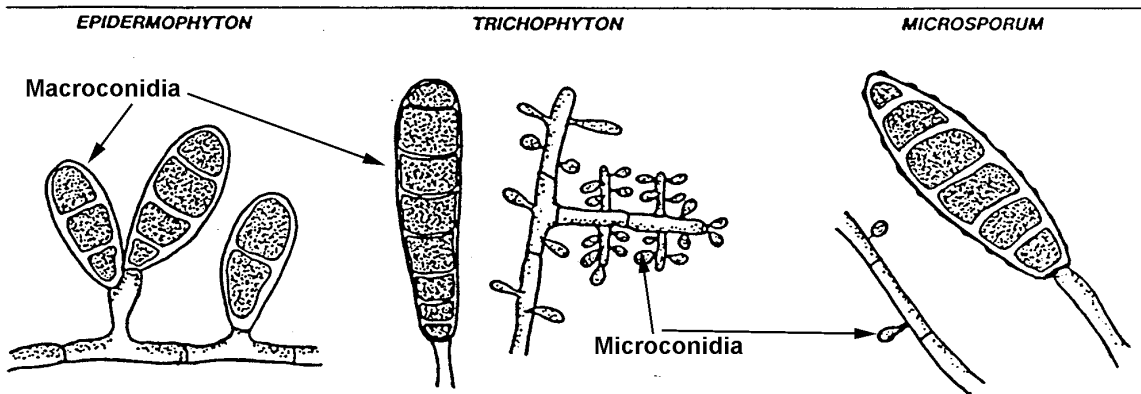
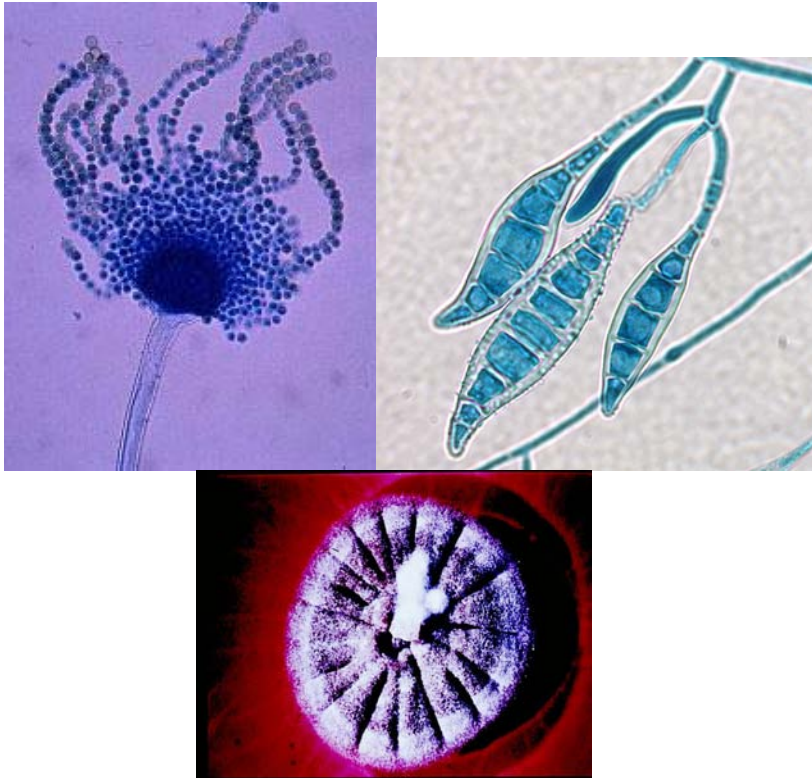


The University of Texas at El Paso
College of Health Sciences

Clinical Laboratory Science Program

CLSC 3159 – Opportunistic & Parasitic Infection Laboratory



Fall 2009

The University of Texas at El Paso
College of Health Sciences
Clinical Laboratory Science Program

CLSC 3159 – Opportunistic & Parasitic Infection Laboratory
Monday or Wednesday* at CHS 608

Instructor: *Dora E. Meraz M.T. (ASCP), M. Ed.*
Office: College of Health Sciences (CHS) 618
e-mail: (Web-CT account)

Phone: 747-7243
Fax: 747-7207

OFFICE HOURS: Mondays and Wednesdays 11:00 am - 12:00 pm

If you are unable to see me at this time, you may arrange an appointment at another time. You may schedule meetings by WebCT, telephone, or in person after lab session. Please use office hours to clarify lecture objectives, special interests or career goals at the earliest convenience for both parties. The best time to reach me by phone is during posted office hours. If I am unable to answer your call, please leave a detailed message and I'll return your call as soon as possible.

