Laboratory Co-ordinator; Dr. Shelley Brunt  
brunt@utsc.utoronto.ca
office hours Wednesday 11 am to noon
Thursday noon to 1 pm

Please Check the Intranet Course Page for the room number of your Lab Section and for the name and e-mail address of your TA. Announcements will be made on the Intranet Course Page, so please check this page weekly.

The laboratory component of BI C17 requires 3 hours of laboratory per week. The Laboratory component represents 41% of your final grade. This laboratory will provide hands-on experience in basic microbiology skills. 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 objective of the laboratory is to provide you with a comprehensive introduction to basic techniques and concepts required for understanding bacterial physiology with an emphasis on bacterial impact on human health. On completion of this course the student will understand concepts of microbial growth, control of microbial growth and medical microbiology. This background will be provide the foundation for forth year courses in microbiology including BIOD17, 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 question, graphs and calculations) 20%
Lab participation /preparation/performance (TAs will assign flow charts/mind maps prep/summaries/class presentations/class write ups) 6%

Attendance is mandatory: you require UTSC medical certificate for illness or a acceptable reason (cleared prior to the lab by Dr. Brunt) for absence from the instructor. If you miss a lab which are an not excused you may not hand in the assignment. Two unexcused absences result in loss the 6% performance grade One unexcused absence 2.5%. If you miss 3 laboratories you forfeit all grades related to the lab work (26%). Lab assignments will not be accepted
late.

**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) of the
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 on her/his hand. Please, don’t pile them up on the front bench.

**Use of TURNITIN**

"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.”

**READ the academic integrity section below**

Academic integrity is essential to the pursuit of learning and scholarship in a university, and to ensuring that a degree from the University of Toronto is a strong signal of each student’s individual academic achievement. As a result, the University of Toronto’s Code of Behaviour on Academic Matters (http://www.governingcouncil.utoronto.ca/policies/behaveac.htm) outlines the behaviours that constitute academic dishonesty and the processes for addressing academic offences. Potential offences include, but are not limited to:

In papers and assignments:

- Using someone else’s ideas or words without appropriate acknowledgement.
- Submitting your own work in more than one course without the permission of the instructor.
- Making up sources or facts.
- Obtaining or providing unauthorized assistance on any assignment.

On tests and exams:

- Using or possessing unauthorized aids.
• Looking at someone else’s answers during an exam or test.
• Misrepresenting your identity.

In academic work:

• Falsifying institutional documents or grades.
• Falsifying or altering any documentation required by the University, including (but not limited to) doctor's notes.

All suspected cases of academic dishonesty will be investigated following procedures outlined in the Code of Behaviour on Academic Matters. If you have questions or concerns about what constitutes appropriate academic behaviour or appropriate research and citation methods, you are expected to seek out additional information on academic integrity from your instructor or from other institutional resources (see http://www.utoronto.ca/academicintegrity/).

For each laboratory Data Sheets associated with each particular Exercises are found in the section 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 ffixam
Laboratory Assignments Winter 2012
Data Sheets associated with the Exercises listed below, or lab reports will be handed in and graded (point value as shown).

**Assignment 1** (7%) due Feb 4, 2013
Exercise 3-12 Morphological Unknown
Data Sheet 1 %
Slides (3 slides are handed in):
Gram stain 2 %
Acid-fast stain 2 %
Spore stain 2 %

**Assignment 2** (2.5 %) Due March 4, 2013
JS1 bacterial growth: introduction, graphs and generation time determination and short discussion

