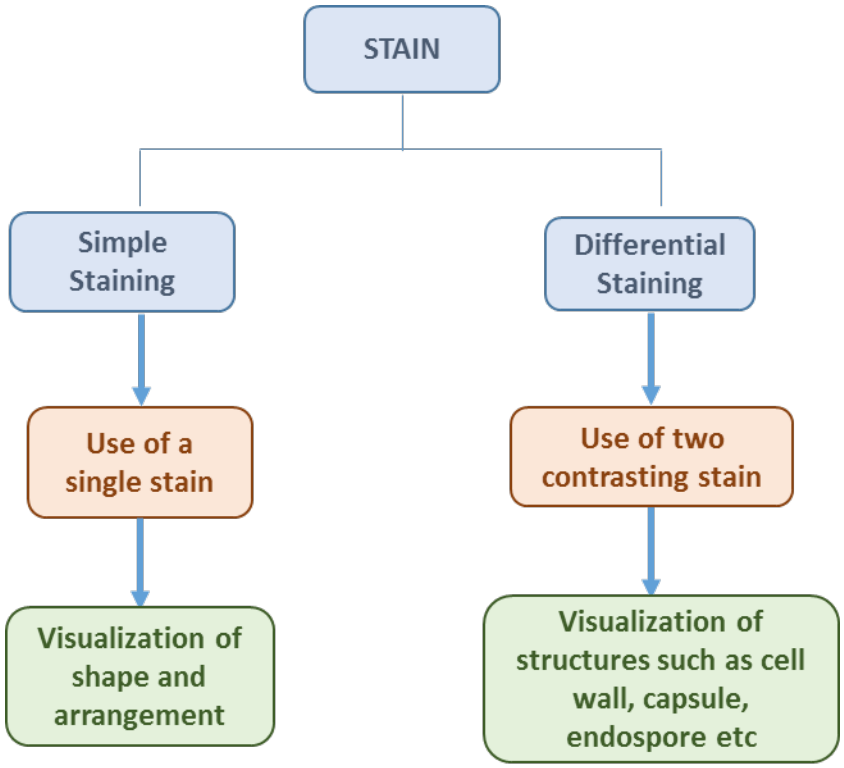
BIOS242 Lab 4

Name:

Lab 4: Simple Staining

Learning Objectives:

- To learn about principle of staining
- To learn the process of simple stain
- To prepare wet mount slides



In light microscopy, colored dyes, called stains are used to improve contrast between the specimen and the background surrounding the specimen. Staining makes visualization of the specimen easier due to increased contrast. This is crucial for specimens that are mostly transparent. Bacteria, due to their size are difficult to visualize under light microscope without staining. Stains contain chromophores of a specific color. In addition, the stains may bind to cells based on the charges present.

In simple staining, only one type of stain is used. Use of single stain allows the user to observe size, shape and arrangement of bacteria. Acidic stains are negatively charged and bind to positively-charged structures in the cells. Basic stains are positively charged and bind to negatively-charged structures, such as nucleic acids and many cell wall components. Basic stains such as Crystal violet, Safranin, and Methylene

blue are some of the common stains used in a Microbiology laboratory. Differential staining uses two or more similar stains in a way that it allows observation of specific features of bacteria which often becomes basis of identification of the bacteria.

In this lab, we will carry out simple staining and subsequent observation of size, shape and arrangement on bacteria and yeast. We will also make wet-mount slides of yeast and cheek cells.

Exercise 1: Simple

Stain Materials:

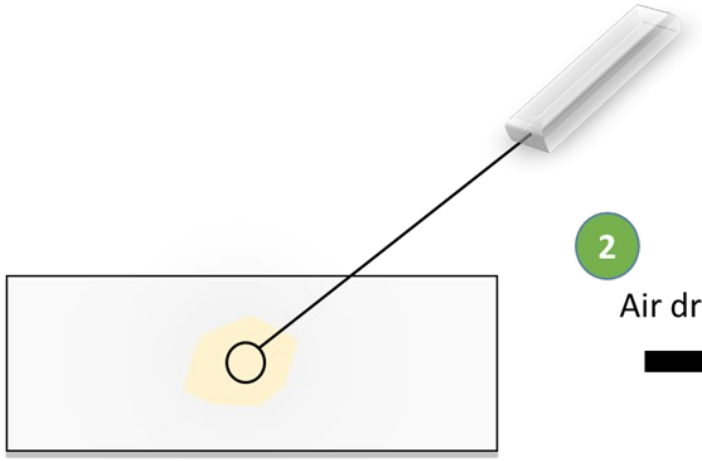
Overnight cultures of *S. epidermidis*, *B. subtilis*, *S. cerevisiae* (yeast) , inoculating loop, glass slides, incinerators, crystal violet or any other stain chosen by instructor; tongs, staining racks/trays, immersion oil, lens paper, bibulous paper, microscope

Note to students: Wear gloves and use PPE before starting the lab work. Use aseptic technique to prevent contamination. Use only 1-2 drops of stain per slide. Avoid using excess.

