Microbiology 102 "Sample Final"
(Most questions are taken from a recent final exam.)

I. MULTIPLE TRUE/FALSE. In the blank by each statement, place a **+** for a **true** statement or a **O** for a **false** statement. There can be **any number** of **+** or **O** statements. Be sure to **indicate a clear response in each blank**, as "hybrid responses" will be marked incorrect. **Please do not change or qualify the wording of any statement in any way.** Each is either true or false as stated.

Example: Some things we made note of along the way:

__+__ No organism (large or small) "makes its own food" as is expressed all too often with regard to autotrophs. All organisms obtain nutrients appropriate for their kind from their environment.
__O__ The terms "media" and "bacteria" can be used either as plural or singular terms.
__+__ Agar does not contribute nutrients to any significant degree in a bacteriological medium and is important only as a solidification agent.

1. Regarding terminology related to plating bacteria:

_____ The terms "colony-forming unit" and "colony" mean <u>exactly</u> the same thing and can be used interchangeably.
_____ A colony-forming unit can be made up of one or more cells, while a colony is visible to the naked eye and can be made up of millions of cells.
_____ In the quantitation of bacteria, it is important to know the amount of medium in the petri dish.

2. A sample of lake water was diluted to **10^{-3}** (that is, 1/1000). <u>Then</u>, **0.1** ml of this dilution was plated, giving rise to 42 colonies after incubation.

_____ In this problem, when we plated 0.1 ml of the 10^{-3} dilution, it was equivalent to plating **1 ml** of a **10^{-4}** dilution of the same sample.
_____ In this problem, when we plated 0.1 ml of the 10^{-3} dilution, it was equivalent to plating **10^{-4} ml** of the undiluted sample.
_____ Theoretically, plating 1 ml (instead of 0.1 ml) of the 10^{-3} dilution would give rise to ten times as many colonies on the plate.
_____ To calculate the number of CFUs that were present per ml of the original undiluted lake water sample, one would multiply 42 by **10^{-4}**.

3. In performing the dilution plating procedure:

_____ One can prepare a 1/10 dilution in a variety of ways, but the proportion of the **amount to be diluted** to the **amount of diluent** is **always 1 to 9**.
_____ It is OK to have the dilution tubes uncapped and upright in the test tube rack while making transfers between them.
_____ In spreading the inocula over the surface of plates with a sterile hockey stick, one can start with the most dilute inoculum and move up to the more concentrated inoculum without having to re-sterilize the hockey stick.

4. We can determine the following from a properly-streaked plate (such as what is accomplished by our three-phase streaking procedure with the loop):

_____ Finding whether or not a supposedly-pure culture is contaminated.
_____ Isolation of the individual components of a mixed culture.
_____ Determination of colonial characteristics.
_____ The number of colonies that will help us determine the no. of CFUs per ml of the culture.

5. The following are good lab practices:

_____ Dropping the caps of the tubes to the table top when making transfers between the tubes.
_____ Making the thickest possible smear for the gram stain such that it can be easily focused upon.
_____ Having one person hold the tubes while another person makes transfers between them.
_____ Eating and drinking in the lab.
_____ **Always** discarding wet mounts and pipette tips into disinfectant and no where else.

6. Regarding bacterial growth and motility:

_____ When doing a colony count, it is OK to omit the weird-looking and very small colonies.
_____ The amount of cells in a given colony is important in calculating CFU/ml.
_____ Cloudiness (turbidity) in a broth medium is a sign of microbial growth.
_____ Bacterial flagella can be seen with our laboratory microscopes.

