



CYTOLOGY REFERENCE GUIDE

Dechra Veterinary Products



CYTOLOGY

Cytology is a quick, simple-to-perform method to achieve useful information about the possible etiology of cutaneous lesions. This reference guide was created as a source of step-by-step recommendations to assist the veterinary team in proper collection and preparation of appropriate cytological samples. Dechra's goal is to allow you to confidently diagnose and prescribe utilizing cytology.

Many thanks go out to the many veterinarians who helped write and contribute photos.

ROUTINE STAIN MAINTENANCE:

1. Microorganisms can grow in stain solutions which can lead to artifact of bacterial overgrowth on slides. Recommended to maintain two separate staining set-ups: 'clean' cytology for blood smears and effusion cytology and 'dirty' cytology for otic, cutaneous impression and fecal smears. In a busy practice, stains may require changing weekly.
2. If samples are staining poorly, the stains should be replaced. Stain jars should be thoroughly cleaned and dried before filling with fresh stain solution.
3. Keep the lids closed on stain jars, especially the light blue fixative (first solution), as it evaporates quickly.

NOTE: Keep all unstained slides away from formalin as exposure will interfere with staining.

SKIN SCRAPING

(For ectoparasite identification)

MATERIALS NEEDED:

- Mineral oil
- Dull #10 scalpel blade
- Microscope slides
- Cover slips
- Microscope

STEPS:

1. Apply one to two drops of mineral oil onto the microscope slide. Smear scalpel blade in mineral oil and also apply small amount of mineral oil to area to be scraped.
2. Hold blade at a 45 degree angle to the skin surface and with moderate pressure, repeatedly scrape the lesion in the direction of hair growth and away from your hand. It may be necessary to clip the hair over the area to facilitate adequate sample collection.
 - a. Be careful not to press down on the blade, which could cut the skin.
3. Spread the collected sample uniformly in the mineral oil on the glass slide. Place a coverslip. Scan the slide with 4X and 10X objective lens. To improve identification of mites, increase the contrast (4a).
4. Methodically scan the entire sample by repeatedly moving from one side of the slide to the other side.
 - a. To enhance visualization of mites using the 4X and 10X objective lens, partially close the microscope's iris diaphragm and lower the condenser to provide increased contrast.

(Skin Scraping continued on next page)

SKIN SCRAPING

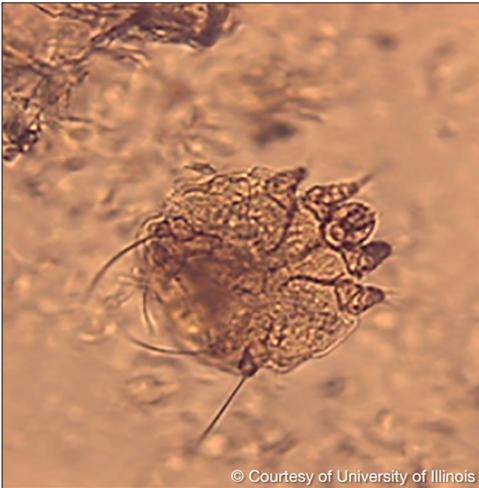
5. Two methods of skin scrapings are employed dependent on the mite suspected.
 - a. **Superficial skin scrapings** are performed when looking for mites that live on or just below the skin surface i.e., *Cheyletiella* spp, *Demodex gatoi*, *Demodex cornei*, *Sarcoptes* spp, *Notoedres* and trombiculoides mites. Using a mineral oil coated scalpel blade, superficially scrape a wide area of skin collecting a large amount of scale and crust. No need to cause bleeding when looking for surface-dwelling mites. Transfer sample to mineral oil on microscope slide. Place a coverslip over sample. Recommend scraping more than one location.
 - b. **Deep skin scrapings** are performed when looking for *Demodex canis* and *Demodex cati* they reside in hair follicles and sebaceous glands. Smaller areas are scraped but scrapes are deeper and repeated until slight bleeding (capillary ooze) is noted.

