguish as little as 10 pg of IgG added to a well. We have subsequently adapted this ELISA procedure to detect allergen-specific IgG subclasses and other isotypes with minimal modification of the protocol.²⁹ This success suggests that the indirect ELISA method may be widely applicable with a variety of monoclonal antibodies.

In direct comparison of ELISA and SPRIA results, there were good agreements in 41 of the 51 sera (80.4%), although 10 of them (19.6%) elicited consistently different potency estimates in the two assays (Fig. 3). Generally, there was a tendency for SPRIA to elicit higher values than ELISA (Figs. 3 and 4). From a practical point of view, these differences, usually $<35\%$, are unlikely to affect materially clinical or research findings. Most, but not all, discrepancies may be explained by more efficient insolubilization of the whole array of RWE allergens by covalent coupling with cyanogen bromide compared with passive adsorption to plastic wells.^{16, 17, 36-37} With complex allergenic mixtures, optimization of the ELISA assay is essential for increasing its sensitivity and for minimizing possible discrepancies compared to the immunoassays involving covalent binding, because complex allergenic mixtures may vary appreciably re-

garding the ability of major allergens to adsorb effectively to the plastic surfaces. In general, allergenic extracts with high protein content, such as pollens, work well in ELISA, whereas many heterogeneous mixtures, including glycoproteins and carbohydrates (e.g., foods and molds), perform less well.^{17, 36}

In conclusion, the IgG ELISA with McAb and fluorogenic substrate generally elicits comparable results to agarose-based SPRIA for ragweed-specific IgG in most sera. The discrepancies observed are relatively minimum, insignificant clinically, and technically unexplained. Because of its technical appeal, the RWE-IgG ELISA will be an attractive alternative to SPRIA for laboratory evaluation of patients undergoing ragweed immunotherapy. The general application of the method to a wide variety of allergenic extracts awaits further study.

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Persisting airway obstruction in asymptomatic children with asthma with normal peak expiratory flow rates

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*Twice-daily symptom scores and peak expiratory flow readings were compared with spirometric values (FEV₁ and forced expiratory flow rate between 25% and 75% of FVC [FEF₂₅₋₇₅]) measured at 2-week intervals in assessing airway obstruction in 20 children with asthma studied during 16 weeks. Of 56 2-week periods during which symptoms were absent, peak flow was decreased in 30 (54%), FEV₁ in 20 (36%), and FEF₂₅₋₇₅ in 37 periods (66%). Peak flow readings were normal in 13 of 70 periods (16%) in which FEV₁ was decreased, and in 33 of 113 periods (29%), in which FEF₂₅₋₇₅ was decreased. Of 25 periods in which symptoms were absent and peak flow was normal, 19 (76%) were associated with decreased FEF₂₅₋₇₅. The results confirm previous studies that indicate peak flow readings are a useful addition to symptom diaries. More importantly, they demonstrate that airway obstruction may be present in a large proportion of asymptomatic children with asthma who have normal peak flow rates and suggest that frequent assessment of FEF₂₅₋₇₅ is required, as well as daily monitoring of symptoms and peak flow both in trials of drug therapy and for more optimal assessment of the effectiveness of therapy in clinical practice. (*J ALLERGY CLIN IMMUNOL* 1988;82:19-22.)*

Asthma is the most common chronic disorder of childhood, affecting 7% of children in North America, and its prevalence is increasing.¹ It is a disease characterized by wide variations over short periods of time in resistance to flow in intrapulmonary airways with a spectrum of symptoms ranging from chronic dry

Abbreviations used

FEF₂₅₋₇₅: Forced expiratory flow between 25% and 75% of FVC
PEFR: Peak expiratory flow rate

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coughing to life-threatening respiratory failure. Because symptoms are, by nature, subjective, it is often difficult to relate them to a given degree of airway obstruction, and after an episode of severe acute asthma, airway obstruction may persist for several