

Application of the MAST™ Immunodiagnostic System to the Determination of Allergen-Specific IgE

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The MAST Immunodiagnostic Test System was developed to provide a comprehensive, simple means for the *in vitro* measurement of multiple antigens or antibodies. The first commercial application of the MAST system incorporates several novel features for cost-effective diagnosis of IgE-mediated allergy in a clinical laboratory or a physician's office. The basis of the MAST system is a unique analytical test chamber, which contains cellulose thread as the solid-phase matrix and allows multiple test results from a single assay. This test chamber incorporates both positive and negative controls and requires no volume-dependent pipetting steps. Immunographic exposure onto high-speed Polaroid instant film allows for quantifying results with an automatic recording infrared-transmittance densitometer. Test results are easily interpreted by using a patient test record provided with the system. The MAST system greatly simplifies testing for allergen-specific IgE, while retaining specificity and sensitivity. Currently, with the MAST system one can simultaneously measure picomoles of allergen-specific IgE in up to 35 different allergen classes. In addition to allergy testing, the MAST technology is applicable to other immunodiagnostic profiles.

Additional Keyphrases: allergies · immunographic photography · infrared densitometer · reagins · RAST

Atopic allergy is a hypersensitive immunologic condition mediated by a distinct class of serum antibodies called reagins, which have been identified as immunoglobulin E (1, 2). Immunocompetent B lymphocytes, when stimulated by a specific allergen, produce antibodies to the allergen. IgE antibody binds via its F_c portion to receptors on the surface of mast cells and basophilic leukocytes (3). Subsequent binding of allergen to specific cell-bound IgE triggers cell degranulation and the release of various substances, including vasoactive amines. These substances cause contraction of smooth muscle, itching, swelling, and transmucosal leakage of extracellular fluids. The most common clinical manifestations of this biological process are hay fever, asthma, dermatitis, hives, and anaphylactic shock. Because these clinical manifestations are directly related to IgE concentrations, the assessment of patient allergen-specific IgE is valuable in the diagnosis and treatment of atopic allergy.

The *in vitro* fluorometric measurement of allergen-specific antibody was first described by Millman et al. in 1964 (4), and the radioallergosorbent test (RAST) for the determination of allergen-specific IgE in serum was described by Wide et al. in 1967 (5). The RAST assay is now widely used as an adjunct or replacement for the classical skin-testing procedures used in allergy diagnosis. RAST assays are "sand-

wich"-type immunoassays with allergen covalently connected to a solid phase, usually a paper disk or a microtiter plate (6). After the patient's serum is incubated with the solid-phase allergen, the amount of allergen-specific IgE in the serum is quantified by incubation with labeled anti-IgE. The amount of label attached to the solid phase is measured in a gamma-counter or spectrophotometer and is directly proportional to the allergen-specific IgE in the patient's serum. The RAST procedures, which were designed for use primarily in the clinical laboratory, require the parallel assay of standards.

We report a new system for measuring allergen-specific IgE. The MAST system incorporates many new, unique analytical and design features so that the assay can be easily performed in the physician's office or in the clinical laboratory. The new solid phase used in the system, cellulose thread, allows the simultaneous measurement of multiple allergens in a unique analytical test chamber such that no volume-dependent pipetting steps or separate standards are required; quality controls are internal to each test chamber; the detection system involves immunography onto high-speed Polaroid® instant film; an inexpensive microprocessor-controlled automatic recording infrared transmittance densitometer quantifies results from the immunographic film strip; and a patient-result form allows easy interpretation of the test results. Here we will describe the use of this apparatus to diagnose IgE-mediated inhalant allergic disease.

