

**BIO C17 Summer 2016  
MICROBIOLOGY : THE BACTERIAL CELL  
LAB INFORMATION AND SCHEDULE**

Please Check course time table for the room number of your Lab Section and blackboard for the name and e-mail address of your TA. Announcements and some labs will be posted on Blackboard. Please check this page at a minimum weekly.

**The laboratory component of BIOC17 is an intensive 3 hours of laboratory per week where you will be learning valuable microbiology practical skills while developing your analytical and presentation skills. .**

This laboratory will provide hands on experience in basic microbiology skills applicable to many positions in the workplace. We will supplement the lectures with working examples of concepts discussed in lectures. On occasion concepts that are most easily presented in the laboratory will be addressed in the laboratory only. The Laboratory component represents **41% of your final grade.**

**Learning objectives:**

**The objective of the laboratory is to provide you with a comprehensive introduction to basic techniques and concepts required for understanding bacterial growth and physiology with an emphasis on bacterial impact on human health.** *On completion of this laboratory the student will understand concepts of microcopy, microbial identification, culturing, quantitative analysis of growth, control of microbial growth and medical microbiology.* Students will gain practical skills in use of microscopes, bacterial identification, aseptic technique and producing and maintaining bacterial cultures. This background will provide an excellent companion to immunology (BIOC39) and the foundation for fourth year courses in microbiology including BIOD17, BIOD21 BIOD26. BIOD29. Skills acquired will be useful in laboratory based jobs in industry, government microbiology laboratories, blood services and medical microbiology laboratories. Skills will also provide the foundation for graduate work in fields utilizing microorganisms.

**GRADE DISTRIBUTION FOR THE LABORATORY**

Laboratory component of final exam; During exam period TBA (included with lecture exam)	15%
Lab reports (Data Sheets and questions, graphs and calculations)	17.5%
Lab participation /preparation/performance ( TAs will assign flow charts/mind maps prep /summaries/class presentations/ class write ups/reflective practice/class data presentation	8.5%

**Lab assignments will not be accepted late.**

**Attendance is mandatory:** you must provide a UTSC medical certificate for illness . If you have an acceptable reason (cleared prior to the lab by Professors'

Terebiznik or Brunt) for absence you will be excused.

### **Consequences for missing laboratories**

If you miss a lab for which you were not excused you may not hand in the assignment. Two unexcused absences result in loss of the 8.5% performance grade and all related grades for the missed lab. One unexcused absence 3 % and all related grades. If you miss 3 laboratories you forfeit all grades related to the in lab work (26%).

### **Use of TURNITIN for Lab report**

*"Normally, students will be required to submit their course essays to Turnitin.com for a review of textual similarity and detection of possible plagiarism. In doing so, students will allow their essays to be included as source documents in the Turnitin.com reference database, where they will be used solely for the purpose of detecting plagiarism. The terms that apply to the University's use of the Turnitin.com service are described on the Turnitin.com web site".*

#### **For the formal report follow this procedure:**

The day your report is due, hand in a hard copy to your TA and submitted to turnitin

Each page of the report must be number and labeled with the student's name. In addition, you **must initial all the pages** of your hard copy. The total number of pages must be indicated on the cover of your report. The hard copy must be received by the TA in her/his hand. Please, don't pile them up on the front bench.

The entire report including figure legends must be submitted to turnitin in a format that can be read by the program. It is your responsibility to ensure turnitin has evaluated your submission. Format required is word or PDF no screen shots or scanning of text are accepted. If your report can not be evaluated by turnitin or is not submitted than you receive a zero on your report

You must inform Professor Brunt in writing by the second week of classes if you wish to opt out and you must then provide an electronic copy of your report to Dr. Brunt along with all rough work and hard copies of all references.

