The Medical Mycology Handbook

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Foreword

Medical mycology is a fascinating aspect of biological study that has been handicapped because of the lack of well-trained laboratory personnel. Nevertheless, fungi were observed and described long before other types of microorganisms. Unfortunately, bacteria proved more exciting to investigators following the monumental works of earlier bacteriologists and virologists. In the early nineteenth century mycology was progressing very well until the Pasteur-Koch era, at which time it was pushed back into the "dark ages." Then, with the publishing of *Les Teignes* by Sabouraud in 1910, the emphasis on mycology was increased. However, the rapid development of virology again led to less activity in mycology. During World War II, with the increased mobility of individuals, infections caused by mycotic organisms began to be recognized more frequently, and many more people were attracted to the specialization of medical mycology. The rapid expansion of laboratory mycology throughout the world resulted in the use of personnel with little or no training in medical mycology.

Because of mycology's history and because the discipline is still expanding but sorely neglected, there is a place for a manual that has evolved from many years of practical experience. The authors have assembled basic concepts necessary to understanding clinical mycology as well as current information. Morphologic, physiologic, and taxonomic principles are presented in a concise and understandable manner. It is apparent that the authors' many years at the bench have been invaluable in their efforts to develop a practical approach to the isolation and identification of mycological agents.

Throughout the handbook the authors have drawn heavily on their personal experiences, providing accurate, to-scale drawings and individual descriptions of pathogenic and contaminating fungi. There are many refreshing innovations that make this handbook an excellent tool for a person new to the field of medical mycology; the book will also be appreciated as a superior review for the established laboratory worker. The *Handbook* has been prepared in a practical and sincere

manner, with the objective of making laboratory mycology a precise and informative branch of science as well as that of recruiting motivated and dedicated people to the authors' field of specialization. There is little doubt that, by mastering the procedures, beginning or experienced laboratory workers will find many opportunities to use their training and qualifications. There remains a dire need for highly qualified medical mycology laboratory workers if mycology is to reach the position it so richly deserves within the medical sciences.

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Preface

We face an ever-increasing threat from fungal disease. Normal defenses against fungi have been lowered by long-term therapies such as antibiotic administration, steroid treatment, immunosuppressive drugs used with organ transplants, and cytotoxic drugs used in cancer treatment. Common yeasts and molds that have always seemed harmless are now becoming opportunistic pathogens. This means that workers in clinical microbiology laboratories are asked to identify a bewildering new assortment of fungal isolates, in addition to the few well-known major pathogens.

Those of us who have developed expertise in this field are largely self-trained. Our bible has consisted of medical mycology texts and laboratory manuals. From these books we have developed a good working knowledge of the major pathogenic fungi and several common molds. Now, with so many unusual isolates coming into our laboratories, we are being led to discover other sources. We are learning exciting new ways of looking at the fungi, a new terminology and new nomenclature, and we are beginning to come to grips with fungal taxonomy.

We are expected to train other technologists to do this work, usually in a very few days. These workers cannot be asked to read so much material in a short time. Neither can the work be shortchanged by oversimplifying the field. In response to the needs of the many technologists and other students who come through our laboratories, we have developed a system of training that follows the rhythm of a clinical laboratory while providing basic background information. This handbook has been written as an aid in this training and as a resource for all workers in this field.

Part 1 sets the stage for the working part of the book. Besides the general characteristics of fungi, new systems of taxonomy are described and compared with earlier systems still used in many texts. With the work of Barron as a background, it has been exciting to follow the proceedings of the first Kananaskis conference (The First International Specialists's Workshop Conference on Criteria and Termi-

nology used in the Classification of Fungi Imperfecti). We hope we have made these systems easily available to our readers. A general description of individual fungal diseases is given, with references to the more thorough clinical descriptions (written specifically for physicians) in the basic mycology texts. A quick look at Table 3, which lists medically significant fungi by disease classification, gives some idea of the range of significant organisms that might be isolated in a mycology laboratory.

