



Comparison of Intradermal Skin Test and Multiple Allergen Simultaneous Test Results in Canine Atopic Dermatitis

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Abstract Intradermal skin test (IDST) is generally considered a useful tool in identifying causal allergens in canine atopic dermatitis. Currently, multiple allergen simultaneous test (MAST), an in vitro testing method for allergen-specific immunoglobulin E, is being used as an alternative method. However, there are no reports comparing the IDST and MAST results in the same dogs. This study compared the results of both tests to evaluate the agreement and correlation between them. The sensitivity, specificity, and accuracy of the MAST were 76.2%, 64%, and 66.7%, respectively. Moderate positive predicted value (PPV, 50-75%) or high sensitivity (80-100%) were identified for indoor allergens, such as cat epithelia, house dust, and house dust mites. In contrast, high negative predicted value (NPV, 93.3-100%) and specificity (60-100%) were observed for environmental allergens and fungi. Although the agreement between IDST and MAST for all allergens was fair ($\kappa = 0.301$), that for each allergen was poor ($\kappa < 0.01$), except for birch ($\kappa = 0.158$). Spearman's rank correlation analysis revealed a low correlation between the MAST and IDST results ($\rho = 0.308$, $p = 0.001$). As compared to the IDST results, the MAST results did not identify the causative allergens sufficiently. IDST may not be performed for environmental allergens and fungi with high NPV and specificity if the MAST result is negative, but it may have to be performed for indoor allergens with moderate PPV and high sensitivity when the MAST result is positive.

Key words canine, atopic dermatitis, intradermal skin test, multiple allergen simultaneous test.

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Introduction

Canine atopic dermatitis (CAD) is one of the most common skin disorders affecting dogs. It is defined as a genetically predisposed inflammatory and pruritic allergic skin disease that presents a characteristic clinical feature associated with immunoglobulin (Ig) E antibodies against environmental allergens (5). The diagnosis of CAD is based on excluding other skin conditions with clinical signs similar to or overlapping with those of CAD and detailed interpretation of the medical history and clinical features (7).

IgE-mediated allergies account for majority of the clinically significant environmental, food, and medication allergies. Skin testing is an important component in the diagnosis of IgE-mediated allergies. Once CAD has been tentatively diagnosed based on the clinical criteria, allergen-specific IgE tests can help to confirm the diagnosis.

In veterinary medicine, the intradermal skin test (IDST) is a useful tool in identifying causal allergens. However, this method has several disadvantages, such as requirement of sedation or anesthesia and high cost (12). Moreover, this test could be affected by sex, age, season, and administration of anti-allergic drugs (8,12). Serum allergic tests (SAT) have been commercialized since the 1980s and are widely used to diagnose and treat allergic skin disorders. SAT detecting allergen-specific IgE against inhalant and food allergen components include various methods such as the radioallergosorbent test (RAST), serological enzyme-linked immunosorbent assay (ELISA), ImmunoCAP™ system (Pharmacia Diagnostics AB; Uppsala, Sweden), and multiple allergen simultaneous test (MAST) (6,9,11,13). MAST-chemiluminescent assay (MAST-CLA) has been widely utilized because it does not use radioactive materials or high-cost equipment. MAST-immunoblot, a more upgraded MAST assay, is faster, simpler, and requires a smaller volume of serum samples as compared to MAST-CLA (10,13). This test can simultaneously measure allergen-specific IgE for over 35 common allergens in IgE-mediated skin diseases (14).

To date, there have been no reports comparing the results of MAST and IDST in dogs. Therefore, this study aimed to evaluate the reliability, accuracy, and agreement between MAST and IDST results and identify the diagnostic value of MAST in dogs.

