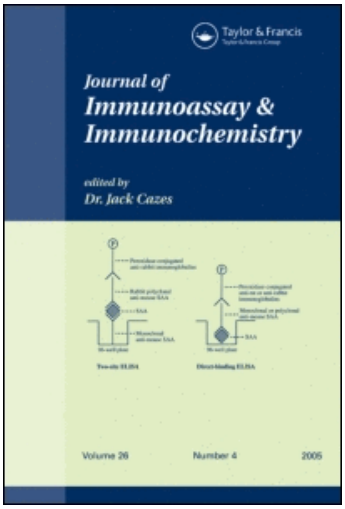


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# DEVELOPMENT OF THE ABBOTT MATRIX™ AERO ASSAY FOR THE MEASUREMENT OF SPECIFIC IGE

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## ABSTRACT

An enzyme immunoassay has been developed for the quantitation of specific immunoglobulin E (IgE) in human serum to a panel of allergens. The assay system, called the Abbott MATRIX™ Aero, includes an instrument, reagents and test cell disposables. Each test cell contains fourteen airborne allergens individually localized on a nitrocellulose solid phase. Individual calibration curves for each allergen are established by the manufacturer and included in barcode form with each test kit. Stable factory calibration eliminates the need to establish a calibration curve with each assay run. The instrument automatically incubates, washes, and reads the test cell and prints each result, which ensures assay reproducibility and provides ease-of-use. Analysis of test results shows good agreement with another in vitro assay for specific IgE. The Abbott MATRIX™ Aero is a sensitive, reproducible and easy-to-use system for the measurement of specific IgE to a panel of fourteen allergens simultaneously using a single, small volume of serum. (KEY WORDS: Enzyme immunoassay, specific IgE, allergens, Abbott MATRIX™).

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## INTRODUCTION

The role of immunoglobulin E in type I hypersensitivity diseases is well established (1,2). Hence, the determination of total and allergen-specific IgE in serum is an important aid in the diagnosis of atopic allergy. In vivo tests, such as the skin-prick test, have been used to identify the causative allergens and have been shown to correlate with clinical symptoms and provocation tests (3). However, skin tests require that the patient discontinue anti-allergic drugs prior to the test (4,5). In vitro tests, such as the radioallergosorbent test (RAST), have been used to directly measure allergen-specific IgE antibody in serum (6) and have been shown to correlate well with skin tests (7,8,9,10). However, the RAST is time consuming and labor intensive in screening sera for specific IgE to a large panel of allergens. Multiple-antigen RAST assays (11) have been developed which screen sera for the presence or absence of specific IgE to a panel of allergens on one disk, but these assays do not quantitate specific IgE to each of the allergens present on the disk. The MAST assay (12) quantitates specific IgE to each of a panel of allergens, but the assay requires a number of manipulations and each assay is not calibrated individually. In this study, we describe the performance characteristics of an enzyme immunoassay, called the Abbott MATRIX™ Aero, which measures the level of allergen-specific IgE to a panel of fourteen allergens simultaneously using a small volume of serum. The assay is easy to perform and is unique in that each specific IgE assay is individually calibrated at the factory. Comparisons of the results of MATRIX Aero panel with RAST demonstrate that the former is useful in measuring allergen-specific IgE antibody.



## MATERIALS AND METHODS

### Instrument System

The Abbott MATRIX™ instrument system has been previously described (13). Briefly, the instrument consists of a carousel in which up to 10 test cells, each containing a unique specimen, may be processed. The carousel is controlled by a random-access positioning

mechanism for placement of test cells during the addition of reagents and for the washing and reading steps. The reader measures diffuse reflectance and provides a printout of results based upon internally stored calibration information.

Preparation of the Solid Phase

Preparation of the solid phase is shown in Figure 1. The solid phase consists of a nitrocellulose membrane embossed to form a 5 X 6 matrix of multiple isolated spots, each of which is 2.5 mm in diameter. One to 2.5 microliters of each allergen is added to a separate spot on the membrane and dried. Unreacted sites on the membrane are blocked by incubating in 10% horse serum (heat-inactivated at 56° C for 60 min) in 10 mM Tris-buffered saline (TBS) at 37° C for 60 min, the membrane is washed with TBS and stored dry until used. Also bound to the membrane are: 1) rabbit antibody to goat immunoglobulins (Dako Corp, Santa Barbara, CA), which serves as a procedural control, indicating whether the antibody-enzyme conjugate (Reagent B) and enzyme substrate (Reagent C) have been added in the correct order; and 2) horse serum, which serves as a negative control, indicating the nonspecific binding of assay components to the membrane. Each solid phase is assembled into a plastic test cell (Figure 2). The evaporation of liquid reagents is prevented during the incubation periods by a plastic cover which is applied to the top of the test cell. The cover is equipped with a self closing port through which sample and reagents are added and waste solutions are removed.



