Lesson 1:
The Scientific Method

Agricultural biotechnology is a rapidly expanding area of research. This growth makes the documentation of research methods, procedures, and results vitally important. With so many researchers exploring the applications of agricultural biotechnology, proper research methods and proper documentation of those methods is necessary. The scientific method helps researchers organize their experiments and allows other researchers to duplicate their work, which adds credibility to the results. Patent applications have been rejected or slowed due to poor documentation of research or poor research methods, both of which make replicating the research difficult. This lesson will outline the proper method of designing biotechnology research efforts.

The Scientific Method

The scientific method is a way of addressing scientific questions that provides a rational and structured system for research. A single biotechnology research effort may cost hundreds of thousands of dollars or more. A solid research approach is necessary to ensure the wise use of funds. Biotechnology researchers use the time-tested structure of the scientific method when conducting research.

The scientific method used by biotechnology researchers has six major parts. The first step in using the scientific method is to identify the problem to be investigated in a problem statement. The problem statement expresses the general purpose of the research. Knowledge about the problem or question for which an answer is being sought is required. The problem must be stated in such a way that it will lead to experimentation that will solve the problem. A sample problem statement is “Some plants in a corn field have yellow leaves.”

After the problem has been identified, the second step is an investigation into previous research to identify alternate explanations or solutions to the problem. This activity leads into the third step of the scientific method, which involves formulating a hypothesis, or educated guess, about the anticipated outcome of the research. The hypothesis is essential, since experiments are developed to validate or invalidate this statement. It must be a focused and detailed statement that can be tested for accuracy. A hypothesis for the problem statement in the preceding paragraph is “Nitrogen deficiency causes the corn plants’ leaves to be yellow.” Since the hypothesis will direct the methods of experimentation, the hypothesis is the most important statement made by the researcher.

The fourth step is to design an experiment that will accurately test the hypothesis. This step is also a critical step. If the hypothesis is correct but poor experimental methods are used to test it, the chance of obtaining usable results is reduced. Selecting experimental methods for research requires knowledge of the available testing procedures and their advantages and limitations.

The fifth step of the scientific method is to conduct the experiment and collect data. When experimentation is complete, the final step is to draw conclusions about the success of the experiment by analyzing the information it provides. Based on this information, the researchers will accept or reject the hypothesis. The results of the experiments are examined, and evidence about answers to the original problem is detailed. As a part of this step, the limitations of the research results must also be explained. The conclusions collected must be supported by evidence in order to be valid.

Many researchers must add an additional step to this process. After the experiments have been chosen to test the hypothesis, researchers generally have to write a proposal. This proposal is directed at one or more funding agencies that provide funding to support research efforts. Researchers who work for private companies do not normally write a formal proposal, but they must still convince the company that the proposed research is worthwhile.

Importance of the Scientific Method

A logical question to ask when discussing the scientific method is simply, "Why is the scientific method important for research?" Three main reasons for its importance exist. First, the scientific method provides a logical approach to solving a problem. Scientific research, including research in biotechnology, is essentially a search for the unknown. The search for an unknown requires a basic, comprehensive search beginning from a given point. The scientific method helps researchers to analyze the known information and to select the best way to find the desired answers. Second, the scientific method is important because it helps force researchers to examine their research objectively. The structure of the scientific method aids in identifying alternative answers to research questions since the researchers must carefully examine existing research in the area of interest. A final reason that the scientific method is important is that its use allows other researchers to repeat the experiments. Other researchers need to be able to duplicate the results of an experiment before its validity can be fully established. Research that follows the scientific method is more easily understood than research that does not follow that structure. Research must follow the scientific method to gain professional credibility.

Laboratory Notebooks

Laboratory notebooks serve an important function in biotechnology research. Notebooks provide a detailed account of the day-to-day activities of the experimental process. These notes are vital in examining directions for future research and for proving that specific research was done at a set time, which is required for patent applications. When research is not successful, researchers often review laboratory notebooks so that new research efforts can better address the problem. A notebook should be bound and written using permanent ink. It should also be complete, covering actual activities and all observations made. The laboratory notebook should have a cover that identifies the subject of the research and a table of contents.