Part 2 starts by addressing the question "Where do I begin?" Chapter 4 follows the accepted mycologic techniques now in use, from specimen collection to final identification of fungal isolates. It is written for workers new to mycology who are trained in routine bacteriologic techniques. Setting up of media, microscopic preparations of specimens, what to do when something grows, and systems for identification of yeasts, dermatophytes, systemic and opportunistic fungi are described. Many flow charts and tables (with drawings) are included. While the morphologic recognition of a yeast or mold is always a critical factor in making a fungal identification, several differential tests have been developed, particularly for yeast identification. Procedures for all these tests are precisely written for use in a clinical laboratory. Our system contains elaborate safeguards to protect inexperienced workers from possible inhalation of infectious fungal spores and from accidentally discarding a major pathogenic fungus that "doesn't look very important." While only a handful of fungi are normally pathogenic, they are similar in many ways to those that may be routinely isolated as common molds. A mycology technologist needs to be familiar with all of these, and our system takes this into account. Besides the few primary pathogens, the clinical mycologist is aware that we live in a world of opportunistic fungal disease. Any fungal isolate in a clinical laboratory is potentially significant.

Chapter 5 begins with two guides. A general guide (Table 20) to gross characteristics is arranged primarily by color. The guide to microscopic characteristics, with small key drawings (Table 21), is arranged by kinds of spore or conidium production. Individual descriptions of over 100 fungi are easily located by alphabetical arrangement. Most of these descriptions are illustrated with clear line drawings, done to scale by Joyce Stewart, exactly as seen from clinical isolates. Written gross and microscopic descriptions are given and measurements of critical structures are stated in micrometers or millimeters. Several references are given with each description. Brief statements of known pathogenicity and any known serologic tests are given. While the time-honored system of "matching the isolate with the picture in the book" is still the easiest way to begin to make an identification, we are increasingly aware of similarities between major pathogens and many common molds. We are now better able to differentiate these and we are feeling more confident as we learn to use new skills and better tools for observing these fascinating organisms.

In the appendix, which includes formulae for stains, reagents, and media, emphasis is placed on measures for quality control. We find that even in this highly interpretive field, it is possible to develop a program that leads to quality assurance. This program includes the use of clinical material in testing staining reagents and the use of clinical and stock strains of fungi and bacteria for testing media. We believe that our final and most important quality-control measure is a series of review questions for technologists who are assigned to do this work. This kind of review gives confidence to workers on the bench and reassurance to supervisors.

It is our hope that this manual will help to bring the isolation and identification of medically significant fungi within the easy reach of all clinical laboratories. If we have made it possible for mycotic disease to be more easily recognized and diagnosed, we will feel that our time has been well spent.

Mary C. Campbell
Joyce L. Stewart

Acknowledgments

Grateful acknowledgments of those who have encouraged and supported us in this project are in order. We feel privileged that the distinguished medical mycologist, Dr. Howard Larsh, has agreed to write the Foreword. We thank him for his endorsement of our book and for sharing with our readers his own perspective of this field. We also want to mention specifically Dr. Carlyn Halde, who opened many doors; Dr. Michael McGinnis, who responded quickly and generously to questions; and Dr. Richard Thompson who outlined the material that led to the development of Part 1. Dr. Bryce Kendrick has given a helpful criticism of our drawings of conidium ontogeny.

We are fortunate that several experts in medical mycology and related fields have been willing to take time to review some of our material. While we have gratefully accepted most of their suggestions, they are not responsible for any inaccuracies or oversights. Betty Russell has read drafts of all the chapters. In addition, various parts of the material have been reviewed by Mary Bauman, Sharon DeLong, Nancy Gerhardt, Leanor Haley, Abdel Rashad, Greg Raugi, Michael Saubolle, and Annette Youngberg.

We are grateful to the many dermatologists who have studied with us over the years, beginning with Dr. Jacob Swartz at the Massachusetts General Hospital. We particularly want to mention Dr. Leon Ray, who led in the development of a mycology teaching program at the University of Oregon Medical School and who is the senior author of our first published teaching manual, the *Fungus Syllabus.* We are also grateful to our fellow laboratory workers, whose patience, good ideas, and many questions have led to the writing and organization of much of this material.