Allergen Optimization

Several concentrations of allergen are applied to the nitrocellulose and evaluated with positive test samples. The optimum allergen concentration is selected such that the maximum end point signal is obtained before significant background reactions are detected (see example in Figure 3). Each allergen has its own optimal concentration which varies from one allergen to the next, and which must be verified for each new lot of allergen. The end point

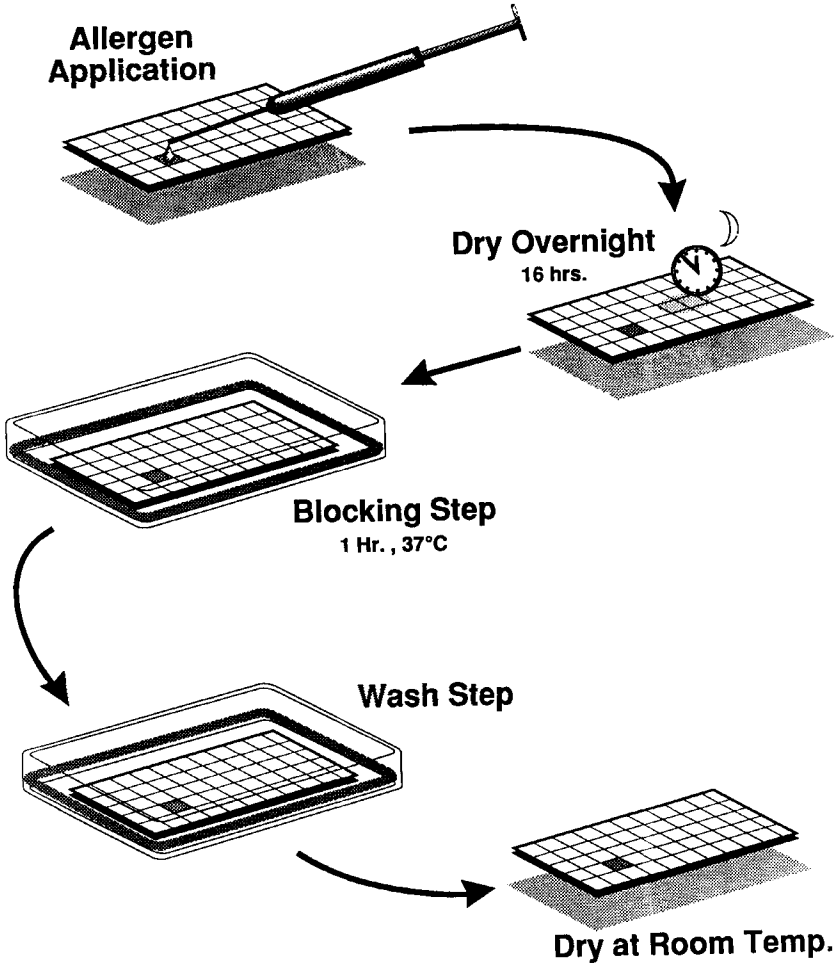


FIGURE 1. Preparation of the solid phase.

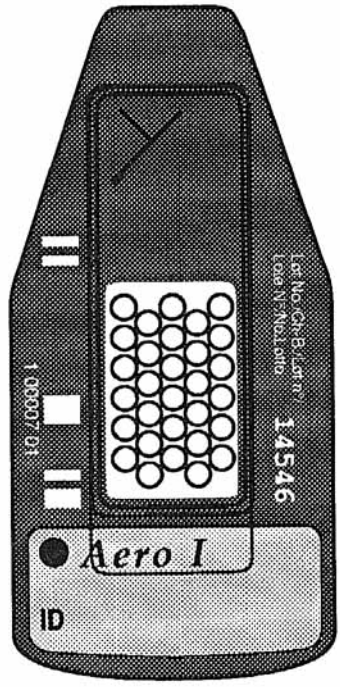


FIGURE 2. Test cell for the MATRIX Aero assay.



signal is measured by reflectance densitometry and recorded as D(r) which is calculated according to the following equation (14): $D(r) = (1-R)^2/2R$ where R is the ratio of reflected light intensity to incident light intensity from the light emitting diode source in the instrument. This transformation converts reflectance to values which are linear with concentration.

Assay Optimization

Each step of the assay was studied to determine the optimum incubation time. The test specimens included a clinical sample which was negative for all allergens, to determine the effect of incubation times on non-specific reactivity, as well as dilutions of a pool of sera

