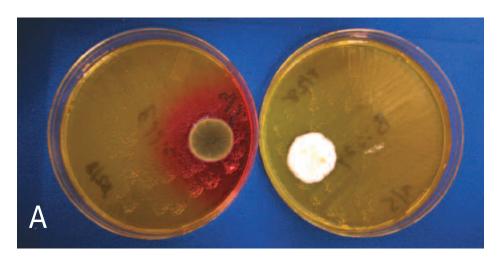
Karen A. Moriello, DVM, Diplomate ACVD, University of Wisconsin-Madison

Microscopic Examination of Fungal Cultures

Although there are many pathogenic dermatophytes, the 3 most common pathogens encountered in small animal practice are *Microsporum canis*, *M gypseum*, and *Trichophyton*. On occasion, other pathogens may be isolated but this is rare.

he use of mediums such as dermatophyte test medium (DTM) is encouraged because it contains a color indicator and antimicrobial agents that inhibit most, but not all, contaminant growth. The number of colonies that need to be sampled and examined microscopically can be limited by focusing on white- to buff-colored colonies producing a red color change in the medium; the red color change must occur simultaneously with the appearance of grossly visible colony growth. Plates should be examined daily—once the entire plate turns red, the value of the color indicator as a "red flag" is gone. Some contaminants will have the right gross appearance and produce the simultaneous red color change, so all suspect colonies should be checked microscopically.

M canis, M gypseum, and *Trichophyton* have rather characteristic microscopic morphologies; however, if help is needed in identification, see www.doctorfungus.org. For future use in identification, pictures of organisms can be photographed directly through the microscope eyepiece using a digital camera. Many of the pictures in this article were taken with this technique.



Fungi versus Contaminants—Plates

Both colonies on these plates (A) can be identified as contaminants by gross examination alone. The white colony (right) is not producing a red color change as it is growing and the colony that is producing a color change (left) is pigmented. Pigmented colonies are contaminants.

White colonies producing a red color change in the medium around them as they grow (B) should be examined microscopically. Note that one colony growing on the plate does not have a red color change surrounding it, which indicates that it is a contaminant.

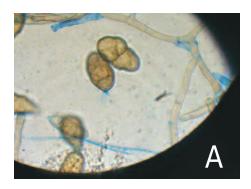


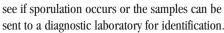
 $\mathsf{DTM} = \mathsf{dermatophyte} \ \mathsf{test} \ \mathsf{medium}$

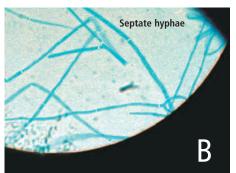
continues

Fungi versus Contaminants—Slides

Slides are first examined at 10× to look for hyphae and sporulation. Once a site has been found, the slide is examined at 40× to determine if it shows a possible pathogen. Microscopic colonies can be eliminated as contaminants if the colonies are pigmented (dematiaceous) (A) or if the hyphae are aseptate; pathogens are hyaline or pale and have septate hyphae (B). However, diagnosis of pathogens cannot be based on septate hyphae alone; they must also have macro- and/or microconidia. If these are not present, the sample can be incubated longer to







Microsporum

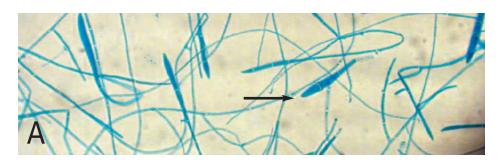
The "boat" shape of the macroconidia (arrow) is characteristic of the genus Microsporum and apparent at low magnification (10×) (A).