NOTE: Always use a new scalpel blade between patients to prevent transfer of infectious organisms such as methicillin-resistant *Staphylococcal* spp.

6. Tips to Increase Recovery of follicular *Demodex canis* and *Demodex cati* Mites
 - a. Look for newly formed lesions. Gently squeeze or pinch skin to express mites closer to the skin surface from deep within the hair follicle. Repeatedly scrape area until superficial bleeding is noted. Transfer sample to microscope slide.
 - b. Recommend scraping more than one lesion.
 - c. If lesions are in body locations that are difficult to scrape (around the eye or interdigitally), plucking hairs from the lesion with forceps can be performed. Place hairs in mineral oil on glass slide, apply coverslip and scan slide with 4X objective.
7. Tips to identify *Sarcoptes* Mites:
 - a. Scabies mites live in the stratum corneum (superficially) and are often few in number; sampling multiple lesions and larger areas can be helpful.
 - b. Obtain multiple, wide, superficial scrapings of crusted, or alopecic lesions on elbows, pinnal margins, and the ventral trunk.

SKIN SCRAPING

8. Tips to identify *Cheyletiella* Mites (“walking” dandruff):
 - a. *Cheyletiella* are relatively large surface-dwelling mites that are often found along the dorsum and are quite visible on 40X magnification.
 - b. Obtain wide, superficial scrapings of scaly lesions, and place these scrapings in mineral oil for microscopic examination.
 - c. *Cheyletiella* Mites can also be found using multiple applications of clear (not frosted) acetate tape onto scaly areas, or collected through the use of a flea comb.



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Adult canine sarcoptic (scabies) mite --
Sarcoptes scabiei



© Courtesy of University of Illinois

Canine *Sarcoptes scabiei* eggs
(*Sarcoptes scabiei* var. *canis*)

SKIN SCRAPING



Cheyletiella Mites



Adult feline sarcoptic mites Notoedres cati



Adult and nymph Demodex Mites



Demodex egg

ACETATE TAPE IMPRESSIONS

(For identification of surface mites i.e., cheyletiellosis and lice and microbes)

MATERIALS NEEDED:

- Clear (not-frosted) acetate tape or packing tape
- Microscope slides
- Microscope

STEPS:

1. Press sticky side of tape to hair and skin repeatedly covering a wide area. Take several samples from different affected areas.
2. Apply sticky side of tape to microscope slide. Examine entire slide with 40X and 100X magnification (4X and 10X lens).



© Courtesy of University of Illinois

Acetate tape impression

TRICHOGRAM (Hair Examination)

(Useful sampling technique for examination of morphology of hair shaft, identification of dermatophyte infection and ectoparasite infestation, and determination of phases of hair growth cycle.)

MATERIALS NEEDED:

- Mineral oil
- Curved small hemostats (Note: recommend not collecting samples with fingertips due to possible zoonotic potential of some diseases)
- Microscope slides
- +/- Coverslip
- Microscope

STEPS:

1. Grasp a small number of hairs and epilate in the same direction as hair growth to prevent breakage.
2. Lay hairs in mineral oil on glass slide in same orientation as plucked. Place coverslip.
3. Examine entire length of hair from root to tip. Lower condenser +/- partially close iris diaphragm to improve contrast while scanning the sample.
 - a. Hairs have either anagen (growing phase of hair cycle) or telogen (resting phase of hair cycle) bulbs – healthy adult animals have a mixture of both
 - i. Anagen bulbs – rounded, smooth, glistening, often pigmented and soft, the root may be curved
 - ii. Telogen bulbs – club- or speared-shaped, rough-surfaced, non-pigmented, and generally straight

TRICHOGRAM

3.
 - b. A normal hair is uniform in diameter and tapers gently towards the distal tip.
 - c. Melanin granules (hair pigmentation) should be relative uniform in size and evenly distributed.



