Bacteriology 102
Final Lab Exam
May 12, 2002

score: /100

NAME _____

I. MULTIPLE TRUE/FALSE (44 points). 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. *Do not change or qualify the wording of any statement in any way.* Each is either true or false as stated. (1/2 point for each blank.)

1. Regarding the terms **cells**, **colony-forming units** and **colonies**:

_____ A "colony-forming unit" (CFU) is **microscopic** and can be made up of one or more cells.
_____ A "colony" is visible to the naked eye and arises from a "colony-forming unit" during the incubation of the plate**.**
_____ The terms "colony-forming unit" and "colony" can always be used interchangeably.
_____ In the quantitation of bacteria, it is important to know the number of cells in the average-sized colony.
_____ Endospores can be "colony-forming units."
_____ A "plaque" is the bacteriophage equivalent of a "colony-forming unit."

2. A sample of hamburger was diluted to $\mathbf{10^{-2}}$ (that is, 1/100). **One ml** of this dilution was plated, and **300 colonies** were counted after incubation.

_____ When one prepares a 1/100 dilution of a sample, there is always a ratio of 1 part of sample to 100 parts of diluent.
_____ To determine the number of CFUs which had been present **per gram** of the undiluted hamburger sample, one must multiply 300 by $\mathbf{10^{2}}$, rather than by $\mathbf{10^{-2}}$.
_____ Theoretically, plating one-tenth ml (instead of one ml) of the 10^{-2} dilution would give rise to **30** colonies.
_____ Plating one ml of a 10^{-2} dilution of the hamburger is theoretically equivalent to plating one-tenth ml of a 10^{-1} dilution of the same sample.
_____ Plating one ml of a 10^{-2} dilution of the hamburger is theoretically equivalent to plating 10^{-2} gram (i.e., 0.01 gram) of the undiluted hamburger.

3. The following are consistent with good "aseptic technique":

_____ Having one person hold the tubes while another person makes transfers between them.
_____ Leaving culture tubes unplugged and upright in the test tube rack while making inoculations between them.
_____ Placing the top lids of petri dishes on the table top while making transfers or observing colonies.
_____ Touching the wire of a recently-flame-sterilized inoculating loop to your hand to check if it is cool enough to touch the loop to the inoculum.
_____ Pouring plates from a bottle of melted agar medium which was not wiped off after being taken from the water bath, as we expect the water in a 50°C water bath to be sterile.
_____ Doing the slide catalase test on an uncovered slide and then observing it closely for bubbles.

4. The capsule

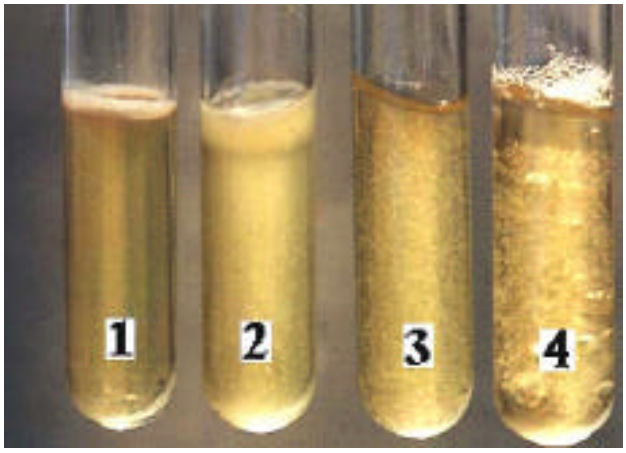
_____ can inhibit proper decolorization of a gram-negative cell in the gram-staining procedure.
_____ is an integral layer of the cell envelope of both gram-positive and gram-negative bacteria.
_____ takes up the India ink in the "capsule stain" we did in lab and is thus stained a dark color.

