Name: _____

Microbiology
Kirby-Bauer Susceptibility Testing (Modified)

Materials required:

- Misc. equipment and supplies as needed
- Trypticase soy broth (TSB), 1 ml
- Mueller Hinton Agar (MH) 2
- Sterile swab
- Antibiotic disks, as per culture type
- Metric ruler/caliper
- *Staphylococcus aureus* ATTC 25922 and *Escherichia coli* ATTC 25923

What is the Clinical Purpose of performing a Susceptibility Test: (1 pt)

Procedure: DAY 1

1. Using your inoculating needle touch 3-5 well isolated colonies of the organism to be tested. Use the needle to inoculate a TSB. Incubate at 35° C for one hour. The broth should be noticeably turbid. (A turbidity standard is normally used.)
2. Label your plates as directed. Soak a sterile swab in the TSB for a few seconds. Using the spread plate technique, cover the entire MH plate using overlapping streaks. Turn the plate 90 degrees and repeat. Turn the plate 45 degrees and repeat for a third time. The last inoculating step requires running the swab around the inner edge of the plate. The plate must be covered with a uniform lawn of the bacteria to be tested. Repeat this plate inoculation on a second plate.
3. Place the antibiotic dispenser on the MH plate. Dispense the antibiotic disks. Place no more than 4 discs on a plate. See reference chart below as to determine which discs to place on each plate. Using the back side of a loop gently tamp the disks onto the media. Good contact is essential for accurate results. If a disk is out of position on the media do not move it. Incubate at 35° C for 18-24 hours.
4. **DAY 2:** Observe your MH plate.

   a. If there were obvious gaps in the inoculum, what would this indicate; other than the zones of inhibition? (0.5 pt)

   b. If there were two or more morphological differences present, what would this indicate? (0.5 pt)

5. On the table provided, list the antibiotics and their abbreviations. Using a metric rule or caliper, measure the zone of inhibition in millimeters for each antibiotic. Record your measurements.
6. Using the provided chart interpret the results as: S (sensitive), I (intermediate), or R (resistant). Record your results on the table below.

Name: _____

Susceptibility results:		***S. aureus***		***E. coli***	
Antibiotic	**Abbr.**	**Zone**	**Interp** (0.5 pt)	**Zone**	**Interp** (0.5 pt)
Ampicillin	AMP				
Chloramphenicol	C				
Erythromycin	E				
Gentamicin	CN				
Penicillin	P				
Streptomycin	S				
Tetracycline	TE				
Sulfonamides	SF				

Zone Diameter Interpretive Standards:

Antimicrobial Agent	Resistant	Intermediate	Susceptible
Ampicillin (gram neg)	≤ 13		≥ 17
Ampicillin (Staph)	≤ 28		≥ 29
Chloramphenicol	≤ 12	13-17	≥ 18
Erythromycin	≤ 15	14-22	≥ 23
Gentamicin	≤ 12	13-14	≥ 15
Penicillin (Staph)	≤ 28		≥ 29
Streptomycin	≤ 11	12-14	≥ 15
Tetracycline	≤ 14	15-18	≥ 19
Sulfonamides	≤ 12		≥ 26

Organism Identification (Either Staph or E. coli)**: _____**

Type your answers to the following questions on a separate sheet of paper and attach.

1. Your experimental results should have shown that some of the antibiotics have a broad range of activity against bacteria. Why do these same antibiotics have no activity against eukaryotic cells such as fungi? (1 pt)

2. All aspects of the Kirby-Bauer (KB) test are standardized to assure reliability.
   a. What might the consequence be of pouring a plate 2 mm deep instead of 4mm? (1 pt)

   b. The plates are supposed to be used within a specific time after their preparation and should be free of visible moisture. What to you surmise as the reason for these directions? (1 pt)

3. In clinical applications of the KB test, diluted cultures must be used within thirty minutes. Why is this important? (Hint: look at generation times as explained in chapter 7). (1 pt)

4. *E. coli* and *S. aureus* were chosen to represent Gram negative and Gram positive bacteria, respectively. For a given antibiotic, is there a difference in susceptibility between Gram negative and Gram positive bacteria? Explain what the difference(s) is(are) between these two organisms. (1 pt)

5. Suppose you do this test on a hypothetical *Staphylococcus* species with the antibiotics penicillin (P-10) and chloramphenicol (C-30). You record zone diameters of 20 mm for the chloramphenicol disc and 25 mm for the penicillin disc. Which antibiotic would be the most

effective against this organism? What does this tell you about comparing zone diameters to each other and the importance of the interpretive chart? (2 pt)