Yeast, molds, and cell types in 2 benchtop guides

Anne Ford

April 2013—During his six years overseeing fungal proficiency testing and assembling a library of fungal images on behalf of the CAP's Microbiology Resource Committee, Gordon L. Love, MD, D(ABMM), noticed some-thing: Molds are surprisingly lovely.

"Fungi are the most beautiful of microorganisms, and molds are truly the flowers of the laboratory," says Dr. Love, who is medical director of Quest Diagnostics' Los Angeles laboratory in West Hills, Calif., medical director for the western region of Quest Diagnostics, and clinical professor of pathology at University of California, Davis, School of Medicine. He points to *Chaetomium*, a dematiaceous mold whose spined fruiting bodies float in the air and could be said to suggest dandelions.

And then there's *Aspergillus terreus*. A mold with narrow, hyaline hyphae, it produces a cinnamon-tinted colony that looks sort of . . . delicious. "It almost looks like a cookie," he says.

Not convinced? Thanks to the *Mycology Benchtop Reference Guide: An Illustrated Guide for Commonly Encountered Fungi*, a guide Dr. Love created on behalf of the CAP Microbiology Resource Committee as a user-friendly reference for laboratorians, you can judge for yourself (starting with the cover, which features *Chaetomium* in all its spined and fruiting glory).

Available since January, the guide describes essential characteristics—such as growth rates, colonial and microscopic morphology, and ecology—of 74 yeast and molds. The accompanying color photographs include colony and microscopic morphology for molds, as well as microscopic morphology for yeast. Also available since January is the *Body Fluids Benchtop Reference Guide: An Illustrated Guide for Cell Morphology*. Produced by six members of the CAP's Hematology/Clinical Microscopy Resource Committee, it comprises photographs and discussions of 36 types of cells found in body fluid.

Like the CAP's *Hematology Benchtop Reference Guide*, published in January 2012, the mycology and body fluids books are small, durable, spiral-bound volumes with water-resistant pages. They are designed to stand up to the wear and tear of benchtop use while providing users with a concise guide to the information most likely to be needed on a daily basis.

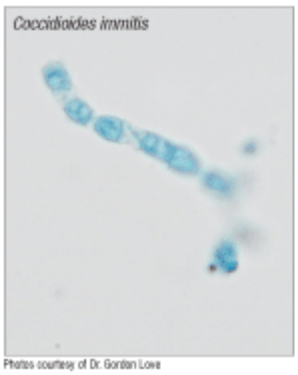
"We tried to make them resistant to fluids, et cetera, so they hold up at the bench, as opposed to reference books that don't always do that," says Joan E. Etzell, MD, who helped create the body fluids book. Dr. Etzell, chair of the Hematology/Clinical Microscopy Resource Committee, is a member of Contra Costa Pathology Associates and a medical director at John Muir Health's MuirLab, Concord, Calif., and clinical professor of laboratory medicine at the University of California in San Francisco.

Every organism in the mycology guide, Dr. Love points out, has been not only morphologically identified but also sequenced and identified by molecular means. "I think that sets this book apart from other texts," he says, "in that everything in here has been identified precisely." Then, too, the guide alerts users to the terminology changes that many fungi have recently undergone. For example, the zygomycete formerly known as *Absidia* now bears the name *Lichtheimia corymbifera* complex. And while the *Cladosporium* genus once comprised pathogenic as well as nonpathogenic molds, the pathogenic molds have been moved primarily into the *Cladophialophora* genus.

The benchtop guide Dr. Love created describes the essential characteristics of 74 yeast and molds. “I tried to indicate those particular molds or yeast that have been recognized as potential problems to handle in the microbiology laboratory,” he says.

In addition, “I tried to indicate those particular molds or yeast that have been recognized as potential problems to handle in the microbiology laboratory,” Dr. Love says. Among those is *Cladophialophora bantiana*, a dematiaceous—pigmented—mold and emerging fungal pathogen that’s been found to cause brain lesions in individuals with otherwise normal immune systems. If you find that alarming, you’re not the only one. “In the book, I caution that all molds should be processed only under laminar-flow, biological safety cabinets, but handling of this and certain other molds should be limited. In particular, slide cultures should not be made,” he stresses. “You just don’t want to disseminate it. If it’s inhaled, the medical technologist handling the culture could be at risk.”

