BIO C17 Summer 2013
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 BI C17 requires 3 hours of laboratory per week. The Laboratory component represents **41% of your final grade**. This laboratory will provide hand 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 the6% performance grade and all related grades to missed lab. One unexcused absence 3 % and all related grades. 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)
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".

You must inform Dr Brunt in writing if you wish to opt out and you must then provide an electronic copy of your report to Dr. Brunt

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 treats cases of cheating and plagiarism very seriously. 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 Summer 2013

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 June 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 (4%) Due July 2, 2013

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

Assignment 3 (9%) formal research paper on Use of temperature , salt and UV in control of growth of microorganisms. (Paper copy due at the beginning of your lab July 23 2013, and to turnitin by 5 pm on the day of your lab)

Ex.7-3 antibiotics
Ex 2-14 disinfectants
Ex. JS2 β-lactamase test

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 for full details
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 . 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**.
- **If you are caught eating or drinking you will be asked to leave your lab: associated lost grades**
- If you are immunocomprimised you must see me before the lab begins

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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 the custom text book 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.

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FOR THE FIRST LABORATORY

Before coming to the first lab, please read the Introduction pages 1 to 9 on Safety and laboratory quidlines, and Section 1 on fundamental skills as well as 2-12 theory 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

Week :		**Exercise**
1.	**May 14**	***Section 3 Microscopy and Staining*** 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 : ***Bring to lab a summary of the major components of the microsope. 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. **May 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. **May 28** ***Section 3 Microscopy and Staining, continued***

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

4. **June 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 pages 141-143
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. **June 11** record results from week 4 Ex 1-4 and 1-5

Section 6 Quantitative Techniques

Set up:

Refer to chapter 5 pages 149-156 of your text book
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 30C and 37C. 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 June18

6. **June 25** Record viable counts for JS1 and plaque counts (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
Ex 6-6: Thermal death time versus decimal reduction value

Exercise

7. **July 2** ***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
Ex. 6-6 Thermal death time versus decimal reduction value: Lab 2

Set up :

Refer to chapters 6 and 7 in your text
Ex. 2-14 Effect of disinfectants, Lab 1 (part of formal report)

Ex. 7-3 Antimicrobial susceptibility (affect of antibiotics), Lab 1 (part of formal report)
Ex. 5-5 Catalase test, Lab 1 (obtain results)

Ex. 5-15 Gelatinase test, Lab 1
Ex 5-24 Bacitracin Novobiovin and Optochin Susceptibility test , Read only ,understand the theory
Ex. JS2 β-lactamase test (obtain results); lab posted on blackboard (part of the formal report)
Ex 4-4 growth on Mannitol salt agar
Ex 7-1 Snyder Test lab 1

8. July 9 ***Microbial Growth : Factors Affecting Growth etc***

Data analysis*:*

Ex. 2-14 Effect of disinfectants, Lab 2 (formal report)
Ex. 7-3 Effect of antibiotics, Lab 2 (formal report)
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**
Ex. 2-13 Ultraviolet radiation: lethal effect , Lab 1
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. July 16 ***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 lab2
Ex 5-6 DNase test, lab 2

Set up: Differential tests /Medical Medical Microbiology

Refer to chapter 4 of your text book
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 (refer to chapter 9 of your text book

10. **July 23** **Sec*tion 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)
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

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

11. **July 30** ***Section 8 Microbial Genetiscs***
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

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

Correct	**Incorrect**
Staphylococcus aureus	Staphylococcus aureus
Staphylococcus aureus	
S. aureus	*Staph. aureus*
Staphylococcus spp. (where species is not known)	

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:

proper name	**adjective**	**cell shape**	**general arrangement**
Bacillus	bacillus	rod	single or in short chains
Diplococcus	diplococcus	coccus	in pairs
Streptococcus	streptococcus	coccus	in chains
Staphylococcus	staphylococcus	coccus	in clusters