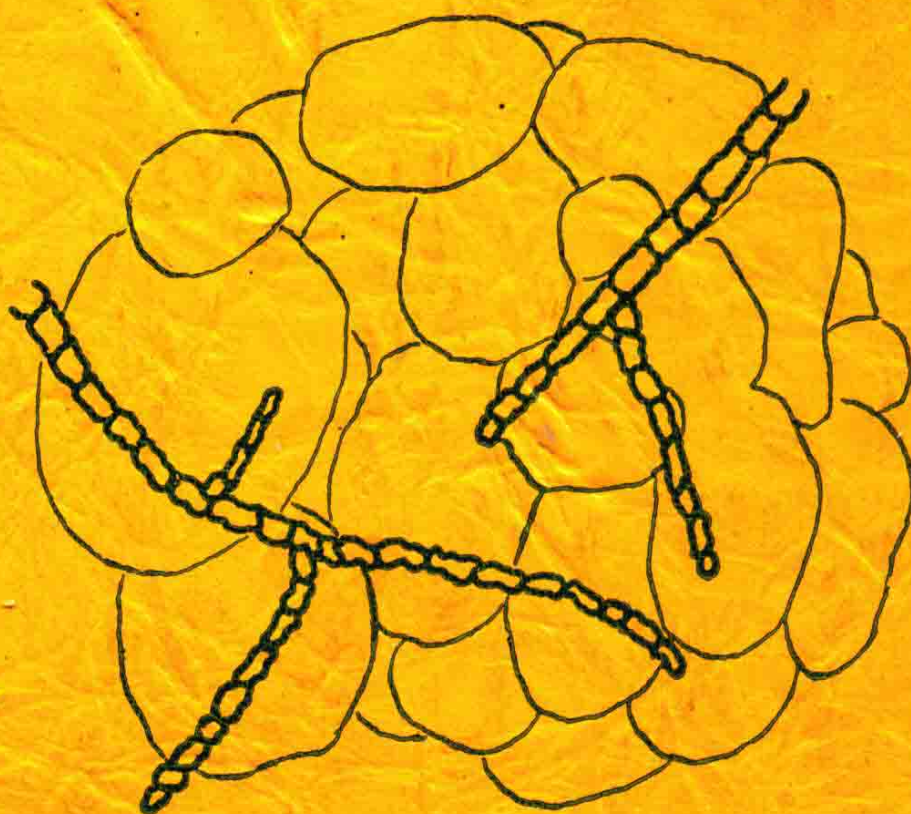


MEDICAL MYCOLOGY

LABORATORY MANUAL

E. S. BENEKE



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Laboratory Manual

by

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BURGESS PUBLISHING COMPANY

426 South Sixth Street — Minneapolis 15, Minnesota

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THIRD PRINTING 1962

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Printed in the United States of America

PREFACE

This manual is primarily intended for use by laboratory technicians, microbiologists, and students in medical mycology courses as a guide in the study and laboratory diagnosis of pathogenic fungi in men and animals. For more detailed information on symptomology, pathology, differential diagnosis, prognosis and treatment, reference should be made to anyone of several textbooks or references.

The material in this manual has been arranged so that the student trained in bacteriological procedures, with little or no training in mycology is introduced to some of the essential characteristics of fungi and some of the common contaminants before handling any of the pathogenic fungi. Subsequent to becoming acquainted with some of the common contaminants, it has been found that the student receives valuable training while trying to isolate and identify as many unknown fungi as possible on culture media from various sources such as air borne spores, dust in attics, soil or dung.

Most of the pathogenic fungi that have been reported to occur as clinical cases in North America are included in this laboratory manual. Some of the fungus diseases included in this manual are usually found to be more prevalent in other parts of the world, but may occur in North America when people travel so rapidly from one continent to another. Each disease contains information on type of material that may contain the fungus, procedures for direct microscopic examination of the material, staining technics, culture procedures, macroscopic appearance of the organism if grown in culture, microscopic appearance of the fungus, pathological studies and animal inoculation procedures where applicable. A selection of some of the current references are included for additional information on technics and mycological aspects of the organism. This manual has been arranged so that the student or the medical technician can readily refer to the essential characteristics of each disease which should aid in a more rapid laboratory diagnosis of the etiological agent.

