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## BETTER RESULTS FROM FUNGAL CULTURES

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Dermatophyte culture is the indispensable diagnostic test in feline practice. Cultures should be performed to screen, diagnose, and monitor patients presenting for a variety of skin lesions. Definitive identification avoids false empirical diagnosis of dermatophytosis and subsequent failure to identify and treat the true cause of disease. Culture also helps avoid missed diagnosis in cases with atypical presentation, as well as subsequent unpleasant conversations with angry clients. Culture allows for specific identification of dermatophyte and therefore assists in recognition of the potential source of infection. From a practice management perspective in-house fungal culture can be a profit center for the hospital, as well as a source of pride for the technical staff.

Unfortunately many veterinarians have been frustrated by the high number of negative tests associated with fungal cultures. This may be because of common errors resulting in false negatives; however, a high rate of negatives should be expected if practitioners are collecting cultures frequently enough. In dermatology referral practices less than 10-15% of dermatophyte cultures are positive.

### WHEN TO CULTURE?

In certain regions of the USA (warm, humid; South) where dermatophytosis is prevalent, culture by DTM is part of the minimum dermatologic data base for any cat presenting for any skin disease. For the rest of the USA, and other low incidence regions, cultures should be performed on any cat with clinical signs compatible with dermatophytosis, including erythematous papules, pustules, or alopecia. Less common presentations include hyperpigmentation, milium dermatitis, wide-spread exfoliative erythroderma, symmetrical non-inflammatory alopecia ("psychogenic alopecia"-like lesions), eosinophilic plaques, indolent lip ulcers, chin acne, clawbed disease, pruritus and inflammation of the concave pinnae. Because dermatophytes occasionally cause unusual lesions, culture should be submitted any case that failed to respond to appropriate therapy for initial diagnosis. Likewise, consider culture on the first visit for any dermatology case for which you are the second opinion. Because of the zoonotic potential, dermatophyte culture should also be used to screen cats belonging to owners diagnosed with ringworm by their physician, hospice/therapy cats, or cats in households with high risk humans. Because of the difficulty in managing ringworm in multicat homes and catteries, collect samples routinely to screen newly acquired cats or kittens prior to introduction to household. Active show cats should be cultured at least every 2-3 months.

### WHAT TO COLLECT?

There are two common methods for collecting culture material: the hair pluck and the toothbrush. Hair plucking consists of inoculating media with hairs plucked with hemostats from lesional skin. Ideally collect 10-20 hairs to maximize the chance of a positive culture. Collect hairs from the periphery of new or expanding lesions. Hairs with erythematous papules at their base are good targets. The ideal sample is hair that fluoresces under a wood's lamp.

Push the hairs gently into the media to get good contact, but avoid burying them. The toothbrush technique is used for animals without lesions or non-classic lesions. A new toothbrush wrapped in plastic is selected and brushed against the direction of hair growth near the base of hairs. Approximately 30 strokes are sufficient; stop earlier if large amount of hair or scale builds up. Press the bristles into the culture media. Alternatively clip the bristles from the brush and inoculate the media with them as if they were hairs.

### WHAT MEDIA TO USE?

Several commercial culture systems are available. My preference is for a flat culture plate, such as Derm-Duet or Sab-Duet (BactiLab). These plates contain two separate compartments with different media: Dermatophyte test media (good selection for dermatophytes) and Sabouraud's dextrose agar (produces good macroconidia for microscopic evaluation). These plates are easy to inoculate by either technique, easy to sample for microscopic identification, and cannot be closed tightly. Screw top culture systems are difficult to inoculate with toothbrush, harder to sample later, and if closed tightly they are prone to overgrowth with yeast or bacteria.

### HOW TO STORE?

The ideal environment for growing dermatophytes, is a dark drawer, cabinet or cardboard box, kept at 75.2 – 80.6°F (24-27°C), which is slightly warmer than room temperature. A recent study suggested that room temperature could result in slower growth and false negative samples. This warmth can be provided by a light bulb or ambient "hot rocks" designed for use in pet reptiles. Check the temperature to be sure it is not too hot. Ultra-violet light is the biggest enemy of dermatophyte cultures. Never leave cultures exposed on a window sill or other direct light for longer than needed to examine or sample the plates. Also prevent cultures from drying by placing a small dish of water in the drawer, cabinet, or box.

Most dermatophyte species will form colonies within 2-7 days. In general Trichophyton species take a bit longer to grow than Microsporum species. Slow growing variants, particularly in animals receiving antifungal therapy may take even longer. Keep cultures for at least 21 days. Tossing plates that have not "changed colors in 5 days," as suggested by some culture system literature, will reliably result in false negative cultures, especially in samples kept at room temperature.

### DERMATOPHYTE OR CONTAMINANT?

There are three critical features to evaluate when determining if that fuzzy stuff on your culture plate is a pathogen or a contaminant by non-pathogenic saprophyte. First, evaluate colony morphology. Dermatophyte colonies tend to be fluffy and white; occasionally slightly pink or off-white. Saprophytes can be white, but many of the common ones are green, black, or brown. If a colony is heavily pigmented it is likely a contaminant.

Second, evaluate media color change. Color change is an area that creates a lot of confusion and false-positive diagnoses. Color change is the result of a pH indicator that turns red when protein is metabolized by the fungal colony. Since dermatophytes, which grow on keratin, preferentially use protein in the media, the color change should occur simultaneously with colony growth. Saprophytes, such those

that commonly turn your bread green before you finished the loaf, prefer carbohydrates and don't start metabolizing protein until the carbohydrate is exhausted. Consequently color change will occur with contaminants, just not at the time of initial colony growth. For this reason, plates must be inspected daily. If a colony appears, turn the plate over and look through the clear bottom; if the area immediately under the colony has changed colors then the colony is likely dermatophyte; if the media is still yellow the young colony is likely saprophyte. Color change itself is not diagnostic for pathogen; collar change simultaneous with colony growth is strongly supportive.

The third and most conclusive feature to evaluate is microscopic morphology of macroconidia produced by the colony. *Microsporum* species form canoe shaped macroconidia with multiple parallel septae. *Trichophyton* may be more difficult to recognize, but are described as cigar-shaped. Common contaminants express a variety of different shapes: dandelion burst patterns, irregular bulbs, non-uniform incomplete septated macroconidia, etc. The best way to collect a sample for microscopic identification is to place a drop of lactophenol cotton blue stain directly on a glass slide. Take a piece of clear cellophane tape (not frosted) and gently touch the sticky side to the top of the colony (try that with a screw-top culture). Place the tape, sticky side down, directly on to the slide and examine through the tape. Note that phenol can damage microscope lens, so avoid direct contact between excess stain and the objective.

## SUMMARY

Take frequent culture samples. Use flat culture plates. Store the cultures properly in a warm, dark, moist environment; window sills are a big mistake. Examine your garden daily. Color change alone is not a diagnostic criteria; color change simultaneous with colony growth is. Don't toss your plates too soon. Practice identification of contaminants and dermatophytes under the microscope.

## RECOMMENDED READING

1. Moriello, KA. Symposium on feline dermatophytosis. *Veterinary Medicine* 98(10): 844-891. 2003.
2. Davise H. *Medically Important Fungi: Guide to Identification*, 4<sup>th</sup> ed, American Soc Microbiology, 2002.
3. St-Germain G. *Identifying Filamentous Fungi: Clinical Laboratory Handbook*. Star Publishing, 1995.