# Introduction to General Microbiology

## Biology 362, Lecture and Laboratory

## Course Description and Schedule

Chaminade University
Winter 2000
5:45 -10:00 p.m. (M, W)

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**This is an accelerated introductory course to general microbiology with major emphasis on clinical (medical) microbiology. This course is directed toward upper-division biology majors. A basic understanding of general biology and organic chemistry is required. Although more inclined toward the clinical field, the concepts learned in this course can be applied to other areas of microbiology (environmental, industrial, etc.). Students must be enrolled in both the lecture and laboratory portions of this course.**

**The following texts will be used for the lecture and laboratory portions of the course.**

1. **Jensen, M.M., Wright, D.N., and Robison, R.A. 1997. Introduction to Microbiology for the Health Sciences, 4th edition. Prentice Hall, New Jersey.**

2. **Leboffe, M.J., and Pierce, B.E. 1999. A Photographic Atlas for the Microbiology Laboratory, 2nd edition. Morton Publishing Co., Colorado.**

**The student may also consult the following references:**

1. **Henry, J. 1996. Clinical Diagnosis and Management by Laboratory Methods, 19th edition. W.B. Saunders Co., Philadelphia.**

2. **Golub, E. (recent edition). The Cellular Basis of the Immune Response. Sinauer Associates, Inc., MA.**

3. **Nisonoff, A., Hopper, J., and Spring, S. 1975 (or more recent editions). The Antibody Molecule. Academic Press, New York.**

4. **Manual of Clinical Microbiology. 1999. American Society for Microbiology, 7th edition. ASM Press, Washington, D.C.**

5. **Any of the Cumitech series published by the American Society for Microbiology.**

6. **Benenson, A. 1995. Control of Communicable Diseases in Man, 16th edition. American Public Health Association, Washington, D.C.**

**In any scientific field (e.g. biology, chemistry), old theories are losing their popularity and new ideas are being introduced. As a result, updated and additional information that may not be found in the required texts will be introduced to the student during the lecture and laboratory periods. Please be reminded that due to the accelerated nature of this course, you will be exposed to a great deal of information in a short period of**

time. **Therefore, you are discouraged from missing any lecture or laboratory sessions. In the event that you do, it will be your responsibility to obtain notes from fellow classmates. Laboratory sessions missed cannot be made-up (including the lab racticals . You will discover that a great deal of commitment and discipline will be required to excel in this course. You will also discover that each lecture and laboratory session tend to build on one another (or lay a foundation for the next session). If you find yourself missing several class sessions due to certain obligations (e.g. military, family, other classes, etc.) you might want to consider taking this course at a more opportune time.**

**At the conclusion of the course, it is hoped that students will be able to:**

1. **Describe the differences between procaryotic and eucaryotic microorganisms in terms of taxonomic, ecological, and metabolic differences.**

2. **Describe the methods of controlling the growth and spread of microorganisms.**

3. **Understand the role of host defense mechanisms in combating pathogenic microorganisms and other foreign bodies.**

4. **Have a general knowledge of various host-parasite relationships among different groups of infectious agents.**

5. **Demonstrate aseptic techniques and safety in a microbiology laboratory.**

6. **Demonstrate some of the methods by which microorganisms are isolated and identified.**

7. **Understand the importance of quality control and quality assurance in a clinical microbiology laboratory.**

**In a microbiology laboratory, safe must always be on the mind of the student. All microorganisms used in this teaching laboratory have the potential of causing disease. Be reminded that the concentrations of microorganisms used in this laboratory are normally not found in such high numbers in the natural world. It is highly recommended (not required) that students possess a laboratory gown for this course.**

**Students will be required to keep a laboratory notebook containing test procedures performed, observations, conclusions (significance), etc. Students should also bring a pencil, china-marker, and Sharpie permanent marker to the lab.**

**Dates to Remember**

**Lecture Exam dates: 1/26, 2/14, 3/6, 3/20**
**(There will be no cumulative final)**

**Laboratory Practical Exam dates: 2/28 and** 3/1
**(All laboratory practicals will come to an end on the evening of 3/1.**

**Laboratory written exams: 2/16 and 3/22**

**Holidays: 1/17 and 2/21**

**EXAM POLICY**

**In studying for written exams, you will be responsible only for what is covered in the lecture sessions. However, this does not mean that you should ignore your textbook. The textbook is a means of clarifying and reinforcing the information covered in the lectures.**

**All written exams will contain "short answer or essay-type questions. There will be no multiple-choice questions! You will be given one hour to complete the written exams. Every effort should be made to take the written exams or laboratory practicals on the required date. If you have to work late on an exam night (including the last exam night) you should show up even if it's late. I will be here until 10:00 p.m.**

**If you do miss an exam date, a make-up exam will be scheduled only if a reasonable excuse can be given (illness, family emergency, "tough" employer). In the event of a job-related excuse, a written note (which includes the name and phone number of your supervisor) must be obtained from your employer. This will be your ticket for the make-up exam.**

**It will be the responsibility of the student to contact the instructor in order to arrange a time to take the make-up exam. The make-up exam should be taken either before or during the next class session following the required exam date (example: if the exam is originally scheduled for 1/26/00, the make-up exam should be taken either on 1/27, 1/28, or 1/31). If you anticipate missing classes in the future due to employer obligations (e.g., training, duty/drills, deployment, etc.), please contact me quickly so that some schedule can be worked out. If you are unable to contact me at my office (292-5496), you can always leave a message for me at Chaminade University at 735-4837.**

**Grading of Exams/Course**

**Although you will be graded according to a class curve, the following standard format should be used as a guide to your performance in this course.**

| Percentile Range | Letter Grade |
|---|---|
| 100 - 89 | A |
| 88-79 | B |
| 78-69 | C |
| 68-59 | D |
| 58 and under | F |

**Minimum Suggestions for Passing this Course**

1. **If you don't understand, ask questions. There is no such thing as a "stupid" question. Arrangements outside of class time can be made to answer any questions that you may have.**

2. **Review your lecture notes daily and keep up with your reading. Reading the text-book is essential in understanding the lecture notes.**

3. **Read your lab atlas, lab agendas, pertinent handouts, and lab schedule prior to entering the lab session for that day.**

4. **Try as much as possible not to be absent for the lecture or lab sessions.**

5. **Read exam questions very carefully before answering.**

**Please note that no "incompletes" will be given for this course. This course will be officially over on the evening of 3/22/00. No make-up exams will be given after this date.**

**Your continued attendance in this course will imply that you agree to adhere to the policies of this course.**

LECTURE OUTLINE
(Subject to change without notice)

I. Introduction to Microbiology
   A. Definition
   B. Subdisciplines in clinical microbiology
   C. Common terms used in clinical microbiology

II. Methods to Study Microorganisms (Chapter 3 and 7)
   A. Light microscopy
      1. parts of microscope
      2. resolving power vs. magnification
   B. Types of microscopy
      1. brightfield
      2. darkfield
      3. phase contrast
      4. fluorescence
      5. electron
   C. Staining of microorganisms-also see pgs. 21-34 of Atlas
      1. simple stain
      2. negative stain
      3. differential stain
   D. Counting microorganisms-also see pgs. 83-88 of Atlas
      1. direct counts
      2. plate (viable) counts
      3. indirect counting methods

III. Scope of Microbiology (Chapter 2)
   A. Cell as the basic unit of living systems
   B. Basic cell types
      1. procaryotes
      2. eucaryotes
   C. Classification (taxonomy) of microorganisms
   D. Nomenclature (binomial)
   E. Viruses, viroids, and prions (see pgs. 115-120 of Atlas)

IV. Cellular Structures (Chapter 4)

A. Procaryotic cell
1. size
2. shapes
3. structures
a. cell envelope (differences between Gram negative and Gram positive bacteria)
b. capsule
c. cell wall
d. flagella
e. fimbriae
f. pili
g. cytoplasmic membrane
h. mesosome
i. cytoplasm
-ribosomes
-nuclear region
j. endospores

B. Eucaryotic cell

V. Metabolic Functions (Chapter 5)

A. Energy metabolism
1. catabolism vs. anabolism
2. reduction vs. oxidation
3. fermentation vs. oxidative metabolism

B. Monomers and Polymers

C. Proteins
1. amino acids as monomers of proteins
2. structure of proteins
a. primary structure
b. secondary structure
c. tertiary structure
d. quarternary structure
3. enzymes - functional proteins
a. enzymes as catalysts
b. specificity of enzymes
c. substrate of an enzyme
d. enzymes as a means of identifying bacteria
e. factors affecting the function of enzymes

VI. **Synthesis of Macromolecules (Chapter 6)**

- **A. DNA (deoxyribonucleic acid)**
  - **1. structure**
  - **2. replication**
- **B. RNA (ribonucleic acid)**
  - **1. structure**
  - **2. types of RNA**
  - 3. **synthesis (transcription)**
- **C. Protein synthesis**
  - **1. triplet code**
  - **2. translation**
- D. **Alterations in genetic information**
  - 1. mutations
    - **a. point mutations**
    - **b. frameshift mutations**
  - **2. mutagens**
- **E. Genetic exchange among bacteria**
  - 1. transformation
  - **2. transduction**
  - **3. conjugation**
- **F. Significance of mutations and gene transfer**
  - **1. antibiotic resistance**
  - **2. virulence factors**

VII. **Growth and Nutrition of Microorganisms (Chapter 7)-overlap with lab**

- **A. Significance of understanding growth and nutrition in bacteria**
- **B. Nutritional requirements of bacteria**
  - **1. heterotrophs**
  - **2. culture media**
    - a. **characteristics of culture media**
    - **b. complex vs. synthetic media**
    - **c. selective vs. differential media**
    - **d. used to eventually obtain pure cultures**
- **C. Microbial growth**
  - **1. definition of microbial growth**
  - **2. growth curve in a closed system**
    - **a. lag phase**
    - b. l**ogarithmic phase**
    - **c. stationary phase**
    - **d. death phase**
  - **3. environmental influences on microbial growth**

**VIII. Sterilization and Disinfection (Chapter 8)**
- **A. Definitions**
- **B. Physical methods to control microbial growth**
- **C. Factors affecting disinfectant action**

**IX. Antimicrobial Agents (Chapter 9)**
- **A. The ideal antimicrobial agent**
- **B. Problems associated with fungal, parasitic, and viral agents**
- **C. Synthetic agents**
- **D. Antibiotics**
- **E. Antifungal agents**
- **F. Antiviral agents**
- **G. Microbial resistance and sensitivity (see pgs. 39, 93-94 of Atlas)**

**X. Host-Parasite Interactions (Chapter 13)**
- **A. Normal microbial flora (sites)**
- **B. Transmission of microorganisms**
  1. **exit of microorganisms from the host**
  2. **routes of entry into host**
  3. **endogenous spread**

**XI. Nonspecific Host Defense Mechanisms (Chapter 10)**
- **A. External defense mechanisms**
  1. **bacterial interference (normal flora)**
  2. **physical barriers**
- **B. Nonspecific internal defense mechanisms (see pgs. 101-104 of Atlas)**
  1. **WBC**
  2. **other blood components**
  3. **lymphatics/RES**
  4. **inflammation and phagocytosis**

**XII. Acquired Immune Responses (Chapter 11)-see handout by instructor**
- **A. Concept of immunity**
  1. **humoral response**
  2. **cell mediated immune response**
- **B. Antigens (Ags)**
  1. **soluble vs. particulate antigens**
  2. **antigenic determinants (epitopes)**
  3. **haptens and carriers**
- **C. Complement**

D. Antibody (Ab) forming and cell mediated cells
   1. origin of such cells
      a. stem cell
      b. progenitor cell
      c. primary and secondary lymphoid organs
   2. "T" and "B" lymphocytes
      a. surface characteristics
      b. helper and effector function
   3. role of the macrophage in Ab response

E. Humoral antibody response
   1. Ab structure and classes
   2. mechanism of Ab production: clonal selection theory
   3. primary and secondary Ab responses
   4. effects of Abs on microorganisms
      a. opsonization
      b. neutralizing Abs (antiviral/antitoxin)
      c. binding of complement
   5. cross-reactivity of Abs

F. Cell mediated responses (GMR)
   1. effector and helper cells of CMR
   2. non-T and non-B cells
   3. examples of CMR
      a. delayed type hypersensitivity (DTH)
      b. graft vs. host (GVH)
      c. allograft rejection
      d. cell mediated lympholysis (CIVIL)
      e. mixed lymphocyte reaction (MLR)
   4. immediate type hypersensitivity (IHS) vs. DTH
   5. major histocompatibility complex (MHC) of man
      a. regions of the HLA complex
      b. Class I and Class II molecules
         -structure
         -significance
      c. means to detect HLA antigens
         -serologically defined antigens
         -lymphocyte defined antigens
   6. cytokines
      a. definition
      b. function

X111. **Applications of the Immune Response (Chapter 12)**

**A. Types of immunity**

1. **natural activity immunity**
2. **artificial active immunity**
   a. **killed vaccines**
   b. **attenuated vaccines**
   c. **toxoids**
3. **natural passive immunity**
4. **artificial passive immunity**

**B. Measurement of antibodies**

1. **titer**
2. **reasons for measuring Ab levels**
3. **acute and convalescent Ab levels**

**C. Ab detection (serological) tests as a clinical tool**

1. **types of Ab detection tests**
   a. **agglutination tests-also see pgs. 108-109 of Atlas**
   b. **precipitin/flocculation tests-also see pgs. 106-109 of Atlas**
   c. **neutralization**
   d. **complement fixation (CF)**
   e. **fluorescent Ab see pg. 112 of Atlas**
      **-direct methods**
      **-indirect methods**
   f. **hemagglutination inhibition (HI)**
   g. **radioimmunoassay (RIA)**
   h. **enzyme immunoassay (EIA)-also see pgs. 110-111 of Atlas**
2. **sensitivity vs. specificity of Ab tests (see handout by instructor)**

**XIV. Introduction to Infectious Diseases (Chapter 13)**

**A. Characteristics of a successful pathogen**

**B. Virulence factors**

1. **attachment/invasiveness**
2. **circumvention of defense mechanisms**
3. **induction of tissue damage or malfunction**

**C. Koch's postulates**

1. **to determine etiology**
2. **problems associated with postulates**

XV. **Introduction to Parasitology (Chapter 40)**

**XVI. Selected Topics**

# LABORATORY SCHEDULE

Note: Laboratory sessions will be modified to fit the focus and resources of this course. Consult media handouts from syllabus and Photographic Atlas for description of media, reagents, and techniques.

| DATE | TOPIC |
|---|---|
| 01/10/00(M) | 1. Maintaining a laboratory notebook.<br>2. Laboratory safety orientation (see handout from instructor).<br>3. Use and Care of the Microscope (see handout from instructor).<br>4. Labeling of tube media..<br>5. Transfer of slant culture to a slant and broth (to be demonstrated by instructor). To be performed by each student. Incubate in 35 C. incubator. Loosen all caps on tubes. The slant used is called a Tryptic Soy Agar (TSA) slant and the broth is called a Todd-Hewitt broth. See media handout from syllabus for a description of these two media.<br>8. Labeling of plate media.<br>7. Streaking for isolation (to be demonstrated by instructor). See pg. 9 of Atlas. Streak broth culture (provided by instructor) onto Blood Agar (BA) plates. These plates are also known as TSA + 5% sheep blood (SB) plates. To be performed by each student. Incubate plates upside down at 35 C. For a description of Blood Agar plates, see media handouts from syllabus and pg. 41-42 of Atlas. |
| 01/12/00 (W) | 1. F/U (Follow-up) on culture transfers from previous period. Make observations of your slant and broth tubes (see pg. 5 -6 Atlas) from previous lab period.<br>2. Make observations of your streaked blood agar plates. See pg. 9 of Atlas.<br>3. Read media handouts from syllabus for 1 4 lab session. Pay close attention as to what ingredients make a particular medium selective and/or differential. Also, recognize what kind of reactions occur on a given agar plate when certain bacteria act on it. Also read pgs. 7, 8, 14, 15, 20, and 41 in the Atlas.<br>4. Lab lecture on media and incubation systems to be used on 01&4/00. |
| 01/19/00 (W) | 1. Lab lecture on media and Incubation systems to be used on 01/4100, |
| 01/24/00 (M) | 1. Streak the folllowing broth cultures onto the prescribed media listed on the chart on the next page.<br>2. Observe how each bacterial species grows in the broth tube. |

| | BROTH CULTURE | MEDIA TO BE INOCULATED | | |
|---|---|---|---|---|
| a. | Staphylococcus aureus | BAP[1] | MaC[2] | MSA[3] |
| b. | Staphylococcus egidermidis | BAP | Mac | MSA |
| c. | Streptococcus py enes[4] | BAP | Mac | |
| d. | Streptococcus pneumoniae[5] | BAP | Mac | |
| e. | Bacillus subtilis | BAP | Mac | |
| f. | erichia coli | SAP | Mac | |
| g. | Enterobacter cloacae | BAP | Mac | |
| h. | Proteus vulgaris | BAP | Mac | |
| i. | Salmonella typhimurium | BAP | Mac | XLD[6] |
| j. | Pseudomona aeruginosa | BAP | Mac | |

3. Incubate all media In a 35 C. incubator. Loosen all caps on tube media and invert plates.
4. Streak out an anaerobe (Clostridium perfringens) from a thioglycollate tube onto a Blood Agar plate (remove growth from thioglycollate tube with a transfer pipette and place a small drop of inoculum onto the corner of a Blood Agar plate for streaking). Incubate in anaerobe jar (see pgs. 7- 8 of Atlas) at 35 C. Notice how anaerobe grows in thioglycollate tube (see pg. 7 of Atlas).
5. Streak out a Neisseria meningitidis onto a Lewis-Martin Agar plate. Incubate in candle jar.
6. You might want to prepare a data chart for the next lab period to describe all your observations.

1 Blood Agar plate (TSA + 5% sheep blood)
2 MacConkey Agar plate (with lactose as only sugar)
3 Mannitol Salt Agar plate
4 stab BAP along streak and incubate BAP in candle jar
5 stab BAP along streak and incubate BAP in candle jar
6 xylose-lysine-desoxycholate agar plate

01/26/00 (W)

1. Using pages 1 - 4 of the Photographic Atlas, describe cultural characteristics of streaked cultures of **01/24/00.**
   a. Note similarities and differences of colony morphology and plate reactions **between** the same and different genera of bacteria.
   b. Note odors and growth requirements **between** the different genera of bacteria. Compare growth on BAP vs. Mao-Conkey's vs. XLD vs. MSA, etc., for each isolate. Observe growth on Lewis-Martin.
2. Prepare bacterial **smears (demonstrated** by instructor) of all your isolates (anaerobic and candle jar isolates included). You will be Gram staining (see pgs. 27 and 28 of Atlas and media handouts) your smears during the next lab period.
3. Perform the oxidase test (see media handout and pgs. 71 and 72 of Atlas) on all isolates from your BAP or Lewis-Martin media.
4. Make a smear of a **Candida** albicans (yeast) culture. Gram stain next lab period.

01/31/00 (M)

1. Gram stain all your smears (pgs. 23 - 28 of Atlas and **media** handout).
2. Look at demo acid fast stained (Kinyoun's stain) slide of **Mycobacterium** tuberculosis (see pg. 29 - 30 of Atlas and media handout).
3. Gram stain your Candida **albicans** smear.

02/02/00 **(W)**

1. Discuss results of previous **lab period.**
2. Lecture on next lab period of 2/07/

02107100 (M)

1. **Presumptive identification of pathogenic Staphylococcus** aureus. The coagulase tube test. (see pg. 46 of Atlas).
2. Presumptive identification of Group A streptococci (to be demonstrated by **instructor).**
3. Presumptive identification of Stre **tococcus pneumoniae** (to be demonstrated by instructor).
4. Read media handout in preparation for next lab. Also read pgs. 37, **38, 45, 47-48, 52-55, 62-63,** 68-70, 73-74, and and 80-82 of Atlas. Read API 20E **instructions.**
5. You will be provided with 24 - 48 hour cultures of the 5 following **organisms.**

   a. **Esch richia** coli
   b. **Enterobacter cloacae**
   c. Proteus **vulgaris**
   d. **Salmonella tv imurium**
   e. Pseudomonas **aeruginosa**

Each organism will be inoculated into the following biochemical test media set.

a. Triple Sugar Iron Agar (TSI) slant
b. Lysine Iron Agar (LIA) slant
c. API 20E Identification system'strip

In all, you and your partner will need 5 sets of the above media. PLAN AND ORGANIZE!

02/09/00 (W)

1. Look at BAP with bacitracin disk (0.04U).
2. Look at BAP with optochin disk.
3. F/U on the previous lab period: Biochemical testing on 5 organisms inoculated on 02/07/99. Reading and documenting of reactions in API 20E strips.
4. What do these reactions tell you about the growth characteristics of these different genera of bacteria?

02/14/00 (M) - 02/16/00 (W)

1. Discussion of lab results obtained from 02/09/00 lab session.
2. Lecture on 02/23/00 lab session dealing with mycology.
3. First Written Laboratory Exam on 02/16/00.

02/23/00 (W)

1. Read pgs. 143-150 of Atlas. Presumptive identification techniques for Candida albicans (yeast).
   a. germ tube test (to be demonstrated by instructor)
   b. pseudohyphae and chlamydospores in corn meal agar with Tween 80 (to be demonstrated by instructor). See media handout on corn meal agar.
2. Techniques used in identification of filamentous fungi (molds).

   WARNING: Normally, all laboratory work on filamentous fungi should be done in a biosafety cabinet (at least Classl).

   a. Observe fungal culture growing on Sabouraud agar slant with antibiotics (cycloheximide and chloramphenicol). See media handout.
   b. Do a slide (micro) culture (to be demonstrated by instructor).
3. Incubate all yeast and molds at room temperature.
4. Gram staining practice.
5. Lab lecture for next laboratory session of 02/28/00.

02/28/00 (M) - 03/01/00 (W)

1. First Laboratory Practical Exam: Gram staining of unknowns. You may use your lab notes. Answer sheet will be provided.
2. Beginning of Second Laboratory Practical Exam: Identifica-

tion of bacterial unknown. Identify your unknown to the Genus level. Your unknown will be a gram negative rod that is oxidase negative and a **facultative anaerobe**. Write the name of your unknown on the answer sheet provided. You may use your lab **notes**.

3. All practicals will come to an end on the evening of 03101/00. Tum in your answer sheet for your bacterial **unknown** on the evening of 03/01/00.

03/01/00 (W)

1. Observe con meal agar plates for pseudohyphae and chlamydospores.
2. Observation of fungal slide **cultures,** etc.
3. Demo **slides** of Coccidioides, Histoplasma, and Aspergillus.
4. Lecture on laboratory period for 03/06/00.

03/06/00 (M)

1. Salmonella serotyping
2. Salmonella phase **reversal**
3. **Indirect Fluorescent antibody (IFA) test** for *Toxoplasma gondii*

03/08/00 (W)

1. Continuation of Salmonella serotyping

03/13/00 (M)

1. Read pgs. 151-175 of Atlas before coming to this lab period.
2. Microscopic examination of parasitic specimens.

03/22/00 (W)

ast Laboratory Written Exam