**Bio233 Lab. Gram stain.**

**Safety issues**

1. Tie your hairs behind in the lab, especially when using Bunsen burners.
2. Wear gloves when handling samples with live bacteria.
3. Avoid staining to skins and clothes.
4. Beware that some bacteria are pathogens.

**Prepare the smears**

1. Place a very small drop of distilled water on each slide. (Make sure the slides are clean. Wipe off with 70% ethanol if necessary).

2. Flame an inoculating loop and the mouth of the culture tube.

3. Remove a small quantity of bacteria from the slant.

4. Flame the mouth of the tube and replace the cap.

5. Mix the bacteria with the water on the slide and spread thinly. Reflame the loop.

6. Denote the type of bacteria on each slide with a wax pencil. Record the dates and your initials or full names.

7. Allow the smear on the slide to air-dry. (This should take <5 minutes. If it takes too long, redo another smear with less water.)

8. Pass the slide, smear side up, through a flame quickly 3 times to fix the bacterial cells. Fixing kills the bacterial cells and causes them to stick to the slide.

9. Allow the slides to cool.

10. Turn off the Bunsen burner.

**Gram stain**

1. Flood the fixed smears with crystal violet for 1 minute.

2. Rinse the slide gently with tap water until the water runs off clear.

3. Flood the smear with Gram iodine for 1 minute.

4. Rinse with tap water.

5. Decolorize with 95% ethanol. Allow the ethanol to stream across the slide until the runoff is clear.

6. Rinse with tap water.

7. Flood with safranin for 1 minute.

8. Rinse with tap water.

9. Carefully blot, covered with a cover slip, and observe under microscope. (Put a tiny bit of water on the edge of the cover slip, so it will stick.)

**Clean up**

* Turn off power of microscope. Clean oil from the lens.
* Turn off Bunsen burner and gas.
* Return staining set, samples, and tools to their original places.
* Wash your hands with soap!

**Tools and materials**

* Distilled water
* Slides, cover slips
* Bunsen burners and lighter
* Inoculating loops
* Microscope (optional oil-immersion lenses)
* Lens wipers
* Bibulous papers (Absorbent papers)
* Wax pencil
* Crytal violet
* Gram iodine
* Safranin
* 95% EtOH
* Dropping pipettes
* Shape collection box
* Staining rack
* Lab coat is recommended.

**Sample Strains (Strain may vary depending on availability).**

* + *Bacillus subtilis*: A Gram-positive, rod-shaped, and catalase-positive bacterium commonly found in soil. It has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. It is also a model organism for bacterial genetics and cellular development. Its genome has been sequenced. *B. subtilis* grows at 30◦C on nutrient agar and needs two days to form colonies.
  + *Micrococcus luteus*: A Gram-positive, spherical, saprotrophis bacteriam found in soil, dust, water air, and as part of the normal flora of the mammalian skin. It also colonized human mouth, mucosae, oropharynx and upper respiratory tract. *M. luteus* grows at 30◦C on nutrient agar.
  + *Escherichia coli*: A Gram negative rod-shaped bacterium.

**Report (Your report should be written in WORD and submitted to SpelELearn).**

1. Describe your experimental observations and results for the bacterial species. Did your experiment work? If not, what could be problems?
2. What is the purpose of Gram’s iodine in this experiment?
3. Normally, Gram stain works better for freshly grown bacteria. Briefly explain why old bacterial cultures tend not to give good Gram stain results.
4. (Optional) What happens if we used 95% EtOH to wash off safranin at the end of the Gram stain?