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Atopic Dermatitis Allergy Testing and Management Introducing the Heska Allercept® IgE Test

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Contents

Introduction

Gribbles Veterinary Pathology in conjunction with Heska Corporation is delighted to make available to the veterinary practitioners of Australia the Allercept® IgE Test.

This document contains an overview of atopic dermatitis in the dog, allergy testing and management and comparative data on the Allercept® IgE Test. Gribbles Veterinary Pathology gratefully acknowledges the support of Specialist Veterinary Dermatologists in Australia in the development of the test for Australian conditions and for their assistance in providing information and presentations on atopic dermatitis.

Details on submitting samples for testing on the Allercept® IgE test are provided at the end of this document. For further information please contact Gribbles Veterinary Pathology on **1300 307 190**.

Atopic Dermatitis in the Dog: Allergy Testing and Management

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Definitions

Atopic dermatitis (AD): a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features including pruritus and secondary infections. It is associated most commonly with IgE antibodies to environmental allergens.

Allergen-specific immunotherapy (ASIT): the practice of administering gradually increasing quantities of an allergen extract to an allergic subject to ameliorate the symptoms associated with subsequent exposure to the causative allergen

'Allergy' testing: a convenient but inaccurate phrase referring to intradermal testing or allergen-specific IgE serology.

Intradermal testing (IDT): The process of introducing small quantities of allergen into the dermis of atopic patients to try and assess specific hypersensitivities. Positive reactions may be classed as immediate phase or late phase.

Allergen-Specific IgE Serology (ASIES): Measurement of serum immunoglobulin E concentrations that can bind to specific allergens.

Pathophysiology of atopic dermatitis

Overview

Atopy is a multifactorial disease in which genetically predisposed dogs may exhibit a combination of cutaneous IgE-mediated immediate and late-phase reactions to environmental allergens and exhibit a myriad of immunological changes leading to pruritus. Immunological abnormalities, antigenic stimuli, altered physiological and pharmacological reactions and genetic predisposition all play a role in the pathogenesis. Atopy can be considered in two phases; the acute phase and the chronic phase.

In the acute phase an epidermal barrier defect could facilitate contact of environmental allergens and microbes with epidermal immune cells at skin sites of trauma and friction, and areas with increased surface humidity (interdigital areas, external ear canals etc.). There are epidermal dendritic cells that partake in allergen processing and initiation and augmentation of the inflammatory response. Leukocytes, keratinocytes are all involved in the inflammatory reaction. There is an early influx of granulocytes and an allergen specific Th2 response, thus promoting IgE production.

In the chronic phase there is a significant role from microbes, self-trauma and neuromediators all of which contribute to the chronic inflammation. Chemokines are released in a continuous allergen-dependent and independent cycle, with subsequent influx and activation of leukocytes and further chemokines and mediator release. A Th1 profile may then predominate. The failure to down-regulate pro-inflammatory mechanisms leads to self-perpetuating cutaneous inflammation. Exaggeration of the barrier dysfunction in lesional skin may result in non-immunologically mediated pruritus due to environmental proteases and water loss (dry skin) as well as predispose to further allergen absorption and irritable skin. Furthermore inflammation results in upregulation of cellular and neurological receptors that result in increased sensitivity of neural pruritic pathways

Role of Genetics and Heritability

Heritability is a measure of the degree to which inheritance plays a part in the aetiology of the disease and if this approaches 1, then the trait is considered to be entirely genetically determined. A study in 429 Labradors and Golden retrievers revealed a heritability of 0.47. This means there is a significant contribution from the environment and from genetic factors. In humans, the calculations vary from 0.425 to 0.495. A precise mode of inheritance has not been found, but it is considered a polygenic disorder.

The Hygiene Hypothesis

The hygiene hypothesis of atopic disease in humans suggests that environmental changes in the industrialised world have lead to a decrease in microbial contact at an early age and thus resulted in the growing epidemic of atopic eczema, allergic rhinoconjunctivitis, and asthma over and above what genetics alone can explain. More recently a modified version of this original hypothesis has been developed. This suggests that it is not just a decreased exposure to all infections that increases risk of atopic disease, but more specifically is related to establishment of intestinal microbiota and subsequent development of oral tolerance in the infant. There is evidence to suggest antibiotics early in life can risk allergic development.

Role of Leukocytes

There is a complex interplay between a variety of cell types such as mast cells, T cells, dendritic cells, eosinophils, neutrophils, B cells, and macrophages through release of chemokines or inflammatory mediators.

Dendritic cells
Langerhans cells are the principle antigen presenting dendritic cell of the epidermis. There are higher numbers in lesional and non-lesional AD skin. Langerhans cells express FcεRI (receptor for IgE) and process allergen and present this to T cells. Subsequently, epidermal (including inflammatory dendritic epidermal cells or IDECs in humans), as well as dermal dendritic cells may also have a significant role in skewing the T-cell response to the absorbed antigen and may therefore be critical to the initiation of the atopic state.

T lymphocytes
T lymphocytes play a major role in lesional skin of dogs with AD. There are two subsets recognised in the pathogenesis of atopic dermatitis; Th1 and Th2. In early lesions a Th2 profile tends to predominate and the cytokines produced promote IgE production. It is considered that a Th1 profile predominates with chronicity. ASIT has been shown to trigger a switch to a Th1 profile and induce regulatory T cells that can downregulate the severity of the allergic reaction.

B lymphocytes
B cells are important in that they synthesise antigen specific antibodies esp. IgE and IgG. They are not a major component of the atopic inflammatory infiltrate though so immunoglobulin synthesis probably occurs in extracutaneous sites such as lymph nodes, spleen or bone marrow.

Mast Cells
Mast cells (MC) exert their effect by synthesising and releasing inflammatory mediators, e.g. histamine, proteases and cytokines. In AD this is triggered by binding and crosslinking of allergen-specific IgE to MC bound IgE-specific receptors (FcεRI). Upon degranulation, the granule contents cause the signs of allergic inflammation by interaction with microvasculature and other inflammatory cells. Mast cells in the atopic individual have a higher releasability by IgE-mediated degranulation than normal dogs

Mast cells are found in highest numbers where the body interfaces with the environment such as around blood vessels of pinnae, interdigital areas and lowest density is on nasal planum. Mast cell numbers are also higher in atopic lesional skin, but otherwise there is no difference between non-lesional atopic skin and normal skin.

Eosinophils
Eosinophils play a role in chemotactic stimuli in the inflammatory reaction. They have receptors on their cell surface for IgG, IgE and other inflammatory mediators. Eosinophil cationic protein is a major pruritic mediator in human AD.

Keratinocytes
They can release cytokines and chemokines (chemotactic cytokines) that recruit leukocytes to the site of inflammation. It is proposed in humans this may occur following aeroallergen or detergent exposure. Activated keratinocytes can release mediators, including proteases that directly induce pruritus.

Role of Antibodies

IgE is not considered to be the mainstay of development or perpetuation of atopic dermatitis. In humans they describe two groups of atopics, those who have high levels of allergen specific IgE (extrinsic atopics) and those that do not (intrinsic atopics). It is also well accepted in dogs that not all atopic dogs will display allergen-specific IgE either. Furthermore, allergen-specific IgE can occur in the normal dog, IgE concentrations do not necessarily drop with successful allergen-specific immunotherapy and AD has been recognised in human atopics with agammaglobulinaemia!

Allergen specific IgG has been identified in dogs with AD, but concentrations are generally higher in normal individuals than atopics, so IgG may be playing a protective role. However, there are also IgG receptor bindingsites on mast cells, just as there are for IgE, and binding of immunoglobulins to either can induce degranulation of the mast cell.

Role of Barrier Dysfunction

Loss of the epidermal barrier facilitates penetration of bacteria, allergens and chemical irritants that further enhance the inflammatory process. This may explain the high clusters of Langerhans cells at the site of allergen penetration. Barrier defects have been well described in humans and there is evidence to suggest similar intercellular lipid defects in the stratum corneum of atopic dogs and probable barrier defects.

The skin consists of epidermis and dermis and provides the protective barrier to the environment. The outermost layer of the epidermis is the stratum corneum (cornified layer) and disruption to this layer reduces the barrier to pathogens and allows moisture loss. Disruption to the outer layer may result from environmental trauma or from certain skin disorders. Repeated epidermal insults from scratching lead to an increase in epidermal turnover and consequently to epidermal hyperplasia. This in turn leads to inflammation where there is an increased migration of active Langerhans cells and other inflammatory cells into the epidermis.

The epidermis is an extremely active site of lipid synthesis. Lipids are important in barrier function, stratum corneum water holding, cohesion and desquamation of corneocytes and control of epidermal proliferation and differentiation. The three most abundant lipid species in the stratum corneum are cholesterol, ceramides and free fatty acids. Ceramides are the most important lipid component for the lamellar arrangement of the stratum corneum and consequently as a barrier function as they allow stretching and bending. Polyunsaturated fatty acids (such as the omega-6 linoleic acid contained in sunflower oil) are incorporated into the ceramides.

There are differences in the lipid content of skin depending on the anatomic region. Water-soluble antigens (e.g. plant pollens) are commonly in contact with the lipid-depleted sites. The skin in atopic dermatitis is known to have a paucity of stratum corneum lipids and is therefore more readily sensitised to hydrophilic antigens. Immunological abnormalities in the atopic individual also contribute to differences in sensitisation thresholds.

