

Assignments General Directives

The first page must include the following information:

- The assignment number
- Course code :BIO3126
- Your name or names
- Your group number
- The date

Assignments may be done and handed in individually or in groups of two (you and your partner). Assignments must be typewritten except for calculations, which can be done by hand. Tables and graphs must be computer generated, clear and concise. Tables and graphs must have an appropriate title and a caption if appropriate

Only hard copies of the assignments will be accepted. DO NOT EMAIL assignments. Assignments must be handed in to the appropriate teaching assistant at the indicated due date BEFORE you leave the lab.

A 10% per day penalty will be imposed on late assignments. (Weekends will be counted as one day). If a valid reason, such as a medical reason, is given, you will be exempted from doing the required assignment.

PowerPoint presentations:

- Use the following black and white template format for your PPT presentations
- Pictures must be in color
- Save as a PDF file with one slide per page
- Submit on Brightspace in the appropriate folder

<div style="border: 1px dashed black; padding: 10px; margin-bottom: 20px;">Indicate an appropriate title here</div> <div style="border: 1px dashed black; padding: 10px; margin: 0 auto; width: 80%;">Indicate your name or names Your group number The course code The date</div>
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<div style="border: 1px dashed black; padding: 5px; margin-bottom: 10px;">Indicate an appropriate title here</div>	
<ul style="list-style-type: none">• Add your picture here	<ul style="list-style-type: none">• Indicate the required information here

Grading scheme for tables

(Each cell is worth 0.25 point)

Presentation	Table 1	Table 2	Table 3	Table 4
• Legend, column headings, and row headings are presented				
• Appropriate data is presented				
• Tables are computer generated				
• Spaces and/or lines are used wisely to group appropriate data or to separate different components of the table				
• Reading of the table (independent, no unnecessary text, clear and simple)				
Legend				
• Starts with the number of the table				
• The first sentence is a specific and complete title				
• All symbols and non-standard abbreviations are explained in the legend				
Totals	/2.0	/2.0	/2.0	/2.0
Grades are converted according to the points assigned on the respective assignments				

Grading scheme for graphs

(Each cell is worth 0.25 point)

Presentation	Fig 1	Fig 2	Fig 3
• Correct type of graph			
• The appropriate variables are presented			
• The graph was computer generated			
• Appropriate page layout – the graph occupies 1/2 to 2/3 of the page			
• The legend occupies about a third of the page and is below the graph			
Data			
• Units of axes (the intervals for the abscissa and the ordinate cover the data range)			
• Identification of axes (appropriate for the chosen variables)			
• Type of curve adjustment (the best trend is illustrated)			
Legend			
• Starts with the number of the graph			
• The first sentence is a specific and complete title			
Totals	/2.5	/2.5	/2.5
Grades are converted according to the points assigned on the respective assignments			

Grading scheme for microscopic images
(Each cell is worth 0.25 point)

Presentation	
• The required format is respected and the correct number of images is provided	
• A slide with the title including all the information requested is included	
Data: Image quality	
• Images are in focus	
• Good smear, uniform spread	
• Uniform staining (color)	
• Good staining (correct color)	
Legend	
• Starts with a figure number	
• Provides a specific and complete title	
• Staining technique and stain used if appropriate	
• Cell shape	
• Aggregation	
• Magnification	
Totals	/3.0
Grades are converted according to the points assigned on the respective assignments	

Assignment 1

Part I: Problems. Solve the following problems. You are not required to show your calculations. Only submit your final answers. Indicate your answers to two significant digits after the decimal. Note: DO NOT round off the results of your calculations until the final answer. **(2.5 points/question)**

1. What is the molarity of a solution of ammonium chloride prepared by diluting 150.0 mL of a 2.0 M NH_4Cl solution to 3.0 L? **0.1M or 100mM**
2. A student takes a sample of a 2.1 M KOH solution and dilutes it by adding 135.00 mL of water. The student then dilutes this solution 3X and determines that the concentration of the final solution is 0.025 M KOH. What was the volume of the original sample? **5 mL**
3. A microbiologist wants to prepare a stock solution of H_2SO_4 so that samples of 5.0 mL will produce a solution with a concentration of 0.10 M when added to 100.0 mL of water. What should the molarity of the stock solution be? **2.1 M**
4. What volume of water should be added to 10.0 mL of a 5.0 M acetic acid solution in order to obtain a final concentration that is 0.25 M acetic acid? **190 mL**
5. Three solutions "A", "B", and "C" are mixed to obtain the following ratio: A:B:C = 1:2:8. Each of these solutions was diluted by what factor? A: **11X**, B: **5.5X**, C: **1.38X**
6. A microbiologist has three microbial cultures: *E.coli* at a density of 5×10^9 cells/mL, *B. subtilis* at a density of 5×10^8 cells/mL, and *P. notatum* at a density of 1×10^6 cells/mL. From these, he wishes to prepare a single mixed culture containing 5×10^8 cells/mL of *E.coli*, 1.25×10^8 cells/mL of *B. subtilis*, and 2×10^5 cells/mL of *P. notatum* in a final volume of 10mL. What volume of media and of each of the original cultures should be used to achieve this?
Media 4.5mL, coli 1 mL, subtilis 2.5 mL, and notatum 2 mL.
7. The microbiologist mentioned in the previous problem realizes that he only has 2.25 mL of media. Given this information, what is the maximum volume of the microbial mixture previously described that can be prepared? **5 mL**
8. Three parts of water are added to 2 parts of a 2.0 M solution of FeSO_4 and 4 parts of a 1.0 M solution of FeSO_4 . What is the percentage (m/v) of FeSO_4 in the final solution? (MM of FeSO_4 : 151.91 g/mole) **13.50%**
9. A microbiologist prepares 100 mL of a 0.3 M solution of $\text{K}_2\text{Cr}_2\text{O}_7$ in water. A week later, 30 mL of water has evaporated. How much water must the microbiologist add to the evaporated solution in order to obtain a 0.5% (m/v) solution of $\text{K}_2\text{Cr}_2\text{O}_7$? (MM of $\text{K}_2\text{Cr}_2\text{O}_7$: 294.19 g/mole)
1.69L
10. A chemical test has determined the concentration of a 100 mL solution of an unknown substance to be 2.0 M. The solution is completely evaporated, leaving 20 g of crystals of the unknown solute. What is the molar mass of the unknown substance? **100 g/mole**

