ANSWERS TO LABORATORY SYLLABUS QUESTIONS:

Introduction and Significance of Hand Washing:

1. Why are microbiology students advised to never eat or drink in the Microbiology laboratory?
   Some of the bacteria used in this laboratory are potential gastrointestinal pathogens capable of causing severe illness if ingested. Eating or drinking while working with live bacteria is foolish and in violation of State and Federal Safety Standards.

2. Assuming you have a liquid microbial culture (broth culture) that you must dispose of. What are you expected to do with it?
   Broth cultures ready for disposal are to be placed in the discard area in one of the large multi-tube racks. They must be maintained in a vertical position, and may not be placed into the metal, discard bins. Never discard broth cultures in the sink drains.

3. While working in this laboratory, you will encounter a number of potentially harmful microorganisms. Aside from these, what are some of the hazards associated with work in the microbiology lab?
   Fire is a significant hazard; use caution when working with and around lit Bunsen Burners. Some of the chemicals used in this laboratory are toxic or flammable, and represent hazards if not handled/used appropriately. Be careful with chemicals (stain reagents, solvents, etc.). Glass culture tubes, beakers, flasks, etc. can be broken, resulting in the formation of sharp objects (sharps) with injury potential to students. Be careful with glass. Students behaving inappropriately pose a significant hazard in the microbiology laboratory. Be alert and avoid actions that might result in injury to yourself or others.

4. According to the CDC, handwashing is ___________________________________. **According to the CDC, handwashing is the single most important procedure for preventing nosocomial infection.**

5. Did the application of hand sanitizer effectively eliminate or prevent the growth of the "transient" flora provided? How do you know? **The answer will be variable here and is dependent on the type of "transient" flora used. The presence of colonies, not similar to those observed on the "before" quadrant would indicate the "transient flora" survived the sanitizing process, cells were transferred to the plate, and reproduced there forming colonies visible to the naked eye.**

6. Did thoroughly scrubbing the hands reduce the number and variety of cells present? How do you know? **The answer will be variable here and is dependent on the type of soap solution used and the length/thoroughness of the washing process. The presence of colonies indicates that some bacteria associated with the skin as "normal flora" were exposed, were transferred to the plate and reproduced forming visible colonies.**

7. Did the data you obtained meet your expectations, or did the results differ from what you expected?
   The answer will be variable here and is dependent on data collected as well as student expectation.

8. Were variations in growth consistent throughout the laboratory, or did they vary with different individuals, solutions used and/or times used for washing? **Some variation is expected because different students will have different quantities and types of organisms present on their hands, and because different organisms respond differently to the soap solutions, etc. used.**

Microscopic Technique:

1. What is microbiology?
   Microbiology is the science or study of organisms too small to be observed with the naked eye, i.e., the study of microscopic organisms/microorganisms or microbes.

2. How many objective lenses does your microscope have, and what is their power of magnification (alone and when used in combination with the ocular lens)?
   There are four objective lenses with powers of 4x, 10x, 45x, and 100x. When used in combination with the ocular lenses (that are 10x), the powers of magnification increase to 40x, 100x, 450x, and 1000x respectively.

3. When changing from low power to oil immersion, it will be necessary to _________ (increase or decrease) the amount of light used in order to see clearly. **Increase.**

4. If you wish to keep a specimen within the field of view as you increase magnification, you must position it where within the viewing field? Why is this so?
   Objects must be placed at the center of the field of view. If the specimen is not positioned at the center of the field of view, there is a high probability that it will not be visible when the magnification is increased. This is because the diameter of the field of view decreases as magnification is increased.

5. You should always begin focusing using the ___________________________ objective lens. Why is this so?
   Always begin focusing using the scanning or low power objectives. These lenses are short enough that there is no danger of making contact between the lens and the glass of a slide or coverslip while focusing. It will prevent damage to lenses and prepared slides.

6. Why is it not advisable to use the coarse focus adjustment knob when observing specimens with the oil immersion lens?
   Use of the coarse focus adjustment knob while using the oil immersion lens is a good way to break coverslips, particularly on prepared slides. Coverslips are very thin, break easily, and make viewing extremely difficult when broken. Prepared slides are expensive to replace. Depending on how the microscope is adjusted, it might also be possible to bring the oil immersion lens into contact with the substage-condenser lens. This might cause damage to both lenses. Objective lenses are very expensive!

7. Why should the high-dry objective be rotated away from a drop of oil on a slide or cover slip rather than through it?
   If the high dry objective is moved through an oil droplet on the stage it will probably contact the oil and become coated. This layer of oil will have to be cleaned off before the lens can be used for viewing, and if it is not cleaned off, will cause permanent damage to the objective. It is to your advantage to avoid bringing the high dry objective into contact with immersion oil.

8. What happens to depth of field as magnification is increased? **Depth of field decreases as magnification is increased. Threads observed with the 4X objective all appear to be in focus at the same time because the depth of field is great. Threads observed with the 45X objective appear to be arranged in layers (which they are) because the depth of field has been significantly decreased.**

Microscopic Measurement:

1. What is the significance of calibration and how is it accomplished in this laboratory?
   Calibration is the process of comparing a measuring device to a known standard, and is necessary if you wish to assign units to the measuring device. It is necessary to calibrate the ocular micrometer (compare it to the known units on the stage micrometer) because the divisions represented on the ocular micrometer are arbitrary in size, and cannot be expressed in known units without calibration.

2. Why must the ocular micrometer be recalibrated for each new objective used?
   Recalibration is necessary at each new magnification, because the distances between the lines on the ocular micrometer change as magnification is changed.

3. In figure 2.3 above, what is the absolute length in microns covered by 5 ocular divisions?
   Five microns.

4. Does the scanning (4X) lens actually magnify objects 4 times?
   No, data show that most of our 4X objectives magnify objects 4.5 times.

5. Approximately, what is the size of a *Paramecium* (width and length)?
   The size is somewhat variable, but for *Paramecium caudatum* the range is around 50 microns in width, and 150-200 microns in length.

6. What is the size of a rod-shaped bacteria cell (width and length)?
   The size is variable depending upon the type of cell, but large bacilli (genus *Bacillus*) are around 1-1.5 microns in width, and may be up to 7 microns in length. Bacteria such as *E. coli* are about .5 microns wide and 1-1.5 microns in length.

7. What type of cell does a *Paramecium* have, and what type does a bacterium have? Which cell type (eukaryotic or prokaryotic) is generally larger?
   The *Paramecium* is a type of eukaryotic cell, and the bacterium is an example of a prokaryotic cell. Prokaryotic cells are generally much smaller than eukaryotic cells.

Culture of Microorganisms and Media Preparation:

1. What advantage is there to growing microorganisms in broth or liquid media?
   Bacteria grown in broth media have more consistent contact with the nutrients available to them, so may grow faster. It is often easier to obtain an accurate representation of cell shape and arrangement when bacteria are grown in broth or liquid media. Flagellated forms are more likely to form flagella in broth than on solid media.

2. What general type of organism might you expect to produce a pellicle growth form, a clouded medium, or sediment at the tube bottom? (Refer to the section of your textbook covering oxygen requirements and microbial growth.)
   Obligately aerobic organisms will tend to produce pellicle growth forms, facultatively anaerobic organisms will produce clouded media and those that are obligate anaerobes or microaerophils will tend to produce sediments; however, motility also influences growth form to some extent, and so does the type of medium used.

3. What is agar, and why is it an ideal solidifying agent for microbiological media?
   Agar is a polysaccharide produced by marine algae (Phylum Rhodophyta), and is added to liquid media to make solid media. It is ideal for laboratory use because most bacteria will not utilize it as a food source (will not digest it) and because it is solid at room and most incubator temperatures.

4. In what ways do defined media differ from complex media?
   Defined media contain nutrients in relatively pure chemical form and in specified amounts while complex media contain nutrients in crude form and in unknown amounts. Because defined media typically include a large number of constituents, they are often more time consuming to prepare than are complex media.

5. Why are culture media normally sterilized prior to use?
   Culture media are normally sterilized prior to use to insure that there are no unknown organisms living in or on them prior to inoculation. This allows investigators to work with pure cultures of known types of organisms (assuming they use aseptic technique).

6. How many different types of bacteria appear to be growing on the plate you exposed to air?
   The answer here is quite variable and will be influenced by the environment (indoors VS outdoors, pets and children present or not, etc.), and how long the plate was actually exposed. If the medium dries out due to excessive exposure, growth will be limited.

Aseptic Technique and Streak Plate Preparation

1. What is Aseptic technique? When do we use it in this laboratory?
   Aseptic = without sepsis - This means essentially without infection or contamination. In this laboratory, it is a technique used to manipulate desired microbial cultures without introducing unknown contaminants and without infecting students. We use it <u>always</u> when we are working with living bacterial or fungal cultures.