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Trichogram Prep



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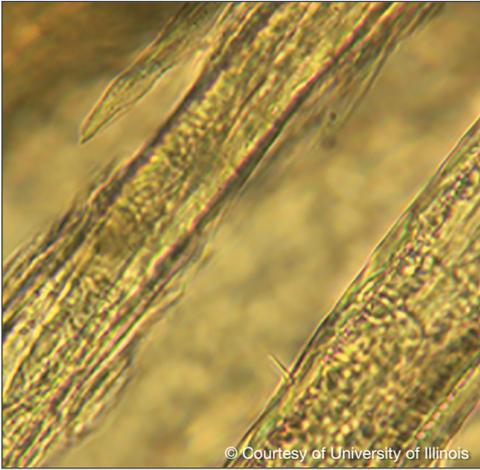
Anagen Bulb



© Courtesy of Kimberly Coyner, DVM, DACVD

Telogen Bulb

TRICHOGRAM



© Courtesy of University of Illinois

Refractile macroconidia and hyphae in dermatophyte-infected hair



© Courtesy of Kimberly Coyner, DVM, DACVD

Frayed distal tip of hair secondary to excessive grooming (self-inflicted alopecia)

TRICHOGRAM

1. Hair plucks can be collected from areas difficult to scrape, such as paws when investigating for demodicosis. *Demodex* Mites can be found clinging to the hairs, especially the lower portion of the hair shaft. May see parasitic eggs (lice, *Cheyletiella* spp) attached to hair.
2. If suspect dermatophytosis, look for abnormal or thickened hairs with irregular borders. Use 40X objective lens to look for refractile arthroconidia and hyphae. Microscopic identification of dermatophytes is quite difficult and can be easily missed.
3. Large and/or irregular clumps of melanin within the hair shaft are suggestive of color dilution alopecia.
4. Keratin casting around a hair is common in sebaceous adenitis.

SKIN SURFACE CYTOLOGY

(Important test to evaluate for bacterial and yeast infections, and to characterize the inflammatory infiltrate)

NOTE:

- Neutrophilic or pyogranulomatous inflammation suggests an infectious or inflammatory process.
- Eosinophilic infiltrate suggests a hypersensitivity or parasitic dermatitis, but can also be seen in other dermatoses, such as some drug reactions.
- Acantholytic (single keratinocytes that have lost their attachment to neighboring keratinocytes and become rounded) cells are found most commonly with pemphigus complex but can also be seen with chronic bacterial or dermatophyte infections. Skin biopsies for histopathology should be performed when rafts of acantholytic cells are found.

MATERIALS NEEDED:

- Hair clippers or scissors
- Microscope slides
- Modified Wright's stain (Diff-Quik)
- Cotton-tipped applicator
- 25 gauge needles
- Acetate tape
- Dull No. 10 scalpel blade

STEPS:

1. If needed, carefully clip or scissor hairs off lesions. Remember, that fluid-filled lesions are fragile and will possibly rupture with aggressive hair removal.
2. There are several different techniques to obtain cytological samples. Method selection is dependent on types of lesions present. More than one sampling method may be used in different areas on the patient.

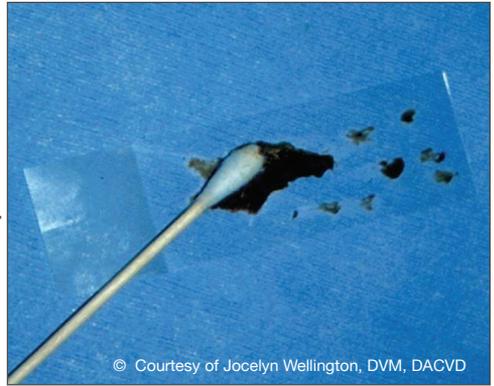
(Skin Surface Cytology continued on next page)

