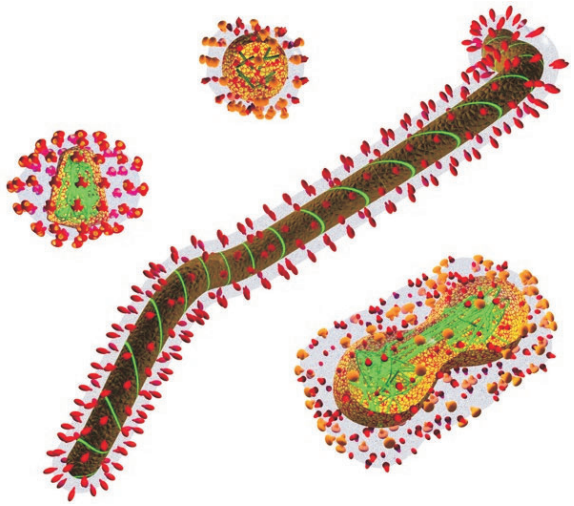
Using Aseptic Techniques in the Biotechnology Laboratory

THE LAST NATURAL CASE of smallpox occurred in Somalia in 1977. In 1978, an English photographer—who worked on the floor above a laboratory doing work with the smallpox virus—was exposed to smallpox due to inadequate safety procedures in a lab. She developed smallpox. Since no one suspected smallpox, she was not given the proper treatment until it was too late. Her death was the last smallpox death before the disease was declared eradicated.



Objective:

Describe the importance of basic aseptic techniques in the biotechnology laboratory.

Key Terms:

aerosols
aseptic techniques
autoclave
biological entities
disinfectant
HEPA
laminar airflow hood
media
sterilized

Understanding Aseptic Techniques in Biotechnology Laboratories

Aseptic techniques are the procedures used to keep biological materials contained. Aseptic literally means "without infection," but it is often used as a synonym for "sterile" or "without microorganisms." Aseptic techniques are used to protect the people who work with potentially harmful organisms and to protect other people as well as the environment from exposure. Keeping the work materials pure for successful, meaningful scientific work is important.

IMPORTANCE OF ASEPTIC TECHNIQUES

Protecting the worker, others, and the environment primarily relies on keeping the **biological entities** (microorganisms, cells, or significant sub-cellular parts) in containers. When they need to be transferred or treated, the process must be done under controlled conditions.

Protecting the Worker and the Environment

Workers are protected by protective equipment (e.g., lab coats, gloves, and safety glasses), which is used to prevent exposure. The laboratory work area should be cleaned with a **disinfectant**—a harsh chemical that kills or prevents the growth of microorganisms. Transfer devices need to be sterilized after transfers to prevent additional contact with people or the environment. Containers of biological entities should be kept closed. When opened for transfer, the container should be opened as infrequently and for as short a time as possible. Keep any agitation to a minimum to prevent the formation of **aerosols**—clouds of liquid particles in the air that can easily escape when you open containers. All materials and equipment exposed to biological entities must be **sterilized** (made free of live microorganisms) before reuse or disposal. When necessary, a **laminar airflow hood**—a device to move air through a filter capable of removing biological entities—should be used. Also, eating and drinking are prohibited in the laboratory.

FIGURE 1. Disinfectants are commonly used to clean laboratories.

FURTHER EXPLORATION...

ONLINE CONNECTION: Safety for Laboratory Workers

For laboratory workers to be safe in a biotechnology laboratory, they must be trained to perform aseptic techniques. In addition, they must use proper aseptic techniques every time. The potential for serious consequences is very real.

Working in educational settings, researchers and technicians learn the techniques with relatively harmless materials. As they progress to more serious laboratory work, the potential for life-threatening accidents becomes probable. The need for continued training and insistence on upholding proper technique is critical.

To find out more about possible consequences of flawed techniques or inadequate training, visit the link below:

http://www.sunshine-project.org/publications/pr/pr030707.html

Protecting the Work

Protecting the work or maintaining the purity of the biological entities is important to science. It is critical to know that the organisms or cells being worked on are of one type or too many variables may be introduced. Keeping the culture pure is an extremely important part of the aseptic technique.

A laminar airflow hood that filters the air before it reaches the work is useful in keeping the cultures pure. This workstation should be disinfected before and after work to minimize the chances for contamination. All culture vessels and **media** (the water, food, and nutrients to support culture growth) need to be sterile. Transfer tools (e.g., loops and pipettes) need to be sterile before use. For many biotechnology applications, materials (e.g., reaction tubes) need to be sterile and free of outside DNA, RNA, and any chemicals that might interfere with the intended process.

