Laboratory Exam I (Example I)

1. Define:

   Transient microbiota – Transient microbiota are microorganisms found living in or on the surface of another organism, such as a human, but are residing there only temporarily, i.e., they are not permanent residents, so are not considered normal microbiota (normal flora). Transient microbiota can be transferred from one person to another, and if they are pathogens, can cause serious problems within clinical settings.

   Microbial enrichment – Microbial enrichments are used to promote the growth of certain types of microorganisms, while inhibiting the growth of others. Enrichments can involve specific types of culture media, e.g. the *Pseudomonas* and *Azotobacter* enrichment media used in our laboratory, or they can involve procedures (pasteurization, desiccation, radiation exposure, etc.), that will kill or damage some cells, leaving others to grow without competition.

   Cultured food – A cultured food is one that contains live microbial cultures at the end of its initial processing steps. Foods made from milk or cream that has been inoculated with specific microbial cultures (cheeses, yogurts, buttermilk, etc.), are common examples. Cultured foods typically contain lactic acid bacteria, but sometimes contain other bacteria types as well as fungi.

2. Students are advised to clean their section of the bench top before and after any laboratory session involving the manipulation of live microbial cultures. Proper application of disinfectant will remove unwanted bacteria and fungi that might contaminate cultures, and will also remove dust mites. Dust mites allowed to enter culture plates will destroy pure cultures by walking over the plate surface feeding on and contaminating whatever bacteria are present.

3. Many of the bacteria cultured in this laboratory are gastrointestinal pathogens, highly likely to make students sick if they are accidentally ingested. The "no food or drink in the laboratory" rule is designed to prevent illness.

4. Never leave the burner unattended (walk away from the work station) when it is lit, keep the burner near the center of the work station (away from any edge), keep flammable materials (hair, paper, clothing, alcohol, etc.) away from the lit burner, pay attention to where burners are located and avoid reaching across open flames. If fire spreads onto the bench surface for any reason, **TURN OFF THE GAS**!

5. If crystal violet had splashed in a student's eyes, the student should notify the instructor, and go to one of the eyewash stations (green and yellow striped pole at the back of the lab, or in the corner of the prep room directly behind the chalk board). Once there, the student should turn on the water fountains (using hand or foot pedal), and rinse the affected eye for at least 15 minutes.

6. Building-wide evacuation as indicated by the fire alarm sounding, or directions received through the emergency phone (at the front of the laboratory).

7. The answers will be variable here depending on the materials present. Used Petri plates, cotton swabs, plastic pipettes and pipette tips, toothpicks, contaminated paper towels and other materials to be autoclaved, should be placed in the large metal bins on the bottom shelf inside the discard cabinet. Glassware (beakers, finger bowels, etc.) that has been used but not exposed to live cultures should be placed on top (on the bench surface) of the discard cabinet. Glass culture tubes (large and small, with or without lids), should be placed in the plastic racks (vertical position, open end up), on the upper shelf inside the discard cabinet, and used glass slides should be placed into the small metal bin inside the discard cabinet.

8. Handwashing/ Joseph Lister and Ignaz Philipp Semmelweis

9. Objective/ ocular/ Total magnification possible is 40x, 100x, 450x and 1000x.

10. Immersion oil/ focus

11. Diameter/ depth

12. Refraction (bending or scattering) of light/ Kimwipes or VWR light-duty tissue wipers can be used to clean oil off the microscope stage, glass slides and the bench surface should oil be spilled there. These should never be used on any microscope lenses.

13. The low power or 10x objective, because it provides enough magnification to see where bacteria are on a slide, but is still short enough that it cannot contact a slide resting on the stage when the coarse focus knob is in use.

14. Substage condenser lens/ If the light intensity is too high, images will appear "washed out", i.e., colors and details will not be visible. Also viewing specimens with too much light will cause eyestrain and headaches, making your laboratory experience miserable.

15. Ocular micrometer/ calibration

16. Cell size is variable and will be influenced by the degree of magnification used. Remember that if the 100x objective is being used, each small division of the ocular micrometer is equal to 1 micrometer, if the 45x objective is being used, each small division = 2.2 micrometers, if the 10x objective is being used each small division = 10 micrometers, and if the 4x objective is being used, each small division is equal to 22.2 micrometers.

17. Culture medium (pleural is media)/ defined medium/ The answer is variable here and will require that you read the label on the medium container provided. Remember that 1 Liter is equal to 1000 ml, so you will have to take this into consideration.

18. Nitrogen/ carbon

19. Aseptic technique/ Wire loops should be flamed until red hot along their entire length before and after each microbial transfer. Petri plates should be opened only briefly when cultures are being removed or applied to the agar surface, otherwise they should be kept closed. Culture plates left open-to-the-air are exposed to organisms suspended in the air and can readily become contaminated (remember what air-plates typically look like). It is difficult to maintain a pure culture when air exposure is a common occurrence.

20. Flaming the tube mouth will kill any microorganisms on the glass, and will create convection currents that will help prevent air-borne microorganisms from entering while the tube is open./ Hold them in the hand holding the wire loop or transfer instrument, do not set them down on the bench surface.

21. This answer is variable here, but properly streaked plates have well-isolated colonies, i.e., colonies that are separated from one another on the medium surface./ The plate label must be placed on the plate bottom (agar side), not on the lid, and must contain the student's name (first initial plus full last name), the date, the medium type, and the culture identification.