SKIN SURFACE CYTOLOGY

2. a. Direct smears:
 - i. Usually performed for fluid-containing lesions (i.e., pustules, blisters, vesicles)
 - ii. Fluid or exudate, aspirated from a lesion with a sterile 25 gauge needle, is placed on a microscope slide. The sample is spread on the slide by placing a second slide perpendicular to the first slide and with gentle pressure, sliding it along the length of the first slide (squash preparation). Applying minimal pressure to the slide will reduce damage to fragile cells while creating a good monolayer of cells.
 - iii. The sample is allowed to air dry. Once dry, it is stained with modified Wright's stain (i.e. Diff-Quik).
- b. Impression or Touch Preparations:
 - i. Used for exudative or greasy lesions, after removal of a crust or scab, fluid expressed from a lesion or after rupture of a pustule or vesicle with a sterile 25 gauge needle.
 - ii. Multiple imprints are made on the same slide by repeatedly touching the slide to the lesion.
 - iii. Can touch cut-surface of biopsy sample repeatedly to a microscope slide after first blotting off blood and other fluid.
- c. Swab Cytology:
 - i. Typically used for examination of debris and/or exudate from an otic canal or fistulous tracts.
 - ii. Moistening the swab with sterile saline prior to use can improve sample collection from skin lesions. Rub applicator over skin lesion.
 - iii. Insert the cotton-tipped applicator into the draining tract or otic canal and gently rotate.
 - iv. Smears are prepared by gently rolling swab over glass slide. Do not smear or rub the swab in a back and forth motion as this will cause cell damage (lysis).
- d. Dry Skin Scraping (no mineral oil):
 - i. Use dull scalpel blade or medical spatula to collect samples from under a crust, surface of exudative or seborrheic skin lesion or a claw fold.
 - ii. Scrape surface of lesion with scalpel blade.
 - iii. Gently smear sample in one direction onto glass slide.
 - iv. Samples collected by the above techniques are either air-dried or heat-fixed and then stained with a Romanowsky-type quick stain (e.g., Diff-Quik).

SKIN SURFACE CYTOLOGY

2. e. Acetate tape preparations:
 - i. Useful technique where lesions are dry or flaky or in difficult to access areas such as interdigital webs or skin folds. Very useful for evaluating for *Malassezia* spp.
 - ii. Use either clear acetate tape or packing tape. Tear strip of tape slightly shorter than the length of a microscope slide. Firmly press tape to skin surface and then remove.
 - iii. Place tape adhesive side down onto a microscope slide. Lift edge of tape and apply one or two drops of the final stain (purple) of Diff-Quick to the microscope slide. Read soon after stain applied as the adhesive may separate from the tape making it difficult to clearly examine the sample.
3. Samples that are greasy, flaky or collected from otic canals may be heat-fixed before staining. A lighter is placed near the bottom of the slide for 1-2 seconds. Wipe black residue from the slide before staining. Alternatively, slide can be placed on a warming plate for several minutes.
4. Staining Procedure for Any Quick Stain
 - a. Dip slide in Solution I (fixative – methanol) 5-8 times, for one second each time. Allow excess fixative to drain off into jar, then touch end of slide to paper towel to wick away excess stain. This step prevents dilution of the next stain jar. Do not rinse. Methanol fixes cells to microscope slide.
 - b. Dip slide in Solution II (red or pink) 5-8 times for one second each time. Allow excess stain to drain off into jar, then touch end of slide to paper towel to wick away any remaining stain. Do not rinse.
 - c. Dip slide in Solution III (blue) 5-8 times for one second each time. Allow excess to drain off into jar.
 - d. Rinse slide by dipping multiple times in distilled water or turn slide over and place under gentle stream of tap water.
 - e. Allow slide to air dry or use hand-held hair dryer on low heat to speed drying time.

