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# 208 MICROBIOLOGY

## SECTION 3

### LABORATORY WEEK 12

- THE ENTEROBACTERIACEAE
- GASTROINTESTINAL PATHOGENS
- **EXERCISE 37.** DIFFERENTIATION OF AEROBIC -VS- FACULTATIVE GRAM NEGATIVE RODS
- **EXERCISE 38.** RAPID METHODS OF BACTERIAL IDENTIFICATION, MULTIMEDIA SYSTEMS FOR BIOCHEMICAL PROFILING
- **EXERCISE 39.** YEAST AND MOLDS

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### THE ENTEROBACTERIACEAE

Members of genera belonging to the Enterobacteriaceae family have earned a reputation placing them among the most pathogenic and most often encountered organisms in clinical microbiology. These **Gram- negative rods** are usually associated with intestinal infections, but can be found in almost all natural habitats. They are the causative agents of such diseases as meningitis, bacillary dysentery, typhoid, and food poisoning. As well as being **oxidase negative**, all members of this family are glucose fermenters and nitrate reducers. In most cases, the pathogenicity of a particular enteric bacterium can be determined by its ability to metabolize lactose. Non-utilizers are usually pathogenic while the lactose utilizers are not. Because many different species in this family can cause similar symptoms, biochemical tests are crucial to the identification, diagnosis, and treatment of infection. In this exercise we will discuss genera of the Enterobacteriaceae family which are most commonly encountered in the clinical laboratory:

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### GASTROINTESTINAL PATHOGENS

#### SALMONELLA

*Salmonella* bacteria are instigated in more than 50,000 cases of bacterial **food infection** in the United States every year. Transmission of this microbe is usually through **uncooked meats and eggs**. **Chickens are a major reservoir of *Salmonella***, which explains its ubiquitous presence in poultry products. Ingesting foods contaminated with significant amounts of *Salmonella* can cause intestinal infection (**Gastroenteritis**) which eventually leads to **diarrhea, vomiting, chills, and headache**. The 2200 known serotypes of *Salmonella* are classified according to their surface antigens. The capsular properties of this Gram-negative rod can cause serious complications in immunosuppressed individuals such as HIV/AIDS patients. In the United States, *S. typhimurium* and *S. enteritidis* are the two leading causes of salmonellosis (inflammation of the intestine caused by *Salmonella*).

While most *Salmonella* are carried by animals, *S. typhi* is unique because it is only carried by humans. This intracellular parasite can cause typhoid fever (enteric fever) which is characterized by **fever, diarrhea, and inflammation of the infected organs.**

### **SHIGELLA**

*Shigella* is the primary causative agent of **bacillary dysentery** throughout the developing world. The disease is spread via the fecal-oral route and requires very low cell numbers to initiate infection (as few as 10 cells). In many cases, a *Shigella* infection will lead to diarrhea accompanied by fever. *Shigella* is also an **invasive pathogen** which can be recovered from the bloody stool of an infected host. Invasive pathogens colonize the host's tissues as opposed to growing on tissue surfaces. The four species in this genus are sometimes referred to by a letter designation based on their serological antigen:

Serotype A- *S. dysenteriae*

Serotype B- *S. flexneri*

Serotype C- *S. boydii*

Serotype D- *S. sonnei*

Strain D is the causative agent of most cases of *Shigella*-related diarrhea (shigellosis), while strain C species are rarely encountered in the laboratory. Anyone can get shigellosis but it is recognized more often in young children. **Those who may be at greater risk include children in day care centers, foreign traveler to certain countries, institutionalized people, and active homosexuals.** Animals are not infected with nor do they carry *Shigella*.

**Hemolytic uremic syndrome (HUS)** is a predominantly pediatric condition that consists of the simultaneous triad of hemolytic anemia, thrombocytopenia and acute renal failure, it is the most common cause of acute renal failure in children and is fatal in a small percentage.