5. Cells of *Mycobacterium* are termed "**acid-fast**" organisms.

_____ "Acid-**fast**" refers to the **rapid production of acid** from the fermentation of sugars.
_____ Cells successfully stained with carbol fuchsin hold the stain "fast" and are therefore **not decolorized** when treated with the acidic decolorizing agent.
_____ The principles underlying the gram staining procedure (why it works the way it does) are the same as those for the acid-fast staining procedure.

total negative points for page:

6. A test we did in Experiment 5.1 involved the use of Thioglycollate Medium (shown at right):



_____ This test is designed to determine the "oxygen relationship" of chemoheterotrophic bacteria that can grow well in this medium.

_____ An organism which can grow anaerobically in the medium is one which can obtain energy by the process of **anaerobic respiration**.

_____ An organism capable of **aerobic respiration** would be able to grow in the presence of oxygen and also give a catalase-positive reaction.

_____ If the organism shown in Tube #1 can reduce nitrate, and if we were to add nitrate to the medium, we would expect the organism to grow throughout the tube instead of just on top (as shown).

7. In two experiments involving bacterial quantitation early in the semester, you were provided with a number of tubes of melted Plate Count Agar (PCA) in a 50°C water bath for use in preparing plates by the "pour-plate" method.

_____ Plate Count Agar is an example of a medium that can support the growth of all known kinds and types of bacteria.

_____ We would expect (theoretically) to obtain the **same number** of colonies on our plates if the tubes each contained **twice** as much medium.

_____ If you were to keep a tube out at room temperature too long and the medium solidifies, you can put it back into the 50°C water bath and the medium will become 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.

8. For growth curves in general:

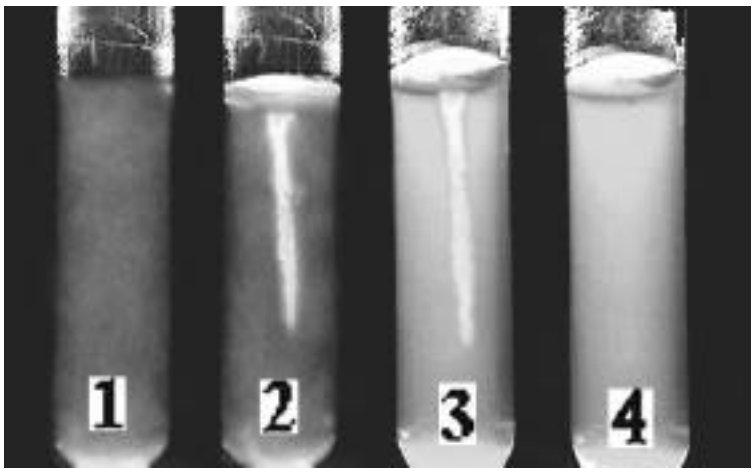
_____ One plots the CFUs/ml for each time point and then draws a line such that all of the dots are **on the line** and are thus "connected."

_____ The growth curve will always be the **same** for any particular species of bacteria, no matter what medium or incubation conditions we use.

_____ If a culture doubles its population every 15 minutes, the **growth rate** is therefore four generations per hour.

_____ If an actively-growing culture has 1.0 X 10^6 CFUs/ml at 8:00 AM and 4.0 X 10^6 CFUs/ml at 9:00 AM, the **generation time** is 30 minutes.

9. For the Motility Medium tubes shown at right: Note that Tube #1 is an uninoculated control, #2 and #3 show a visible "stab line," and #3 and #4 are both cloudy throughout the medium. Therefore, the following tubes are considered "positive" for motility:



_____ Tube #2

_____ Tube #3

_____ Tube #4

10. The **genotype** possessed by a cell can be changed by

_____ mutation.

_____ recombination.

_____ altering environmental conditions in which the organisms are being incubated (temperature, oxygen availability, etc.).

total negative points for page:

11. To isolate a certain kind of organism from a natural source, using the enrichment and isolation principles we learned about in our experiments:

_____ It is best to utilize an **all-purpose medium** to isolate as many different kinds of bacteria as possible. Then we can study all of the colonies obtained and choose which ones we want to continue with.
_____ We always wish to compare our results to a **general key** to find out if we isolated the correct strains and got the correct determination of CFUs per gram of sample.
_____ We always try to **duplicate the original habitat** of the organisms in the laboratory as much as possible.
_____ We expect each different kind of colony on a plate to represent a **different genus**.