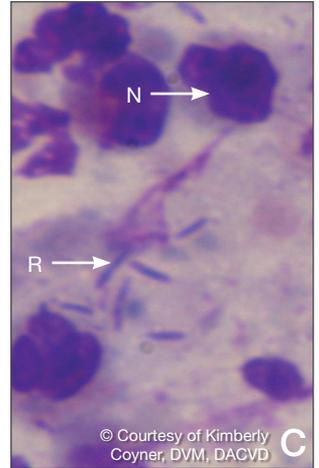
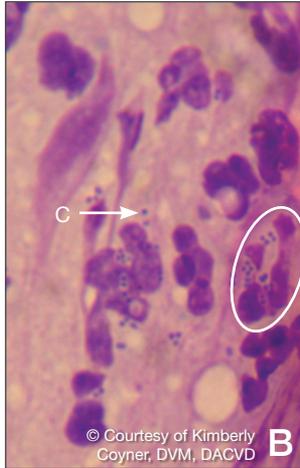
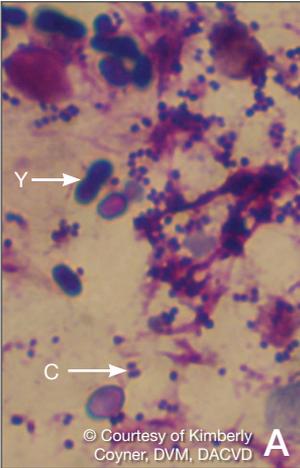


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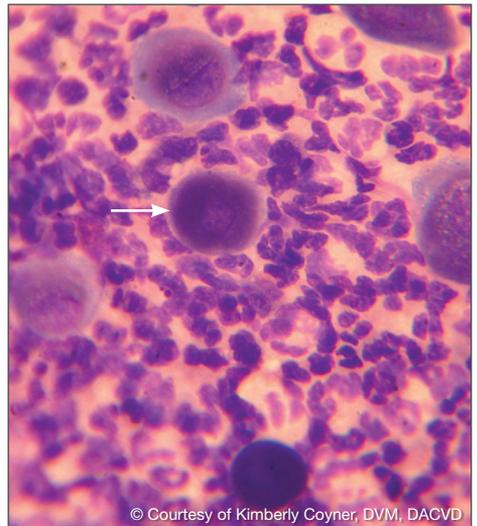
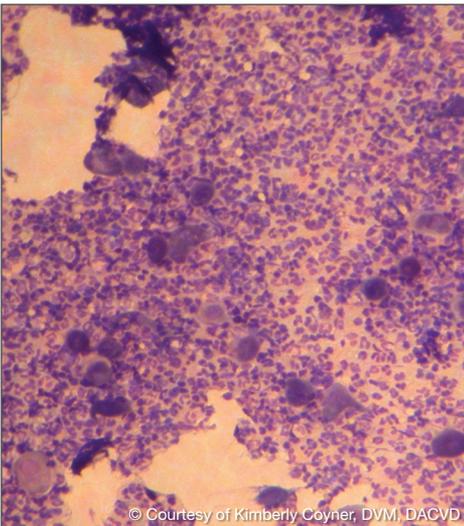
Collected otic debris on the slide

SKIN SURFACE CYTOLOGY

5. Scan sample with 4X to 10X lens to find a representative area (area with concentration of debris or cells), and examine that area with 40X to 100X lens.



Diff-Quik stained skin impression smears with: A) yeast and cocci; B) neutrophils and intracellular cocci; C) neutrophils and rod bacteria



Diff-Quik stained impression smears showing neutrophils and acantholytic cells at 100X (left) and oil-immersion magnification (right)

OTIC CYTOLOGY

(To evaluate for mites, microorganisms and inflammatory cells)

MATERIALS NEEDED:

- Microscope Slides
- Mineral Oil
- Cotton-tipped applicators (swabs)
- Coverslips

TWO TECHNIQUES:

EVALUATION FOR OTODECTES (EAR MITES):

1. Use cotton-tipped applicator to gently remove large amount of debris from otic canal. Mix sample evenly in mineral oil on glass slide. Place coverslip.
2. Scan entire slide with 4X or 10X lens for mites or eggs.

EVALUATION FOR BACTERIAL OR YEAST INFECTIONS

1. Insert cotton-tipped applicator into otic canal and gently rotate to remove exudate and debris. Roll sample onto glass slide. Samples should be collected from each ear.
2. Sample can be heat-fixed to prevent sample loss during staining when primarily ceruminous debris collected.
3. Stain slide with Diff-Quik similar to other cytology preparations. Let air dry or dry with hand-held hair dryer on low heat.