### **Academic integrity**

The University of Toronto's Code of Behaviour on Academic Matters applies to all University of Toronto Scarborough students. The Code prohibits all forms of academic

dishonesty including, but not limited to, cheating, plagiarism, and the use of unauthorized

aids. Students violating the Code may be subject to penalties up to and including

suspension or expulsion from the University. See material in the course outline

**tips sheet provided on the web site**

<http://www.utoronto.ca/~vpdean/documents/AcademicIntegrity-Tipsheet2015.pdf>

**For each laboratory Data Sheets** associated with each Exercise are found in at the back of the Lab Manual "**Microbiology: Laboratory Theory and Application**" custom edition M. Leboffe and B. Pierce. You are strongly encouraged to fill in a data sheet for each laboratory completed during this course for preparation for the lab exam

## **Laboratory Assignments Summer 2016**

**Assignment 1 (5 %) due May 31, 2016 at the beginning of lab**

**Exercise 3-12 Morphological Unknown**

Data Sheet 1 %

Slides ( 2 slides are handed in):

Gram stain 2 %

Spore stain 2 %

**Assignment 2 (3%) due June 28<sup>th</sup> 2016 at the beginning of lab**

**JS1 bacterial growth: introduction, graphs and generation time determination and short discussion**

**Assignment 3 (9.5%) formal research paper on environmental factors that affect growth. due July 12, 2016 at the beginning of lab**

**Hand in hard copy at the beginning of the laboratory and to turnitin by 5 pm on the day of your laboratory**

Ex. 2-9 Effect of temperature, Lab 1

Ex. 2-10 Effect of pH, Lab 1

Ex. 2-11 Effect of osmotic pressure

TAs will go over specific requirements for assignments. An outline of the requirements for the formal report will be posted on blackboard closer to the due date

## Background information about laboratory protocol

This course deals with potentially dangerous, generally unseen living organisms. Therefore, there are strict rules for working in the lab. Compliance with these rules is taken into account when the lab participation grade is determined.

- **If you eat or drink or chew gum you will be asked to leave your lab: associated lost grades**
- **If you are immunocompromised you must see the instructor of the course before the lab begins**

### Lab RULES (failure to follow the rules can lead to removal from the lab)

- **no cell phone use in the laboratory. With TAs permission you may use your phone to take a picture**
1. Do not bring coats, hats, etc. into the laboratory.
  2. Personal safety equipment is required:
    - lab coat (done up) with the sleeves rolled down,
    - closed toed shoes
    - hair tied back
    - Goggles are required when staining
  3. Do not eat or drink in the laboratory.
  4. Keep paper, pencils, fingers, etc. out of your mouth.
  5. Wash benches down with 70% alcohol at the beginning and end of lab as described by your TA (see below)
    - . At the beginning and end of each lab session, tidy up your work area as follows: **first shut off bunsen burners**. Squirt /pour an S-shape of 70% isopropanol onto the lab bench. Spread the alcohol with a paper towel to disperse over the bench top, but **do not dry it with the towel**. , **allow the alcohol to evaporate**. Throw the paper towel in the dry waste bag
  6. Follow directions for disposal of all material used in the laboratory. All material that has been in contact with microorganisms must be disposed of in disinfectant or autoclave bags.
  7. Discard pipettes point-down, in the upright plastic pipette holders. Make sure the pipette tips are covered with disinfectant.
  8. Place all *test tubes* containing living cells in the racks in autoclave basins;
  9. Place all *flasks* in an upright position in the discard pans.
  10. All pipette tips for micropipettors must be disposed in buckets provided
  11. **Microscopes** must be cleaned before being put away following TA's instructions for the correct way to put away your microscope. (see below)
  12. **Wash hands thoroughly with soap and water** once or twice during the lab, at any time you come in contact with live cells and also **just before leaving the laboratory**.
  13. When leaving the lab, REMOVE YOUR LAB COAT and store it in a locker in the lab ( if possible). Although not recommended, it is allowable to wear the lab

coat in another lab course. However, **DO NOT UNDER ANY CIRCUMSTANCES** wear your lab coat in the cafeteria or in any other public place (e.g. the Meeting Place, Library, etc.).

### **HANDLING OF MICROSCOPES**

- 1)** Each microscope and the storage space for that particular microscope, will have the same identifying number
  - 2)** Your TA will assign you to a specific microscope . You are responsible for this particular microscope.
  - 3)** When finished for the day, please do the following:
    - a)** Center the mechanical stage
    - b)** Lower the light intensity to the minimum and shut off the light
    - c)** Using a cotton swap and alcohol wipe of the lens and the stage. Then wipe with lens paper
    - d)** Place the lowest objective (4x) over the stage
    - e)** Wrap the electric cord around the base
    - f)** Turn the nosepiece relative to the base (if the nose piece swings), in the direction instructed by the TA  
(there is only one way that the microscope will fit into the storage space).
    - g)** Hold the microscope with two hands-(one hand should be under the base of the microscope) while transporting it to the cabinet ( and away from the cabinet)
    - H)** Place in the cubby hole assigned to that particular microscope
- The TAs will check that each microscope has been put away properly.
  - Points will be deducted if the microscopes are not put away properly.