Materials and Methods

Case selection

Among the patients visiting the veterinary teaching hospital from January 2013 to June 2020, 92 dogs were diagnosed

with CAD, and 35 of them underwent IDST. Of these 35 patients, MAST was performed in 16 dogs using their stored serum. CAD was diagnosed based on the fulfillment of at least five of Favrot's criteria (onset of signs below 3 years of age; mostly living indoors; glucocorticoid-responsive pruritus; pruritus without lesions at onset; front feet affected; ear pinnae affected; unaffected ear margins; and unaffected dorso-lumbar area) and rejection of other possible pruritic causes, such as microbial and fungal infection, parasite burdens, adverse food reactions, and endocrine disorders (3,4).

IDST

Among the atopic patients, 16 dogs underwent IDST for 29 allergens (Greer Labs Inc) according to the guidelines currently in use (Table 1) (8). Drugs that could adversely affect the IDST results, such as glucocorticoids and anti-histamines, were not administered for at least 4 weeks before IDST was scheduled. The dogs were sedated with intravenous administration of 20 µg/kg medetomidine (Domitor; Pfizer). Intradermal injections of 0.1 mL of each allergen extract were administered using insulin syringes (BD Ultra-Fine, Becton, Dickinson and Company; USA). Histamine phosphate (Histatrol; Alk Abello) and 0.9% phosphate-buffered saline were used as positive and negative controls, respectively. The skin test reactions were evaluated at 0 and 15 min after injection and scored 0-4 by measuring the diameter or area of erythema or wheal. Scores 0 and 4 corresponded to the reactions of negative and positive controls, respectively. Any reaction with a score ≥ 2 was classified as positive. Sedation was reversed with 125 µg/kg atipamezole (Antisedan; Pfizer) administered intramuscularly.

MAST-immunoblot assay

MAST-immunoblot, EUROBlotOne (Euroimmun AG; Lübeck, Germany), was used to analyze the serum allergen-specific IgE concentrations. Serum samples were collected from January 2013 to June 2020. Peripheral blood was collected by jugular venipuncture and centrifuged for 10 min at 4,500 rpm. Serum samples were stored at temperatures below -70°C until the test was performed. This test kit consists of test strips with 127 allergens, including inhalation and food profile panels. Among them, only 12 allergens common with the IDST allergens were used for comparative analysis (Table 2).

To read the results, the specific IgE antibody value for each specific allergen was analyzed using a test device and divided into classes 0-6 by allergen-specific IgE concentrations according to the manufacturer's instructions; those below class 1 (<0.35 kU/L) were considered negative.

Table 1. Allergens used for intradermal skin test (total 29 allergens matched)

Group	Allergens	Concentration
Pollen	Bermuda grass	1,000 PNU/mL
Weeds	Cocklebur	1,000 PNU/mL
	Goldenrod	1,000 PNU/mL
	Lamb's Quarter	1,000 PNU/mL
	Pigweed. Rough/Red root	1,000 PNU/mL
	Plantain, English	1,000 PNU/mL
	Sage Mix	1,000 PNU/mL
Trees and shrubs	11 Tree Mix	1,000 PNU/mL
	Birch Mix	1,000 PNU/mL
	Pine Mix	1,000 PNU/mL
Molds	<i>Candida albicans</i>	1,000 PNU/mL
	<i>Fusarium solani</i>	1,000 PNU/mL
	<i>Penicillium</i> Mix	1,000 PNU/mL
	<i>Mucor</i> Mix	1,000 PNU/mL
	<i>Rhizopus</i> Mix	250 PNU/mL
Yeast	<i>Malassezia pachydermatis</i>	1,000 PNU/mL
Epidermis and inhalants	Cat epithelia	1,000 PNU/mL
	Cotton seed	1,000 PNU/mL
	Pyrethrum	1,000 PNU/mL
	Silk	500 PNU/mL
	Mixed feather	1,000 PNU/mL
	Human dander	1,000 PNU/mL
House dust	House dust mixture	100 PNU/mL
House dust mites	<i>Dermatophagoides farina</i>	1:5,000 w/v
	<i>Dermatophagoides pteronyssinus</i>	1:5,000 w/v
Insects	Flea	1:1,000 w/v
	Mosquito	1,000 PNU/mL
	Cockroach	1,000 PNU/mL
	House fly	1,000 PNU/mL
Positive control	Histamine Phosphate	0.0275 mg/mL
Negative control	0.9% phosphate buffered saline	

PNU, protein nitrogen unit; w/v, weight/volume.

