Laboratory Exam I (Example II) Spring, 2013

1. Define:

   Calibration – Calibration is the process of comparing a measuring device to a known standard in order to determine the values of the units present. In this laboratory students used a known standard called a stage micrometer to determine the value of the smallest measuring units on the ocular micrometers (inside the eyepieces of their microscopes), with each different power of magnification used.

   Fungi – Fungi are achlorophyllus, eukaryotic organisms represented by yeasts (single-celled forms), molds (microscopic filamentous forms), and fleshy fungi (macroscopic organisms formed by masses of filaments). All are chemoheterotrophs, most are saprotrophs, and many live symbiotically with other organisms (some beneficial, some parasitic and a few opportunistic pathogens). In this class, all "fungi" are categorized within the domain Eukarya and Kingdom fungi (mycetae).

   Fermented food – A fermented food is one containing the fermentation products of microorganisms and influenced (flavor and texture), by those products. In this class we considered beverages such as wine and beer to be foods, along with sauerkraut, pickles and sourdough breads. Fermented foods can contain live microorganisms as finished products, but most often do not because the organisms responsible for the fermentation have either been removed or killed by processing steps.

2. Laboratory disinfectant

3. Eat or drink

4. Bunsen burners/ keep flammable materials such as long hair, fuzzy sweaters, alcohol, paper etc. away from the flames. Also avoid reaching across open flames, and if fire were to spread onto the bench surface, to **TURN THE GAS OFF!**

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. Turn to the left in the main hallway (toward the double doors nearest the theater building), proceed carefully down the steps, cross the sidewalk and gather on the open lawn area outside the building (between Sewell Hall and the pavement strip).

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. Ignaz Philipp Semelweis/ nosocomial infections, i.e., those originating within a hospital.

9. Transient

10. Ocular/ objective/ 40x, 100x, 450x and 1000x

11. Immersion oil/ focus

12. As magnification is increased both the depth and diameter of the viewing field decrease.

13. Low power or 10x/ It prevents the refraction (bending or scattering) of light.

14. The answers will vary here depending on the materials present, but only optical lens wipes should be used to clean any microscope lenses. Kimwipes and VWR light-duty tissue wipers can be used to clean oil from the microscope stage, and prepared slides./ Check the instrument carefully before answering this question – The light switch must be off, the mechanical stage centered, the revolving nosepiece positioned such that the 4x object is directly over the sub-stage condenser lens, and the cord is wrapped properly (up through the eyepieces, around under the stage).

15. Ocular micrometer/ Cell size is variable. 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.

16. Iris diaphragm/ sub-stage condenser/ eyestrain and headaches.

17. Culture media/ 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./ Defined media contain nutrients in pure chemical form and in specified amounts, so any component of the medium (again you must read the label), that is an extract or infusion (yeast extract, beef extract, brain heart infusion, etc.) makes the medium complex. Peptone, tryptone, trypticase, and other protein breakdown products contains unknown types of amino acids, so make the medium complex.

18. Nitrogen/ carbon

19. Aseptic technique/ Wire loops should be flamed until red hot along their entire length before and after each microbial transfer. Glass culture tube mouths should be passed through (rolled in) the flame of the Bunsen burner after removing the cap, before and after each transfer, and before replacing the cap. Plastic snap-on tube caps should be held by the hand holding the wire loop and not placed on the bench surface.

20. Air present in the laboratory contains numerous airborne organisms (recall what air plates look like), so minimal exposure to air will help to prevent cultures from being contaminated.

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 the bacteria present there to spread and form a confluent mass rather than isolated colonies. Even non-motile bacteria will spread over the surface if the agar is wet.

23. Pure/ 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/ This answer will be variable, be sure to 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. Swim out of focus or leave the viewing field/ infection (if they were potential pathogens).

26. Negative or indirect/ direct

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-muramic acid/ lipopolysaccharide

32. Remove the color from the thin-walled, Gram-negative cells./ Nothing, because the Gram-negative cells have been decolorized, but have not been counter-stained with safranin. They will be colorless and extremely difficult to see.

33. If the answer here is indicated by a letter (A, B,C, etc.), it is variable, but the correct answer is 3% potassium hydroxide (KOH)./ Thin-walled cells will break open when exposed to 3% KOH, and when their cell contents mix with the solution, it becomes viscous and slimy within a few seconds (will string from a loop lifted away from a glass slide).

34. The answers are variable here – review cell morphology as presented in the lab syllabus. Remember that 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 the location is either central or terminal.

38. 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.

39. 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.

40. Bacteria/ Cyanobacteria/ The examples used will be selected from the prepared slides available.

41. Yeasts/ molds

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. *Plasmodium*/ Sporozoea

50. Phylum is Platyhelminthes, and the other answers are variable but apply to flatworms.

51. Phylum is Aschelminthes, and the other answers are variable but apply to roundworms.

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

56. 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.

57. *Streptococcus thermophiles*/ acetaldehyde

58. *Escherichia coli*, *Salmonella enterica*, *Vibrio parahaemolyticus*, and *Shigella dysenteriae* are all important foodborne pathogens, while *Staphylococcus aureus* and *Clostridium botulinum* can cause dangerous entoxication.