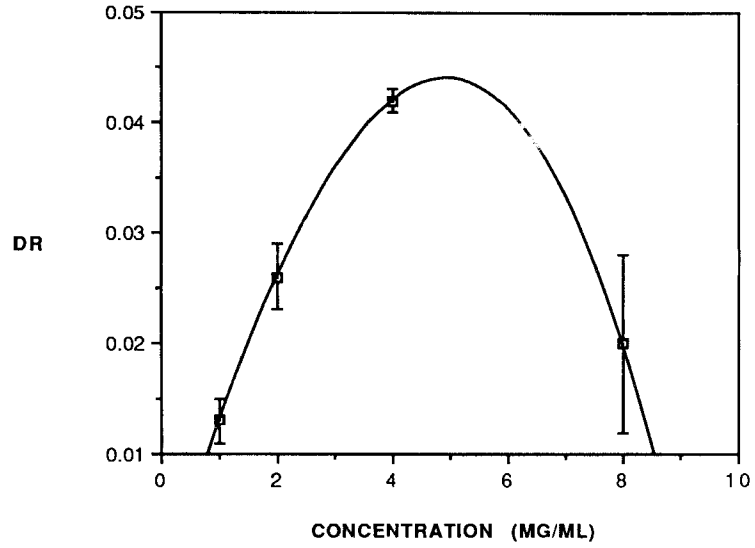


FIGURE 3. Optimization of Alternaria allergen extract. The signal produced fromthe reaction of a positive serum pool with several different concentrations of Alternaria indicates that the maximum signal is produced when approximately 60 ug/mL of Alternaria allergen protein is applied to the nitrocellulose. Data shown is the mean ± the standard deviation of the mean for triplicates of a serum pool that contains IgE to Alternaria.



positive to several allergens. The assay kinetics were determined with these samples and various reagent formulations. The optimum formulation and incubations achieved the maximum signal while still maintaining minimal background. The signal of the negative control spot was followed closely to insure that it did not rise above a level of 0.250 D(r). This insures a sufficient dynamic range with the Abbott MATRIX™ reader, which is linear to 50 D(r).

## Reagents

Allergens are obtained from Greer Laboratories (Lenoir, North Carolina) or Nelco Laboratories (Deer Park, New York) in lyophilized

form and stored at 4° C. Allergens are resuspended at their optimum concentration in $H_2O$ just prior to application to the nitrocellulose.

Specimen dilution buffer (Reagent A) is 10% (v/v) horse serum (heat-inactivated at 56° C for 1 hour) in 10 mM TBS. Enzyme conjugate (Reagent B) contains affinity purified goat-anti-human-IgE labelled with alkaline phosphatase by the method of Nakane and Kawaoi (15). The substrate (Reagent C) is 5-bromo-4-chloro-3-indolylphosphate at 1 mg/mL in 0.1 M 2-amino-2-methyl-1-propanol. All reagents contain azide as preservative.

## Assay Protocol

The protocol for the assay is shown in Figure 4. One-half mL of Reagent A and 0.5 mL of patient specimen are added to a test cell and incubated at 35° C in the Abbott MATRIX™ analyzer for 16 hours. Good contact between allergens on the solid phase and liquid reagents is assured by the flow of reagents across the solid phase. The carousel is tilted at 10 degrees and as the test cell rotates, the fluid flows across the solid phase. The test cell is automatically washed 3 times after the first, and each subsequent, incubation with one mL of 10 mM TBS. One mL of conjugate (Reagent B) is then added by the operator. After a 90 minute incubation, the test cell is washed and the operator adds one mL of substrate (Reagent C). After another 90 minute incubation period, the test cell is washed and the lid is removed by the operator. The test cell is dried in the instrument for 25 minutes and the blue color of the reaction is measured by the reflectance densitometer in the instrument. Results are calculated and printed by the instrument (see example of printout in Figure 5).

## Calibration

Individual standard curves are determined by the manufacturer for each allergen on each lot of test cells with standards made from dilutions of human plasma pools positive to that allergen. The negative control signal is subtracted from the signal for each allergen to establish a delta D(r). The delta D(r) at the cutoff between a positive and negative result is assigned a value of 0.25 sIgE and is categorized as

