

SELECTED QUESTIONS FROM OLD MICRO 102 QUIZZES
PART I – EXPERIMENTS 1 THROUGH 7

Question numbers refer to the applicable experiment. Questions with blanks are **multiple true-false** questions unless otherwise indicated.

1A. Petri dish cultures

- are always labeled on the bottom lid and incubated in an inverted position.
- are examined only through the bottom lid (i.e., the lid containing the medium).
- are always incubated upside down (medium side up).
- are always prepared by streaking a culture with a loop in order to obtain isolated colonies.

1B. The following terms are always plural, never singular:

- bacteria
- bacillus
- media

1C. A colony-forming unit (CFU)

- and a colony are the same thing.
- is microscopic, as is a cell.
- is composed of one or more cells which will be able to utilize the medium (on which they are placed) and eventually produce one colony through the process of cellular division.
- is a term used only when we are quantitating bacteria, such as determining the number of CFUs per ml or per gram of a sample.

2A. The gram stain

- classifies bacteria according to the structure of the cell envelope.
- is an alternative to the acid-fast stain; a positive reaction for each stain will mean the same thing.
- gives the best results when an old culture (grown 2 or more days) is used.
- results in red or pink cells for a gram-negative organism.
- should be performed on an extremely thick smear, such that the gram reaction can be seen by the naked eye.
- may give a false reaction for encapsulated cells, as the presence of a capsule may inhibit proper decolorization (i.e., a gram-negative cell may appear as a gram-positive cell).

2B. “Gram-variability”

- is a term which can be used where two gram reactions are seen due to an error in the staining procedure.
- applies to an organism which changes its cell wall structure from the gram-positive type to the gram-negative type as the culture ages.
- applies to what is ultimately seen when cells in a culture of gram-positive bacteria lose the ability to retain the primary stain during the decolorization process.
- indicates a mixed (i.e., impure) culture.

2C. For an “acid-fast” organism, the word “acid” refers directly to

- acid produced from fermentation.
- mycolic acids associated with the cell wall.
- the resistance of a stained cell to an acid decolorizing agent (such as acid alcohol).

2D. Examples of differential stains include

- the gram stain.
- the acid-fast stain.
- the single application of crystal violet to a smear.

2E. Give two reasons why an organism which is gram-positive may give a gram-negative staining reaction.

2F. A wet mount will become a smear if it dries out without having had a coverslip placed on it. T/F

2G. Why is a smear heat-fixed prior to staining?

2H. Total magnification of a microscope is calculated by adding the magnifications of the ocular and objective lenses. T/F

3A. Regarding aseptic technique, the following are acceptable procedures:

- Leaving open tubes upright in the test tube rack while transfers are made between them.
- Sterilizing only the very tip of the loop or needle prior to making transfers.
- Taking the lids off petri plates and placing them elsewhere on the bench while streaking or examining the plate.
- Flaming the open ends of plugged tubes after opening and before closing them.
- Discarding capsule stains and other wet mounts into the container meant for discarding stained smears and broken glassware.

3B. When inoculating a tube of sterile medium from a colony on a plate,

- one should use a needle, not a loop.
- one would pick from only one colony, especially if the plate contains colonies of different species of bacteria.
- one must transfer the entire colony in order for the inoculation to be successful.

4A. You made several dilutions of a food sample and plated 1 ml of a 10^{-4} dilution which resulted in 250 colonies. Your neighbor performed the same procedure with the same sample, but only 13 colonies resulted.

- It is likely that either one of you failed to mix the dilutions well before making further dilutions and platings.
- If the procedure were such that you were using a selective medium and your neighbor were using an all-purpose medium such a difference in the colony count would not seem impossible.
- Based on your results, there were 2.5×10^6 CFUs per gram of the food.

4B. In the determination of the concentration of living bacteria in a sample, we determine the number of colony-forming units (CFUs) per ml (or gram) rather than the number of cells per ml (or gram), because:

- There may be cells which cannot grow on the medium used and/or at the conditions of incubation.
- Any colony counted may have arisen originally from one or more cells.
- The colony count always includes a certain proportion of contaminants which were not present in the sample.

