

Bacterial Culture and Antibiotic Susceptibility Testing

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Abstract: *Complicated bacterial infections should prompt clinicians to pursue a definitive diagnosis. Two methods of bacterial culture and antibiotic susceptibility testing are commonly used in veterinary medicine: (1) the disk diffusion technique and (2) the broth dilution technique. Both methods identify the infecting pathogen and the antibiotics that are likely to inhibit its growth. The broth dilution test also provides the minimal inhibitory concentration, which can help in making the best antibiotic choice.*

Bacterial infections in companion animals are frequently self-limiting, causing discomfort but not serious illness. The types of bacteria responsible for these infections are frequently predictable. The choice of antibiotics to treat simple infections can usually be made based on historical data and the clinician's experience.

More serious bacterial illness should prompt clinicians to identify the responsible pathogen and determine the medication most likely to be efficacious in inhibiting or killing the bacteria. Bacterial culture and antibiotic susceptibility testing should be considered for life-threatening illnesses; recurrent, nonresponsive, or chronic infections; and illnesses with a history of previous antibiotic therapy.

Reasons for Bacterial Culture and Antibiotic Susceptibility Testing

Bacterial culture and antibiotic susceptibility testing are important for confirming the presence of bacterial infection, identifying the responsible pathogen, and directing antibiotic choices.¹ Additional variables such as route and frequency of administration, cost, and potential adverse effects can then be considered when choosing the most appropriate antibiotic for the patient. Using susceptibility testing to help in choosing the most effective antibiotic can reduce the expense and client frustration that may occur with blind antibiotic trials; lower the risks of complications and of promoting antibiotic resistance; and improve the chance and speed of a patient's recovery.

Specimen Collection

Samples must be collected and handled properly to obtain reliable results. Poor collection techniques may result in lack

of bacterial growth or abundant growth of contaminants. To avoid contamination, aseptic technique is necessary when obtaining samples. Submission of fluid, effusion, exudate, and tissue samples is preferred to simply submitting swabs of these samples.²

Collection of samples early in the disease process is recommended to reduce the possibility of pathogenic bacteria dying or being overgrown by other bacteria.² Samples should be collected before antibiotic therapy to assure the best growth of the pathogen. If antibiotic therapy has already been instituted, samples should be collected just before the next dose is administered.²

Identifying Bacteria

When submitting samples to a laboratory for bacterial culture and antibiotic susceptibility testing, the clinician should include information about the site of sample collection and the type of lesion. This information assists the microbiologist in deciding which nutrient media and growth conditions to use. Samples for bacterial culture are applied to plates of various growth media with a sterile loop, effectively spreading bacterial organisms over the surface of each plate in a single layer.³ Once inoculated, the plates are incubated in an environment with controlled temperature, humidity, and oxygen and carbon dioxide levels that are optimum for replication of the suspected bacteria.

Each bacterial organism grows into a small cluster, called a *colony*, and individual colonies are inoculated onto new, separate media, creating pure samples.³ Identification of the cultured bacteria is based on the characteristics of colony growth and appearance as well as biochemical testing of the individual colonies.³