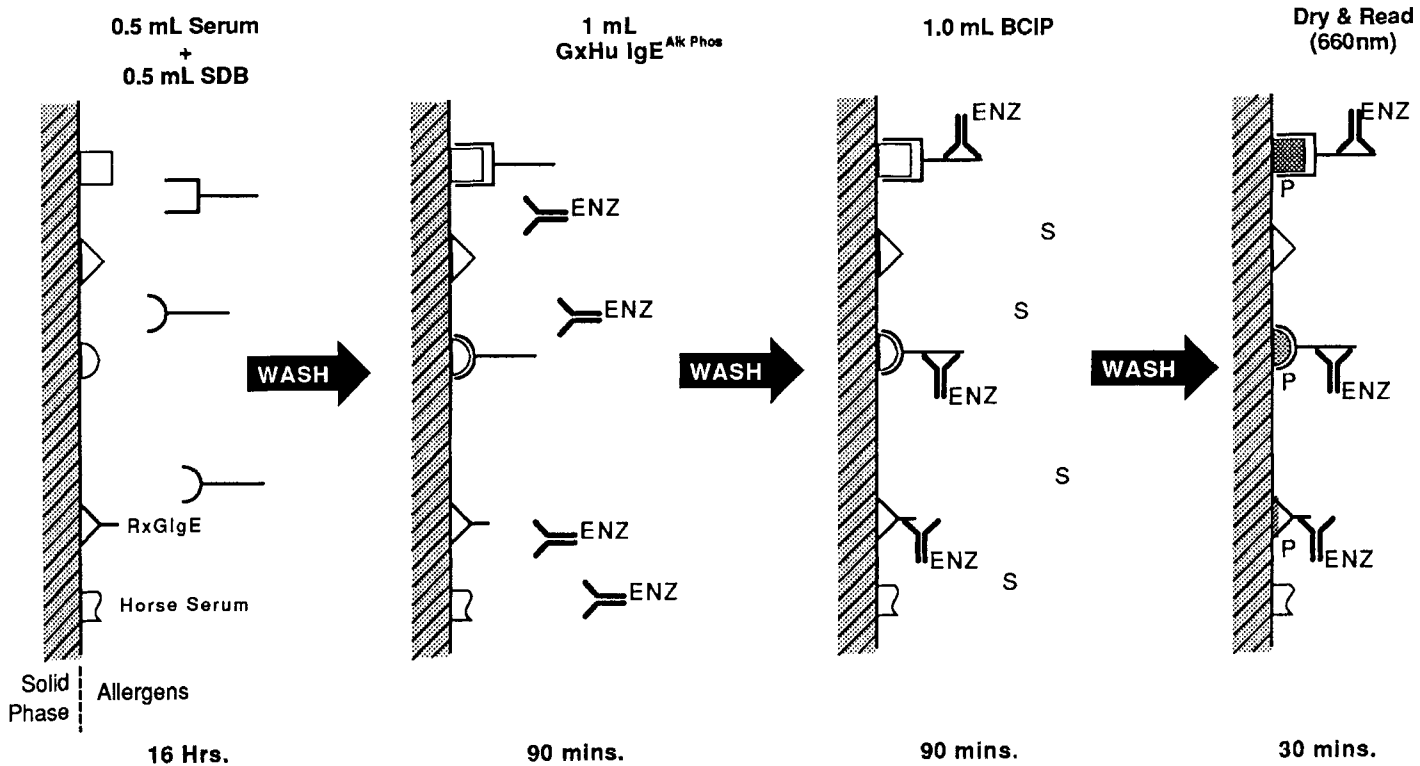


FIGURE 4. Assay protocol.

Date: 12-JUN-1990
Time: 12:30
Position: 1

Sample ID 1: 111-22-3333
Sample ID 2: 12345
Sample ID 3:

AERO

Controls:

| Procedure | Valid |
|---|---|
| Negative | Valid |

| Allergen | sIgE | Class | |
|---|---|---|---|
| DUST/MITES | | | |
| D. farinae | 3.90 | 3 | * |
| D. pter. | 12.5 | 4 | * |
| EPIDERMALS | | | |
| Cat | 2.81 | 3 | * |
| Dog | 0.40 | 1 | * |
| MOLDS | | | |
| Alternaria | <0.25 | 0 | |
| Aspergillus | <0.25 | 0 | |
| Penicilium | <0.25 | 0 | |
| GRASSES | | | |
| Bermuda | 1.22 | 2 | * |
| Timothy | 2.48 | 2 | * |
| TREES | | | |
| Birch | 0.31 | 1 | * |
| Olive | <0.25 | 0 | |
| WEEDS | | | |
| Mugwort | 0.33 | 1 | * |
| Parietaria | <0.25 | 0 | |
| Plantain | 1.18 | 2 | * |

*(Actual Printout May Vary)*



FIGURE 5. Sample printout of Abbott MATRIX™ Aero Assay.

class score 1. The class score 2 cutoff is 0.5 sIgE, the class 3 cutoff is 2.5 sIgE, and the class 4 cutoff is 12.5 sIgE. The sIgE values are established at the time the standard curves are calibrated and this calibration information is stored on barcoded data sheets which accompany each kit and are unique for each master lot. The operator enters the calibration data using the barcode wand of the instrument which stores the data in a structured database located in battery-backed RAM memory. When an assay is initiated, the identification barcode on the test cell is read by the instrument and the software links this identification number to the correct calibration in the database in order to perform data reduction and report test results.



Interfering Substances

Several substances with the potential for interfering with assay results, such as allergy medications, total IgE up to 2500 IU/mL, and elevated levels of hemoglobin, bilirubin and triglycerides, were added to two specimen pools to determine their effect upon sIgE values. A list of these substances and the concentration tested are shown in Table 1. Concentrations were chosen based upon the National Committee for Clinical Laboratory Standards guidelines (16) and represent ten times the highest reported concentration following a therapeutic dose.

Reference Testing

Serum was collected from 162 patients who were undergoing evaluation for allergies at a number of allergy clinics from across the United States. Allergen-specific IgE was measured using the Phadebas RAST kit (Pharmacia Diagnostics Ltd, U.K.) according to the manufacturer's instructions under Standard Protocol. Briefly, 50 uL of serum was incubated with an allergen-coated disc for 3 hr at 20° C. The discs were washed and incubated with 50 uL of radio-labelled anti-human IgE for 18 hr at 20° C. The discs were washed and the radioactivity was measured with a gamma counter. Allergen-specific IgE was expressed as PRU/mL with values greater than 0.35 PRU/mL being considered positive.