We also gratefully acknowledge the release given by the Oregon Department of Higher Education for permission to use the laboratory procedures developed by Mary C. Campbell at the University of Oregon, Health Sciences Center, Department of Clinical Pathology. Permission to use copyrighted materials has been given by Dr. Frank Swatek and by Dr. Constantin John Alexopoulos and the John Wiley and Sons publishers. Thanks go to Dr. Kirtikant Sheth for the use of his photomicrographic camera and to Phoebe Rich and Jim Phillips for photographic help. Jonathon Newman's wise counsel has been invaluable. We have had the excellent help of several typists, including Fran Hawkins, Amy Nutter, Rob Bellin, and Margaret Campbell.

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M.C.C.
J.L.S.

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PART I

UNDERSTANDING IT

1. Introduction

BRIEF HISTORY

Fungal invasion of human tissue was recognized in the early 1800s, well before the science of bacteriology was developed. The fascinating and often complex fungal structures could be studied under the microscope with simple magnification, either in human tissue or in laboratory cultures. Even today, with all of the histologic, biochemical, nutritional, and serologic tests available to us, the identification of a pathogenic fungus is often made by recognition of characteristic structures seen in culture using a low-power objective of the microscope.

As pathogenic fungi were recognized by early physicians, they were described in the literature and given names, many of which were based on the clinical, not the cultural, characteristics. Often the same fungus would be given a new name each time it was isolated in a new clinical setting. This led to a tremendously complicated nomenclature, which is now, happily, becoming unraveled. We owe a great debt of gratitude to the dedicated workers who have been studying and redescribing these organisms and classifying them in agreement with the classification of fungi in general. The laboratory identification of pathogenic fungi is much simpler today than it was 50 years ago.

GENERAL CHARACTERISTICS

Morphologic Features (Yeasts and Molds)

The fungi seen in a medical laboratory are referred to either as *yeasts* or *molds.* These are descriptive, not formal, taxonomic terms. Characteristically, the yeasts have moist-to-waxy colonies in culture, with a predominance of budding cells (3 to 5 μm in diameter). The molds have leathery-to-velvety, powdery, granular, or cottony colonies. These mold colonies are made up of hyphae (tubular cells 2 to 20 μm in diameter), which grow by elongation at the tips or by lateral branching, forming a tangled mass of mycelium. Many kinds of spores and spore production are seen in the molds.

The word *dimorphic* means *two forms.* Five of the major systemic pathogenic fungi are described as dimorphic. Four of the major systemic pathogenic fungi grow as molds in culture at room temperature, as yeasts in culture at 37°C, and also as yeasts in tissue. These are *Blastomyces dermatitidis, Histoplasma capsulatum, Paracoccidioides brasiliensis,* and *Sporothrix schenckii.* A fifth major pathogen, *Coccidioides immitis,* grows as a mold in culture both at room temperature and at 37°C. It grows in a spherule form in tissue and, under special conditions, in culture, at 37° to 40°C. A *spherule* is a structure, bounded by a membrane, in which spores are produced. At maturity, when spores are completely formed, it is called a *sporangium.* Many other fungi are found to have different forms under different conditions. A good example of this is the yeast *Candida albicans,* which can produce either a yeast or a hyphal form, depending on available nutrients and other factors, whether in culture or in tissue. Other fungi have a yeast form in early culture growth and develop a hyphal form as they mature. We want to emphasize that, while it is necessary to demonstrate dimorphism in order to identify some of the major pathogens, dimorphism is not limited to these organisms.

A vexing feature of many of the fungi seen in a medical laboratory is their ability to mutate. On repeated subcultures, characteristic spores will be lost, and only sterile hyphae will remain. The word *pleomorphic* has been used to describe such cultures. They would be more accurately described simply as sterile mutants.

