1. (3 pts) Pseudomembranous colitis is caused by *Clostridium difficile*. Under what circumstances does this illness arise?

   After administration of antibiotics which knock off the normal flora.

List two ways in which normal flora may inhibit the growth of pathogenic bacteria.

A. Normal flora may secrete products (i.e. acid) which make the local environment inhospitable.

B. Normal flora may sequester necessary nutrients

2. (2 pt.) What is an ID$_{50}$, i.e., infectious dose 50?

   This is the dose of bacterial inoculum required to establish an infection 50% of the time in test subjects/animals. It measures infectivity.

   What is an LD$_{50}$, i.e., lethal dose 50?

   This is the dose which kills subjects/animals 50% of the time. It measures lethality.

3. (1 pt) An outbreak of intestinal illness, sometimes accompanied by systemic manifestations, occurred among second year medical students at a medical school in Providence, RI. Urine, stool, throat, blood and cerebral spinal fluid cultures were taken from one patient. A gram-negative rod, *Bacterium X*, grew out of his stool culture. Which of the following observations would give the strongest indication that *Bacterium X* is a true pathogen?

   A. DNA hybridization analysis shows that *Bacterium X* is genetically related to a known pathogen.

   B. *Bacterium X* was also isolated from the CSF of this patient.

   C. *Bacterium X* was isolated from the stool of all other afflicted students.

   D. The strain of *Bacterium X* isolated from the stool culture produces endotoxin.

   E. The strain of *Bacterium X* isolated from the stool culture is resistant to multiple antibiotics.

4. (2 pts.) What would you have to show to rigorously demonstrate *Bacterium X* is the causative agent of the intestinal illness?

   Follow Koch's Postulates:

   1) Isolate the suspected agent from diseased patients

   2) Culture the agent

   3) Show that introducing the cultured agent causes disease in test patients/animals

   4) Reisolate the agent and show it's the same microbe that you originally isolated.
Questions 5 through 8 (1 pt. each). A gifted medical student in your lab, under your guidance, does indeed rigorously demonstrate that Bacterium X is the etiological agent. You have identified one gene, vir1, which is required for Bacterium X to colonize the human intestine, i.e., are present 10 days after ingestion. In vitro (i.e., in the test tube), vir1 is expressed by Bacterium X only in presence of lactose and under high [Mg”] conditions. You are interested in identifying as many other vir genes (i.e., genes required to for intestinal colonization) of Bacterium X as possible. You are considering a number of approaches, but want to think hard about the inherent limitations of each approach.

For each statement below, indicate which of the following approaches applies best. Each answer can be used once, more than once, or not at all.

Approaches:

A. Clone 25 kilobase fragments of DNA into a plasmid to generate a set of clones that together represent the entire chromosome. Transform your clones into a bacterial strain that does not colonize the intestine. Take your whole population of resulting bacteria and feed to human volunteers. Isolate all the bacteria that successfully colonize, i.e., are present in the intestine 10 days later.
B. Make random transposon insertions onto the Bacterium X genome. Screen individual mutants for their ability to colonize the intestine.
C. Use TnphoA (i.e., a transposon that allows you to determine if the gene that the transposon has inserted into encodes a secreted product) to make random insertions onto the Bacterium X genome. Screen individual mutants for the ability to secrete the phoA gene product (i.e., give blue staining when plated on indicator plates).
D. Use TnlacZ (i.e., a transposon that allows you to determine the regulation of genes that the transposon has inserted into) to make random insertions onto the Bacterium X genome. Screen individual mutants for turning on lacZ (e.g., give blue staining) only in presence of lactose and under high [Mg”] conditions.

5. (1 pt.) This approach is the most labor-intensive approach that makes the fewest assumptions as to the nature of other vir genes.

6. (1 pt.) If successful, this genetic approach results in the selection or enrichment of vir genes, and has the potential to be the quickest and least labor intensive.

7. (1 pt.) This approach relies on the observation that many virulence-related genes are coregulated.

8. (1 pt.) This approach will work only if the factors required for colonization are encoded by genes that are physically linked (i.e., relatively close together) in the genome of Bacterium X.

9. (1 pt.) You decide to take approach A. To maximize your chances of success, what microorganism should you transform your clones into? (Circle one).

A. A gram-positive bacterium that cannot colonize the intestine.
B. A gram-negative bacterium that cannot colonize the intestine.
C. The yeast Saccharomyces cerevisiae, a laboratory microorganism that is easy to manipulate genetically.
10. (2 pts.) The same medical student in your laboratory performs electron microscopic studies of Bacterium X, and finds that this bacterium has filamentous "organelles" that protrude from its surface. Identify two functions that these organelles might serve:

A. Attachment to the epithelium, while keeping the bacterium at some distance.
B. Motility (cella)

11. (1 pt.) To determine the function of these organelles, you want to identify the genes that encode this organelle. Of the approaches (A through D) given on previous page, which one is best (i.e., most specifically) suited to the identification of such genes?