Information from individual experiments is recorded on laboratory sheets. These sheets contain six major sections. The first section is for the title of the experiment, the date, and the name(s) of the investigator(s). The second section should briefly describe the purpose of the experiment. A list of materials needed should be included next. The procedures for the experiment should be outlined in the fourth section. These procedures should be detailed and include specific quantities of substances used, as well as the precise methods of using them. The next section should be a record of experimental results in the form of data and observations. Finally, the conclusions drawn from the research are recorded at the end of the laboratory sheet.

Summary

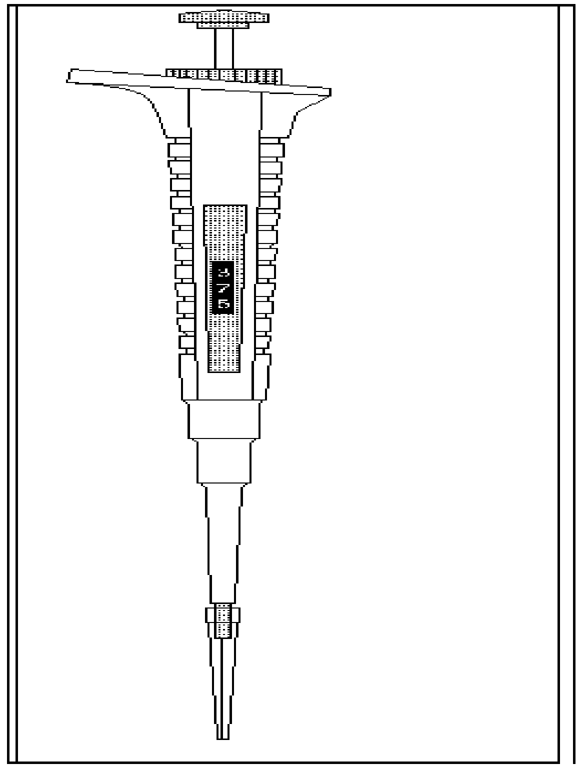
The scientific method is an important tool in research for biotechnology. Without the logical structure of the scientific method, research would involve a blind search for answers. The six parts of the scientific method help researchers organize their research efforts and increase the likelihood of success. The laboratory notebook is an important source of documentation for researchers. This notebook should be a complete, day-to-day account of the research.

Credits

Peterson, Dennis R., and Thomas Relberger. *Biotechnology in Agriculture*. Stillwater, Okla: Mid-America Vocational Curriculum Consortium, Inc., 1992.

Raven, Peter H., and George B. Johnson. *Understanding Biology*. St. Louis: Mosby, 1988.

Lesson 2:
Laboratory Equipment and Techniques



The heart of biotechnology is the research laboratory. Biotechnology laboratories are equipped with many types of tools and instruments. Individuals involved in biotechnology should be familiar with the equipment used in these laboratories as well as special procedures for developing and maintaining suitable working conditions that are free of contaminants.