FIGURE 1

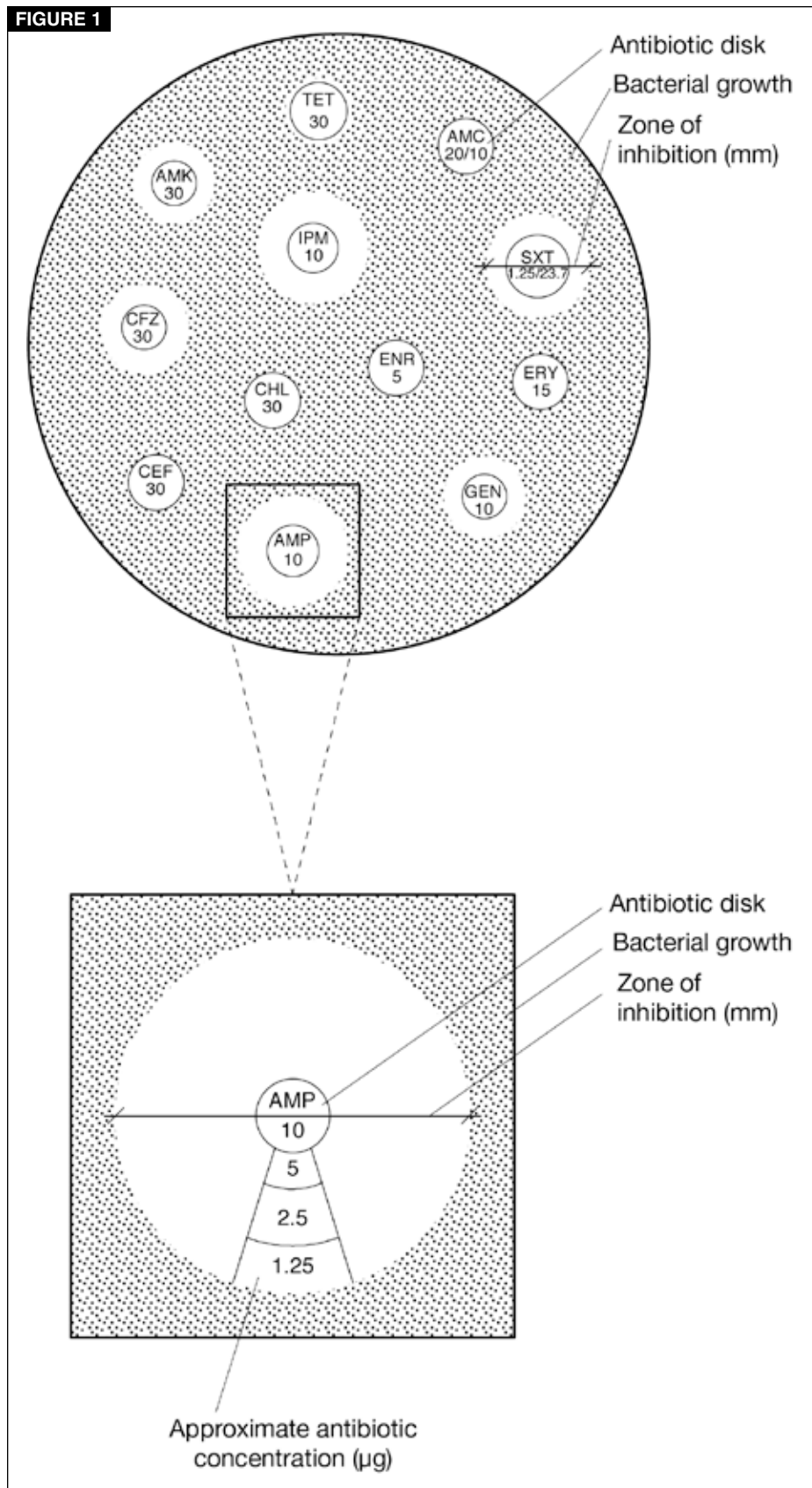


Diagram of the disk diffusion method. The antibiotic concentration in the agar decreases as the drug diffuses away from the disk.

Testing Bacteria for Antibiotic Susceptibility

Once identified, the bacteria undergo testing to identify the antibiotics most likely to inhibit their growth. The most common methods of antibiotic susceptibility testing used in veterinary laboratories are the disk diffusion and broth dilution techniques.⁴

Disk Diffusion Technique

The disk diffusion technique (Kirby-Bauer method) historically has been and continues to be the method most commonly used in the veterinary field for determining antibiotic susceptibility. In this technique, a fixed volume of nutrient broth containing a standard concentration of bacteria is smeared evenly onto the surface of an agar plate.⁵ Next, disks of filter paper, each impregnated with a standard concentration of an antibiotic, are applied to the plate surface.⁶ The plate is incubated, and as the bacteria grow on the surface of the plate, the antibiotics diffuse from the paper disks out into the agar. Each antibiotic diffuses at a different rate, achieving different concentrations in the surrounding agar based on its molecular size and chemical properties. The concentration of antibiotic in the agar decreases as the antibiotic diffuses further from the disk. Eventually, the antibiotic concentration in the agar drops below that needed to inhibit the growth of the bacteria. The area around the disk in which the antibiotic concentration is high enough to inhibit bacterial growth is called the *zone of inhibition* (FIGURES 1 and 2).

The zone of inhibition is measured in millimeters by a laboratory technician. The edge of this zone correlates with the antibiotic concentration that inhibits the growth of the bacteria. The width of the zone is compared to a standard

table of predetermined zone widths representing antibiotic concentrations in the agar that correlate with the concentration of the antibiotic achievable in the plasma of a patient using the manufacturer's recommended dosage. If the zone of inhibition is wider than the predetermined zone, the bacterial species is considered to be susceptible (S) to the antibiotic. If bacteria grow within the predetermined zone width, the species is considered resistant (R). An intermediate (I) designation is used if the zone of inhibition approximates the predetermined zone width.