12. (4 pts.) You use this approach to identify a gene, vir2, which you suspect is important for colonization and disease. Identify the steps you need to perform in order to rigorously prove that vir2 is important for infection.

Koch's Molecular Postulates:
1) Isolate the gene (done)
2) Mutate the gene
3) Show that such mutants have reduced infectivity /virulence.
4) Show that restoration to wt, or genetic complementation with a wt plasmid restores infectivity /virulence.

13. (1 pt.) Sequencing of the entire genome of Bacterium X by a company that is interested in developing novel antibiotics reveals that Bacterium X encodes a "two-component regulatory system", i.e., a system that is used in other bacteria to coordinately control large numbers of genes. Of the approaches (A through D) given on previous page, which one is best (i.e., most specifically) suited to the identification of genes that fall under the control of this two-component regulatory system?
14. A. (2 pts) Endotoxin. Is endotoxin considered a conventional toxin? Why or why not?
No, it is unconventional because it is not a protein and it is not secreted into the microenvironment.

B. (1 pts.) What class of host inflammatory mediators are released in response to endotoxin?
Cytokines (TNFα/IL-1/IL-6)

C. (2 pts.) Paradoxically, endotoxin can be considered beneficial or harmful to the host. Under what circumstances is the host response to endotoxin beneficial to the host? Under what circumstances harmful?
Beneficial: Acts as a non-specific "alarm" to notify the immune system of a localized infection.
Harmful: Under conditions of massive infection/bacteremia, the LPS response may induce septic shock.

15. Gram positive and gram negative bacteria. (4 pts.)

A. How many membranes are present?

B. Is peptidoglycan found in one, both or neither class(es)?

C. What is the color of each upon gram staining?
Purple  Pink

D. Name two classes of pathogenic bacteria that are neither gram negative nor gram positive.
Mycobacterium
Chlamydiae
16. A. (2 pts.) Phagocytes. What are the two most important types of professional phagocytes?
   1. Neutrophils
   2. Macrophages

B. (3 pts.) Outline the typical sequence of events by which these cells kill microorganisms.
   - Encounter
   - Phagocytosis
   - Oxidative Burst or Phagolysosome Formation
   - Apoptosis is necessary.

C. (1 pt.) Which of the above steps does opsonization facilitate?
   Phagocytosis

D. (2 pts.) What are the two most important opsonins?
   C3b & IgG

17. (3 pts.) Describe three of the strategies that intracellular pathogens use to grow within phagocytes.
   A. Escape from the phagolysosome.
   B. Ability to live within the phagolysosome (i.e. catalase)
   C. Prevention of fusion of the phagosome & lysosome

18. (2 pts) What anatomical location does Salmonella typhi colonize in order to establish a chronic carrier state? What particular property does S. typhi possess to allow it to survive here?

   Location. Gall bladder
   Bacterial property. Capsule which renders it immune to bile salts.

19. Neisseriae (5 pts.).
   A. (1 pt.) Recall that Neisseriae gonorrhoeae grown in the lab is sensitive to serum killing, whereas N. gonorrhoeae taken from a patient is not. What component of serum is responsible for the killing of in vitro-grown bacteria?
      Complement

   B. (1 pt.) Recall that a component of N. gonorrhoeae is modified by the addition of a sugar to allow this organism to avoid serum killing. What is the component of N. gonorrhoeae that is modified? The capsule glycoprotein.
C. (EXTRA CREDIT 1 pt.) What is the sugar?  

\[ \text{Sialic Acid} \]

D. (1 pt.) The sugar is transferred from a donor molecule that consists of CMP linked to the sugar. Is the donor molecule synthesized by the human host or from the bacterium?

\[ \text{Human Host} \]

E. (1 pt.) Is the enzyme that catalyzes this modification of human or bacterial origin?

\[ \text{Bacterial (Sialyltransferase)} \]

Questions 20 through 24 (1 pt each):

A. Antigenic variation
B. Phase variation
C. Both
D. Neither

\[ \text{A} \rightarrow 20. \text{ Refers to the expression of variants of a microbial structure.} \]

\[ \text{B} \rightarrow 21. \text{ Refers to the expressing or not expressing a microbial structure.} \]

\[ \text{C} \rightarrow 22. \text{ Can contribute to immune evasion by microbial pathogens.} \]

\[ \text{A} \rightarrow 23. \text{ Occurs in } N. \text{ gonorrhoeae} \]

\[ \text{C} \rightarrow 24. \text{ Occurs in } N. \text{ meningitidis} \]

25. A patient is admitted to the hospital with septic shock (i.e., overwhelming bacteremia that is associated with vascular shock and multisystemic organ failure).