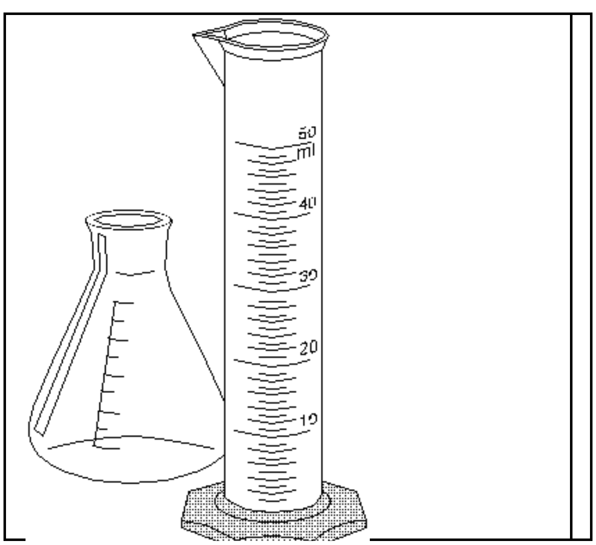
Laboratory Equipment

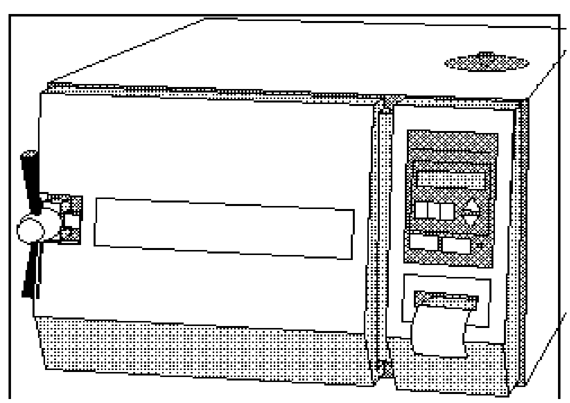
Laboratories where biotechnology research is conducted contain many different types of laboratory equipment. The specific equipment found in a given laboratory will vary based on the type of research being done. However, most laboratories have a basic set of equipment.

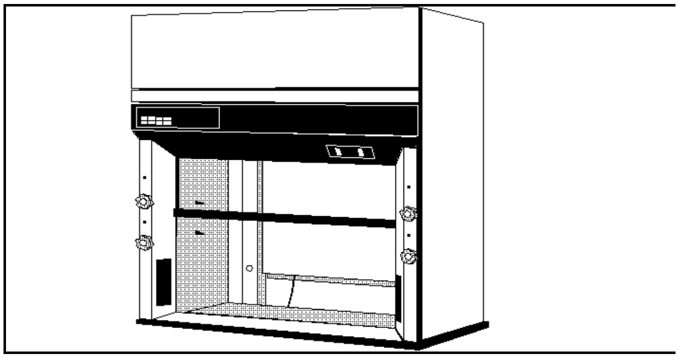
The pipettor (Figure 2.1), which is used to measure and transfer amounts of liquid smaller than one milliliter (ml), is a common tool in biotechnology laboratories. Pipettors are available in three main sizes: zero to 20 microliters (μl), 20 to 200 μl, and 200 to 1,000 μl.

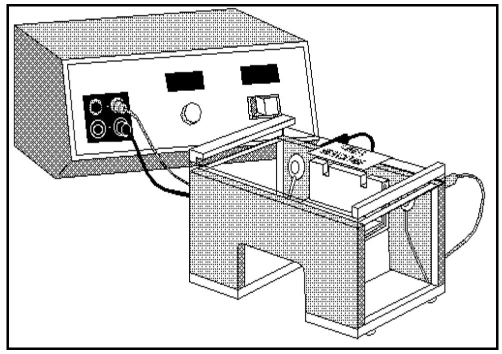
A variety of plastic ware and glassware is used in the biotechnology laboratory. Pipette tips, test tubes, and centrifuge tubes are examples of plastic ware used in a laboratory. Petri dishes made of plastic or glass are the containers most commonly used for growing bacteria or tissue cultures. Glassware used in laboratories includes beakers, flasks, graduated cylinders, and test tubes. Figure 2.2 shows some examples of plastic ware and glassware.

Researchers sterilize most of the plastic ware and glassware in an autoclave, which is illustrated in Figure 2.3. An autoclave uses steam under high pressure for sterilization.









A fume hood (Figure 2.4) is an enclosure that vents air to the outside. Fume hoods allow researchers to use chemicals with dangerous or noxious fumes. They are commonly sterilized with ultraviolet light or a 70 percent alcohol solution when researchers do tissue culture and other sensitive procedures.

Another piece of equipment needed for tissue culture and the propagation of bacteria is an incubator, which is shown in Figure 2.5. An incubator maintains a preset temperature that provides an optimum climate for cell cultures to grow.

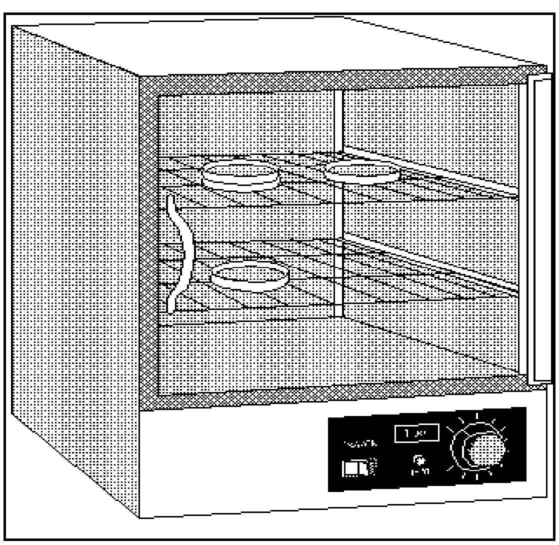
An electrophoresis unit (Figure 2.6) is a common piece of equipment. Electrophoresis separates DNA fragments by size using an electric current. The electrophoresis unit is like a sieve for separating these microscopic fragments. The fragments are shown on an electrophoresis gel.