FIGURE 2. A researcher is making a transfer and is using aseptic techniques.

BASIC ASEPTIC TECHNIQUES

There are a number of basic aseptic techniques for working in the biotechnology laboratory. It is necessary to think about how each technique keeps the biological entity where the researcher wants it to be and not anywhere else.

Laboratory Workers and Workstations

When you enter a biotechnology laboratory, you should first remove any jewelry you are wearing, and then wash your hands. Next, put on a lab coat or apron, safety glasses, and gloves. Your workstation should be sprayed with a disinfectant (e.g., sodium hypochlorite or bleach, ethyl alcohol, or Lysol) and wiped with a paper towel. Let the surface dry before using it. In addition, do not use alcohol around an open flame.

When using potentially harmful microorganisms, tumor-based animal cells, any human cells, or most plant cells, it is necessary to work in a laminar airflow hood. The hood uses a **HEPA** or high energy particle air filter that will remove particles larger than 0.3 μm to prevent any potentially harmful exposure to the work or the environment. Basic biotechnology work with nonpathogenic organisms can be performed without the use of a hood.

Tools

Containers and transfer devices need to be sterile before use. Glassware can be heated dry in an oven at 325°F (163°C) for two hours or sterilized in an autoclave at 250°F (121°C) for 20

minutes. The **autoclave** is a pressure vessel that heats steam above 212°F (100°C) by increased pressure. Plastic containers may be autoclavable or disposable. The disposable containers usually are sterilized by ethylene oxide gas. The sterility needs to be guarded by handling as little as possible.

FIGURE 3. A medical autoclave.

Transfer devices can be presterilized and carefully dispensed disposables (e.g., plastic loops, pipettes, and micropipette tips). More permanent metal transfer devices (e.g., loops) need to be heated to sterilize them before and after each transfer. This can be accomplished by flaming in a Bunsen burner. You must be careful after a transfer to heat the loop slowly to dry any liquid so it does not splatter. An alternative heating device is a Bacti Cinerator—an enclosed electric heater that will heat the device hot enough to sterilize and will contain any splatter.

To maintain an aseptic process, all procedures should be planned to facilitate opening containers infrequently and for short periods of time.

Media

All media and treatment solutions must be sterile. Most culture materials can be autoclaved to sterilize. Depending on the container, the liquids may be autoclaved in their containers. For small containers (e.g., petri plates), the media must be autoclaved in a larger vessel and poured into the sterile plates under aseptic conditions.

Some specialized nutrients or growth stimulators cannot be heated to autoclave temperatures. These materials are sterilized by running them through a very fine filter. The solution is then added to sterile media, again with aseptic procedures.

Disposal

All materials must be sterilized before disposal. Glassware—with or without media—is autoclaved before media disposal, and then glassware is washed. Disposable equipment is chemically disinfected or placed in autoclavable bags and sterilized before disposal.

Summary:

Aseptic techniques are the procedures used to keep biological materials contained. Aseptic techniques are used to protect the people working with potentially harmful

organisms, other people, and the environment. The techniques also keep the work materials pure for successful, meaningful scientific work.

Through the use of proper tools and procedures, potentially harmful biological entities are kept contained. These entities must be transferred with care. Starting with a clean environment and carefully executing each movement ensures that everything stays where it should be. All containers, media, and transfer devices must be sterile before use. In addition, all tools and materials must be sterilized after use to dispose of them safely.

Checking Your Knowledge:

1. What is the aseptic technique?
2. What are aseptic techniques designed to protect?
3. What must you never do in a biotechnology laboratory?
4. How are media sterilized prior to use?
5. What must be done to laboratory materials before they are cleaned for reuse or disposal?

Expanding Your Knowledge:

Prepare a short set of interview questions. Then contact a local hospital or a large clinic to determine the approximate percentage of time and money a laboratory spends on aseptic procedures.

Web Links:

Aseptic Technique
http://en.wikipedia.org/wiki/Aseptic_Technique

Protocol: Sterile Techniques
http://www.protocol-online.org/prot/General_Laboratory_Techniques/Sterile_Technique/

Good Practices
http://www.eurobiobank.org/common_docs/Good_practices_for_cell_culture_techniques.doc

Agricultural Career Profiles
http://www.mycaert.com/career-profiles