TABLE 1

List of Potential Interfering Substances and Levels Added to Specimens.

| | |
|---|---|
| 1) Acetominophen | 20 mg/dL |
| 2) Acetylsalicylic acid | 50 mg/dL |
| 3) Ascorbic acid | 3 mg/dL |
| 4) Bilirubin | 40 mg/dL |
| 5) Caffeine | 10 mg/dL |
| 6) Chlorpheniramine maleate | 15 mg/dL |
| 7) Cimetidine | 10 mg/dL |
| 8) Ephedrine | 100 mg/dL |
| 9) Epinephrine | 0.5 mg/dL |
| 10) Hemoglobin | 100 mg/dL |
| 11) Heparin | 8000 units/dL |
| 12) Ibuprofen | 40 mg/dL |
| 13) Nicotine | 2 mg/dL |
| 14) Phenobarbital | 15 mg/dL |
| 15) Prednisone | 6 mg/dL |
| 16) Protein (albumin) | 6 g/dL |
| 17) Terfenadine | 1.2 mg/dL |
| 18) Theophylline | 25 mg/dL |
| 19) Triglycerides | 3000 mg/dL |



## RESULTS

### Allergen and Assay Optimization

The allergen protein concentrations which were tested ranged from 0.05 to 170 mg/mL. An example of results from a typical experiment to determine optimal protein concentration for the Alternaria allergen is shown in Figure 3. The most effective concentration range for each of the allergens tested is shown in Table 2.

Incubation times for each step in the assay were varied. Results indicate that the signal does not increase for any allergen after 14 hours of incubation for the first step in the reaction (see examples in Fig. 6). Sixteen hours was chosen as the incubation time for the first step in order to accomodate the work schedule of most laboratories for an overnight incubation. The second and third steps of the assay were incubated 30, 60, 90, 120 and 240 minutes. Results from these

TABLE 2

List of allergens with a range of optimum coating concentrations.

| Allergen | Protein Concentration (mg/mL) |
|---|---|
| Dermatophagoides farinae | 2.8 - 11.2 |
| Dermatophagoides pteronyssinus | 2.4 - 21.0 |
| Alternaria alternata | 0.06 - 2.0 |
| Penicillium notatum | 0.4 - 2.4 |
| Aspergillus fumigatus | 2.0 - 25.0 |
| Cat | 2.4 - 10.3 |
| Dog | 2.6 - 19.2 |
| Phleum pratense (timothy grass) | 0.2 - 3.3 |
| Cynodon dactylon (Bermuda grass) | 1.6 - 20.2 |
| Parietaria officianalis | 2.0 - 20.0 |
| Plantago lanceolata (English plantain) | 3.4 - 25.0 |
| Artemisia (mugwort) | 1.2 - 22.5 |
| Betula (birch) | 0.4 - 3.0 |
| Olea europea (olive) | 2.0 - 17.4 |



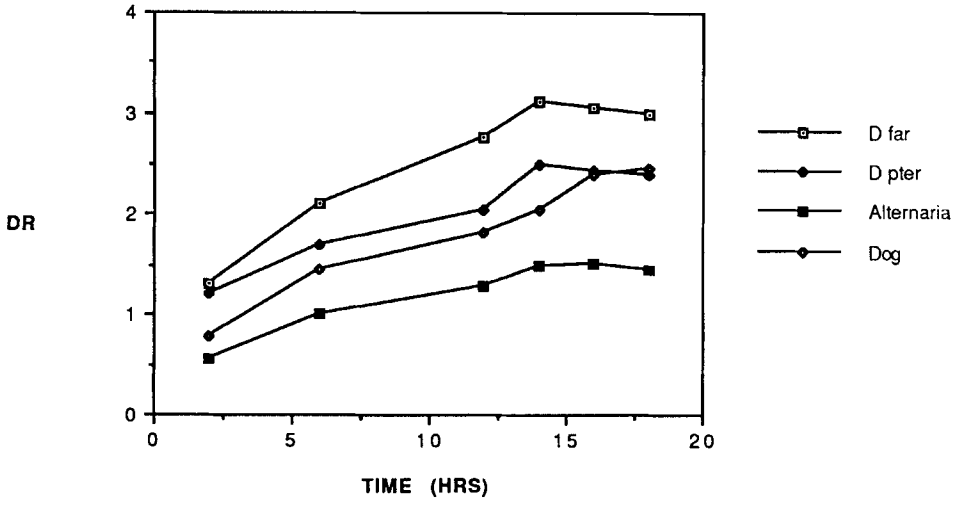


FIGURE 6. Optimization of incubation time for the first step of the assay. The data indicates that the signal increases very little after 14 hours.