2. What is the function of flaming the mouths of tubes prior to and following inoculation?
   Flaming the mouths of culture tubes will kill any microbes present on the surface of the glass, and also creates convection currents (air currents) that act to keep possible air contaminants out of the tubes while they are open.

3. What is the objective of streaking microbial cultures over a broad agar surface?
   The function of streaking microbial cultures over a broad agar surface is to dilute the cultures, or reduce the number of cells in any given area. Since each cell in contact with the medium has the potential of producing a colony (is a colony-forming unit), well-isolated colonies can only be obtained if the cultures are properly diluted.

4. Why are Petri plates normally incubated bottom-side up?
   Plates are incubated bottom-side up because water from the medium (which is poured while hot) condenses on the plate lid. If the plates are incubated lid-side up, this water can drip onto the agar surface causing confluent growth in the place of well-isolated colonies.

5. Where are labels applied to plates containing microbial cultures and why?
   Labels are always applied to the bottom of the plate (not the lid). If the lid should be removed, and replaced with a different lid, the label will stay with the culture, and not be mixed up. Since plates are incubated bottom-side up, the labels are visible during incubation and labels on the bottom do not block the view of colonies on the agar surface.

Pure Culture Technique

1. What is a pure culture and what method is most commonly used to obtain a pure culture?
   A pure culture is one containing only one type of organism (one species). The method most commonly used to obtain pure cultures is to streak agar plates, look for variation in colony morphology and to streak new plates using cells taken from well-isolated colonies.

2. What is a bacterial colony and what is meant by colony morphology?
   A bacterial colony is a mass of cells growing on solid medium (in most cases), and readily visible to the naked eye. Individual cells cannot be seen, but the colonies they form when they reproduce, can. Colony morphology refers to the cultural characteristics of colonies (what they look like), including form, margin, elevation, surface texture, optical character, pigmentation and size.

3. What does the term punctiform refer to?
   The term punctiform refers to colony form, and applies to tiny colonies that appear as pinpoints on the medium surface. If any colonies produced by a pure culture are 0.5-1.0 mm or larger in diameter, the culture's form is not punctiform.

4. Under what circumstances would you know if the optical character of a colony was fluorescent or bioluminescent?
   Fluorescent colonies will show their optical character only when exposed to ultraviolet light, and the light produced by bioluminescent colonies is only visible when the colonies are observed in the dark.

5. Which method of streak plate preparation (streak from a broth mixture or directly from a colony on agar) seemed to yield the best results? What explanation can you give for the variation observed?
   The answer is variable; however, cultures that contain high populations of viable cells will often be more readily isolated if they are diluted in broth before being streaked onto an agar plate. Bacteria that arrange themselves in chains may also be isolated more readily if the chains are broken up in broth before a plate is streaked.

Staining of Microorganisms – Part A, Direct VS Indirect Staining:

1. How do direct stains differ from indirect stains in terms of ionic composition (the charge associated with their chromophore) and their interactions with cell surfaces, i.e., how they work? **Direct stains have color associated with cations (have positively-charged chromophores) while indirect stains have color associated with anions (have negatively-charged chromophores). Direct stains color the cells, leaving the background uncolored while indirect stains color the background areas between cells, leaving the cells uncolored.**

2. Why is it important to form bacterial smears by mixing cells with a small amount of liquid and spreading them over a slide surface? **In order to accurately determine cellular morphology (shape, size and arrangement), it is necessary to see numerous individual cells separated from one another by a contrasting background. If the cells are too densely packed or piled on top of each other, their morphological features cannot be observed.**

3. What are two functions of heat-fixing bacterial smears prior to staining? **Heat-fixing kills bacteria, so if the culture contained potential pathogens, the cells are dead and no longer infectious. Heat-fixing also sticks the cells to the glass so they will not rinse off during staining procedures.**

4. What is the approximate size relationship between the prokaryotic and eukaryotic cells found in a cheek cell preparation?
   The eukaryotic (cheek cells) are much larger (100x) than the prokaryotic (bacteria) cells.

5. Were all the cells associated with your morphological unknown the same shape? Were they all the same size? Was their arrangement consistent? **Answers will be variable here, but the following points should be considered. Although many types of bacteria tend to have consistent shapes (all cocci or all bacilli), some bacteria are pleomorphic and able to form cells of various shapes. The cells of a bacterial population will show variation in size (just as other living organisms vary in size) and arrangements are often inconsistent. It is important to observe multiple regions of your unknown slide preparation before answering these questions.**

Staining of Microorganisms – Part B, Gram Stain:

1. What is a differential stain and when might such a stain be used?
   A differential stain is one that causes cells to look different even though all cells are treated the same way. They allow for the differentiation of cells (bacteria or other microorganisms) on the basis of their chemical or physical properties. This type of staining is very useful in the identification of unknown bacteria, and is used routinely in this lab.

2. What is peptidoglycan, what is its composition and where is it found? **Pedptidoglycan is a complex macromolecule composed of N-acetyl muramic acid, N-acetyl glucosamine and amino acids, some of which are unique in nature, i.e., not found in proteins or in other types of organisms. Peptidoglycan is found in the cell walls of many types of bacteria but is not found in archaea or eukaryotic organisms.**

3. How does the cell wall of a typical Gram-positive bacterium differ from that of a typical Gram-negative bacterium? **The cell walls of Gram-positive bacteria contain thick peptidoglycan and have no outer membrane, while the cell walls of Gram-negative bacteria have thin peptidoglycan layers and are surrounded by an outer membrane of complex composition.**

4. What is an important feature of the lipopolysaccharide (LPS) found in association with Gram-negative cell walls? **The Lipopolysaccharide found in the outer membrane of many Gram-negative bacteria is toxic to mammals and can cause disease symptoms even after the bacteria are dead.**

5. What is the function of a mordant?
   A mordant is a substance that increases the affinity or attraction between cells and the dye being used.

6. What would you observe if you forgot to apply a counterstain while making a Gram stain of a Gram-negative bacterial culture? **You would probably not be able to see any cells, because they would all be colorless. Gram-negative cells that have not been counterstained will not contrast greatly with their white background and so will be very difficult to observe.**

7. What were the Gram stain characteristics and morphological features of the cultures you observed? **The answer is variable; however, if you followed procedures accurately, you should have seen Gram-positive cocci and large bacilli (with endospores), plus tiny Gram-negative bacilli.**

Staining of Microorganisms – Part C, Acid-Fast stain:

1. In what ways is the acid-fast stain similar to the Gram stain?
   Both the Gram stain and the acid-fast stain are basic stains and both are differential stains that cause differences in cell wall composition to be visible as color differences.

2. What is mycolic acid and how does it influence staining?
   Mycolic acid is the wax-like lipid present in the walls of acid-fast cells. This material makes cells resistant to staining, but also makes them resistant to being decolorized once they have been successfully stained.

3. Are Gram-negative bacteria likely to be acid-fast?
   All acid-fast bacteria are Gram-positive, so no, Gram-negative bacteria are not likely to be acid-fast.

4. What would you expect to observe if you decolorized an acid-fast stain preparation with acetone-alchol instead of acid-alcohol?
   Acetone-alcohol is the decolorizing reagent used in the Gram stain. If this material is used to decolorize cells stained with carbol fuchsin, the red color will not come out of any cells, and non-acid-fast cells will appear to be acid-fast. It is important to use the correct decolorizing reagents when preparing different types of differential stains.

5. What color do the acid-fast cells appear when stained with the acid-fast stain preparation?
   Acid-fast cells stain red, while other cells appear blue (when methylene blue is used as the counterstain).

Staining of Microorganisms – Part D, Endospore Stains, Capsule Stains & Flagella:

1. What are bacterial endospores? What shape were the endospores you were able to observe, and where were they located within the cells? (Central or terminal)
   Endospores are dormant structures produced within certain types of bacteria, and are very resistant to heat, drying, radiation and various chemical agents. They allow bacteria to survive conditions that would be lethal to vegetative cells. Endospores generally appear as round (spherical) or oblong (ellipsoidal) structures within cells. They may be located centrally or may be terminal, and sometimes distend the cell to a noticeable extent.

2. What is a sporangium? Were the sporangia visible in your preparation swollen, or did they retain the shape of non-sporulating vegetative cells?
   A sporangium is a cell that has undergone sporulation and contains an endospore (See pg. 1106-1107 Bergey's Manual Vol. 2). The answer is variable depending upon what types of cells were observed.

3. What is a glycocalyx? How do bacterial capsules and slime layers differ from one another?
   A glycocalyx is a layer of material deposited outside the bacterial cell wall. They are often composed of polysaccharides, glycoproteins, or polypeptides, and are not heat stable. Capsules tend to be dense and well organized (readily stained) while slime-layers are loose and diffuse. Capsules are present only on certain bacteria and may or may not be formed depending upon the type of medium the organisms are grown in or on. *Klebsiella pneumonia* grown in milk or on lactose agar usually produces visible capsules.