11. Antibiotic administration in children who are 3 months old or less is typically done on a per weight basis. For example, the recommended dosage of amoxicillin is 30 mg/kg. How many milliliters of a 0.1g/mL amoxicillin solution should be administered to a child weighing 7 Kg?
2.1 mL
12. You wish to prepare a solution that contains 300g of a compound "A" and that is at a final concentration of 2 M compound "A". What volume of water should you add to 300 g of compound "A" in order to obtain the desired solution (MM of compound "A": 50g/mole; density: of compound "A": 1.5g/mL)? **2.8L**

The following diagram represents two solutions (A and B) separated by a water permeable membrane which is impermeable to solutes. Use this as the initial condition to answer questions 13-17.

A. $\frac{800 \text{ osmoles}}{10 \text{ litres}}$	B. $\frac{250 \text{ osmoles}}{5 \text{ litres}}$
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13. What are the osmolarities of solutions A and B in the original condition?
80 Osm and 50 Osm
14. What is the osmotic relationship of solution A relatively to solution B?
Hyperosmotic
15. What is the tonic relationship of solution A relatively to solution B?
Hypertonic
16. What will be the osmolarity in compartment A once osmosis is complete?
70 Osm
17. What will be the volume in compartment A once osmosis is complete?
11.42L
18. Calculate the osmolarities of the following solutions : (Note: All solutes are impermeable except for urea)
- 0.25 M Na_2CO_3 + 1M urea **1.75 Osm**
 0.02 M $\text{Al}(\text{NO}_3)_3$ **0.08 Osm**
 0.125 M LiBr **0.25 Osm**
 0.05 M glucose + 0.2M NaCl **0.45 Osm**
19. Indicate the osmotic and tonic relationship of red blood cells, whose internal osmolarity is 250 mOsm relative to each of the indicated solutions.

Solution	RBC Vs Solution	
	Tonicity	Osmolarity
0.05 M Na_2CO_3 + 1M urea	iso	hypo
0.02 M $\text{Al}(\text{NO}_3)_3$	hyper	hyper
0.125 M LiBr	iso	iso
0.05 M glucose + 0.2M NaCl	hypo	hypo

20. How many grams of CaCl_2 must you dissolve in 1 L of water to obtain a solution that is isosmotic to red blood cells? (Osmolarity of RBC: 250 mOsm; MM of CaCl_2 : 110.98 g/mole)
9.25g

PART 2: EXPERIMENTAL DATA AND THEIR INTERPRETATION (5 POINTS/QUESTION)

EXERCISE 1.0: GENERATING A STANDARD CURVE AND DETERMINING AN UNKNOWN CONCENTRATION OF METHYLENE BLUE

1. Show your calculation as to how 5 mL of a 0.4mM solution of methylene blue was prepared from a stock solution of 0.26% (m/v).
2. Submit a standard curve generated with Excel which represents the relationship between the concentration of methylene blue (% m/v) and the absorbance at 550nm. Present the line of best fit. Indicate the equation of the line and the R² coefficient.
3. According to your results what was the unknown concentration of methylene blue. Indicate the concentration obtained from each of the two dilutions. Show your calculations.

EXERCISE 1.1: DIFFUSION, OSMOSIS AND TONICITY IN RED BLOOD CELLS

4. Complete the following table :

	Molarity (M)	Osmolarity (OsM)	% Hemolysis
Sucrose			
NaCl			
Glycerol			

5. Submit a graph that represents the relationship between the osmolarities of the different solutions and the percent hemolysis of red blood cells. Present the line that illustrates the trend. Do not include a line of best fit.

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6. According to your results, what is the approximate internal osmolarity of the red blood cells? Justify your answer.
7. According to your results, the cell membrane is most permeable to which solute? Explain how you reached this conclusion.
8. Briefly define the terms crenation and hemolysis as they relate to osmosis.

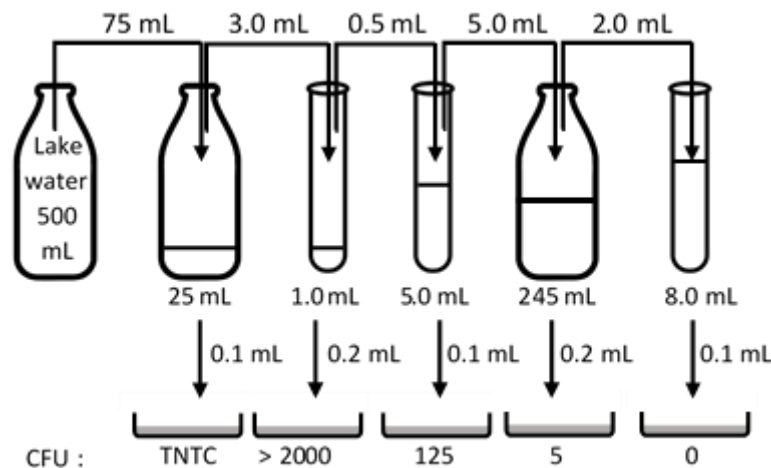
EXERCISE 1.2: BACTERIAL GROWTH VS SOLUTE CONCENTRATION

9. Among the different growth condition used in this lab exercise, which one (s) if any, would allow the growth of a moderate halophile. Justify your answer.
10. Among the different growth conditions used in this lab exercise, which one (s) if any, would allow the growth of a moderate osmophile. Justify your answer.