12. Selective media include the following:

_____ Blood Agar, as only **hemolytic** bacteria will grow on Blood Agar.
_____ A medium in which all carbon compounds are excluded.
_____ A medium which allows us to differentiate between glucose fermenters and those which do not ferment glucose.

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

_____ Organisms without extracellular enzymes would find it difficult or impossible to grow and produce colonies on the initial isolation medium we used.
_____ "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 obtain pure isolates or (at least) isolates that could be easily distinguished and separated from any contaminants.
_____ *Streptomyces* is an antibiotic.

14. Regarding antibiotics:

_____ For an organism to be considered "susceptible" to a particular antibiotic in the **antibiotic disc sensitivity test**, the zone of inhibition seen around a disc of the antibiotic can be of any size – just as long as some inhibition is seen on the plate.
_____ An antibiotic-resistant organism is **always** a mutant, as all bacteria are normally antibiotic-sensitive.
_____ Antibiotics can be used as **selective agents** in bacteriological media.
_____ Antibiotics and antibodies are the same thing; the two terms are interchangeable.
_____ Antibiotics **are bacteria** that inhibit certain other bacteria.

15. 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

_____ do not utilize sulfur for any purpose.
_____ 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 would unfortunately be virtually 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.

16. When we heat-shock a suspension of soil, we expect

_____ immediate production of endospores by vegetative cells of *Bacillus*.
_____ to kill reproductive spores of such soil organisms as *Streptomyces* and molds.
_____ to kill vegetative cells of non-endosporeforming microorganisms.
_____ to kill vegetative cells of *Bacillus*.

total negative points for page:

17. You are studying a gram stain of a black colony obtained from a plate of Vogel-Johnson Agar, and your partner decided to make a wet mount of it. After finishing observations, you and your partner both discard your slides into the red bucket on the stage (designated for broken glass and stained smears). After awhile, you decide to retrieve your slide and – while looking for it in the red bucket – you cut your hand on the cover slip that came off your partner's wet mount. The next day you have a nasty infection on your hand.

_____ This episode helps to confirm the rule that wet mounts must be discarded **only** into the disinfectant and **not** into the red bucket with the stained smears.
_____ **Any** black colony on Vogel-Johnson Agar must be *Staphylococcus aureus*, and further steps to identify it would really be unnecessary.
_____ Just doing a **catalase** test would confirm a black colony on Vogel-Johnson Agar to be *Staphylococcus aureus.*

18. In the production of fermented milk products, such as yogurt and the "pseudo-yogurt" we made in lab:

_____ We can use **any** species of lactic acid bacteria, as they **all** ferment lactose.
_____ We depend on bacteria already present in the milk to participate in the production.
_____ **Slime** produced by the organisms was the reason for the solid consistency of the product.
_____ We noticed in our titrations that more base (NaOH) was used to neutralize the milk after incubation than before.

19. Regarding the group of organisms known as the lactic acid bacteria (the "lactics"):

_____ They are all strict anaerobes.
_____ They are gram-positive rods and cocci that form substantial amounts of lactic acid from fermentation of sugars.
_____ They include the enteric group of bacteria.
_____ All lactic acid bacteria ferment lactose and produce slime from sucrose.

20. Regarding the group of organisms known as the enteric bacteria (the "enterics"):

_____ They are found only in the intestinal tract.
_____ They are all facultative anaerobes.
_____ They all ferment glucose and lactose.
_____ The group includes *Neisseria* and *Pseudomonas*.
_____ The group includes *Salmonella* and *E. coli*.

21. 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 *Pseudomonas*.

22. 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.