4. Describe the size and location of an endospore and a capsule relative to a vegetative cell.
   An endospore occurs inside a cell and may or may not cause the sporangium to be swollen (spores are often slightly smaller in diameter than the cells in which they form, but may attain a size about half again as wide). A capsule is found outside the cell wall. Capsules vary in thickness, but often appear to be about twice the thickness of the cell they surround. Microcapsules (which are much thinner) may also be observed.

5. What were the arrangements of the flagella you observed?
   Flagellar arrangements are variable; however, polar (amphitrichous) flagella occur at the ends of a cell while peritrichous flagella are distributed fairly uniformly all over the cell surface.

Enrichment for Selected Bacteria from the Environment and Introduction to Cyanobacteria:

1. What is an enrichment procedure or medium? **An enrichment procedure or medium is one designed to promote the growth of certain organisms while inhibiting the growth of others.**

2. Can you explain the enrichment procedures used in this exercise to culture three different genera of bacteria from a single soil sample? (Be able to!)
   A typical soil sample contains a wide variety of bacteria. It is possible to enrich for specific types of organisms by utilizing selective media or techniques as follows:
   a) For the *Pseudomonas* enrichment we used a medium containing sodium benzoate as the only carbon source. Sodium benzoate is used as a preservative, and is a carbon source few bacteria can utilize because it is toxic. Many species of *Pseudomonas* can use sodium benzoate as a carbon source because they have such a wide nutritional range, i.e., can use many unusual organic compounds for carbon and energy. In at least some species, genes carried on plasmids determine this ability.
   b) For the *Azotobacter* enrichment, we used a medium lacking nitrogen. This is because *Azotobacter* are capable of "fixing" nitrogen from the atmosphere, something relatively few bacteria can do.
   c) The enrichment for *Bacillus* involved the use of pasteurization, because bacteria in the genus *Bacillus* form endospores that are thermoduric (can tolerate exposure to heat), while vegetative cells are killed. *Bacillus* endospores can survive pasteurization and new vegetative cells will grow from them once the soil is cooled and the proper nutrients are made available.

3. Were members of the genus *Azotobacter* the only types of bacteria observed to be growing on the nitrogen-free medium?
   The answer will be variable here, but generally no. It is more common to find multiple different genera of nitrogen-fixing bacteria on our nitrogen-free medium.

4. Do you think *Pseudomonas*, *Azotobacter* and *Bacillus* were the only types of bacteria present in your soil sample? Why or why not?
   No, the above-mentioned bacteria were not the only ones present in the soil samples. Soil typically includes a wide variety of genera including *Streptomyces*, *Mycobacterium*, *Clostridium*, *Corynebacterium*, *Arthrobacter* and many other forms.

5. What is bioluminescence and what enzymes are associated with this phenomenon?
   Bioluminescence is the ability of certain organisms to convert chemical energy into light energy and thereby emit light. The enzymes associated with this phenomenon are generally called luciferases, after Lucifer, the angle of light.

6. How do the cyanobacteria differ from most of the eubacteria you have been working with in lab?
   Generally the cyanobacteria are larger than most other bacteria (eubacteria). In addition to this, they often form unique features such as trichomes (cells in parallel chains) heterocysts and akinetes. In our slide boxes the cyanobacteria are stained variously to appear greenish, blue, purple or pink. If Gram stained, they would all appear pink (Gram-negative).

7. How do the cyanobacteria differ from eukaryotic algae?
   The cyanobacteria are prokaryotic cells, so they tend to be smaller, and they lack membrane bound organelles such as chloroplasts and nuclei.

8. Do any of the cyanobacteria you observed display motility? Do they possess flagella?
   Wet mounts of *Oscillatoria* are usually motile, but they have a gliding motility, and do not swim. The cells of cyanobacteria are not flagellated.

Introduction to Fungi (mycology):

1. What are hyphae, rhizoids, haustoria, mycorrhizae and conidiophores?
   Hyphae (singular hypha) are the microscopic thread-like filaments that make up the mycelium of a mold-type fungus. Rhizoids are specialized (root-like) hyphae that attach some fungi to their substrate or to surfaces such as glass. They secrete digestive enzymes and absorb nutrients. Haustoria are specialized hyphae of parasitic fungi that penetrate the cells of host organisms and absorb nutrients from them. Mycorrhizae are specialized hyphae of fungi that form symbiotic relationships with plant roots. Mycorrhizae help plants obtain minerals and water from soil. Conidiophores are specialized hyphae that support conidiospores.

2. What are the distinctive features of the various phyla of fungi observed?
   The fungi phyla observed in this laboratory can be distinguished on the basis of the types of sexual reproductive structures produced. Examples of four different types of sexual reproductive structures were provided, each of which was quite distinctive from the others.

3. From your observations, would you conclude that the formation of spores occurs more frequently among the molds or the yeasts?
   Mold-type fungi produce spores more frequently than yeast-type fungi since yeasts reproduce primarily by budding and not by forming spores (buds can also be called blastospores).

4. The yeasts used in baking (*Saccharomyces*) belong to which phylum of fungi? ***Saccharomyces* belong to the phylum Ascomycota.**

5. If you had a mixed culture of fungus and bacteria, what selective plating procedure could you use to isolate each in a pure culture?
   In order to separate fungi and bacteria it would be helpful to use more than one type of medium, and to prepare a number of streak plates. Media that are somewhat acidic would tend to select for fungi, while neutral media would tend to promote the growth of bacteria. If you were selecting for spore-forming bacteria you could pasteurize the inoculum before it was streaked onto a plate.

6. Which do you think you would be more likely to observe in the natural environment, cellular or true slime molds?
   True slime molds.

An Introduction to Microscopic Algae:

1. Which of the living algae you observed demonstrated motility? What was their mechanism of locomotion?
   Living *Volvox*, *Haematococcus* and *Euglena* are motile via flagella, diatoms move by means of a gliding motion produced by protoplasmic streaming.

2. Which of the algae are unicellular in form? Which are filamentous?
   ***Euglena*, *Haematococcus*, *Chlamydomonas*, *Chlorella*, and the various diatoms are typically unicellular. *Spirogyra*, *Ulothrix*, and *Oedogonium* are filamentous. *Cladophora* form branching filaments, *Volvox* is colonial, and *Hydrodictyon* is net-like.**

3. Which of these algae contain green chlorophyll pigments?
   All algae contain chlorophyll pigments.

An Introduction to Protozoa:

1. Most protozoa are nutritionally categorized as ____________________ but some forms are capable of using light energy and inorganic carbon. Which protozoa often contain chloroplasts and function as photoautotrophs? **Most protozoa are nutritionally categorized as chemoheterotrophs and feed on other organisms much as animals do. The euglenoids often contain chloroplasts and function as photoautotrophs.**

2. Protozoa are single-celled organisms with eukaryotic cells. What types of organelles are readily visible within or on these organisms?
   Organelles visible usually include nuclei, food vacuoles, contractile vacuoles, cilia, and flagella.

3. In which living forms are contractile vacuoles evident? What is their function? **Living forms such as *Amoeba*, *Paramecium*, and a number of other protozoa typically contain contractile vacuoles, but these structures are most readily observed only in the larger, slower moving forms. Contractile vacuoles are excretory in function, allowing organisms living in hypotonic environments to rid themselves of excess water. They also aid intracellular circulation of fluids and aid excreation of liquid waste.**

4. Organisms called *Trichonympha* are categorized within which protozoan phylum? Where are these organisms found?
   The *Trichonympha* belong to the phylum Parabasala and are found in termite guts.

5. Which protozoa have more than one nucleus? **The Diplomonadids have two equal-sized nuclei and many ciliates have both macro and micronuclei.**

Introduction to Multicellular Parasites:

1. Microscopic invertebrates are similar to protozoa and algae in that they are composed of ________________________ type cells.
   Microscopic invertebrates are similar to algae and protozoa in that they are composed of eukaryotic type cells.

2. What types of organisms fall into the category generally identified as helminthes?
   The term helminthes refers to organisms that are classified as Platyhelminthes (flatworms) or Aschelminthes (roundworms).

3. Name two general types of flatworms (Platyhelminthes) that are known to infect humans.
   Flukes (Class Trematoda) and tapeworms (class Cestoda) are two general types of flatworms that infect (infest) humans.

4. What is an endoparasite; what is an ectoparasite, and which of the parasites listed falls into each category?
   Endoparasites live primarily within their hosts. They inhabit various regions of the body (in various organisms) during various stages of their life cycles. Flatworms and roundworms are endoparasites. Ectoparasites live outside the hosts, and usually penetrate the skin in some manner in order to obtain nutrients. Fleas, ticks, lice, and mites are examples of the ectoparasites observed in this laboratory. Mosquitoes and various other flies are also ectoparasites.

Food Microbiology - Fermentation - Sauerkraut, Wine, & Rootbeer:

1. The type of fermentation a food or beverage undergoes is determined primarily by? .
   The pH and carbohydrate content of the food or juice as well as the organisms present. In the case of sauerkraut, the salt content is also important because the hypertonic environment restricts the growth of many organism types.