### **ESCHERICHIA COLI**

*E. coli*, is the most encountered bacterium in the clinical laboratory. Besides being the number one cause of human urinary tract infections, *E. coli* has been linked to diseases in just about every other part of the body. Pneumonia, meningitis, and traveler's diarrhea are among the many illnesses that pathogenic strains of *E. coli* can cause. As part of the normal flora of the human intestinal tract, *E. coli* plays a crucial role in food digestion by producing vitamin K from undigested material in the large intestine. Pathogenic strains of *E. coli*, however, can cause severe cases of diarrhea in all age groups by producing a powerful endotoxin. Treating

Strains of *E.coli* that cause gastroenteritis and dysentery in humans have been grouped into many categories, four of which are: enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic(ETEC). Pathogenic *E. coli* are serotyped on the basis of their **O (somatic), H (flagellar), and K (capsular) surface antigen** profiles (laboratory exercise 40). Each of the categories listed above has a different pathogenesis and comprises a different set of O:H serotypes .

In Florida, gastrointestinal illness caused by <i>E. coli</i> is reportable in two categories: <i>E. coli</i> O157:H7 or <i>E. coli</i> , other. In 1997, 52 cases of <i>E. coli</i> O157:H7 and seven cases of <i>E. coli</i> , other (known serotype), were reported to the Florida Department of Health.
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**Enteropathogenic *E. coli* (EPEC)** is the oldest recognized category of diarrheogenic *E. coli*. EPEC cause either a watery or bloody diarrhea and has been linked to infant diarrhea. Sporadic outbreaks of EPEC diarrhea have occurred for half a century in infant nurseries and is a major cause of infant diarrhea in developing countries. Breastfeeding may protect against disease, experiments have shown human milk (and colostrum) to strongly inhibit the adhesion of EPEC to Hep-2 cells in vitro. Transmission of EPEC is via the fecal-oral route with an incubation period as short as nine hours.

**Enteroinvasive *E. coli* (EIEC)** strains were first shown to cause diarrhea in volunteer studies conducted in 1971. The organisms invade the epithelial cells of the intestine and cause a watery diarrhea. In a minority of patients, EIEC may produce an illness that is similar to shigellosis (bacillary dysentery). Dysentery caused by EIEC usually occurs within 12 to 72 hours following ingestion of contaminated food and is self-limiting. The illness is characterized by the appearance of blood and mucus in the stools, abdominal cramps, vomiting, fever, chills and malaise.

**Enterohemorrhagic *E. coli* (EHEC)** While the main EHEC serotype is *E. coli* O157:H7 other serotypes such as O111:H8 and O104:H21 are diarrheogenic in humans. EHEC excrete potent toxins called verotoxins or Shiga toxins; so called because of their close resemblance to the Shiga toxin of *Shigella dysenteriae*. This group of organisms is often referred to as Shiga toxin-producing *E. coli* (STEC). STEC organisms can be identified by several methods including the use of DNA probes that identify genes that code for toxins. The diarrhea may range from mild and nonbloody to bloody stools with no leukocytes. STEC strains can cause **hemolytic-uremic syndrome**.

**Enterotoxigenic *E. coli* (ETEC)** strains elaborate heat-labile toxin (LT), heat-stable toxin (ST), or both toxins. A variety of laboratory techniques can be used to identify ETEC: gene probes designed to detect the toxins or the toxin genes, tissue culturing, or immunochemical tests. The organisms colonize the surface of the small bowel mucosa where they elaborate their enterotoxins. The incubation period is short (14 to 50 hours). The diarrhea is watery, usually without blood or mucus. Fever may not be present and the disease is usually self-limiting. Infection with ETEC may cause diarrhea which is similar to that caused by *Vibrio cholerae*.

### **YERSINIA**

Two important species are included in the *Yersinia* genus: *Y. enterocolitica* and *Y. pestis*. *Y. enterocolitica* is the most often encountered species of *Yersinia* in the lab. This bacterium is an invasive pathogen which can penetrate the gut lining and enter the lymphatic system and the blood. Infection, which is usually through ingestion of contaminated foods, can cause a severe intestinal inflammation called yersiniosis. **Swine have been implicated as the principal animal reservoir for human pathogenic *Y. enterocolitica*. Release of its enterotoxin can cause severe pain similar to that found in patients with appendicitis (Mesenteric Lymphadenitis).** *Y. enterocolitica* is easy to identify because it is able to grow in cold temperatures and is motile at room temperature. Antibiotic treatment can consist of aminoglycosides, chloramphenicol, or tetracycline.

