A. Multiple Choice. 1 pt each.

The following choices are used for questions 1 – 5.

(a) Proteobacteria  (d) Cyanobacteria
(b) Firmicutes    (e) Spirochaetes
(c) Actinomycetes

1. This phylum of bacteria is characterized by oxygenic photosynthesis.
2. This phylum of bacteria is characterized by having axial filaments and a membrane called an outer sheath.
3. This phylum of bacteria is characterized by an outer membrane with lipopolysaccharide and porin, and it includes the Enterobacteriaceae and the Pseudomonadaceae.
4. This phylum of bacteria is the “High GC Gram-positive” bacteria, and it includes Corynebacterium, Micrococcus, and Mycobacterium.
5. This phylum of bacteria is the “Low GC Gram-positive” bacteria, and it includes Streptococcus, Staphylococcus, Bacillus, and Clostridium.

The following choices are used for questions 6 – 10.

(a) lithotroph   (d) auxotroph
(b) heterotroph (e) psychrotroph
(c) mixotroph

6. This term describes an organism that can grow at 0 – 10°C, but typically has optimum growth between 20 – 30°C and a maximum around 35°C.
7. This term describes an organism that requires an organic source of carbon for growth.
8. This term describes an organism that uses an inorganic source or reduction potential for growth.
9. This term describes an organism that can use either an organic source of carbon or CO₂ as its carbon source, depending on its growing conditions.
10. This term describes a genetic mutation that has lost the ability to grow on a minimal medium, and requires the addition of a specific nutritional supplement for growth.
The following choices are used for questions 11 – 14.
(a) Simple diffusion directly across a phospholipid bilayer
(b) Facilitated diffusion across a membrane
(c) Active transport across a membrane
(d) Both (a) and (b)
(e) Both (b) and (c)

11. Small hydrophobic and polar molecules (such as O₂, CO₂, N₂, H₂O, and glycerol) can pass through a membrane by this mechanism, but larger uncharged polar molecules and ions (such as glucose, Na⁺, and K⁺) cannot pass through a membrane by this mechanism.
12. A membrane protein must mediate this process.
13. This process requires the expenditure of the cell’s energy, usually in the form of ATP hydrolysis.
14. In this process, the substance being transported moves from a high concentration to a low concentration until equilibrium is reached.

The following graph pertains to questions 15 – 17. It shows the growth of *E. coli* in batch culture.

15. What is the approximate mean generation time of this culture?
(a) 10 min  (d) 100 min
(b) 20 min  (e) 120 min
(c) 40 min

16. Which term best describes the time between 90 min and 120 min on the graph?
(a) Lag phase  (c) Stationary phase
(b) Log or exponential phase  (d) Death phase

17. The growth rate for this culture
(a) is the time required for the culture to recover from the lag phase.
(b) is the total time of the exponential phase.
(c) is the total time of the exponential phase by the time of the lag phase.
(d) is 1/(mean generation time).
(e) is determined by fitting the entire curve to a quadratic equation, then fitting the linear portion of the exponential phase to a linear equation, and solving the simultaneous equations.
18. Suppose that this graph represents a batch culture of *E. coli* grown at its optimal growth temperature. If a similar flask of *E. coli* were grown at a temperature below its optimal growth temperature, with all other parameters being the same (including the same amount of starting inoculum, in approximately the same conditions of nutrient depletion and stress), how do you predict the curve for the second flask would be different?

(a) In the graph of the culture grown at the suboptimal temperature, the lag phase is expected to be significantly longer.

(b) In the graph of the culture grown at the suboptimal temperature, the slope of the curve at mid-exponential phase is expected to be larger (a steeper slope).

(c) In the graph of the culture grown at the suboptimal temperature, the slope of the curve at mid-exponential phase is expected to be smaller (a less steep slope).

(d) In the graph of the culture grown at the suboptimal temperature, the maximum absorbance reached is expected to be less.

(e) In the graph of the culture grown at the suboptimal temperature, the maximum absorbance reached is expected to be greater.

The following choices are used for questions 19 – 23. For each question, you should choose the term that gives the **best** fits the definition given.

(a) Sterilization  
(b) Disinfection  
(c) Antiseptic  
(d) Bactericide  
(e) Bacteriostat

19. An agent that kills bacteria.

20. A agent that reduces numbers of potentially pathogenic microbes and is mild enough to be applied to skin surfaces.

21. A treatment that reduces the total number of microbes on an object or surface, but does not necessarily remove or kill all of the microbes.

22. An agent that inhibits bacterial growth.

23. A treatment that kills or removes all living cells, including viruses and spores, from a substance or object.

The following choices are used for questions 24 – 28.

(a) Thermal death point  
(b) Thermal death time  
(c) Decimal reduction time  
(d) z value  
(e) F value

24. The time in minutes at a specific temperature (usually 121.1°C or 250 °F) needed to kill a population of cells or spores.

25. The time required to reduced a population of microbes by 90% (a 10-fold, or one decimal, reduction) at a specified temperature and specified conditions.

26. Lowest temperature at which a microbial suspension is killed in 10 minutes; misleading because it implies immediate lethality despite substrate conditions.