2. The fermentative bacteria responsible for the formation of sauerkraut are?.
   According to texts, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, and *Lactobacillus plantarum* are found in sauerkraut, but other fermentative organisms including *Lactococcus* species may be present.
   What fermentation product is responsible for the flavor of the sauerkraut?
   The fermentation product responsible for the sour flavor of sauerkraut is lactic acid.

3. The fermentative organisms used in the production of our "wine" and root beer were? Are these organisms homofermentative or heterofermentative?
   ***Saccharomyces cerevisiae* are the organisms involved and they are heterofermentative.**

4. The metabolic pathway used by *Saccharomyces* in the fermentation of wine and root beer yields what end products?
   ***Saccharomyces* yield carbon dioxide and ethyl alcohol (ethanol) when fermenting sugars used in the production of wine and rootbeer. They also form acetaldehyde, but it serves as a final electron acceptor and is converted into ethanol.**

Food Microbiology - Cultured Foods - Cheese and Yogurt:

1. What is rennet, and what is it used for in the production of cheese?
   Rennet is a commercially prepared product containing the enzyme rennin, which catalyzes the breakdown of the milk protein casein. In cheese production, this enzyme is added to milk in order to initiate coagulation (the formation of curds) so that most of the liquid can be removed.

2. What are curds and whey? What is casein?
   Curds are the solid portions of the milk formed during coagulation. The whey is the liquid material that remains. Casein is an important protein associated with milk.

3. What is the difference between a ripened and an unripened cheese?
   A ripened cheese is one that is allowed to age for a period of time following the initial processing steps, so the organisms present are allowed to grow and continue to ferment. Ripened cheeses also often have the growth of various surface flora (e.g., fungi) encouraged. These organisms can greatly influence the flavor, aroma, and texture of the cheese. An unripened cheese is one that is a finished product as it comes from the initial processing steps.

4. What types of bacteria are characteristically present in commercially produced yogurt?
   Yogurt typically contains *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Note – The Bacilli are now identified as *Lactobacillus delbrueckii bulgaricus* in some references.

5. What bacterial products give yogurt its characteristic flavor and aroma?
   Lactic acid and acetaldehyde give yogurt its distinctive flavor and aroma.

6. What are the apparent health benefits of consuming cultured foods?
   Some investigations indicate that yogurt may decrease the problems associated with lactose intolerance, may reduce serum cholesterol, and may have anticancer effects.

7. Which food-borne bacteria can cause infection, and which can cause intoxication?
   Foodborne bacteria genera including *Escherichia, Salmonella, Vibrio* and *Listeria* cause infection, while *Staphylococcus, Bacillus* and *Clostridium* cause intoxication.

Determination of Microbial Numbers:

1. Why would a scientist/technician want to know how many viable bacterial cells are present in a sample? Give one example of a situation in which this information would be important to know.
   Viable cell counts are preformed routinely on materials intended for human use or consumption; water and milk for example. Though low numbers of certain bacteria are considered acceptable in milk arriving from various daries, *Salmonella* species are not tolerated. Viable cell counts are also used in research and clinical laboratories.

2. If the initial inoculum used at step 4 (above) was 1.0ml rather than 0.1ml, what degree of dilution would be reached in the third water blank?
   The third water blank would contain a 10^{-6} dilution.

3. Approximately how many viable bacterial cells were present in 1 ml of your original bacterial culture?
   The answer is variable, but is determined by multiplying the number of colonies on the plate by the dilution factor of the plate.

4. Do all bacterial cultures (in the stationary phase of growth) contain similar numbers of cells? What explanation might be given for the variation in population observed?
   Data suggest that there is variation in the maximal concentration (m-concentration) of cells present in one ml of broth culture, but typical numbers would be in the range of 10^8 or 10^9 cfu/ml. Cell size is one factor that can influence the number of cells an environment can support. The carrying capacity of the environment (and therefore the m-concentration of the culture) will be lower if the cells being grown are large.

Use of Selective and Differential Media & Replica Plating Techniques:

1. Which types of bacteria can grow on MacConkey agar, on EMB, on T-7? Why?
   MacConkey, EMB, and T-7 agars are all designed for use in the differentiation of Gram-negative bacteria. Most types of Gram-positive organisms will not grow on these media because they are inhibited by one or more of the substances present. MSA is used for the differentiation of various genera of *Staphylococcus*. This medium is too hypertonic for the growth of most microorganisms because it contains 7.5% salt.

2. What morphological differences can you detect between the colonies growing on MacConkey's agar and EMB? What do these morphological differences indicate?
   Gram-negative bacteria growing on MacConkey's agar appear opaque pink if they are lactose-positive (can ferment lactose), and "clearish" if they are not. Colonies on EMB appear dark, or have dark centers, often with a metallic green sheen (iridescent green), if they are lactose-positive, and are "clearish" if they are not. These differences demonstrate that morphological features (phenotypes) of organisms can be influenced by their environment.

3. Which media would you use to isolate the following organisms: *Staphylococcus*, *Streptococcus*, *Shigella* and *Neisseria*.
   Mannitol Salt Agar (MSA) is often used in the isolation and differentiation of various genera of *Staphylococcus*. This medium is too hypertonic for the growth of most microorganisms because it contains 7.5% salt. Streptococci will not usually grow on this medium; however, several species in the genus *Micrococcus* will. Staphylococci and Streptococci will grow nicely on blood agar and can usually be distinguished from one another on the basis of colony size, optical character and hemolysis reaction. *Shigella* species can be isolated on either HEK or XLD since these media inhibit Gram-positive bacteria and tend to promote the growth of *Shigella*. *Neisseria* are often isolated on chocolate agar since this medium provides the nutrients necessary for growth of these fastidious organisms. In this laboratory we typically use either blood agar or Brain Heart Agar for the growth of *Neisseria* because we do not have chocolate agar.

4. What aspect of blood agar makes it a differential medium?
   Blood agar contains whole RBCs and the degree to which these are acted upon by various types of organisms makes it a differential medium. Cells that can lyse the RBCs will form clear zones in the agar (β-hemolysis), those able to convert hemoglobin to met-hemoglobin will turn the agar greenish in color (α-hemolysis). Bacteria that have no influence on the RBCs show no hemolysis (γ-hymolytsis).

Physiological Characteristics of Bacteria - Carbohydrate Metabolism, MR-VP, Catalase, and Oxidase:

1. How would you interpret the results of an O/F test if the sealed tube showed no change in color and the unsealed tube was yellow only near the surface of the medium and not within the Durham tube?
   The organisms present are obligately aerobic and cannot utilize the carbohydrate present under anaerobic conditions (they cannot ferment). They are able to produce some aerobic acid when utilizing the carbohydrate by means of respiration.

2. How can you determine if or not organisms are able to ferment a specific carbohydrate with the production of acid and gas in a tube of solid media containing phenol red as the pH indicator?
   Agar tubes containing phenol red are red when the medium is neutral (prior to inoculation) and turn yellow if the pH is reduced (acids are produced). There is no way to determine exactly what type of acid/acids are being produced; we only know that there has been a significant reduction in the pH if the medium turns yellow. Gas production is indicated by the formation of cracks or bubbles in the agar or if the agar has been lifted off the tube bottom. A shiny layer along the stab line can also indicate gas production (gas may be CO_2 or H_2).

3. How would you determine that gas production had taken place if you were using a broth medium?
   Gas formation within a tube of broth medium is usually determined by placing a small inverted glass tube (Durham tube) within the medium. Gas is indicated by the presence of bubbles within this tube. As gas is produced, it rises toward the surface, and some is trapped within the Durham tube.

4. What carbohydrate fermentation tests could you use to test for the formation of acetylmethylcarbinol or acetoin?
   The medium used in this laboratory to test for the formation of acetylmethylcarbinol (acetoin) is Methyl Red - Voges Proskauer (MR-VP) medium. The carbohydrate fermented is glucose, and the test reagents used are Barritt's solutions A and B.

5. What is esculin and what happens to a slant of esculin agar if the organisms growing there can hydrolyze esculin?
   Esculin is a glycoside and if organisms growing on an esculin slant can hydrolyze esculin the slant will turn dark gray or black in color.

6. Describe the reaction you observed when you subjected your culture to 3% hydrogen peroxide. Is *Staphylococcus aureus* catalase-positive or catalase-negative?
   Samples of the bacteria *Staphylococcus aureus* subjected to 3% hydrogen peroxide usually bubble vigorously such that the cells are dispersed into the reagent, and the visible mass of bacteria disappears. This is a positive catalase reaction.

7. What is being tested for in the oxidase test? Is *Escherichia coli* oxidase-positive or oxidase-negative? How do you know?
   The oxidase test is designed to test for the presence of cytochrome C, an enzyme essential to oxidative metabolism. *Escherichia coli* are oxidase-negative. This is made evident by the lack of reaction observed when a mass of *E. coli* is brought into contact with the oxidase test reagent. No color change is observed.