TABLE 3

The Effects of Incubation Times of Steps 2 and 3 on the Signal, in D(r), for 4 Allergens and the Negative Control.

| Time | D. far | D. pter | Alt | Dog | Neg Con |
|---|---|---|---|---|---|
| 30/30 | 1.171 | 0.694 | 0.275 | 0.329 | 0.063 |
| 30/60 | 1.626 | 0.858 | 0.313 | 0.465 | 0.068 |
| 30/90 | 2.928 | 1.295 | 0.497 | 0.855 | 0.101 |
| 90/30 | 2.381 | 1.251 | 0.518 | 0.780 | 0.066 |
| 60/90 | 7.192 | 3.146 | 1.170 | 3.051 | 0.127 |
| 90/90 | 9.914 | 4.253 | 1.664 | 6.219 | 0.186 |
| 240/60 | 8.485 | 4.111 | 1.748 | 6.495 | 0.260 |



studies indicate that the signal continues to increase through four hours, but the negative control value also begins to increase beyond an acceptable level after 90 minutes of incubation (Table 3). Therefore, 90 minutes was chosen as the time of incubation for steps 2 and 3 to maximize signal while keeping the negative control below 0.25 D(r).

Additional studies indicate that the results are unaffected by a 3 hour delay in the addition of reagent B after step 1, a 1 hour delay in the addition of reagent C after step 2, or a 1 hour delay before removing the lid after step 3.

Procedural and Negative Controls

The procedural control serves as an internal check that reagents B and C are added in the correct order. If the reflectance of the procedural control drops below a preset minimum, the printout records "invalid" for the procedural control and no assay results are printed. To verify that improper addition of reagents will produce an invalid procedural control, reagents A, B, and C were omitted or added in the improper sequence. Only when reagents B and C are added in the proper order is the procedural control result "valid" (Table 4). However, this control cannot detect the omission of either reagent A or the sample.

TABLE 4

Performance of the Procedural Control.

| Condition | Range of D(r) Values |
|---|---|
| Recommended Procedure | 21.634 - 34.169 |
| Sample Omitted | 36.459 - 44.623 |
| Reagent A Omitted | 20.270 - 29.024 |
| Reagent B Omitted | <0.001 |
| Reagent C Omitted | <0.001 |
| Reagent B Added Twice | <0.001 |
| Reagent C Added Twice | <0.001 |
| Order of Addition Reversed (ie., C added step 2, B added step 3) | <0.001 |



The negative control serves as an internal check that the non-specific background reaction is below a preset maximum and when it is, it also serves as a background correction. Figure 7 shows a graph of the range of negative control values plotted against total IgE values for all samples tested, as well as dilutions of a pool of specimens with a total IgE value of 2500 IU/mL. The negative control increases only slightly with increasing concentrations of total IgE which suggests that blocking the nitrocellulose with 10% heat-inactivated horse serum is effective in eliminating nonspecific binding and that the automated wash procedure is sufficient to remove non-specific IgE and other assay components which may lead to an elevated background signal and aberrant results. If the reflectance reading for the negative control rises above a preset maximum (0.250 D(r)), the printout records "invalid" for the negative control and no assay results are printed. An invalid negative control was observed in only one of the 162 specimens tested.

Interfering Substances

Results from these studies indicate that none of the substances tested, including drugs often taken to relieve allergy symptoms, levels of total IgE up to 2500 IU/mL, and elevated levels of hemoglobin,

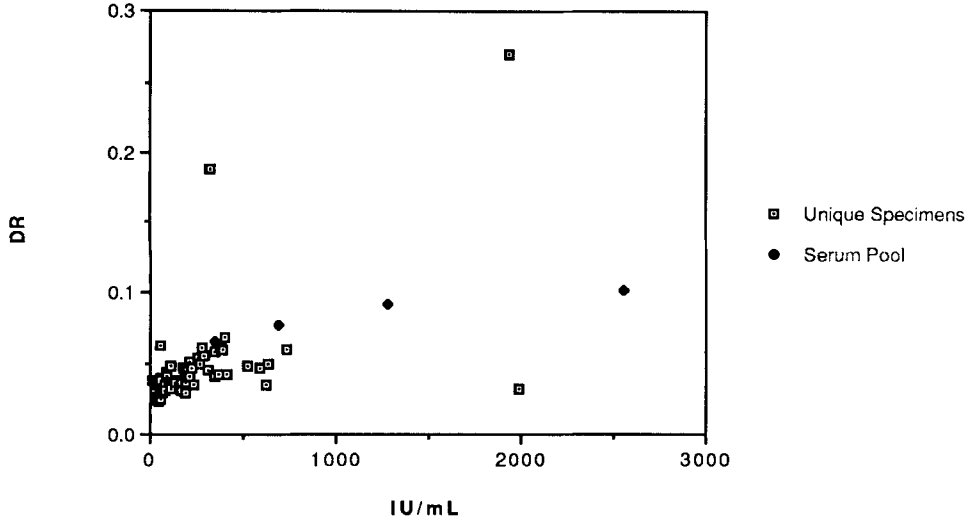


FIGURE 7. Negative control values vs level of total IgE.



bilirubin and triglycerides, changed sIgE values for any of the allergens by more than 20% (see example in Figure 8).

