Saving Fungi and Saving Money. . .

How to Preserve Fungal Cultures

By James Harris, PhD

James Harris has had an interest in fungi for more than 50 years.

Dr. Harris completed his graduate work in mycology at Texas A&M University following his undergraduate degree at the University of Texas at Austin.

Currently, he is retired from the Laboratory Services Section of the Texas Department of State Health Services where he served as the Laboratory Training Coordinator.

Dr. Harris has presented mycology workshops in over 20 states and the District of Columbia

Microbiology laboratories in government, industry and in universities have a need to maintain healthy, viable fungal cultures – sometimes for many months or years. These laboratories focus on clinical microbiology, plant pathology, food science, and pharmaceutical research.

The mycological literature in the 1930's described sterile water preservation of pathogenic fungal cultures. Still, I frequently meet laboratory personnel who have not used the easy and economical technique for culture maintenance described seventy years ago and referenced frequently in the literature.

Preservation methods currently used, other than sterile water storage, include:

1. Periodic serial subcultures
2. Freezing
3. Freeze drying, and
4. Sterile mineral oil overlay

Figure 1: Sterile Water (Hardy Cat. no. K187)

Some disadvantages of various alternative procedures are the:

1. Induction of abnormal forms, or pleomorphism
2. Bothersome and very messy culture retrieval
3. Potential thawing of frozen specimens due to accidental power failure

It is noteworthy that all of these methods are more expensive

and complex than the sterile water storage discussed here.

To preserve fungi in sterile water, one simply dips the end of a sterile applicator stick in sterile distilled water and rolls the moistened stick over the surface of a mature culture of the desired fungus to collect spores or conidia. These propagules are swirled from the stick into sterile water (3-5ml) in a small screw cap test tube. After replacing and tightening the tube cap, the suspension is stored at room temperature in a dust free area.

The fungus may be re-grown by dipping a sterile applicator stick into the water suspension, thereby retrieving some fungal cells, and aseptically inoculating them into a desired culture medium. After appropriate incubation, the inoculated medium should contain fungal growth resembling the original stored culture; pleomorphism is rarely seen in cultures retrieved from water storage.

When preparing the storage suspension, always take care to collect and transfer to the water only fungal growth without fragments of culture medium. Tightly cap the storage tubes, since evaporation to dryness usually results in death of the stored cells. It is important to occasionally inspect the tubes and to add sterile water to any showing water loss.

This technique is highly successful. Many fungi (including filamentous molds, yeasts and yeast-like fungi) will survive for a decade or more in sterile water. Some zygomycetous fungi may be less stable in water storage, surviving for only a few months, but most fungi commonly encountered in clinical laboratories or in plant pathology laboratories remain viable for years. In addition, plant pathologists have long used this method to preserve important bacterial pathogens for research.

"Many fungi will survive for a decade or more in sterile water."

For added security, after several years of storage, some mycologists prepare new sterile water storage tubes of collected and sub-cultured fungi. This can extend preservation of the culture with a minimal amount of subculture, thus minimizing induction of pleomorphism in the fungus. Those few fungi failing to survive long term water storage may be replaced from a reputable culture source. Because the great majority of commonly encountered fungi, especially those used in teaching institutions, survive very well in sterile water, this method can significantly reduce culture storage expense.

There is no "perfect" method of preservation appropriate for all laboratories and applicable to all fungal organisms. However, if cost and difficulty of storage are major considerations, the sterile water storage method is an excellent choice for simple storage of many commonly encountered fungi.

James Harris, PhD
Austin, Texas

Editor's note: For a complete selection of starter cultures, see the complete catalog of MBL organisms available from Hardy Diagnostics. For a listing of all fungal media and supplies, see our Mycology Catalog. at www.HardyDiagnostics.com.