

Brief Communication

Evaluation of a point-of-care immunodot assay for predicting results of allergen-specific intradermal and immunoglobulin E serological tests

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Abstract Immunotherapy to prevent recurrence of clinical signs of atopic dermatitis (AD) is based on intradermal or serological tests that assist in identifying allergen-specific immunoglobulin E hypersensitivities. Unfortunately, the results of such tests can be negatively influenced by several factors, which include the age of the patients, the season of testing and the administration of anti-allergic drugs. Screening to predict when these expensive tests will be useful would benefit owners of dogs with AD. The objectives of this study were to determine whether a point-of-care allergen-specific immunodot assay (Allercept E-Screen©, Heska Corp., Ft Collins, CO, USA) could predict results of either intradermal or Allercept© full panel serological tests in atopic dogs. Thirty dogs living in the south-eastern USA were diagnosed with AD in accordance with current standards. Allergen-specific intradermal, serological and E-Screen© tests were performed in all subjects. For flea, house dust mite and pollen allergens altogether, results of the E-Screen© assay agreed with those of intradermal and serological tests in 26/30 dogs (87%) and 25/30 dogs (83%), respectively. In this group of dogs, the probabilities of obtaining intradermal or serological tests positive for these allergens were 70 and 67%, respectively. If either skin or serum tests were performed only in dogs with positive E-Screen© tests, the probability of obtaining positive results would be increased from 70 to 95% and from 67 to 90%, respectively. In this population of dogs with AD, results of the E-Screen© point-of-care immunodot assay was found to often agree with those of allergen-specific intradermal or Allercept© tests for selected allergen groups.

INTRODUCTION

Canine atopic dermatitis (AD) is a common condition that has been recently redefined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features. It is associated most commonly with immunoglobulin (Ig)E antibodies to environmental allergens.¹ The treatment of canine AD involves a multifaceted approach that includes allergen avoidance, antimicrobial and anti-inflammatory pharmacotherapies as well as allergen-specific immunotherapy (ASIT).² Indications for ASIT have been proposed recently by the World Health Organization,³ and these have been adopted by the ACVD Task Force on Canine Atopic Dermatitis.² Both organizations recommended that ASIT be performed on patients with demonstrable evidence of IgE antibodies to clinically relevant allergens. To reveal such IgE hypersensitivity, allergen-specific intradermal testing (IDT) and/or IgE

serology (ASIS) are performed. Unfortunately, several factors, such as season of testing, age of patient or concurrent drug administration are known to negatively affect results of either intradermal or serological tests.^{4,5}

If an inexpensive screening test were designed to predict whether IDT or ASIS would yield positive or negative results, it would be beneficial to dog owners, as a negative screening test would delay the performance of costlier procedures.

The Allercept E-Screen© point-of-care immunodot assay has been developed recently by the Heska Corp. (Ft Collins, CO, USA).⁶ The 'testing' dot contains a proprietary mixture of flea, house dust mites and pollen allergens, whereas the 'control' dot contains canine IgE. The patient's serum is deposited onto the dots, and the presence of allergen-specific IgE is revealed using enzyme-tagged monoclonal antibodies directed against canine IgE.⁷ A colorimetric reaction is triggered by the addition of a chromogenic enzyme substrate. After verification of the presence of a purple-coloured 'control' dot, any similar colour change of the 'testing' dot is considered suggestive of elevated allergen-specific IgE serum levels. The test can be completed in less than 5 min. Unpublished studies performed by the Heska Corp. revealed that the Allercept E-Screen© had positive

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and negative predictive values of 91 and 80% for their full Allercept® panel, respectively.⁶

The objectives of this study were to determine whether results of the Allercept E-Screen® point-of-care immunodot assay agrees with those of either IDT or Allercept® full-panel ASIS in dogs with AD living in the south-eastern USA.

Thirty dogs were diagnosed with AD following current guidelines.⁸ These patients belonged to 20 breeds; there were 13 males and 17 females; the age of testing ranged from 0.6 to 9.0 years (median: 3.8 years), and the duration of their atopic signs prior to testing varied from 0.3 to 8.0 years. Fourteen dogs (47%) had perennial or persistent signs, three dogs (10%) exhibited seasonal or intermittent disease, and twelve dogs (40%) suffered from perennial signs with seasonal exacerbation. In all subjects, the decision to perform ASIT was agreed upon by both investigators and owners provided that IDT and ASIS would reveal allergen-specific IgE hypersensitivities matching the pattern of clinical signs.