Laboratories using the disk diffusion technique report the bacteria isolated, a list of the antibiotics tested, and the designation S, I, or R. A disadvantage of the disk diffusion technique is that the exact concentration of the antibiotic that inhibited bacterial growth is not known.

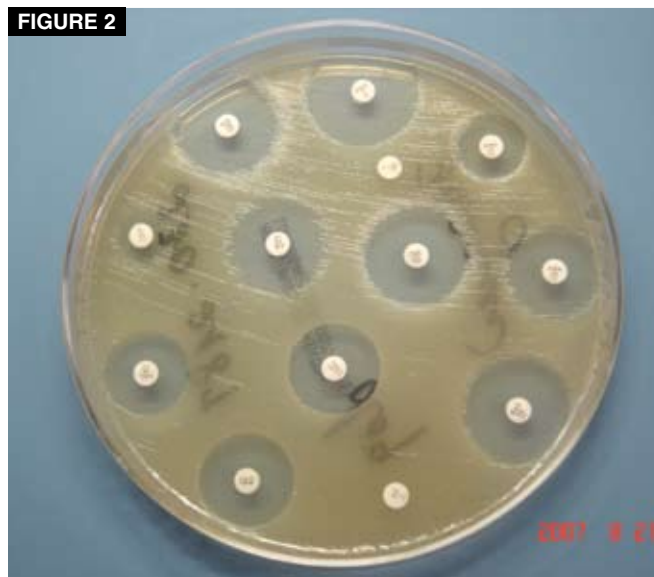
Broth Dilution Technique

The broth dilution technique of antibiotic susceptibility testing is also known as the *minimal inhibitory concentration (MIC)* technique. Test tubes or wells containing increasing concentrations of each antibiotic to be tested, from 0.0312 to 512 µg/mL, are inoculated with a fixed volume of nutrient broth containing a standard concentration of bacteria.⁷ The concentration of the antibiotic in each tube is double that in the previous tube. Very few laboratories evaluate bacterial growth at the full range of antibiotic concentrations.⁸ More often, a smaller range of dilutions is used to evaluate bacterial growth based on previous experience with antibiotic susceptibility testing for the particular pathogen.

The tubes are incubated and examined for turbidity. A turbid sample is an indication of bacterial growth, whereas a clear sample is an indication of inhibition of bacterial growth (FIGURE 3). The MIC is the lowest concentration of the antibiotic being tested that inhibits the growth of the bacteria, resulting in a sample that lacks turbidity (FIGURE 4). To determine whether the pathogen is susceptible, intermediate, or resistant, the MIC is compared with the concentration of antibiotic that can be achieved in the plasma of a patient using the manufacturer's recommended dosage. (The concentration of antibiotic achievable in the plasma using the recommended dosage is also sometimes called the *breakpoint* or MIC_{BP}). Ideally, the clinician should have the breakpoint values available when evaluating the test results (TABLE 1⁹).

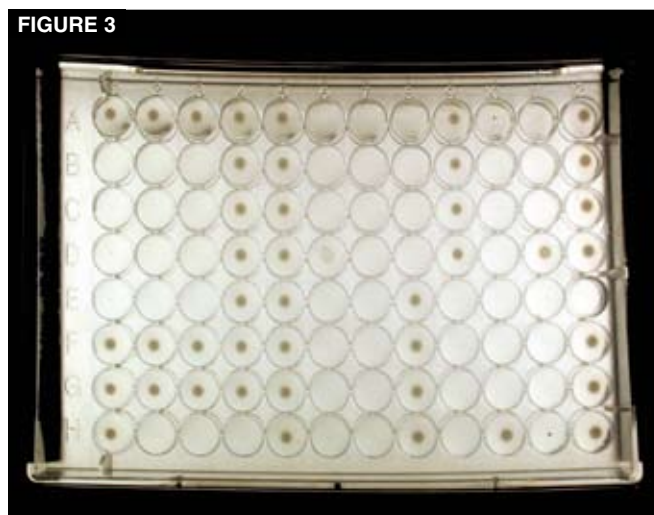
When reporting broth dilution results, laboratories typically note the species of bacteria isolated, the antibiotics tested, the MIC for each antibiotic tested, and a final interpretation of S, I, or R for each antibiotic. A less than (<) notation is used if the MIC was less than the range of antibiotic concentration tested (i.e., there was no bacterial growth even at the lowest antibiotic concentration tested), whereas a greater than (>) notation is used if the MIC was greater than the range of antibiotic concentration tested (i.e., there

FIGURE 2



Disk diffusion plate. Courtesy of Cynthia Essmyer, MD, DABIM, DABPMM, Saint Luke's Hospital, Kansas City, Missouri

FIGURE 3



Broth microdilution tray. Courtesy of William Fales, PhD, DACVM (Hon), University of Missouri Veterinary Medical Diagnostic Laboratory

was bacterial growth in all of the antibiotic concentrations tested). Some laboratories also report the antibiotic dosage used to make the interpretation of susceptibility.

