

# EXCERPTS FROM THE LAB MANUAL RELEVANT MATERIAL FOR THE FIRST WEEK OF THE SEMESTER

## Experiment 1 – Microorganisms in the Environment

Although microorganisms are present in or on nearly everything, it is usually not possible to demonstrate their presence by direct microscopic observation unless their density is high. However, if sterile culture media are exposed to air or inoculated with substances such as soil or lake water, a variety of microorganisms will multiply in the media and can be examined subsequently. To prove that microorganisms are in or on a substance, it is necessary that all media and equipment used be **sterile** and that **aseptic technique** be employed in performing inoculations and transfers.

The following procedures are meant to demonstrate **colony** formation by microbial cells inoculated onto a petri dish medium. Each **vegetative cell** which can utilize the medium as a source of nutrients and can tolerate the physical conditions present (temperature, pH, atmosphere, etc.) should multiply – resulting, during **incubation**, in a visible **colony** of identical cells. Differently-appearing colonies imply different species of microorganisms; colony appearance is often used in the characterization of bacterial species.

When we observe colonies, we cannot assume each arose from just one cell originally planted on the medium, however. A pair, chain or cluster of cells or individual cells which “land” on the medium in close proximity to each other can multiply and produce a single colony. Thus, we use the term **colony-forming unit** when we consider the **common origin** for the cells of a colony.

Besides vegetative cells, certain bacterial species can produce another type of cell known as a **spore**. This semester we will be seeing and working with **endospores** and **reproductive spores**. A spore is a dormant type of cell whose main advantage is being able to withstand harsh environmental conditions, and it will **germinate** to become a vegetative cell which can then multiply and form a colony of vegetative cells. Thus, **endospores and reproductive spores also qualify as colony-forming units**.

Another term we will often use is **culture** which is simply a large population of living cells. Examples include a colony (above), a flask of organisms in a liquid medium, and a tube of slanted agar medium on which organisms are growing. A culture of cells, dividing every 20 minutes, can begin with one “new” cell and result in 16,777,216 (i.e.,  $2^{24}$ ) cells after just 8 hours! A **pure culture** is composed of **identical** cells (except for occasional mutants) which are all of the same **strain**. (“Strain” refers to a specific kind of organism and is further discussed on page 124 in this manual.) A **mixed culture** contains two or more different kinds of organisms. A culture is considered mixed even when it contains different strains of the same species as we will see in Experiment 8A.

We often refer to “**young**” and “**old**” **cultures**, depending on how long they have been **incubating** since **inoculation**. We **do not, however, refer to “young” and “old” individual cells in the same way**, as the cells of many of the species we work with undergo division every 20 minutes. Thus an “old cell” – just about to divide into two “new cells” – may be less than a half-hour old!

### LABORATORY PROCEDURE *Period 1*

#### Materials

- Hay infusion
- 3 plates of Plate Count Agar (PCA)
- 2 sterile cotton swabs
- 1 tube of sterile saline (3-4 ml)

#### Procedure

##### **Wet mount of a hay infusion.**

1. A hay infusion was prepared by adding lake or pond water to hay or straw and incubating the mixture at 30°C for 5-7 days. Examination of this infusion will reveal the vast numbers and different kinds of microorganisms in the environment! To visualize the creatures inhabiting and reproducing in the hay medium, it is necessary to make “wet mounts” and observe them with the microscope. The procedure for making a wet mount is shown in the box on the right.

#### PROCEDURE FOR THE PREPARATION OF WET MOUNTS

- Using a sterile inoculating loop (or a Pasteur pipette if you are examining a sample from nature), pick up a **small** drop of liquid and place it on a microscope slide.
- With a toothpick, put a **thin** layer of Vaseline (about two cm square) on the palm of your hand. Take a cover slip, held by the edges, and run each edge through the vaseline such that the cover slip is rimmed with a small amount of Vaseline.
- Place the cover slip on the drop such that that a few air bubbles are trapped. Gently press down on the cover slip. The other end of your loop works well for this.

2. Take your prepared slide to one of the **phase-contrast microscopes** (commonly called “phase scopes”) that are set up in the room and examine the specimen. Heed the instructor’s directions on the use of the microscope (focusing and illumination).

**As a general rule, the phase-contrast microscopes are set up with the 40X lens in place along with the light set near maximum illumination. Start with the lens almost touching the cover slip; then focus back until a sharp image of the specimen appears. See instructor if you need to move to a higher magnification.**

Please treat the microscopes with care as they are very expensive precision instruments.