Assignment 2

Part I: Problems. Solve the following problems. You are not required to show your calculations. Only submit your final answers. Indicate your answers to two significant digits after the decimal. Note: DO NOT round off the results of your calculations until the final answer. **(3.0 points/question)**

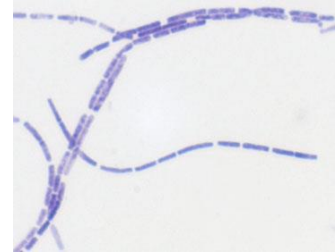
- Which of the methods used in this lab would be best to determine the total number of bacteria in one milliliter of lake water? Briefly explain why this method was chosen and why the other ones were not. **Direct count gives total number. Turbidity and viable counts would not allow growth of all bacteria since there are no universal media.**
- Turbidity measurements were taken of three different bacterial culture. One of these was a culture of *Escherichia coli* that has the following characteristics: A diameter of $\approx 1\mu\text{m}$, a length of $\approx 2\mu\text{m}$, and a volume of $\approx 1\mu\text{m}^3$. The second was a culture of *Bacillus subtilis* that has the following dimensions: A diameter of $\approx 1.0\mu\text{m}$, a length of $\approx 10\mu\text{m}$, and a volume of $\approx 5\mu\text{m}^3$. The third was a culture of *Micrococcus* that has the following dimensions: A diameter of $\approx 1.0\mu\text{m}$ and a volume of $\approx 0.5\mu\text{m}^3$. The three cultures had the same turbidity measurement. Indicate the order, from highest to lowest cell number /mL of the three cultures. Indicate the reasoning of the chosen order. **Micrococcus-Escherichia-Bacillus. Smallest volume = least light scattering**
- To assess the number of bacteria in ground beef, a 5 g sample of meat is homogenized in 35 mL of water resulting in a total volume of 40 mL. A 2 mL sample of the meat suspension is then diluted by a factor of 10^3 X. 1mL of the final dilution is then added to 2.5 mL of saline from which 0.1mL was plated. If 100 colonies were observed on the plate, what was the original number of bacteria/g of ground beef? **1.2×10^7**
- You perform the following serial dilutions: 15X, 1/4, and 1:4. What is the final dilution achieved by this series? **1/300**
- Consider the following dilution scheme:



Report the total number of CFUs in the **entire** 500 mL amount of the original lake water sample. (TNTC=too numerous to count.) **1.55×10^7**

6. A brand of probiotic yogurt claims to contain approximately 10^{15} viable bacteria in 30 mL. You wish to verify this claim by performing a viable count. What tenfold dilutions would you plate if you only had two plates available and wished to plate 0.1 mL samples? 10^{-10} and 10^{-11}

7. A viable count of a culture of the bacteria shown in this image was performed in two different ways. In the first case, 0.1 mL of a 10^{-6} dilution was plated and gave rise to 115 CFU. In the second case, the culture was first treated in a homogenizer after which 0.1 mL a 10^{-7} dilution was plated. In this case, 102 CFU were observed. Briefly explain the difference in the viable counts obtained. **Before homogenization 1 CFU = to more than one cell.**

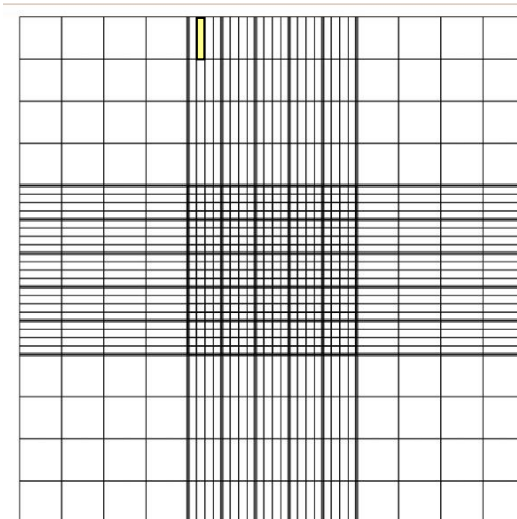


8. You perform viable counts on a culture of *Escherichia coli* (a small rod shaped bacteria) and a culture of *Micrococcus luteus* (a cocci shaped bacteria), which each contain 10^{10} bacteria in 100 mL. How many CFUs would be expected if you plated 0.2 mL of 10^{-5} dilutions of each culture? **coli : 200, luteus: 50**
9. You setup an MPN to determine the number of bacteria/gram of Camembert cheese. To do so, 100 g of cheese are homogenized in a final volume of 1L. The suspension is then used to perform a three tubes MPN assay. Tubes containing 10 mL of broth were inoculated with 1 mL from each of the respective dilutions. You obtain the results indicated in the table below.

Dilution	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
# of positive tubes	3	3	2	2	0	1

What is the most probable number of bacteria/g of cheese? **Accept 2.8×10^6 or 2.9×10^7**

10. You also perform a viable count from the same original suspension presented in the previous question. What dilution of the suspension should you plate to confirm the MPN result assuming that you plate 0.1 mL? Indicate the dilution and the expected number of colonies. **(10^{-4} 29 CFU or 10^{-3} : 290 CFU) or (10^{-3} 28 CFU or 10^{-4} : 280 CFU)**
11. Two milliliters of an *E. coli* culture is mixed with 8 mL of dye. A drop (approximately 0.1 mL) of this dilution is applied to a hemocytometer slide's counting chamber. Three squares (yellow colored square on the image below) are counted giving the following results: 36, 44, and 40 bacteria. How many bacteria are there per mL in the original sample? **1.6×10^8**



12. You perform a three-tube MPN assay on a 100 mL sample of milk. Tubes containing 5 mL of broth were inoculated with 1 mL from each of the respective dilutions. You obtain the results indicated in the table below.

Dilution	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
# of positive tubes	3	3	3	3	2	1	0

What average number of bacteria would you expect to observe in one square of a hemocytometer slide? The dimensions of the square chosen are 0.05 mm X 0.05 mm X 0.1 mm. **37 or 38**

13. The table below indicates the approximate diameters of the field of vision observed with the different microscope objectives.