Physiological Characteristics of Bacteria Continued - Amino Acid Decarboxylation, Citrate Utilization, and Coagulase Test:

1. Why is it necessary to run a control tube containing a carbohydrate base without any amino acid whenever you are testing a cultures' ability to decarboxylate amino acids?
   The control tube contains the same carbohydrate that is present within the amino acid tube, but lacks the amino acid. For this reason, it gives a clear indication of whether or not the bacteria being tested can ferment the carbohydrate. If the carbohydrate cannot be fermented (control tube stays purple) the amino acid test is invalid, because there is no way to determine if the purple color is due to amine formation, or if it is due to no reaction at all. Note that it is essential to inoculate both the control tube and the amino acid tube with a visible amount of the bacteria being tested, and that both tubes must be sealed with vaspar.

2. What is vaspar. Why is it necessary to use this material whenever an amino acid decarboxylation test is being conducted?
   Vaspar is a mixture of Vaseline and paraffin (at a ratio of about 50:50). It is used during amino acid decarboxylation tests to seal the tubes and thus prevent the escape of volatile amines. Without some type of seal, most of the amines produced will leave the solution, and the test results will appear negative. The vaspar seal also keeps oxygen out of the medium, so the environment within is anaerobic. This will force the culture present to ferment if they are capable of doing so.

3. What is the pH indicator present in Simmons Citrate agar? What happens to the pH of this medium when the citric acid is utilized? What enzyme is involved?
   The pH indicator present in Simmons citrate agar is bromothymol blue. This indicator appears greenish in neutral media, but turns a deep blue if the pH increases. This medium contains sodium citrate as the only carbon source. If bacteria are able to take in citrate there is a related reaction resulting in the formation of ammonia (thus a rise in pH) and the agar turns blue. The enzyme involved in citrate transport is called citrate permease.

4. What is coagulase? What does a positive coagulase reaction look like?
   Coagulase is an enzyme produced by certain bacteria that catalyzes the coagulation of blood plasma. Since blood plasma is normally liquid, a positive reaction is indicated by a change in the plasma from liquid to solid.

5. What is the CAMP test and how does it work?
   The CAMP test is a diagnostic test used to identify certain types of pathogenic bacteria. It indicates if or not the hemolytic ability of these bacteria is increased when they are exposed to the β-hemolysin of *Staphylococcus aureus.* Organisms that show enhanced hemolytic ability in the presence of β-hemolysin are said to be CAMP-positive. The zone of enhanced hemolysis typically shows up as an arrowhead-shaped clear area within the region being partially hemolyzed by the β-hemolysin when *Staphylococcus* and other organisms are placed on a blood agar plate as described in this exercise.

Physiological Characteristics of Bacteria Continued - Gelatin Liquefaction, Urea Hydrolysis, TSI and SIM:

1. Hydrogen sulfide (H_2S) is produced by certain bacteria that are able to catabolize what type of organic compounds?
   Hydrogen sulfide is produced by certain bacteria that are able to catabolize sulfur-containing amino acid molecules, e.g., cysteine and methionine.

2. The presence of hydrogen sulfide is indicated in TSI and SIM media by the formation of what?
   The black precipitate present is iron sulfide and forms when the sulfur from H_2S binds with iron in the medium.

3. Indole is produced when the amino acid ____________________ is degraded by certain bacteria?
   Tryptophan
   What enzyme is involved? **Tryptophanase**
   What reagent is used to test for the presence of indole? **Kovac's reagent**

4. How can you determine if or not organisms are motile by observing their growth pattern in SIM media?
Bacteria that are non-motile cannot "swim" through the SIM medium, and so grow only along the line of inoculation. If a distinct line is observable, the organisms in question are not motile. Motile organisms will distribute themselves throughout the medium, and no line of inoculation will be visible. If motile organisms form H_2S, iron sulfide will be throughout the medium.

5. What pH indicator is present in urea agar? What chemical change is responsible for the color change apparent in this medium when urea is catabolized? What enzyme is involved?
Urea agar contains the pH indicator phenol red. This indicator appears as a peach color prior to inoculation (pH 6.8), and becomes "hot" pink if the urea present is catabolized. The hydrolysis (catabolism) of urea yields ammonia, which is basic (pH 8.1 or greater), and this is responsible for the color change. The enzyme involved is called urease.

6. What do some bacteria use nitrate for?
Some respiratory organisms, e.g., *Pseudomonas aeruginosa*, can use nitrate as a final electron acceptor in the place or molecular oxygen.

7. What two enzymes can be tested for with the Nitrate reduction test? What types of end products are formed by the catalytic activity of these enzymes?
The two enzymes being tested for are nitrate reductase and nitrite reductase. The catalytic activity of nitrate reductase results in the formation of nitrite from nitrate, and the activity of nitrite reductase results in the formation of nitrogenous gasses.

8. What is the effect of zinc on nitrate?
Zinc particles added to nitrate medium will cause the reduction of nitrate to nitrite.

Application of the Polymerase Chain Reaction in Bacterial identification

1. What is the PCR and what is it being used for in this exercise?
The polymerase chain reaction is a unique process that allows specific segments of DNA to be amplified (replicated millions of times) in vitro within a few hours. It is an extremely powerful tool with multiple applications in biotechnology. In this exercise, the PCR is being used to amplify segments of 16S ribosomal DNA from bacteria.

2. What are oligonucleotide primers, and why are they necessary in the PCR, i.e., what function do they serve?
Oligonucleotide primers are short segments of DNA (or RNA) usually 18-20 bases in length, that are able to hybridize with (anneal to) specific regions of DNA. They provide the free 3' ends required by Taq polymerase to build new complementary strands of DNA. They also make the DNA a duplex, which is necessary in order for the DNA polymerase to bind and build new DNA. They also determine which segments of DNA will be amplified and their composition determines the anneal temperature to be used during the PCR.

3. What is *Taq* polymerase and where does it come from?
***Taq* polymerase is a type of enzyme (DNA-dependent DNA polymerase) produced by hyperthermophilic bacteria (*Thermus aquaticus*) that live in the hotsprings of Yellowstone National Park. This enzyme is frequently used to replicate DNA in polymerase chain reactions.**

4. Why must the DNA polymerase used in PCRs be thermostable?
The polymerase chain reaction requires that DNA be heated to around 94°C, multiple times (around 30-35) during processing. Exposure to such high temperatures will often inactivate (denature) enzymes. If the *Taq* polymerase were not thermostable, the PCR would not work.

5. When the polymerase chain reaction is used to amplify DNA in vitro, the components of the reaction mixture are subjected to three different temperature settings during each reaction cycle. These temperatures cause DNA to be denatured, to anneal and then to extend. Explain what is occurring during each of these steps.
When DNA is denatured, the hydrogen bonds between complementary bases break and the two strands of the double helix separate (come apart). Primers anneal to template DNA, which means they hydrogen-bond with or hybridize with the specific regions of DNA they are complementary to. Hydrogen bonds form between bases of primer and template DNA. Taq polymerase is responsible for extension. These enzymes add new nucleotides to the 3' ends of primers, so build new DNA strands in the 5' to 3' direction.

6. Why is amplification of 16S ribosomal DNA likely to yield a product that can be used in the identification/classification of unknown bacteria?
All known bacteria contain ribosomes and produce 16S ribosomal DNA. This DNA is highly conserved (has not changed much through evolutionary time) so serves as a good indicator of the evolutionary relationships between bacteria.

Automated Nucleotide Sequencing and Electropherogram Evaluation

1. What is automated nucleotide sequencing?
Nucleotide sequencing is the process of determining the sequence (arrangement) of nucleotide bases in a nucleic acid (DNA or RNA) molecule. Automated sequencing is accomplished by instruments called sequencers. These highly sophisticated instruments use the PCR to perform sequencing reactions with multiple samples (48-96) all at once, and will provide sequences 500 bp or more in length, with 98% accuracy, within a few hours.

2. What are dideoxynucleoside triphosphates (ddNTPs) and how do they affect DNA replication if they are incorporated into growing nucleotide strands?
A dideoxynucleoside triphosphate is an analog of a nucleoside triphosphate (e.g., ATP, GTP, CTP, etc.) that lacks a hydroxyl group (OH^-) on the #3 carbon of its sugar. When these molecules are incorporated into growing nucleotide strands during replication, they terminate the strand (stop the replication process) because new, incoming bases cannot form phosphor-diester bonds with them (they do not have the required 3' OH^- group).

3. What is an electropherogram?
An electropherogram is a four-colored chromatogram generated by an automated sequencing instrument. Each electropherogram displays sequencing results as a series of peaks accompanied by a computerized interpretation of this data presented as a text file.

