



A new automated multiple allergen simultaneous test-chemiluminescent assay (MAST-CLA) using an AP720S analyzer

Sungsil Lee ^a, Hwan Sub Lim ^b, Jungyong Park ^a, Hyon Suk Kim ^{a,*}

^a Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea

^b Kwandong University College of Medicine, Kangwon-Do, Republic of Korea

ARTICLE INFO

Article history:

Received 9 October 2008

Received in revised form 16 January 2009

Accepted 17 January 2009

Available online 27 January 2009

Keywords:

Multiple allergen simultaneous test (MAST)

Chemiluminescent assay (CLA)

Automated analyzer AP720S

Allergy screening, allergen-specific IgE

ABSTRACT

Background: In the diagnosis of atopic diseases, allergen detection is a crucial step. Multiple allergen simultaneous test-chemiluminescent assay (MAST-CLA) is a simple and noninvasive method for *in vitro* screening of allergen-specific IgE antibodies.

Methods: The Korean Inhalant Panel test on 20 patients and Food Panel test on 19 patients were performed using the conventional manual MAST-CLA kit and the new automated MAST-CLA method (automated AP720S system for the Optigen Assay; Hitachi Chemical Diagnostics, Inc., USA) simultaneously. The results were evaluated for positive reactivity and concordance.

Results: The results of inhalant panel gave a relatively higher class level result than the food panel. The 8 patients out of 20 (40%) of the inhalation panel, and 9 patients out of 18 (47.4%) of the food panel showed 100% concordance between the 2 systems. Eighteen patients (90%) of the Inhalation Panel and sixteen patients (84.2%) of the Food Panel showed more than 91% concordance.

Conclusions: These results suggest that the MAST-CLA assay using the new, automated AP720S analyzer performs well, showing a high concordance rate with conventional MAST-CLA. Compared to manual MAST-CLA, the automated AP720S system has a shorter assay time and uses a smaller serum volume (500 µl) along with other conveniences.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

As environmental substances in modern society change, the number of people diagnosed with an allergic disease has gradually increased. Usually, the diagnosis of an allergic disease depends mainly on the patient's chief complaints in the context of past history and a physical examination. Before treating the patient, however, the physician should look objectively at the causes of the complaints, and consider whether they result from the expected allergen or might be from unexpected allergens [1].

The skin prick test, widely used by clinicians, has a high concordance rate with total serum IgE, the allergen-specific IgE level and the bronchial provocation test, and has good reproducibility and sensitivity [2–5]. Nonetheless, the skin prick test has the following limitations: 37–50% of healthy subjects give a positive response to a given allergen [6]; certain medications, especially antihistamines, can

change test results; and tests cannot be performed on patients with skin disease [7,8].

In the mid-1960s, Ishizaka et al. [9] discovered that IgE antibodies cause the allergic hyper-response. Wide et al. [10] developed the radioallergosorbent test (RAST), which measures the serum allergen-specific IgE antibody, and has been used for examination of allergens. This test is expensive, however, because of the need for an individualized RAST for each allergen, making it difficult to search through all possible allergens that might be causing the symptoms. In addition, the cost of the radioactive isotope detection equipment is high and the examiner is at risk for radiation exposure. Accordingly, Miller et al. [11] developed the multiple allergosorbent test (MAST)-radioimmunoassay (RIA), which could examine several allergens simultaneously, and Brown et al. [12] developed the MAST chemiluminescent assay (MAST-CLA) which substituted chemiluminescent substances for radioactive isotopes.

The MAST-CLA[®] allergy system test was introduced in Korea in the 1990s and used with the MAST-CLA Korean allergen-specific IgE Panel to classify the 35 most common allergens in Korea since 1995. Since then, the MAST-CLA Korean allergen-specific IgE Panel has been divided into the Korean Food Panel (food panel) and the Korean Inhalant Panel (inhalant panel). Both the food and inhalant panels include different 35 allergens each [13]. Hitachi Chemical Diagnostics Inc., developed the automated MAST-CLA AP720S system with a new

Abbreviations: A, automated AP720S; Food Panel, the Korean Food Panel; Inhalant Panel, the Korean Inhalant Panel; M, manual MAST-CLA; MAST-CLA, multiple allergen simultaneous test-chemiluminescent assay; RAST, radioallergosorbent test.