4C. In doing the plate count procedure:

- One can prepare a 1/10 dilution in a variety of ways, but the proportion of the amount to be diluted to the total amount is always 1 to 10.
- 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 resterilize the hockey stick.
- The plates are always incubated in an inverted or upside-down position.

4D. The following could result in recording a lower CFU count than what one should get:

- Failing to mix a dilution thoroughly before using it to inoculate a plate or make another dilution.
- Actually plating 0.1 ml of a dilution when one believes one is plating 1 ml.
- Failing to cool the hockey stick after flame-sterilizing it.

4E. If one milliliter (1 ml) of a 10^{-4} dilution of a sample is inoculated into a plate of an all-purpose medium and 300 colonies result,

- one would expect that the number of viable (living) cells in the one ml amount would be equal to or greater than 300.
- one would expect, theoretically, that 0.1 ml of the same dilution would have resulted in 30 colonies.
- one can calculate the CFU/ml of the original, undiluted sample to be 3.0×10^{-2} .

4F. In the “total aerobic plate count” of a sample, such as what we determined in the hamburger experiment, one may make several dilutions of the sample and then inoculate plates from the dilutions. Then, after incubation,

- plates with greater than 300 colonies or less than 30 colonies are not used.
- only colonies of a certain size are counted.
- the colonies we see are only of strictly aerobic bacteria.

5A. Fermentation

- results in production of acid and possibly gas from the breakdown of sugars.
- is associated with the type of growth of facultative anaerobes in Thioglycollate Medium where growth is less dense in the anaerobic region.
- is generally associated with a positive catalase reaction for an organism.

5B. Respiration

- by an organism implies the ability of the organism to produce catalase.
- explains why a facultative anaerobe grows better in the presence of air than under anaerobic conditions.
- which involves the breakdown of a sugar is indicated by a large amount of acid and gas.

5C. A facultative anaerobe

- respire in the presence of oxygen.
- ferments in the absence of oxygen.
- and an aerotolerant anaerobe can be differentiated by the catalase test.

5D. Chemoheterotrophic organisms

- derive their energy from chemical reactions; light is not involved.
- obtain carbon from carbon dioxide in the atmosphere.
- always obtain energy by means of respiration.

5E. Regarding bacteriological media:

- An all-purpose medium is one which will support the growth of all species of bacteria.
- A differential medium is defined as one which supports the growth of some organisms but not others.
- One would not include any carbon compound in the medium if one wanted to grow a heterotroph, as these organisms can obtain carbon from the carbon dioxide in the atmosphere.
- One must include a siderophore in a medium in order to support the growth of a siderophore auxotroph.
- Anything which can be used as a nutrient is called a growth factor.

5F. A complex medium

- is one in which all the different elements and compounds are known quantitatively.
- can be a chemically-defined medium to which a known amount of a hay infusion is added.
- can be a chemically-defined medium to which a known amount of purified NaCl is added.

5G. An “all-purpose medium”

- supports the growth of all bacteria.
- is not formulated purposely to inhibit any organism.
- would be something like Plate Count Agar, the medium used for the “total aerobic plate count” in the hamburger experiment.
- includes agar as an essential source of nutrients.

5H. In Glucose O/F Medium and Glucose Fermentation Broth,

- an organism needs to utilize glucose for carbon and energy in order to grow at all.
- one which grows and shows an acidic reaction throughout the medium may be a facultative anaerobe.
- glucose and the pH indicator are “differential agents.”

5I. Circle the correct choices:

- a. An organism would be expected to produce less siderophore in an environment containing a relatively high/low (circle one) concentration of iron compounds.
- b. Circle the term which always represents the plural form: bacterium/bacteria.
Circle the term which always (and only) represents the singular form: medium/media.
- c. Organisms which can respire are facultative anaerobes/aerotolerant anaerobes/
strict aerobes(circle two).
- d. Organisms which can ferment are facultative anaerobes/aerotolerant anaerobes/
strict aerobes(circle two).