Objective	Diameter (mm)
4x	4.0 mm
10x	2.0 mm
100x	0.2 mm

A bacterium (A) is visualized with the 10x objective and observed to be a rod whose length is approximately $1/10^{\text{th}}$ of the field of vision. Another bacterium (B) is visualized with the 100x objective and observed to be a rod whose length is approximately $1/5^{\text{th}}$ of the field of vision. Which bacterium is longer? (Hint: Check out the next question) **Bacterium A is longer**

14. A specimen is examined under the microscope with an ocular of 4X and an objective of 10X. A measurement of the specimen within the field of vision was 0.2 mm. What is the original size of the specimen? **0.005mm**

15. A specimen is examined using two different microscopes. In one case (A), the specimen was visualized using an ocular with a magnification of 4x and an objective of 40x. In the second case (B), the specimen was visualized with an ocular of 10x and an objective of 10x. Which of the following statements is (are) true? Indicate all the true statements.

- A. The **observed** size of the specimen would be the same in both cases.
- B. The **observed** size of the specimen would be greater in B as compared to A.
- C. **The fraction of the field of vision occupied by the specimen would be greater in A as compared to B.**
- D. **The size of the field of vision would be smaller in A as compared to B.**

Part 2: Experimental data and their interpretation (5 points/question unless otherwise indicated)

EXERCISE 2.0: TURBIDITY MEASUREMENTS (Continued from exercise 1.2)

1. Complete the following tables:

Growth of <i>S. aureus</i> as a function of osmolarity				
Solute concentration (%m/v)	Osmolarity (Osm/L)		O.D. 600	
	Sucrose	NaCl	Sucrose	NaCl
0.5				
5				
10				
15				

Growth of <i>E. coli</i> as a function of osmolarity				
Solute concentration (%m/v)	Osmolarity (Osm/L)		O.D. 600	
	Sucrose	NaCl	Sucrose	NaCl
0.5				
5				
10				
15				

2. Submit bar graphs illustrating the percentage of growth obtained under each condition as compared to the broth with no added solute. To determine the relative percentage of growth divide the O.D.600 of the condition being assessed by the O.D. 600 of the broth without any added solute and the multiply by 100. Submit one bar graph for each solute tested.

EXERCISE 2.4: DIRECT COUNT OF A YEAST SUSPENSION

3. Submit your calculation for the determination of the number of yeast cells per milliliter in the original suspension. Make sure to indicate the following information: Dimensions of the squares from which the counts were obtained, the three counts, the average number of cells per square, and your calculation determining the original number of yeast cells/mL.

EXERCISE: 3.0 VIABLE COUNTS (Continued from exercise 2.1)

4. Submit a bar graph illustrating the viable counts as a function of osmolarity.

5. Submit a graph illustrating the relationship between the O.D600 readings obtained for the *E.coli* culture grown under the different conditions and the corresponding viable counts obtained. Include the line of best fit and the R² coefficient.

EXERCISE 3.1: VIABLE COUNTS OF A SOIL SAMPLE (Continued from exercise 2.2)

6. Submit your calculations for the determination of the number of bacteria per gram of soil.

EXERCISE 3.2: MPN OF A SOIL SAMPLE (Continued from exercise 2.3)

7. Complete the following table. Below the table, indicate the number combination used to determine the MPN index. Submit your calculations showing the determination of the MPN per g of soil.

Dilution	Number of tubes out of three with growth
10^{-3}	
10^{-4}	
10^{-5}	
10^{-6}	
10^{-7}	
10^{-8}	

MPN Combination used to determine MPN index:

MPN index:

MPN /g of soil (Show calculation):

COMPARISON OF VIABLE COUNTS

8. Determine the percentage variation between the two methods used to determine the number of bacteria in the soil sample (MPN vs viable count). Show your calculations. Briefly discuss any difference observed.

EXERCISE 3.3, 3.5 and 3.6: MICROSCOPIC VISUALIZATION OF BACTERIA (15 points)

9. Submit a PPT presentation, which includes the following pictures. Make sure to follow the instructions indicated in the general directives.

- Pictures of three different colonies- Indicate colony morphology
- Simple stain of *E.coli*
- Three simple stains of colonies obtained from soil sample
- Three negative stains of colonies obtained from soil sample

Assignment 3

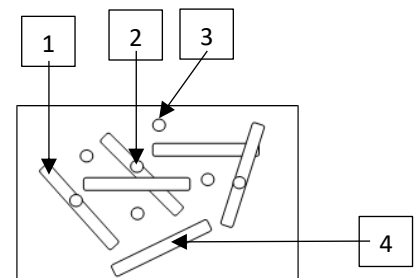
Part 1: Problems. Solve the following problems. You are not required to show your calculations. Only submit your final answers. Indicate your answers to two significant digits after the decimal. Note: DO NOT round off the results of your calculations until the final answer. **(3.0 points/question)**

1. What color would bacteria of the genus *Bacillus* and bacteria of the genus *Salmonella* be if the Gram's iodine step was omitted in the Gram staining procedure?

- A. Purple and red respectively
- B. Red and purple respectively
- C. Blue and red respectively
- D. Both would be red
- E. Both would be purple

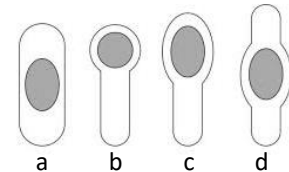
2. This picture represents a schematic of a bacterial smear of *Bacillus subtilis*. What color would each of the structures identified in this picture be following a typical Gram stain? Identify the structure and indicate the expected color.

2 + 3 colorless; 1 + 4 purple



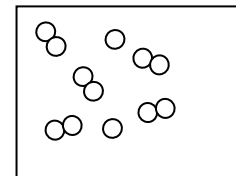
3. Which of these spore morphologies is typical of bacteria of the genus *Bacillus*?

a



4. The bacteria in this image appeared red after a typical Gram stain. According to this information, they probably belong to which genus?

- a. *Moraxella*.
- b. *Escherichia*.
- c. *Mycobacteria*.
- d. *Streptococcus*.



