

## Microbiology Laboratory Safety and Basic Procedures

Safety in a microbiology laboratory is important in the prevention of infection that might be caused by the microorganisms being studied. This laboratory does not require the use of virulent human pathogens. However, many types of microorganisms are *potentially* pathogenic. This means that, although they would not cause disease in a normal healthy host, they might possibly do so if a large enough quantity of the microbes came into contact with a compromised host, such as by wounds and cuts.

In addition to microorganisms, there are some chemicals used in this laboratory that are potentially harmful. Many procedures involve glassware, open flames, and sharp objects that can cause damage if used improperly.

The following precautions should be taken to avoid the problems that could potentially occur.

1. **The laboratory procedures must be read prior to attending that laboratory session.** By being knowledgeable on the procedures, you will assure the safety of yourself and of your fellow laboratory students.
2. **Lab coats are required.** Wearing old clothing is also desirable, since many reagents can produce permanent stains on clothes. Anyone wearing shorts must wear a **mid-calf length** lab coat.
3. **Safety glasses are required and must be worn in this lab except when using the microscope or computer or at other times as designated by the TA or instructor.**
4. **Students may not wear sandals or open toed or canvas shoes** because of the constant danger of cuts and infections from broken glass found on the lab floors and the possibility of chemical spills. Anyone wearing inappropriate footwear will not be allowed to work in the lab.
5. Long hair **must** be tied back to minimize fire hazard or contamination of experiments.
6. Smoking, eating, and drinking in the laboratory are absolutely prohibited.
7. Upon entering the laboratory, coats, books, and other paraphernalia should be placed in specified locations and never on bench tops (except for your lab manual).
8. At the beginning and termination of each laboratory session, bench tops are to be wiped with a disinfectant solution.
9. Observe strict personal hygiene. Wash your hands with soap at the start of the laboratory session before performing any procedures **and** before leaving the laboratory at the end of the session.
10. All cultures should be handled as being potentially pathogenic and the following precautions should be observed at all times:
  - a. Cultures must always be carried in a test tube rack when moving around the laboratory.
  - b. Cultures must be kept in a test tube rack on the bench tops when not in use.
  - c. Broth cultures must never be pipetted by mouth. Always use a suction aid (never use your mouth) when filling a pipet or use a pipetter with a biological or chemical reagent.

- d. Spilled cultures should be covered with paper towels and then saturated with disinfectant solution. Following 15 minutes of reaction time, the towels should be removed and disposed of in the manner indicated by the instructor.
11. Spills, cuts and other accidents should be reported to the instructor or TA in case further treatment is necessary.
12. Aerosols should be avoided by use of proper technique for flaming the inoculating loops and needles and by performing any mixing of cultures and reagents in such a way as to avoid splashing. Proper use of these procedures will be demonstrated.
13. Removal of media, equipment, and cultures from the laboratory is prohibited unless so directed by the instructor or TA.
14. During and at the end of each lab period, used pipets should be discarded into the designated trays. Other used glassware should normally be placed into discard trays located in the back of the lab. Plasticware such as petri plates and pipetter tips should be discarded in the marked wastebasket at the back of the lab. Used paper should be discarded into wastebaskets at the end of the lab period. **Broken** glassware is discarded into the box marked “Broken Glassware”, **not** into wastebaskets.
15. If you need help, dial 123 on the lab phone to get the Campus Emergency Office. Other useful numbers are:

<b>MTU Public Safety</b>	<b>487-2216</b>
<b>Dr. Bagley’s Office</b>	<b>487-2385</b>
<b>Microbiology Teaching Lab</b>	<b>487-2458</b>
<b>Biological Sciences Office</b>	<b>487-2025</b>
<b>Outside Emergency Agencies</b>	<b>8-911</b>

16. **You must report to your supervisor (checkout) prior to leaving the laboratory setting.**

## GENERAL INFORMATION ON USE OF THE MICROSCOPE

The following general information is provided for reference purposes on the use and care of the compound light microscopes used in the Microbiology Teaching lab. Diagrams of the microscopes are in the lab drawers. **Additional information on using the phase contrast vs. brightfield capabilities when observing unstained specimens is presented on the next page.**