5J. [In a virtual experiment], we considered a strain of *Arthrobacter flavescens* which was unable to grow on a complex medium. The main purpose of the experiment was to demonstrate how

- A. flavescens* produces iron which other organisms can utilize.
- an organism can grow better when supplied with a required growth factor.
- all siderophores are pigmented and glow under an ultraviolet light.

5K. For growth curves in general:

- The growth curve will always be the same, no matter what medium, incubation conditions or organism we use.
- Dead and living cells both contribute to the absorbance value.
- In the stationary phase, all cell division ceases.

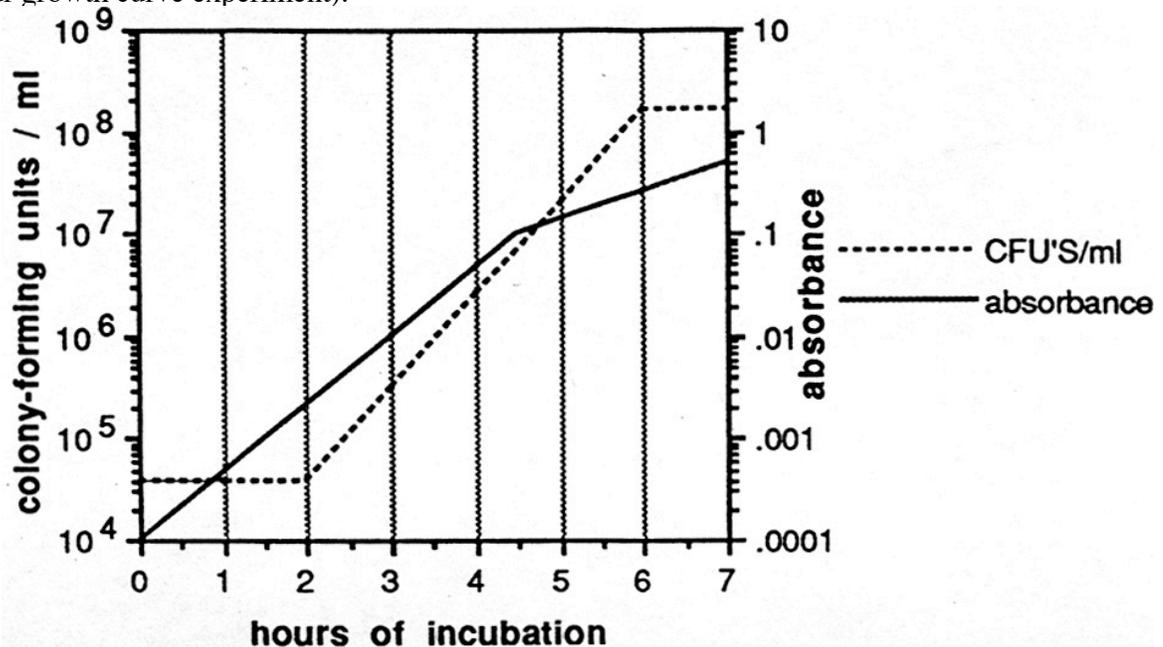
5L. In determining the growth rate and generation time for a bacterial species,

- we consider points only on the best straight line drawn among the data points for the exponential phase of the growth curve.
- we can use any two CFU/ml values recorded from plates prepared during the exponential phase.
- we necessarily assume that each cell will divide into two cells, not three or more.

5M. (This problem can be done without the use of the formulas!) At 2 A.M., a flask of a liquid medium is inoculated with 1×10^3 bacterial cells. The lag phase lasts a half hour. At 7:30 A.M., the culture enters the stationary phase with a population of about 1×10^6 cells in the flask.

- Approximately 10 generations were produced by 7:30 A.M.
- We can estimate the **generation time** from the data given.
- If the cells did not separate after division during the incubation period (perhaps they formed chains or clusters), we would get the same plate count for the 7:30 A.M. sample as for the 2 A.M. sample.
- In the stationary phase, we do not expect any division or death of any cell in the culture.