Growth Requirements

Generally speaking, all fungi need a protein source and a carbohydrate source. Sabouraud's dextrose agar, the standard medium for support of yeast and mold growth, contains only dextrose and peptone as nutritional sources. Many common molds and yeasts grow well on fruits, vegetables, grains, breads, and meats. The formation of enzymes that break down complex organic substances causes changes recognized as decay. Some fungi are able to grow better in soil composed of decaying vegetable debris. This kind of material is the source of most pathogenic fungi, many of which prefer specific kinds of organic debris. *Histoplasma capsulatum*, for example, is found most often in association with the excreta of chickens, bats, or starlings. Some fungi may, under special conditions, colonize areas of human tissue, but their growth requirements are better met in the soil where animal excreta or animal or vegetable debris are found. On the other hand, *Candida albicans* is isolated primarily as a parasite on a human host. Different fungi have different nutritional requirements, some of which may be used as an aid in their identification. The ability of yeasts to use combinations of carbohydrates unique to each species provides major criteria for yeast identification.

The fungi are able to tolerate a wide range of pH; many of them can grow in media that have a pH of anywhere from 2.0 to 10.0. Although they prefer a neutral pH, the ability of some fungi to grow under such conditions is useful in developing selective media that inhibit the growth of bacteria and allow the growth of the fungi.

Moisture is necessary for the growth of molds and yeasts. Moist cellars and damp camping gear are two examples of places where molds may be expected to grow. When the cellar or the camping gear dries out, the spores will survive, but the mold will not grow unless moisture is supplied again.

The yeasts and molds prefer moderate temperatures, but the ability of molds to grow on leftover food in the refrigerator demonstrates the fact that they can also grow at lower temperatures. All the fungi isolated routinely in a medical laboratory grow well at room temperature. Some grow well or better at 37°C. *Aspergillus fumigatus* can tolerate temperatures as high as 56°C. Some, as already described, will produce a mold form at room temperature and a yeast form at 35° to 37°C. Some will not grow at all at 35° to 37°C. Generally speaking, a fungus that is able to grow at body temperatures (35° to 37°C) has a greater chance of invading deep human tissue than one that is inhibited at these temperatures.

Reproduction

ASEXUAL (VEGETATIVE) REPRODUCTION

Fungi will grow and reproduce indefinitely so long as a proper food supply is available. The *thallus,* the name given to the main body of the fungus, extends either by repeated budding of yeast cells, by elongated budding of pseudohyphae, or by continued elongation and branching of the true hyphae. Pseudohyphae are distinguished by constrictions between each cell, resulting from the budding process. The appearance has been likened to a chain of sausages. (The pseudohyphae and budding cells of *Saccharomyces cerevisiae* are shown in Fig. 1). True hyphae are recognized by the lack of constrictions. In septate hyphae (which are 2 to 8 μm across) straight cross walls are laid down and branching occurs (Fig. 2). In the much wider aseptate hyphae (which are 5 to 15 μm across), branching and "cleavage lines" may occur, but true cross walls are absent (Fig. 3). As the culture ma-

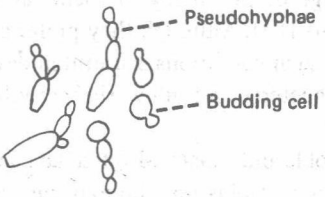

Figure 1. Pseudohyphae and budding cells.

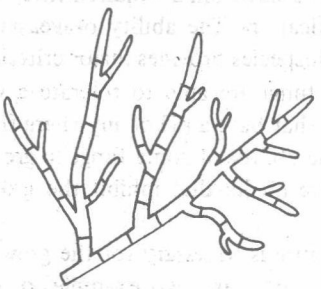

Figure 2. Septate hyphae.

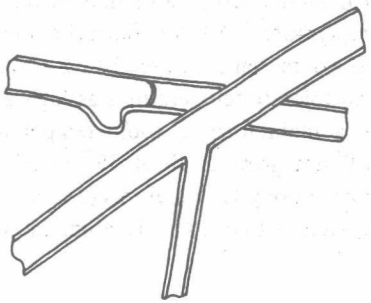

Figure 3. Aseptate hyphae.