

Application of the dot immunobinding assay to allergy diagnosis

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The recently developed dot immunobinding assay is operationally simple and facilitates performance of multiple simultaneous assays. Here, its use as the basis for determination of total and allergen-specific IgE is established. For total IgE, the same commercially available polyclonal anti-human IgE was used on the solid phase and as a peroxidase conjugate in the liquid phase. After incubation with a chromogenic substrate, IgE was determined from the color intensity of the resulting dots with a scanning reflectance densitometer. The limit of sensitivity was 50 pg/ml of IgE. Standardized conditions gave the dynamic range 50 to 2500 IU/ml in serum. The IgE measured was not subject to interference by serum components, was labile at 56° C, was soluble at 30% saturation (NH₄)₂SO₄, and was unaffected by anti-human immunoglobulins of other specificity. Coefficients of variation were 0.05 within run, and were 0.1 between run. Comparison with data on sera obtained with the PRIST method yielded a correlation coefficient of 0.96 and a linear regression of slope 1.15. Assays for allergen-specific IgE were established with bee venom and dust mite allergens in the solid phase. The same peroxidase-conjugated antibody was used as for total IgE. Comparisons with comparable RAST assays were performed. (J ALLERGY CLIN IMMUNOL 74:85, 1984.)

Since the establishment of the role of immunoglobulin E in type I hypersensitivity reactions,^{1, 2} measurements of both specific and total IgE have become a part of the armory of allergy diagnosis. Assays for specific³ and total IgE⁴ have been commercially available for some years. However, questions concerning handling of radioactive materials, technical inconvenience, and diagnostic cost-effectiveness have tended to limit their application and prompted attempts to develop alternative procedures.⁵⁻¹⁶ This laboratory has recently developed immunobinding assay procedures on the basis of nitrocellulose¹⁷ where the component of the solid phase is in the form of a dot.¹⁸ This methodology has already been successfully applied to viral serology¹⁸ and autoimmune diseases.^{19, 20} The methodology has the merits of

Abbreviations used

IgE, IgG, IgA, or IgM:	Immunoglobulin E, G, A, or M
BSA:	Bovine serum albumin
TBS:	Tris-buffered saline

combining extreme simplicity in operation with high sensitivity and the ability to carry out multiple independent assays simultaneously on the same analyte. It therefore lends itself obviously to application in allergy diagnosis. We have established the conditions for applicability to total and allergen-specific IgE determinations. The results are the subject of this work.

MATERIAL AND METHODS

Antisera and patient sera

Rabbit anti-human, ϵ chain-specific, anti-IgE and rabbit anti-human (IgA, IgG, IgM) were obtained from DAKOPATTS (Copenhagen, Denmark). Patient sera with known total IgE or dust mite-specific IgE were kindly provided by Dr. Brunner, Institut-Dr.-Viollier, Basel. The sera-containing, bee venom-specific IgE were kindly provided by Dr. K. Blaser of Bern University, Biochemistry Institute.

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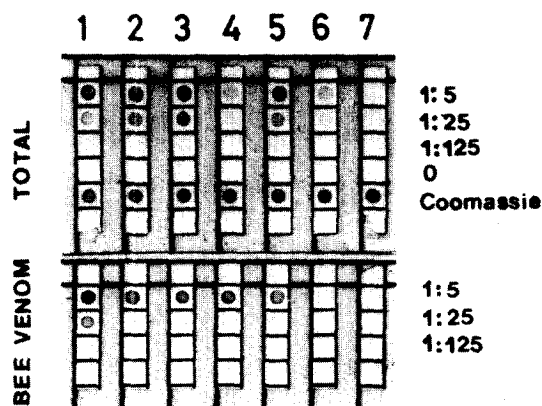


FIG. 1. Physical presentation of assay. Total IgE was performed with dots of the indicated dilutions of rabbit anti-human IgE, and bee venom specific IgE was with the indicated dilutions of venom. For more details of sera 1 through 6, see Table V. No. 7 is the control with no serum. The procedures for total and specific IgE were as described in "Material and Methods."