Sebum provides an oily layer over the stratum corneum that helps a little retain moisture. It also helps form a chemical and physical barrier against potential pathogens. Resident bacteria of the skin and hair follicles produce lipase which breaks down sebum resulting in fatty acid products that have antibacterial properties.

In atopic dermatitis in dogs and humans there have been abnormalities associated with the epidermal barrier. This leads to three basic problems:

- microfissures form, which then provide a portal of entry to pathogens and allergens
- increase in scale in dry skin presents a greater surface area for bacterial and yeast adhesion, which leads to secondary infection
- increases in allergic and non-allergic itch (see management for details)

Role of Infections in Atopic Dermatitis

During inflammation, antimicrobial peptides such as defensins and cathelicidins are released by inflammatory cells and keratinoctytes. In atopic humans there is a lower level of these antimicrobial peptides. This, together with the fact that there is a greater adherence of staphylococcal bacteria to atopic keratinocytes, localised defects in barrier function, altered microenvironmental humidity, a TH-2 skewed immune response suppressing an effective anti-microbial immune response makes atopic individuals more prone to skin infections. Bacterial exotoxins can act as superantigens and augment the inflammatory response and worsen pruritus. In dogs, hypersensitivity to *Malassezia* spp. has also been recognised in recent years. All this leads to more severe pruritus and skin trauma.

Role of Inflammatory Mediators

There are many chemical inflammatory mediators that play a role in the pathogenesis of atopic dermatitis in triggering inflammation and pruritus and modify the perception of pruritus. It is important to realise that the most talked about one of these, histamine, is only one of many. There are also several neuropeptides, phosphodiesterases, eicosanoids, proteases, opioids, kinins, neutrotrophins, endovanniloids and endocannabinoids that all play a role.

Role of Toll-like Receptors

Toll-like receptors (pattern recognition receptors in the immune response to infections) may also play a role in the pathogenesis of atopic dermatitis. Whether or not there is TLR polymorphism or whether TLR activation and augmentation of an autoimmune response plays a role, is not yet fully elucidated.

Diagnosis of Atopic Dermatitis

A diagnosis of atopic dermatitis is made based on a history and clinical criteria consistent with AD and ruling out other differential diagnoses. The skills then required to make this diagnosis are:

a) a thorough understanding of all differential diagnoses and their basic pathogeneses,
b) sound history taking and clinical examination skills
c) sound cytological skills, and importantly
d) a logical diagnostic approach.

History and Clinical Signs

Atopic dermatitis presents with variable clinical signs in both dogs and humans. While the diagnosis may be far more likely in the West Highland White terrier that chews its paws for the same three months per year every year, many cases are not so straightforward, and there is not one single historical or clinical feature that, if present, indicates the presence of AD. Several schemes have been proposed to better define clinical criteria (see Table 1 next page) and while helpful as a guide none are perfect.

Table 1: Clinical Criteria Proposed for Diagnosis of Atopic Dermatitis in Dogs

Willemse (1986, 1988)	Prelaud (1998)
Major Features (must have at least 3) • Pruritus • Facial and/or digital involvement • Lichenification of the flexor surface of the tarsal joint and/or extensor surface of the carpal joint • Chronic or chronic-relapsing dermatitis • Individual or family history of AD and/or breed predilection *Minor Features (must have at least 3)* • First appearance of signs < 3 years • Facial erythema and cheilitis (inflammation of the lips) • Bacterial conjunctivitis • Superficial staphylococcal pyoderma • Hyperhidrosis • IDT positive • ASIES positive • Elevated serum allergen-specific IgGd	*Major Criteria (must have at least 3)* • Corticosteroid-sensitive pruritus • Pinnal erythema • Bilateral cranial erythematous pododermatitis • Cheilitis • First appearance of signs 6 months to 3 years

Differential Diagnoses and Ruling Them Out

The challenge in the pruritic dog where AD is a consideration is that there are potentially numerous differential diagnoses which may or may not be concurrent with a diagnosis of AD. Add to this confounding factors such as environmental exacerbation and secondary infections (Table 2) and the challenge becomes considerable in some cases. A complete physical examination and history taking are mandatory for this reason in all cases, and some differential diagnoses may be able to be ruled out by these alone.

Table 2: Factors that can cause or contribute to pruritus (not exhaustive)

Primary Disease	Modulating Factors	Amplifying Factors
• Atopic dermatitis	• Dry skin	• Bacterial colonisation, folliculitis, furunculosis • *Malassezia* dermatitis • Psychogenic / temperament factors
• Flea bite hypersensitivity • Insect bite allergy (e.g. Mosquito)	• Heat • Boredom	
• Food adverse reaction • Contact allergy / irritation • Scabies • *Cheyletiella*	• Anxiety • Stress • Humidity • Maceration	
• Demodicosis (rarely pruritic on it's own, but common cause for pruritic secondary infection, can be concurrent in some AD-susceptible breeds or following GC therapy) • Neoplasia (e.g. some cases of mast cell tumour, epitheliotrophic t-cell lymphoma) • Autoimmune disease (e.g. some cases of pemphigus foliaceous)		

Once a list of differential diagnoses fitting with the clinical presentation and history is formulated, specific tests (Table 3) can be performed to help rule them in or out. If a disease is confirmed then this should be treated appropriately and the patient then reassessed to see if AD is still a consideration.

Table 3: Tests used to help make a diagnosis of atopic dermatitis.

Test	Rationale
Superficial & Deep Skin Scrapings	Diagnosis of parasitic skin diseases
Tape Cytolog	
Otic Cytology	Diagnosis of bacterial or yeast skin infections
Pustule / Papule Cytology	Diagnosis of bacterial or yeast ear infections or inflammation
Trichogram	Diagnosis of bacterial, demodex infections; may be supportive of contact dermatitis, pemphigus foliaceous Diagnosis of demodicosis, dermatophytosis; can help differentiate traumatic from non-traumatic hair loss in cases where pruritus is not directly observed
Flea Control Trial (>2 weeks)	Diagnosis of flea bite hypersensitivity
Scabies Treatment trial (>2-4 weeks)	Diagnosis of scabies
Elimination Diet Trial (>6 weeks, novel protein)	Diagnosis of adverse food reaction
Contact Avoidance trials (>10-14 days)	Supportive of contact dermatitis
Skin Biopsy	Rarely useful in most cases of pruritic skin disease because histopathological changes are often non-specific; however it is important for confirmation of the diagnosis of neoplastic or autoimmune diseases

It is then only at this stage, once differential diagnoses have been identified and managed and AD clinically confirmed that consideration should be given to allergy testing.

Case Selection for Allergy Testing

If AD is a diagnosis of exclusion where a compatible history and clinical presentation exist, it means that most patients should be able to be clinically confirmed as suffering atopic dermatitis without ever having had a test for airborne allergens performed. So what is the role for allergy testing if it is not appropriate or necessary for making a diagnosis of AD?

The only reasons that allergy testing needs to be performed in atopic animals are:
- to identify airborne allergens that may be avoidable
- to identify airborne allergens for inclusion in allergen-specific immunotherapy (aka ASIT, allergy vaccine)
- where an owner 'just wants to know' for their peace of mind

This means that if symptomatic therapy for pruritus and / or infections is to be the only therapy employed in a clinically confirmed AD patient where the owner has no interest in the fine details of triggering allergens, then any form of allergy testing in that patient is redundant. Furthermore, even if allergens may be able to be identified on allergy testing, if a patient or client is not an appropriate case for avoidance or ASIT, then again any form of allergy testing in that patient is redundant because these cases will need to be managed symptomatically only. However, if a client and patient are good candidates for possible avoidance strategies and / or immunotherapy, then allergy testing is strongly recommended, as this can lead to treatment options that can minimise or eliminate drug usage (and concurrent adverse drug reactions) while still maintaining patient comfort.

Case Selection for Avoidance Strategies

Avoidance of airborne allergens is difficult and not practical in many cases. However there are some specific cases where avoidance of the allergens in atopic dermatitis may be possible as a sole therapy, and in these cases allergy testing is required to confirm the allergen that needs to be avoided. These cases are described in more detail below in the management section.

Case Selection for Allergen-Specific Immunotherapy

ASIT is a useful tool in the management of many cases of AD (Table 4). However, not all cases are suited to this mode of therapy.

There are two critical stages during the work-up of an atopic dog where the appropriateness of ASIT needs to be considered.

1. *Prior to allergy testing.* ASIT (and therefore allergy testing) is indicated for a patient when a clinical diagnosis of atopy is made, where suspected allergens are unavoidable, and where symptomatic therapy is ineffective or associated with unacceptable side effects (e.g. glucocorticoids). Importantly, there are client factors as well that must be satisfied: the ability to either give injections or get them done, the ability for regular rechecks, the ability to understand the process, the ability to support the up front costs of testing and vaccine, and the ability to communicate effectively

Table 4: Advantages and disadvantages of ASIT

Advantages	Disadvantages
• Less frequent administration than symptomatic therapy • No long term side effects reported • Acceptance of injections compared to oral medication • Often more cost effective especially medium to large breeds • Preventative, not reactive treatment	• Owner fear of giving injections • Possible poor patient tolerance of injections • Risk of anaphylaxis • Initial up front cost • **Client education, patient follow up and support essential to the success of ASIT**

2. *After allergy testing.* ASIT is strongly indicated where the allergens identified in testing match with the presence of the allergens in the environment and the seasonality of the dog, and where avoidance is impossible. If these criteria (esp. the first two) cannot be fulfilled, the appropriateness of ASIT needs to be reconsidered because the response is more likely to be suboptimal.

Options for Allergy Testing

The major options currently commercially available for diagnosis of relevant allergens in AD are the intradermal test, or allergen-specific IgE serology. Where allergy testing is elected for, it is important for the clinician to understand the principles, benefits and limitations of the tests that are available to be able the make the right recommendations for the patient and client. It is also important to realise that no allergy test is completely sensitive and specific and clinically normal animals can have positive reactions and clinically diagnosed atopics can have negative reactions on testing.

Intradermal Testing

How it works. A panel of 40-70 suspect allergens relevant for the geographic area of the patient are injected into the dermis under sedation. Where allergen-specific IgE is present on tissue mast cells in the epidermis, the allergen leads to cross-linkage of Fc RI molecules, which induces release of preformed granules (containin histamine and other enzymes) and immediate production of lipid mediators (inc. prostaglandin D_2, leukotriene C_4, D_4, E_4, platelet activating factor) and some pro-inflammatory cytokines. The subsequent wheal and flare reaction though is almost totally histamine mediated and H1 receptor agonists (i.e. antihistamines) can block the reaction almost completely.

Histamine binds to the venular endothelial cells leading to endothelial cell synthesis and release of mediators that cause vascular smooth muscle relaxation and a red injection site from local accumulation of RBCs. Endothelial cells subsequently retract leading to plasma extravasation and swelling of the injection site (the “wheal”). Finally the blood vessels at the margins of the wheal dilate (augmented by the nervous system) and become engorged causing the characteristic red rim (the “flare”). This immediate phase reaction occurs within 5- 30 minutes of injection. A ‘positive’ reaction is one that shows erythema, induration and a diameter greater than halfway between the negative (saline) and positive (histamine) controls. Late phase reactions may also be seen 4-24 hours later, and these result from the inflammatory cell infiltrate caused by the chemotactic factors from mast cells.

Pros and Cons. To date this is still considered the ‘gold standard’ test for identification of relevant allergens in dogs with AD as it assesses the afferent (i.e. IgE) as well as the efferent (i.e. mast cells, mediators and response of the blood vessels) pathways for a type I hypersensitivity.

The test though is not without limitations. Performing and reading such test requires a high level of skill and storage and handling of allergens must adhere to strict guidelines to maintain potency. Tests must be performed at least once weekly to fortnightly to be economically viable, and also to maintain skill in interpreting in administering and reading the test. Drug withdrawal times must be observed in most cases and chronic skin changes can make interpretation impossible. Patients must be sedated and an area clipped for the test. False positive reactions can occasionally occur but recent studies on the correct ‘threshold’ concentrations of allergens to use have reduced this problem. Clinically normal animals can show rare positive reactions also and it has been considered that these may suggest sub-clinical hypersensitivity, or be a harbinger of future allergies.

There is a rare risk of anaphylaxis with IDT, and the patient will need to be able cope with light sedation for the test.

Table 5: Limitations of Intradermal Testing

False Positive Reactions	False Negative Reactions
• Improper technique (poor test site selection / preparation, injections too close, volume too big, traumatic injections) • Irritant test allergens (excessive concentration, contamination, harbinger of future allergy?) • Irritable skin (infections, nonspecific mast cell degranulation) • Urticaria • Dermatographism	• Improper technique (insufficient volume, air or SC injection, late reading time) • Insufficient allergen (poorly manufactured, non-standardised or old extract, solution too dilute) • **Drug interference (glucocorticoids & progestagens, high dose CyA, ACP, opiates,** antihistamines, immunosuppressive drugs, adrenergic compounds Bronchodilators Theophylline, long term EFAs, **immunotherapy**) • **Host Factors** (pigmentation, stress, reproductive status, age, chronic skin changes, mast cell exhaustion, hypothyroidism, hyperadrenocorticism, **low IgE producer**, lack of tissue IgE) • **Incorrect antigen selection or relevant antigens not available for testing** • **Test performed at wrong time (i.e. seasonal variation)** • Monovalent epitopes

Allergen-Specific IgE Serology

How it works. Traditional ELISA testing for allergen-specific IgE begins with binding of a fixed amount of allergen to a plastic microtitre well (though some techniques start with the allergen in a liquid phase). The serum containing the IgE is then applied to the plate, and antibodies in the serum bind to the allergen. A concentrated solution of non-interacting protein (a 'blocker') such as casein is used to block non-specific adsorption of other proteins to the plate. The excess serum is then rinsed away and the IgE detection reagent is added. This detection reagent may be a polyclonal or monoclonal anti-IgE antibody, or the recombinant high-affinity IgE receptor (FcεRI). The detection reagent binds to the IgE that has already bound to allergen on the plate. The specificity of binding to IgE is critical because antibodies other than IgE will also have bound to the allergen in most cases. The plate is washed again to remove any unbound detection reagent. After this wash, only the detection reagent-antigen complexes remain attached to the well. Additional steps allow the quantification of bound detection reagent by use of a chromogenic or fluorogenic signal. The amount of colour should correlate with the amount of IgE bound in the well. The results are then compared with ranges based on historical positive and negative controls.

Pros and Cons. This test assays the presence of allergen-specific IgE in the serum, but this is not always indicative of clinical allergy. IgE serology tests historically have frequently resulted in large numbers of positive results because of cross reactions with IgG and the fact that this can occur in clinically normal (non-atopic) dogs supports these as false positive reactions. Advances in technology have meant that initially monoclonal antibody and now Fcε receptor based tests have become available both utilizing variously solid phase and liquid phases, both of which have increased the positive predictive value compared with the older polyclonal tests.

ASIES are subject to some of the limitations of IDT, and in addition also laboratory error. They are however less subject to many host factors and not at all subject to issues associated with preparation, administration or interpretation of IDTs. A problem specific to all ASIES tests is that of large numbers of false positive reactions to irrelevant botanical carbohydrate epitopes in pollen allergic dogs. This has been reported to occur in a small number (~5-10%) of cases. Furthermore, the majority of ASIES test for fewer allergens than IDT, meaning a greater chance of missing possible allergens for inclusion in ASIT, which may affect the outcome of therapy.

Clinicians must therefore have a solid understanding of the various serology tests, their specificity, sensitivity, methodologies and understand their short-comings and benefits if they are to be able to correctly use these tests.

Table 6: Limitations of allergen-specific IgE serology

False Positive Reactions	False Negative Reactions
• IgG binding to allergens • Laboratory error • Laboratory cut-offs • IgE to non-biologically active botanical carbohydrate epitopes	• Reacting phase of allergen (liquid vs solid) • Non-standardised allergen • Loss of IgE in transit (4h at 56°C) • Laboratory error • Laboratory cut-offs • **Drug interference (glucocorticoids & progestagens, high dose CyA, immunotherapy)** • **Host Factors (age, low IgE producer,** • low serum allergen specific IgE) • **Incorrect antigen selection or relevant antigens not available for testing** • **Test performed at wrong time (i.e. seasonal variation)**

Controversies in Allergy Testing

Why do some dogs with clinical AD have negative allergy tests?.

Up to 16-20% of dogs clinically diagnosed with AD can have negative IDTs, and in one study about half of these also had negative ASIES[13]. Most had no definable problems with the testing. The cause for these cases where there is no identification of causative allergens and no obvious reason for failure of the test has not been well defined but there are three major possibilities.

- The dog is allergic to allergens not in the tests
- The dog has low levels of IgE
- The dog has 'atopy-like disease' (ALD). In humans a proportion of cases of atopic dermatitis are described as 'intrinsic' and having no allergen-specific IgE. While clinically indistinct from 'extrinsic' IgE mediated cases, there have been subtle differences in the pathogenesis reported. Analogous cases may exist in dogs.

What's wrong with allergen mixes in testing?

Allergens used in most testing are crude antigens that likely contain numerous epitopes. Some of these (but probably not all) are likely shared with other plants within the same genus but the problem is that this has not been proven in dogs. So while allergen mixes of similar species of plant may appear harmless, it can lead to dilution of individual epitopes leading to false negative reactions. Furthermore, inclusion of irrelevant allergens in ASIT may lead to subsequent sensitisation. Allergen mixes are thus best avoided.

Why is there a lack of correlation between IDT and ASIES?

It is not surprising that there is not complete correlation between IDT and ASIES for at least two reasons. Firstly, the tests are not measuring the same thing (tissue-bound IgE vs. serum IgE), and secondly the tests are not standardised compared with each other (i.e. the cut-offs for positive and negative reactions are not comparable)

Is IDT or ASIES better at identifying allergens for ASIT in seasonal allergic dogs?

This question was addressed recently in a study by Rosser[14]. While IDT has been previously considered the 'gold standard' for allergen identification, in reality the only relevant standard is the history and clinical signs of the patient. If any allergy test results do not match the seasonality of the patient then the test results are wrong not the patient!

This study was conducted in Michigan, where there are distinct pollination periods for trees, grasses and weeds. Twenty-nine seasonally atopic dogs underwent IDT and ASIES (FcεRI-based) to try and determine relevant antigens for ASIT and results were compared with the seasonal histories of the patients. Combining IDT and ASIES results correlated well in 27 of 29 dogs (93%) for all groups of allergens. These findings strongly supported the simultaneous use of both IDT and ASIES for the selection of aeroallergens for ASIT in dogs with atopic dermatitis.

Does outcome of immunotherapy depend on the test used?

Numerous studies have been performed in the last 20-30 years to try and answer this question with the findings suggesting at least a 50% improvement in response to ASIT in 50-100% of dogs irrespective of the allergy test used to select allergen for the vaccine. However, the bulk of studies were open and uncontrolled, and methodologies were not comparable. Interestingly two reports (one anecdotal and one unpublished) suggested similar to Rosser[14] that better results may be had using a combination of IDT with ASIES. Further studies to confirm these and Rosser's findings are needed.

What are the effects of glucocorticoids and cyclosporin on allergy testing?

Glucocorticoids (GCs) have numerous potential effects on allergy testing. In vitro, rat and mouse studies show a decrease in allergen-specific IgE with GC use, though a paradoxical increase in total IgE may be seen. Furthermore GCs also decrease mast cell cytokine and histamine production, mast cell IgE receptors, and blood vessel permeability and neurogenic oedema. GCs do not interfere though with mast cell degranulation.

Recommendations: These changes will interfere with both ASIES and IDT and withdrawal periods should be observed for best results (see below). Note though that response to GCs is individualistic and the amount of IgE suppression can vary considerably between individuals.

The effect of cyclosporin A (CyA) on allergy testing is less straightforward. Laboratory and human clinical studies have shown that large doses (~25mg/kg/day) suppress IgE production but lower doses (5-10mg/kg/day) have either little impact or possibly increase IgE production. This is supported by a case report in humans where good clinical response was noted but allergen-specific IgE remained essentially unchanged. In vitro CyA can also decrease mast cell degranulation but this is not supported in clinical studies in humans and dogs where positive prick or IDT have been noted following 6-16 weeks of therapy at 5mg/kg/day with little change from initial testing.

Recommendations: CyA therefore can likely be used at standard doses with minimal interference on IDT for up to 16 weeks. CyA may not interfere with ASIES but this is yet to be substantiated in the dog.

Clinical Decision Making: IDT vs. ASIES

Having made the decision to perform allergy testing, the clinician is faced with the options of what test to choose.

When to perform allergen specific IgE serology with a view to in-house ASIT or avoidance.

- When IDT is not accessible
- When sedation or clipping is not possible
- Where the AD is uncomplicated and the only symptom is non-lesional pruritus

NOTE:

- Testing should not be performed in dogs <5-6 months of age
- **Drug withdrawal must be observed**
  - 3-8 weeks off prednisolone
  - 3-4 months off methylprednisolone acetate, progestagens
  - 2 weeks off topical glucocorticoids, antihistamines, and clomipramine (latter up to 4 weeks)
- Test < 8-30 days after end of the patient's pollen season (and preferably the middle to end of pollen season)
- Testing should not be performed unless the managing veterinarian is experienced with immunotherapy / atopic management
- Apoquel® and Cytopoint® are fine to continue using as they will not affect the test

When to refer for further workup or IDT.

- When the managing veterinarian is not experienced with immunotherapy / atopic management
- When referral is accessible
- When AD is not able to be confirmed clinically
- When the ASIES test result does not correlate with clinical history of the dog
- Where there are significant skin lesions, as these dogs will have lots of non IgE mechanisms involved and are less likely to respond to "recipe book" straightforward immunotherapy
- When patient is 'not quite right' – either looks wrong or is not responding to medications as it should if straightforward AD was present

Management of the atopic patient.

Atopic dermatitis (AD) is a common diagnosis in the dog, but our ideas of what an atopic dog is have changed over the years. Twenty years ago we thought atopic dermatitis was an IgE mediated disease due to ***inhaled*** allergens. Ten years ago we thought atopic dermatitis was an IgE mediated disease due to ***percutaneously*** absorbed allergens. Now we realise it is a far more complex disease and that more than one pathophysiological pathway can create the "atopic" clinical picture. Barrier dysfunction, irritable skin, microbial proteases, IgE and non-IgE mechanisms with genetic and environmental modification all combine to create the atopic dog. It appears that we have to re-define our definition of canine AD: it is not "just an allergy".

The major clinical symptom in atopic dermatitis is pruritus. This can impact not only on the quality of the dog's life but also on the enjoyment of owning a dog. If we are to successfully manage dogs with atopy we need to address ALL the mechanisms of itch including itch due to:

- barrier dysfunction (itch from dry skin, irritants, environmental proteases),
- infections (IgE mechanisms, compliment cascade, microbial proteases), and
- allergy (IgE and non IgE mechanisms and allergen dependent and independent upregulation)

Pruritus then becomes a complex interplay of numerous neurochemical mediators. We must also be cognisant that the contribution of barrier dysfunction, infections and allergy will be variable among our patients and that barrier dysfunction may be acquired due to inflammation. Identifying what mechanism(s) are involved in each individual patient so as to formulate a treatment rationale addressing each aspect of these is the art of dermatology.

Even though the signal to make you feel itchy usually starts in the skin the signal must be carried by afferent nerve fibres from the skin to the spine, then up the spine to the brain. Ultimately it is the brain that itches not the skin itself. This means that not only is the generation of the pruritic signal complex but also spinal and central processing will affect the quality of the itch and the patient's temperament will affect the response to that itch.

The pruritic response (lick, scratch, rub, roll, chew etc) will vary with the intensity of the itch and the temperament of the patient and be modified by other competing environmental signals. Pain fibres and itch fibres compete for the same pathways in the brain and so the more painful the pruritic behaviours the longer the itch relief will be.

If pruritus is the symptom that we are trying to control then we need to try and establish an objective baseline measure of itch so we can assess efficacy of our treatments. This is remarkably hard to achieve as our clients tend to be very subjective. As a general rule, a comfortable level of itch is a dog who exhibits pruritic behaviour of low intensity, terminates that behaviour spontaneously after 15 to 60 seconds, gets relief and therefore does not need to repeat that behaviour, at the same site, for several hours. This level of itch does not require intervention. Where the itch signal is strong enough for a dog to interrupt their walk, play, eating or sleeping behaviour to scratch OR where the pruritic episode is intense and includes vocalisation or needs to be physically interrupted to terminate, OR is prolonged > 60 seconds OR frequently repeated, then intervention is required.

Unfortunately there is no one treatment that addresses all the mechanisms of itch. So if we are to achieve our goals of having a comfortable patient while minimising steroid reliance and hopefully avoiding the need for life-long, non-specific immunosuppressive therapies, then treatment is always going to be a multifaceted attack and also needs to consider the patient's temperament.

1. Improving the epidermal barrier

Epidermal barrier dysfunction in lesional and non-lesional skin is well recognised in people with atopic eczema. Changes including reduced lipid (ceramide) levels and elevated chymotryptic enzyme activity (leading to premature breakdown of corneodesmosomes that hold the stratum corneum together to create the barrier) result in reduced barrier function. This is further reduced by soaps/shampoos (increasing skin pH increases chymotryptic enzyme activity) and microbial and environmental proteases (inc. dust mite proteins). In dogs, abnormalities in essential fatty acids contributing to the skin barrier and in the intercellular lipid lamellae have been identified.

The outcome of poor epidermal barrier function is ALLERGIC itch associated with increased percutaneous allergen absorption, and NON-ALLERGIC itch associated with liberation of inflammatory cytokines from the epidermis (irritable skin) PLUS itch associated with dry skin PLUS itch associated with environmental proteases PLUS itch due to increased microbial proteases. Improving the epidermal barrier can lessen the severity of the atopic disease.

Moisturising the skin.

A. Hydrotherapy

Moisture can be returned to the skin by bathing in water. A long rinse cycle when shampooing helps hydrate the stratum corneum. This will rapidly dehydrate unless moisturises are applied post rinsing to hinder evaporation. The mechanical action of washing can be useful for removal of allergens/irritants from the skin, reduction of microbial adhesion sites and reducing the itch due to cooling of the skin.

B Topical Moisturising Agents

Formulations may be shampoos, rinses, lotions, sprays, creams, emulsions and ointments. Each formulation has advantages and disadvantages and selection depends on the type of condition being treated, the surface area involved and the presence of hair.

Shampoos. Shampoos are generally drying because of their detergent action but some formulations have sustained-release microvesicles that contain active ingredients. These vesicles have an outer lipid membrane that will bind to hair and skin and breakdown to release their contents having a moisturising effect. Ingredients in shampoos that may help moisturise are fatty acids, lipids, urea, glycerin, colloidal oatmeal and chitosanide e.g. Epi-soothe Spherulites® (Virbac) which incorporate oatmeal and chitosanide; Allergroom S® (Virbac) with spherulites that contain glycerine, lactic acid, urea and chitosanide. Shampoos should not be relied upon as the sole moisturising treatment except in very mild cases. It should also be remembered that even "non-irritant" shampoos may irritate skin with a dysfunctional epidermal barrier. Not all dogs are improved by shampooing!

Lotions, creams, and ointments. Lotions are liquids with the active ingredients dissolved or suspended. If mixed in an alcohol base, they are drying (e.g. Dalacin T). Moisturising lotions often contain propylene glycol and water but may also deliver therapeutic agents. An example is Resisoothe Lotion® (Virbac) which incorporates oatmeal. Lotions have a longer action than shampoos but shorter duration then creams and ointments. For lichenified skin they are probably inadequate. Alpha keri lotion (fractionated lanolin and mineral oil) is very useful for application after shampooing. It is easily applied to large areas.

Dry, lichenified skin is best treated with Hydraderm (sorbolene cream and hygroscopic agent glycolic acid). As the water evaporates, it leaves an oily film on the skin and glycolic acid will draw water from the dermis and trap it in the stratum corneum.

Ointments and oily creams are more occlusive, which facilitates hydration of stratum corneum and increases penetration of the incorporated active ingredient. Their disadvantage is that they occlude the pilosebaceous orifice and may produce a folliculitis and are too greasy to apply effectively on hairy skin.

Fatty Acids. Fatty acid (FA) additives in topical preparations can decrease transepidermal water loss. The skin also incorporates some circulating lipids such as plant sterols, essential fatty acids, polyunsaturated fatty acids and arachidonic acid. An example is Megaderm® (Virbac) which contains omega 3 and 6. Dietary supplementation of omega 6 fatty acids may assist barrier repair although appropriate studies to validate this are currently lacking. There is evidence though that the addition of both omega 3 and omega 6 fatty acids to the diet may reduce steroid reliance in atopic dogs. Omega 3 fatty acids become incorporated in the T-cell TCRs and may modulate T-cells directly. The action of fatty acids may be more than the presumed down regulation of pro- inflammatory eicosanoids. Interestingly, inhibition of PGD2 by fatty acids may have a negative effect on barrier repair. Maximum benefit from fatty acid supplementation in most studies has taken 8 to 10 weeks. The best reported 'effective' doses of omega 3 as a sole therapy range from 66-85mg/kg combined docosahexanoic and eicosapentanoic acids or up to 126 mg/kg α-linolenic acid.

Future treatments for barrier repair.
Ceramide moisturisers that mimic the ceramide, cholesterol and free fatty acid levels of human skin are already available and showing great promise in reducing steroid reliance in human atopic dermatitis and should be useful for dogs. Phytosphingosine shampoos/sprays and spot on treatments are marketed in Europe for the treatment of barrier dysfunction in dogs. Although our experience with these products is limited they do appear to be promising

2. Controlling infections

Both human and canine atopic patients show a tendency to more commonly develop secondary infections. Dogs commonly develop secondary bacterial (particularly *S. intermedius*) and *Malassezia* (particularly *M. pachydermatis*) infections. Microbial colonisation may exacerbate the inflammation via numerous mechanisms including IgE responses to bacterial and fungal proteins, T-cell activation by bacterial superantigens, protease liberation and microbial PAMPs (pathogen associated molecular patterns) activating the immunological response via TLRs (toll-like receptors).

Clinical experience indicates that controlling microbial colonisation and active infection in some atopic dogs can dramatically reduce the intensity of the atopic lesion and reduce pruritus and reduce steroid reliance. Management of infection falls into two main parts.

Control of current infections

The immediate priority with infection is in controlling the current infection and assessing the contribution of infection to the overall clinical picture. Total pruritus in a dog is the result of pruritus due to infection AND pruritus due to allergy AND pruritus from barrier dysfunction. Controlling the infection with antimicrobials will allow you to assess how important pruritus from infection is to your atopic patient (remember this will vary among atopics). If infection is a major contributor to the pruritus then preventing relapses will be a priority to successful management.

a. Cytology and clinical evaluation is needed to determine the nature of the infection and the depth of the infection. Depth of the infection determines whether systemic or topical treatment is needed and the duration of treatment

b. Antimicrobials with good antistaphylococcal activity (cephalexin 22mg/kg bid, clindamycin 5.5mg/kg bid and the fluoroquinolones) are good empirical choices for superficial infection (i.e. folliculitis). Antimicrobials should be continued for a minimum of 3 weeks (7 days beyond clinical resolution) but improvement should be noted within 4 to 7 days and 14 days is usually adequate length of time to evaluate the response (or lack thereof) in the pruritus.

c. For deep infections where other bacteria may be an issue antibiotics that have anaerobic activity should be considered (e.g. clindamycin) or adding metronidazole 20mg/kg sid to the cephalexin or fluoroquinolone regimen. Minimum duration of treatment in deep infections is at least 4 to 8 weeks (2 weeks beyond clinical resolution). Due to the duration of treatment needed, deep tissue culture (NOT swabs of draining sinuses) should be considered in some cases prior to treatment and always performed if there is a failure of initial empirical treatment.

d. Surface bacterial infections (bacterial overgrowth) can be dry and exfoliative or moist and exudative. The latter are often in intertriginous areas and associated with marked self-trauma. The exfoliative lesions can do poorly with systemic antibiotics and topical treatments e.g. Dalacin T® (clindamycin 1% lotion) can be more effective for these. Dalacin T is drying due to alcohol in the vehicle and should not be used more than twice daily or for longer than 7 to 14 days, and should be discontinued if irritancy occurs. Exudative lesions require antibiotic and steroid combinations e.g. Fuciderm® and should resolve rapidly with twice daily application. Fuciderm is a carbomer gel and is preferred for exudative lesions over lotions or ointments that can be macerating.

e. *Malassezia* overgrowth where generalized or associated with lichenified skin should be treated with systemic azoles such as ketoconazole 5mg/kg sid-bid or itraconazole 5mg/kg sid. Both should be given with food and discontinued if anorexia, hyporexia or vomiting occurs. Azoles should not be given to pregnant animals and can interact with other medications. Clinicians should familiarise themselves with the drug interactions and potential side effects with these drugs if not using these routinely. Mild and localised *Malassezia* overgrowth may respond to shampoo therapy (azoles or 3-4% chlorhexidine), miconazole (Micotopic®) or clotrimazole lotions (Canesten®), or 2% acetic acid / 2% boric acid (Malacetic®) wipes.

Maintenance, minimisation of recurrent infections

a. If there is a good response to antimicrobials then prevention of recurrence of microbial overgrowth or infection is vital. For recurrent superficial bacterial infections pulsed antibiotics (weekend dosing) can be useful together with the use of leave on chlorhexidine lotions. For surface bacterial infections leave on chlor hexidine lotions and use of chlorhexidine based shampoos can be effective.

b. For *Malassezia* overgrowth regular use of antifungal shampoos together with treatment of regionalised areas (e.g. interdigital areas, lip folds) with Malacetic Wipes® can be effective in short haired dogs. For dogs with hairy feet the Malacetic otic® applied with a cotton ball is a better option to penetrate the haircoat. Maintenance frequency will vary with the patient from daily to twice weekly for regional treatment and weekly to fortnightly for shampoos.

3. Controlling the allergy

Avoidance

Avoidance is not likely to be effective for airborne allergens like pollen as these allergens are widely dispersed in both indoor and outdoor environments. Climatic conditions including wind velocity, humidity and variables such as pollution levels and environmental activity (including vehicular movements) can all affect pollen dispersion. We have no control over these factors. Avoidance may be possible though for dogs with only dust mite allergies.

Based on studies in dogs and humans, it is be possible to control dust mite allergies with aggressive control of dust mites in the environment, or total avoidance of going indoors at all. However, this is not always practical in the domestic situation. It could be expected though, that if exposure of allergic pets to dust mites can be reduced at least partly, then response to other therapies, including ASIT, is likely to be better. Strategies include:

- complete exclusion from human bedrooms as dust mite counts are high in bedrooms.
- pets sleeping at night in a non carpeted room to try and reduce nightly dust mite exposure.
- pets sleeping on a suspension bed with a synthetic material (e.g. 'shade cloth' type with aluminium frame) as they do not accumulate mites.
- pet bedding using cotton sheets only. Rugs and blankets can harbour dust mites, and even the dead bodies of the mites can still trigger the allergies in most dogs. Mite bodies are more easily washed from sheets.
- weekly hot wash (> 55°C) of pet bedding. This kills the dust mites.
- if > 55°C is not possible with modern hot water systems then eucalyptus oil in a load of washing at a 0.4% rate, with a 60 minute soak followed by a cool rinse kills 99% of mites.
- weekly vacuuming of the home with a vacuum cleaner with a fine particle filter (e.g. HEPA filers or double bagged vacuum cleaners). Dust mites pass through the filters of average vacuum cleaners. There is no benefit to vacuuming more frequently.
- steam cleaning of the carpets and cleaning the ducted heating at the commencement of the process may also be advantageous.

Avoidance may also be useful for dogs with certain types of "grass allergy". Clients will sometimes observe that the dogs flare quickly after contact with grass. The distribution affected correlates with contact areas so the distribution may vary in dogs depending on coat type. Although well recognised, the actual pathomechanism is poorly understood and probably involves IgE mediated, DTH and irritant contact pathways. Avoidance may be useful in these cases e.g. environmental isolation versus lycra suits.

Allergen specific immunotherapy (ASIT)

The first reported successful use of ASIT in the dog was in 1941. Currently, this intervention is one of the mainstays of the management of AD, and there have been numerous studies examining this mode of therapy in the last 38 years. However, a wide variety of non-standardised trials and a lack of focus of case selection criteria have left us none the wiser as to the true efficacy nor optimal protocols of ASIT.

Mechanism of action. The induction of peripheral T cell tolerance appears to be the most essential step in ASIT. Initial T-cell anergy is followed by T-cell re-activation with Th-2 to Th-1 subversion together with IgG interference of CD23 mediated T-cell activation. These changes are initiated by multiple suppressor factors including IL-10 and/or TGF-β produced by ASIT activated allergen-specific regulatory T (T_{reg}) cells. T_{reg} cells also directly or indirectly influence effector cells of allergic inflammation, such as mast cells, basophils and eosinophils

Goals of ASIT. The goals of ASIT are to have a comfortable dog that requires LESS steroid medications to be comfortable. ASIT will sometimes allow a cessation of oral medications, and very rarely may put an animal into long term remission (<1% of cases). These outcomes are extremely important to convey to clients prior to allergy testing and commencement of ASIT.

It is important to remember that immunotherapy does not address innate barrier dysfunction or all the mechanisms that lead to bacterial and yeast colonisation in atopic dogs. Realistically if ASIT is successful we expect the typical dog to show a 60 to 80% reduction in medication reliance during the first 12 months on immunotherapy.

Efficacy of ASIT. Efficacy of ASIT has been examined in many nonstandardised studies and significant response has been reported in 52-100% of dogs. The truth is likely to lie somewhere in the middle of this range. Many dogs that fail ASIT are due to poor selection of the patient, poor owner compliance, poor management of concurrent problems or an inability to identify the important allergens.

Time to effectiveness. Response times vary from 2 months to 12 months but study comparisons are difficult due to lack of standardisation of response assessment criteria. If there is no change with ASIT after 9-12 months the patient should be re-evaluated for unaddressed concurrent problems. If none are found then consideration should be given to repeat allergen testing and modification of vaccine if there is evidence that important allergens have been missed. If this is not the case the vaccine should be discontinued.

Predictive factors for efficacy. Standard ASIT involves subcutaneous administration of native allergens. The key though is accurate selection of allergens. The differences between intradermal skin testing and serum IgE testing have already been discussed. Regardless of the test used, immunotherapy is unlikely to be successful unless the patient is a good candidate for immunotherapy (disease, patient and client factors), that the allergens are selected based on knowledge of the patient's environment, plant pollination periods and seasonality of disease. The best number of allergens to use is not known but one report suggested ASIT with more allergens (11-20) in dogs with >10 reactions increased the response rate.

Experience of the managing veterinarian also has a significant impact on the case. Nuttal et al 1998 described cases managed by the dermatology practice had a significantly better response to ASIT than externally managed cases (95% versus 60%). This is likely a result of a better understanding of the disease, management and also just increased communication time with the clients. In our practice we spend several hours every day talking to clients on the telephone in addition to longer than standard face to face consultations. Compliance was also better for referral managed patients. Similar outcomes have been shown in studies of human allergy patients where compliance is poorer by patients managed by the referring practice (11% versus 34% for non- compliance rate).

Inherent patient factors are less clear, but some studies have variously suggested that dogs that had clinical signs for 3-5 years or more before commencing ASIT were less likely to respond, that dogs over 5 years of age were less likely to respond, and that certain breeds may be more (Golden retrievers, Shar Peis, Australian Shepherds) or less (WHWT, Cairn and Yorkshire terriers, Bichon Frises) likely to respond. More studies are required to clarify these.

ASIT protocols. There is no "recipe book" for ASIT and manipulation of allergy vaccine protocols to get the best results requires experience. The allergen dose may play a critical role to outcome with individual cases requiring sometimes more or less than the 'standard' dose. A detailed examination of protocols available for ASIT are beyond the scope of these notes and the reader is referred to Scott et al 2001[2] and 22nd Proceedings of the North American Veterinary Dermatology Forum p.55 for more on this subject.

Assessment of Efficacy. Assessment of effectiveness of ASIT is determined by improvement in clinical signs (pruritus, infection recurrence), and / or reduction or elimination of maintenance medications such as glucocorticoids and antibiotics. It is important to document the level of drug reliance at the start of immunotherapy so that we have an objective measure of ASIT response. It is also important to give the owners realistic expectations to the outcome of ASIT such as target levels for drug reliance, and also to inform them at what point do we make a determination as to whether this is a satisfactory outcome. We typically compare both drug dependence and clinical signs 12 months after starting to minimize the possible confounding factors of seasonal variation. Many clients struggle with the concept that atopic dermatitis is a life-long disease and that management will likewise for most cases be life-long. The real benefits of drug minimisation with ASIT are the long-term health (and often financial) benefits.

Duration of ASIT. Persistence of benefit after discontinuation has not been evaluated in a controlled study so results vary from 4% to 35%. Such information needs to be reevaluated in a controlled trial and so ASIT should be considered life ling until such data is published.

The future of ASIT.

Immunotherapy is not a static "science". Altering ASIT to maximise the beneficial immunological response to administered allergens is the future focus of ASIT. CpG motifs (from bacterial DNA) conjugated to antigens can enhance Th1 maturation, and suppress Th2 responses and inhibit IgE production. Epitope mapping and cloning of allergens allows for production of specific antigenic epitopes that can be modified to maintain T cell epitopes but not to allow IgE binding. This may overcome the greatest risk of immunotherapy, namely anaphylaxis. Anaphylaxis is a real risk in humans on ASIT and a minor risk for dogs however it is the PERCEPTION of risk that will deter some clients from pursuing ASIT.

Non - allergen specific treatments (Anti-IgE immunotherapy)

IgE plays a pivotal role in allergic disease. Anti-IgE therapy can be both passive (vaccination with antibodies that target the variable portion of allergen specific IgE or the Fc portion of all IgE) or it can be active (immunisation to induce antibody production against IgE in either an allergen specific or allergen non-specific, targeting Fcε, manner). Down regulating IgE responses without cross-linking IgE (and hence triggering possible anaphylaxis) is the aim.

Passive immunisation with high dose monoclonal antibodies (mAb) directed against Fcε epitope Cε3 (to minimise the risk of anaphylaxis) can reduce serum IgE levels, inhibit IgE binding to mast cells and basophils by FcER1 and other effector cells and dendritic cells via FcεRII, and inhibit IgE production and induce apoptosis in IgE expressing B cells. Immune complexes to mAb Cε3 are small and do not activate compliment or accumulate in tissues. However, with passive therapies there is no sustained response after treatment is discontinued.

Bacteriophage immunization expressing Cε3 may lead to active anti-Cε3 therapies in the future. Safety studies are pending. It should be noted that anti-IgE therapy is only recommended currently as an adjunctive therapy in human asthma as response can be variable. Similarly anti-IgE vaccines for dogs are eagerly awaited to be used TOGETHER with ASIT but it is likely to be less effective then ASIT if used as a monotherapy. Remember the main mechanism of action of ASIT (and of AD itself) is via allergen specific T-cells.

Non - allergen specific treatments (pharmacotherapy)

Pharmacotherapy is usually required together with ASIT, at least in the short to medium term, and may still be needed long term. Clients must also be made aware of HOW medications work and why some of the safer medications (e.g. antihistamines) are less likely to be effective. Remember that histamine is a small player in the big overall picture of pruritus and there are a paucity of pharmacokinetic studies done in dogs for the antihistamines we use.

Of the drugs that have good evidence for their use in atopic dermatitis we have only glucocorticoids and cyclosporin while there is moderate evidence for the use of pentoxifylline and misoprostol and little objective support for the use of antihistamines. The side effects of glucocorticoids are problematic where supraphysiological levels (> 0.1 to 0.2mg/kg) are given frequently and cyclosporin causes broad spectrum suppression of T-cell functions making it less ideal for life long therapy and probably not compatible with ASIT.

Recently there has been a re-birth of interest in topical therapies for dogs with regional atopic lesions where the coat type allows for topical application. Topical glucocorticoids can be effective and offer more targeted treatment with les systemic side effects. Local side effects of cutaneous thinning and comedone formation can be problematic especially in thin skin areas like the axillae and inguinal folds. Currently Gentocin spray® (Schering Plough) is the only registered topical steroid spray for animals available in Australia however there are others in the USA and Europe that will likely be available in Australia in the future. For some axillary and inguinal regions topical pimecrolimus can be effective. It is a topical immunomodulator that causes localised cutaneous immunosuppression is a similar mode to cyclosporin without the atrophy of glucocorticoids.

Conclusion

When starting ASIT great care should be taken to improve barrier function with moisturisers, fatty acid supplementation and appropriate shampoo protocols, control recurrent infection if present with systemic and topical antimicrobial strategies if required, then control the atopic itch with allergen/irritant reduction strategies, topical therapies if possible and where the itch is generalised, systemic prednisolone. A minimum effective prednisolone dose is determined (prednisolone reliance) and this is continued but should gradually be reduced as the ASIT takes effect. Dogs with high prednisolone reliance at the outset of ASIT will often also receive antihistamines or occasionally pentoxifylline for steroid sparing effect. Once prednisolone reliance is determined this is a useful objective guide to ASIT response - the better the response to ASIT the lower the ongoing prednisolone reliance. Immunosuppressive therapies (cyclosporin, azathioprine, chlorambucil) are used where ASIT is not possible or does not effectively reduce the prednisolone reliance to an acceptable level within 12 months of compliant ASIT. Immunosuppressive therapy is only undertaken with owner informed consent of known and unknown risks and adherence to appropriate monitoring protocols.

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Why are accurate results important in IgE testing ?

To show the implications of accurate test results in the treatment decision process, a study using sera from atopic laboratory beagles sensitised to a single allergen has been performed.

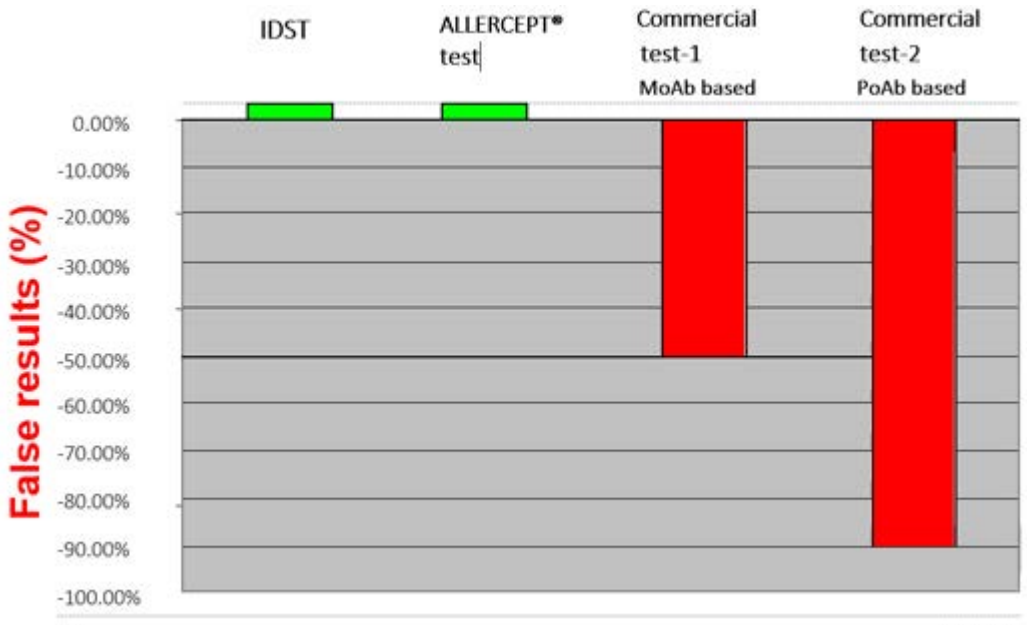
Methodology:

High IgE responder beagles were bred and maintained in an indoor facility. Puppies were sensitized to house dust mites, a grass pollen, or a weed pollen, by an injection of allergen extract, given once per month for 6 months, the first administration starting within 10 days of birth. Sera were obtained from these dogs at 6 month of age or later. In addition, sera were obtained from laboratory beagles, which had been experimentally sensitized to flea bite and showed classic symptoms of flea allergy dermatitis (FAD) upon flea infestation.

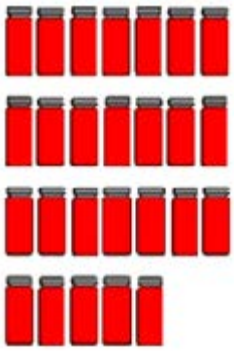
Serum from barrier raised specific pathogen-free (SPF) laboratory dogs was purchased from a commercial supplier. Sera from dogs sensitized to only a single allergen were aliquoted, coded and submitted in a blinded fashion to the Heska® Veterinary Diagnostic Laboratory, and to other commercial testing laboratories. After serum collection, the dogs were intradermally skin tested with a panel of 12 allergens by a Board-certified veterinary dermatologist who did not know the dog's sensitization status. In vitro test results for IgE to the allergen group mites, fleas, grass pollens and weed pollens were compared. A result was considered "false" if it was not in agreement with the known sensitization status of the dog.

FLEA-ALLERGIC DOG: IgE to flea only*

Test results



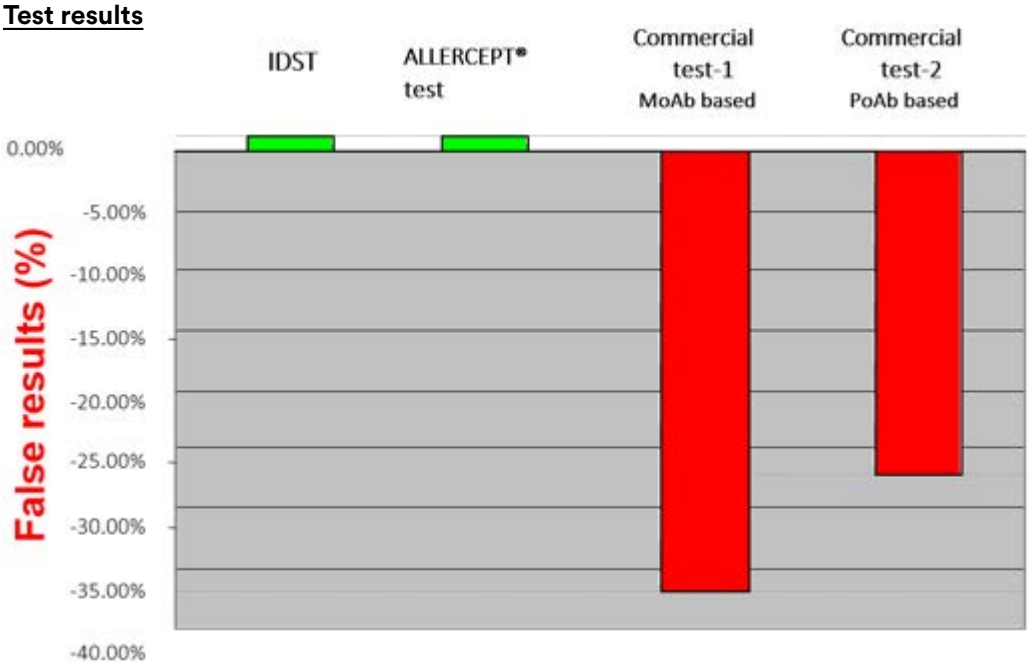
Potential treatment consequences based on test results

	IDST	ALLERCEPT test	Commercial test-1, MoAB based	Commercial test-2, PoAB based
Allergens tested	12	48	52	45
Correct results	1	1	0	0
False results	0	0	26	40
Flea allergen detection	YES	YES	NO	NO
Recommended immunotherapy treatment	NONE	NONE	YES	YES
Potential allergens to include in immunotherapy Green = correct Red = false				

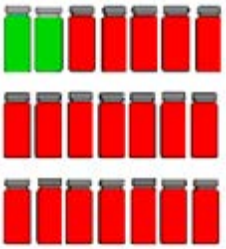
* This study was made using sera from laboratory beagles sensitized only to flea bite.

MITE-ALLERGIC DOG: IgE to mites only*

Test results



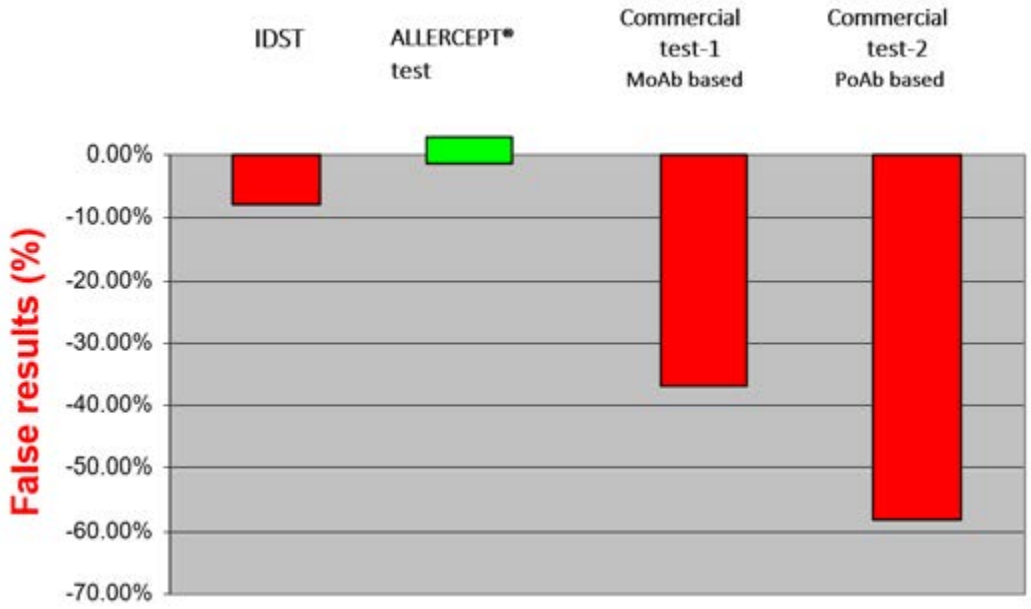
Potential treatment consequences based on test results

	IDST	ALLERCEPT test	Commercial test-1, MoAB based	Commercial test-2, PoAB based
Allergens tested	12	48	52	45
Correct results	2	2	2	2
False results	0	0	19	12
Mite allergen detection	YES	YES	YES	YES
Recommended immunotherapy treatment	YES	YES	YES	YES
Potential allergens to include in immunotherapy Green = correct Red = false				

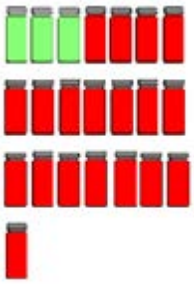
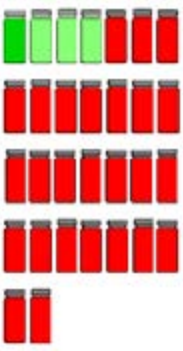
* This study was made using sera from atopic laboratory beagles sensitized to a single allergen.

GRASS-ALLERGIC DOG: IgE to grasses (Meadow fescue) only*

Test results



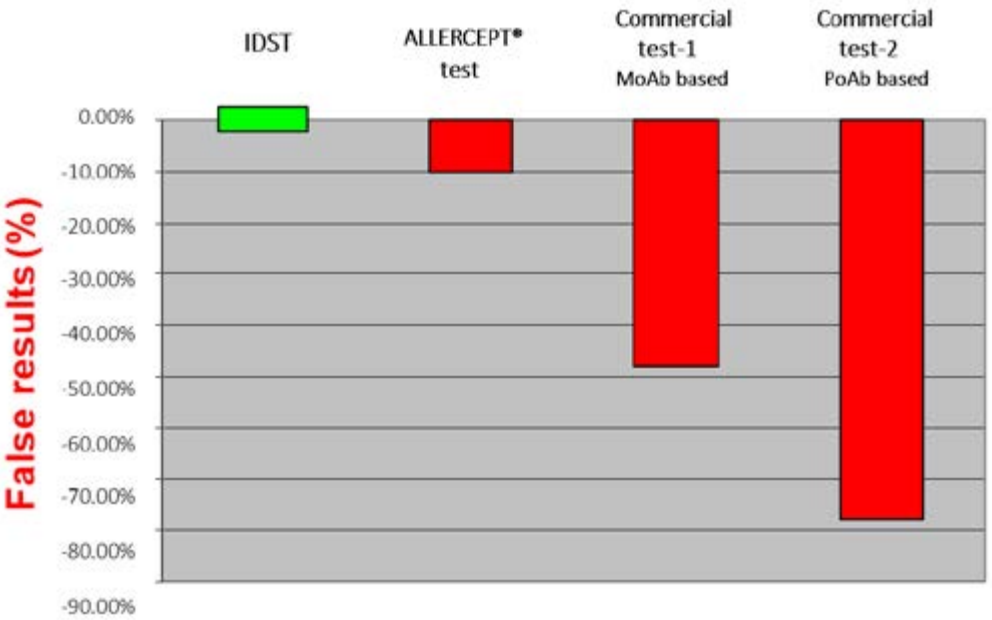
Potential treatment consequences based on test results

	IDST	ALLERCEPT test	Commercial test-1, MoAB based	Commercial test-2, PoAB based
Allergens tested	12	48	52	45
Correct results (cross-reacting grasses)	1 (1)	1 (5)	0 (3)	1 (3)
False results	1	0	19	26
Grass allergen detection	YES	YES	YES	YES
Recommended immunotherapy treatment	YES	YES	YES	YES
Potential allergens to include in immunotherapy Green = correct Light-green = cross- reacting grass Red = false				

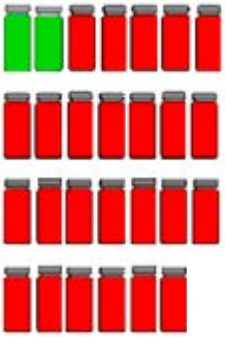
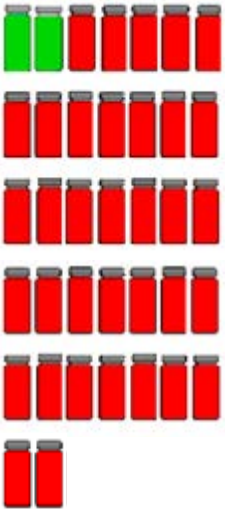
* This study was made using sera from atopic laboratory beagles sensitized to a single allergen.

WEED-ALLERGIC DOG: IgE to weeds (Yellow Dock/Sorrel) only*

Test results



Potential treatment consequences based on test results

	IDST	ALLERCEPT test	Commercial test-1, MoAB based	Commercial test-2, PoAB based
Allergens tested	12	48	52	45
Correct results	1	2	2	2
False results	0	5	25	35
Weed allergen detection	YES	YES	YES	YES
Recommended immunotherapy treatment	YES	YES	YES	YES
Potential allergens to include in immunotherapy Green = correct Red = false				

* This study was made using sera from atopic laboratory beagles sensitized to a single allergen

Leading Veterinary Reference Laboratories Worldwide use the Heska® ALLERCEPT® Allergy program

Europe

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Mörikestrasse 28/3
71636 Ludwigsburg
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Axiom Veterinary Laboratories
The Manor House Brunnel Road,
Newton Abbot
Devon TQ12 4PB,
UK
www.axiomvetlab.com

Laboklin
Prinzregentstrasse 3
97688 Bad Kissingen
Germany
www.laboklin.de

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Synlab Labor
Leitershofer Starsse 25
86157 Augsburg
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Kawaramachi-dori, Nakagyo-ku
Japan
www.falco.co.jp

Hitachi Chemical Co., Ltd.
13-1 Higaschi-cho-4-chome
Hitachi-shi
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www.hitachi-chem.co.jp

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Takatsujidori
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Submission of Samples for Allercept® IgE Test

1. Serum sample required (min 1mL) collected in a plain collection tube. Mix the tube thoroughly by gentle inversion approximately 5 to 6 times. Samples should be stored at 2-8°C before delivery. Deliver to the laboratory as soon as possible.

2. In cases whereby the sample(s) cannot be dispatched to Gribbles Veterinary i.e. collected over the weekend or during public holidays like Easter, Christmas, remove the clot from the sample by either centrifugation or standing samples until the serum separates and pour off the serum into a blood tube or plain tube NOT a blood collection tube. Store both serum and clot at 2-8°C until delivery day.

3. It is recommended that you use permanent marker pen to label all sample containers with client/animal identity. This is best done on the body of the container and not on the lid. Please detail date collected.

4. A completed submission form must accompany each case submission. Phone 1300 307 190 to order these forms. For companion animals the small animal submission form is required. It is essential that the forms be filled out correctly and completely. A full but concise history helps us give you the best information from the laboratory results.

5. Package the individual specimens into biohazard bags containing a separate section for the submission form. Samples are to be transported in an esky packed with an absorbent impact proof protective layer and clearly labelled Gribbles Veterinary Pathology. The use of protective bubble wrap and coolant pads is recommended for specimens, especially those travelling some distance.

6. Gribbles Veterinary operates a courier service in Victoria, NSW and South Australia. In regional areas contract couriers are utilised. Please contact Gribbles Veterinary Pathology Help Desk on 1300 307 190 for further information.

When to perform allergen specific IgE serology with a view to in-house ASIT or avoidance.

- When IDT is not accessible
- When sedation or clipping is not possible
- Where the AD is uncomplicated and the only symptom is non-lesional pruritus

NOTE:
- Testing should not be performed in dogs <5-6 months of age
- **DRUG WITHDRAWAL MUST BE OBSERVED**
  - 3-8 weeks off prednisolone
  - 3-4 months off methylprednisolone acetate, progestagens
  - 2 weeks off topical glucocorticoids, antihistamines, and clomipramine (latter up to 4 weeks)
- Test < 8-30 days after end of the patient's pollen season (and preferably the middle to end of pollen season)
- Testing should not be performed unless the managing veterinarian is experienced with immunotherapy / atopic management
- Apoquel® and Cytopoint® are fine to continue using as they will not affect the test

The Heska ALLERCEPT® Allergy IgE test Specific for Australia

WEEDS	TREES	GRASSES	MITES/INSECTS	MOULDS
Curled dock	Elm	Paspalum	Flea saliva	Alternaria
Sheep sorrel	Maple	Couch (bermuda grass)	Ant	Aspergillus
Red root amaranth	Grey birch	Bent grass (red top)	Cockroach	Cladosporium
Lamb's tongue	Red oak	Phalaris (canary grass)	Dermatophagoides pteronyssinus	Penicillium
Fat hen	Silver poplar	Sweet vernal	Dermatophagoides farinae	
Perennila ragweed	Liquidamber	Kentucky blue grass	Tyrophagus putrescentiae	
Mugwort	Plane tree	Orchard grass		
Mustard week	Privet	Broome grass		
Dandelion	Peppercorn	Timothy grass		
Daisy	Cypress	Perennial grass		
	Black widow	Yorkshire fog		
	Pine mix	Johnson grass		
	Eucalyptus			
	Melaleuca			
	Casuarina			
	Wattle			

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