4. Why is it necessary to edit nucleotide sequence data that has been obtained from an automated sequencer? **Although automated sequencers generate both peak data and text files, they cannot verify that their interpretation of peak data is correct, i.e., that the text file is a correct interpretation of the peak data. Human evaluation and editing is necessary to insure that sequencing results are correct.**

5. What are IUB-Codes and what do they represent when present within a nucleotide sequence? **IUB-Codes, determined by the International Union of Biochemistry, are single letter representations for individual nucleotide bases and the possible combinations of different bases in twos or threes. These make up the text file associated with electropherograms, and represent either single peaks (A, G, C, and T) or overlapping peaks (R, W, H, etc.). The letter N is used to represent any nucleotide.**

6. What portions of the data presented in an electropherogram are most likely to contain errors and therefore to require careful evaluation?
Data at the beginning of a sequence may lack peaks, or contain overlapping peaks, and data toward the end of a sequence contains poorly defined broad peaks with valleys that do not return to the baseline. Both of these regions tend to contain miscalled bases and must be carefully evaluated.

7. Why was it necessary to "flip" or "reverse" the 1492-Reverse (or 1530-Reverse) sequence before copying it to the word file? What would occur if you failed to do this?
Reverse sequences are constructed on primers that anneal to DNA strands complementary to those used for building Forward sequences. For this reason, unless Reverse sequences are "flipped" or "reversed" they cannot be combined with Forward sequences. It would be impossible to obtain a contiguous sequence of maximum length if the Reverse sequence was not "flipped" or "reversed" before it was copied.

Genomics, Proteomics and Bioinformatics.

1. What is a genome and how is it related to genomics?
The term genome is usually defined as the total DNA content of the chromosome(s) within a cellular organism. It may be RNA in some viruses and is RNA in viroids. Genomics is the study of genomes from multiple different organisms as a single functional unit, i.e., all at once.

2. What is a proteome and how is it related to proteomics?
The term proteome refers to the entire protein complement of a cell, an organism or a tissue type. Proteomes are subject to change over time. Proteomics is the study of proteomes from multiple organisms all at once and involves comparing proteins from different sources, along with their composition and functions.

3. What is the relationship between computer technology and a new science known as bioinformatics?
Bioinformatics is a new science that involves the application of computer technology to the study of various aspects of living organisms. Genomics and proteomics involve the analysis and comparison of tremendous volumes of information, and would not be possible without computer technology.

4. What do the letters NCBI stand for in association with this exercise, and what kinds of information are available at the NCBI website?
NCBI is the National Center for Biotechnology Information and is a public database containing a tremendous volume of information e.g., nucleotide and amino acid sequences obtained from a wide variety of organisms, the human genome project and scientific articles from multiple publications, many freely accessible.

5. What do the letters BLAST stand for and how was the BLAST algorithm used in association with this exercise?
BLAST = Basic Local Alignment Search Tool. The BLAST is an algorithm and was used to compare student nucleotide sequences with those available in the public database of NCBI.

The Miniscreen - Rapid Isolation of Plasmid DNA:

1. How does plasmid DNA differ from chromosomal DNA?
   Plasmid DNA occurs in loops that are much smaller than the loops formed by bacterial chromosomal DNA. (A plasmid model the size of a hula-hoop would compare fairly accurately to a chromosome the size of a standard college-size track).

2. What are cloning vectors, and what are they used for in genetic manipulation experiments?
   Cloning vectors (also called cloning vehicles) are pieces of DNA that can initiate their own replication (are replicons) when inserted into host cells (bacteria, yeast, or others). They typically carry multiple cloning sites (regions where they can be cut with restriction enzymes so that new genes can be added). Their function is to carry DNA into cells and reproduce it there. Small plasmids and bacteriophage are the most commonly used cloning vectors.

3. Why is it possible to separate plasmid DNA from chromosomal DNA using fairly simple centrifugation steps?
   Particles of significantly different sizes will tend to separate when subjected to centrifugation, because the larger, heavier particles settle to the tube bottom more quickly than will the smaller, lighter particles.

4. Why might investigators want to remove plasmid DNA from bacterial cells, i.e., what purpose does this procedure serve?
   Bacterial plasmids can be used as cloning vectors. Typically, investigators remove plasmid DNA from host cells, cut the plasmids open using restriction enzymes, insert pieces of DNA (genes) that are being investigated, and then introduce the plasmids into new cells. Since the plasmids replicate themselves (and because their host cells replicate rapidly), they can be used to produce large quantities of the particular genes being studied. Removing plasmid DNA from host cells is often the first step in genetic engineering or gene manipulation experiments.

Gel Electrophoresis of DNA Samples:

1. What is gel electrophoresis? What is this procedure used for?
   Gel electrophoresis is a procedure that uses an electrical current to separate DNA fragments into discrete size categories. In gel electrophoresis, the DNA fragments are first loaded into wells in a slab of agarose (or polyacrylamide) gel, and then subjected to an electrical current. The smaller DNA fragments travel faster through the gel, and so tend to move away from the larger fragments. The procedure has many applications, but in this laboratory is used to determine the degree to which restriction enzyme cleavage has been successful, to indicate the sizes of PCR product and plasmid samples, and to determine the sizes of restriction fragments carried by those plasmids.

2. Why do DNA molecules migrate when subjected to an electric current? In which direction do they travel (toward the anode or cathode)?
   DNA molecules migrate because they have a net negative change. They tend to travel toward the positive pole or anode.

3. Which of the following DNA fragments would you expect to travel a greater distance from the loading well within a given period of time; a 500 base pair fragment, or a 300 base pair fragment? Why?
   The 300 base pair fragment will travel the greater distance because it is smaller, and can more readily pass between the molecules in the gel.

4. What were the approximate sizes of the bacteriophage lambda DNA fragments generated during this exercise? Which of the various plasmids used is larger in size?
   Lambda DNA cut with HindIII will yield eight fragments that are 23,130, 9,146, 6,557, 4361, 2322, 2027, 564 and 125 base pairs in length. The plasmid pGEM is larger than pUC19, but pGLO is larger than both.

5. Is the sample of PCR product DNA larger or smaller than the plasmid samples used?
   The PCR product DNA is smaller than all plasmids used. It is only around 1500 bp in length.

6. Is it possible to distinguish between cut and uncut samples of plasmid DNA by observing these in an electrophoresis gel? If so, which will appear larger, and why?
   It is often possible to distinguish between cut and uncut plasmid samples in an electrophoresis gel because uncut plasmids can supercoil and cut plasmids cannot. Supercoiled DNA will often travel through a gel faster than an uncut sample, so will move a greater distance down the gel in the same amount of time. The uncut sample will appear to be larger.

7. What is the relationship between RFLP and DNA fingerprints?
   RFLP = Restriction Fragment Length Polymorphism, and is a technique or method that involves cutting DNA samples into multiple fragments with restriction enzymes and then running the fragments in a gel to generate a banding pattern. The pattern generated may be called a RFLP pattern, or a DNA fingerprint.

8. Did the number and sizes of RFLP bands observed in your gel match the expected number and size as determined through nucleotide sequence analysis? If not, suggest a reason why they did not?
   Answer is variable. Assuming the PCR product in the gel is the same size as the one provided on the Website , the fragment sizes should be the same; however, patterns generated in the gel will have less resolution. If fragments are of similar size (e.g., 210bp and 220bp), they will appear as one band instead of two in the gel. Also fragments of 75-50bp or smaller are likely to be invisible, i.e., they will not show in the gel.

Restriction Endonuclease Digestion of DNA and RFLP:

1. What are restriction endonucleases? Where are they normally found?
   Restriction endonucleases are enzymes that catalyze the "site-specific" cleavage of DNA molecules (they break phosphodiester bonds). They are normally produced, and therefore found, within bacteria cells.

2. What function do restriction endonucleases have in vivo?
   In vivo, these enzymes appear to be involved in the protection of bacteria against viral invasion and in the maintenance of species specificity.

3. What name would be given to the second restriction endonuclease system found in association with *Bacillus stearothermophilus* strain ET?
   Such an enzyme would be called *Bst*EII.

4. What function do restriction enzymes have in recombinant DNA technologies?
   Restriction endonucleases are used to "cut" chromosomal DNA into small fragments and to cut loops of plasmid DNA so that the small fragments of chromosomal DNA (carrying genes) can be inserted into them.

5. What are the differences between blunt and cohesive termini? Which of these is produced when DNA is cut with EcoRI and which when DNA is cut with AluI?
   Blunt ends are formed when a restriction enzyme cuts both strands of a DNA molecule at the same location (straight across), leaving no unpaired bases. The enzyme *Alu*I forms blunt ends.

   Cohesive termini are formed when a restriction enzyme cuts the two strands of a DNA molecule at different points within the recognition sequence leaving one strand longer than the other by one or more bases. The unpaired bases form the cohesive termini or sticky ends (because these can bind to complimentary strands through hydrogen bonding). The enzyme *Eco*RI forms cohesive termini four bases in length.

