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Fungal Cultures for Diagnosing Dermatophytosis

Dermatophytosis is the most common infectious and contagious skin disease of dogs and cats. Its 3 pathogens of importance are *Microsporum canis*, *M gypseum*, and *Trichophyton* species. Dermatophytosis has some classic clinical presentations, but the disease is pleomorphic. A fungal culture is a reasonable core diagnostic test in any animal presenting with hair loss and/or follicular skin disease.

Dermatophytosis is highly contagious and easily transmitted, and in the author's practice is seen increasingly in dogs that go to dog parks or "doggy day care," in animals being boarded, and those from shelters or rescue organizations. With respect to the latter, a fungal culture is as important as screening new arrivals for infectious viral or parasitic diseases.

What You Will Need

- Individually wrapped toothbrushes
- Sterile forceps
- Alcohol wipes
- Sterile cotton-tipped swabs
- Fungal culture medium
- Acetate tape (clear or frosted)
- Glass coverslips
- Lactophenol cotton blue or new methylene blue stain
- Markers
- Plastic self-closing sandwich-size bags
- Clear plastic storage box
- Inexpensive digital fish tank thermometer

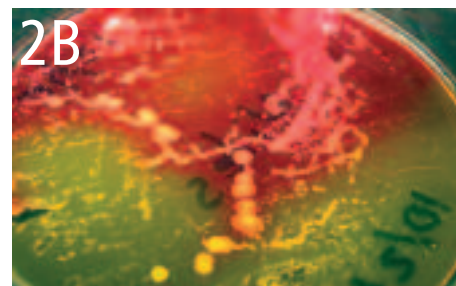
STEP BY STEP FUNGAL CULTURES

Culture Area

1 Designate a site for in-house fungal cultures. Cultures must be examined daily, so the site should be near the clinic's main laboratory. It is strongly recommended that culture plates be kept in plastic containers. Inexpensive shoe-box-size storage containers are ideal because they are small enough to fit in most laboratories and can be disinfected regularly. Keeping cultures in a single site minimizes the possibility that they will get lost or not be examined and the plates can be checked daily. Also, the temperature inside the boxes will be warmer than the ambient room temperature, helping incubate the plates.

Preventing Incubation Problems

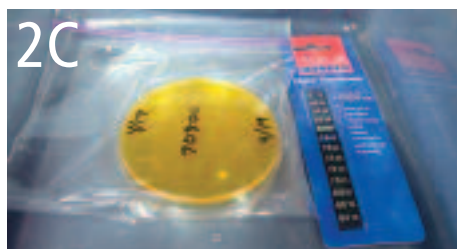
2 Several problems are commonly encountered with in-house incubation of fungal cultures. These include dehydration of plates; too cold an ambient temperature, which results in poor sporulation; and contamination of plates with media mites. Problems with dehydration can be minimized by keeping plates in an airtight container (A). Placing individual plates in a self-sealing sandwich bag can help solve this common problem and also prevent and/or contain media mite infestations. Media mites are microscopic mites associated with food or free-living in the environment. Possible sources of contamination include the animal



itself, food sources in the clinic, and environmental debris. The source is often not identified. In the author's laboratory, media mite infestations are commonly associated with cultures from outdoor animals or large animals. One telltale sign is a serpiginous line of red on the fungal culture medium (B).

It is widely recommended that cultures be incubated at "room temperature," but room temperature varies among clinics and seasonally. Ideally, incubation temperature should remain at 75°F to 80°F. A small, inexpensive digital fish tank thermometer is a good tool for monitoring the temperature (C; see page 56). In my own laboratory, the thermometer is

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left in its original packaging for easy cleaning, but another option is to keep the thermometer in a plastic bag to prevent contamination.

Medium Selection

3 Many commercial culture media are available for use in veterinary practice. In my opinion, the criteria for selection should be: getting the most volume for the money; the ease in which plates can be inoculated and sampled; whether plates have a red color indicator; and shelf life. In general practice, I recommend the use of dermatophyte test medium (DTM). A glass jar can be used with this medium if the jar's opening allows for insertion of a toothbrush for inoculation and easy sampling. Jars can be problematic, however, because closing them too tightly often leads to an overgrowth of bacteria or yeast. Plates with small surfaces should be avoided because they make recognizing suspect colonies more difficult—the entire surface of the plate will often turn red very rapidly, making it hard to monitor colonies. Note that plates that require refrigeration are at risk of being ruined if they are accidentally frozen; store the plates on the refrigerator door or in the vegetable crisper.

