1. Many organisms that are used in this lab are classified as opportunistic pathogens. They are called this because they …
   a. can cause disease in all individuals.
   b. can cause disease in children only.
   c. can cause disease in those who may be, for various reasons, immunocompromised.
   d. are not part of the normal flora associated with the body.
   e. None of the above.

2. To prevent contamination of surrounding environment and ourselves, we use
   a. Aseptic technique.
   b. Pure cultures only.
   c. Mixed cultures.
   d. A wire loop.
   e. Lysol

3. To most easily isolate individual colonies of a non-fastidious aerobe or facultative anaerobe, one would use
   a. A pour plate.
   b. A streak plate.
   c. A broth culture.
   d. An agar slant.
   e. None of the above

4. Why do the more isolated colonies on a streak plate tend to grow bigger?
   a. They have longer time to grow.
   b. They are more aggressive than the other colonies.
   c. They don’t have to compete as much for nutrients.
   d. The nutrients in agar plates migrate towards more isolated colonies.
   e. None of the above

5. Phase contrast microscopy allows the viewer to see more contrast because
   a. it increases the wavelength of light transmitted through samples.
   b. the microscope converts differences in refractive index into differences in intensity of light.
   c. it adds color to unstained bacteria.
   d. it does not work any better than brightfield illumination.
   e. none of the above

6. What two bacteria made up the Gram Stain Control used in class?
   a. Bacillus megaterium and Edwardsiella tarda
   b. Streptococcus pneumoniae and Clostridium perfringes
   c. Staphylococcus aureus and Escherichia coli
   d. Serratia marcescens and Escherichia coli
   e. None of the above
7. In the Oxidation-Fermentation Test, what is the purpose for sealing one tube w/ mineral oil, while leaving the other tube unsealed?
   a. The mineral oil is added to see if the organism can metabolize mineral oil.
   b. The mineral oil promotes anaerobic growth, while the unsealed tube allows aerobic growth.
   c. The mineral oil promotes aerobic growth, while the unsealed tube allows anaerobic growth.
   d. Both A & B are correct
   e. Both A & C are correct.

8. The principle behind a positive stain is . . .
   a. to outline cells by staining the background and the cells themselves are unstained.
   b. the cells stand out because they are stained and the background is unstained.
   c. the cells are outlined such that you can observe their 3-D structure.
   d. Hey it stained! That’s a positive!
   e. None of the above

9. What is the correct sequence of reagents used in a Gram Stain?
   a. Gram’s iodine, acetone-alcohol, safranin, crystal violet
   b. Crystal violet. Gram’s iodine, safranin, acetone-alcohol
   c. Safranin, Gram’s iodine, acetone-alcohol, crystal violet
   d. Crystal violet, Gram’s iodine, acetone-alcohol, Safranin
   e. None of the above

10. Gram’s iodine is the ________ of the Gram stain.
    a. Primary stain
    b. Decolorizer
    c. Mordant
    d. Counterstain
    e. None of the above
11. Which component of cell wall in bacteria confers the difference between Gram positive and Gram negative stains?
   a. Lipopolysaccharide
   b. Peptidoglycan
   c. Phospholipid
   d. Protein
   e. None of the above

12. Which of the following reasons may cause a false Gram negative result?
   a. Forget to do heat fixation
   b. Excess staining time
   c. Inadequate time of decolorization
   d. Excess time of decolorization
   e. None of the above

13. Selective media:
   a. favors the growth of strains with certain characteristics
   b. inhibits the growth of certain strains
   c. produces visibly distinct signals based on the growth of organisms which possess or lack a particular physiological trait
   d. kills all bacteria
   e. none of the above

14. What other information can Gram staining yield beyond positive or negative reactions?
   a. Cell morphology and arrangement
   b. Colony morphology
   c. Presence or absence of bacterial capsule
   d. The production of gas by the strain tested
   e. None of the above