6. What is RFLP and what role does this technique play in bacterial identification?
   RFLP (Restriction Fragment Length Polymorphism) is a technique or method used in the analysis of DNA, and involves cutting a sample of DNA with a restriction enzyme and subjecting the resulting fragments to gel electrophorsis. The pattern created is sometimes called a DNA fingerprint, but may also be referred to as a RFLP pattern.

Characterization of Restriction Fragments:

1. What is a cloning vector? What is a gene library?
   Cloning vectors (also called cloning vehicles) are pieces of DNA that can initiate their own replication (are replicons) when inserted into host cells (bacteria, yeast, or others). They typically carry multiple cloning sites (regions where they can be cut with restriction enzymes so that new genes can be added). Their function is to carry DNA into cells and reproduce it there. Small plasmids and bacteriophage are the most commonly used cloning vectors. A gene library (also called a gene bank) is a collection of restriction fragments carried by cloning vectors that have been inserted into cells.

2. What were the sizes of the restriction fragments present within your DNA samples?
   The sizes of restriction fragments are quite variable, but those we worked with were between 500 and 1500 base pairs in length.

3. If you were looking for a gene that contained 1500 base pairs (1.5 Kb), could this gene be present in any of the recombinant molecules you were working with?
   Yes, this gene could be present in some of our fragments.

Calcium Chloride Procedure for Making Competent Cells/Transformation:

1. What are competent cells?
   Competent cells are those able to take in DNA from the environment. Although in nature such cells usually take in DNA from closely related cells, under laboratory conditions cells can be made to take up DNA from any source.

2. Why does treatment with ice-cold calcium chloride increase the efficiency of transformation?
   When present at temperatures at or near 0° C, calcium chloride (or other cations) will bind to the negatively charged phosphate groups of the cell membrane, thus shielding the negative charge on the surface of the cell. Since DNA is a negatively charged particle, it will now be attracted to the cell surface rather than repelled by it.

3. What percentage of cells in a bacterial population are usually made competent by treatment with ice cold calcium chloride?
   Only about 10% of the viable cells in a population are made competent to receive DNA.

4. What is transformation?
   Transformation (as it occurs naturally) is the process by which DNA is transferred from dead donor cells to living recipient cells. Under experimental conditions the DNA being transferred is artificially extracted and often modified prior to being introduced into new host cells. The efficiency of transformation is also greatly increased.

   Why is plasmid DNA frequently used in transformation procedures?
   Plasmid DNA is fairly easily removed from cells via miniscreen procedures, and so is readily available. Plasmids can be used to carry genes into host cells and will reproduce themselves there, thus replicating the genes they carry.

5. The plasmids pUC19, pGEM and pGLO carry marker genes that encode resistance to what type of antibiotic?
   The plasmids pUC19, pGEM and pGLO carry genes encoding resistance to ampicillin.

6. Were more bacteria able to grow on the ampicillin plates, or on the TSA plates? Why would you have expected these results?
   More bacteria grew on the TSA plates. This would be expected since only those cells that had been transformed (had picked up the plasmid DNA) would be able to grow on the ampicillin plates. All cells should be able to grow on the TSA plates.

7. If a 100-ml sample of log phase *E. coli* cells typically contains about one million ($1x10^6$) cells, approximately what percent of your competent cells were transformed?
   The answer is variable, but in most cases, only about 10% of the cells will be transformed.

8. How do *E. coli* colonies expressing GFP genes differ from *Photobacterium* colonies expressing lux genes? What optical characters are being displayed by these colonies?
   The *E. coli* colonies expressing GFP genes were displaying an optical character called fluorescence, while the *Photobacterium* colonies were displaying bioluminescence.

Introduction to Viruses and Phage Typing

1. What is a bacteriophage; a coliphage, a virion; a plaque?
   A bacteriophage is a virus that infects bacterial cells, while a coliphage is a virus that can infect only *E. coli* cells. A virion is a virus particle as it exists outside the host cell, or free in the environment. It is the complete, infective form of a virus. A plaque is a cleared area or "window" visible in a lawn of bacteria on a plate. It is due to cell lysis caused by a cytolytic bacteriophage present in the culture.

2. What is phage typing, and why can viruses be used to identify specific types of bacteria?
   Phage typing is a bacterial identification method that involves the use of known bacteriophages (viruses that infect bacteria) to identify unknown bacteria. Viruses can be used to identify specific types of bacteria because they are host specific, i.e., each type of virus will infect only a certain type of host cell.

3. How do the plaques formed by the coliphage X174 differ from those formed by the coliphage T2?
   The plaques formed by the coliphage X174 are larger than those formed by the coliphage T2, and therefore easier to see.

4. Which of the bacteria strains tested was a better host for the coliphage X174, and which was a better host for the coliphage T2? How did you know? ***E. coli* strain C was the better host for the coliphage X174 as indicated by the plaques formed when a specified concentration of virions were used to infect a population of cells. When the host strain *E. coli* B was exposed to the same virus, no plaques were formed. *E. coli* strain B is the optimum host for the coliphage T2, but this virus type was also supported by *E. coli* strain C.**

5. Which of the four plates contain *E. coli* strain C and which plates contained *E. coli* strain B? How did you know?
The answer is variable here. The plates showing small plaques were both infected with the coliphage T2, so can contain either *E. coli* strain B or strain C. The plate showing a large number of X174 plaques was inoculated with *E. coli* strain C. The plate showing no plaques was inoculated with *E. coli* strain B, because *E. coli* strain B cannot be infected by X174.

Isolation and Purification of Coliphages from the Environment:

1. How many different types of plaques were visible on your phage isolation plate? How many different types of virus appear able to infect *E. coli* strain C?
Phage isolation plates will show considerable variation, however at least 3-4 different types of plaques are likely. Each different looking plaque type represents a different type of virus. The *E. coli* strain C can be infected by multiple different types of coliphage because it is a restriction-negative strain, i.e., it does not carry restriction enzymes.

2. What is lysogeny? Did any of your bacteria become lysogenic? What is the condition of the viruses within cells that have undergone lysogeny?
Lysogeny is a condition or relationship between a bacteriophage and its host cell in which the virus has become incorporated into the host cell's chromosome. You may or may not see evidence of lysogeny. If you see evidence of lysogeny (turbid plaques), the cells growing within these are carrying viruses that have become prophages, i.e., have become incorporated into the host cell's chromosome.

3. How many virions are transferred by stabbing a plaque with a wire needle?
The results are somewhat variable, but usually about 1000 virions are transferred by a wire needle stabbed into the middle of a plaque.

Bacteriophage Reproduction and Plaque Formation:

1. How do viruses reproduce themselves within host cells?
Viruses vary considerably in terms of composition and host cell preference, but all reproduce themselves in a similar manner. They use host cell enzymes and ribosomes to transcribe and translate their viral genes (sometimes with the aid of viral enzymes carried into the cell), and they reproduce their nucleic acid and protein components as separate units. Once synthesized, these components are then assembled into new virions.

2. What is the latent period (burst time) as described in this exercise and how is it determined?
The latent period or burst time is the time required for a virus to complete its life cycle within a host cell, and escape from that cell. Technically, the latent period begins only after a virion has penetrated its host, i.e. after the nucleic acid core is inside, and ends just prior to assembly of the new virions.

However, since adsorption, penetration, assembly and release (liberation) occur fairly quickly, most of the time associated with viral replication is associated with the latent period (time required for transcription, translation and replication of specific viral genes, m-RNA and the complete viral genome respectively). To determine the latent period, look for a sharp increase in the number of PFU/mL as plotted on semilog paper. The time elapsed between time zero and the point of this sharp increase is the latent period.

3. What is burst size (burst number) as described in this exercise, and how is it determined?
   The burst size or burst number is the number of free virions released from each infected cell in the population. It is determined by dividing the maximum number of PFU/mL present at the end of the latent period by the initial number of PFU/mL (infected bacterial cells) present at the start of the exercise. In order to determine these numbers, it is necessary to know the dilution factor for each plate being counted, and to recognize that one PFU (plaque forming unit) can be either a free virion or an infected cell

4. According to the data obtained in the laboratory, what is the approximate latent period and burst size for the coliphage T2 when grown on *E. coli* strain B cells?
   According to our data (which is quite variable) the coliphage T2 on *E. coli* strain B has a latent period of less than 20 minutes, and a burst size of around 100 phage particles per infected cell. The burst size usually associated with cytolytic viruses is 150-200 virions per infected cell. The coliphage X174 grown on *E. coli* strain C yields similar results.

Microbial Control - Effects of Temperature, UV Light and Chemicals:

1. What is the name of the red-colored pigment produced by *Serratia marcescens*?
   The non-water soluble pigment is called prodigiosin.