Other material

Horse radish peroxidase was grade I from Boehringer (Mannheim, West Germany), nitrocellulose type HAWG from Millipore Corp. (Bedford, Mass.), 4-chloronaphthol from Merck (Darmstadt, West Germany), pure IgG from Nordic (Tillburg, The Netherlands), BSA from Serva (Heidelberg, West Germany), and normal horse serum from GIBCO (Paisley, United Kingdom). Pure myeloma IgE (PS) was a gift from Dr. K. Ishizaka (Baltimore, Md.). *Dermatophagoides pteronyssinus* was kindly provided by Dr. K. Mumcuoglu (Zürich) as a live culture. Dust mite allergen was then further purified as described.²¹ This extract gave positive skin reactions in dust mite-sensitive individuals. Bee venom was prepared by removal of the stings and collection of the venom. Material from 10 bees was pooled. PRIST kits for total IgE and RAST kits for *D. pteronyssinus* specific or whole bee venom-specific IgG were from Pharmacia (Uppsala, Sweden). Index cards with lines printed for orientation of strips in the densitometer were made to our specifications.

Determination of total IgE by sandwich dot immunobinding assay

Various options for dot immunobinding assays were described earlier.^{18, 19} The optimized procedure for IgE determination is given here.

Various dilutions of the rabbit anti-human IgE were made in TBS: 0.15M of NaCl, 0.01M of Tris-Cl, pH 7.6 containing 1 mg/ml of BSA and applied as dots organized within the grid printed on the nitrocellulose. In the earlier part of this work, the dots were applied with Drummond (Broomall, Pa.) Microdispensers or with Brand (Wertheim, West Germany) Transferpettors, both modified with calibration in 0.5 μ l intervals up to 5 μ l. In the latter part of this work, a Hamilton (Bonaduz, Switzerland) Microlab M controlled by a foot switch and programmed to deliver volumes repetitively in the range 0.1 to 0.5 μ l was used. With

use of the latter, dots of 0.2 μ l could be made with high precision and consequently gave higher accuracy in the densitometry as they were better resolved from the grid lines on the nitrocellulose. However, for the sake of uniformity, dots of 0.5 μ l were used throughout this work. Typically, antibody dilutions in the dots were 1:5, 1:25, and 1:125. Additionally, where indicated, a dot of Coomassie brilliant blue (0.2 mg/ml in TBS, 1 mg/ml of BSA) was applied as an optical marker. The color intensity of the stain remained unchanged throughout the procedure. The excess protein-binding capacity of the nitrocellulose was blocked by incubating the nitrocellulose for 2 hr at 37°C in TBS containing 10% horse serum. At this stage, the nitrocellulose can be stored for at least 3 mo in a dry state at ambient temperature.

The nitrocellulose was cut into suitable strips and rehydrated with TBS followed by incubation with 1 ml of a 1:50 dilution of patient serum in TBS containing 10% horse serum for 2 hr at ambient temperature with gentle agitation in slots of Dynatec (Alexandria, Va.) Multi-reservoir trays. The strips were washed five times in TBS in situ and further incubated for 2 hr in 1 ml of a 10,000-fold dilution of peroxidase-conjugated anti-human IgE (conjugated by the two-step glutaraldehyde procedure as described by Avrameas and Ternynk²² with no modification). The same batch of rabbit anti-human IgE was used for the conjugation as for application to the dot. When sera from different species or even different individual rabbits were used, problems with high backgrounds were frequently encountered.

Washing was repeated as above and followed by addition of 1 ml of freshly mixed peroxidase substrate solution consisting of 0.6 volumes of 4-chloronaphthol (3 mg/ml in methanol), 10 volumes of TBS, and 0.004 volumes of stock H₂O₂ (30%). The color reaction proceeded for 15 min, after which time no further color developed. The strips were washed with H₂O, blotted on paper towel, glued to index cards with rubber cement and, if necessary, scanned by use of a CAMAG (Muttens, Switzerland) reflectance densitometer at 600 nm. The data were recorded with a Spectra Physics (San Jose, Calif.) SP4100 integrator. The peak height values were used for quantitation. Values are either given as densitometric units of absorbance, or data were processed further by use of a Hewlett Packard 9845B desk top computer with statistics software extended and modified by Dr. J. Maeder (Friedrich Miescher-Institute). Secondary standards were constructed from a high titer IgE serum, previously standardized with the PRIST assay and standards.

Determination of allergen-specific IgE

Essentially the same procedure was used as above for the total IgE except that the dots contained the allergen preparations in a similar dilution series in TBS containing 1 mg/ml of BSA. Patient sera were diluted 1:5 in TBS containing 10% horse serum, and the primary incubation was overnight at room temperature.