15. The definition of generation time is:
   a. The time in which the cells are growing in log phase
   b. The time it takes for the cell concentration to double
   c. The rate at which cells are doubling during log phase
   d. The period or phase of growth when cell concentration increases exponentially
   e. None of the above
16. The recently discovered archaea bacteria Strain 121 can grow at 121°C (temperature normally used to sterilize media) with a generation time of 24 hours. What is its growth rate at 121°C?
   a. 24 doublings x hour$^{-1}$
   b. 1 doubling every 24 hours
   c. 1 / 24 hours = 0.04 hours$^{-1}$
   d. 1 / 24 hours = 0.04 hours
   e. None of the above

17. A bacterial culture grown in a slightly hypotonic environment (slightly more salt in the bacteria than in the surrounding environment) is shifted to an extremely hypotonic environment (distilled water). What is a likely result of this shift in conditions?
   a. Water will rapidly leave the cell resulting in cell lysis (bursting).
   b. Water will rapidly enter the cell resulting in cell lysis.
   c. Water will rapidly leave the cell resulting in plasmolysis (cell shrinkage).
   d. Water will rapidly enter the cell resulting in plasmolysis.
   e. None of the above

18. Which of the following statements is FALSE?
   a. All bacteria need oxygen to grow.
   b. Oxygen can damage bacterial components.
   c. Some bacteria can respire using compounds other than O$_2$.
   d. Oxygen is reduced during aerobic respiration.
   e. None of the above

19. The suffix “-cide” refers to
   a. which side of the plate you treat with an antibiotic
   b. a substance that prevents the growth of microbes without killing them
   c. an antibiotic
   d. a substance that kills microbes
   e. none of the above

20. Minimum inhibitory concentration (MIC) is a measure of the
   a. antibiotic resistance in humans
   b. effectiveness of an antimicrobial agent against a microbe
   c. how well a media selects for microbial growth
   d. the best temperature for a specific microbes growth
   e. none of the above
21. The _______ method is one technique used to determine MIC
   a. paper disk
   b. antibiotics
   c. bactericide
   d. antimicrobial agent
   e. all of the above

22. To determine the MIC of an antibiotic, _______ are often used to establish a uniform confluent growth of a bacterial strain for the test.
   a. pour deeps
   b. pour slants
   c. pour plates
   d. rich broth
   e. minimal broth

23. When making a graph to determine MIC, you plot...
   a. ln(disk) on the Y axis VS (corrected radius)^2 on the X axis
   b. corrected radius on the Y axis VS ln(disk) on the X axis
   c. length of zone of inhibition on the Y axis VS (corrected radius)^2 on the X axis
   d. ln (disk) on the Y axis and length of the zone of inhibition^2 on the X axis
   e. none of the above

24. The skin is a good defense against potential infection. This is because the outer layer of skin is composed of:
   a. living cells that play a role in the immune system.
   b. dead cells interconnected with keratin fibers.
   c. living cells interconnected with keratin fibers.
   d. dead cells which slough off once colonized by bacteria.
   e. none of the above

25. Sweat glands provide a potential entry route for pathogens. Your body protects itself by:
   a. excreting a mildly antibacterial aqueous solution from the sweat glands.
   b. excreting lactose substances from the sweat glands.
   c. a response that closes the openings to glands if invaded by a pathogen.
   d. a and c
   e. none of the above
26. Which population(s) of bacteria is easiest to remove by handwashing?
   a. transient bacteria
   b. hidden bacteria
   c. resident bacteria
   d. none of the above
   e. all of the above

27. While plating for the handwashing lab you dispensed 1 mL of the rinse water into a sterile petri dish and 0.1 mL into another. Both amounts of rinse water were taken from the same basin. You add molten agar and allow the plates to harden. After 24 hours of incubation at 37 degrees Celsius you count 200 colonies of various types on the 1 mL plate. Ideally, you would expect how many colonies on the 0.1 mL plate?
   a. 2
   b. 20
   c. 200
   d. 2000
   e. $2 \times 10^5$

28. When examining the Gram stain of your unknown mixture in Lab 11 it is important to observe all of the following except:
   a. Motility
   b. cell shape
   c. cell growth arrangements
   d. Gram stain reaction
   e. none of the above

29. Isolation and identification are important procedures for studying bacteria from samples of _____.
   a. human blood
   b. soil
   c. pond water
   d. all of the above
   e. none of the above