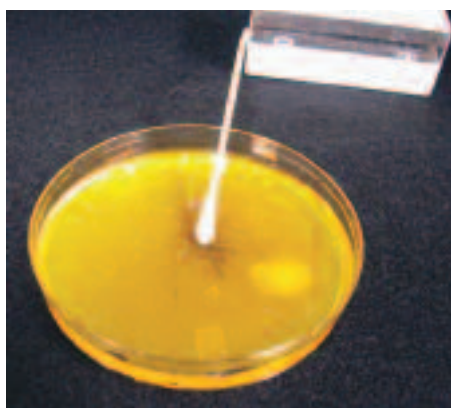


DTM = dermatophyte test medium

Inoculation Options

4 There are 2 methods for sampling lesions or animals for dermatophytosis. One is to pluck individual hairs and the other is to use a toothbrush or equivalent for sampling the entire body or a site. Individual hair sampling is best suited for Wood's-lamp–positive hairs or hairs that are grossly abnormal. If the area is heavily contaminated, wiping it with alcohol may decrease contaminant growth. Individually wrapped toothbrushes, which are mycologically sterile, can be purchased in bulk at dollar discount stores or from hotel supply companies. Alternatively, sterile gauze sponges with a tight weave can be used.

- **Plucking individual hairs:** Grasp the hair in the direction of growth and tug gently, trying to get the root bulb. Gently press the hair onto the growth medium.

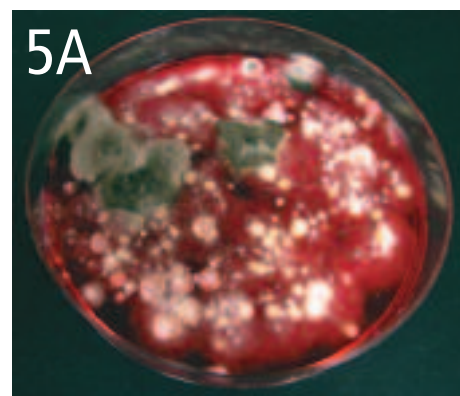


- **Toothbrush or gauze sponge sampling:** This technique can be used to sample a large lesion or the entire body of an animal. Comb or wipe the coat aggressively until hair is clearly visible on the surface or in the bristles. Be sure to sample near the eyes, in the ears, and between the toes. When inoculating a plate with a toothbrush or gauze, gently stab the bristles or press the gauze over the entire surface of the plate. Be careful not to press too hard or the medium will be pulled off by the toothbrush. If the hair coat is visibly contaminated with debris, wipe it with a cloth to minimize contaminant growth swarming the culture plate.

Caution: When using a whole-body sampling technique, always sample suspect lesional areas *last* to avoid mechanical spread of infectious material.

Monitoring Growth & Incubation

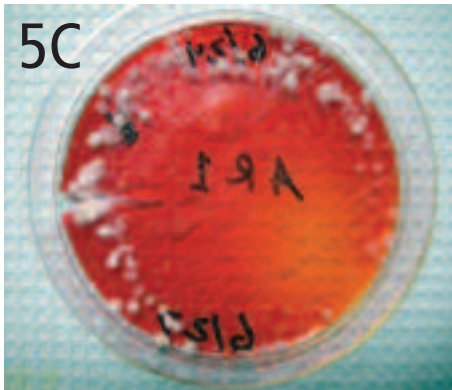
5 Plates should be checked daily for fungal culture growth. A good practice is to designate a technician to check cultures daily and keep a log of color change and growth on the plates. When using DTM, look for white or buff-colored colonies (A) that develop a red color change around them as they grow. This is a key characteristic of pathogens. Fungal culture colonies that are grossly or microscopically pigmented (as are the brown areas in A) are not pathogens, they are contaminants. All pale or buff-colored colonies should be microscopically examined. Cultures from animals that live outdoors or in heavily contaminated environments may be rapidly overgrown with contaminants, masking or making it difficult to identify a pathogen. Close inspection of this plate (B) revealed *M canis* was present, but as the colonies were swarmed by contaminants, this



finding could easily have been missed. New cultures should be performed in such cases.

Note that while most pathogens will grow and sporulate within 7 to 10 days, *Trichophyton* cultures often take up to 14 days. Plates should be kept for at least 14 days and preferably for 21 days.

In my experience, fungal culture growth in animals undergoing treatment can be delayed, often developing during week 3 of incubation. In addition, colonies may look grossly atypical. The colonies will still be pale, but sporulation may be significantly delayed. The plate pictured (C) is from a cat receiving topical therapy. Note the ring of white colony growth on the margins of the plate. Microscopically, these were identified as *M canis* colonies, which did not grow until more than 15 days after inoculation.



