Diagnostic Testing

Dermatologic Diagnostic minimum data base includes skin scrapes, otic swabs, and cutaneous cytology. The goal should be to identify all secondary infections (pyoderma, demodicosis, dermatophytosis, otitis, Malassezia dermatitis, and pododermatitis) then formulate a diagnostic plan to identify and control the underlying/primary disease (ie. allergies, endocrinopathies, keratinization defects, and autoimmune skin diseases).

Skin Scrapes

Skin scrapes are one of the most common dermatologic diagnostic tests. This relatively simple and quick test can identify many types of parasitic infections. Although not always diagnostic, the relative ease and low cost makes it an essential test in a dermatological minimum data base.

Many practitioners reuse scalpel blades; however, with our increasing awareness of transmittable diseases (Bartonella, Rickettsii, FeLV, FIV, herpes, and papilloma virus), the reusing of scalpel blades for skin scrapes should be stopped.

Procedure:

<u>Superficial skin scrapes</u> (for Sarcoptes, Notoedres, Demodex gatoi, Cheyletiella, Otodectes, chiggers)

A dulled scalpel blade is held perpendicular to the skin and used with moderate pressure to scrape in the direction of hair growth. If the area is haired it may be necessary to clip a small window to access the skin. In an attempt to find the relatively few sarcoptic mites that may be present on a dog, large areas are scraped (1-2 inches). Applying mineral oil directly to the skin to be scraped helps dislodge debris and makes it easier to collect the scraped material. Since these mites do not live deep in the skin, it is not necessary to visualize capillary oozing or blood. The most productive sites for sarcoptic mites include the ear margin and lateral elbows. Anecdotal reports suggest that Demodex gatoi in cats may be more easily found on the lateral shoulder. Usually several slides are needed to spread the collected material thinly enough for microscopic examination.

Deep skin scrapes (for Demodex spp. except D. gatoi)

A dulled scalpel blade is held perpendicular to the skin and used with moderate pressure to scrape in the direction of hair growth. If the area is haired (usually alopecic areas caused by folliculitis are selected) it may be necessary to clip a small window to access the skin. After several scrapes, the skin should become pink with the capillaries becoming visible and oozing blood. This assures that the material collected is from deep enough within the skin to collect the follicular Demodex mites. Most people also squeeze (pinch) the skin to express the mites from deep in the follicles into a more superficial area so that they are more easily collected. If the scraping failed to collect a small amount of blood, then the mites may have been left in the follicle resulting in a false negative. In some situations (shar peis or deep inflammation with scarring) it may be impossible to scrape deep enough to harvest the Demodex mites. These cases are few in number but

require biopsy to identify the mites in the hair follicles. Hair-plucks from a area of lesional skin may be used to help find mites but the accuracy of this technique compared to skin scrapes is unknown.

Regardless of the collection technique, the entire slide should be searched for mites using low power (usually a 10X objective). Searching the entire slide ensures that if there were only 1-2 mites (typical of scabies infections) the user will likely find them. It may be helpful to lower the microscope condenser which makes the mites contrast better increasing their visibility. (Make sure to raise the condenser before looking for cells or bacteria on stained slides)

Mites	Diagnostic test	Accuracy	Other tests	
Demodex	Deep scrape	HIGH	Biopsies may	
canine			be needed in	
			extremely	
			thickened	
			lesions	
Demodex cati	Deep scrape	HIGH		
Demodex gatoi	Superficial scrape	LOW	Lime sulfur dip	
		May be hard	trial, response	
		to find mites	to treatment	
Sarcoptes	Superficial scrape	LOW	Response to	Pinnal pedal
		(only 20%)	treatment	reflex (80%)
Otodectes	Otic mineral oil	HIGH		
	prep, superficial			
	scrape			
Cheyletiella	Flea comb, tape	MODERATE	Vacuum	Fecal flotation
	prep, superficial		collection	may identify
	scrape, vacuum		techniques are	mites
			preferred by	
			some	
			veterinarians.	
Lice	Usually grossly	HIGH		
	visible, tape prep			
Notoedres cati	Superficial scrape	HIGH		
Trombiculosis	Targeted scrape on	MODERATE		
	focal lesion			

Cutaneous cytology

Cutaneous cytology is the second most frequently employed dermatological diagnostic technique. Its purpose is to identify bacteria or fungal organisms (yeast), the infiltrating cell types, neoplastic cells, or acantholytic cells (typical of pemphigus complex).

