2024 UT Austin Invitational

Microbe Mission B/C Exam

Instructions and Scoring:

- **THIS IS A CLASS SET. DO NOT WRITE ON THE EXAM.**
- You will have 50 minutes for this exam. You may bring one 8.5” x 11” cheat-sheet and two non-graphing calculators. Calculators that can compute logarithms will be useful, though not necessary.
- There are a total of 100 numbered questions and **371** points available on the exam. Point values for questions are written in (parentheses) after the question statement.
- The six sections are roughly ordered by increasing difficulty, but the questions in each section are not. You *should* skip around the test - it’s OK not to finish!
- There are 17 multiple true-false questions on this exam, worth 6 points each.
    - Division B competitors will be awarded 1 point per correct response.
    - Division C competitors will be awarded 6 points for 6 correct responses, 4 points for 4 or 5 correct responses, 2 points for 2 or 3 correct responses, and no points otherwise.
- **You will be awarded 1 point for every long answer question you leave blank.** Long answer questions have word limits, and answers which exceed the word limit will not be graded.
- Ties will be broken by scores on sections 6, 5, 4, 3, 2, and 1, in that order.

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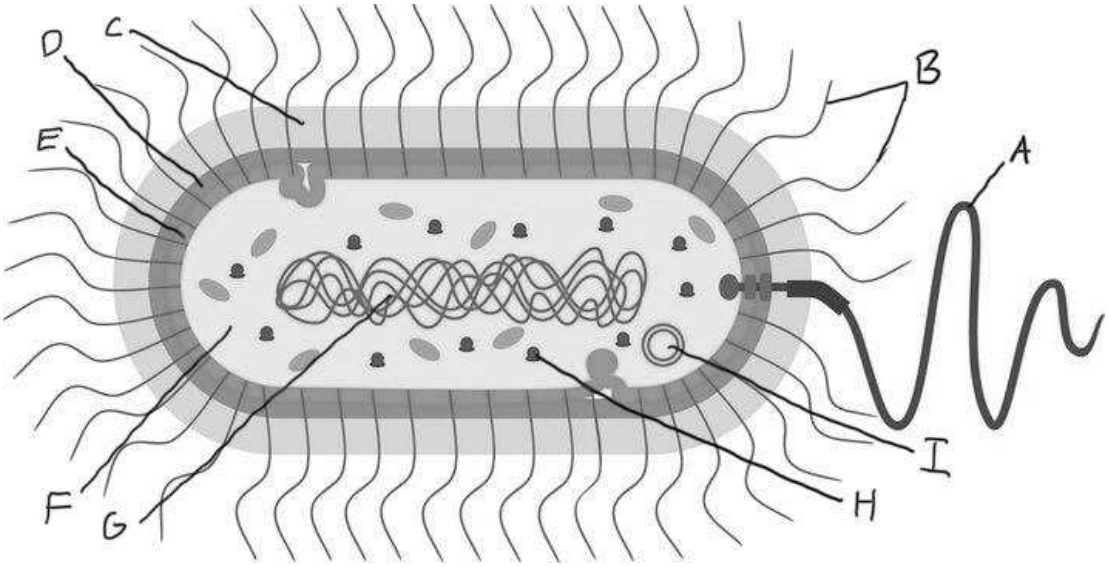
(Any questions after the conclusion of this event may be emailed to abora@mit.edu)

Section 1
Life Lessons (60)



1) Is the cell above an animal cell, a plant cell, or a bacterial cell? (1)
   A. Animal Cell
   B. Plant Cell
   C. Bacterial Cell

2) Label the parts of the cell pictured above on your answer sheet! (9)

3) Assign each of the following 10 parts of the cell to the most appropriate part of the venn diagram on your answer sheet. (10)

   A. Cell Wall
   B. Centriole
   C. Chloroplasts
   D. DNA
   E. Cillia
   F. Mitochondria
   G. Nucleoid
   H. Nucleus
   I. Plasma Membrane
   J. Ribosomes

4) Sketch a simple picture of the shape of the bacteria *vibrio cholerae* (no need to draw any internal structure). What is the genus of this bacteria? What disease does this bacteria cause in humans? (4)

5) Sketch a simple picture of the shape of the bacteria *bacillus anthracis* (no need to draw any internal structure). What is the genus of this bacteria? What disease does this bacteria cause in humans? (4)

Please DO NOT write on this exam!

6) Both of the above microbes were discovered by which German scientist, sometimes called the father of bacteriology? (2)

A. Leeuwenhoek
B. Koch
C. Mahler
D. Richter

7) Escherichia virus lambda is a virus that infects bacteria. What is the specific name for viruses that infect bacteria? (2)

8) Viruses are … (1)

A. Living
B. Nonliving

9) Bacteria are … (1)

A. Living
B. Nonliving

Escherichia virus lambda has a very interesting way of choosing what stage of the viral replication cycle it follows. It relies on two proteins, cro and cI, binding to sequences in DNA which control the production of specific proteins.

10) When cro is bound, the virus makes many copies of itself and bursts open the host cell. What stage is this? (2)

A. Lytic
B. Lysic
C. Lysogenic
D. Lytogenic

11) When cI is bound, the virus is latent, and the cell is growing. What stage is this? (2)

A. Lytic
B. Lysic
C. Lysogenic
D. Lytogenic

12) From the information above, Is this virus temperate or virulent? (2)

A. Temperate
B. Virulent

13) Cro and cI are repressors. What part of an operon would repressors bind to? (2)

A. Promoter
B. Operator
C. Exon
D. Terminator

14) Suppose that a virus copies some genetic material from some bacteria and inserts it into another one. What form of horizontal gene transfer has occurred? (2)

