MICROBIOLOGY LABORATORY (BIOL 310L) SCHEDULE

Spring 2017

Lecture Professor: Dr. Susan Morrison
Lab Instructors: Ms. Tracy Hirsch
Dr. Susan Morrison

Required:

(1) Leboffe & Pierce, Microbiology Laboratory & Theory Application, Brief, 3rd edition.
(2) Coursepack for BIOL310 and BIOL310L
(3) Sharpie marker, Safety Glasses, Lab Coat; Ms. Hirsch also requires a **bound** composition notebook.

"Pack" pages refer to the laboratory portion of BIOL 310 coursepack from SAS-E-Ink.
The Leboffe & Pierce manual pages are listed as "Leboffe."
Everything that is listed must be read before coming to class.

I January 23-January 26 INTRODUCTION; SAFETY; ASEPTIC TECHNIQUE; USE OF MICROSCOPES; OBSERVATION OF PREPARED SLIDES; ENVIRONMENTAL SAMPLE; EPIDEMIC

Leboffe pages 1--8; Pack pages L7-24 **Safety & Laboratory Guidelines**
(read thoroughly, understand and apply throughout the semester)
Leboffe Ex. 1-4 **Common Aseptic Transfers & Inoculation Methods**
Leboffe pages 59-60 **Microbial Growth, Ubiquity & Diversity** (read)
Leboffe Ex 2-1 **Ubiquity of Microorganisms**
& Pack pages L29-30 **Distribution of Microorganisms in the Environment**
Leboffe pages 141-142 **Microscopy & Staining** (read)
Leboffe Ex 3-1 **Introduction to the Light Microscope**
Pack pages L25-28 **Observation of Prepared Slides of Bacteria** (3 bacterial morphologies at 3 magnifications each)
Leboffe Ex. 7-4 **Epidemic Simulation** (time permitting)

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Notes:

- *+ indicates exercise for which follow-up will be necessary. The time in brackets [ ] indicates the approximate time span at which follow-up should be done.*
- *[9]"Pack" pages refer to the BIOL 310 coursepack from SAS-E-Ink. Leboffe refers to the manual by Leboffe & Pierce.*
- *Appendix = an appendix in the lab manual. You should familiarize yourself with it, but do NOT memorize it. It is for reference only.*
- ***NOTE:**In addition to the questions for exercises which you do, you are also responsible (on tests and in your notebooks) for questions in exercises requiring only reading and for results and questions for exercises done as demonstration. You are also responsible for all parts of the exercises done from the coursepack or handouts, as well as from the lab book.*
- ***Important Note: If the schedule needs to be shifted because of class cancellation for a hurricane, influenza, or other emergency during the term, the date of the lab final and/or other activities may change.***
- ***Lab books may be collected and graded at ANY time during the semester; this could occur once or more than once and may be announced OR unannounced. You should come to class at all times with your lab book(s) organized, complete and up-to-date.***

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II January 30-February 2 ASEPTIC TECHNIQUE, ENVIRONMENTAL SAMPLE & CULTURE CHARACTERISTICS (continued); PREPARATION OF SLIDES & OBSERVATION OF SIMPLE STAINS & NEGATIVE STAINS; STREAK PLATES; PREPARATION OF CULTURE MEDIA; THE AUTOCLAVE

Leboffe Ex. 1-5 & Pack L36-37 **Streak Plate Methods of Isolation**
Leboffe Ex. 1-4 **Review** Common Aseptic Transfers & Inoculation Methods

Leboffe pages 173-176 **Bacterial Structure & Simple Stains** (read)
Leboffe Ex 3-4 **Simple Stains** (includes making a bacterial **smear**)
Leboffe Ex 3-5 **Negative Stains**

Listing for this day continued on next page

II January 30-February 2 (*continued*)

Leboffe page 87 (Top)	**Environmental Factors Affecting Microbial Growth**
Leboffe Ex 2-1	**Continue**: Ubiquity of Microorganisms
& Pack L29-30	**Continue:** Distribution of Microorganisms in the Environment
Leboffe Ex 2-2	**Colony Morphology**
Leboffe Ex. 2-3	**Growth Patterns on Slants**
Leboffe Ex. 2-4	**Growth Patterns in Broth**
Leboffe Ex 1-3 & Pack L31-33	**Nutrient Broth & Nutrient Agar Preparation** [read & understand; we will not be able to carry this out because our temporary lab is not adequately equipped.]
Leboffe Ex 2-5	**Evaluation of Media** (read only)
Leboffe Ex 2-11	**Steam Sterilization** (read only)

III February 6-9 PATHOGEN POSTER PROJECT SIGN-UP; BEGIN IDENTIFICATION OF "UNKNOWN" BACTERIAL CULTURE; GRAM STAIN; USE OF SPECIAL PURPOSE MEDIA (SELECTIVE & DIFFERENTIAL); ISOLATION OF PURE CULTURES

Pack L57-62	Sign up & begin **pathogen poster project** ---Organize teams of **4** students; select pathogen & area of body for normal microbiota
Leboffe Ex. 1-5 & Pack pp. L36-37	**Streak Plate Methods of Isolation** (use for Unknown culture)
Leboffe page 545	**Identification of Unknowns** (read)
Pack L38-L50	**Identification of Unknown Bacterial Cultures**
Leboffe Ex 3-12	**Morphological Unknown (read)**
Leboffe page 187 (top)	**Differential & Structural Stains** (read)
Leboffe Ex. 3-6	**Gram Stain**
Leboffe pp. 227-229	**Selective Media & Differential Media**
Leboffe Ex. 4-1	**β-phenylethylalcohol Agar**
Leboffe Ex. 4-2	**Columbia CNA Agar** (read only, pay particular attention to figure)
Leboffe Ex. 4-3	**Mannitol Salts Agar**
Leboffe Ex. 4-4	**MacConkey Agar**
Pack L60	**CLED Agar**
Leboffe Ex 2-2	**Cultural Characteristics of Microorganisms & Colony Morphology** Use this to describe Environmental Samples & for all future observations
Leboffe Ex 2-3	**Growth Patterns On Slants**
Leboffe Ex 2-4	**Growth Patterns In Broth**
Pack L34-35, L44	**Dichotomous key practice**—begin today (& continue in subsequent weeks)

IV February 13-16 DETERMINATION OF OXYGEN REQUIREMENT; METHODS FOR GROWING ANAEROBES; BIOCHEMICAL TESTS; HYDROLYTIC ENZYMES; CATALASE; NITRATE TEST; SPORE STAIN

DEADLINE: for **pure working cultures** of your unknown; **reserve & working** culture slants of unknown

Leboffe page 95 (top)	**Aerotolerance** (read) & **Oxygen Requirements**
Pack pages _____	**Determination of Atmospheric Oxygen Requirements** (using Agar Deep Stabs)
Leboffe Ex 2-6	**Fluid Thioglycollate Medium** for culturing anaerobes [Read]
Leboffe Ex 2-7	**Anaerobic Jar** for culturing anaerobes [Read]
Leboffe pages 267-270	**Differential Tests** (Read)
Leboffe Ex. 5-4	**Catalase Test**
Leboffe page 331	**Tests Detecting Hydrolytic Enzymes** (read)
Leboffe Ex. 5-10	**Starch Hydrolysis** (Amylase Test)
Leboffe Ex. 5-13	**Casein Hydrolysis** (Caseinase or Casease Test)
Leboffe Ex. 5-6	**Nitrate Reduction Test** [24-48 hr] (Biochemical Test)

Continue or complete ongoing exercises & identification of unknown cultures

Pack L34-35, 44	Continue Dichotomous Key practice
Pack L57-62	Continue group work on Pathogen Poster & Normal Microbiota

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V February 20-23 BIOCHEMICAL TESTS—SUGAR FERMENTATIONS; OTHER BIOCHEMICAL TESTS (IMViC); SULFIDE; MOTILITY (using a SEMI-SOLID AGAR); CONTROL OF MICROBIAL GROWTH---with ULTRAVIOLET LIGHT

Leboffe pages 267-270	**Differential Tests** (Review)
Leboffe page 279 (Top)	**Fermentation Tests**
Leboffe Ex. 5-2	Carbohydrate Fermentation using **Phenol Red Fermentation Broth** [24hr]
Leboffe Ex. 5-19	Carbohydrate Fermentation using **Triple Sugar Iron Agar** **[18-24hr]**
	IMViC Test Battery
Leboffe Ex. 5-3	**Methyl Red Test**
Leboffe Ex. 5-7	**Citrate Test**
Leboffe Ex. 5-18	**SIM Medium: Motility, Indole and Hydrogen Sulfide Test**
Leboffe Ex. 2-12	**The Lethal Effect of Ultraviolet Radiation on Microbes**

Pack L34-35, 44	Continue Dichotomous Key practice
	Continue or complete ongoing exercises & identification of unknown cultures
Pack L57-62	Pathogen Poster/Normal Microbiota Project continued

IMPORTANT SCHEDULE NOTE: You may need to return to the lab the next day (ideal) or the day after to read these test results. If reading of the results is delayed and they can't be properly stored, they won't be accurate. Your lab instructor will give you directions.

