

**SCB260: General Microbiology**  
**Labs 10-11-12**

**UNKNOWN CULTURE**

**PURPOSE:**

Identifying an unknown will provide the opportunity not only to apply previously learned skills but requires that you make judgments, critically analyze data, and make logical conclusions.

**PROCEDURES:**

- 1) Choose one numbered test tube, containing your "unknown culture."
- 2) Write your name and the number of your culture on the sheet provided by the instructor.

Once you choose your specimen, it is your responsibility. Strict adherence to aseptic technique is crucial to identification.

**POINTS TO REMEMBER:**

- 1) Before you begin to work, carefully think through a sequential plan you wish to follow. Reviewing all previous lab data will help.
- 2) Before you perform each procedure, Read and Reread all instructions in your manual carefully. If you have procedural questions, ask.
- 3) Perform only those tests that aid in confirming the identity of your specimen.
- 4) Be sure to allow for appropriate incubation times.
- 5) Always keep a viable stock culture on hand. Remember the bacterial growth curve!! Once the organisms deplete their sources of nutrients and waste products accumulate, they will die.
- 6) Cultures that you wish to save are always stored in the refrigerator – not in your incubator.
- 7) Record your data on the unknown report sheet as soon as you obtain them.
- 8) Your laboratory technique will be evaluated during these labs.
- 9) Report is due at the end of Lab 12.

## RESOURCES:

Laboratory Manual

Bergey's Manual

Laboratory Incubators and Refrigerators

Culture Media and Antibiotic Discs\*

Test Reagents

\*Be sure you inoculate correctly and use the correct reagents to test for end products.  
Media supplies are limited.

\*In addition to the materials used in class, the following are available:

- 6.5% broth sodium chloride broth
- Phenol red mannitol broth
- Phenol red fructose broth
- Bile Esculin Slants
- Novobiocin Discs

## LIST OF POSSIBLE UNKNOWNNS

Alcalignes faecalis  
Bacillus cereus  
Bacillus megaterium  
Bacillus subtilis  
Bacillus stearothermophilus  
Citrobacter freundii  
Clostridium perfringens  
Clostridium sporogenes  
Enterobacter aerogenes  
Enterococcus faecalis  
Escherichia coli  
Lactobacillus brevis  
Lactococcus lactis  
Micrococcus luteus  
Micrococcus roseus

Micrococcus varians  
Mycobacterium phlei  
Proteus vulgaris  
Proteus mirabilis  
Pseudomonas aeruginosa  
Pseudomonas fluorescens  
Serratia marcescens  
Staphylococcus aureus  
Staphylococcus epidermidis  
Staphylococcus saprophyticus  
Streptococcus faecalis  
Streptococcus agalactiae  
Streptococcus salivarius

## UNKNOWN REPORT

Student Name \_\_\_\_\_

Unknown # \_\_\_\_\_

Due Date \_\_\_\_\_

- 1) Morphology  
(shape and arrangement) \_\_\_\_\_
- 2) Gram Stain Reaction \_\_\_\_\_  
(organisms)
- 3) Endospore Formation \_\_\_\_\_
- 4) Temperature Optimum \_\_\_\_\_
- 5) Oxygen Requirement \_\_\_\_\_
- 6) Culture Characteristics
  - TSA Broth \_\_\_\_\_
  - TSA Slant \_\_\_\_\_
  - Streak Plate  
(Colony Morphology) \_\_\_\_\_  
\_\_\_\_\_

Based on These Data:

Genera and Species  
Eliminated

Genera and Species  
Probable or Possible

Biochemical characteristics (indicate your results using a flow chart/dichotomous key; start flow chart with the results of your gram stain reaction and morphology).

Genus species name of unknown \_\_\_\_\_

## EVALUATION OF UNKNOWN PROJECT

Inclusion of essential data	25%
Correct/logical interpretation of observation/data*	25%
Correct genus identification	20%
Correct species identification	10%
Correct flow chart format	10%
Grammar/spelling	5%
Clear, clean copy – overall neatness	5%
<u>Penalties:</u> Late submission of report	-20%

\*Reduction in grade is proportional to error made (i.e., an incorrect gram stain interpretation is considered more serious than an error made due to an unstable genetic trait).