A. (1 pt.) Which Neisseriae species (\( N. \text{ gonorrhoeae} \) or \( N. \text{ meningitidis} \)) is more likely to be the causative agent?  

\[ N. \text{ meningitidis} \]

B. (1 pt.) What component of that bacterium explains its greater invasive potential?

\[ \text{Capsule} \]

C. (1 pt.) What host defense mechanism does this component impair?

\[ \text{Complement opsonization in the bloodstream} \]

Z. (1 pt.) What body compartment or location must *H. influenzae* type B colonize in high numbers in order to cause meningitis? Blood (which allows it to reach the CSF)

AA. (1 pt.) What feature of *H. influenzae* type B is most responsible for its ability to achieve high numbers in this compartment or location? Polyribosome

BB. (1 pt.) What group of individuals are most susceptible to invasive *H. influenzae* type B infection? (Circle one)

- Neonates (e.g., 0-6 months).
- Infants age 6 months to three years.
- Children age 4 y to 14.
- Adults.
- Elderly.

D. (2 pts.) Explain the above susceptibility.

After six months, the passive immunity conferred by maternal Ab wears off. Prior to three years, we can't make Ab to polysaccharides very well.

E. (2 pts.) What intervention in the last ten years or so has resulted in a ~90% decrease in the incidence of invasive *H. influenzae* type B infection?

Vaccination of the capsule conjugated to a protein carrier to all infants.
27. Match each term in column A to the **BEST AND MOST SPECIFIC** definition in column B. Write the number from column B in the blank space to the left of column A (8 pts.).

<table>
<thead>
<tr>
<th>Column A</th>
<th>Column B</th>
</tr>
</thead>
<tbody>
<tr>
<td>8. toxin</td>
<td>1. Gram-positive lipopolysaccharide</td>
</tr>
<tr>
<td>9. endotoxin</td>
<td>2. binds to surface of target cell</td>
</tr>
<tr>
<td>12. exotoxin</td>
<td>3. digests target cell membrane</td>
</tr>
<tr>
<td>6. enterotoxin</td>
<td>4. active in target cell cytoplasm</td>
</tr>
<tr>
<td>4. A subunit</td>
<td>5. receptor on target cell surface</td>
</tr>
<tr>
<td>2. B subunit</td>
<td>6. active in the GI tract</td>
</tr>
<tr>
<td>11. type III secretion system</td>
<td>7. aerobic spore-forming rods</td>
</tr>
<tr>
<td>10. Clostridia</td>
<td>8. protein that has adverse effects on cells or host organisms</td>
</tr>
<tr>
<td></td>
<td>9. Gram-negative lipopolysaccharide</td>
</tr>
<tr>
<td></td>
<td>10. anaerobic spore-forming rods</td>
</tr>
<tr>
<td></td>
<td>11. injects toxin into target cell cytoplasm</td>
</tr>
<tr>
<td></td>
<td>12. toxin that is secreted into the medium by the bacterium</td>
</tr>
</tbody>
</table>

28. Toxic shock syndrome toxin. Fill in the blanks. (8 pts.)

a. What single word best describes the mechanism of toxic shock syndrome toxin (TSST), i.e., what class of toxins does TSST belong to?  
**Superantigen**

b. What two host cell types are required for TSST action?  
**T-Cells**  
**Macrophages**

c. What two host cell molecules are required for TSST action?  
**TcR for antigen**  
**XBU (signal transduction)**

d. What class of host molecule is increased by TSST action?  
**Cytokines**

e. What organism makes TSST?  
**Staph. Aureus**

f. What are its shape and Gram-staining properties?  
**Gram B  Cocccus**
29. Circle the best answer for each question (6 pts.).

a. What is the site of action of cholera toxin?
   - large intestine
   - liver
   **small intestine**
   - stomach

b. What is the structure of cholera toxin?
   - 5B+5A
   - 1B+5A
   **5B+1A**
   - 1B+1A

c. What is the host molecule that is targeted by cholera toxin?
   - Gsβγ
   - GTP
   **Gsα**
   - Gox

d. What host cell molecule is increased after intoxication by cholera toxin?
   - ATP
   - GTP
   **cAMP**
   - cGMP

e. What is the route of entry for *Vibrio cholerae*?
   - inhalation of aerosols
   - puncture wound
   **ingestion of contaminated water**
   - abrasion of skin

f. What is the most appropriate therapy for cholera patients?
(ORT = oral rehydration therapy)
   - ORT with K⁺ and glucose
   - ORT with Mg²⁺ and glucose
   **ORT with Ca²⁺ and glucose**
   - ORT with Na⁺ and glucose