Table 2. Allergens used in the comparative analysis between intradermal skin test and multiple allergen simultaneous test

Group	Allergens
Weeds	Goldenrod Lambenrodaller Pigweed (Rough/Red root)
Trees and shrubs	Birch Mix Pine Mix
Molds	<i>Penicillium</i> Mix
Yeast	<i>Malassezia pachydermatis</i>
Epidermis and inhalants	Cat epithelia
House dust	House dust mixture
House dust mites	<i>Dermatophagoides farina</i> <i>Dermatophagoides pteronyssinus</i>
Insect	Cockroach

Statistical analysis

Considering the IDST results as true, dogs with positive and negative IDST results were defined as the patient group and the control group for MAST, respectively. The sensitivity, specificity, positive predicted values (PPV), negative predicted values (NPV), and accuracy of MAST were compared with that of IDST as the standard. Spearman's rank correlation coefficient (ρ) and Cohen's kappa coefficient (κ) were used to evaluate associations and multiple levels of agreement between IDST and MAST for dichotomous parameters (qualitative and positive-negative), respectively. The predicted value was arbitrarily categorized as low (<50%), moderate (50-69%), high (70-89%), or very high ($\geq 90\%$). The ρ values were interpreted as follows: $\pm \geq 0.9$ = very high, $\pm 0.7-0.9$ = high, $\pm 0.5-0.7$ = moderate, $\pm 0.3-0.5$ = low, and $\pm < 0.3$ = little, if any relationship. The κ values were interpreted

ed as follows: 0.81-1.00 = almost perfect agreement, 0.61-0.80 = substantial, 0.41-0.60 = moderate, 0.21-0.40 = fair, 0.01-0.20 = slight, and <0.01 = no agreement (2). When p-value was <0.05, the probabilities were considered statistically significant.

Results

After excluding other skin conditions and fulfillment of the clinical criteria, 35 dogs with CAD underwent IDST. Among these 35 dogs, the sera of 16 dogs were subjected to MAST. Data pertaining to breed, sex, and initial onset age of the dogs diagnosed with CAD are summarized in Table 3. The most common breeds were Shih Tzu (25%) and Maltese (25%). The mean age of onset of the CAD-related clinical signs was 2.4 years (range, 6 months-7 years). The percentage of patients with an initial onset age below 3 years was 68.7% (n = 11).

Comparison of IDST and MAST results

A comparison of the IDST and MAST results for each allergen has been summarized in Table 4. All 16 dogs undergoing the two tests presented at least four positive responses in IDST. The most common allergen group was house dust mites (HDM), followed by epidermis and inhalants, mold, and house dust. Among the individual allergens, *Dermatophagoides farinae* (75%) presented the highest positivity, followed by *D. pteronyssinus* (62.5%) and house dust mix (50%).

Using a serum IgE cut-off of ≥ 0.35 kU/L for MAST, the allergens showing the most prevalent positive response and the highest detection rate were *D. farinae* (100%), HDM

(100%), and cat epithelia (100%). The lowest positivity was observed for the *Penicillium* mix (IDST, 0%; MAST, 0%). When the results of both tests were compared, the concordance and discordance between them varied depending on the type of allergen (Fig. 1). When IDST was positive, MAST was positive in 0-75% and negative in 0-18.75%; when IDST was negative, MAST was positive in 0-87.5% and negative in 0-100%.