ACKNOWLEDGMENTS

The author is most grateful to The Upjohn Company and especially to Dr. A. Garrard Macleod and Dr. Tim Rey, Editor and Associate Editor, respectively, of Scope for making possible the publication of color plates showing characteristic structures of pathogenic fungi in tissue and culture. The engravings for the color illustrations and the two folded inserts used in this manual were supplied through the courtesy of The Upjohn Company, Kalamazoo, Michigan. Deep appreciation is also expressed to Dr. John Hammer, Kalamazoo, Michigan for his aid in the preparation and publication of the color illustrations. The author is also indebted to Dr. Emma S. Moss, Director, Department of Pathology, Charity Hospital of Louisiana, and Dr. Albert L. McQuown, Pathologist, Our Lady of the Lake Sanitarium, Baton Rouge, Louisiana for permission to use the photographs of the deep mycoses published in Scope, and to Dr. Arthur E. Schultz, East Lansing, Michigan for use of the photograph of Aspergillosis infection in the eye. He is also indebted to Dr. A. P. Ulbrich for making available some of the photographs of the dermatomycoses.

The author is especially grateful to Mrs. Claire L. Taschdjian, Department of Pediatrics, Maimonides Hospital, Brooklyn, New York, for critically reading the manuscript and offering valuable suggestions. He is indebted to Dr. John Lockwood and Dr. Samuel M. Ringel for reading portions of the manuscript, and to Dr. Seth Mizuba for assistance with the bibliography.

The inspiration and encouragement of Dr. Const. J. Alexopoulos and Dr. C. W. Emmons have been greatly appreciated during the preparation of the manual. Some of the slide cultures used for photomicrographs were prepared with the able assistance of Mrs. Mary E. Joy. The acquaintance and association with Dr. N. F. Conant has been an inspiration in the study and teaching of medical mycology.

The author is indebted to many colleagues and students for suggestions in the development of the manual.

E. S. Beneke, Ph. D.

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CHARACTERISTICS OF FUNGI

INTRODUCTION

In order to become acquainted with some of the fungi among many thousands in the world, an introduction to a systematic arrangement of these organisms is a necessity. After the introduction of a few basic concepts on terminology, the major classification system, and a few organisms, the identification of fungi becomes much easier. After becoming acquainted with some of the contaminants and the pathogenic fungi, the medical bacteriologists, medical technologists, physicians, and veterinarians, will have a better concept about the behavior of fungi when pathogenic in man or animals.

Fungi have somatic or vegetative structures that may be one-celled or tubular in form. These tubes or threads are known as hyphae or a single thread is a hypha. The hyphae branch and may have cross walls or septa. The hyphae develop from spores which vary considerably in one organism or another. A large mat of hyphal growth is known as mycelium.

Two general types of reproduction occur in the fungi: asexual and sexual. Asexual reproduction is the most common in the pathogenic fungi which in most cases do not reproduce sexually. Asexual reproduction may be by fragmentation of hyphae, cells in the hyphae may break up into their component cells which are called arthrospores (oidia), cells may become thick-walled and are known as chlamydospores, while the most common type of asexual reproduction is by means of spores. Yeasts may multiply by budding (blastospores), or by cell division.

CLASSIFICATION

The same categories or groupings are used in fungi as well as in plants to separate the organisms. The categories include: Kingdom, Phylum, Class, Order, Family, Genus and species (see chart). Although there are variations in classification systems, there are four classes in the true fungi or Eumycophyta:

1. Phycomycetes include a number of one-celled organisms, but most of the group is filamentous. The mycelium lacks cross walls in most cases, and organisms reproduce asexually by means of sporangia which contain motile or nonmotile spores. Sexual reproduction is by means of a thick-walled resting spore such as a zygospore or oospore. Only a few human pathogens occur in this group.