PROCEDURE PEARL

Fungal culture plates are easier to inoculate if they are allowed to warm up to room temperature beforehand.

PROCEDURE PEARL

When inoculating a plate with a toothbrush, be careful not to press too hard or the medium will be pulled off by the toothbrush.

PROCEDURE PEARL

Allowing stained samples to sit for 5 to 10 minutes before examining them allows for intracellular accumulation of stain and easier identification.

Sampling for Microscopic Identification

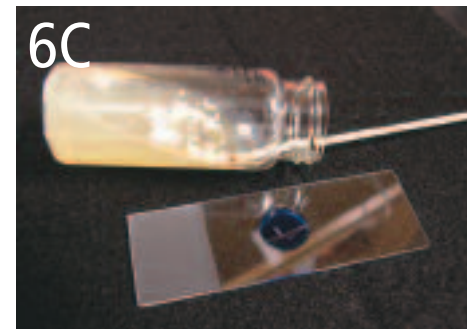
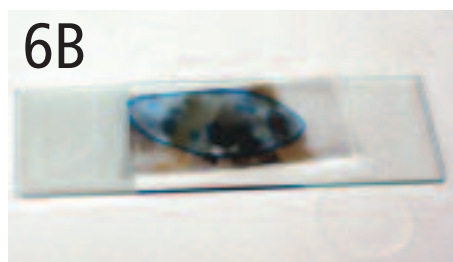
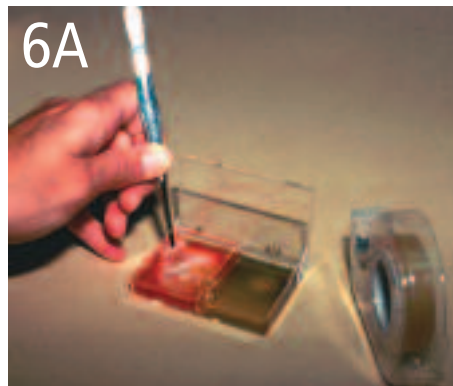
6 The most common pathogen encountered in private practice is *Microsporum*, and this genus is relatively easy to identify microscopically. *Trichophyton* species can be more difficult to determine. A valuable resource to aid in identification is www.doctorfungus.org.

Acetate tape is most commonly used to collect samples (A). Either clear or frosted tape can be used, but the latter requires more manual dexterity. The key to good visibility is sandwiching the sample between 2 drops of stain. Place a drop of stain on a slide and press the sticky side of the tape to the edge of the colony. If clear acetate tape is used, the tape is placed sticky-side down over a drop of stain, another drop of stain is added to the

nonsticky side, and then the glass coverslip is placed. If frosted tape is used, the tape is placed sticky-side up, a drop of stain is added on top of the tape, and the glass coverslip is added (B). The coverslip can be placed directly over the tape. These upside-down preparations with frosted tape work as well as those with clear tape. To prevent the tape from adhering to the microscope, the entire surface of the tape should be coverslipped. Lactophenol cotton blue stain is recommended because the phenol will kill the spores; however, new methylene blue stain can also be used.

When it is not possible to sample the plate with acetate tape, a small sample of the colony will need to be collected using a sterile inoculating loop, small forceps, or even a stick. The sample is transferred to a glass slide where a drop of stain has been placed (C, D).

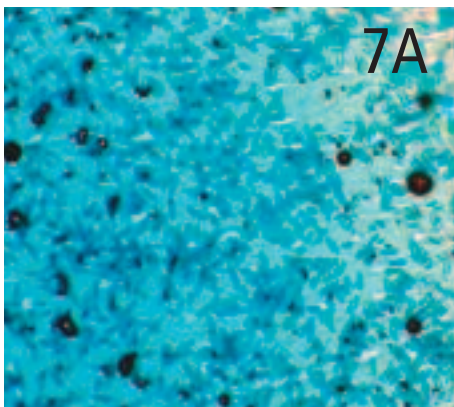
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Microscope Technique

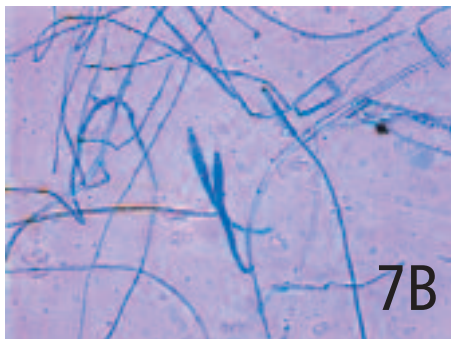
7 I examine all slides at low power in order to find areas where the hyphae have stained well; then move to a higher power for final examination. *M. gypseum* produces large numbers of macroconidia (A). *M. canis* produces fewer macroconidia (B), and the walls are thicker and rough.