* Corresponding author. Department of Laboratory Medicine, Yonsei University College of Medicine, 134 Shinchondong, Seodaemunku, Seoul, 120-752, Republic of Korea. Tel.: +82 2 2228 2443; fax: +82 2 364 1583.

E-mail address: kimhs54@yuhs.ac (H.S. Kim).

Table 1
Age distribution of subjects

Panel	Inhalant Panel	Food Panel	Total
Age	No.* tested	No. tested	No. tested
<10	2	5	7
10≤<20	10	5	15
20≤<30	3	3	6
30≤<40	0	3	3
40≤<50	1	0	1
50≤<60	3	2	5
≥60	1	1	2
Total	20	19	39

No.*: number.

MAST Optigen reagent in 2007, reducing the required patient sample volume, and changing some of the allergens. Automation was needed for allergy testing due to increasing the test volume. But, there's no data for evaluation for the new automated system. We compared the AP720S using MAST Optigen reagent with the previous MAST-CLA assay, by applying the 2 methods to identical samples.

2. Materials and methods

2.1. Subjects and samples

Randomly selected patients ($n=39$) who were clinically suspected to have an allergic disease were tested using the MAST-CLA inhalant panel or food panel. For a comparison study, the specimen volume and status should be satisfactory. Twenty patients from the inhalant panel and 19 patients from the food panel were included in the study for minimum number of statistics. Nineteen subjects were male and twenty were female. The age distribution was from 1 to 70 years (mean 24.1 years, Table 1). Samples were simultaneously tested with the manual MAST-CLA kit and the automated AP720S.

According to the general principles of immunoserologic tests, venous blood of the subjects was collected into serum separation tubes (SST) and centrifuged at 3500 rpm for 15 min to separate serum. Both manual and automated tests were performed on the collection day, at the same time, as soon as possible after serum separation. If necessary, serum samples were stored at 4 °C for a maximum of two days and centrifuged again before testing to remove fibrins or red blood cells.

2.2. Manual MAST-CLA method

The MAST-CLA method used previously with the food and inhalant panel was performed with a MAST-CLA® allergy system kit (Optigen™ assay, Hitachi Chemical Diagnostics, Inc, Mountain View, CA). Thirty-eight cellulose threads coated with allergens were arranged on the test chamber called MAST pette. The positive control, the negative control and the anti-total IgE antibody were attached on the first, second and third lines, respectively. Each of the 35 allergens was attached on subsequent threads.

MAST pettes were filled with 1.4 ml of patient serum and incubated at room temperature for 16–24 h. Each was then washed 3 times with wash buffer. Samples were incubated for 4 h with anti-IgE enzyme labeled with horseradish peroxidase and washed 3 times. After the addition of substrate and reaction for 30 min at room temperature, signal was detected by a MAST-CLA luminometer. The results were inter-

Table 3
Frequency of multiple positives per patient

No. of allergen-specific IgE	Number of patients (%)			
	Inhalant Panel		Food Panel	
	MAST-CLA	AP720S	MAST-CLA	AP720S
1	0 (0.0)	6 (33.3)	1 (11.1)	3 (50.0)
2	5 (27.8)	1 (5.6)	4 (44.4)	0 (0.0)
3	5 (27.8)	7 (38.9)	0 (0.0)	1 (16.7)
4	3 (16.7)	3 (16.7)	2 (22.2)	0 (0.0)
5	3 (16.7)	1 (5.6)	0 (0.0)	1 (16.7)
6	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
8	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)
9	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)
10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
>10	1 (5.6)	0 (0.0)	2 (22.2)	0 (0.0)
Total	18 (100.0)	18 (100.0)	9 (100.0)	6 (100.0)

preted as classes 1, 2, 3 and 4 according to the reactive density. Class 1 is minimal 1+ reactivity and class 4 is strong 4+ reactivity. Based on hospital experience, results from standards, and other data [14] that showed that class 1 results might be observed in healthy subjects who lacked clinical evidence of allergic disease, we decided to make class 2 the positive result cut-off point.