## LAB INFORMATION AND SCHEDULE

The lab Exercises (Ex. #) are as designated in the custom Lab Manual. There are additional labs which will be provided as files posted to blackboard at least a week prior to the laboratory these are designated JS.

You are responsible for **all the introductory material** in each relevant **Section** and **Exercise** in the lab manual, (even if it appears on pages preceding the actual exercise). **You are also responsible for all Sections/Exercises, etc. of Exercises listed as "read", "read only" or "reading assignment".**

- Supporting information from the custom text book is also indicated where appropriate to assist with understanding laboratory material
- Lab reviews will be held outside of the laboratory in the last week of classes.

Links for helpful pictures, videos and animations:

### Gram staining

gram stain video

<http://www.microbelibrary.org/library/gram-stain/3018-the-gram-stain-an-animated-approach>

<http://www.microbelibrary.org/library/disease/3049-animation-of-peptidoglycan-synthesis-in-gram-positive-bacteria-and-inhibition-by-antibiotics>

<http://www.microbelibrary.org/library/gram-stain/2833-gram-stain>

gram stain picture: opens to Gram positive rods but at the end of the page you can click to find negative rods or positive/negative rods

<http://www.microbelibrary.org/component/resource/gram-stain/2864-gram-stain-gram-positive-rods>

### capsule stain

<http://www.microbelibrary.org/component/resource/laboratory-test/3040-capsule-stain>

### endospore stain:

<http://adwww.microbelibrary.org/component/resource/laboratory-test/3134-endospore-stain>

<http://www.microbelibrary.org/library/laboratory-test/2803-spore-stain-tutorial>

### acid fast stain

<http://www.microbelibrary.org/library/2-associated-figure-resource/3481-acid-fast-stain-of-mycobacterium-smegmatis-and-staphylococcus-aureus>

## FOR THE FIRST LABORATORY

**Before** coming to the first lab, please read the following in the custom lab Manual: The Introduction pages on Safety and laboratory guidelines, and Section 1 on fundamental skills which includes pgs 14-24, as well as 2-12 theory on steam sterilization from the lab manual. Look carefully at the Figures that describe aseptic technique. You will need to be proficient at these techniques to function in the Microbiology laboratory. **You are responsible for the theory of READ only laboratories**

## LAB SCHEDULE

### Week of Laboratory Exercise(s) and Assignments

*Before each laboratory read and make notes on introductory materials. We do not use all the organisms listed so your TA will go through the organisms each week*

<b>Week :</b>	<b>date</b>	<b>Exercise</b>
<b>1</b>	<b>May 10</b>	<b>Section 3 Microscopy and Staining</b> read Lab manual pages 1-19 Chapter 3 Microscopy, staining and Classification of your text book  Ex. 3-1 The light microscope read and understand : <b><i>Bring to lab a summary of the major components of the microscope and a description of how to calibrate a microscope (see below). Hand in at the beginning of class for participation credit</i></b> Ex. 3-2 Calibration of the ocular micrometer . <b>You must show your TA the set up and how to calibrate your microscope for participation credit</b> Ex. 3-5 Smear preparation and simple stains Ex. 3-6 The negative stain Ex. 3-12 Wet mount and hanging drop preparations: motility vs. brownian movement Ex. 3-13 Flagella staining - demonstration slides only
<b>2.</b>	<b>May 17</b>	<b>Section 3 Microscopy and Staining, continued</b> <b><i>Prepare flow charts prior to lab for each of the three staining techniques and have your TA initial . Use these to do your staining</i></b>  Ex. 3-7 Gram stain Ex. 3-8 Acid fast staining <b>READ AND LOOK AT PREPARED SLIDES. You are responsible for the theory of acid fast staining</b> Ex. 3- 9 Capsule staining

3.            May 24            **Section 3 Microscopy and Staining, continued**  
**Make flow chart for endospore stain (TA initials)**  
**and for the unknown identification (see posted**  
**table for organisms) to include with the data**  
**sheet when you had it in next week**  
 Ex. 3-10 Endospore stain  
 Ex. 3-14 Morphological unknown (begin)  
 Inoculate Nutrient Broth and motility tube see EX 5-28
4.            May 31            **Section 3 Microscopy and Staining,**  
**continued**  
  