2. What variation in pigment production was visible when *Serratia marcescens* was grown at 25° C and at 37° C?
   ***Serratia marcescens* produce more red pigment when grown at 25° C than they do when grown at 37° C.**

3. What is the overall effect of high temperatures (heat) on bacteria suspended in fresh water? Does exposure time influence this effect?
   The overall effect of high temperatures (heat) is to denature proteins and stop metabolic activity. If temperatures are high enough, the exposed organisms will die. After one minute of exposure to boiling water, most vegetative cells were killed, but endospore-forming bacteria and some other forms survived. After five minutes of exposure, few if any of the bacteria present remained viable. Exposure time does influence the effects of temperature.

4. The device used in this lab. to sterilize media and glassware is called a/an ________________ and uses what physical factors to kill microbes?
   The device is an autoclave, and uses moist heat (steam) under pressure to kill bacteria.

5. Name one test medium or experimental procedure that demonstrates the effects of (A) pH and (B) osmotic pressure on microbial growth.
   The effects of pH can be demonstrated by media that have different initial pH levels, such as bacteria culture media and fungi culture media. Fungi media are typically a little more acidic, and tend to inhibit bacterial growth. The effects of osmotic pressure are readily demonstrated by MSA (a medium containing high levels of salt) and the production of sauerkraut (where salt was used to form the brine). Once lactic acid has been produced in the sauerkraut container, the acid (low pH) prevents the growth of other organisms, thus preserving the food.

6. What is Ultra Violet radiation? What wavelength of UV radiation is most effective in controlling microorganisms?
   Ultra Violet (UV) radiation is electromagnetic radiation with a wavelength of less than 400 nm. (usually designated as 4-400 nm). Ultra violet radiation with a wavelength of 260-270 nm is considered to be the most effective against microorganisms because it is known to be strongly microbicidal, mutagenic, and carcinogenic.

7. Were the chemical agents you tested more effective in the control of Gram-negative organisms or Gram-positive organisms? What explanation can you give for the variation in results obtained?
   In general, Gram-negative organisms are less sensitive to chemicals than are Gram-positive organisms (because their outer membranes prevent chemicals from entering), but different chemicals will have variable effects on the organisms being tested.

Antimicrobial Sensitivity Testing - Agar Diffusion or Kirby-Bauer Test:

1. What are antimicrobial agents? Which of these would be effective for controlling *Staphylococcus aureus, Escherichia coli, Proteus mirabolis,* or *Pseudomonas aeruginosa*?
   Antimicrobial agents are a group of chemotherapeutic agents that can be used systematically in the treatment of infectious disease. The second part of this question has variable answers depending upon which antimicrobial agents are used (also age of discs).

2. What is a zone of inhibition, and what is meant by minimum inhibitory concentration?
   The zone of inhibition is the cleared region around the sensitivity/susceptibility disc where microbial growth is inhibited. The minimum inhibitory concentration is the lowest concentration of the drug being tested that will inhibit the growth of the microbe in vitro.

3. What are antibiotics? How do broad and a narrow spectrum drugs differ?
   Antibiotics are antimicrobial agents that, at least initially, were produced by living organisms. Broad-spectrum antibiotics will kill or inhibit the growth of a wider range of microorganisms (more different types) than will narrow spectrum drugs.

4. The concentration of an antimicrobial drug that is the minimum required to inhibit the growth of the microbe in question, i.e., the Minimum Inhibitory Concentration (MIC) is found where on the test plate?
   The MIC is found at the outermost edge of the zone of inhibition.

5. What are breakpoints and how do they relate to therapeutic dose?
   Breakpoints are discriminatory antimicrobial concentrations used in the interpretation of the results obtained during susceptibility testing to define isolates as susceptible (sensitive), intermediate or resistant (MICs are breakpoints). Clinical breakpoints refer to those concentrations (MICs) that separate strains where there is a high likelihood of treatment success from those where treatment is more likely to fail, and are based on results obtained during multiple clinical trials (these change over time). The therapeutic dose, i.e., the concentration of an antimicrobial drug expected to yield clinical control of a particular pathogen within the human body, must integrate drug potency against specific populations of potential pathogens with the pharmacokinetics (PK)/pharmacodynamics (PD) of antimicrobial agents (what happens to drugs inside the body due to metabolic processes). The accurate determination of therapeutic dose must take into account in vivo complexities such as dosing schedule, the site of infection, likely PK/PD of the drug within a specific individual, adequacy of host defenses and a range of other factors.

Bacteriological Examination of Water:

1. Why are the tests used in the bacteriological examination of water designed to test for *E. coli*, a relatively non-pathogenic type of bacteria, rather than some of the more serious pathogens?
   ***Escherichia coli* are used for the bacteriological examination of water tests basically because they are very common gut inhabitants (uniformly present in man and other animals) that do not normally grow in the natural environment outside of their host organisms. They are easy to grow on artificial media, and are easy to test for because they are lactose positive (can ferment lactose to produce acid and gas) a characteristic not common among bacteria.**

2. What three tests are included in the "Standard Methods" for the bacteriological examination of water.
   The steps of the Standard Methods tests include the presumptive test, the confirmatory test, and the completed test.

3. Why is lactose containing broth and agar use to detect bacterial contaminants in water?
   Lactose containing media are used because *E. coli* are lactose positive, and lactose fermentation is a good test for the differentiation of bacteria in the family Enterobacteriaceae from other types.

Composition of Blood and White Cell Study:

1. What are erythrocytes, leukocytes and thrombocytes? How many different types of leukocytes are normally found in a blood sample.
   Erythrocytes are red blood cells (erythro = red, cyte = cell), leukocytes are white blood cells (leuko = white) and thrombocytes or platelets are cell fragments involved in blood clotting (Thrombo = clot). There are five different types of leukocytes recognized including monocytes (macrophages), lymphocytes, neutrophils (polymorphonuclear leukocytes), eosinophils, and basophils. You should be able to recognize all five of these, since although basophils are present in small numbers, they can be found on most slides.

2. What is neutrophilia, and what is it indicative of?
   Neutrophilia is a high neutrophil count, and is indicative of an acute or localized infection such as an abscess or appendicitis.

3. What condition might result in eosinophilia?
   Eosinophilia (high eosinophil count) might be the result of an allergic reaction to some chemical substance or to the presence of some parasite.

4. Which type of leukocyte are you least likely to observe in a typical blood smear?
   You are least likely to observe basophils because they make up only 1% of the total leukocyte population and are therefore rather rare in blood smears.

Diagnostic Immunology :

1. What is serology?
   Serology is the study of antibody and antigen interactions in vitro.

2. When applied to antibodies, what does isotype mean, and what is an anti-isotype?
The term isotype is applied to immunoglobulin or antibody molecules that can be distinguished antigenically but are found in all normal humans. The five "classes" of immunoglobulins (IgG, IgM. IgA, IgD and IgE) are different isotypes. Immunoglobulins within the same class, but having different light chain components (kappa vs lambda) are also different isotypes. Antibodies that have been produced in response to a specific isotype are referred to as anti-isotype antibodies.

3. What type of serological reaction is involved in the Ouchterlony test?
The Ouchterlony test involves a precipitation reaction.

4. What are agglutinogens and how do they relate to blood type?
Agglutinogens (agglutinating antigens) are antigenic groups or epitopes found on the surfaces of RBCs. They are responsible for the differences in blood we recognize as blood groups.

5. What are haemagglutinins (agglutinins or isohaemagglutinins)?
Haemagglutinins are antibodies (immunoglobulins) produced by the body and which are able to bind with specific agglutinogens. These antibodies are produced by modified B-lymphocytes called plasma cells and are found in the circulation (in blood plasma and in serum).

6. What types of agglutinogens would a person have if their blood type were A+? What type of haemagglutinins would this person produce?
This person's RBCs would carry type A agglutinogens, and type D or Rh, they would be able to produce Anti-B haemagglutinins.

7. What is antiserum and what was it used for in the blood typing exercise?
Antiserum is blood serum (plasma minus the clotting elements) that contains a high titer of some specific antibody or antibodies. In this exercise it was used to initiate agglutination reactions, and thereby to indicate blood type.

8. Hemagglutination due to IgM is useful as a diagnostic test, but what would happen to such cells inside the body, and why?
Cells bound by IgM will clump together, and this could cause circulatory problems because the clumps could block capillaries; however, a more critical problem would involve complement factors. The binding of antibodies to cells in vivo will trigger the complement cascade, initiating the membrane attack complex, and will cause lysis of the cells involved.

9. What is an ELISA and what is this type of procedure used for?
An ELISA is an enzyme-linked immunosorbent assay and is a very sensitive method used in the detection of serum antibody (or antigen). There are many applications for ELISAs at the present time, detection of anti-HIV antibody being only one of them.

10. Is an ELISA more or less sensitive than the Ouchterlony test, and why?
The ELISA is much more sensitive. This is because 1) the layering of secondary antibody enhances the effect of the initial antigen-antibody complexing, and 2) even minute amounts of conjugated enzyme bound in a well will eventually act on the substrate to bring about a detectable color change.

11. What is epidemiology?
Epidemiology is the quantitative study of disease occurrence and the factors influencing the frequency and distribution of disease.