The broth dilution method is considered to be superior to the disk diffusion method because it provides the MIC in addition to an interpretation of S, I, or R.⁵ Comparing the MIC to the achievable antibiotic plasma concentration allows consideration of the relative susceptibility of the bacteria to each antibiotic. The lower the MIC compared with the achievable antibiotic plasma concentration (MIC_{BP}), the more likely the therapy is to be effective.

Interpretation of Antibiotic Susceptibility Results

The Clinical Laboratory Standards Institute (CLSI) is respon-

FIGURE 4

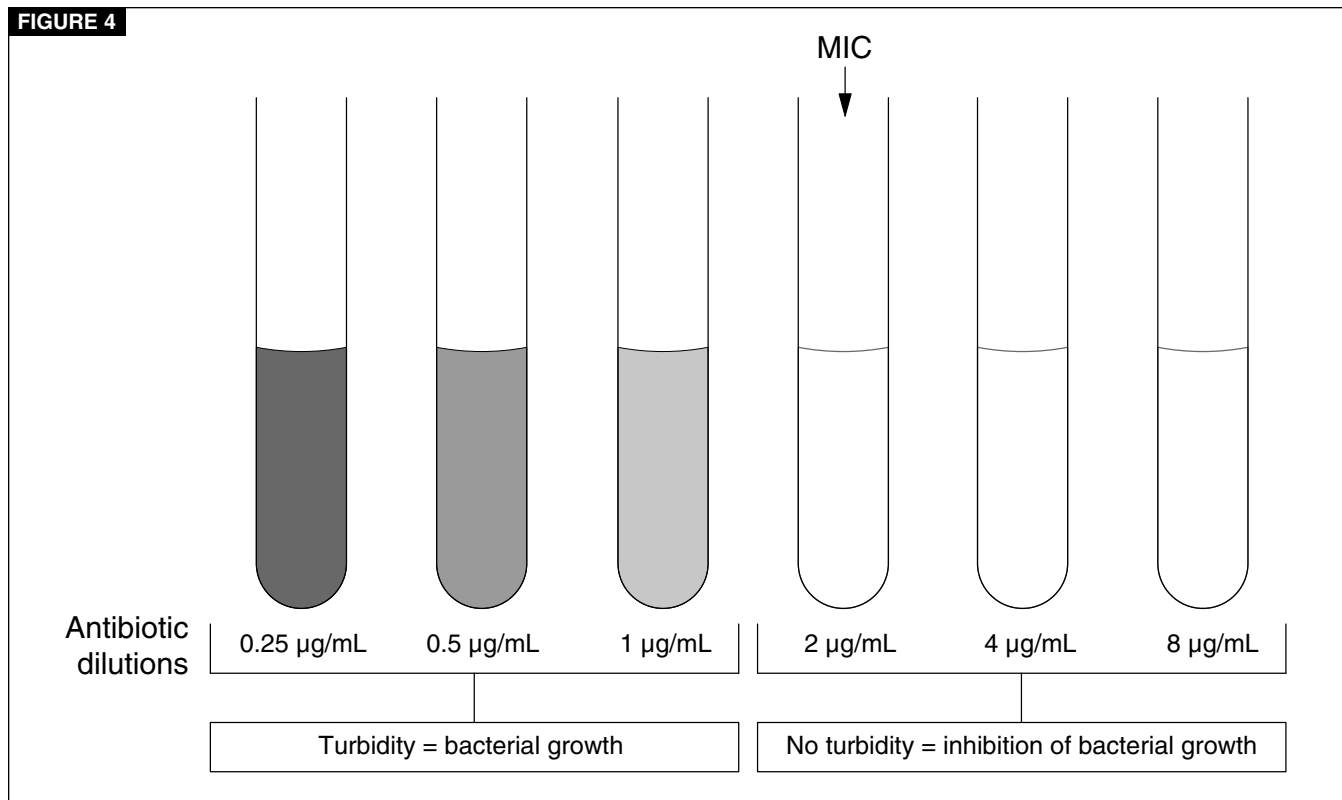


Diagram of the broth dilution method. The MIC is the lowest concentration of antibiotic that lacks turbidity (i.e., has no bacterial growth).