VI February 27-March 2 **ACID-FAST STAIN; ENDOSPORE STAIN; BIOCHEMICAL TESTS; MOTILITY (using WET MOUNTS); BIOCHEMICAL I.D. SYSTEMS**

Leboffe Ex. 3-7	**Acid Fast Stain** (a differential stain)
Leboffe Ex. 3-9	**Endospore Stain** (a structural stain)
Leboffe Ex. 3-10	**Wet Mount & Hanging Drop Preparations**
Leboffe Ex. 9-5	**Identification of Enteric Microorganisms Using Computer-Assisted Multi-Test Microsystems (demonstration)**
Leboffe L71-74, Pack L57-L62	Pathogen Poster project continued
Pack L34-34, 44	Continue Dichotomous Key practice
Pack L38-50	Continue or complete ongoing exercises & identification of unknown cultures
------	**Continue identification of unknowns** [See message below about media requests.] **Review** for next week's test

*******Friday March 3--noon; Monday, October 10-noon*--*Deadline** for requesting supplemental media for unknown culture identification.*** You may request new media not previously used, and will be advised whether it can be provided. All requests must be **in writing or by e-mail to your instructor using the subject line : Special Media Request.** Please explain why this medium is of value for identification of your unknown. For previously used media, you should indicate why it is necessary for you to repeat the test now if you did not repeat a test immediately after first reading the results. It may take 3-4 days to get these media prepared. Forgetting to come in to read test results is NOT a valid reason to request more media. **HINT**: Request media sooner than this date to permit more time to apply those results.*

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VII March 6-9 Spring Break-Lab Will Not Meet

VIII March 13-16

****** ****LABORATORY TEST (closed book)** [practical set-ups & written only sections]**
****** ****PRACTICAL TESTS IN ASEPTIC TECHNIQUE; PLATE STREAKING; MICROSCOPE FOCUSING****
----- Continue or complete ongoing exercises & identification of unknown cultures

REMINDER: Lab books may be collected and graded at ANY time during the semester; this could occur once or more than once and may be announced OR unannounced. You should come to class at all times with your lab book(s) organized, complete and up-to-date.

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IX March 20-23 Work on Pathogen Poster Project; Dilutions; *SENIORS HAVE SPECIAL ASSIGNMENT*

Special Assignment for Seniors

Posters L57-L62	Continue work on **pathogen poster & normal microbiota project** with your team
Leboffe Ex. 7-3	**Morbidity & Mortality Weekly Report (MMWR) Assignment** (use your pathogen poster microbe)
Pack pp. L63-72	**Dilutions Tutorial** [to prepare for Viable Count (Standard Plate Count) next week]

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X March 27-30 UNKNOWN REPORTS DUE; DILUTIONS & PLATE COUNTS; WATER QUALITY TESTING (MPN); CONTROL OF MICROBIAL GROWTH---with ANTIBIOTICS, ANTISEPTICS & DISINFECTANTS

Deadline For Submitting Unknown Reports
Lab reports due at the beginning of your lab section; 10% penalty for each day late, including each weekend day; reports over 10 days late will not be accepted.

Leboffe pages 441-442	**Medical, Environmental & Food Microbiology** (read)
Leboffe Ex. 7-2	**Antimicrobial Susceptibility Test: Disk Diffusion (Kirby-Bauer) Method**
Leboffe Ex. 2-13	**Effectiveness of Chemical Germicides: The Use-Dilution Test for Disinfectants & Antiseptics**
Leboffe pages 405-406	**Quantitative Techniques** (read)
Leboffe Ex. 6-2	**Standard Plate Count (Viable Count)**
& Pack L63-72	
Leboffe Ex. 7-6+	**Multiple Tube Fermentation Method for Total Coliform Determination**
	A. Presumptive Test: Determination of the Most Probable Number (Demo);
+	B. Confirmed Test
+	C. Completed Test
Pack pages L63-72	Methods for Preparation of **Dilutions & Dilution Problems** (work)
Handout	Continue exercise on pathogenic bacteria and normal microbiota
-----	Continue or complete ongoing experiments

XI April 3-6 PATHOGEN POSTER PRESENTATIONS; MEDICAL MICROBIOLOGY; NORMAL MICROBIOTA; EPIDEMIC; CHECKOUT & CLEANUP

Student presentations	**PRESENTATION of Pathogen Posters**
Assemble composite poster showing **Normal Microbiota** of human body	
Leboffe page 441	**Medical Microbiology-Introduction (read)**
Leboffe Ex. 7-4	**Epidemic Simulation** (if not done earlier in the semester)
Leboffe Ex. 7-3	**Morbidity & Mortality Weekly Report (MMWR) Assignment** (about your pathogen poster microbe)
-----	Complete ongoing exercises
-----	**Laboratory Checkout and Cleanup**
See Week XII	**Sign up** for your Food Microbiology lab food selection

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XII April 10-13 Food Microbiology (Demonstration & Presentation)

Leboffe page 491 (top)	**Microbiology of Food**
Pack L77-78	**Microbial Production of Food Products**
Pack 77-78	****Advance Assignment**** Sign up for the food item on sign-up sheets posted in the lab.
Leboffe Ex. 7-8	**Making Yogurt**

XIII April 17-20 ********Cumulative LABORATORY TEST #2 (closed book)**********