30. In interpreting Oxidation Fermentation Test results, which of the following conclusions is correct if the sealed tube is green (no change) and the unsealed tube is yellow (acidic)?
   a. The organism is able to ferment the sugar present in the medium.
   b. The organism is able to oxidize the sugar present in the medium.
   c. The organism is able to both ferment AND oxidize the sugar present in the medium.
   d. The organism is not able to metabolize the sugar at all, and is therefore non-saccharolytic.
   e. The organism is highly motile and consumes the sugar in the process
31. In interpreting the Triple Sugar Iron (TSI), which conclusion is correct if the following results were observed: red (basic) slant, yellow (acidic) butt, no bubbles, no black color.
   a. The organism is able to ferment ONLY glucose, and not sucrose/lactose
   b. The organism is able to ferment BOTH glucose and sucrose/lactose
   c. The organism cannot ferment ANY of the sugars present
   d. The organism produces gas from fermenting the sugar(s)
   e. The organism produces $\text{H}_2\text{S}$

32. Why shouldn't you use colonies from a Sheep’s Blood Agar (SBA) plate to test for the catalase reaction?
   a. Any organism that is catalase positive will not be able to grow on SBA.
   b. Enzymes in SBA deactivate any catalase enzymes that the organism has, leading to false negative results (the organism really does have catalase enzymes (is positive), but the test shows that it doesn't have the enzymes (is negative)).
   c. Sheep’s blood has its own set of catalase enzymes so that it may lead to false positive results.
   d. SBA is often highly contaminated with many different organisms, so there is no way to tell if a certain organism is catalase positive.
   e. a & b above

33. Transfer 0.1mL culture to 9.9mL dilution A. Transfer 0.1mL from dilution A to 9.9mL dilution B. Transfer 0.1ml from dilution B to 9.9mL dilution C. Spread 0.1mL from C onto three plates (replication). After incubation, the three plates had 41, 42, and 43 colonies respectively. What is the culture’s CFU/ml?
   a. $4.2 \times 10^8$ CFUs / ml
   b. $4.2 \times 10^7$ CFUs / ml
   c. $4.2 \times 10^6$ CFUs / ml
   d. Not enough colonies for viable count
   e. None of the above

34. If the percent transmittance on a spectrophotometer is reading 0%, what is the OD value? (OD = $2 - \log(\%\text{Transmittance})$).
   a. 2.0
   b. $-2.0$
   c. 0
   d. Cannot calculate
   e. None of the above
35. What is the best method for determining viable cells?
   a. Plate count
   b. Turbidity
   c. Bacterial conjugation
   d. Paper disk method
   e. None of the above

36. Enrichment media:
   a. favors the growth of strains with certain characteristics
   b. inhibits the growth of certain strains
   c. produces visibly distinct signals based on the growth of organisms which
      possess or lack a particular physiological trait
   d. kills all bacteria
   e. None of the above

37. The 7.5% NaCl in Mannitol Salt Agar (MSA) is used as:
   a. an enrichment source
   b. a selective agent
   c. an anaerobic indicator
   d. a differential agent
   e. a conjugation enhancer

38. The Phenol Red indicator and lactose (fermentable carbon source) in MacConkey
    agar serve as:
   a. enrichment sources
   b. selective agents
   c. anaerobic indicators
   d. differential agents
   e. conjugation enhancers

39. *E. coli* and *E. tarda* are streaked on MacConkey agar plates. MacConkey agar
    contains lactose and Phenol Red. *E. coli* can ferment lactose, *E. tarda* cannot.
    After incubation, the *E. coli* colonies would appear ___________ , and the *E. tarda*
    colonies would appear ___________.
   a. white, white
   b. white, red
   c. red, white
   d. yellow, yellow
   e. green, clear
40. What is the mechanism by which genes acquired from a Hfr donor can be inserted into the chromosome of a recipient cell?
   a. independent replication of the transferred nucleic acid
   b. recombination
   c. transcription into mRNA
   d. this cannot occur (such traits cannot be maintained)
   e. reverse transcription into DNA