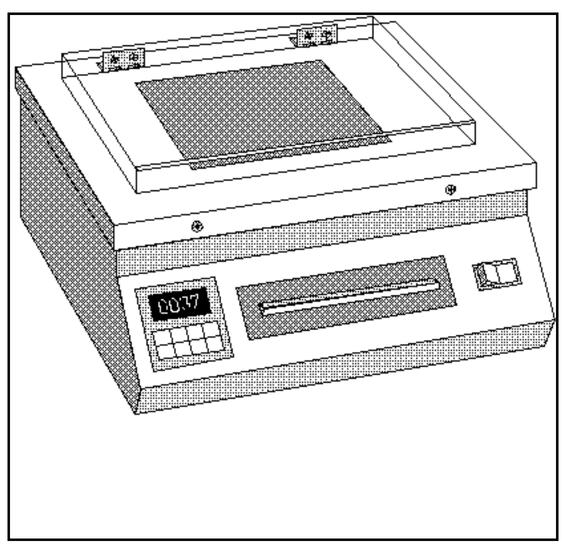
A A transilluminator, shown in Figure 2.7, is used to view an electrophoresis gel. A transilluminator illuminates the gel by passing a shortwave ultraviolet light through it. Regular light will not show the dyes used to stain the gel.

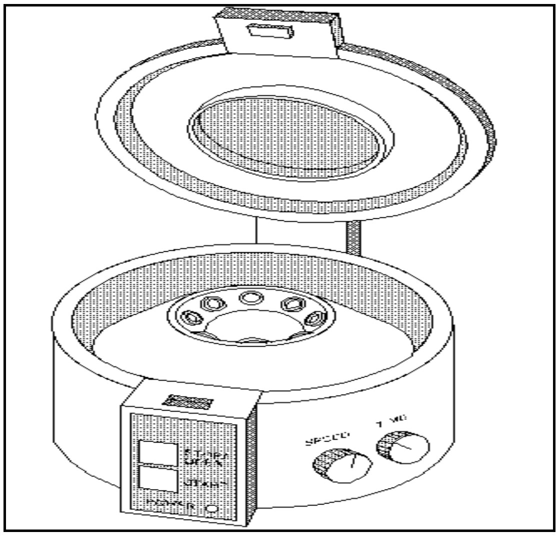
Polymerase chain reaction (PCR) equipment is also found in biotechnology laboratories. PCR is a method of increasing the quantity of DNA in a sample by heating and cooling the DNA to break it down and force it to replicate. PCR equipment takes various forms, from a series of water baths to the newest automated form, the thermocycler (Figure 2.8).

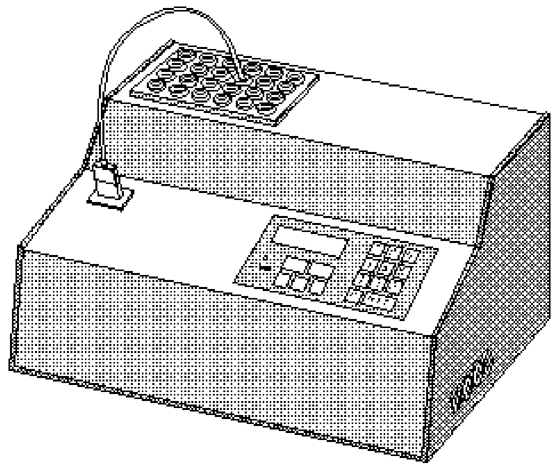
DNA is separated from a liquid by using a microcentrifuge, which is illustrated in Figure 2.9. A microcentrifuge is essentially a high power spinner that uses centrifugal force to separate solids, such as DNA, from a liquid.

A vortex is a vibrating mixer used on test tubes. It mixes a solid or liquid with a liquid. A test tube is placed on a small rubber cap that vibrates in a circular motion, which causes the contents of the test tube to mix.