7. These are some things about a culture that we can learn from its Gram stain:

_____ colony characteristics
_____ Gram reaction
_____ shape of the cells

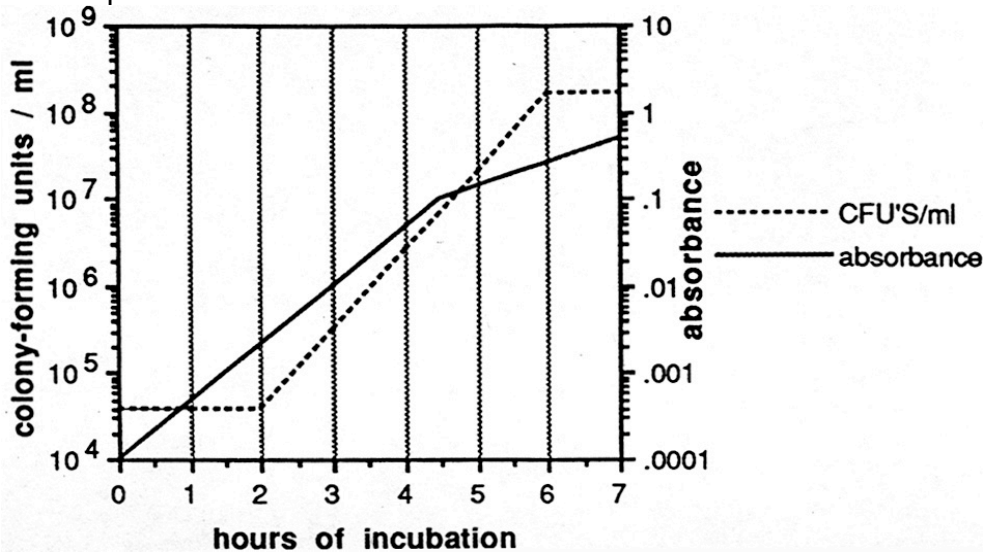
8. These are some ways that we can make a medium "selective" for certain kinds of bacteria:

_____ Only include essential nutrients that certain organisms can break down and utilize.
_____ Leave out an important element such as nitrogen.
_____ Add an antibiotic to an all-purpose medium.
_____ Add lactose to an all-purpose medium.

9. You are provided with a number of tubes of a melted all-purpose medium in a 50°C water bath for use in pouring plates.

_____ If you were to keep a tube out at room temperature too long and the medium solidifies, you can put the tube back in the 50°C water bath and the medium will be liquid again.
_____ After pouring the plates, it is best to keep the petri plate lids **off** so the medium will cool off and solidify faster.
_____ An all-purpose medium is defined as one that supports the growth of **all species** of bacteria.

10. Note the following growth curve of a typical organism such as the strain of *E. coli* we used in our growth curve experiment:

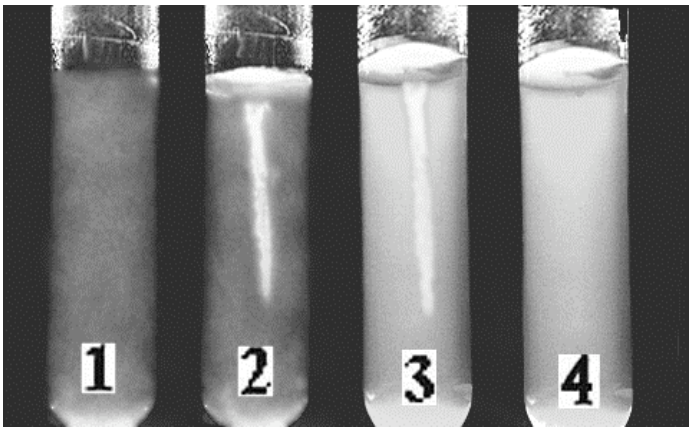


_____ The stationary phase begins at six hours.
_____ The exponential phase lasts 4.5 hours.
_____ One would determine the **generation time** from this graph by finding the time difference between $1X10^6$ CFUs/ml and $1X10^7$ CFUs/ml.

11. In Experiment 7, we studied some characteristics of twelve known species of bacteria, and we also identified an unknown. Furthermore we noticed that all of the organisms grew to a greater or lesser degree in all of the media and in the presence of air (i.e., with oxygen present).