- 5N. If the growth rate for a particular organism was found to be 4 doublings per hour, the generation time would be:
- 15 minutes
 - 30 minutes
 - impossible to determine without more information
- 5P. Note the following growth curve of a typical organism (such as the strain of *E. coli* we used in our growth curve experiment):



- ___ To generate the growth rate, one can choose points on the CFU/ml graph corresponding to 1 hour and 4 hours.
- ___ One possible reason for the difference (flat vs. slanted) in the two graphs between 0 and 2 hours is that the cells/ml may be increasing but the CFUs/ml are not.
- ___ The death phase begins at 4.5 hours.
- ___ One could determine the generation time from a graph such as this by finding the time difference between 1×10^6 CFUs/ml and 2×10^6 CFUs/ml.
- 6A. When observing a wet mount, true motility of a culture is indicated when one sees
- cells which are moving around on their own, independently of each other.
 - Brownian motion.
 - all of the cells moving together in the same direction.
- 6B. A negative result in Motility Medium
- is indicated if growth occurs only along the line where the medium was stab-inoculated.
 - should be confirmed by a wet mount of a young culture of the same organism.
 - may exhibit growth over the surface of the medium.
 - may occur for strictly aerobic, motile organisms.
- 7A. You have been found most worthy to be the recipient of a generous gift of 1000 cultures. But here's the catch: You must identify each culture to genus and species! So, having been given free room and board at the lab, you proceed to do the following:
- Immediately perform a large number of differential tests (gram stain, motility, lactose fermentation, starch hydrolysis, indole, nitrate reduction, etc.) on each culture.
 - Test each culture with a few "primary" tests, such as the those to determine gram reaction, glucose fermentation and catalase, before considering which further tests to do.
 - Determine the generation time and growth rate of each organism under a variety of media and growth conditions.
 - Not do the gram stain at all, as it is too tricky and of no real importance.

7B. In Experiment 7, we could determine the probable oxygen relationship of each of the twelve organisms without inoculating the standard test medium for O₂ relationship (Thio. Medium).

_____ We know enough about how the cultures grow such that we can rule out their being strict anaerobes.

_____ We can use the results from the glucose fermentation and catalase tests to differentiate between strict aerobes, facultative anaerobes and aerotolerant anaerobes.

_____ We can use the results from the lactose fermentation and amylase tests to differentiate between strict aerobes, facultative anaerobes and aerotolerant anaerobes.

7C. In the test to determine whether or not starch was broken down,

_____ we add iodine to a plate of Starch Agar after incubation, and we expect the iodine to react with amylase to give a noticeable reaction.

_____ growth on a plate of Starch Agar indicates an organism's ability to break down starch, and we only do the test with iodine to confirm this fact chemically.

_____ we could perform the same test on Heart Infusion Agar as on Starch Agar.

7D. In the test for nitrate reduction,

_____ growth and gas indicate fermentation.

_____ a red color – which is obtained only after the additions of the reagents and the zinc – means that one can record a positive reaction for the organism.

_____ we may be able to determine whether or not a “strict aerobe” can grow anaerobically, using nitrate in place of oxygen.

7E. The streak plate method (i.e., done with the loop) helps us to achieve the following:

_____ determination of the number of colony-forming units per ml of the culture

_____ determination of the purity of the culture

_____ isolation of the different organisms in a mixed culture

_____ determination of colonial characteristics

7F. When testing an organism to see if it can break down a particular substance,

_____ we may see growth of the organism in a medium containing the substance – whether or not the substance is being broken down.

_____ we must have a pH indicator in the medium.

7G. Aerotolerant anaerobes generally produce colonies which are smaller than those of the usual facultative anaerobes and strict aerobes.

_____ This is related to the fact that aerotolerant anaerobes do not respire and therefore are not as efficient in their metabolism as the other types.

_____ These organisms can be differentiated from the other types by the catalase test.