COURSE DESCRIPTION:

Welcome to Clinical Microbiology I Lab

The purpose of this course is to provide a basic and practical introduction to the fields of Medical Mycology and Parasitology. This course will present an overview of the mycology laboratory including safety procedures, specimen collection and processing, isolation and identification of fungi and parasites and diseases caused by these organisms.

COURSE GOAL: At the end of this course the student will be able to perform basic laboratory techniques in both parasitology and mycology laboratories. In addition, the student will apply basic knowledge to make decisions and solve problems in the clinical setting.

COURSE OBJECTIVES:

A. Cognitive

Upon completion of this course, the student should be able to:

1. Learn basic terms in mycology such as: mycosis, mycelium, hypha, septate, aseptate, spore, mold, yeast, etc.
2. Define and identify clinically significant fungi in the following groups: cutaneous mycoses, subcutaneous mycoses and systemic mycoses.
3. Learn key procedures used in specimen collection
4. Learn safety procedures and biosafety levels in the laboratory
5. Describe the composition and use of the following fungal media:
 - a. Sabouraud Dextrose Agar
 - b. Mycosel or Mycobiotic
6. Describe or accurately perform a KOH preparation from skin, hair, or nail scrapings.
7. Accurately describe the growth rate, pigmentation, and colonial topography of commonly encountered fungal contaminants.
8. Demonstrate acceptable technique in the preparation and interpretation of tease mounts and scotch tape preps of fungal contaminants.
9. Describe and demonstrate proper technique in the performance of the slide culture technique for fungal contaminants.

10. Describe the composition and function of lactophenol cotton blue stain for hyaline fungi; demonstrate the proper usage of this stain in the preparation of fungal wet mounts.
11. Describe the usage and demonstrate proper technique in the preparation of India ink preps for fungal detection.
12. Describe and accurately perform a germ tube test for yeast identification.
13. From fungal colonies, microscopic preparations, or 35mm slides, accurately identify the following fungi:

1. <u><i>Microsporium canis</i></u>	2. <u><i>Microsporium gypseum</i></u>	3. <u><i>Rhizopus sp.</i></u>	4. <u><i>Trichophyton rubrum</i></u>	5. <u><i>Alternaria sp.</i></u>
6. <u><i>Trichophyton tonsurans</i></u>	7. <u><i>Trichophyton mentagrophyte</i></u>	8. <u><i>Curvularia sp</i></u>	9. <u><i>Coccidioides immitis</i></u>	10. <u><i>Penicillium sp</i></u>
11. <u><i>Blastomyces dermatitidis</i></u>	12. <u><i>Histoplasma capsulatum</i></u>	13. <u><i>Aspergillus niger,</i></u>	14. <u><i>Aspergillus fumigates</i></u>	15. <u><i>Cryptococcus neoformans</i></u>
16. <u><i>Fusarium sp.</i></u>	17. <u><i>Candida albicans</i></u>	18. <u><i>Dreschlera</i></u>	19. <u><i>Scopulariopsis</i></u>	20. <u><i>Absidia</i></u>
21. <u><i>Bipolaris</i></u>				

14. Describe the recommended procedure for collection, preservation, and processing of stool samples for parasitic examination.
15. Describe proper technique in the following parasitology methods:
 - a. Direct examination of fecal material with saline and iodine.
 - b. Ritchie fecal concentration method
 - c. PVA-Trichrome staining for protozoan parasites
 - d. Blood smear examination for parasites
 - e. Scotch tape prep for *Enterobius* ova
 - f. Acid fast stain for *Cryptosporidium*
16. Accurately identify the following parasites in formalin samples, trichrome stained smears, acid fast stained smears, or color slides:

<u><i>Entamoeba histolytica</i></u>	<u><i>Giardia lamblia</i></u>	<u><i>Trichomonas vaginalis</i></u>	<u><i>Trypanosoma brucei</i></u>
<u><i>Trypanosoma cruzi</i></u>	<u><i>Leishmania donovani</i></u>	<u><i>Cryptosporidium species</i></u>	<u><i>Blastocystis hominis</i></u>
<u><i>Plasmodium species</i></u>	<u><i>Hookworm species</i></u>	<u><i>Schistosoma species</i></u>	<u><i>Hymenolepis species</i></u>
<u><i>Taenia species</i></u>	<u><i>Diphyllobothrium latum</i></u>	<u><i>Ascaris lumbricoides</i></u>	

B. Affective

To show the appropriate responsible behaviors, students will demonstrate:

1. A positive attitude by being prepared for lecture and laboratory sessions, completing assigned tasks on time and displaying self-motivation.
2. Organization by utilizing time effectively, sequencing and prioritizing tasks for completion with time constraints and maintaining a neat clean work.
3. Attention to detail by diligently pursuing accuracy and documenting data accurately and legibly.
4. Problem solving ability by explaining purpose of each step in diagnosis, interpretation, procedure, recognizing discrepancies in techniques or procedures and repeating necessary lab tests when necessary.

5. Dependability by following directions, working independently after being given directions.
6. Stability and self-confidence by approaching and performing routine tasks confidently without assistance and maintaining composure.
7. Appropriate interpersonal skills by cooperating and communicating effectively with classmates and instructors and displaying courteous, considerate behavior and appropriate appearance.
8. Ethical behavior and integrity by respecting confidentiality of patient information, complying with professional standards and code of ethics, adhering to safety policies and abiding by all rules and regulations of the institution.

NO ONE WILL BE ALLOWED IN THE LABORATORY WITHOUT PROPER PERSONAL PROTECTIVE COVERING. UNIVERSAL PRECAUTIONS WILL BE OBSERVED AT ALL TIMES. AT THE INSTRUCTORS DISCRESSION, THE INSTRUCTOR MAY DISMISS A STUDENT WHO DOES NOT HAVE THE PROPER PERSONAL PROTECTION.

COURSE POLICIES:

1) Required Text: Fisher, F. and N.B. Cook. 1998. Fundamentals of Diagnostic Mycology. W.B. Saunders, Philadelphia, PA.

Leventhal, R. and Cheadle R. 2002. Medical Parasitology: A Self-Instructional Text. F.A., Davis, 5th ed.

Suggested readings:

Forbes, B.A., Sahm D.F., and Weissfeld, A.S. 2002. Finegold. Bailey & Scott's Diagnostic Microbiology 9th ed. Mosby.

Garcia, L.S. and D.A. Bruckner, 1996. Diagnostic Medical Parasitology, 3rd ed. ASM Washington, D.C.

Kwon-Chung, K.J. and J. Benett 1992. Medical Mycology. Lea and Febiger, Phi PA.

2) Class Attendance: The student is expected to attend ***all lab sessions and be on time***, wear protective equipment, and actively participate. It is responsibility of the student to notify the instructor of any absence and to provide legitimate documentation of absence to abide to University regulations. The instructor reserves the right to drop a student due to tardiness or absences when in the judgment of the instructor, a student has been absent to a degree as to impair his or her status relative to credit for the course. The instructor may drop the student from the class with a **W** before the course drop deadline or with an **F** after the course drop deadline. If a student is 10 minutes late this will be recorded as a tardy.

3) Instructional Strategies: The laboratory is competency based. The student must demonstrate their competency to perform the lab procedure at the designated level before they can progress to the next lab. Lab assignments must be written up and the procedures performed within the standard deviation for the procedure and to the satisfaction of the instructor for a pass/fail grade. If they receive a fail grade, the lab must be repeated until the student receives a pass grade. In a competency based program you are either competent to perform the procedure or you are not. No one progresses until they are competent. **THERE WILL BE NO MAKEUP LABS.** All competencies and repeats must be taken the day they are assigned unless extenuating circumstances occur. The student must take the initiative in this course and see that everything is learned and completed. A written exam will also be given for a letter grade and **MUST** be passed with at least the minimum passing grade of 75%.