**Assignment 3** (2%) data sheets due week March 18, 2013
- Ex.7-3 antibiotics
- Ex 2-14 disinfectants

**Assignment 4** (8.5 %) formal research paper on Use of temperature, salt and UV in control of growth of microorganisms. (Due day of your lab April 1, 2013, to turnitin by 5 pm on the day of your lab)
- Ex. 6-6 thermal death time versus decimal reduction
- Ex. 2-11 Effect of osmotic pressure,
- Ex. 2-13 The lethal Effect of Ultraviolet radiation on Microbial growth
- Ex 4-4 Mannitol salt agar.
TAs will go over specific requirements for assignments. An outline of the requirements for the formal report will be posted
Lab RULES
1. Do not bring coats, hats, etc. into the laboratory.
2. Always wear a lab coat (done up) in the laboratory with the sleeves rolled down and closed shoes. See additional laboratory requirements
3. Do not eat or drink in the laboratory.
4. Keep paper, pencils, fingers, etc. out of your mouth. Avoid using paper labels. If used, moisten with water, not your tongue.
5. Wash benches down with 70% alcohol and the beginning and end of lab as described in 12
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 micropipetters must be disposed in buckets provided
11. Microscopes must be cleaned before being put away. See earlier pages and TA’s instructions for the correct way to put away your microscope.
12. 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. Instead, allow the alcohol to evaporate. Throw the paper towel in the dry waste bag.
13. 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.
14. 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 per pair. Together 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  

4) The TAs will check that each microscope has been put away properly.  
5) Points will be deducted if the microscopes are not put away properly.  

**Additional laboratory requirements:**  
- **Lab coats** and **closed shoes** (no sandals) are mandatory. If you arrive with inappropriate foot attire and/or no lab coat you will not take part in the lab and it will be considered an unexcused absence with a los of 2.5% of your grade.  
- Goggles during staining and when requested by the TA.  
- Hair that can fall forward must be tied back  
- Please wear sleeves of the lab coat **rolled down** and **remove your lab coat when class is over**. Do not wear your lab coat in public areas e.g. the cafeteria. Please also bring a **dark -coloured, waterproof, superfine (S) marker** e.g. **SHARPIE** for writing on Petri dishes. Regular markers (for paper) and pens, do not write well on plastic Petri dishes and tubes. **Please wash your hands using soap, once or twice during lab time and at the end of the lab before leaving the room.**  
- **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.** I  
- **If you are caught eating or drinking you will be asked to leave your lab:**  **associated lost grades**
LAB INFORMATION AND SCHEDULE
The lab Exercises (Ex. #) are as designated in the Lab Manual. There are additional labs which will be provided as files posted 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 Principles in Prescott is also indicated where appropriate to assist with laboratory material
- Lab reviews will be held outside of the laboratory before the final exam and room permitting earlier in term.
- Prescott’s Principles is a very good reference for lab related background it is highly suggested you read the relevant sections.

FOR THE FIRST LABORATORY
Before coming to the first lab, please read the Introduction pages 1 to 9, and Section 1 on fundamental skills as well as 2-12 theory. 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.

Week : week of  
1. Jan 14 

Exercise
Section 3 Microscopy and Staining
read Lab manual pages 1-19 and section one

Ex. 3-1 The light microscope read and understand: Bring to lab a summary of the major components of the microscope. Hand in at the beginning of class for participation credit.
Ex. 3-2 Calibration of the ocular micrometer. You must show your TA the set up for participation credit

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
Ex. 5-28 Motility test (read only)
Ex. 3-3 Examination of eukaryotic microbes (read only)

2. Jan 21  
Section 3 Microscopy and Staining, continued

Ex. 3-7 Gram stain
Ex. 3-8 Acid fast staining procedures
Ex. 3-9 Capsule staining

3. Jan 28  
Section 3 Microscopy and Staining, continued

Ex. 3-10 Endospore stain
Ex. 3-14 Morphological unknown (begin)
Inoculate NB and motility tube see 5-28

4. Feb 4  
Section 3 Microscopy and Staining, continued

Ex. 3-14 Morphological unknown, completion —hand in data sheet and summary, Gram stain, acid fast stain and endospore stain at the end of lab

Section I Fundamental Skills
Refer to Prescott: chapter 6 (6.7-6.8)

Ex. 1-2 Nutrient agar and nutrient broth preparation (read )
Ex. 1-3 aseptic transfer (read only);
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. Feb 11  
record results from week 4 Ex 1-4 and 1-5

Section 6 Quantitative Techniques
Set up:
Refer Prescott section 6.8 7.2, 7.3
Read Appendix C, D, E
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

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

Comparison of growth at 30°C and 37°C. 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 your assignment 2

