Allergy Testing: Which Test and How to Interpret

Wayne Rosenkrantz, DVM, ACVD
Animal Dermatology Clinic
Tustin, CA

The results of allergy tests in cases of clinical atopic dermatitis (AD) should be used more as an aid in allergen avoidance and allergen selection than as a diagnostic test. Emphasis on the diagnosis of AD should be based more on rule outs of other pruritic differentials and the presence of clinical historical and physical signs. A variety of diagnostic inclusion criteria has been established, and this topic has previously been discussed in detail in this lecture series.

General considerations for allergy testing

Most veterinary dermatologists and allergists consider intradermal skin testing (IDST) the standard for evaluating a patient for AD. It has the advantage of testing the organ that is directly affected by the disease and has a lower incidence of clinically irrelevant positive reactions that vitro testing. In addition to detecting cutaneous IgE reaginic antibody, IDST can detect IgGd-sensitized mast cells. Many specialists also like the test because they can perform it quickly, interpret the test directly, and start immunotherapy the same day. The major disadvantages include the influence of drugs, stress, and hormones on test results; the need to purchase and maintain allergens for testing; learning the techniques of performing and interpreting the test; inability to test dogs with severe skin disease; the need to sedate and shave the patient; and in some cases the lack of sensitivity in classic AD cases.

Allergens come in different measurement units, such a protein nitrogen units, weight/volume, or biologic units. For testing, aqueous allergens are generally used and may be mixed with glycerin, propylene glycol, and alum precipitate, all of which are methods to improve absorption or prolong immunologic stimulation. There have been differences seen with various allergen sources, but unfortunately very little information regarding dogs and cats is available. Government regulation on quality is limited or lacking, even in human medicine. In general, testing should be done for relatively limited individual- or species-specific testing, as opposed to group testing. Even when selecting a serum in vitro testing (SIVT) company, be sure to consider the quality of allergens used in the test. Other items to consider are the cost per test or per item tested, cost of treatment per month from the company, success of treatment, number of items tested, and customer service. It is also important to inquire whether a veterinary dermatologist, veterinarian, or technician will provide consultation.

Negative allergy test results can occur with IDST or SIVT. Always make sure you have a compatible case and have ruled out other pruritic diseases, especially food allergy. Negative results in classic cases of atopic dermatitis (AD) may be explained by the concept that AD does not have to be IgE-mediated or that antibodies may not be present in the skin at detectable concentrations at the time of testing. Some have suggested that testing is preferred during the allergy season or just after. However, very inflamed or scarred skin may also lead to negative results. A major concern is the issue of inadequate drug withdrawal times, although there are no studies documenting this. General rules to follow include no oral glucocorticoids for 3 to 6 weeks, no topical glucocorticoids for 10 to 21 days, no injectable glucocorticoids for 8 to 10 weeks, and no antihistamines for 7 to 10 days. However, the author has seen cases requiring much longer withdrawal periods than the time frames given here. It has recently been established that cyclosporine can be used up until or even during IDST and not affect moderate to severe IDST reactions. Other causes for negative results include injection technique; outdated testing solutions; other drugs that affect or lower blood pressure, such as some tranquilizers; stress; off-season testing; so-called anergy (testing in a peak time during excessive mast cell degranulation); and breed poor reactivity (greyhounds). Some SIVT may yield slightly more false-negative results due to the specificity of their testing technique. In particular, it is speculated that the FcR- receptor technique increases the number of false-negative results.

Intradermal testing specifics

Allergens

There are many universally important allergens. These include house dust, human dander, feathers, molds, weeds, grasses, and trees; however, the most important allergens are the house dust mites (Dermatophagoides farinae and D. pteronyssinus). Other house dust mites and storage mites (Blomia tropicalis and Tyrophagus putrescentia) and other insects (i.e., cockroaches) can also be very important depending on the area where you practice.

If IDST is used, be sure to select allergens according to the region where the animal lives. This information can be obtained from the company from which you are purchasing allergens, from a local allergist, or by reviewing pollen counts in your area. In the US, you can search your area via the Internet on the National Allergy Bureau. Allergens should be obtained from a reputable allergen source that deals with veterinary testing. Another excellent source of information on allergen selection and for IDST is in Allergic Skin Diseases of Dogs and Cats, 2nd edition, by Reedy and colleagues. The author uses Greer Labs (Lenoir, NC) for help with allergen selections and supplies. These companies can help you set up appropriate testing concentrations and provide you with information on basic maintenance of your testing solutions. Testing concentrations vary depending on what allergens you are testing for. Many studies have evaluated what the correct threshold concentrations are. The optimal threshold concentration is the highest

concentration that produces a positive reaction in 10% of the normal dogs. Most allergens are tested at 1000 pnu/ml, but house dust and house dust mite allergens are tests at much lower concentrations (62.5pnu/ml). Testing with mixes is usually not recommended, as mixes can give false-negative results because they are too diluted In addition, the patient may be allergic only to one item in the mix, potentially resulting in unnecessary allergens in your immunotherapy, which could affect your success rates.