4) Test Policy: Proficiency testing and quizzes will be given at various intervals on the material covered. No make ups will be offered. If you cannot attend a test for a legitimate reason (death, illness etc.) inform me as soon as possible and we will arrange a time to my schedule. **5% of final grade will be removed if the student misses any of the scheduled assessments for a legitimate reason.** (Make ups exams/quizzes, while they may

cover the same material may differ from the exam/quiz taken by the rest of the class in organization, format, or specific item data.) Students should maintain a 75% or above average to continue in the program. Student participations will be taken into account for grade determination. The final grade for the laboratory will be calculated as follows:

a) Proficiency and mid-term exam	30%	(50% exam + 50% practical)
b) Quizzes	30%	
c) Attendance & Participation	10%	
d) Final exam	30%	

5) Grading Scale: 90 – 100 =A, 80 – 89 =B, 75 – 79 =C, 70 or below =F

6) Laboratory Safety Requirements: Wearing a laboratory coat is mandatory and open shoes are not allowed in the laboratory. Students are to follow the following general safety precautions at all times:

- a. The practice of personal cleanliness is important in any laboratory. It is a safe practice to wash your hands frequently and always before leaving the laboratory.
- b. Eating, drinking and applying cosmetics in the laboratory are strictly forbidden as a precaution against accidental infection.
- c. Your working area should be free of extraneous articles ie: books, purses, etc. With the 5% disinfectant solution provided, wet a sponge and wipe the table top area before and after work.
- d. Please do not wear open sandals to the laboratory. If you drop or break a culture tube you may get infectious material and glass splinters in your feet.
- e. No organisms are to be removed from the laboratory at any time.
- f. If infectious material is spilled on the table top or floor, flood the entire contaminated with 5% bleach. Cover immediately with paper towels and allow to stand for 10 min. Carefully collect the paper towels and dispose them in the biohazard container. Notify the instructor at once of any accident. Instruction for the decontamination of cloths and shoes will be given if needed. Caution nearby workers to avoid the contaminated area until it is properly cleaned and disinfected.
- g. Always dispose all contaminated material in the biohazard container. These containers are not to be used for regular trash or paper.
- h. Since you are responsible for the safety of other students as well as yours own, failure to observe these guidelines may result in **dismissal** from the class.

7) Specific Laboratory Standards:

- a) Data must be recorded directly into a laboratory notebook binder. DO NOT record data on paper towels.
- b) Data must have a proper title and subheadings.
- c) When appropriate, the following information should accompany recorded information:
 1. Genus and Species or identification number of the organism
 2. Source of isolate
 3. Isolation medium
 4. Incubation temperature and time of incubation (hours, days, weeks)
 5. Colony description
 6. Drawings of all stained preparations
 7. Tabulate all biochemical data. Use flow charts for organism identification as recommended.
- d) All tubes and petri dishes must be properly labeled when incubated
- e) Discard all tubes and petri dishes in the appropriate container as soon as possible when finished.
- f) Any film, field trip or conference will be summarized in the data book in a special section

PARASITOLGY/MYCOLOGY TENTATIVE LABORATORY SCHEDULE
CHS 608 MONDAY OR WEDNESDAY
11AM-1PM

Aug 24/26	Intro to Mycology Lab / Lab Safety / Specimen collection & processing
August 31/ Sept. 02	Opportunistic Fungi – Set up environmental cultures
Sept 07/ 09	Colony morphology and Tease mounts/ Microscopic morphology
Sept 14-16	Set up slide cultures / Microscopic morphology
Sept 21-23	<i>Dermatophytes</i> – colony morphology/ microscopic morphology / hair perforation test
Sept 28-30	Yeast morphology/Germ tube production/Biochemical Identification of yeast
Oct. 5-7	Mid- Term
Oct. 12-14	Dimorphic Fungi- Systemic Mycosis-Thomason Laboratory*
Oct 19-20	Dimorphic Fungi – Systemic Mycosis-Qui
Oct 26-28	Intro to parasitology Lab
Nov 02-04	Concentration procedures for Stool Specimens
Nov. 9-11	Microscopic examinations from concentrations of <i>Helminths</i>
Nov 16-18	Permanent slides blood and tissue parasites
Nov 23-25	Permanent slides blood and tissue parasites
Nov 30-Dec 2	Permanent slides and tissue parasites
Dec 7-9	Review/Final Exam