IDT was performed with a panel of ≈60 allergens according to current guidelines.⁴ For the purpose of this study, the IDT was considered positive if any injection of allergens represented in the E-Screen® (fleas, house dust mites, pollens) yielded an erythematous wheal of diameter superior or equal to the average of diluent and histamine control injections.

Blood was drawn for performance of serological tests. One millilitre of serum was kept for in-house Allercept E-Screen® testing, and the remainder was submitted to the Heska Corp. for full-panel Allercept® ASIS. For the purpose of this study, and similarly to the interpretation of the IDT, the full-screen Allercept® was considered positive only when allergens represented in the E-Screen® (fleas, house dust mites, pollens) had corresponding enzyme-linked immunosorbent assay (ELISA) units superior or equal to 150 EA.

According to the manufacturer's recommendations, the E-Screen® was considered positive when any purple colour change was visible in both the 'testing' and 'control' dots within minutes of chromogen application.

In the following sections, predictive values, likelihood ratios, pre- and post-E-Screen® probabilities and odds were calculated according to Friedland *et al.* and Cockcroft & Holmes.^{9,10}

EVALUATION OF E-SCREEN® TO PREDICT IDT RESULTS

Both tests yielded positive results in 18/30 dogs (60%), both were negative in 8/30 dogs (27%), and they diverged in 4/30 dogs (13%) (Table 1). The rate of concordance between these tests was 26/30 or 87%.

In this group of 30 dogs with AD, the probability of obtaining a positive IDT was 70% (pre-E-Screen® probability of positive IDT). If testing were to be restricted to dogs with positive E-Screen®, the probability of obtaining a positive IDT would increase to 95%

Table 1. Agreement between E-Screen® and intradermal test results

	IDT +	IDT –	TOTAL
E-Screen® +	18 (a)	1 (b)	19
E-Screen® –	3 (c)	8 (d)	11
Total	21	9	30

IDT, intradermal testing. E-Screen® predictive value for positive IDT: $a/(a + b) = 95\%$. Pre-E-Screen® probability of a positive IDT: $(a + c)/(a + b + c + d) = 70\%$. Pre-E-Screen® odds of positive IDT: $\text{probability}/(1 - \text{probability}) = 2.33$. Likelihood ratio of a positive IDT (LR +): $\text{sensitivity}/(1 - \text{specificity}) = (a/a + c)/(1 - [d/b + d]) = 7.71$. Post-E-Screen® (+) odds of positive IDT: $\text{pre-test odds} \times \text{LR} + = 17.96$. Post-E-Screen® (+/–) probability of positive IDT: $\text{post-test odds}/(\text{post-test odds} \pm 1) = 95\%$. E-Screen® predictive value for negative IDT: $d/(c + d) = 73\%$. Pre-E-Screen® probability of a negative IDT: $(b + d)/(a + b + c + d) = 30\%$. Pre-E-Screen® odds of negative IDT: $\text{probability}/(1 - \text{probability}) = 0.43$. Likelihood ratio of a negative IDT (LR –): $(1 - \text{sensitivity}/\text{specificity}) = (1 - [a/a + c]/[d/b + d]) = 0.16$. Post-E-Screen® (–) odds of negative IDT: $\text{pre-test odds} \times \text{LR} - = 0.07$. Post-E-Screen® (–) Probability of negative IDT: $\text{post-test odds}/(\text{post-test odds} \pm 1) = 6\%$.

(post-E-Screen® probability of positive IDT, which is identical to the positive predictive value) (Table 1).

In contrast, in this group of dogs with AD, the probability of obtaining a negative IDT was 30% (pre-E-Screen® probability of negative IDT). In dogs with negative E-Screen®, the probability of obtaining a negative IDT would decrease to 6% (post-E-Screen® probability of negative IDT) (Table 1).

EVALUATION OF E-SCREEN® TO PREDICT SEROLOGICAL TEST RESULTS

Both tests yielded positive results in 17/30 dogs (57%), both were negative in 8/30 dogs (27%), and they diverged in 5/30 dogs (16%) (Table 2). The rate of concordance between these tests was 25/30 or 83%.