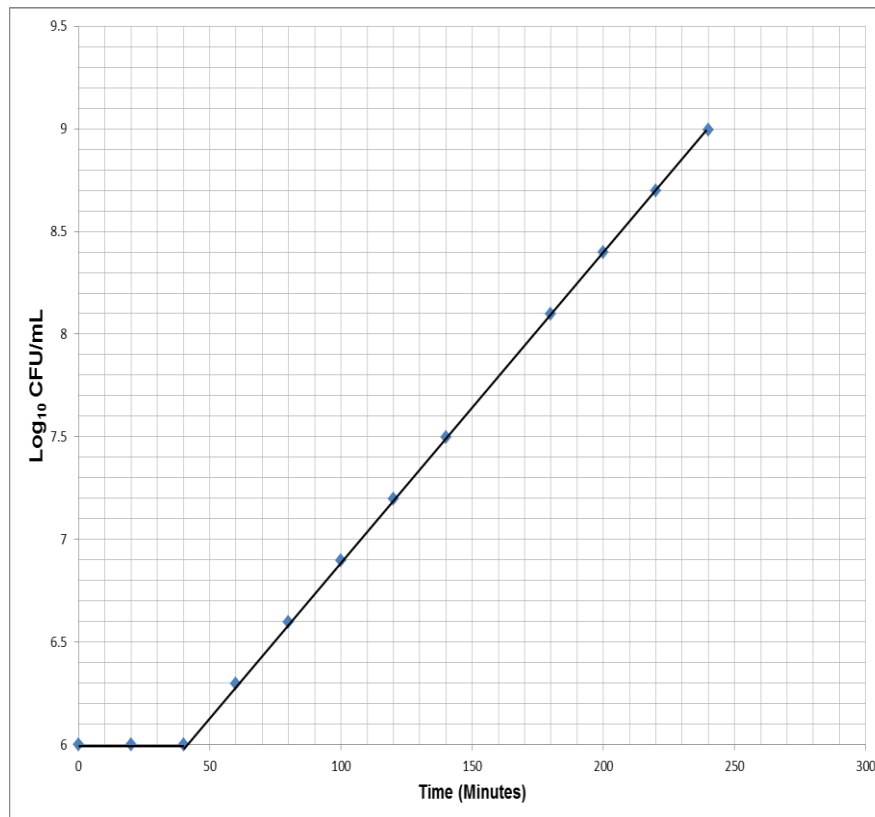
5. The cells in the above image would be what color following a Gram stain where the ethanol wash was omitted?

- a. Blue.
- b. Purple
- c. Red.
- d. Colorless.

6. A bacterial culture is sampled at two time points representing an interval of 70 minutes. 3.2×10^6 cells/mL were present in the first sample and 5.12×10^7 cells/mL in the second sample. How many times did the population double within the time span examined?

4 times

7. Cultures from the tissues of a patient with syphilis are done on samples taken at an interval of one week. The first sample had 2000 CFUs, whereas the second sample had 22627 CFUs. Four days later, a third sample had 90509 CFUs. Did the speed at which the bacterium divides change between the first and second sampling as compared to between the second and third sampling. Justify your answer. **No, same doubling time.**
8. A biologist determines that the relationship between cell number and optical density of a given bacteria is one O.D. unit per 8×10^8 cells. A broth is inoculated at time $t = 0$ h with an unknown number of bacteria. After 2 hours of growth, the cell number was determined to be 2.56×10^9 . If the generation time of these bacteria under these conditions is twenty minutes, what must have been the initial optical density at time zero? **(0.05)**
9. The growth rate constant (k) of a bacterial population is 0.5 generations/hour. If a broth is inoculated with 1000 bacteria, how many bacteria would there be after one day? **4.1×10^6**
10. You initiate two bacterial cultures, "A" and "B". Culture "A" initially has one hundred cells and a growth rate (μ) of 0.01 cells/minute. Culture "B" initially has four cells and a generation time of 23 minutes. Which culture would reach the highest cell number after 3 hours of growth? **B**
11. The culture illustrated in the following graph represents one liter of medium containing a limiting amount of glucose (0.2 grams). At time zero the culture contained 0.001g of cells. Given that the stationary phase starts after 260 minutes of exponential growth, what would be the growth yield of this culture (Y_g ; Grams of bacteria/gram of glucose)? **10.24**



Growth of bacteria as a function of time. The Log_{10} of the numbers of cells/mL are presented (Y axis)

12. Consider the information given in the previous question. Assume that the same growth profile was obtained with 0.2g of glucose (MW 180g/mole) or 0.2g of lactose (MW 342g/mole). Which carbon source is most efficiently used (Highest Y_m)? **Lactose**
13. What color would an O-F tube overlaid with oil and one without oil be for bacteria with each of the following metabolic characteristics
- | | | |
|---|-------------|-------------|
| | +oil | -oil |
| • Strict anaerobe, which ferments glucose | yellow | green |
| • Strict anaerobe, which uses glucose by anaerobic respiration | yellow | green |
| • A facultative anaerobe, which can ferment and respire glucose | yellow | yellow |
| • A strict aerobe, which can use glucose as a carbon source | green | yellow |
14. Yeast is a facultative anaerobe that can metabolize glucose through ethanolic fermentation or oxidize glucose through glycolysis and the Krebs cycle. How many moles of CO_2 would each of these metabolic pathways generate from three moles of glucose? **Fermentation: 6, oxidation: 18**
15. Consider the information given in the previous question. How many moles of NADH would be oxidized by the metabolism of three moles of glucose aerobically and anaerobically (fermentation)? **aerobically: 30 anaerobically: 6**

Part 2: Experimental data and their interpretation (5 points/question)

EXERCISE 5.0: *E. COLI* GROWTH CURVE

1. Submit a graph with two growth curves (M9 + glucose medium at 37°C with shaking and M9 + glucose + 1% tryptone at 37°C with shaking) which respects the following criteria :
 - Changes in optical density as a function of time
 - Each curve must have 32 time points representing the interval T0 – T16h
 - Consider the cultures inoculated at 10 o'clock = T0, those inoculated at 11 o'clock = T1h, those inoculated at 12 o'clock = T2h and those inoculated at 1 o'clock = T2h
2. Determine from your profiles which phases were observed and the time span of each of these.
3. Determine from your profiles the generation time (g) and the growth rates (μ) of each culture. Show how you arrived at these values.
4. Determine from your profiles the number of times the populations doubled (n). Show how you arrived at these values.