He points, too, to *Coccidioides immitis*, a dimorphic fungus that’s “considered one of the very difficult molds to work with, the reason being that as the mold form grows, arthroconidia are produced in which hyphae break up into small segments,” he says. “These arthroconidia break off and can be inhaled into the lungs and cause disease. It’s been suggested that as few as 12 *Coccidioides immitis* arthrospores can cause disease in an immunocompetent individual. So this is a dangerous mold to work with.



Photos courtesy of Dr. Gordon Love

“Now, in the microbiology laboratory,” he continues, “laminar-flow, biological safety cabinets are used, and the recommendation is that all molds are worked up in them. But these cabinets aren’t perfect; they can’t protect 100 percent of the time. When only a few arthroconidia can cause infection, *Coccidioides immitis* really has to be handled carefully. The handling of *Coccidioides immitis* should be limited to the minimum amount required to make the diagnosis.”

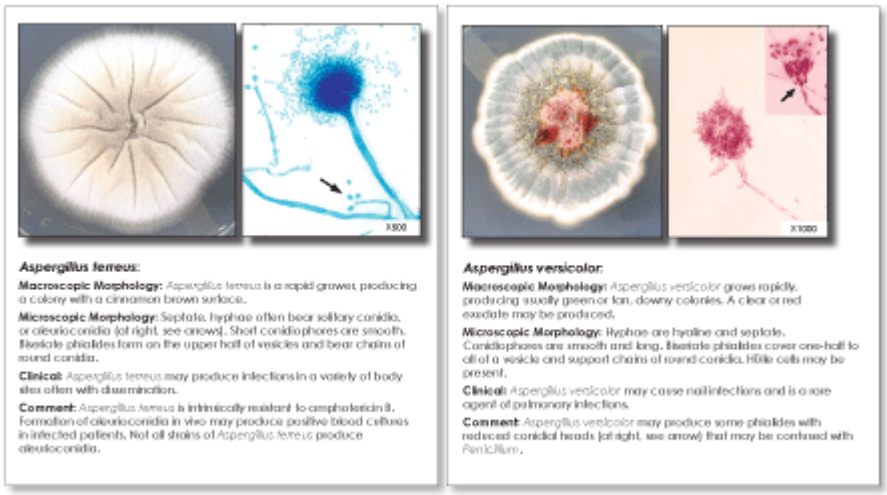
Coccidioides immitis is especially potentially dangerous because it’s easy to confuse with *Malbranchea*, a mold with narrow, hyaline hyphae that’s not associated with human disease. Dr. Love calls it “basically a contaminant.” But, like *Coccidioides immitis*, it produces alternating arthroconidia. While *Coccidioides immitis* arthroconidia are more swollen and produce thicker walls than those of *Malbranchea*, “in practice, that distinction may be difficult to establish with certainty,” he says. That’s why, as he writes in the guide, “Processing a potential Malbranchea culture carefully under a biological safety cabinet would be prudent.”

Another potentially alarming pathogen: *Aspergillus terreus*, the mold that produces that attractive cookie-like

colony mentioned earlier. “It’s being seen increasingly as a hospital-acquired infection,” Dr. Love says. “In some hospitals, it’s been found to colonize water systems. The issue with *Aspergillus terreus* is that if it causes an infection, it may have the ability to produce conidia directly from hyphae inside the body. This is in distinction to the rest of the species of *Aspergillus*, which produce fruiting heads with conidia only when there’s air contact as in molds growing on plated media or sometimes in pulmonary or sinus mycetomas.” While *Aspergillus terreus* also produces fruiting heads with conidia when in contact with air, conidia growing directly from hyphae inside the body can then circulate in the blood. As a result, *Aspergillus* terreus is the only species of Aspergillus in which positive blood cultures are readily produced in patients. “This ability to produce conidia inside the body is a pathogenic mechanism that’s very alarming,” Dr. Love says.