Agreement between IDST and MAST results

The analyses of the agreement between the IDST and MAST results are summarized in Table 5. As compared to

Table 3. Signalment of sixteen dogs diagnosed with canine atopic dermatitis

Signalment	Classification	Number of dogs (%)
Breed	Shih Tzu	4 (25)
	Maltese	4 (25)
	Yorkshire Terrier	1 (6.25)
	Dachshund	1 (6.25)
	Cocker Spaniel	1 (6.25)
	French bulldog	1 (6.25)
	Golden Retriever	1 (6.25)
	Mixed breed	3 (18.75)
Sex	Male	9 (56.25)
	Female	7 (43.75)
Age of onset (years)	<1	1 (6.25)
	1-3	10 (62.5)
	3-5	4 (25)
	≥ 5	1 (6.25)

Table 4. Detection number and percentage of positive cases in 16 dogs with atopic dermatitis in intradermal skin test and multiple allergen simultaneous test with 12 allergens

Group	Allergen	IDST		MAST IgE	
		No. (%) PR	No. (%) PR	MV (kU/L) (R)	
Weeds	Goldenrod	1 (6.25)	1 (6.25)	1.1 (1.1)	
	Lamb's Quarter	1 (6.25)	0 (0)	0	
	Pigweed. Rough/Red root	1 (6.25)	0 (0)	0	
Trees and shrubs	Birch mixture	1 (6.25)	7 (43.75)	1.09 (0.6-2.0)	
	Pine mixture	1 (6.25)	0 (0)	0	
	<i>Penicillium</i> mixture	0 (0)	0 (0)	0	
Molds	<i>Malassezia pachydermatis</i>	1 (6.25)	15 (87.5)	1.73 (0.7-2.4)	
Yeast	Cat epithelia	3 (18.75)	16 (100)	3.35 (3.1-3.0)	
Epidermis and inhalants	House dust mixture	8 (50)	16 (100)	2.96 (0.5-3.7)	
House dust	<i>Dermatophagoides farinae</i>	12 (75)	16 (100)	1.78 (0.5-4.9)	
House dust mite	<i>Dermatophagoides pteronyssinus</i>	10 (62.5)	14 (87.5)	2.35 (0.5-5.8)	
Insect	Cockroach	3 (18.75)	3 (18.75)	0.93 (0.4-1.9)	

IDST, intradermal skin test; MAST, multiple allergen simultaneous test; IgE, immunoglobulin E; PR, positive reactions; MV, mean value; R, range.

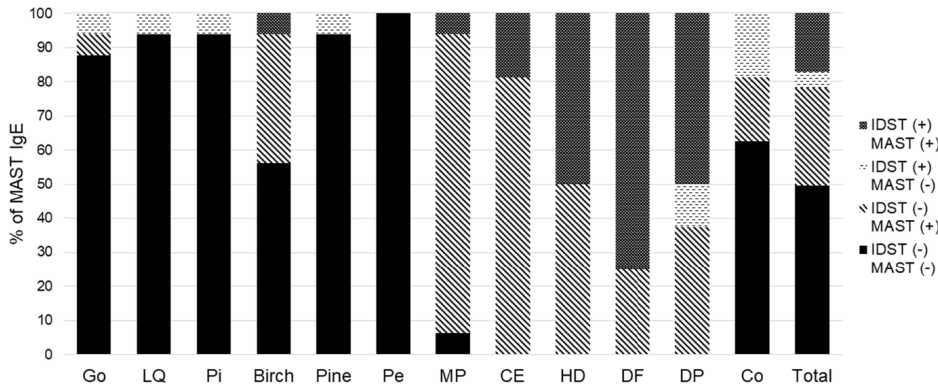


Fig. 1. Concordance and discordance of the results between intradermal skin test and multiple allergen simultaneous test. +, positive response; –, negative response; IDST, intradermal skin test; MAST, multiple allergen simultaneous test; CE, cat epithelia; Co, cockroach; DF, *Dermatophagoides farinae*; DP, *Dermatophagoides pteronyssinus*; Go, Goldenrod; HD, house dust; LQ, Lamb’s quarters; MP, *Malassezia pachydermatis*; Pe, *Penicillium*; Pi, pigweed.