The microscope is an important device for enlarging and viewing organisms or specimens that are not visible to the naked eye. Two basic types of microscopes are used in biotechnology laboratories. The dissecting microscope is a low-power microscope that magnifies 10 to 100 times. This microscope is used in embryo transfer and tissue culture techniques. The second type of microscope is a general laboratory microscope that magnifies 100 to 1,000 times.

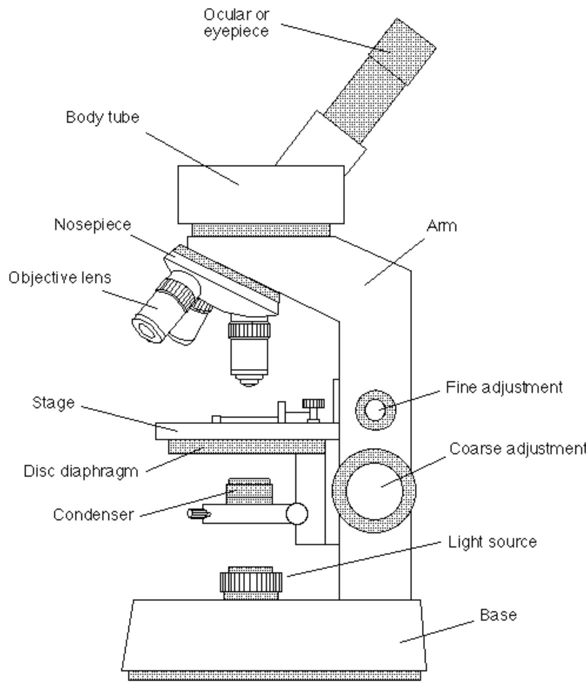
The Microscope

A microscope (Figure 2.10) has many parts. The ocular, or eyepiece, is the initial point for viewing a specimen and contains the first lens system, which normally magnifies the specimen 10 times (10X). The second lens system is called the objective. It projects the magnified image up through the ocular. Most microscopes have two or three objectives that vary in their degree of magnification. A rotating piece called the nosepiece holds the objectives.

The body tube of the microscope holds the ocular and the objectives the correct distance apart. The arm is the curved support that connects the body tube to the base. The base is the stand on which the microscope rests. Slides containing the specimens to be observed are placed on the stage, which has clips to hold the slide in place.

The disc diaphragm contains a series of different sized openings that control the amount of light shining on the specimen. The light comes from a light source like a mirror or small electric lamp. On microscopes with a lamp, a condenser focuses the light on the specimen.

The two main dials for adjusting a microscope are the coarse adjustment and the fine adjustment. The coarse adjustment is the larger of the two dials; it is always used first to focus on a specimen with the low power objective. The fine adjustment refines the focus. With the high-power objective, only the fine adjustment is used to focus the microscope.



Procedures for Manipulating Microscopic Specimens

A specimen viewed under a microscope must be very thin since it is placed, or mounted, on a glass slide. If a wet mount is needed, a drop of water should be added to the specimen. Usually, a cover slip is placed on top of the specimen; gently pressing on the cover slip will remove air bubbles. To view some specimens correctly, they must be stained. The correct staining procedures vary greatly from specimen to specimen.

Once the slide is prepared, it is placed on the stage and secured by the clips. Next, the light source must be turned on and adjusted so that light passes through the specimen. The low-power objective is then selected, and the coarse adjustment is used to focus the specimen's image. If more magnification is needed, the high-power objective is selected, and the fine adjustment is used to focus the image. The fine adjustment should only be used to move the objective up and away from the stage. If either the coarse adjustment is used or the fine adjustment is adjusted toward the specimen slide while using the high-power objective, damage to the objective can result.

Aseptic Techniques

Aseptic techniques are procedures used to create and maintain a work area free of bacteria and other microorganisms that might contaminate delicate experiments. A sterile environment is necessary for procedures such as tissue culture or the propagation of bacteria. Some aseptic techniques are described below.

- *Controlled air movement* - The researcher works in an enclosed chamber that allows the flow of air to be controlled.

- *Disinfection* - The work area is disinfected with a 10 percent bleach solution. Then the instruments and work area are sprayed with a 70 percent ethanol solution and allowed to air dry.