If the laboratory temperature is too cold, *M. canis* may not sporulate and all that will be seen are hyphae with thick, finger-like projec-



tions on the ends (C). This is evidence that the colony is trying to sporulate. Increasing the temperature in the laboratory will help speed the process. ■

Article archived on www.cliniciansbrief.com



Baytril® (enrofloxacin)

Antibacterial Tablets For Dogs and Cats

BRIEF SUMMARY:

Before using Baytril Tablets, please consult the product insert, a summary of which follows:

CAUTION:

Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.
Federal law prohibits the extralabel use of this drug in food-producing animals.

INDICATIONS:

Baytril® (brand of enrofloxacin) Antibacterial Tablets are indicated for the management of diseases associated with bacteria susceptible to enrofloxacin. Baytril Antibacterial Tablets are indicated for use in dogs and cats.

CONTRAINDICATIONS:

Enrofloxacin is contraindicated in dogs and cats known to be hypersensitive to quinolones.
Dogs: Based on the studies discussed under the section on Animal Safety Summary, the use of enrofloxacin is contraindicated in small and medium breeds of dogs during the rapid growth phase (between 2 and 8 months of age). The safe use of enrofloxacin has not been established in large and giant breeds during the rapid growth phase; large breeds may be in this phase for up to one year of age and the giant breeds for up to 18 months. In clinical field trials utilizing a daily oral dose of 5.0 mg/kg, there were no reports of lameness or joint problems in any breed. However, controlled studies with histological examination of the articular cartilage have not been conducted in the large or giant breeds.

ADVERSE REACTIONS:

Dogs: Two of the 270 (0.7%) dogs treated with Baytril® (brand of enrofloxacin) Tablets at 5.0 mg/kg per day in the clinical field studies exhibited side effects, which were apparently drug-related. These two cases of vomiting were self-limiting.

Post-Approval Experience: The following adverse experiences, although rare, are based on voluntary post-approval adverse drug experience reporting. The categories of reactions are listed in decreasing order of frequency by body system.
Gastrointestinal: anorexia, diarrhea, vomiting, elevated liver enzymes
Neurologic: ataxia, seizures

Cats: No drug-related side effects were reported in 124 cats treated with Baytril® (brand of enrofloxacin) Tablets at 5.0 mg/kg per day for 10 days in clinical field studies.

Post-Approval Experience: The following adverse experiences, although rare, are based on voluntary post-approval adverse drug experience reporting. The categories of reactions are listed in decreasing order of frequency by body system.
Ocular: Mydriasis, retinal degeneration (retinal atrophy, attenuated retinal vessels, and hyperreflective tapeta have been reported), loss of vision. Mydriasis may be an indication of impending or existing retinal changes.
Gastrointestinal: vomiting, anorexia, elevated liver enzymes, diarrhea
Neurologic: ataxia, seizures

Behavioral: depression, lethargy, aggression

For medical emergencies or to report adverse reactions, call 1-800-422-9874.

ANIMAL SAFETY SUMMARY:

Dogs: Adult dogs receiving enrofloxacin orally at a daily dosage rate of 52 mg/kg for 13 weeks had only isolated incidences of vomiting and inappetence. Adult dogs receiving the tablet formulation for 30 consecutive days at a daily treatment of 25 mg/kg did not exhibit significant clinical signs nor were there effects upon the clinical chemistry, hematological or histological parameters. Daily doses of 125 mg/kg for up to 11 days induced vomiting, inappetence, depression, difficult locomotion and death while adult dogs receiving 50 mg/kg/day for 14 days had clinical signs of vomiting and inappetence.

Adult dogs dosed intramuscularly for three treatments at 12.5 mg/kg followed by 57 oral treatments at 12.5 mg/kg, all at 12 hour intervals, did not exhibit either significant clinical signs or effects upon the clinical chemistry, hematological or histological parameters.
Oral treatment of 15 to 28 week old growing puppies with daily dosage rates of 25 mg/kg has induced abnormal carriage of the carpal joint and weakness in the hindquarters. Significant improvement of clinical signs is observed following drug withdrawal. Microscopic studies have identified lesions of the articular cartilage following 30 day treatments at either 5, 15 or 25 mg/kg in this age group. Clinical signs of difficult ambulation or associated cartilage lesions have not been observed in 29 to 34 week old puppies following daily treatments of 25 mg/kg for 30 consecutive days nor in 2 week old puppies with the same treatment schedule.

Tests indicated no effect on circulating microfilariae or adult heartworms (*Dirofilaria immitis*) when dogs were treated at a daily dosage rate of 15 mg/kg for 30 days. No effect on cholinesterase values was observed.