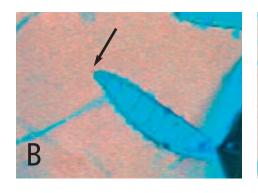
M Canis

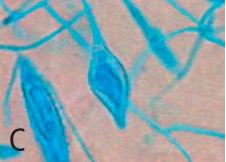
M canis colonies tend to have fewer macroconidia than M gypseum in the early stages of growth. Macroconidia of M canis are thickwalled and spindle-shaped and can have up to 15 cells, thick walls with spines on the surface, and a knob or hook at the end of a spindle (arrow) (B). If it is difficult to see the thickness of walls or other details of the organism, set the slide aside for at least 30 minutes. This will allow the stain to concentrate intracellularly, making visualization easier. Cultures from animals undergoing treatment may be slow to sporulate or have distorted macroconidia (C) (original magnification 1000×).

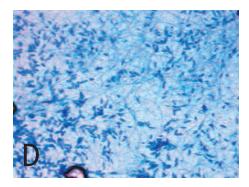


M gypseum grows rapidly and readily sporulates. It has the boat-like characteristics of the genus Microsporum but the walls are thinner, it has 6 or fewer cells, and the ends of the macroconidia are rounded. At 10× magnification, large numbers of macroconidia are readily visible (D). A large number of thin-walled macroconidia is the most reliable characteristic for identification (E) (original magnification 40×).











practice hotline

Vaccine Licensing Agreement

The Animal Health Division of Bayer HealthCare AG (www.bayer.com) and Juvaris BioTherapeutics, Inc (www.juvaris.com) have joined in a licensing agreement under which Bayer will have complete veterinary access to the Juvaris monoimmunotherapy and vaccine technology being developed for human health care.

-Press release 3/4/08

Whole Body Imaging System

DIGITAL-VETTEK (AFP Imaging, www.afpimaging.com) has introduced a 16-megapixel whole-body imaging system that the manufacturer promises will "provide hospital-quality images and higher resolution at very competitive prices." The system includes a full 14-inch by 17-inch imaging area and high-resolution touch-screen monitor and can be retrofitted to existing tables.—*Press release 3/17/08*

New Product

Hartz Feline Vitamin Recall

Hartz Mountain Corporation (www.hartz.com) has voluntarily recalled a second lot of Hartz Vitamin Care for Cats (739 bottles, lot SZ 22771), in which FDA detected the presence of *Salmonella*. Another lot was recalled last November due to similar concerns. Contact Hartz at 800-275-1414 for more information.—*Press release 3/7/08*

Butler Animal Health Opens Denver Warehouse

New Facility

Veterinary product distributor Butler Animal Health Supply (www.accessbutler.com) has opened a 22,000-square-foot warehouse branch in Denver. The company expects the new facility to help solve problems related to shipping during adverse weather conditions in Colorado and southeastern Wyoming.—*Press release 2/19/08*

Trichophyton

Trichophyton species are the most difficult of the common pathogens to identify. These colonies are often slow to grow and it is recommended to observe the plate for 21 days, especially if the sample is from a highly suspect patient (eg, small mammal, small animal exposed to farm animals, hedgehogs). Suspect colonies may require subculture if the colony is at risk for being swarmed by overgrowth. Many species require specific incubation temperatures and enriched mediums; however, the more common species (eg, *T mentagrophytes* and *T verrucosum*) will grow on DTM.

Key characteristics include the presence of large numbers of spherical micronidia (*red arrows*), rare macroconidia (*arrow*), and spiral hyphae (*arrowheads*) (**A and B**). Spiral hyphae can be found in other dermatophytes such as *M persicolor*; this organism can look very similar to *T mentagrophytes* but a few characteristics help differentiate the two. First, *T mentagrophytes* will produce macroconidia without special subculturing. While macroconidia are not present in high numbers, they can be found in most specimens with careful examination; however, *M persicolor* often has to have macroconidia induced via special subculture. In addition, *M persicolor* does not grow well at 37°C but *T mentagrophytes* does.

A complete discussion on preparing fungal cultures and slides for microscopic examination (Procedures Pro: Fungal Cultures for Diagnosing Dermatophytes) was published in the October 2007 issue of Clinician's Brief, which is archived on www.cliniciansbrief.com.

Article archived on www.cliniciansbrief.com