Test procedure

The actual testing procedure is usually performed over the lateral thorax, which, with a no. 40 blade, is carefully and gently clipped to avoid irritating the skin. The skin surface is marked with a semi-permanent marker for reference injection sites, and the allergens are injected intradermally. Sedation is often administered before the testing. Dexmedetomidine (Dexdormitor) or xylaxine and atropine are common sedatives for dogs at the author's practice. Other acceptable forms of sedation include propofol (with or without maintenance with isoflurane) and tiletamine-zolazepam. A positive control histamine is used at a 1/100,000 dilution, and a negative control of saline is used. The histamine should react with at least a 10-mm wheal within 10 to 15 minutes; if this does not occur, postpone the testing for at least 1 week. Injections are made with the bevel completely within the epidermis and between the layers of the skin. You should be consistent with the volume injected at each site (about 0.05 ml). The test should usually be read within 5 to 20 minutes. Reactions are graded based on size, erythema, and induction of the wheal. These reactions are determined on a 0 to 4+ scale that, for the most part, is subjective and based on your positive and negative controls. Your histamine serves as your 4+ and the saline as a negative control. Most specialists believe that 2+ or greater reactions are significant, but should always be correlated to the patient's history of exposure to that particular allergen. The size of the wheal does not necessarily correlate with their clinical importance. In some dogs, the IDST site can become very pruritic after testing and topical glucocorticoids can help to eliminate this problem.

False-positive reactions do not occur very often if dilutions for the allergens are correct. Some of the other possible causes for false-positive reactions include the presence of irritants or contaminated test allergens, improper technique, dermatographism, substances that cause nonimmunologic histamine release (physical urticaria or opioids), and irritable skin that is more commonly seen in Chinese Shar-Peis and boxers.

IDST requires strict attention to detail and significant time and expense to set up and maintain. It is the preferred method of testing canine AD but should not be considered the most sensitive indicator of AD, as classic cases can test negative. However, the incidence of irrelevant positives with IDST is considered lower than with SIVT, making this test preferred by most veterinary dermatology specialists.

Serum in vitro testing specifics

Several laboratories have developed in vitro tests for diagnosis and treatment of canine AD. These in vitro tests measure allergen-specific IgE that is present in the patient's serum. The radioallergosorbent test (RAST) and enzyme-linked immunosorbent assay (ELISA) attach the allergens to be tested to a solid substrate, such as a paper disk or a polystyrene well. The liquid-phase immunoenzymatic assay does not use a solid phase initially but mixes a labeled allergen with the patient's serum. The combined labeled allergen-antibody complex is subsequently bound by the label to the plastic well. This method in humans reduces the incidence of false-positive results due to background, nonspecific IgE. This liquid-phase also reduces the conformational distortions of antigens and hidden epitopes that are more common with solid-phase techniques. Some companies use monoclonal antibodies against IgE, whereas others use polyclonal antibodies or mixtures of monoclonal antibodies. Monoclonal antibodies are completely uniform and always bind to the same site of the IgE molecule. However, if that portion of the IgE is hidden, binding will not occur, leading to false-negative results. With polyclonal antibodies, there is binding to several sites of the IgE molecule, and this method may therefore have a greater chance of binding and detecting IgE. However, polyclonal antibodies may not always be 100% specific for IgE. This is one thought on the reason for false-positive reactions seen with some of the tests, and this could also affect repeatability of the test. One company uses a recombinant high-affinity receptor to detect allergen-specific IgE. This should theoretically provide more specificity for IgE detection and has been associated with better correlation to IDST with some antigens tested. Better correlation with IDST has also been seen with companies using monoclonal or mix monoclonal tests and liquid-phase immunoenzymatic techniques and that process patient's serum through a column of ascarid antigen to remove parasitic IgE and through a column with protein A to remove IgG. With all the newer techniques, results have improved and false-positive results have been reduced. However, in some situations newer techniques have reduced the number of positive results in classically AD cases. Looking at total IgE levels is also controversial. Total levels of IgE in dogs is much higher than in humans—mean levels in healthy dogs are 190 μg/ml, which is very similar to what is found in dogs with AD. As a result, the author finds little to no value in running total IgE levels as a screening test. In vitro testing should be reserved for selection of allergens in dogs that already have a clinical diagnosis of AD.

Major advantages of in vitro tests are lack of risk, sedation, shaving, and discomfort to the patient; quantitative results; easily done and readily available to all veterinarians; can be performed on patients with widespread cutaneous inflammation or dermographism; and the success of allergen-specific immunotherapy (ASIT) is similar to what is seen based on IDST. Claims have been made that glucocorticoids do not affect in vitro test results, and this statement has been supported by studies using low-dose and short courses of

oral steroids. However, most manufacturers caution veterinarians that glucocorticoid therapy may affect test results by potentially reducing class reactivity. The disadvantages of these tests are cost and the incidence of false-positive or clinically insignificant results, making them inappropriate to diagnose atopic disease.