Procedure:

Direct Impression Smear

Moist exudate is collected from pustules, erosions, ulcers, or draining lesions. Alternatively, crusts can be lifted revealing a moist undersurface. Papular lesions can be traumatized using the corner of a glass slide or a needle and then squeezed to express fluid. Yeast dermatitis can be sampled by repeatedly sticking the slide onto lichenified lesions or by using a dry scalpel blade to collect material which is then smeared onto a dry slide. Regardless of which technique is used, the moist exudate collected on the slide is allowed to dry. The slide is then stained using a commercially available cytology stain (modified Wright's stain: Diff Quik is the most common) and gently rinsed. A low power objective is used to scan the slide and select ideal areas for closer examination. High power (40X objective or preferably 100X oil objective) is used to identify individual cell types as well as bacteria or fungal organisms.

Fine Needle Aspirate Method

Use a needle (22-25 ga) and 6cc syringe to aspirate the mass. Clean the area if necessary with alcohol or chlorhexidine. Immobilize the lesion; insert the needle into the nodule aiming for the center of the lesion; pull back on plunger to apply suction; release and redirect, pull back on plunger again, stop if you see any blood in the hub of the needle as this will dilute the cellular sample. Release negative pressure before you remove the needle from the lesion. An alternative technique repeatedly inserts the needle without the syringe into the lesion redirecting several times. This latter technique (without negative pressure) decreases the frequency of inadvertently diluting the sample with blood and works best for soft masses. Once the sample is collected, express the material onto a microscope slide by blowing a syringe-full of air through the needle to spray the cells onto the slide. Smear the material gently to thin the clumps of cells and stain with cytology stain. The slide should be scanned using low power (4X - 10X) to find a suitable area for closer examination. High power (40X objective) may be used to identify the infiltrating cell type and cellular atypia.

Acetate tape preparations

Tape preps are used to evaluate a variety of different conditions. The basic technique involves using crystal clear tape (single or double sided) to collect a sample of hair or superficial skin debris.

Tape Preps for Mites:

Tape preps can be an effective method of collecting and restraining Cheyletiella and lice for microscopic examination. The mites are usually large enough to visualize so that a piece of tape can be used to capture a specimen. The tape then prevents the creature from escaping.

Tape Preps for hair (trichogram):

Tape is used to secure the hair sample in position on a glass slide. The sample is examined using low power (4X-10X objective). See Trichogram section for

analysis techniques. Oil may be a better media for trichograms.

Tape Preps for yeast:

Tape preps for yeast dermatitis are one of the most efficient and effective methods for identifying Malassezia skin infections. Although not as reliable and quantitative as impression cultures using Sabouraud media, the speed and ease make the tape prep the most common technique employed to identify Malassezia. The lichenified lesion (elephant skin on the ventral neck or ventrum) is sampled by repeatedly applying the sticky side of the tape onto the lesion. The tape is then adhered to a glass slide and stained with a cytology stain (omitting the first alcohol stain solution). The tape serves as a cover slip and can be examined using high power (100X oil immersion) to identify Malassezia organisms. This technique is useful but false negative results are common with all yeast collection techniques.

Otic Swabs

Otic swabs are useful to determine if a normal appearing ear canal actually has exudate deep in the ear. If a cotton swab is used to gently collect a sample and if relatively clean then the ear is most likely normal. If the sample demonstrates a black waxy exudate, a mineral oil prep should be performed to identify any mites (Otodectes, Demodex). If the sample is light brown or demonstrates a purulent exudate, cytology should be performed to identify bacteria or yeast.