*The date/visit is subject to change according to the availability of clinical specimens

Clinical Microbiology I Laboratory

Clinical Mycology/Parasitology Objectives

MYCOLOGY

Week1-2 **I. Introduction to Mycology Laboratory/ Set up of Environmental Cultures**

Objectives:

- a. Discuss the concept of universal precautions
- b. Differentiate among the different types of biosafety levels
- c. Explain how to clean up after a culture spill
- d. State the advantages and disadvantages of using slant tubes and petri dishes for fungal cultures
- e. Discuss selection and preparation of specimen collection for the Mycology lab

Week 3 **II Colony Morphology and Tease mounts**

Objectives:

- a. Discuss the importance of direct examination of specimens submitted for fungal culture
- b. Explain when and why each of the following wet mounts should be performed:
 - lactophenol cotton blue
 - KOH
 - india ink
 - Methenamine silver
 - Periodic Acid Schiff
- c. Perform and explain the purpose of the tease mount
- d. Identify basic fungal structures such as: hypha, type of hypaha sepatated, aseptated, hyaline or dimatiaceous; conidia, phialides, raquet hypha, spores, and yeast cells.
- e. Describe the colony morphology of fungal cultures including texture, topography and pigmentation

Week 4 **III Set up Slide Cultures**

Objectives:

- a. Outline the steps in performing a slide culture
- b. Discuss the advantages and disadvantages of performing a slide culture vs. a tease mount
- c. Compare and contrast the following techniques: tease mount, cellophane tape and slide culture
- d. List the media most commonly used in the mycology laboratory

Week 5 **IV Microscopic Morphology of Opportunistic Fungi**

Objectives:

- a. Define / explain “opportunistic fungi”.
- b. Review and describe the microscopic morphology of the following organisms including the following information: type of hypha, sepatation, macroconidia, microconidia, annelloconidia, hyaline, dimatiaceous, phialide, racquet hypha

Alternaria
Curvularia

Penicillium
Aspergillus

Fusarium
Absidia
Bipolaris

Rhizopus
Dreschlera
Scopulariopsis

- c. Use colony morphology and conidia patterns to recognize the above organisms.
- d. Discuss the increased importance of opportunistic pathogens in the medical field.
- e. Correlate clinical data, microscopic morphology and etiologic agent.

Week 6 **V Dermatophytes Colony morphology, Microscopic morphology/ Hair perforation tests**

Objectives:

- a. Draw and describe the morphological features of the following dermatophytes: *Microsporum*, *Trichophyton* and *Epidermophyton*
- b. Perform tease mounts and slide cultures of *Microsporum canis*, *Trycophyton rubrum*, *Trycophyton mentagrophytes* and *Epidermophyton floccosum*
- c. Describe colony morphology of the organisms mentioned above.
- d. List two media which enhance conidation.
- e. Construct a chart including the diseases caused by dermatophytes.
- f. Define ectothrix, endothrix and favic infection.
- g. Given the clinical data and microscopic morphology, identify the etiologic agent and suggest the possible diagnosis.
- h. Perform the hair perforation test and indicate which organism is positive *T. rubrum* or *T. mentagrophytes*.