RESULTS

Format of the tests

When the dot immunobinding assays for total- or allergen-specific IgE has been performed according to

TABLE I. Comparison of titers obtained visually and by densitometry

Serum number	IgE (units per milliliter)	
	Visual	Densitometer
1	38	35
2	50	53
3	62	59
4	75	105
5	250	180
6	250	219
7	375	273
8	500	374
9	500	444
10	500	398
11	750	542
12	1000	974

The sera were selected from the same group as were used in the correlation study (see Fig. 5). The visual titers were determined by comparison with the same standards as were used for the calibration of the densitometry (0, 5, 10, 15, 20, 25, and 30 U/ml). The sandwich dot immunobinding assays for total IgE were performed as was described under "Material and Methods" except that assays were done with both 1:5 and 1:50 dilutions of the sera. The dilutions that gave a value lying in the range of the standards were used for the calculation both in the case of the visual comparison and the densitometric determination. For details of calibration curve fit, see Table IV.

the methods described in the preceeding section, the nitrocellulose strips may be mounted on index cards. This provides a permanent record. Fig. 1 illustrates such a card with a series of tests for total IgE and bee venom-specific IgE on the same set of sera. The card can be conveniently used for reference purposes (the color is stable under ambient conditions for at least 3 yr).

Quantitation of total IgE in serum by the sandwich dot immunobinding assay

The titer can be estimated by visual matching of the color intensity with a set of standards or by scanning densitometry followed by peak height analysis. A comparison of the two methods of evaluation is demonstrated in Table I. It can be observed that visual estimation yields values of sufficient precision for diagnostic purpose, readily distinguishing sera with values below or above approximately 200 IU/ml.^{23, 24} However, for larger numbers of samples, automatic evaluation and higher precision with the scanner are preferable.

Precision of the assay for total IgE

A detailed evaluation of the precision of the densitometric quantitation was made. The results are illus-

TABLE II. Precision of dot immunobinding assay for total IgE

Anti-IgE in dot	Serum diluted					
	Within run		Daily		Stock at 4° C	
	$\bar{X}$	C	$\bar{X}$	C	$\bar{X}$	C
1:5	57.7	0.043	68.9	0.091	61.9	0.031
1:25	43.9	0.033	53.7	0.109	46.3	0.075
1:125	21.6	0.056	25.7	0.117	21.5	0.120
1:625	4.4	0.146	4.8	0.188	3.5	0.272
Optical marker	39.2	0.037	44.2	0.026	37.9	0.045

The standard assay (see "Material and Methods") was performed with a pool of high titer sera. For the within-run precision, a 10 ml sample of the diluted serum was distributed between 10 assay strips. Dilutions were also made daily from aliquots of a pool stored at -80°C . Aliquots were freshly thawed for each run. Duplicate samples were run on successive days. In the case of the stock held at 4°C , one dilution was made in TBS, 10% horse serum, 0.02% NaN_3 , and duplicate 1 ml samples were assayed on 6 successive days. The optical marker was 0.2 mg/ml Coomassie blue G250 in TBS, 1 mg/ml of BSA. Systematic variations of the marker between the three groups are due to slight variations in the settings of the densitometer. These values can be used to correct the corresponding systematic variations in the immunoperoxidase stains. $\bar{X}$ = mean in densitometer units of absorbance; C = coefficient of variation.

trated in Table II. The coefficient of variation of the scanning system, independently of any antibody reaction, was 0.03 to 0.04 as revealed by the optical marker Coomassie blue. Further evaluation was obtained with a series of anti-IgE dots with decreasing amounts of antibody in the dots. Approximately the same coefficient of variations as above was obtained within-run for the higher intensity dots. This increased with decreasing intensity and was 0.15 for the borderline detectable dots at 1:625 antibody dilution. For between-run variation, the corresponding values ranged from 0.1 to 0.3. This performance compares well with that obtained with ELISA readers measuring optical transmission.

Sensitivity of the assay for total IgE

The limits of sensitivity of the assay have been explored by use of pure myeloma IgE. Overnight incubation of the sample results in a detection limit of approximately 100 pg/ml total IgE (Fig. 2, A). For the standard assay conditions with 2-hours incubation, this limit was approximately 1 ng/ml (Fig. 2, B). Since these determinations were performed with pure myeloma protein, it is important to verify whether serum components will disturb the assay under the standard conditions. For this purpose, a serum was selected that demonstrated no detectable IgE with the dot immunobinding assay. This was then mixed with