 Ex. 3-14 Morphological unknown, completion –  
**interpret 5-28 and hand in flow chart (data sheet**  
**and summary, Gram stain and endospore stain at**  
**the end of lab**  
  
**Section I Fundamental Skills**  
 Refer to pages 141-148 of your text book  
 Ex. 1-2 Nutrient agar and nutrient broth preparation  
 (read ONLY )  
 Ex 1-3 aseptic transfer (read only); Hava discussion  
 with your bench for five minutes and per bench  
**HAND IN AN EXPLANATION OF WHY YOU USE**  
**THIS TECHNIQUE**  
 Ex. 1-4 Spread-plate technique, organisms to be  
 used will be assigned in lab  
 Ex. 1-5 Streak-plate technique, mixed population to  
 assigned in class read appendix B and C
5.            June 7            record results from week 4 Ex 1-4 and 1-5  
**What is the purpose of these two plating techniques?**  
  
**Section 6 Quantitative Techniques**  
**Set up:**  
 Refer to chapter 5 pages 149-156 of your text book  
 Read Appendix C , D, E of manual  
  
 Ex. 6-3 Direct count  
 Ex. 6-1 Standard viable (plate) count (read only /data  
 provided to do calculations)  
 Ex. 6-4 Closed system growth (read only)  
 Ex. 6-5 plaque assay, refer to page 450-451 in  
 manual (construct a flow chart and show to your TA at  
 the start of the lab)

**Ex. JS1 Bacterial growth in a closed system (assignment), Present flow chart to TA at the beginning of the laboratory part of your assignment participation**

comparison of growth of *E.coli* at 30C and 39C.

Exercise JS 1 will be posted as a file one week prior to the lab. Record O.D. measurements as described. Plates from viable counts will be incubated 24 to 48 hrs., and then stored next week . **This is assignment 2**

**Reading week June 14**

**6. June 21** Record viable counts for JS1, plaque counts (Ex 6-5)

***Section 2 Microbial Growth : Factors Affecting Growth***

**Set up:**

Refer to pages 131-140 in your text

Ex. 2-9 Effect of temperature, Lab 1

Ex. 2-10 Effect of pH, Lab 1

Ex. 2-11 Effect of osmotic pressure, Lab 1

The above three labs comprise your lab report

Ex 6-6: Thermal death time versus decimal reduction value

**7. June 28** **Hand in Generation time exercise**  
***Section 2 Microbial Growth : Factors Affecting Growth killing effect and differential tests***

**Data analysis :**

Ex. 2-9 Effect of temperature, Lab 2

Ex. 2-10 Effect of pH, Lab 2

Ex. 2- 11 Effect of osmotic pressure, Lab 2

Data the above three exercises is needed for formal lab report

Ex. 6-6 Thermal death time versus decimal reduction value: Lab 2 (discuss as a class).

**Set up :**

Refer to chapters 6 and 7 in your text

Ex. 2-14 Effect of disinfectants, Lab 1

Ex. 7-3 Antimicrobial susceptibility (affect of

antibiotics), Lab 1  
Ex. 5-5 Catalase test, Lab 1 (obtain results) relate the results to EX 2-14  
Ex. 5-15 Gelatinase test, Lab 1  
Ex 5-24 Bacitracin Novobiocin and Optochin Susceptibility test , Read only , understand the theory  
Ex. JS2  $\beta$ -lactamase test (obtain results); lab posted on blackboard relate results to Ex 7-3  
Ex 4-4 growth on Mannitol salt agar  
Ex 7-1 Snyder Test lab 1