1. Take the microscope out of the cabinet and use both hands to lift it to your work area. Do not tilt the instrument.
2. Keep the microscope stage parallel to the table surface.
3. Move the condenser all the way up to the microscope stage and then lower it by a one-eighth turn of its control knob.
4. Note the three objectives: **low power** (10X), **“high dry”** (40X), and **oil immersion** (100X). They can be distinguished from each other by the number that is etched into each objective.
5. Rotate the low power objective (10X) into place and lower it as far as it will go with the coarse adjustment knob.
6. Before turning on the light source, make sure that the intensity control is set to zero. Turn on the light and adjust the light source to project maximum light into the lens system.
7. Adjust the eyepieces in order to use **both** eyes when viewing specimens.
8. You can easily learn how to focus the microscope by marking an "X" on a clean slide. Place it on the stage and then use the low power objective to focus on the "X".
9. The 40X and 100X objectives on these microscopes are **spring-loaded AND parfocal** (i.e., **what is in focus with one objective will be in focus with all other objectives**). Only **minor adjustments of the fine adjustment knob** should be needed to bring the subject into clear view with these objectives.
10. Place a drop of immersion oil on the subject before examining it with the oil immersion objective (100X). Swing that objective into place and use the fine adjustment knob to bring the subject into focus. Use **only** the fine adjustment with oil immersion lenses. Higher magnification requires more light so you may need to increase the amount of light.
11. **NOTE:** In general practice in this lab, you will focus the microscope on your specimen using the low power (10X) objective and then **DIRECTLY** move to the 40X or oil immersion (100X) objective. When studying bacteria, you will typically **NOT** need to use the 40X objective.
12. Turn off the light source and set the intensity control to zero.
13. Always clean the microscope before returning it to its cabinet, especially the lenses and stage. Wipe any dust or oil off the instrument. **Use only lens paper and the cleaning fluid to clean the lenses.** Rotate the low power objective into place before putting the microscope back into the cabinet.
14. Always examine your microscope carefully at the start of each lab period; report any problems with the microscope to the TA immediately. **Check your microscope before the end of each lab period (during which you have used the microscope) to ensure that it has been cleaned and prepared for storage properly.**

## WET MOUNT PREPARATION

1. Flame a loop to redness in the burner flame and then allow it to cool without contaminating it (i.e., don't set it down on the lab bench).
2. For examining a liquid, use the cooled, sterile loop to place a loopful of the liquid to be examined on a clean slide. Resterilize the loop and set it aside. For a culture on solid media, first place one or two loopfuls of saline or water on the slide; then transfer a small amount of the culture to the slide with a needle.
3. Place a coverslip over the drop of fluid. Try to avoid the formation of air bubbles under the coverslip. Remember to place a drop of immersion oil on the top of the coverslip if you plan to use the oil immersion objective.
4. For **brightfield microscopy**, the slider under the stage is in the middle position. A **blue (daylight) filter** is placed above the light source for observation of these unstained specimens. (The blue filter is stored in the drawer under the lab bench.)
5. For **phase contrast microscopy**, the color-coded (black or red) end of the slider under the stage is pushed (left or right) into the open portion of the stage when using the 100X objective; this slider is pushed from the opposite (unmarked) end when using the 40X objective. A **green filter** is placed above the light source in order to create the phase contrast conditions for observations of these unstained specimens. (The green filter is also stored in the drawer under the lab bench.)
6. Discard the slide in the appropriate location.

## OBSERVATION OF LIVING MICROORGANISMS

The line of demarcation that exists between the world of microorganisms and the larger organisms more often studied in general biology is not distinct; there is basically a continuum of different organisms ranging in size and complexity from viruses, rickettsias, and mycoplasmas to whales and redwoods. By convention, however, microorganisms are usually considered to have relatively simple cell structures and are easily observed only with the aid of the light microscope, i.e., too small to be seen with the unaided eye. There are many exceptions: many microbes can be seen with the unaided eye and certain fungi and algae form relatively complicated structures composed of many cells.

### PURPOSE

To demonstrate the shape of *Corynebacterium glutamicum* in pure and mixed cultures.

### MATERIALS

Cultures of *C. glutamicum* plus other microorganisms, slides, immersion oil, lens paper, coverslips, loops and needles, and phase contrast microscopes.

### PROCEDURE

1. Review the use of the microscope. Be sure to ask questions if you are not familiar with the microscope assigned to you.
2. Use a sterile loop to transfer a droplet of one of the microbial broth (liquid) *C. glutamicum* culture to a clean slide. Prepare a wet mount (add a cover slip) and examine it using the low (10X), high dry (40X), and oil immersion (100X) objectives using **both** brightfield **and** phase contrast microscopy. Determine the general cell shape.
3. For a *C. glutamicum* culture on solid media, first place one or two loopfuls of saline or water on the slide. Then, transfer a small amount of the culture to the slide with a needle. Mix the culture with the fluid before placing a coverslip onto the liquid.
4. Examine the wet mounts set-up on the demonstration microscopes to determine which contains a pure culture of *C. glutamicum*.