Mineral oil can be used to dissolve the black waxy material collected from an otic swab. The swab should be stirred in the oil to remove the exudate and make the sample suitable for examination. Examine the entire slide using low power (4X or 10X objective) to identify any mites. Usually Otodectes mites are easy to visualize but dropping the condenser and scanning the entire slide may help assure obtaining the diagnosis.

Otic cytology is used to identify secondary yeast and bacterial otitis externa. Collect debris with a cotton swab. An easy and quick technique is to roll the swab from the right ear on the right side of the slide and the swab from the left ear on the left side of the slide assuming that the slide has markings to identify which direction is up. If the material is very waxy, the end of the slide should be heated to help melt the wax and allow the stain to penetrate the sample better. The sample must be stained with cytology stain (modified Wright's stain: Diff Quik®). Examine the slide at low power (10X objectives) to find a cellular area that will likely have organisms. Then use the high power objective (40X or 100X oil immersion objectives) to identify the organisms causing the secondary otitis.

Otic cytology is necessary to identify the type of secondary infections to best select medical therapy. Additionally, otic cytology is useful to evaluate a patient's response to treatments, especially if the otitis has not completely resolved. In these cases, otic cytology will be able to determine if the number and mixture of organisms is improving. This determination is crucial to prevent premature discontinuation or switching of treatments which leads to increased antimicrobial resistance.

DTM fungal cultures

DTM fungal cultures are used to isolate and identify dermatophyte organisms. Dermatophyte test media is made with special ingredients that inhibit bacterial growth and turn red when dermatophytes grow.

Procedure:

The area to be sampled is usually cleaned by gently applying alcohol to the hair and skin. The alcohol must dry prior to collecting the specimen. Samples of hair, crust or scale are collected from lesional skin using a sterile forceps. Using a Wood's lamp to collect fluorescing hairs may increase the diagnostic accuracy. The collected material should be gently applied to room temperature DTM media being careful not to bury the sample within the media. Having the media at room temperature prior to placing the sample on the media helps hasten fungal growth. Fungal culture plates with a large removable or flip-up lid make sample deposition much easier (standard Petri dish or Bactilabs culture plates). For animals without lesions (resolving infections or subclinical carriers) a new tooth brush can be used to brush the entire hair coat. The collected sample is then distributed onto the culture plate. Claws can be cultured by clipping an affected nail and grinding or shaving the surface to produce small particles that are deposited on the media. Dermatophytes grow in the keratin structure of the claw causing the distinctive onychodystrophy.

The DTM culture plates should be examined daily for 2-3 weeks. With dermatophytes, the medium will change color as soon as a white/buff colored fluffy colony is visible on the medium. Some contaminants (usually black, grey, and green) will be able to change the medium to red but only after growing for several days. If the culture plate has not been evaluated daily, then it will be impossible to determine when the color change occurred in relationship to the appearance of the fungal colony growth.

Once the fungal colony has been growing for several days it will begin to produce macroconidia. Keeping the culture warm and in a humid environment facilitates conidia formation. The macroconidia should be sampled and microscopically examined to determine the dermatophyte species. Clear acetate tape is touched to the surface of the fungal colony to be evaluated. The tape is then adhered to a glass slide and a drop of cytology stain is applied. The macroconidia are usually apparent using a low power (10X objective). This is especially important in dogs because the identification of Microsporum canis may indicate the presence of an infected asymptomatic cat. The identification of trichophyton or microsporum gypseum suggests an environmental source of the dermatophyte infection (rather than a cat).

Some fungal species, which cause deep infections/ cellulitis (Blastomycosis, Pythiosis, Histoplasmosis, Coccidiomycosis, etc.) represent a zoonotic hazard when grown as in-house cultures. If they are suspected, swab samples and tissue specimens should only be submitted to and cultured by well equipped microbiology laboratories.