### KLEBSIELLA

The most clinically important species of this genus is *Klebsiella pneumoniae*. This large, non-motile bacterium produces large sticky colonies when plated on nutrient media. *Klebsiella*'s pathogenicity can be attributed to its production of a heat-stable enterotoxin. *K. pneumoniae* infections are common in hospitals where they cause pneumonia (characterized by emission of bloody sputum) and urinary tract infections in catheterized patients. In fact, *K. pneumoniae* is second only to *E. coli* as a urinary tract pathogen. *Klebsiella* infections are encountered far more often now than in the past which is probably due to the bacterium's antibiotic resistance properties.

The following list of genera are included for their role as normal intestinal flora and opportunistic infections of the urinary tract.

### EDWARDSIELLA

### CITROBACTER

### ENTEROBACTER

### SERRATIA

### PROTEUS

### MORGANELLA

### PROVIDENCIA

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#### **Case Study. Exam Review**

1. A 16-year-old female is admitted to the hospital with severe abdominal cramps, bloody diarrhea and a fever of 102F. She has been experiencing symptoms for the past 48 hours, since after eating at a fast food restaurant with a group of her friends three days ago. She recalls that the hamburger she ate was not very well cooked. (It is later learned that the meat being used in that restaurant to prepare hamburgers has been recalled due to bacterial contamination.) All of the following organisms can cause diarrhea, but which is the most likely cause of her illness? □

a. □ species of *Salmonella*

b. □ species of *Shigella*

c. □ *Escherichia coli* O157:H7

d. □ *Staphylococcus aureus*

e. □ species of *Yersinia*

2. Which of the above pathogens would you choose if the patient were 6-years-old and the restaurant were serving under cooked or mishandled chitterlings?

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**Case Study. Exam Review**

3. In June 2002, over the space of 3 days, 14 children were admitted to the Children's Hospital of Los Angeles city. All the children were between the ages 3 to 5 and all with the same symptoms. Their illness began with diarrhea, bloody stool and fevers of 102 –104F. 3 days later two of children's kidneys failed and they both died. Their history was significant that they all attended the same day care facility. All of the above organisms can cause diarrhea, but which is the most likely cause of this outbreak and death?

4. How would the following case history change your answer in #3 above?

"The children's history was significant that they all belonged to the same first grade class that had attended a local dairy farm and that they all had been walking through the barn yard with milk cows"

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### Exercise 37. DIFFERENTIATION OF AEROBIC -VS- FACULTATIVE GRAM NEGATIVE RODS

The **Enterobacteriaceae** is a large diverse family of bacteria commonly referred to as the **fermentative, gram-negative, enteric bacilli**, indicating that they are gram-negative rods that can **ferment sugars**. Many are normal flora of the intestinal tract of humans and animals. Some infect the intestinal tract. Members of this family have the following five characteristics in common:

1. They are **gram-negative rods**
2. If motile, they possess a **peritrichous arrangement of flagella**
3. They are **facultative anaerobes**
4. They are **oxidase negative, catalase positive**
5. All species **ferment the sugar glucose** but otherwise vary widely in their biochemical characteristics.

### PSEUDOMONAS AND OTHER NONFERMENTATIVE GRAM-NEGATIVE BACILLI

Nonfermentative gram-negative bacilli refer to gram-negative rods or coccobacilli that **cannot ferment sugars**. The nonfermentative gram-negative bacilli are often normal inhabitants of soil and water. They may cause human infections when they colonize immunosuppressed individuals or gain access to the body through trauma. However, less than one-fifth of the gram-negative bacilli isolated from clinical specimens are nonfermentative bacilli. **The most common gram-negative, nonfermentative rod that causes human infections is *Pseudomonas aeruginosa*.**

*Pseudomonas aeruginosa* is an obligate aerobic, **opportunistic pathogen**. It is a common cause of nosocomial infections and can be found growing in a large variety of environmental locations. In the hospital environment, for example, it has been isolated from drains, sinks, faucets, water from cut flowers, cleaning solutions, medicines, and even disinfectant soap solutions. It is especially dangerous to the debilitated or immunocompromised patient. Like the opportunistic Enterobacteriaceae, *Pseudomonas* is a gram-negative rod, it is frequently found in small amounts in the feces, and it causes similar opportunistic infections: **urinary tract infections, wound infections, pneumonia, and septicemia**.

*P. aeruginosa* is responsible for 12 percent of hospital-acquired urinary tract infections, 16 percent of nosocomial pneumonia cases, and 10 percent of the cases of septicemia. In addition, *P. aeruginosa* is a significant cause of **burn infections** with a 60 percent mortality rate. **It also colonizes and chronically infects the lungs of people with cystic fibrosis**. Like other opportunistic gram-negative bacilli, *Pseudomonas aeruginosa* also releases endotoxin and frequently possesses R plasmids. A number of other species of *Pseudomonas* have also been found to cause human infections.

## CULTURE

You are given two unknown gram negative rods on Nutrient Agar labeled “A” and “B”.

## PROCEDURE

Step 1. Each station has been provided with filter paper and Oxidase test reagent. Conduct an Oxidase test on unknowns A and B to determine which belongs to the Enterobacteriaceae.

UNKNOWN GRAM NEGATIVE RODS	OXIDASE TEST RESULTS
unknown A	
unknown B	

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## Exercise 38. RAPID METHODS OF BACTERIAL IDENTIFICATION

Gram-negative non-fermenting bacteria are widely distributed in the environment, often acting as opportunistic pathogens. These bacteria are frequently associated with many nosocomial infections and tend to be resistant to available drug therapies. The need to control and prevent these nosocomial infections has created a high demand for a quick and accurate method of **identification** for these bacteria.

**Hospital microbiology laboratories** typically employ **fully automated computerized methods** for the identification and classification of enteric bacterial species. One example of such a system is the Automicrobic System (bioMerieux Vitek, Inc.). The system uses disposable polystyrene cards that include 30 reaction micro-wells. **Each microwell contains biochemical substrates or dehydrated antibiotics.** A filling module automatically fills each microwell with the test bacterium and seals the card. An incubation and reading module optically scans the cards on a 1-hour rotational basis as they are incubated. A computer module records the biochemical reactions and performs identification analyses. **The final identification report can be available with as little as 6 hours.**

**Miniaturized Multimedia Systems** for Bacterial Identification have been developed and utilized successfully when **speed as well as cost effective Identification is needed.** In some instances, it is possible to identify a bacterium correctly by using only a few tests, but more often an extensive biochemical “profile” must be attained in smaller facilities such as academic laboratories, local clinics and reference or quality control laboratories. These commercially available test kits are especially useful for identifying the common enteric bacteria. One type of kit, the **Enterotube II**, is a tube of 12 compartmentalized, conventional agar media that can be inoculated rapidly from a single isolated colony on an agar plate. The media provided indicate whether the organism ferments the carbohydrates glucose, lactose, adonitol, arabinose, sorbitol and dulcitol; produce H<sub>2</sub>S and/or indole; produces acetylmethylcarbinol; deaminates phenylalanine; splits urea; decarboxylates lysine and /or ornithine; and can use citrate when it is the sole source of carbon in the medium.

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### MULTIMEDIA SYSTEMS FOR BIOCHEMICAL PROFILING

#### IDENTIFYING MEMBERS OF THE ENTEROBACTERIACEAE WITH THE ENTEROTUBE II

The Enterotube II contains 12 different media that can be used to carry out 15 standard biochemical tests.
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Color plate 12.5

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# THE ENTEROTUBE II

## CULTURE

Each group will receive one of the following:

1. Unknown member of the Enterobacteriaceae isolated on Blood Agar and inoculated on to the Enterotube II rapid I.D. system.
2. Instruction booklet.
3. Color code chart.

## PROCEDURE

**Step 1.** The instructor will demonstrate proper set up and inoculation of the Enterotube II.

**Step 2.** You are given an Enterotube II that has been inoculated with an Oxidase negative, Gram negative rod; it has been incubated at 37C for 24 hours.

**Step 3.** Interpret the results of your EnterotubeII using the instructions below. For more detail on the 15 biochemical tests in the Enterotube II see package insert .

**compartment 1.** Interpret the results of **glucose** fermentation .  
any yellow = +; red or orange = -  
If positive, circle the number 2 under glucose on your Results page.

**compartment 1.** Interpret the results of **gas** production .  
wax lifted from agar = +; wax not lifted from agar = -  
If positive, circle the number 1 under gas on your Results page.

**compartment 2.** Interpret the results of **lysine** decarboxylase.  
any purple = +; yellow = -  
If positive, circle the number 4 under lysine on your Results page.

**compartment 3.** Interpret the results of **ornithine** decarboxylase.  
any purple = +; yellow = -  
If positive, circle the number 2 under ornithine on your Results page.