In this group of 30 dogs with AD, the probability of obtaining a positive ASIS was 67% (pre-E-Screen®

Table 2. Agreement between E-Screen® and serological test results

	ASIS +	ASIS –	TOTAL
E-Screen® +	17 (a)	2 (b)	19
E-Screen® –	3 (c)	8 (d)	11
Total	20	10	30

ASIS, allergen-specific IgE serology. E-Screen® predictive value for positive ASIS: $a/(a + b) = 89\%$. Pre-E-Screen® probability of a positive ASIS: $(a + c)/(a + b + c + d) = 67\%$. Pre-E-Screen® odds of positive ASIS: $\text{probability}/(1 - \text{probability}) = 2.03$. Likelihood ratio of a positive ASIS (LR +): $\text{sensitivity}/(1 - \text{specificity}) = (a/a + c)/(1 - [d/b + d]) = 4.25$. Post-E-Screen® (+) odds of positive ASIS: $\text{pre-test odds} \times \text{LR} + = 8.62$. Post-E-Screen® (+/–) probability of positive ASIS: $\text{post-test odds}/(\text{post-test odds} \pm 1) = 90\%$. E-Screen® predictive value for negative ASIS: $d/(c + d) = 73\%$. Pre-E-Screen® probability of a negative ASIS: $(b + d)/(a + b + c + d) = 33\%$. Pre-E-Screen® odds of negative ASIS: $\text{probability}/(1 - \text{probability}) = 0.49$. Likelihood ratio of a negative ASIS (LR –): $(1 - \text{sensitivity}/\text{specificity}) = (1 - [a/a + c]/[d/b + d]) = 0.19$. Post-E-Screen® (–) odds of negative ASIS: $\text{pre-test odds} \times \text{LR} - = 0.09$. Post-E-Screen® (–) probability of negative ASIS: $\text{post-test odds}/(\text{post-test odds} \pm 1) = 8\%$.

Table 3. Evolution of post-test probabilities following variation of pre-test probabilities

Positive IDT		Negative IDT		Positive ASIS		Negative ASIS	
Pre-test probability (%)	Post-test probability (%)	Pre-test probability (%)	Post-test probability (%)	Pre-test probability (%)	Post-test probability (%)	Pre-test probability (%)	Post-test probability (%)
10	46	10	2	10	32	10	2
20	66	20	4	20	52	20	4
30	77	30	6	30	65	30	7
40	84	40	10	40	74	40	11
50	89	50	14	50	81	50	16
60	92	60	19	60	86	60	22
70	95	70	27	70	91	70	30
80	97	80	39	80	94	80	43
90	99	90	59	90	97	80	63

Example of interpretation: if the probability of obtaining a positive IDT is only 10% in a given clinic, then performing IDT in dogs with positive E-Screen© tests would increase this probability to 46% (first row, first two columns). Similarly, if the probability of obtaining a negative IDT is only 10%, then performing IDT in dogs that do not have negative E-Screen© tests would reduce this probability to 2% (first row, third and fourth columns).

probability of positive ASIS). If such test was performed only in dogs with positive E-Screen©, the probability of obtaining a positive serology would increase to 90% (post-E-Screen© probability of positive ASIS) (Table 2).

Altogether, the probability of obtaining a negative ASIS was 33% (pre-E-Screen© probability of negative ASIS). If we eliminated from testing dogs with negative E-Screen©, the probability of obtaining a negative ASIS would be reduced to 8% (post-E-Screen© probability of negative ASIS) (Table 2).

There are several limitations that could impact the generalization of these findings. First, the sample size was relatively small, yet it was felt that the breed distribution, age of onset and duration of the disease were representative of those seen in our referral population.

Second, all dogs lived in North Carolina, a southeastern American state with distinctive pollen seasons. Approximately half of the subjects of this study exhibited perennial signs, whereas the other half presented with intermittent seasonal disease or with perennial manifestations and seasonal exacerbation of pruritus and skin lesions. Whether the findings of this study could be extrapolated to patients living in other geographical locations with different climates, or to dogs with different disease seasonality patterns is unknown. Moreover, if the prevalence (i.e. pre-test probabilities) of positive/negative IDT and ASIS was different at other settings compared with those seen at North Carolina State University, then probabilities of positive/negative IDT and ASIS tests after performing E-Screen© immunoassays (i.e. post-test probabilities) also would be different (Table 3).