Table 5. Sensitivity, specificity, positive predicted value, negative predicted value, and accuracy of multiple allergen simultaneous test against intradermal skin test

Group	Allergen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Weed	Goldenrod	0	93.3	0	93.3	87.5
	Lamb’s Quarter	0	100	0	93.8	93.8
	Pigweed. Rough/Red root	0	100	0	93.8	93.8
Trees and shrubs	Birch mixture	100	60	14.3	100	62.5
	Pine mixture	0	100	0	93.8	93.8
Molds	<i>Penicillium</i> mixture	0	100	0	100	100
Yeast	<i>Malassezia pachydermatis</i>	100	6.7	6.7	100	12.5
Epidermis and inhalants	Cat epithelia	100	0	18.8	0	18.8
House dust	House dust mixture	100	0	50	0	50
House dust mite	<i>Dermatophagoides farinae</i>	100	0	75	0	75
	<i>Dermatophagoides pteronyssinus</i>	80	0	57.1	0	50
Insect	Cockroach	80	0	57.1	0	50
Total		76.2	64	37.5	91.3	66.7

PPV, positive predicted value; NPV, negative predicted value.

Table 6. Analyses of agreement and correlation between multiple allergen simultaneous test and intradermal skin test

Group	Allergen	Agreement		Correlation	
		κ^a	ρ^b	p value	
Weed	Goldenrod	0	0	0	
	Lambrododn ski	0	-0.067	0.806	
	Pigweed. Rough/Red root	0	0	0	
Trees and shrubs	Birch mixture	0.158	0.185	0.492	
	Pine mixture	0	0	0	
Molds	<i>Penicillium</i> mixture	0	0	0	
Yeast	<i>Malassezia pachydermatis</i>	0.008	-0.368	0.161	
Epidermis and inhalants	Cat epithelia	0	0	0	
House dust	House dust mixture	0	0	0	
House dust mite	<i>Dermatophagoides farinae</i>	0	0	0	
	<i>Dermatophagoides pteronyssinus</i>	0	0	0.271	
Insect	Cockroach	0	0	0.393	
Total		0.301	0.308	0.001	

^aCohen’s kappa coefficient: ≤ 0 = poor, 0.01-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial, and 0.81-1.00 = almost perfect agreement.

^bSpearman’s rank correlation coefficient: $\pm < 0.3$ = little if any relationship, $\pm 0.3-0.5$ = low, $\pm 0.5-0.7$ = moderate, $\pm 0.7-0.9$ = high, $\pm \geq 0.9$ = very high.

IDST, the sensitivity, specificity, accuracy PPV, and NPV of MAST for all allergens were 76.2%, 64%, 66.7%, 37.5%, and 91.3%, respectively. Particularly, allergens with very high NPV (93.3-100%) included weeds (Goldenrod, Lamb's quarter, and pigweed), trees and shrubs (birch and pine), molds (*Penicillium*), and yeast (*Malassezia pachydermatis*). The NPV of all indoor allergens (cat epithelia, house dust, *D. farinae*, and *D. pteronyssinus*) was 0%.

Although the agreement between IDST and MAST for all allergens was fair ($\kappa = 0.301$), that for each allergen was poor ($\kappa \leq 0.008$), except for birch ($\kappa = 0.158$) that showed slight agreement (Table 6). Spearman's rank correlation analysis revealed a low correlation between the MAST and IDST results ($\rho = 0.308$, $p = 0.001$).

Discussion

This study evaluated the agreement and correlation between MAST and IDST results as well as the reliability of MAST in diagnosing CAD. The sensitivity, specificity, and accuracy for all allergens were 76.2%, 64%, and 37.5%, respectively, with MAST. The environmental allergens and fungi, except for birch, presented high specificity, NPV, and accuracy, while indoor allergens showed high sensitivity, moderate PPV, and low to moderate accuracy. Moreover, the MAST results for all allergens showed low agreement and correlation with IDST results and non-acceptable reliability. The agreement for each allergen ranged from poor ($\kappa = 0$) to slight ($\kappa = 0.158$), and the correlations were negligible ($\rho = 0.185$) or low ($\rho = -0.368$). Therefore, IDST may need to be conducted when the MAST result is positive for indoor allergens, but not when it is negative for environmental allergens and fungi.