8. July 5 ***Microbial Growth : Factors Affecting Growth***  
**Data analysis:**  
**Refer to pg 145 of text for types of media**  
Ex. 2-14 Effect of disinfectants, Lab 2  
Ex. 7-3 Effect of antibiotics, Lab 2  
**Bench data presentation and discuss data sheets**  
Ex. 5-17 Gelatinase test, Lab 2  
Ex 4-4 growth on Mannitol salt agar lab 2  
Ex 7-1 Snyder Test Lab2
- Set up** **refer to page 175 of your text book for background**  
Ex. 2-13 Ultraviolet radiation: lethal effect , Lab 1 (formal report)  
Ex. 10-5 Ultraviolet radiation: damage and repair, lab1  
*Ex. 5-16 DNase test, lab 1 (negative control inoculation)*
- Medical microbiology***
- Ex 7-4 Clinical Biofilms  
Ex. JS3 Effectiveness of hand scrubbing, Lab 1, lab posted  
Ex 7-6 Epidemic simulation lab 1
9. July 12 ***Section 2 Microbial Growth/ section 7 Medical microbiology***  
**Formal lab report due**  
**Data analysis Benches present data for 2-13:**  
Ex. JS4 Effectiveness of hand scrubbing, Lab 2  
Ex. 2-13 Ultraviolet radiation: lethal effect of, Lab 2  
Ex. 10-5 Ultraviolet radiation damage/ repair, Lab 2  
Ex. 7-4 Biofilms lab 2

Ex 7-6 Epidemic simulation lab2 class discussion of result

Ex 5-6 DNase test, lab 2 (compare results to figure posted on the bulletin board in lab)

**Set up: Differential tests /Medical Microbiology**

**prepare a flow chart in lab as a group for lysozyme assay: show to TA prior to starting**

Ex JS5 blood agar week 1 (lab will be posted)

Ex 7-2 lysozyme Assay (completed today)

Ex 4-5 MacConkey Agar lab 1

Ex 5-27 Coagulase test **Read and understand the concept**

**Section 10 Microbial Genetics**

Ex. 10-3 Bacterial transformation (**flow chart is required**) : the pGLO system

Lab 1 (refer to chapter 9 of your text book)

10. July 19

**Section 8 Microbial Genetics**

refer to chapter 9 of your text book

**Data analysis:**

Ex. 10-3 Bacterial transformation : the pGLO system, Lab 2 (**class participation: lab bench analysis, hand in data sheet at the end of lab**)

Ex JS5 blood agar Lab 2

Ex 4-5 MacConkey Agar Lab 2

**Set up: Microbial Genetics/differential blood microscopy**

**Ex. JS6** Bacterial conjugation: the transfer of antibiotic resistant plasmids between cells. Exercise will be posted

**Section 9 Hematology and Serology**

Ex. 11-1 Differential blood count

11. July 26

**Section 8 Microbial Genetics**

**Data analysis:**

Ex . JS6 Bacterial conjugation: the transfer of antibiotic resistant plasmids between cells, Lab 2.

**Work per group hand in your interpretation and then discuss**

**Set up**

**Section 9 Hematology and Serology**

Ex. 11-4 Slide agglutination

Lab review for lab component of the final exam by your TA

## **BACTERIAL NOMENCLATURE**

The proper name of an organism is composed of two words. The first word of the name refers to the *genus* to which the organism belongs, and the second word is the “specific epithet”. In most cases the specific epithet used is the *species*. If the specific species has not been designated, then the second term used is the abbreviation “spp.” or “sp”. The name is italicized (or underlined in typewritten manuscripts). The genus name starts with a upper case letter, while the species name starts with a lower case letter (e.g. *Staphylococcus aureus*).

The first time that a microbial name is used in a manuscript (e.g. lab report), both names should be used. Thereafter in the manuscript, when the species is cited, the word that refers to the genus may be abbreviated by using the italicized ( or underlined) capital letter, followed by a period (e.g., *S. aureus*). If confusion might result from using only the single letter to represent the genus name, the entire name should be used.

Table 1. Proper and improper usage of bacterial nomenclature in a formal report

### **Correct**

*Staphylococcus aureus*

Staphylococcus aureus

*S. aureus*

*Staphylococcus spp.* (where species is not known)

### **Incorrect**

Staphylococcus aureus

*Staph. aureus*

The genus names of certain bacterial groups are used **(a)** as proper names as above and **(b)** as adjectives to describe the morphology of certain cell types.

When a genus name is used as an adjective it is not capitalized, italicized nor underlined. For example:

<b><u>proper name</u></b>	<b><u>adjective</u></b>	<b><u>cell shape</u></b>	<b><u>general arrangement</u></b>
<i>Bacillus</i>	bacillus	rod	single or in short chains
<i>Diplococcus</i>	diplococcus	coccus	in pairs
<i>Streptococcus</i>	streptococcus	coccus	in chains
<i>Staphylococcus</i>	staphylococcus	coccus	in clusters