- *Scrubbing up* - The researcher scrubs his or her hands and arms thoroughly and allows them to air dry. He or she then sprays them with a 70 percent ethanol solution.

- *Sterilization* - Researchers use an autoclave to sterilize all materials and instruments. An ultraviolet light kills microorganisms in the work area.

A researcher may maintain an aseptic work area by using a shield to avoid breathing on an experiment. He or she should also avoid sneezing or coughing in the work area. When using a fume hood, researchers should use the rear portion of the enclosed area to reduce exposure to bacteria that might enter the area.

Importance of Aseptic Techniques

Experimental procedures like tissue culture and most DNA analysis techniques require proper aseptic techniques to be successful. Contaminants will destroy many biotechnology experiments, so the work environment must be free of them. Bacteria, viruses, and other microbes can interfere with many laboratory procedures.

Summary

Many different types of laboratory equipment are used in biotechnology research. A basic piece of equipment is the microscope, so understanding its parts and their functions is important. Researchers must also know the procedures for manipulating microscopic specimens. Researchers in biotechnology should practice aseptic techniques to prevent the contamination of their work.

Credits

Peterson, Dennis R., and Thomas Rehberger. *Biotechnology in Agriculture.* Stillwater, Okla.: Mid-America Vocational Curriculum Consortium, 1992.

Williams, O., E. Bonde, and N. Younggren. *Laboratory Exercises for Biological Sciences*. Philadelphia: Lea & Febiger, 1963.

Armitage, Kenneth B. *Investigations in General Biology.* New York: Academic Press, 1970.

Lesson 3:
Biotechnology Laboratory Safety

Biotechnology researchers use many types of equipment, chemicals, and specimens in their work; the correct use of all three is vital to the safety of the researchers. Laboratory safety is a very high priority in biotechnology laboratories. The ability to handle chemicals and specimens safely and to use equipment correctly is necessary for employment. A knowledge of emergency procedures for chemical spills and fires is also critical. This lesson highlights some of the most important safety precautions and concerns.

Common Biotechnology Laboratory Safety Concerns

Common biotechnology laboratory safety concerns fall into five major categories: microorganisms, chemicals, radioactivity, electrical hazards, and physical hazards. The use of bacteria and fungi is common in biotechnology laboratories. Some microbes, called pathogenic microbes, are dangerous because they are capable of causing disease. These types of microbes require special containment laboratories and are not used in school laboratories. However, all microorganisms should be handled properly, since even nonpathogenic microorganisms can be harmful in certain cases. Care should be taken to follow aseptic techniques strictly in order to contain microorganisms.

Many types of chemicals are used in biotechnology laboratories, including solvents, enzymes, dyes, and buffers. These chemicals are safe if handled correctly. Care must be taken to avoid contact with the skin. Many chemicals can be absorbed through the skin or spread onto other surfaces by contact. Most of the dyes and buffers are toxic if ingested. Use of these chemicals should be limited if a suitable alternative is available.

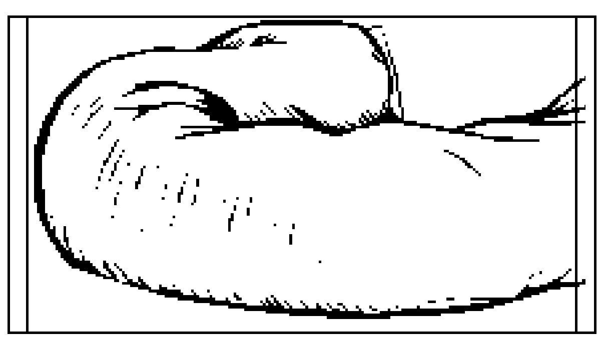
Radioactivity is used in biotechnology laboratories for probes or markers that verify the transfer of DNA segments. A special permit is required to use radioactive materials. To obtain a permit, researchers must complete training about the safe handling and disposal of radioactive substances. Radioactive materials are not used in school laboratory settings.

Electrophoresis equipment can be an electrical hazard. This equipment is safe if used properly. However, if safety precautions are not observed, electrical shock can occur. An individual should never touch the gel solution while the machine is on.