In this study, the sensitivity and specificity for all allergens in the MAST were 76.2% and 64%, respectively. In human medicine, the skin prick test (SPT) is considered the gold standard for the diagnosis of AD. The MAST is also widely used, and several studies have reported its efficacy in diagnosing and treating allergic diseases (9,13). Even in veterinary medicine, this test is preferred by veterinarians and clients; nevertheless, only three reports have compared MAST with IDST in horses with allergic diseases (15-17). A previous report suggested that MAST shows good detection performance for three of five allergens with substantial diagnostic capability, but requires careful clinical analysis for few other allergens (17). The mean values of sensitivity and specificity for each allergen, depending on manufacturer's cut-off value, were 81% (range, 44-89%) and 56% (range, 60-100%), respectively. The overall sensitivity and specificity values noted in

this study are similar to those of a previous study.

Low NPV and specificity with moderate PPV and high sensitivity were identified in indoor allergens, including house dust, *D. farinae*, and *D. pteronyssinus*. Contrasting results were observed for environmental allergens and fungi. These results suggest that the frequency of exposure affects the production of allergen-specific IgE. Because all dogs in this study lived indoors, they were exposed to indoor allergens more often; hence, they may have produced more allergen-specific IgE. Therefore, IDST can be performed when the MAST result is positive for indoor allergens. However, IDST may not be needed when a negative MAST result is obtained for environmental allergens or fungi.

In this study, the accuracy of MAST relative to IDST was between 12.5% and 100% for each allergen and 66.7% for total allergens. In human medicine, a study comparing MAST and SPT in patients with chronic rhinitis reported an overall accuracy of 63.92% for total allergens and 68.39-100% for each allergen (10). In veterinary medicine, similar results were obtained in horses with AD with a mean accuracy of 73%, based on the manufacturer's cut-off value, and ranged between 50% and 93% for each allergen (17). In previous studies of CAD comparing in vivo skin tests and in vitro assays such as RAST and ELISA, the accuracy for each allergen was 12.5-82% for RAST (6) and 43-64% for ELISA (11). Therefore, the overall accuracy of MAST in this study is similar to that of previous studies.

The agreement and correlation for all allergens between MAST and IDST were identified as fair ($\kappa = 0.301$) and with low positive correlation ($\rho = 0.308$), respectively. In human medicine, the agreement between MAST and SPT was reported as substantial ($\kappa = 0.672$) for total allergens (1). In another study comparing MAST and IDST results in horses, there was fair ($\kappa = 0.432$) to substantial ($\kappa = 0.689$) agreement for three of the five allergens (*D. farinae*, *Acarus siro*, and *D. pteronyssinus*) and a moderate to high positive correlation ($\rho = 0.657-0.870$) for two of the five allergens (*D. farinae* and *Acarus siro*) (17). The agreement in the present study result is significantly lower than that of the previous result of the studies on humans and horses, and the correlation is lower than that in horses. Therefore, the reliability of the MAST has been identified as non-acceptable in dogs.

This study has several limitations. First, the sample size included and the number of allergens compared is too small to generalize the results. Therefore, further studies using larger cohorts with more allergens will be necessary in the future. Second, the frozen serum used in this study was stored for different periods. Since MAST assays have not been evaluated for CAD, the potential influence of frozen serum is un-

clear.

In conclusion, the present study recommends the clinical utility of MAST as a screening test for CAD. In comparison with IDST, MAST presents different sensitivities and specificities for each allergen, with poor agreement and no correlation. However, for total allergens, MAST shows fair agreement with IDST, non-acceptable reliability, and a low positive correlation. MAST is not suitable as a method for distinguishing causative allergens of CAD, but it may be used to determine whether IDST should be performed. When a negative MAST result is obtained for environmental allergens and fungi, the IDST procedure may not be necessary. In contrast, IDST may be performed when a positive MAST result is obtained for indoor allergens.

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Conflicts of Interest

The authors have no conflicting interests.

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