41. From a mating mix of donor E. coli 785 (str$^+$/F$^+$lac+) and recipient E. coli 1177 (str$^-$lac$^-$/F$^-$), “apparent transconjugants” as observed on MacConkey agar with streptomycin, can include any of the following EXCEPT:
   a. E. coli str$^+$lac$^-$/F$^+$lac+
   b. E. coli str$^-$lac$^-$/F$^+$lac+
   c. E. coli str$^-$lac$^+$/F$^-$
   d. E. coli str$^-$/F$^+$lac+
   e. None of the above

42. 2 mL of donor culture at $2.1 \times 10^7$ CFU/mL and 4 mL of recipient culture at $7.5 \times 10^8$ CFU/mL are combined in a mating mix (total vol = 6 mL). What is the dilution factor from the original donor culture to the mating mix?
   a. 0.5
   b. 2
   c. 1.5
   d. 3
   e. 20

43. What is the cell density of the donor in the mating mix above?
   a. $6.3 \times 10^7$ CFU/mL
   b. $3.0 \times 10^7$ CFU/mL
   c. $7.0 \times 10^6$ CFU/mL
   d. $4.2 \times 10^6$ CFU/mL
   e. $5.0 \times 10^5$ CFU/mL
44. A bacterial cell which is kan^lac^-/F'^lac^+ ("kan" refers to the antibiotic kanamycin):
   a. can transfer kanamycin resistance to other cells via transfer of the kan gene on the F' plasmid
   b. would lose the ability to use lactose if it lost the plasmid it’s carrying
   c. is susceptible to kanamycin
   d. would produce a white colony on MacConkey agar
   e. is resistant to lysis by lactose

45. Differential media:
   a. favors the growth of strains with certain characteristics
   b. inhibits the growth of certain strains
   c. produces visibly distinct signals based on the growth of organisms which possess or lack a particular physiological trait
   d. kills all bacteria
   e. none of the above

46. You are conducting an experiment to determine the rate of growth of a recent bacterial strain that you isolated from soil. The experiment requires that you dilute your bacterial culture of known concentration (CFUs/ml) growing in nutrient broth down to a concentration of exactly $2 \times 10^6$ CFUs/ml in a final volume of 200 ml of nutrient broth. After dilution you will incubate the flask containing the 200 ml of culture and monitor its growth. The dense culture that you will dilute from contains cells at a concentration of $8 \times 10^8$ CFUs/ml. What is the correct dilution scheme.
   a. 1 ml of dense culture into 199 ml of nutrient broth
   b. 2.0 ml of dense culture into 198 ml of nutrient broth
   c. 0.5 ml of dense culture into 199.5 ml of nutrient broth
   d. 0.2 ml of dense culture into 199.8 ml of nutrient broth
   e. 5.0 ml of dense culture into 195 ml of nutrient broth
47. You have been working on a bacterial isolate and you have discovered a lytic bacteriophage that attacks it. The plaques that this bacteriophage produces are very similar to the phage plaques that you saw in the 302 laboratory. To complete a series of experiments on the bacteriophage and its life cycle in your bacterial isolate, you need to conduct an experiment where you infect a 100 ml culture of your bacteria (at $3 \times 10^8$ CFU/ml) with the bacteriophage. According to published protocols describing a similar bacteriophage, it is recommended that the cells be infected at an MOI of 3.0. MOI is the acronym for Multiplicity of Infection and refers to the ratio of bacteriophage:cell at the time of the initial infection, i.e. when you add the bacteriophage to the cells. That is to say that for every cell that you have, you need to add 3 phage particles to the culture to begin the infective process. You have prepared a bacteriophage stock and determined that the concentration of bacteriophage in your stock is $3.6 \times 10^{11}$ phage/ml. What volume of the phage stock do you need to add to your 100 ml culture to get the desired MOI?
   a. 0.1 ml
   b. 0.15 ml
   c. 0.2 ml
   d. 0.25 ml
   e. 0.5 ml

48. Immersion oil improves ______ of light microscope by increasing the numerical aperture.
   a. contrast
   b. resolution
   c. magnification
   d. wavelength
   e. none of the above

49. Which of the following techniques would you use to prepare your samples to best visualize the motility of bacteria?
   a. Wet mounts or gram staining.
   b. Hanging drops or gram staining.
   c. Wet mounts or hanging drops.
   d. Heat or chemical fixation.
   e. All of the above.

