

Aseptic Techniques in Mammalian Cell Culture

Introduction

We're going to discuss one of the most critical aspects of working with mammalian cell cultures—maintaining sterility to prevent contamination. Whether you're working in research, biotechnology, or pharmaceuticals, understanding and mastering aseptic techniques is crucial for successful and reproducible experiments.

In this lecture, we'll explore essential sterility practices in cell culture. This includes proper usage of laminar flow hoods, sterilization of equipment, and maintaining a sterile environment to ensure the integrity of your cell cultures. Let's begin by understanding why sterility is so important in mammalian cell culture.

Why Sterility is Crucial in Cell Culture

Sterility in mammalian cell culture ensures that the cells you are growing are not contaminated by unwanted microorganisms such as bacteria, fungi, or viruses. Contamination can cause:

- **Cell death:** Uncontrolled microbial growth can outcompete your cells for nutrients and space.
- **Altered results:** Contaminants may affect your experimental outcomes by introducing unwanted variables.
- **Resource wastage:** Contaminated cultures may need to be discarded, leading to lost time, effort, and resources.

Aseptic Techniques: The Foundation of Sterility

Aseptic techniques are the practices used to prevent contamination of cultures. These practices are designed to limit the exposure of your cell cultures to harmful microorganisms. To perform them correctly, you must be diligent, methodical, and aware of your surroundings.

1. Proper Use of Laminar Flow Hoods

Laminar flow hoods are the cornerstone of sterile cell culture work. They provide a sterile working environment by using high-efficiency particulate air (HEPA) filters that remove airborne contaminants.

Types of Laminar Flow Hoods:

- **Class I:** Air flows inward to protect the worker, but not the sample. Primarily used for powder handling or non-sterile procedures.

- **Class II:** Air flows inward and down over the work surface, protecting both the worker and the sample. This is the most common type used in cell culture.
- **Class III:** Completely enclosed and provides the highest level of protection for both the sample and the worker, typically used for highly hazardous materials.

Key Steps for Using Laminar Flow Hoods Correctly:

- **Preparation:** Before beginning work, clean the hood with 70% ethanol or an appropriate disinfectant. Always start by turning on the flow hood for at least 10-15 minutes to allow the air to stabilize.
- **Organizing your workspace:** Place only necessary items inside the hood, ensuring that there's minimal disruption to airflow. Avoid overcrowding to maintain laminar airflow.
- **Movement:** Work slowly and deliberately. Sudden movements disrupt the sterile airflow. Keep your hands and tools inside the hood and avoid waving your arms around or crossing over sterile surfaces.
- **Sterile Technique:** Always disinfect tools, such as pipettes, forceps, and bottles, with 70% ethanol before use. Maintain the sterile zone within the flow hood and avoid reaching across the work surface.

Common Mistakes to Avoid:

- Talking over the hood or touching the front grill, which can introduce contaminants.
- Using unsterilized items or failing to properly disinfect tools before use.

2. Sterilization of Equipment

Sterilization is critical for ensuring that no microorganisms are introduced into your cultures via tools, media, or containers.

Types of Sterilization Methods:

- **Autoclaving:** The most common method for sterilizing equipment in cell culture labs. Autoclaving uses high-pressure steam to kill microbes. Typical parameters are 121°C for 15-30 minutes, but these can vary depending on the size of the equipment.
 - **Items to autoclave:** Glassware, pipettes, plasticware, and media containers.
 - **Caution:** Ensure that materials are autoclave-safe. Some plastics may deform or melt at high temperatures.
- **Chemical Sterilization:** This involves using chemicals like ethylene oxide (EO) or formaldehyde gas for sterilizing equipment. Chemical sterilization is often used for heat-sensitive items, such as certain plastics or electronic components.

- **UV Sterilization:** UV light is commonly used for sterilizing workspaces, especially in laminar flow hoods or biosafety cabinets. However, it does not penetrate deep surfaces, so it should not be relied on as the sole method of sterilization.
- **Filtration:** For heat-sensitive liquids or gases, filtration is used to remove microorganisms. Membrane filters with a pore size of 0.2 μm are typically used for filtering culture media, buffers, and air.

Key Considerations for Sterilization:

- Always check if items are labeled “autoclave-safe” or not, and ensure the sterilization cycle is correctly set.
- Regularly inspect your equipment for signs of wear or damage, particularly filters and seals.
- Store sterilized items in a clean, sealed area to avoid re-contamination.

3. Maintaining a Sterile Environment

In addition to using laminar flow hoods and sterilizing equipment, maintaining a sterile environment in the entire lab is essential.

General Sterile Lab Practices:

- **Cleanliness:** Keep the lab well-organized and free of dust, debris, and spills. Regularly wipe down surfaces with 70% ethanol or other disinfectants.
- **Proper Attire:** Always wear lab coats, gloves, and masks when working with cell cultures. This helps to prevent contamination from human hands, hair, or respiratory droplets.
- **Air Quality:** Use air filtration systems to maintain clean air in the lab. Consider using biological safety cabinets (BSCs) for more sensitive or hazardous work.
- **Refrigerators/Freezers:** Store reagents, media, and cells in properly labeled, clean refrigerators or freezers. Prevent contamination by regularly cleaning these storage areas.

Critical Techniques for Keeping Cultures Free from Contamination:

- **Opening Vessels:** When opening culture vessels (like flasks or Petri dishes), do so slowly and in a sterile environment. Always flame the neck of the container briefly before opening to sterilize it.
- **Handling Cultures:** When transferring cells or media, use sterile pipettes and tips. Always flame the opening of bottles and vials before use, and minimize exposure to open air.
- **Waste Disposal:** Dispose of contaminated materials in biohazard containers and autoclave them before disposal to ensure microbial containment.

4. Handling Contamination

Despite best efforts, contamination can still occur. Here are steps to follow if you notice any contamination:

- **Identification:** Observe any changes in cell appearance or growth patterns. Common contaminants include bacterial growth (cloudy media), fungal contamination (white or greenish growth), or mycoplasma (subtle, often undetectable without specific tests).
- **Isolation:** Immediately isolate contaminated cultures to prevent the spread to other cultures. Dispose of them in biohazard bins after autoclaving.
- **Clean the Lab:** After contamination is detected, thoroughly disinfect all surfaces, equipment, and the laminar flow hood.

Conclusion

Maintaining sterility in mammalian cell culture is vital for ensuring the success of your experiments and the validity of your results. By mastering aseptic techniques such as proper use of laminar flow hoods, sterilization of equipment, and maintaining a clean, sterile environment, you create the conditions necessary for healthy and uncontaminated cell cultures.

Remember, sterile practices are not just about technical skill—they're about building habits and attention to detail. It's the diligence you put into every step, from cleaning the hood to disinfecting tools, that will protect your cultures from contamination and ensure that your work is reproducible and reliable.