REMINDER: Be sure you indicated **+** for true and **O** for false. Graders only look at these answers and do not consider any modifications or additions you may have written.

total negative points for page:

II. MATCHING (14 points).

1. Place the letter of the best, most appropriate item from column **b** in each blank by each statement in column **a**. *Only one letter per blank.* Any letter may be used any number of times or not at all. Some statements in column **a** have more than one blank. (One-half point for each blank.)

a	**b**
____ ____ **Two** processes that are important in determining an organism's "oxygen relationship."	**A**. aerobic respiration
____ ____ **Two** processes which are possessed by the purple non-sulfur photosynthetic bacteria. We used a test for these which was similar to the test we did in Experiment 5.1 for "oxygen relationships."	**B**. anaerobic respiration
____ ____ ____ **Three** processes associated with **anaerobic** growth.	**C**. fermentation
____ The process responsible for creating anaerobic conditions in our photosynthetic enrichments and also in the enteric identification media which were overlayed with mineral oil.	**D**. oxygenic phototrophy
____ Process not dealt with in any of our Experiments in 102.	**E**. anoxygenic phototrophy
____ Process associated with nitrate reduction.	

2. Place the letter of the best, most appropriate organism from column **b** in each blank by each organism in column **a**. *Only one letter per blank.* **Any letter may be used <u>only once</u>**. (One-half point for each blank.

a	**b**
____ We incubated our *Bacillus* isolation plates under aerobic conditions in order to inhibit this anaerobic endospore former.	**A**. *Mycobacterium*
____ Gram-negative coccus.	**B**. *Neisseria*
____ Aerobic nitrogen-fixer.	**C**. *Escherichia coli*
____ Genus of acid-fast bacteria.	**D**. *Azotobacter*
____ Many species produce antibiotics.	**E**. *Clostridium*
____ Primary example of a fecal coliform.	**F**. *Streptomyces*

3. Place the letter of the best, most appropriate item from column **b** in each blank by each statement in column **a**. *Only one letter per blank.* Any letter may be used any number of times or not at all. (One-half point for each blank.)

a	**b**
____ We must not forget to include **this** in a tube of Glucose Fermentation Broth if we intend to detect whether or not an organism can ferment glucose.	**A**. amylase
____ Detects insoluble gas produced in fermentation or denitrification.	**B**. autotroph
____ Addition of iodine detects the presence of **this** in Starch Agar.	**C**. blood
____ Extracellular enzyme that coagulates plasma.	**D**. coagulase
____ A structure or activity in a cell with which an antibiotic can interfere.	**E**. differential
____ Organism which obtains carbon only from carbon dioxide (CO_2) is **defined** as being **this**.	**F**. Durham tube
____ Organic compounds required in small amounts by certain organisms which are unable to synthesize them.	**G**. growth factors
____ These are required by bacteria and tend to be present in media ingredients as chemical contaminants, so one usually does not need to consider adding them when making media.	**H**. heterotroph
____ Medium formulated purposely to inhibit a certain type of organism.	**I**. lithotroph
____ Growth of an isolate on a slant of Nitrogen-Free Agar and also on a slant of an all-purpose medium indicate this type of organism.	**J**. nitrate-reducer
____ A specific type of *Salmonella* based on antigen-antibody tests.	**K**. nitrogen-fixer
____ Chemical released by a cell into the environment to assist in iron uptake.	**L**. pH indicator
	M. selective
	N. serotype (serovar)
	O. siderophore
	P. species
	R. starch
	S. target site
	T. trace elements

total negative points for page:

III. SHORT ANSWER (25 points).

1. (2 points) Give **one reason** why a cell which has a gram-positive type of cell envelope may give a gram-negative staining reaction – other than too much alcohol-acetone was used or some other error in technique. (Saying "gram-variability" is too general; please give a further explanation.)