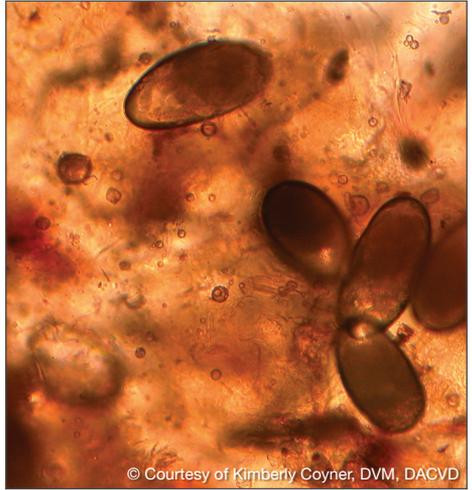
OTIC CYTOLOGY

4. Scan slide on 100X magnification (10X objective lens) for representative areas. Yeast and bacteria are often found in clumps of keratinocytes.
5. Identify leukocytes, red blood cells, cornified epithelial cells, bacteria and yeast with 40X objective.
6. Further evaluate cells and microorganisms with higher magnification oil immersion lens (100X) to enhance visualization of the morphologic characteristics of bacteria and to identify phagocytosis by neutrophils.
7. Examine 5-10 areas to estimate the numbers of bacteria, yeast and/or leukocytes. Record estimates in medical file at each evaluation to monitor response to therapy at subsequent examinations.

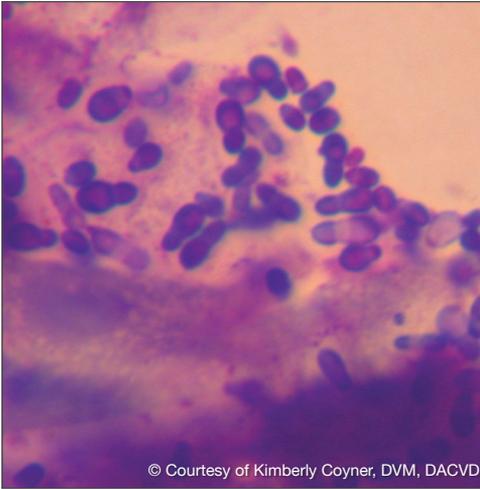
OTIC CYTOLOGY



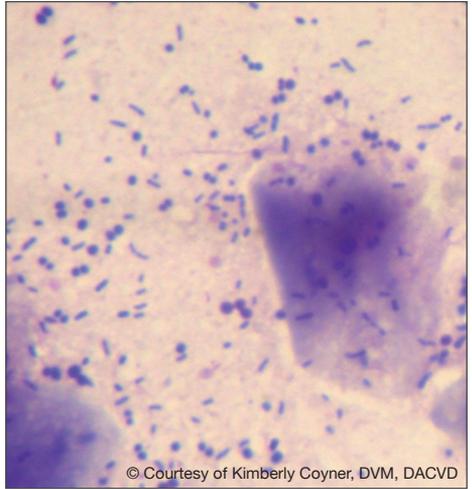
Ear Mites



Ear Mite Eggs



Otic cytology (Diff-Quik stain) showing yeast.



Otic cytology (Diff-Quik stain) showing cocci and rods (mixed) bacterial population.

DIASCOPYY

(A simple quick test to determine if cutaneous erythema due to vascular dilation or extravasation of red blood cells)

MATERIALS NEEDED:

- Microscope Slide

METHOD:

1. Press microscope slide firmly over erythemic area.
2. If skin blanches (whitens), erythema due to inflammation.
3. If skin does not blanch, erythema is due to hemorrhage (i.e., vasculopathy)

USING THE MICROSCOPE - GOLDEN RULES

STEPS:

1. Routine microscope cleaning and maintenance is important.
2. Use immersion oil sparingly and ONLY use with the oil immersion objective. If you are unsure which lens this is, look carefully at the lens – on most modern microscopes the oil immersion lens will actually bear the word 'Oil' on it. It is usually 100X, but some microscopes may also have a 50X oil immersion objective.
3. Do not leave the oil immersion lens in oil for any longer than necessary. If left in oil overnight, irreparable damage can occur.
4. Do not put the 4X, 10X or 40X objectives into the immersion oil. If this occurs, immediately clean the lens.
5. Clean the lens with proper lens tissue immediately after use.
6. Leave the low power objective in place when finished viewing and lower the stage.
7. If you spill solvents or chemicals, including mineral oil, they should be cleaned away immediately.

