Bio 504 Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Lab

**Gram Staining and Bacteria**

**Aim:** To identify the following bacterial cultures: *Bacillus subtilus*, *Micrococcus luteus* and *Aquaspirillum serpens*, in terms of shape and Gram stain.

**Hypothesis:** (2)

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**Material:**

Per class:

MicroLIVE culture, *Bacillus subtilus*

MicroLIVE culture, *Micrococcus luteus*

MicroLIVE culture, *Aquaspirillum serpens*

Dispensing tube, Gram crystal violet

Dispensing tube, Gram iodide

Dispensing tube Safranin counterstain

Hotplate

Marking pencil

Waste container

Per group:

Compound microscope

3 toothpicks

Large plastic cup

Small plastic cup filled with distilled water

Decolorizing solution dropper

Microscope slide

Pipet

Paper towels

Protective gloves

Stop watch

**Procedure:**

Making Bacteria Smears

1. Draw 3 numbered staining circles on the glass microscope slide using the marking pencil as shown below:

 1

 1 2 3

1. Place on the top right hand corner of your slide your group identification #.
2. Hold the dispensing tube near the tip and exert gentle pressure to dispense 1 small drop of *Bacillus subtilus* culture in the first circle of your slide.
3. Hold the dispensing tube near the tip and exert gentle pressure to dispense 1 small drop of *Micrococcus luteus* culture in the second circle of your slide.
4. Hold the dispensing tube near the tip and exert gentle pressure to dispense 1 small drop of *Aquaspirillum serpens* culture in the third circle of your slide.
5. Using one toothpick for each bacterial culture, spread the drop of bacteria outward so it covers the entire staining circle area but does not extend beyond.
6. Discard the contaminated toothpick in the waste container.
7. Place the slide on the hot plate that is set at low to heat-fix the bacteria for a few minutes.
8. Remove the slide after all the water has evaporated.

Gram Staining Procedure

1. Place the slide over the large cup. This is your “cup-sink”.
2. Apply a single drop of Gram crystal violet stain to the centre of each dried smear.
3. Allow the stain to contact the smear for 1 minute.
4. Use the pipet to withdraw water from the small cup.
5. Apply it, drop-wise, to the stain washing it off the slide until the water runs clear.
6. Apply a single drop of Gram iodine mordant to the centre of each staining circle.
7. Allow the reagent to act for 1 minute.
8. Repeat steps 4 and 5.
9. Use the small plastic dropper to apply decolourizer, drop-wise, over the 3 staining circles until no more colour runs off the slide.
10. Apply a single drop of safranin counterstain to the centre of each staining circle.
11. Allow the stain to act for 1 minute.
12. Repeat steps 4 and 5.
13. Place the slide on the hot plate to evaporate any remaining water.
14. Remove the slide after the water has evaporated.

Observing the Stained Smear

1. Set the microscopes at the lowest power.
2. Scan the staining circles to locate an optimal area for exploration.
3. Progressively increase the magnification of the microscope to observe the cells.
4. Draw the cells observed at the highest magnification.
5. Record observations in Table 1.

Evaluation

1. Hand in the microscope slide to your teacher for evaluation. (10)

**Results:** (10)

Figure 1 - \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Magn. = \_\_\_\_\_\_\_\_

Figure 2 - \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Magn. = \_\_\_\_\_\_\_\_

Figure 3 - \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Magn. = \_\_\_\_\_\_\_\_

Table 1 - \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |
| --- | --- | --- |
| bacterial culture | shape | stain |
| *Bacillus subtilus* |  |  |
| *Micrococcus luteus* |  |  |
| *Aquaspirillum serpens* |  |  |

**Conclusion:**

1. Identify the shape and stain (Gram-positive or Gram-negative) of each of the bacterial cultures. (6)

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1. Differentiate between the cell walls of each of the bacteria studied. (2)

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1. In terms of medicine, why is the Gram stain so important? (3)

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