## CULTURE MEDIA FOR MICROORGANISMS

### MEDIA CLASSIFIED ACCORDING TO COMPOSITION

**Synthetic Media.** These are prepared according to exact specifications with the precise chemical composition known. These chemically defined media are usually used for very specific purposes such as enrichment of autotrophs or in vitamin assay work.

**Non-Synthetic Media.** These are complex media prepared from raw materials such as meat extracts, peptones, yeast extract, and various sugars. They support the growth of a wide variety of microorganisms.

### COMMON USES FOR MEDIA

**General Purpose Media.** These support the growth of a large variety of microbes, particularly chemoheterotrophs. For example, nutrient agar consists of beef extract, peptone, and agar all dissolved in water; it can be used to culture many commonly encountered organisms. Certain other organisms have more complicated requirements for certain compounds and are called fastidious chemoheterotrophs. These latter organisms must be cultured on general purpose media which have been enriched or supplemented with the particular growth factors needed by the specific organisms.

**Differential Media.** These media often contain reagents or chemicals that produce differences in growth that allow the observer to differentiate between types of microbes. For example, if a mixture of organisms is cultured on blood agar medium, some of the bacterial colonies may hemolyze the blood cells and produce clear areas in the agar. This permits one to distinguish between hemolytic and non-hemolytic bacteria. pH indicator dyes may also be used to distinguish between growth producing acidic, neutral or alkaline end products.

**Selective Media.** These media allow for the growth of only certain types of organisms due to addition of inhibitors or a specific carbon source. For example, addition of sodium azide to a medium would inhibit the growth of organisms using respiration but would permit growth of organisms using only fermentation. Enrichment culture techniques exploit the principle of survival of the fittest to favor the growth of certain organisms. These techniques often involve the use of media that permit one type of organism to outgrow all others. For example, if a particular medium contains lactose as the sole fermentable carbohydrate, then the lactose-fermenting organisms present in a mixed culture would tend to outgrow the other types.

### INGREDIENTS COMMONLY USED IN MEDIA

Familiar compounds such as sodium chloride and hydrochloric acid are used both in the chemistry lab and in media making, but some less familiar ingredients are commonly used in microbiological media preparation. Some of these are:

**Beef Extract.** Beef is boiled and the resulting broth is concentrated into a paste or dried to a powder. It contains amino acids, vitamins and minerals.

**Peptone.** Protein is converted into proteoses, peptides and amino acids by means of enzymatic hydrolysis.

**Yeast Extract.** This is the water soluble portion of autolyzed yeast and contains amino acids, nucleic acids, vitamins and other growth factors.

**Agar.** This is a complex carbohydrate material refined from marine algae and used to produce semi-solid media. It solidifies at temperatures between 40 °C and 45 °C and will not remelt until it is boiled. It is not used as nutrient material except by some marine microbes.

## PURE CULTURE TECHNIQUE

When a **single** viable bacterial cell is deposited on a solid agar medium, it normally reproduces to form a cluster or colony of cells. Usually an isolated colony constitutes a pure culture, since it is considered to consist of similar cells all derived from the same original cell. Isolated colonies can be obtained to produce the pure culture that is needed before making a detailed study of any given organism. The **streak plate method** is one simple and useful technique for isolating bacteria and other microorganisms in pure culture and is routinely used in all types of microbiology.

### PURPOSE

To isolate a pure culture of bacteria by using the streak plate method.

### MATERIALS

One pre-dried plate of solid media and a bacterial culture on solid or in liquid media,.

### PROCEDURE

1. The plates have been pre-dried to reduce the amount of spreading by motile organisms. Mark the bottom of the plate with your initials.
2. Use a loop to transfer culture from liquid media or a needle to transfer culture from solid media to sector 1.
3. Streak the plate, using a method similar to the one shown at the right. Try to streak as much of the plate surface as possible. The objective is to streak fewer organisms on each of the four consecutive sectors in order to obtain isolated colonies. Use a needle for the remaining sectors. Take care to flame the needle **after** streaking each sector. **Lightly** re-inoculate the needle into the previous sector before streaking the next one.
4. Invert the inoculated plate and incubate it the indicted temperature.
5. Examine the plates to determine if well-isolated (i.e., pure) colonies are present.