<u>Trichogram</u> (for the evaluation of the hair tips, shafts, and roots) A trichogram is used to visualize the hair for evidence of pruritus, fungal infection, pigmentation defects, and growth phase.

Procedure:

A small amount of hair to be examined is epilated. Tape or mineral oil is used to secure the hair sample in position on a glass slide. The sample is examined using low power (4X or 10X objective)

Hair Tips:

The hair tips are usually evaluated to determine if a patient is pruritic (especially cats) or if there is a non-traumatic cause of the hair loss (endocrine disease or follicular dysplasia). Pruritic animals will break the tips off the hairs leaving a broken end that can easily be detected. This determination is especially useful in feline patients when the owners are not convinced that the patient is pruritic due to the secretive nature of some cats.

Hair Roots:

The hair roots may be examined to identify anagen and telogen hairs in an attempt to determine if the hair follicles are cycling normally. In most breeds, the majority of hairs will be in the telogen stage but some anagen hairs should be identifiable. In breeds with prolonged growth periods (poodles), most of the hairs may be in anagen with relatively few hairs in the telogen stage. In telogen defluxion, all of the hairs epilated are in telogen.

Hair shafts:

Dermatophyte ectothrix can sometimes be visualized in patients with dermatophytosis. Identifying the ectothrix can be difficult and may require KOH and cytology stain to help dissolve the excessive keratin. The cortex of the hair will appear swollen and damaged and if broken the ends will appear frayed (like a broom). The organisms (small spherical structures) may be clumped around the damaged region of the hair shaft. Hair shafts can be examined for pigmentary clumping which would be suggestive of color dilution alopecia and follicular dysplasia. Ectoparasites eggs may be visible attached to the hair shat in pediculosis and Cheyletiellosis. Other hair shaft abnormalities have been reported but are extremely rare.

Wood's lamp exam

A Wood's lamp is a special UV light source that uses a wave length of 340-450mm (UV A spectrum that does not hurt the skin or eyes). This unique combination causes

tryptophan metabolites produced by some strains of Microsporum canis to fluoresce a bright apple green color. Unfortunately, not all Microsporum strains will produce the cell product making the Wood's lamp useful in only approximately 50% of Microsporum canis infections. This technique is not able to identify Trichophyton species or Microsporum gypseum.

It is important to allow the light source to warm up so that the appropriate wave length of light is produced. Many false positives can be observed due to the fluorescence of scale and certain topical medication. A true dermatophyte infection will have the apple green fluorescence on the root of the hair shafts. All dermatophyte infections should be confirmed with a fungal culture.

Biopsy

Cutaneous biopsy evaluation can be frustrating for the practitioner and pathologist. The diagnostic reliability of skin biopsies can be improved through the proper selection of lesions for biopsy, the use of a dermatopathologist, and by providing the pathologist with a complete clinical differential diagnosis list.

Cutaneous biopsy has the potential to provide the most information in the shortest period of time. Even if cutaneous histopathology can not identify the exact etiology, the pathologist should be able to classify the cutaneous changes into 1 of 6 general categories:

Neoplasia	
Infectious (folliculitis, cellulitis)	
Immune mediated (autoimmune, vasculitis,	
drug reaction, etc.)	
Endocrine-like (Hypothyroidism,	
Cushing's, follicular dysplasia, etc.)	
Keratinization defects (Primary seborrhea,	
sebaceous adenitis, ichthyosis, etc.)	
Allergy	

The diagnostic efficiency of cutaneous biopsies can be improved by:

Obtaining several skin biopsies from different representative lesions. Biopsy everything that looks different.

Provide the pathologist with a detailed list of differential diagnoses based on clinical lesions, patterns, and response to treatment.