27. The change in temperature, in °C, necessary to cause a tenfold change in the D value of an organism under specified conditions.
28. Shortest time needed to kill all organisms in a suspension at a specified temperature under specific conditions; misleading because it does not account for the logarithmic nature of the death curve (theoretically not possible to get down to zero).

The following choices are used for questions 29 – 33.

(a) Hexachlorophene  
(b) Betadyne  
(c) Cetylpyridinium chloride  
(d) Silver nitrate  
(e) Copper sulfate

29. This quaternary ammonium compound is the antibacterial ingredient in several brands of mouthwashes (e.g. Scope).

30. Use of this phenolic as an antiseptic is limited now because it is suspected of causing brain damage.

31. Drops of this substance once were used in preventing ophthalmic gonorrhea in infants, but in recent years have been replaced by less toxic antibiotics.

32. The active ingredient of this commercial antiseptic is iodine chelated to an organic carrier that makes the iodine less toxic and stains less.

33. This substance has been used as an algicide in swimming pools.

34. Ultraviolet irradiation at a wavelength of 260 nm

(a) requires a cobalt-60 source.
(b) is a powerful sterilizing agent useful for sterilizing heat-sensitive plasticware and other labware.
(c) damages DNA by causing the formation of thymine dimers.
(d) can be used to sterilize “shelf milk” (e.g., individual coffee creamers at restaurants) so that it doesn’t need refrigeration.
(e) is an effective way of preserving microbial cultures by reducing the chances of genetic mutation in the culture.

35. The temperature of an ultracold freezer unit is approximately

(a) 5°C  
(b) 0°C  
(c) -20°C  
(d) -80°C  
(e) -200°C

B. Short Answer and Short Essay Questions

1. Compare and contrast conventional pasteurization, flash pasteurization, and ultrahigh-temperature sterilization. (6 pt)
2. Briefly describe each of the following examples of active transport systems in bacteria. (3 pt each)

(a) ATP-binding cassette transporter systems

(b) Lactose transport by lactose permease in *E. coli*

(c) Phosphoenolpyruvate: sugar phosphotransferase systems

(d) Iron transport by siderophores
3. The following questions pertain to the method for aerobic plate counts given in the *FDA Bacteriological Analytical Manual*. (2 pt each)

(a) What are “spreaders,” (hint: they are not what you use to spread the drop on a spread plate) and how are spreaders dealt with in the FDA aerobic plate count procedure?

(b) When none of the plates from any of the dilutions in an aerobic plate count have CFUs, exactly what results are reported?

4. The following data were collected in a serial dilution and plate counting experiment to determine the CFU/ml of a broth culture. For each dilution, duplicate plates were prepared, with 0.1 ml plated by spread-plating on each plate. “TNTC” means to numerous to count. Compute the CFU/ml in the broth culture, using the method given in the *FDA Bacteriological Analytical Manual* as applied in class and in the Quantitative Bacteriology lab. (5 pt)

<table>
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<th>Dilution Factor</th>
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<th>Count on Plate 2</th>
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<tr>
<td>$10^{-7}$</td>
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</table>
5. Turbidity, or light scattering, is a convenient method of measuring bacterial concentration. However, there is an issue with turbidity measurements at higher absorbance values above about 0.5. Very briefly, what is this issue, and how can it be overcome (you need not go through the entire derivation, just state how you do the process). (4 pt)

6. How is it possible to relate measurements of turbidity to other measurements of bacterial concentration, such as CFU/ml or gm/ml? (3 pt)

7. Briefly describe the BBL “Gas-Pak” system, and state what it is used for. (4 pt)

8. Briefly describe the specific role that acyl homoserine lactone plays in regulating growth in many gram-negative bacteria, and the mechanism by which it does this. (4 pt)
9. The $D_{60}$ value of *Staphylococcus aureus* in chicken a la king (delicious bits of chicken meat in a rich chicken gravy) is about 5.3 min, while the $D_{60}$ value for *Staph. aureus* in turkey stuffing (seasoned breading, moistened with turkey broth then baked) is about 15.4 min. Suggest why there is such a large difference between the two values. (3 pt)

10. In *Practical Guide to Autoclave Validation* by R.G. Lewis, the author states, “Determining the bioburden and D-value for all items to be sterilized in a load can be quite time-consuming and costly.” Instead, the author describes the alternative practical approach to autoclaving and autoclave validation that in fact is almost always employed. What is this approach called, and briefly describe the basis for autoclave validation based on this approach, as discussed in Lewis’ article. (4 pt)

11. The $D_{60}$ value of *Staphylococcus aureus* in milk is 0.9 min, with a $z$ value of 9.5°C. How long would it require to reduce a population of *Staph. aureus* cells in milk from $10^6$ to $10^0$ cells at 60°C, at 55°C, and at 65°C? (6 pt)
12. How could a heat-sensitive solution be most easily sterilized in a typically equipped microbiology laboratory (i.e. ours). (2 pt)

13. Briefly describe two separate methods used commercially to sterilize disposable plastic labware such as petri dishes. (4 pt)

14. Briefly describe the method of lyophilization, and state what it is used for with respect to microbial cultures. (4 pt)