Clinical Evaluation

Sera from 162 patients, with specific IgE levels which covered the range of positive results, were evaluated using the Pharmacia RAST and the Abbott MATRIX™ Aero assay. If RAST is used as the reference, the overall percent sensitivity and specificity of the MATRIX Aero assay is 87% and 85% respectively, for those allergens where at least 35 RAST positive samples are available (Table 5a). If MATRIX is used as the reference and RAST is measured against MATRIX, the overall sensitivity and specificity of the RAST is 79% and 89% respectively (Table 5b). The sensitivity and specificity of both assays exceeds 95% for all allergens when 3 and 4 + positive specimens are compared.

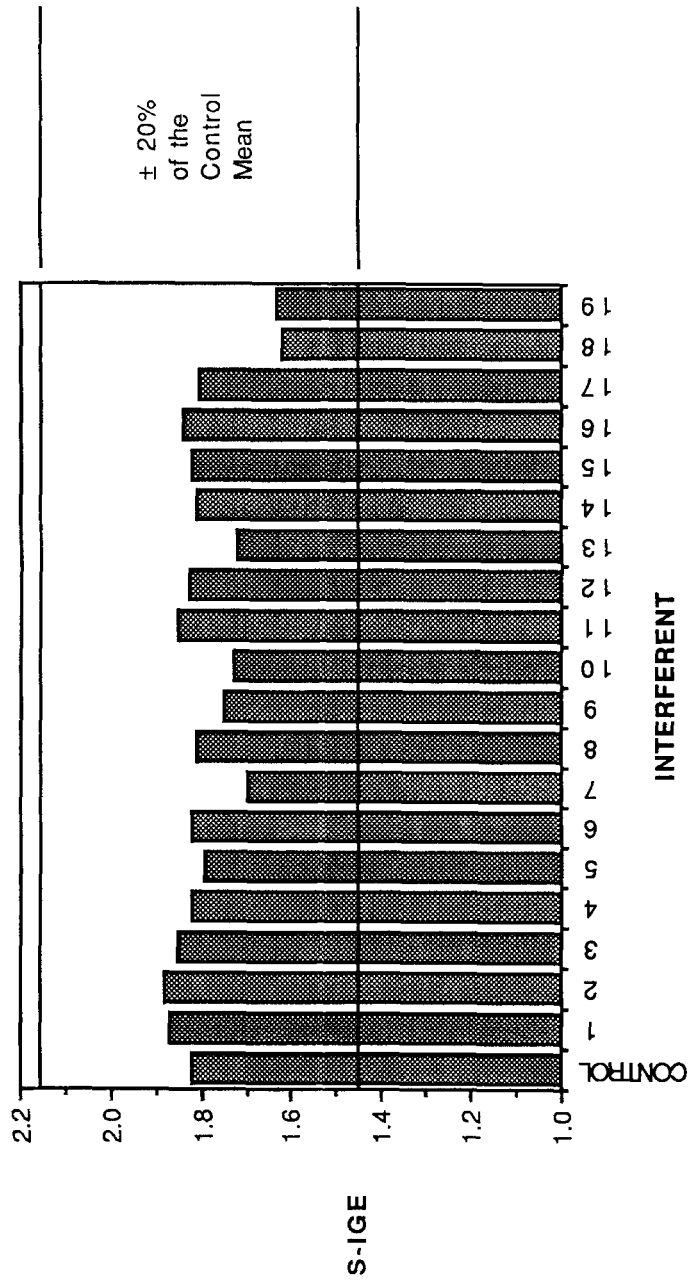


FIGURE 8. The effects of several potential interferents upon sIgE values for Alternaria. The horizontal lines indicate ± 20% of the mean for the control. The number of the interferent is identified in Table 4 together with the concentration at which it was tested. None of the interferents caused any of the results to fall outside of these limits.

TABLE 5a

Sensitivity and Specificity of MATRIX versus RAST (Reference)

| Allergen | # True Positive[a] | # RAST Positive | % Sens. | # True Negative | # RAST Negative | % Spec. |
|---|---|---|---|---|---|---|
| D pter | 57 | 62 | 92 | 96 | 100 | 96 |
| D far | 53 | 56 | 95 | 95 | 106 | 90 |
| Cat | 75 | 83 | 90 | 69 | 79 | 87 |
| Bermuda | 66 | 88 | 75 | 58 | 74 | 78 |
| Timothy | 84 | 92 | 91 | 64 | 70 | 91 |
| Alt | 38 | 45 | 84 | 108 | 117 | 92 |
| Mugwort | 37 | 47 | 79 | 94 | 115 | 82 |
| Plantain | 38 | 40 | 95 | 81 | 122 | 66 |
| Overall | 448 | 513 | 87 | 665 | 783 | 85 |