_____ We can conclude that none of the organisms were strict anaerobes.
_____ Aseptic technique was not important when working with the known cultures as pure cultures were provided.
_____ Our best gram stains were made from cultures that had been incubating for a week.
_____ To identify a bacterial culture, we needed to include the CFUs/ml of a typical broth culture.

12. For the Motility Medium tubes shown on the right: Tube 1 is an uninoculated control, Tubes 2 and 3 show a visible "stab line," and Tubes 3 and 4 are both cloudy throughout the medium. Therefore, a **non-motile** organism would be indicated for which of the following tubes? (Disregard any growth on the surface.)



_____ Tube #2
_____ Tube #3
_____ Tube #4

13. *Escherichia coli* (i.e., good old *E. coli*) is characterized as being able to respire aerobically (with oxygen) and anaerobically (with nitrate), and – given a suitable organic substrate such as glucose – it can also ferment. It derives its energy only from chemical reactions (i.e., light is not involved) and it cannot use carbon dioxide as a source of carbon. From this description, we can say the following:

_____ *E. coli* should be able to grow anaerobically in a tube of an all-purpose liquid medium supplemented with glucose and/or nitrate.
_____ *E. coli* can be described as an organotroph as well as a heterotroph.
_____ This example shows that the terms organotroph and heterotroph mean exactly the same thing; one is synonymous with the other.
_____ Because of its ability to respire aerobically and also ferment, it would be considered a facultative anaerobe.

14. Bacteriophages

_____ are **viruses** which infect bacteria.
_____ are **bacteria** that act like viruses and infect humans.
_____ can form colonies which may appear indistinguishable from bacterial colonies.
_____ are to **plaque-forming units** as bacterial cells are to **colonies**.

15. In the isolation of *Streptomyces* from soil,

_____ Antibiotics were included in the isolation medium, as we expect *Streptomyces* to be resistant to all known antibiotics.
_____ "Penassay Agar" selected specifically for antibiotic-producing bacteria and inhibited others.
_____ When we picked colonies off of the initial isolation medium, we streaked onto an all-purpose medium, as we hoped to encourage the growth of any contaminants present in order to make them easily avoidable.
_____ We consider **antiobiotics** to be **bacteria** that inhibit certain other bacteria.

16. When looking for purple non-sulfur photosynthetic bacteria, we had to make use of certain medium and incubation requirements in order to isolate them most efficiently, as these organisms

_____ produce easily-distinguished pigmented colonies when the plates are incubated under anaerobic conditions and in the light.
_____ produce non-pigmented colonies if the plates are incubated under aerobic conditions, and the organisms could be indistinguishable from chemotrophs.
_____ may be overrun (crowded out) by respiring chemotrophs if the plates are incubated under aerobic conditions.
_____ may be overrun (crowded out) by fermenting chemotrophs if incubated under anaerobic conditions on a medium containing glucose as a carbon source.

17. Regarding our isolation procedure for *Bacillus* (which involved the heat-shocking process):

_____ We expected vegetative cells to **produce** endospores **during** the heat-shocking process.
_____ When we make endospore stains from colonies on our isolation plates, we should expect to see **no** vegetative cells, because if we did, we would think our heat-shocking technique was faulty or perhaps some contaminants got in.
_____ We expect to find relatively more vegetative cells of *Bacillus* in moist, nutrient-rich soil and relatively more endospores in a sample of the same soil which was allowed to dry out.
_____ We expect to see fewer endospores at the edge of a growing *Bacillus* colony than at the center.
_____ We incubated our plates under aerobic conditions in order to inhibit the strictly anaerobic endospore-formers.

18. Enrichment and isolation procedures for the "enterics"

_____ are aided by media which contain one or more selective agents that inhibit gram-positive bacteria.
_____ may involve media which contain **lactose** as the only fermentable sugar, even though many enterics do not ferment lactose.
_____ often results in the isolation of non-enterics such as *Pseudomonas*.