Insist on a histopathology report that includes a thorough description of

the skin sections as well as a discussion of how these findings rule-in or rule-out the differential diagnoses provided by the submitting veterinarian.

Use a dermatopathology service to take advantage of the special interests and training of these pathologists.

Lesion selection

Primary cutaneous lesions (pustules, vesicles, petechia, erythematous macules, papules) are preferred for sampling. Secondary lesions (crust, alopecia, scale, ulcers, and erosions) may be useful but typically have less diagnostic impact. A good strategy is to sample several sites (at least 3) making sure to get a range of lesion types. Generally, biopsy samples should be obtained from every area that appears different.

The nose and foot pads are particularly painful to biopsy; however, these areas are very often affected by autoimmune skin diseases and should be sampled. Heavy sedation or general anesthesia may be required to biopsy the nose or foot pads.

Procedure:

Once the areas to be biopsied are selected, the lesions should be left untraumatized. Do not prep or clean the areas as this will remove the superficial crust and scale which may be essential to determine the diagnosis. A local anesthetic (lidocaine, articaine, novocaine, etc) may be injected into the subcutaneous tissue making sure not to inject too superficially. Lidocaine may decrease the viability of some infectious organisms; therefore, its use in biopsy samples destined for minced tissue culture should be avoided.

A disposable Bakers punch (0.4mm - 0.8mm) should be used to perform the biopsy. The biopsy punch is placed on the lesions and moderate pressure is applied while twisting the biopsy punch. Once the punch penetrates the full thickness of the skin, the punch is removed leaving the skin sample attached to the subcutaneous fat. With great care not to traumatize the skin sample, forceps should be used to grasp the sample by the deep fat (this prevents forceps marks in the epidermis which decrease the diagnostic potential of the sample). The subcutaneous fat can be cut to release the skin biopsy sample. If the skin is thin or if it is critical for the pathologist to be able to orient the sections, the sample should be placed on a firm substrate (piece of index card or tongue depressor). The sample should be submitted in 10% formalin.

An alternate method uses a scalpel to obtain an excisional biopsy sample using a classic elliptical shaped excision. This is the preferred technique for large lesions.

Nails can be biopsied using one of two techniques. If the nail is soft, an 8mm

Bakers punch to biopsy a lateral portion of the nail, nail bed, and nail base. This technique only works if the nail is soft enough to be sectioned. If the nail is hard (a more normal state), amputation of the third phalanx is required. Obviously, this is not the ideal sample collection technique as many owners are extremely reluctant to permit digit amputation. Often a dew claw can be harvested to minimize the impact of diagnostic amputation.

Once the sample is removed, the wound should be closed with suture or cutaneous staples.

Cultures (for bacterial or fungal infections)

Bacterial and fungal cultures are an important part of dermatology diagnostics. Any deep cellulitis-like lesions, especially with draining tracts, should be cultured for bacterial and fungal organisms. Nodules and tumors should be cultured if infectious etiologies are on the differential list.

Procedure:

Culturette swabs are useful for collecting moist exudates for culture. All superficial purulent exudate should be removed and the lesion cleaned with nonpreserved saline or water. Fresh exudate can then be expressed and collected with the swab for submission to the microbiology laboratory. This cleaning technique helps reduce the number of contaminant organisms.

Otic cultures should be obtained prior to any lavage or cleaning procedures.

For deep skin cultures, the preferred technique utilizes a sterile biopsy procedure to collect a piece of skin for submission to the microbiology laboratory. The skin should be surgically prepped and rinsed well with non-preserved saline or water. This prevents the disinfectant solution from killing the pathologic organisms. Once the skin sample is harvested, it should be placed in a culture swab or sterile container (with a drop of nonpreserved saline), refrigerated, and shipped overnight. Caution should be taken to avoid freezing the skin samples intended for culture as this will decrease the efficacy. The lab should then perform a minced tissue culture to isolate the organisms within the dermis.