2.3. Automated AP720S method with MAST Optigen reagent

The automated AP720S method steps were similar to the manual MAST-CLA. The major differences were a decrease in the required sample amount and a decrease in the test duration. The AP720S required 500 µl of serum sample for the 35 allergens and replaced cellulose threads with a polystyrene solid phase in the MAST pette chamber. Serum sample reaction time was reduced from 16–24 to 2 h. Reaction time with the photoreagent was shortened from 30 to 10 min due to improvements of the new reagents. The total incubation time of the manual MAST-CLA method was 20 h and 30 min, whereas that of the automated AP720S method was 4 h and 20 min.

2.4. Comparison of automated AP720S results with the manual MAST-CLA results

We compared the manual MAST-CLA and automated AP720S results for each subject's serum and determined the concordance rate using a luminometer. All 20 MAST pettes were examined simultaneously within 1 h after addition of chemiluminescent agent, to eliminate observational lumino-density errors.

3. Results

3.1. Frequency of positives for allergen-specific IgE antibodies

Twenty-six subjects (66.7%) assayed using the manual MAST-CLA and 24 subjects (61.5%) assayed with the AP720S with MAST-Optigen system were observed to have allergen-specific IgE antibodies above class 2 for at least 1 allergen. The frequency of positives by sex were 13 male subjects (68.4%) and 14 female subjects (70.0%) with the manual MAST-CLA, and 12 male subjects (63.2%) and 12 female subjects (60.0%)

Table 2
Frequency of positives for allergen-specific IgE antibodies by age group

Panel	Inhalant Panel			Food Panel			Total		
	No. tested	No. (%) of positivity of MAST-CLA		No. tested	No. (%) of positivity of MAST-CLA		No. tested	No. (%) of positivity of MAST-CLA	
		Manual	AP720S		Manual	AP720S		Manual	AP720S
<10	2	2 (100.0)	2 (100.0)	5	2 (20.0)	1 (20.0)	7	3 (42.9)	3 (42.9)
10≤<20	10	10 (100.0)	10 (100.0)	5	3 (60.0)	2 (60.0)	15	13 (86.7)	12 (86.7)
20≤<30	3	3 (100.0)	3 (100.0)	3	2 (66.7)	1 (33.3)	6	5 (83.3)	4 (66.7)
30≤<40	0	0	0	3	1 (33.3)	1 (33.3)	3	1 (33.3)	1 (33.3)
40≤<50	1	1 (100.0)	0 (0.0)	0	0	0	1	1 (100.0)	0 (0.0)
50≤<60	3	1 (33.3)	2 (66.7)	2	1 (50.0)	1 (50.0)	5	2 (40.0)	3 (60.0)
≥60	1	1 (100.0)	1 (100.0)	1	0 (0.0)	0 (0.0)	2	1 (50.0)	1 (50.0)
Total	20	18 (90.0)	18 (90.0)	19	9 (47.4)	6 (31.6)	39	26 (66.7)	24 (61.5)

Table 4
Frequency of positives for allergen-specific IgE antibodies by allergen

Inhalant Panel			Food Panel		
Allergen	% of positivity		Allergen	% of positivity	
	MAST-CLA	AP720S		MAST-CLA	AP720S
<i>D. farinae</i>	85%	65%	<i>D. farinae</i>	31.6%	26.3%
<i>D. pteronyssinus</i>	70%	55%	<i>D. pteronyssinus</i>	31.6%	15.8%
Housedust	70%	55%	Dog	31.6%	5.3%
Dog	35%	15%	Housedust	21.1%	10.5%
Cat	20%	15%	Cat	21.1%	5.3%
Mugwort	20%	15%	Cockroach mix	10.5%	10.5%
Cockroach mix	20%	0%	Short ragweed	10.5%	5.3%
Dandelion	10%	0%	<i>Alternaria</i>	10.5%	0%
			<i>Aspergillus</i>	10.5%	0%
			White oak	10.5%	0%
			Rye	10.5%	0%
			Mugwort	5.3%	10.5%
			Crab	0%	10.5%

with the AP720S with MAST-Optigen. Analysis of the subject-age distribution of positives showed that the 10–30 year-old group had the highest frequency of positives from the inhalant panel, and the 20–30 year-old group had the highest positives from the food panel (Table 2).