The guide also contains many discussions of far less dangerous organisms, such as *Aspergillus versicolor*. A rapidly growing mold, *Aspergillus versicolor* can sometimes be confused with *Penicillium*. That’s because, as Dr. Love explains, *Aspergillus versicolor* differs from other *Aspergillus* species in that some of its fruiting heads have conidial heads so reduced in size that they’re almost absent: “In these cases, the structure looks just like *Penicillium*, which does not have fruiting heads.”

So how to make the correct identification? Start with the book’s color photographs, Dr. Love suggests. “For *Aspergillus versicolor*, you want to look for a green or tan downy colony,” he says, “though in very mature colonies, there is a wine-red exudate that may be produced. Then look at the microscopic morphology. You want to see long, smooth conidiophores supporting vesicles. One-half or more of vesicle surfaces are covered by double rows of cells that support chains of conidia.”



Sample pages from the *Mycology Benchtop Reference Guide*. *Aspergillus terreus*, left, is “being seen increasingly as a hospital-acquired infection,” Dr. Love says. *Aspergillus versicolor* can be confused with *Penicillium*.

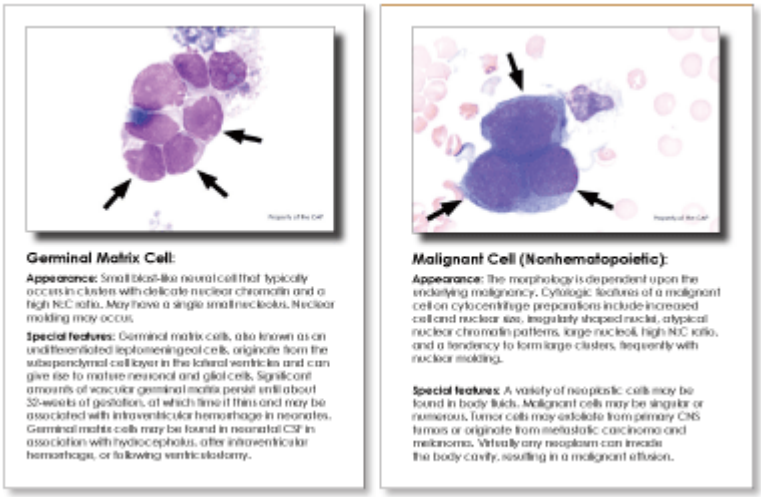
Of course, the guide also offers much information about yeast and yeast-like fungi. Among the former is *Cryptococcus neoformans*, which produces round, budding yeast without hyphae, and which can produce systemic infection in immunocompromised patients. “I saw it extensively in the HIV epidemic in the 1980s, before effective retrovirals were available,” says Dr. Love. “What fascinates me about *Cryptococcus neoformans* is that it can show a wide range of sizes. In tissue and body fluids, it can grow as small as two to three microns in diameter, or as large as, say, 25 micrometers or more in diameter. In the mid-1980s, I did an autopsy on a patient who had a brain lesion due to *Cryptococcus neoformans*, and the yeast cells—excluding the capsule—were 30 micrometers or more in diameter. But when I grew the *Cryptococcus neoformans* in the microbiology laboratory, they were only about three to six micrometers in diameter. That yeast has always been fascinating to me.”

Dr. Love believes that the mycology guide will continue to be useful even as more and more laboratories embrace molecular techniques for identification. While “ultimately, molecular technique will predominate,” in his view “having a command of the morphology will continue to be important because you can select the most appropriate, least expensive molecular method if you are guided by morphology.”

Somewhat smaller in size, but just as comprehensive, is the *Body Fluids Benchtop Reference Guide*, which Dr. Etzell created with Martha R. Clarke, MD; George Girgis, MT(ASCP); Alice L. Werner, MD; Anna K. Wong, MD; and Tracy I. George, MD, all members of the CAP’s Hematology/Clinical Microscopy Resource Committee.