EXERCISE 5.1: GROWTH YIELDS AS A FUNCTION OF CARBON SOURCE

5. Submit a table containing the following information: Growth yield (O.D. values), mass of carbon source, moles of carbon source.
6. Submit a bar graph that compares growth yields as a function of the masses of the different carbon sources.
7. Submit a bar graph that compares growth yields as a function of the moles of the different carbon sources.

EXERCISE 5.3: YEAST FERMENTATION BIOASSAY

8. Submit a graph with four curves illustrating the moles of ethanol produced as a function of time with each of the quantities of glucose.
9. Submit a table presenting the rates of ethanol production (moles ethanol/min.) as a function of grams of glucose.
10. Submit a bar graph that compares ethanol yields (in grams) as a function of the different amounts of glucose (grams).
11. Submit a bar graph that compares ethanol yields (in moles) as a function of the different amounts of glucose (grams).

Assignment 4

Part 1: Theory (2.5 points/question)

1. When *Staphylococcus epidermidis* is inoculated on Phenolethanol agar, individual cells generate visible colonies whereas *Escherichia coli* dies. Phenolethanol agar is an example of what type of medium?
 - A. A general purpose medium
 - B. A differential medium
 - C. A selective medium
 - D. A complex medium
2. What is agar?
 - A. A polysaccharide polymer derived from an algae.
 - B. A nitrogen source in solid media.
 - C. An amino acid polymer derived from tendons.
 - D. A polymer that can be degraded by the majority of bacteria of medical importance.
3. Which compound can be used as both a carbon and nitrogen source in the phenol red broth?
 - A. Sucrose.
 - B. Phenol red.
 - C. Amino acids.
 - D. Ammonium sulfate.
4. Which statement possibly describes the fermentation of a carbohydrate in the phenol red broth?
 - A. The medium is clear and turned yellow.
 - B. The medium is cloudy and turned dark red.
 - C. The pH indicator changed to a color indicating the presence of an alkaline product.
 - D. The medium is cloudy and shows the accumulation of a gas in the inverted vial.
5. Which of the following statements concerning the utilization of amino acids in culture media is **true**?
 - A. Amino acids can be used as a carbon source
 - B. Amino acids can be used as a nitrogen source.
 - C. Amino acids can be used as a source of electrons.
 - D. All the statements (A – C) are true.
 - E. Statements A and B are true but not C.
6. Which characteristic (s) is (are) common to the phenol red broth, the urea slant, and the TSI slant?
 - A. These media contain phenol red.
 - B. They are media that allow the detection of fermentation.
 - C. They are media that allow the detection of acid production.
 - D. They are media that allow to determine the nitrogen source used.

7 – 10 match the appropriate answer to each of the statements. Some answers may be used more than once and some may not be used at all.

- A. Phenol red sucrose B. Nitrate broth C. Simmon's citrate slant
D. Phenylalanine agar slant E. SIM

7. Used to verify the degradation of tryptophan. **E**

8. Used to verify the production of phenylpyruvic acid. **D**

9. Used to verify the generation of nitrogen gas. **B**

10. Used to verify the degradation of cysteine. **E**

11 – 14 match each of the reagents or compound with its appropriate goal. Some answers may be used more than once and some may not be used at all.

- A. Kovacs reagent B. Ferric sulfate C. Sterile mineral oil
D. Zinc chloride E. Yeast extract

11. Used to detect the presence of H₂S. **B**

12. Used to reduce the availability of oxygen. **C**

13. Used as a source of vitamins, amino acids and other nutrients. **E**

14. Catalyst that reduces nitrates. **D**

15 – 20 Match each of the bacteriological tests with the observations or reaction indicating a positive test. Some answers may be used more than once and some may not be used at all.

- A. OF media B. Simmon's citrate C. Lysine iron agar
D. Nitrate broth E. Decarboxylase broth F. Urea slant

15. The presence of alkaline by-products turns the medium to a pink color. **F**

16. The presence of alkaline by-products turns the medium to a blue color. **B**

17. Reduction of sulfur generates a black precipitate. **C**

18. The pH indicator bromcresol purple becomes purple in when an alkaline product is generated. **E**

19. Green medium in the absence of sugar metabolism. **A**

20. Yellow medium because of sugar fermentation. **A**

21. The abundant production of acid by fermentation of glucose leads to a decrease in pH such that the pH of the medium falls below 4.5. Which of the following test can detect this?
- A. Sim medium
 - B. Methyl red test
 - C. Simmon's citrate
 - D. Voges-Proskauer
 - E. Phenol red broth
22. Which of these conclusions may be valid for a red nitrate broth? Choose all that apply.
- A. Nitrate was reduced to nitrite.
 - B. Nitrate was not reduced.
 - C. Nitrite was reduced to ammonia and nitrogen gas.
 - D. The test organism can perform anaerobic respiration.
23. Which of these tests can detect the reduction of sulfur? Choose all that apply.
- A. SIM's medium.
 - B. TSI.
 - C. Lysine iron agar.
 - D. O-F medium
24. Which of these tests can detect the breakdown of cysteine but not the breakdown of tryptophan? Choose all that apply.
- A. SIM's medium.
 - B. TSI.
 - C. Lysine iron agar.
 - D. O-F medium.
25. Which of these tests would necessarily be positive for a fecal coliform? Choose all that apply.
- A. Phenol red glucose.
 - B. Phenol red lactose.
 - C. Urea slant.
 - D. O-F medium.
 - E. Nitrate broth.
26. Which of these tests, if positive, would indicate the fermentation of glucose without the production of acids? Choose all that apply.
- A. Phenol red glucose.
 - B. Methyl red test
 - C. Vogues Proskauer test.
 - D. O-F medium.
 - E. Ornithine decarboxylase test.

27. Which of these bacteria is not a coliform? Choose all that apply.

- A. *Bacillus subtilis*.
- B. *Proteus miribalis*.
- C. *Staphylococcus aureus*.
- D. *Listeria monocytogenes*.
- E. *Serratia marcescens*.

28. Which of these bacteria belongs to the *Enterobacteriaceae* family and can metabolize proteins and cysteine? Choose all that apply.