A. Conjugation
B. Transduction
C. Transformation

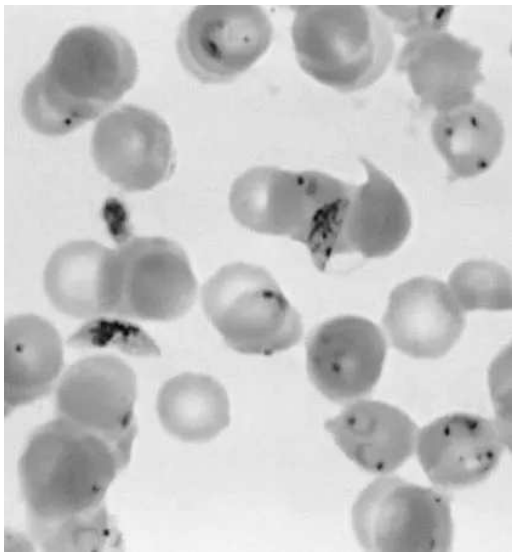
15) Suppose that a bacteria's plasma membrane is weakened by the presence of calcium chloride, causing the bacteria to take up DNA from the external environment. What form of horizontal gene transfer has occurred? (2)

A. Conjugation
B. Transduction
C. Transformation

16) Frederick Griffith discovered what form of horizontal gene transfer? (2)

A. Conjugation
B. Transduction
C. Transformation

17) What is the scientific name of the apicomplexan (from the microbe list) that is present in the blood smear to the right? (4)



18) This organism is responsible for what dangerous human disease commonly associated with mosquitoes? (4)

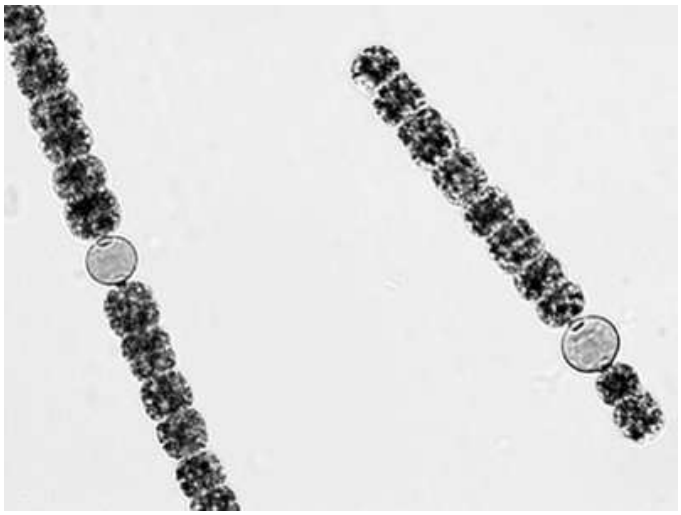
19) Human hepatocytes play a key role in this organism's life cycle. In what human organ are hepatocytes found? (2)

20) This organism is a parasite which has two hosts - humans and mosquitoes. What is the genus of the mosquitoes which transmit this disease? (2)

Section 2

Lean Mean Green Machine (43)

Dr. Ene Ockamat, a leading marine ecologist, has encountered the organism pictured on the next page, Anabaena, in his research. Anabaena is a cyanobacteria, a photosynthesizing prokaryote.



1) What pigment is primarily responsible for the green color in *Anabaena*? Be specific for full credit. (2)

2) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. *Anabaena* is an obligate anaerobe.
2. *Anabaena* is a facultative anaerobe.
3. *Anabaena* is an obligate aerobe.
4. *Anabaena* is a photoautotroph.
5. *Anabaena* is a photoheterotroph.
6. *Anabaena* is a chemoheterotroph.

Dr. Ockamat tells you that the two bigger cells in the image above are called heterocysts. Heterocysts are cells specialized for nitrogen fixation.

3) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. Oxidative photosynthesis provides usable energy sources to organisms.
2. Nitrogen fixation consumes organisms' energy reserves.
3. Nitrites and Nitrates are the end-products of nitrogen fixation.
4. Nitrosomonas and Nitrobacter are types of bacteria that perform the same step in the nitrogen cycle as Heterocysts.
5. The heterocysts have thicker cell walls than the other bacteria pictured.
6. Heterocysts have a lower concentration of the pigment from question 1 in this section than the other cells in the picture.

A key difficulty of performing nitrogen fixation is that the enzyme that performs nitrogen fixation functions best in cells with very low concentrations of oxygen.

4) What metabolic process in *Anabaena* and many other organisms (including humans) results in the <u>decrease</u> of oxygen concentrations in a cell over time and produces usable energy for the cell? (1)

5) What is the name for the enzyme/class of enzymes which catalyze the reduction of diatomic nitrogen (N_2)? (3)

For species like Anabaena that perform photosynthesis, it can be a challenge to create an oxygen-free environment.

6) How many photosystems are involved in the process of photosynthesis? (1)

7) Which photosystem catalyzes the splitting of water and production of oxygen? (1)

8) What is the name for the process in which one extracts energy from light WITHOUT using the photosystem in the above question? Does this process result in the production of oxygen? (3)

Anabaena employs an interesting strategy to solve this problem. Some individuals develop into heterocysts, which focus primarily on nitrogen fixation, while the rest of the cells focus on photosynthesis. Then, the two different types of cells in the population "trade" resources, so every cell can satisfy its metabolic needs.

9) What general class of macromolecules are imported into Heterocysts from the other cells in the greatest quantities? (2)

A) Carbohydrates
B) Lipids
C) Proteins
D) Nucleic Acids

10) Nitrogen fixed in heterocysts is transported to the other cells in the form of what monomers? Hint: it is easier to transfer more stable monomers than less stable ones. (2)

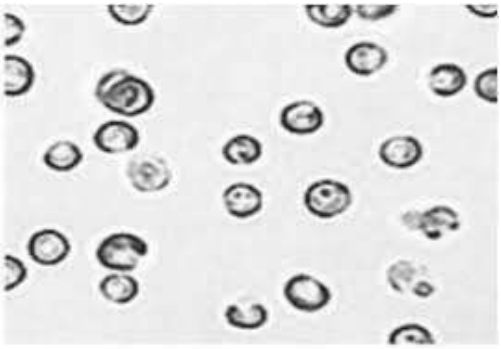
A) Simple Sugars
B) Fatty Acids
C) Amino Acids
D) Nucleotides

11) The relation between the heterocysts and the other *Anabaena* cells is an example of what ecological interaction? (2)

A) Mutualism
B) Commensalism
C) Parasitism
D) Protocooperation

Dr. Ockamat is intrigued by Anabaena's unique symbiotic relationships. In order to find similar organisms to study, he sequences the 16S ribosomal RNA of Anabaena and compares it to that of organisms on the microbe list. When he does so, he is extremely surprised to find a close match to a Eukaryotic species!!

