Name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Period\_\_\_\_

**LAB: #3 Positive Stains & Lab #4 Negative Stains**

**Purpose:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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**Data & Observations:**

* Bacteria are ALWAYS viewed under Oil Immersion.
* Use COLORED pencils
* Now that we are only observing bacteria, your drawings do not need to show the relative size compared to the field of view. INSTEAD, draw the bacteria **large enough** to clearly show **color & morphology & arrangement**
* NOTE – we are NOT doing a gram stain, so GP & GN are not appropriate terms for this lab.

|  |  |  |
| --- | --- | --- |
| *Staph***Positive Stain** | *Bacillus***Positive Stain** | *Aquaspirillum***Positive Stain** |
| Morphology:Arrangement: | Morphology:Arrangement: | Morphology:Arrangement: |
| *Staph* **Negative Stain** | *Bacillus***Negative Stain** | *Aquaspirillum***Negative Stain** |
| Morphology:Arrangement: | Morphology:Arrangement: | Morphology:Arrangement: |

**Procedure Questions:**

1. Why should you reflame the loop if it sizzles when you obtain a colony for staining?
2. Why is it necessary to spread the bacteria around on the slide, rather than leaving in a small concentrated spot?

**Questions:**

1. What are 2 purposes/benefits of fixing the smear?
2. In heat fixing, what might happen if too much heat were applied?
3. Compare (similarities) and contrast (differences) 4 or more characteristics of the appearance of *Bacillus* when viewed by positive vs. negative staining.
4. Size is more accurate in a negative stain than a simple stain. Why? Give 2 or more reasons.

**Critical Thinking:**

1. Methylene blue can be prepared as a basic stain or an acidic stain. Describe how the view through the microscope would vary in the two stains. Be specific.
2. Methylene blue is not the only dye that can be used for simple staining.
	1. List the type of dye required for a simple stain.
	2. Explain the mechanism of this type of dye. (Why does it stain the way it does?)
	3. List 2 other dyes discussed in class that could be used as a simple stain.
3. Nigrosin is not the only dye that can be used for negative staining.
4. List the type of dye required for a negative stain.
5. Explain the mechanism of this type of dye. (Why does it stain the way it does?)
6. What type of microscope would give an appearance similar to that of a negative stain? Explain.
7. Bacteria can be seen without staining. Why then are fixing and staining important?