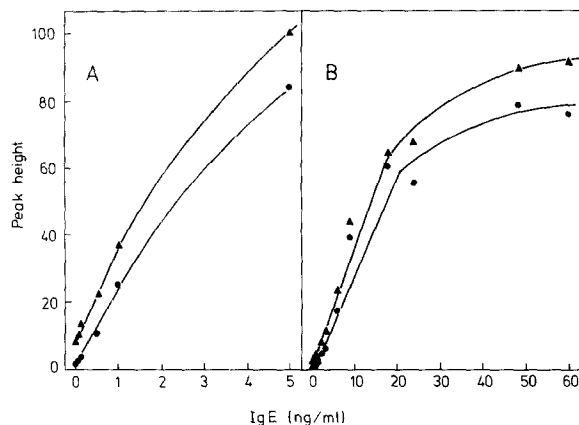


FIG. 2. Sensitivity of the assay for total IgE. Pure myeloma IgE was diluted to the indicated concentrations, and assayed (B) as under "Material and Methods" or (A) with overnight incubation with the IgE dilutions. The results are given for dots with anti-IgE diluted 1:5 (Δ — Δ) or 1:25 ($\bullet$ — $\bullet$).

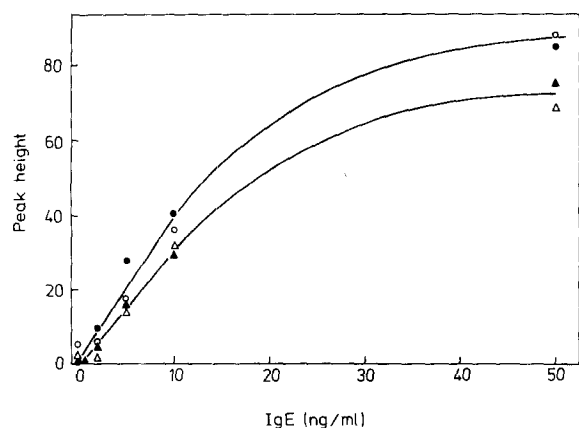


FIG. 3. Effect of serum components on the assay for total IgE. The assay was as in Fig. 2, B (open symbols) or with the addition of 1:50 dilution of a human serum with no detectable IgE (closed symbols). Anti-IgE in the dots was 1:5 ($\circ$ — $\circ$) or 1:25 (Δ — Δ).

various amounts of the myeloma protein. The results in Fig. 3 illustrate that values obtained were not significantly different in the presence or absence of the standard 1:5 serum dilution. There is no loss of sensitivity caused by interference by unknown serum components.

Specificity of the assay for total IgE

Because of the high sensitivity of this assay, it is theoretically possible that a false positive signal might arise from a serum component or caused by the presence of up to 10^9 -fold excess of other immunoglobulins that might have some weak cross-reactivity. In order to confirm absolute ϵ chain specificity, the following experiments were performed.

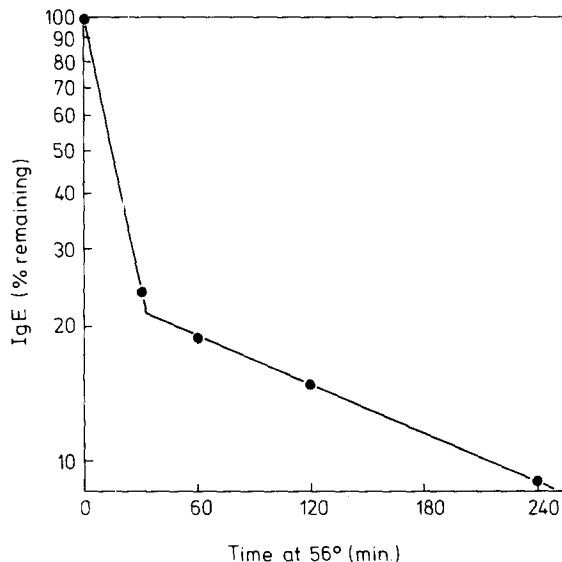


FIG. 4. Thermal stability of total IgE. A pool of high titer IgE sera was heated for the times indicated at 56° C, centrifuged, and the total IgE was determined. The decay curve demonstrated two phases. The slope of the earlier phase corresponds to a half life of 15 min, and of the later phase corresponds to a half life of 150 min.

First, the distinguishing thermolability of IgE^{25, 26} was used as demonstrated in Fig. 4. The signal for total IgE in the dot immunobinding assay decayed at 56° C with apparently biphasic kinetics. More than 90% was inactivated within 4 hr.

Second, as previously reported, IgE is not precipitated in the presence of 30% saturation ammonium sulfate unlike other immunoglobulins.²⁷ This is confirmed in Table III. Interestingly, the same results were obtained whether the ammonium sulfate supernatant was added directly to the assay or whether it was dialysed first. The failure of 30% saturation ammonium sulfate to inhibit the assay potentially provides a simple one-step procedure for removing other immunoglobulins. So far, the effect of interference by IgG in specific-IgE assays has not been investigated. This feature will be especially useful in the measurement of allergen-specific IgE where there may be inhibition of the assay by high levels of the corresponding allergen-specific IgG.^{16, 27, 28} Results in Table III also demonstrate that the assay is effectively titrated out by addition of small amounts of exogenous-competing anti-IgE but not by large amounts of oligo-specific anti-IgG, IgA, or IgM.