2. DEFINITIONS (12 points) Briefly and effectively **define four of the following five terms.** (If you answer all five, we can only then grade the first four.) **Place an X by the term not defined.** *Please do not give only an example or analogy as a definition.*

   a. bacillus (the **general term**; not the genus *Bacillus*)

   b. antibiotic

   c. starter culture

   d. chemoorganotroph (Note the 2 parts to this word and the fact that we are not asking about carbon source in this definition.)

   e. any bacteriological term of your choice that you feel has been under-represented in this exam.

tube in light tube in dark

3. (2 points) The same organism was inoculated into these two Succinate Agar tubes. Note that there is surface growth in each tube, and the tube on the left has heavy, red-pigmented anaerobic growth. The **best term** to describe this organism would be facultative anaerobe / facultative phototroph / succinate fermenter (circle one). The red color is due to photosynthetic pigments / the reaction of a pH indicator / contamination by *Serratia* (circle one).

4. (2 points) With numbers (1-4; 1 is the most important), list the priorities of the following procedures with regard to the identification of bacterial cultures:

   _____ Determination of glucose fermentation and catalase reactions.
   _____ Determination of the generation time of a typical culture.
   _____ Determination of gram reaction and the shape and arrangement of the cells.
   _____ Determination of lactose fermentation and amylase reactions.

total negative points for page:

5. (2 points) With numbers (1-4; 1 is the "best"), arrange the following media according to how you think they would support the growth of a variety of different bacteria.

_____ MacConkey Agar
_____ MacConkey Agar + sodium azide
_____ Heart Infusion Agar
_____ Heart Infusion Agar + yeast extract

6. (2 points) With numbers (1-4; 1 is the first), arrange the following procedures which are involved in the isolation and identification of enterics:

_____ Inoculate source material into the selective enrichment medium.
_____ Streak plate for isolated colonies.
_____ Inoculate Kligler Iron Agar (KIA) tubes with pure cultures.
_____ Eliminate any tubes which show the obvious presence of non-enterics (such as *Pseudomonas*) and run a number of different biochemical tests on the various cultures.

7. (3 points) You have cultures of five organisms as listed below. However, the labels of the tubes have come off and you need to re-label the tubes correctly! First, you consider the various reactions you know for the organisms in question:

genus	gram reaction	shape	catalase reaction	glucose fermentation	lactose fermentation	phenylalanine deaminase	citrate utilization
Bacillus	+	rod	+	+ or –	**?**	**?**	**?**
Staphylococcus	+	coccus	+	+	**?**	**?**	**?**
Enterobacter	–	rod	+	+	+	–	+
Morganella	–	rod	+	+	–	+	–
Pseudomonas	–	rod	+	–	–	–	**?**

a. The results obtained from **what <u>one</u> specific laboratory procedure** will differentiate *Bacillus* and *Staphylococcus* from each other and also from the remaining three genera?

b. The remaining three genera can <u>each</u> be distinguished by the result of <u>one</u> test. Indicate a test (<u>just the test</u>, not the reaction) which will give the distinguishing reaction for each of the organisms:

Enterobacter _____

Morganella _____

Pseudomonas _____

IV. PROBLEMS, PROBLEMS, PROBLEMS (17 points).

1. (2 points) The **same dilution** can be obtained in **each** of the following situations:

a. The addition of 1 ml of a sample to 4 ml of sterile diluent.

b. The addition of _____ ml of the same sample to 44 ml of diluent.

c. The addition of 10 ml of the same sample to _____ ml of diluent.

2. (1 point) The inoculation of 1 ml of a 10^{-3} dilution of hamburger is theoretically equivalent to the inoculation of _____ gram(s) of the undiluted hamburger.

total negative points for page:

3. A sample of lake water was diluted and inoculated into plates of **Plate Count Agar** (PCA) and tubes of **Lactose Lauryl Tryptose Broth** (with Durham tubes). After incubation, the results were obtained as indicated on the following table. (Note: **Each** of the three columns of results on this table shows the observations for **two plates** and **three tubes**.)

dilution **of lake water**		10^0	10^{-2}	10^{-2}
amount inoculated		0.1 ml	1.0 ml	0.1 ml
colony count on PCA		too numerous	88 & 84	9 & 6
# of tubes showing	growth	3	2	2
	gas bubble	3	2	1

a. (3 points) Determine the number of CFUs **per ml** of the lake water.

b. (2 points) Determine the presumptive, most probable no. of coliforms **per ml** of the lake water.