sible for determining the standards used to interpret bacterial culture and antibiotic susceptibility results. Antibiotic pharmacokinetic and pharmacodynamic studies performed by the CLSI, independent researchers, and antibiotic manufacturers provide the information used to formulate these standards. These standards differ between humans and animals; therefore, it is important that the laboratory use the standards created for animals.

The CLSI is responsible for reporting the concentration of each antibiotic to which a bacterial pathogen is to be considered susceptible or resistant. For bacteria to be considered susceptible to an antibiotic, their growth must be inhibited at a concentration lower than the plasma concentration achievable using the manufacturer's labeled dosage. A bacterial pathogen is considered resistant to a particular antibiotic if its growth is inhibited at a concentration that is higher than the concentration that can be reasonably achieved in plasma.⁵ An intermediate classification suggests that the concentration of antibiotic needed to inhibit the growth of the organism is very close to the plasma levels achievable at the recommended dosage.

Occasionally, manufacturers provide a dosage range to treat infections. If a dosage range for a drug is given, the highest dose in the range is used to determine whether the pathogen is considered susceptible, intermediate, or resistant to the respective antibiotic.

Guidelines for Evaluating Antibiotic Susceptibility Results

Ideally, clinicians should always choose a drug to which the identified bacteria are considered susceptible and should avoid agents to which they are intermediate or resistant. If MIC information is available, an antibiotic with an MIC that is much lower than the achievable antibiotic plasma concentration should be considered because it is much more likely to be effective in treating the infection.¹⁰

Antibiotic doses can also be altered based on MIC values. If the MIC is much lower than achievable antibiotic plasma concentrations based on the recommended manufacturer's dosage, a lower dose or dosing interval may still be effective. If the MIC is near the achievable antibiotic plasma concentration, a higher dose or dosing interval should be used.

Limitations of Bacterial Culture and Antibiotic Susceptibility Testing

Selecting antibiotics based on susceptibility data does not guarantee clinical success. Susceptibility tests are conducted in vitro and cannot completely predict the behavior of the pathogen or the antibiotic in vivo. The determination of S, I, or R is based on the expected concentration of the drug in the plasma, not in the tissue at the site of infection.⁹ The concentration of bacteria at the site of infection may be higher than that used during the antibiotic susceptibility

testing, resulting in a reduced effectiveness of the drug. False-positive results can occur if normal flora or nosocomial bacteria are isolated. False-negative results are possible if the sample is improperly collected or stored for culture or if the patient has previously received antibiotic therapy. Factors such as the cost of therapy, pharmacodynamics and pharmacokinetics of the drug, and location and character of the infection must also be taken into account when choosing the best antibiotic for each particular patient.⁵

Conclusion

The broth dilution and disk diffusion techniques are the most commonly used methods of bacterial culture and antibiotic susceptibility testing in veterinary medicine. Both methods can be used to identify the likely pathogen involved in a bacterial infection and the antibiotic most likely to inhibit the bacteria.

References

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TABLE 1 Antibiotic Breakpoints of Common Antibiotics

| Antimicrobial | Susceptible (µg/mL) ^a | Resistant (µg/mL) |
|-------------------------------|----------------------------------|-------------------|
| Amikacin | ≤16 | ≥64 |
| Amoxicillin-clavulanic acid | ≤8/4 ^b | ≥32/16 |
| Ampicillin | ≤8 ^b | ≥32 |
| Cefazolin | ≤8 | ≥32 |
| Cefotaxime | ≤8 | ≥64 |
| Cefoxitin | ≤8 | ≥32 |
| Ceftazidime | ≤8 | ≥32 |
| Cephalothin ^c | ≤8 | ≥32 |
| Chloramphenicol | ≤8 | ≥32 |
| Ciprofloxacin | ≤1 | ≥4 |
| Clindamycin | ≤0.5 | ≥4 |
| Difloxacin | ≤0.5 | ≥4 |
| Enrofloxacin | ≤0.5 ^d | ≥4 |
| Erythromycin | ≤0.5 ^b | ≥8 |
| Gentamicin | ≤2 | ≥8 |
| Imipenem (and meropenem) | ≤4 | ≥16 |
| Marbofloxacin | ≤1 | ≥4 |
| Orbifloxacin | ≤1 ^d | ≥8 |
| Oxacillin ^e | ≤2 | ≥4 |
| Rifampin | ≤1 | ≥4 |
| Tetracycline | ≤4 | ≥16 |
| Ticarcillin | ≤64 ^b | ≥128 |
| Trimethoprim-sulfamethoxazole | ≤2/38 | ≥4/76 |
| Vancomycin | ≤4 | ≥32 |

^aValues between the susceptible and resistant ranges are interpreted as intermediate.