Calibration of the assay for total IgE

The data in the preceding part of this work have been expressed simply as arbitrary peak height units obtained from the integrator. In order to obtain an

TABLE III. Specificity of dot immunobinding assay for total IgE

Treatment	Ig class determined	Percent of control
Ammonium sulfate supernatant	E	66
Dialyzed ammonium sulfate supernatant	E	58
Ammonium sulfate supernatant	G	11
Serum plus rabbit anti-human IgE 10 nl	E	49
Serum plus rabbit anti-human IgE 100 nl	E	13
Serum plus rabbit anti-human IgE 1 μ l	E	0
Serum plus rabbit anti-human IgG, IgM, IgA 5 μ l	E	89
Serum plus rabbit anti-human IgG, IgM, IgA 50 μ l	E	96

The ammonium sulfate treatment was performed by addition of 0.5 volumes of saturated ammonium sulfate, pH adjusted to 8.0, precipitation during 30 min at 4° C, and centrifugation for 30 min at 5000 $\times$ g at 4° C. Part was assayed directly, and part was dialysed against three changes of TBS. The IgG in the original serum and supernatant was determined with dots of a series of dilutions in the range 1:500 to 1:5000 and calibration with known standards of pure human IgG. The indicated volumes of competing antibodies were added to the serum dilution and incubated at room temperature for 24 hr before the incubation with the strips. Values are expressed as a percent of the corresponding immunoglobulin in an untreated control.

accurate assay, it is desirable to express the data in International Units. It is possible to standardize the assay with "internal standards" of known amounts of pure IgE applied in separate dots to the same strip, as we have done elsewhere for IgG and IgM assays.¹⁸⁻²⁰ However, because of the nonavailability of sufficient quantity of pure IgE, "external standards" were used as follows. A high titer serum was standardized with the PRIST assay and corresponding standards, and that serum was then used as a secondary standard for the dot immunobinding assay. Polynomial regression analysis demonstrated that the curves so obtained gave an extremely good fit to a quadratic function. The parameters are listed in Table IV, and the least squares fit is also demonstrated. These parameters of Table IV were used to transform data to International Units in order to facilitate comparison with standard assay methods.

Comparison of total IgE determination with dot immunobinding and PRIST assays

The total IgE in International Units per milliliter was determined for a set of 35 sera of suspected allergy patients, both by dot immunobinding assay and

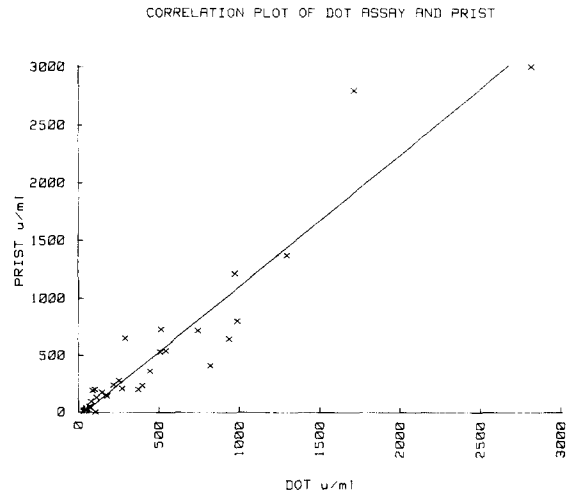


FIG. 5. Regression analysis of PRIST and dot immunobinding assay data for total IgE. A series of 39 sera with titers spanning the normal and pathologic ranges were assayed by both the methods indicated.

TABLE IV. Parameters of fit of calibration curves to a quadratic function

Parameter	Antibody dilution in dot		
	1:5	1:25	1:125
a	-0.0643	-0.0226	0.00548
b	3.34	1.85	0.239
c	1.68	0.665	0.559
R ²	0.988	0.993	0.957

The secondary standard serum was diluted to 5, 10, 15, 20, 25, 30 IU/ml, and the assay for total IgE was performed as described in "Material and Methods." The parameters are taken from the best fit to the equation $y = ax^2 + bx + c$ where x International Units is the IgE in the assay and y is the response in densitometer units. R² is the least squares fit coefficient of the experimental points to the curve defined by the equation.