PCR Assays

PCR (polymerase chain reaction) assays use laboratory methods to amplify DNA within a sample. PCR is many times more sensitive and specific than other diagnostic tests for the identification of viral, bacteria and fungal organisms. In the future, PCR will become a powerful tool for the diagnosis of most cutaneous infections. At this writing, most diagnostic laboratories provide testing for mycobacteria, and some deep fungal organisms. Since this technology is evolving extremely rapidly, it may be helpful to contact the diagnostic laboratory for testing availability and sample requirements.

Serology

The detection of antibodies for select infectious agents may provide useful information regarding the patient exposure, active infection, and resolution of some fungal, rickettsial, and protozoal diseases. This diagnostic test may be most useful for rickettsial diseases and cryptococcus.

Immunostaining techniques

Direct immunofluorescence provides a unique methodology for the diagnosis of autoimmune skin diseases. Direct immunofluorescence has been used in veterinary dermatology for over 30 years; but the accuracy and repeatability of this diagnostic test has been questioned. The effect of body region can greatly influence the results of direct immunofluorescence with 11% - 78% of normal foot pads or nasal samples demonstrating false positive results. Additionally, diagnostic laboratories can demonstrate poor reproducibility with duplicate samples. More recent techniques including immunoperoxidase and monoclonal antibodies seem to provide more accurate results; however, their use is limited.

Procedure:

Skin samples are collected through traditional biopsy techniques. If immunofluorescence will be used, Michelle's preservative is required. The need for special media has made immunofluorescence fall from favor with many clinicians and diagnostic laboratories. Immunoperoxidase offers the same diagnostic value but can be performed on formalin fixed tissue eliminating the need for additional biopsy samples preserved in Michelle's preservative. The diagnostic laboratory should be contacted for testing availability and sample requirements.

Diascopy

Diascopy is a simple technique that involves placing a glass slide over an erythematous lesion and applying moderate pressure. The skin under the slide will either blanch (turn white as the blood is squeezed out) or remain erythematous. This test is useful to differentiate vasodilatation from ecchymosis. Urticarial lesions are caused by dilated blood vessels that leak fluid but not red cells; therefore, the red lesion will blanch when pressure is applied. Ecchymosis (typical of vasculitis) is caused by red blood cells leaking out of the vessels. These erythematous lesions will not blanch since the cells are in the dermis.

Allergy Testing

Serum immunoglobulins rise in allergic dogs making it possible to identify and measure antigen specific antibody levels. These tests are readily available from several different companies and can be easily performed in any practice environment. In general, the patient does not need to be withdrawn from medications that would interfere with traditional intradermal allergy testing; however, since these tests do measure a component of the immune response, anti-inflammatory medications may alter the results. Discontinuing all steroid containing medications, as one would do before intradermal allergy testing, should be considered before the patient's serum sample is acquired. Some companies include steroid withdraw in their patient preparation requirements.

Intradermal Allergy Testing has been considered the gold standard for diagnosing and treating canine atopy for many years and remains the primary testing method used by most veterinary dermatologists. Intradermal allergy testing allows us to test the skin where the allergic response is occurring. Most animals tolerate the procedure well and results are immediately available. Animals should be sedated to minimize any anxiety or stress and must be withdrawn from antihistamines for 10-14 days and from all steroid-containing medications for at least 4 weeks. The antigens used must be carefully stored and maintained to assure high quality testing materials and appropriate antigen stock for immunotherapy vaccine formulation. Generally, at least 40 allergens should be included to assure that a large enough spectrum of regional allergens is incorporated into the test.

