Exercise 6-Assignment
Morphological Unknown
(30 Points Possible)

The purpose of this exercise is to familiarize students with some of the procedures used in determining the morphological characteristics of unidentified (unknown) bacteria. **Morphology** may be defined as the science or study of structure and form without regard to function. In order to accurately determine morphology, the culture being tested must be pure; therefore, another purpose of this exercise will be to test student knowledge of aseptic technique and streak plate preparation. Upon completion of this exercise, students (as a class) will have been introduced to a number of common bacteria with unique morphological features.

Having obtained a culture containing organisms of a specific type, each student will be expected to determine and record the following:

1. The **number** of the morphological unknown tube as marked (available at the beginning of the exercise), and the technical name of the organisms present, **genus** and **specific epithet** (available at the end of the exercise).

2. The **cell size** (or range in size) as taken from solid or liquid media (specify which). Cell size should be expressed in micrometers (microns). Remember that if you are measuring rods or spirilla your size must include both diameter and length.

3. The **cell shape** - rods (bacilli), cocci, spirilla or other.

4. The **cell arrangement** - diplo, strepto, staphylo, tetrads, sarcinae, etc.

Note: Cell size and shape will be more accurately determined if a negative or indirect staining technique is used. Cell arrangement is often more readily determined by observing live bacteria in a wet mount OR if a broth culture is used to make a smear rather than taking cells from a colony growing on solid media.

5. The cultures's response to differential staining – **Gram-stain** quality (Gram-positive or Gram-negative) and if or not the organisms present are **acid-fast**.

6. Presence and appearance of special structures such as **capsules** and/or **endospores**. Note also the location of endospores if present (**central** or **terminal**), their shape (**spherical** or **ellipsoidal**) and if or not the **sporangium** (cell containing the spore) is swollen.

7. Whether or not the organisms are **motile**. This may be determined by preparing a wet mount of cells taken from a **fresh** live culture. Motility may be observed with either the high dry or oil immersion objective.

8. The **colony morphology** of your organisms as they appear on a properly streaked plate. The cultural characteristics of colonies includes **form** (shape), **margin** (edge), **elevation** (height), **surface texture**, **optical character** (light transfer or refraction), **pigmentation** (color in colonies and/or in the medium), **size** (in mm), and any other distinguishing characteristic of the colonies that develop (crystal formation, odor, etc.). Please specify the type of medium used for your streak plate.

9. **Taxonomy** – Record the **technical name** and **taxonomic lineage** of your unknown, reference **gene** and **author** information as determined through the **NCBI**. Be sure you are accessing the correct section of this database before you record your information. All morphological unknowns are **bacteria**, not humans, mice, cows, or other types of organisms.

In addition to the above information, each student will be required to include an illustrated record of cell morphology as determined during the observation of stained preparations. Illustrations must be in color and must accurately indicate the size, shape and arrangement of the cells as **magnified 5000X** (1 micron under 1000x magnification = 5 mm on paper). Draw several cells to indicate cell arrangement for each preparation represented. All illustrations are to be made on unlined, white paper (8 1/2 X 11).

At the completion of this exercise, each student will be required to submit the following items:

1. A completed copy of the **Morphological Unknown Report Form** (see below). Make certain all of the required information has been included, and that you know the difference between cellular and colony morphology.

2. A **one-page (one side) color illustration** indicating the morphological characteristics of the unknown culture as determined with a Gram-stain, an indirect stain, an acid-fast stain, a malachite green endospore stain and a capsule stain.

3. One **properly streaked plate** of solid media (appropriately labeled) containing well-isolated colonies of the unknown organisms in pure form, i.e., the plate should contain **no contaminants**.

NOTE – If when you access NCBI to learn the identity of your unknown culture, you find that the organism type described is *Boss torus* (cow) or *Rattus rattus* (rat), you are using the wrong accession number. All of the morphological unknown cultures are bacteria, not mammals.

Name: _____ Lab Section: _____

Morphological Unknown Report Form - 30 points

The number of my unknown was _____.

A properly streaked plate containing the correct culture was submitted (3 points)

The culture submitted was pure, i.e., there were no contaminants present. _____ (1.5 pts.)
The culture was properly streaked, i.e., the colonies were well isolated. _____ (0.5 pt.)
The plate was properly labeled, correct location _____(0.5 pt.) correct content _____(0.5 pt.)

Colony Morphology (5 points) Hint: For full points, do not leave any portion of this blank!

Medium used to grow the colonies: (1 pt.)	Optical Character: (0.5 pt.)
Form: (0.5 pt.)	Size range in mm: (0.5 pt.)
Margin: (0.5 pt.)	Surface Texture: (0.5 pt.)
Elevation: (0.5 pt.)	Pigmentation: (0.5 pt.)
Other notes (odor, crystal formation, changes in morphology with time, etc.): (0.5 pt.)	

Cellular Morphology, Part 1 (2 points):

Shape:	Size range in microns as determined by the indirect stain (diameter OR length & diameter):
Arrangement:	Were the organisms motile in a wet mount? (Remember to use FRESH cells!)

Cellular Morphology, Part 2: Stains & KOH Test (10 points):

Stain or Test	Data (What did you see?)	Result (+ or -)	Conclusion (What does it mean?)
KOH test			
Gram Stain			
Acid-Fast Stain			
Endospore Stain (see pg. 80, step #2 of procedure for all required info.)			
Capsule Stain			

Conclusions and Comments: (2 points)
Use this space to describe the **cellular morphology** of your unknown using **COMPLETE SENTENCES**. For full points, include the conclusions reached from the data and results obtained.

Taxonomy as determined through the NCBI (3 points)

Database Accession # _____

To complete this section, access the National Center for Biotechnology Information (NCBI) online. You may do this by typing NCBI into a Google search window, by typing in this URL (http://www.ncbi.nlm.nih.gov/), or by using the link provided on the Microbiology web page. Search the nucleotide database using the **accession number** you were given. Here's how: 1) select "Nucleotide" under "Popular Resources"; 2) type or paste your accession number into the search window; 3) click "Search"; 4) The identity of your morphological unknown (genus and specific epithet in blue) will appear just to the right of the word ORGANISM (it also appears at the top of the page).
Record the following:
Genus and specific epithet: _____
Taxonomic lineage: _____
_____
Name of gene being investigated: _____
Name of first author: _____ Country: _____

Illustrations (5 pts.)

Scientific illustrations of your unknown must include *each* of your **FIVE** stains (Indirect, Gram stain, Acid-Fast, Malachite green Endospore, and Capsule stains). Include enough cells to accurately indicate the cellular arrangement characteristic of your culture.

Illustrations must be completed on ONE side of one, unlined sheet of white paper, and your cells must be magnified 5000X (5mm on paper for every micron). This means that if your cells are 1 micron in diameter, you will draw them 5mm in diameter. **Illustrations must be in color and must match your descriptions!**

For full credit, be sure to label each illustration appropriately (total magnification, type of stain, structures visible, etc.).