12) Why would Dr. Ockamat initially be surprised at finding a close match between the 16S ribosomal RNA sequence in a prokaryotic species and the rRNA of a eukaryotic species? (4)



The particular Eukaryotic species he finds a match to is shown to the left.

13) What is the **genus** of this species? (4)

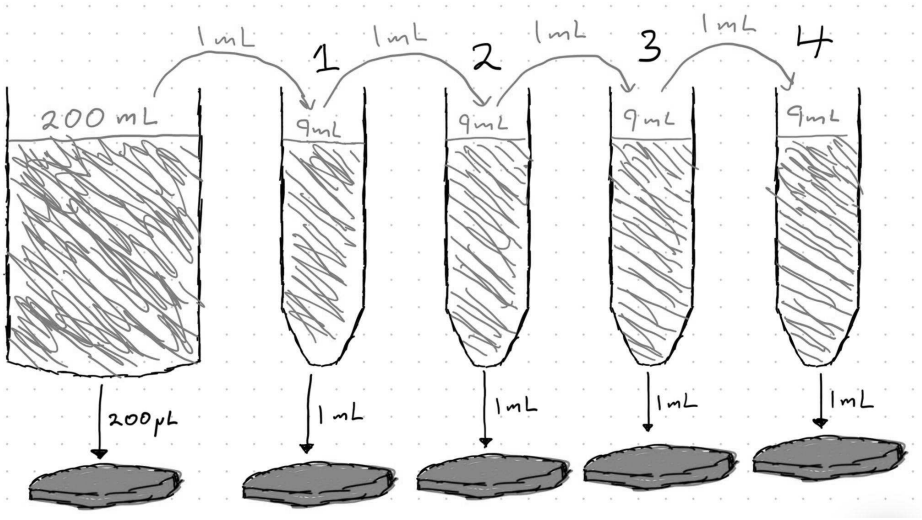
14) What particular organelle in this species is most likely responsible for its green color? (2)

15) Explain why Dr. Ockamat found such a close match between the ribosomal RNA of *Anabaena* and this species. (4)

Section 3

(My Concentration is) Falling Off (56)

*Minecraft youtuber Sill Bun is rather concerned about the *culture* of his followers. After seeing some troubling online videos regarding people emerging from toilets, he has decided to test his local bathroom to determine whether it has safe levels of the dangerous microbe P. Esports, which is known to induce *neurological decay*.*



In the image above, the extracted sample (containing P. Esports) is the 200 mL in the leftmost flask. The concentration of a solution is the number of bacteria/CFUs present per milliliter.

All the test tubes are initially filled with 9 mL of pure, distilled water (no bacteria).

His experimental procedure is as follows:

1. *First, transfer 1 mL of solution from the 200 mL solution to the test tube marked "1".*
2. *Repeatedly extract a small volume of solution from test tube "1" and dispense it back into this vial.*
3. *Transfer 1 mL of the solution in test tube 1 to the petri dish (red, agar plate), and spread it evenly across the surface.*
4. *Transfer 1 mL of the solution in test tube 1 to test tube 2.*
5. *Repeat steps 2-4 for the rest of the test tubes.*

1) Between which steps (1&2, 2&3, 3&4, 4&2) would it be important to change one's pipette tip? HINT: You don't want to contaminate a solution that has a low concentration of bacteria with a pipette tip that has a high concentration of bacteria. (4)

2) Which steps (1-4) change the concentration of the solution in vial 1? (4)

3) What is the name of the procedure Sill is executing? (4)

4) What is the purpose of step 2? (4)

Sill needs to make sure he has the correct lab equipment at hand, and knows how and when to use them. Match the following instruments to their name, function and best intended purpose in this lab. Use ONLY CAPITAL LETTERS on your answer sheet.

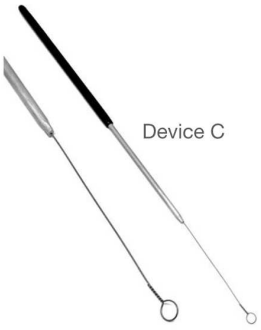
Choices:

A. Micropipette
B. Serological Pipette
C. Inoculating Loop
D. Cell Spreader

E. Transfer precise, small amounts of liquids
F. Transfer larger amounts of liquids
G. Spread solutions evenly on plates.
H. Extract single bacterial colonies

I. All Steps
J. Only step 3
K. Fill the initial 9 mL of water in each test tube
L. Not used at all in the lab.


Device A


Device C

Please DO NOT write on this exam!

5) The device in image A is a _____ and is generally used to _____. In this procedure, it will be used in _____. (3)

6) The device in image B is a _____ and is generally used to _____. In this procedure, it will be used in _____. (3)

7) The device in image C is a _____ and is generally used to _____. In this procedure, it will be used in _____. (3)

8) The device in image D is a _____ and is generally used to _____. In this procedure, it will be used in _____. (3)

9) Calculate the ratio between the concentration of bacteria in vial 4 and the initial beaker. (3)

10) Calculate the ratio between the expected number of bacterial colonies on plate 4 and the expected number of colonies on the leftmost plate. (5)

After performing his procedure and incubating the bacteria for some time, Sill counts 37 colonies on plate 3.

11) Calculate the concentration of bacteria in CFUs/mL in the original sample. (5)

12) What does CFU stand for? (1)

13) How many colonies would you expect Sill to see on the other plates? Why would Sill choose to report the count of this particular plate as opposed to other ones? (6)

After arriving at a count of the bacteria in his sample, Sill is now highly concerned. He thinks P. Esports is about as detrimental to human health as some strains of E. Coli.

14) According to the WHO, what is the recommended limit on the concentration of E. Coli in drinking water? (2)

A) 0 CFUs/100 mL
B) 1 CFU/100 mL
C) 10 CFUs/100 mL
D) 100 CFUs/100 mL

Now aware of the serious problem at hand, Sill needs to find out more about how fast P. Esports grows! This requires being able to quickly count the number of bacteria in particular samples.

15) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. When Sill plates a small solution on agar, he will need to incubate his plate before he can start to see colonies of bacteria forming.
2. For the purposes of counting, 1 colony on the plate would be treated as 1 CFU.
3. An optical density measurement is proportional to the concentration of microbes in solution.
4. The reading on an optical density measurement is proportional to ten to the power of the number of microbes in the solution.
5. It would be appropriate to measure the optical density of a solution of E. Coli using light with a wavelength of 600 nm.
6. Removing 10 mL of solution from the 200mL flask, assuming that it is evenly mixed, will NOT change the measured optical density.

[Note: some answers from section 3 may be useful in completing section 4]

Section 4
A Cultured Individual (52)

Now that he understands how to count bacteria, Sill needs to figure out how to grow them. He needs to decide between culturing his P. Esports on an agar plate and culturing it in a liquid medium.

Below is the composition of a MacKonkey agar plate (MAC):

- *Peptone – 17 g*
- *Proteose peptone – 3 g*
- *Lactose – 10 g*
- *Bile salts – 1.5 g*
- *Sodium chloride – 5 g*
- *Neutral red – 0.03 g*
- *Crystal violet – 0.001 g*
- *Agar – 13.5 g*
- *Water – 1 liter; adjust pH to ~7.0*
- *Sodium taurocholate - 0.05%*

HINT: Neutral red is a dye that turns pink in acidic solutions and yellow in basic solutions.

1) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. MAC is a defined medium.
2. Crystal violet binds to and inhibits the growth of gram positive bacteria.
3. This is a selective medium.
4. Lactose is the only nutrient source in the plate.
5. Bacteria which ferment lactose will turn the plate pink.
6. This is a differential medium.

Below is the composition of LB broth, the liquid medium Sill is considering:

- *Tryptone - 10 g*
- *Yeast Extract - 5 g*
- *Sodium chloride - 10 g*
- *Water - 1 liter; adjust pH to ~7.0 with NaOH*

(Autoclave the mixture for 25 min at 120°C before use).

2) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. This is a defined medium.
2. The pH of this medium is expected to fluctuate significantly once bacteria are introduced because NaOH is not a buffer.
3. This is a selective medium.
4. LB broth performs exceptionally in culturing E. Coli bacteria.
5. The purpose of autoclaving the mixture is to get rid of any bacteria that may have been introduced into it during the preparation process.
6. This is a differential medium.

Now, Sill can assess the growth of P. Esports. He takes a small sample from the 200mL beaker from before and adds it to a solution of LB broth in a test tube. For this part of the lab experiment, Sill will use an optical density measurement to assess the number of bacteria in the tube over time.

***Note that Sill does something unusual for this experiment** - he keeps adding new nutrients to the broth and filters out toxic waste products from it.*

3) What phase of the bacterial growth curve do you expect to be absent under these conditions? (2)

Sill completes his experiment and records the following data:

Time (min)	Optical Density (OD)
1	0.02
3	0.05
5	0.2
7	0.7
9	0.9
11	0.9
13	0.9
15	0.8

Sill tests the original 200 mL solution and finds that it has an optical density of 0.4. Use either your calculated value from problem #11 of Section 2 or the conversion below to plot the bacterial growth curve below (remember to check what the axes are).

> ***Conversion****: If you are unsure about your answer to Section 3, #11, you may use the conversion that 2,000 bacteria (CFUs) per milliliter corresponds to an OD of 0.4 for up to 75% partial credit.*

(For those of you who may not have brought an appropriate calculator, it may be helpful to know that log(2) = 0.3, log(5) = 0.7, log(7) = 0.85, log(8) = 0.9, log(9) = 0.95)

4) Plot the data from the table above, then draw a curve of best fit (note the axes!). (12)

5) Estimate the doubling time of *P. Esports* at its maximum growth rate. Compare this value to typical bacterial doubling times. (3)

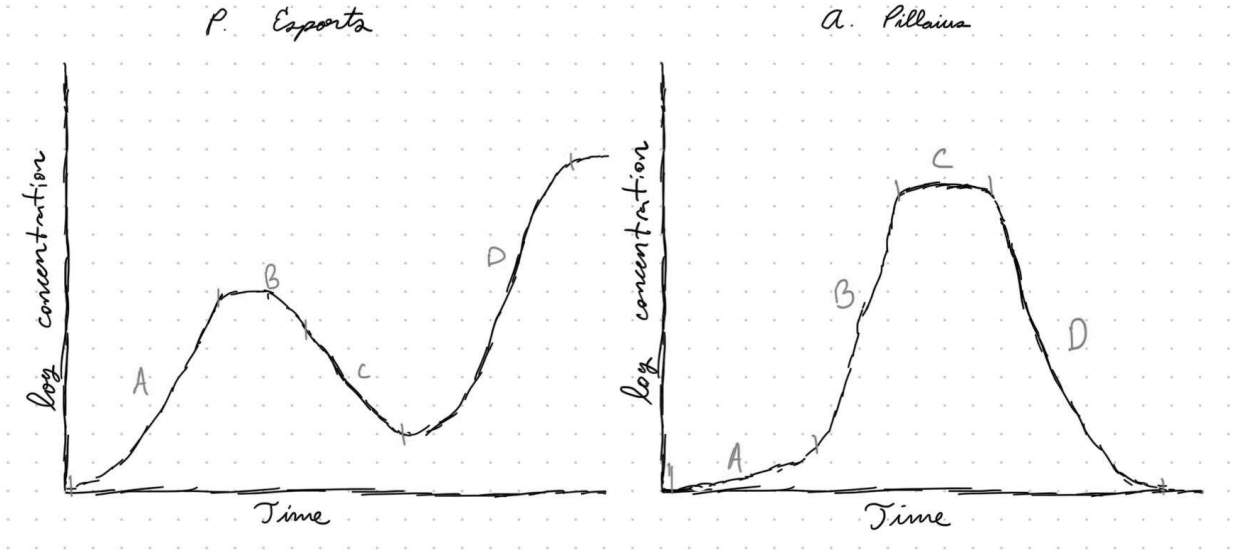
Sill notices that something is off with the above graph. At some point in time T, his lab assistant Baryan No-aura must have accidentally added a high concentration of an antibiotic!