Assessed by reactivity, using the manual MAST-CLA gave 90% positives from the inhalant panel, and 47.4% positives from the food panel. The inhalant panel gave 90% positives, and the food panel gave 31.6% positive using the automated AP720S. More frequent positivity were noticed from inhalant panel (Table 2). The highest number of positive allergens from the inhalant panel for both the manual MAST-CLA and the automated AP720S was 3 allergens. Three allergen positive samples were 27.8% for the manual MAST-CLA and 38.9% for the automated AP720S. The highest number of positive allergens from the food panel was 2 allergens with the manual MAST-CLA (44.4%), and 1 allergen using the automated AP720S (50.0%, Table 3).

farinae (*D. farinae*) was the most frequently positive allergen on the inhalant panel and on the food panel, by both manual MAST-CLA and automated AP720S. The inhalant panel showed relatively higher positivity than the food panel (Table 4).

3.2. Allergen-specific IgE antibodies for each subject by manual MAST-CLA and automated AP720S

Table 5 summarizes the allergens that showed class differences of >2 levels for the manual MAST-CLA and the automated AP720S. Those allergens that changed are described in Table 6. All results from the Inhalant Panel and the Food Panel are schematized by subject in Figs. 1

Table 5
Allergens showing a class difference of more than two levels

No. of patients	Inhalant Panel allergen	Food Panel allergen
1	Rye cultivated, Pigweed mix, Dandelion, Short ragweed, <i>Alternaria</i> , <i>Aspergillus</i> , <i>Cladosporium</i> , <i>Penicillium</i>	Egg white, Shrimp, Tuna, Garlic, <i>Cladosporium</i>
2		Crab, White oak, Rye, Short ragweed, <i>Alternaria</i> , <i>Aspergillus</i> , Cat, Cockroach mix, Housedust
3	<i>D. farinae</i>	<i>D. farinae</i> , <i>D. pteronyssinus</i>
4	Dog, Cockroach mix, <i>D. pteronyssinus</i>	
5		Dog
6	Housedust	

Table 6
Allergens that showed a change

Inhalant Panel		Food Panel	
MAST-CLA (old)	AP720S (new)	MAST-CLA (old)	AP720S (new)
Poplar mix	Cottonwood east	Citrus mix	Orange
		Onion mix	Buckwheat

and 2, respectively. They suggested that subjects' results tended to be lowered by 0.5–1 class level when the automated AP720S using MAST Optigen system was used. These results were considered as a favorable improvement because the nonspecific weak reactivity was reduced.

3.3. Concordance rate of positive results between the manual MAST-CLA and the automated AP720S

The food panel consisted of 33 allergens and the inhalant panel consisted of 34 allergens were compared and evaluated by both systems. Using class 2 as the cut-off level for a positive result, Table 7 shows the concordance rate of positive results between the manual MAST-CLA and the automated AP720S. From the food panel, 9 subjects (47.4%) showed the same result, or a 100% concordance, for all items from the 2 equipment systems, and 8 subjects (40.0%) showed 100% concordance on Inhalant Panel items. Sixteen subjects (84.2%) had a concordance rate of greater than 91% for food panel allergens, and 18 (90.0%) for inhalant panel items. No subject had a concordance rate of <50%.

4. Discussion

The immunologic mechanisms by which allergen diseases cause inflammatory reactions are IgE antibodies, mast cells, inflammatory mediators, IgG or IgM immune-complexes, complement, neutrophils, and effector T lymphocytes or lymphokines. The MAST-CLA assay measures allergen-specific IgE antibodies [15]. The exposure routes of allergens that cause allergen diseases are inhalation, injection, ingestion, direct contact, among others; primarily, inhalation results in the production of IgE antibodies [16].