Procedure:

The patient is withdrawn from all medications containing steroids and antihistamines. The patient is sedated to avoid excess stress and cortisol release. An area on the lateral thorax is clipped with a #40 blade. The skin should not be traumatized or cleaned. A permenant marker is used to indicate sites of injections. A special syringe is used to administer 0.5-0.1ml of each allergen that has been prediluted to 1000-1500 PNU (for most allergens). The test should be completed within a 30minute window at which time the initial injections should be ready to read. Each injection site is evaluated for erythema and swelling. Histamine and saline controls are used to help determine the range of reactivity and a 0-4 scale is used to assign the relative reactivity of each injection site. A good positive should look like a bee sting with a sharp ridge at the peripheral margin of the reaction. Negative reactions may have some noticable swelling from the injected volume of fluid but erythema and the distinctive sharp ridge on palpation are absent.

Which is the better test? There are few clinical studies that directly compare the patient response rates to immunotherapy formulated based on each of the allergy testing methods. The limited information that is available suggests that the average response rate to immunotherapy vaccine based on serologic allergy testing is about 60% (55%-60% of the dogs treated show good to excellent response); however, if the immunotherapy vaccine is based on intradermal allergy testing about 68% (50%-86%) of treated dogs demonstrate good to excellent response. Perhaps the ideal allergy test would combine the information provided from both an intradermal and serologic allergy test to render a more complete representation of the dog's allergic condition. Indeed, some veterinary dermatologists have started performing both tests in every animal they evaluate for atopy.

Patch Testing

Patch testing is the method of choice for identifying allergens in humans; however, due to the limitations of veterinary species and the artificial dermatitis created by the occlusive bandaging needed, patch testing animals is extremely problematic and unreliable.

<u>Therapeutic trials</u> are often needed to eliminate an etiology as a cause of a patient's lesions.

<u>Flea allergy dermatitis</u> is one of the most common skin disease in animals. Many patients are extremely effective at removing fleas and flea dirt by grooming making it difficult to prove the existence of a flea infestation. Therefore, dogs with lumbar dermatitis and all pruritic cats should be treated aggressively for possible flea allergy dermatitis. Fipronil, imidicloprid, and selamectin work exceptionally well. Due to grooming and limitations of each product, treatments should be applied every 2-3 weeks in flea allergic animals. In heavily infested environments, it may take several weeks to reduce the number of emerging fleas. Owners may perceive this as lack of efficacy when in fact it is caused by the large number of fleas in the pupal stage.

<u>Feline Demodicosis</u> caused by Demodex gatoi is emerging as a contagious, pruritic feline alopecic dermatosis especially in the Southern United States. Demodex gatoi may be difficult to find. Therefore, a therapeutic trial consisting of lime sulfur dips applied weekly for 4 to 6 weeks is needed to eliminate Demodex gatoi as a possible etiology. Interestingly, alternative treatments do not seem to be efficacious for this parasite.

<u>Sarcoptiform mites</u> (Scabies, Notoedres, Cheyletiella, etc.) are uncommon but demonstrate regional variation in infection rates. Most mites are readily found on skin scrapes; however, in some cases mites may be difficult to find. A therapeutic trial with an effective therapy serves to eliminate this etiology as a differential.

<u>Food Allergy Trial</u>: currently, a dietary food trial is the only way to confirm or eliminate food allergy dermatitis as a cause of pruritus. There are no in vitro testing methodologies that correlate with clinical disease.

Limited ingredient commercial diets offer the benefit of being balanced and are suitable for long-term management. If the patient refuses to eat a variety of commercially available diets, a home cooked diet can often be used successfully. During the 12 week trial phase, the patient should be fed a simple diet consisting of one or two ingredients. It is important that the patient does not receive any additional treats or allowed access to wild game (hunting). After the 12 week trial, the patient should be assessed for overall improvement. It is usually best to definitively confirm or rule out food allergy by challenging the patient with its previous diet. A food allergic patient should demonstrate improvement during the 12 week food trial and relapse within hours to days of exposure to its previous diet. Once it is determined that the patient is food allergic, the patient should be transitioned to a balanced diet for long-term control. A balanced diet can be achieved by adding supplements to a home cooked diet or selecting a commercially prepared diet based on ingredients successfully used to control the allergy.