Laboratory Report 31

Student: _____

Date: _____ Section: _____

31 Antimicrobic Sensitivity Testing: The Kirby-Bauer Method

A. Results

1. List the antimicrobials that were used for each organism. After measuring and recording the zone diameters in millimeters for each antimicrobial, consult table 31.2 for interpretation of its sensitivity. Record the degrees of sensitivity (R, I, or S) in the rating column. Exchange data with other class members to complete the entire chart.

	ANTIMICROBIAL	ZONE DIA.	RATING (R, I, S)	ANTIMICROBIAL	ZONE DIA.	RATING (R, I, S)
S. epidermidis						
S. marcescens						
P. fluorescens						
E. coli						

2. Which antimicrobials would be suitable for the control of the following organisms?

   S. epidermidis: _____

   E. coli: _____

   S. marcescens: _____

   P. fluorescens: _____

3. Based on class data only, which antimicrobial has the broadest spectrum? Which one has the narrowest spectrum?

   _____

   _____

B. Short-Answer Questions

1. Differentiate between the following and provide one example of each:

   a. antibiotics and antimicrobial drugs

   _____

   b. broad- and narrow-spectrum antimicrobials

   _____

2. What factors influence the size of the zone of inhibition for an antimicrobial?

   _____

3. Why are certain gram-negative bacteria more resistant than gram-positive bacteria to antimicrobials that attack cytoplasmic targets?

   _____

   _____

4. Why are gram-positive bacteria typically more resistant than gram-negative bacteria to antimicrobials that disrupt plasma membranes, such as polymyxin B?

   _____

   _____

5. If a bacterial isolate shows intermediate to moderate resistance to an antimicrobial, how might this drug still be successfully used in the treatment of this microbe?

   _____

   _____

6. What specific medium must be used in testing the effectiveness of antimicrobial drugs, and why is it preferable?

   _____

Antimicrobic Sensitivity Testing: The Kirby-Bauer Method (continued)

7. If an infectious agent is sensitive to several antimicrobial drugs, what other considerations might be used to determine the best treatment option?

8. Describe the five cellular targets of antibiotics, and explain why antibiotics are effective against bacteria but not viruses.

Laboratory Report 32

Student: _____

Date: _____ Section: _____

32 Evaluation of Antiseptics and Disinfectants: The Filter Paper Disk Method

A. Results

1. List the disinfectants/antiseptics that you tested in the table below. With a metric ruler, measure the zones of inhibition in millimeters on all four plates, and record this information in the table below.

DISINFECTANT/ANTISEPTIC	Zone of Inhibition (mm)	
	Staphylococcus epidermidis	*Escherichia coli*

2. Which chemical was the most effective for inhibiting the growth of *S. epidermidis*? Of *E. coli*?

_____

3. Which chemical was the least effective for inhibiting the growth of *S. epidermidis*? Of *E. coli*?

_____

_____

4. What do your results indicate about the relative chemical resistances of these two species?

_____

_____

B. Short-Answer Questions

1. Differentiate between antiseptic and disinfectant. Include examples of each in your answer. Indicate whether any chemicals can be used as both.

_____

_____

Evaluation of Antiseptics and Disinfectants: The Filter Paper Disk Method (continued)

2. What factors influence the size of the zone of inhibition produced by a chemical?

3. How might the physical differences between gram-positive and gram-negative bacteria contribute to differences in chemical resistances?

4. What are the modes of action for some of the antiseptics and disinfectants you used? Explain how they work to control the growth of microorganisms.

Laboratory Report 53

Student: _____

Date: _____ Section: _____

53 The Staphylococci: Isolation and Identification

A. Results

1. **Tabulation**

After examining your mannitol salt agar plate, record the presence (+) or absence (−) of staphylococcus colonies in the appropriate columns. After performing coagulase tests on the various isolates, record the results as (+) or (−) in the appropriate columns. Collect data from your classmates to complete the chart.

FOMITE			NOSE	
Item	Colonies on MSA	Coagulase	Colonies on MSA	Coagulase

CULTURE	TOTAL TESTED	TOTAL COAGULASE POSITIVE	PERCENTAGE COAGULASE POSITIVE	TOTAL COAGULASE NEGATIVE	PERCENTAGE COAGULASE NEGATIVE
Fomite					
Nose					

2. **Microscopy**
   Provide drawings here of your various isolates as seen under oil immersion (Gram staining).

UNKNOWN CONTROL	NOSE	FOMITE

3. **Record of Culture and Test Results**
   Record your observations for all tests in the table below. Using Table 53.1, determine the likely identity of each isolate.