Method:

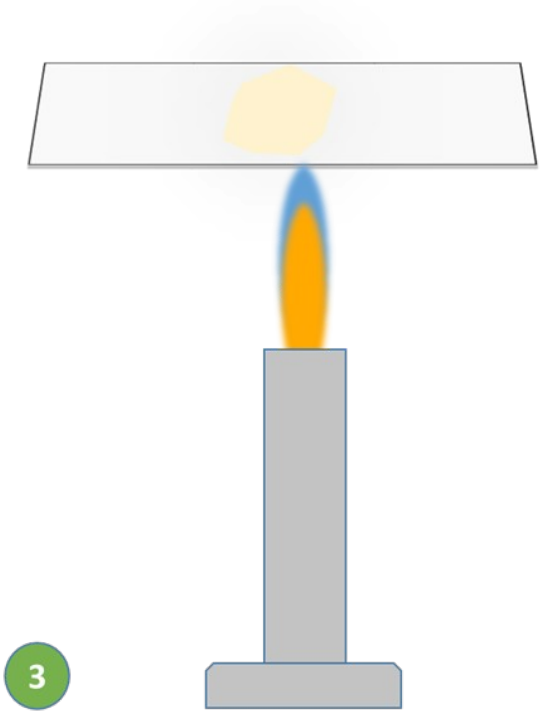
1. Obtain a clean glass slide, loop and overnight cultures. Hold the glass slides from the edges to avoid transferring grease or oil from fingers to the slide.
2. Using aseptic technique, transfer a loop full of *S. epidermidis* culture at the middle of the glass slide. Spread it in a thin layer.
    a. It is important to make a thin smear. A thick smear prevents light to pass through it, may have cells too close to each other or as clumps which results in poor observation. A thin smear allows better observation of cells by allowing the light to pass through it.
3. Allow the smear to air dry completely. Once the smear is completely dry, hold the glass slide using tongs and quickly pass the slide 1-2 times through flame of Bunsen burner to heat fix the bacteria.

    a. Completely air drying the specimen on the slide is crucial. Complete air drying of the specimen allows the cells to attach to the glass slide without boiling of the wet smear. Boiling will cause fragmentation/shrinkage of bacteria resulting in poor specimen quality and observation.
    b. Once heat fixed, cells become attached to surface of the glass slide and will not get washed off during the staining process.
4. Once bacteria is heat fixed, allow the glass slide to cool to room temperature.
5. Add 1-2 drops of diluted stain and wait for 1 minute.
6. Remove the stain and gently wash it off using minimal amount of DI water.
7. Blot dry the slide and observe using 10X and then 40X lens of a compound microscope.
8. Record your observations at 40X lens in Lab report.
    a. Process to use Microscope is described in appendix A.
    b. Check with your instructor if 100X lens is needed. If 100X lens is used, don't forget to use oil.
    c. Record results in the lab report.
9. Repeat the method using cultures of *B. subtilis* and *S. cerevisiae*. Record observations in Lab report and answer questions.



1 Using aseptic technique, transfer a loopfull of culture to a clean glass slide and spread the culture as a thin layer

2 Air dry completely



3 Fix the bacteria to slide by quickly passing the slide through flame 1-2 times

4 Cover the smear with stain; wait for 1 minute. Rinse the stain, blot dry and observe using microscope

Exercise 2: Wet-mount

Slides Materials:

Overnight cultures of *S. cerevisiae* (yeast), inoculating loop, sterile swabs, glass slides, cover slips, incinerators, diluted solution of crystal violet or any other stain chosen by instructor; tongs, staining racks/trays, immersion oil, lens paper, microscope

Note to students: Wear gloves and use PPE before starting the lab work. Use aseptic technique to prevent contamination. Use only 1-2 drops of stain per slide. Avoid using excess.

