Tissue culture Laboratory Safety

Introduction

A cell culture laboratory has a number of specific hazards associated with handling and manipulating human or animal cells and tissues, as well as toxic, corrosive, or mutagenic solvents and reagents.

Common hazards are accidental punctures with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, and inhalation exposures to infectious aerosols.

The fundamental objective of any biosafety program is to reduce or eliminate exposure of laboratory workers and the outside environment to potentially harmful biological agents. The most important element of safety in a cell culture laboratory is the strict adherence to standard microbiological practices and techniques.

1.1 Lab Safety

Safe Laboratory Practices

- Lab coats
- Safety glasses
- Proper footwear
- Hair back
- No food or drink in the laboratory

1.2 Biohazards

An agent of biological origin that can cause disease in humans such as:

✤ Microorganism , Toxin and Allergen

1.3 Biosafety

The combined use of laboratory practices, laboratory facilities and safety equipment. Used biosafety practices to protect workers/students , products/experimental results and environment/laboratory classroom.

1.3.1 Biosafety Levels

Biosafety Level 1 (BL-1) is the basic level of protection and is appropriate for agents that are not known to cause disease in normal, healthy humans. This level is used in the most research and clinical laboratories (Fig.1).

Biosafety Level 2 (BL-2) is suitable when working with agents of moderate-risk that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure (Fig.2). It is different from the BSL-1 in that

(1) Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists.

(2) Access to the laboratory is limited when work is being conducted.

(3) Extreme precautions are taken with contaminated sharp items.

(4) Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

Biosafety level 3 (BL-3) is suitable when working with agents that known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections and that are indigenous or exotic in origin. All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features (Fig.3).

Biosafety Level 4 (BL-4) is the appropriate level when working with Dangerous and exotic agents that poses a high risk of life-threatening disease by infectious aerosols and for which no treatment is available. The agents such as all viruses include Marburg virus, Ebola virus. The Biosafety Level 4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment. The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied positive-pressure personnel suit (Fig.3).



Figure2: A typical Biosafety Level 2 laboratory (Graphics reproduced from CUH2A, Princeton, NJ, USA). (http://www.pakbiosafety.com/topicofthemonth.htm)





Figure4: A typical Biosafety Level 4 laboratory

1.3.1.1 Biosafety Level 2

Biosafety Level 2 is suitable for tissue culture lab.

Examples of BL-2 agents:

- Human blood or body fluids
- E. coli 0157:H7
- Retroviral vectors
- Human cells in cell culture

Standard Work Practices

- 1. Access to the laboratory is limited or restricted when experiments are in progress.
- 2- Prohibiting the use of pipettes mouth and must use mechanical pipetting devices.
- 3- Wash hands frequently
- 4- Decontaminate work surfaces daily
- 5- Handle wastes properly
- 6- Maintain insect and rodent control program

Special Practices

1- The laboratory supervisor puts laboratory policies and procedures whereby identifying people who are allowed to enter the lab.

2- A biohazard sign must be posted on the entrance to the laboratory.

3-Place used slides and coverslips in sharps containers, never in any other receptacle.



Personal Protective Equipment (PPE)

- 1- Lab coat or apron
- 2- Face protection (Safety glasses, goggles or mask...)
- 3- Gloves

4- Biosafety cabinet biological safety cabinets, preferably Class II, or other appropriate personal protective equipment.

Laboratory Facilities

1- The laboratory is designed so that it can be easily cleaned and contain sink for hand washing

- 2- Adequate illumination to avoiding reflections that could impede vision.
- 3- An eyewash facility is available.
- 4- Autoclave available.

5- Locate biological safety cabinets away from doors, windows and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.

6- Lab must be separated from public areas.

1.4 Decontamination

Sterilization: destroy all microbial life, including spores.

Disinfection: destroy a majority of microbial life, but not necessarily spores.

Antiseptic: germistat used on skin to inhibit growth of microorganisms.

Methods:

Heat: steam heat, dry heat, incineration.

Chemical: bleach, ethanol, hydrogen peroxide, ethylene oxide, paraformaldehyde.

Radiation (UV).

1.5 Biological Waste

Types:

Cultures, Sharps, Pipettes, tips and weigh boats

1.5.1 Solids

- 1- Collect and package in clear autoclave bags
- 2- Autoclave to sterilize

3- Dispose of in regular trash dumpster (as long as no chemical or radioactive contaminants are present)

1.5.2 Liquids

- 1- Collect in containers with lids
- 2- Autoclave or treat with 10% bleach to sterilize

3- Dispose of down the drain (as long as no chemical or radioactive contaminants are present)

1.5.3 Sharps (needles, syringes, scalpel blades, slides, blood vials, pasteur pipettes)

- 1- Collect in approved sharps container
- 2- Autoclave to sterilize
- 3- Dispose of in medical waste boxes