	GROWTH ON MSA (+/–)	FERMENTATION OF MANNITOL (+/–)	HEMOLYSIS ON BAP (α/β/γ)	COAGULATION OF PLASMA (+/–)	DNASE (+/–)	NOVOBIOCIN (S/R)	SPECIES
Unknown							
Nose							
Fomite							

B. Short-Answer Questions

1. Describe the selective and differential properties of mannitol salt agar (MSA) for the isolation and identification of staphylococci.

   _____

   _____

2. Describe the differential property of blood agar for the isolation and identification of staphylococci.

   _____

   _____

3. Why is the coagulase test considered to be the definitive test for *S. aureus*?

   _____

   _____

4. What is the role of coagulase in the pathogenesis of *S. aureus*?

   _____

   _____

5. What is the role of α-toxin in the pathogenesis of *S. aureus*?

6. What are healthcare-associated infections?

7. Why are the staphylococci among the leading causes of healthcare-associated infections?

8. Why are staphylococcal infections becoming increasingly difficult to treat?

9. Why might hospital patients be tested for nasal carriage of *S. aureus*?

10. Describe results from a coagulase, DNase, and novobiocin test that would suggest a mixed culture was used for the tests, as opposed to a pure culture.

11. Why is MRSA not only transmitted in hospitals?

Laboratory Report 54

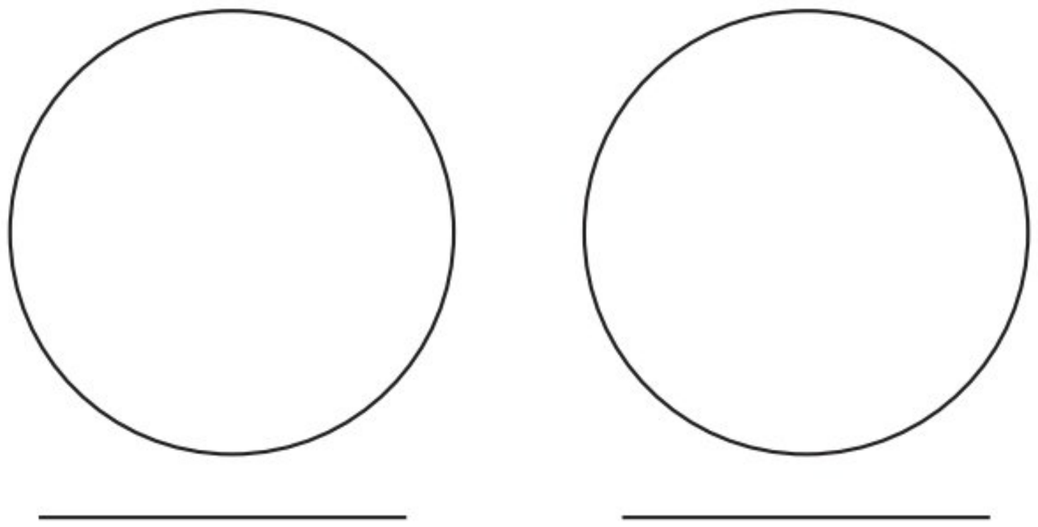
Student: _____

Date: _____ Section: _____

54 The Streptococci and Enterococci: Isolation and Identification

A. Results

1. Microscopy
   Provide drawings here of your isolates and unknowns as seen under oil immersion (Gram staining).



_____ _____

2. **Record of Test Results**
   Record here all information pertaining to the identification of pharyngeal isolates and unknowns.

SOURCE OF UNKNOWN	Hemolysis	Bacitracin Susceptibility	CAMP Reaction	SXT Sensitivity	Bile Esculin Hydrolysis	Tolerance to 6.5% NaCl	Optochin Susceptibility

3. **Final Determination**
   Record here the identities of your group's various isolates and unknowns:

   Pharyngeal isolates: _____

   _____

   Unknowns: _____

   _____

   _____

B. Short-Answer Questions

1. When bacteria from a throat swab are streaked on blood agar, why is the agar stabbed several times with the loop?

2. Differentiate between alpha- and beta-hemolysis.

3. What compound in the cell wall is the basis for the Lancefield classification?

4. In the CAMP reaction, which organism produces the CAMP factor? What substance does the CAMP factor react with to cause enhanced breakdown of red blood cells?

5. Humans may carry both staphylococci and streptococci as normal microbiota. How might you easily differentiate between the two genera?

6. Name two tests that are useful in differentiating *S. pyogenes* and *S. agalactiae*.

7. Name two tests that are useful for the differentiation of pneumococci and oral viridans streptococci.

8. What test can be performed to differentiate the enterococci from other group D streptococci?

9. What test can be performed to differentiate between group A and group C streptococci?

10. Describe the appearance of an *S. agalactiae* colony grown on blood agar. Describe how that colony would differ in appearance from a colony of *S. pyogenes*.