**compartment 4.** Interpret the results of **H<sub>2</sub>S** production.  
true black = +; beige = -  
If positive, circle the number 1 under H<sub>2</sub>S on your Results page.

**compartment 4.** **Indole** production.  
Your instructor will give you the Indole test results of your unknown.

- compartment** 5. Interpret the results of **adonitol** fermentation.
- compartment** 6. Interpret the results of **lactose** fermentation.
- compartment** 7. Interpret the results of **arabinose** fermentation .
- compartment** 8. Interpret the results of **sorbitol** fermentation.

any yellow = +; red or orange = -

If positive, circle the points granted for each carbohydrate given on your Results page.

- compartment** 9. **Voges-Praskauer** test .

This test is not used unless a final VP confirming test is later called for

- compartment** 10. Interpret the results of **dulcitol** fermentation.

yellow = +; any other color = -

If positive, circle the number 1 under dulcitol on your Results page.

- compartment** 10. Interpret the results of **PA** deaminase .

black or smoky gray = +; any other color = -

If positive, circle the number 4 under PA on your Results page.

- compartment** 11. Interpret the results of **urea** hydrolysis in

red or purple = +; beige = -

If positive, circle the number 2 under urea on your Results page.

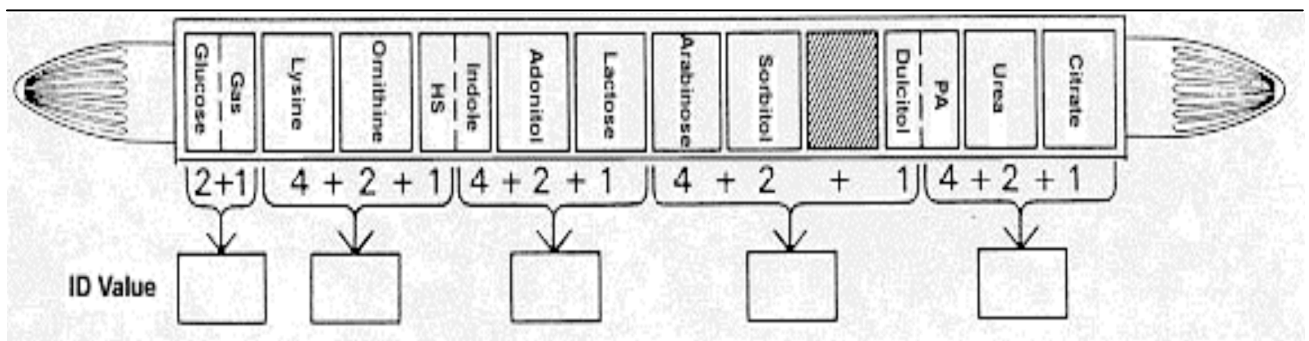
- compartment** 12. Interpret the results of **citrate** utilization .

any blue = +; green = -

If positive, circle the number 1 under citrate on your Results page.

**Add all the circled numbers in each bracketed section and enter the sum in the space provided below the arrow on your Results page.**

Locate the 5 digit number in the **Computer Coding and Identification System** booklet and find the best identification in the column entitled "ID Value."



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## EXERCISE 39. YEAST AND MOLDS

Medical mycology is concerned with the study and identification of the pathogenic yeasts and molds, collectively called *fungi* (sing. *Fungus*). You should be familiar with a number of important mycotic diseases.

**Yeasts are unicellular fungi that reproduce by budding**, that is, by forming and pinching off daughter cells. Yeast cells are much larger (about 5 to 8 times larger) than bacterial cells. The best-known (and most useful) species is “baker’s yeast,” *Saccharomyces cerevisiae*, used in bread making and in fermentations for wine and beer production.

**Molds are multicellular, higher forms of fungi.** They are composed of filaments called *hyphae*, abundantly interwoven in a mat called the *mycelium*. Specialized structures for reproduction arise from the hyphae and produce asexual spores, each of which can germinate to form new growth of the fungus. The visible growth of a mold often has a fuzzy appearance, because the mycelium extends upward from its vegetative base of growth, thrusting specialized hyphae that bear conidia into the air. This portion is called the *aerial mycelium*. You have often seen this on moldy bread or other food, and you have probably also noted that different molds vary in color (black, green, yellow), because of their spore pigment.