Yeast Culture:

1. Obtain *S. cerevisiae* (yeast) culture, two glass slides, two cover slips, inoculating loop, and stain.
2. Wear gloves before handling glass slides and coverslips needed to make wet-mount slides.
3. Handle glass slides and cover slips by holding from sides rather than between your fingers to avoid getting the slides dirty.
4. Transfer a small drop of *S. cerevisiae* (yeast) culture on both slides using an inoculating loop.
    a. Don't forget to use aseptic techniques while handling the cultures.
5. On one of the slides, add a drop of stain (in case of stained specimen), while do not use stain on the second slide (unstained specimen).
6. Put a coverslips on both slides by starting with one side of coverslip touching to the liquid culture on the specimen and then dropping the coverslip at an angle. This technique is useful in preventing catching of air bubbles underneath the coverslip.
7. Use wipes to carefully remove excess liquid from the edges of the coverslip.

8. Observe slides using 10X, and 40X lenses. Record observations made using 40X lens in the Lab report.

Cheek Cells:

9. Obtain glass slide, cover slip, stain, sterile swab.
10. Swab inside of your cheek using sterile swab and rub the surface of the glass slide.
11. Add 1-2 drops of diluted stain.
12. Put a coverslip at an angle by first touching one side of coverslip to the liquid on the slide and then dropping the coverslip at an angle. This technique is useful in preventing catching of air bubbles under the coverslip.
13. Use wipes to carefully remove excess liquid from the edges of the coverslip.
14. Observe using 10X and 40X lenses. Record observations made using 40X lens in the Lab report.

Lab Report:

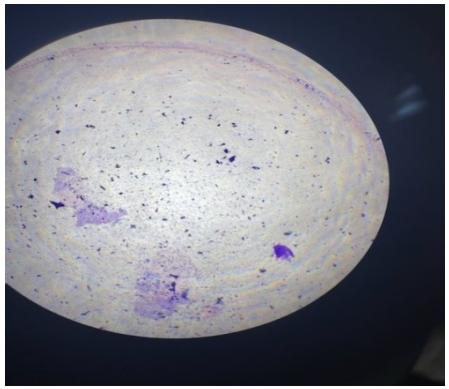
Purpose: Describe the purpose of the lab.

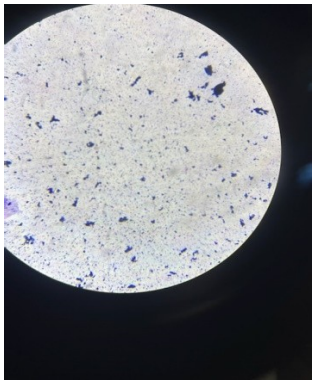
Results:

Exercise 1: Simple Stain

Draw the observation inside the circles provided. Label your image appropriately. Write the magnification at which the observation was made.

Bacteria: *B. subtilis*





Additional observation/notes:

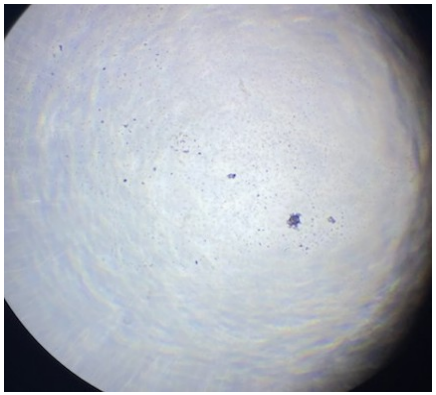
Bacteria: *S. epidermidis*

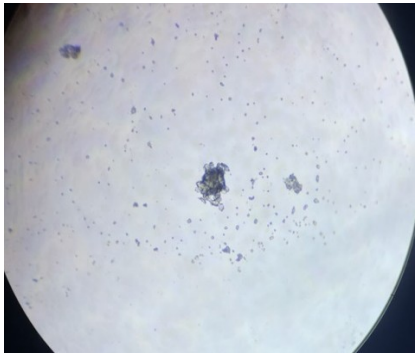
Additional observation/notes:

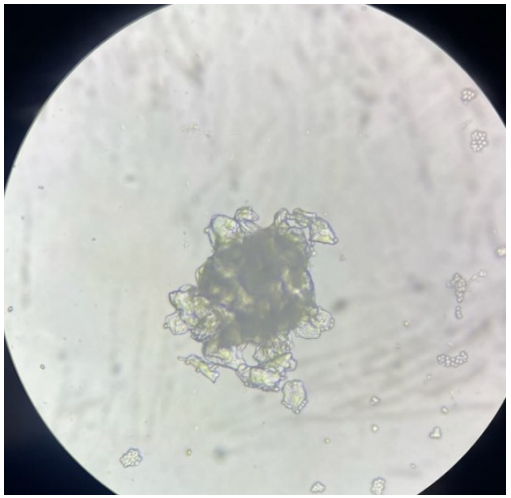
Exercise 2: Wet-mount Slides

Draw the observation inside the circles provided. Label your image appropriately. Write the magnification at which the observation was made.

S. cerevisiae: Unstained

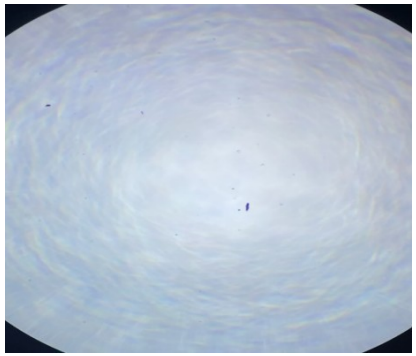


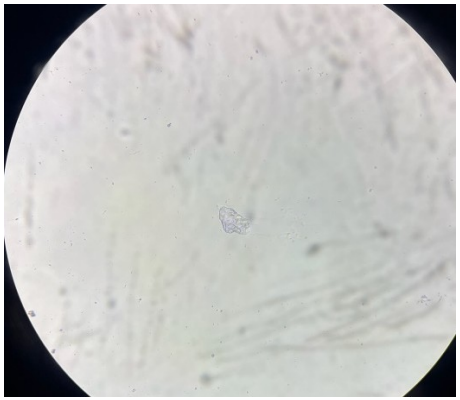




Additional observation/notes:

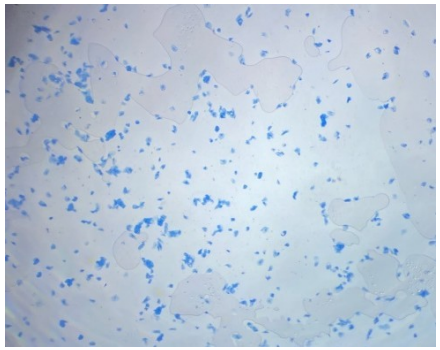
S. cerevisiae: Stained

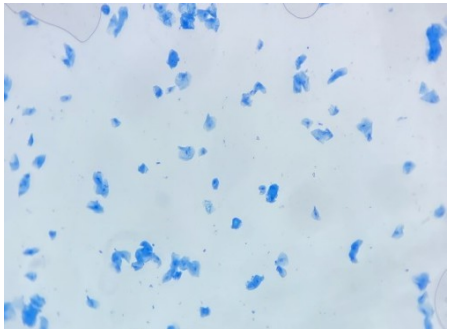


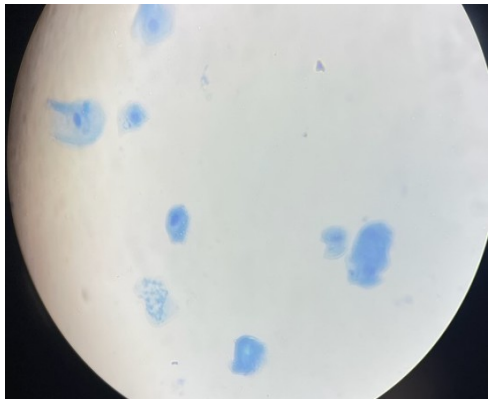


Additional observation/notes:

Cheek Cells:







Additional observation/notes:

Complete the following table:

	Type of Cell	Shape	Size	Arrangement
S. epidermidis	bacteria	cocci	1-2mm	Grape-like clusters
B. Subtilis	bacteria	Bacillus	4-10mm (micrometers)	Rod shaped
S. cerevisiae	yeast	Round to ovoid	5-10mm	
Cheek Cells	Eukaryotic			Distinct nucleus

Questions:

1. Describe differences between a wet mount slide and simple staining.

   Simple staining is used to increase contrast on bacterial image, allowing us to observe cell size, morphology, and arrangement. Wet mounts do not heat fix bacteria cells so, the procedure doesn't damage or kill the bacteria.

2. Why is air-drying important before heat fixing the specimen? Air-drying ensures that the smear is thin enough to stain.

3. If you forgot to heat fix the specimen, how will it impact observation of specimen?
   The bacteria in the smear would be washed off the slide during the staining and decolorization steps. You would not see anything on the slide under the microscope

4. You observed unstained and stained yeast specimen. Which one was easier to observe? Explain the reason? The stain slides were a lot easiser to observe. I think this is because of the colors that would show up.

Grading Rubric:

Activity	Deliverable	Points
Experimental Set up and Observation	Obtain/Set up the slides as directed in the lab or by instructor; Observe all assigned slides using microscope and record observation in lab report	6
Lab Report and Questions	Complete lab report and answer ALL questions • Purpose (1 point) • Table (4 points) • Questions (4 points)	9
All Lab Deliverables	Complete ALL lab work and lab report	15