SKIN CONDITION & DECHRA TOPICAL SOLUTION

Dechra Topical	Yeast	Bacteria	Inflammation	Pruritus	Barrier Repair	Greasy, Waxy	Follicular Condition	Seborrhea
MICONAHEX + Triz® Shampoo, Spray & Wipes	2% Miconazole	2% Chlorhexidine, 2% Miconazole, USP TrizEDTA®			Ceramide Complex			
MAL-A-KET® Shampoo & Wipes	1% Ketoconazole, 2% Acetic Acid	2% Chlorhexidine, 2% Acetic Acid				2% Acetic Acid		
MAL-A-KET® Plus TrizEDTA® Spray Conditioner	1% Ketoconazole	2% Chlorhexidine, USP TrizEDTA						
MALACETIC® Shampoo, Spray & Wipes	2% Acetic Acid, 2% Boric Acid	2% Acetic Acid, 2% Boric Acid				2% Acetic Acid, 2% Boric Acid		
MALACETIC ULTRA® Shampoo & Spray	0.15% Ketoconazole, 1% Acetic Acid, 2% Boric Acid	1% Acetic Acid, 2% Boric Acid	1% Hydrocortisone	1% Hydrocortisone	Ceramide Complex	1% Acetic Acid, 2% Boric Acid		
TrizCHLOR® 4 Shampoo Spray & Wipes	4% Chlorhexidine	4% Chlorhexidine, USP TrizEDTA						
TrizCHLOR® 4HC Shampoo & Spray	4% Chlorhexidine	4% Chlorhexidine, USP TrizEDTA	1% Hydrocortisone	1% Hydrocortisone				
DERMABENS® Shampoo		2.5% Benzoyl Peroxide, 1% Sulfur, 1% Salicylic Acid		2.5% Benzoyl Peroxide	Ceramide Complex	2.5% Benzoyl Peroxide, 1% Sulfur	2.5% Benzoyl Peroxide	2.5% Benzoyl Peroxide, 1% Sulfur, 1% Salicylic Acid

CONDITION PRESENT IN THE EAR & CHOICE OF DECHRA OTIC

<i>Dechra Otic or Flush</i>	<i>Yeast</i>	<i>Bacteria</i>	<i>Inflammation</i>	<i>Pruritus</i>	<i>Greasy, Waxy</i>	<i>Moist</i>	<i>General Debris</i>
MALACETIC ULTRA® Otic Cleanser	Ketoconazole, Acetic Acid	Acetic Acid	Hydrocortisone	Hydrocortisone, Ceramide Complex	Acetic Acid	Acetic Acid	
TrizULTRA +KETO® Flush	Ketoconazole	USP TrizEDTA®	Non-acidic, Non-stinging				
MALACETIC® Otic Cleanser	Acetic & Boric Acid	Acetic & Boric Acid			Acetic & Boric Acid	Acetic & Boric Acid	
MAL-A-KET® Plus TrizEDTA® Flush	Ketoconazole	Chlorhexidine; USP TrizEDTA					
TrizEDTA® Aqueous Flush		USP TrizEDTA	Non-acidic, Non-stinging				
EPIKLEAN® Ear Cleanser						Denatured Alcohol, Salicylic Acid	Propylene Glycol
TrizCHLOR® Flush		Chlorhexidine; USP TrizEDTA					
KLEAROTIC Ear Cleanser					Squalane		



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Photos courtesy of Dr. Kimberly Coyner, DVM, DACVD, Dr. Jocelyn Wellington, DVM, DACVD and the University of Illinois.

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