Third, for the purpose of this study, IDT and ASIS were considered positive only in cases demonstrating hypersensitivity to flea, house dust mite or pollen allergens, which were represented in the E-Screen© testing dot. As this point-of-care test was not designed to detect mould, mammalian or storage mite hypersensitivity, it was felt that it should not and could not be evaluated for prediction of IDT and ASIS results for such allergens.

Fourth, only results of Heska's Allercept© full panel were considered for the serological arm of this study. As other commercially available ASIS tests employ various IgE antibody detection systems, it is likely that the value of the E-Screen© to predict results of these other serum tests could be markedly different than those shown herein.

Finally, one should keep in mind that, in ~15% of the patients enrolled in this study, the result of the E-Screen© test did not agree with those of IDT or Allercept© ASIS. For these patients, basing the decision of performance of IDT or ASIS tests following E-Screen© results would be inaccurate.

In conclusion, the results of this study suggest that the E-Screen© point-of-care immunodot assay is useful for predicting results of IDT or Allercept© ASIS. If IDT or full Allercept© ASIS were to be performed only in dogs with positive E-Screen© results, the probability of positive tests would be refined from 70 to 95% and from 67 to 90%, respectively, compared with the performance of such procedures without previous immunodot screening. Similarly, if we eliminated from testing dogs with negative E-Screen© results, the probability of obtaining negative IDT or Allercept© ASIS test results would be refined from 30 to 6% and from 33 to 8%, respectively.

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Résumé L'immunothérapie dans le traitement de la dermatite atopique (AD) est basée sur des tests intradermiques ou sérologiques qui permettent d'identifier les hypersensibilités liées aux IgE spécifiques d'allergènes. Malheureusement, les résultats de ces tests peuvent être faussement négatifs, à cause de facteurs variés, comme l'âge du patient, la saison de test et l'administration de molécules anti-allergiques. Un dépistage qui permettrait de prédire si ces tests coûteux seront utilisables serait intéressant pour les propriétaires de chiens à AD. Les objectifs de cette étude étaient de déterminer si un test spécifique d'allergènes (Allercept E-Screen[®], Heska Corporation, Ft Collins, Colorado) peut prédire les résultats soit des tests intradermiques soit d'un ensemble de tests sérologiques chez le chien atopique.

Resumen La inmunoterapia para prevenir las recidivas clínicas de la dermatitis atópica (DA) se basa en pruebas intradérmicas o serológicas que permiten identificar hipersensibilidades por IgEs alérgeno-específicas. Desafortunadamente, los resultados de tales pruebas pueden verse alterados por varios factores, que incluyen la edad de los pacientes, la estación de la prueba y la administración de fármacos antialérgicos. La investigación para predecir cuándo estas pruebas costosas pueden ser útiles beneficiaría a los dueños de perros con DA. El objetivo de este estudio fue determinar si un análisis alérgico-específico inmunodot del punto-de-cuidado (Allercept E-Screen[®], Heska Corporation, Ft Collins, Colorado) podría predecir resultados de las pruebas serológicas intradérmicas o del panel completo Allercept[®] en perros atópicos.

Zusammenfassung Immuntherapie zur Vermeidung klinischer Anzeichen atopischer Dermatitis (AD) basiert auf intradermalen oder serologischen Tests, die bei der Identifikation von allergen-spezifischen IgE-Überempfindlichkeitsreaktionen helfen. Unglücklicherweise können die Resultate solcher Tests durch verschiedene Faktoren, wie das Alter der Patienten, die Jahreszeit bei Durchführung des Tests und die Verabreichung anti-allergischer Medikamente negativ beeinflusst werden. Suchtests für die Voraussage, wann diese teuren Tests nützlich sind, wären für den Besitzer von Hunden mit AD nützlich. Ziel dieser Studie war es festzulegen, ob eine patientennahe allergen-spezifische Immunodot-Untersuchung (Allercept E-Screen[®], Heska Corporation, Ft Collins, Colorado) entweder die Ergebnisse des Intradermaltestes oder des vollständigen Allercept[®] serologischen Tests bei atopischen Hunden voraussagen kann.