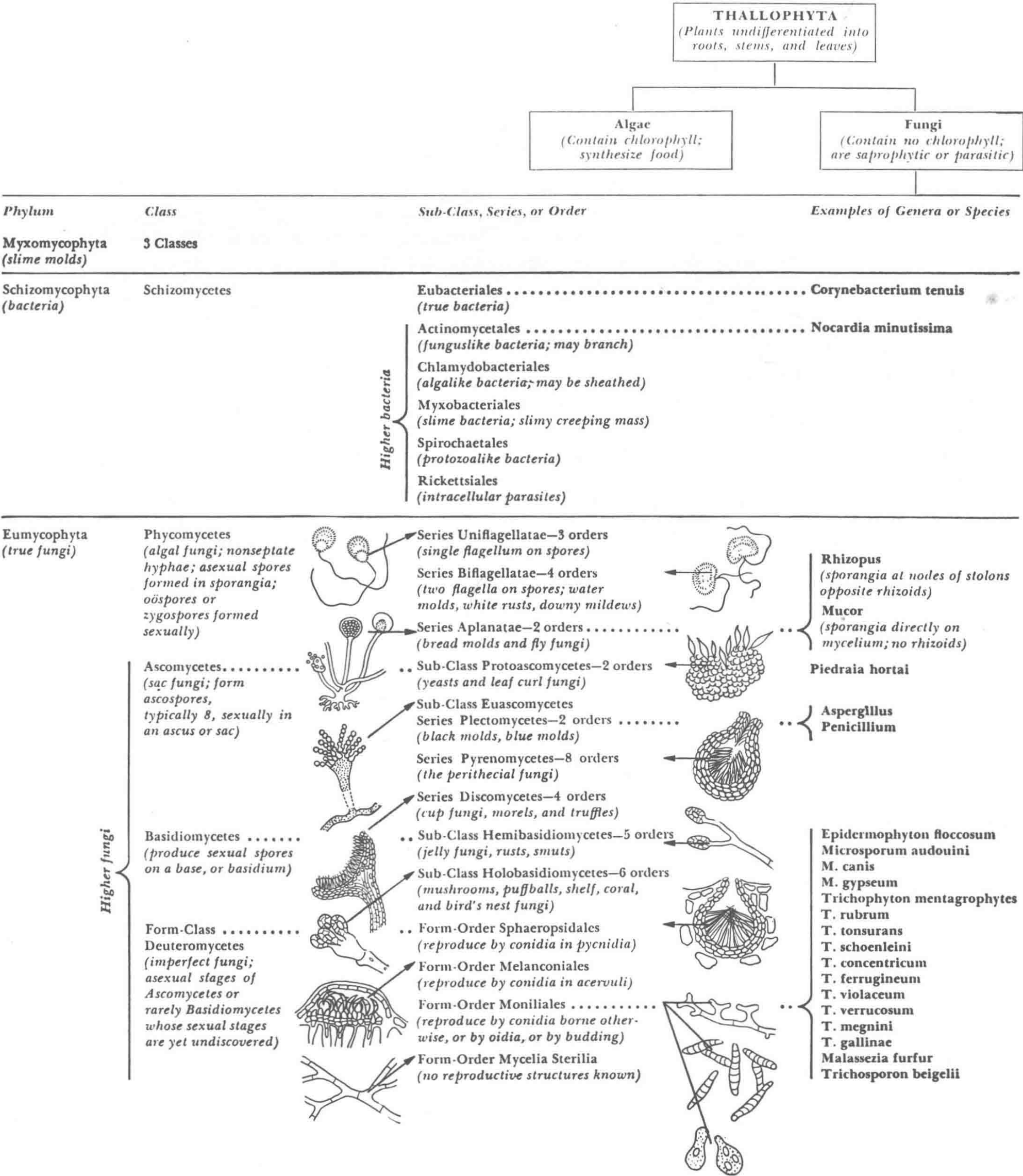
2. Ascomycetes have cross walls or septa in the mycelium. Asexual reproduction varies from budding cells, cell division, breaking up of cells in the hyphae, to a specialized branch or conidiophore bearing conidia. The typical reproductive structure for the entire group is the ascus, a sac-like structure containing usually 8 ascospores as a result of sexual reproduction. Not many human pathogens belong to this group.
3. Basidiomycetes have septate mycelium with uninucleate or binucleate cells. The binucleate mycelium which arises from sexual reproduction usually forms clamp connections or bulges on the side of the cells where the nuclei divide in the formation of new cells. The typical reproductive structure is the basidium, a club-shaped structure usually bearing 4 basidiospores on the surface. This group is of little importance in causing human or animal diseases. At times mushroom poisoning is of importance in medical practice.
4. Deuteromycetes (Fungi Imperfecti) are an artificial group of fungi which reproduce by asexual means only. Many apparently would be Ascomycetes or in some cases Basidiomycetes if the sexual stage were found to occur. Most of the pathogenic fungi in man and animals belong to this class.

In order to develop a better background concerning fungi, it is suggested that reference be made to a textbook on mycology such as "Introductory Mycology" by Const. J. Alexopoulos. There are a number of other reference books or textbooks that may be utilized in the study of fungi.

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RELATIONSHIP OF THE VARIOUS CATEGORIES OF THE FUNGI



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A SUGGESTED LABORATORY SCHEDULE

The following schedule is based on two-hour laboratories. Additional demonstrations, materials and audiovisual aids are used when available (see sources of materials in the back of the manual). Whenever possible the following fungi should be grown on slide cultures for semipermanent mounts and in bottle or plate cultures by the students (exceptions: Coccidioides immitis and possibly a few other systemic fungi).