PRIST. The regression plot (Fig. 5) yielded a correlation coefficient of 0.95. The two assays are thus in good accordance with each other. The best fit straight line had a slope of 1.15 and passed through the origin. The correlation between the two methods is thus qualitatively and quantitatively excellent.

Allergen-specific IgE determinations by the dot immunobinding assay

The use of the peroxidase-conjugated anti-IgE demonstrated a high sensitivity and specificity in the determination of total IgE where the species in the solid phase was also anti-IgE. Since nitrocellulose is capable of binding a wide variety of macromolecular species, we reasoned that the methodology would also be attractive for the determination of allergen-specific IgE. In order to establish the feasibility of this, we set

TABLE V. Dot immunobinding assays for bee venom specific IgE

Serum group	Serum no.	RAST score	Absorbance values with allergen in dot at		
			1:5	1:25	1:125
Known bee venom allergy	1	++++	60.8	29.0	6.1
	2	++++	29.9	10.8	0.9
	3	+++	31.4	9.1	1.4
	4	++++	32.8	9.5	0.9
	5	++++	29.6	7.8	0.0
No known bee venom allergy	6	++	8.2	2.5	0.0
	7	0	3.4	1.2	0.0
	8	++	48.5	25.5	14.6
	9	+++	1.5	0.0	0.0
	10	++	3.4	1.5	0.0
	11	++	4.8	2.6	0.0
No serum	—	—	0	0	0

The bee venom was taken directly from the stings of 10 local bees and diluted in TBS-BSA as indicated with no other treatment. The first group were from patients with a known clinical picture of allergy against bee venom. Sera 6, 7, and 8 were +++, +++++, and ++ in RAST for dust mite allergen, sera 9 and 10 were of elevated total IgE with unknown specific allergy, and sera 11 was from a normal donor with low IgE and no allergy.

up assays in principle similar to those for total IgE, only with the specific allergen immobilized on the solid phase. We have therefore performed exploratory studies with bee venom and house dust-mite extract as allergens. The allergens were prepared as described under Material and Methods.

The results with the bee venom are demonstrated in Table V. It can be observed that essentially all sera of the group with known bee venom allergy revealed high positive signals in both the RAST and the dot immunobinding assays. Exceptions were serum number 3 that was not +++ in the RAST technique, and serum number 8, which, although not known to be from an allergic patient and revealing only ++ in the RAST, revealed a high value by dot immunobinding.

The sera tested for house dust mite-specific IgE were from a group previously submitted to a clinical laboratory for specific-IgE determination by the RAST method. The results in Table VI again demonstrate the close relationship between the RAST scores and the dot immunobinding data. The following discrepancies are noted: serum number 4 was ++++ by RAST but had low dot immunobinding. Serum 7 was +++ by RAST but had high dot immunobinding. Strikingly, all 29 RAST-negative sera were also negative by dot immunobinding. In both allergy groups in Tables V and VI the discrepancies can be accounted for by the different sources of allergen used by us here

compared with the Pharmacia RAST kits. The results here nevertheless demonstrate that, in principle, allergen-specific assays can be established by application of the allergen preparation as a dot to nitrocellulose and detection of IgE binding with peroxidase coupled anti-IgE.

DISCUSSION

The results in the preceding section demonstrate the utility of the dot immunobinding assay for measurement of total IgE and the potential for measurement of allergen-specific IgE. The assay has advantages over conventional in vitro allergy diagnostic methods. No expensive equipment such as gamma counters or fluorimeters are required. The results can either be assessed by visual comparison or quantitated with a densitometer, if desired. Although the currently used densitometric equipment is expensive (each \$15,000) and perhaps too sophisticated for routine clinical use, there is no inherent difficulty in designing and constructing a simple dedicated photometer, similar to the Ames Reflectance Colorimeter, Elkhart, Ind. With the equipment used here, the quantitation is both precise and sensitive. A positive signal can be obtained when the stain is on the borderline of visibility.

The assay is based on materials of which all are readily available commercially except for allergen preparations. The polyclonal antibody used is of extraordinarily high titer so that one batch can be used for millions of assays. The assay also has the potential to be performed with monoclonal antibodies against human IgE. However, at present, the polyclonal antibody used here offers no serious disadvantages. The assay is very simple to perform in practice and requires very little laboratory time.

Being a modified ELISA assay, the method here also has all of the advantages of the classical ELISA, including compatibility with multipipetting equipment designed for ELISA, since the geometry of the multiwell trays corresponds to the standard microtiter plate format. It has the additional advantages accruing from the high adsorption capacity of nitrocellulose and focusing of the reaction in a dot, as detailed elsewhere,²⁹ as well as inherently providing an attractive, permanent, easily stored record.