Most of the thousands of species of yeasts and molds that are found in nature are saprophytic and incapable of causing disease. Indeed, many are extremely useful in the processing of certain foods (such as cheeses) and as a source of antimicrobial agents. *Penicillium notatum*, for example, is the mold that produces penicillin.

## MYCOTIC DISEASES AND THEIR AGENTS

Fungal disease fall into three clinical patterns: *superficial* infections on the surface epithelial structures (skin, hair, nails), *systemic* infections of deep tissues, and *subcutaneous* infections.

### **Superficial Mycoses**

The pathogenic fungi that cause infections of the skin, hair, or nails are often referred to collectively as *dermatophytes*. There are three major genera of dermatophytes:

#### **Trichophyton:**

This genus contains many species (e.g., *T. mentagrophytes*, *T. rubrum*, *T. tonsurans*) associated with “**ringworm**” infections of the scalp, body, nails and feet. “Athlete’s foot” is perhaps the most common of these infections.

#### **Microsporium:**

There are three common species of this genus: *M. audouini*, *M. canis*, and *M. gypseum*. These fungi cause **ringworm** infections of the hair and scalp, and also, of the body.

*Microsporum canis* is a **zoophilic dermatophyte** of world-wide distribution which is a frequent cause of ringworm in humans, especially children. Cats and dogs are the main sources of infection. Invaded hairs show an **ectothrix infection\*** and fluoresce a bright greenish-yellow under **Wood's ultra-violet light\***.

**Epidermophyton:**

One species, *E floccosum*, causes ringworm infection of the body, including athlete's foot. It does not affect hair or nails.

These superficial fungal infections are called ringworm, because the lesions are often circular in form. The medical term for ringworm is *tinea*, followed by a word indicating the area, e.g., *tinea capitis* (scalp), *tinea corporis* (body), or *tinea pedis* (feet).

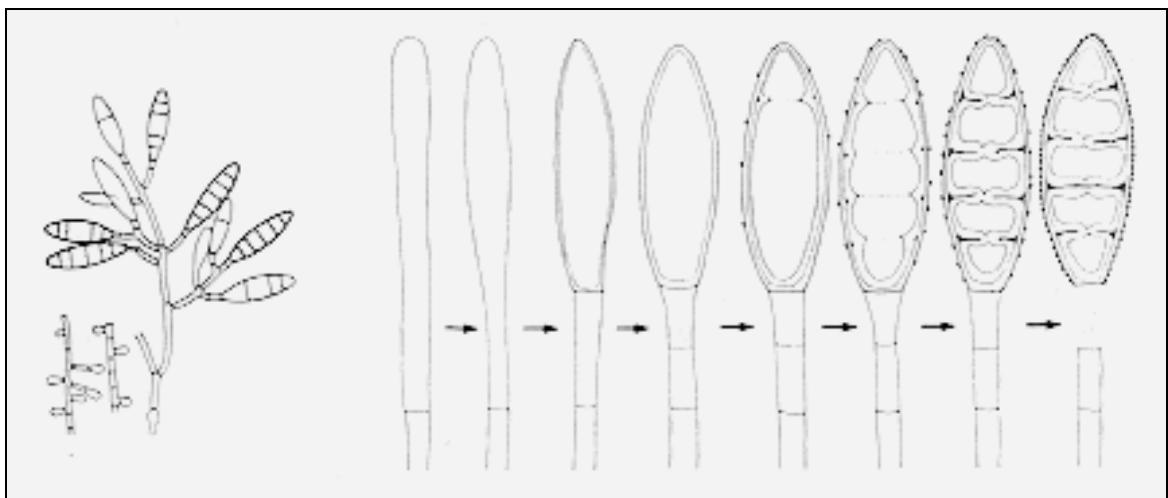
**\*Ectothrix infection**

Ectothrix hair invasion is due to infection with *M. canis*, *M. audouinii*, *M. distortum*, *M. ferrugineum*, *M. gypseum*, *M. nanum*, and *T. verrucosum*. The fungal branches (hyphae) and spores (arthroconidia) cover the outside of the hair. Ectothrix infections can be identified by **Woods light** (long wave ultraviolet light) examination of the affected area. Your physician or veterinarian may use the Woods Lamp in diagnosis.

**Endothrix infection**

Endothrix invasion results from infection with *T. tonsurans*, *T. violaceum* and *T. soudanense*. The hair shaft is filled with fungal branches (hyphae) and spores (arthroconidia). Endothrix infections do not fluoresce with Woods light.