^bThere are exceptions for interpreting some pathogens: for ampicillin, susceptibility is ≤0.25 µg/mL for staphylococci and streptococci; for amoxicillin-clavulanate, susceptibility is ≤4/2 µg/mL for staphylococci; for ticarcillin, susceptibility is ≤64 µg/mL for *Pseudomonas* spp and ≤16 µg/mL for enteric gram-negative bacteria; for erythromycin, susceptibility is ≤0.25 µg/mL for streptococci.

^cCephalothin is used as a marker to test for susceptibility to cephalixin and cefadroxil.

^dFor enrofloxacin and orbifloxacin, the intermediate category may require higher doses.

^eIf organisms are resistant to oxacillin, they should be considered also resistant to other β-lactam antibiotics.

Adapted with permission from Papich MG, Bidgood T. Antimicrobial drug therapy. In: Ettinger SJ, Feldman ED, eds. *Textbook of Veterinary Internal Medicine*. 6th ed. St. Louis: Elsevier Saunders; 2005:498-503.

CE TEST

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- 1. Bacterial culture and susceptibility testing would be important to perform in a patient with**
 - a. a life-threatening illness.
 - b. a recurrent infection.
 - c. an illness previously treated with antibiotic therapy.
 - d. any of the above
- 2. Which sample is ideal for submission for bacterial culture and antibiotic susceptibility testing?**
 - a. a syringe of whole blood in an EDTA tube obtained during routine venipuncture
 - b. a syringe of purulent exudate collected during surgery
 - c. a swab of voided urine collected in a bowl
 - d. a swab of a superficial skin wound
- 3. Samples for bacterial culture and sensitivity testing should ideally be collected**
 - a. as early in the disease process as possible.
 - b. after the first dose of antibiotic has been given.
 - c. after the animal has become febrile.
 - d. when the white blood cell count is elevated.
- 4. Which characteristic is indicative of bacterial growth using the broth dilution technique?**
 - a. visualization of bacteria in the sample using light microscopy
 - b. turbidity of the sample
 - c. viscosity of the fluid
 - d. none of the above
- 5. Which laboratory finding can be used to help predict the most effective antibiotic selection?**
 - a. diameter of the zone of inhibition (disk diffusion technique)
 - b. number of bacteria per high-power field on direct microscopic examination (broth dilution technique)
 - c. MIC (broth dilution technique)
 - d. characteristics of colony growth on the agar plate (disk diffusion technique)
- 6. Which antibiotic would be most likely to result in successful treatment of a bacterial infection at the MIC given?**
 - a. enrofloxacin; MIC = 0.25 µg/mL
 - b. cefazolin; MIC = 1 µg/mL
 - c. ampicillin; MIC = 16 µg/mL
 - d. clindamycin; MIC = 1 µg/mL
- 7. Based on the disk diffusion technique, the most effective antibiotic is likely to be**
 - a. the one with the largest zone of inhibition.
 - b. the one the smallest zone of inhibition.
 - c. designated S.
 - d. the one with the lowest MIC.
- 8. Which of the following is not a limitation of bacterial culture and antibiotic susceptibility testing?**
 - a. They are in vitro tests.
 - b. They assume equal plasma and tissue concentrations of antibiotic.
 - c. False-positive and false-negative results are possible.
 - d. They can limit the chance of antimicrobial resistance.
- 9. When a manufacturer supplies a dosage range for an antibiotic, susceptibility testing is based on**
 - a. the low end of the dosage range.
 - b. the middle of the dosage range.
 - c. the high end of the dosage range.
 - d. a dose 10% above the high end of the dosage range.
- 10. The susceptibility or resistance of a pathogen is determined by comparing the MIC with the**
 - a. achievable plasma drug concentration.
 - b. diameter of the zone of inhibition.
 - c. minimum bactericidal concentration.
 - d. characteristics of colony growth.