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FACULTY OF ENGINEERING & TECHNOLOGY

LT.7 Laboratory safety and biohazard

Outline

1. Laboratory safety
2. General precautions
3. Risk associated with tissue culture laboratory
4. Safety Regulations
5. Biohazard
6. Bio-safety & Bio-safety level
7. Containment

Laboratory Safety

•Working safely to avoid personal as well as injury to other laboratory members working around you is of utmost important.

•Every laboratory has their set of guidelines designed according to national and regulatory bodies guidelines

•The most important element of safety in a cell culture laboratory is the strict adherence to standard microbiological practices and techniques

Common safety risks in cell culture laboratory

•Electrical & Fire hazard

•Exposure to contaminating animal cells, viruses and pathogenic bacteria

•Exposure to toxic, corrosive, or mutagenic solvents and reagents.

•accidental punctures with syringe needles or other contaminated sharps

•inhalation exposures to infectious aerosols

Some of the general precautions for the safety of a tissue culture laboratory

- Strict adherence to recommendations of regulatory bodies.
- Periodical meetings and discussions of local safety committees.
- Regular monitoring of the laboratories.
- Periodical training of the personnel through seminars and workshops.
- Print and make the standard operating procedures (SOPs) available to all staff.
- Good record keeping.
- Limited access to the laboratory (only for the trained personnel and selected visitors).
- Appropriate waste disposal system for biohazards, radioactive wastes, toxins and corrosives.

Risk associated with tissue culture laboratory

Risk associated with tissue culture laboratory can be categorized in following category :

General	Broken glass, Liquid nitrogen, cylinder , tubing
Burns	Autoclaves, hot air ovens, hot plates
Fire	Flaming particularly in association with alcohol
Radiological	Radioisotopes in sterile cabinet, Irradiation of culture
Biological	Importation of cell lines and biopsies, Genetic manipulation, Propagation of viruses, position and maintenance of laminar flow hoods.

Safety Regulation

•Safety regulations are formulated by regulatory authorities to guide safe handling of equipments and safety of personnel working in animal tissue culture laboratory.

•These guidelines are country specific and may not be applicable to all countries.

Safety Regulations

Topic	United States	United Kingdom (generally harmonized with EC Directives)
General	Occupational Safety and Health Administration [OSHA, 2009]	Management of Health and Safety at Work Regulations [1999]
Equipment	Occupational Safety and Health Administration [OSHA, 2009]	Provision and Use of Work Equipment [HSE, 1998]
Chemical	Occupational Safety and Health Administration [OSHA, 2009] National Institute for Occupational Safety and Health [CDC-NIOSH, 2009]	Control of Substances Hazardous to Health [Health and Safety Commission [HSE-COSHH]]
Biological	CDC NIH: Biosafety in Microbiological and Biomedical Laboratories [CDC/OHS, 1999]	Health & Safety Executive, Infections at Work and Genetically Modified Organisms [HSE, 2008a]; Advisory Committee on Dangerous Pathogens [ACDP, 2003, 2005]
Radiological	US Nuclear Regulatory Commission. Medical, Industrial, and Academic Uses of Nuclear Materials [U.S .NRC, 2008]	HSE Radiation Protection publications [HSE, 2008b]
Disposal	National Research Council [2009] Appropriate State Legislature	Department of Health [DoH, 2006]

(Adapted from *Culture of Animal Cells* by Ian Freshney, CRC Press)

Biohazard

The accidents or the risks associated with the biological materials / agent of biological origin capable of causing disease in humans are regarded as biohazards or biological hazards.

Examples:

•Pathogenic microorganisms,

• Toxins, allergens,

•Tissues samples

• Cultures with human pathogens

•Cells subjected to various genetic manipulations (genetically modified organisms),

•Developments of primary cultures,

•Cell lines.

Biohazard waste categories

•Solid Bio-hazrdous waste (non-sharp)

•Solid collection, treatment & Disposal

•Liquid bio-hazrdous waste

•Sharps

Symbol of Biohazard

Control of Biohazard

Biohazards can be controlled to a large extent by strict adherence to the regulatory guidelines and maintenance programmes. Some important aspects are listed.

•Microbiological safety cabinet or biohazard wood with pathogen trap filters have been developed.

•Vertical laminar-flow hood (instead of horizontal laminar-flow hood) reduces direct exposure of the operator to the samples/processes.