In this study, the most frequent allergens that gave positive IgE results were dermatophagoides and housedust, consistent with an earlier report [17]. Using the manual MAST-CLA, seven subjects were found to have a positive reaction against cat allergen, and 85.7% of these were positive for dermatophagoides and housedust. If the cut-off level for *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) was lowered to class 1 [18], all subjects who were cat allergen-positive would also test positive for dermatophagoides and housedust. When analyzed with the automated AP720S, four subjects had a cat allergen-positive response, and of these, 75% showed positive results for dermatophagoides and housedust. Similarly, if the cut-off level for *D. pteronyssinus* was lowered from class 2 to class 1, three subjects (75%) who were positive for cat allergen would also be positive for dermatophagoides and housedust allergens. The class level results for each subject using the automated AP720S and the manual MAST-CLA are listed in Table 8. These results suggest cross-reaction with dermatophagoides. This is supported by a previous study that suggested that the P1 antigen of the major allergen of *D. pteronyssinus* has a common antigenic epitope with the major antigen of *D. farinae* [19]. In addition, most subjects with cat allergen-positive results showed a positive reaction against dermatophagoides. There have been reports of cat allergens contaminated with dermatophagoides and housedust [20,21], which our study could not rule out. To resolve this, increasing the subject number and using more specific evaluation methods to detect the allergen without cross-reactions and contamination are needed.

The purpose of this study were to evaluate 2 methods, the manual MAST-CLA and the automated AP720S using MAST Optigen reagent for

Food Allergen	Pt 1		Pt 2		Pt 3		Pt 4		Pt 5		Pt 6		Pt 7		Pt 8		Pt 9		Pt 10		Pt 11		Pt 12		Pt 13		Pt 14		Pt 15		Pt 16		Pt 17		Pt 18		Pt 19	
	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M		
Total IgE	0	2	1	3	0	3	0	3	0	2	1	3	0	1	0	3	0	2	1	3	0	2	1	3	0	3	1	3	0	2	2	4	2	4	2	3	0	3
Soybean	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Milk	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cheddar Cheese	0	0	0	1/0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Egg White	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Crab	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Shrimp	0	0	0	1/0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Tuna	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Codfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Salmon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Pork	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Chicken	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Beef	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
A: Orange, M: Citrus mix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Peach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Wheat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Rice	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Barley	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Garlic	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
A: Buckwheat, M: Onion mix	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Peanut	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Yeast/Bakers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Birch/Alder	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
White Oak	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Rye	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Mugwort	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Short Ragweed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Alternaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Aspergillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Cladosporium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Cat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Dog	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Cockroach Mix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Housedust	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Mite-farinae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Mite-pterony	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

Fig. 2. Results of the Food Panel by subject.

concordance rate and examiner convenience. We found that to optimize the performance of the automated AP720S, an alarm system is needed to determine if each pette is completely and correctly installed. When a pette was installed, visual confirmation was required by the examiner which introduced uncertainty. Also, the buffer and waste bottles were not large enough for general hospital use, since the bottles must be emptied every 30 pipettes. To improve the convenience and test performance, we recommend changes to the above limitations.

This study showed that most food and inhalant panel allergens showed a concordance rate of >90% between the automated AP720S and the manual MAST-CLA systems. The AP720S decreased the total performance time by eliminating 80% of the incubation period, which improved convenience to the examiner. Moreover, it is expected that the false positive rate will decrease for the food panel items. In the near future, through multi-directional studies of patients with allergic diseases, and increases in sample numbers, more exact conclusions can be made.

When total IgE value measured by RIA and by MAST-CLA were evaluated by regression analysis, the r^2 were 0.333 and 0.311, respectively [22]. Because of this statistical insignificance, the total IgE was not analyzed by MAST-CLA in this study.

The establishment of a standard cut-off class for positive results should be resolved for future studies. In this work, class 2 was used as the positive result cut-off level, following Park et al. [23], and in keeping with the current trend to use class 2 as the general clinical adjusted cut-off level. Some reports have used the adjusted class 1 as the positive cut-off level [24–26], increasing the positive rate of allergy disease patients dramatically. In class 1 adjusted reports [26], however, even non-allergic disease patient groups showed a 37% positive rate. A high positive rate like this in the non-allergic group requires considerable statistical correction. Clearly, using class 1 as a cut-off level requires correction for false-positives.