6) Given that the antibiotic was added exactly after an even number of minutes had passed, what is T, the time when the antibiotic was added? (2)

7) Was the antibiotic bacteriostatic or bactericidal? (1)

Sill then added a small sample of a new microbe, A. pillaius, to this colony. This microbe has a plasmid containing resistance genes to the antibiotic Sill added, but is less efficient in exploiting the nutrient source in the broth than P. Esports.

The growth curves for P. Esports and A. pillaius in the broth are shown below.



8) Label the parts of the growth curve for the *A. Pillaius*. (4)

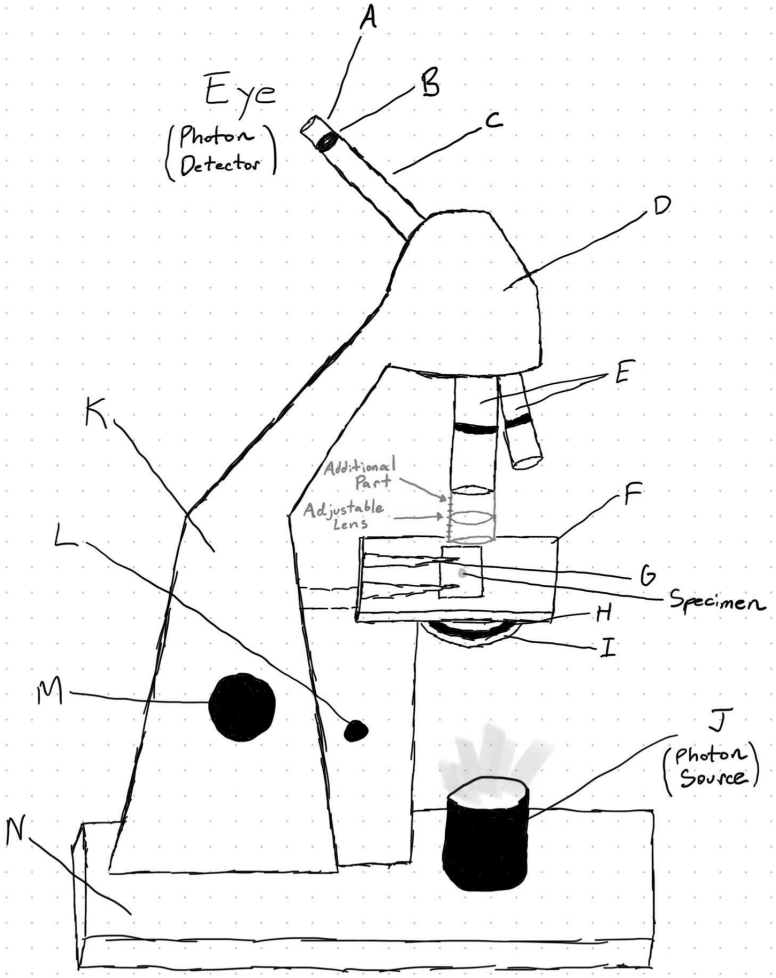
9) Give 2 examples of community interactions that are demonstrated between *P. Esports* and *A. Pillaius*. Explain (4).

10) Give reasoning for why the growth curves of *P. Esports* and *A. Pillaius* look like they do. Be sure to explain what triggers the changes in concentrations of the microbes in each stage. Note that Sill adds the antibiotic from question #6 at some point in time. Indicate what events trigger the changes between stages. (12)

Section 5

Super?? Resolution Microscopy (53)

Legendary Professor Chavid Den has long been known as one of the world's greatest experts in cryptography, aviation engineering, and forensic science. However, Chavid is also one of the world's leading experts in microscopy. Help the professor analyze one of their new prototype microscopes!



Chavid's work centers around light microscopy, which has some unique advantages over electron microscopy.

1) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. Light microscopy has a higher resolution than electron microscopy.

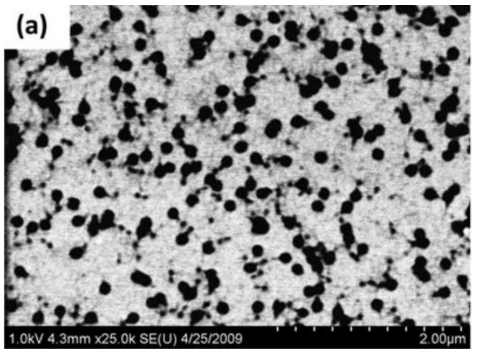
2. Sample preparation for light microscopy is usually faster than sample preparation for electron microscopy.
3. Light microscopy can be used to visualize live specimens, while electron microscopy cannot.
4. Electron microscopy is much more capable of generating 3D images of specimens than light microscopy.
5. The vast majority of dyes and dyeing procedures in electron microscopy are lethal to specimens, while the vast majority of dyes and dyeing procedures used in light microscopy are not lethal.
6. The majority of dyes used in electron microscopy can also be used effectively in light microscopy.

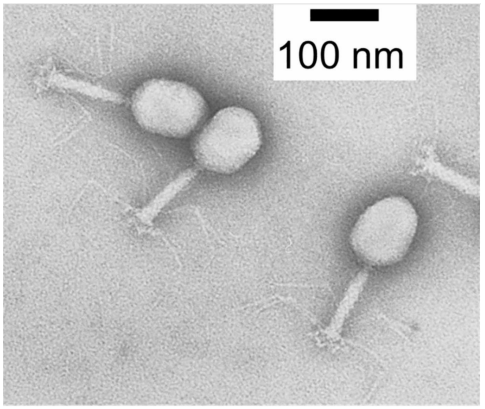
2) Identify the parts of the microscope in Professor Den's sketch of the microscope above. Ignore the red and blue parts of the sketch. Provide a more specific name than "photon source" for the component labeled "J". (7)

Note that in traditional light microscopy, the specimen is located between the photon source (part J) and the photon detector (one's eye), as is pictured in Prof. Den's sketch.

3) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. In TEM, the electron detector is located in between the electron source and the specimen.
2. In SEM, the specimen is located in between the electron source and the electron detector.
3. The wavelength of a photon in Light Microscopy is typically shorter than the De Broglie wavelength of an electron used in electron microscopy.
4. The images at right depict T4 Phages.
5. The top image at right is from a SEM, while the lower image is from a TEM.
6. The lower image is about 5000 times more magnified than the upper image.