Washing is done on a simple "squirt and pour" basis, as the strips remain in place by surface tension. No elaborate washing equipment is required. The actual laboratory time involved is therefore only that time needed to make the dilutions and addition of single components to the trays. The overall assay time can be typically 5 hr or overnight with 2½ hr the following morning. The 5-hour assay was selected as being the most comfortable for total IgE, although,

since there is excess sensitivity (1:50 dilution of the serum), faster protocols are possible with lower dilutions. This applies in addition to the peroxidase-conjugated antibody incubation (1:10,000 dilution), which can also be shortened if a lower dilution factor is used. A further advantage of the method is the very small amount of serum required: 200 μ l for specific-allergen IgE and 20 μ l for total IgE with the conditions described here. The full potential for use with a variety of allergens and combined simultaneous assays can now be explored.

The assay is also sufficiently simple and stable so that it can be performed in remote areas with no technical facilities. The resulting dots are stable in color and can be sent as letter mail with no need for special protection, if precise quantitation is to be performed at a central facility. The conjugates are stable at 4° C for years, according to the suppliers specifications. The dots of antibody or allergens on nitrocellulose are also stable if they are maintained dry at room temperature. Large numbers of samples can be handled with no difficulty. When the primary incubation is overnight, the sensitivity of the assay for total IgE (50 pg/ml) is also appropriate for measurement of IgE in lymphoid cell culture supernatants. When the primary incubation is for 2 hr, as in the standard assay conditions, the sensitivity range is such as to be appropriate for obtaining meaningful determinations with cord blood serum as well as in the normal range.^{24, 25}

The assay is also sufficiently versatile so that by suitable choice of conjugate, it can be used to measure specific-IgG and IgM antibody populations, as has already been done for viral serology (J. Gordon, in preparation), and human autoimmune diseases.²⁰ This opens up the possibility of allergen-specific IgG assays for monitoring desensitization therapy. The results in Tables V and VI also demonstrate the feasibility of dot immunobinding assays for allergen-specific IgE. Although there is not complete agreement with the data from dot immunobinding and the commercial RAST assays, the discrepancies reveal the difficulties inherent in the standardization of allergen preparations and do not reflect on the validity of either methodology as such. The results obtained here warrant a further investigation with larger numbers of patient sera and with a wider variety of purified specific allergens. The methodology thus provides a strategy for simple kits that can contain any desired spectrum of allergen-specific IgE assays combined with total-IgE assays. It therefore offers the possibility of procedures for in vitro allergy diagnosis that would be less expensive and complex than are currently available.

TABLE VI. Dot immunobinding assays for dust mite-specific IgE on serum samples from suspected dust mite-allergic patients

Serum no.	RAST score	Absorbance values for dots with allergen diluted		
		1/1	1/5	1/25
1		39.4	29.3	19.9
2		37.5	25.0	9.4
3		66.2	38.1	16.2
4	++++	9.0	3.0	1.2
5		23.1	16.1	6.5
6		17.7	12.2	5.4
7		63.8	45.4	23.8
8	+++	6.7	5.4	2.2
9		3.4	0.9	0.0
10		27.3	13.4	4.3
11	++	4.0	2.8	0.9
12		3.9	2.9	0.0
13	+	2.3	0.0	0.0
14 to 32	0	3.2 $\pm$ 1.2	1.7 $\pm$ 1.5	0.6 $\pm$ 1.1
None		3.3	1.5	1.3

The dot assay for dust mite-specific IgE was performed as described under "Material and Methods" with the dust mite extract at 5 mg/ml of protein and diluted as was indicated before application of the dot. The individual serum values are grouped according to the RAST values for *D. pteronyssinus*. The means and standard deviations only are illustrated for the RAST-negative sera nos. 14 to 32.

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REFERENCES

1. Ishizaka K, Ishizaka T, Hornbrook MM: Physicochemical properties of reaginic antibody. V. Correlation of reaginic antibody. *J Immunol* 97:840, 1966
2. Johansson SGO, Bennich H: Studies on a new class of human immunoglobulins. I. Immunological properties. In: Killander J, editor: Nobel Symposium 3. Stockholm, 1967, Almqvist and Wiksell, p 1973
3. Wide L, Bennich H, Johansson SGO: Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* 2:11057, 1967
4. Ceska M, Lundkvist U: A new and simple radioimmunoassay method for determination of IgE. *Immunochemistry* 9:1021, 1972
5. Adkinson NF: The radioallergosorbent test: Uses and abuses. *J ALLERGY CLIN IMMUNOL* 65:1, 1980
6. Adkinson NF: The radioallergosorbent test in 1981—limitations and refinements. *J ALLERGY CLIN IMMUNOL* 67:87, 1981
7. King WP: Efficacy of a screening radioallergosorbent test. *Arch Otolaryngol* 108:781, 1982
8. Thompson RA, Bird AG: How necessary are specific IgE antibody tests in allergy diagnosis? *Lancet* 1:169, 1983
9. Herbert FA, Weimar N, Salkie ML: RAST and skin test