Week 7 **VI Yeast Morphology/ Germ Tube production**

Objectives:

- a. Describe the colony morphology of yeast cultures
- b. Compare and contrast mold cultures vs. yeast cultures including: growth rate, texture, topography and pigmentation
- c. List at least 4 culture media for the isolation of yeast
- d. Prepare an india ink wet mount, discuss the principle of the technique and differentiate a positive vs. a negative test
- e. Perform a germ tube test and explain what the purpose and significance is of this test
- f. Define the following terms: pseudohypha, germ tube, chlamydiospore, blastoconidia, budding

VII Biochemical Identification of Yeast

Objectives:

- a. Define the following terms: assimilation, fermentation, colonization, infection
- b. Design a flow chart for the presumptive identification of yeast
- c. Compare and contrast the identification scheme for moulds vs. yeast
- d. List the media used to enhance chlamydiospore formation
- e. Perform Gram stains of the following: *Cryptococcus neoformas*, *Candida albicans* and *Candida krusei* and describe the microscopic morphology.
- f. Inoculate a biochemical bioset for the identification of yeast, interpret the results and identify the organism in question.
- g. Correlate clinical data, microscopic morphology and biochemical testing with etiologic agent.

Week 8 **MID TERM**

Week 9 Thomason Laboratory (Tentative visit)

Week 10 Dimorphic Fungi: Systemic Mycosis

Objectives:

- a. Briefly explain what is a dimorphic fungi
- b. Differentiate between mycelial vs yeast phase
- c. List the fungi that cause systemic mycoses
- d. Define systemic infection
- e. Design a flow chart for the identification of the following fungi:
H. capsulatum, *C. immitis*, *B. dermatitides*, *P. brasiliensis*
- f. Outline the procedure for converting any dimorphic fungi from one phase to the other
- g. Describe the symptoms of primary pulmonary infections caused by systemic fungi

PARASITOLOGY

Week 11 VIII Artifacts that can be confused with Parasitic Organisms

Objectives:

- a. Name non-human elements that can be confused with parasites
- b. List 2 characteristics by which *E. Histolytica* can be differentiated from macrophages
- c. Outline the steps in the preparation of an iodine wet mount
- d. Define: cyst, trophozoite, pseudopodia,
- e. Recognize the morphological features of *E. coli* and *E. histolytica* cysts
- f. Perform an iodine wet mount from a stool concentration and identify the following material: vegetable fibers, meat fiber, calcium oxalate crystals, starch, pollen granules, meat fiber, yeast, and bacteria.

Week 12 IX Concentration Methods for Stool Specimens

Objectives:

- a. State the purpose of the concentration methodology
- b. Describe at least 2 collection kits and indicate which fixatives are used and which procedures can be performed from specimens preserved in each fixative
- c. Prepare 5 iodine wet mounts from positive concentration mix and draw the parasitic forms encountered in each sample
- d. Differentiate protozoan cysts vs. helminth eggs
- e. Construct a chart containing the egg morphological features of the following organisms: *Trichuris trichura*, *Taenia solium/saginata*, Hookworms, *Ascaris* fertile and unfertile, *E. vermicularis*.
- f. Compare and contrast the cysts of *E. histolytica* and *E. coli*.
- g. Recognize the cysts/trophs of the following: *Iodoamoeba butchlii*, *Giardia lamblia*, *Endolimax nana*,

Week 13 X Permanently stained Mounts

Objectives:

- a. Discuss the pros and cons of the two most commonly used permanent stains.
- b. Describe the use of the following stains in the examination of parasites: Modified acid fast, Trichrome, Iron hematoxilin
- c. Examine 6 permanent stained mounts and locate the parasitic forms

- found in each slide
- d. List the reagents of the trichrome stain
 - e. Outline the major steps in the trichrome stain procedure
 - f. Discuss the clinical significance and identification of the following:
Toxoplasma gondii, *Cryptosporidium parvum*

Week 14

XI Blood Permanent Smears

Objectives:

- a. Explain the importance of preparing thick and thin blood smears
- b. List 5 blood parasites and describe their morphology
- c. Define Xenodiagnosis
- d. Name the stain of choice for the identification of blood parasites
- e. List 3 concentration procedures for the recovery of blood parasites
- f. Examine 5 blood permanent smears and identify the blood parasite in each one
- g. Explain the importance in quantifying blood parasites
- h. Given clinical and epidemiological data as well as morphological characteristics of a parasite evaluate the case and give diagnosis/therapy

Week 15

FINAL EXAM