**Lab REPORT #3**

**Identification of Unknown Bacteria**

**Differential Staining and Specialized Bacterial Growth Media**

* Differential Staining (Gram, Acid-fast, Endospore)
* Interpret Unknown on MacConkey’s & Mannitol Salt Media
* Identification of Unknown

Name:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Lab Partner:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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### Lab Day and Time:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

### IMPORTANT!! For any micrograph photos that you take, make sure that the scope camera is set to capture the image as the smallest file size possible. If you don’t, the file size of your Word .doc will be huge, possibly too big to upload. Also, make sure the photos you import into this document are large enough on the page for me to see details of the image (such as bacterial cell shape, color and arrangement).

### a. Unknown Identity: Number \_\_\_\_\_

b. Record the results of your Gram stain reaction and morphology from last week:

### Take micrograph photos of your Acid Fast observations viewed with the oil immersion lens. Make sure your photo is large enough, and detailed enough so that I can see cell shape and arrangement. If it is not, use the draw function in Word to also draw what it looks like.

Insert photo of the **Acid Fast positive control** below.

Name of genus and species: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Color of stain retained: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Stain reaction (acid-fast or non-acid fast) and cell morphology\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Insert photo of **Acid Fast negative control** below.

Name of genus and species: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Color of stain retained: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Stain reaction and cell morphology\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Insert photo of your **Acid Fast stained unknown** below.

Color of stain retained: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Stain reaction and cell morphology\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

### Take micrograph photos of your Endospore stain observations viewed with the oil immersion lens. Make sure your photo is large enough, and detailed enough so that I can see cell shape and arrangement. If it is not, use the draw function in Word to also draw what it looks like.

Insert photo of **Endospore stain positive control** below. Use an arrow to point to an endospore in the photo.

Name of genus and species: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Color of stain retained: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Stain reaction (endospore + or endospore -) and cell morphology\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Insert photo of **Endospore stain negative control below**.

Name of genus and species: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

Color of stain retained: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

Stain reaction and cell morphology\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Insert photo of your **Endospore stained unknown** below.

Color of stain retained: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Stain reaction and cell morphology\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. Compare and contrast Gram stain, Acid Fast stain & Endospore stain*.*

*(Because this question is more involved than the others, it is worth 2x the points of the other questions.*

|  |  |  |  |
| --- | --- | --- | --- |
| Item | Gram stain | Acid Fast stain | Endospore stain |
| What are the 2 controls for each stain? Indicate which species was used as the positive, and which species the negative control. |  |  |  |
| What is the expected stain reaction (+ or -) and shape of each control? |  |  |  |
| What color(s) is the control that retains the primary stain? |  |  |  |
| What color(s) is the control that retain the counter stain? |  |  |  |
| What **cellular attribute** is identified in the staining process? *(In other words, specifically what do we learn about the bacteria cell & structures by using the differential stain?)* |  |  |  |

5. What is the difference between a vegetative cell and an endospore? Name two groups of bacteria that form endospores.

1. Specifically what two things will we learn about our unknown by plating it on MacConkey’s? Your answer should describe in detail the selective and differential aspects of this specialized medium.

1. Specifically what two things will we learn about our unknown by plating it on Mannitol Salt? Your answer should describe in detail the selective and differential aspects of this specialized medium.
2. What is likely to happen if we tried to Gram stain an Acid fast cell? Why?
3. What is likely to happen if we tried to endospore stain and acid fast cell? Why?

This material is adapted from the Applied Microbiology Laboratory Manual by Cynthia Schauer. For Power Point slides that correspond to this lab material, see the Virtual Microbiology Classroom of the [Science Prof Online](http://www.scienceprofonline.com) website.