50. A light microscope has an objective lens with a magnification of 100x and an ocular lens with a magnification of 10x. What is the total magnification of the image?
   a. 90x
   b. 100x
   c. 110x
   d. 1000x
   e. 1100x
51. Agar plates should be inverted for incubation so that
   a. bacteria will grow better because of the gravity.
   b. bacteria will obtain more oxygen for growth.
   c. the chance of contamination will be reduced.
   d. the agar will not dry up during incubation.
   e. condensation on the lid of the plate will not drip onto the agar surface to spread and mix colonies.

52. Which technique of sample preparations do the following figures illustrate?
   a. Simple staining.
   b. Negative staining.
   c. Capsule staining.
   d. Wet mounts.
   e. Hanging drops.

53. What is most likely to happen if you forgot to flame your inoculating loop after each streak during plate streaking for colony isolation?
   a. There would be more chance to get isolated colonies.
   b. There would be less chance to get isolated colonies.
   c. No colony will grow at all.
   d. Colonies tend to grow bigger.
   e. It wouldn't make any difference.

54. Three different dilutions of a bacterial culture (A, B and C) produced a reading of 75%, 50% and 20% transmittance respectively in spectrophotometer. Which of the three dilutions would give the highest OD (optical density) value?
   a. A
   b. B
   c. C
   d. Can't be determined.
   e. None of the above.
55. To determine the cell density of a bacterial culture, you made four different dilutions of the culture and spread 1 ml of each on TSA plates. The plates were cultured at 37°C for 24 hours and the colonies on each plate were counted. Which of the following colony count is considered LEAST reliable for calculation of the bacterial concentration of the original culture?
   a. 50  
   b. 100  
   c. 150  
   d. 250  
   e. 500

56. The following bacterial growth curve showing the four typical phases of growth. When did the exponential phase start?
   a. After ~ 0 hr  
   b. After ~ 1.5 hr  
   c. After ~ 3.5 hr  
   d. After ~ 5 hr  
   e. After ~ 10 hr

57. Which of the following tests can be used to distinguish between two major types of fermentation?
   a. Urea hydrolysis and nitrate reductase tests.  
   b. Indole and oxidase tests.  
   c. Hemolysis and coagulase tests.  
   d. Triple sugar iron and ornithine decarboxylase tests.  
   e. Methyl-Red and Voges-Proskauer tests.
58. One way to determine bacterial motility is to stab a strain into a tube of semi-solid agar and then incubate the samples. If the following figures represent the results of five different samples of bacteria from the motility test, which of them suggests a motile strain? (vertical line represents the stab line; black dots are bacterial growth)

![Motility Test Figures]

a. A  
b. B  
c. C  
d. D  
e. E

59. Which of the following techniques is almost always the first performed for the identification of bacteria?
   a. Acid-fast staining  
   b. Gram staining  
   c. Oxygen tolerance test  
   d. Hydrogen sulfide production test  
   e. Citrate utilization test

60. Typically, Gram-positive bacteria appear _____ and Gram-negative ones appear _____ in Gram staining reactions.
   a. dark purple; pink  
   b. pink; dark purple  
   c. dark purple; dark blue  
   d. dark blue; dark purple  
   e. pink; orange
61. __________ are substances produced by a strain of microbe that kill or inhibit the growth of cells of another strain or group of strains.
   a. Disinfectants
   b. Antiseptics
   c. Antimicrobial agents
   d. Antibiotics
   e. Hormones

For the following five questions, refer to the figure of the microscope (next page).

62. Identify the part labeled A.
   a. ocular lens (eyepiece)
   b. condenser
   c. iris lever
   d. focus knob
   e. objective lens

63. Identify the part labeled B.
   a. ocular lens (eyepiece)
   b. condenser
   c. iris lever
   d. focus knob
   e. objective lens

64. Identify the part labeled C.
   a. ocular lens (eyepiece)
   b. condenser
   c. iris lever
   d. focus knob
   e. objective lens

65. Identify the part labeled D.
   a. ocular lens (eyepiece)
   b. condenser
   c. iris lever
   d. focus knob
   e. objective lens

66. Identify the part labeled E.
   a. ocular lens (eyepiece)
   b. condenser
   c. iris lever
   d. focus knob
   e. objective lens