Centrifuges and ultraviolet lamps used in biotechnology laboratories are considered physical hazards. A centrifuge should have a lock that prevents the lid from opening while the machine is spinning. This safety feature prevents fingers from being caught in the rotating machine, which can cause serious injuries. With prolonged exposure, ultraviolet radiation from a transilluminator can damage retinas and bare skin.

Cleaning Up Spills

The possibility of a spill exists whenever chemicals are handled. When the spilled chemical is known, clean up procedures for that specific chemical should be used. The proper procedures are outlined on a Material Safety Data Sheet (MSDS). Chemical suppliers develop these sheets and ship them with all chemicals. The sheets must be kept in a specific notebook or file for easy reference. The MSDS provides a variety of information about the chemical, including its toxicity level, first aid measures, required personal protective equipment, and disposal procedures.



When specific clean up procedures are not available or the content of the spill is unknown, special procedures must be followed. A spill pillow (Figure 3.1) is used to absorb any liquid chemical. The used spill pillow should be regarded as hazardous waste and disposed of appropriately. If the spill is a solid or a powder, it can be gently swept into a glass container and disposed of as hazardous waste. The spill area should be cleaned with a disinfectant and an ethanol solution to ensure that any remaining traces of the chemical are removed.

Disposal of Biotechnology Laboratory Waste

Biotechnology laboratories commonly produce waste products classified as hazardous waste, which must be disposed of appropriately. Simply dumping everything down the drain is not acceptable. Some of the waste generated by a biotechnology laboratory can be decontaminated and thrown away with other trash to be placed in a landfill. All cultures and equipment that have come in contact with infectious microbes must be autoclaved or disinfected with hospital-type disinfectants before being thrown away. Examples of chemicals requiring special disposal are organic solvents and highly toxic chemicals. The proper disposal method for a chemical substance is found on the chemical's MSDS. Improper disposal of hazardous waste endangers the environment, and a company may receive large fines if OSHA or the EPA discovers that proper disposal procedures are not being followed.

Emergency Fire Procedures

Biotechnology laboratories, like all laboratories, must be prepared for emergencies. A fire exit plan should be posted in the laboratory. In a research laboratory, just as in a classroom laboratory, the fire exit plan should be followed if a fire breaks out and should be practiced during fire drills. Everyone should know the location of the fire extinguisher, the fire blanket, and the fire alarm switch. If a fire occurs in a classroom lab, students should immediately notify the instructor and begin exiting the room.

Personal Protective Equipment

Personal protective equipment (PPE) can help prevent injury to laboratory workers. All laboratories require workers to wear safety glasses or goggles while in the laboratory. They also require latex or other appropriate types of gloves for most laboratory work. Normally, workers use disposable latex gloves. Lab coats or aprons should be worn to protect clothing. Shorts, short skirts, and sandals are not permitted because they expose too much skin to the laboratory environment.

Injuries in the Laboratory

Anyone working in a laboratory should know what to do if an injury occurs. Simple first aid procedures, like applying pressure to stop blood loss or flushing skin or eyes with water if they come in contact with chemicals, should be done immediately. In a classroom lab, the instructor should also be notified without delay so that he or she can follow the school procedure for emergencies. Students should always read and follow any precautions noted in a laboratory exercise to help avoid injury.

Laboratory Ventilation

Most biotechnology laboratories do not require special ventilation. A fume hood vented to the outside is necessary when using chemicals that produce bad odors or harmful vapors. Because it is enclosed, the fume hood may also be used to prevent contamination by serving as a sterile environment for certain laboratory procedures. Some chemicals may only be stored in a lockable ventilated storage cabinet. Ventilated storage cabinets are designed to prevent the buildup of gases that can cause an explosion or fire.

Summary

To work in a laboratory, biotechnology researchers must be able to handle microorganisms, chemicals, radioactivity, electrical hazards, and physical hazards safely. Researchers must wear personal protective equipment and be aware of laboratory hazards.

Credits

Collins, C. H., ed. *Safety in Biological Laboratories*. New York: John Wiley & Sons, 1985.

Peterson, Dennis R., and Thomas Rehberger. *Biotechnology in Agriculture.* Stillwater, Okla.: Mid-America Vocational Curriculum Consortium, 1992.

National Centre for Biotechnology Education. “Safety Guidelines.” http://134.225.167.114/ncbe/safety (7 September 1997).