M I N I M P N T A B L E

number of positive tubes			MPN per inoculum of middle tubes
first set	middle set	last set	
3	3	3	>24
3	3	2	11.0
3	3	1	4.6
3	3	0	2.4
3	2	2	2.1
3	2	1	1.5
3	2	0	0.93
3	1	1	0.75
3	1	0	0.43
2	2	1	0.28
2	2	0	0.21
2	1	0	0.15
2	0	0	0.091
1	1	0	0.073
1	0	0	0.036
0	0	0	<0.036

4. (1 point) Consider this situation: 20 Hfr cells are mixed with 20 F^- cells, and 8 pairs of Hfr and F^- cells undergo conjugation. If 2 F^- cells each incorporate DNA from an Hfr cell into its chromosome, the "recombination frequency" would then be: (Circle the letter of the one correct choice.)

A. 40% **C**. 10%

B. 20% **D**. 5%

5. (3 points) Exactly **one-half** of a **100-gram chunk of cheese** was mixed with 450 ml of sterile diluent, and the other half was placed in the refrigerator for future consumption. Two successive 1/100 dilutions were then made. From the last (most dilute) dilution, one-tenth ml was plated onto each of 2 plates of PCA. After incubation, 48 colonies were counted on one plate and 52 were counted on the other.

Calculate the number of CFUs we expect to be present in the **entire, other half** of the chunk of cheese which is still in the refrigerator.

6. You are given two strains of *E. coli* – strain "A" and "B" – and a 5 ml tube of the phage P1. Given these materials, you proceed to determine the concentration of phage in the tube by the following methods:

First you make **eight consecutive dilutions** of the original phage stock, adding 1 ml of the previous dilution to 9 ml of sterile diluent. Then, you take cultures of both strains of *E. coli*, each grown to a density of 1.0 X 10^9 cfu/ml. You add 0.1ml of your final phage dilution to 0.1ml of each of the two *E. coli* cultures in separate tubes of melted top agar. Then you plate the top agar tubes, each onto a separate plate of PCA, an all-purpose medium. You incubate both plates overnight at 37°C.

The next day, you discover that there are no plaques on the plate with *E. coli* A, but there are 79 plaques on the plate with *E. coli* B.

a. (1 point) What is a likely reason there are no plaques on the plate where *E. coli* A was added? Assume there was no problems with experimental technique.

b. (2 points) What is the concentration of the original phage stock, i.e. the concentration in the original tube of P1?

c. (2 points) Predict what a plate would look like if **no** *E. coli* cells were added to the top agar, and explain why you predict this.

V. EXTRA CREDIT (2 points).

1. You isolated an enteric from beautiful Lake Splammo, and your Kligler Iron Agar culture of the isolate has a **red (alkaline) slant** and a **yellow (acidic) butt** after one day of incubation at 37°C. To begin the identification process, you consider the reactions on the following table:

KEY TO GRAM-NEGATIVE RODS COMMONLY FOUND IN L. SPLAMMO

TEST	coliforms	*Shigella*	*Morganella*	*Providencia*	*Proteus*	*Citrobacter*	*Pseudomonas*
glucose fermentation	+	+	+	+	+	+	–
lactose fermentation	+	–	–	–	–	–	–
mannitol fermentation	+	+	–	–	–	+	–
H_2S production	–	–	–	–	+	+	–
ornithine decarboxy-lation	±	–	+	–	±	±	–

a. From the results of just the pH-related reactions, you know the isolate would not be identified as _____ or _____ , and you can also see that the isolate is positive / negative (circle one) for H_2S production.

b. Your lab partner has been experimenting with differential media and has **added a somewhat large amount of mannitol and ornithine** to her KIA formulation, and she also got the **same result** with the same isolate. According to the table, the organism would be identified as this genus: _____ .

total negative points for page:

total bonus (+) points for page: