SELECTED QUESTIONS FROM PREVIOUS MICRO 102 QUIZZES
PART II – EXPERIMENTS 8 THROUGH 17

For the following questions, the numbers refer to the applicable experiments. Questions with blanks are multiple true-false questions unless otherwise indicated; these are generally answered + for true and – for false.

8A. Consider an organism which has undergone mutation such that it is resistant to an antibiotic while normal cells of the same species are sensitive to the antibiotic.

_____ The antibiotic must have caused the mutation.
_____ The target site for the antibiotic may have been altered such that the antibiotic is no longer effective at that site.
_____ The cell membrane may have been altered such that the antibiotic is restricted entry into the cell.

8B. Conjugation and recombination

_____ refer to two separate events, one following the other.
_____ result in the potential for up to half of a population of cells to undergo a genotypic change, if the population is made up of equal numbers of "donor" and "recipient" cells.
_____ will probably lead to no detectable recombinants, if the donor and recipient cells are otherwise genetically identical to each other.
_____ always involves two living cells.
_____ involve donor and recipient cells fusing into one cell.

8C. The genotype possessed by a cell

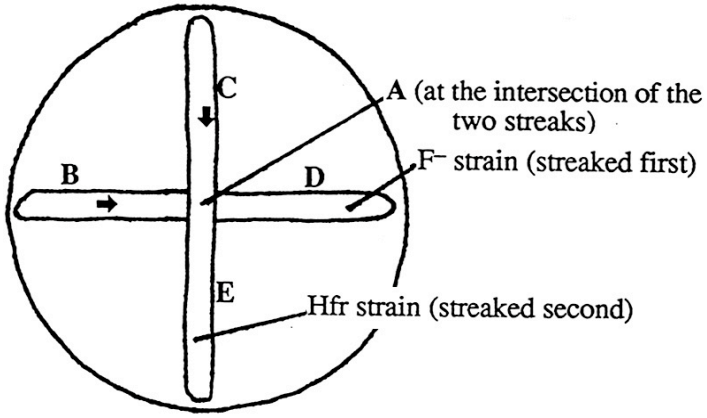
_____ is always identical to the genotype of all other cells in its species.
_____ can be changed by mutation.
_____ can be changed by recombination.
_____ is readily changed by altering environmental conditions (temperature, O_2, etc.).

8D. You have a set of 10 *E. coli* cells. Five are Hfr cells and 5 are F^- cells. Four pairs of the cells undergo conjugation. Only one of the F^- cells incorporates new DNA into its chromosome. Therefore, the **recombination frequency** is (circle the letter of the **one** correct choice):

a. 1/10 (i.e., 10%)
b. 1/5 (i.e., 20%)
c. impossible to determine without more information

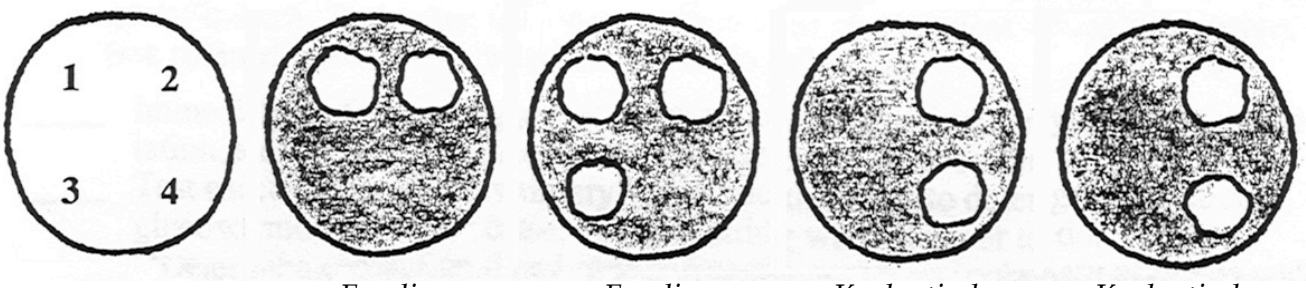
8E. From a concentrated suspension of *Staphylococcus epidermidis* (1 x 10^{10} CFUs/ ml), you take one ml and add it to 9 ml of sterile saline. You then plate one ml onto Nutrient Agar containing 100μg streptomycin/ml of medium. Forty colonies arise during incubation. What is the **mutation frequency**? (You may express as it as a fraction or percent.)

8F. In our qualitative recombination experiment, we cross-streaked Hfr and F^- strains of *E. coli* on a plate of Minimal Medium as follows:



We expected growth of **recombinants** in these regions (CIRCLE TWO): **A B C D E**

9A. Phages **1**, **2**, **3** and **4** are spot-inoculated onto plates of various bacteria as indicated below. The first diagram shows the position of each phage on each plate.



E. coli strain A | *E. coli* strain B | *K. planticola* strain C | *K. planticola* strain D

For the following, circle the designation(s) of the correct phage(s):

a. Which phage(s) is (are) "strain-specific" (within a species)? **1** **2** **3** **4**

b. Which phage(s) is (are) specific to one species? **1** **2** **3** **4**

c. Which phage(s) infect(s) all organisms tested? **1** **2** **3** **4**

9B. Bacteriophages

_____ cannot be seen with the microscopes we use in lab.
_____ are viruses which utilize bacterial cells as their "medium" for replication.
_____ may be utilized in some bacterial identification procedures.
_____ when added to a medium in a sufficient quantity, can make the medium selective against organisms which are sensitive to the particular bacteriophage used.
_____ may reproduce in the absence of bacterial cells and form colonies on media much like bacterial colonies.
_____ are to plaque-forming units (PFUs) as bacterial cells are to colony-forming units (CFUs).

10A. In the antibiotic disc sensitivity test, we were testing the sensitivity of

_____ various antibiotics to *Streptomyces*.
_____ various antibiotics to a test organism.
_____ a test organism to various purified antibiotics.
_____ a test organism to antibiotics produced by a *Streptomyces* isolate growing on a paper disc.

10B. In the antibiotic disc sensitivity test,

_____ each disc contains a certain amount of a particular antibiotic which is expected to make the medium selective in the area around the disc.
_____ more than one test organism is used in each plate.
_____ any zone of inhibition indicates that the antibiotic is inhibiting the test organism at the "target site."

10C. You have isolated the dreaded skin-degrading bacterium *Gnarlibacter grodium*. You then inoculate a pure culture over the surface of a plate of an all-purpose medium and apply four antibiotic disks. After incubation, you measure the diameters of the zones of inhibition around the disks and record the following: **Bacitracin – 9 mm**; **Colistin – 8 mm**; **Streptomycin – 20 mm**; **Tetracycline – 20 mm**. Looking closely, you see many tiny colonies in the "zone of inhibition" surrounding the Streptomycin disk!

ZONE SIZE INTERPRETATIVE TABLE

ANTIBIOTIC ON DISK	INHIBITION ZONE DIAMETER TO NEAREST mm		
	RESISTANT	INTERMEDIATE	SUSCEPTIBLE
Bacitracin	8 or less	9-12	13 or more
Colistin	8 or less	9-10	11 or more
Streptomycin	11 or less	12-14	15 or more
Tetracycline	14 or less	15-18	19 or more

a. Why is Streptomycin **not** the antibiotic of choice in controlling this infection?

b. Which would be the preferred antibiotic to administer to the patient?

10D. Dr. X had a patient suffering with an infection caused by a particular strain of *Neisseria gonorrhoeae*, the causative agent of gonorrhea. By a quantitative test on a broth culture of *N. gonorrhoeae* which was isolated from the patient, it was found that the culture was sensitive to penicillin except for one penicillin-resistant mutant for every one million cells. Dr. X went ahead and gave his patient penicillin. At this point – given these facts – one may expect

_____ a cure.
_____ eventually the probability of isolating *Neisseria gonorrhoeae* cells from the patient which are penicillin-resistant.
_____ the need to employ a truly effective antibiotic.

11A. When inoculating plates to obtain a desired type of organism from a natural source (or from a liquid enrichment of a natural source),

_____ we must make an effort to obtain isolated colonies as we cannot expect the desired organism to be present as a pure culture in the inoculum.
_____ one may see different kinds of colonies corresponding to different species of the desired type of organism.
_____ one always expects that the plating medium used will select for the desired type of organism such that all other types are inhibited from growing.

11B. Fill in the following table concerning growth conditions and related items regarding the variable metabolism of purple non-sulfur photosynthetic bacteria:

grown as –>	photoautotroph	photoheterotroph	chemoheterotroph
nature of carbon source			
most likely nature of electron donor			
mode of energy generation			
light required?			
aerobically or anaerobically incubated?			

11C. When we set up our broth enrichment for purple non-sulfur photosynthetic bacteria,

_____ various respiring organisms, including purple non-sulfur photosynthetic bacteria, utilize the oxygen initially dissolved in the medium, thus creating an anaerobic environment.
_____ we incubated the bottle under fluorescent – rather than tungsten – light, in order to provide the wavelengths of light which these organisms prefer.
_____ the stopper prevents additional oxygen from getting into the medium.

11D. 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 the plates are incubated under anaerobic conditions with glucose included in the medium.

11E. When we heat-shock a suspension of soil, we expect

_____ immediate production of endospores by vegetative cells of *Bacillus* and *Clostridium*.
_____ more endospores will germinate than would otherwise (if we had not heat-shocked the soil).
_____ killing of most or all of the reproductive spores of such soil organisms as *Streptomyces* and molds.
_____ killing of most or all of the vegetative cells of non-endosporeforming microorganisms.
_____ killing of most or all of the vegetative cells of *Bacillus*.

11F. A heat-shocked suspension of soil is streaked onto Plate Count Agar, an all-purpose medium that includes glucose, a fermentable sugar. When we incubate this plate under aerobic conditions, we expect the growth of

_____ strictly aerobic species of *Bacillus*.
_____ facultatively anaerobic species of *Bacillus*.
_____ *Clostridium*.

11G. If we were to take the plate prepared above and incubate it under anaerobic conditions, we would expect the growth of

_____ strictly aerobic species of *Bacillus*.
_____ facultatively anaerobic species of *Bacillus*.
_____ *Clostridium*.

11H. Concerning bacteriophages, antibiotics and endospores:

_____ Living vegetative cells are required for their development.
_____ The first two, if added to a bacteriological medium (such as what may be in a plate), will make that medium selective for bacteria which are resistant to them.
_____ The last two can be produced by certain organisms in soil.
_____ Bacteriophages and endospores each contain nucleic acids which are essential to their continuing existence.

11I. The endospores we see in stained slides made from *Bacillus* colonies

_____ are the very same spores which were originally inoculated onto the plate.
_____ come from colonies where endospores are growing and dividing.

11J. *Assume for the purposes of this problem that the cells are not grouped in any arrangement such as chains or clusters, and we can consider each cell as one CFU and vice versa. Also the soil sample under consideration contains many* ***cells of each type*** *as we learned in the introduction to Experiment 11C: vegetative cells, endospores and reproductive spores.*

You prepare a suspension of soil in a screw-cap tube as you did in Period 1 of Experiment 11C. You then inoculate 0.1 ml onto a plate of Nutrient Agar which we will call Plate A. After heating the soil suspension at 80°C for 15 minutes, you plate 0.1 ml of the soil suspension onto a second plate of Nutrient Agar which we will call Plate B.

After appropriate incubation, you count 150 colonies on Plate A and 75 colonies on Plate B.

What percentage of colony-forming units in the soil sample were endospores?

11K. Nitrogen-fixing organisms

_____ can be the source of nitrogenous compounds for non-nitrogen-fixing organisms when both are growing in nitrogen-free media.
_____ are those which produce gaseous nitrogen from various chemical reactions involving nitrogen-containing compounds.
_____ include a wide variety of different microorganisms such as fungi, algae and protozoa – not just bacteria.

11L. The following are associated with *Streptomyces*:

_____ photosynthesis
_____ geosmins
_____ coccus morphology of vegetative cells
_____ extracellular enzymes
_____ antibiotics

11M. In our isolation of *Streptomyces*, we used a medium which includes

_____ large-molecular weight compounds which must be broken down by extracellular enzymes in order for microorganisms to grow on the medium.
_____ a selective agent which selects against molds as much as possible.
_____ antibiotics such as streptomycin.
_____ agar.

11N. In the *Streptomyces* isolation experiment, we looked for antibiotic production

_____ by one or more of the test organisms streaked up to the *Streptomyces* growth.
_____ by the way in which the test organisms grew in the vicinity of *Streptomyces*.
_____ which caused inhibition of the growth of *Streptomyces*.

11O. You have found a strain of *Grodybacter gnarlii* which is resistant to all of the antibiotics which can be tested! Briefly (preferably with a diagrammed flow chart) explain how you might look for a strain of *Streptomyces* which will produce an antibiotic against *G. gnarlii*.

11P. For the following set of matching terms, place the letter of the correct item from column **b** in the blank by each statement in column **a**. **Some statements have two or three blanks.** *Only one letter per blank*. Any letter may be used any number of times or not at all.

a

_____ _____ **Two** processes that ultimately determine an organism's "oxygen relationship" designation. (Remember that the catalase test and the glucose fermentation test are relevant to oxygen relationships.)
_____ _____ _____ **Three** processes associated with **anaerobic** growth.
_____ _____ _____ **Three** processes that are classified as **chemotrophic**.
_____ _____ **Two** processes that are classified as **phototrophic**.
_____ Process not dealt with in any of our experiments. Typically, plants, algae and cyanobacteria perform this process.
_____ Process responsible for causing anaerobic conditions to form in the photosynthetic enrichments and in the decarboxylation test media.
_____ A different compound is used as an electron acceptor where one would normally expect to see **oxygen** utilized as such.

b

A. aerobic respiration
B. anaerobic respiration
C. fermentation
D. oxygenic phototrophy
E. anoxygenic phototrophy

11Q. For each of the types of organisms in column **a**, indicate **two** important things from column **b** that are involved in the **successful <u>isolation</u>** of the organism. *Only one letter per blank*. Any letter may be used any number of times or not at all.

a

_____ _____ Purple non-sulfur photosynthetic bacteria
_____ _____ *Streptomyces*
_____ _____ *Bacillus*
_____ _____ Coliforms (Exp. 15A)
_____ _____ Strictly aerobic nitrogen-fixing bacteria

b

A. Aerobic incubation.
B. Anaerobic incubation in the dark.
C. Anaerobic incubation in the light.
D. Antibiotic production.
E. Endospores.
F. Ferment lactose to acid and gas.
G. Growth in media that inhibit Gram-positive bacteria.
H. Obtain nitrogen from the atmosphere.
I. Provision of a carbon source not generally utilized by other organisms.

12A. Regarding the lactic acid bacteria (also known as the "lactics"):

_____ They are gram-positive and may be rods or cocci.
_____ All produce slime from sucrose and acid from the fermentation of lactose.
_____ Some are utilized in the production of fermented dairy products such as yogurt and cheese.
_____ Their metabolic activities in foods and in (or on) the human body can be a deterrent against many pathogenic organisms.

12B. Efficient isolation of lactic acid bacteria can be accomplished by:

_____ heat-shocking the inoculum.
_____ the use of a plating medium containing a fermentable sugar and an abundance of growth factors.
_____ a cytochrome inhibitor in the plating medium.
_____ the use of aerobic incubation of the isolation plates in order to inhibit strictly anaerobic bacteria which may also grow on plating media used for lactic acid bacteria.

12C. The hot-loop test

_____ distinguishes between homo- and heterofermentative lactic acid bacteria.
_____ distinguishes between lactic acid bacteria and other groups of bacteria.
_____ performs the same function as the Durham tube – i.e., a gas bubble in a Durham tube and a positive hot-loop reaction indicate the same thing.

13A. In the isolation of *Staphylococcus aureus* by the use of Vogel-Johnson Agar,

_____ the black color seen for any colony is due to hydrogen sulfide production.
_____ we expect any black colony to be *Staphylococcus aureus*.
_____ black colonies which are catalase-positive and consist of gram-positive cocci in clusters are probably of the genus *Staphylococcus*.
_____ suspected colonies must be tested for the coagulase reaction before any identification of *Staphylococcus aureus* is made.

13B. The candle jar

_____ provides an elevated level of carbon dioxide in its atmosphere.
_____ provides an environment suitable for strictly anaerobic bacteria.
_____ will only support the growth of autotrophic organisms.

13C. Hemolysis

_____ is termed "alpha" when the blood cells and hemoglobin (the pigment) are completely wiped out in the area adjacent to a colony of a *Streptococcus* on Blood Agar.
_____ can be used to differentiate between *Streptococcus* and other organisms.
_____ can be observed on Heart Infusion Agar.

14A. Regarding the enteric bacteria (also known as the "enterics"):

_____ They are facultatively anaerobic.
_____ They are bacteria which are found only in the intestinal tract.
_____ They all produce hydrogen sulfide.
_____ They include such organisms as *Pseudomonas* and *Neisseria*.
_____ They include such organisms as *Salmonella* and the true coliforms.
_____ They may be found in the early part of a sauerkraut fermentation.

14B. Enrichment and isolation procedures for enteric bacteria

_____ are aided by media which inhibit gram-positive bacteria.
_____ may result in the isolation of *Pseudomonas*.
_____ may involve media which contain lactose as the only fermentable sugar even though many enterics do not ferment lactose.

14C. Kligler Iron Agar (KIA)

_____ tests for glucose fermentation.
_____ tests for lactose fermentation.
_____ tests for hydrogen sulfide production.
_____ can differentiate enterics from strict aerobes like *Pseudomonas*.
_____ gives the most reliable results for pH-related reactions when examined at just one day of incubation (not at two or more days).
_____ gives reliable results when used for gram-negative rods such as *Pseudomonas*, *E. coli* and other enterics, and it isn't a recommended medium for other types of organisms (e.g., gram-positive cocci).
_____ is an example of a differential medium where both alkaline and acid-producing activities by an organism may be found.
_____ – if incubated under anaerobic conditions – should show the same result for any organism as it would when incubated under aerobic conditions.

14D. When mineral oil is placed on a broth medium inoculated with an enteric,

_____ creation of anaerobic conditions in the medium is achieved by respiration of the organism rather than by any immediate action of the mineral oil.
_____ the mineral oil prevents additional oxygen from getting into the medium.
_____ mineral oil is necessary to supply trace elements to the medium.

14E. The methyl red test

_____ can be done by adding the methyl red reagent to a slant or plate of any all-purpose medium.
_____ is done after at least two days of incubation of the culture.
_____ distinguishes between fermenting and non-fermenting bacteria.

14F. A methyl red-negative reaction in MR-VP Broth

_____ is associated with enterics which produce "neutral products."
_____ means that the pH is in the alkaline range (above pH 7).
_____ would be expected for a non-fermenter such as *Pseudomonas*.
_____ for a particular lactose-fermenting enteric will help to explain why the slant of a KIA culture would change from yellow to red when incubated over a two-day period.

14G. The "slide agglutination test"

_____ can be used along with the API-20E tests to identify an organism as a particular kind of *Salmonella*.
_____ involves the use of known antibiotics to test for certain antigens on the cells of the unknown organism.
_____ detects catalase.
_____ detects coagulase.

14H. Using numbers (1-4), put the following procedures in the correct order. These procedures deal with the isolation and identification of enterics.

_____ Inoculate preliminary biochemical test media such as Kligler Iron Agar (KIA).
_____ Streak plates of selective-differential media for isolated colonies.
_____ Obtain source material.
_____ Inoculate various biochemical test media (Lactose Fermentation Broth, MIO, Simmons Citrate Agar, etc.).

14I. You wish to exploit certain properties of the difficult-to-isolate bacterium ***Excalibacterium*** (an enteric) in order to help you detect and isolate it from samples which are highly-contaminated with other enterics. You decide to start with MacConkey Agar which you know (from Bact. 102!) contains **lactose** as the only fermentable sugar. **Peptone** is another medium ingredient which you recall; it contains a mixture of various amino acids – none in any especially high amount. Following is a table showing important organisms to consider in this situation:

genus	fermentation of					decarboxylation of	
	glucose	maltose	lactose	sucrose	mannitol	lysine	arginine
Edwardsiella	+	+	–	–	–	+	–
Aquamonas	+	+	–	–	–	+	+
Excalibacterium	+	–	–	+	–	+	–
other enterics	+	+	+ or –	+ or –	+ or –	+ or –	+ or –

a. On MacConkey Agar, what would you expect the net pH reaction would be for any of the three genera specifically listed on the table above? (Circle one) **ACID ALKALINE**

b. As these three genera do not ferment or respire lactose, how can they grow on MacConkey Agar? (That is, what is a likely energy source and how might they utilize it?)

c. What would be the best choice for a sugar to add to MacConkey Agar which will assist greatly in the differentiation of *Excalibacterium* colonies from the others on the table? (Circle one) **GLUCOSE MALTOSE SUCROSE MANNITOL**

d. If lysine were to be included in the medium in a relatively large amount, what effect would this have on the pH reaction associated with *Excalibacterium* colonies? (Circle one)

MORE ACIDIC MORE ALKALINE

14J. What effect (acidic or alkaline) does each of the following microbial processes have in a medium containing the underlined ingredients?

a. **Aerobic deamination** of one or more amino acids in the medium.

b. **Fermentation** of the sugar in the medium.

c. **Anaerobic decarboxylation** of an amino acid which is specifically added to the medium in a relatively large amount.

15A. Among the features 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.

15B. In the bacteriological examination of water, one looks for coliforms, because

_____ they indicate the possibility of various pathogenic organisms also being present.
_____ they should be present in any "normal" supply of water, and if they are not there, then something is "wrong" with the water.
_____ they are organisms which are easy to enrich for and detect.

15C. In the presumptive test for coliforms, a positive result

_____ is recorded when growth is seen, whether or not gas is present.
_____ indicates that the tube contains a pure culture of the organisms being detected.
_____ confirms the presence of *Escherichia coli*.
_____ can be the result of growth of one coliform originally inoculated into the medium from the sample or a dilution of the sample.
_____ can be produced by organisms other than true coliforms which can withstand the selective agents in the medium.

15D. The most probable number (MPN) method of counting bacteria

_____ may be used to estimate the number of bacteria per ml of sample, if an all-purpose broth medium is inoculated from various dilutions of the sample.
_____ may be used to estimate the number of indole-producing bacteria per ml of a sample, if Tryptone Broth is inoculated from various dilutions of the sample and then tested with Kovacs reagent after incubation.
_____ gives the estimated number of fecal coliforms per ml of water from the results of the Presumptive Test.

15E. Fecal coliforms

_____ are any bacteria found in the enteric (intestinal) canal.
_____ and coliforms are two separate (non-overlapping) groups of bacteria.
_____ and enterics are two separate (non-overlapping) groups of bacteria.
_____ can grow and produce gas in Brilliant Green Lactose Bile (BGLB) Broth.
_____ include *Escherichia coli*.

15F. In the enrichment, isolation and identification process for coliforms, the "completed tests"

_____ include the isolation and identification procedures.
_____ include the "total aerobic plate count."
_____ begin with the streaking of Eosin-Methylene Blue Agar from tubes of a selective enrichment.

15G. The following media are selective for organisms which are gram-negative:

_____ Brilliant Green Lactose Bile (BGLB) Broth
_____ Blood Agar
_____ MacConkey Agar
_____ EMB (Eosin-Methylene Blue) Agar
_____ Vogel-Johnson Agar

15H. When isolating coliforms from water, why is there a two-step enrichment process (presumptive and confirmatory tests)? That is, why not just inoculate the water into the confirmatory media?

16A. Koch's Postulates

_____ are applied in bacteriological water analysis.
_____ associate a certain organism with a certain disease.
_____ are a list of procedures used to identify an unknown culture.

17A. You have cultures of five organisms as listed below. However, the labels of the tubes have come have come off, and you need to re-label them correctly! First, you consider the various reactions you know for the organisms in question:

genus and species	gram reaction	shape	catalase reaction	glucose fermentation	lactose fermentation	phenylalanine deaminase	citrate utilization	H_2S production
Bacillus lactis	+	rod	+	+	+	–	–	–
Enterococcus faecalis	+	coccus	–	+	+	–	–	–
Citrobacter freundii	–	rod	+	+	+ or –	–	+	+
Morganella morganii	–	rod	+	+	–	+	–	–
Pseudomonas haywardii	–	rod	+	–	–	–	+ or –	–

a. The results obtained from what **one** specific laboratory **procedure** will differentiate *Bacillus lactis* and *Enterococcus faecalis* from each other and also from the remaining three organisms? This can be answered in no more than **two words**. (Remember, it's a procedure, not a reaction.)

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

Citrobacter freundii _____

Morganella morganii _____

Pseudomonas haywardii _____

17B. You isolate a pure bacterial culture and decide to do some tests. You find that the organism is catalase -positive, but it does not ferment glucose. It grows very well on MacConkey Agar, and the colonies do not show any lactose fermentation. In nitrate broth, you see no gas, but there is a positive reaction (red color) when you add the nitrite reagents.

a. Recall our test for "oxygen relationship" and the reasons for the various growth patterns associated with each different designation. What **oxygen relationship** designation would you indicate for your organism? Circle **one**:

strict aerobe facultative anaerobe aerotolerant anaerobe strict anaerobe

b. What type of **energy-yielding metabolism** is the isolate capable of? Circle **any number** of the following:

aerobic respiration anaerobic respiration fermentation

c. What would be the likely **Gram reaction** for this isolate? Circle one: + −