These tests are being marketed to veterinarians with various claims about accuracy, reliability, and usefulness in diagnosis and treatment of canine atopy. Again, these tests should be reserved for allergen selection and management of AD, not diagnosis. Most specialists are seeing ASIT success with these in vitro tests, often with equal responses to that of IDST.

Deciding on which serum in vitro test to use should be based on several considerations. The author recommends individual testing, as group testing can lead to inclusion of allergens in treatment solutions to which the animal has no IgE. Screening tests, although inexpensive, can be misleading and should be avoided. All serum in vitro testing should not be used for diagnosis but as a tool to select allergen treatment. Although there are technologic differences between the companies, no comparisons based on ASIT have been made with most companies and individual researchers report very similar successes with many of the commercial companies. Based on this information, selection should be based heavily on client service, support staff by qualified veterinary specialists, regional allergen testing, number of items tested, and list of ASIT extracts.

Interpretations of results

Ultimately, the value of allergy testing is in correlating the results with the pet's clinical history. By combining history and positive reactions, you can get the most out of your allergy testing regarding avoidance and allergen selection for immunotherapy. Avoidance, allergen reduction, and immunotherapy should be combined to get the most value from your test results.

Avoidance or reduced allergen exposure

Common allergens that lend themselves to avoidance or reduced exposure include epithelial allergens (i.e., cats, rabbits, feathers, wool), house dust and storage mites, and insects (ants, flies, moths, cockroaches, mosquitoes, fleas). With airborne allergens, such as mold and tree, grass, and weed pollens, the pet is less likely to benefit from avoidance. Mold control can be of value if there is a specific area of the home or yard that obviously has mold overgrowth. Despite suggestions for avoidance of these allergens, complete avoidance may be impractical or impossible. For example, in a mixed household of dogs and cats, it may not be possible to separate or prevent cat dander exposure. Wool carpets, blankets, or clothing may be avoided depending on the owner's willingness to remove or replace such items. Down pillows, comforters, or birds in the environment can be a source of feathers, and again, some of these may be more readily removed than others. House dust mites (Dermatophagoides farinae and pteronyssinus) and storage mites (Tyrophagus, Blomina, Acarus) are impossible to completely eliminate in the home. However, measures to reduce allergen exposure can be implemented. Common suggestions for house dust mite reduction include washing bedding in hot water (>130○ F) to reduce house dust allergen, elimination of carpeting, replacing cloth upholstery with leather, frequent vacuuming, and use of house dust mite miticidal agents, such as borates (Ecology Works® DustMite and Flea Control) or benzyl benzoate (Acarosan DustMite Spray®). For storage mite control, it is best to keep dry dog food and treats in cool, dry environments and avoid storage in areas of higher humidity. These mites tend to proliferate more when microscopic mold begins to grow on food. Ideally, food should be refrigerated. For insect allergies, premise control for such insects, as ants and cockroaches and repellents for mosquitoes or other biting flies can be helpful. The author commonly uses permethrin-based products, such as Vectra 3 D (Summit Pharmaceuticals/Ceva) or Advantix (Bayer), as they have some value in areas with greater mosquito exposure. Flea control is the logical approach for flea-allergic patients, and maintaining a high level of control is essential. More frequent administration of products is often indicated in flea-allergic patients than what is recommended for non–flea-allergic patients. Keeping pets off the grass or away from specific trees or weeds is rarely helpful and usually impractical. However, occasional avoidance of a specific grass, tree, or weed may be helpful if the owners can implement this avoidance.

Correlation of allergens with environmental exposure

When selection of allergens for immunotherapy is considered, it is essential to correlate reactions with clinical history and exposure of allergens. Knowing the pets lifestyle (indoors, outdoors), when signs are worse (morning, evening, or after being outdoors), and seasonality can help when selecting which allergens to include in your recipe. Most commercial in vitro labs run allergy screens based on pollen counts that are known allergens in your specific regions. However, allergen loads within specific areas can vary, and checking your local pollen counts is a good idea (National Bureau of Allergy http://www.aaaai.org/nab/index.cfm?p=pollen). It is often helpful to go over the results with the owner to discuss specific tree, grasses, flowers, and weeds that the pet reacted to, as some owners are knowledgeable of common plants in their local environment. Knowing this information can help you preferentially place certain allergens into your allergen recipe over others. It is also extremely important to know the seasonality of patient exposure to certain types of pollen. For example, tree pollens tend to be more common in the spring and weed reactivity in late summer or fall. Again, there will be geographic variability and you need to be familiar with your area's seasonal pollens. The presence or absence of cats in the home may influence selection of this allergen if a dog is allergic to cats. Year-round atopic dogs commonly have house dust reactions, and if positive reactions to these mites are detected they should be included in your ASIT recipe.

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