4) Calculate the volume, in mL, of ONE individual pictured in the pictures to the right. Answers within a factor of 100 of the true answer will get full credit, and partial credit will be awarded only if work is shown. (4)

Professor Den is impressed by the magnification of the images above. However, they think that they can do even better with his light microscope. Chavid notices that the objective lenses on his microscope are labeled 4x, 10x, 40x, and 100x.

5) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. Higher magnification objectives are usually shorter than lower magnification objective lenses.
2. The objective which states “4x” actually magnifies the image by a factor of 40.
3. Most modern microscopes have a coaxial fine adjustment and coarse adjustment knob (coaxial means that the knobs rotate around the same axis)
4. If one uses microscope oil immersion, it is important to apply the oil to the 100x objective lens before turning it directly at the stage as otherwise it could contaminate the specimen.
5. Kohler illumination is a more recent technique then Critical/Nelsonian illumination, and results in very even lighting of samples, which can increase contrast.
6. Decreasing the numerical aperture of an objective lens increases the theoretical limit of its resolution.

Professor Den has come up with a brilliant new idea to increase resolution! They will attempt to directly increase the magnification of their light microscope by attaching a new, adjustable objective to the previous 40x objective (red/blue parts on diagram)!

6) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. If Professor Den’s adjustable objective magnifies the image by a factor of 20, the combined 40x/adjustable objective lens will have the same magnification as a 60x objective.
2. There is precisely one location of the adjustable lens for which the stage is a focal plane (i.e., the image will only be perfectly “in focus” for one location of the lens)
3. If Chavid took multiple pictures of the specimen through the microscope, each with slightly varied positions of the adjustable lens, deconvolution software could be used to create a much clearer image of the specimen.
4. If Chavid looked at a specimen moving up and to the right under an unmodified light microscope, it would appear to be moving down and to the left.
5. If Chavid looked at a specimen moving up and to the right through his modified light microscope, it would appear to be moving down and to the left.

6. If Chavid looked at a specimen moving up and to the right through a stereo microscope, it would appear to be moving down and to the left.

7) Explain why or why not an electron microscope would generate an inverted image. (3)

8) Describe THREE (theoretical) benefits of an “adjustable objective lens” as described above, and explain how modern light microscopes can provide similar benefits. (6)

Professor Den adopts a new microscopy technique, and sees the image below:

9) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. The image was taken through Phase Contrast Microscopy.
2. The image was taken through Differential Interference Contrast Microscopy.
3. Differences in density are used to emphasize contrast in the image.
4. The microscopy technique pictured can be used to visualize live cells.
5. The microbe pictured is a eukaryote.
6. The microbe pictured can exist in both haploid and diploid forms.

10) Describe 3 modifications Chavid could have made to their microscope to produce the above image. Feel free to name *single parts* of the microscope that can be added/swapped out. (3)

Section 6 (106)

It’s the resistance!

Intro biochemistry Teacher's Assistant (TA) Roon has got fed up with ID events and wants to destroy all the microbes on earth before Bacteriology becomes a thing. However, she likes birds, insects, and trees, so she wants to minimize the effect of her antibiotics on eukaryotic cells. She first targets mechanisms of bacterial locomotion.

1) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. Eukaryotic flagella use sliding filaments to generate movement, while prokaryotic flagella use a rotary motor.
2. Eukaryotic flagella and motile cilia both have a 9+2 arrangement of microtubules.
3. The basal bodies of prokaryotic flagella have eight distinct ring-based layers.
4. Lophotrichous bacteria have all their flagella concentrated at a single spot on the bacterial surface
5. Peritrichous bacteria have flagella pointing in all directions.
6. *E. Coli* is a Lophotrichous bacteria.

She thinks that flagella are a better target for a medicine than cilia because some human cells also have motile cilia, making them potentially vulnerable to the drug!

2) Give an example of a human cell that has motile cilia. Hint: there are some examples among cells in the brain and lungs. (2)

From her extensive studies of biology, TA Roon remembers that there already exist natural immune mechanisms to target proteins in bacterial flagella! The green protein in the diagram to the right is Zebrafish Toll-Like Receptor 5.

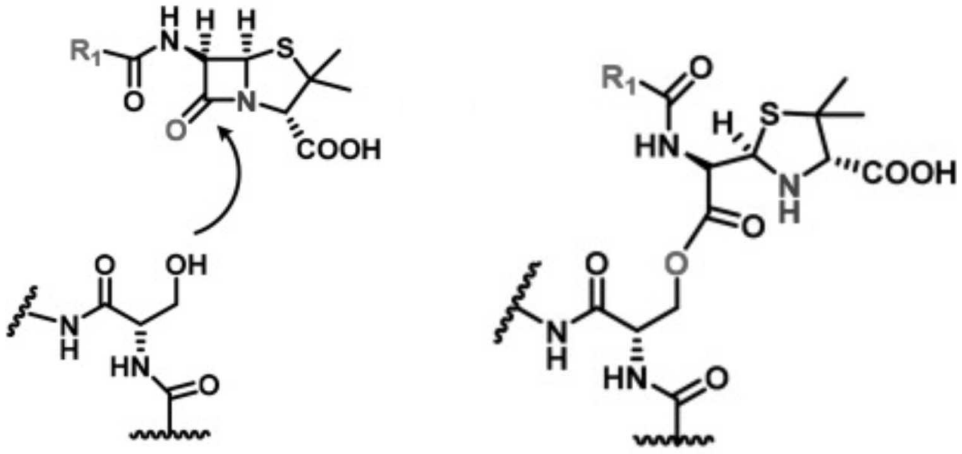
3) What is the name of the orange protein in the above diagram, which is a key part of bacterial flagella? (2)

4) What type of representation is used to depict the proteins in the diagram to the right? (1)

A) Wire model
B) Ribbon model
C) Ball-and-Stick model
D) Ramachandran Plot

5) The orange protein has a large number of what common secondary structural motif? (1)

Satisfied with the human body's innate ability to target key parts of bacterial locomotion, TA Roon turns her attention to other key differences between bacterial and eukaryotic cells and potential targets for his antibiotics. She starts off by considering penicillins and their mechanism of action.