Reading week week of Feb 18

6. Feb 25

Record viable counts for JS1 and plaque counts (6-5)

Section 2 Microbial Growth: Factors Affecting Growth
Prescott section 7.5 and 8.2
Read Ex 2-2, 2-3, 2-4, 2-12 theory

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

Exercise

7. March 4

Section 2 Microbial Growth: Factors Affecting Growth killing effect and differential tests

Data analysis: (for these exercises a class participation presentation of data
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 (part of the formal report)
Ex. 6-6 Thermal death time versus decimal reduction value: Lab 2 (This is part of your formal report)

Set up:
Refer Prescott section 8.1 8.3, 8.5 8.6, 31.2, 31.3, 31.4
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)
Ex. 5-15 Gelatinase test, Lab 1
Ex 5-24 Bacitracin Novobiocin and Optochin Susceptibility test , Read only, understand the theory
Ex. JS2 β-lactamase test (obtain results); lab posted on the intranet/blackboard
Ex 4-4 growth on Mannitol salt agar (This is part of your formal report)
Ex 7-1 Snyder Test lab 1

8. March 11

Microbial Growth : Factors Affecting Growth etc
Data analysis:

Ex. 2-14 Effect of disinfectants, Lab 2 (part of assignment 3)
Ex. 7-3 Effect of antibiotics, Lab 2 (part of assignment 3)
Ex. 5-17 Gelatinase test, Lab 2
Ex 4-4 growth on Mannitol salt agar lab 2
Ex 7-1 Snyder Test Lab 2

Set up

Ex. 2-13 Ultraviolet radiation: lethal effect, Lab 1 (part of your formal report)
Ex. 10-5 Ultraviolet radiation: damage and repair, lab1
Ex. 5-16 DNase test, lab 1

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. March 18

Section 2 Microbial Growth/section 7 Medical microbiology

Data analysis:
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 lab 2
Ex 5-6 DNase test, lab 2

Set up: Differential tests /Medical Medical Microbiology

See Table 2.4 in Prescott pg 489

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 data bring answers to questions for participation credit due at the beginning of lab

Section 10 Microbial Genetics

Ex. 10-3 Bacterial transformation : the pGLO system
Lab 1

10. March 25 Section 8 Microbial Genetics
refer Prescott section 14.6, 14.7, 16.4-16.8

Data analysis:
Ex. 10-3 Bacterial transformation : the pGLO system, Lab 2 (class participation: lab bench analysis)
Ex JS5 blood agar Lab 2
Ex 4-5 MacConkey Agar Lab 2

Set up: Microbial Genetics/differential test
Ex. JS6 Bacterial conjugation: the transfer of antibiotic resistant plasmids between cells, Lab 1. Exercise will be posted
Ex 5-30 Enterotube II- multiple test system week 1 (read section 24.2 of Brock)

Section 9 Hematology and Serology
Ex. 11-1 Differential blood count

11. April 1 Section 8 Microbial Genetics
Data analysis:
Ex. JS6 Bacterial conjugation: the transfer of antibiotic resistant plasmids between cells, Lab 2. Work per group and hand in your interpretation prior to class discussion
EX 5-30 Enterotube: week 2 interpret
Set up
Section 9 Hematology and Serology
Ex. 11-4 Slide agglutination
Lab review for lab component of the final exam by your TA Formal review will be held in separate session by Dr. Brunt

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

<table>
<thead>
<tr>
<th>Correct</th>
<th>Incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>S. aureus</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Staph. aureus</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp. (where species is not known)</td>
<td></td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>proper name</th>
<th>adjective</th>
<th>cell shape</th>
<th>general arrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus</td>
<td>bacillus</td>
<td>rod</td>
<td>single or in short chains</td>
</tr>
<tr>
<td>Diplococcus</td>
<td>diplococcus</td>
<td>coccus</td>
<td>in pairs</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>streptococcus</td>
<td>coccus</td>
<td>in chains</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>staphylococcus</td>
<td>coccus</td>
<td>in clusters</td>
</tr>
</tbody>
</table>