19. Among the requirements of a good "indicator organism" is/are the following:

_____ the ability to cause the problem being examined.
_____ the ease in which it is detected.
_____ the ability to remain in the contaminated environment indefinitely.

II. MATCHING. Place the letter of the **most appropriate item** from column **b** in the blank by each statement in column **a**. *Only one letter per blank.* **Any letter may be used only once!**

a	**b**
_____ Primary stain in the gram stain procedure.	**A**. 1000X
_____ A type of medium that purposely inhibits a certain type of organism.	**B**. 110X
_____ Term referring to the prevention of cross-contamination between the environment and the culture being studied.	**C**. agar
_____ Motility response of an organism to the presence or absence of light. (Hint: Recall our microscopic demonstration involving *Rhodospirillum*.)	**D**. amylase
_____ Component of media that acts as a solidifying agent.	**E**. aseptic technique
_____ A type of cell that can remain viable for many years and resist extremes of heat and cold, but cannot increase its numbers by dividing as does the usual type of bacterial cell.	**F**. broth
_____ Total magnification achieved with the use of a 10X ocular lens and a 100X objective lens.	**G**. chemotaxis
_____ Another term for a liquid medium.	**H**. conjugation
_____ Reagent which detects catalase.	**I**. crystal violet
_____ Reagent which detects the presence of starch.	**J**. differential
_____ Detects gas produced from fermentation or denitrification.	**K**. Durham tube
_____ Essential for determining whether fermentation occurred in a tube of Glucose Fermentation Broth.	**L**. endospore
_____ A dense film of bacteria growing over the surface of a plate.	**M**. growth curve
_____ A circular clearing in this film (above) of lysed bacterial cells.	**N**. hydrogen peroxide
_____ Process where a bacteriophage infects a bacterial cell and subsequent lysis of the cell occurs.	**O**. iodine
	P. lawn
	R. lytic cycle
	S. pH indicator
	T. phototaxis
	V. plaque
	W. selective
	X. sterilization

III. SHORT ANSWER.

1. MICROSCOPY-RELATED QUESTIONS.

   a. What is **resolution**?

   b. Why must **immersion oil** be used with the high-powered (100X) objective lens?

   c. What must be done to a dried bacterial smear before it can be stained? (Just two words are sufficient).

   d. Give one reason why a cell which has a gram-positive type of cell envelope may give a gram-negative staining reaction – other than a mistake made in the staining/decolorizing procedure.

   e. The proper kind of microscope to use for observing stained smears is (**circle one**):

      the phase-contrast microscope the microscope found in the cabinets

2. VIRTUAL EXPERIMENTS. Answer any three of the following questions.

   a. In the Antibiotic Disc Sensitivity Test, a **zone of inhibition** around a disc of Streptomycin is seen to contain lots of small colonies in the otherwise clear zone. What do these colonies represent (they are not contaminants!), and would it be OK to utilize Streptomycin to treat an individual who has an infection caused by the organism growing on the plate?

   b. How can we prove whether or not an **isolate** obtained in the "Enrichment & Isolation of Nitrogen-Fixing Bacteria" experiment can actually utilize atmospheric nitrogen (N_2) as its source of nitrogen?

   c. What is the **Slide Agglutination Test**, and how can it be used to identify a specific kind of *Salmonella*?

   d. What is **Brownian motion**, and is it an example of true motility?

   e. Briefly explain **phage-typing**, and how it can be used to help us identify bacteria?

   f. Explain **Koch's Postulates**.

   g. What does **acid-fast** mean, and what are mycolic acids?

   h. What is a **siderophore**, and how does it help concerning bacterial nutrition?

   i. Distinguish between **mutation** and **recombination**.