**FIGURE 12.1 *Microsporum canis* MACROCONIDIA  
LARGE THICK-WALLED, 6-15 DIVISIONS IN MATURE FORMS**



The drug **griseofulvin** is a naturally occurring compound and so is a true antibiotic with antifungal properties. It binds to the proteins involved in microtubule formation and prevents separation of chromosomes at mitosis. Why griseofulvin does not affect human cells is not known. It is used in the treatment of ringworm and other fungal infections of the skin or nails.

### Systemic and Subcutaneous Mycoses

Many of the fungi involved in systemic and subcutaneous infections are either yeasts or display *both* a yeast and a mold phase (they are said to be **dimorphic** because of this). **The yeast phase of dimorphic fungi grows best as 35 to 37°C, whereas their mold phase grows optimally at a lower (25°C) temperature.**

### Opportunistic Mycoses

Under ordinary circumstances, fungi are of low pathogenicity and have little ability to invade the human body. However, when the host's immune defense mechanisms are decreased by illness (leukemias, lymphomas, acquired immune deficiency syndrome or inherited immune deficiencies) or by drugs (steroids, cancer chemotherapeutics, transplantation drugs), fungi (as well as other microorganisms) find the opportunity to invade and establish disease. Because few antimicrobial agents are available to combat fungal infections, these represent among the most serious opportunistic illnesses and are frequently the direct cause of the patient's death. Some opportunistic fungi, such as the yeasts ***Candida*** and ***Cryptococcus*** are not always associated with immunosuppression, but others, especially species of *Aspergillus* and *Mucor*, infect only "disabled" hosts. Because the latter organisms are also widespread in the environment, health care personnel must be certain that specimens obtained from immunocompromised patients are always placed in sterile containers and in such a manner as to avoid contamination with airborne fungal spores. The microbiology technologist must also protect culture plates and broths from such contamination so that any molds that grow out are known to come from the patient and not the environment.

**Cryptococcosis:** *Cryptococcus neoformans* is the causative agent of cryptococcosis. Given the neurotropic nature of the fungus, the most common clinical form of cryptococcosis is meningoencephalitis. The course of the infection is usually subacute or chronic. Cryptococcosis may also involve the skin, lungs, prostate gland, urinary tract, eyes, myocardium, bones, and joints.

**Candidiasis:** Fungal disease caused by *Candida albicans*. This yeast is found among the normal flora of the mouth, digestive tract, and vagina of perfectly healthy people, but under some circumstances, and for reasons unknown, it may cause severe and even fatal infections, with lesions and eruptions of the skin, nails, mouth, bronchial tubes and lungs.

**Blastomycosis:** Infection apparently comes from spores or mycelium of *Blastomyces dermatitidis* and *B. brasiliensis*, in the soil and any part of the body may become infected through access of wound on body. Infections form localized lesions, but may spread throughout body forming extensive ulceration. *see table 12.1*

**Coccidioidomycosis:** Systemic mycosis, also known as Valley Fever. Contracted through inhalation of spores of *Coccidioides immitis*,<sup>¶</sup> from soil, causing respiratory problems in animals. However, may spread throughout the body by way of the bloodstream and cause pathologic changes - lesions of one sort or another - in just about all tissues in all parts of the body, and may be fatal in such cases. *see table 12.1*

**Histoplasmosis:** Systemic mycosis, usually occurring in people and dogs. Contracted through inhalation of spores of *Histoplasma capsulatum*, that occur in droppings of birds, and bats as well as other rodents. May only cause localized respiratory infection, but can spread through the blood and become fatal. This mycosis is particularly endemic in Kentucky. *see table 12.1*

**Sporotrichosis:** refers to the infection caused by the dimorphic fungus *Sporothrix schenckii*. The disease has been described worldwide, however it is more common in tropical and subtropical America. Sporotrichosis is acquired through direct inoculation into the skin (generally, via rose thorns) and rarely via inhalation of conidia. As a consequence, the majority of cases are localized lesions affecting the skin and subcutaneous tissues with minimal if any systemic manifestation. *see table 12.1*