Laboratory Period

Organism for Study

- 1 Introduction, Classification of fungi, Equipment.
- 2 Penicillium sp.; Aspergillus sp.; demonstration of slide culture methods, culture procedures.
- 3 Paecilomyces sp.; Scopulariopsis sp.; Gliocladium sp. Media for fungi.
- 4 Trichoderma sp.; Verticilium sp.; Cephalosporium sp.; Fusarium sp.
- 5 Isolation of air-borne fungi, etc.; Alternaria sp.; Hormodendrum sp.; Helminthosporium sp.
- 6 Nigrospora sp.; Pullularia sp.; Mucor sp.; Syncephalastrum sp.;
- 7 Rhodotorula sp.; Saccharomyces sp.; Streptomyces sp.; Phoma sp.
- 8 Patch test; Complete report on identification of air-borne fungi; Nocardia tenuis. Unknown for identification.
- 9 Piedraia hortai; Trichosporon biegelii; Malassezia furfur. Hotchkiss - McManus stain.
- 10 Methods for culture of dermatophytes; Use of Wood's light; Microsporum audouini; Microsporum gypseum.

Laboratory Period	Organism for Study
11	<u>Microsporum canis</u> ; <u>Epidermophyton floccosum</u> ; isolation of fungus from tissue, hair; determination of fungus in skin; film strips on "Histopathology of Human Fungus Infections" (4 in series).
12	Kodachromes of the dermatophytes; <u>Trichophyton mentagrophytes</u> ; <u>Trichophyton rubrum</u> ; <u>Trichophyton violaceum</u> . Unknown pathogenic fungus.
13	<u>Trichophyton tonsurans</u> ; <u>Trichophyton schoenleini</u> , <u>Trichophyton ferrugineum</u> ; other dermatophytes.
14	Completion of slides. Review.
15	Spot test.
16	Injection of animals with <u>Sporotrichum schenckii</u> ; <u>Histoplasma capsulatum</u> ; <u>Blastomyces braziliensis</u> ; <u>Cryptococcus neoformans</u> .
17	<u>Hormodendrum pedrosoi</u> ; <u>Hormodendrum compactum</u> ; <u>Phialophora verrucosa</u> ; Kodachromes.
18	<u>Monosporium apiospermum</u> ; <u>Nocardia asteroides</u> ; <u>Nocardia madurae</u> ; <u>Nocardia braziliensis</u> .
19	Actinomycosis; culture of organism; tissue sections.
20	<u>Candida albicans</u> ; <u>C. tropicalis</u> ; <u>C. pseudo-tropicalis</u> ; <u>C. krusei</u> ; <u>C. parakrusei</u> ; injection of animals; stain smears; tissue sections; fermentations; chlamydospore culture; color movie on candidiasis.
21	Lecture by a dermatologist.
22	<u>Cryptococcus neoformans</u> ; staining of organism; injection of animals with <u>Coccidioides immitis</u> . <u>Geotrichum</u> sp., stained smear.
23	Aspergillosis; infection of chickens with aspergillosis. <u>Sporotrichum schenckii</u> ; staining of tissue.

Laboratory Period

Organism for Study

- | | |
|----|---|
| 24 | <u>Blastomyces dermatitidis</u> ; <u>B. brasiliensis</u> ; staining of tissue. |
| 25 | <u>Coccidioides immitis</u> ; staining of tissue; film strips on <u>Blastomycosis</u> and <u>Coccidioidomycosis</u> . |
| 26 | <u>Histoplasma capsulatum</u> ; staining of tissue, Rhinosporidiosis; Haplomycosis. |
| 27 | Reports on current literature. |
| 28 | Completion of slides, review. |
| 29 | Spot test. |

COMMON CONTAMINANTS LABORATORY PROCEDURES

INTRODUCTION

In order to become acquainted with the characteristics of growth, colonies and appearance of the somatic (or vegetative) and reproductive structures in fungi, a study of the common contaminants utilizing some of the more readily identifiable fungi at first is one good way to become acquainted with the handling of these organisms by the laboratory technician. The atmosphere usually contains many of the fungi that cause contamination in cultures. Some of these may occasionally cause allergies or be pathogenic in rare situations. In a few cases some of the contaminants may be confused with the pathogenic fungi. Both contaminants and pathogenic fungi may be isolated from skin lesions, sputum or other sources of materials from a patient.

The first few lectures or laboratory periods may be devoted to an introduction to the major classification systems and terminology of the fungi utilizing specific examples to illustrate the different classes in case there is a lack of previous background in mycology.

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