•Pathogen containing samples are treated in separate rooms with separate facilities

•(centrifuge, incubator, cell counting etc.).

• Sterilization of all wastes, solid glassware etc. and their proper disposal.

•Facilities for change of clothing while entering and leaving the rooms.

•Strict adherence to the access of designated personnel to the culture rooms

Disposal of biohazard waste

The disposal of biohazards should be as per the guidelines of local regulatory authority.

- Potentially bio-hazardous materials must be sterilized before disposal by autoclaving
- By immersion in a sterilizing agent such as hypochlorite
- Hypochlorite is often used at 300 ppm of available chlorine, but some authorities demand 2500 ppm (a 1:20 dilution of Chlorine).

Biosafety

Definition

•*The discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials.*

• Alternatively, it can also be defined as “The application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards”.

•*Biosafety* defines the containment conditions under which infectious agents can be safely manipulated.

•It describes the microbiological practices, safety equipment, and facility safeguards for the corresponding level of

risk associated with handling a particular agent.

•Depending upon handling of risk associated with agents in tissue culture, there are four ascending levels of containment, referred to as biosafety levels 1 through 4.

Containment principle

The action of keeping something harmful under control or within limits.

Safety in the laboratory is achieved by application of layered, containment principles applied in accordance with the risk assessment to prevent exposure of laboratory workers to a pathogen or the inadvertent escape of a pathogen from the microbiological laboratory.

Safety layers in containment

Safety layers include primary and secondary containment.

Primary: Primary containment provides immediate protection to workers in the biological laboratory from exposure to chemical and biological hazards.

•Primary barriers include biological safety cabinets, fume hoods and other engineering devices used by laboratory technicians while working with a biological hazard.

Secondary Containment: Secondary containment is intended to protect the laboratory worker, the community and the environment from unintended contamination with a biological hazard.

•Secondary containment consists of architectural and mechanical design elements of a facility that prevent worker contamination and escape of pathogens from the laboratory into the environment.

Biosafety Levels

Biosafety Level 1 (BSL-1)

BSL-1 is the basic level of protection common to most research and clinical laboratories, and is appropriate for agents that are not known to cause disease in normal, healthy humans. E.g. Saccharomyces cerevisiae, E.coli K-12 And non-infectious bacteria

Biosafety Level 2 (BSL-2)

BSL-2 is appropriate for moderate-risk agents known to cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. Most cell culture labs should be at least BSL-2, but the exact requirements depend upon the cell line used and the type of work conducted. E.g. *Hepatitis A virus*, *Salmonella, Streptoccoccus pyrogens.*

Biosafety Level 3 (BSL-3)

BSL-3 is appropriate for indigenous or exotic agents with a known potential for aerosol transmission, and for agents that may cause serious and potentially lethal infections. E.g. *Hanta virus*, *Mycobacterium tuberculosis, rabies virus, West Nile Virus, SARS, Corona Virus*

Biosafety Level 4 (BSL-4)

BSL-4 is appropriate for exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available. These agents are restricted to high containment laboratories. E.g. *Ebola virus*, *small pox virus*

MCQs

1. What are risk associated with tissue culture laboratory?
    a. Fire Risk
    b. Biological risk
    c. Burns
    d. All of the above
2. Containment is required to
    a. Protect exposure of pathogenic microbes and virus to laboratory worker
    b. To prevent advertent release of pathogen to environment
    c. Both (a) and (b)
    d. None of the above
3. Bio-safety describes the microbiological practices, safety equipment, and facility safeguards for the corresponding level of risk associated with handling a particular agent. (True / False)
4. Which of the following safety regulation describes the biological safety
    a. CDC/OHS, 1999
    b. CDC NIOS 2009
    c. OSHA 2009
    d. HSE-COSHH

References & Suggested Reading

1. https://www.chabsa.org/index.php?option=com_content&view=article&id=42&Itemid=218
2. https://biosafety.utk.edu/biosafety-program/biosafety-manual/2-principles-of-biosafety/

Further reading

1. Watson, J.D., Gilman, M., Witowski J.and Zoller, M. Recombinant DNA, 2nd ed., Scientific American Books, 1983
2. Glick, B.R. and Pasternack, J.J. Molecular Biotechnology, 3rd ed., ASM Press, 2003
3. Davis J.M. Basic Cell Culture: A Practical Approach, IRL Press, 1998
4. Freshney R.I. Animal Cell Culture a practical approach, 1987