- A. *Bacillus subtilis*.
- B. *Proteus miribalis*.
- C. *Pseudomonas aeruginosa*.
- D. *Enterobacter aerogenes*.
- E. *Serratia marcescens*.

29. A bacterial unknown gave the following results for various biochemical tests: Blue citrate slant, yellow slant and butt for TSI with cracks in the medium, yellow urea slant, yellow phenol red lactose broth with gas accumulation. What is the probable identity of this bacterium? Choose all that apply.

- A. *Escherichia coli*.
- B. *Proteus miribalis*.
- C. *Pseudomonas aeruginosa*.
- D. *Enterobacter aerogenes*.
- E. *Serratia marcescens*.

30. Which of these bacteria is a strict aerobe? Choose all that apply.

- A. *Escherichia coli*.
- B. *Proteus miribalis*.
- C. *Pseudomonas aeruginosa*.
- D. *Enterobacter aerogenes*.
- E. *Serratia marcescens*.

Part 2: Experimental data and their interpretation (5 points/question)

EXERCISE 6.8: BACTERIAL METABOLISM - OXIDATION FERMENTATION

1. Indicate the following information for each of the unknowns:

- Type of metabolism of glucose (fermentative or oxidative) for each of the three unknowns.
- Type of metabolism of lactose (fermentative or oxidative) for each of the three unknowns.
- Oxygen requirements of each of the three unknowns (anaerobic, aerobic or facultative).

2. Indicate the Identity of each of the three unknowns (A, B and C).

EXERCISE 6.0-6.6: BIOCHEMICAL TESTS

3. According to your results, what are the identities of each of the bacterial unknowns (A, B, C, and D)?

EXERCISE 6.7: ENTEROPLURI TEST

4. Complete the following table for the unknown you were assigned (A, B, C, or D):

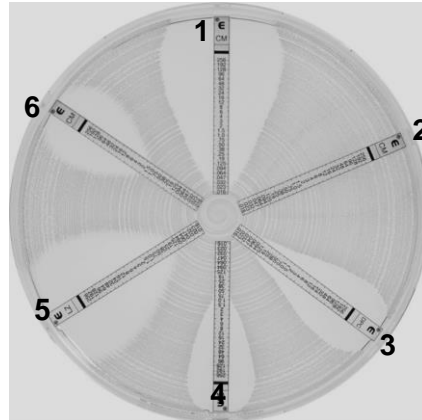
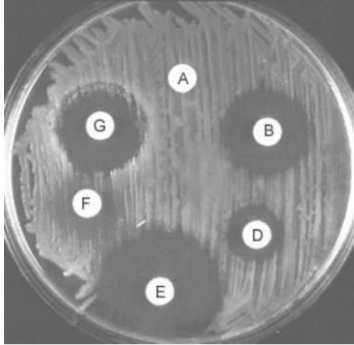
Unknown assigned:

	Group 1			Group 2			Group 3			Group 4			Group 5		
Test	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1
Positivity code															
Result															
Sum of the codes															
Numerical code															

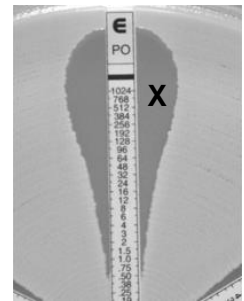
5. Indicate the enteropluri numerical code and the identity of each of the three unknowns (A, B, C, or D).

Assignment 5

Part 1: Problems and theoretical questions. You are not required to show your calculations. Only submit your final answers. Indicate your answers at two significant digits after the decimal. (2 points/question)



- According to the Kirby Bauer assay illustrated, which antibiotics have the highest and lowest MICs? **highest: A lowest: E**
- According to the E-test illustrated, which strip on the E-test corresponds to antibiotic “D” on the Kirby Bauer assay? **4**
- According to the E-test illustrated, what is the concentration of the antibiotic tested in the area indicated by the “X”?
 - Approximately 50 μ g/mL.
 - Greater than 50 μ g/mL.**
 - Less than 50 μ g/mL.
 - Approximately 1024 μ g/mL.
- The lower the _____, the better the antibiotic.
 - therapeutic index
 - toxic dose**
 - therapeutic dose
 - selective toxicity
 - action spectrum
- An antibiotic that targets which of the following would be expected to have the lowest selective toxicity?
 - Synthesis of bacterial DNA.
 - Synthesis of bacterial RNA.
 - Synthesis of bacterial cell walls.
 - Bacterial plasma membrane.**
 - Bacterial ribosomes.

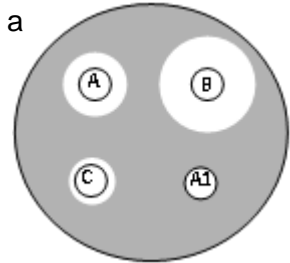


6. Which of the following is a synthetic antibiotic with a beta-lactam ring?
- A. Cephalosporin
 - B. Penicillin
 - C. Tetracycline
 - D. Chloramphenicol
 - E. Streptomycin
7. Which of these antibacterial drugs inhibit protein synthesis? (Choose all that apply)
- A. Aminoglycoside
 - B. Tetracycline
 - C. Ampicillin
 - D. Chloramphenicol
 - E. Erythromycin
8. Antibiotics that inhibit the synthesis of the bacterial cell wall are active only against what type of cells?
- A. Gram negative
 - B. Dormant
 - C. Actively growing
 - D. Endospores
 - E. Gram positive
9. Which of the following antibiotics would be effective in treating a fungal infection?
- A. Cephalosporin
 - B. Tetracycline
 - C. Chloramphenicol
 - D. Streptomycin
 - E. None of them
10. Which of the following antibiotics is dependent on osmotic pressure to kill the cells?
- A. Penicillin
 - B. Tetracycline
 - C. Chloramphenicol
 - D. Streptomycin
 - E. None of them
11. Which of these drugs has the highest selective toxicity?
- A. Cephalosporin
 - B. Tetracycline
 - C. Chloramphenicol
 - D. Streptomycin
 - E. Cyanide
12. Which of these drugs must penetrate the cell in order to be effective? (Choose all that apply)
- A. Cephalosporin
 - B. Tetracycline
 - C. Chloramphenicol
 - D. Penicillin

13. Lysozyme and penicillin are both antimicrobial compounds, which act on the cell wall. These compounds would be effective against what type of cells?