Recently, it was hypothesized that the ROC (receiver operating characteristic) curve should be used to determine a reliable MAST-CLA positive cut-off level, based on statistical considerations [18]. When the ROC curve was adjusted for the three most common allergens, the best sensitivity and specificity results were as follows: *D. farinae*, class 2; *D. pteronyssinus*, class 1; and cockroach mix, trace. Therefore, the positive cut-off level for considering all allergens should be at least class 2, to minimize false positives.

Because of the absence of a reference method and reference material for the allergen test, positive results cannot be absolutely confirmed for any method. In the near future, quality control materials and an external quality control survey will be introduced to minimize differences between laboratories [27]. Accuracy and reproducibility are essential for all clinical laboratory tests, and *in vitro* multiple-allergen screening tests with serum should also be verified.

In conclusion, the results from the automated AP720S showed a good concordance rate with those from the manual MAST-CLA kit, and

Table 8

D. farinae, *D. pteronyssinus* and housedust allergens results for a subject with a positive cat allergen result

Patient	Automated AP720S class/manual MAST-CLA class			
	Cat	<i>D. farinae</i>	<i>D. pteronyssinus</i>	Housedust
Inhalant Pt. 11	3/4	4/4	3/4	2/3
Inhalant Pt. 12	4/4	0/3	0/trace	0/4
Inhalant Pt. 18	4/4	4/4	4/4	4/4
Food Pt. 10	0/2	0/2	0/2	0/1
Food Pt. 12	1/2	0/trace	0/1	0/1
Food Pt. 16	0/2	4/4	4/4	4/4
Food Pt. 17	4/4	4/4	4/4	4/4

Abbreviation: Pt, patient.

the AP720S system was more convenient. For specimens that are below the class 2 level, the new automated MAST-CLA system decreased reactivity by 0.5–1 class. Other improvements over the manual MAST-CLA kit were minimal false positive results, almost complete automation of the test, and a lower specimen volume requirement, which was especially useful for testing children.