3. DEFINITIONS. **Briefly** define each of the following four terms. *Please do not give an example or analogy as your only answer*. Examples are not required, and incomplete sentences are OK. **Please do not go beyond the definition with additional descriptive information** – for example, we are not asking about source of electrons (reducing power) for the first term.

   a. photoheterotroph

   b. bacillus (This is the **general** term; do not describe the genus. Just 3 words are sufficient.)

   c. colony-forming unit

   d. extracellular enzyme

4. CIRCLE THE CORRECT CHOICE

   a. When we stoppered our photosynthetic enrichments and poured mineral oil over our Decarboxylase Broth (in the enteric experiment), we expected anaerobic conditions to be achieved immediately / by aerobically-respiring organisms / by anaerobically-respiring organisms.

   b. One always incubates petri dishes in a(n) inverted / non-inverted position.

   c. As a rule, one should study the colony characteristics of an organism by looking through the bottom of the plate / on the top surface of the medium.

   d. Motility Medium is always inoculated with the loop / needle.

   e. The medium bacteriophages utilize for replication is the host culture / top agar / bottom agar.

5. Using numbers (1-4; 1 is most important), what priorities do you place on these tests when identifying unknown organisms.
   _____ Determination of lactose and amylase reactions.
   _____ Determination of catalase and glucose reactions.
   _____ Determination of generation time for a typical broth culture.
   _____ Determination of gram reaction and morphology.

6. Using numbers (1-4; 1 is first), indicate the order of a typical enrichment/isolation procedure.
   _____ Streak for isolated colonies on the appropriate plating medium.
   _____ Inoculate isolates onto slants of an all-purpose medium.
   _____ Inoculate sample into appropriate broth medium.
   _____ Run tests for identification.

7. SEMI-PRACTICAL. **Use the table below this question to answer the following:**
You are given an unknown from a very limited list of possible organisms, and you perform several tests. You find out that it is Gram-positive and non-motile. When you inoculate it into Glucose Fermentation Broth, you observe a color change due to an acidic reaction throughout much of the tube with no generation of gas. The same result is seen with Lactose Fermentation Broth.

a. Based only on the results listed above, list three possible genera for your isolate.

b. As you try to further narrow down the genus of your isolate, why would it not be helpful to inoculate starch agar?

c. You remember from your gram stain that your isolate has spherical-shaped cells. Which one of the three possible genera does this eliminate?

d. You next perform the catalase test and discover your isolate is positive. What is the identity of your isolate?

TABLE OF POSSIBILITIES FOR THE ABOVE QUESTION

Genus	Gram Reaction	Cellular Shape	Motility	Catalase	Glucose Fermentation	Lactose Fermentation	Breakdown of Starch
Amylobacillus	+	rod	+	+	A	–	+
Enterococcus	+	coccus	–	–	A	A	–
Staphylococcus	+	coccus	–	+	A	A	–
Lactobacillus	+	rod	–	–	A	A	–
Klebsiella	–	rod	–	+	AG	AG	–

A=acid; AG=acid and gas

IV. PROBLEMS.

1. Five ml of sample were added to 14 ml of diluent. The dilution thus made can be expressed as (circle one):

1/5 5/14 5/19 1/100

2. You are interested in obtaining bacteriophages from sewage which infect *E. coli* strain B. You are given a 10^{-2} dilution of filtered sludge from the sewage treatment plant, and you add 0.1 ml of this dilution to a tube containing about 4.5 ml of melted Top Agar. To this mixture you add a few drops of host culture. You then dump the entire contents of the Top Agar tube onto a plate of Bottom Agar. After incubation, you count 91 plaques on the plate.

Determine the number of plaque-forming units (PFUs) **per ml** of the **undiluted** sewage sample. (Be sure to **show your work** so we can follow your calculations.)

3. In another phage isolation experiment – this time using lake water – you find 180 plaques on a plate that was inoculated with **0.1 ml** of the lake water sample. Therefore, there were _____ PFUs/ml of the sample.