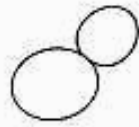
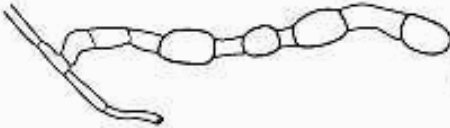
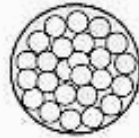
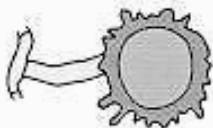
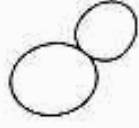
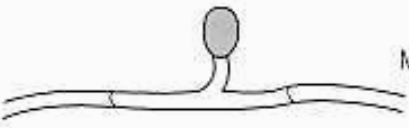


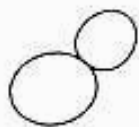
## **LABORATORY DIAGNOSIS**

For the laboratory diagnosis of mycotic diseases, the causative fungi may be isolated from a variety of clinical specimens representing the focus of infections (sputum, spinal fluid, pus aspirated from lymph nodes or other lesions, skin scrapings). They can often be visualized in wet mounts of such specimens. Stains are usually not required for these preparations, but potassium hydroxide solution is used to clear away tissue cells and debris, making fungi more prominent.

**Fungi are identified by the morphology of their reproductive spores and mycelial features and some of their metabolic properties.** They grow slowly in culture, producing large colonies whose gross appearance is also helpful in identification. Some are yeasts or yeast-like, and some are dimorphic or diphasic, as mentioned previously. The latter grow as yeast at 35°C in the host and as molds at 25°C in laboratory culture.

Fungi are most commonly cultured on **Sabouraud's Dextrose Agar**. This media contains 4% Dextrose which is slightly hypertonic in scale and an adjusted pH of 5.6. These properties make SDA selective for Yeast and Molds since bacteria prefer an environmental pH close to neutral and sugar concentrations adjusted to 1%.

**TABLE 12.1 CLINICALLY IMPORTANT DIMORPHIC FUNGI**

Fungus	In vitro (25° C)	In vivo (37° C)
<i>Blastomyces</i>	 Mold	 Yeast
<i>Coccidioides</i>	 Mold	 Spherule
<i>Histoplasma</i>	 Mold	 Yeast
<i>Paracoccidioides</i>	 Mold	 Yeast
<i>Sporothrix</i>	 Mold	 Yeast

## **CULTURE**

*Saccaromyces cerevisiae* Sabourauds Dextrose Agar slant.

Prepared Slide of *Aspiggillus*, *Rhizopus*, *Penicillium*.

Demonstration plate cultures containing *Aspiggillus*, *Rhizopus*, *Penicillium*.

## **PROCEDURE**

Step 1. Prepare a Gram stained smear of *Saccaromyces cerevisiae*.

Step 2. Examine the prepared slide containing three mold types: *Aspiggillus*, *Rhizopus*, *Penicillium*.

Step 3. Examine the plate cultures of *Aspiggillus*, *Rhizopus*, *Penicillium*.

Step 4. Record your observations in results table 12.1

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## RESULTS TABLE 12.1

<b>YEAST AND MOLDS</b>				
	<i>S.cerevisiae</i> GRAM STAIN	<i>R. nigraficans</i> PREPARED SLIDE	<i>A. niger</i> PREPARED SLIDE	<i>P. notatum</i> PREPARED SLIDE
DIAGRAM SLIDE PREPARATION				
CELL COLOR AND ARRANGEMENT				
PLATE CULTURE DISCRIPTION	X			

See Color plates 12.1,12.2,12.3,12.4

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## TERMS AND QUESTIONS FOR STUDY

1. For each of the diseases listed, indicate the type of specimen(s) that should be collected for laboratory diagnosis.

Cryptococcosis

Tinea capitis

Thrush

Histoplamosis



11. What is meant by the term “Enteric Pathogen”?
12. Describe two mechanisms by which enteric pathogens produce disease.
13. Describe a Miniaturized Multimedia System for rapid identification.
14. Why is it important to differentiate glucose nonfermenters from Enterobacteriaceae?
15. What is Hemolytic Uremic Syndrome?
16. What would be considered a major reservoir of *Salmonella*? From what foods would you expect this type of infection?
17. Discuss why *Shigella* is involved in day care outbreaks more so than *Salmonella* or *Yersinia*?
18. What do the acronyms EPEC, ETEC, EIEC and EHEC represent?
19. What would be considered a major reservoir of *Yersinia*?
20. What roll does the Oxidase test play in gram negative rod differentiation? in gram negative cocci?