11. Vaginal swabs are taken from pregnant women in their third trimester. Which streptococcal species is the focus of the investigation, and why is this test conducted?

12. Which streptococci are implicated in the development of dental caries? What is the mechanism of their formation?

13. Why is *S. pneumoniae* not able to be classified by the Lancefield system?

Laboratory Report 55

Student: _____

Date: _____ Section: _____

55 Gram-Negative Intestinal Pathogens

A. Results

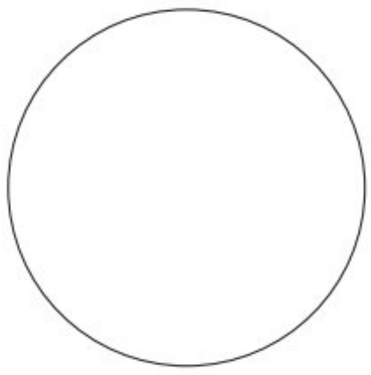
1. Unknown Number _____

   Record your observations and result (positive or negative) for each of the assays completed on your unknown.

	FERMENTS LACTOSE	FERMENTS GLUCOSE	H_2S PRODUCED	MOTILITY	INDOLE	UREASE
Observations						
Result						

2. Microscopy

   Provide a drawing of your unknown as seen under oil immersion (Gram staining).



2. Using your results and the separation outline in figure 55.1, what was the genus of your unknown?

_____

3. Check with your instructor to determine if you have identified your unknown correctly. If your identification was correct, describe the important steps you took to ensure success. If you did not identify the genus correctly, list some potential sources of error that could have led to this result.

_____

_____

_____

_____

_____

4. Now that you know the genus of your unknown, what further steps would you follow to characterize and identify the species?

_____

_____

_____

B. Short-Answer Questions

1. Name three enteric pathogens of primary medical importance.

2. The ability of *Salmonella* to produce H_2S is one characteristic that helps differentiate it from *Shigella*. List the three opportunities you had in this exercise to determine whether or not your unknown produced H_2S.

3. What selective agents are added to media to preferentially grow enterobacteria for study? What type of growth is inhibited?

4. What characteristic separates *Salmonella* and *Shigella* from most of the other *Enterobacteriaceae*? What media can be used for this differentiation?

5. What two characteristics separate *Salmonella* from *Shigella*? What media can be used for this differentiation?

6. Which coliform bacteria are the most difficult to distinguish from the *Salmonella* or *Shigella* pathogens? What is the primary characteristic used to differentiate them?

7. How can acid production by glucose and lactose fermentation be differentiated in the same tube?

8. What is alkaline reversion? Explain why this condition gives a false negative result.

9. In this lab exercise, were the results of the indole test necessary to differentiate between *Salmonella* and *Shigella*? Explain why or why not.

10. What food is a common source of *Salmonella* infections?

Laboratory Report 47

Student: _____

Date: _____ Section: _____

47 Temperature: Lethal Effects

A. Results

Examine your group's plates, looking for evidence of growth. Record the results in the table below by indicating the presence or absence of growth as positive (+) or negative (−). Collect results from the other groups to complete the chart.

ORGANISM	60°C					70°C					80°C					90°C					100°C				
	C*	10	20	30	40	C*	10	20	30	40	C*	10	20	30	40	C*	10	20	30	40	C*	10	20	30	40
S. epidermidis																									
E. coli																									
B. megaterium																									

*control (no-heat) tubes

1. If they can be determined from the above information, record the **thermal death point** for each of the organisms.

   S. epidermidis: _____ *E. coli:* _____ *B. megaterium:* _____

2. From the table shown above, determine the thermal death time for each organism at the tabulated temperatures and *record this information in the table below.*

ORGANISM	THERMAL DEATH TIME				
	60°C	70°C	80°C	90°C	100°C
S. epidermidis					
E. coli					
B. megaterium					

B. Short-Answer Questions

1. What is the importance of inoculating a control plate in this experiment?

   _____

   _____

2. To measure the culture temperature, why is the thermometer placed in a tube separate from the culture?

   _____

   _____

Temperature: Lethal Effects (continued)

3. *Bacillus megaterium* has a high thermal death point and a long thermal death time, but it is not classified as a thermophile. Explain.

_____

_____

4. Give three reasons why endospores are much more resistant to heat than are vegetative cells.

a. _____

b. _____

c. _____

5. List four diseases caused by spore-forming bacteria.

a. _____

b. _____

c. _____

d. _____

6. Give two reasons why this experiment can fail to give the appropriate results.

_____

_____