- screening in the investigation of asthma. *Ann Allergy* 49:311, 1982
10. Bruynzeel PLB, Houben LAMJ, Berrens L: A modified sandwich technique for the measurement of IgE. *Clin Chim Acta* 91:67, 1979
 11. Scott ML, Thornley MJ, Coombs RRA, Bradwell AR: Measurement of human serum IgE and IgA by reverse passive antiglobulin hemagglutination. *Int Archs Allergy Appl Immunol* 64:222, 1981
 12. Blanchard GC, Gardner RE: A solid phase fluoroimmunoassay for human IgE. *J Immunol Methods* 52:81, 1982
 13. Magnusson CGM, Masson PL: Particle counting immunoassay of immunoglobulin E antibodies after their elution from allergosorbents by pepsin: An alternative to the radioallergosorbent test. *J ALLERGY CLIN IMMUNOL* 70:326, 1982
 14. Kendell C, Ionescu-Matiu I, Dreesman GR: Utilization of the biotin/avidin system to amplify the sensitivity of the enzyme-linked immunosorbent assay (ELISA). *J Immunol Methods* 56:329, 1983
 15. Rao PVS, McCartney-Francis NL, Metcalfe DD: An avidin-biotin micro ELISA for rapid measurement of total and specific IgE. *J Immunol Methods* 57:71, 1983
 16. Metzger WJ, Butles JE, Swanson P, Reinders E, Richerson HB: Amplification of the enzyme-linked immunosorbent assay for measuring allergen-specific IgE and IgG antibody. *Clin Allergy* 11:523, 1981
 17. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose: Procedure and some applications. *Proc Natl Acad Sci USA* 76:4350, 1979
 18. Hawkes R, Niday E, Gordon J: A dot immuno-binding assay for monoclonal and other antibodies. *Anal Biochem* 119:142, 1982
 19. Rordorf C, Gambke C, Gordon J: A multidot immunobinding assay for autoimmunity and the demonstration of novel antibodies against retroviral antigens in the sera of MRL mice. *J Immunol Methods* 59:105, 1982
 20. Gordon J, Rosenthal M: The diagnostic potential of a multidot immunobinding assay for autoimmunity. (in preparation)
 21. Chapman MD, Platts-Mills TAE: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus* antigen P1. *J Immunol* 125:587, 1982.
 22. Avrameas S, Ternynck T: Peroxidase-labeled antibody and FAB conjugates with enhanced intracellular penetration. *Immunochemistry* 8:1175, 1971
 23. Nye L, Merrett TG, Landon J, White RJ: A detailed investigation of circulating IgE levels in a normal population. *Clin Allergy* 5:13, 1975
 24. Kjellman NIM, Johansson SGO, Roth A: Serum IgE levels in healthy children by a sandwich technique (PRIST). *Clin Allergy* 6:51, 1976
 25. Johansson SGO: The clinical significance of IgE. In: Franklin EC, editor: *Clinical Immunology Update*. ??, pp 124, 1982
 26. Zimmermann EM, Yunginger JW, Gleich GJ: Interference in ragweed pollen and honeybee venom radioallergosorbent tests. *J ALLERGY CLIN IMMUNOL* 66:386, 1980
 27. Lynch NR, Dunand P, Newcomb RW, Chai H, Bigley J: Influence of IgG antibody and glycopeptide allergens on the correlation between the radioallergosorbent test (RAST) and skin testing or bronchial challenge with *alternaria*. *Clin Exp Immunol* 22:35, 1975
 28. Gordon J, Hawkes R, Niday E, Rordorf C, Rosenthal M, Towbin H: Dot immunobinding and Western blotting as diagnostic tools. In Avrameas S, Druet P, Masseyeff R, Feldmann G, editors: *Immunochemical techniques*. Amsterdam, 1983, Elsevier Science Publishers BV, p 303
 29. Isersky C, Kulczycki A Jr, Metzger H: Isolation of IgE from reaginic rat serum. *J Immunol* 112:1909, 1974

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