In the above diagrams, the penicillin molecule is the one with two rings. When it binds to a particular amino acid on a particular protein (the other molecule in its images), its beta-lactam ring is destroyed.

6) What is the hybridization of the blue nitrogen atom in penicillin BEFORE binding to the amino acid (HINT: consider resonance structures)? (2)

7) The beta lactam ring is a cyclic form of what biologically important functional group? (1)

8) What enzyme/class of enzymes, critical for cell wall construction in gram-positive bacteria, is inactivated when it binds to penicillin? (4)

9) What is the name of the particular amino acid which binds to the penicillin molecule? (1)

10) Is the bond between the protein and the penicillin molecule covalent, ionic, or neither? (1)

11) Who discovered penicillin? What year did they get the nobel prize in? (2)

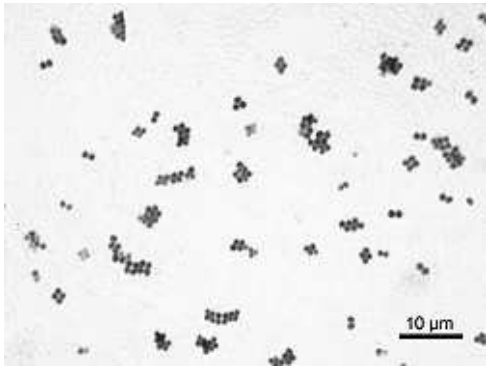
12) Modern biology labs use bioengineered yeast instead of bioengineered bacteria to produce penicillin, but bacteria are used to produce a wide variety of other compounds. Why do we use yeast instead of bacteria to produce penicillin? (4)

13) Is penicillin more effective against gram positive or gram negative bacteria? (1)

TA Roon wants to test whether penicillin would be effective against a particular bacteria which is a common human pathogen. She performs a gram stain on this bacteria!

14) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. Carbol Fuchsin is a common primary stain in gram staining.
2. Crystal Violet will be washed out of gram positive cell walls if a mordant is not added.
3. Acetone is a commonly used mordant in gram staining.
4. Gram staining is ineffective on bacteria without cell walls.
5. Safranin is a common counterstain used in gram staining.
6. Safranin will only stain gram negative cells, even if crystal violet is not applied first.



15) Estimate the field of view of the microscope image above. (4)

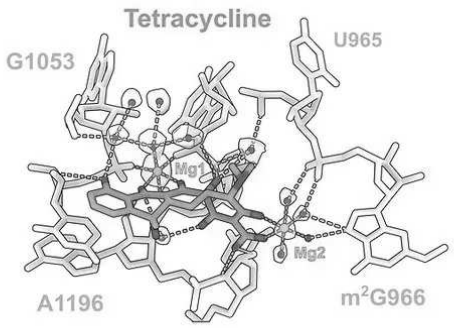
16) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. The bacteria pictured in the above image have a coccus shape.
2. The bacteria pictured in the above image are gram negative.
3. The bacteria pictured in the above image are Acid-Fast.
4. The bacteria pictured above are *S. Aureus*.
5. The bacteria pictured above are *M. Aeruginosa.*
6. The bacteria pictured above has a multi-layered cell wall which protects it from penicillin.

When applying penicillin V to a particular strain of this bacteria, TA Roon is disappointed to see that it has no effect. TA Roon then treats this strain of bacteria with Amoxicillin and Cephalexin, other Beta-lactams, and still sees no result!

17) What is the name for this particular strain of bacteria, a dangerous antibiotic-resistant human pathogen (feel free to abbreviate)? (4)

Now, TA Roon turns her attention to tetracyclines.

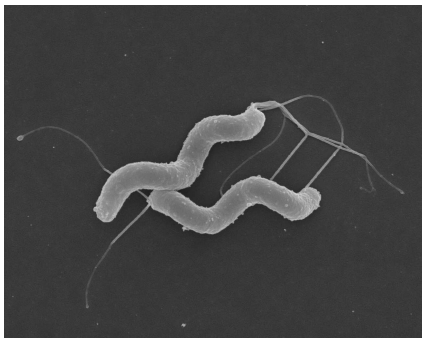


Tetracycline is an antibiotic that interferes with ribosomes! Consider the diagram of the tetracycline-ribosome interaction above.

18) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. The carbon backbone of tetracyclines are generally composed of four 6-membered rings
2. Tetracycline selectively binds to hydrophobic portions of proteins
3. Tetracycline selectively binds to DNA
4. Tetracycline requires cobalt cofactors for proper action
5. Post-transcriptional modifications like m^2G are unique to Eukaryotes and Archaea
6. m^2G modifications promote tRNA - Ribosome interactions

Nowadays, tetracycline has been used so much in practice that many bacteria are developing methods of antibiotic resistance to the drug, like the one pictured to the right!



19) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. A bacteria with a plasmid that codes for Tetracycline efflux proteins (proteins which pump Tetracycline outside of the cell) would likely survive when exposed to Tetracycline.
2. A bacteria in a mildly acidic environment (pH 4~6) would likely survive when exposed to Tetracycline.
3. A bacteria with a plasmid that codes for proteins which inhibit Tetracycline-ribosome binding would likely survive when exposed to Tetracycline.
4. The organism pictured on the previous page is a bacteria.
5. Highly basic environments aid the organism on the previous page in breaking down tetracycline.
6. The image of the organism on the previous page was taken with a Scanning Electron Microscope.

20) Some strains of the organism pictured above actually have mutations in their 16S rRNA which makes them resistant to tetracycline. Write out the scientific name of the organism pictured on the previous page. (4)

Specifically, bacteria with an AGA -> TTC mutation in nucleotides 926-928 have demonstrated resistance to Tetracycline.

In the early 2000s, researchers invented a Real-Time PCR screen for 16S rRNA mutations associated with the organism pictured on the previous page.