References

- [1] Brown CR, Higgins KW, Frazer K, Schoelz L, Dyminski JW, Marinkovich VA, et al. Simultaneous determination of total IgE and allergen specific IgE in serum by the MAST chemiluminescent assay system. Clin Chem 1985;31:1500–5.
- [2] Norman PS, Peebles RS. In vivo diagnostic allergy testing methods. In: Rose NR, editor. Manual of Clinical Laboratory Immunology, 5th ed. Washington, D.C.: ASM Press; 1997. p. 875–80.
- [3] Nelson HS. Diagnostic procedures in allergy. I. Allergy skin testing. Ann Allergy 1983;51:411–8.
- [4] Indrajana T, Spieksma FT, Voor horst R. Comparative study of the intracutaneous, scratch and prick tests in allergy. Ann Allergy 1971;29:639–50.
- [5] Scolozzi R, Boccafogli A, Vicentini L, Baraldi A, Bagni B. Correlation of MAST chemiluminescent assay (CLA) with RAST and skin prick tests for diagnosis of inhalant allergic disease. Ann Allergy 1989;62:193.
- [6] Kang YM, Huh YG, Choi GY, Kim NS, Kim HJ, Kim NS. Allergic skin test reactivity to five common aeroallergens in nonallergic population. Korean J Allergy 1989;9:378–84.
- [7] Pipkorn U. Pharmacological influence of antiallergic medication on in vivo allergen testing. Allergy 1988;43:81–6.
- [8] Reddy PM, Nagaya H, Pascual HC, Lee SK, Gupta S, Lauridsew JI, et al. Reappraisal of intracutaneous tests in the diagnosis of reagenic allergy. J Allergy Clin Immunol 1978;61:36–41.
- [9] Ishizaka K, Ishizaka T, Hornbrook MM. Physico-chemical properties of human reagenic antibody. IV. Presence of a unique immunoglobulin as a carrier of reagenic activity. J Immunol 1966;97:75–85.
- [10] Wide L, Bennich H, Johansson SG. Diagnosis of allergy by an *in vitro* test for allergen antibodies. Lancet 1967;2:1105–7.
- [11] Miller SP, Marinkovich VA, Riege DH, Sell WJ, Baker DL, Eldredge NT, et al. Application of the MASTTM immunodiagnostic system to the determination of allergen-specific IgE. Clin Chem 1984;30:1467–72.
- [12] Brown CR, Higgins KW, Frazer K, Schoelz LK, Dyminski JW, Marinkovich VA, et al. Simultaneous determination of total IgE and allergen-specific IgE in serum by the MAST chemiluminescent assay system. Clin Chem 1985;31:1500–5.
- [13] Yoon YO, Lee MK, Park HS, Park SS, Hong CS. The skin test reactivity and the level of total IgE in allergic patients. Korean J Allergy 1989;9:385–98.
- [14] Nepper-Christensen S, Backer V, DuBuske LM, Nolte H. *In vitro* diagnostic evaluation of patients with inhalant allergies: summary of probability outcomes comparing results of CLA- and CAP-specific immunoglobulin E test systems. Allergy Asthma Proc 2003;24:253–8.
- [15] Stites DP, Terr AI, Parslow TG, editors. Basic & Clinical Immunology. 8th ed. Appleton & Lange; 1994. p. 314–46.
- [16] Marsh DG, Meyers DA, Bias WB. The epidemiology and genetics of atopic allergy. N Engl J Med 1981;305:1551–9.
- [17] Yang WK, Kim HR, Lee MK, Park AJ. Evaluation of the MAST-chemiluminescent assay system for the diagnosis of allergic rhinitis. J Allergy Med J 1997;22(1):25–33.
- [18] Park JS, Nam HS, Kim YB, Choi YJ, Lee SH, Kim SH. Validity of MAST-CLA for diagnosis of arthropod allergy using receiver operating characteristic (ROC) analysis. Korean J Allergy 2007;45:239–43.
- [19] Platts-Mills TAE, Heymann PW, Chapman MD, Hayden ML, Wilkins SR. Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: development of a radioimmunoassay for antigen P1 equivalent in housedust and dust mite extracts. J Allergy Clin Immunol 1986;78:398–407.
- [20] Ann YM, Ihm SH, Park HS. Comparison of the skin prick test positivities of three commercial cat fur extracts and study on the possibility of contamination with house dust mites in cat fur extracts. Korean J Allergy 1990;10:235–42.

Table 7

Concordance rate of positive results between the manual MAST-CLA and the automated AP720S

Concordance rate (%)	Food (no. of subject)	Inhalant (no. of subject)
100	9	8
91–99	7	10
81–90	1	1
71–80	2	0
61–70	0	1
51–60	0	0
41–50	0	0
31–40	0	0
21–30	0	0
11–20	0	0
1–10	0	0
0	0	0
Total	19	20

- [21] Park HS, Lee MK, Hong CS. Cat-induced respiratory allergy: relationship between cat allergen and commercial extracts of house dust. *Korean J Allergy* 1989;9:10–9.
- [22] Yang SE, Oh HB, Hong SJ, Moon DH, Chi HS. Analysis of MAST chemiluminescent assay (MAST CLA) results performed in Asan Medical Center. *Korean J Lab Med* 1998;18:660–6.
- [23] Park HS, Kim JW, Chung DH, Kim YJ. Relationship between skin prick test, radioallergosorbent test, and chemiluminescent assay in allergic patients. *Korean J Allergy* 1991;11:347–54.
- [24] Ownby DR, Bailey J. Comparison of MAST with radioallergosorbent and skin tests for diagnosis of allergy in children. *Am J Dis Child* 1986;140:45–8.
- [25] Ogino S, Bessho K, Harada T, Irifune M, Matsunaga T. Evaluation of allergen-specific IgE antibodies by MAST for the diagnosis of nasal allergy. *Rhinology* 1993;31:27–31.
- [26] Lim HS, Yoon JK, Lee HH. Allergen patterns using MAST CLA test in Korean pediatric patients. *Korean J Lab Med* 2001;21:292–7.
- [27] Lim HS, Kim HS, Oh H. Current status of serum allergen tests in Korea. *Korean J Lab Med* 2008;28:124–9.