3. When finished, **discard the slide into the disinfectant container** as it contains living specimens.

### Sampling various environments.

4. Take one of the PCA plates and remove the lid. Expose the surface of the medium to the air for 15-30 minutes and then replace the cover. **Label the plate with your name, the date and what the experiment is (“air exposure”) on the bottom lid.** (This is standard procedure for labeling petri plates.)
5. For the remaining plates, various sites can be sampled with sterile cotton-tipped swabs moistened with the sterile saline. Each swab is then streaked across the entire surface of the medium in a petri plate and then discarded into disinfectant. (Who knows for sure if we are picking up any pathogenic organisms?) Examples of various items which you and your neighbors can sample include skin, the lab bench, a doorknob, a cell phone, shoes (highly recommended!), and various objects in the vicinity. **Discard the tube of saline** into the slanted tray on the discard cart.
6. Incubate the plates by placing them in an **inverted** (medium side up) position in the 30°C incubator. Note: As a rule, we will always incubate our plates in an inverted position. Otherwise, moisture collecting on the top lid may drop down on the developing colonies, causing them to run together.

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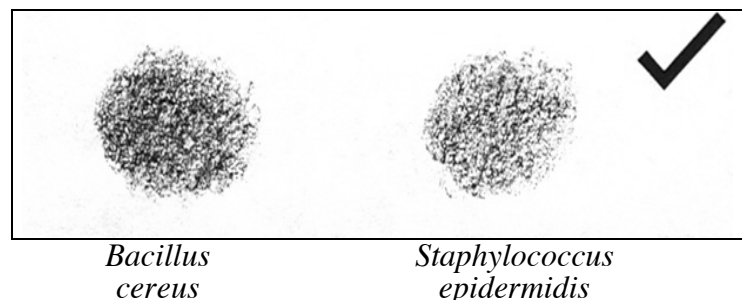
## Experiment 3A – Basic Bacteriological Staining Methods

COMPLETE INTRODUCTION TO EXPERIMENT 3 IS IN THE LAB MANUAL

Preliminary identification of bacteria is usually based upon their cell morphology and grouping and the manner in which they react to certain staining procedures. The results of the Gram Stain procedure are utilized as an indication of which general grouping an unknown organism belongs, such that relevant tests can be performed for the ultimate identification. The Simple Stain procedure is utilized to simply make the cells visible such that the shapes and arrangements of the cells can be observed and recorded.

### Materials

Slide with smears of two species of bacteria:



### Procedure

#### Simple stain.

1. You are provided with a microscope slide which is already prepared with two smears as illustrated above. According to the instructor’s explanation and demonstration, **heat-fix the slide**, making sure that the slide goes through the flame smear-side up.

2. Holding the slide with a clothespin over the sink, cover the slide with crystal violet for one minute. (These steps are also given in the simple stain procedure in **Appendix F**, page 116.)
3. Carefully rinse off the dye with tap water, and blot the slide dry with paper towel.
4. Using **two** hands, obtain the light microscope from the cabinet between you and your partner. This is the type of microscope that we will always use to observe **stained smears**.

**Be sure to heed the instructor's comments** on the handling and use of the microscope. Detailed directions concerning illumination and focusing are given on a handout, and **Appendix A** includes some theory concerning microscopy.

5. Place the slide on the stage such that it is oriented as illustrated above. Make sure the clips on the stage hold the slide securely on three sides.
6. Begin your observations with the *Bacillus cereus* smear, referring to the handout and the instructor's comments which expand on the following steps. **Terminology associated with cellular shapes and arrangements is listed on page 122.**
  - a. **Focus the smear initially with the 10X objective lens.** As you focus up and down with the 10X lens, you may see dirt and other deposits on the condenser lens and the bottom surface of the slide come into view, but you will need to **focus on the smear** which is on the top surface of the slide. Unless you are focusing on the smear, you may be unable to switch to the higher-power lens for more detailed viewing of the cells. Consider why this may be so, and also why it is highly advisable to make sure the condenser lens and the bottoms of the slides are **clean**.
  - b. Once the smear is in focus, do not move the focusing knobs but swing the 10X lens to the side, **add a rather large drop of immersion oil directly on the smear**. Then **swing the 100X lens into place**, making sure it "clicks" and becomes immersed in the oil as you do so. It is absolutely necessary that the 100X lens is immersed in the oil, as the oil is needed to contain the light (i.e., prevent its scattering) between the slide and the lens.
  - c. With minimal "fine-tuning" with the fine-adjustment knob you should be able to see the cells come into sharp focus. *Bacillus cereus* cells are relatively large and rod-shaped ("bacilli") and are generally observed in chains. Record your observations in the table below.
7. Repeat this procedure with the *Staphylococcus epidermidis* smear. Cells of this organism are spheres ("cocci") which are usually arranged in clusters ("staphylococci") and pairs.
8. When you are through, be sure the microscope is put away properly (i.e., all oil wiped off, 10X objective lens in place, stage centered). **Slides are always discarded in the broken-glass container (the red bucket)**, and it is never necessary to put stained smears into disinfectant.

## TODAY'S OBSERVATIONS

(These can be transferred to the lab manual when convenient to do so.)

### WET MOUNT OF HAY INFUSION

### SIMPLE STAIN

	Observed under 10X objective (100X total magnification)	Observed under 100X objective (1000X total magnification)
<i>Bacillus cereus</i>		
<i>Staphylococcus epidermidis</i>		