No adverse effects were observed on reproductive parameters when male dogs received 10 consecutive daily treatments of 15 mg/kg/day at 3 intervals (90, 45 and 14 days) prior to breeding or when female dogs received 10 consecutive daily treatments of 15 mg/kg/day at 4 intervals: between 30 and 0 days prior to breeding, early pregnancy (between 10th & 30th days), late pregnancy (between 40th & 60th days), and during lactation (the first 28 days).
Cats: Cats in age ranges of 3 to 4 months and 7 to 10 months received daily treatments of 25 mg/kg for 30 consecutive days with no adverse effects upon the clinical chemistry, hematological or histological parameters. In cats 7-10 months of age treated daily for 30 consecutive days, 2 of 4 receiving 5 mg/kg, 3 of 4 receiving 15 mg/kg, 2 of 4 receiving 25 mg/kg and 1 of 4 nontreated controls experienced occasional vomiting. Five to 7 month old cats had no side effects with daily treatments of 15 mg/kg for 30 days, but 2 of 4 animals had articular cartilage lesions when administered 25 mg/kg per day for 30 days.

Doses of 125 mg/kg for 5 consecutive days in adult cats induced vomiting, depression, incoordination and death while those receiving 50 mg/kg for 6 days had clinical signs of vomiting, inappetence, incoordination and convulsions, but they returned to normal.

Enrofloxacin was administered to thirty-two (8 per group), six- to eight-month-old cats at doses of 0, 5, 20, and 50 mg/kg of body weight once a day for 21 consecutive days. There were no adverse effects observed in cats that received 5 mg/kg body weight of enrofloxacin. The administration of enrofloxacin at 20 mg/kg body weight or greater caused salivation, vomiting, and depression. Additionally, dosing at 20 mg/kg body weight or greater resulted in mild to severe fundic lesions on ophthalmologic examination (change in color of the fundus, central or generalized retinal degeneration, abnormal electroretinograms (including blindness), and diffuse light microscopic changes in the retina.

DRUG INTERACTIONS:
Compounds that contain metal cations (e.g., aluminum, calcium, iron, magnesium) may reduce the absorption of some quinolone-class drugs from the intestinal tract. Concomitant therapy with other drugs that are metabolized in the liver may reduce the clearance rates of the quinolone and the other drug.
Dogs: Enrofloxacin has been administered to dogs at a daily dosage rate of 10 mg/kg concurrently with a wide variety of other health products including anthelmintics (praziquantel, febantel, sodium disphenol), insecticides (fenthion, pyrethrins), heartworm preventatives (diethylcarbamazine) and other antibiotics (ampicillin, gentamicin sulfate, penicillin, dithyrostreptomycin). No incompatibilities with other drugs are known at this time.
Cats: Enrofloxacin was administered at a daily dosage rate of 5 mg/kg concurrently with anthelmintics (praziquantel, febantel), an insecticide (propoxur) and another antibacterial (ampicillin). No incompatibilities with other drugs are known at this time.

WARNINGS:
For use in animals only. In rare instances, use of this product in cats has been associated with Retinal Toxicity. Do not exceed 5 mg/kg of body weight per day in cats. Safety in breeding or pregnant cats has not been established. Keep out of reach of children.

Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposure. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight.

For customer service or to obtain product information, including Material Safety Data Sheet, call 1-800-633-3796.

PRECAUTIONS:
Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures.

Quinolone-class drugs have been associated with cartilage erosions in weight-bearing joints and other forms of arthropathy in immature animals of various species.

The use of fluorquinolones in cats has been reported to adversely affect the retina. Such products should be used with caution in cats.

U.S. Patent No. 4,670,444
Bayer HealthCare LLC
Animal Health Division
Shawnee Mission, Kansas 66201 U.S.A.
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October, 2004

practice hotline

Insurance

Chronic and Recurring Illnesses

Embrace Pet Insurance, Mayfield Village, Ohio, now covers chronic and recurring illnesses that frequently show up in middle-aged and older pets including skin allergies, osteoarthritis, epilepsy, hyperthyroidism, and pancreatitis. An additional benefit is that these chronic conditions are not excluded when a policy renews. For additional information, visit www.embracepetinsurance.com.—Press release 8/29/07

Relief Efforts

Aid for Flood Stricken Animals

The World Society for the Protection of Animals (WSPA) mounted an emergency relief operation to deliver food and veterinary care to tens of thousands of livestock belonging to impoverished families in Bangladesh. Prior to the WSPA's intervention, consideration of the animals had been only on economic grounds.—Press release 8/23/07