- A. Gram negative
- B. Actively growing
- C. Endospores
- D. Gram positive
- E. B and D

14. This image represents a Kirby Bauer assay comparing the sensitivity of a bacterium to different antibiotics. All these antibiotics are from a natural source except for "A1" which is a chemically modified version of "A". According to these results, what conclusion can be drawn about antibiotic "A1"?



- A. The modification reduces the MIC.
- B. The modification increases the MIC.
- C. The modification increases the MBC.
- D. The modification increases the action spectrum.
- E. None of the above

15. According to the results of the Kirby Bauer assay illustrated above, which antibiotic has the highest selective toxicity?

- A. A.
- B. A1.
- C. B.
- D. C.
- E. This conclusion cannot be drawn from these results.

16. If all these antibiotics have the same toxic dose, which one must have the lowest therapeutic index?

- A. A.
- B. A1.
- C. B.
- D. C.
- E. This conclusion cannot be drawn from these results.

17. A broth containing 1×10^9 cells of *E.coli* is autoclaved at 120°C for 5 minutes. How many cells will remain?

D_{110} of *E. coli*: 5.0 minutes

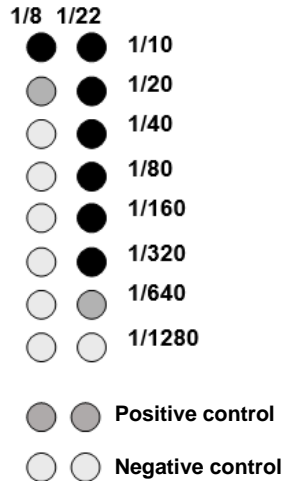
Z of *E. coli* = 10°C

1 X 10⁻¹ or 0.1 cells

18. Consider the information presented in the previous question. What is the death rate constant (k) at 120°C of the culture?

-4.61 cells/min.

An ELISA was done on the serum of a patient to detect the presence of antibodies (IgG) against HPV. Wells were coated with HPV and then treated with different dilutions of the patient's serum. A secondary enzyme conjugated antibody was then used for detection purposes. Serum samples were taken at two different dates over a two week interval. The concentration of antibodies for the positive control was $1\mu\text{g/mL}$.



19. What is the approximate anti-HPV IgG concentration for patient C as of 1/22?

- A. $1\mu\text{g/mL}$
- B. $640\mu\text{g/mL}$
- C. $320\mu\text{g/mL}$
- D. $160\mu\text{g/mL}$
- E. $250\mu\text{g/mL}$

20. Over the two week interval, the IgG concentration in patient C's serum increased by what factor?

- A. 8 Times
- B. 12 Times
- C. 16 Times
- D. 32 Times
- E. 64 Times

Part 2: Experimental data and their interpretation (5 points/question)

EXERCISE 8.0: KIRBY-BAUER DIFFUSION ASSAY

1. Submit a graph that shows the relationship between the antibiotic concentration and the log of the diameter of the zone of inhibition (mm). Your graph should include both bacteria assayed with the assigned antibiotic.

EXERCISE 8.2: DETERMINING THE THERAPEUTIC DOSE - MIC

2. Complete the table below:

- Determine the percent reduction of growth of each bacterial species obtained at each of the concentrations of each of the antibiotics. $((O.D. \text{ without antibiotic} - O.D. \text{ with antibiotic}) / O.D. \text{ without antibiotic}) \times 100$
- Determine the MIC ($\mu\text{g/mL}$) of each of the antibiotics for each of the bacterial species (the lowest concentration resulting in a reduction of at least 50%)
- Determine the therapeutic indices for each of the antibiotics for each of the bacterial species. (MIC /toxic dose)

Antibiotic	C_{\max} and C_{\min} ($\mu\text{g/mL}$)	LD50 ($\mu\text{g/mL}$)
Ampicillin	34 – 1.8	530
Kanamycin	12 – 2	400
Nalidixic acid	4.5 – 0.2	204
Erythromycin	8.3 – 2.7	460

Antibiotic	MIC ($\mu\text{g/mL}$)		Therapeutic indices	
	<i>S. faecalis</i>	<i>E.coli</i>	<i>S. faecalis</i>	<i>E.coli</i>
Ampicillin				
Kanamycin				
Naladixic acid				
Erythromycin				

3. According to the results presented, indicate in the table below whether each bacteria is sensitive, of intermediate sensitivity or resistant to each of the antibiotics.

Antibiotic	Sensitivity	
	<i>S. faecalis</i>	<i>E.coli</i>
Ampicillin		
Kanamycin		
Naladixic acid		
Erythromycin		

R : resistant; S : sensitive; IR : intermediate resistance

4. According to the data presented above which antibiotic (s) would be recommended to treat an infection by each of the bacteria. Justify your answer.

5. According to the data presented above, which antibiotic has the broadest action spectrum? Justify your answer.

EXERCISE 8.3: SENSITIVITY OF YEAST TO A DISINFECTANT

6. Submit a graph that shows the death profile of yeast as a function of time to the disinfectant. Express viability as the log of the number of viable cells/mL.
7. According to the data presented previously, what is the D value of the disinfectant at the assigned temperature? Show your calculation.
8. According to the data presented previously, what is the death rate constant (k) of the disinfectant? Show your calculation.

EXERCISE 8.4: SENSITIVITY OF YEAST TO HEAT

9. Indicate the decimal reduction time (D value) at the temperature you were assigned. Show your calculations.
10. According to the data presented previously, what is the approximate z value? Show your calculation. **Omit; give points to everyone.**

EXERCISE 8.5: ELISA OF OVALBUMIN

11. Submit a standard curve showing the relationship between the known ovalbumin concentrations assayed and the absorbance reading obtained.
12. According to the data presented previously, what is the approximate concentration in the commercial egg white preparation? Show your calculation.