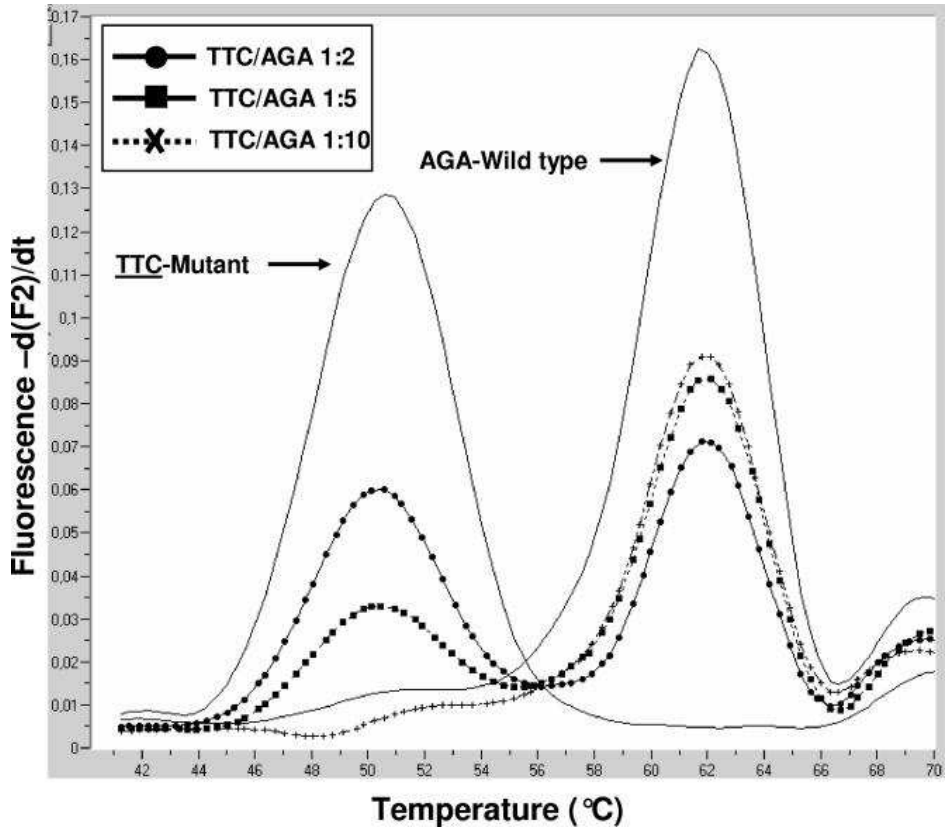
They designed the following hybridization probes to test for the presence of mutant strains. The probes only differ in the underlined section:

Probe 1: 5′-AAG GTT CTT CGT GTA TCT TCG-3′
Probe 2: 5′-AAG GTT CTT CGT GTA GAA TCG-3′

21) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. The minimum inhibitory concentration of tetracycline needed to prevent the growth of bacteria with the mutation is higher than the minimum inhibitory concentration needed to prevent the growth of wild-type bacteria.
2. PCR can be used on bacteria that are no longer alive, which is a major benefit over many other screening techniques.
3. PCR requires the design and use of RNA primers to be effective.
4. DNA polymerase from *Pyrococcus Furiosus* is more accurate when used in PCR than DNA polymerase from *Thermus Aquaticus*.
5. Probe 1 binds to mutant strains.
6. Probe 2 binds to wild-type strains.

To perform the screen, the researchers attached a fluorescent indicator to ONE of the probes, and let it bind to target sequences. Probes release fluorescent signals immediately <u>after they separate</u> from the target sequence. The below figure shows the fluorescence of the samples with the indicated ratios of mutant to wild-type DNA being tested.



22) Multiple Select: Identify whether each of the following statements are true 'T' or false 'F'. (6)

1. If a probe is an imperfect match to the DNA sequence it is binding to, it will have a lower force of attraction, and hence a lower melting point, than a probe that is a perfect match to the DNA sequence it is binding to.
2. The probe-wild type sequence interaction is weaker than the probe-mutant sequence interaction.
3. The probe used in this experiment is probe 2.
4. This test can detect the presence of mutants with high sensitivity if at least 50% of the bacteria in a particular sample have the mutant allele.
5. This test can detect the presence of mutants with high sensitivity if at least 20% of the bacteria in a particular sample have the mutant allele.
6. This test can detect the presence of mutants with high sensitivity if at least 5% of the bacteria in a particular sample have the mutant allele.

After all those PCR questions, TA Roon wants a break from DNA replication. She knows that very high concentrations of Nalidixic Acid will affect bacterial replication, but doesn't know exactly how it works. She knows that it inhibits some mystery protein crucial to bacterial genome replication. To figure out exactly which protein Nalidixic Acid inhibits, TA Roon uses cool techniques to identify the DNA sequences that the mystery protein binds to. She first thinks that the mystery protein may be DnaA.

23) Which of the following best describes the function of DnaA? (2)

A. Initiate genome replication
B. Relieve negative genomic supercoils
C. Transcribe DNA into RNA
D. Terminate genome replication

Fill in the blanks on your answer sheet:
24) DnaA binds to a specific sequence called the _____ ____ _____ (abbreviated DUE), (2)
25) which is located within the _____ ____ _____ (abbreviated oriC) (2)

TA Roon's cool techniques reveal that the mystery protein binds to sequences with very high GC content (lots of G-C base pairs).

26) Would you expect this region to be easier or harder to separate into two strands than the rest of the genome, on average? Does this support the conclusion that DnaA is the mystery protein? (4)

A. Easier, Yes
B. Harder, Yes
C. Easier, No
D. Harder, No

TA Roon figures out that the mystery protein has both ligase and nuclease activity, and that Nalidixic Acid selectively inhibits the ligase activity of this protein.

27) How would you expect the number of <u>double-strand breaks</u> in the genome to change as a result of Nalidixic Acid exposure? (2)

28) Is Nalidixic Acid bacteriostatic or bactericidal? (2)

TA Roon reminds you that this antibiotic selectively targets <u>bacterial</u> genome replication.

29) What is the mystery protein? (12)

30) Nalidixic Acid is an example of what bicyclic class of antibiotics? (4)

Congrats! You survived Microbe Mission B/C!

(Note the "Bonus" section on your answer sheet)

Please DO NOT write on this exam!