TABLE 5b

Sensitivity and Specificity of RAST versus MATRIX (Reference)

| Allergen | # True Positive | MATRIX Positive | % Sens. | # True Negative | MATRIX Negative | % Spec. |
|---|---|---|---|---|---|---|
| D pter | 57 | 61 | 93 | 96 | 101 | 95 |
| D far | 53 | 64 | 83 | 95 | 98 | 97 |
| Cat | 75 | 85 | 88 | 69 | 77 | 90 |
| Bermuda | 66 | 82 | 80 | 58 | 80 | 73 |
| Timothy | 71 | 77 | 92 | 64 | 85 | 75 |
| Alt | 36 | 40 | 90 | 113 | 122 | 93 |
| Mugwort | 37 | 58 | 64 | 94 | 104 | 90 |
| Plantain | 38 | 79 | 48 | 81 | 83 | 98 |
| Overall | 433 | 546 | 79 | 670 | 750 | 89 |

[a] Indicates those samples which tested positive by the test assay (MATRIX) which were also positive by the reference assay (RAST). # True Negative indicates those samples which tested negative by the test assay (MATRIX) which were also negative by the reference assay (RAST).

% Sensitivity = # True Positive/# Reference Positive X 100.

% Specificity = # True Negative/# Reference Negative X 100.

TABLE 6

Assay Precision.

HIGH POSITIVE SAMPLE

| Allergen | Mean sIgE | Within Day* % CV | Between Day* % CV | Total % CV |
|---|---|---|---|---|
| D farinae | 1.427 | 17 | 0 | 17 |
| D pteron. | 14.769 | 11 | 9 | 14 |
| Alternaria | 1.664 | 8 | 3 | 8 |
| Aspergillus | 37.571 | 20 | 13 | 24 |
| Penicillium | 8.438 | 9 | 10 | 14 |
| Dog | 7.041 | 15 | 0 | 15 |
| Cat | 30.688 | 18 | 8 | 19 |
| Timothy | 12.000 | 12 | 0 | 16 |
| Bermuda | 33.583 | 16 | 5 | 17 |
| Plantain | 36.000 | 13 | 6 | 14 |
| Mugwort | 26.607 | 10 | 12 | 16 |
| Olive | 8.807 | 12 | 7 | 14 |
| Birch | 16.769 | 14 | 0 | 14 |
| Parietaria | 2.500 | 11 | 5 | 12 |

LOW POSITIVE SAMPLE

| Allergen | Mean sIgE | Within Day* % CV | Between Day* % CV | Total % CV |
|---|---|---|---|---|
| D farinae | 0.193 | 17 | 8 | 19 |
| D pteron. | 0.370 | 12 | 0 | 12 |
| Alternaria | 0.398 | 6 | 7 | 9 |
| Aspergillus | 0.409 | 21 | 9 | 23 |
| Penicillium | 0.277 | 24 | 3 | 24 |
| Dog | 0.927 | 12 | 6 | 14 |
| Cat | 0.667 | 13 | 5 | 14 |
| Timothy | 1.764 | 16 | 9 | 18 |
| Bermuda | 0.805 | 20 | 0 | 20 |
| Plantain | 0.256 | 12 | 5 | 13 |
| Mugwort | 0.527 | 15 | 12 | 19 |
| Olive | 0.269 | 21 | 17 | 27 |
| Birch | 0.263 | 14 | 8 | 16 |
| Parietaria | 0.627 | 6 | 1 | 12 |

*Components of variance, not precision estimates (17).

Assay precision was determined by testing two specimen pools having varying quantities of specific IgE to several of the allergens. Specimens were assayed in triplicate using two instruments over 10 days. Results from these studies, shown in Table 6, indicate that the total variation (%CV) of each assay is generally less than 20%.

## DISCUSSION

The Abbott MATRIX™ Aero in vitro assay tests serum specimens for specific IgE to a panel of allergens simultaneously using 0.5 mL of serum. Results from optimization experiments show that the protein concentration of the allergen solution applied to the nitrocellulose is unique for each allergen and critical to assay performance. The built-in procedural and negative controls, which flag assays which may produce aberrant results, are additional features designed to improve the reliability of assay results. The incubation time of each step of the assay is also important for optimal assay performance and was chosen to maximize signal-to-noise ratio. After each wash step, the assay is stable such that Reagent B can be added up to 3 hrs after incubation 1, Reagent C up to 1 hour after incubation 2, and the lids removed up to 1 hr after incubation 3. This feature gives flexibility to the operator while maintaining accuracy. Results are unaffected by elevated levels of drugs and other common blood proteins which may be present in patient specimens, and total IgE to at least 2500 IU/mL. Analysis of test results shows good agreement with RAST.

Factory calibration of each allergen assay eliminates the need to establish a standard curve with each assay run and provides unique answers for each allergen. Automated timing of incubations and washing of test cells minimizes the time necessary for the technician to run the assay and assures reproducibility of results. Reproducibility of results is also aided by the instrument display, which prompts the technician as to when and which reagent to add.

In summary, the Abbott MATRIX™ Aero test is an easy-to-use, accurate, and reproducible method for measuring specific IgE antibodies to a panel of allergens.

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