22. Up-side down or agar-side up./ Water dripping onto the agar surface will cause bacteria to move (even if they are not motile), causing colonies to run together in a confluent mass. Isolation is impossible under these circumstances.

23. A pure culture is one that contains only one type of organism, or one population (all the cells present are of the same species)./ This answer is variable. Check to see if there is variation in the colonies present. Remember, a pure culture contains only one type of microorganism, and the colonies present will have consistent cultural characteristics (morphology).

24. Morphology/ Review the cultural characteristics of colonies in the lab syllabus and consider describing colony form, margin, elevation, surface texture, optical character, pigment production and size in millimeters.

25. Contrast/ Dead cells cannot swim/move around (leave the viewing field or move out of focus) and dead cells are no longer able to cause infection (if they were pathogens while alive).

26. Direct/ indirect or negative

27. Cations (positively charged particles)/ Cell surfaces have a slight negative charge and will attract the cations (because particles with opposite charges will attract one another). The glass slide surface does not have a negative charge.

28. Size and shape

29. Air dried and heat fixed/ differential

30. Eukaryotic/ prokaryotic (bacteria)

31. Peptidoglycan/ N-acetyl glucosamine/ lipopolysaccharide

32. Gram's iodine is a mordant, and causes the crystal violet to bind with the cell surface./ Nothing, because the Gram-negative cells would be colorless and therefore not visible.

33. Cells with thick walls will display no change in viscosity when mixed with 3% KOH, because the cell contents are not released.

34. The answers are variable here – review cell morphology as presented in the lab syllabus. Recall, Gram-positive cells stain dark purple and Gram-negative cells stain light pink.

35. Acid-fast/ mycolic acid

36. The answers are variable here, but unique structures observed in association with bacteria in this laboratory included flagella (amphitrichous and peritrichous), capsules, endospores, heterocysts and akinetes.

37. The answer is variable here, but endospores were stained with either carbol fuchsin or malachite green. Endospore shape is either spherical or ellipsoidal, and location is either central or terminal.

38. The three types of bacteria expected in the soil enrichment samples were *Pseudomonas*, *Azotobacter* and *Bacillus*.

    Our enrichment medium for *Pseudomonas* (broth and solid media) contained sodium benzoate as the only carbon source, and this material is toxic to most bacteria (is commonly used as a preservative). Bacteria in the genus *Pseudomonas* are unique in their ability to use a wide range of unusual organic compounds as nutrient (sources of energy and carbon). They have unique enzymes allowing them to catabolize sodium benzoate, so they were able to grow in this medium while most other types of bacteria could not.

    For the *Azotobacter* enrichment, we used nitrogen-free media (broth and solid) because only nitrogen-fixing bacteria could grow on this, and other types would be inhibited. Bacteria in the genus *Azotobacter* are able to take molecular nitrogen (N_2) from the air and use it to make the organic compounds necessary for growth (they can fix nitrogen), therefore they were readily able to grow. Note – multiple types of nitrogen-fixing bacteria other than *Azotobacter* grew on the nitrogen-free media.

    For the *Bacillus* enrichment we placed a small sample of soil into a glass tube with 5 ml of water, mixed the tube contents, and then placed the tube in a beaker of boiling water for 1 minute to pasteurize the contents. Bacteria in the genus *Bacillus* are endospore-formers, and endospores are highly resistant to heat (are thermoduric). Most vegetative cells were killed by this treatment, but the thermoduric endospores of various *Bacillus* species present survived. A small sample of the pasteurized liquid was then streaked on nutrient agar, the spores germinated, and new *Bacillus* organisms grew. Note – Bacteria in the genus *Clostridium* are also capable of forming thermoduric endospores, but most *Clostridium* are anaerobic and will not grow on media exposed to air.

39. Bacteria/ Cyanobacteria/ The genera present will vary.

40. Yeasts/ molds

41. Anamorph

42. These answers are variable, and apply to fungi

43. These answers are variable and apply to fungi

44. These answers are variable and apply to fungi

45. Phylum is Chlorophyta, the other three answers are variable but apply to algae

46. These answers are variable and apply to algae

47. Kingdom is Protista, unique wall or skeletal material is glass, and organisms with glass coverings are Diatoms and Radiolarians (their order of placement is variable).

48. These answers are variable and apply to protozoa

49. *Plasmidium*/ Sporozoea

50. The phylum is Platyhelminthes. The other answers are variable depending on the specific organism types being considered.

51. The phylum is Aschelminthes, and the other answers are variable.

52. The kingdom is Animalia, the phylum is Arthropoda, and the other answers are variable.

53. *Leuconostoc*/ *Lactobacillus*/ lactic acid

54. Alcohol/ carbon dioxide/ *Saccharomyces cerevisiae*

55. Lactic acid

56. *Lactococcus lactis* (subspecies *lactis* and *cremoris*)

57. Curds and whey

58. Water/ Ripened cheeses are aged and sometimes have a secondary flora added to them (often fungi in the genus *Penicillium*). Unripened cheeses are finished products at the end of their initial processing steps.

59. *Streptococcus thermophiles*/ acetaldehyde

60. Regular consumption of fresh yogurt can help people maintain gut flora that are beneficial and help prevent the growth of pathogens, regular consumption of fresh yogurt has been shown to reduce serum cholesterol levels, yogurt contains calcium needed for bone maintenance, and yogurt consumption may help prevent colon cancer.