Materials and Methods

Apparatus

MASTpette™ test chamber. Each MASTpette test chamber contains as many as 38 threads in a parallel array (Figure 1). Currently, the test chamber can be used to assay a comprehensive profile of up to 35 clinically relevant inhalant allergen classes, each allergen bound covalently to a separate cellulose thread. Each thread can have a single allergen or a mixture of immunologically cross-reactive

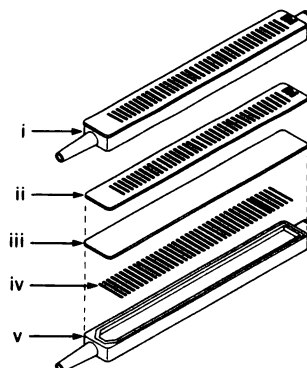


Fig. 1. The MASTpette test chamber: (i) the intact test chamber; (ii) metal grid; (iii) polystyrene coverslip; (iv) individual cellulose threads; and (v) polystyrene test chamber body

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allergens coupled to it. In addition to the allergen threads, the MASTpette test chamber contains two control threads. A positive-control thread with human IgE covalently attached provides a measure of how much ^{125}I -labeled anti-IgE should be bound in the assay. A negative-control thread devoid of allergen serves to measure nonspecific binding in the assay and compensates for film variation.

MAST photocassette. The photocassette (Figure 2) provides a means for developing up to five MASTpette test-chamber images per exposure, a process we call "immunography." The photocassette contains a special rare-earth phosphor screen that emits light when excited by gamma-radiation. A permanent photographic print is produced by sandwiching a Polaroid Type 57 (high-speed) film packet between the MASTpette test chambers inserted in the photocassette lid and the phosphor screen mounted in the photocassette lower member. After exposure, the film is processed like other Polaroid instant film, by pulling the packet between stainless-steel rollers also located in the photocassette.

MAST densitometer and reader card. The reader card guides the positioning of the individual immunographic strip or "immunograph" for proper insertion into the densitometer, and the densitometer scans the strip to determine the film transmittance (Figure 3). Film transmittance is measured through the use of a light-emitting diode as the source illuminator (peak wavelength, 890 nm), and an infrared-sensitive phototransistor is the detector. An analog-to-digital converter in the densitometer converts the analog voltage (0 to 5.0 V) from the phototransistor to a digital signal for use by the microprocessor.

After the reader card is inserted, the densitometer automatically determines the transmittance for each thread and translates the result into reaction levels corresponding to the concentration of allergen-specific IgE in the patient's serum; no further calculations are necessary. The positive-control thread provides an internal check on the viability of the radiolabeled antibody; if the transmittance of this

thread is too low, a double asterisk will appear on the resulting printout. The negative thread provides a control for nonspecific binding of patient IgE or radiolabeled antibody. The transmittance of the negative-control thread (nonspecific binding) is subtracted from the gross transmittance for each allergen thread; a single asterisk on the printout indicates abnormally high nonspecific binding. After the net voltage is determined, the densitometer converts the reading to a MAST class (see below).

Other apparatus. Other useful apparatus, peripheral to the equipment necessary to the MAST test system, are the MAST workstation, wash buffer dispenser, film splitter, and allergy test record (not shown). The MAST workstation supports as many as five MASTpette test chambers and facilitates the filling, incubation, and washing steps of the test procedure. The wash buffer dispenser allows the rapid wash of the test chamber without requiring the attachment and reattachment of a syringe. The MAST film splitter slices a film print into five immunographs, each representing the photographic image of one MASTpette test chamber. The MAST allergy test record is used in conjunction with the reader card, patient immunograph, and densitometric printout to present results to the patient in an easily understood fashion. It lists the allergens present on the test chamber threads, has a guide for interpreting MAST classes, and provides a convenient way to store the results in the patient's chart or file.

For certain radioactivity measurements, we used a Packard Multi-PriasTM gamma counter.

Materials

Additional materials included stopcocks, 10-mL and 3-mL Luer-lock syringes, and Polaroid Type 57 film. Pharmacia Phadebas[®] RAST reagents were obtained from Pharmacia Diagnostics, Piscataway, NJ.

Allergens. Raw allergens were obtained from Hollister-Stier, Inc., Spokane, WA; Sharp and Sharp, Inc., Everett, WA; Biopol, Inc., Spokane, WA; Berkeley Biologicals, Inc.,

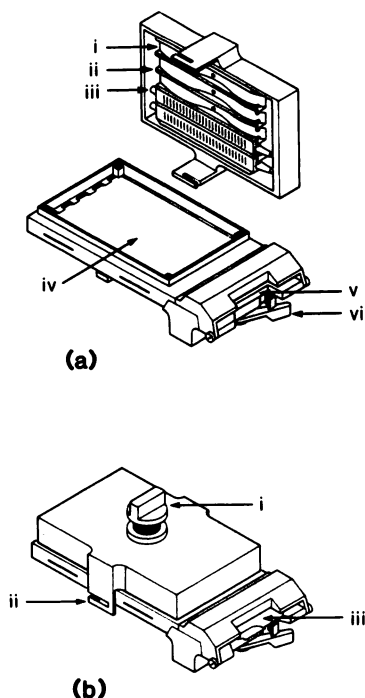


Fig. 2. a, The separated MAST photocassette lower member and lid: (i) platform spring; (ii) platform; (iii) test chamber; (iv) phosphor screen; (v) film slot; (vi) control lever. b, Assembled MAST photocassette: (i) control knob; (ii) latch; (iii) film envelope

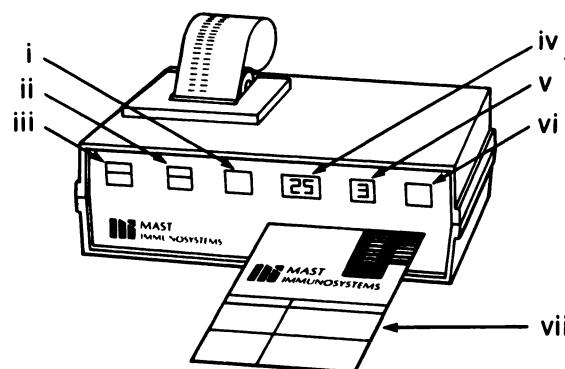


Fig. 3. Details and schematic of the MAST densitometer

(i) Paper feed; (ii) printer switch; (iii) on/off switch; (iv) thread number display; (v) MAST class display; (vi) manual advance; (vii) reader card. A flashing display alerts the operator when control voltages are outside specified limits

Berkeley, CA; and Greer Laboratories, Inc., Lenoir, NC.

Sera. Serum samples were obtained from donor patients. Generally blood was drawn and allowed to clot at 5 °C. Serum was removed and either used fresh or stored at -70 °C until needed.

Antibody. The antibody to a human myeloma IgE was raised in horse and affinity-purified. The anti-IgE was then labeled at 6.5 Ci/g with ^{125}I by using Iodo-GenTM from Pierce Chemical Co., Rockford, IL, in a modification of the method as described (7, 8).

Wash buffer. The wash buffer was 20 mmol/L phosphate buffer, pH 7.2, containing 0.15 mol of sodium chloride, 0.5 mL of Tween 20, and 0.5 sodium azide per liter.

Procedures

Preparation of allergen-coated threads. Each allergen was obtained defatted from the vendor and thereafter solubilized in water by blending or sonication. We then centrifuged and filtered the allergen extracts prior to coupling to the thread as described (9).

Comparison procedure. Results by the MAST system were compared with those by the Phadebas RAST, as described in the product insert, with use of the double-overnight procedure. Concentration of IgE is expressed in arbitrary units, as defined in the RAST product insert.

MASTpette test chamber assay. Before each test, we label the individual MASTpette test chambers with patient identification, drain them of storage buffer, and attach a stopcock to the top. After insertion into the workstation, approximately 1.3 mL of patient's serum is drawn up to fill the test chamber by means of a 3-mL syringe attached to the stopcock. The stopcock is then closed and the bottom of the test chamber is sealed with a rubber cap. After overnight (16–24 h) incubation at room temperature, the cap is removed, the stopcock opened, and the MASTpette test chamber washed three times by the sequential application of 10 mL of wash buffer, voiding the test chamber of wash buffer each time into the workstation. The ^{125}I -labeled anti-IgE is then drawn up to fill the MASTpette test chamber, the stopcock closed, and the bottom of the test chamber capped as before. After incubation for 16–24 h at room temperature, the test chamber is drained, washed, removed from the workstation, and loaded into the upper member of the photocassette. After insertion of a packet of Polaroid Type 57 film into the lower member of the photocassette body, the upper and lower parts are connected. To begin exposure, we withdraw the film envelope and turn the control knob on the upper member down fully to assure contact of the test chambers with the film negative. The photocassette is then placed in an airtight plastic bag and kept at 2–8 °C for 16 h or -20 °C for 8 h.

After the exposure is complete, we remove the photocassette from the plastic bag, turn the control knob completely up, reinsert the film envelope into the photocassette, and separate the photocassette's upper and lower members. The film is brought to room temperature (10–20 min) and processed as follows: first, set the control lever on the photocassette body to "P", then rapidly pull the film packet out between the rollers, and develop the film for 60 s. Separation of the envelope reveals the positive print, which is print-coated and allowed to dry.

We then place the film in the film splitter and slice it into five individual immunographic strips. Each immunograph is aligned in a reader card with the top band (positive control) within the top window marked "Pos," and all other bands aligned within the reader-card windows. The reader-card is fed into the densitometer, which automatically reads each immunograph and provides a readout for each band.

Results

Immunography. In the current MAST system, the photographic image that results from the assay is a direct function of the amount of radioactive antibody bound to the thread. Figure 4 presents the voltage readings of 600 threads vs their corresponding radioactivity in counts/min. The standard exposure time on high-speed film (16 h at 5 °C) allows sensitive detection of allergen-specific IgE from approximately 0.03 to 8 kilo-int. units/L. Patient samples that exhibit thread voltages in excess of class 4 (see below, Figure 6) may be rephotographed for a shorter period to yield relative reactivities above the initial reading range.

MASTpette test chamber metal grid. Figure 5 shows the utility of the metal grid attached to the face of the MASTpette test chamber. "Cross-talk" between radioactive threads in the immunograph taken without a grid is prevented when the metal grid is in place.

System response. We calibrate the MAST system with a reference serum containing 15 kilo-int. units of birch aller-

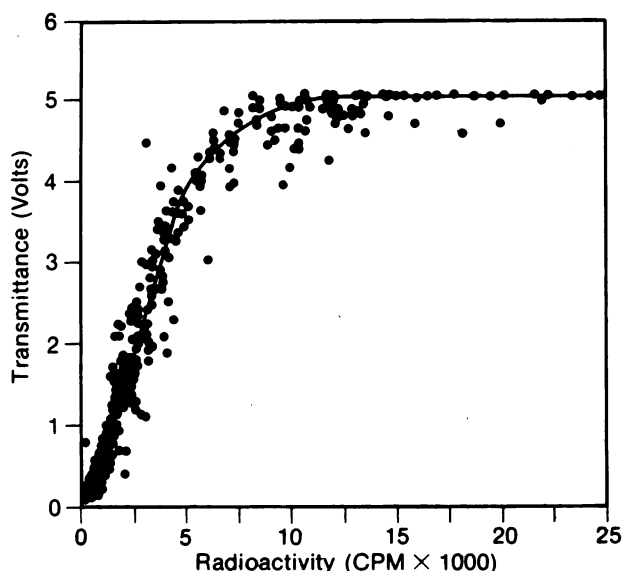


Fig. 4. Correlation between immunography and radioactivity counts for 20 different patients' sera

Immunography was performed at 5 °C for 18 h, the 20 immunographic images resulting in 600 separate transmittance determinations. After immunography, the test chambers were disassembled and each thread was counted separately for radioactivity

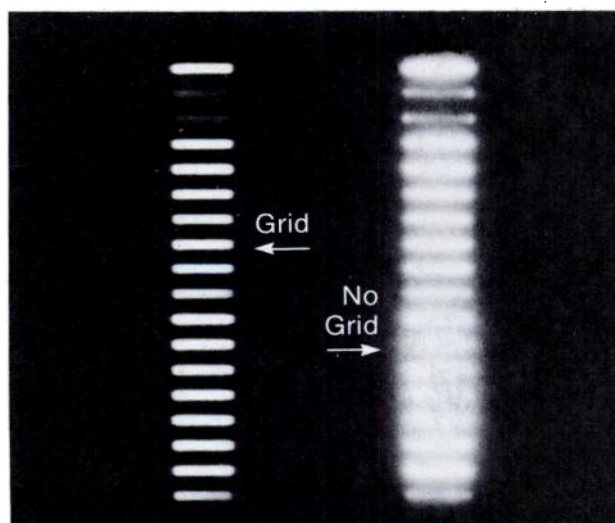


Fig. 5. Effect of using metal grid to read immunographic results of MASTpette test chambers

gen-specific IgE per liter. This serum was sequentially diluted in nonallergic human serum and assayed to obtain a standard curve of film transmittance vs IgE concentration. The results of this analysis are presented in Figure 6, which also shows the definitions of MAST classes. Assuming 2.4 ng/unit, the class 1 lower limit is equal to 0.45 μ g of allergen-specific IgE per liter, and the lower limit of the equivalence class is 80 ng/L.

Serum incubation time. MASTpette test chambers were filled with patients' sera and incubated at room temperature for various times as shown in Figure 7 (top); subsequent steps in the assay were as described above. These typical results for each MAST class show that the rate of binding of IgE to allergen is apparently independent of the concentration of allergen-specific IgE, because the different class reactivities plateau at the same time. Generally, results are consistent after 6 to 8 h; however, sensitivity does increase slightly with overnight incubation.

Antibody incubation time. Figure 7 (bottom) presents typical results for selected MAST classes when antibody incubation times were varied. Optimal sensitivity is achieved after overnight incubation (16–24 h).

Exposure time and temperature. The film exposure time is a function of the radioactive and biological decay of the ^{125} I-labeled anti-IgE and the temperature of exposure. However, during the two-month shelf-life of the antibody there is very little biological degradation. Radioactive decay necessitates that, as the antibody ages, the time of exposure of the MASTpette test chamber to the film must also increase to achieve the same voltage transmittance; therefore, each MAST test kit contains an exposure schedule corresponding to the antibody lot. We followed an exposure schedule over the shelf-life of one antibody lot with three patients' serum samples; the results were consistent with those of the between-run precision (shown below).

Temperature also has an effect on exposure time. The rare-earth phosphor screen in the MAST photocassette converts the radioactive gamma emission into visible light that can be detected by the film, and this transition occurs more efficiently at lower temperatures (10). Figure 8 demonstrates transmittance vs radioactivity for an 8-h exposure at various temperatures. Less exposure time is required to reach a given transmittance voltage with a given radioactive thread at -20°C than 5°C . Because most physicians

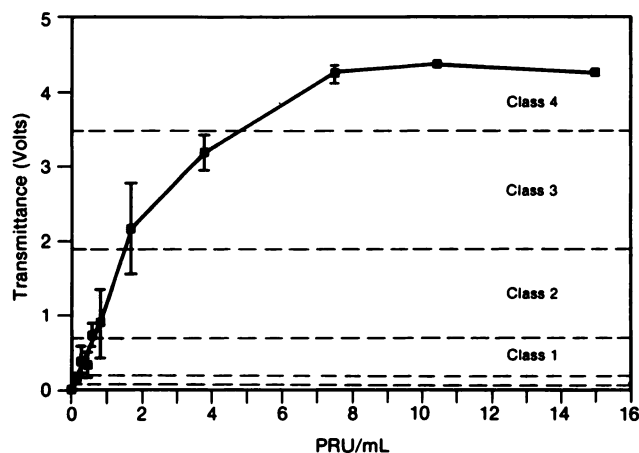


Fig. 6. Linearity of system response to a human serum containing a known concentration of birch-specific IgE diluted sequentially with nonallergic human serum

Each dilution was assayed in quadruplicate in MAST test chambers containing birch thread and in triplicate with Pharmacia birch disks. MAST results are presented as average (± 1 SD) net voltage; RAST results are presented as PRU/mL from the double-overnight procedure. Dotted lines indicate MAST class borders

and clinical laboratories have access to refrigerators and freezers set at these two temperatures, the roughly twofold difference in exposure time between the -20°C and 5°C enables the planning of convenient exposure times.

Precision. Within-run variation was estimated by assay-

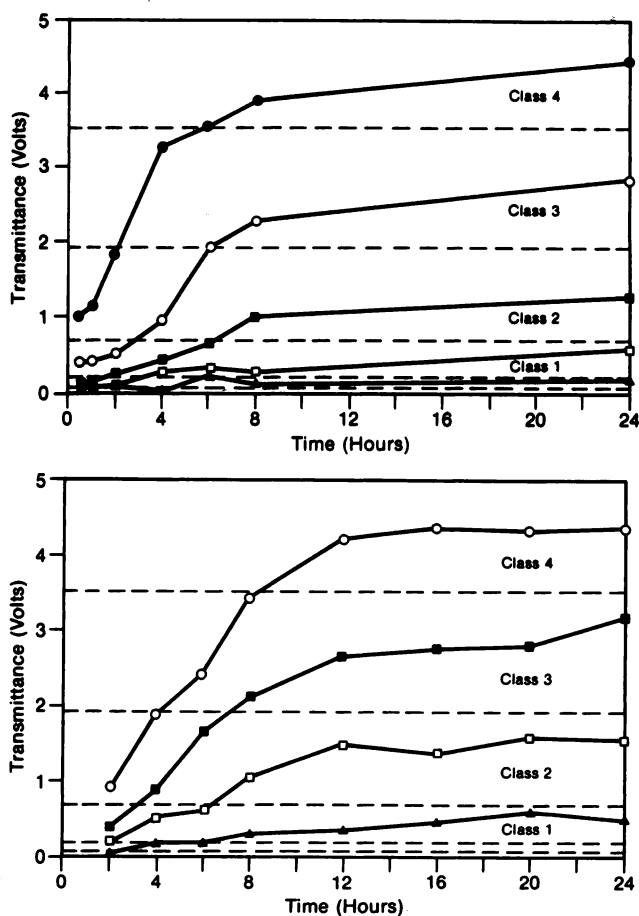


Fig. 7. Effect of time of incubation of serum (top) and antibody (bottom) Top, MASTpette test chambers were filled with a patient's serum in duplicate at the time points indicated and incubated at room temperature. Radiolabeled antibody was added at zero time and incubated at room temperature for 18 h. Bottom, MASTpette test chambers were filled with a patient's serum and incubated for 21 h at room temperature. Radiolabeled antibody was added to all test chambers at the same time, and duplicate test chambers were washed at the indicated time points. In both cases, film was exposed for 16 h at 5°C . Net voltage for each thread was determined, and the volts of representative threads from each MAST class were graphed vs incubation time

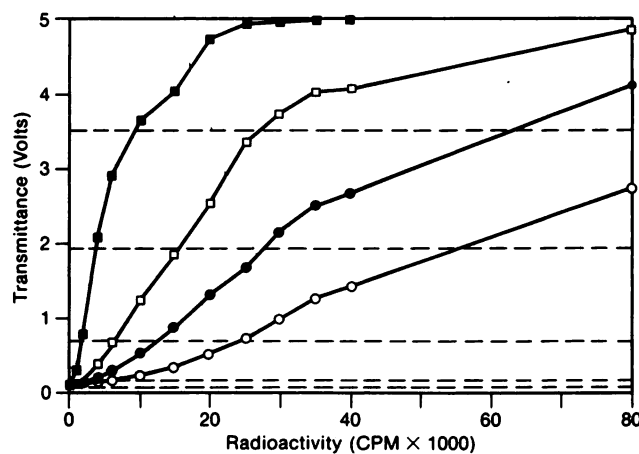


Fig. 8. Effect of temperature on immunography

Five MASTpette test chambers constructed with threads of known radioactivity were exposed for 8 h on successive days at 25°C ($\circ$), 5°C ($\bullet$), -20°C ($\square$), and -70°C ($\blacksquare$). The average voltage for each radioactivity level is shown. Test chambers photographed without a phosphor screen show very low transmittance even after exposure for much longer periods at these temperatures

ing a positive serum pool in five MASTpette test chambers on the same day using the same reagents; the results are presented in Figure 9 (top). The average CV for all threads in these assays was approximately 18%. Between-run CV, estimated by assaying the same serum pool on different days with test chambers and reagents from six different production lots over a period of four months (Figure 9, bottom), was again approximately 18%.

Because MAST test results are a direct function of radioactive antibody bound to thread, the antibody must have the same specific radioactivity, lot-to-lot. Using the iodine-labeling method described above, we obtained an average specific activity of 6.5 (SD 0.5) Ci/g in more than 50 separate labeling procedures.

Densitometer precision was estimated by reading, 20 times, one immunograph showing all classes of reactivity. The average standard deviation was 0.021 V (CV 1.86%). Results were similar when we used four different densitometers to read the same immunograph.

Correlation studies. For 20 patients' serum samples we compared results by the MAST Inhalant Profile assay with those by the Pharmacia Phadebas RAST for six allergens (Figure 10): oak, birch, *Atriplex*, sage, grass, and cat dander. The data were correlated in terms of the standard RAST scoring system. The slopes, y-intercept, and correlation coefficients for the individual allergens are summarized in Table 1. The correlation was good. Discrepant results may be due to the use of allergens from different sources to

produce the Pharmacia disks and the MAST threads, differences well documented in the allergy-testing literature (11).

Discussion

There are many immunoassay techniques currently available, including homogeneous and heterogeneous immunoassays with both radiolabeled and enzyme-labeled developing reagents (12). The current MAST system involves a heterogeneous immunometric sandwich technique with a multiple solid-phase array.

The MAST system was designed to bring several technologies together into a unique and sensitive diagnostic system. The use of cellulose thread for the solid phase allows the construction of a test chamber for performing multiple assays in a single test with several controls. The immunographic detection system combines the technology of the phosphor screen and high-speed instant film for cost-effective quantification of test results by measuring the transmittance of the film image with a low-cost infrared transmittance densitometer. In addition, the data-processing capabilities of the densitometer allow very easy interpretation of the test results with no transcription errors.

The first application of the MAST system has been the semi-quantitation of allergen-specific IgE. The current test allows the simultaneous measurement of specific IgE to allergens in as many as 35 different classes. There are no volume-dependent pipetting steps involved; the chamber is simply filled first with serum and then with labeled anti-IgE. This is an advantage over RAST procedures, which do

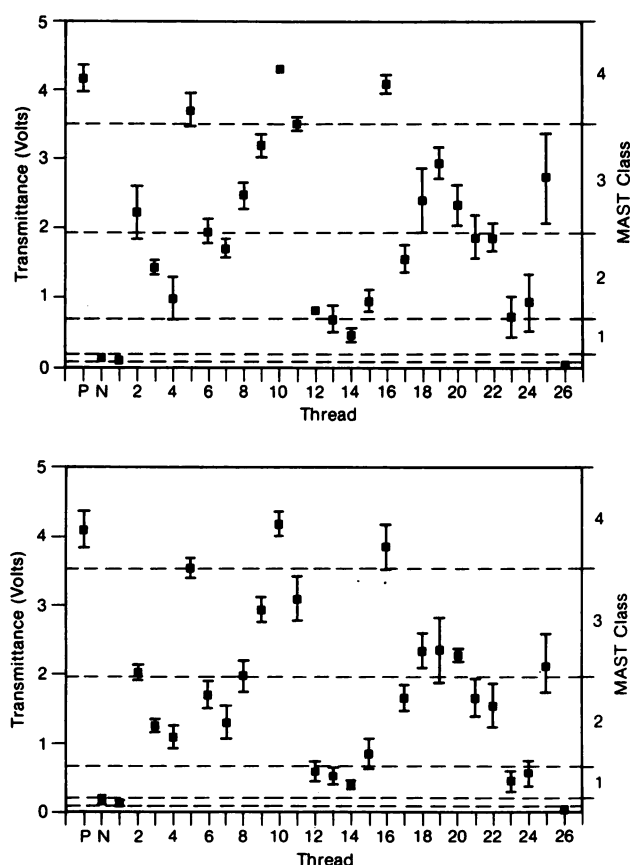


Fig. 9. Within-run (top) and between-run (bottom) precision

Top. Five MASTpette test chambers were used to assay a positive serum pool on the same day with the same reagents. Absolute voltage was determined for each thread; results are presented as average absolute volts $\pm$ 1 SD. Class borders, indicated by dotted lines, are not adjusted for net voltage. Bottom. Six different lots of MASTpette test chambers and reagents were used to assay the same positive serum pool over a four-month period. The absolute ($\pm$ 1 SD) voltage values for each lot were calculated from five MASTpette test-chamber assays run the same day and these values were used to calculate the average and standard deviation for the six lots

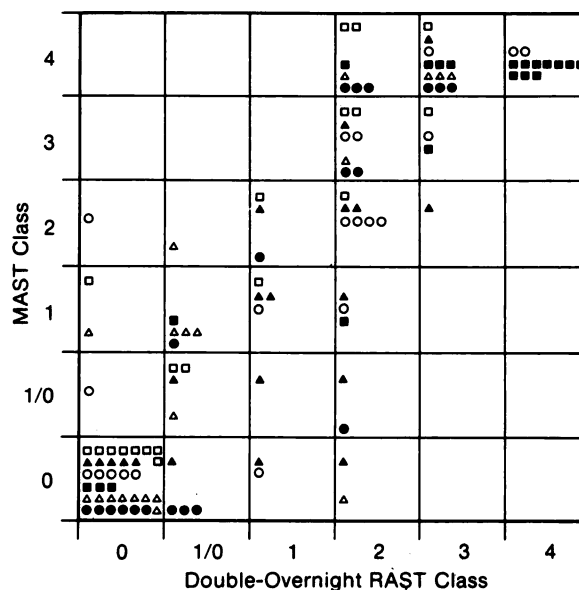


Fig. 10. MAST vs RAST correlation for 30 atopic human serum samples assayed with one MASTpette test chamber each and duplicate RAST disks for six allergens

$r = 0.88$; y-intercept = 0.15; and slope = 1.07. ● = Oak; △ = birch; ■ = grass; ○ = cat dander; ▲ = sage; □ = *Atriplex*

Table 1. MAST vs RAST

Allergen	n	y-intercept	Slope	Correlation
Oak	20	-0.075	1.459	0.903
Birch	20	0.207	1.221	0.873
Grass	20	0.318	0.967	0.924
Cat	20	0.229	0.964	0.889
Sage	20	-0.074	0.874	0.745
<i>Atriplex</i>	20	0.135	1.332	0.927
Combined	120	0.149	1.071	0.883

require volumetric pipetting. Positive- and negative-control threads are included in the test chamber to assure proper test performance and account for nonspecific binding to the cellulose threads. The resulting immunographic image allows for further quality control because the film can be visually scanned and compared with the densitometer print-out. In addition, the immunograph is a permanent record that can be repeatedly viewed for easy reference.

The assay can be performed without a standard curve because of the stringent quality control of the antibody labeling, film exposure, and densitometer precision, combined with a defined system of immunometric assay. The assay sensitivity is equal to that of other radioimmunoassays for measuring allergen-specific IgE, and is useful for concentrations of allergen-specific IgE from 0.08 to 12.5 ng/mL.

Results by the MAST system also correlate very well with those by RAST assays. Differences in specific results can often be accounted for by the use of different sources of allergen by different manufacturers (11).

The incorporation of several technologies has yielded an integrated diagnostic system that is very sensitive, is simple to perform, and allows for easy interpretation of results. Future developments are being directed toward other important diagnostic assays for which multiple determinations from a single test will provide accurate, cost effective results.

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