“We included the most common cell types that are seen in a variety of body fluids as well as some of the cell types

that can be difficult to distinguish," says Dr. Etzell. "In addition, we've included some examples of malignant cells that can occur in body fluids."



Germinal Matrix Cell:

Appearance: Small blast-like neural cell that typically occurs in clusters with delicate nuclear chromatin and a high N:C ratio. May have a single small nucleolus. Nuclear molding may occur.

Special features: Germinal matrix cells, also known as an undifferentiated leptomeningeal cells, originate from the subependymal cell layer in the lateral ventricles and can give rise to mature neuronal and glial cells. Significant amounts of vascular germinal matrix persist until about 32-weeks of gestation, at which time it thins and may be associated with intraventricular hemorrhage in neonates. Germinal matrix cells may be found in neonatal CSF in association with hydrocephalus, after intraventricular hemorrhage, or following ventriculostomy.

Malignant Cell (Nonhematopoietic):

Appearance: The morphology is dependent upon the underlying malignancy. Cytologic features of a malignant cell on cytocentrifuge preparations include increased cell and nuclear size, irregularly shaped nuclei, atypical nuclear chromatin patterns, large nucleoli, high N:C ratio, and a tendency to form large clusters, frequently with nuclear molding.

Special features: A variety of neoplastic cells may be found in body fluids. Malignant cells may be singular or numerous. Tumor cells may exfoliate from primary CNS tumors or originate from metastatic carcinoma and melanoma. Virtually any neoplasm can invade the body cavity, resulting in a malignant effusion.

From the *Body Fluids Benchtop Reference Guide*, two pages that help users distinguish germinal matrix from malignant cells.

The guide organizes body fluids into the following categories: erythroid series, lymphoid series, myeloid series, mononuclear phagocytic series, lining cells, miscellaneous cells, crystals, and microorganisms. A section called "Miscellaneous Findings" includes a discussion of mitotic figures, which, as the book notes, can be difficult to distinguish from degenerating cells.

"Mitotic figures tend to have some structure to them," Dr. Etzell explains. "In the photograph in the book, you can see some linear structures within the mitotic figure. But sometimes, if the mitotic figure is more condensed, it can look more like degenerating nuclear chromatin. Both can have a very deep purple homogeneous structure. As a cell degenerates, the nucleus degrades as well, and it may not always retain the round shape; there may be some irregularities to the nuclear membrane."

Also in the "Miscellaneous Findings" section is a discussion of starch granules, which occasionally show up as a contaminant from the powder on medical gloves. As the guide notes, these granules typically have a diameter four to six times that of a red blood cell, and appear irregularly round with a central slit or indentation. "I'd say it's less than 10 percent of the time that we see them, but we certainly do see them," Dr. Etzell says, "and it's important not to confuse them with a cellular type or a true crystal."

One important distinction she hopes the guide will help users make is that between blast cells and normal lymphocytes. "The presence of blasts in body fluid often means involvement by acute leukemia or another hematopoietic neoplasm," she explains. "The photomicrograph in the guide nicely illustrates blasts, and illustrates that they're larger than normal lymphocytes. They do have more dispersed chromatin with a nucleolus, and this is certainly a very important cell type for technologists and pathologists to be able to reliably identify, since involvement of fluids by leukemia or other hematopoietic neoplasms is diagnostically quite important."

She hopes, too, that the guide will prove useful to users who are attempting to distinguish germinal matrix cells from malignant cells. "Germinal matrix cells," she reminds readers, "typically will occur in neonates, and they have many features that resemble those of malignant cells—including high nuclear-to-cytoplasmic ratio, some nuclear irregularities, and finely dispersed chromatin. So, as with most things that we do in the clinical lab, it's important to correlate the patient's age and history with what we're seeing on the slide to ensure that we don't confuse something like germinal matrix cells with a malignancy."

Anne Ford is a writer in Evanston, Ill. To order the reference guides, call 800-323-4040 or 847-832-7000 option 1. Each of the two benchtop reference guides is $78.